In Situ Product Recovery of Butanol from the Acetone Butanol Ethanol Fermentation

A thesis submitted by

Victoria Outram

For the award of

Engineering Doctorate in Biopharmaceutical Process Development

Biopharmaceutical and Bioprocess Technology Centre

April 2018

Abstract

From 1916 the “acetone butanol ethanol”, or “ABE”, fermentation process was the main production method for n-butanol. It was superseded in the 1950s by a more economical petrochemical process, causing the majority of plants to cease operation. In the fermentation, product inhibition led to low productivity and high energy demand in the downstream processing, making the process unable to compete with the petrochemical route. Overcoming these problems could revive the ABE industry and promote a bio-based economy.

In situ product recovery (ISPR) can be applied to the fermentation process to counteract the effects of product toxicity. Productivity increases of greater than 300% are theoretically possible. Many ISPR techniques have been applied to the ABE process at laboratory scale, but a direct comparison of the different techniques has been hindered by experimental inconsistencies. Here, a techno-economic analysis was performed to compare the most developed ISPR techniques, with process simulations providing comparative data on the separation efficiency and energy demand. All the techniques were found to be economically viable, with profit increases compared to an equivalent batch plant of 110-175% and payback times of 2.2-4.5 years. In addition to generating the most profit and having the shortest payback time, perstraction was the only technique to lead to a reduction in overall plant energy demand, by ~5%, compared to a traditional ABE process. Thus perstraction warrants further investigation for application to the ABE process.

Perstraction is significantly underdeveloped compared to other ISPR techniques. It was originally designed to overcome various problems associated with liquid-liquid extractions, including solvent toxicity. Here, experiments focused on the use of high-distribution toxic extractants with commercially available membranes. Results showed that high-distribution toxic extractants (1-pentanol, 1-hexanol, 1-heptanol, 1-octanol and 2-ethyl-1-hexanol) have a larger mass transfer coefficient than oleyl alcohol (the main non-toxic extractant), although chemical structure differences, such as branching, can have a greater impact on mass transfer than distribution coefficient. Unfortunately, all extractants investigated here were transferred across the membrane to some extent, which would limit perstraction to non-toxic extractants. However, differences in membrane type have a greater impact on mass transfer than the choice of extractant. Porous membranes have a mass transfer coefficient 10 times greater than non-porous membranes, which would see a factor of 10 reduction in

membrane size and cost. Overall, this work has confirmed that perstraction is technically viable and compared options for process improvements through membrane and extractant selection.

Acknowledgements

Firstly, I would like to thank Professor Adam Harvey and Dr. Jonathan Lee for their academic input and supervision. Also, thank you to Carl-Axel Lalander and Dr. Tim Davies for their industrial input and supervision.

Secondly, for the financial support from EPSRC and Green Biologics, as without their funding this research would not have been possible.

Many thanks to everyone Green Biologics, who made my EngD experience thoroughly enjoyable. A particular thanks to the Analytical team for running all my samples, especially Rachel and Mohini when I wanted a new chemical analysed. Also, the Process Development team for general lab support.

Thank you to GE for the flat membranes that were tested during the experimental work.

Last, but not least, to friends and family for their support and encouragement throughout the EngD.

Contents

List of Figures

List of Tables

Abbreviations

ABE	Acetone Butanol Ethanol
CNT-PDMS	Carbon nanotubes filled PDMS
DOE	Department of Energy
GBL	Green Biologics Ltd.
ISPR	*In situ* product recovery
LLE	Liquid-liquid extraction
MSA	Mass separating agent
NL	Nylon (polyamide)
NRTL	Non-random two liquid
NT	Non-toxic
PAN	Polyacrylonitrile
PDMS	Polydimethylsiloxane
PE	Polyethylene
PES	Polyethersulfone
PDMS	Polydimethylsiloxane
PPD	1,3-Propanediol
PPG	Polypropylene glycol
ppm	Parts per million
PTFE	Polytetrafluoroethylene
RC	Regenerated cellulose
RO	Reverse osmosis
SDS	polystyrene-*b*-polydimethylsiloxane-*b*-polystyrene
STR	Stirred tank reactor
T	Toxic
TRL	Technology readiness level

vvm	volume per volume per minute

Nomenclature

A	Membrane area for mass transfer (m^2)
C_{iA}^{b}	Concentration of *i* in the bulk aqueous phase (g m^{-3})
C_{iA}^{*}	Concentration of *i* in the aqueous phase in equilibrium with the concentration in the extractant phase (g m^{-3})
C_{im}^{A}	Concentration of *i* in the membrane at the interface with the aqueous phase (g m^{-3})
C_{iE}^{b}	Concentration of *i* in the bulk extractant (g m^{-3})
C_{iE}^{*}	Concentration of *i* in the extractant phase in equilibrium with the concentration in the aqueous phase. (g L^{-1})
C_{im}^{E}	Concentration of *i* in the membrane at the interface with the extractant (g m^{-3})
C_{iA}^{m}	Concentration of *i* in the aqueous phase at the membrane interface (g m^{-3})
C_{iE}^{m}	Concentration of *i* in the extractant at the membrane interface (g m^{-3})
ΔC_i	Concentration gradient of component *i* (g m^{-3})
c_i	Molar concentration of *i* (mol mol^{-1})
C	Capital cost in U.S. dollars, U.S. Gulf Coast, Jan 2010 basis
D	Mass diffusivity (m^2 s^{-1})
D_i	Diffusion coefficient (m^2 s^{-1})
D_{iE}	Diffusion coefficient of *i* in the extractant (m^2 s^{-1})
D_{im}	Mutual diffusivity at infinite dilution of *i* in the membrane (cm^2 s^{-1})
D_{id}	Maximum inner flask diameter (m)
J_i	Flux of *i* across the membrane (g h^{-1} m^{-2})
K_d	Distribution coefficient (or partition coefficient)
$K_{di,A}^{E}$	Distribution of *i* between the aqueous phase and extractant

$K_{di,A}^{m}$	Distribution of *i* between the aqueous phase and the membrane
$K_{di,E}^{m}$	Distribution of *i* between the extractant and the membrane
K_{ov}	Overall mass transfer coefficient (m s^{-1})
$K_{ov,A}$	Overall mass transfer coefficient based on the aqueous side (m s^{-1})
$K_{ov,E}$	Overall mass transfer coefficient based on the extractant (m s^{-1})
k	Convective mass transfer film coefficient (m s^{-1})
k_A	Aqueous phase film mass transfer coefficient (m s^{-1})
k_E	Extractant film mass transfer coefficient (m s^{-1})
k_m	Membrane mass transfer coefficient (m s^{-1})
L	Characteristic length (m)
L_i	Coefficient of proportionality
M_m	Molar mass of membrane (g mol^{-1})
N	Rotational speed, revolutions per second (s^{-1})
N_i	Number of moles of *i* (mol)
P	Pressure (Pa)
Q	Plant capacity in tonnes per year (t yr^{-1})
R	Gas constant (J mol^{-1} K^{-1})
Re	Reynolds number
Re_N	Reynolds number in an agitated system
s	Reactor conversion (biological yield) (g product g^{-1} substrate)
Sc	Schmidt number
Sh	Sherwood Number
T	Temperature (°C)
ΔT_{min}	Minimum temperature difference (°C)
t_m	Membrane thickness (m)
U	Number of functional units

V_A	Volume of aqueous phase (m^3)
V_E	Volume of extractant (m^3)
V_F	Fermentation volume (m^3)
V_i	Molar volume of *i* at normal boiling point ($m^3\ mol^{-1}$)
v_i	Molar volume of *i* ($m^3\ mol^{-1}$)
$\boldsymbol{v}$	Mean velocity of the fluid ($m\ s^{-1}$)
γ_i	Activity coefficient of *i*
ϵ_m	Membrane porosity
μ_i	Chemical potential of *i* ($J\ mol^{-1}$)
$\frac{d\mu_i}{dx}$	Chemical potential gradient of component *i* ($J\ mol^{-1}$)
η_m	Dynamic viscosity of membrane (Pa s)
ρ	Density ($kg\ m^{-3}$)
τ_m	Pore tortuosity (actual pore length divided my membrane thickness)
ϕ	Association factor ($\phi = 1$ hydrophobic compounds)

Chapter 1. Introduction

1.1: Bio-Based Chemicals

One of the greatest challenges for society is to become more sustainable, moving away from using non-renewable sources such as fossil fuels (Sheldon *et al.*, 2015). The importance of this is highlighted by organisations such as the European Commission developing and adopting a bioeconomy strategy to focus on this need and channel research efforts to address the common problem of sustainable production and use of biological resources (European Commision, 2012).

In 2010 the majority of chemicals were derived from petrochemical sources, with only 4% from renewable sources. Renewably produced chemicals include polylactic acid produced by Cargill, Nebraska, USA, Bio-PE (polyethylene) by Braskem, Brazil and Bio-PDO (1,3-Propanediol) by DuPont, Tennessee, UAS (Golden *et al.*, 2015). The Department of Energy (DoE) produced a list of 12 bio-based building block chemicals that could be derived from sugar to displace the petrochemical supply chain. These chemicals were 1, 4 succinic, fumaric and malic acids, 2, 5 furan dicarboxylic acid, 3 hydroxy propionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid, levulinic acid, 3-hydroxybutyrolactone, glycerol, sorbitol, xylitol/arabinitol (Werpy *et al.*, 2004) . The development of bio-based products is not a new concept; as up until the 1930's bulk chemicals, such as butanol, ethanol, acetic acid, citric acid and lactic acid, were typically produced from biomass (Willke and Vorlop, 2004). The re-commercialisation of these processes and development of new bio-products from renewable raw materials will fit into the bioeconomy strategy.

1.2: Microbial n-Butanol and the ABE Fermentation

In 2012 the global market for n-butanol was estimated to be approximately $7 billion (3.8 million tons), with 4.6% growth predicted between 2013 and 2018 (Jiang *et al.*, 2015). It is currently produced through oxo-synthesis of propene, but was originally produced by the acetone butanol ethanol (ABE) fermentation, with production dating back to World War I (Uyttebroek *et al.*, 2015). The original process was considered uneconomical to run compared to the petrochemical-based process. Therefore the microbial production of n-butanol eventually ceased in the 1980s (Green, 2011), although production was maintained in China, until 2004 (Chiao and Sun, 2007). There was the opportunity for re-emergence of

the ABE fermentation in China due to butanol consumption increasing by 6.7% per year. This increase could not be met by petrochemical production, therefore ABE plants were restarted or built, with a total solvent production of 210,000 tons achieved by September 2008 (Ni and Sun, 2009). Biobutanol production has also, since, commenced in Brazil at the HC Sucroquímica sugarcane biorefinery (Mariano *et al.*, 2013).

Since the ABE process stopped there have been significant technology developments that may make it possible to develop a competitive ABE process. This includes better understanding of the microorganism, through genome sequencing, allowing the development of specific genetic modification tools (Green, 2011). It also includes learning from process developments in ethanol fermentation in the 1980s, such as *in situ* product recovery research, and applying them to other fermentations (Stark and von Stockar, 2003).The challenges associated with the ABE fermentation process are well-documented. Dürre (2011) and Green (2011) presented both the academic and industrial challenges, respectively, associated with microbial n-butanol production. These have been summarised in Table 1.1, below. The key overlapping challenges are high feedstock cost, low butanol yield and product recovery. These problems can be overcome through both strain and process development to create a more competitive process.

Table 1.1: Summary of challenges facing ABE fermentation.

Academic perspective (Dürre, 2011)	**Industrial perspective (Green, 2011)**
Substrates are expensive and compete with food	High feedstock cost
Formation of by-products	Low butanol titres (increase recovery cost)
Culture degeneration	Low butanol yield
Phage contamination	Low volumetric productivities
Low solvent yield	Solvent recovery is energy intensive and expensive
Low solvent tolerance	High water usage
Butanol recovery	

1.3: *In Situ* Product Recovery

One way to improve the ABE fermentation process is the application of *in situ* product recovery (ISPR). This involves integrating the primary product recovery step with the fermentation. Van Hecke *et al.* (2014) stated the advantages of ISPR as:

- Reducing downstream product recovery costs, due to an increased product concentration

- Improved volumetric recovery, due to reduction in product toxicity to the microorganism
- Reduction in waste water, as a concentrated feed can be used
- Improved yield, due to removal of product, which reduces unwanted side reactions and toxicity effects

ISPR should help to overcome many of these challenges and those presented in Table 1.1.

Although ISPR has been investigated for many bioprocesses, research is still required to aid the transition from lab-based research to industrial implementation. Woodley *et al.* (2008) suggest that research should focus on the method of integration of the separation process, robustness of the ISPR process, rapid ISPR assessment methods and the degree of product enhancement achieved by the ISPR method. In a more recent review of ISPR technology, Van Hecke *et al.* (2014) stated that information is missing on the energy consumption, for both ISPR techniques and the overall process, which limits economic analysis. They also state that both the energy consumption and the economic analysis can be aided by the use of chemical process simulation software. Van Hecke *et al.* (2014) also suggested that research should focus on the scalability of ISPR processes, the long term robustness of the process, and maximising the product recovery through the ISPR method, with the overall aim of industrial implementation.

1.4: Research Project

The research project presented in this thesis focuses on the application of ISPR to the ABE fermentation for an industrial process. As stated by Uyttebroek *et al.* (2015):

"Biomass for sustainable fuels and chemicals will be the only resource for future generations. The efficiency of the biobutanol production can be improved by altering upstream processes, by metabolic engineering, by decreasing by-product formation and by improving in situ product recovery"

The application of ISPR to the ABE fermentation is likely to have a major impact on whether it is possible to recommerciallise the ABE fermentation and introduce more bio-based/bio-derived products into the market. Overcoming the current limitations with ISPR, as discussed above, will see improvements in ISPR technology and help make decisions to improve the ABE process.

To date, various ISPR methods have been investigated for their ability to reduce solvent inhibition, and to improve the yield and productivity of the fermentation. ISPR should also improve the economics and energy efficiency of the plant. So far this work has all been performed at laboratory scale, with very little consideration for application to commercial large-scale ABE processes. Consequently, there is no obviously superior technique for *in situ* recovery of ABE. This is compounded by the continuous developments occurring within each technique, such as membrane or adsorbent development for pervaporation and adsorption. These developments mean that it is difficult to assess within an *in situ* recovery technique what the best method is, let alone comparing these developments with other *in situ* recovery techniques.

1.4.1: Industrial Partner – Green Biologics Ltd.

This project has been carried out in collaboration with Green Biologics Ltd. Green Biologics are a renewable chemicals company with a primary focus on developing green alternatives to everyday products. The company has facilities in both the UK and USA to develop a commercial ABE process. Green Biologics has developed technology for the production of butanol through clostridial ABE fermentations, and are continuously looking to develop their process through both strain and process development. Through development of an advanced fermentation technique, such as *in situ* recovery of the butanol, it will be possible to improve the characteristics of the fermentation, such as yield, productivity and fermentation duration due to avoiding product inhibition of the microorganisms, enabling enhanced butanol production.

1.5: Thesis Aims and Objectives

The overall aim of the project is to develop an ISPR technique for the ABE fermentation that would be applicable to a commercial process. This technique should increase the yield and productivity of the fermentation as product concentration inhibitory to the microorganism are not reached. The recovery technique should produce a concentrated stream, which will reduce the load on downstream separation, increasing the energy efficiency of the process and improving the process economics.

To achieve this aim the following objectives were defined:

1) Assess existing ISPR research for the ABE fermentation, to determine the current state of various ISPR techniques with regards to commercial application.

2) Perform a techno-economic analysis of ISPR techniques for the ABE fermentation, using process simulations to give a quantitative comparison, and select the most economic technique for further investigation.

3) Experimental development of chosen technique, with respect to application to a commercial ABE process.

1.6: Research Contributions

This aim of this research was to assess and develop an ISPR technique for integrating with an ABE fermentation. The following list outlines the main research contributions in this work, achieved whilst working towards this aim:

- This work provided the first quantitative review of existing literature focusing on integrating ISPR with ABE fermentation at laboratory scale. A direct comparison across the wide range of research has been difficult, both within ISPR techniques and across all possible techniques. This is due to differences in fermentation protocols, including strain and media used, reactor configuration, etc. This limited previous comparisons to a qualitative analyses. To overcome this, here, a comparison was made based on the improvements seen between the integrated fermentation and the non-integrated control fermentation. This allowed for differences in the fermentation protocol to be minimised in the comparison. This work was published in the peer-reviewed journal Biotechnology Progress.
- A techno-economic analysis of seven ISPR techniques for the ABE fermentation was completed. These techniques were:
    - Gas stripping: recovery into the gas phase through sparging anaerobic gas through the fermentor to selectively recover ABE. See sections 2.3.1.1 and 3.2.1
    - Pervaporation: selective recovery across a permeable membrane into the gas phase. See sections 2.3.1.1 and 2.3.2

- Vacuum fermentation: application of a vacuum to the fermentor to “boil off” ABE at fermentation temperature. See sections 2.3.1.1 and 3.2.2
- Flash separation: similar to vacuum fermentation but performed in an external separation vessel. See sections 2.3.1.1.
- Liquid-liquid extraction: selective recovery of ABE into a second liquid phase. See sections 2.3.1.2 and 3.2.4
- Perstraction: selective recovery of ABE into an extractant across a membrane separating the two liquids. See sections 2.3.1.2 and 3.2.5
- Adsorption: Removal of ABE via capture onto a solid surface. See sections 2.3.1.3 and 3.2.6

This combined development of process simulations to understand the separation efficiency and energy demand of the process, followed by an early-stage economic analysis for application of ISPR to a batch ABE process. This is the first comparative assessment of this nature, and was designed to fill in the gaps in ISPR research for the ABE fermentation as raised by Van Hecke *et al.* (2014). This work was published in the peer-reviewed journal Bioresource Technology.

- Based on the outcomes of the techno-economic analysis, perstraction was selected as the best ISPR technique for integration with the ABE fermentation. This conclusion was largely based on perstraction being the only technique to reduce the overall plant energy demand compared to the traditional process (in our simulations).
- An in-depth analysis into perstraction was performed, focusing on commercially available materials and taking advantage of the proposed advantages of perstraction over liquid-liquid extraction. To the best of the author’s knowledge this is the most comprehensive, comparative analysis of membranes for the extraction of ABE. The analysis included comparisons between porous and non-porous membrane, and hydrophilic and hydrophobic membranes. This analysis focused on the use of high-distribution extractants, which were toxic to the fermentation. This is important as the use of toxic extractants is one of the proposed advantages of perstraction, due to the membrane isolating the toxic extractant from the microorganism. This provided greater insight into extractant selection. In particular, whilst a higher distribution coefficient leads to a high extraction rate it does not guarantee it. Instead, other factors, such as chemical structure, need understanding to be taken into account.

This outcome provides a new perspective on extractant selection that had not previously been discussed, particularly in relation to the ABE fermentation.

- This research confirms suspicions by other researchers that oleyl alcohol can transfer across a membrane. A gas chromatography method for detection of oleyl alcohol was developed as part of this work. The detection/measurement of oleyl alcohol in the aqueous phase had not been performed for any previous perstraction or liquid-liquid extraction work with the ABE fermentation.

1.7: Thesis Structure

This thesis is broken down into six chapters in total. The others are, as follows:

- Chapter 2 provides an overview of the ABE fermentation and ISPR, providing the background information and stating the key requirements required for the ABE fermentation and successful ISPR integration. This includes an overview of all possible ISPR techniques associated with the ABE fermentation and an initial assessment of the current state of each technology. This chapter also provides an overview of the current intellectual property associated with ISPR and butanol isomers, along with other commercialisation activities involving ISPR, butanol and the ABE fermentation.
- Chapter 3 is presented in the form of a review paper for Biotechnology Progress (Outram *et al.*, 2017), this has led to some intended repetition with the previous chapter. It is a comprehensive review of ISPR that has been applied to the ABE fermentation, with the aim of selecting the best ISPR technique to carry forward for further development.
- Chapter 4 is a techno-economic analysis of seven ISPR techniques compared to a traditional ABE process. It is a research paper for Bioresource Technology (Outram *et al.*, 2016). The results of this chapter lead to the selection of perstraction for further development.
- Chapter 5 is focused on perstraction development. It includes information on perstraction theory, and a more detailed assessment of perstraction in relation to it as an ISPR technique in general, and more specifically to the ABE fermentation and the next steps required for further development. This is followed by experimental work to develop perstraction focusing on the use of toxic extractants and

commercially available membranes. The chapter finishes with an assessment of how the experimental results impact the economics of applying this technique.

- Chapter 6 provides the conclusions from the work presented in this thesis and how it complies with the objectives stated in Chapter 1, along with suggestions for future work.

Chapter 2. Literature Review

2.1: Biobutanol

Butanol is a commodity chemical which has four isomers, with n-butanol (or 1-butanol) the focus of this thesis. n-Butanol has many industrial uses, in particular as a feedstock for other chemicals such as acrylate and methacrylate esters. These n-butanol derivatives are often found in surface coatings, adhesives, textiles, polymers and many more (Dürre, 2008). Currently, the main production route for n-butanol is propylene oxo synthesis, which is petrochemical based. n-Butanol can also be produced through fermentation producing so-called "biobutanol" (Green, 2011). Until the 1950s, fermentative production of n-butanol was the main production route, responsible for 66% of n-butanol worldwide (Dürre, 2008). One advantage of biobutanol over petrochemical butanol is that it can be used as a biofuel, as well as a replacement for petrochemical butanol. As a biofuel, butanol can be a direct replacement for gasoline, or blended with gasoline or diesel (Dürre, 2007; Liu *et al.*, 2013b). Biobutanol offers advantages over bioethanol as a biofuel as it is less corrosive, has a lower vapour pressure, will not absorb water and can be used in the existing infrastructure for gasoline (Dürre, 2008; Liu *et al.*, 2013b). Furthermore, butanol has density lower heating value (MJ/kg) 1.2 times that of ethanol, so lower fuel consumption is possible (Jin *et al.*, 2011).

Since the oil crisis in 1973, oil prices have dramatically increased. This has caused the chemical industry to look to non-fossil fuel sources for chemicals and fuels (Ni and Sun, 2009). Biobutanol has attracted significant interest, as it can be used in both the chemical and fuel industry (Dürre, 2008).

In this thesis, butanol will mean n-butanol. All other butanol isomers will be described by their full name.

2.2: Acetone Butanol Ethanol (ABE) Fermentation

The acetone butanol ethanol (ABE) fermentation was developed by Chaim Weizmann, between 1912 and 1914, and implemented on an industrial scale in 1915 to produce acetone, for use in the manufacture of cordite (a smokeless explosive) as part of the war effort (Gibbs, 1983; Jones and Woods, 1986). After World War I, the demand for butanol

rapidly increased due to the need for a quick-drying lacquers for the rapidly developing car industry, of which butanol and its ester, butyl acetate, were suitable (Dürre, 2008). When Weizmann's patent expired in the 1936, production of ABE rapidly expanded worldwide with plants in USA, Japan, India, Australia and South Africa (Jones and Woods, 1986; Zverlov *et al.*, 2006). Two thirds of the world's butanol was produced via fermentation (Dürre, 2008). In the 1950s the process began to decline, although production was sustained in USSR and South Africa until the 1980s (Dürre, 2008), and until 2004 in China (Chiao and Sun, 2007). The decline was due to the low crude oil prices and the increasing price of the main feedstock for the fermentation process, molasses, as this was also being used as an animal feed. This meant that the ABE fermentation could not compete, economically, with the cheaper petrochemical butanol (Gibbs, 1983; Jones and Woods, 1986; Dürre, 2008). Production has since restarted in China in 2007, using predominantly corn, although other feedstocks include molasses, cassava and corn stover (Jiang *et al.*, 2015). Ni and Sun (2009) and Jiang *et al.* (2015) provide detailed accounts on the re-emergence and status of the ABE fermentation in China.

2.2.1: ABE microorganisms

The ABE process is an anaerobic fermentation typically performed using strains of *Clostridium* bacteria. The most commonly used industrial species are *Clostridium acetobutylicum, Clostridium beijerinckii, Clostridium saccharobutylicum* and *Clostridium saccharoperbutylacetonicum* (Ni and Sun, 2009; Dürre, 2011). *C. acetobutylicum* is the most widely studied strain, originally isolate from starchy substrates; whereas the other species prefer molasses (Dürre, 2011). *C. acetobutylicum* typically produces ABE in the ratio 30:60:10 (McCoy and McClung, 1935), but *C saccharoperbutylacetonicum* can achieve butanol ratios of 73-85% (Keis *et al.*, 2001). *C. beijerinckii* strains often produce isopropanol instead of acetone (Dürre, 1998).

A wide range of substrates can be used to grow the microorganism, with all strains able to utilise arabinose, xylose, glucose, maltose and lactose (Keis *et al.*, 2001). Other substrates can be used, but it is strain-dependant, for example *C. saccharobutylicum* cannot utilise pectin unlike the other three strains (Keis *et al.*, 2001). Keis *et al.* (2001) and Rainey *et al.* (2015) provide more detailed information on strain-specific substrate utilisation and other strain characteristics.

The microorganisms typically, e.g. *C. acetobutylicum*, have a biphasic metabolism: in the growth phase acids (acetic and butyric) are produced (acidogenic phase), then a metabolic shift occurs into the solventogenic phase, in which the acids are re-assimilated and converted into ABE. Although, some organisms such as *C. saccaroperbutylacetonicum* are able monophasic and produce solvents during the growth phase. The production pathway for *C. acetobutylicum* in Figure 2.1 displays the metabolic reactions responsible for this. Jones and Woods (1986) provide a detailed description of the fermentation pathway and the ABE fermentation.

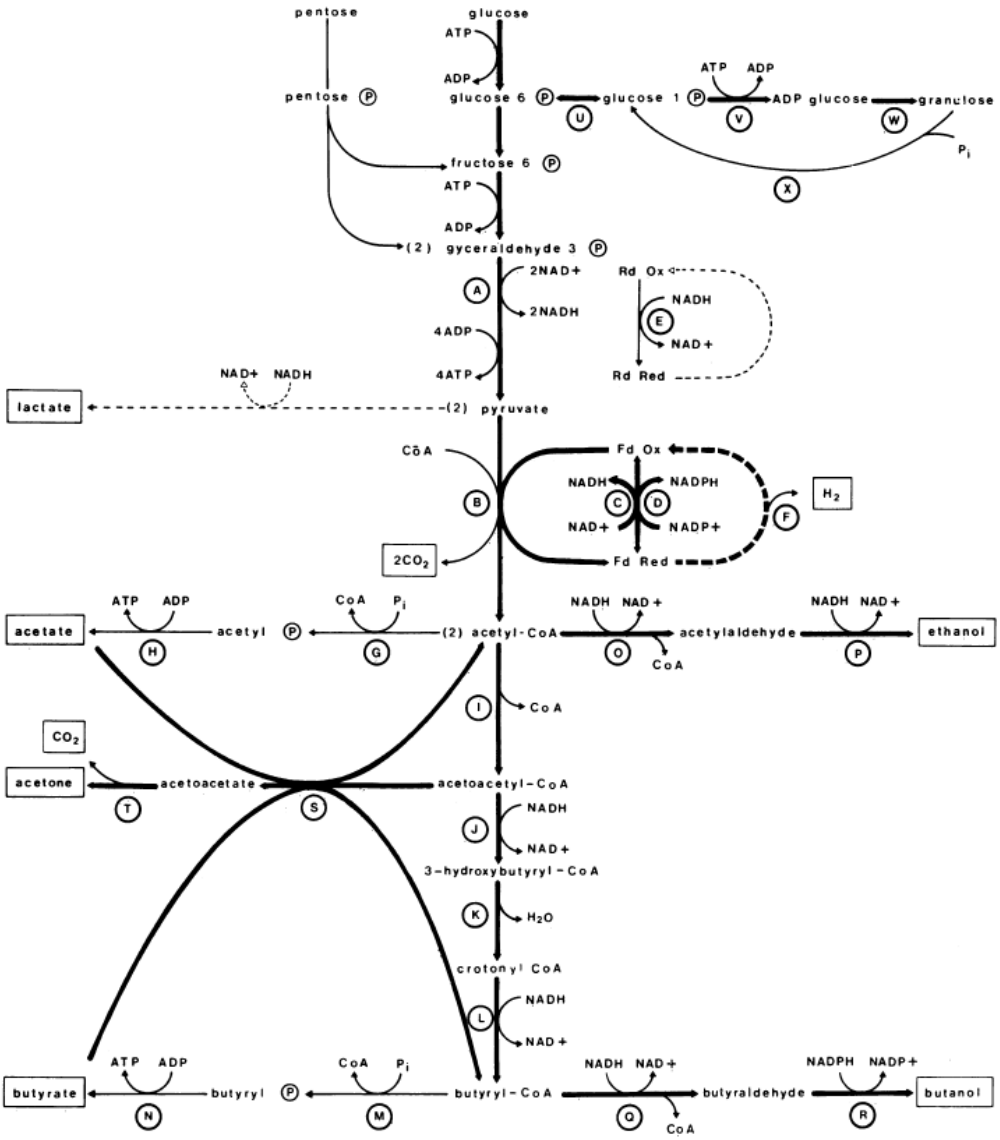


Figure 2.1: *C. acetobutylicum* fermentation pathway (Jones and Woods, 1986)

Two key parameters used to compare fermentations are yield, equation 2.1, (g ABE produced g^{-1} substrate consumed) and productivity, equation 2.2, (g ABE produced L^{-1} fermentation broth h^{-1}).

$$Yield = \frac{mass\ ABE\ produced\ (g)}{mass\ substrate\ consumed\ (g)} \quad \textbf{(2.1)}$$

$$Productivity = \frac{mass\ ABE\ produced\ (g)}{volume\ of\ fermentation\ broth\ (L).\ fermentation\ duration\ (h)} \quad \textbf{(2.2)}$$

For an industrial ABE fermentation, typical yields and productivities are 0.28-0.33 g ABE g^{-1} substrate consumed and <0.3 g ABE h^{-1} L^{-1}, respectively (Kumar *et al.*, 2012).

2.2.2: Downstream Separation

The most common route for downstream separation of ABE fermentations is that described by Mariano and Filho (2011). Figure 2.2 shows a diagram of this process, which involves five distillation columns and a decanter. The first stage after the fermentor is a beer stripper in which the ABE is concentrated and removed through the top of the column, then the acetone and ethanol are removed in the next two columns, with the bottom product of the ethanol column being fed to a decanter. The decanter facilitates the split of the butanol-water heterogeneous azeotrope, with the butanol-rich phase going to one column for a butanol product stream and the water-rich phase going to the other column for a wastewater product stream. The top of the butanol and water columns is at azeotropic conditions, therefore recycled back to the decanter to ensure maximum butanol recovery. (Mariano and Filho, 2011).

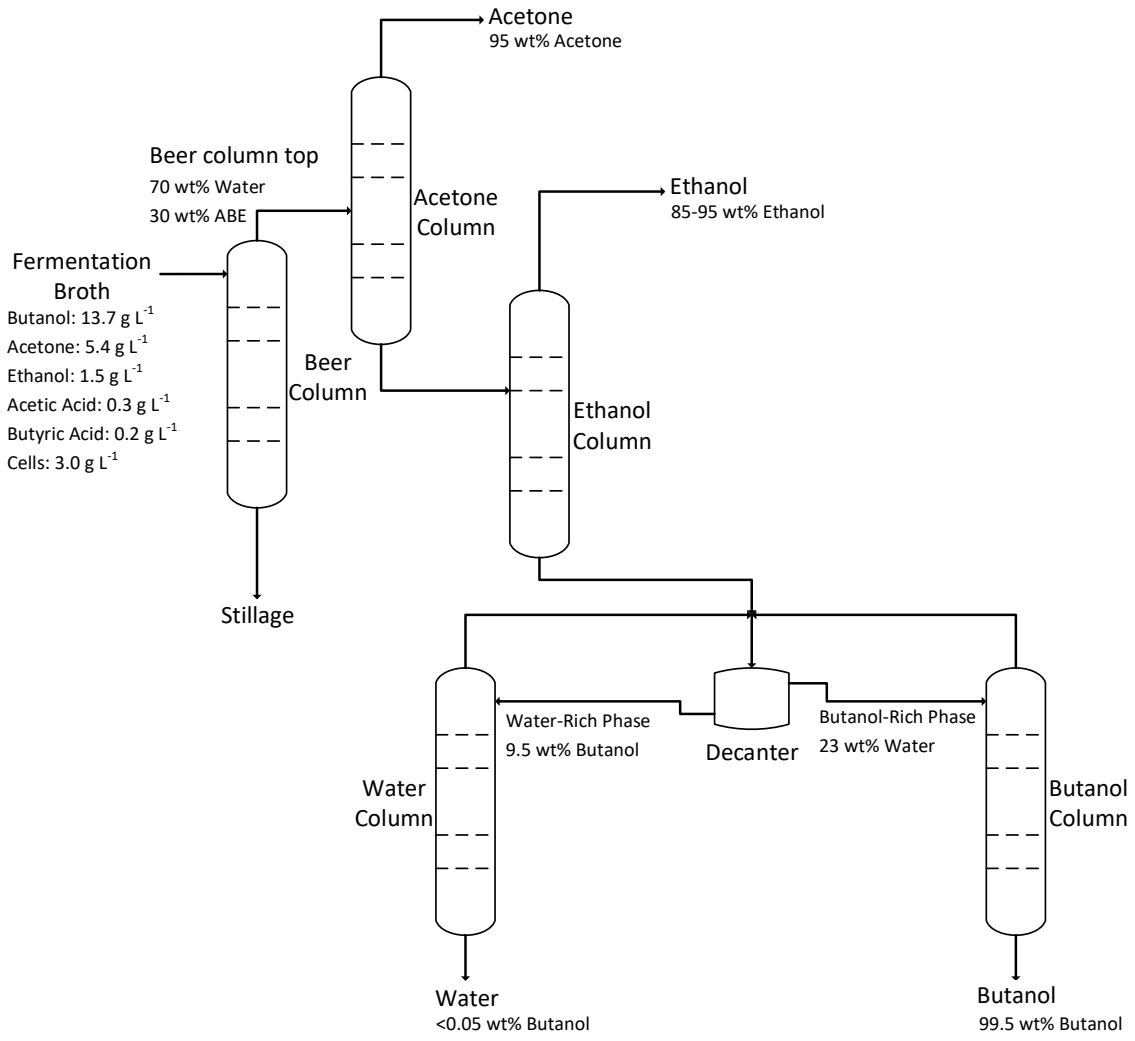


Figure 2.2: Schematic of downstream ABE separation process (Mariano and Filho, 2011; Roffler *et al., 1987)*).

The process is very energy intensive due to the low concentrations of ABE (less than 2wt%) in the fermentation broth stream. Mariano and Filho (2011) developed an Aspen Plus® simulation of this process and found that more energy is required to separate the ABE fermentation broth than the energy content of butanol itself, which is 36 MJ/ kg butanol. Increasing the concentration of butanol entering the downstream separation would significantly reduce the energy demand of the plant, and the associated operating costs of the downstream processing.

2.2.3: Problems with the Fermentation

The demise of ABE fermentation occurred because it could not compete economically with the petrochemical production of butanol. There were a range of contributing factors, which

1were compiled by (Dürre, 2011). The main factors were the high substrate cost, low solvent yield and the high energy requirement in recovering the butanol by distillation (Dürre, 2008; 2011).

The cost of substrate was thought to contribute approximately 60% of the process operating costs (Jones and Woods, 1986). This, therefore, had a major influence on the cost of production. The substrate cost varies significantly with demand. Ideally an inexpensive substrate would be used that did not compete with other market uses, particularly food (Dürre, 2011). Using a pure glucose substrate would cost 1.11 \$ kg^{-1}, a first generation feedstock such as corn (0.19 \$ kg^{-1}) or sugarcane (0.047 \$ kg^{-1}), whereas a second generation feedstock (for example bagasse or corn stover, both 0.033 \$ kg^{-1}) are cheaper as they are typically seen as agricultural wastes (Kumar *et al.*, 2012). Another possibility is third generation feedstocks, such as carbohydrate-rich alae, but significant development is required before this could be considered an industrial applicable feedstock (Wang *et al.*, 2017). The substrate costs could also be reduced if the fermentation could maintain production at the maximum yield.

The fermentation, typically, has a low production yield of 0.33 g ABE g^{-1} substrate (Jones and Woods, 1986). The low yield is inherent to the fermentation due to multiple product (ABE) and by-product (H_2 CO_2, acetate and butyrate) formation (Tashiro *et al.*, 2013). Furthermore, butanol concentrations greater than 15 g L^{-1} are toxic to the bacteria, which causes the fermentation to stop (Oudshoorn *et al.*, 2009). This also influences the quantity of substrate used, as very dilute concentrations must be used, leading to large volumetric capacity batch fermentations (Roffler *et al.*, 1987c).

The low product concentrations increase the energy demand of the downstream processing, via distillation, to recover the acetone, butanol and ethanol (Ezeji *et al.*, 2004a). The downstream separation makes up approximately 20% of the operating costs, the highest cost after feedstock (Jones and Woods, 1986). This is due to the low boiling point heterogeneous azeotrope, at 93°C, between butanol and water. This results in a large quantity of water being retained with the butanol, as shown at the top of the beer column in Figure 2.2. Consequently, a large quantity of water needs to be boiled to achieve acceptable purity levels of butanol (Dürre, 2008; Oudshoorn *et al.*, 2011). Solutions to these problems could significantly improve the economics of the process, allowing biobutanol to re-enter the chemical market.

2.2.4: ABE process improvements

Two methods for improving the ABE fermentation have received substantial interest. The first is genetic modification of the microorganism to increase butanol tolerance and/or to increase the yield of butanol produced, preferably by removing the acetone and ethanol production capabilities of the microorganism. These targets are complementary, as, if the butanol concentration is increased, the bacteria would have to be able to tolerate it (Dürre, 2008).

The other method is through process development, in particular developing an advanced fermentation process that can remove butanol as it is produced, from the fermentation broth (Dürre, 2008). This will reduce butanol inhibition by maintaining the butanol concentration below inhibitory levels, consequently allowing an improvement in the yield. This is due to less substrate being required for cell maintenance and growth because of toxic fermentation conditions, instead it can be directed towards ABE production. It would also allow for the use of fed-batch fermentations, which would reduce the volume of fermentation broth (for a fixed annual production) to be treated and improve the productivity (Ezeji *et al.*, 2004a).

The two methods (strain and process development) are complementary and if performed in tandem could generate significant improvements in the fermentation process. This thesis is concerned with the process development, specifically the application of *in situ* product recovery to the ABE fermentation.

2.3: *In Situ* Product Recovery

In situ product recovery techniques are applied to bioprocesses to increase the productivity of inhibited fermentations, reduce the wastewater treatment costs (due to reduced process volumes used because fed-batch fermentations with a concentrated can now be used, assuming a fixed annual production) and minimise product degeneration (Roffler *et al.*, 1984). The aim of ISPR techniques are to remove the product from the vicinity of the cell as soon as it is formed (Freeman *et al.*, 1993). Freeman *et al.* (1993) stated three methods through which increasing the productivity and yield is possible:

1. Minimising the effect of product accumulation, allowing for maximal product production.
2. Minimising product loss through interaction with the cell or environmental conditions that can create uncontrolled removal of the product, for example through evaporation.
3. Reduce the number of downstream processing steps following fermentation.

It is the first of these effects that is the most important for the ABE fermentation, as, due to inhibition by butanol, the maximum concentration possible is approximately 15 g butanol L^{-1} (Oudshoorn *et al.*, 2009). The second point also contributes to low yield, and can be used to explain the low yield achieved in some fermentations (Mariano *et al.*, 2011b). Mariano *et al.* (2011a) predicts, that for an ABE plant, the percentage losses of ABE in the gas stream are 1.7, 0.2 and 1.4% of the total amount produced, therefore minimising these losses will be beneficial.

Several reviews have been produced that give a comprehensive analysis of ISPR techniques covering a wide range of products. Stark and von Stockar (2003) tabulated various whole cell fermentation (rather than enzyme based processes) ISPR techniques. The most frequently investigated products were alcohols, in particular ethanol, closely followed by butanol. Although there are differences in the properties of butanol and ethanol, the techniques that have been well developed for ethanol, such as the BIOSTILL process developed in the 1980s (Groot *et al.*, 1992), are a good starting point for the investigating the *in situ* removal of butanol. (Stark and von Stockar, 2003) divided up the different techniques into four categories; extractive, evaporative, immobilisation and size. These classifications agree with those proposed by Freeman *et al.* (1993). Cen and Tsao (1993) reviewed four main techniques: adsorption, gas stripping, extraction and membrane separation, all of which fit into the categories suggested by (Stark and von Stockar, 2003). Cen and Tsao (1993) focussed on the use of a mass separating agent (MSA), which facilitates separation of the product, e.g. an extractant or an adsorbent. Cen and Tsao (1993) state that the MSA has to be:

- Biocompatible, so as to not inhibit the fermentation
- Have a high selectivity and separation factor for the desired product
- Easily separated from the reaction broth
- Not cause degradation of the product or contaminate the product

- Easy to recover product from the MSA and allow the MSA to be recycled
- A commercially available product

Schügerl and Hubbuch (2005) reviewed integrated bioprocess techniques, but the products and techniques reviewed were biopharmaceutical-related products, such as inclusion bodies and higher molecular weight products.

Most recently, Van Hecke *et al.* (2014) produced a follow-up review to Stark and von Stockar (2003). 32% of the ISPR literature produced between 2003-2013 focused on recovery of organic solvents. The key requirements for industrial implementation of ISPR were discussed and found to be:

1. Simple technology for straightforward scale up
2. Demonstration of long-term robustness and scalability
3. Demonstration of decreased energy demand
4. Demonstration of maximum product recovery
5. Techno-economic analysis of integrated techniques

However, the critical parameters needed to assess these requirements are often missing from publications (Van Hecke *et al.*, 2014). They also state that the application of ISPR will become more important industrially, because, as metabolic engineering advances, there is the potential for a wider range of "bio-products", introducing more toxicity/processing problems.

ISPR has been applied to the ABE processes with many different techniques investigated or suggested. The next section will give a brief explanation of the techniques that have been proposed for the *in situ* removal of butanol from the fermentation broth.

2.3.1: Overview of techniques for the ABE fermentation

The techniques have been divided into the four categories of *in situ* recovery techniques: evaporative, extractive, immobilisation and size, proposed by Stark and von Stockar (2003). To avoid confusion with immobilised fermentations, the immobilisation ISPR category will be termed "solid phase separations".

2.3.1.1 Evaporative recovery techniques

Evaporative techniques make use of the volatility differences between the product and the fermentation, separating the product through a liquid to gas phase transition. The techniques that fall into this category are gas stripping (Ezeji *et al.*, 2003), flash separation (Mariano *et al.*, 2008), vacuum fermentations (Mariano *et al.*, 2011b), pervaporation (Groot *et al.*, 1984b) and vapour permeation (Vane and Alvarez, 2008). Acetone, butanol and ethanol can all be removed from the fermentation broth in the gaseous phase. Removal of all three products makes evaporative techniques attractive. The downside to these techniques is the complete recovery of the products from the gaseous phase (Groot *et al.*, 1989; Ezeji *et al.*, 2004a; Mariano *et al.*, 2011b): large cooling duties are required to capture all the products, especially acetone.

Gas stripping is the most widely researched evaporative technique for the ABE fermentation. It is a simple process, consisting of sparging an anaerobic gas through the fermentation broth, without harming the bacteria, whilst selectively removing the ABE. This means that it can be applied to a wide range of reactor configurations and to batch, fed-batch and continuous fermentations (Mollah and Stuckey, 1993; Ezeji *et al.*, 2003; 2004a; Richter *et al.*, 2012; Ezeji *et al.*, 2013). The downside to gas stripping is the large volumes of gas required to maintain solvent concentrations in the fermentation broth below inhibitory levels. In a typical fermentation a gas flow rate of 2-3 vvm is required (Ezeji *et al.*, 2003; Xue *et al.*, 2012), which, when scaled up, means a large compressor and condensing duty will be required. The practicality of these large gas volumes also needs to be considered through thorough mechanical design of equipment. This includes considerations around the injection pressure of the gas to overcome the static head of the fermentation broth and the additional cost this would add to the fermentor design. Alternative process design, such as using an external stripper need to be considered if it would reduce the economic impact of applying gas stripping. A reduction in condensing duty may be possible if vapour permeation is applied. This is a process in which the gaseous stream leaving the fermentor is concentrated by passing across a membrane that removes the solvents (Vane and Alvarez, 2008). Due to its simplicity and ease of implementation gas stripping is one of the more favoured techniques, recent developments have seen gas stripping used as a tool to maintain solvent concentrations below toxic levels to investigate other factors affecting the fermentation.

Pervaporation (and membrane evaporation (Gapes *et al.*, 1996)) is similar to gas stripping, but the gas and liquid phases are separated by a membrane. Pervaporation is, typically, performed with a circulation loop from the fermentor to an external pervaporation unit, with the solvent-depleted retentate being returned to the fermentor. The gas phase can either be a vacuum (Matsumura *et al.*, 1988) or a sweep gas (Groot *et al.*, 1984a). Pervaporation has been shown to successfully reduce the fermentation broth solvent concentration allowing for extended fermentations and increased substrate usage (Groot *et al.*, 1984a), as with gas stripping. The biggest decision, with pervaporation, is the choice of membrane, as a high flux and selectivity for ABE is desired, but these two factors are inversely related (Gapes *et al.*, 1996). This is due to both the flux and selectivity being functions of the membrane permeability. For example, porous membranes allow for high fluxes due to the ease of transport through the pores, relying more on the evaporative properties of the product for separation. Whereas, non-porous membranes offer higher selectivity as the product has to diffuse through the membrane, therefore membrane diffusivity will have a greater influence (Gapes *et al.*, 1996). The downsides to pervaporation are that it is more complex than gas stripping, even though it allows for a reduced gas flow rate (through the application of a vacuum), and has the potential for fouling by the bacteria and fermentation broth components (Qureshi and Blaschek, 1999a; Van Hecke *et al.*, 2012).

The final set of evaporative techniques applies a vacuum to the fermentation broth to "boil off" the solvents at fermentation temperature. This can be implemented through either an external flash separator (Mariano *et al.*, 2008), or *in situ* to the fermentation (vacuum fermentation) (Mariano *et al.*, 2011b). All investigations into these techniques have been carried out by the same research group (Mariano *et al.*, 2008; 2010; 2011b; 2012b). Investigations into external flash separation have only been performed using mathematical modelling of the scenario, with experimental work performed on vacuum fermentations (Mariano *et al.*, 2008; 2012b). Whilst experimental work has been performed, and shown to reduce the solvent concentration below 5 g L^{-1} removing product inhibition in the fermentor, it is limited to only batch fermentations (Mariano *et al.*, 2011b; 2012a). Considerations are needed into the economics associated with applying a vacuum at 6.5 kPa, similar to Mariano *et al.* (2012a), as the fermentor will need to be reinforced to cope with this reduced pressure.

2.3.1.2 Extractive recovery techniques

Extractive recovery techniques remove the ABE from the fermentation broth to a secondary liquid phase. This is predominantly achieved via liquid-liquid extraction (Roffler *et al.*, 1988), with a range of extractants (Li *et al.*, 2010; Dhamole *et al.*, 2012; Garcia-Chavez *et al.*, 2012), perstraction (Qureshi and Maddox, 2005), aqueous two phase extraction (Kim and Weigand, 1992) and liquid demixing (Oudshoorn *et al.*, 2011).

The most developed extractive technique is liquid-liquid extraction (LLE), and is a common technique in the processing industries. It exploits the difference in solubilities of ABE in two immiscible liquids. Typically an organic solvent, which is immiscible with water, is used, providing it is non-toxic to the bacteria. Ishii *et al.* (1985) published a comprehensive list detailing key extractant criteria. This is discussed further in Chapter 3. For the ABE fermentation, oleyl alcohol is the most commonly used extractant, and has been used to prove that product inhibition can be reduced (Roffler *et al.*, 1987c; b). Compromises have to be made when selecting the extractant, as it was found that extractants with higher partitions were toxic to the bacteria (Ishii *et al.*, 1985; Roffler *et al.*, 1987b). One solution to this has been to use a mixed extractant, such as decanol/oleyl alcohol, to combine the high partition of decanol with the non-toxic nature of oleyl alcohol (Evans and Wang, 1988a). This has also seen investigations into the use of an ionic liquid (Ha *et al.*, 2010; Garcia-Chavez *et al.*, 2012) or a non-ionic surfactant (cloud point extraction) (Dhamole *et al.*, 2012). Biodiesel has also been investigated as an alternative extractant, as the biodiesel/ABE mixture could be used directly as a biofuel (Ishizaki *et al.*, 1999; Li *et al.*, 2010). The distribution coefficient for biodiesel was 1.23 (Li *et al.*, 2010), lower than that of oleyl alcohol therefore more extractant would be required to remove the same quantity of butanol. The downside to biodiesel extractants is that the distribution coefficient of butyric acid is 1.62 (Li *et al.*, 2010), so it is preferentially extracted over butanol which will cause a reduction in yield due to incomplete re-assimilation of butyric acid to butanol. A techno-economic analysis is required to compare the use of biodiesel as an extractant without product recovery for biofuel to oleyl alcohol with product recovery for the chemicals market.

Perstraction is a development from LLE, similar to how pervaporation is a development of gas stripping. Perstraction separates the fermentation broth from the extractant using a membrane. The ABE transfers across the membrane into the extractant solvent. In theory it should enhance the fermentation more than LLE as it solves the problems caused by mixing

the extractant and fermentation broth i.e., extractant toxicity, incomplete phase separation and emulsion formation (Qureshi and Maddox, 2005). Against this, the presence of the membrane adds another mass transfer step for the ABE removal, which could slow down the overall process. The membrane also introduces the possibility of fouling which would require an increase in membrane area to maintain the desired flux.

Generally, perstraction experiments so far have been relatively simple, performed using silicone tubing immersed in the fermentation broth or extractant, with the other passing through it. Whilst perstraction is supposed to overcome limitations, such as toxicity of the extractant, the main extractant to have been used is (non-toxic) oleyl alcohol (Qureshi *et al.*, 1992). Limited research into perstraction membranes have been performed, with silicone (Qureshi and Maddox, 2005) and polypropylene (Grobben *et al.*, 1993) membranes being predominantly used. The development appears to follow a similar process to that of pervaporation membranes, so it would be expected, with continuing developments in membrane technology, that better membranes would become available.

Aqueous two phase extraction is an alternative to traditional LLE, as it creates two aqueous phases which have different affinities for the biomass and products. This is done by the addition of soluble polymers, for example polyethylene glycol and dextran to the fermentation broth. Aqueous two phase extraction has been used in the biotechnology industry where an organic solvent would damage the biomolecules (e.g. proteins) to be extracted (Mattiasson, 1983). When paired with the ABE fermentation, it has been shown that increased yields and solvent concentrations were possible compared to a standard batch fermentation (Kim and Weigand, 1992). Research has not progressed further as butanol is not affected through use of an organic solvent as an extractant; instead research has focused on LLE.

Liquid demixing separates the ABE from the fermentation broth by altering the liquid-liquid equilibrium, causing the formation of a butanol-rich organic phase, typically through the addition of salts to the mixture. This is one of the newer techniques for the separation of ABE, and has not yet been proven *in situ* for the fermentation, as the required concentration of the additive would inhibit the bacteria (Oudshoorn *et al.*, 2011). The salt concentrations required are over 160 g/L of sodium chloride; this creates a large salt gradient across the cell membrane, therefore more energy would be required by the cell to maintain this at the expense of ABE production (Oudshoorn *et al.*, 2009). However, the idea of being able to

separate the ABE into its own distinct phase whilst in fermentation conditions is appealing. Preliminary research into liquid demixing of ABE from water deemed it to be uneconomical due to the cost of the salt required, although this did not take into account recycling the salt for use in multiple fermentations (Oudshoorn *et al.*, 2011).

Whilst research has been performed on the extraction process, limited information is available on the most economical and least energy intensive process to remove the ABE from the extractant. The removal of ABE and regeneration of the extractant add an extra unit operation to the process, compared to the traditional distillation based separation presented in section 2.2.2; assuming that the beer column is considered as the primary separation equivalent to the ISPR technique. This, therefore, adds additional operation and capital costs to the ABE process that need to be taken into account alongside the primary separation step.

2.3.1.3 Solid phase separations

Solid phase separations involve the transition from the liquid to the solid phase. This category is centralised around adsorption techniques to remove the solvents from the broth (Groot and Luyben, 1986), although the newly developed technique of freeze crystallisation for separation can also be considered a solid phase separation technique (Oudshoorn *et al.*, 2009).

Adsorption is the oldest technique investigated for ISPR of ABE from the fermentation broth: it was first investigated by Weizmann *et al.* (1948). It involves the bonding of the ABE to a solid surface, as the fermentation broth flows over it. The exiting broth stream will have a reduced ABE concentration reducing the solvent inhibition. This is typically a batch process, as once all the binding sites are occupied, no more ABE will be removed from the broth, therefore regeneration of the adsorbent via removal of the ABE is a key part of the process (Maddox, 1982). Research into adsorption has focused on the wide range of adsorbents available, including activated carbon (Weizmann *et al.*, 1948), silicalite-based zeolites (Ennis *et al.*, 1987) and polymeric resins (Groot and Luyben, 1986). The major problem with adsorption as a technique is that it removes acetic and butyric acid from the broth (Yang *et al.*, 1994). This reduces the yield of the fermentation as the acids cannot be converted to solvents.

Freeze crystallisation is a newly proposed separation technique for ABE, originating from the purification of waste water and process streams (van der Ham *et al.*, 1998; Oudshoorn *et al.*, 2009). Eutectic freeze crystallisation works by freezing the bulk water, and removing the butanol (which has a significantly lower freezing point, of -88.6°C, than water) as a concentrated solvent stream. This is a more energetically favourable transition than the liquid to gas phase change. As no research has been performed into this, some investigation would be required, such as into adverse biological effects and the introduction of solid handling equipment (Oudshoorn *et al.*, 2009). Another challenge would be processing the ice/solvent stream. The concentration of ABE in the fermentation broth would be about 1 wt% therefore very little liquid will need to be removed creating a highly viscous slurry.

2.3.1.4 Size-based separation techniques

The last category for ISPR is size, in which ABE is removed from the fermentation broth based on size of the molecule. This is predominantly achieved via the use of a membrane, such as via ultrafiltration (Ferras *et al.*, 1986) or reverse osmosis (Garcia *et al.*, 1986). Although, pervaporation and perstraction are also membrane-based techniques, the phase change across the membrane is the primary means of separation therefore they are not considered size-based separation techniques.

Ultrafiltration has two roles with ISPR, firstly as a method to remove the ABE from the vicinity of the cells and secondly as a cell concentration tool. Ferras *et al.* (1986) investigate coupling ultrafiltration to a fermentation to facilitate continuous fermentation. This proved successful, and high biomass densities were generated, although there was increased fouling of the membrane. The application of ultrafiltration is unlikely to be used on an industrial scale due to loss of nutrients in the permeate and it does not significantly increase the solvent concentration in the permeate. Instead ultrafiltration could be used to increase the biomass concentration in the fermentor and to remove the cells from solvents before an alternative recovery technique, which is harmful to the microorganism, could be applied (Qureshi *et al.*, 2005; Mariano *et al.*, 2008).

Reverse osmosis is a technique commonly used to purify water, and has a lower cut off point than ultrafiltration so only water will pass through the membrane. When applied to the ABE process, the ABE will be retained in the retentate with the permeate returning to the fermentor. Garcia *et al.* (1986) tested the applicability of reverse osmosis to the ABE

fermentation and found that, when in combined with an ultrafiltration membrane, reverse osmosis could separate butanol from the aqueous phase. The downside to this is that the reverse osmosis membrane also separated glucose, acetic acid and butyric acid from the aqueous phase, with only the water being returned to the fermentor. This would have a negative impact on the fermentation as ideally glucose, acetic acid and butyric acid would be returned to the fermentor for conversion into solvents. The ultrafiltration membrane was required to eliminate fouling of the reverse osmosis membrane but rapid fouling was seen on the ultrafiltration membrane (Garcia *et al.*, 1986). Realistically, reverse osmosis is not ideally suited for coupling to the ABE fermentation as it isolates the water rather than the ABE. Reverse osmosis is possibly better suited to end-process concentration of the solvents to the desired purity.

2.3.1.5 Hybrid Separation Strategies

More recent work has focused on the use of a combination of techniques or stages to improve the separation. These have been termed "hybrid" strategies by Xue *et al.* (2014d). With ISPR this has focused on the use of two or more stages to increase product concentration such as Xue *et al.* (2014b) with two-stage gas stripping, combined gas-stripping pervaporation (Xue *et al.*, 2016b) or Lu and Li (2014) with the combination of LLE and gas stripping. Generally these are initial investigations, requiring further work to support the claims made with regards to energy improvements.

Furthermore, the definition of two-stage or hybrid strategies needs further clarification. Typically the first stage of the ISPR process removes ABE from the fermentation broth, the second stage further concentrates the product collected during the first stage. This could be considered analogous to recovery by LLE followed by distillation as proposed by Roffler *et al.* (1988), yet this is not considered a hybrid strategy. In practice, only the first separation stage is the ISPR; further stages can be considered as further purification steps. There are many different routes for separation of product from the fermentation broth, condensate or separating agent. These options need to be thoroughly investigated alongside each technique to understand which gives the best performance. The application of a hybrid separation strategy will, ultimately, increase the complexity of the process. This will need to be considered from both an operational and economical standing, compared to single-stage strategies, before too much development is performed.

2.3.2: Technique Applicability

Table 2.1 summarises the current state of research of each technology. All of the techniques can be found in other industries, but are normally applied as end-of-process techniques. There is very little data concerning process optimisation, energy consumption and economic analyses of industrial-scale ISPR operation. This is no doubt due to the complexity and time-consuming nature of designing a suitable plant, then developing process simulations to establish energy demand and sizing of plant equipment for a detailed economic analysis.

Table 2.1: Comparison of state of single-stage ISPR technologies.

	Type of technique	ISPR Fermentations Performed?	Mode (Batch, Fed-batch, Continuous)	Optimised at laboratory Scale?	Separating Agent Regeneration?	Energy Data Available?	Economic Analysis	Plant Applicable Design
Flash	Evaporative	No	n/a	No	n/a	Yes (Mariano *et al.*, 2011a)	No	Yes (Mariano *et al.*, 2011a)
Vacuum	Evaporative	Yes	Batch (Mariano *et al.*, 2011b)	Yes (Mariano *et al.*, 2012a)	n/a	Yes (Mariano *et al.*, 2012a)	No	Yes (Mariano *et al.*, 2012a)
Gas Stripping	Evaporative	Yes	All (Ezeji *et al.*, 2003; 2004a; 2013)	Yes (Ezeji *et al.*, 2005)	n/a	No	No	Yes (Groot *et al.*, 1989)
Pervaporation	Evaporative	Yes	All (Qureshi *et al.*, 1992; Wu *et al.*, 2012)	Partially (Van Hecke *et al.*, 2013)	n/a	Yes (Matsumura *et al.*, 1988; Van Hecke *et al.*, 2012)	No	Yes (Van Hecke *et al.*, 2012)
Vapour Permeation	Evaporative	No	n/a	No	n/a	No	No	Yes (Vane and Alvarez, 2008)

	Type of technique	ISPR Fermentations Performed?	Mode (Batch, Fed-batch, Continuous)	Optimised at laboratory Scale?	Separating Agent Regeneration?	Energy Data Available?	Economic Analysis	Plant Applicable Design
LLE - Organic	Extractive	Yes	Batch and Fed-Batch (Roffler *et al.*, 1987c; b; Qureshi *et al.*, 1992)	No	Yes (Roffler *et al.*, 1988)	Partially (Kurkijärvi *et al.*, 2014)	Yes (Roffler *et al.*, 1987a)	Yes (Roffler *et al.*, 1988)
LLE - Ionic Liquid	Extractive	No[1]	n/a	No	No	Yes (Garcia-Chavez *et al.*, 2012)	No	No
Cloud Point Extraction	Extractive	Yes	Batch (Dhamole *et al.*, 2012)	No	No	No	No	No
Perstraction	Extractive	Yes	All (Qureshi *et al.*, 1992; Grobben *et al.*, 1993; Tanaka *et al.*, 2012)	No	No	No	No	No
Liquid Demixing	Extractive	No	n/a	No	No	No	Rudimentary (Oudshoorn *et al.*, 2011)	Yes (Oudshoorn *et al.*, 2011)
Aqueous Two Phase	Extractive	Yes	Batch (Kim and Weigand, 1992)	No	No	No	No	No

[1] Ionic liquids have been tested in shake-flask fermentations for toxicity (Cascon *et al.*, 2011), but no LLE-fermentation data has been published.

	Type of technique	ISPR Fermentations Performed?	Mode (Batch, Fed-batch, Continuous)	Optimised at laboratory Scale?	Separating Agent Regeneration?	Energy Data Available?	Economic Analysis	Plant Applicable Design
Adsorption	Solid phase	Yes	All (Ennis *et al.*, 1987; Yang *et al.*, 1994; Wiehn *et al.*, 2014)	No	Yes (Oudshoorn *et al.*, 2012)	Rudimentary (Qureshi *et al.*, 2005)	No	Yes (Yang and Tsao, 1995)
Freeze Crystallisation	Solid phase	No	n/a	No	n/a	No	No	No
Ultrafiltration	Size based	Yes	Continuous (Ferras *et al.*, 1986)	No	n/a	No	No	Yes (Ferras *et al.*, 1986)
Reverse Osmosis	Size based	Yes	Fed-Batch (Garcia *et al.*, 1986)	No	n/a	No	No	Yes (Garcia *et al.*, 1986)

Four of the techniques suggested have not yet been tested with the ABE fermentation, Table 2.1. Liquid demixing and freeze crystallisation were both suggested by Oudshoorn *et al.* (2009), and while some follow-up work occurred with liquid demixing (Oudshoorn *et al.*, 2011), the techniques are still just concepts rather than a practical solution to ABE removal. Flash fermentations have been studied to a greater extent, with a plant scale design and energy analysis (Mariano *et al.*, 2011a), but the concept has not been experimentally tested with an ABE fermentation. It could be inferred that this concept is likely to work via the research into vacuum fermentations by the same research group (Mariano *et al.*, 2011b). Though differences in recovery could be possible, as vacuum fermentations are aided by the fermentation gases stripping ABE from the solution having a cumulative effect on ABE removal (Mariano *et al.*, 2011b), the same effect might not be seen in an external flash separation unit. Vapour permeation is a combination of pervaporation and gas stripping, but so far has only had a preliminary investigation with model solutions, spent fermentation broth and process simulations (Vane and Alvarez, 2013; Vane *et al.*, 2013). Until all these techniques have been experimentally validated it is not possible confirm their applicability at an industrial scale.

As a category, evaporative ISPR techniques are the most developed, Table 2.1. This is due to the simplicity of these techniques, and because minimal additional equipment is required to test them, in particular vacuum fermentations and gas stripping. These techniques have dilute ABE concentrations in the gas stream, therefore the process has suffered from insufficient capture of the product. Unless the product can efficiently be removed from the fermentor and form two phases on condensing it will not provide any advantage over the traditional distillation (Xue *et al.*, 2014b). Incomplete product capture will have a negative effect on the economics as the overall plant yield will decrease, which is counterproductive to the strain and fermentation developments to improve bacterial yield. Pervaporation is one of the more complicated evaporative techniques and the most widely researched ISPR topic for the ABE fermentation. Most of the research in this area is concerned with the development of new membrane materials, and not all of the research is tested with actual fermentation broth. These materials show promise for selective removal of ABE, but unless more work is done on the commercialisation of these membranes and an economic study using them for ISPR of butanol they will remain an academic study.

LLE was the first technique to be considered for an industrial process (Roffler *et al.*, 1987a). The majority of the work since has focused on finding or developing a biocompatible extractant that is selective for ABE. From literature it would appear that finding a non-toxic extractant has been prioritised over the development of an extraction process ideal for combining with the ABE fermentation. There has been limited research focusing on the use of a more industrial-style setup, such as an external extraction column (Roffler *et al.*, 1988). Ionic liquids have been suggested for use with the ABE as an alternative to organic extractants (Ha *et al.*, 2010). The problem with ionic liquids is their limited commercial availability on a large enough scale to be used with the fermentation. The proposed benefit of ionic liquids as an extractant is that they can be customised for a specific purpose (Ha *et al.*, 2010), which will add to the difficulty of acquisition and the cost. Cloud point extraction and aqueous two-phase extraction have both been tested as a possibility for application with the fermentation, but no further development has occurred.

Perstraction was developed to overcome some of the potential problems with LLE and to enable the use of an extractant with a more favourable partition for ABE. It has been used in all fermentation operating modes, but there has been no research into the energy and economic impact on the ABE fermentation, Table 2.1. Research appears to have stagnated, with a primary focus on feasibility and extractant selection. No research has looked at process optimisation or the effects of different membrane/extractant combinations. With the membrane developments that have occurred for pervaporation (Liu *et al.*, 2013a), it could be assumed that the same developments would be applicable to perstraction. Perstraction results have shown promise for application to the ABE fermentation (Qureshi and Maddox, 2005), but more work is required to properly understand the process at a molecular level and to determine whether the membrane or extractant is dominant in extraction of the product. This is not to say that while perstraction can still be considered a relatively new technique it cannot be developed for commercial processes, as TNO have developed a modular ISPR perstraction unit which has been proven for a phenol fermentation (Heerema *et al.*, 2011b).

Adsorption is the only immobilisation-based technique that has been combined with fermentation. It is one of the more developed techniques for ISPR, but there is still a lot to be researched with regards to practical operation before it can be applied at industrial scale. There has been little research into how the process would be combined with the

fermentation. Many experiments have directly added the adsorbents to the stirred tank reactor (STR) or shake flask where the fermentation was occurring (Yang *et al.*, 1994). There have only been a couple of examples where the adsorbent had been combined with the fermentation in an external column configuration (Yang and Tsao, 1995; Wiehn *et al.*, 2014; Xue *et al.*, 2016a). Lee *et al.* (2015) found that the bacteria were negatively impacted by the addition of the adsorbent to the STR, through the abrasive contact of the adsorbent on the cell, resulting in the fermentation acid crashing. This indicates the importance of finding an adsorptive process that does not compromise the microorganisms' integrity. Wiehn *et al.* (2014) are the only researchers to have investigated an alternative process using expanded bed adsorption to reduce fouling of the adsorbent and harm to the bacteria. In contrast, the mechanism of adsorption is well understood, with many different adsorbents tested for efficient removal of ABE. Not all of these adsorbents have been tested with an actual ABE fermentation (Eom *et al.*, 2012a; 2012b). The adsorbents need to be tested with the ABE fermentation and investigations into the energy and economics are required.

Amongst the size-based techniques, it is possible to rule reverse osmosis out as a suitable, standalone, ISPR technique. For reverse osmosis to be technically viable it would have to be combined with a cell retention unit prior to it, similar to that performed by Garcia *et al.* (1986). Ultrafiltration can be used in combination with the ABE fermentation, but it would be more beneficial to the fermentation to combine it with another downstream unit allowing a recycle of any unused substrate and nutrients. Minier *et al.* (1990) demonstrated this by coupling the permeate with distillation, selectively removing the ABE from the broth and recycling any the ABE-free fermentation broth back to the fermentor. This makes ultrafiltration a cell retention unit operation rather than an ISPR technique, as it is the downstream technique that removes the ABE from the fermentation broth. Utilising a cell retention method, such as ultrafiltration or an immobilised bioreactor, could allow for better separation conditions to be used during ABE removal via other techniques.

From the data in Table 2.1, vacuum fermentations, gas stripping, pervaporation, LLE with an organic extractant and adsorption could be considered the most developed techniques. All these techniques would require a degree of development to ensure optimal performance within an industrial-based process. Pervaporation and adsorption are more complex and would require more development than the other techniques due to the use of a membrane or adsorbent.

2.3.3: Technology Readiness Levels

To better assess and rank the development of each ISPR technique for the ABE fermentation an assessment of the technology readiness level (TRL) was performed. This was based on the Horizon 2020 TRL scale (EARTO, 2014). Each ISPR technique was assigned a TRL, shown in Table 2.2. No techniques have been categorised as TRL7 or higher, progression to this level would be more fitting of a commercial/industrial focused company rather than a research organisation or university.

Table 2.2: Technology readiness levels of ISPR techniques.

TRL Scale	Horizon 2020 Definition	ISPR Techniques
1	Basic principles observed	Freeze crystallisation
2	Technology concept formulated	Cloud point extraction Liquid demixing Vapour permeation
3	Experimental proof of concept	LLE – ionic extractant Ultrafiltration Reverse osmosis Flash separation Aqueous two phase extraction
4	Technology validated in lab	Adsorption Vacuum fermentation Perstraction
5	Technology validated in relevant environment	Gas Stripping
6	Demonstration in relevant environment	LLE – organic extractant Pervaporation
7	System prototype demonstration in an operational environment	
8	System complete and qualified	
9	Actual system proven in operational environment	

It is not surprising that the conclusions from Table 2.1, that vacuum fermentations, gas stripping, pervaporation, LLE with an organic extractant and adsorption are the most developed techniques, are in agreement with the TRLs shown in Table 2.2. This is because both have been formulated based on the same literature. Interestingly, based on Table 2.2,

perstraction is considered to be at the same level as adsorption and vacuum fermentation. This is largely because perstraction for ISPR has been tested in batch, fed-batch and continuous operation with successful results.

2.3.4: Commercial/Industrial Scale Biobutanol Production

Currently, most research into *in situ* butanol removal has been performed at laboratory scale. There are very few reports of advancement of ISPR technologies to pilot or commercial scale (Van Hecke *et al.*, 2014). Green (2011) stated that product recovery via integrated fermentations was an attractive process option for commercial biobutanol production. Visioli *et al.* (2013) noted that between 2008 and 2012 there was an increasing trend of patents being registered for ISPR and butanol/isobutanol production, increasing the percentage of patents focused on ISPR to 20%. However, there is still a greater focus on substrate choice and treatment, and organism development (28% and 27%, respectively) (Visioli *et al.*, 2013). Many biobutanol-focused companies include ISPR as part of their technology portfolio: this indicates the importance of ISPR technology in progressing renewable chemical and biofuel production, below is a summary of their outputs.

The most publicised integrated fermentation technology is the GIFT ™ process developed by Gevo Inc. In 2013, this process started operating at a 1 million litre-scale fermentation at Gevo's plant in Luverne, Minnesota, USA (Gevo Inc, 2013). The process used an *ex situ* flash separation to remove isobutanol from the fermentation broth before returning the isobutanol-depleted broth to the fermentor. The vapours from the flash separation were condensed and phase separation was allowed to occur to obtain a bio-isobutanol-rich phase (Evanko *et al.*, 2012). This process would be applicable to the ABE fermentation and is very similar process to that modelled by Mariano *et al.* (2008; 2011a), although it does not include the cell separation step. The difference is that Gevo Inc. is that it is producing bio-isobutanol, rather than n-butanol through the ABE fermentation. Gevo are targeting both the chemical and fuel market with an initial focus on four key markets: solvents, gasoline blends, jet fuel and isooctane (Gevo Inc., 2017).

Butamax™ Advanced Biofuels LLC. , a BP and DuPont joint venture, is also focusing on the production of bio-isobutanol, using liquid-liquid extraction to remove the product from the fermentation (Grady *et al.*, 2009). From the Grady *et al.* (2009) patent, it could be surmised that the extractant to be used is oleyl alcohol, which is in agreement with Ishii *et al.* (1985)

and Roffler *et al.* (1987b), who found that oleyl alcohol is the best extractant for butanol recovery.

Cobalt Technologies were investigating the production of bio-n-butanol via a continuous immobilised biofilm concentrate fermentation (Wilson, 2011). Interestingly, they have not acknowledged the use of an ISPR process. Instead they have modified the traditional distillation process to be more energy- and cost-efficient. Cobalt Technologies achieved this through the design of a vapour compression distillation system that can reduce the separation energy by 50-75% (Contag, 2008; Kaufman *et al.*, 2010). This demonstrates that the microorganism and fermentation operating mode are a major factor in process design, and an integrated fermentation might not be best suited to all fermentation processes.

Green Biologics Ltd. (GBL) is headquartered in Abingdon, UK with a plant in the USA for the production of n-butanol and acetone. They have developed expertise in both synthetic biology and advanced fermentation process technology to produce bio-n-butanol through a *Clostridium* biocatalyst using a range of sustainable feedstocks (Davies, 2013; Green Biologics Ltd, 2014). GBL have included ISPR as part of their process development with a patent focusing on a single-stage fermentation with controlled product removal (Green *et al.*, 2014).

Optinol Inc, based in San Fransisco, USA have focused on process development of n-butanol using a non-genetically modified organism, rather than developing a high-butanol producing strain (Optinol Inc, 2014). As part of their process development an ISPR process was created extracting the butanol into an oil phase, with the oil removed from the fermentor as a foam. When the foam is broken an aqueous phase containing 4% butanol is formed. Optinol have stated that for a fermentation broth with a butanol concentration of 16g/L, 34% of butanol can removed through this method (Day *et al.*, 2013). While the oil used for extraction has a low affinity for butanol compared to oleyl alcohol, this extraction technique exploits the oil-water phase properties to allow for a simple separation of the two phases. Since 2013 there has been no further news about the progress of this company.

VITO, a European research organisation in Belgium has also investigated ISPR in relation to the ABE process. They have focused on membrane technology, in particular pervaporation, publishing a number of research articles on the subject (Van Hecke *et al.*, 2012; 2013; 2015). Membrane technology has also been a focus of the Dutch research organisation TNO, who have investigated perstraction for ISPR of phenol (Heerema *et al.*, 2011a; 2011b). While their

process has not been investigated with the ABE fermentation, developments within both these companies have used commercially available membranes. This indicates that the industrial use of membrane technology is likely to be possible in the near future.

ISPR has the potential to significantly improve fermentation, with productivity increases over 300% observed (Ezeji *et al.*, 2004a). There is a large commercial interest as biobutanol-focused companies all have ISPR included in their product portfolio. This is further supported by research organisations having an interest in the application of ISPR, particularly as the application of ISPR will be relevant to other fermentation processes as well. The techniques used by industrial biotechnology companies are similar to the most researched techniques in Table 2.1, with the use of a vacuum for low temperature separation and LLE methods being the most popular. Both these techniques are well understood separation techniques found in other industries. Utilising techniques that are already understood has meant a quicker speed of development. For example LLE was the first fully developed technique, by Roffler *et al.* (1987a) in 1987 with a full process design and economic analysis.

2.3.5: Progressing ISPR

Research into ISPR and the ABE fermentation can be divided into two classes: advanced, meaning approaching or in commercial operation at some scale, and preliminary, meaning still very much in the research phase. The techniques which have been investigated most thoroughly are gas stripping, vacuum fermentations, pervaporation, LLE, perstraction and adsorption. Other techniques, such as flash separations, extractive fermentations using alternative extractants such as ionic liquids, liquid demixing, aqueous two phase extraction and freeze crystallisation are at a considerably earlier development stage. Reverse osmosis has greater applicability as an end of process dehydration technique rather than to remove ABE from the fermentation. It is debatable whether ultrafiltration can be classified as an ISPR technique as it has greater potential as a biomass concentration or removal step within the integrated fermentation process. Cell retention could be considered as an ISPR aid, to be used in conjunction with techniques considered harmful to the microorganism, and would include ultrafiltration and immobilised fermentations.

For the “real” impact of ISPR research, the application and scale up needs to be considered. Researchers need to consider how applicable a laboratory method is at scale and if performance will be hindered by a more industrial applicable method. More work is also

required on the energy benefits and economic impact of each technique. There are many statements indicating ISPR “should” or “could” reduce the energy demand without research to confirm this. Research also needs to focus on the use of commercially available and cost effective materials. The development of new materials for separation is desirable and necessary, but ISPR technology will be left behind if materials are not easily accessible when processes are being designed.

2.4: Summary

This chapter has provided an overview of the ABE fermentation, ISPR, and an overview of all techniques proposed for combination with ABE fermentation. The proposed techniques have been shown to be at a range of different development stages. The next steps are to further investigate the most developed ISPR techniques and their application to the ABE fermentation. As already stated, these techniques are gas stripping, pervaporation, vacuum fermentations, liquid-liquid extraction, perstraction and adsorption. A greater understanding of these techniques from a fermentation, energy and economic assessment is required. This will help satisfy the key requirements of ISPR as stated by Van Hecke *et al.* (2014). The following chapter provides an in-depth assessment of these techniques and their impact on the fermentation, to determine whether there is an optimum technique or which information is missing and required to enable this assessment.

Chapter 3. Applied *In Situ* Product Recovery in ABE Fermentation

This chapter is a modified version of a review article originally published in Biotechnology Progress (Outram *et al.*, 2017), therefore there is some intended repetition with the previous chapter.

3.1: Introduction

This chapter focuses on the application of ISPR to the ABE fermentation and the impact it has. The primary focus has been free cell (not immobilised or biofilm based) fermentations in a stirred tank reactor (STR), to allow for comparison of the various ISPR techniques. Other reactor configurations, such as immobilised fermentations, have been considered where STR fermentations have not been performed. The techniques that have been experimentally combined with the ABE fermentation are gas stripping, vacuum fermentations, pervaporation, liquid-liquid extraction, perstraction and adsorption.

It must be noted that the application of ISPR to the ABE fermentation is the primary separation stage. It is therefore additional to the further downstream process, which is typically distillation, to separate individual products and achieve the desired purities for sale.

3.2: *In Situ* Product Recovery

The aim of ISPR techniques is to remove the product from the vicinity of the cell as soon as it is formed (Freeman *et al.*, 1993), this should lead to increased productivity and overall titres for fermentations in which product inhibition occurs and reduced waste water treatment costs (Roffler *et al.*, 1984). There have been several comprehensive reviews covering ISPR for a wide range of fermentations and products. Van Hecke *et al.* (2014) provided the most recent review, which considered developments in ISPR between 2003-2013. They conclude that more research is required to prove scalability, long-term robustness and stability of the ISPR technology, decreased energy consumption and to maximise the product recovery (Van Hecke *et al.*, 2014).

Since 2012, there has been a dramatic increase in the number of reviews focusing on the ISPR from the ABE fermentation. Abdehagh *et al.* (2014), focussed on the separation ability of the

technique, rather than improvements in production. This study concluded that pervaporation and adsorption show the most promise for ISPR. Huang *et al.* (2014) provide an overview of gas stripping, vacuum/flash separations, liquid-liquid extraction, membrane techniques and adsorption, with a focus on novel separating agents such as ionic liquids and composite membranes. Xue *et al.* (2014d) qualitatively compares ISPR techniques to conventional distillation, concluding that no ISPR technique will be able to concentrate the products to reagent grade. The most recent review was by Staggs and Nielsen (2015), which focused on the mode of application of the ISPR technique i.e. direct contact or recirculation in an external contactor. To complement these reviews this review's focus is on the effect of each technique on the fermentation.

The ISPR techniques were compared in terms of the amount of substrate utilised, productivity and yield. The "substrate utilised" is defined here as the total amount of substrate consumed during the fermentation. The productivity is defined here as the mass (g) of ABE produced per litre of reactor volume per hour. The yield is the mass (g) of ABE produced per mass (g) of substrate consumed (Qureshi and Blaschek, 2001a). The equations for yield and productivity are supplied in section 2.2.1. The % substrate utilised, productivity or yield increase is the percentage difference between the substrate utilised, productivity or yield for the integrated *in situ* recovery fermentation and the non-integrated (control) fermentation. These parameters have been selected as they are generally considered to be the main parameters of comparison in experiment-based literature, particularly productivity and yield. Substrate utilisation was selected to demonstrate the improvements in the fermentation, particularly fermentation longevity due to reduced toxicity. Measurement of substrate can be considered more reliable than product concentration, which can be highly inaccurate due to varying product separation methods. Furthermore, the concentrated product is not always directly measured, sometimes being inferred from model solution data or assuming the yield is the same as the control fermentation (de Vrije *et al.*, 2013). Also, the final concentration varies based on volume used for calculation, i.e. fermentation volume (which is variable during the fermentation) through to the condensate concentration post separation, particularly with evaporative techniques. Productivity and yield provide a standardised measure of the fermentation performance. By comparing the % increase compared to the control fermentation, the effects of various

differences in experimental methods should be negated, or at least minimised, allowing trends relating to the impact of the ISPR technique on the fermentation can be observed.

This review differs from the previous reviews by taking a quantitative approach to the comparing the experimental data of the ISPR techniques and their impact on the fermentation. This review also considers the final concentration from each ISPR technique that will enter the downstream distillation process, where possible.

3.2.1: Gas Stripping

Gas stripping is a separation technique that involves the removal of solvents via dissolution into a gas passing through the fermentation broth. This technique was studied by a range of authors from the mid 1980s (e.g. Ennis *et al.* (1986)) , through to the present day (e.g. Xue *et al.* (2016b)). Numerous publications cover all operation modes and a range of bioreactor configurations (Ezeji *et al.*, 2003; Lu *et al.*, 2012; Chen *et al.*, 2014a; 2014b).

Gas stripping for ABE fermentations involves the recycling of the fermentation gases (carbon dioxide and hydrogen), or application of other anaerobic gases such as oxygen-free nitrogen (Ennis *et al.*, 1986; 1987; Groot *et al.*, 1989), through the fermentor via a condenser to remove the ABE from the gas stream (Qureshi *et al.*, 1992). As it can be performed *in situ*, at laboratory scale, without the need for expensive equipment and reactor modifications gas stripping is considered a simple technique (Qureshi and Blaschek, 2001b). This cannot be assumed to be the same at commercial plant scale. Based on data from Ezeji *et al.* (2003), the concentration in the gas stream is very dilute at approximately 1.7 mg L^{-1}, meaning that large condensing duties will be required, which will significantly increase operating costs. Additionally the compressor duty to supply gas at flow rates of 2-3 vvm of a plant-scale reactor is energy intensive (Ennis *et al.*, 1986). On top of the large condensation capacity, the plant design needs to ensure that all equipment is capable of withstanding the high pressures required for the circulating gasses to overcome the static head in the fermentor. The design, particularly for compressors, also needs to account for any safety issues that might arise from compressing hydrogen to ensure flow through the fermentor, this will see an increase in equipment cost.

A wide range of studies have been performed for gas stripping, with Ezeji's body of work (Ezeji *et al.*, 2003; 2004a; 2005; 2007a; 2013) being the most comprehensive. A general conclusion to be drawn from this data is that the productivity of the fermentation is improved through the application of gas stripping. The productivities in Table 3.1 show an increase moving from batch to fed-batch. It must be noted that the productivity increase seen by Maddox *et al.* (1995), 357%, is due to the low productivity of the control fermentation (0.07 g ABE L^{-1} h^{-1}). This demonstrates that relieving product inhibition has a significant positive effect on the fermentation. This removal of product toxicity has allowed for more substrate to be consumed, with more than a 100% increase in substrate utilization possible.

Table 3.1: Free cell ABE fermentation outcomes with gas stripping in an STR.

Mode	Microorganism	Substrate (Concen-tration for ISPR)	% Substrate Increase for ISPR (vs. control)	ABE Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g ABE g^{-1} Substrate)	% Yield Increase (vs control)	Gas[a]	Ref.
Batch	*Clostridium acetobutylicum* P262[b]	Lactose (58 g L^{-1})	101%	0.31	41%	0.27	-31%	N_2	(Ennis *et al.*, 1986)
	C. acetobutylicum P262[b]	Whey Permeate/ Lactose (199 g L^{-1})	542%	0.32	357%	0.35	35%	CO_2+ H_2	(Maddox *et al.*, 1995)
	Clostridium beijerinckii BA101	Glucose (162 g L^{-1})	263%	0.6	107%	0.47	21%	CO_2+ H_2	(Ezeji *et al.*, 2003)
	C. beijerinckii BA101	Liquefied Corn Starch (LCS) (55 g L^{-1})	23%	0.31	107%	0.43	5%	CO_2+ H_2	(Ezeji *et al.*, 2007a)
	C. beijerinckii BA101	Saccharified Liquefied Corn Starch (SLCS) (64 g L^{-1})	41%	0.4	74%	0.41	2%	CO_2+ H_2	(Ezeji *et al.*, 2007a)
	C. beijerinckii CC101	Wood Pulp Hydrolysate (33 g L^{-1})	36%	0.17	55%	0.39	18%	CO_2+ H_2	(Lu *et al.*, 2013)
	C. beijerinckii NRRL B593[c]	Glucose/ Xylose (60 g L^{-1})	77%	0.29	81%	0.32[d]	-	CO_2+ H_2	(de Vrije *et al.*, 2013)

Mode	Microorganism	Substrate (Concen-tration for ISPR)	% Substrate Increase for ISPR (vs. control)	ABE Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g ABE g^{-1} Substrate)	% Yield Increase (vs control)	Gas[a]	Ref.
Fed- Batch	*C. beijerinckii* BA101	Glucose (500 g L^{-1})	1001%	1.16	300%	0.47	21%	CO_2+ H_2	(Ezeji *et al.*, 2004a)
	C. beijerinckii BA101	Saccharified Liquefied Corn Starch (SLCS) (226 g L^{-1})	395%	0.59	157%	0.36	-10%	CO_2+ H_2	(Ezeji *et al.*, 2007a)
	C. acetobutylicum P262[b]	Whey Permeate (183 g L^{-1})	576%	0.26	271%	0.38	19%	CO_2+ H_2	(Qureshi *et al.*, 1992)
Continuous	*C. beijerinckii* BA101	Glucose (1125 g L^{-1})[e]	2278%	0.92	229%	0.41	5%	CO_2+ H_2	(Ezeji *et al.*, 2013)
	C. beijerinckii NRRL B593[c]	Glucose/ Xylose (52 g L^{-1})[f]	56%	0.93	40%	0.30[c]	-	N_2	(de Vrije *et al.*, 2013)
	C. beijerinckii NRRL B593[c]	Glucose/ Xylose (41 g L^{-1})	88%	1.3	65%	0.30[c]	-	N_2	(de Vrije *et al.*, 2013)

[a] CO_2 and H_2 represent recycling of the gases produced during fermentation

[b] *C. acetobutylicum* P262 has since been reclassified as *Clostridium saccharobutylicum* P262 (Keis *et al.*, 2001)

[c] *C. beijerinckii NRRL B593* produces isopropanol instead of acetone (de Vrije *et al.*, 2013)

[d] Yield has been assumed equal to the yield of the batch for calculations of the productivity (de Vrije *et al.*, 2013)

[e, f, g] The dilution rate was 0.003 h^{-1}, 0.06 h^{-1} and 0.11 h^{-1}, respectively

Interestingly, there is a decrease in productivity when moving to a continuous fermentation compared to the fed-batch fermentation, but it should be noted that this comparison is based upon only one strain *C. beijerinckii* BA101. In the continuous fermentations performed by Ezeji *et al.* (2013), this decrease in productivity cannot be related to the decrease in yield (0.92 g L^{-1} h^{-1} and 0.41 g g^{-1} for continuous (Ezeji *et al.*, 2013) compared to 1.16 g L^{-1} h^{-1} and 0.47 g g^{-1} for fed-batch (Ezeji *et al.*, 2004a)), because if the yield was the same as in the fed-batch fermentation the productivity would still be lower. Low productivity is a result of the cyclic fermentation profile, switching between the acidogenic and solventogenic phase, which means that a true steady state is not attained. The reduced yield is due to the removal of some of the nutrients from the process, in the reactor bleed, meaning 100% sugar utilisation was not possible (Ezeji *et al.*, 2013).

There are some results in Table 3.1 which show the yield of the gas stripping process being greater than the theoretical yield of the bacteria, which is 0.40 g g^{-1} (Ezeji *et al.*, 2013). In the work by Ezeji *et al.* (2003; 2004a; 2013) the increased yield, 0.41-0.47 g g^{-1}, is contributed to the consumption of other carbon sources present in the complex medium used, such as sodium acetate. It is also suspected that less substrate is used for biomass maintenance, therefore a greater product yield is possible (Ezeji *et al.*, 2004a). No reason was provided for the higher than expected yield in the case of Ezeji *et al.* (2007a). In some cases a decrease in yield compared to the non-integrated fermentation is seen, for example Ennis *et al.* (1986) observed a 31% decrease in yield; but this is probably related to inefficient condensing capability, meaning that not all solvents are captured and are consequently not accounted for when calculating the yield, as seen by Groot *et al.* (1989) and Ezeji *et al.* (2004a) who take into account solvent losses when calculating the overall yield. This inability to capture all the solvents has a knock-on effect, meaning that the productivities cannot be assumed to be accurate, adding further uncertainty to any comparison between operating modes. de Vrije *et al.* (2013) overcame the loss of products by assuming the same yield as the control fermentation, 0.30-0.32 g g^{-1}, and used this to calculate the productivity. This calculation method is likely to provide an inaccurate result as it assumes that the ISPR technique has no negative or positive effect on the microorganism's performance.

Gas stripping has limitations due to the low ABE concentration in fermentation broth, large quantities of water removed and high gas flow rates required (Xue *et al.*, 2012). Xue *et al.* (2012) proposed that operating at higher butanol concentrations, 8 g butanol L^{-1} compared

to 5 g butanol L^{-1}, would increase the concentration of product in the vapour and reduce the energy for separation. The downside to this is if 8 g butanol L^{-1} is often inhibitory to the bacteria. Xue *et al.* (2012) tested this idea in an immobilised fermentation using an intermittent gas flow rate. The gas flow only operated while the butanol concentration in the broth was greater than 8 g butanol L^{-1}. This gas stripping regime saw a 33% increase in productivity compared to the control, while the yield remained constant. Stripping at a higher concentration saw a condensate concentration of 195.9 g ABE L^{-1}, compared to 76.8 g ABE L^{-1} achieved by Ezeji *et al.* (2007a) in a fed-batch free cell fermentation with saccharified liquefied cornstarch with the butanol maintained no higher than 5 g butanol L^{-1}.

3.2.1.1 Hybrid Gas Stripping

Since 2013 there has been a flurry of investigations into hybrid separations. A major focus of the hybrid separation processes has been improving the efficiency of gas stripping. This has included investigations into multi-stage gas stripping processes, increasing the temperature at which gas stripping is performed and hybrid gas-stripping pervaporation processes (de Vrije *et al.*, 2013; Setlhaku *et al.*, 2013; Xue *et al.*, 2013a; Chen *et al.*, 2014b; Xue *et al.*, 2014b; 2016b), with the aim of reducing the energy requirements for further separation of the condensate. Oudshoorn *et al.* (2009) estimated the selectivity of gas stripping for butanol to be between 4 and 22, which is low compared to distillation with an estimated selectivity of 72, therefore the recovered solution is not very concentrated. It has been widely noted that to achieve significant decreases in the energy for downstream purification, two-phase separation needs to be observed in the recovered ABE solution (Lu *et al.*, 2012; 2013; Chen *et al.*, 2014b). To achieve this phase separation, it has been suggested that the butanol concentration in the fermentor should be greater than 8 g L^{-1}, but concentrations this high start to impact on the fermentation performance (Chen *et al.*, 2014b; Xue *et al.*, 2014b).

To achieve this higher concentration Xue *et al.* (2013a) proposed a two-stage gas stripping process. The aqueous phase condensate from the first stripping stage, 153 g ABE L^{-1}, being subjected to gas stripping to achieve a more concentrated solution, 447 g ABE L^{-1}. When combined with the organic phase from the first stripping stage the final product solution was 532 g ABE L^{-1} (Xue *et al.*, 2013a). The first stage reduced inhibition in the fermentor, while the second stage increased the concentration of condensate. Xue *et al.* (2014b) proceeded

to further optimise the process and achieved a final product concentration of 671 g ABE L^{-1}, predicting a 50% decrease in operational energy to 7-15 MJ kg^{-1} butanol (Xue *et al.*, 2014d).

de Vrije *et al.* (2013) discussed the use of increasing the temperature while gas stripping to improve the selectivity of the process. de Vrije *et al.* (2013) utilised the bacteria's natural sporulation cycle for a repeated batch process. The broth was heated to 70°C at the end of a batch to remove the products via enhanced gas stripping and heat shock the spores to restart the fermentation with fresh media added. The final condensate concentration, nor total product formation was not stated so this cannot be compared to the two-stage process proposed by Xue *et al.* (2014b). Chen *et al.* (2014b) also investigated the use of a higher stripping temperature, but combined the fermentation with an immobilised cell bioreactor. Immobilisation of the cells allowed the fermentation medium to be heated to 70°C without impacting the viability of the bacteria. This saw condensate concentrations of 703 g butanol L^{-1} in the organic phase and 78 g butanol L^{-1} in the aqueous phase. The combined concentration was 150 g butanol L^{-1} (Chen *et al.*, 2014b), indicating that a two-stage stripping system will offer better performance.

Gas stripping has also been combined with pervaporation, using a carbon nanotube filled polydimethylsiloxane (CNT-PDMS) membrane (Xue *et al.*, 2016b). Gas stripping was first performed on the fermentation broth to relieve ABE toxicity. Pervaporation was then performed on the aqueous phase portion of the condensate to further increase the final product concentration. This method produced a final product concentration of 623 g ABE L^{-1} (Xue *et al.*, 2016b), which is slightly lower than that achieved using the two-stage gas stripping process (671 g ABE L^{-1}) (Xue *et al.*, 2014b). Xue *et al.* (2016b) predict that the energy for the pervaporation step will be as low as 4 kJ kg^{-1} butanol due to the starting solution containing 80 g L^{-1} butanol. The overall two-stage gas stripping-pervaporation process would require ~20 MJ kg^{-1} butanol. Compared to two-stage gas stripping a hybrid gas stripping-pervaporation process is more complex, producing a lower product concentration and requires more energy for this stage of the process.

These hybrid techniques apply a second/enhanced separation stage to the fermentation. Other than de Vrije *et al.* (2013), have all focused on immobilised fermentations. It would be useful to see the potential impact these hybrid techniques (if possible) could have when combined with free cell fermentations. Liu *et al.* (2004) and van der Merwe *et al.* (2013) proposed flowsheets with alternative product concentration techniques to distillation for

the ABE fermentation. It would be advantageous to complete a similar analysis for the various hybrid options to help decide which further concentration techniques would be best suited for an industrial process.

3.2.2: Vacuum Fermentation

Vacuum fermentations have a reduced pressure in the fermentor, causing the ABE to "boil off" at fermentation temperature. Vacuum fermentations were first used in the ethanol industry to selectively remove ethanol from fermentation broths. The use of a vacuum for an ABE fermentation should be more straightforward than for an ethanol fermentation, as the *Clostridium sp.* used are strict anaerobes (Mariano *et al.*, 2011b). The viability of vacuum fermentations was experimentally tested by Mariano *et al.* (2011b; 2012a; 2012b).

Mariano *et al.* (2011b) demonstrated that it is possible to recover ABE from fermentation broths under vacuum on a laboratory scale with no adverse effects on the bacteria. The system was initially characterised using a model ABE solution, with concentrations ranging from 5-15 g butanol L^{-1}, but this was found to be unrepresentative of real fermentation broths in which the gas created by the bacteria expands under reduced pressure, stripping the solvents from the broth. This effectively creates a hybrid gas stripping-vacuum system. Mariano *et al.* (2012b) reported that under constant vacuum conditions the rate of removal of butanol was approximately 10 times higher than that found by Ezeji *et al.* (2003) using gas stripping, which could reduce the butanol concentration by up to 68.5% (Mariano *et al.*, 2012b). Performing the fermentation under vacuum was able to achieve butanol concentrations of less than 1 g L^{-1} in the fermentation broth (Mariano *et al.*, 2011b). More recently vacuum fermentation was also proven to be effective with combining with simultaneous saccharification, fermentation and recovery (Qureshi *et al.*, 2014b). Qureshi *et al.* (2014b) successfully demonstrated the combined process using 86 g L^{-1} corn stover as a feedstock, in simultaneous saccharification, fermentation and recovery. The ability to utilise lignocellulosic feedstocks as well as combining feedstock treatment with the fermentation and recovery should also see a reduction in operational costs.

Two vacuum modes have been investigated: constant and cyclic. Cyclic vacuum fermentations were found to be considerably more competitive in terms of energy demand than traditional distillation. The cyclic vacuum process allows the concentration of butanol to build up, then reduces the concentration rapidly by applying a vacuum for 2 hours,

repeating this process throughout the fermentation (Mariano *et al.*, 2012a). This is the only variation in operation that has been investigated. Currently all trials have been on batch fermentations (Table 3.2) with a maximum applied vacuum time of 30 hours (Mariano *et al.*, 2011b), so whether vacuum fermentation can be extended for improved productivity is unknown. Whether this extended time at reduced pressure would impact microbial performance is also unknown (Mariano *et al.*, 2011b; 2012a; 2012b).

Table 3.2: ABE fermentation performance with *in situ* vacuum recovery in an STR.

Mode	Micro-organism	Substrate (Concen-tration for ISPR)	% Substrate Increase for ISPR (vs. control)	ABE Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield (g ABE g^{-1} Substrate)	% Yield Increase (vs. control)	Operating Temp-erature (°C)	Vacuum Range (mmHg)	Vacuum Operating Mode	Ref.
Batch	*C. beijerinckii* P260	Glucose (62 g L^{-1})	38%	0.34	31%	0.24	-31%	37	711-737	Inter-mittent	(Mariano *et al.*, 2011b)
	C. beijerinckii NCIMB 8052	Glucose (66 g L^{-1})	56%	0.37	54%	0.34	-8%	35	711-737	Inter-mittent	(Mariano *et al.*, 2012a)
	C. beijerinckii P260	Glucose (58 g L^{-1})	29%	0.28	8%	0.22	-37%	37	711-737	Contin-uous	(Mariano *et al.*, 2011b)
	C. beijerinckii NCIMB 8052	Glucose (65 g L^{-1})	54%	0.43	79%	0.29	-22%	35	711-737	Contin-uous	(Mariano *et al.*, 2012b)
	C. beijerinckii P260	Corn Stover (83 g L^{-1})	7%	0.34	55%	0.39	30%	35	584	Contin-uous	(Qureshi *et al.*, 2014b)

All of the literature focussing on vacuum fermentations for the ABE process is a product of the same researchers (Mariano *et al.*, 2011b; 2012a; 2012b; Qureshi *et al.*, 2014b). In each of these studies inefficient condensation resulted in low ABE capture with ABE condensate concentration of 16-49 g L^{-1}, lower than the concentration required for spontaneous phase separation (Mariano *et al.*, 2011b). Consequently the yield has been underestimated as demonstrated by the negative yield increases seen in Table 3.2. Qureshi *et al.* (2014b) accounted for losses of ABE in the system, based on previous work, and therefore achieved a positive yield increase of 30%. This appears to be a common flaw in evaporative techniques, in particular vacuum fermentation and gas stripping (Ezeji *et al.*, 2004a; Qureshi *et al.*, 2014b). Another potential problem with the use of vacuum fermentations, highlighted by Mariano *et al.* (2011b; 2012a), was that a small concentration of acids (up to 0.4 g L^{-1}) was detected in the condensate. As the fermentation utilises acids as intermediates, acid removal is undesirable during ISPR as it will reduce the yield of the process.

Mariano *et al.* (2012a) assessed the energy requirement for the addition of a vacuum to the fermentation. They showed that the use of a vacuum reduced the downstream distillation energy requirement by 11.2 MJ kg^{-1} butanol for a continuous vacuum and 15 MJ kg^{-1} butanol for intermittent vacuum. When combined with the energy required for the vacuum fermentation the total energy requirement became 32.4 and 22.0 MJ kg^{-1} butanol for continuous and intermittent vacuum, respectively. For a comparable batch process without ISPR, the energy requirement was 26.8 MJ kg^{-1} butanol. The use of a continuous vacuum will see an increase in the plant energy demand, defeating one of the main purposes of adding ISPR. The use of an intermittent vacuum sees an 18% decrease in energy. Mariano *et al.* (2011a) have demonstrated that as the butanol concentration in the first distillation column increases the energy requirements rapidly decrease. This can be inferred as the reason for the intermittent vacuum fermentation requiring less energy than the continuous vacuum fermentation, where the condensate concentrations were 51.5 g ABE L^{-1} and 33 g ABE L^{-1} respectively (Mariano *et al.*, 2012a).

Generally, the conclusions over application of vacuum to ABE fermentations are not definitive, as there is not enough data (Table 3.2). The application of a vacuum can increase the substrate utilisation and productivity of the fermentation, but without an efficient product capture step the benefits are not observed, as significant product is lost. The decreased yield would have a negative effect on the process economics, impacting the

amount of feedstock required. Additionally, the practicality of scaling up a vacuum fermentation system needs to be considered. This process would require a reinforced fermentor to withstand the continuous reduced pressure that the fermentor would be operating at, this would increase the required capital cost. The duration that the vacuum is operational for will also need to be taken into account to ensure the equipment is suitably rated for these conditions. Consideration is also required as to the potential hazards if air was to leak into the system, and potential effects when mixed with the fermentation gasses, in particular hydrogen, and the increased risk of explosion.

3.2.3: Pervaporation

Pervaporation utilises a membrane between the fermentation broth and the gaseous phase (Groot *et al.*, 1984a). A simplified schematic is shown in Figure 3.1. Pervaporation renders the flowsheet more complex, as generally an external unit is required to perform the separation. It is possible for pervaporation to be performed within the bioreactor (Larrayoz and Puigjaner, 1987; Cho and Hwang, 1991), but this would be an unusual configuration.

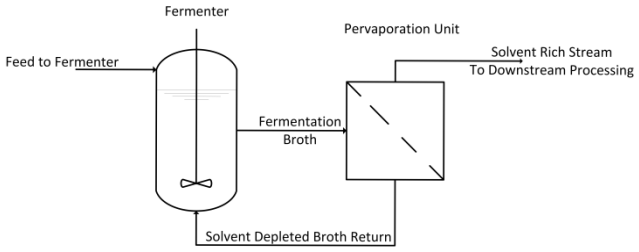


Figure 3.1: Diagram of a fermentation with pervaporation.

Pervaporation has been shown to increase the substrate utilisation, productivity and yield of ABE fermentations (see Table 3.3). It is the most widely researched area in relation to ISPR and the ABE fermentation. Within this body of research, there is a greater focus on membrane performance than integrated fermentation performance (Liu *et al.*, 2013a). Of the work performed where the ABE fermentation is coupled to a pervaporation system no definitive conclusion can be drawn on fermentation or pervaporation operating conditions.

Table 3.3: Free cell ABE fermentation with *in situ* recovery by pervaporation in an STR.

Mode	Microorganism	Substrate (Concen-tration for ISPR)	% Substrate Increase for ISPR (vs. control)	Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g ABE g^{-1} Substrate)	% Yield Increase (vs. control)	Mem-brane	Driving Force	Driving Force Rate	Ref
Batch	*C. beijerinckii* BA101	Glucose (60 g L^{-1})	5%	0.5	47%	0.42	42%	Silicone tubing	Air	2-18 L min^{-1}	(Qureshi and Blaschek, 1999c)
	C. acetobutylicum XY16	Glucose (60 g L^{-1})	15%	0.3	50%	0.37	29%	PDMS/ ceramic composite	Vacuum	<400Pa	(Wu *et al.*, 2012)
	C. saccharobutylicum P262	Whey Permeate/ Lactose (211 g L^{-1})	424%	0.43	207%	0.37	19%	Silicone	N_2	40-42 L hr^{-1}	(Qureshi *et al.*, 2014a)
	C. acetobutylicum DP217	Cassava (70 g L^{-1})	0%	0.51	21%	0.36	9%	Silicalite-PDMS/PA N	Vacuum	280 Pa	(Li *et al.*, 2014)
Fed-Batch	*C. acetobutylicum* ATCC824	Glucose (199 g L^{-1})	138%	0.66	47%	0.27	-18%	PDMS	Vacuum	200 Pa	(Shin *et al.*, 2015)
	C. acetobutylicum ATCC824	Glucose (276 g L^{-1})	231%	0.94	109%	0.28	-15%	SDS	Vacuum	200 Pa	(Shin *et al.*, 2015)
	C. acetobutylicum XY16	Glucose (200 g L^{-1})	285%	0.21	5%	0.28	-3%	PDMS/ ceramic composite	Vacuum	<400Pa	(Wu *et al.*, 2012)
	C. acetobutylicum XY16	Glucose (200 g L^{-1})	285%	0.25	25%	0.3	3%	PDMS/ ceramic composite	Vacuum	<400Pa	(Wu *et al.*, 2012)

Mode	Microorganism	Substrate (Concen-tration for ISPR)	% Substrate Increase for ISPR (vs. control)	Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g ABE g^{-1} Substrate)	% Yield Increase (vs. control)	Mem-brane	Driving Force	Driving Force Rate	Ref
Fed-Batch	*C. beijerinckii* BA101	Glucose (384 g L^{-1})	568%	0.98	180%	0.43	-2%	Silicone	Air	2-18 L min^{-1}	(Qureshi and Blaschek, 2000)
	C. acetobutylicum ATCC824	Glucose (445 g L^{-1})	950%	0.18	33%	0.35	21%	Silicalite-Silicone	Vacuum	266-666 Pa	(Qureshi *et al.*, 2001)
	C. acetobutylicum DP217	Cassava (736 g L^{-1})	951%	0.76	80%	0.38	15%	Silicalite-PDMS/PAN	Vacuum	280 Pa	(Li *et al.*, 2014)
	C. acetobutylicum P262[a]	Whey Permeate (123 g L^{-1})	355%	0.14	100%	0.34	6%	Polyprop-ylene	N_2	10-20 L min^{-1}	(Qureshi *et al.*, 1992)
	C.acetobutylicum ATCC 55025	Glucose (172 g L^{-1})	144%	0.46	15%	0.32	3%	Zeolite-PDMS	Vacuum	<1kPa	(Xue *et al.*, 2015)
Continuous	*C. acetobutylicum* ATCC824	Glucose (100 g L^{-1})/ Xylose (50 g L^{-1})[b]	201%	0.65	126%	0.30	67%	PDMS (Pervatech)	Vacuum	960 Pa	(Van Hecke *et al.*, 2015)

[a] *C. acetobutylicum* P262 has since been reclassified as *C. saccharobutylicum* P262 (Keis *et al.*, 2001)

[b] The overall dilution rate was 0.0017 h^{-1}

For pervaporation the major decision to be made is the choice of membrane, as the ideal membrane should selectively allow the transfer of ABE while retaining butyric acid, acetic acid, water and nutrients. The membrane also needs to be minimally fouling, so that it is not blocked by cells adhering to the membrane surface. Table 3.3 shows that a range of organophilic membranes have been tested, all of which show an improvement in the productivity of the fermentation. While silicone (including polydimethylsiloxane, PDMS) has been the most investigated membrane, as it is commercially available, inexpensive, and offers easy manipulation for the development of laboratory-scale pervaporation units (Groot *et al.*, 1984a; 1984b; Larrayoz and Puigjaner, 1987; Qureshi and Maddox, 1992; Qureshi and Blaschek, 1999c; Xue *et al.*, 2014c; Shin *et al.*, 2015; Van Hecke *et al.*, 2015) other membrane choices, including polypropylene (Qureshi *et al.*, 1992), oleyl alcohol liquid membrane on a polypropylene support (Matsumura *et al.*, 1988), polystyrene-*b*-polydimethylsiloxane-*b*-polystyrene (SDS) (Shin *et al.*, 2015)and PDMS supported ionic liquid membranes (Izák *et al.*, 2008) have been investigated. More recently there has been a move towards the use of composite membranes, utilising a combination of materials for improved selectivity and flux performance. This has included silicalite-silicone composite membrane (Qureshi *et al.*, 2001) silicalite-PDMS/polyacrylonitrile (PAN) membrane (Li *et al.*, 2014), PDMS/ceramic composite (Wu *et al.*, 2012), zeolite-mixed PDMS (Xue *et al.*, 2015) and carbon nanotube filled PDMS (CNT-PDMS) (Xue *et al.*, 2014a). A wider range of membranes have been investigated for butanol/water or ABE/water solutions, but this does not necessarily transfer to the performance in conjunction with a fermentation (Liu *et al.*, 2013a).

The membrane choice affects the selectivity and diffusion rates of the ABE, which will determine the concentration of the ABE in the permeate. Selectivity and flux are both functions of the membrane. Careful selection of the membrane material and increasing membrane thickness can improve the selectivity. In contrast, the flux increases with a decrease in membrane thickness. Other improvements in flux can be made to pervaporation through the use of higher temperatures, for example, but this would require an additional step of microorganism removal prior to heating the pervaporation feed stream (to 65-78°C), and cooling of the retentate prior to re-addition to the bioreactor (Van Hecke *et al.*, 2012; Cai *et al.*, 2013). Other factors such as feed concentration and composition, biomass concentration and sweep gas flow rate also influence the membrane flux (Qureshi and Blaschek, 1999a; 1999c). Qureshi and Blaschek (1999c) and Gapes *et al.* (1996) state that the application of a vacuum on the permeate side increased the flux compared to the

application of a sweep gas, which explains why recent research has focussed on vacuums (Van Hecke *et al.*, 2012; Wu *et al.*, 2012; Van Hecke *et al.*, 2013).

The use of a composite membrane has also been proposed as a method of achieving non-competitive flux and selectivity. Polymers have high flux, are relatively cheap and easy to fabricate into a membrane, but are prone to aging over a long time. Inorganic materials are often highly selective for butanol and have good strength but are expensive. By combining both materials together the membrane should be more selective with a sufficient flux while not having a prohibitive cost for scale-up (Huang *et al.*, 2014). Li *et al.* (2014) used a silicalite-PDMS/PAN membrane and were able to achieve an average permeate concentration of 201 g ABE L^{-1}, resulting in spontaneous phase separation. Xue *et al.* (2014a) used a CNT-PDMS membrane. They were able to achieve a butanol separation factor of 16.6 and butanol titres over 100 g butanol L^{-1} in the permeate, when the membrane consists of 10% carbon nanotubes. This membrane has not been directly applied to the ABE fermentation. Zeolite-PDMS were also tested by Xue *et al.* (2015), with an 80% zeolite loaded PDMS membrane being combined with a free cell fermentation. The condensate collected contained 253 g ABE L^{-1}, which formed an organic phase containing over 600 g butanol L^{-1}. The membrane surface was smooth and non-porous, this reduced fouling by the bacteria as there were no pores or imperfections for the bacteria to stick to (Xue *et al.*, 2015). The addition of a second material to increase the selectivity and flux significantly improves the compatibility with the ABE process. Combine this with achieving high purity ABE (greater than 250 g ABE L^{-1} in the total permeate) means the downstream energy required for product recovery would decrease (Li *et al.*, 2014). Currently these membranes have only been made for research purposes. An economic study is required to understand the impact the use these novel membranes would have on the process.

As pervaporation is an evaporative ISPR technique, the product is captured through condensation. The inefficiencies of condensation of ABE have already been discussed in the sections concerning gas stripping and vacuum fermentation. In the papers regarding these techniques the inefficiencies of complete product capture are acknowledged, but this has not been the case for pervaporation. In Table 3.3 it can be observed, for fed-batch fermentations, a negative yield increase compared to the control fermentation. The fed-batch fermentations are not inhibited by ABE or limited by available substrate, as an increase in substrate utilisation is observed, yet there is a decrease in yield compared to the

control fermentation. This indicates the potential loss of product, possibly through incomplete condensation. Incomplete product capture appears to be an inherent issue where the ABE is transferred to the vapour phase and needs to be considered when designing the fermentation process. As the recovery of solvents (ABE) from inert gasses is known to be problematic, particularly with water-cooled systems (Frank, 2000), it should be considered a key area of focus for researchers looking at evaporative techniques. It is possible to use a refrigerant-cooling system (Frank, 2000), below ambient temperatures but a detailed energy and economic analysis would be required to develop the most efficient solvent-capture system.

To date pervaporation research for the ABE fermentation has predominantly focused on glucose based media, Table 3.3. On this type semi-defined media (e.g. glucose with corn steep liquor), Qureshi and Blaschek (1999b) found that the pervaporation membrane was not fouled by direct contact with the fermentation broth. The closest example to using an industrial applicable feedstock is cassava by Li *et al.* (2014), and they also reported that their pervaporation did not experience any fouling. As more second generation lignocellulosic feedstock's are introduced for bioprocess, the feed mixtures tend to be more complex with a higher solid content. This could see an increase in fouling, and requirements for larger membrane areas but thorough testing is required.

3.2.4: Liquid-Liquid Extraction

Liquid-liquid extraction (LLE) is a common technique in the processing industries. It exploits the differences between relative solubility of a compound in two immiscible components. Typically the solvent used is an organic liquid which is immiscible in water, therefore when applied to a fermentation broth the product will preferentially transfer from the aqueous phase into the organic phase.

There are a variety of key parameters that need to be considered, described in Table 3.4. Davison and Thompson (1993) also stated that the operability of the LLE system and the method of contacting must be considered, especially for plant or pilot plant operation, which was only considered by Roffler *et al.* (1988). One of the biggest challenges with LLE is finding an extractant, which can satisfactorily meet all of the key characteristics for the extractant.

Table 3.4: Key Characteristics for LLE extractant.

Key Characteristic	**Ref.**
Non-toxic to the microorganism	(Ishii *et al.*, 1985; Davison and Thompson, 1993)
Have a high partition coefficient (high capacity)	(Ishii *et al.*, 1985; Davison and Thompson, 1993)
Immiscible with water and not emulsion-forming with aqueous phase	(Ishii *et al.*, 1985)
Favourable physical properties, for example a low viscosity and a large density difference compared to water	(Ishii *et al.*, 1985; Weilnhammer and Blass, 1994)
High chemical stability, particularly at high temperatures (to ease extractant renewal)	(Weilnhammer and Blass, 1994)
Sterilisable	(Ishii *et al.*, 1985)
Commercially available at low cost	(Ishii *et al.*, 1985; Weilnhammer and Blass, 1994; Li *et al.*, 2010)

Ishii *et al.* (1985) and Roffler *et al.* (1987b) performed a series of extractive batch fermentations to find a suitable extractant for the ABE fermentation, and both concluded that oleyl alcohol is an acceptable extractant for butanol. It is non-toxic to the microorganisms and has a good distribution coefficient with an average distribution coefficient for butanol of 4.4 (Roffler *et al.*, 1987b). Table 3.5 shows that oleyl alcohol increases both yield and productivity compared to a non-integrated fermentation, unlike other tested extractants. As a consequence of this, oleyl alcohol is the most widely studied extractant, with Roffler *et al.* (1987b) performing batch fermentations and fed-batch fermentations (Roffler *et al.*, 1987c), before moving to a scaled down industrial fed-batch system (Roffler *et al.*, 1988) (the outcomes of these are shown in Table 3.5) and an economic assessment of a commercial process of a continuous ABE fermentation with an inline recovery unit using oleyl alcohol (Roffler *et al.*, 1987a). The fed-batch fermentations demonstrate an improvement over integrated batch fermentations, with substrate utilization and productivity increases over 100% being achieved (Roffler *et al.*, 1987c). Roffler *et al.* (1987c; b) reported final organic phase butanol concentrations of 24-30 g butanol L^{-1}. This is a small concentration increase, especially compared to those seen in the condensate for the evaporative techniques. ABE separation from the oleyl alcohol should be less intensive than separation from water, as there is no water-butanol/water-ethanol azeotrope formations, but the low concentration will impact the energy requirement.

Table 3.5: Liquid-Liquid Extraction coupled with free cell ABE fermentation in an STR.

Mode	Microorganism	Substrate (Concen-tration for ISPR)	% Substrate Increase for ISPR (vs. control)	Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g ABE g^{-1} Substrate)	% Yield Increase (vs. control)	Extractant	Ref.
Batch	*C. acetobutylicum* ATCC824	Glucose (97 g L^{-1})	18%	0.69	19%	0.17	-6%	Kerosene	(Roffler *et al.*, 1987b)
	C. acetobutylicum ATCC824	Glucose (78 g L^{-1})	-5%	0.53	-9%	0.17	-6%	50wt% Dodecanol in kerosene	(Roffler *et al.*, 1987b)
	C. acetobutylicum ATCC824	Glucose (89 g L^{-1})	9%	0.43	-26%	0.16	-11%	30wt% Tetradecanol in kerosene	(Roffler *et al.*, 1987b)
	C. acetobutylicum ATCC824	Glucose (100 g L^{-1})	22%	0.72	24%	0.19	6%	Oleyl Alcohol	(Roffler *et al.*, 1987b)
	C. acetobutylicum ATCC824	Glucose (100 g L^{-1})	22%	0.71	22%	0.17	-6%	50wt% Oleyl alcohol in decane fraction	(Roffler *et al.*, 1987b)
	C. acetobutylicum ATCC824	Glucose (100 g L^{-1})	22%	0.74	28%	0.18	0%	50wt% Oleyl alcohol in benzyl benzoate	(Roffler *et al.*, 1987b)
	Clostridium saccharoper-butylacetonicum N1-4	Potato glucose (75 g L^{-1})	34%	0.52	2%	0.38	0%	Oleyl Alcohol	(Ishizaki *et al.*, 1999)
	C. saccharoper-butylacetonicum N1-4	Potato glucose (74 g L^{-1})	32%	0.55	8%	0.4	5%	Methylated crude palm oil	(Ishizaki *et al.*, 1999)
	C. acetobutylicum BCRC10639 (ATCC824)	Glucose (unknown)		0.27	28%	0.21	15%	Biodiesel	(Yen and Wang, 2013)

Mode	Microorganism	Substrate (Concen-tration for ISPR)	% Substrate Increase for ISPR (vs. control)	Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g ABE g^{-1} Substrate)	% Yield Increase (vs. control)	Extractant	Ref.
Batch	*C. acetobutylicum* ATCC824	Glucose (86 g L^{-1})	51%	0.36	9%	0.36	-8%	Oleyl Alcohol	(Lu and Li, 2014)
	C. acetobutylicum ATCC824	Glucose (117 g L^{-1})	105%	0.46	39%	0.37	-4%	Oleyl Alcohol with gas stripping	(Lu and Li, 2014)
Fed-Batch	*C. acetobutylicum* ATCC824	Glucose (155 g L^{-1})	91%	0.9	55%	0.24	33%	Oleyl Alcohol	(Roffler *et al.*, 1987c)
	C. acetobutylicum ATCC824	Glucose (218 g L^{-1})	169%	1.5	159%	0.22	22%	Oleyl Alcohol	(Roffler *et al.*, 1987c)
	C. acetobutylicum ATCC824	Glucose (303 g L^{-1})	274%	1.3	124%	0.21	17%	Oleyl Alcohol	(Roffler *et al.*, 1987c)
	C. acetobutylicum ATCC824	Glucose (86 g L^{-1})	60%	0.17	-23%	0.23	-21%	PPG1200	(Barton and Daugulis, 1992)
	C. acetobutylicum BCRC10639 (ATCC824)	Glucose (unknown)		0.30	37%	0.31	65%	Biodiesel	(Yen and Wang, 2013)
	C. acetobutylicum P262 [a]	Whey Permeate (68.6 g L^{-1})	154%	0.15	114%	0.35	9%	Oleyl Alcohol	(Qureshi *et al.*, 1992)
	C. acetobutylicum ATCC824	Glucose (300 g L^{-1})	270%	1	72%	0.19	6%	Oleyl Alcohol	(Roffler *et al.*, 1988)

[a] *C. acetobutylicum* P262 has since been reclassified as *C. saccharobutylicum* P262 (Keis *et al.*, 2001)

Other solvents have been tested with fermentations, such as decanol, dibutylphthalate, 2-butyl-1-octanol and polypropylene glycol 1200 (PPG) (Eckert and Schügerl, 1987; Wayman and Parekh, 1987; Evans and Wang, 1988a; Barton and Daugulis, 1992; Qureshi and Maddox, 1995; Bankar *et al.*, 2012; González-Peñas *et al.*, 2014a). Decanol has a high distribution coefficient for butanol, 6.2, but is toxic to the bacteria and dissolves into the fermentation broth (Eckert and Schügerl, 1987; Evans and Wang, 1988a). Evans and Wang (1988a) investigated a mixture of decanol-oleyl alcohol as an extractant, where it was observed that a mixture containing 40% of decanol was detrimental to fermentation. On the other hand, Bankar *et al.* (2012) did perform a successful continuous fermentation with a 20% decanol, 80% oleyl alcohol mixed extractant with a two-stage immobilised reactor. A maximum solvent productivity of 2.07 g L^{-1} h^{-1} was achieved in the second stage reactor at a dilution rate of 0.5 h^{-1}.The downside of this was the final product concentration only reached 25.32 g ABE L^{-1}. Dibutylphthalate was used as an extractant by Wayman and Parekh (1987) but Roffler *et al.* (1987b) ruled out its use in a fermentation due to the density being very similar to water making the removal of the extractant from the fermentation broth difficult. Barton and Daugulis (1992) screened 63 organic solvents and decided that PPG 1200 was the best extractant. This was largely due to the high partition coefficient and biocompatibility of the extractant. Unfortunately, PPG 1200 did not show the same promise in fed-batch fermentations as a reduction in both productivity (-23%) and yield (-21%) was seen, Table 3.5. The authors have associated this with the extraction of acids and intermediates into the PPG as the glucose uptake rate had increased by 60% compared to the control, with only a 26% increase in solvent formation (Barton and Daugulis, 1992). Extraction of acids is not a desirable trait in the extractant, as the acids cannot be assimilated into the desired products. 2-butyl-1-octanol was suggested as an extractant as a result of González-Peñas *et al.* (2014b) extractant screening method. It was selected as it was shown to be biocompatible, with an increase in yield over the control experiment. Surprisingly, González-Peñas *et al.* (2014b) found 2-butyl-1-octanol to be a better extractant than oleyl alcohol which has been the most commonly used extractant for ISPR. 2- butyl-1-octanol produced a yield of 27.43 w/w% compared to 25.5 w/w% for oleyl alcohol. It also had a greater distribution coefficient (6.76) and selectivity (644) for butanol compared to oleyl alcohol (4.57, 295) (González-Peñas *et al.*, 2014b).

With LLE the achieved yields are very low (Table 3.5), especially compared with evaporative techniques like gas stripping and pervaporation (Tables 3.2 and 3.3). With evaporative

techniques the average yield is 0.35 g ABE g^{-1} substrate which is close to the theoretical yield. The average yield for LLE is 0.25 g ABE g^{-1} substrate, which is approximately 40% lower than evaporative techniques. This reduction in yield between techniques is likely to be related to the contact of extractant with fermentation broth. Either substrate or acids is being removed from the broth into the extractant or the extractant is having a toxic effect. While these extractants have been selected as they are biocompatible, the sustained contact over the duration of the fermentation could be having a negative impact. In contrast, there is a general improvement in productivity over the control fermentation; therefore further work would be required to understand why the yield is lower than other techniques.

One of the proposed advantages of ISPR techniques is the increased product titres, reducing the downstream separation energy demand. Unfortunately the product concentration in the organic phase is rarely stated; rather the total product quantity/concentration based on the fermentor volume is specified. Without this concentration it is difficult to assess the extraction efficiency in the same manner as evaporative techniques, where the final condensate concentration is stated.

For an economically viable process it is essential that the extractant is recyclable, usually meaning that the removal of ABE from it is straightforward. Unfortunately, removal of ABE from the extractant and regeneration of the extractant have not been discussed much in the literature. Roffler *et al.* (1987a) developed a steam stripping or distillation system for the removal of ABE from oleyl alcohol as part of their economic assessment. This was successful, as oleyl alcohol has a boiling point of 282-349°C, significantly higher than that of butanol. This technique has not been subjected to rigorous testing to understand the process and effects on the oleyl alcohol (Roffler *et al.*, 1987a). Vacuum distillation followed by flash separation has been suggested as an alternative method of separation, but this has not been proven experimentally (Shi *et al.*, 2005). However, this was a very general investigation into the fermentation performance and energy requirements, and did not provide any indication whether flash separation of ABE from oleyl alcohol would be beneficial to the process. Lu and Li (2014) have suggested applying gas stripping to the extractant phase inside the fermentor. This regenerates the extractant while it is still in contact with the fermentation broth, removing the need for external distillation. The results of a "bottle experiment" have proven to be promising as the gas-stripped extractant experiment had greater productivity and yield than the standard LLE experiment. The results are shown in Table 3.5. The

productivity and yield increase is related to maintaining a product concentration gradient between the fermentation broth and extractant. Although, it must be noted that the yield decreases between the control fermentation and ISPR fermentations. The authors do not acknowledge why there is this decrease in yield, creating difficulties in assessing the true impact of applying gas stripping to the extractant phase. The ABE concentrations in the condensate from gas stripping stage ranged between 166-204 g ABE L^{-1}. This is a significant increase in product concentration compared to the butanol concentration in the extractant of 40 g butanol L^{-1} oleyl alcohol (Lu and Li, 2014), although it is lower than the concentrations exhibited by the two-stage gas process (500-700 g ABE L^{-1} (Xue *et al.*, 2013a; 2014b)). Lu and Li (2014) did not report any energy requirements for this system and Roffler *et al.* (1988) did not report the concentration of ABE after separation from the extractant, making a comparison with distillation or other ISPR techniques difficult.

To circumvent the re-extraction step both Ishizaki *et al.* (1999), Li *et al.* (2010) and Yen and Wang (2013) have investigated the use of an extractant that would allow for direct use as a biofuel while in the extracted form. The use of biodiesel (methylated fatty acids e.g. methylated crude palm oil) was shown to reduce the need for recovery from the extractant (Ishizaki *et al.*, 1999; Li *et al.*, 2010; Yen and Wang, 2013), as it produces an ABE-enriched biodiesel, which significantly improves the quality of biodiesel with an increased cetane number and a reduced cold filter plugging point (Li *et al.*, 2010). The disadvantage with biodiesel is that it preferentially removes butyric acid from the fermentation, which is required by the bacteria to produce butanol (Li *et al.*, 2010). Although, Yen and Wang (2013) still observed an increase in yield over the control fermentation by 15% in a batch 65% in fed-batch fermentation using biodiesel as an extractant, Table 3.5. Ishizaki *et al.* (1999) demonstrated that the use of methylated crude palm oil is competitive in terms of fermentation characteristics compared to an extractive fermentation using oleyl alcohol, Table 3.5; with an 8% improvement in productivity and 5% yield improvements compared to 2% and 0% respective improvements with oleyl alcohol. Direct extraction with biodiesel will also see acetone transferred into the proposed fuel. Chang *et al.* (2014) suggests that the addition of acetone will not have a negative impact on the use of ABE-enriched biodiesel in a combustion engine. Equivalent results were seen when acetone was mixed with gasoline and tested a spark ignition engine (Elfasakhany, 2016). If ABE-enriched fuel was to be introduced, long term testing on engine performance and wear would be required. The idea of using biodiesel as an extractant to create a superior biofuel is attractive and could form

part of an ABE-based biorefinery creating multiple products (García *et al.*, 2011). If this were the case, the end use of the ABE produced needs to be considered when choosing an extractant.

3.2.5: Perstraction (Membrane Extraction)

Perstraction is a development from liquid-liquid extraction. It works on the same principles of mass transfer of ABE from an aqueous phase to an organic solvent, but the organic solvent and fermentation broth are separated by a membrane. The ABE transfers across the membrane into the organic phase. It is very similar to pervaporation, but has a liquid on the permeate side to provide the "driving force" rather than a gas or vacuum. If the key criteria for LLE, outlined in Table 3.4, are not achieved, then LLE is not possible with the ABE fermentation. A membrane separating the two process streams can in principle overcome these problems (Jeon and Lee, 1987).

The main technique has been extraction into oleyl alcohol across a silicone membrane, as this has favourable partition characteristics for butanol (Jeon and Lee, 1987; 1989; Qureshi *et al.*, 1992; Grobben *et al.*, 1993; Qureshi and Maddox, 2005). Qureshi and Maddox (2005) demonstrated in a batch fermentation that perstraction could increase the substrate utilisation by 694%, productivity by 163% and yield by 33%, Table 3.6. However Grobben *et al.* (1993) investigated the use of fatty acid methyl esters from sunflower oil, which would allow for the direct use of the extractant and biobutanol as a biofuel (this is similar to the work by Li *et al.* (2010) and Ishizaki *et al.* (1999)). The use of fatty acid methyl esters did not match the performance by oleyl alcohol with a 40% decrease in productivity compared to the control fermentation and only a 16% increase in substrate utilisation, Table 3.6. This could be due to the lower distribution coefficient for ABE in the fatty acid methyl esters (0.45, 1.1 and 0.05 respectively) compared to oleyl alcohol creating a lower driving force across the membrane. Jeon and Lee (1987) investigated two other solvents for the use of perstraction; polypropylene glycol and tributyrin. Table 3.6 shows that while the alternative solvents show improvement over the non-integrated fermentation (productivity increases of 68% for polypropylene glycol and 42% for tributyrin) oleyl alcohol remains the best extractant for the recovery of ABE. When choosing possible extractants for perstraction it seems that the same criteria for selecting an extractant for LLE were used in case of any back-extraction of the solvent into the fermentation broth (Qureshi *et al.*, 1992).

Table 3.6: Free Cell ABE Fermentation in an STR with *in situ* recovery via perstraction.

Mode	Microorganism	Substrate (concentration for ISPR)	% Substrate Increase for ISPR (vs. control)	Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g ABE g^{-1} Substrate)	% Yield Increase (vs. control)	Membrane	Extractant	Ref
Batch	*C. acetobutylicum* P262[a]	Lactose/Whey Permeate (227 g L^{-1})	694%	0.21	163%	0.44	33%	Silicone tubing	Oleyl Alcohol	(Qureshi and Maddox, 2005)
	C. saccharoperbutylacetonicum N1-4	Potato Glucose (89 g L^{-1})	50%	0.32	-16%	0.21	-23%	PTFE	Oleyl Alcohol	(Tanaka *et al.*, 2012)
	C. saccharoperbutylacetonicum N1-4	Potato Glucose (86 g L^{-1})	45%	0.39	3%	0.23	-13%	PTFE	1-Dodecanol	(Tanaka *et al.*, 2012)
Fed-Batch	*C. acetobutylicum* ATCC824	Corn Mash/ Glucose (601 g L^{-1})	902%	1.02	113%	0.36	23%	Silicone tubing	Oleyl Alcohol	(Jeon and Lee, 1987)
	C. acetobutylicum ATCC824	Corn Mash/ Glucose (422 g L^{-1})	603%	0.81	69%	0.35	21%	Silicone tubing	Polypropylene glycol	(Jeon and Lee, 1987)
	C. acetobutylicum ATCC824	Corn Mash/ Glucose (155 g L^{-1})	158%	0.68	42%	0.32	9%	Silicone tubing	Tributyrin	(Jeon and Lee, 1987)
	C. acetobutylicum DSM1731 (ATCC8529)	Potato powder (92 g L^{-1})	26%	1.00	59%	0.35	35%	Polypropylene	Oleyl Alcohol/ Decanol	(Grobben *et al.*, 1993)
	C. acetobutylicum DSM1731 (ATCC8529)	Potato powder (85 g L^{-1})	16%	0.38	-40%	0.32	23%	Polypropylene	Fatty acid methyl esters	(Grobben *et al.*, 1993)

Mode	Microorganism	Substrate (concentration for ISPR)	% Substrate Increase for ISPR (vs. control)	Productivity for ISPR (g ABE L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g ABE g^{-1} Substrate)	% Yield Increase (vs. control)	Membrane	Extractant	Ref
Fed- Batch	*C. acetobutylicum* P262[a]	Whey Permeate (123 g L^{-1})	355%	0.24	243%	0.37	16%	Silicone tubing	Oleyl Alcohol	(Qureshi *et al.*, 1992)
Continuous	*C. acetobutylicum* ATCC824	Corn Mash/ Glucose (2134 g L^{-1})[b]	unknown	2.27	305%	0.33	2%	Silicone tubing	Oleyl Alcohol	(Jeon and Lee, 1989)

[a] *C. acetobutylicum* P262 has since been reclassified as *C. saccharobutylicum* P262 (Keis *et al.*, 2001)

[b] Dilution rate was 0.2 h^{-1}

There have been two examples, Shukla *et al.* (1989) and Tanaka *et al.* (2012), where toxic solvents have been used in a perstraction system. Shukla *et al.* (1989) chose 2-ethyl-1-hexanol as an extractant. 2-ethyl-1-hexanol is known to be toxic to bacteria, but was considered less toxic than 1-octanol therefore would be an acceptable extractant (Shukla *et al.*, 1988). The results of the toxicity tests of 1-octanol or 2-ethyl-1-hexanol were not published, so the degree of toxicity under the conditions described is unknown. Shukla *et al.* (1989) used a hollow fibre polypropylene membrane and did not report any ill effects from the use of this extractant, although an immobilised *C. acetobutylicum* on wood chips was used for the fermentation, this could reduce the chances of the bacteria coming into contact with the solvent at the membrane interface. The only acknowledgement of using a toxic extractant was by Tanaka *et al.* (2012), who chose 1-dodecanol as an extractant. It was selected based on a high partition coefficient of 5.14, but it is unknown why 1-dodecanol was chosen over other high-distribution, toxic extractants such as 1-octanol with a distribution coefficient of 5.6-7.33 (Kim *et al.*, 1999).The same levels of bacterial growth were seen when using 1-dodecanol and oleyl alcohol as an extractant for perstraction. While the same levels of growth was seen and there was a slight increase in productivity (3%) there was a 13% decrease in yield compared to the control fermentation. The authors have not commented on this, as the maximum butanol productivity for both oleyl alcohol and 1-dodecanol, 0.979 g L^{-1} h^{-1} was 1.25 times higher than the maximum butanol productivity for the control, 0.817 g L^{-1} h^{-1}.

One of the problems of LLE was the trade-off between having a high partition coefficient and being non-toxic to the bacteria. It was thought that the use of a membrane would allow for the use of extractants with higher partition coefficients. As seen in Table 3.6 from the description above most researchers have chosen extractants known to be non-toxic to the bacteria. The earlier research appeared to indicate that some extractant is leaching across the membrane into the aqueous phase (Groot *et al.*, 1990). Groot *et al.* (1990) believed that this was related to sorption of the solvent to the membrane. Some tests they performed using hexanol and silicone exhibited toxic effects to the fermentation, although data confirming this is not shown (Groot *et al.*, 1990). Jeon and Lee (1987) stated that tributyrin had a partial inhibitory effect over time; therefore the fermentation with perstraction could not be run for only 84 hours, consuming 154 g L^{-1} glucose compared to the oleyl alcohol based fermentation which operated for 209 hours consuming 601 g L^{-1} glucose. Resulting in the conclusion that non-toxic solvents had to be used for perstraction. If this is true, one of

the motivations for research into perstraction has been incorrect, meaning there might be very little advantage of using perstraction rather than LLE. In contrast, Qureshi and Maddox (2005) suspected that back diffusion was a possible reason for the fermentation stopping, but later disregarded it, as it was shown that the fermentation stopped due to nutrient depletion. Combining this with the successful fermentations performed by Shukla *et al.* (1989) and Tanaka *et al.* (2012) using toxic extractants, there is no conclusive evidence that higher partition coefficient extractants cannot be used.

The majority of research has used silicone tubing as the membrane, Table 3.6 (Jeon and Lee, 1987; 1989; Groot *et al.*, 1990; Qureshi *et al.*, 1992; Shah and Lee, 1994; Qureshi and Maddox, 2005), because it is widely available and has acceptable mass transfer characteristics. It is also possible that it was chosen because it is readily available in the laboratory and easy to configure into an appropriate system (Jeon and Lee, 1987; 1989; Groot *et al.*, 1990; Qureshi *et al.*, 1992; Qureshi and Maddox, 2005). Jeon and Lee (1987) chose it as it has high permeability for butanol and acetone, can be autoclaved, has high mechanical strength, is easy to handle, biologically inert, compatible with many organic solvents and had been used for pervaporation with the ABE fermentation (Jeon and Lee, 1987). Qureshi and Maddox (2005) stated similar reasons for using a silicone membrane, including that silicone had been proven not to foul and there was no dead space for bacterial growth on the tubing. There has been very little comparison in terms of other membrane options. Only Groot *et al.* (1990) have compared possible membrane options, which were silicone, neoprene and latex. Based on the mass transfer coefficient, silicone had the highest coefficient for all extractants tested compared to neoprene and latex. For hexanol, the corresponding mass transfer coefficient was 5.2×10^{-7} m s^{-1} for silicone, 0.4×10^{-7} m s^{-1} for neoprene and 0.3×10^{-7} m s^{-1} for latex; meaning that the membrane choice will have a significant impact on the mass transfer in the system. No comparisons of polypropylene hollow fibre membranes and silicone have been performed. Grobben *et al.* (1993) have used an alternative polypropylene membrane. Polypropylene membranes appear comparable to silicone tubing (Table 3.6), but different bacterial strains and substrates have been used for the fermentation. Shukla *et al.* (1989) used a Celgard X20, a hydrophobic microporous hollow fibre membrane. It is suspected that this is also a polypropylene membrane which was commercially available at the time of research. Tanaka *et al.* (2012) chose a polytetrafluoroethylene (PTFE) membrane as it is more hydrophobic than other membranes that have been used, therefore it should be more selective for ABE. The supposed increased

selectivity due to the PTFE membrane could not be validated as was not compared with alternative membrane materials. It is evident that membranes for perstraction need to be optimised, as membrane development continues and become more commercially available, it is likely that more sophisticated industrially applicable membranes will become available (Qureshi and Maddox, 2005).

Comparing the fermentations in Table 3.6 with oleyl alcohol LLE fermentations in Table 3.5, not much difference can be seen in fermentation performance. Perstraction appears to enable higher fermentation productivities. Perstraction does show a greater, more consistent increase in yield, between the ISPR and control fermentation compared to the LLE fermentations; achieving an average yield of 0.33 g g^{-1} consumed for integrated fermentations. This could be because the membrane reduces transfer of key nutrients and intermediates into the extractant phase. Similar to LLE, the recovery of ABE from the extractant is not considered, nor is the product concentration in the organic phase consistently reported. Qureshi and Maddox (2005) reported that the butanol concentration never exceed 10 g butanol L^{-1} oleyl alcohol, although the extractant was replaced with fresh extractant 5 times during the fermentation. The extractant was replaced to limit the product build up in the fermentor, but this concentration is lower than that reported for LLE at 40 g butanol L^{-1} oleyl alcohol (Lu and Li, 2014). Unless the extractant concentration can be increased or optimised for better fermentation performance this lower extractant concentration is likely to increase the energy for distillation. In this scenario the use of perstraction with non-toxic extractants will have to be suitably justified to be applied to the ABE fermentation.

3.2.6: Adsorption

Adsorption is the binding of a compound onto the surface of a solid adsorbent or resin. It is the oldest technique investigated for the use of ISPR from ABE fermentations. In 1948, Weizmann *et al.* (1948) first investigated butanol adsorption to relieve product inhibition and reduce the energy demand due to distillation.

A wide range of adsorbents have been used in conjunction with the butanol and the ABE fermentation, and this list is continually evolving as new, more complex adsorbents become available. Some of the adsorbents used are activated carbon (Weizmann *et al.*, 1948; Groot and Luyben, 1986; Cousin Saint Remi *et al.*, 2012; Xue *et al.*, 2016a), silicalite or silicalite-based zeolites (Milestone and Bibby, 1981; Maddox, 1982; Ennis *et al.*, 1987; Cousin Saint

Remi *et al.*, 2012) and polymeric resins (Groot and Luyben, 1986; Ennis *et al.*, 1987; Nielsen *et al.*, 1988; Nielsen and Prather, 2009; Xue *et al.*, 2016a). The initial conclusion from Qureshi *et al.* (2005) was that silicalite adsorbents improved the fermentation the most, as they have the ability to concentrate fermentation broth from 5 g butanol L^{-1} to 810 g butanol L^{-1}, but more recent work exhibits a tendency towards polymeric resins (Nielsen and Prather, 2009; Eom *et al.*, 2012a; Lin *et al.*, 2012a; 2012b). Over time the adsorbents used have become increasingly complex with Cousin Saint Remi *et al.* (2012) recommending ZIF-8, a metal organic framework adsorbent from Sigma-Aldrich as a superior adsorbent to silicalite. The most recent work published on adsorption has moved back to the use of commercially available resins, along with selecting an activated carbon resin Norit ROW 0.8 to be combined with the fermentation broth, rather than a polymeric resin such as Dowex Optipore L-493 and SD-2 (Xue *et al.*, 2016a).

The majority of the adsorbents have not been tested in an ABE fermentation, rather using a model ABE solution instead. Thus there is a small amount of fermentation data to compare in Table 3.7. Yang *et al.* (1994) are one of the few who have investigated an adsorbent in conjunction with fermentation. They demonstrated that the addition of 30% resin to a fermentation can achieve 130% increase in productivity of the fermentation. When this was adapted to a fed-batch fermentation with external column the productivity increased by 233% for a single cycle adsorption, and 323% with multiple adsorption cycles.

Table 3.7: Free cell ABE fermentation in an STR with adsorption recovery of products.

Mode	Micro-organism	Substrate (Concen-tration for ISPR)	% Substrate Increase for ISPR (vs. control)	Productivity for ISPR (g L^{-1} h^{-1})	% Productivity Increase (vs. control)	Yield for ISPR (g g^{-1})	% Yield Increase (vs. control)	Adsorption Mode	Adsorbent	g adsorbent mL^{-1} liquor	Ref
Batch	*C. aceto-butylicum* ATCC824	Glucose (92 g L^{-1})	22-47%	0.53	33%	0.32	3%	Batch (*In situ*)	Polyvinyl-pyridine (PVP) resin	5%	(Yang *et al.*, 1994)
				0.63	58%	0.32	2%			10%	
				0.74	85%	0.31	1%			20%	
				0.92	130%	0.32	3%			30%	
Fed-Batch	*C. aceto-butylicum* ATCC824	Glucose (190 g L^{-1})	334%	1.33	233%	0.32	2%	Single cycle (*ex situ* column)	Polyvinyl-pyridine (PVP) resin	n/a	(Yang and Tsao, 1995)
	C. aceto-butylicum ATCC824	Glucose (1199 g L^{-1})	2636%	1.69	323%	0.32	4%	Cyclic (*Ex situ* column)	Polyvinyl-pyridine (PVP) resin	n/a	(Yang and Tsao, 1995)
	C. acet-obutylicum ATCC824	Glucose (180 g L^{-1})	67%	0.72	14%	0.28	65%	Expanded Bed Adsorption (*Ex situ)*	Poly (styrene-co-divinylbenzene) (Dowex Optipore L493)	n/a	(Wiehn *et al.*, 2014)
	C. aceto-butylicum JB200	Glucose (158 g L^{-1})	267%	0.34[a]	-3%	0.22[a]	0%	Single cycle (*ex situ* column)	Activated Carbon (Norit ROW 0.8)	n/a	(Xue *et al.*, 2016a)

[a] productivity of butanol production and butanol yield, rather than for total products

In the past two years more research has focused on combining adsorption with the fermentation. Liu *et al.* (2014) combined the adsorption using KA-I resin with an immobilised biofilm reactor. A membrane was used to ensure no biomass came into contact with the adsorbent. Two adsorption modes were investigated, selective for butanol and co-adsorption of acetone, both these methods observed a reduction in productivity and yield by 37% and 9% for the butanol selective adsorption and 9% and 7% when acetone was co-adsorbed. Lee *et al.* (2015) added the adsorbent directly to the fermentor. Fouling was not observed, but the authors commented on the potential physical interaction between the biomass and adsorbent being detrimental to the fermentation. The reasons suggested for this appear to be tenuous, but the mode of adsorption should be considered to minimise impact on the bacteria. This work demonstrated that in batch fermentations, using a modified *C. acetobutylicum* ATCC 824, the product concentration could be increased due to the adsorption of products, reducing toxicity. The final broth concentration reached 10 g butanol L^{-1} this is the same as the fermentation with no ISPR, and the same yield was observed in both fermentations (Lee *et al.*, 2015).

Follow up work by Lee *et al.* (2016) using an *ex situ* adsorption column, combined with a fed-batch fermentation using *C. beijerinckii* NCIMB 8052, saw no detrimental effects to the fermentation. The integrated and non-integrated fermentation both had the same yield of 0.31, but the integrated fermentation had an increased total product concentration of 26 g ABE L^{-1} compared to 18.5 g ABE L^{-1}. No productivity was supplied for these fermentations (Lee *et al.*, 2016). Wiehn *et al.* (2014) proposed an expanded bed adsorption process. This allows the fermentation broth to pass through the adsorbent without the need for microbial separation, due to the increased voidage space in the bed. A reduced biomass concentration was observed, compared to the control fermentation, but the overall fermentation metrics appeared positive with increases in both yield and productivity by 14% and 65% respectively, Table 3.7. A limited degree of fouling was observed in the 72 hour experiment, this could be a bigger issue in longer fermentations (Wiehn *et al.*, 2014). Xue *et al.* (2016a) used an activated carbon resin in both free cell STR fermentations and an immobilised bioreactor. The results of the free cell fermentation are shown in Table 3.7 with a productivity decrease of 3% and no change in the yield. The immobilised cell fermentation had a productivity increase of 22% and the same yield as the control fermentation. The batch fermentation only used a single cycle adsorbent, whereas the immobilised fermentation had 3 adsorbent cycles enabling greater product removal hence a higher productivity. The immobilised

fermentation eventually ceased due to a build-up of acetone to 18 g L^{-1} in the fermentation broth, inhibiting the bacteria (Xue *et al.*, 2016a).

Although the limited integrated adsorption-ABE fermentation generally indicates a good compatibility between the two processes, the real industrial feedstock's need to be considered. Industrial feedstock's will have a considerably higher solids content compared to laboratory feeds such as glucose-based media, this will increase the likelihood of fouling, particularly from feed components such as proteins. Additionally, feedstocks which are lignocellulosic would typically undergo a hydrolysis step prior to fermentation, this can release additional chemicals e.g. phenols which could adsorb to the resin, competing the butanol and reducing the adsorbent capacity for the desired product. This would increase the mass of adsorbent required per fermentor volume, and potentially interfere with the desorption step and further downstream processing.

Weizmann *et al.* (1948), in agreement with Yang *et al.* (1994) and Lin *et al.* (2012b) observed that the adsorption is competitive. Butanol is adsorbed in preference to acetone, for example. The order of preference for adsorption was ethanol as the weakest, followed by acetone, then butanol as the strongest (Lin *et al.*, 2012b). Yang *et al.* (1994) found that the order was ethanol, acetone, acetic acid, butanol then butyric acid. This order is undesirable, as the butyric acid displaces the butanol, the desired product to be removed, and hinders the conversion of the butyric acid to butanol. Additionally Xue *et al.* (2016a) experienced the increased concentration of other products in the broth causing the fermentation to stop. This also raises questions as to whether any key nutrients are adsorbed during the process, which would be highly undesirable.

A downside of adsorption is that it is inherently a batch process, as the ABE has to bind to the adsorbent and then, once it has reached capacity, desorption has to occur. In many experiments, batch adsorption was performed (Maddox, 1982; Groot and Luyben, 1986; Nielsen *et al.*, 1988; Yang *et al.*, 1994), meaning that once the adsorbent has reached capacity it can no longer relieve product inhibition. This indicates that the ratio of adsorbent to broth needs to be optimised, as the productivity varies with the quantity of adsorbent, Table 3.7. The alternative is operating a minimum of two external packed bed columns in a cyclic manner, allowing for one column to be adsorbing, while the other is desorbing (Ennis *et al.*, 1987; Yang and Tsao, 1995; Qureshi *et al.*, 2005; Lin *et al.*, 2012a). Operation in this cyclic manner with a fed-batch fermentation yielded a favourable fermentation productivity,

Table 3.7, (Yang and Tsao, 1995; Xue *et al.*, 2016a). This operating mode would reduce the adsorbent inventory required per fermentation, although the product removal would have to occur externally from the bioreactor. Ideally the development of a continuous adsorption process (e.g. simulated moving bed adsorption) would be best suited, to allow the simplest removal of ABE and regeneration of adsorbent.

Once the adsorbent has reached its capacity the ABE needs to be removed and the adsorbent regenerated. The main methods of adsorption are through, increasing the temperature (Cousin Saint Remi *et al.*, 2012; Xue *et al.*, 2016a) displacement with steam (Lee *et al.*, 2015) or another solvent, e.g. methanol (Yang *et al.*, 1994; Lin *et al.*, 2012b), or through vacuum evaporation(Nielsen and Prather, 2009; Wiehn *et al.*, 2014). The recovered butanol titres ranged from 43-167 g L^{-1} (Lee *et al.*, 2015; Xue *et al.*, 2016a). If the higher concentrations are consistently achievable then the desorbed titres are similar to that achieved by LLE (Lu and Li, 2014), but still lower than the concentrations achieved in gas stripping (Xue *et al.*, 2013a).

3.3: Comparison of Techniques

Currently no review has compared all possible ISPR techniques for ABE fermentations. One challenge is that it is difficult to make accurate comparisons between work performed by different groups, as different methods and procedures have been followed. This includes the use of different strains, media, reactor configurations and mode of operation. To make a valid comparison between the different ISPR techniques they have principally been compared in terms of their fermentation performance in STRs (see Tables 3.1-3.1, 3.5-3.7). The downside of this approach is that not all techniques have been coupled to an ABE fermentation in an STR. Furthermore, there is significant variability in the data extracted within each ISPR technique.

From batch fermentations it can be observed that every technique tested (gas stripping, vacuum fermentations, pervaporation, liquid-liquid extraction, perstraction and adsorption) can have a positive effect on the fermentation. This is due to the removal of the butanol inhibition allowing for complete utilisation of the substrate and the possibility of prolonged fermentations. This is seen by the significant increase in substrate utilisation in Tables 3.1-3.3 and Tables 3.5-3.7. Only in two batch fermentations was an increase in substrate not observed, Table 3.3 and Table 3.5. In the case of pervaporation, no additional substrate was fed to the reactor and both the control and integrated fermentation consumed all the

substrate supplied (Li *et al.*, 2014). For LLE, a decrease in substrate utilisation was observed with a 50 wt% dodecanol in kerosene extractant. There was also a decrease in productivity and yield, which could be related to toxicity of dodecanol to the bacteria (Roffler *et al.*, 1987b; Tanaka *et al.*, 2012). The most prominent ISPR techniques for batch fermentations are gas stripping (see Table 3.1) and pervaporation (Table 3.3). It is difficult to compare different adsorption processes, as a major factor in the improvement in productivity is the quantity of adsorbent added to the broth, but this is not always reported, Table 3.7.

Where overcoming product inhibition has been successfully demonstrated for any technique, the next step is to perform a fed-batch fermentation which increases substrate loading and fermentation time giving higher productivity. Increases of substrate consumption between 100-900% compared to the control fermentation are common, Tables 3.1, 3.3, 3.5-3.7. Fed batch data is only reported for: gas stripping, pervaporation, liquid-liquid extraction, perstraction and adsorption. Adsorption, Table 3.7, enables the greatest improvement in productivity compared to standard batch fermentations, though the mechanism of adsorption contact has changed. This is closely followed by gas stripping, but the data in Table 3.1 has a degree of unreliability, as the productivity was calculated including estimated solvent losses (Ezeji *et al.*, 2004a). In all cases a condenser was not sufficient to capture all the solvents produced, so the true productivity of the fermentation is unknown. This is a common problem with evaporative techniques due to the highly volatile nature of acetone and the high dilution of the vapours. Liquid-liquid extraction, however, appears to perform well with relatively repeatable increases in productivity and yield, Table 3.5 (Roffler *et al.*, 1987c).

In literature there have been some discrepancies between what is considered a fed-batch and continuous process. There have been several occurrences where a fed-batch fermentation has been called continuous in literature (Li *et al.*, 2014; Shin *et al.*, 2015). The continuous process described by Shin *et al.* (2015) and Li *et al.* (2014) is the same as the fed-batch process described by Wu *et al.* (2012) and Qureshi and Blaschek (2000). It must be recognised that for ISPR typical process definitions no longer match the process. With a fed-batch ISPR process, there is a feed in but there is also a product stream out. The product stream is not representative of the fermentor, in the same way it would be for a traditional continuous process, and the fermentor cannot be considered steady state as the

concentrations (particularly biomass) change, although, often a concentrated feed is used to maintain a constant fermentation volume.

Continuous fermentations can be performed, but the continuous removal of fermentation broth leads to a lower increase in yield than for fed-batch fermentations, (Table 3.6). In spite of this reduced yield, greater consistency in improved productivity is seen. The reduction in yield for continuous fermentations is due to substrate removal via the outlet stream being accounted for as substrate consumption. This can have a significant effect on the process economics, as the substrate has the largest contribution to the cost of production (Jones and Woods, 1986). The dilution rate of a continuous fermentation controls the growth rate of the bacteria. From the limited data comparing continuous free cell fermentations, the dilution rate does not appear to affect the ISPR. Only gas stripping, pervaporation and perstraction have been combined with continuous fermentation, with a greater focus on the application of fed-batch fermentations. Interestingly perstraction, Table 3.6, shows the greatest improvement in productivity for continuous fermentations closely followed by gas stripping, Table 3.1.

In recent years, there has been a growing trend towards immobilised fermentations. A good example of this is Xue *et al. (2012; 2013a; 2016a; 2016b)* who have combined immobilised bioreactors with gas-stripping and adsorption. Immobilised fermentations have also been considered for combination with pervaporation and LLE (Friedl *et al.*, 1991; Qureshi and Maddox, 1995; Bankar *et al.*, 2013). This has included a range of reactor configurations from a packed fermentor with circulating feed to a fluidised bed reactor and a range of support material from bonechar to sugarcane bagasse (Friedl *et al.*, 1991; Qureshi and Maddox, 1995; Bankar *et al.*, 2013). Immobilised fermentations are not yet a realised commercial technology for the ABE fermentation (van der Merwe *et al.*, 2013), but in combination with an ISPR could allow for enhanced separation conditions, e.g. high temperatures (Chen *et al.*, 2014b), without being detrimental to the bacteria. Experimental comparisons of immobilised fermentations with ISPR need to be investigated to understand if immobilised bacterial fermentations should have an increased focus compared to free cell fermentations.

One of the factors driving the application of ISPR to the ABE fermentation is the potential energy reduction, due to increased ABE concentration going to downstream processing. The energy associated with separation is generally not considered alongside the experimental results. Xue *et al.* (2013b) suggest that energy reductions will not be observed if the ISPR

technique cannot concentrate the ABE to more than 40 wt%. This would allow removal of the beer stripper from product purification, which has the largest energy demand, concentrating the fermentation broth from 2 wt% ABE (Xue *et al.*, 2013b). None of the single-single stage techniques have suggested they can concentrate the product to this concentration. The hybrid, two-stage separation processes combined based upon an initial gas stripping stage are the only techniques to create an ABE solution greater than 40 wt%, with concentrations over 650 g ABE L^{-1} possible (Xue *et al.*, 2014b). To date, hybrid systems have only considered two-stage gas stripping (Xue *et al.*, 2014b), combined gas-stripping and pervaporation (Xue *et al.*, 2016b) and extractive gas stripping (Lu and Li, 2014). There is the potential for many more hybrid or two-stage separation systems to be designed. The economics of two-stage systems will need to be considered and compared to a traditional batch and single-stage ISPR process. The application of a single stage ISPR and beer stripper is effectively a two-stage process; therefore the cost of implementations and operation could be a deciding factor for commercial implementation.

The review has compared 6 ISPR techniques based on available experimental data focusing on free cell fermentations. To mitigate the effects of various experimental methods the % increase of substrate utilised, productivity and yield was considered. The experimental data has successfully demonstrated that ISPR has a positive impact on the fermentation. The generation of models to represent the fermentation with integrated ISPR could help speed up developments in ISPR. They can help focus developments to the techniques that would provide the greatest improvements to the process. Experimental work can then be completed to validate the model results and confirm there are no biocompatibility issues. This will reduce the time and expense of testing every ISPR possibility experimentally, and provide a comparable baseline. The publication of experimental results can also be improved to aid the comparison of different studies. This can be done by ensuring that enough data is provided to enable a mass balance of the process to be calculated, hence enabling an easier comparison; see Appendix A for a suggestive list of data to be included.

3.4: Conclusion

From the comparison of STR fermentations it is possible to say that all techniques exhibit improvements in fermentation productivity and that for different operating modes different techniques appear to be superior. For batch fermentations, gas stripping and pervaporation were favourable, for fed-batch: adsorption and gas stripping, and continuous perstraction

has the greatest improvement. The use of novel two-stage or hybrid techniques also needs to be considered, particularly their compatibility with free cell STR fermentations. This means that the decision on which technique to apply will be based on additional data such as energy consumption and an economic analysis. These should be considered alongside the fermentation data, and this would help to categorically state which is the best ISPR technique. Future work should include process optimisation as part of trying new feedstocks, improved strains or separating agents (e.g. membranes, adsorbents, and extractants).

3.5: Summary

While all techniques show improvements in the fermentation, there is not enough information to make an opinion as to which technique should be of primary focus for industrial application. Important information regarding the energy demand and the economic impact of ISPR is missing. As indicated by Van Hecke *et al.* (2014) the use of a techno-economic analysis through process modelling would provide useful data for making this decision. Focusing on the techniques discussed in this chapter, along with flash separation due to its simplicity and similarity to vacuum fermentations, a techno-economic analysis has been completed in Chapter 4.

Chapter 4. Techno-Economic Analysis

A comparison of the energy use of in situ product recovery techniques for the Acetone Butanol Ethanol fermentation. This chapter is a research article published in Bioresource Technology (Outram *et al.*, 2016).

4.1: Introduction

Butanol is a commodity chemical that can be produced via the Acetone Butanol Ethanol (ABE) fermentation. The fermentation is limited by high product toxicity especially of butanol, therefore only reasonably dilute product concentrations (~20 g ABE L^{-1}) are attained (Green, 2011). This affects the energy requirement for product separation and purification (Dürre, 2008), which is important as the energy cost for the process was the second highest overall production cost, contributing 14%, behind that of the feedstock (79%) in a conventional batch ABE process (Pfromm *et al.*, 2010; Green, 2011). To overcome product toxicity, *in situ* product recovery (ISPR) has been applied and extensively researched for the ABE fermentation at laboratory scale (Abdehagh *et al.*, 2014). ISPR also provides the potential to increase the plant's production capacity from the same fermentor volume, through increased fermentation productivity and the use of fed-batch fermentations (Ezeji *et al.*, 2004a). While ISPR has been proven to increase the productivity and yield of the ABE fermentation, a definitive identification of the optimum technique has remained elusive.

A wide range of ISPR techniques have been investigated for compatibility with the ABE fermentation process. They can be compared using three key criteria. These are: the technique's ability to remove the product from the fermentation broth, the energy requirement and the economic impact. A meaningful comparison of the various techniques based on the extant literature is difficult for various reasons: differences in experimental method, ranging from media composition and microorganism strain to reactor configuration, for example. Only a few techniques have been subject to a comprehensive energy and economic analysis. The most mature techniques for ISPR are gas stripping, pervaporation, vacuum (and flash) fermentations, liquid-liquid extraction (LLE), perstraction and adsorption, which have been highlighted in the recent review by Staggs and Nielsen (2015). All of these techniques have been proven to reduce product inhibition, but none are sufficiently developed for application to an industrial process (Van Hecke *et al.*, 2014).

The effects of application of the various modes of ISPR on the process have been widely studied, generally demonstrating improvements in productivity and the reduction of product toxicity (Ezeji *et al.*, 2010), whereas the associated energy demand of ISPR processes are often absent from literature (Van Hecke *et al.*, 2014). This paper focuses on the energy demand associated with ISPR techniques. A rough economic assessment of each process is made to compare the payback time associated with adding an ISPR technique to an existing batch plant.

It is known that ISPR can reduce downstream process energy demand, but the effect on the whole ABE production process has not been accounted for (Van Hecke *et al.*, 2014). Some limited energy analysis has been performed (see Table 4.1). A wide range of values have been calculated, based upon varying assumptions and process designs. The assumptions and process designs have been stated in Table 4.1's legend. These substantial differences between the various process designs and calculation methods make it extremely difficult to compare results across techniques.

Table 4.1: Comparison of energy information in literature for ISPR.

Technique	Oudshoorn *et al.* (2009)[a]	Qureshi *et al.* (2005)[b]	Groot *et al.* (1992)[c]	Nielsen and Prather (2009)[d]	Mariano *et al.* (2011a)[e]	Mariano *et al.* (2012a)[e]	Salemme *et al.* (2016)[f]
	(MJ kg^{-1} BuOH)	(MJ kg^{-1} BuOH)	(MJ kg^{-1} ABE)	(MJ kg^{-1} BuOH)	(MJ kg^{-1} BuOH)	(MJ kg^{-1} BuOH)	
Flash					4.4-6.5		
Vacuum						10.2-15.6	
Gas Stripping	14-31	22	21				15.3
Pervaporation	2-145	14	9				
Liquid-liquid Extraction	7.7	9	14				9.9
Perstraction	7.7						
Adsorption	1.3-33	8	33	7.8			

[a] Energy required for separation of butanol from water. Calculations on steady state flow and enthalpy changes in the system. Using thermodynamic data from NRTL property package in Aspen Plus 12.1 (Oudshoorn *et al.*, 2009).
[b] Energy requirement for butanol separation from broth. Unknown calculation method.
[c] Estimated total heat of recovery for the overall heat process. Based upon recovering by a two column system. Unknown calculation method.
[d] For adsorption and desorption process. Calculated from mass balance.
[e] Electrical energy only for in situ separation, based on process simulations.
[f] Energy requirement for separation technique and a four column distillation set up. All energy was made homogenous by expressing as fuel equivalents. No units were given by Salemme *et al.* (2016), but the specific energy demand for butanol is calculated as the total energy rate supplied divided by the mass flowrate of butanol in the system and the lower heating value of butanol.

In this work, comparative simulations have been performed for all ISPR techniques presented in Table 4.1. Simulations for perstraction were considered independently from liquid-liquid extraction, unlike that performed by Oudshoorn *et al.* (2009). The process

simulations allowed for comparison of both the effectiveness of the ISPR techniques, and the energy demand for both the ISPR technique and the downstream processing. The simulations were also beneficial for an economic assessment of the techniques.

4.2: Process Simulations

Process simulations were produced for a 50,000 te yr^{-1} ABE production plant with IPSR, using UniSim Design (Honeywell). A 50,000 te yr^{-1} plant was simulated being representative of a retrofit of a medium sized bioethanol plant for the production of biobutanol. The plants were compared on a production rate basis. This was based on a constant feed rate and a fixed conversion rate, accounting for the increased productivity of an ISPR fermentation, allowing for inefficiencies in the ISPR technique to be observed. The fermentation concentration was controlled to a maximum of 5 g L^{-1}, in the stream returning to the fermentor after every ISPR technique. This is below the inhibitory concentration of butanol, allowing every ISPR process to have the same production rate hence the same fixed conversion rate. It is assumed that the ISPR techniques have no physiological impact on the bacteria. The thermodynamics of the process were described using the Extended-NRTL (Non-Random Two Liquid) model, based on previously being used for simulations of ABE and ethanol fermentations (Wooley and Putsche, 1996; Oudshoorn *et al.*, 2009; van der Merwe *et al.*, 2013). This model is good for multicomponent, azeotropic, dilute systems, like that experienced with the ABE fermentation. The Extended-NRTL version of the model is better suited to the wide temperature and concentration ranges present in the ISPR systems, utilising interaction parameters as a function of temperature. Two models were used; one with the binary coefficients estimated using UNIFAC vapour-liquid equilibrium, the other using UNIFAC liquid-liquid equilibrium. The model used depended on the mass transfer occurring in each unit operation. Model applicability was confirmed by comparing it to experimental data from Stockhardt and Hull (1931), see Figure 4.1. Good correlation between the experimental data and model, especially for the low butanol concentrations (3-8 g L^{-1}) when ISPR occurs with the average error between bubble and dew point temperatures being 0.59% and 0.75%, respectively. Additionally the model was compared to experimental, ternary ABE data (Perelygin, 1980), where the model predicted the equilibrium concentrations with good accuracy. The average error in bubble point temperatures was 0.46%, and an error of 8% for predicted acetone and ethanol

concentrations. The error for butanol was larger at 16% but the concentration ratio of butanol was significantly less than that experienced in the fermentation broth.

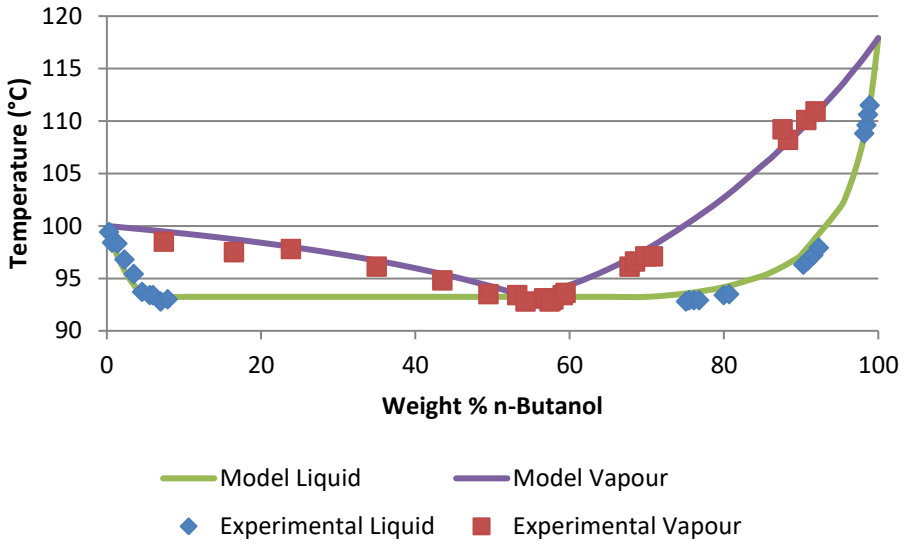


Figure 4.1: Vapour-liquid equilibrium data at 101.3 kPa for butanol water comparing the extended-NRTL model from UniSim Design with experimental data from Stockhardt and Hull (1931).

The fermentations and downstream separations via distillation were based upon the work of Mariano *et al.* (2011a), using the same stoichiometric reactor model and downstream processing route. However, one alteration to the downstream processing route was made by adding the ISPR ABE-rich stream *after* the beer column rather than before. The beer column is used to concentrate the ABE in the fermentation and remove any unwanted components from the product stream, such as substrate, acetic acid and butyric acid that have not been converted to products, and biomass. For ISPR to have a positive impact the product recovery should be a concentrated ABE stream with no contaminants, therefore it will be not need to be pre-processed before ABE purification negating the need for it to be passed through the beer column. This agrees with Huang *et al.* (2014), with acetone being removed first, followed by ethanol then butanol and water. The beer column is still present in the simulations for processing the remaining fermentation broth at the end of the fermentation.

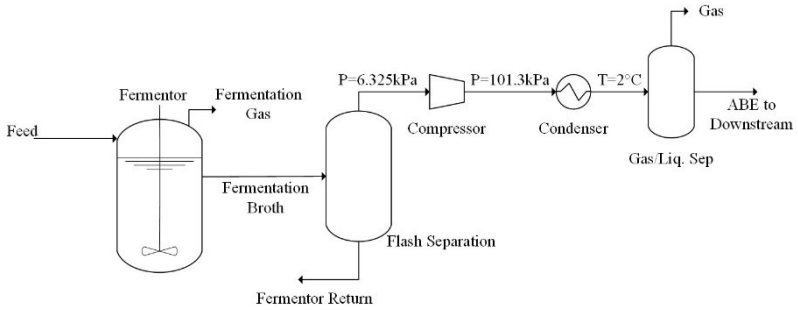
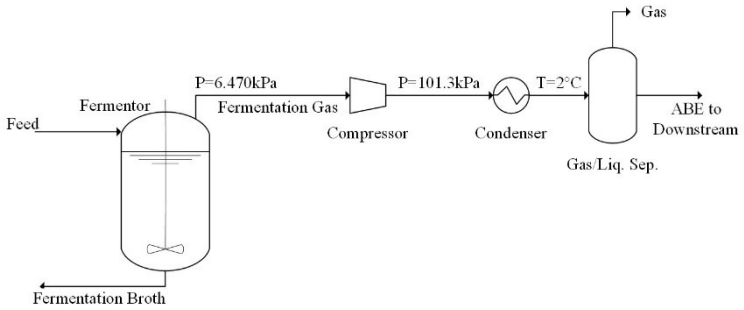
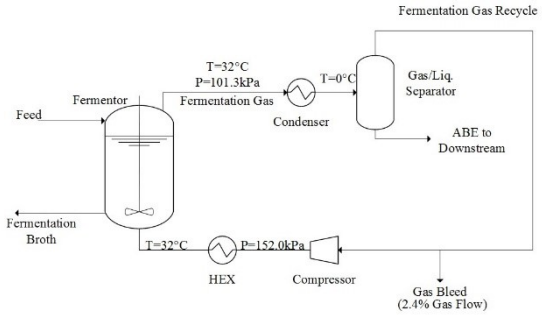
The reactor conversion was based on an ABE product ratio of 23:75:2 (wt%), this considers a process using a high-butanol-producing strain. This is similar to the ratios seen by Tanaka *et al.* (2012). 80% of the product is produced via ISPR methods with a constant substrate concentration of 20 g L^{-1} in the reactor. During the ISPR process the reactor conversion was set based on the amount of substrate converted to each product, ABE, or intermediate, acetic and butyric acid, based upon the stoichiometric reactions provided by Mariano *et al.* (2011a). The percentage of substrate converted into acetone, butanol, ethanol, butyric acid,

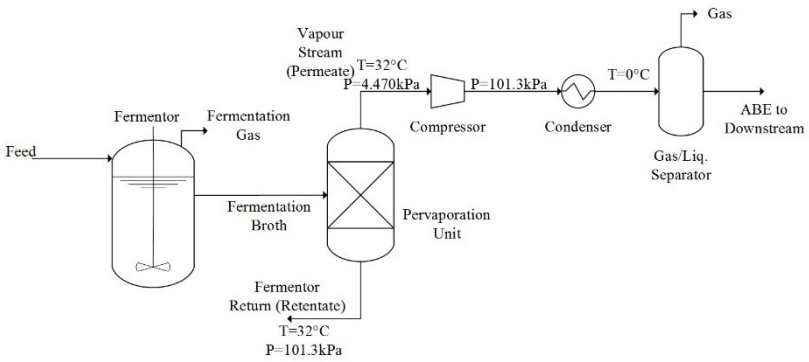
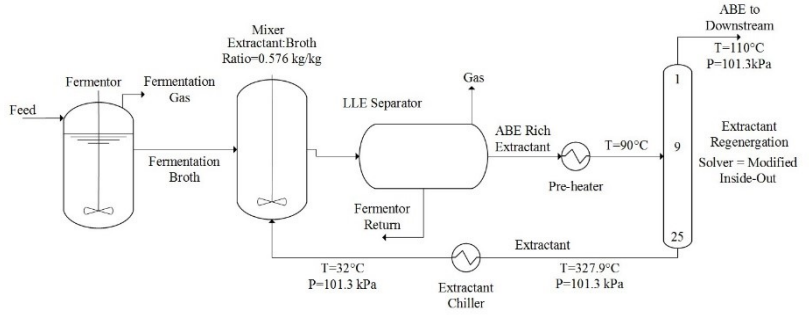
acetic acid and biomass were 6.24%, 15.9%, 0.34%, 2.24%, 1.64%, 46.7%, respectively. Carbon dioxide and hydrogen were also produced during the fermentation. These are represented as by-products of these reactions. As the simulation is performed under steady-state conditions the conversions ensure that there is a uniform concentration of substrate, products and biomass. For this reason, the fermentation broth is not returned to the fermentor after ISPR has been applied, although in real fermentations the fermentation broth would be returned to the fermentor for further processing.

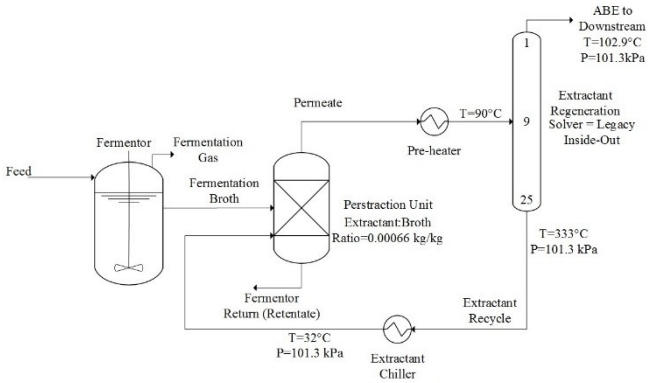
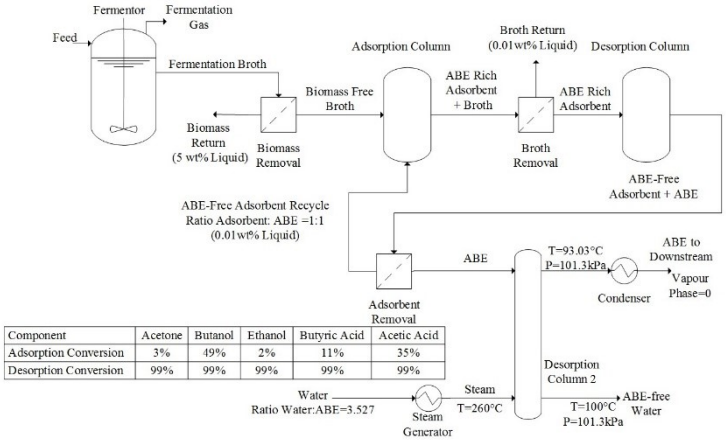
The reactor was assumed to be continuous. Whilst this is not a direct representation of the ABE process, which would typically be a batch or fed-batch fermentation, it allows for a direct comparison of the energy demand for the ABE production process with different ISPR methods. This was due to the simulation software only being able to simulate a steady-state bioprocess. Other reactor types such as biofilm or immobilised reactors have been demonstrated at laboratory scale but have not yet been demonstrated commercially for the ABE fermentation therefore have not been considered for simulation (van der Merwe *et al.*, 2013). An additional continuous reactor was added to the process to represent the batch culture produced at the end of the fermentation. For the batch process the reactor conversion based on substrate consumption for acetone, butanol, ethanol, and biomass production was 14.2%, 36.2%, 0.78% and 48.9%, respectively. It was assumed that no acids would be present at the end of the batch phase, as they would have been re-assimilated into ABE to achieve the maximum yield possible. Hence, the whole process energy could be calculated, allowing comparison to standard batch fermentations.

The ISPR techniques simulated were flash fermentation, vacuum fermentation, gas stripping, pervaporation, liquid-liquid extraction, perstraction and adsorption. These methods of ISPR were selected as they are the most developed techniques and performance information is available in conjunction with the ABE fermentation. Key process conditions were based on experimental data from literature. The process flow diagrams used for the simulations in UniSim Design are shown in Table 4.2 and Figure 4.2, along with the specific details relating to each ISPR technique.

Table 4.2: Description of ISPR technology simulations.

ISPR Technique	Diagram	Sensitivity Analysis Changes	
		Variable	**Range**
Flash Fermentation (Mariano *et al.*, 2008)		Vacuum Pressure (kPa) Condenser Temperature (°C)	0.5925-6.9575 0-4
Vacuum Fermentation (Mariano *et al.*, 2011b)		Vacuum Pressure (kPa) Condenser Temperature (°C)	5.823-7.117 0-4
Gas Stripping (Ezeji *et al.*, 2003; 2004a)		Compressor Pressure (kPa) Condenser Temperature (°C) Gas Bleed	101-202 -2 - 2 2.4-12.2%

Pervaporation (Wu *et al.*, 2012)	 Component | Acetone | Butanol | Ethanol | Butyric Acid | Acetic Acid | Dextrose | Biomass | Water Permeate Split | 0.62 | 0.46 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 Retentate Split | 0.38 | 0.54 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.98	Vacuum Pressure (kPa) Condenser Temperature (°C) Component Split	3.47-5.47 -2 – 2 ±10%
Liquid-liquid Extraction (Roffler *et al.*, 1987a)		Extractant: broth ratio Preheater Temperature (°C)	0.5184-0.6336 80-100

Perstraction (Qureshi and Maddox, 2005)	 Component | Acetone | Butanol | Ethanol | Butyric Acid | Acetic Acid | Dextrose | Biomass | Water Permeate Split | 0.85 | 0.45 | 0.85 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 Retentate Split | 0.15 | 0.55 | 0.15 | 0.93 | 1.00 | 1.00 | 1.00 | 1.00	Extractant: broth ratio Preheater temperature (°C) Component split	0.000594-0.000726 80-100 ±10%
Adsorption (Yang *et al.*, 1994)	 Component | Acetone | Butanol | Ethanol | Butyric Acid | Acetic Acid Adsorption Conversion | 3% | 49% | 2% | 11% | 35% Desorption Conversion | 99% | 99% | 99% | 99% | 99%	Steam temperature (°C) Adsorbent: broth ratio Water: adsorbent ratio	250-270 0.9-1.1 3.177-3.877

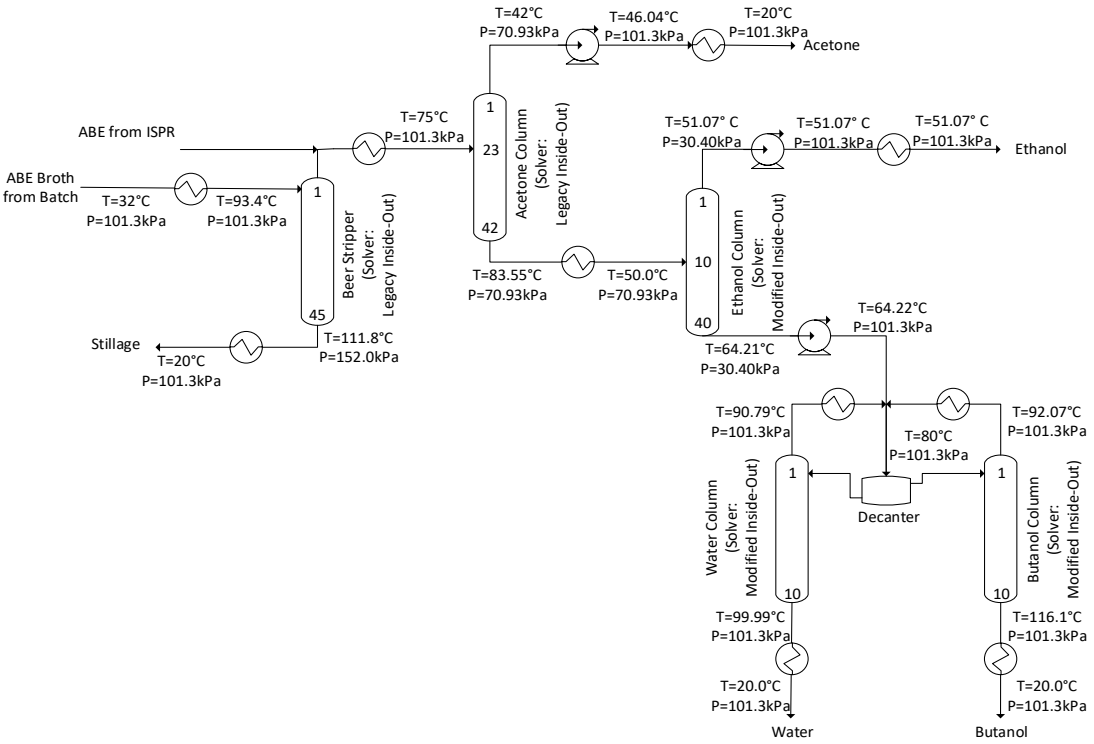


Figure 4.2: Downstream distillation stream conditions for simulation

In addition to simulating the ABE fermentation with ISPR, a conventional batch ABE fermentation was also simulated. As the application of ISPR increases the productivity of the plant, hence annual production, the batch plant capacity was assumed to be 18,000 te yr^{-1}. This is representational of the same capacity plant, without the ISPR technique applied. The annual production is reduced due to batch fermentations, but the annual number of fermentations per year is increased due to shorter fermentation times. This accounts for a 63% decrease in production capacity, compared to the 50,000 te yr^{-1} basecase.

Van Hecke *et al.* (2014) suggested that there are limitations to current simulation software with regards to its ability to incorporate ISPR technologies into the simulations. The limitations, such as the inability to effectively simulate a membrane or adsorption process, stem from the software being designed for the traditional petrochemical industry. Perhaps this explains the lack of information surrounding process energy consumption. As UniSim Design was designed for the chemical industry, it is not wholly suited to simulating a bioprocess, although it is ideal for simulating separation processes of ABE from water. To overcome the limitations of the software, the following assumptions were made:

- Stoichiometric, continuous, steady-state simulation of the fermentation. The continuous stream from the fermentor was assumed to represent the desired

conditions in the reactor. The flow leaving the reactor is representation of the conditions and concentrations that would be observed during a fed-batch, ISPR fermentation

- The NREL database was used to provide properties for biomass (Wooley and Putsche, 1996). The chemical composition of biomass was defined as $CH_{1.57}N_{0.23}O_{0.39}S_{0.0035}$ allowing the molecular weight to be calculated as 24.6 kg $kmol^{-1}$ (Wooley and Putsche, 1996). This allowed for creation of a biomass component the same as that used by Mariano *et al.* (2011a)
- Acetic acid and butyric acid were produced as reaction intermediates. They were assumed to be present in the broth in their dissociated form, as the pH of the broth (minimum pH 5 (Qureshi and Maddox, 2005)) is greater than the pKa of the acids (4.76-4.88). This reduces acid removal from fermentation broth during ISPR, particularly during evaporative techniques, to ensure no contamination of the final product
- The rate of product formation is assumed to be equivalent to that observed at low solvent titres, ~5 g ABE L^{-1}, this concentration is considered non-inhibitory to the fermentation (Ezeji *et al.*, 2004a)
- Membranes can be simulated as component splitters, using membrane flux data (from literature, Table 4.2) to calculate retentate and permeate component fractions. The pressure drop across the membrane was not simulated, though the pressures are controlled in the streams leaving the component splitter. This is particularly important for pervaporation
- The extractant oleyl alcohol, for LLE and perstraction, is simulated as a hypothetical component, using UNIFAC to calculate component properties such as molecular weight, critical temperature, critical pressure, critical volume and acentricity. UNIFAC was used as no experimental data was available for these values; experimental data would have provided greater accuracy
- Adsorption and desorption were simulated as conversion reactions, with carbon representing the adsorbent. As the process is simulated as a series of conversion reactions, carbon forms an ideal basis for matrix, as it is present within UniSim's component database and activated carbon has been used as an adsorbent for ABE (Groot and Luyben, 1986)

- Evaporation-based techniques utilised a gas scrubber to reduce the ABE concentrations in the gas emission streams to capture product and ensure the plant's gas emission were within workplace exposure limits. The limits were acetone, 500 ppm, butanol, 50 ppm, and ethanol, 1000 ppm, based upon United States Department of Labor Occupational Safety and Health Administration (OSHA, 2014)
- Final product concentrations for acetone, butanol, ethanol and water were 99.5 wt%, 99.5 wt%, >80 wt% and 99.5 wt% respectively
- No losses of product in the downstream distillation process.
- Two distillation column solvers were used. The legacy inside-out solver, which is a general purpose solver for most general problems. For more complex conditions such as near azeotropes the modified inside-out solver was used to allow better calculation of factors such as heat exchange inside the column flowsheet. All solvers used are stated in Table 4.2 and Figure 4.2.
- All recycle streams were simulated by inclusion recycle operator. This was done to ensure successful convergence of the simulations.

Further to this, no energy integration was applied to the simulations, to allow for an equal comparison of maximum energy demand across each technique. Using process stream information from the simulations, grand composite curves were developed for each ISPR technique, to understand if any energy can be saved from heat integration. These were developed following the method provided by Towler and Sinnott (2013a).

4.2.1: Economic Analysis Method

An economic analysis was performed based on the results of the simulations. The fixed capital costs were estimated using an updated Bridgewater's method (4.1), described in Towler and Sinnott (2013b).

$$C = 380{,}000U\left(\frac{Q}{s}\right)^{0.3} \quad \textbf{(4.1)}$$

Where:

C is the capital cost in U.S. dollars, U.S. Gulf Coast, Jan 2010 basis

U is the number of functional units

Q is the plant capacity in tonnes per year

s is the reactor conversion (biological yield)

The variable operating costs were based upon the mass balance and relative energy requirements extracted from the process simulations. For the economic analysis the product yield was assumed to be 0.32 kg ABE kg^{-1} substrate, 0.1 kg biomass kg^{-1} substrate and 0.58 kg gas kg^{-1} substrate. The feedstock and product values were taken from Kumar *et al.* (2012) and Qureshi *et al.* (2013). The sale of fermentation gases (H_2 and CO_2), corn oil, corn protein and fibres and biomass was included as additional products. The process simulations were developed to represent a retrofit of a medium sized bioethanol plant. The economic analysis considers the impact of adding ISPR to the plant, by calculating the additional fixed capital required, the additional profit made and associated payback times due to the addition of ISPR.

4.3: Results and Discussion

The process simulations were designed to assess the process energy demand for ABE fermentations incorporating ISPR. The separation efficiency was used to compare how well each ISPR technique can remove ABE from the fermentation broth. The maximum energy demand and separation efficiencies are shown in Figures 4.3 and 4.4, respectively Figure 4.3 shows the upstream and downstream contributions to the total energy, where the upstream is in relation to the fermentation and ISPR process shown in Table 4.2 and downstream is the distillation purification process shown in Figure 4.2.

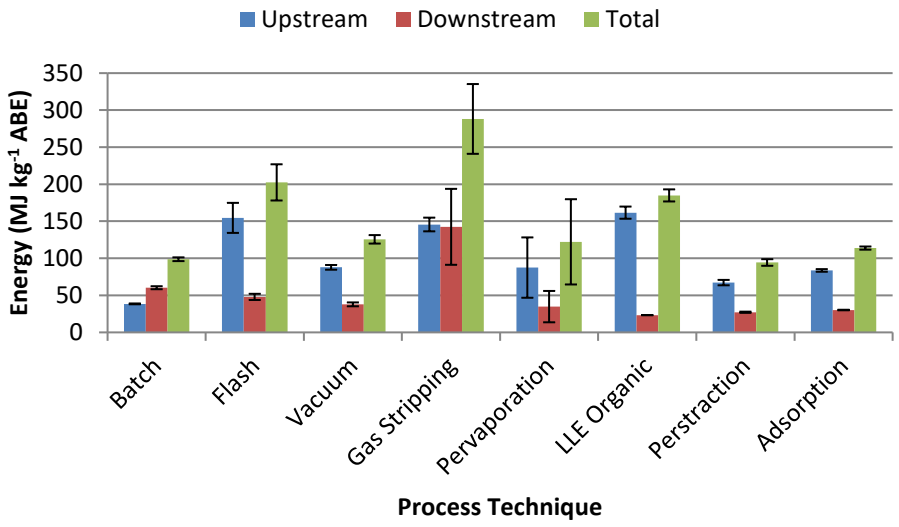


Figure 4.3: Maximum energy requirement for seven ISPR techniques (production rate 50 kt yr^{-1}), representing individual upstream (blue), downstream (red) and total requirements (green), compared to a batch process (production rate 18 kt yr^{-1}).

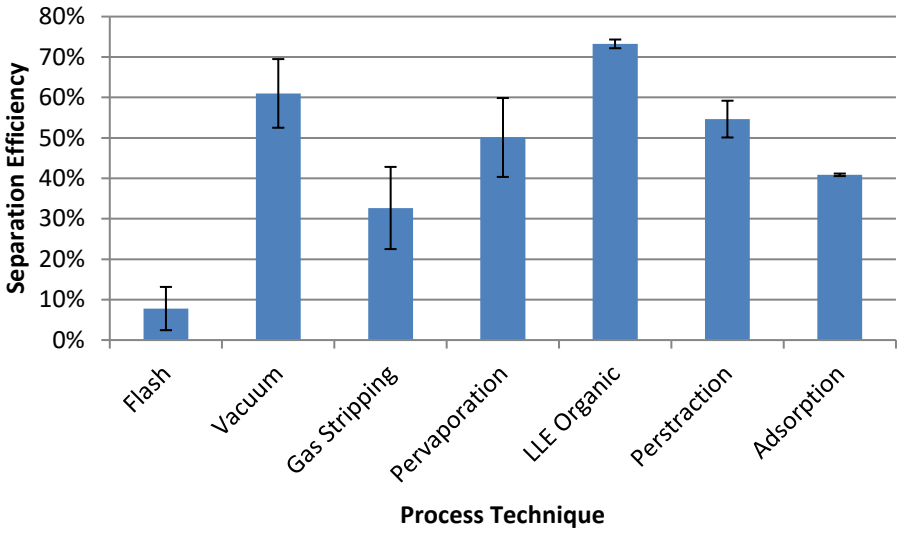


Figure 4.4: Separation efficiency for each ISPR technique.

The simulation results provide insight into both the energy required for the fermentation and ISPR technique, and the impact the ISPR method has on the downstream energy consumption. All energy uses were included in the analysis, including the energy required for heating, cooling and electrical pump duty. This is in contrast to previous work, such as Mariano *et al.* (2011a; 2012a), where only the energy for the vacuum pumps is considered. A sensitivity analysis was performed to assess the reliability of the simulations and the assumptions on which they were based. The variables tested and their ranges are shown in Table 4.2.

4.3.1: Upstream Energy

The "upstream energy" is the energy required for the fermentation and *in situ* separation step, which are shown in Table 4.2. Figure 4.3 shows a wide range of energy demands for this section of the process. Of all the ISPR techniques, perstraction has the lowest upstream requirement, and LLE the highest. The high LLE energy demand is due to the large volume of extractant used for removal of the butanol from the fermentation broth. More extractant means more energy is required for butanol removal via distillation and sterilisation of the extractant before coming in contact with the fermentation broth. The recovery of ABE from any separating agent and renewal of the separating agent has been included as part of the upstream energy demand. For LLE this makes a significant contribution to its high energy demand. Oleyl alcohol is also used as the extractant in the perstraction process, but, as a membrane separates the fermentation broth and extractant phases, smaller volumes of oleyl alcohol are required, less energy is required for renewal of the extractant and no energy is required for dispersion and coalescence of the two phases.

4.3.2: Evaporative Techniques

In general, evaporative techniques have a high upstream energy demand. This is largely due to the low concentration of ABE in the recovered stream and the associated difficulties of capturing the gas from this stream. For example in gas stripping, the ABE is heavily diluted as the gas flow rate required to maintain the butanol concentration in the fermentation broth below inhibitory levels is substantial. In these simulations the butanol concentration in the bioreactor was maintained at 3.5 g L^{-1}, based on the concentrations seen by Ezeji *et al.* (2004a). Water is also removed from the fermentation broth with evaporative techniques, further diluting the vapour stream. Energy was then required to capture the ABE from the vapour phase. It is difficult to capture 100% of the solvents at 1-2°C due to the low vapour pressures of the ABE, in particular acetone, which has a vapour pressure of 0 kPa at approximately -50°C. This was also remarked upon by Ezeji *et al.* (2004a) in their laboratory-scale trials. It was not possible to capture the entire product from the vapour phase within the temperature ranges for condensers seen in literature. Clearly, final product titres, therefore yields and productivities, for evaporative-based experimental techniques will

contain substantial errors. This is why the evaporative techniques data have larger error bars in Figures 4.3 and 4.4.

Pervaporation has the smallest evaporative downstream energy. The separation is largely achieved by a non-porous membrane. This reduces the amount of water being removed from the fermentation broth. Less energy will be required for evaporation and condensation as less water is being removed from the fermentation broth. For all other evaporative techniques there is direct contact between the liquid and vapour phase.

The flash and vacuum techniques are very similar processes, but they exhibit different upstream energy requirements due to the location of the separation. Flash separation is performed in an external vessel, whereas vacuum separation takes place inside the fermentor. The application of a vacuum to the fermentor is likely to have an additive effect to stripping of the ABE by the gasses produced in the fermentation. Mariano *et al.* (2011b) came to this conclusion when comparing vacuum and flash separations. This also goes some way to explain the differences seen in the separation efficiencies between the two techniques, Figure 4.4. The results show that vacuum fermentation is superior to flash separation, but the cost of implementing this needs to be considered particularly when considering the size of the fermentation vessel that needs to withstand 6.5 kPa vacuum. In practice, sensibly achieving a 6.5 kPa vacuum is going to be unattainable, particularly on a retrofit plant where the original fermentor would not have been designed to withstand a constant vacuum.

The evaporative techniques are more sensitive to changes in the simulations. This is probably due to the amount of solvent removed from the broth being a direct result of the energy applied, unlike other techniques that use a separating agent to remove the product from the fermentation broth. All changes to the process occurred in the upstream section, but significant variance is seen in the downstream process, particularly with gas stripping.

4.3.3: Downstream Separation

The downstream energy is related to the purification of the ABE by a sequence of distillation steps, as seen in Figure 4.2. In contrast to the upstream energy demand, LLE has the lowest downstream energy requirements for ISPR techniques. This low energy requirement means that, overall, LLE is not the most energy intensive ISPR technique. Rather, it is gas stripping,

which has one of the highest upstream energy requirements and the highest downstream energy requirement. The downstream energy is almost identical to the energy required for upstream processing.

The four largest ISPR downstream energy demands are for the evaporative separation techniques. Due to the large amounts of water removed from the fermentation broth during the *in situ* recovery step. The downstream energy requirement is heavily dependent on the concentration of ABE entering the downstream system. The ISPR techniques with the lowest downstream energy demands have much higher concentrations of ABE at this point. LLE produces the most concentrated stream from *in situ* recovery. It is therefore not surprising that LLE and perstraction have the lowest downstream processing energy demands, as they both utilise a non-polar organic extractant for separation. The extractant's affinity for water is extremely low, hence the high product concentration and low downstream energy demand.

Compared to a standard conventional batch ABE fermentation, it can be seen that all ISPR processes have a greater upstream energy, Figure 4.3. This is to be expected, as at least one additional unit operation is being added to the upstream process. In contrast, all ISPR techniques, other than gas stripping, have a lower downstream energy than the standard batch process. This is expected due to the increased concentration of ABE in the downstream section of the process, resulting in lower downstream energy demand. Interestingly, however, when the energy demand for the whole process is considered, only perstraction has a lower total energy than the batch process. The reduction is nearly 5%.

4.3.4: Separation Efficiency

From the simulations it was also possible to quantify a comparative degree of recovery. This is not evident in the existing literature. The separation efficiency is a comparison of the amount of ABE separated from the fermentation broth during ISPR compared to the amount of ABE present in the fermentation. As all ISPR simulations had the same feed flow rate and reaction scheme, an assessment of the techniques' abilities to remove ABE from the fermentation broth can be made (see Figure 4.4). The simulation results show that the highest separation efficiency is achieved by LLE, then by vacuum fermentation. Based on the simulations the minimum separation efficiency required to achieve a maximum fermentation broth concentration of 5 g ABE L^{-1} is 36%. This minimum threshold is achieved

by all techniques other than flash separations and gas stripping. This is due to the difficulty of capturing the ABE from the dilute vapour phase generated as part of the ISPR method. Adsorption has the potential to be a very selective technique for the recovery of ABE from the fermentation broth. The downside is that product recovery and adsorbent regeneration both require steam. The steam is a means of direct heat application for separation, although some water vapour will be transferred into the product recovery stream to downstream processing. Other methods of adsorbent regeneration are possible, such as chemical recovery, for example with methanol, but the ABE would then need to be recovered from the methanol, further increasing the number of process steps (Yang *et al.*, 1994).

4.3.5: Membrane Technologies

Based on the results shown in Figures 4.3 and 4.4, the effect of using a membrane technology can be observed. In the case of LLE and perstraction, it can be seen that the membrane option (perstraction) has a lower degree of recovery and energy demand for upstream processing although it has a slightly higher downstream energy demand. The comparison for evaporative technologies is slightly more difficult, as both flash separation and vacuum separation are equivalent to pervaporation. As vacuum separation is a significantly more effective technique than flash separation this will be used here as the comparison with pervaporation. Pervaporation has a lower degree of recovery and lower energy demand than vacuum separation. The energy differences between vacuum separation and pervaporation are small compared to those calculated for the extractive processes.

From these simulation results it is clear that the use of membranes currently hinders the effectiveness of the separation, as both membrane techniques have a lower separation efficiency compared to vacuum fermentations and LLE. It is also worth noting that with current membrane technology the mass transfer differences across the membrane, between pervaporation and perstraction, do not have a large impact on the separation efficiency. The relatively large error associated with the separation efficiency of pervaporation and perstraction was expected due to the software's inability to directly simulate a membrane operation; therefore there was greater reliance on published experimental data, and there is very limited information available in relation to the membrane permeability and diffusion rates, particularly for perstraction.

In terms of energy of the process, the use of membranes reduces the energy demand of the process although it is questionable whether the energy reduction for evaporative techniques is enough to justify pervaporation over vacuum fermentation. Vacuum fermentations and pervaporation both require 87 MJ kg^{-1} ABE for the upstream separation and the downstream separation for pervaporation is 3 MJ kg^{-1} ABE less than that required for vacuum fermentations. The greater separation efficiency corresponds to an increase in ABE recovered, which is likely to generate a bigger profit than any savings made from reducing the energy by 3 MJ kg^{-1} ABE. These results can probably be improved through membrane development and optimisation to make membrane techniques more widely used.

4.3.6: Heat Integration

Heat integration can be used to reduce the energy demand of the process. González-Bravo *et al.* (2016) have previously investigated heat integration for downstream biobutanol separation, assessing two distillation systems and two hybrid liquid-liquid extraction/distillation systems. Their results indicated that the energy demand can be reduced through heat integration but they did not consider applying it alongside ISPR. Using process stream information from the simulations it was possible to assess the potential for reducing the heating and cooling demand. Grand composite curves were developed for each simulation, so the minimum energy for heating and cooling could be calculated, using a ΔT_{min} of 20°C. An example grand composite curve is presented in Figure 4.5. The calculated minimum energy does not include energy for electrical equipment such as pumps and compressors or energy required to maintain a constant temperature in the bioreactor or other equipment (for example membrane units).

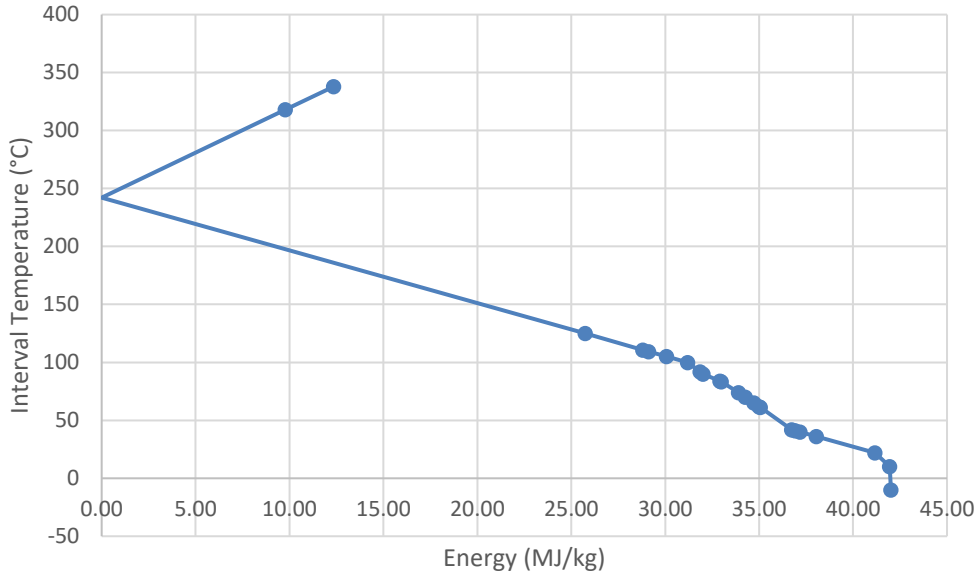


Figure 4.5: Example grand composite curve for liquid-liquid extraction flowsheet

The minimum heating and cooling energy required for a batch process was 19.7 MJ kg^{-1} ABE, this was similar to that required for gas stripping (18.3 MJ kg^{-1} ABE) and flash separation (18.9 MJ kg^{-1} ABE). These ISPR techniques did not achieve the minimum separation efficiency of 36%, therefore the concentrations of ABE in the downstream separation section are going to be equivalent to that of a batch system so similar minimum energies is not unexpected.

The techniques which achieved a separation efficiency greater than 36% all had significantly lower minimum energies for heating and cooling, apart from liquid-liquid extraction with a minimum energy of 54.3 MJ kg^{-1} ABE. This was the largest minimum energy of all the techniques, and is largely related to the energy required to recover the ABE from the extractant and subsequently cool the extractant for reuse. Vacuum fermentation, pervaporation, adsorption and perstraction all have similar minimum heating and cooling energies of 9.5 MJ kg^{-1} ABE, 6.3 MJ kg^{-1} ABE, 5.5 MJ kg^{-1} ABE and 4.1 MJ kg^{-1} ABE, respectively. Having minimum heating and cooling requirements lower than the batch process means that heat integration applied to the ISPR processes will further reduce the process energy demand. Applying heat integration would increase the capital cost due to heat exchanger requirements. Further work would be required to fully understand the possible heat exchange networks and to optimise the energy savings that could be made, alongside the additional capital cost for installation of the heat exchange network.

4.3.7: Literature Comparisons

It is difficult to make comparisons between the simulation results presented here and previously published results in the literature. As previously stated, the results from literature in Table 4.1 rely on assumptions that are different in each source and vary from some of the assumptions used in these simulations. The major difference with this work is that the data presented in Table 4.1 are primarily concerned with the energy required for just the separation technique, rather than the entire upstream and downstream process. The biggest difference seen between the literature results and this is the energy requirements for LLE. The overall energy, 185 MJ kg^{-1} ABE (Figure 4.3), was one of the highest where as in previous studies the energy demand for LLE (7-9 MJ kg^{-1} BuOH) was one of the lowest (Table 4.1). It is suspected that the previous studies did not include the energy required to separate the ABE from the extractant (Groot *et al.*, 1992; Qureshi *et al.*, 2005; Oudshoorn *et al.*, 2009). This difference can have a significant impact on the viability of the process from an energy demand assessment.

In contrast, other authors such as Salemme *et al.* (2016) have only considered alternative downstream processing routes, replacing the traditional batch column with either a gas stripper or LLE unit using 2-ethyl-1-octanol. The results provided by Salemme *et al.* (2016) include heat integration, therefore could be compared to these minimum energy demand results presented in this work. For gas stripping they achieved a specific energy requirement of 15.3 MJ kg^{-1}, this is very similar to the minimum energy demand of 18.3 MJ kg^{-1} ABE achieved in this work; indicating that that gas stripping is very similar to a distillation column. The results for LLE vary greatly with Salemme *et al.* (2016) achieving 9.9 MJ kg^{-1} whereas this work presents a minimum energy requirement of 54.3 MJ kg^{-1} ABE. It must be noted that this paper has an additional distillation column and the primary separation is occurring at concentrations less than 10 g L^{-1} whereas the feed concentration used by Salemme *et al.* (2016) is 30 g L^{-1}. This difference in concentration can have a large impact on the energy required for separation as demonstrated by Mariano *et al.* (2011a).

4.3.8: ISPR versus Batch Processing

It is well-documented that for the traditional ABE fermentation process the energy-intensive nature of solvent purification by distillation is one of the biggest challenges to be overcome (Ezeji *et al.*, 2004b; Dürre, 2007; Green, 2011). The information that is available tends to

compare the energy for ISPR with other ISPR techniques, as shown by Table 4.1, with hardly any comparison to the conventional batch process, particularly in terms of the whole process. One of the significant results is that whilst the downstream energy requirement can be reduced through the application of an ISPR technique, increases in whole process energy are usually observed. This is confirmed by Mariano *et al.* (2012a), in which the energy for a vacuum fermentation is considered. The same result was observed here (Figure 4.3) for all ISPR techniques, apart from perstraction, the whole process energy is greater than that for the batch process. This means that whilst the use of ISPR can reduce the downstream energy, it shifts the associated costs to an alternative part of the process.

It is clear that, generally speaking, non-evaporative processes will provide less intensive recovery and a greater annual production because of the high condensing power required for acetone capture. The target output for the simulations was 50,000 tonnes ABE $year^{-1}$. In no ISPR technique was the entire product produced recovered, indicating that no technique is 100% efficient for recovery. As the substrate inputs were constant across every ISPR technique, the losses were used to assess the effectiveness of each technique through the separation efficiency, Figure 4.4. The losses are due to the lack of development, in terms of scale up and optimisation, with the application of ISPR to the ABE fermentation. The only technique to have shown any systematic process development is LLE, which the simulations show has the highest separation efficiency (Roffler *et al.*, 1987a; 1988). These losses need to be assessed for each technique. Minimisation of these losses will see an increase in product recovery, and a reduction in the energy requirement.

The results in Figure 4.4 show that the more developed techniques, vacuum fermentation and LLE, exhibit some of the highest recoveries of ABE from the fermentation broth. However, this is probably a result of their higher technology readiness levels than a fundamental phenomenon. Ideally, the results would have shown that the technique with the lowest energy demand also had the highest separation efficiency clearly indicating the best ISPR technique. As this is not the case, it means that a compromise is required when deciding on an appropriate technique. From the results presented here, perstraction appears to be the most favourable technique, as it is the only ISPR technique that gives an improvement over the batch process in terms of overall energy demand. Furthermore, it has the third highest separation efficiency, indicating good separation characteristics. Another possible advantage is that it is at a relatively early stage of development, so could yet prove

to be more effective, if membrane technology advances or if economies of scale are realised for this technology.

Information concerning the development of these techniques from laboratory scale upwards is limited (Van Hecke *et al.*, 2014). The simulations are only a representation of the process, and advances in these technologies could significantly alter the results presented here. Improvements to the process could come in the form of membrane, adsorbent or extractant development. Development of these technologies at larger scales, thereby providing scale-up data would have a significant impact on the ABE process, particularly for adsorption and perstraction.

4.3.9: Economic Assessment

An economic comparison of the application of multiple ISPR techniques to the ABE fermentation has not previously been described in literature (Abdehagh *et al.*, 2014). This is due to the early stage of development of most ISPR techniques and scarcity of information about associated costs, such as membranes and adsorbents. An economic assessment has been completed by Roffler *et al.* (1987a) for LLE using oleyl alcohol, compared to a batch plant to produce the same quantity of butanol, indicating that 20% less capital was required due to reduced broth volume required and higher fermentation productivity. Abdi *et al.* (2016) have provided a comparison of a non-integrated ABE fermentation with an ABE fermentation integrated with flash separation at a pressure of 7kPa in an external vessel. This analysis demonstrated how the application of ISPR could significantly increase the profitability of the process, meaning the ABE fermentation would be more able to cope with fluctuations in the market price of butanol. van der Merwe *et al.* (2013) primarily focused on varying the downstream processing route, but found the application of ISPR, using gas stripping, with LLE as the first downstream processing step to be profitable. This is similar to the economic assessment performed by Liu *et al.* (2004), which considered the use of LLE or gas stripping replacing the initial beer column, with varying distillation routes. Results shown in Table 4.3 compare the extra capital required and extra profit generated by utilising ISPR.

The fixed capital cost was estimated using an updated Bridgewater's method (Towler and Sinnott, 2013b). It provided an estimate of the upstream fixed capital required for each ISPR technique based on the number of functional units in the process, for a plant in the U.S. Gulf Coast, January 2010. The downstream process capital expenditure was not considered as it is

identical for every technique, due to having the same process configuration (Figure 4.2). This method agrees with other literature values for both a batch ABE plant and a plant with ISPR. Kumar *et al.* (2012) calculated that the capital cost for a batch plant producing 10,000 tonnes of butanol per year from corn to be $10.3 million. Nilsson *et al.* (2014) estimated the capital cost for an ABE plant with gas stripping to be approximately $62 million. Both values are similar to the results shown in Table 4.3, indicating a good estimate of the capital cost.

The operating costs were based on the annual production and the relative energy requirements (MJ kg^{-1} ABE) from the process simulations for each technique. The yield for the ISPR techniques has been assumed to be equivalent to a batch process, therefore each ISPR process has the same annual production and feedstock consumption. In reality the overall annual production would vary for each technique due to different efficiencies of the recovery; however for optimal economic efficiency all products need to be recovered at some point during the process, allowing the overall process yield to match the bacterial yield. Product and feedstock values ($ kg^{-1}) were taken from Kumar *et al.* (2012) and Qureshi *et al.* (2013). The payback time for the addition of ISPR to an existing ABE fermentation plant was calculated and shown in Table 4.3. This compares the extra capital cost required for ISPR and the extra profit generated through the use of an ISPR technique. This method agrees with the method used by Abdi *et al.* (2016) who observed operating costs for the integrated fermentation increase by 181%, this is similar to the results presented here whereby the operating costs increased by 182% for vacuum separation and 205% for flash separation.

Similar to the results in existing literature, the use of ISPR increases the profitability of a fixed volume plant. In terms of comparing ISPR techniques, perstraction and adsorption have the joint lowest payback time, closely followed by vacuum fermentation. These three options also have the greatest increase in profitability over a batch process. The major variable affecting the profitability is the energy cost, which was determined from the process simulations. It is unsurprising that the techniques with the lowest energy demand also had the shortest payback time.

All ISPR techniques assessed could increase plant profitability (Table 4.3) through increasing capacity. The ISPR-based plants produce 2.75 times more product than that of the batch plant. Perstraction is the only ISPR technique to produce an equivalent increase in the profit. Gas stripping was the least profitable of all the ISPR techniques, only increasing the batch profit by 110%. This is due to the high energy demand of the process, (Figure 4.3), which,

when combined with the increased capital cost, increases the additional payback time of the plant. The application of flash fermentation to the ABE process has a similar effect.

The economics of the process needs to be considered alongside the separation efficiency. Vacuum fermentation and LLE have the greatest separation efficiencies but fall in the middle of the range in terms of profit increase, due to the higher energy demand. These are also the most developed techniques, therefore have limited scope for development. Perstraction, adsorption and pervaporation have the greatest increase in profitability, but there is a much greater scope for development to improve the separation efficiencies of these ISPR techniques, making the techniques more attractive for commercial ABE production.

The potential reduction in energy has been one of the driving forces for research into the application of ISPR to the ABE process. Perstraction is the only technique which has an energy demand lower than that of a batch process. It is a very similar technique to LLE and should be able to overcome some of the problems that occur when using LLE, specifically extractant toxicity and the volume of extractant required. The biggest difference between LLE and perstraction is the amount of product recovered, (Figure 4.4). Increasing the separation efficiency will help to make perstraction an ideal ISPR technique for the ABE fermentation. However, it has one significant disadvantage: its relative lack of development. Currently, perstraction exhibits low extractant rates, as there has been no systematic optimisation of the extractant and membrane materials vs. flow rates (Qureshi and Maddox, 2005). Significant membrane development occurred for the use of pervaporation in ABE pervaporation between Groot *et al.* (1984a)'s use of silicone tubing and Van Hecke *et al.* (2012)'s use of commercially available pervaporation modules from Pervatech. If similar developments can occur for perstraction, it could prove to be the most cost-effective ISPR technique for the ABE fermentation.

Table 4.3: Economic assessment of the application of ISPR to an ABE production plant.

			Batch	Flash	Vacuum	Gas Stripping	Pervaporation	LLE	Perstraction	Adsorption
	Capital Cost	$ (million)	**10.1**	**68.4**	**54.7**	**68.4**	**68.4**	**54.7**	**54.7**	**54.7**
Annual Production	Acetone	kt yr^{-1}	3.9	10.7	10.7	10.7	10.7	10.7	10.7	10.7
	Butanol	kt yr^{-1}	13.7	37.5	37.5	37.5	37.5	37.5	37.5	37.5
	Ethanol	kt yr^{-1}	0.4	1.1	1.1	1.1	1.1	1.1	1.1	1.1
	TOTAL	kt yr^{-1}	18.0	49.3	49.3	49.3	49.3	49.3	49.3	49.3
Operational Cost	Feedstock Cost	$ yr^{-1} (million)	9.6	26.4	26.4	26.4	26.4	26.4	26.4	26.4
	Energy Cost	$ yr^{-1} (million)	1.4	8.1	5.0	11.5	4.8	7.4	3.8	4.5
	Other	$ yr^{-1} (million)	2.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
	TOTAL	$ yr^{-1} (million)	13.4	40.8	37.7	44.2	37.5	40.1	36.4	37.2
Product Sales	Acetone	$ yr^{-1} (million)	4.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
	Ethanol	$ yr^{-1} (million)	0.3	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	Butanol	$ yr^{-1} (million)	16.2	44.3	44.3	44.3	44.3	44.3	44.3	44.3
	Additional Products	$ yr^{-1} (million)	4.1	11.3	11.3	11.3	11.3	11.3	11.3	11.3
	TOTAL	$ yr^{-1} (million)	25.1	68.9	68.9	68.9	68.9	68.9	68.9	68.9

			Batch	Flash	Vacuum	Gas Stripping	Pervaporation	LLE	Perstraction	Adsorption
Profit		**$ yr^{-1} (million)**	**11.8**	**28.1**	**31.2**	**24.7**	**31.3**	**28.8**	**32.4**	**31.6**
	Extra Capital	$ (million)		58.3	44.6	58.3	58.3	44.6	44.6	44.6
	Extra Profit	$ yr^{-1} (million)		16.3	19.4	12.9	19.6	17.0	20.7	19.9
Additional Payback Time		**yr**		**3.6**	**2.3**	**4.5**	**3.0**	**2.6**	**2.2**	**2.2**
% Profit Increase				**139%**	**165%**	**110%**	**166%**	**145%**	**175%**	**169%**

4.4: Conclusions

Previously, no ISPR technique has been identified as being the most profitable for removal of ABE from fermentation broth. This study shows that ISPR generally *increases* the overall energy requirement of the plant. The one exception found in this study is perstraction, which had the lowest overall energy requirement, leading to a 175% increase in profit over a conventional batch plant. However, perstraction does not provide the greatest separation efficiency. This was achieved by LLE, which is a more mature technique. However, developments in perstraction membrane technology it could be possible to recover more ABE, matching the separation efficiency of LLE, in which case perstraction would be the best performing process by most metrics.

4.5: Summary

This focus of this chapter has been a techno-economic analysis to compare seven ISPR techniques suggested for the ABE fermentation. A comparison of this nature has not previously been performed, and is not possible based on an analysis of literature alone. Key findings from this analysis are that the application of ISPR does reduce the downstream energy requirements for ABE separation, in accordance with previously published statements. However, the overall energy of the plant increases as a more "intensive" separation is required; separating ABE at a lower concentration, 5-8 g butanol L^{-1}, compared to 15 g butanol L^{-1}, while maintaining viability of the bacteria. As stated by Van Hecke *et al.* (2014), analysis of the whole process had not been completed, therefore these results help provide a greater understanding of the impact ISPR can have on a process.

Perstraction has been identified as the most promising technique for combining with the ABE fermentation, as it has the potential to reduce the overall energy demand below that of an equivalent sized batch process while achieving the minimum separation required to reduce the impact of product toxicity. As summarised in both Chapters 2 and 3, perstraction is a relatively immature ISPR technique compared to the other ISPR techniques investigated in this chapter. A better understanding of both the impact of membrane and extractant choices available for perstraction is required. The next chapter in this thesis is concerned with the application of perstraction to the ABE process.

Chapter 5. Perstraction

5.1: Introduction

Perstraction is a technique which was developed to tackle the weaknesses of liquid-liquid extraction (LLE). LLE was found to be an effective technique for ISPR but there were several problems which hindered the fermentation (Jeon and Lee, 1987; Roffler *et al.*, 1987b; c). The problems associated with LLE are:

1. Majority of extractants are toxic to the bacteria, limiting the duration of the fermentation (Jeon and Lee, 1989; Qureshi and Maddox, 2005)
2. Reduces toxic effects from butanol, but replaces it with toxic effects of the extractant
3. Accumulation and inactivation of cells at the extractant interface (formation of a rag layer) (Jeon and Lee, 1989; Qureshi and Maddox, 2005)
4. Extraction of product intermediates (acetic acid and butyric acid) (Qureshi and Maddox, 2005)
5. Formation of an emulsion, which is difficult to separate (Qureshi and Maddox, 2005)
6. Loss of extractant due to inefficient phase separation (Jeon and Lee, 1989; Qureshi and Maddox, 2005)
7. High energy demand required for sterilisation of the extractant (Jeon and Lee, 1989)

To overcome these problems a membrane was placed between the fermentation broth and the extractant to separate the phases, Figure 5.1, reducing the problems seen with LLE (Abdehagh *et al.*, 2014). This ISPR process is called perstraction (Ezeji *et al.*, 2007b). It can also be called membrane-assisted solvent extraction, membrane solvent extraction, membrane extraction or pertraction (although pertraction can also be used to described processes using a liquid membrane, rather than transfer into an extractant). Perstraction is similar to the technique pervaporation, where products are transported across the membrane into a vapour phase.

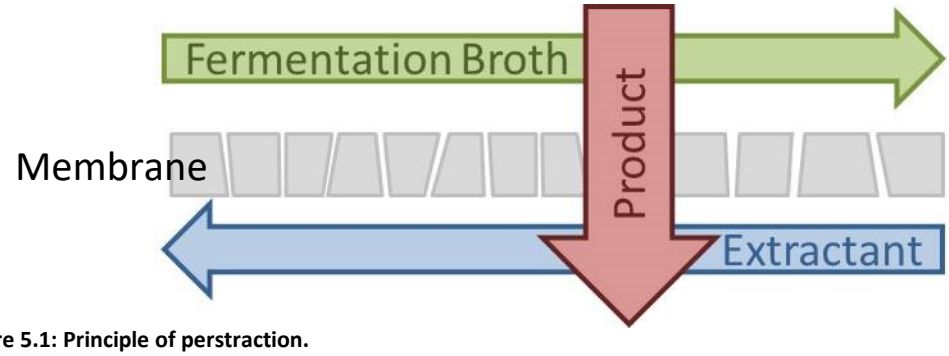


Figure 5.1: Principle of perstraction.

During perstraction the fermentation products diffuse through the membrane into the extractant (Huang *et al.*, 2014). The other fermentation broth components (e.g. biomass, substrate, nutrients and intermediates) are retained in the retentate and returned to the fermentor (when an external perstraction unit is used). The membrane provides a large fixed surface area for mass transfer. In LLE, energy has to be supplied to the process to disperse the extractant in the broth, for a large mass transfer area (Abels *et al.*, 2013). The downside of using a membrane is the additional resistance it adds to the extraction process and the potential for fouling (Cen and Tsao, 1993; Ezeji *et al.*, 2007b; Abels *et al.*, 2013; Abdehagh *et al.*, 2014; Huang *et al.*, 2014).

In LLE, a compromise between distribution coefficient (the ratio of the concentration i in the organic phase to the concentration of i in the aqueous phase) and toxicity to the microorganism is required. When using perstraction an extractant with a more favourable distribution coefficient, that is toxic to the microorganism or forms a stable emulsion with the fermentation broth, can be selected (Daugulis, 1988). Commonly, an organic extractant is used due to their availability, but other liquids such as ionic liquids could also be used (Abels *et al.*, 2013). The selection of an extractant with a high selectivity for ABE could also help retain some fermentation broth components in the retentate (Abdehagh *et al.*, 2014; Xue *et al.*, 2014d).

Perstraction also has several additional parameters which can influence the mass transfer and rate of product removal, such as flow rate and membrane area (Tanaka *et al.*, 2012; Abels *et al.*, 2013). Understanding of these variables and how they affect mass transfer would allow perstraction to be easily scalable and transfer to continuous operation (Abels *et al.*, 2013).

5.2: Examples of Perstraction – Industrial and Research

Perstraction, under its many guises, has been used or investigated in several industries. It has been proposed for product removal from several inhibitory fermentations such as ethanol (Matsumura and Märkl, 1986), phenol (Heerema *et al.*, 2006; 2011a; 2011b), butyric acid (Zigová *et al.*, 1999; Wu and Yang, 2003) and the ABE fermentation (Jeon and Lee, 1987; 1989; Grobben *et al.*, 1993; Qureshi and Maddox, 2005; Tanaka *et al.*, 2012). The application of perstraction to fermentation has, generally, not proceeded past proof of concept stage, Table 2.1. Currently the only example of further developments is with a recombinant phenol fermentation by TNO, the Netherlands Organisation for applied scientific research (Heerema *et al.*, 2011a). The production of phenol was engineered into a solvent-tolerant *Pseudomonas putida* S12, but phenol still has an inhibitory effect on the bacteria, hence the need for ISPR. Perstraction was chosen as the method of ISPR to minimise contact between the microorganism and extractant and prevent emulsion formation. The use of perstraction was demonstrated to increase the fermentation productivity 132% (Heerema *et al.*, 2011b). Compared to the ABE fermentation, the phenol fermentation has a low productivity of approximately 0.007 g L^{-1} h^{-1} (0.072 mM h^{-1}) (Heerema *et al.*, 2011b). This means that the rate of extraction does not need to be as fast as that required for the ABE fermentation, where productivities greater than 0.21 g L^{-1} h^{-1} are observed in a batch system (Qureshi and Maddox, 2005). Whilst increased perstraction rates are possible by increasing the membrane area this will adversely affect the economics of perstraction.

Perstraction has been suggested for other separation processes where methods such as distillation would be energy intensive, such as the separation of two organic solvents, for example 2-propanol/n-heptane mixtures (Papadopoulos and Sirkar, 1993). It has also been proposed for the treatment of liquid radioactive waste. LLE is often used to remove metals from a waste stream with an organic extractant, but similar disadvantages to using LLE in fermentations are observed. The use of a membrane can overcome these problems, and is advantageous due to the precisely defined interfacial area (Zakrzewska-Trznadel, 2013).

Perstraction is also used for the production of low-alcohol beverages (Diban *et al.*, 2008; Wollan, 2008; De Francesco *et al.*, 2014). The highly selective nature of perstraction, due to membrane and extractant combinations, means that only the alcohol is removed. This means that the final product resembles the alcoholic version, as closely as possible; something that is difficult to achieve with other dealcoholisation techniques (De Francesco

et al., 2014). De Francesco *et al.* (2014) reduced the ethanol content in beer to less than 1% (v/v) in four hours of operation with a polypropylene hollow fibre membrane and deaerated water as the extractant.

5.3: Perstraction Theory

The mechanism for mass transfer in perstraction is related to the concentration of the solute on either side of the membrane and the mass transfer across the membrane.

5.3.1: Liquid-Liquid Equilibrium

The separation is equilibrium-based, as for LLE. A concentration gradient is established between two phases and, ideally, the solute has a preference for extracting phase. This can be defined by the distribution coefficient (or partition coefficient), K_d:

$$K_d = \frac{C_{iE}^*}{C_{iA}^*} \quad \textbf{(5.1)}$$

Where:

C_{iE}^* is the concentration of *i* in the extractant phase in equilibrium with the concentration in the aqueous phase (g L^{-1}).

C_{iA}^* is the concentration of *i* in the aqueous phase in equilibrium with the concentration in the extractant phase (g L^{-1}).

When $K_d > 1$ the solute *i* has a greater affinity for the extractant, than the aqueous phase. This is desired for the extraction of products from the fermentation broth as it will increase the product concentration, making separation of product from extractant easier.

It is assumed that the distribution coefficient for a liquid-membrane-liquid system will be the same as that for a liquid-liquid system at equilibrium, as the liquid-liquid equilibrium distribution coefficient has been used as the main extractant selection parameter in ABE perstraction research (Jeon and Lee, 1987; Tanaka *et al.*, 2012). This is true for a porous membrane system, as there is a direct liquid-liquid interface which is immobilised by the membrane. The direct liquid-liquid interface would mean that it is possible for a concentration equilibrium, equivalent to a LLE system, to be established between the two phases. In a non-porous system, multiple equilibriums are established. Firstly, between the

aqueous phase and the membrane and, secondly, between the membrane and extractant phase. For equilibrium to occur, all phases must be at equilibrium including the aqueous phase with the extractant phase, as described by (5.1). The maximum value for distribution coefficient possible will be equal to that for a LLE system, therefore this value of distribution coefficient has been used to select high capacity extractants.

5.3.2: Membrane Mass Transfer

5.3.2.1 Types of Membrane

There are three types of membrane that could be considered for perstraction, Figure 5.2:

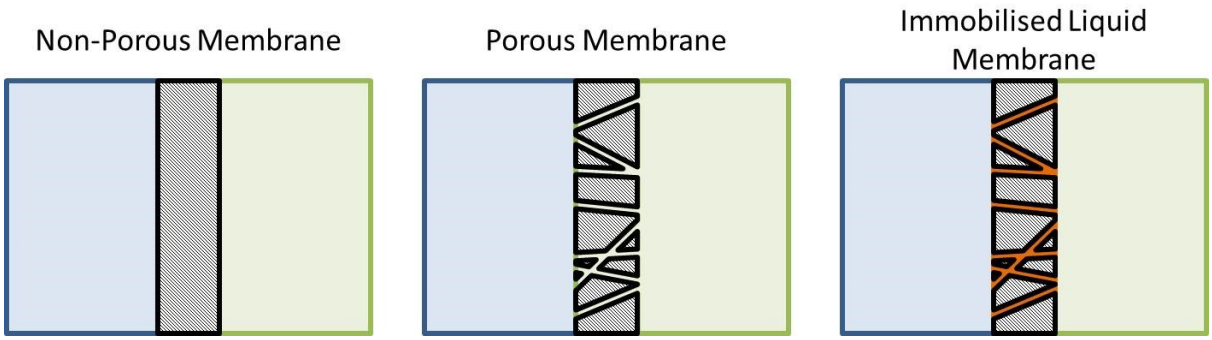


Figure 5.2: Types of membrane for perstraction.

1) Non-porous membranes rely on the absorption of solute into the membrane and desorption out of the membrane into the extractant.
2) Porous membranes, where the membrane acts as a support to immobilise the interface between the fermentation broth and the extractant. For example, when using a hydrophobic membrane, the non-polar extractant will fill the pores and the solute will dissolve into the extractant, and transfer through the pores into the bulk solution.
3) Immobilised liquid membrane, a liquid is immobilised in the pores of the membrane allowing an alternative liquid to be used as an extractant. The difficulty with this membrane is selecting a liquid which is immiscible in both the fermentation broth and extractant. Otherwise this membrane is not too dissimilar from using a porous membrane.

It is assumed that all membrane types are incompressible. With porous membranes the pores are filled with a liquid, thereby there will be no volume change with pressure changes.

There are two main models that can be used to describe mass transfer across a membrane: the solution-diffusion model for non-porous membranes or the pore-flow model for porous membranes (Wijmans and Baker, 1995). In the solution-diffusion model the solute diffuses through the membrane based on a concentration gradient. The pore-flow model is based on a pressure-driven flow through the pores, this relies on the membrane excluding some permeants from passing through (Wijmans and Baker, 1995). As the perstraction process is not typically pressure-driven, the solution-diffusion model is best suited to describe the mass transfer across the membrane.

5.3.2.2 Solution-Diffusion Model

As previously stated, the transfer of solute across the membrane is governed by the equilibrium of solute on both the aqueous and extractant phase; defined by equation (5.1). When considering the concentration profile across the membrane, with $K_d > 1$ the concentration of the solute should be higher in the extractant, Figure 5.3. This can be related to the chemical potential in the system, and this is the overall driving force, producing a gradient over the membrane (Wijmans and Baker, 1995). This is an assumption of the solution-diffusion model: it ensures that there is a continuous gradient over the membrane, Figure 5.3. The solution-diffusion model also relies on the assumption that the rates of adsorption and desorption are higher than the transport through the membrane, allowing the transport characteristics to be described solely by the membrane (Wijmans and Baker, 1995).

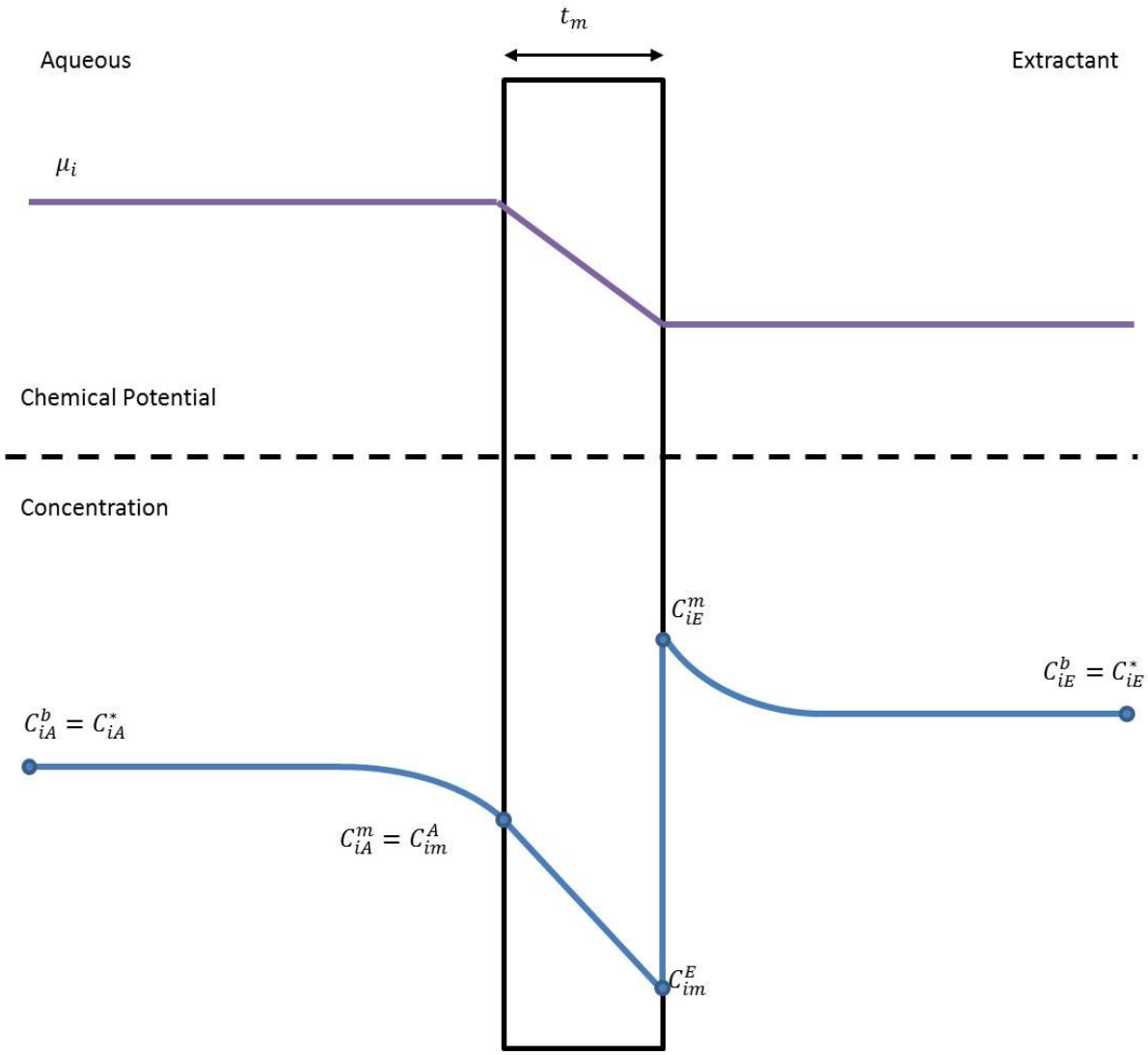


Figure 5.3: Schematic of chemical potential and concentration profiles, at equilibrium, during perstraction with a hydrophobic membrane and an extractant with a K_d>1. *μ_i is the chemical potential of component i, t_m is the membrane thickness, C^b_{iA} is the bulk concentration of i in the aqueous phase, C^*_{iA} is the concentration of i in the aqueous phase in equilibrium with the extractant, C^m_{iA} is the concentration of i in the aqueous phase at the membrane interface, C^A_{im} is the concentration of i in the membrane at the interface with the aqueous phase, C^E_{im} is the concentration of i in the membrane at the extractant interface, C^m_{iE} is the concentration of i in the extractant at the membrane interface, C^b_{iE} is the concentration of i in the bulk extractant, C^*_{iE} is the concentration of i in the extractant in equilibrium with the aqueous phase.*

Based on the review by Wijmans and Baker (1995), the mass transfer across the membrane can be related to Fick's Law using the following derivation. Assuming a constant chemical potential gradient across the membrane, the flux of the solute *i*, J_i, can be described by:

$$J_i = -L_i \frac{d\mu_i}{dx} \quad \textbf{(5.2)}$$

Where:

$\frac{d\mu_i}{dx}$ is the gradient in chemical potential of component *i*

L_i is a coefficient of proportionality

As the concentration is considered the main driving force between two phases, the chemical potential, μ_i, can be related to the concentration via:

$$d\mu_i = RT\, d\ln(\gamma_i c_i) + v_i dp \quad \textbf{(5.3)}$$

Where:

R is the gas constant (J mol^{-1} K^{-1})

T is the temperature (K)

γ_i is the activity coefficient of *i*

c_i is the molar concentration of *i* (mol mol^{-1})

v_i is the molar volume of *i*

P is the pressure

For incompressible phases, such as liquids and solids the volume does not change with pressure. It is also assumed that there is no pressure gradient across the membrane, so the volume/pressure term can be disregarded in equation (5.3) (Wijmans and Baker, 1995). Assuming that a dilute solution is considered, the solution follows Henry's law and the activity coefficient is constant. Therefore equations (5.2) and (5.3) can be combined to give:

$$J_i = -\frac{RTL_i}{c_i}\frac{dc_i}{dx} \quad \textbf{(5.4)}$$

This has the same form as Fick's law, where the diffusion coefficient, D_i is:

$$D_i = \frac{RTL_i}{c_i} \quad \textbf{(5.5)}$$

$$J_i = -D_i\frac{dc_i}{dx} \quad \textbf{(5.6)}$$

Integrating (5.6) over the membrane thickness, l:

$$J_i = \frac{D_i\left(C_{im}^A - C_{im}^E\right)}{l} \quad \textbf{(5.7)}$$

Where:

C_{im}^A is the concentration of *i* in the membrane, at the interface with the aqueous phase

C_{im}^E is the concentration of *i* in the membrane, at the interface with the extractant

Using this relationship the mass transfer rate through the membrane can be described. The difficulty with isolating the flux through the membrane is understanding the concentration profile across the thickness of the membrane. Shukla *et al.* (1989) found that for a hydrophobic hollow fibre membrane, the shell side (aqueous phase) had a considerably greater mass transfer resistance than the extractant side and that of the membrane, therefore the overall mass transfer from the bulk aqueous phase to the bulk extractant phase needs to be considered.

5.3.3: Overall Mass Transfer

Taking the principles of Fick's law, and applying it across the whole perstraction system (bulk aqueous phase, C_{iA}^{b}, to bulk extractant phase, C_{iE}^{b}, Figure 5.3), the flux and mass transfer can be described by (Boontawan and Stuckey, 2005):

$$J_i = K_{ov}[\Delta C_i] \quad \textbf{(5.8)}$$

Where:

J_i is the flux of component *i* across the membrane (g h^{-1} m^{-2})

K_{ov} is the overall mass transfer coefficient (m s^{-1})

ΔC_i is the concentration gradient of component *i* (g L^{-1})

Assuming a system where the solute is transferring from the bulk aqueous phase to the bulk extractant phase, the concentration gradient can be described as the difference between the concentration in the aqueous phase and the concentration of the extractant phase in equilibrium with the aqueous phase (Boontawan and Stuckey, 2005):

$$[\Delta C_i] = C_{iA}^{b} - \frac{C_{iE}^{b}}{K_d} \quad \textbf{(5.9)}$$

$$[\Delta C_i] = C_{iA}^{b} - C_{iA}^{*} \quad \textbf{(5.10)}$$

Substituting this into equation (5.8), the flux across the system can be described in terms of the bulk solution concentrations and the overall mass transfer coefficient:

$$J_i = K_{ov,A}(C_{iA}^{b} - C_{iA}^{*}) \quad \textbf{(5.11)}$$

This has been derived based on the aqueous side concentrations, hence the mass transfer coefficient is based on the aqueous side ($K_{ov,A}$). If the extractant concentrations had been used then the overall mass transfer coefficient would be represented as $K_{ov,E}$.

In this scenario, the flux is the rate of mass transfer per unit area for mass transfer (g $h^{-1}m^{-2}$)

$$J_i = \frac{1}{A}\frac{dN_{iA}^b}{dt} \quad \textbf{(5.12)}$$

$$J_i = \frac{V_A}{A}\frac{dC_{iA}^b}{dt} \quad \textbf{(5.13)}$$

Where:

A is the membrane area for mass transfer (m^2)

Combining this with equation (5.11), the mass transfer is related to the change in concentration with respect to time:

$$V_A\frac{dC_{iA}^b}{dt} = K_{ov,A}A\left(C_{iA}^b - C_{iA}^*\right) \quad \textbf{(5.14)}$$

By measuring the change in concentration over time, overall mass transfer coefficient can be calculated. The method for this is shown in Appendix B.

5.3.4: Overall Mass Transfer Coefficient

Kiani *et al.* (1984) describe the overall mass transfer coefficient in relation to the individual mass transfer coefficients relating to the stages of mass transfer shown in Figure 5.3.

$$J_i = k_w\left(C_{iA}^b - C_{iA}^m\right) \cong k_m\left(C_{im}^A - C_{im}^E\right) \cong k_E(C_{iE}^m - C_{iE}^b) \quad \textbf{(5.15)}$$

$$J_i = K_{ov,A}\left(C_{iA}^b - C_{iA}^*\right) = K_{ov,E}(C_{iE}^* - C_{iE}^b) \quad \textbf{(5.16)}$$

This relationship is typically described as the overall mass transfer coefficient being the sum of the individual resistances found in the system. This consists of the aqueous boundary layer, the extractant boundary layer and membrane resistance (Kiani *et al.*, 1984; Boontawan and Stuckey, 2005).

$$\frac{1}{K_{ov,A}} = \frac{1}{k_A} + \frac{1}{k_m} + \frac{1}{K_d k_E} \quad \textbf{(5.17)}$$

Or

$$\frac{1}{K_{ov,E}} = \frac{K_d}{k_A} + \frac{1}{k_m} + \frac{1}{k_E} \quad \textbf{(5.18)}$$

5.3.4.1 Membrane Mass Transfer Coefficient

As already shown by the solution-diffusion model (5.7) transfer through the membrane is dependent on the diffusivity of the solute in the membrane, D_i. The mass transfer also depends on the type of membrane used.

Boontawan and Stuckey (2005) discuss the mass transfer coefficient using a non-porous silicone membrane in a perstraction system. Under equilibrium conditions, the system can be thought to have three distinct phases; which can be described by three distribution coefficients:

$K_{di,A}^{E}$ The distribution of *i* between the aqueous phase and extractant

$K_{di,A}^{m}$ The distribution of *i* between the aqueous phase and the membrane

$K_{di,E}^{m}$ The distribution of *i* between the extractant and the membrane

The transfer through the membrane can then be thought of as a relationship between the solubility of *i* in the membrane and its ability to diffuse through the membrane (Boontawan and Stuckey, 2005).

$$\frac{1}{k_m} = \frac{1}{K_{di,A}^{m} D_{im}} \quad \textbf{(5.19)}$$

Groot *et al.* (1991) relate the diffusion through a rubbery material, like silicone, to diffusion through liquids. It is therefore proposed that the Wilke-Chang relation can be used to estimate the diffusion coefficient through the membrane.

$$D_{im} = 7.4x10^{-8} \frac{(\phi M_m)^{0.5} T}{\eta_m V_i^{0.6}} \; cm^2 s^{-1} \quad \textbf{(5.20)}$$

Where

D_{im} Mutual diffusivity at infinite dilution of *i* in the membrane ($cm^2\ s^{-1}$)

ϕ association factor ($\phi = 1$ for hydrophobic compounds)

M_m Molar mass of membrane ($g\ mol^{-1}$)

T absolute temperature (K)

η_m dynamic viscosity of membrane (Pa.s)

V_i molar volume at normal boiling point ($m^3\ mol^{-1}$)

For porous membranes, the pores are filled with either the extractant or aqueous phase, with mass transfer occurring at the liquid-liquid phase interface; it is assumed that this is the only place for mass transfer, i.e. no diffusion through the solid portion of the membrane (Prasad and Sirkar, 1992). Kiani *et al.* (1984) and Prasad and Sirkar (1992)relate the transfer through the membrane to the diffusion of *i* between the two liquids and the pore structure of the membrane.

$$\frac{1}{k_m} = \frac{\tau_m t_m}{D_{iE} \epsilon_m} \quad \textbf{(5.21)}$$

Where

τ_m pore tortuosity (actual pore length divided my membrane thickness)

ϵ_m membrane porosity

Equation (5.21) relies on the assumptions that diffusion of the solute is unhindered, the membrane is symmetric and wetted completely by the desired phase and no two-dimensional effects occur (Prasad and Sirkar, 1992).

5.3.4.2 Boundary Layer Mass Transfer Coefficients

The boundary layer mass transfer coefficients, k_A and k_E, describe the interactions between the liquid phases and the membrane interface. The boundary layer mass transfer coefficients will be affected by the membrane geometry, e.g. whether it is a flat sheet or tubular. Though, in general, the boundary layer mass transfer coefficients can be described by the Sherwood correlation, describing the convective mass transfer film coefficient by relating it to the Reynolds number and Schmidt number of the system (Prasad and Sirkar, 1988; Viegas *et al.*, 1998).

$$Sh = \frac{kL}{D} = f(Re, Sc) \quad \textbf{(5.22)}$$

$$Re = \frac{\rho \boldsymbol{v} L}{\eta} \quad \textbf{(5.23)}$$

$$Sc = \frac{\eta}{\rho D} \quad \textbf{(5.24)}$$

Where:

Sh Sherwood Number

k convective mass transfer film coefficient (m s^{-1})

D mass diffusivity (m^2 s^{-1})

L Characteristic length (m)

Re Reynolds number

η Dynamic viscosity (Pa.s)

ρ density (kg m^{-3})

$\boldsymbol{v}$ mean velocity of the fluid (m s^{-1})

Sc Schmidt number

This relates the mass transfer to the fluid motion through the system and the diffusivity, considering both the momentum (viscosity) and mass diffusivities. This shows that mass transfer in the system is dependent on the fluid properties of both the aqueous and extractant phase.

5.4: Perstraction and the ABE Fermentation

The application of perstraction to the ABE fermentation has not been extensively investigated compared to the other ISPR techniques. Of the research performed 80% of the work was carried out between 1987-1994, with two further studies nearly 10 years apart in 2005 and 2012 (Jeon and Lee, 1987; 1989; Groot *et al.*, 1990; Grobben *et al.*, 1993; Qureshi and Maddox, 2005; Tanaka *et al.*, 2012). An additional further study was carried out in 2014 by Núñez-Gómez *et al.* (2014) but this did not focus on the fermentation and only used a model solution to investigate process parameters.

The impact of perstraction on the ABE fermentation was discussed in Chapter 3, reviewing perstraction alongside other ISPR techniques that have been applied to the ABE fermentations. Table 3.6 provides comprehensive information regarding the impact perstraction has on the fermentation. This data showed that perstraction increased the

productivity and yield of the fermentation, resulting in increased substrate consumption in fed batch and continuous fermentations. This positive impact means that product inhibition can be relieved, increasing the fermentation duration, the substrate concentration and final product titre. The work to date has primarily focused on the use of non-porous silicone membranes or porous hollow-fibre polypropylene membranes, primarily with oleyl alcohol as an extractant, Table 5.1. Other membrane extractant combinations have been tested using a synthetic ABE solution, Table 5.2, but not carried forward for integrating with the ABE fermentation.

Table 5.1: Membrane - extractant combinations integrated with ABE fermentation in literature.

Reference	Membrane (and configuration)	Extractant
Jeon and Lee (1987)	Silicone (tubing)	Oleyl alcohol
		Polypropylene glycol
		Tributyrin
Jeon and Lee (1989)	Silicone (tubing)	Oleyl alcohol
		Water
Shukla *et al.* (1989)	Polypropylene (hollow-fibre)	2-Ethyl-1-hexanol
Groot *et al.* (1990)	Silicone (tubing)	Isopropyl myristate
		Ethylene glycol
Qureshi *et al.* (1992)	Silicone (tubing)	Oleyl alcohol
Grobben *et al.* (1993)	Polypropylene (hollow-fibre)	Oleyl alcohol/Decane (50% v/v)
	Polypropylene (hollow-fibre)	Fatty acid methyl esters
Shah and Lee (1994)	Silicone (tubing)	Oleyl alcohol
Qureshi and Maddox (2005)	Silicone (tubing)	Oleyl alcohol
Tanaka *et al.* (2012)	Polytetrafluoroethylene (PTFE) (sheet)	Oleyl alcohol
		Dodecanol

Table 5.2: Membrane - extractant combinations used with a synthetic ABE solution only in literature.

Reference	Membrane (and configuration)	Extractant
Groot *et al.* (1990)	Neoprene (tubing)	Hexane
		Hexanol
		Isopropyl myristate
	Latex (tubing)	Hexane
		Hexanol
		Isopropyl myristate
	Silicone (tubing)	Hexane
		Hexanol
		Octanol
		Decanol
		Oleyl alcohol
		Dibutyl phtalate
		Diethelene glycol
		Triethylene glycol
		Tetraethylene glycol
		Glycerol
		1,2 Butanediol
		1,3 Butanediol
Núñez-Gómez *et al.* (2014)	PTFE (sheet)	Toluene

Various reports discuss the impact perstraction has on the fermentation, rather than the successes (or failure) of the separation process. Uncoupling the fermentation and perstraction performance is difficult due to fermentation concentration being a controlling factor in the mass transfer of the separation. Tanaka *et al.* (2012) attempted to do this by calculating the butanol productivity per unit membrane area (g L^{-1} h^{-1} m^{-2}). The butanol productivity is a function of the bacteria, and a measure of the average "healthiness" of the cells. By considering the productivity per unit membrane area, the productivity has effectively been standardised around one perstraction parameter. This allows some comparison across fermentations, as performed by Tanaka *et al.* (2012), but it is still connected to the fermentation performance. A potentially better comparison is to consider the rate of butanol transfer across the membrane per unit area, or butanol flux (5.13). This is still dependant on the concentration of butanol present in the system, which is dependent on the bacteria, but in most cases the concentration was maintained below inhibitory levels, so the variation is minimal. Only Grobben *et al.* (1993) state the butanol flux, but for other fermentations it can be calculated from the experimental data provided, Table 5.3. The extraction efficiency was also stated or calculated where possible. The extraction efficiency is defined as the percentage of ABE (or butanol) recovered in the extractant compared to the total ABE (or butanol) produced in the fermentation, (5.25).

$$Extraction\ Efficiency = \frac{ABE\ in\ Extractant}{Total\ ABE\ Produced\ by\ Fermentation}\% \qquad \textbf{(5.25)}$$

Table 5.3: Comparison of integrated perstraction fermentations based on literature data.

Reference	Membrane	Extractant	Butanol Flux (g $h^{-1} m^{-2}$)	ABE Extraction Efficiency
Jeon and Lee (1987)	Silicone	Oleyl alcohol	3.83	93.3%
	Silicone	Polypropylene glycol	3.02	95.2%
	Silicone	Tributyrin	2.40	88.1%
Jeon and Lee (1989)	Silicone	Oleyl alcohol	1.35	77.5%
	Silicone	Water	1.79	83.6%
Qureshi *et al.* (1992)	Silicone	Oleyl alcohol	1.21	51.4%
Grobben *et al.* (1993)	Polypropylene	Oleyl alcohol/Decane (50% v/v)	8.8[a]	-
	Polypropylene	Fatty acid methyl esters	0.46[a]	-
Qureshi and Maddox (2005)	Silicone	Oleyl alcohol	0.96	45.0% (84.5%[b])
Tanaka *et al.* (2012)	PTFE	Oleyl alcohol	37.85	48%[c]
	PTFE	Dodecanol	51.42	52%[c]

[a] Values given by Grobben *et al.* (1993), with average for first 25h for oleyl alcohol/decane mixture and first 10h for fatty acid methyl esters extractant. All other flux values were calculated from literature data

[b] Extraction efficiency if no loss of ABE to environment, assuming all ABE remained in the system

[c] Butanol extraction efficiency, no values for total ABE produced/ extracted stated in literature.

As observed in Table 5.3, a wide range of fluxes were achieved, with the highest achieved by Tanaka *et al.* (2012). These fluxes are in the equivalent range to the butanol productivity per

membrane area they reported (78.6 g L^{-1} h^{-1} m^{-2} for 1-dodecanol and 63.7g L^{-1} h^{-1} m^{-2} for oleyl alcohol), and compared to the work by Jeon and Lee (1987) (3.07 g L^{-1} h^{-1} m^{-2} for oleyl alcohol with a silicone membrane) the order of magnitude difference is still maintained (Tanaka *et al.*, 2012). Tanaka *et al.* (2012) used a porous membrane, whereas the silicone membranes used were non-porous. Other than Grobben *et al.* (1993), all fluxes were calculated with fermentation endpoint data. This is representative of the flux over the course of the entire perstraction duration. Grobben *et al.* (1993) chose to report the flux for the initial start-up stages of perstraction (first 25h for the oleyl alcohol/decane mixed extractant and first 10h for the fatty acid methyl acid extractant). Generally, the butanol concentration in the fermentation broth is typically higher than that observed during steady-state ISPR phase of the fermentation. Comparing the butanol flux to the fermentation productivity, Figure 5.4, there is no obvious correlation indicating that the butanol flux is independent from the productivity. The butanol flux was also compared to the distribution coefficient for the extractant used, Figure 5.5. While there is significant variability in the data, there is a general trend towards the greater the butanol distribution coefficient, the greater the butanol flux.

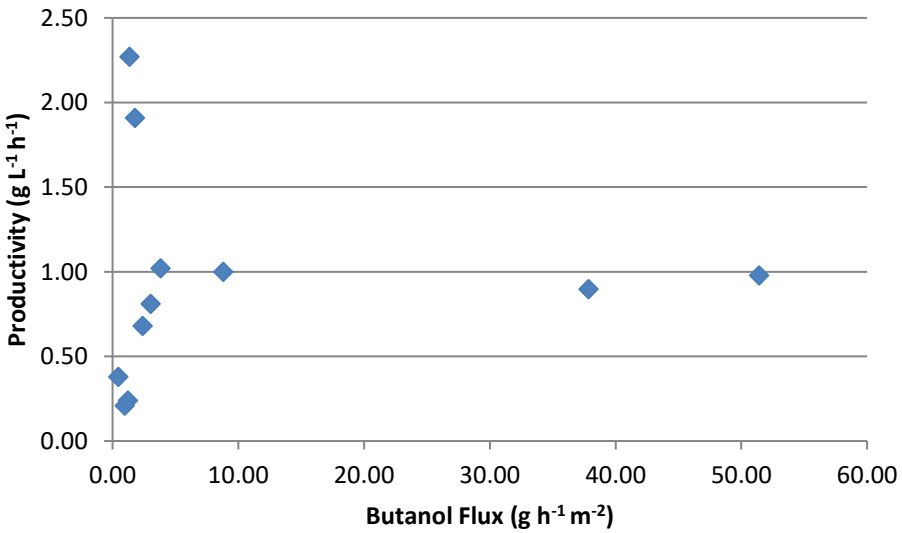


Figure 5.4: Comparison of butanol flux and fermentation productivity from literature data

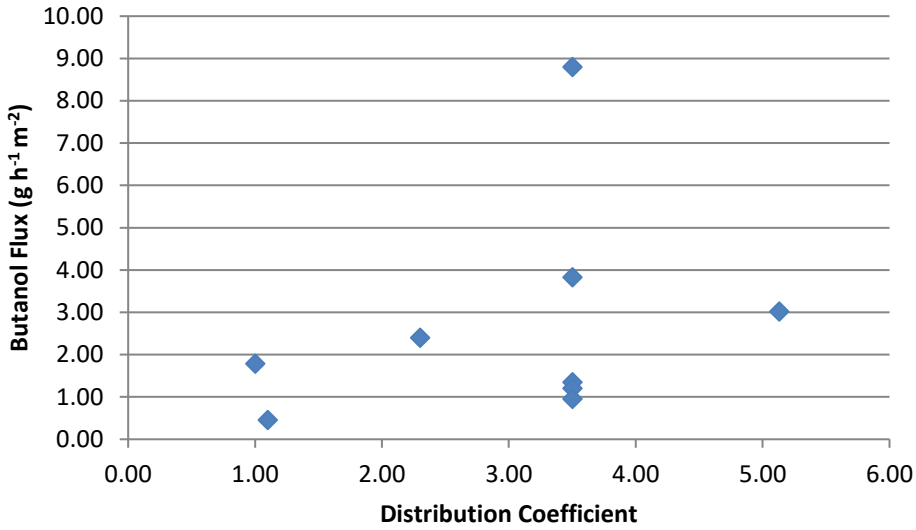


Figure 5.5: Comparison of extractant distribution coefficient with butanol flux from literature data

The variability in the data is likely to be related to different experimental conditions and membrane materials, considering the fluxes for oleyl alcohol as an extractant with a silicone membrane as an example. Jeon and Lee (1987) focused on applying perstraction to the ABE process and removing ABE toxicity from the fermentation, reducing the butanol concentration in the fermentation broth to approximately 4 g L^{-1}. Later work by Jeon and Lee (1989) saw the optimisation of the perstraction process to maintain a relatively stable butanol concentration in the fermentation broth, approximately 6 g L^{-1}, which would lead to a reduction in membrane flux as the same membrane area was used. The research by Qureshi *et al.* (1992) and Qureshi and Maddox (2005) used a complex media consisting of whey permeate and yeast extract, rather than the defined media used by Jeon and Lee (1987), which could increase membrane fouling from unknown media components such as proteins reducing the butanol flux across the membrane.

Table 5.3 also shows that there is a wide range of separation efficiencies: 45-95%. The lowest separation efficiency was measured by Qureshi and Maddox (2005). Although this was not a problem for the fermentation, as the butanol concentration in the broth was maintained below inhibitory limits, consistently achieving a separation efficiency of 95% would enable a reduction in membrane size and/or extractant inventory.

While perstraction can enhance the ABE fermentation, it exhibits high mass transfer resistances due to the membrane. These high resistances mean that there is a low product flux across the membrane. Most researchers acknowledge the high resistances to mass transfer, but there is little understanding of what contributes to the resistances and how to

reduce them. The mass transfer coefficient will affect the extraction efficiencies, as mass transfer is the rate controlling step of the process (Qureshi and Maddox, 2005; Tanaka *et al.*, 2012). Of the three mass transfer resistances, see equation (5.17), Shukla *et al.* (1989) suggest that the aqueous phase had the greatest resistance to mass transfer. As the aqueous phase mass transfer resistance (s cm^{-1}) was 4-6 time greater than the extractant and membrane resistances, which were approximately equal (Shukla *et al.*, 1989). While the high mass transfer resistance can be overcome by increasing the membrane area, this increases the cost of the ISPR technique. Surprisingly, little work has been done to investigate this side of perstraction for the ABE fermentation and to use this knowledge to influence the membrane and extractant choices made. The only demonstration of this is by Groot *et al.* (1990), who tested many extractants with a synthetic ABE solution prior to focusing on one membrane-extractant combination for both an apolar and a polar extractant, Tables 5.1 and 5.2.

One of the reasons for using perstraction was the potential ability to overcome the toxic effects of extractants on the microorganism in LLE. As the distribution coefficient impacts the butanol flux across the membrane, it will be beneficial to use an extractant with as large a distribution coefficient as possible. The extractants with higher distribution coefficients than oleyl alcohol tend to be toxic to the ABE fermentation, therefore overcoming this could improve the effectiveness of perstraction. There have been two published uses of a toxic extractant, Shukla *et al.* (1989) and Tanaka *et al.* (2012), but generally the use of a toxic extractant has been neglected, due to concerns of the extractant leaching into the fermentation broth. Without further investigation it is unknown how far the use of toxic extractants can be pushed for use as an extractant in perstraction. Tanaka *et al.* (2012) performed the most recent research using 1-dodecanol but there was no justification for the use of this extractant and, to date, no follow-up work has been published demonstrating the long term viability of using a toxic extractant with perstraction. Typically the application of ISPR allows for extended fermentation runs through a fed-batch fermentation, whilst it has been shown that perstractive fermentations can be run for over 400 hours (Qureshi and Maddox, 2005), the use of toxic solvents might restrict this. It might also be possible to use an extractant with a higher partition coefficient than 1-dodecanol, which could reduce the extractant inventory on site. The use of an extractant with a higher extractant coefficient could mean the use of a chemical that is more toxic to the bacteria than 1-dodecanol, therefore unfavourable effects might still be seen.

5.5: Progressing Perstraction for use in the ABE Fermentation

Developments are needed to improve the extraction rate across the membrane, so that the extraction rate is higher than the production rate without the need for a disproportionally large membrane compared to the fermentor volume. Clarification is also required on the effect of extractants that are toxic to the fermentation. If it is possible to use a toxic extractant this would, hopefully, allow for a reduced extractant inventory and an increased extraction rate. An increased extraction rate would allow for a smaller membrane area. This would positively impact the efficiency of extraction and the operating costs of the plant. This could lead to perstraction becoming a more desirable ISPR technique than LLE.

In this thesis both the extractant and membrane choice have been experimentally investigated, with the aim of maximising the mass transfer rate of perstraction for application to a commercial ABE process. The use of toxic extractants was one of the proposed advantages of perstraction over LLE. Therefore, it was decided to focus on the use of toxic extractants, as these have a higher distribution coefficient than non-toxic alternatives such as oleyl alcohol. As Figure 5.5 showed, there is a general trend towards a greater butanol distribution coefficient leading to a greater butanol flux, so the toxic extractants should further improve the rate of mass transfer. As discussed above, one of the concerns of using toxic extractants was leaching across the membrane. Therefore, the extractant concentration in the aqueous phase was measured to provide a greater understanding of the transfer of extractant across the membrane. With regards to membrane choice, as shown in Table 5.2, only Groot *et al.* (1990) compared membrane material, but these were all non-porous tubular membranes. Therefore, a comparison of membrane materials was also performed, considering factors such as porosity and hydrophobicity which had not previously been compared.

5.6: Materials and Method

5.6.1: Extractants

Seven extractants were used: 1-pentanol (Acros Organics), 1-hexanol (Acros Organics), 1-heptanol (Acros Organics), 1-octanol (Acros Organics), 2-ethyl-1-hexanol Acros Organics), oleyl alcohol (Acros Organics) and RO (reverse osmosis) water (generated by Sartorius Arium Advanced System).

5.6.2: Membranes

Six membranes were investigated, three tubular membranes and three flat-sheet membranes. The tubular membranes were: silicone (Fisher, with a 0.75mm wall thickness, 3mm internal diameter, 500mm length), non-porous PTFE (Cole Parmer, with a 0.76mm wall thickness, 2.48mm internal diameter, 500mm length), polyethersulfone (PES) (FUD182, Daicen, Japan, 150,000 molecular weight cut-off, approx. 0.01µm pore size. 1.28mm outside diameter, 1.03mm internal diameter, 500mm length). The flat-sheet membranes were: regenerated cellulose (RC55, GE, pore size = 0.45µm, diameter = 50mm), polyamide/nylon (NL17, GE, pore size = 0.45µm, diameter = 50mm) and PTFE (TE36, GE, pore size = 0.45µm, diameter = 47mm).

5.6.3: Microorganism and Medium

The strain *C. saccharoperbutylacetonicum* N1-4 was used in this study. The cultures were stored in lyophilised form at 4°C. The culture was reconstituted in Reinforced Clostridial Medium (RCM) (Oxoid). This was done by mixing 1mL media, taken from a 60mL RCM serum bottle, to suspend the lyophilised culture. Less than 0.1mL (approximately one drop) of the re-suspended culture was added to the 60mL RCM serum bottle and placed in an incubator and grown for 19-20 hours at 32°C. After incubation, the optical density at 600nm (OD_{600nm}), pH and microscopy of the culture was checked, and deemed ready for transfer for fermentation if OD_{600nm} was 2-2.5, pH 5-5.5 and the cells looked healthy and motile. This formed the seed culture

All media components were purchased from Fisher or Sigma at standard laboratory grade. The base media used was prepared by dissolving the following components in of RO water for the desired final concentration: 2.5 g L^{-1} yeast extract, 2.5 g L^{-1} tryptone, 0.025 g L^{-1} $FeSO_4$, 0.5 g L^{-1} $(NH_4)_2SO_4$ and 19.52 g L^{-1} MES (2-(N-morpholino)ethanesulfonic acid) buffer. The medium was adjusted to pH 6.5. A glucose solution was prepared, for a final concentration of 50 g L^{-1}. Both the base media and glucose solution were autoclaved at 121°C for 22 minutes, and then mixed together. Once dispensed the medium was left to equilibrate in the anaerobic cabinet overnight (Whitley A35 anaerobic workstation).

5.6.4: Fermentation – Solvent Toxicity Test

The fermentation occurred in 100mL serum bottles, with 60 mL working volume. 6mL of seed culture was transferred into each serum bottle, and grown under anaerobic conditions (in the anaerobic cabinet) at 32°C for 14-16 hours until the butanol concentration was

approximately 5 g L^{-1}. This corresponds to glucose consumption of 16-20 g L^{-1}, which could be determined through testing a sample with glucose meter. Once the glucose concentration had reached 30-35 g L^{-1}, extractant was then added to the serum bottle to test for solvent toxicity. Five toxic extractants were tested (1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, and 2-ethyl-1-hexanol) at three concentrations 0.5 g L^{-1}, 1 g L^{-1} and 5 g L^{-1}. Samples were collected every 2 hours after extractant addition for measuring ABE concentration, glucose concentration, cell density and microscopy. The cell density was measured using a spectrophotometer at 600nm (Amersham Biosciences Ultrospec 10).

All solvent toxicity experiments were performed in duplicate.

5.6.5: Synthetic ABE Solution

A synthetic acetone (Fisher), butanol (Acros Organics) and ethanol (Fisher) solution was made up with RO water. The final ABE concentration was 16 g ABE/L, at ABE ratio of 22.5%:75%:2.5%.

5.6.6: Perstraction

Perstractive recovery of ABE was tested using two experimental setups, depending on the type of membrane used, 400 mL synthetic ABE solution was used with 200 mL of extractant. The ABE solution has a greater volume to allow an equilibrium to become established more quickly.

For tubular membranes (silicone, non-porous PTFE and PES): The 400 mL synthetic ABE solution was placed in a 500mL bottle, with a three port cap with two tubes for recirculation and a filter to ensure pressure stabilisation. 200mL was placed in a 250 mL bottle, with a three port cap connecting the tubular membrane to the tubing exiting the synthetic ABE solution bottle and a filter on the third port for pressure stabilisation. The tubing was immersed in the extractant with the ABE solution pumped through tubing using a multi-headed peristaltic pump (Watson Marlow 505U) at 150 mL min^{-1} for the silicone and non-porous PTFE membrane, Figure 5.6. A flow rate of 30 mL min^{-1} was used for the PES membrane because of the smaller internal diameter. To ensure good mixing the extractant bottle was agitated at approximately 350 rpm using a magnetic stirrer. The each experiment would last for 150-200 hours and samples were taken periodically throughout the

experiment, for both the synthetic ABE solution and extractant, for analysis of the ABE and extractant concentrations.

Experiments with silicone tubing were performed in triplicate, PTFE in duplicate (due to conclusive results) and in duplicate with the PES membrane.

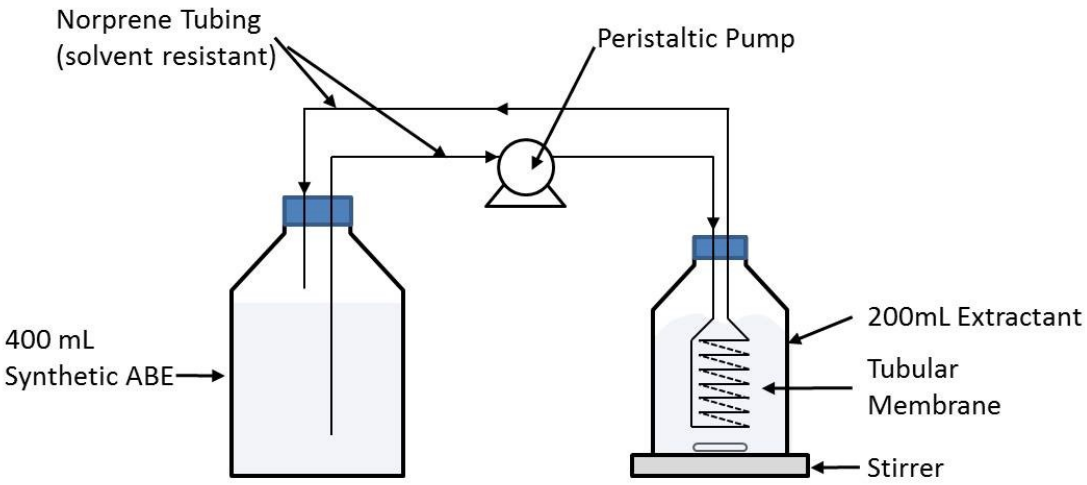


Figure 5.6: Tubular perstraction set up.

For the flat-sheet membranes (RC55, NL17 and PT36), modified Duran bottles were used to contact the two phases, as shown in Figure 5.7. The membrane was sandwiched between two silicone washers then clamped between two glass joints with 40 mm internal diameter. This internal area was the area for mass transfer. 200 mL extractant was placed in the 250 mL bottle and 400 mL synthetic ABE solution was placed in the 500 mL bottle. Both bottles were closed with solid lids (no ports) as shown in Figure 5.7. The bottles were placed in agitated incubator at 32°C and agitated at 200 rpm. The experiment lasted for 150-200 hours. With samples were taken periodically, from both bottles, throughout the experiment, for analysis of ABE and extractant concentration. A technical drawing of the setup is provided in Appendix C.

Due to the limited number of membranes available these experiments were performed in duplicate.

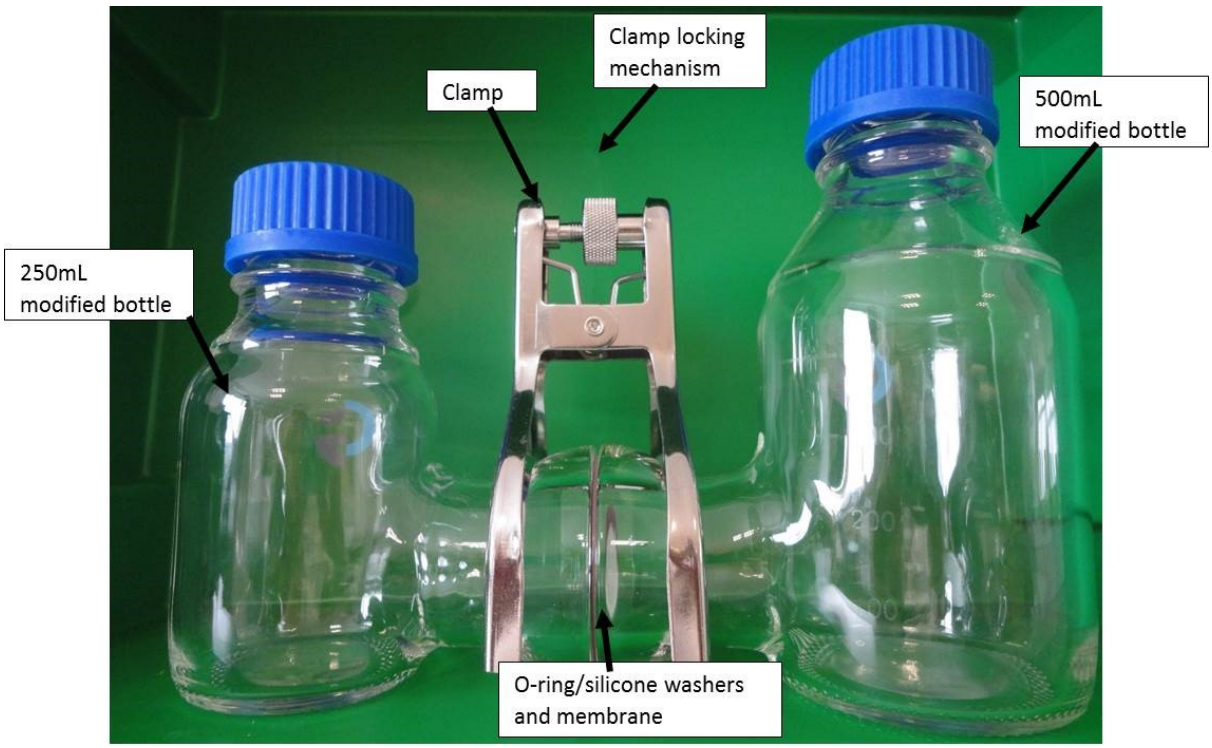


Figure 5.7: Flat-sheet membrane perstraction set up.

The overall mass transfer coefficient was calculated, (5.14), for every tested membrane-extractant combination. It was assumed that both systems were well mixed, therefore a comparison across the two systems can be made.

5.6.7: Chromatographic Analysis

5.6.7.1 ABE and Extractant Concentration

In the aqueous phase samples, concentrations of ABE and extractant were measured by liquid injection gas chromatography (Thermo Scientific Trace 1300). In the extractant phase only ABE concentrations were measured, by headspace gas chromatography (Thermo Scientific Triplus 300 headspace). Both used a split-splitless injector, with a 50:1 ratio, and flame ionisation detector. A HP-FFAP column (50 m, diameter 0.32 mm and film thickness 0.5 µL, Agilent J & W) was used for separation. The carrier gas was nitrogen at a flow rate of 20mL min^{-1}. The oven temperature increased from 80°C to 200°C at a rate of 10°C min^{-1} followed by a 3 minute hold. The injector temperature was 250°C and the refractive index detector temperature was 300°C.

5.6.7.2 Oleyl alcohol concentration

Aqueous phase samples, which potentially contained oleyl alcohol, were prepared for liquid injection gas chromatography by extraction into 1-octanol at a ratio of 800 µL of sample: 1000 µL 1-octanol. The samples were vortexed for 2 minutes, before settling and centrifugation (Sigma 1-14) at 13000 rpm for 5 minutes. 800µL of the organic phase was aliquoted from the sample, ready for analysis. 1-octanol/oleyl alcohol samples were prepared in octanol with 5g L^{-1} palmitoleyl alcohol for internal standard.

Oleyl alcohol was measured using liquid injection gas chromatography (Thermo Scientific Trace 1300), with a HP-FFAP column, split-splitless injector and flame ionisation detector. The carrier gas was nitrogen at a flow rate of 20 mL min^{-1}. The oven temperature started at 175°C and increased to 240°C at a rate of 125°C min^{-1}, with a final hold of 6.5 minutes. The injector temperature was 250°C and the detector temperature was 300°C.

5.6.7.3 Glucose concentration

The glucose concentration was measured, for extraction toxicity samples, using high performance liquid chromatography (Thermo Scientific Dionex Ultimate 3000) with refractive index detector (ERC Refractomax 520). A Rezex RPM column (7.8 x 300 mm, Phenomenex) at 85°C, using HPLC grade water mobile phase at a flow rate of 0.6 mL min^{-1}.

5.7: Results and Discussion

5.7.1: Extractant Choice

In the majority of work to date oleyl alcohol has been the extractant used for perstraction, Table 5.1. This stems from LLE research, where oleyl alcohol was the chosen extractant, as it was non-toxic and had a high distribution coefficient ($K_d \approx 3.5 - 4.7$) (Ishii *et al.*, 1985; Roffler *et al.*, 1987b; Kim *et al.*, 1999).

A wide selection of solvents were tested for use as extractant for acetone, butanol and ethanol. Kim *et al.* (1999) provides the most in-depth assessment of partition coefficients. Dadgar and Foutch (1985) and Ishii *et al.* (1985) have completed the most detailed studies into solvent toxicity for LLE with the ABE fermentation. From the published data in these three studies, there were 21 extractants which has a partition coefficient equal or higher than that of oleyl alcohol for butanol. Of these extractants only three (2-butyl-1-octanol, 2-hexyl-1-decanol and polypropylene glycol) have been confirmed to be non-toxic to the ABE fermentation. This indicates that there is a strong relationship between having a high affinity

for butanol and toxicity to *Clostridium spp*. These 21 solvents formed the starting point for selecting extractants for testing with perstraction, a complete list is provided in Table 5.4. From the 21 solvents, two were ionic liquids (Aliquat 336 and phosphonium); these have been ruled out of selection due to lack on information and accessibility of the liquid. This leaves 19 organic solvents for selection.

Table 5.4: Initial 21 extractants with physical properties and reasons for not selecting.

	Toxicity	Butanol Distribution Coefficient	Molecular Weight	Boiling Point (°C)	Freezing Point (°C)	Density (gcm^{-3})	Viscosity (mPa.s) (at 25C)	Solubility in H2O	Reason for Not Selecting
4-Methyl-2-pentanone	unknown	4.02	100.16	117	-84.7	0.802		1.91 g/100mL	Similar BPT to water
Oleyl alcohol	NT	4.3	268.5	330-360	13-19	0.849		Insoluble	
n-Propyl acetate	T	4.34	102.1	102	-95	0.89		1.89%	Similar BPT to water
3-Pentanone	unknown	4.5	86.1	101.5	-39	0.815		50 g L^{-1}	Similar BPT to water
2-Hexyl-1-decanol (C16 Guerbert Alcohol)	NT	4.5	242.4	195	-18	0.836	41 (at 20 C)	insoluble	High viscosity compared to butanol
Ethyl acetate	unknown	4.62	88.11	77.1	-83.6	0.897		8.3g/100mL	Similar distribution to oleyl alcohol
Oxocol (C14-15)	T	4.7							Similar distribution to oleyl alcohol
Polypropylene glycol	NT	5.13[a]	1000						Non-toxic
1-Dodecanol	T	5.14	186.3	259	24	0.8309	17.2 (at 20°C)	0.004 g L^{-1}	High viscosity compared to butanol
Undecanol	T	5.55	172.3	243	19	0.8298	17.2	Insoluble	High viscosity compared to butanol
1-Octanol	T	5.6-7.3	130.2	195	-16	0.824	7.3	0.460 g L^{-1}	
2-Ethyl-1-hexanol	T	6.09	130.2	180	-76	0.833	6.3	slightly (0.2%)	
1-Decanol	T	6.2	158.3	232.9	6.4	0.8297	10.9	Insoluble	High viscosity compared to butanol

	Toxicity	Butanol Distribution Coefficient	Molecular Weight	Boiling Point (°C)	Freezing Point (°C)	Density (gcm^{-3})	Viscosity (mPa.s) (at 25C)	Solubility in H2O	Reason for Not Selecting
1-Heptanol	T	6.62	116.2	175.8	-34.6	0.8187	5.8	slightly (0.2%)	
2-Butyl-1-octanol	NT	6.76[b]	186.3	147		0.833		insoluble	Non-toxic
Tributyl phosphate	T	7.47[b]	266.3	289	-80	0.9727		1mL/165mL water	Similar Density to Water
1-Pentanol	T	7.48	88.2	137	-78	0.811	3.6	22 g L^{-1}	
Aliquat 336 (ionic liquid)	unknown	8.86[a]	404.16						Ionic Liquid - Lack of information
1-Hexanol	T	9.91	102.1	157.1	-44.6	0.8136	4.6	5.9 g L^{-1}	
Phosphonium (ionic liquid)	unknown	11.55[a]							Ionic Liquid - Lack of information
Phenol	T	24	94.1	181.7	40.5	1.07		8.3 g $100mL^{-1}$	Safety

Toxicity notation: T- Toxic, NT- Non-Toxic

All butanol distribution coefficients from Kim *et al.* (1999), unless specified: [a] González-Peñas *et al.* (2014b) [b] Jeon and Lee (1987)

The list of 19 extractants was further narrowed down based on safety to humans. This saw the removal of phenol, which has the highest partition coefficient of 24 (Kim *et al.*, 1999). If perstraction was to be used in an industrial process, there will be large volumes of extractant present on the plant, therefore any extractant has to be inherently safe, so as not to harm any plant personnel and maintain integrity of the product. Phenol would also incur laboratory safety concerns when experimentally testing the extractant for use with the fermentation.

The list of extractants was considered with respect to the extractants physical properties, such as boiling point, density and viscosity. This allowed for comparison with the physical properties of acetone, butanol and ethanol.

Downstream separation was a contributing factor to the industrial ABE process being discontinued, therefore recovery of the product from the extractant must be considered with regards to complexity of the process, energy demand and cost. In the traditional process, large amounts of energy are required for the downstream separation due to the high water concentration in the fermentation broth, which forms a low boiling azeotrope at 93°C, impacting the operating costs of the process. By comparing the physical properties of the extractant and products, considerations about the ease of separation of the product from the extractant could be accounted for. Ideally, any solvent used for extraction would have a boiling point higher than that of butanol, assuming separation of ABE and the extractant would be by distillation. Next, density was considered. All extractants on the list have densities of around 0.81 g cm^{-3}, similar to that of butanol, hence gravity separation or similar, was not a viable proposition. Lastly, viscosity was considered; as expected, as the carbon chain of the organic solvents increased the viscosity increased. Groot *et al.* (1990) found that maldistribution of the extractant occurred due to the high viscosity of ethylene glycol being used. It was decided that for an initial investigation, solvents with a viscosity of the same order of magnitude as butanol would be considered. As discussed in section 5.3.3, the boundary layer mass transfer coefficients can be described as functions of the Reynolds and Schmidt number (5.22), both of which are functions of viscosity (5.23 and 5.24). It is expected that the viscosity of the extractant will affect the mass transfer of ABE into the product phase, therefore a low viscosity would be preferable for a high mass transfer rate.

From the initial 21 solvents, 5 toxic extractants along with oleyl alcohol and water were selected for testing with the ABE fermentation. The selected extractants and their basic

physical properties and partition coefficients are shown in Table 5.5, below. All the extractants are believed to be toxic to Clostridia.

Table 5.5: Selected extracts for perstraction testing, with distribution coefficient and physical properties.

Extract-ant	Distribution Coefficient (Kim *et al.*, 1999)			Molecular Weight	Boiling Point	Density	Viscosity	Solubility in water
	Acetone	Butanol	Ethanol		(°C)	g cm^{-3}	mPa.s (at 25°C)	g L^{-1}
1-Pentanol	0.88	7.48	0.078	88.2	137	0.811	3.619	22.0
1-Hexanol	Unknown	9.91	1.0-1.2	102.1	157.1	0.8136	4.578	5.9
1-Heptanol	0.65	6.62	0.75	116.2	175.8	0.8187	5.810	1.0
1-Octanol	0.52	5.6-7.33	0.50-0.64	130.2	195	0.824	6.271	<1.0
2-Ethyl-1-Hexanol	0.58	6.09	0.47	130.2	180	0.833	7.288	<1.0
Oleyl Alcohol	0.52	4.3	0.22	268.5	~330	0.850	28.32[a]	Insoluble
Water	-	-	-	18.0	100	1	0.89	n/a

[a] (Blahušiak *et al.*, 2013)

The extractants have been selected based upon the butanol partition coefficient. Whilst there are two other products in the fermentation broth, it is butanol that causes inhibition to the microorganism at the lowest concentration. It must be noted that generally the partition coefficient for acetone and ethanol is less than one for these organic solvents, therefore the acetone and ethanol will disproportionately partition into the aqueous phase rather than the organic extractant. This will limit the amount of acetone and ethanol transferred into the extractant. The toxicity of acetone and ethanol to the Clostridia is unknown, as the product concentrations do not reach inhibitory levels, therefore the effects of the build-up of acetone and ethanol in the fermentation broth is unknown. Although, the distribution coefficient of oleyl alcohol for acetone is 0.52 (Kim *et al.*, 1999), this is similar to the other distribution coefficients for acetone in Table 5.5, and there have been no suggestions of acetone toxicity in LLE fermentations. Oleyl alcohol and RO water were also investigated to provide a comparison to literature data.

5.7.2: Extractant Toxicity

A membrane is used in perstraction as a physical barrier between the two phases, though there may be a direct interface between the phases when using porous membranes. This has

the potential for back transfer of extractant into the aqueous phase. If this does occur, the concentration of extractant in the aqueous phase is expected to be around the solubility limit of the extractant in water, therefore small extractant concentrations, up to 0.05% (w/v), were selected, unlike the toxicity experiments used for LLE where extractant concentrations between 9-85% (w/v) were used (Roffler *et al.*, 1987b; Evans and Wang, 1988b). To compare fermentation performance the yield and productivity of the fermentation were compared. The yields and productivities were normalised against the control fermentation yields and productivities to negate any differences that might be observed in different starting cultures or environmental effects.

The overall yield and productivity are shown in Figures 5.8 and 5.9, respectively. From these graphs it is obvious that both 1-octanol and 2-ethyl-1-hexanol are toxic to the bacteria at all concentrations tested, with a lower yield and productivity than the control fermentation. All three concentrations had the same impact on the fermentation, as the productivity is roughly equal across all three concentrations. 1-Heptanol also exhibited a decrease in yield and productivity at all three concentrations, but not to the same extent as 1-octanol and 2-ethyl-1-hexanol for 0.5 and 1 g extractant L^{-1} concentrations. For 1-heptanol, as the concentration of extractant increased, the yield and productivity both decreased. For 1-hexanol and 1-pentanol a negative impact was only observed at a concentration of 5 g extractant L^{-1}. At concentrations of 1 g extractant L^{-1} or 0.5 g extractant L^{-1}, the productivity was shown to have an increase when compared to the control fermentation, Figure 5.9. This indicates that the presence of 1-hexanol or 1-pentanol at low concentrations has the potential to simulate solvent production.

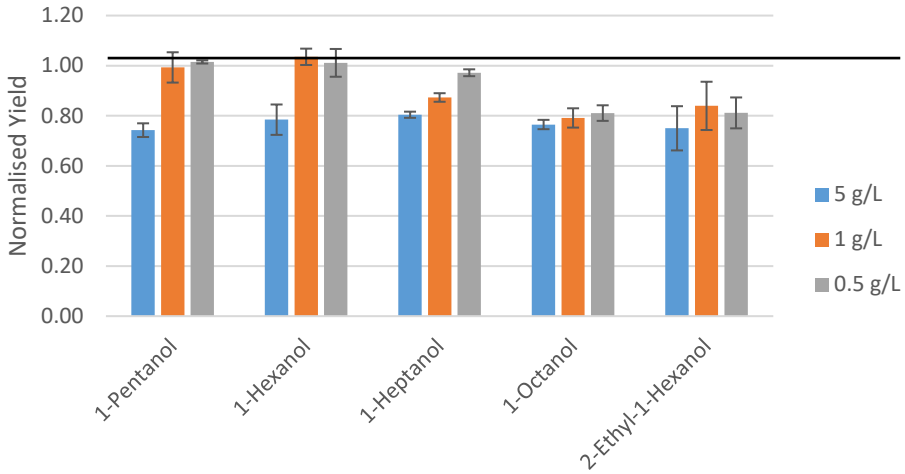


Figure 5.8: Comparison of the normalised overall ABE yield, in 100 mL bottle screen, when five toxic extractants were added at concentrations 5, 1 and 0.5 g L^{-1}, once the butanol concentration had reached 5 g L^{-1}. Performed in duplicate.

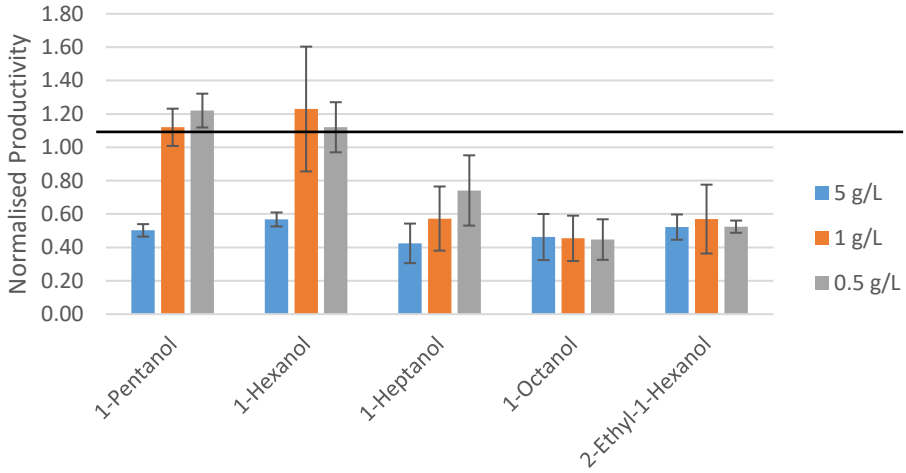


Figure 5.9: Comparison of the normalised overall ABE productivity, in 100 mL bottle screen, when five toxic extractants were added, at concentrations 5, 1 and 0.5 g L^{-1}, once the butanol concentration had reached 5 g L^{-1}. Performed in duplicate.

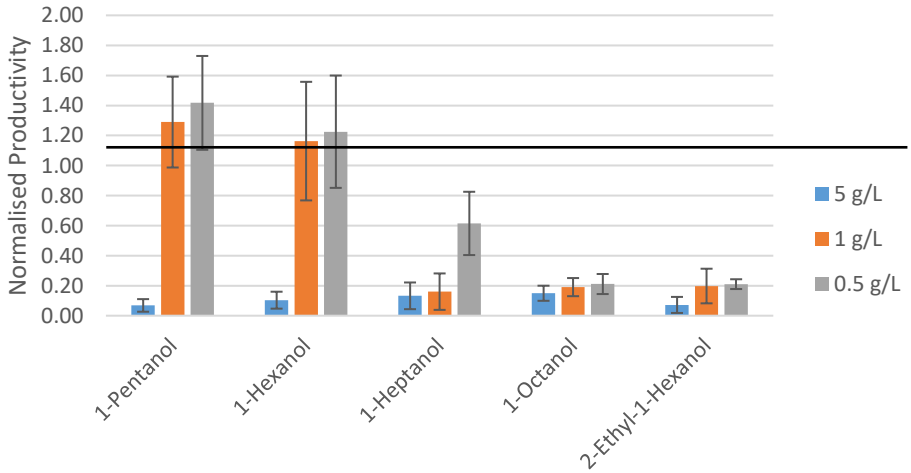


Figure 5.10: Comparison of the normalised ABE productivity post extractant addition, at concentrations 5, 1 and 0.5 g L^{-1}, once the butanol concentration had reached 5 g L^{-1}, in 100 mL bottle screen. Performed in duplicate.

The normalised productivity post extractant addition, as shown in Figure 5.10, confirms the impact that the extractant has on the fermentation. It can clearly be observed that all of the extractants at 5 g extractant L^{-1} have a major impact, reducing the productivity during this stage of the fermentation to less than 20% of that achieved in the control fermentation. As it is known that butanol concentrations higher than 8 g butanol L^{-1} can inhibit the fermentation, the addition of 5 g extractant L^{-1} which is also an alcohol, when the butanol concentration is approximately at 5 g L^{-1}, results in a total alcohol concentration of approximately 10 g L^{-1} in the fermentation broth, therefore an inhibitory effect is expected. This agrees with literature, in that generally alcohols have been found to be toxic to ABE-producing bacteria (Roffler *et al.*, 1987b).

Figure 5.10 also shows how, as the carbon chain increases, so does the toxicity of the alcohol. It is suspected that this is related to the solubility or polarity of the extractant (Bruce and Daugulis, 1991; Kim *et al.*, 1999). Generally, as the carbon chain increases, the molecule becomes more hydrophobic therefore less soluble in water, forming an organic phase. Exposure of the cells to an extractant-based phase has a greater effect on the toxicity compared to when it is only exposed at soluble concentrations (Kim *et al.*, 1999). This is due to toxicity affecting the cells differently at a molecular or phase level. Molecular level toxicity occurs below the extractants solubility level in water. It has been suggested that the organic solvent would dissolve into the cell affecting the cell membrane permeability and enzyme activity. Phase toxicity occurs above the solubility concentration of the organic solvent and is due to the solvent coating the outside of the cells. This would disrupt the uptake of nutrients and removal of products, along with disrupting the cell wall (Bruce and Daugulis, 1991; Kim *et al.*, 1999). It can be surmised that this explains why low concentrations (<1 g L^{-1}) of 1-pentanol and 1-hexanol do not have a negative impact on the fermentation, as all other extractants are insoluble at 1 g extractant L^{-1}. 5 g extractant L^{-1} is below the solubility limit for 1-pentanol and 1-hexanol and the concentration in relationship to the amount of biomass present could be too great, therefore a toxic effect at a molecular level occurs.

5.7.3: Comparison of Perstraction Extractants with a Silicone Membrane

To compare the proposed extractants a perstraction system using a silicone membrane was used. The perstraction system design was based upon that used by Jeon and Lee (1987), Groot *et al.* (1990) and Qureshi *et al.* (1992). Silicone was chosen as it is non-porous, so

should limit the back transfer of extractants into the aqueous phase. Other advantages were ease of use and compatibility with the fermentation, as described by Qureshi and Maddox (2005). Furthermore, it has historically been used for perstraction of ABE from fermentation broth therefore providing a comparison with existing literature. A synthetic ABE solution was used to represent the fermentation broth, with ABE concentrations in the ratios observed in the fermentation broth, providing an idea of the rate of transfer that will be possible from the fermentation broth.

The perstraction system operated for approximately 120 hours, with an average temperature of 23-25°C. This temperature was used as experiment was performed at room temperature due to equipment limitations with regards to mixing and temperature control. Ideally, the system would have operated at fermentation temperature (32°C), operation at this temperature would likely see an increase in the mass transfer rate. It was expected that an equilibrium between the aqueous and extractant phases would be obtained, as Groot *et al.* (1990) stated that they had achieved equilibrium in about 25 hours, using a silicone membrane. Figures 5.11 and 5.12 show the extractant concentration of butanol over the course of the experiment for 2-ethyl-1-hexanol and oleyl alcohol, respectively. These two extractants were the highest and lowest mass transfer rate as inferred by the overall mass transfer coefficient, respectively, for extracting butanol from the aqueous phase. In both cases the butanol concentration in the extractant did not begin to plateau until closer to 120 hours, indicating that an equilibrium was not established in 25 hours. Different membrane thicknesses (0.3mm compared to 0.75mm in this work) and a different extractant (isopropyl myristate) could explain the shorter equilibrium time observed by Groot *et al.* (1990).

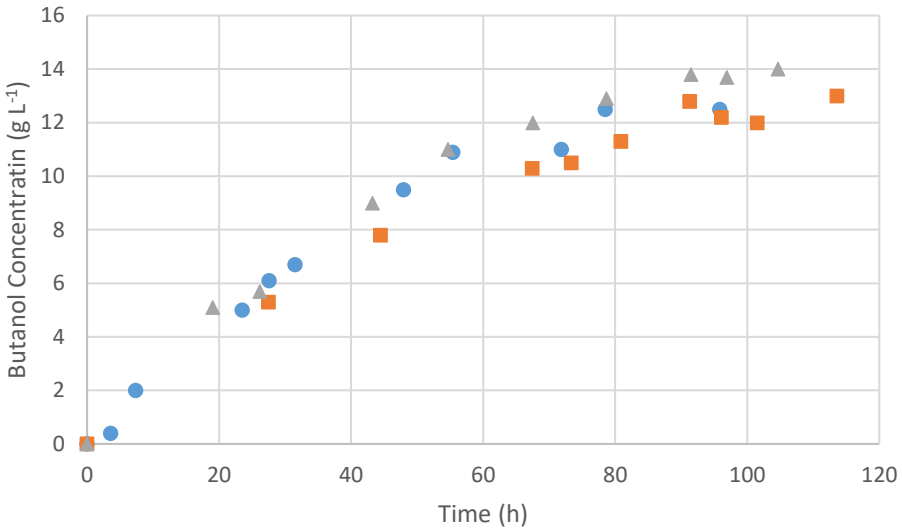


Figure 5.11: Butanol concentration in extractant, 2-ethyl-1-hexanol, over duration of experiment. Performed in triplicate, each colour represents an individual run.

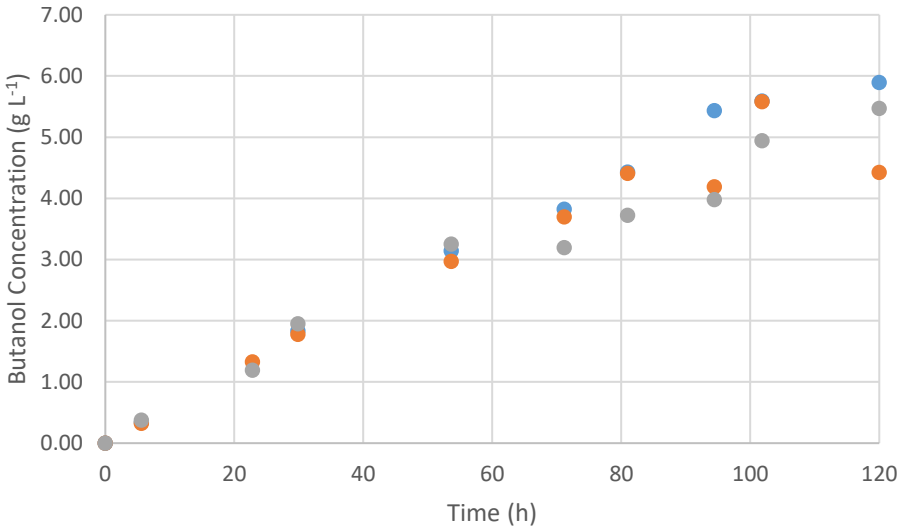


Figure 5.12: Butanol concentration in extractant, oleyl alcohol, over duration of experiment. Performed in triplicate, each colour represents individual run.

To confirm that equilibrium was not achieved, the ratio of concentration of butanol in extractant and aqueous phase over the duration of the experiment was compared. If an equilibrium was achieved, the distribution coefficient of butanol in that extractant should have been achieved. Figure 5.13 demonstrates that this is not the case, as the ratio for all extractants is still increasing at 120 hours. 2-Ethyl-1-hexanol has the fastest rate but still has not achieved the equilibrium partition value of 6 (Table 5.5) observed in liquid-liquid extraction. It is possible that this value will not be attained due to the presence of the membrane, which increases the number of phase interfaces over which the equilibrium needs to establish. If the membrane-extractant distribution is in favour of the membrane then this could reduce the overall distribution coefficient of the system represented by the

aqueous-extractant distribution coefficient. This means that an alternative extractant selection criteria is required, rather than distribution coefficient alone as in Jeon and Lee (1987).

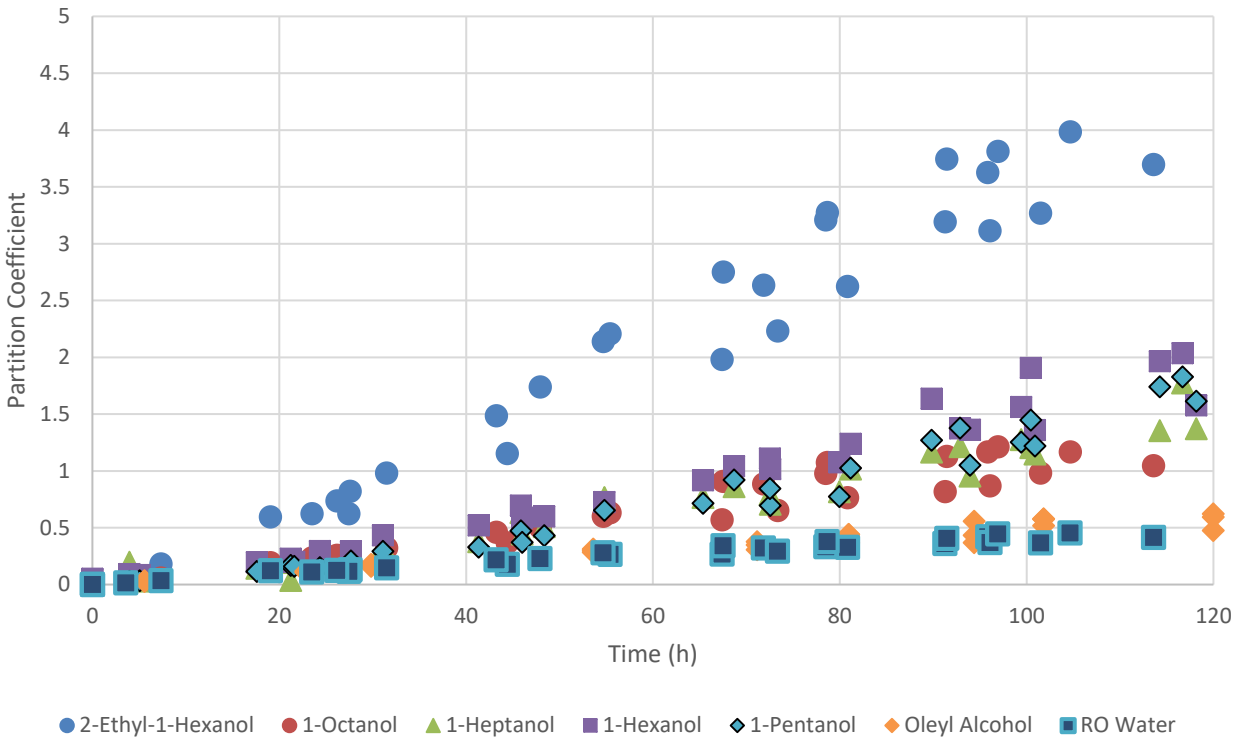


Figure 5.13: Ratio of butanol concentration, partition coefficient, in each extractant during 120 hours perstraction, data points for all three repeats are shown.

From Figure 5.13 oleyl alcohol and water have a similar change in concentration ratio over time. This is interesting as oleyl alcohol has a higher capacity for butanol than water. It is also apparent that the straight chain alcohols (1-octanol, 1-heptanol, 1-hexanol and 1-pentanol) all have very similar concentration ratios over time, forming the middle cluster in Figure 5.13. While these extractants are all relatively close in the butanol concentration ratio, based on the end points (110-120 hours), the order of extractants (highest ratio first) is 1-hexanol, 1-pentanol, 1-heptanol, 1-octanol. This order of distribution coefficients, highest first, in Table 5.5 follows the same order. In fact at approximately 120 hours, all extractants are in the order of their respective distribution coefficients. This highlights how using a high distribution coefficient extractant compared to oleyl alcohol will have a positive impact on the removal of butanol from the fermentation broth.

The data points in Figure 5.13 are from all three repeats for each extractant. It can be seen that the points for each extractant agree well. This gives assurance of the repeatability of the experiments.

To allow for a quantitative assessment of the different extractants, the overall mass transfer for butanol has been assessed. The overall mass transfer coefficient is independent of membrane area, allowing for comparison of this system with others in literature. The overall mass transfer coefficient for butanol was calculated based on transfer of butanol out of the aqueous phase. The aqueous-based overall mass transfer coefficient, $K_{ov,A}$ (5.14), was selected. This was because the primary aim of combining perstraction with the ABE fermentation is to remove the ABE from the fermentation broth and maintain concentrations below inhibitory levels. Only the mass transfer coefficient for butanol was assessed due to butanol being the primary product and the acetone and ethanol having a low affinity for the extractants, as evidenced by the distribution coefficients in Table 5.5.

Figure 5.14 shows the aqueous-based overall mass transfer coefficient for each extractant with a silicone membrane. This graph shows that 2-ethyl-1-hexanol has the highest overall mass transfer coefficient for butanol. The lowest overall mass transfer coefficient for butanol was oleyl alcohol, which was approximately 8 times lower than that of 2-ethyl-1-hexanol. All the other extractants tested had a higher overall mass transfer coefficient than oleyl alcohol. This means that by switching to an organic extractant with a higher distribution coefficient than oleyl alcohol, the rate of transfer will increase and a reduction in extractant volume would be possible. All the other extractants had density and viscosity values closer to that of water than oleyl alcohol. As shown in equations (5.22), (5.23) and (5.24), the density and viscosity affect the film transfer coefficients on either side of the membrane. The order of magnitude higher viscosity of oleyl alcohol perhaps hinders the convective mass transfer.

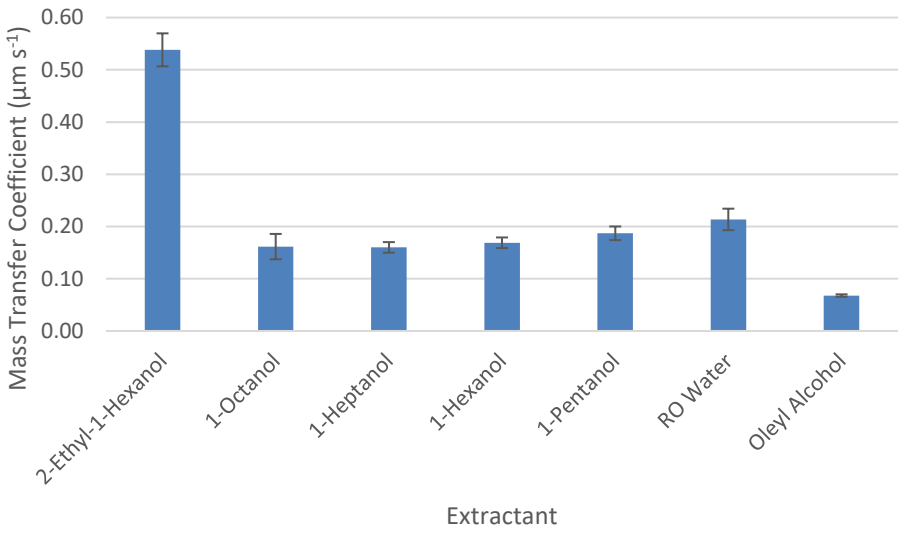


Figure 5.14: Aqueous-based overall mass transfer coefficient of butanol for each extractant using a silicone membrane.

The straight chain alcohols saw an increase in overall mass transfer coefficient as the carbon chain length decreased, though all the overall mass transfer coefficients were very similar in the range of 0.16-0.19 µm s^{-1}. This is approximately 3 times smaller than that of 2-ethyl-1-hexanol. This is particularly interesting, especially for 1-octanol which has the same molecular weight and chemical composition as 2-ethyl-1-hexanol. It is hypothesised that the branched ethyl group increases the extracting capability of the extractant. The same phenomena has been observed with the extraction of ethanol from aqueous solutions, whereby alcohols formed good extractants, but the location of the hydroxyl group and a branched chain increased the distribution coefficient of ethanol (Roddy, 1981). Munson and King (1984) suggested that this was due to the steric effects caused by the branching. Whereby, as the steric hindrance increases the extraction selectivity increases, particularly in C_6 or higher alcohols. This is due to the branched carbon chains hindering electron access to the hydroxyl group, therefore a slight positive charge is present on the hydrogen, and this would be attracted to the polar ethanol molecule (Munson and King, 1984). It is possible that the same effects are occurring with the extraction of butanol from aqueous solution. Where the 2-ethyl-hexanol the carbon chains induce a positive charge which is attracted to the polar hydroxyl group of the butanol, hence 2-ethyl-1-hexanol has a significantly increased overall mass transfer coefficient.

Surprisingly, the use of water as an extractant had a higher overall mass transfer coefficient than all the organic extractants, other than 2-ethyl-1-hexanol. This type of comparison of

water as an extractant for perstraction has not previously been investigated. Jeon and Lee (1989) did test water alongside oleyl alcohol, but different process conditions were used so a direct comparison was not possible. The advantage of using water as a potential extractant is the ease of availability, cost and safety, compared to the organic extractants. The downside is that it will not be possible to increase the concentration of ABE in the aqueous phase, which is one of the key requirements of ISPR (as it reduces downstream energy requirements (Xue *et al.*, 2014b)). This could be overcome by developing it into a hybrid technique, by extracting the ABE from the water with a high distribution, and toxic, extractant such as 2-ethyl-1-hexanol to concentrate the ABE.

Over 120 hours of perstraction the butanol concentration in the water extractant reached approximately 4.5 g L^{-1} this is lower than a typical batch fermentation broth concentration of around 12g L^{-1} (Ezeji *et al.*, 2003). This is because, when equilibrium is reached, the concentration in both the synthetic ABE solution and the extraction should be equal. While it would be possible to control the butanol concentration in the fermentation broth below inhibitory concentrations this would cause an increase in the downstream processing costs, either due to high distillation energy or the addition of another separation step to up concentrate the ABE. It must also be noted that the volume of water required for extraction will be significantly greater than that of an organic extractant which can increase the concentration of the ABE, but this is balance by the cost of water being cheaper than an organic extractant.

The use of overall mass transfer coefficients to decide on the best extractant-membrane combination has not been widely investigated in literature. The earliest perstraction research with the ABE fermentation by Jeon and Lee (1987) did not consider the overall mass transfer to aid selection. Only Groot *et al.* (1990) considered the overall mass transfer coefficient when selecting an extractant. Table 5.6 provides a comparison of all the mass transfer coefficients provided in literature using a silicone membrane and the extractants tested in this work. All the overall mass transfer coefficients are of the same order of magnitude, providing confidence in the results achieved. The differences in temperatures could explain the slight variations in values, as it is known that increased temperatures can increase the mass transfer across membranes (Xue *et al.*, 2014a). The duration of the experiments could also influence the overall mass transfer coefficient. As already stated Groot *et al.* (1990) found equilibrium to be reached around 25 hours, whereas the butanol

equilibrium required at least 120 hours. Comparing the results across this work and Groot *et al.* (1990), oleyl alcohol has the lowest overall mass transfer coefficient in both cases. Also, in both cases the overall mass transfer coefficient of 1-hexanol is greater than that of 1-octanol.

Table 5.6: Comparison of overall mass transfer coefficients of butanol using a silicone membrane in literature.

Extractant	**Groot *et al.* (1990) 30°C ($x10^{-7}$ m s^{-1})[a]**	**Grobben *et al.* (1993) 37°C ($x10^{-7}$ m s^{-1})[b]**	**Jeon and Lee (1989) 35°C ($x10^{-7}$ m s^{-1})[b]**	**This work 23-25°C ($x10^{-7}$ m s^{-1})[a]**
2-Ethyl-1-hexanol				5.38 ± 0.316
1-Octanol	4.0			1.62 ± 0.243
1-Heptanol				1.60 ± 0.101
1-Hexanol	5.2			1.69 ± 0.101
1-Pentanol				1.87 ± 0.131
RO Water				2.14 ± 0.206
Oleyl Alcohol	2.2	~3	0.42 (4.23x10-6 cm s^{-1})	0.67 ± 0.024

[a] Calculated from mass transfer test using synthetic ABE solution
[b] Calculated from fermentation data

In all cases, other than water, the extractant back-transferred across the membrane into the aqueous phase. It was visually evident during these experiments, as the aqueous phase became cloudy, Figure 5.15. This was due to the organic extractant mixing with the synthetic ABE solution and creating a stable emulsion, as when using RO water for the extractant, the ABE solution did not go cloudy. It is expected that a stable emulsion formed due to the fast recirculation of the synthetic ABE solution, 150 mL min^{-1}. Surprisingly, oleyl alcohol also transferred across the membrane into the aqueous phase; something that had not previously been observed.

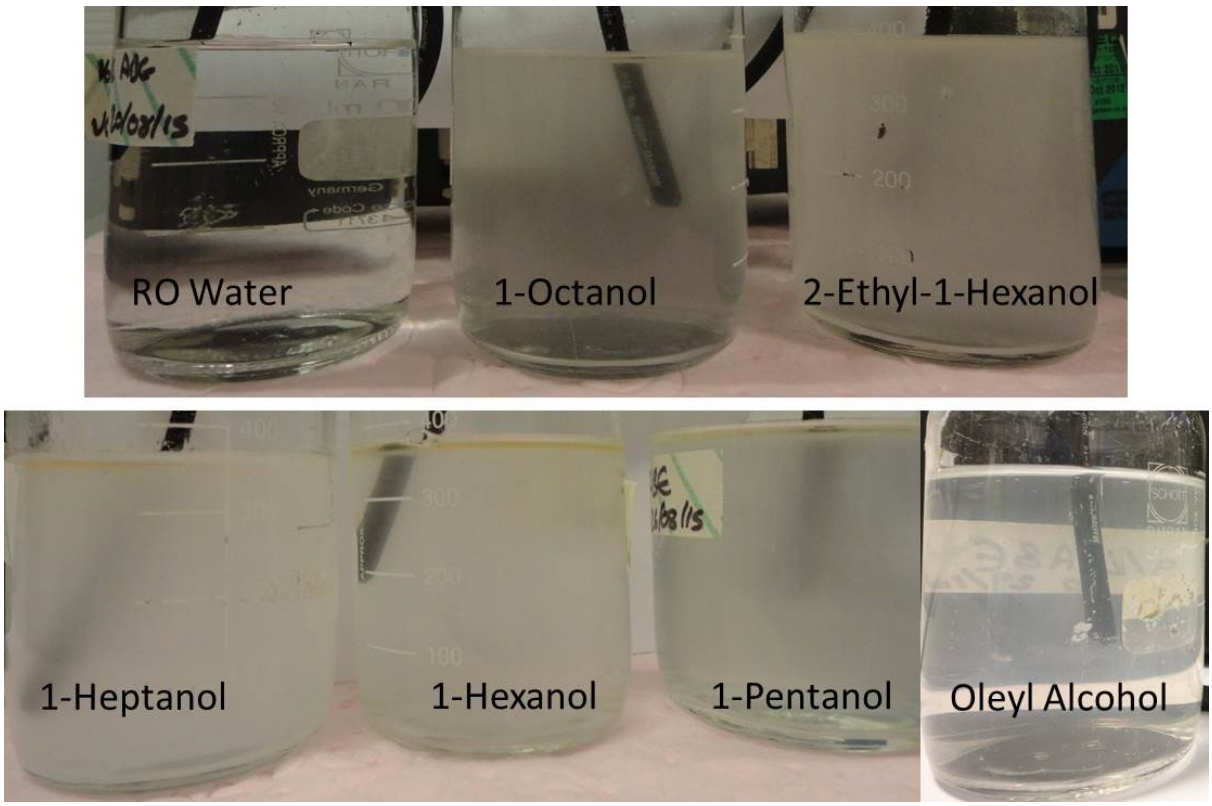


Figure 5.15: Pictures of the synthetic ABE solution after 120 hours perstraction. Extractant used, left to right, top row: RO water, 1-octanol, 2-ethyl-1-hexanol; bottom row: 1-heptanol, 1-hexanol, 1-pentanol, oleyl alcohol.

To better understand the transfer of extractant across the membrane and determine the potential impact on the fermentation, the extractant concentration in the aqueous phase was measured. 2-ethyl-1-hexanol, 1-octanol, 1-heptanol, 1-hexanol and 1-pentanol were measured by gas chromatography. Oleyl alcohol concentration had not previously been measured in either the perstraction or the liquid-liquid extraction literature for the ABE fermentation. To measure the oleyl alcohol an extraction step was required to extract all oleyl alcohol into an organic phase to aid detection on the GC. It was assumed that 100% of the oleyl alcohol was extracted.

Figure 5.16 shows the aqueous phase concentration of extractants during perstraction for all extractants other than oleyl alcohol and RO water. Although oleyl alcohol was analysed using gas chromatography only trace amounts were detected, below the limit of detection for the oleyl alcohol. Example chromatograms showing this are provided in Appendix D. The transfer of oleyl alcohol across a silicone membrane is something suspected by Qureshi *et al.* (1992), though in later work they do not believe diffusion of oleyl alcohol across the membrane would cause the fermentation to stop (Qureshi and Maddox, 2005). It was not possible to detect if water transferred across the membrane, whilst using RO water

extractant, during the experiment but based on volumes of the aqueous and extractant phase it appeared that no water had transferred across the membrane.

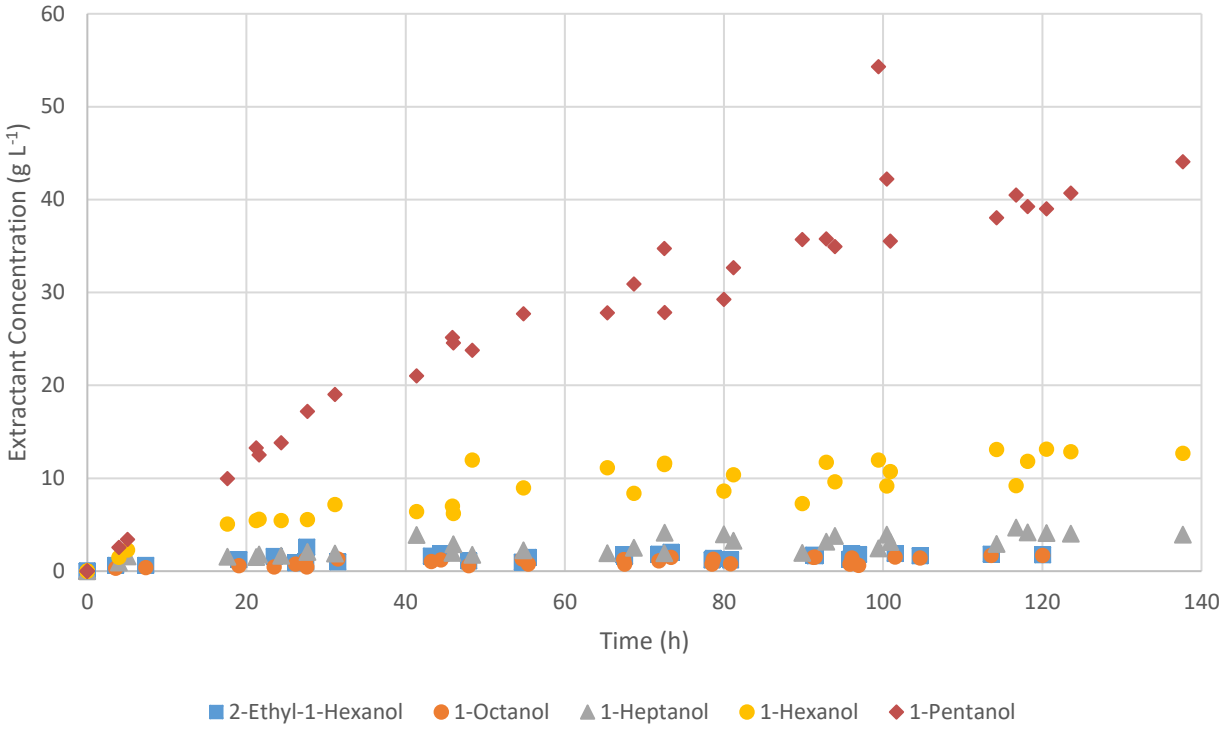


Figure 5.16: Concentration of extractant in the aqueous phase over the course of the perstraction experiments, showing all three repeats for each extractant.

Figure 5.16 shows that the concentration increases with time until it plateaus, establishing a constant aqueous concentration. The equilibrium concentration of 2-ethyl-1-hexanol, and the time profile generally, was approximately the same as that of 1-octanol. Once the aqueous concentration had reached solubility limits any further extractant to transfer across the membrane formed an organic phase layer on top of the aqueous phase, as demonstrated by 1-heptanol in Figure 5.17. This indicates that even though the solubility limit of extractant was obtained, there was still a driving force for transfer of the extractant. The order of concentration from highest to lowest follows that of molecular weight and solubility in water, Table 5.5. The aqueous phase concentration is higher than the maximum solubility in water. This is due to an emulsion forming, Figure 5.15, meaning that complete phase separation has not occurred.

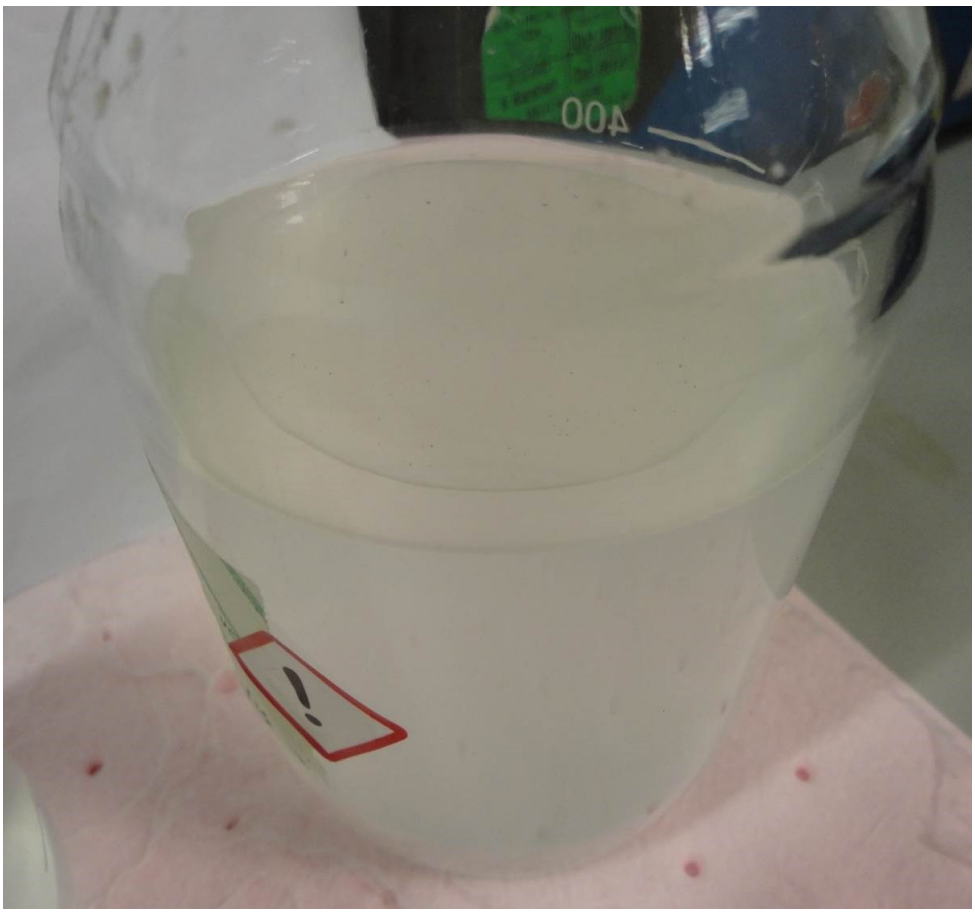


Figure 5.17: Aqueous phase after 120h perstraction with 1-heptanol. A layer of 1-heptanol can be observed on the surface of the synthetic ABE solution.

The transfer of extractant into the aqueous phase would have a negative effect on the fermentation, particularly for 2-ethyl-1-hexanol, which exhibited the highest rate of removal of butanol from the aqueous phase. For all the extractants shown in Figure 5.16, after 24 hours of perstraction the aqueous phase concentration is higher than that tolerable by the bacteria, as determined by the toxicity experiments in section 5.7.2. Other than toxicity, the loss of extractant into the fermentation broth means that the extractant would need to be regularly replenished, thereby increasing the operating costs of the process.

From these results it is evident that perstraction can remove ABE from an aqueous solution, and although oleyl alcohol is a good extractant, the rate of extraction can be significantly increased through the use of extractants with higher distribution coefficients. The downside to this is that these extractants are toxic to the fermentation, and will leach across the membrane, it is therefore imperative to find a membrane-extractant combination which will not allow transfer of the extractant.

5.7.4: Membrane Choice

As demonstrated by Table 5.1 only a limited number of membranes have been tested for perstraction compared to the number of extractants that have been investigated. This is

similar to the early developmental stages of pervaporation where PDMS/silicone and polypropylene membranes were typically chosen (Qureshi and Blaschek, 1999a). It is understood that membrane development is required to improve perstraction characteristics (Qureshi and Maddox, 2005), but for perstraction to be a viable option for ABE fermentation the membrane used needs to be commercially available. There are many novel membrane materials that have been proposed to be combined with the ABE fermentation for other ISPR techniques such as pervaporation (Liu *et al.*, 2013a), for example carbon nanotube based membranes (Xue *et al.*, 2014a), but the lack of commercial availability would hinder fermentation process development.

There is a wide range of commercial membrane material for a wide range of industrial applications of which 6 membranes, Table 5.7, were selected for investigation for perstraction of ABE based upon accessibility and compatibility with organic solvents. Silicone was chosen as a non-porous membrane with successful ABE transfer based on previous perstraction work. Non-porous PTFE is extremely chemically resistant and has different characteristics to silicone. The porous membrane materials were selected based on good affinity for organic solvents, as shown in Figure 5.18. PES, the third membrane evaluated, is recommended for cell culture media as it is low-protein binding. This selection of membranes allows for the comparison of hydrophobic and hydrophilic membrane, porous and non-porous membranes, and differences in membrane configuration.

Table 5.7: Membrane materials and properties of those tested for perstraction of ABE.

Membrane	Configuration	Hydrophobic / Hydrophilic	Porous / Non-Porous	Pore Size (μm)	Area for Mass Transfer (m^2)	Membrane thickness (mm)
Silicone	Tube	Hydrophobic	Non-Porous	n/a	0.00528	0.75
PTFE	Tube	Hydrophobic	Non-Porous	n/a	0.00500	1.52
PTFE	Sheet	Hydrophobic	Porous	0.45	0.00126	0.22
Regenerated Cellulose	Sheet	Hydrophilic	Porous	0.45	0.00126	0.075
Polyamide (Nylon)	Sheet	Hydrophilic	Porous	0.45	0.00126	0.11
Polyether Sulphone (PES)	Tube (hollow fibre)	Hydrophilic	Porous	~0.01	0.00181	0.25

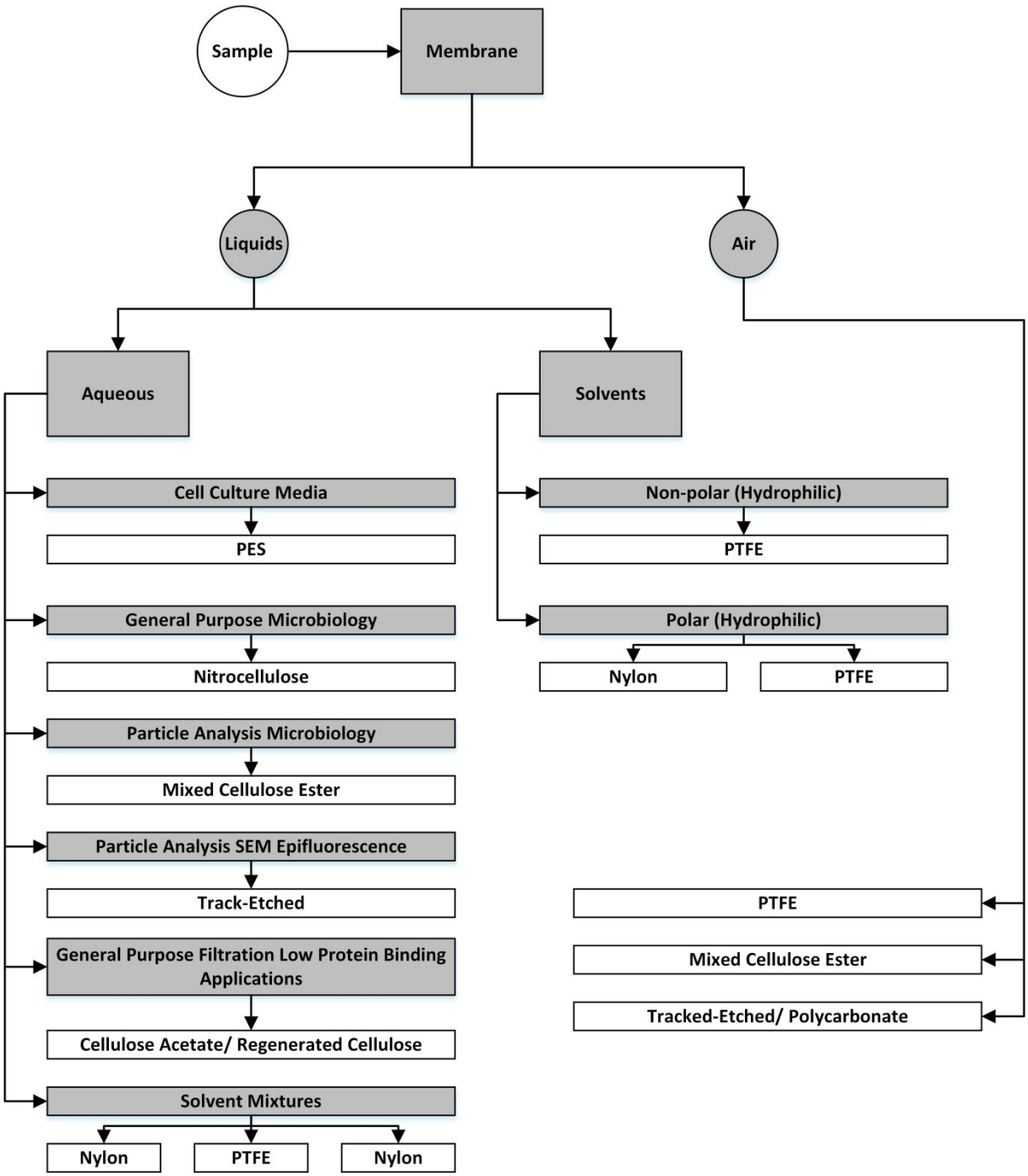


Figure 5.18: A quick reference membrane selection guide for Whatman™ Filters (GE Healthcare, 2016)

The differences in membrane configuration necessitated different perstraction setups, Figures 5.6 and 5.7. As shown in Table 5.7 this led to differing surface areas for mass transfer. The overall mass transfer coefficient was therefore use, to allow for comparison across the different perstraction setups used. To compare any potential effects experienced by the different configurations (notably between a tubular system and flat sheet membranes) the Reynolds number for each system was calculated using equation (5.23).

Table 5.7 also shows that each membrane has a different thickness. This is due to these membranes being commercially available materials, so there is limited or no choice over the membrane thickness. While membrane thickness does have an impact on the membrane mass transfer coefficient, thickness is a manufacturing constraint that would be inherent to an industrial process. Therefore, the membranes have been compared based on the butanol mass transfer rate and the overall mass transfer coefficient.

5.7.5: Perstraction: Membrane Comparison with 2-Ethyl-1-Hexanol and Oleyl Alcohol Extractants

To compare the membranes two extractants were selected: 2-ethyl-1-hexanol and oleyl alcohol. These extractants were chosen because 2-ethyl-1-hexanol provided the fastest mass transfer rate with silicone and oleyl alcohol because it is non-toxic to the bacteria and is the least soluble in the aqueous phase. The overall mass transfer rate was used to compare the membrane and extractant performance.

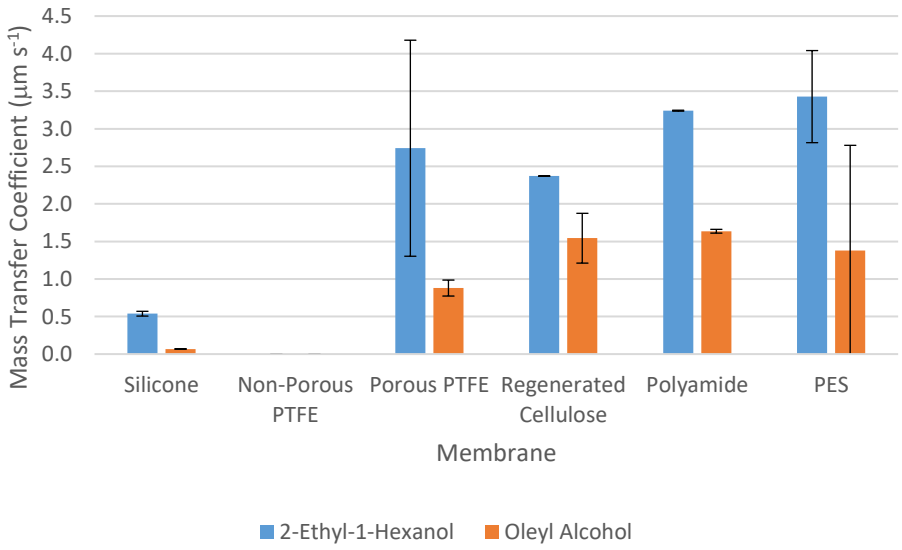


Figure 5.19: Comparison of membrane performance, based on the overall mass transfer coefficient, with 2-ethyl-1-hexanol and oleyl alcohol as an extractant.

The first observation from Figure 5.19 is that 2-ethyl-1-hexanol provides faster mass transfer in all membrane cases (other than the non-porous PTFE membrane) than oleyl alcohol. This illustrates how extractant selection will play a significant part in obtaining an optimum extractant process. Figure 5.19 also shows that the non-porous membranes have an overall mass transfer coefficient 10 times smaller than that of the porous membranes, which illustrates the importance of membrane choice.

No transfer of ABE was observed over a 120 hour period using either extractant when a non-porous PTFE membrane was used, Figure 5.19. PTFE is a more rigid material than silicone, which has a more natural fluidity therefore it is easier for chemicals to diffuse through the material. Consequently, this also meant that it was easier for the extractant to transfer through. It was thought that a material with less natural fluidity like PTFE would have better properties for retaining the extractant, while still allowing the ABE to diffuse through. No transfer of ABE across the membrane indicates the membrane is impermeable or there is no driving force for mass transfer. If the membrane does not allow absorption of either phase (aqueous or extractant) no connection between the two phases is established and there is no driving force for mass transfer. This is graphically demonstrated in part (a) Figure 5.20.

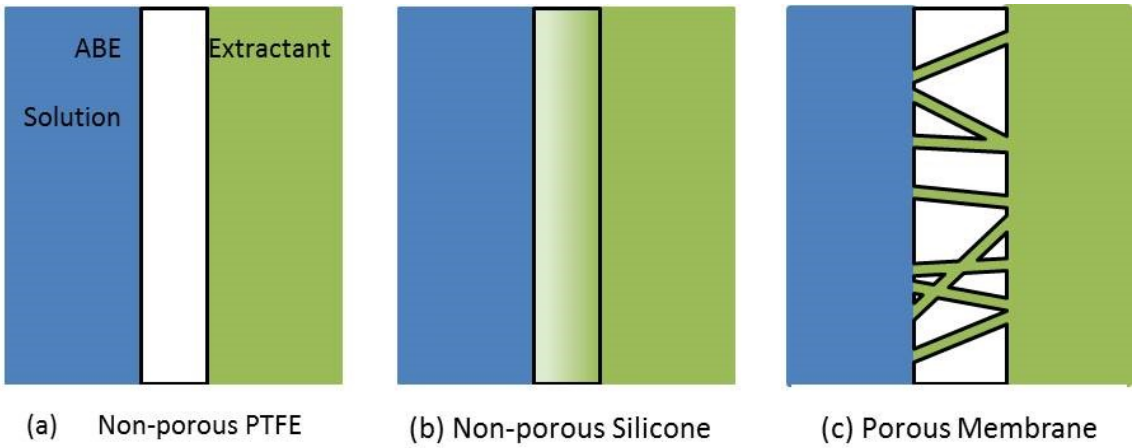


Figure 5.20: Diagram representing extractant concentration with different membrane types. (a) represents a non-porous PTFE membrane, (b) a non-porous silicone membrane and (c) a generic hydrophobic porous membrane.

In contrast to non-porous PTFE, silicone allowed for absorption of the extractant into the membrane, as extractant diffused through and into the aqueous phase, Figure 5.16. This absorption into the membrane creates a connection between the phases, creating the driving force for mass transfer. When a porous membrane is used there is an interface between the two phases therefore the relationship between the two phases is established. This is partially supported by Groot *et al.* (1990) who proposed that the permselectivity of the membrane is affected by an extractant, influencing the transfer of the desired compound, indicating that the extractant plays a dominant role in the transfer across the membrane. With a porous membrane, the extractant is in direct contact with the aqueous phase, therefore considering the localised extractant concentration at the pore mouth can be assumed to be equal to the bulk phase concentration, assuming good mixing of the bulk phase. This is a higher extractant concentration than in the non-porous silicone membrane as indicated by the shade of extractant in Figure 5.20. This higher concentration will relate to

a higher capacity for ABE at the interface, therefore a greater overall mass transfer coefficient, Figure 5.19.

To ensure that the differences were related to the membranes being porous or non-porous, rather than differences in system configuration, the overall mass transfer coefficient was compared on the basis of Reynolds number as shown in Figure 5.21. The tubular system Reynolds number is given in equation (5.23) and the flat-sheet system by equation (5.26).

$$Re_N = \frac{\rho N D_{id}^2}{\mu} \quad \textbf{(5.26)}$$

Where

Re_N is the Reynolds number in an agitated system

N is the rotational speed, revolutions per second (s^{-1})

D_{id} is the maximum inner flask diameter (m)

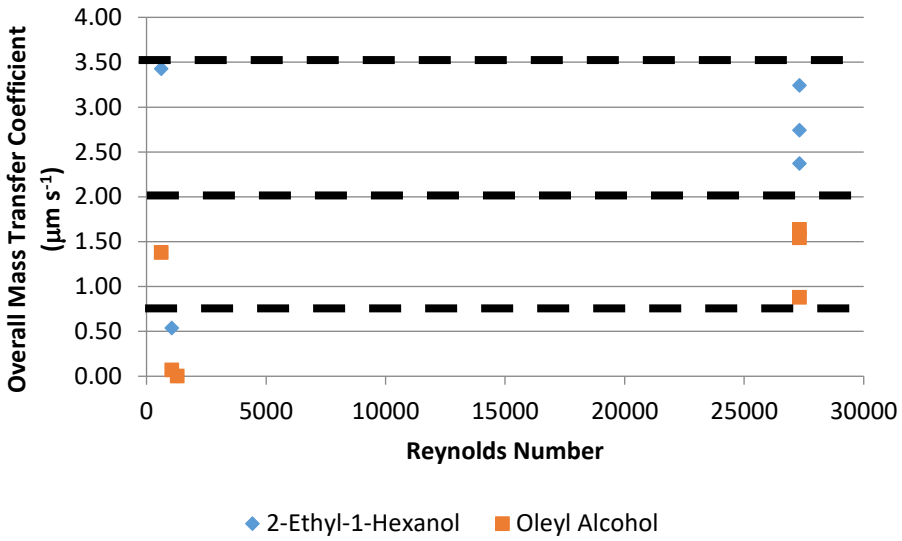


Figure 5.21: Overall mass transfer coefficient compared to the Reynolds number for each membrane. Data for every membrane type shown, categorised by extractant used (blue=2-ethyl-1=hexanol, orange=oleyl alcohol). The dashed black lines separate the different membrane-extractant categories. The top section represents porous membranes with 2-ethyl-1-hexanol, the middle section is porous membranes with oleyl alcohol and the bottom section is non-porous membrane experiments with all extractatants. The data points on left are from the tubular system and the ones on the right from the flat sheet system.

The flat sheet system had a higher Reynolds number than the tubular system, but the mass transfer coefficient for the tubular PES membrane falls within the same numerical range as the flat sheet membranes, indicating that differences in the membrane configuration are not responsible for the differences between the porous and non-porous membranes.

Comparing the different porous membranes to deduce which is the best material of those tested is more complex. With 2-ethyl-1-hexanol PES has the highest overall mass transfer coefficient, closely followed by the polyamide membrane. For oleyl alcohol the polyamide membrane had the highest overall mass transfer coefficient. The PES membrane did not perform as well due to incompatibles between the PES and oleyl alcohol, which saw the membrane dissolve when submerged in the extractant as shown in Figure 5.22. This incompatibility is the reason for the large error bars present on Figure 5.19. The porous PTFE membrane with 2-ethyl-1-hexanol also exhibits a large error. This is also due to membrane-extractant incompatibilities in that small holes appeared in the membrane, as shown in Figure 5.22, below.

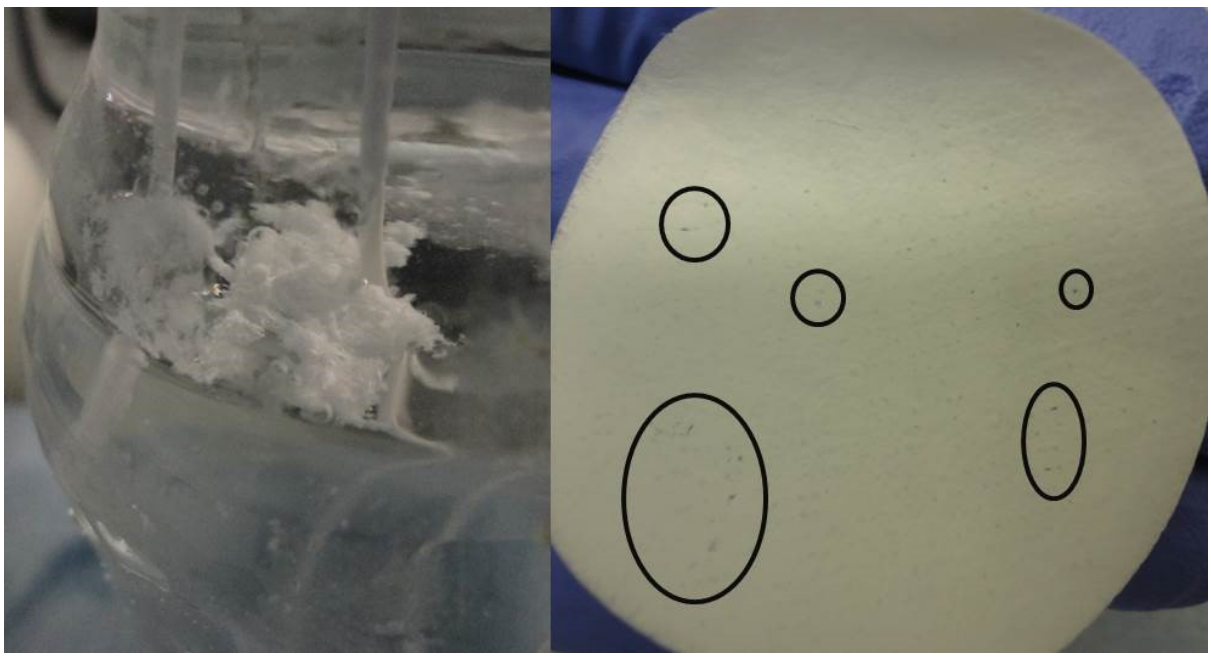


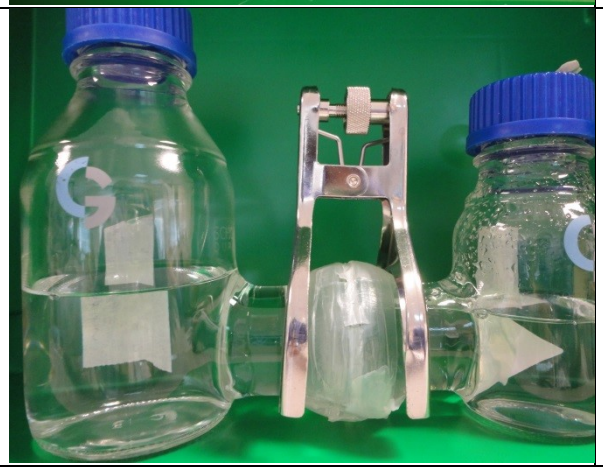
Figure 5.22: Pictures showing membrane-extractant incompatibilities. Left: PES in oleyl alcohol, where membrane pieces are seen floating the extractant. Right: A porous PTFE membrane after contact with 2-ethyl-1-hexanol, the circles show areas of damage, with small holes through the membrane.

Membranes are often mounted on a support material to increase their strength and durability. This material also needs to be chemically compatible with ABE and the extractant. As PTFE is resistant to most chemicals, and the non-porous PTFE showed no weakness to 2-ethyl-1-hexanol, it could be the material used to support the membrane was not chemically compatible with the extractant. Therefore, the membrane support material also needs to be considered for extractant compatibility.

The membranes tested had different affinities for water. As displayed in Table 5.7, the regenerated cellulose, polyamide and PES membrane are hydrophilic, whereas the silicone

and PTFE membranes are hydrophobic. The benefits of a hydrophobic membrane are that the organic components of the synthetic ABE solution will preferentially transfer through, and in relation to porous membranes the pores will be filled with and retain the extractant. With the hydrophilic membranes (all porous) the membrane will be filled with the aqueous phase and form an immobilised phase interface with the extractant. Based on the results in Figure 5.19 it seems that the hydrophilic membranes showed greater promise compared to the hydrophobic membranes. With oleyl alcohol as the extractant using the porous PTFE membrane, the overall mass transfer coefficient was lower than the hydrophilic membranes. With 2-ethyl-1-hexanol; the PTFE membrane had a higher overall mass transfer coefficient than the regenerated cellulose membrane, but when the instabilities of the PTFE membrane with 2-ethyl-1-hexanol are considered, the regenerated cellulose seems to be a better membrane. The downside to the use of hydrophilic membranes was the transfer of water into the extractant. Table 5.8 shows pictures of the flat sheet membrane systems after 120 hours. For the hydrophilic membranes, in particular regenerated cellulose with both extractants and polyamide with oleyl alcohol, two phases can be seen in the 250 mL extractant bottle. The extractant is now resting on top of the aqueous phase that has passed through the membrane. Whilst this allows for easy separation of the solvent, it would reduce the volume of the fermentation and reduce the contact time of the extractant and aqueous phase at the membrane interface. The PTFE membrane system is also shown in Table 5.8 and no additional phase is visible in either bottle. This transfer of the aqueous phase would represent loss of fermentation broth and contamination of the extractant potentially causing difficulties in recovering the ABE from the extractant.

Table 5.8: End-point photos of flat sheet perstraction systems. Aqueous phase = 500mL bottle, extractant =250mL bottle. Red arrow indicates aqueous-extractant phase interface.

	PTFE (Hydrophobic)	Regenerated Cellulose (Hydrophilic)	Polyamide (Hydrophilic)
2-Ethyl-1-Hexanol			
Oleyl Alcohol			

As previously demonstrated in Figure 5.16, it is possible for the extractant to transfer across the membrane. Figure 5.23 compares the extractant transfer across the membrane for the different membranes tested with 2-ethyl-1-hexanol. With all membranes some 2-ethyl-1-hexanol transferred into the aqueous phase, including the non-porous PTFE where very low concentrations of 2-ethyl-1-hexanol (<0.1 g L^{-1}) were present. With every membrane the 2-ethyl-1-hexanol concentration plateaus after approximately 40 hours of operation. After discounting the non-porous PTFE membrane, two distinct bands of data points can be seen in Figure 5.23. The higher band corresponds to hydrophobic membranes, silicone and porous PTFE, and the lower band to the hydrophilic membranes, regenerated cellulose, polyamide and PES. It is not surprising that the hydrophilic membranes have a lower extractant concentration in the aqueous phase as the membranes should have a natural repellent to the extractant, but as shown in Table 5.8 the hydrophilic membranes allow water to pass across. With the hydrophilic membranes the extractant concentration reaches around 0.6-0.7 g L^{-1}, this concentration would still be toxic to the ABE fermentation; based on the results of the toxicity tests (see Figure 5.10).

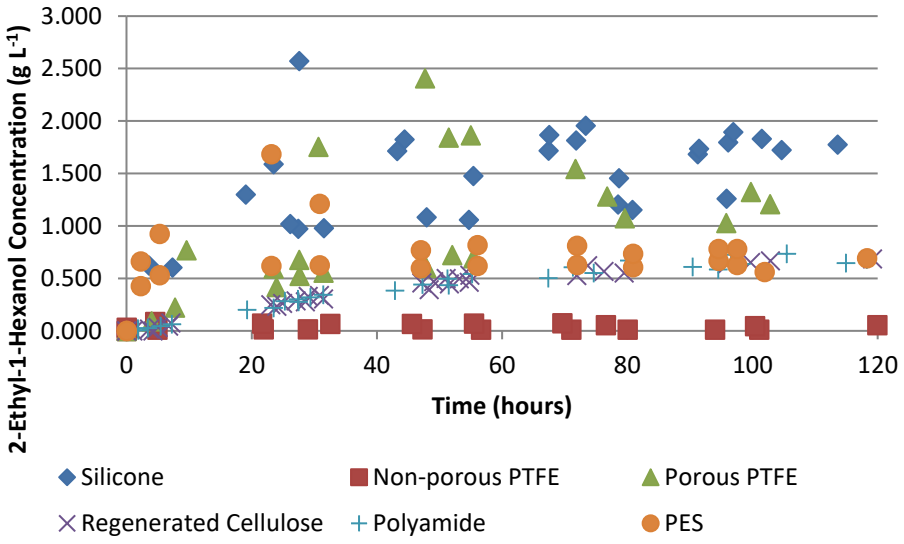


Figure 5.23: Concentration of the extractant, 2-ethyl-1-hexanol, in the aqueous phase over the course of perstraction based on membrane type. Data points for all repeats are shown.

When using oleyl alcohol no transfer of extractant was observed, other than trace amounts with the silicone membrane. Oleyl alcohol is more hydrophobic than 2-ethyl-1-hexanol therefore the properties of the membrane are more effective for retaining the extractant. In particular the porous PTFE was able to retain the oleyl alcohol, but not the 2-ethyl-1-hexanol. Tanaka *et al.* (2012) described the PTFE membrane as more hydrophobic than other hydrophobic membranes used for perstraction. This, combined with the increased

hydrophobicity of oleyl alcohol, could explain why the silicone membrane allowed the oleyl alcohol to pass across but the porous PTFE membrane did not.

When using the silicone membrane an emulsion formed, as shown in Figure 5.15. With the porous membranes an emulsion did not form: the liquid remained clear (see Table 5.8). It is assumed that this is related to the mechanism of transfer across the membrane. With the silicone, the extractant had to absorb into the membrane, then desorb into the ABE solution. When using the porous membrane the two phases were in contact with one another, therefore one solution did not need to desorb into the other hence a better phase separation. The reduction/avoidance of emulsion formation was another one of the disadvantages of LLE that perstraction was supposed to overcome. This observation lends further support to the use of porous membranes over non-porous membranes.

Overall the choice of membrane and extractant needs to be investigated in tandem to ensure no undesired membrane-extractant interactions and the highest possible mass transfer coefficient. The results presented here indicate that porous membranes have a significantly increased overall mass transfer coefficient compared to non-porous membranes. The differences between mass transfer coefficient for porous and non-porous membranes outweigh other physical factors of the system such as agitation, which should also have an impact on the mass transfer. With regards to the transfer of extractant across the membrane into the aqueous phase, hydrophilic membranes are favourable but they do allow the transfer of water into the extractant phase. The presence of water in the extractant would hinder the separation of ABE from the extractant, but if the water-extractant mixture does not form an emulsion and easy phase separation is feasible, then this might be more desirable than higher concentrations of toxic extractant in the fermentation broth.

From the membranes tested in this work the regenerated cellulose, or polyamide, membrane would be most suitable in combination with oleyl alcohol for combining with the ABE fermentation. This is due to porous membranes providing a higher mass transfer rate, whilst hydrophilic membranes reduce the back transfer of extractant into the aqueous phase. Oleyl alcohol would have to be used as the 2-ethyl-1-hexanol still transferred across the membrane into the aqueous phase and was present at a concentration toxic to the bacteria.

5.7.6: Literature Comparison (Impact on Fermentation)

Other than Groot *et al.* (1990) existing literature concerning the application of perstraction to the ABE process, does not focus on the use of synthetic ABE solutions to characterise the system. Instead perstraction is applied directly to the fermentation process. By calculating the butanol flux for each system, using equation (5.13) it is possible to compare the results from this work to the existing literature. The comparison is shown in Table 5.9. The butanol fluxes from literature are the same order of magnitude as those achieved using a synthetic ABE solution in this work. In some cases the flux is higher, but that can be explained by the butanol flux being a function of butanol concentration in the system. In the fermentation butanol is constantly being produced, and the aim of ISPR is for the product removal rate to be equal to the production rate. Therefore, the overall butanol concentration in the fermentation broth should be relatively constant. However, with the synthetic ABE solution the total butanol in the system is constant, therefore the aqueous concentration of butanol decreases through the experiment. This higher average butanol concentration, seen during a fermentation, will lead to a higher butanol flux over the duration of the ISPR stage.

Table 5.9: Comparison of butanol flux between literature and this work.

Membrane	Extractant	Butanol Flux ($g\ h^{-1}\ m^{-2}$)	ABE Medium	Reference
Silicone	Oleyl Alcohol	3.8	Fermentation Broth	(Jeon and Lee, 1987)
Silicone	Polypropylene Glycol	3.0	Fermentation Broth	(Jeon and Lee, 1987)
Silicone	Tributyrin	2.4	Fermentation Broth	(Jeon and Lee, 1987)
Silicone	Oleyl Alcohol	1.4	Fermentation Broth	(Jeon and Lee, 1989)
Silicone	Water	1.8	Fermentation Broth	(Jeon and Lee, 1989)
Silicone	Oleyl Alcohol	1.2	Fermentation Broth	(Qureshi *et al.*, 1992)
Silicone	Oleyl Alcohol	1.0	Fermentation Broth	(Qureshi and Maddox, 2005)
Polypropylene	Oleyl Alcohol /Decane	8.8	Fermentation Broth	(Grobben *et al.*, 1993)
Polypropylene	Fatty acid methyl esters	0.5	Fermentation Broth	(Grobben *et al.*, 1993)
Porous PTFE	Oleyl Alcohol	37.8	Fermentation Broth	(Tanaka *et al.*, 2012)
Porous PTFE	Dodecanol	51.4	Fermentation Broth	(Tanaka *et al.*, 2012)
Silicone	2-Ethyl-1-Hexanol	4.7±0.32	Synthetic ABE	This work
Silicone	1-Octanol	2.7±0.13	Synthetic ABE	This work
Silicone	1-Heptanol	2.7±0.20	Synthetic ABE	This work
Silicone	1-Hexanol	3.1±0.49	Synthetic ABE	This work
Silicone	1-Pentanol	3.4±0.11	Synthetic ABE	This work
Silicone	RO Water	1.5±0.15	Synthetic ABE	This work
Silicone	Oleyl Alcohol	1.6±0.23	Synthetic ABE	This work
Regenerated Cellulose	2-Ethyl-1-Hexanol	19.6±1.93	Synthetic ABE	This work
Regenerated Cellulose	Oleyl Alcohol	15.4±3.05	Synthetic ABE	This work
Porous PTFE	2-Ethyl-1-Hexanol	19.1±0.76	Synthetic ABE	This work
Porous PTFE	Oleyl Alcohol	12.2±2.52	Synthetic ABE	This work
Polyamide	2-Ethyl-1-Hexanol	16.3±2.39	Synthetic ABE	This work
Polyamide	Oleyl Alcohol	15.7±3.07	Synthetic ABE	This work
PES	2-Ethyl-1-Hexanol	13.4±3.38	Synthetic ABE	This work
PES	Oleyl Alcohol	16.2±4.79	Synthetic ABE	This work

Surprisingly, in both examples where a hollow fibre polypropylene membrane was used (Grobben *et al.* (1993)) the flux was more in line with that seen by a non-porous silicone membrane rather than a porous membrane. The PTFE membrane used by Tanaka *et al.* (2012) showed dodecanol having a higher flux than oleyl alcohol. Although dodecanol was not tested in this work, 2-ethyl-1-hexanol has a higher flux than oleyl alcohol; once again confirming that using an extractant with a higher partition coefficient will increase the rate of removal of butanol from the system. When using oleyl alcohol as an extractant Tanaka *et al.* (2012) achieved a flux of 37.8 $g\ h^{-1}\ m^{-2}$, this is 3 times greater than the flux achieved using

a synthetic solution. If this is treated as a uniform scaling factor, then with 2-ethyl-1-hexanol extractant a flux of approximately 57 g h^{-1} m^{-2} could be possible. This flux would be greater than that achieved with dodecanol as the extractant, which would be expected due to 2-ethyl-1-hexanol having a higher distribution coefficient than dodecanol (6.09 compared to 5.14). Tanaka *et al.* (2012) did not discuss the possibility of transfer of extractant into the aqueous phase; and they did not measure the dodecanol concentration in the aqueous phase, therefore it is unknown whether any was present at low concentrations. They did state that no toxic effects were seen during the fermentation and they therefore assumed that there was no transfer, which is not a robust assumption. As shown by this work it is unlikely that there was no transfer of dodecanol, but dodecanol may not be toxic to the bacteria at lower concentrations. Tanaka *et al.* (2012) did demonstrate that dodecanol was toxic when 5mL was mixed with 5 mL of actively growing cells, but Roffler *et al.* (1987b) tested the toxicity by mixing 1 mL of dodecanol with 9 mL of actively growing cells and reported that full bacterial growth was observed. It is unlikely that the extractant concentration will reach 10% (v/v), unless the integrity of the membrane is affected, therefore dodecanol could potentially be a considered a non-toxic extractant for perstraction, but toxic for LLE. The toxicity of any extractant proposed for perstraction needs to be tested at ratios that would be seen during ISPR, as these are likely to be around the solubility limit of the extractant rather than the ratios that would be used during LLE. This has the potential to widen the number of possible extractants that can be used for perstraction.

5.7.7: Updated Techno-Economic Analysis

The results have shown that is possible to increase the rate of butanol removal from an aqueous solution by varying the membrane and extractant used. Very little comparison has previously been performed regarding this, as the techno-economic analysis in Chapter 4 used only data available in literature, and the work by Qureshi and Maddox (2005) was used as the baseline for perstraction. It can now be updated, based on the results presented here to understand the impact these changes could have. Based on the results, the porous regenerated cellulose membrane has been considered and both 2-ethyl-1-hexanol and oleyl alcohol have been considered for the extractant.

For perstraction, there are two main factors that affect the process economics:

1) Membrane area – the larger the membrane area, the larger the CAPEX and OPEX (for membrane replacement)
2) Solvent recovery from extractant: the energy required to recover the ABE from the extractant will depend on concentration (Mariano and Filho, 2011), which is a function of the distribution coefficient of the extractant.

The following sections will show how the results in this chapter have the potential to improve the overall process economics.

5.7.7.1 Membrane Area

The required membrane area and associated cost for each scenario was calculated (see Appendix E), based on the butanol flux. Assuming a desired butanol productivity of the system to be 1 g $L^{-1}h^{-1}$ and a fixed fermentation volume of 100 m^3 (as it is similar to the fermentor sizes used for production, as the largest fermentor size for the ABE fermentation, in the USA, was 189,250L (Dürre, 1998)). This productivity was chosen as it is easily achieved by fed-batch fermentations with perstraction and other ISPR techniques, Table 3.6. The associated membrane cost was calculated based on the membrane cost used by Oudshoorn *et al.* (2010) of 250 € m^{-2}. In reality, every membrane material would have a different cost, but as all materials are commercially available it has been assumed that the cost differences between the different materials would be small. This assumption, therefore, allows for an idea of the impact different membrane sizes would have on the costs associated with perstraction.

The results in Table 5.10, compare both silicone and regenerated cellulose membranes with oleyl alcohol and 2-ethyl-1-exanol from this work, along with the perstraction based case, Qureshi and Maddox (2005), from Chapter 4. The silicone/oleyl alcohol experiment in this work has a slightly higher flux than that observed by Qureshi and Maddox (2005). Increasing the butanol flux of the system reduces the required membrane size. Therefore based on butanol flux, moving from a non-porous membrane to a porous membrane could see a reduction in membrane cost by 90% or more. In comparison, the improvement seen by using an extractant with a higher distribution coefficient for butanol is much smaller; with only a 21% cost reduction by switching from using oleyl alcohol to 2-ethyl-1-hexanol, with a regenerated cellulose membrane. However, the difference between oleyl alcohol and 2-ethyl-1-hexanol with a silicone membrane is greater: a 68% reduction in cost.

Table 5.10: Comparison of membrane area and associated costs based on a fermentation productivity of 1 g butanol L^{-1} h^{-1} for this work and that by Qureshi and Maddox (2005).

Membrane	Extractant	Butanol Flux ($gm^{-2}h^{-1}$)	Membrane Area (m^2)	Membrane Cost (Thousand €)	Reference
Silicone	Oleyl Alcohol	1.0	100000	25000	(Qureshi and Maddox, 2005)
Silicone	Oleyl Alcohol	1.5	66667	16667	This work
Silicone	2-Ethyl-1-Hexanol	4.7	21277	5319	This work
Regenerated Cellulose	Oleyl Alcohol	15.4	6494	1623	This work
Regenerated Cellulose	2-Ethyl-1-Hexanol	19.6	5102	1276	This work

5.7.7.2 Impact of Extractant Loss

The experimental results, in section 5.7.3 and 5.7.5, clearly indicate that extractant will be lost to the fermentation broth during perstraction. Replacing the lost extractant will have an impact on the operating cost of the fermentation. Using the same assumptions as in the previous section (5.7.7.1), of a fixed fermentation volume of $100m^3$, the amount of extractant that needs replacing can be calculated. The approximate cost of 2-ethyl-1-hexanol and oleyl alcohol are 1.41 USD L^{-1} and 8.1 USD L^{-1}, respectively (Alibaba, 2018), converted to Euros at an exchange rate of 1 USD=0.8 Euro. The lost extractant concentrations were based on the experimental results presented here. For oleyl alcohol with a silicone membrane, trace amounts were detected by GC, but the concentration was below the limit of detection. Therefore, the limit of detection (0.01 g oleyl alcohol L^{-1}) will be used to represent this loss. Qureshi and Maddox (2005) did not consider the loss of extractant to the fermentation broth, therefore a comparative cost cannot be calculated. The results are shown in Table 5.11.

Table 5.11: Impact of extractant being lost to the fermentation broth, based on the experimental data presented

Membrane	Extractant	Extractant Concentration in Fermentation Broth (g/L)	Volume lost per 100,000m^3 Fermentation Broth	Cost of Lost Extractant (Thousand €)	Reference
Silicone	Oleyl Alcohol	Unknown	Unknown	Unknown	(Qureshi and Maddox, 2005)
Silicone	Oleyl Alcohol	0.01	1176	7623	This work
Silicone	2-Ethyl-1-Hexanol	1.8	216086	245474	This work
Regenerated Cellulose	Oleyl Alcohol	0	0	0	This work
Regenerated Cellulose	2-Ethyl-1-Hexanol	0.7	84033	95462	This work

Even though oleyl alcohol is nearly 6 times more expensive than 2-ethyl-1-hexanol it would be more economic to operate with oleyl alcohol due to its lower solubility in water. When considering this alongside the toxicity results, which show 2-ethyl-1-hexanol would be toxic at these concentrations, it is beneficial to use a non-toxic extractant with as low as possible solubility in water.

5.7.7.3 Updated Process Simulations

The experimental results were used to update the perstraction process simulation from Chapter 4 (Table 4.2), to understand the impact an alternative membrane or extractant would have on the process.

The perstraction separation was based on the split of ABE in the extractant and that left in the aqueous solution. In the work described in this chapter the perstraction was a batch process, therefore the ABE concentration in the aqueous phase was constantly decreasing until equilibrium was reached, whereas in the fermentation by Qureshi and Maddox (2005) ABE was constantly produced by the bacteria, thereby maintaining a relatively constant concentration in the aqueous phase. This difference in method has had an impact on the ABE splits (see Table 5.12 below) for the silicone – oleyl alcohol combination. This decrease in ABE production causes an increase in the energy requirements for the process, highlighting how important an efficient separation is. If the product flow for this work had been the same as that achieved in the simulation based on the work by Qureshi and Maddox

(2005) then the total energy requirements for the plant would have been approximately the same. The difference in splits will also be related to the differences in membrane areas. The membrane area used by Qureshi and Maddox (2005) was 0.113 m^2, which is 20 times larger than the silicone membranes used in this work, Table 5.7, and 90 times larger than the regenerated cellulose membranes.

Table 5.12: Comparison of energy required for different membrane-extractant combinations, based on updated perstraction process simulation, from Chapter 4, using experimental data.

Membrane	Extractant	Permeate Split			Extractant : Feed Ratio	Product Flow (Tonnes/yr)	Energy (MJ/kgABE)			Source
		Acetone	Butanol	Ethanol			Upstream	Downstream	Total	
Silicone	Oleyl Alcohol	0.85	0.45	0.85	0.00066	33284	52.37	26.19	78.56	Qureshi and Maddox (2005)
Silicone	Oleyl Alcohol	0.10	0.21	0.03	0.00031	17053	123.62	39.43	163.06	This work
Silicone	2-Ethyl-1-Hexanol	0.22	0.68	0.14	0.00066	32476	138.49	25.43	163.92	This work
Regenerated Cellulose	Oleyl Alcohol	0.15	0.53	0.12	0.00078	27171	78.10	28.44	106.54	This work
Regenerated Cellulose	2-Ethyl-1-Hexanol	0.28	0.69	0.23	0.00067	33233	89.87	25.16	115.03	This work

Comparing the data in Table 5.12 for this work, it is possible to see that the use of oleyl alcohol led to a lower annual production rate than 2-ethyl-1-hexanol. This is due to the 2-ethyl-1-hexanol having a greater distribution coefficient than oleyl alcohol. This is particularly noticeable in the decrease in the downstream energy requirement. However, the use of 2-ethy-1-hexanol increases the overall energy demand of the process. The decrease in downstream energy for 2-ethyl-1-hexanol compared to oleyl alcohol is not great enough to negate the increase in upstream energy. This increase in upstream energy is because 2-ethyl-1-hexanol is closer to butanol in terms of physical properties such as boiling point and viscosity. This makes the separation of butanol from 2-ethyl-1-hexanol more complex compared to the separation from oleyl alcohol. This increase in energy could indicate that using an extractant such as oleyl alcohol would be more beneficial, particularly as the same rate of extraction could be achieved by increasing the membrane area. The balance of increased energy to additional membrane cost would need to be considered when selecting the extractant.

A bigger change in energy difference is changing from silicone to a regenerated cellulose membrane. This sees the upstream energy decrease by 40-50 MJ kg^{-1} ABE. This is due to the increased product flow due to the higher membrane flux. This will help to decrease the overall process costs in terms of energy and a smaller membrane; providing further evidence that the use of a porous membrane will be beneficial to establishing an economical perstraction-fermentation process.

5.8: Summary

Perstraction was developed to overcome the problems associated with using LLE as an ISPR technique. The main problem of LLE for this fermentation was the toxicity of the extractant to the bacteria. Even though this was a primary aim of perstraction much of the existing perstraction literature focused on oleyl alcohol, which is non-toxic to the bacteria. This work has focused on using toxic extractants, due to their higher distribution coefficients for butanol.

The experiments presented here have shown that extractants that have a higher distribution coefficient can remove butanol from a water-based solution at a faster rate than oleyl alcohol. Also, the selection of a branched extractant has the potential to increase the overall mass transfer compared to its linear equivalent isomer, as demonstrated with 2-ethyl-1-

hexanol having an overall mass transfer coefficient 3 times greater than 1-octanol. A higher rate of extraction would mean a smaller membrane area, reducing the cost associated with perstraction. The downside was that all extractants studied here transferred across the membrane into the aqueous phase at concentrations toxic to the bacteria, thereby removing one of the supposed advantages of perstraction. It is clear, however, that the degree of toxicity significantly varies with concentration. For instance, here it has been demonstrated that 1-pentanol and 1-hexanol improve the fermentation at 0.5 g L^{-1} but are toxic at 5 g L^{-1}. A similar observation can be made based on the literature for dodecanol. It means that some extractants that have distribution coefficients higher than oleyl alcohol are toxic when used for LLE, but still have the potential to be used for perstraction even if there is a small degree of transfer across the membrane.

Further mass transfer experiments into alternative membranes showed porous membranes had an overall mass transfer coefficient of the order of 10 times greater than that of a non-porous membrane. The porous membranes also exhibited a greater resistance to the transfer of oleyl alcohol into the aqueous phase, although the toxic 2-ethyl-1-hexanol still transferred across the membrane. The advantages in extraction rate seen through the use of a porous membrane are greater than those observed from changing from oleyl alcohol to a toxic extractant such as 2-ethy-1-hexanol. Therefore, it could be possible to further improve the economics of perstraction by using a porous membrane, such as regenerated cellulose, compared to the non-porous silicone.

Chapter 6. Conclusion

The overall aim of this research project was to investigate and develop an *in situ* product recovery technique for application to commercial scale ABE fermentation. The chosen technique should be able to increase the product stream concentration, whilst improving the energy efficiency and economics of the fermentation process.

The main conclusions of this project are summarised below, alongside the main objectives set in Chapter 1. The results matching each successive objective were used to inform the decisions made when meeting the next objective.

6.1: Assessment of Existing ISPR research

The first objective was to assess existing ISPR research, in particular for the ABE fermentation, and to determine whether any technique was in a position to be scaled up for commercial application. Work towards this objective was presented in Chapters 2 and 3.

The conclusions from this work are:

- Fourteen ISPR techniques have been proposed for integration with the ABE fermentation. Unfortunately, none technique has been fully tested or demonstrated to meet all the requirements outlined in section 2.3. This means that there is obvious technique for scale up without further research into applying the technique to the ABE fermentation.
- An assessment of the techniques that have been integrated with the fermentation (gas stripping, vacuum fermentation, pervaporation, liquid-liquid extraction, perstraction and adsorption), demonstrated that ISPR has the capability to improve the fermentation metrics, such as yield, productivity and substrate consumption, as long as the technique does not have a negative impact (e.g. toxic) on the microorganism. As no technique shows a clear dominance in terms of fermentation performance, other factors such as energy demand and economic analysis are required to provide a more insightful quantitative comparison of the different ISPR techniques.
- All of the experimental techniques have only been assessed based on laboratory scale operation. Many of the techniques have not considered how to recover the

product from the separating agent. Unless product capture is considered alongside fermentation integration none of these techniques will be applicable for scale-up.

- As stated previously, development of the techniques has focused on laboratory scale operation; for some of these methods direct scale up is not possible. For example the direct addition of an extractant or adsorbent to the fermentation broth, would not be realistic. Instead, once proof of concept has been demonstrated, research needs to progress to consider scaled down versions of industrial based methods. It must be acknowledged this is a complex task as suitable equipment for most scale down designs does not exist in an "off the shelf" manner.
- Gas stripping, although it looks like an attractive technique at laboratory scale, is not as simple when scaling up. A detailed engineering analysis of the system is required but the compression of fermentation gasses, which includes hydrogen is likely to present a health and safety risk. Combining this with the inability for complete product capture as the current laboratory methods, which can include a liquid nitrogen trap would not be practical at production scale. Most of the new ABE ISPR research still revolves around gas stripping, with researchers stating it is suitable for scale up. Researchers need to address some of these fundamental problems, rather than the current research which involves making the gas stripping process more complex through hybrid-ISPR techniques. Rather than focus on gas stripping as an ISPR for a production process, it should be used as a tool to improve fermentation characteristics

6.2: Techno-economic analysis of ISPR techniques for the ABE fermentation

The second objective developed directly from the outcomes of the literature assessment. By performing a techno-economic analysis of ISPR techniques for the ABE fermentation, through process simulations, comparative information on the separation efficiency and energy impact of each technique was determined, and provided sufficient data for a first stage economic analysis (Chapter 4). The gaps in existing literature highlighted by Van Hecke *et al.* (2014) could be filled in, hence a more complete picture of applying ISPR to the ABE fermentation was developed. Therefore, it was possible to gain a better understanding of the impact these techniques could have on commercial scale processes. The results of the techno-economic analysis were used to select the best technique to carry forward for further investigation.

Seven ISPR techniques were considered: gas stripping, vacuum fermentation, flash separation, pervaporation, LLE, perstraction and adsorption. They were compared to a traditional batch process. The key findings from this work are:

- In the simulations, all techniques other than gas stripping and flash fermentation were able to achieve the minimum separation effectiveness required to maintain a butanol concentration below 5 g L^{-1}, therefore below inhibitory levels to the microorganism. This, generally, supports the experimental findings in literature whereby all techniques saw an improvement in fermentation metrics. Gas stripping, also saw an improvement in fermentation metrics in experimental-based literature but was plagued with product capture inefficiencies. These inefficiencies were apparent in the simulations through a separation effectiveness below the minimum requirement. This is further evidence to support that gas stripping is not as an ideal technique for ISPR, unlike its reputation in literature.
- A reduction in downstream energy, compared to a traditional batch ABE plant, was observed for all techniques other than gas stripping. This confirms one of the fundamental aims of ISPR, to reduce the downstream energy demand as this was the second largest operating cost after feedstock costs.
- The simulation results showed that the upstream energy demand increased compared to the batch process. This initial separation is now occurring at a low product concentration (~5-8 g ABE L^{-1}) rather than end of fermentation concentrations (~15-18 g ABE L^{-1}). This increases the energy required for separation, and therefore the energy demand for the whole process. This means that researchers should focus on process optimisation, rather than developing new, more complex ISPR methods. The techniques assessed in this thesis, have all been successfully demonstrated to improve the fermentation, and see a reduction in downstream energy (other than gas stripping). The only technique that has demonstrated process optimisation, beyond investigating different separating agents, is gas stripping which has been demonstrated to be one of the least applicable techniques for scale up.
- The techno-economic analysis identified perstraction as the best technique for integrating with the ABE fermentation. This is largely based on perstraction being the only technique to have a reduction in overall plant energy demand, compared to a traditional batch process. Perstraction is one of the least developed techniques of

those investigated in the techno-economic analysis, as shown by an assessment of its TRL in Chapter 2. Research into perstraction had shown it to be successful at removing butanol from the fermentation broth, but the research has not progressed beyond a “proof of concept” stage. Meaning, there was no optimisation and limited or no rational decisions behind the operating parameters (extractant and membrane choice). This led to the research performed in Chapter 5.

6.3: Experimental development of chosen technique – perstraction

Perstraction is a membrane extraction technique, whereby the product is transferred across a membrane into an extractant. It was developed to overcome problems observed in LLE, such as product toxicity and emulsion formation. Research prior to this study had predominantly focused on extractant selection, but the main extractant tested was oleyl alcohol, this was the extractant of choice for LLE and non-toxic to the bacteria, therefore no significant advantage of perstraction had been experimentally demonstrated compared to LLE.

In this thesis, research into perstraction was developed by focusing on developments that would be compatible with being integrated at a commercial scale. This included using commercially available membranes, rather than bespoke membranes used in recent academic studies. Another focus of this research was the use of toxic extractants, as they typically have a higher distribution coefficient than non-toxic extractants. A higher distribution coefficient should increase the extraction rate, which was thought to be controlled by the concentration (or chemical potential) difference between the fermentation broth and extractant.

The key findings in this thesis are:

- In previous literature, extractant selection had been based on the distribution coefficient of butanol in the extractant, with a larger distribution coefficient leading to faster mass transfer. This is generally true but other factors are important, such as chemical structure, with regards to mass transfer with perstraction. This is because 2-ethyl-1-hexanol had a mass transfer coefficient approximately 3 times larger than the next fastest toxic extractant, 1-pentanol, which has a larger distribution coefficient of 7.5 compared to 6.1 for 2-ethyl-1-hexanol. Also, the overall mass transfer coefficient for water being the second largest of all the extractants tested. This indicates that

while work to date has focused on selecting extractants based on the distribution coefficient, it might be a good starting point, but it should not be the sole factor used for perstraction, as it has been in previous research.

- This research demonstrated that all the extractants tested, including oleyl alcohol, transferred across the silicone membrane into the aqueous phase. For the toxic extractants, the concentration would have inhibited the bacteria, as demonstrated by the toxicity tests. As well as transferring across the membrane the extractant formed a stable emulsion with the aqueous solution, therefore two of the proposed advantages of perstraction are no longer valid.
- Until this study, there had been no comprehensive comparison of membrane types. The most noticeable effect was that porous membranes can increase the overall mass transfer coefficient by as much as an order of magnitude. This would reduce membrane costs by a factor of at least 10.
- With all membranes, the extractant leached into the aqueous phase, but with porous membranes the concentrations present never exceeded the saturation level and an emulsion did not form. This is still not ideal, as the back extraction will substantially limit the use of toxic extractants. However, it does mean that extractants that are non-toxic below the saturation concentration could be used.
- This study confirmed the need to use a hydrophobic membrane. As hydrophobic membrane would allow the pores to be filled with extractant, while a hydrophilic membrane would be filled with the fermentation broth. The hydrophilic membranes had a larger mass transfer coefficient, but allowed the aqueous solution to pass through the membrane into the extractant. In practice this would not be ideal as it would contaminate the extractant and cause extractant regeneration problems.
- Only batch perstraction experiments were performed. These current methods would not be suitable for scale up. Equipment design for a continuous, scaled process needs to be considered. This includes a continuous perstraction-fermentation recirculation, which will maximise the concentration difference between the fermentation broth and extractant, as well as integrating extractant regeneration into the process.

Perstraction is still in its early stages of development for integration with the ABE fermentation. This research has built upon existing knowledge that perstraction has the ability to remove ABE from the fermentation. This research has demonstrated that

perstraction is a good candidate to be used as an ISPR technique for the ABE fermentation in its own right, rather than building upon LLE knowledge.

6.4: Future Work

Based on the work in this thesis, there is scope for further work to better understand ISPR and its relationship to the ABE fermentation and, in particular, the role perstraction plays in this:

1. A comparison between free-cell and immobilised fermentations with ISPR is required, to understand the differences between the two fermentation modes and the advantages of each technique. This work primarily focused on free-cell fermentations so as to quantitatively compare the impact of ISPR. An immobilised fermentation can increase the range of possible operating parameters for ISPR, therefore a comparison between fermentation operating modes with ISPR could help direct future research.
2. Since the commencement of this work there has been a growing research interest in hybrid or two-stage ISPR processes. A comparative techno-economic analysis, similar to that performed in this work, is required to understand how hybrid processes compare to single-stage techniques and perstraction.
3. To further understand the key factors when selecting an extractant for perstraction. This work could be developed by investigating a wider range of branched extractants, to better understand how they affect the rate of extraction. This should include 2-butyl-1-octanol, which has recently been suggested as a possible alternative to oleyl alcohol for LLE (González-Peñas *et al.*, 2014b).
4. To progress perstraction, a scalable system needs to be developed.
   This could be done in collaboration with a commercial membrane supplier to achieve both best membrane operation and extraction functions.
5. This work has demonstrated that perstraction is unlikely to be successful with a toxic extractant and commercially available membranes. It would be beneficial to complete a parallel LLE and perstraction study with the same extractant and fermentation conditions. This would help to provide more information on the true advantages, if any, of perstraction compared to LLE from an experimental perspective.

6. Further investigation in to the use of water as an extractant. The initial results show water with a silicone membrane to be more favourable than oleyl alcohol. The downside of this is that the use of water as an extractant would not provide a more concentrated ABE solution. However, the use of water would remove any toxicity issues and would be cheaper than an organic extractant. With the growing increase in hybrid ISPR methods, maybe this could provide an alternative first stage.

References

Abdehagh, N., Tezel, F. H. and Thibault, J. (2014) 'Separation techniques in butanol production: Challenges and developments', *Biomass and Bioenergy*, 60: 222-246.

Abdi, H. K., Alanazi, K. F., Rohani, A. S., Mehrani, P. and Thibault, J. (2016) 'Economic comparison of a continuous ABE fermentation with and without the integration of an in situ vacuum separation unit', *The Canadian Journal of Chemical Engineering*, 94: 833-843.

Abels, C., Carstensen, F. and Wessling, M. (2013) 'Membrane processes in biorefinery applications', *Journal of Membrane Science*, 444: 285-317.

Alibaba (2018) *Chinese E-commerce*. Available at: https://www.alibaba.com/ (Last Accessed: 27/03/2018).

Bankar, S. B., Survase, S. A., Ojamo, H. and Granström, T. (2013) 'The two stage immobilized column reactor with an integrated solvent recovery module for enhanced ABE production', *Bioresource Technology*, 140: 269-276.

Bankar, S. B., Survase, S. A., Singhal, R. S. and Granstrom, T. (2012) 'Continuous two stage acetone-butanol-ethanol fermentation with integrated solvent removal using Clostridium acetobutylicum B 5313', *Bioresource Technology*, 106: 110-116.

Barton, W. E. and Daugulis, A. (1992) 'Evaluation of solvents for extractive butanol fermentation with Clostridium acetobutylicum and the use of poly(propylene glycol) 1200', *Applied Microbiology and Biotechnology*, 36: 632-639.

Blahušiak, M., Marták, J., Miranda, F., Schlosser, Š. and Teixeira, J. A. (2013) 'Effect of viscosity of a liquid membrane containing oleyl alcohol on the pertraction of butyric acid', *Chemical Papers*, 67: 1560-1568.

Boontawan, A. and Stuckey, D. C. (2005) 'Mass transfer of terpenes through a silicone rubber membrane in a liquid-liquid contacting system', *Biotechnology Progress*, 21: 1680-1687.

Bruce, L. J. and Daugulis, A. J. (1991) 'Solvent selection strategies for extractive biocatalysis', *Biotechnology Progress*, 7: 116-124.

Cai, D., Zhang, T., Zheng, J., Chang, Z., Wang, Z., Qin, P.-y. and Tan, T.-w. (2013) 'Biobutanol from sweet sorghum bagasse hydrolysate by a hybrid pervaporation process', *Bioresource Technology*, 145: 97-102.

Cascon, H. R., Choudhari, S. K., Nisola, G. M., Vivas, E. L., Lee, D.-J. and Chung, W.-J. (2011) 'Partitioning of butanol and other fermentation broth components in phosphonium and ammonium-based ionic liquids and their toxicity to solventogenic clostridia', *Separation and Purification Technology*, 78: 164-174.

Cen, P. and Tsao, G. T. (1993) 'Recent advances in the simultaneous bioreaction and product separation processes', *Separations Technology*, 3: 58-75.

Chang, Y.-C., Lee, W.-J., Wu, T. S., Wu, C.-Y. and Chen, S.-J. (2014) 'Use of water containing acetone–butanol–ethanol for NOx-PM (nitrogen oxide-particulate matter) trade-off in the diesel engine fueled with biodiesel', *Energy*, 64: 678-687.

Chen, H.-Z., Liu, Z.-H. and Dai, S.-H. (2014a) 'A novel solid state fermentation coupled with gas stripping enhancing the sweet sorghum stalk conversion performance for bioethanol', *Biotechnology for Biofuels*, 7: 1-13.

Chen, Y., Ren, H., Liu, D., Zhao, T., Shi, X., Cheng, H., Zhao, N., Li, Z., Li, B., Niu, H., Zhuang, W., Xie, J., Chen, X., Wu, J. and Ying, H. (2014b) 'Enhancement of n-butanol production by in situ butanol removal using permeating-heating-gas stripping in acetone-butanol-ethanol fermentation', *Bioresource Technology*, 164: 276-284.

Chiao, J. and Sun, Z. (2007) 'History of the Acetone-Butanol-Ethanol Fermentation Industry in China: Development of Continuous Production Technology', *Journal of Molecular Microbiology and Biotechnology*, 13: 12-14.

Cho, C.-W. and Hwang, S.-T. (1991) 'Continuous membrane fermentor separator for ethanol fermentation', *Journal of Membrane Science*, 57: 21-42.

Contag, P. R. (2008) *NREL 21st industry growth forum*. Available at: http://www.nrel.gov/technologytransfer/pdfs/igf21_cobalt_biofuels.pdf (Last Accessed: 14/01/2014).

Cousin Saint Remi, J., Baron, G. and Denayer, J. (2012) 'Adsorptive separations for the recovery and purification of biobutanol', *Adsorption*, 18: 367-373.

Dadgar, A. M. and Foutch, G. L. (1985) 'Evaluation of solvents for the recovery of *Clostridium* fermentation products by liquid-liquid extraction', *Biotechnology and Bioengineering Symposium*, 15: 619-620.

Daugulis, A. J. (1988) 'Integrated reaction and product recovery in bioreactor systems', *Biotechnology Progress*, 4: 113-122.

Davies, E. T. (2013) 'Green Biologics Ltd.: Commercialising bio-n-butanol', *Green Processing and Synthesis*, 2: 273-276.

Davison, B. H. and Thompson, J. E. (1993) 'Continuous direct solvent extraction of butanol in a fermenting fluidized-bed bioreactor with immobilized *Clostridium acetobutylicum*', *Applied Biochemistry and Biotechnology*, 39-40: 415-426.

Day, D. F., Hoogewind, A. K., Randhava, S. S., Oswald, J., Madsen, L. and Kim, M. (2013) *Method for producing butanol and isopropanol*. United States Patent and Trademark Office US2013/0149757. [Online]. Available at: https://www.google.com/patents/US20130149757.

De Francesco, G., Freeman, G., Lee, E., Marconi, O. and Perretti, G. (2014) 'Effects of Operating Conditions during Low-Alcohol Beer Production by Osmotic Distillation', *Journal of Agricultural and Food Chemistry*, 62: 3279-3286.

de Vrije, T., Budde, M., van der Wal, H., Claassen, P. A. M. and López-Contreras, A. M. (2013) '"*In situ*" removal of isopropanol, butanol and ethanol from fermentation broth by gas stripping', *Bioresource Technology*, 137: 153-159.

Dhamole, P. B., Wang, Z., Liu, Y., Wang, B. and Feng, H. (2012) 'Extractive fermentation with non-ionic surfactants to enhance butanol production', *Biomass and Bioenergy*, 40: 112-119.

Diban, N., Athes, V., Bes, M. and Souchon, I. (2008) 'Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor', *Journal of Membrane Science*, 311: 136-146.

Dürre, P. (1998) 'New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation', *Applied Microbiology and Biotechnology*, 49: 639-648.

Dürre, P. (2007) 'Biobutanol: An attractive biofuel', *Biotechnology Journal*, 2: 1525-1534.

Dürre, P. (2008) 'Fermentative butanol production: bulk chemical and biofuel', *Annals of the New York Academy of Sciences*, 1125: 353-362.

Dürre, P. (2011) 'Fermentative production of butanol - the academic perspective', *Current Opinion in Biotechnology*, 22: 331-336.

EARTO (2014) *The TRL scale as a R I policy tool EARTO recommendations final*. [Online]. Available at: http://www.earto.eu/fileadmin/content/03_Publications/The_TRL_Scale_as_a_R_I_Policy_Tool_-_EARTO_Recommendations_-_Final.pdf (Accessed: 16/05/2016).

Eckert, G. and Schügerl, K. (1987) 'Continuous acetone-butanol production with direct product removal', *Applied Microbiology and Biotechnology*, 27: 221-228.
Elfasakhany, A. (2016) 'Performance and emissions analysis on using acetone–gasoline fuel blends in spark-ignition engine', *Engineering Science and Technology, an International Journal*, 19: 1224-1232.
Ennis, B. M., Marshall, C. T., Maddox, I. S. and Paterson, A. H. J. (1986) 'Continuous product recovery by in-situ gas stripping/condensation during solvent production from whey permeate using *Clostridium acetobutylicum*', *Biotechnology Letters*, 8: 725-730.
Ennis, B. M., Qureshi, N. and Maddox, I. S. (1987) 'In-line toxic product removal during solvent production by continuous fermentation using immobilized *Clostridium acetobutylicum*', *Enzyme and Microbial Technology*, 9: 672-675.
Eom, M.-H., Kim, W., Lee, J., Cho, J.-H., Seung, D., Park, S. and Lee, J. H. (2012a) 'Modeling of a biobutanol adsorption process for designing an extractive fermentor', *Industrial & Engineering Chemistry Research*, 52: 603-611.
Eom, M.-H., Kim, W., Lee, J., Lee, J. H. and Park, S. (2012b) 'Kinetic studies on biobutanol recovery process using adsorbent resin', *IFAC Proceedings Volumes*, 45: 125-130.
European Commision (2012) *Innovating for Sustainable Growth: A Bioeconomy for Europe*. European Union.
Evanko, W. A., Eyal, A. M., Glassner, D. A., Miao, F., Aristidou, A. A., Evans, K., R.Gruber, P. and Hawkins, A. C. (2012) *Recovery of higher alcohols from dilute aqueous solution*. United States Patent and Trademark Office US8101808 B2.
Evans, P. J. and Wang, H. Y. (1988a) 'Enhancement of butanol formation by *Clostridium acetobutylicum* in the presence of decanol-oleyl alcohol mixed extractants', *Applied and Environmental Microbiology*, 54: 1662-1667.
Evans, P. J. and Wang, H. Y. (1988b) 'Response ofClostridium Acetobutylicum to the presence of mixed extractants', *Applied Biochemistry and Biotechnology*, 17: 175-192.
Ezeji, T., Milne, C., Price, N. and Blaschek, H. (2010) 'Achievements and perspectives to overcome the poor solvent resistance in acetone and butanol-producing microorganisms', *Applied Microbiology and Biotechnology*, 85: 1697-1712.
Ezeji, T., Qureshi, N. and Blaschek, H. (2007a) 'Production of acetone butanol (AB) from liquefied corn starch, a commercial substrate, using *Clostridium beijerinckii* coupled with product recovery by gas stripping', *Journal of Industrial Microbiology & Biotechnology*, 34: 771-777.
Ezeji, T., Qureshi, N. and Blaschek, H. (2013) 'Microbial production of a biofuel (acetone–butanol–ethanol) in a continuous bioreactor: Impact of bleed and simultaneous product removal', *Bioprocess and Biosystems Engineering*, 36: 109-116.
Ezeji, T. C., Karcher, P. M., Qureshi, N. and Blaschek, H. P. (2005) 'Improving performance of a gas stripping-based recovery system to remove butanol from *Clostridium beijerinckii* fermentation', *Bioprocess and Biosystems Engineering*, 27: 207-214.
Ezeji, T. C., Qureshi, N. and Blaschek, H. P. (2003) 'Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and *in situ* recovery by gas stripping', *World Journal of Microbiology and Biotechnology*, 19: 595-603.
Ezeji, T. C., Qureshi, N. and Blaschek, H. P. (2004a) 'Acetone butanol ethanol (ABE) production from concentrated substrate: Reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping', *Applied Microbiology and Biotechnology*, 63: 653-658.
Ezeji, T. C., Qureshi, N. and Blaschek, H. P. (2004b) 'Butanol fermentation research: Upstream and downstream manipulations', *The Chemical Record*, 4: 305-314.

Ezeji, T. C., Qureshi, N. and Blaschek, H. P. (2007b) 'Bioproduction of butanol from biomass: from genes to bioreactors', *Current Opinion in Biotechnology*, 18: 220-227.

Ferras, E., Minier, M. and Goma, G. (1986) 'Acetonobutylic fermentation: Improvement of performances by coupling continuous fermentation and ultrafiltration', *Biotechnology and Bioengineering*, 28: 523-533.

Frank, O. (2000) 'Solvent Recovery, Condensation', in *Kirk-Othmer Encyclopedia of Chemical Technology*. John Wiley & Sons, Inc.

Freeman, A., Woodley, J. M. and Lilly, M. D. (1993) '*In-situ* product removal as a tool for bioprocessing', *Bio-Technology*, 11: 1007-1012.

Friedl, A., Qureshi, N. and Maddox, I. S. (1991) 'Continuous acetone-butanol-ethanol (ABE) fermentation using immobilized cells of *Clostridium acetobutylicum* in a packed bed reactor and integration with product removal by pervaporation', *Biotechnology and Bioengineering*, 38: 518-527.

Gapes, J. R., Nimcevic, D. and Friedl, A. (1996) 'Long-term continuous cultivation of *Clostridium beijerinckii* in a two-stage chemostat with on-line solvent removal', *Applied and Environmental Microbiology*, 62: 3210-3219.

Garcia-Chavez, L. Y., Garsia, C. M., Schuur, B. and de Haan, A. B. (2012) 'Biobutanol recovery using nonfluorinated task-specific ionic liquids', *Industrial & Engineering Chemistry Research*, 51: 8293-8301.

Garcia, A., Iannotti, E. L. and Fischer, J. L. (1986) 'Butanol fermentation liquor production and separation by reverse osmosis', *Biotechnology and Bioengineering*, 28: 785-791.

García, V., Päkkilä, J., Ojamo, H., Muurinen, E. and Keiski, R. L. (2011) 'Challenges in biobutanol production: How to improve the efficiency?', *Renewable and Sustainable Energy Reviews*, 15: 964-980.

GE Healthcare (2016) 'WhatmanTM Price Catalog'.

Gevo Inc (2013) *Gevo brings second million-liter fermeneter and GIFT® system online*. Available at: http://ir.gevo.com/phoenix.zhtml?c=238618&p=irol-newsArticle&ID=1843687&highlight= (Last Accessed: 14/01/2013).

Gevo Inc. (2017) *Gevo: Our Markets*. Available at: http://www.gevo.com/our-markets/ (Last Accessed: 13/06/2017).

Gibbs, D. F. (1983) 'The rise and fall (… and rise?) of acetone/butanol fermentations', *Trends in Biotechnology*, 1: 12-15.

Golden, J. S., Handfield, R. B., Daystar, J. and McConnell, T. E. (2015) *An economic impact analysis of the U.S. Biobased products industry. A report to the Congress of the United States of America*. A Joint Publication of the Duke Center for Sustainability & Commerce and the Supply Chain Resource Cooperative at North Carolina State University.

González-Bravo, R., Sánchez-Ramírez, E., Quiroz-Ramírez, J. J., Segovia-Hernández, J. G., Lira-Barragán, L. F. and Ponce-Ortega, J. M. (2016) 'Total Heat Integration in the Biobutanol Separation Process', *Industrial & Engineering Chemistry Research*, 55: 3000-3012.

González-Peñas, H., Lu-Chau, T., Moreira, M. and Lema, J. (2014a) 'Assessment of morphological changes of Clostridium acetobutylicum by flow cytometry during acetone/butanol/ethanol extractive fermentation', *Biotechnology Letters*, 37: 1-8.

González-Peñas, H., Lu-Chau, T. A., Moreira, M. T. and Lema, J. M. (2014b) 'Solvent screening methodology for *in situ* ABE extractive fermentation', *Applied Microbiology and Biotechnology*, 98: 5915-5924.

Grady, M. C., Jahic, M. and Patnaik, R. (2009) *A method for producing butanol using two-phase extractive fermentation*. World Intellectual Property Organisation PCT/US2009/046278.

Green Biologics Ltd (2014) *Technology*. Available at: http://www.greenbiologics.com/technology.php (Last Accessed: 28/01/2016).

Green, E., Simms, R. P., Lalander, C. A. M. and Dominguez-Espinosa, R. M. (2014) *Solvent production*. World Intellectual Property Organization PCT/GB2014/051972. [Online]. Available at: http://www.google.com/patents/WO2014207480A2?cl=zh.

Green, E. M. (2011) 'Fermentative production of butanol - the industrial perspective', *Current Opinion in Biotechnology*, 22: 337-343.

Grobben, N., Eggink, G., Petrus Cuperus, F. and Huizing, H. (1993) 'Production of acetone, butanol and athanol (ABE) from potato wastes: Fermentation with integrated membrane extraction', *Applied Microbiology and Biotechnology*, 39: 494-498.

Groot, W. J., Baart de la Faille, T., Donck, P. D., van der Lans, R. G. J. M. and Luyben, K. C. A. M. (1991) 'Mass transfer in silicone rubber membranes for the recovery of fermentation products by pervaporation and perstraction', *Bioseparation*, 2: 261-277.

Groot, W. J., Lans, R. G. J. M. and Luyben, K. C. A. M. (1989) 'Batch and continuous butanol fermentations with free cells: Integration with product recovery by gas-stripping', *Applied Microbiology and Biotechnology*, 32: 305-308.

Groot, W. J. and Luyben, K. C. A. M. (1986) '*In situ* product recovery by adsorption in the butanol/isopropanol batch fermentation', *Applied Microbiology and Biotechnology*, 25: 29-31.

Groot, W. J., Qever, C. E. and Kossen, N. W. F. (1984a) 'Pervaporation for simultaneous product recovery in the butanol/isopropanol batch fermentation', *Biotechnology Letters*, 6: 709-714.

Groot, W. J., Schoutens, G. H., Beelen, P. N., Oever, C. E. and Kossen, N. W. F. (1984b) 'Increase of substrate conversion by pervaporation in the continuous butanol fermentation', *Biotechnology Letters*, 6: 789-792.

Groot, W. J., Soedjak, H. S., Donck, P. B., Lans, R. G. J. M., Luyben, K. C. A. M. and Timmer, J. M. K. (1990) 'Butanol recovery from fermentations by liquid-liquid extraction and membrane solvent extraction', *Bioprocess Engineering*, 5: 203-216.

Groot, W. J., van der Lans, R. G. J. M. and Luyben, K. C. A. M. (1992) 'Technologies for butanol recovery integrated with fermentations', *Process Biochemistry*, 27: 61-75.

Ha, S. H., Mai, N. L. and Koo, Y.-M. (2010) 'Butanol recovery from aqueous solution into ionic liquids by liquid-liquid extraction', *Process Biochemistry*, 45: 1899-1903.

Heerema, L., Roelands, M., Goetheer, E., Verdoes, D. and Keurentjes, J. (2011a) '*In-situ* product removal from fermentations by membrane extraction: Conceptual process design and economics', *Industrial & Engineering Chemistry Research*, 50: 9197-9208.

Heerema, L., Roelands, M., Hanemaaijer, J. H., de Bont, J. and Verdoes, D. (2006) '*In-situ* phenol removal from fermentation broth by pertraction', *Desalination*, 200: 485-487.

Heerema, L., Wierckx, N., Roelands, M., Hanemaaijer, J. H., Goetheer, E., Verdoes, D. and Keurentjes, J. (2011b) '*In situ* phenol removal from fed-batch fermentations of solvent tolerant Pseudomonas putida S12 by pertraction', *Biochemical Engineering Journal*, 53: 245-252.

Huang, H.-J., Ramaswamy, S. and Liu, Y. (2014) 'Separation and purification of biobutanol during bioconversion of biomass', *Separation and Purification Technology*, 132: 513-540.

Ishii, S., Taya, M. and Kobayashi, T. (1985) 'Production of butanol by *Clostridium acetobutylicum* in extractive fermentation system', *Journal of Chemical Engineering of Japan*, 18: 125-130.

Ishizaki, A., Michiwaki, S., Crabbe, E., Kobayashi, G., Sonomoto, K. and Yoshino, S. (1999) 'Extractive acetone-butanol-ethanol fermentation using methylated crude palm oil as extractant in batch culture of *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564)', *Journal of Bioscience and Bioengineering*, 87: 352-356.

Izák, P., Schwarz, K., Ruth, W., Bahl, H. and Kragl, U. (2008) 'Increased productivity of *Clostridium acetobutylicum* fermentation of acetone, butanol, and ethanol by pervaporation through supported ionic liquid membrane', *Applied Microbiology and Biotechnology*, 78: 597-602.

Jeon, Y. J. and Lee, Y. Y. (1987) 'Membrane-assisted extractive butanol fermentation', *Annals of the New York Academy of Sciences*, 506: 536-542.

Jeon, Y. J. and Lee, Y. Y. (1989) '*In situ p*roduct separation in butanol fermentation by membrane-assisted extraction', *Enzyme and Microbial Technology*, 11: 575-582.

Jiang, Y., Liu, J., Jiang, W., Yang, Y. and Yang, S. (2015) 'Current status and prospects of industrial bio-production of n-butanol in China', *Biotechnology Advances*, 33: 1493-1501.

Jin, C., Yao, M., Liu, H., Lee, C.-f. F. and Ji, J. (2011) 'Progress in the production and application of n-butanol as a biofuel', *Renewable and Sustainable Energy Reviews*, 15: 4080-4106.

Jones, D. T. and Woods, D. R. (1986) 'Acetone-butanol fermentation revisited', *Microbiological Reviews*, 50: 484-524.

Kaufman, B. D., Walther, D. C. and Contag, P. R. (2010) *Multistage vapor compression distillation*. World Intellectual Property Organisation WO/2010/126848

Keis, S., Shaheen, R. and Jones, D. T. (2001) 'Emended descriptions of *Clostridium acetobutylicum* and *Clostridium beijerinckii*, and descriptions of *Clostridium saccharoperbutylacetonicum* sp. nov. and *Clostridium saccharobutylicum* sp. nov', *International Journal of Systematic and Evolutionary Microbiology*, 51: 2095-2103.

Kiani, A., Bhave, R. R. and Sirkar, K. K. (1984) 'Solvent extraction with immobilized interfaces in a microporous hydrophobic membrane', *Journal of Membrane Science*, 20: 125-145.

Kim, J., Iannotti, E. and Bajpai, R. (1999) 'Extractive recovery of products from fermentation broths', *Biotechnology and Bioprocess Engineering*, 4: 1-11.

Kim, Y.-J. and Weigand, W. (1992) 'Experimental analysis of a product inhibited fermentation in an aqueous two-phased system', *Applied Biochemistry and Biotechnology*, 34-35: 419-430.

Kumar, M., Goyal, Y., Sarkar, A. and Gayen, K. (2012) 'Comparative economic assessment of ABE fermentation based on cellulosic and non-cellulosic feedstocks', *Applied Energy*, 93: 193-204.

Kurkijärvi, A., Lehtonen, J. and Linnekoski, J. (2014) 'Novel dual extraction process for acetone-butanol-ethanol fermentation', *Separation and Purification Technology*, 124: 18-25.

Larrayoz, M. A. and Puigjaner, L. (1987) 'Study of butanol extraction through pervaporation in acetobutylic fermentation', *Biotechnology and Bioengineering*, 30: 692-696.

Lee, S.-H., Eom, M.-H., Kim, S., Kwon, M.-A., Choi, J.-D.-R., Kim, J., Shin, Y.-A. and Kim, K. H. (2015) 'Ex situ product recovery and strain engineering of Clostridium acetobutylicum for enhanced production of butanol', *Process Biochemistry*, 50: 1683-1691.

Lee, S. H., Eom, M. H., Choi, J. D., Kim, S., Kim, J., Shin, Y. A. and Kim, K. H. (2016) 'Ex situ product recovery for enhanced butanol production by Clostridium beijerinckii', *Bioprocess Biosyst Eng*, 39: 695-702.

Li, J., Chen, X., Qi, B., Luo, J., Zhang, Y., Su, Y. and Wan, Y. (2014) 'Efficient production of acetone-butanol-ethanol (ABE) from cassava by a fermentation-pervaporation coupled process', *Bioresource Technology*, 169: 251-257.

Li, Q., Cai, H., Hao, B., Zhang, C., Yu, Z., Zhou, S. and Chenjuan, L. (2010) 'Enhancing clostridial acetone-butanol-ethanol (ABE) production and improving fuel properties of ABE-enriched biodiesel by extractive fermentation with biodiesel', *Applied Biochemistry and Biotechnology*, 162: 2381-2386.

Lin, X., Wu, J., Fan, J., Qian, W., Zhou, X., Qian, C., Jin, X., Wang, L., Bai, J. and Ying, H. (2012a) 'Adsorption of butanol from aqueous solution onto a new type of macroporous adsorption resin: Studies of adsorption isotherms and kinetics simulation', *Journal of Chemical Technology & Biotechnology*, 87: 924-931.

Lin, X., Wu, J., Jin, X., Fan, J., Li, R., Wen, Q., Qian, W., Liu, D., Chen, X., Chen, Y., Xie, J., Bai, J. and Ying, H. (2012b) 'Selective separation of biobutanol from acetone–butanol–ethanol fermentation broth by means of sorption methodology based on a novel macroporous resin', *Biotechnology Progress*, 28: 962-972.

Liu, D., Chen, Y., Ding, F. Y., Zhao, T., Wu, J. L., Guo, T., Ren, H. F., Li, B. B., Niu, H. Q., Cao, Z., Lin, X. Q., Xie, J. J., He, X. J. and Ying, H. J. (2014) 'Biobutanol production in a Clostridium acetobutylicum biofilm reactor integrated with simultaneous product recovery by adsorption', *Biotechnol Biofuels*, 7: 5-18.

Liu, G., Wei, W. and Jin, W. (2013a) 'Pervaporation membranes for biobutanol production', *ACS Sustainable Chemistry & Engineering*, 2: 546-560.

Liu, H., Wang, G. and Zhang, J. (2013b) 'The Promising Fuel-Biobutanol', in Fang, Z. (ed.) *Liquid, Gaseous and Solid Biofuels - Conversion Techniques*. Rijeka: InTech.

Liu, J., Fan, L. T., Seib, P., Friedler, F. and Bertok, B. (2004) 'Downstream process synthesis for biochemical production of butanol, ethanol, and acetone from grains: Generation of optimal and near-optimal flowsheets with conventional operating units', *Biotechnology Progress*, 20: 1518-1527.

Lu, C., Dong, J. and Yang, S.-T. (2013) 'Butanol production from wood pulping hydrolysate in an integrated fermentation-gas stripping process', *Bioresource Technology*, 143: 467-475.

Lu, C., Zhao, J., Yang, S.-T. and Wei, D. (2012) 'Fed-batch fermentation for n-butanol production from cassava bagasse hydrolysate in a fibrous bed bioreactor with continuous gas stripping', *Bioresource Technology*, 104: 380-387.

Lu, K.-M. and Li, S.-Y. (2014) 'An integrated *in situ* extraction-gas stripping process for Acetone-Butanol-Ethanol (ABE) fermentation', *Journal of the Taiwan Institute of Chemical Engineers*, 45: 2106-2110.

Maddox, I. S. (1982) 'Use of silicalite for the adsorption of n-butanol from fermentation liquors', *Biotechnology Letters*, 4: 759-760.

Maddox, I. S., Qureshi, N. and Roberts-Thomson, K. (1995) 'Production of acetone-butanol-ethanol from concentrated substrate using *Clostridium acetobutylicum* in an integrated fermentation-product removal process', *Process Biochemistry*, 30: 209-215.

Mariano, A. P., Costa, C. B. B., de Angelis, D. D., Maugeri, F., Atala, D. I. P., Maciel, M. R. W. and Maciel, R. (2010) 'Dynamics of continuous flash fermentation for butanol production', *Chemical Engineering Transactions*, 20: 285-290.

Mariano, A. P., de Angelis, D. d. F., Maugeri Filho, F., Atala, D. I. P., Wolf Maciel, M. R. and Maciel Filho, R. (2008) 'An alternative process for butanol production: Continuous flash fermentation', *Chemical Product and Process Modeling*, 3: 1934-2659.

Mariano, A. P., Dias, M. O. S., Junqueira, T. L., Cunha, M. P., Bonomi, A. and Filho, R. M. (2013) 'Butanol production in a first-generation Brazilian sugarcane biorefinery: Technical aspects and economics of greenfield projects', *Bioresource Technology*, 135: 316-323.

Mariano, A. P. and Filho, R. M. (2011) 'Improvements in biobutanol fermentation and their impacts on distillation energy consumption and wastewater generation', *BioEnergy Research*, 5: 504-514.

Mariano, A. P., Filho, R. M. and Ezeji, T. C. (2012a) 'Energy requirements during butanol production and *in situ* recovery by cyclic vacuum', *Renewable Energy*, 47: 183-187.

Mariano, A. P., Keshtkar, M. J., Atala, D. I. P., Maugeri Filho, F., Wolf Maciel, M. R., Maciel Filho, R. and Stuart, P. (2011a) 'Energy requirements for butanol recovery using the flash fermentation technology', *Energy & Fuels*, 25: 2347-2355.

Mariano, A. P., Qureshi, N., Filho, R. M. and Ezeji, T. C. (2011b) 'Bioproduction of butanol in bioreactors: New insights from simultaneous in situ butanol recovery to eliminate product toxicity', *Biotechnology and Bioengineering*, 108: 1757-1765.

Mariano, A. P., Qureshi, N., Maciel Filho, R. and Ezeji, T. C. (2012b) 'Assessment of *in situ* butanol recovery by vacuum during acetone butanol ethanol (ABE) fermentation', *Journal of Chemical Technology & Biotechnology*, 87: 334-340.

Matsumura, M., Kataoka, H., Sueki, M. and Araki, K. (1988) 'Energy saving effect of pervaporation using oleyl alcohol liquid membrane in butanol purification', *Bioprocess and Biosystems Engineering*, 3: 93-100.

Matsumura, M. and Märkl, H. (1986) 'Elimination of ethanol inhibition by perstraction', *Biotechnology and Bioengineering*, 28: 534-541.

Mattiasson, B. (1983) 'Applications of aqueous two-phase systems in biotechnology', *Trends in Biotechnology*, 1: 16-20.

McCoy, E. and McClung, L. S. (1935) 'Studies on anaerobic bacteria', *Archiv für Mikrobiologie*, 6: 230-238.

Milestone, N. B. and Bibby, D. M. (1981) 'Concentration of alcohols by adsorption on silicalite', *Journal of Chemical Technology and Biotechnology*, 31: 732-736.

Minier, M., Grateloup, R., Blanc-Ferras, E. and Goma, G. (1990) 'Extractive acetonobutylic fermentation by coupling ultrafiltration and distillation', *Biotechnol Bioeng*, 35: 861-869.

Mollah, A. H. and Stuckey, D. C. (1993) 'Feasibility of *in situ* gas stripping for continuous acetone-butanol fermentation by *Clostridium acetobutylicum*', *Enzyme and Microbial Technology*, 15: 200-207.

Munson, C. L. and King, C. J. (1984) 'Factors influencing solvent selection for extraction of ethanol from aqueous solutions', *Industrial & Engineering Chemistry Process Design and Development*, 23: 109-115.

Ni, Y. and Sun, Z. (2009) 'Recent progress on industrial fermentative production of acetone-butanol-ethanol by *Clostridium acetobutylicum* in China', *Applied Microbiology and Biotechnology*, 83: 415-423.

Nielsen, D. R. and Prather, K. J. (2009) 'In situ product recovery of n-butanol using polymeric resins', *Biotechnology and Bioengineering*, 102: 811-821.

Nielsen, L., Larsson, M., Holst, O. and Mattiasson, B. (1988) 'Adsorbents for extractive bioconversion applied to the acetone-butanol fermentation', *Applied Microbiology and Biotechnology*, 28: 335-339.

Nilsson, R., Bauer, F., Mesfun, S., Hulteberg, C., Lundgren, J., Wännström, S., Rova, U. and Berglund, K. A. (2014) 'Techno-economics of carbon preserving butanol production using a combined fermentative and catalytic approach', *Bioresource Technology*, 161: 263-269.

Núñez-Gómez, K.-S., López-Mendoza, L.-C., López-Giraldo, L.-J. and Muvdi-Nova, C.-J. (2014) 'Study of acetone, butanol and ethanol liquid extraction from prepared aqueous solutions using membrane contactor technique', *CT&F - Ciencia, Tecnología y Futuro*, 5: 97-112.

Optinol Inc (2014) *Optinol*. Available at: http://www.optinol.com/index.php (Last Accessed: 11/03/2014).

OSHA (2014) *Chemical Sampling Information*. Available at: https://www.osha.gov/dts/chemicalsampling/toc/toc_chemsamp.html (Last Accessed: 20/05/2015).

Oudshoorn, A., Peters, M. C. F. M., van der Wielen, L. A. M. and Straathof, A. J. J. (2011) 'Exploring the potential of recovering 1-butanol from aqueous solutions by liquid demixing upon addition of carbohydrates or salts', *Journal of Chemical Technology & Biotechnology*, 86: 714-718.

Oudshoorn, A., van den Berg, C., Roelands, C. P. M., Straathof, A. J. J. and van der Wielen, L. A. M. (2010) 'Short-cut calculations for integrated product recovery options in fermentative production of bio-bulk chemicals', *Process Biochemistry*, 45: 1605-1615.

Oudshoorn, A., van der Wielen, L. A. M. and Straathof, A. J. J. (2009) 'Assessment of options for selective 1-butanol recovery from aqueous solution', *Industrial & Engineering Chemistry Research*, 48: 7325-7336.

Oudshoorn, A., van der Wielen, L. A. M. and Straathof, A. J. J. (2012) 'Desorption of butanol from zeolite material', *Biochemical Engineering Journal*, 67: 167-172.

Outram, V., Lalander, C. A., Lee, J. G. M., Davies, E. T. and Harvey, A. P. (2017) 'Applied *in situ* product recovery in ABE fermentation', *Biotechnol Prog*, 33: 563-579.

Outram, V., Lalander, C. A., Lee, J. G. M., Davis, E. T. and Harvey, A. P. (2016) 'A comparison of the energy use of *in situ* product recovery techniques for the Acetone Butanol Ethanol fermentation', *Bioresource Technology*, 220: 590-600.

Papadopoulos, T. and Sirkar, K. K. (1993) 'Separation of a 2-propanol/n-heptane mixture by liquid membrane perstraction', *Industrial & Engineering Chemistry Research*, 32: 663-673.

Perelygin, V. M. (1980) 'Vapor-liquid equilibrium data of acetone + ethanol + 1-butanol'. 24/08/2016. Oniitekhim, pp. 1-11. Available at: http://www.ddbst.com/en/EED/VLE/VLE%20Acetone%3BEthanol%3B1-Butanol.php (Accessed: 24/08/2016).

Pfromm, P. H., Amanor-Boadu, V., Nelson, R., Vadlani, P. and Madl, R. (2010) 'Bio-butanol vs. bio-ethanol: A technical and economic assessment for corn and switchgrass fermented by yeast or *Clostridium acetobutylicum*', *Biomass and Bioenergy*, 34: 515-524.

Prasad, R. and Sirkar, K. K. (1988) 'Dispersion-free solvent extraction with microporous hollow-fiber modules', *AIChE Journal*, 34: 177-188.

Prasad, R. and Sirkar, K. K. (1992) 'Membrane-Based Solvent Extraction', in Ho, W. S. W. and Sirkar, K. K. (eds.) *Membrane Handbook*. Boston, MA: Springer US, pp. 727-763.

Qureshi, N. and Blaschek, H. P. (1999a) 'Butanol recovery from model solution/fermentation broth by pervaporation: Evaluation of membrane performance', *Biomass and Bioenergy*, 17: 175-184.

Qureshi, N. and Blaschek, H. P. (1999b) 'Fouling Studies of a Pervaporation Membrane with Commercial Fermentation Media and Fermentation Broth of Hyper-Butanol-Producing Clostridium beijerinckii BA101', *Separation Science and Technology*, 34: 2803-2815.

Qureshi, N. and Blaschek, H. P. (1999c) 'Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation', *Biotechnology Progress*, 15: 594-602.

Qureshi, N. and Blaschek, H. P. (2000) 'Butanol production using *Clostridium beijerinckii* BA101 hyper-butanol producing mutant strain and recovery by pervaporation', *Applied Biochemistry and Biotechnology*, 84-86: 225-235.

Qureshi, N. and Blaschek, H. P. (2001a) 'Recovery of butanol from fermentation broth by gas stripping', *Renewable Energy*, 22: 557-564.

Qureshi, N., Friedl, A. and Maddox, I. S. (2014a) 'Butanol production from concentrated lactose/whey permeate: Use of pervaporation membrane to recover and concentrate product', *Applied Microbiology and Biotechnology*, 98: 1-9.

Qureshi, N., Hughes, S., Maddox, I. and Cotta, M. (2005) 'Energy-efficient recovery of butanol from model solutions and fermentation broth by adsorption', *Bioprocess and Biosystems Engineering*, 27: 215-222.

Qureshi, N. and Maddox, I. S. (1992) 'Application of novel technology to the ABE fermentation process', *Applied Biochemistry and Biotechnology*, 34-35: 441-448.

Qureshi, N. and Maddox, I. S. (1995) 'Continuous production of acetone-butanol-ethanol using immobilized cells of *Clostridium acetobutylicum* and integration with product removal by liquid-liquid extraction', *Journal of Fermentation and Bioengineering*, 80: 185-189.

Qureshi, N. and Maddox, I. S. (2005) 'Reduction in butanol inhibition by perstraction: Utilization of concentrated lactose/whey permeate by *Clostridium acetobutylicum* to enhance butanol fermentation economics', *Food and Bioproducts Processing*, 83: 43-52.

Qureshi, N., Maddox, I. S. and Friedl, A. (1992) 'Application of continuous substrate feeding to the ABE fermentation: Relief of product inhibition using extraction, perstraction, stripping, and pervaporation', *Biotechnology Progress*, 8: 382-390.

Qureshi, N., Meagher, M. M., Huang, J. and Hutkins, R. W. (2001) 'Acetone butanol ethanol (ABE) recovery by pervaporation using silicalite-silicone composite membrane from fed-batch reactor of *Clostridium acetobutylicum*', *Journal of Membrane Science*, 187: 93-102.

Qureshi, N., Saha, B. C., Cotta, M. A. and Singh, V. (2013) 'An economic evaluation of biological conversion of wheat straw to butanol: A biofuel', *Energy Conversion and Management*, 65: 456-462.

Qureshi, N., Singh, V., Liu, S., Ezeji, T. C., Saha, B. C. and Cotta, M. A. (2014b) 'Process integration for simultaneous saccharification, fermentation, and recovery (SSFR): Production of butanol from corn stover using *Clostridium beijerinckii* P260', *Bioresource Technology*, 154: 222-228.

Qureshi, N. Q. and Blaschek, H. B. (2001b) 'Evaluation of recent advances in butanol fermentation, upstream, and downstream processing', *Bioprocess and Biosystems Engineering*, 24: 219-226.

Rainey, F. A., Hollen, B. J. and Small, A. M. (2015) 'Clostridium', in *Bergey's manual of systematics of archaea and bacteria*. John Wiley & Sons, Ltd.

Richter, H., Qureshi, N., Heger, S., Dien, B., Cotta, M. A. and Angenent, L. T. (2012) 'Prolonged conversion of n-butyrate to n-butanol with *Clostridium*

saccharoperbutylacetonicum in a two-stage continuous culture with *in-situ* product removal', *Biotechnology and Bioengineering*, 109: 913-921.
Roddy, J. W. (1981) 'Distribution of ethanol-water mixtures to organic liquids', *Industrial & Engineering Chemistry Process Design and Development*, 20: 104-108.
Roffler, S., Blanch, H. W. and Wilke, C. R. (1987a) 'Extractive fermentation of acetone and butanol: Process design and economic evaluation', *Biotechnology Progress*, 3: 131-140.
Roffler, S. R., Blanch, H. W. and Wilke, C. R. (1984) '*In situ* recovery of fermentation products', *Trends in Biotechnology*, 2: 129-136.
Roffler, S. R., Blanch, H. W. and Wilke, C. R. (1987b) '*In-situ* recovery of butanol during fermentation', *Bioprocess Engineering*, 2: 1-12.
Roffler, S. R., Blanch, H. W. and Wilke, C. R. (1987c) '*In-situ* recovery of butanol during fermentation', *Bioprocess Engineering*, 2: 181-190.
Roffler, S. R., Blanch, H. W. and Wilke, C. R. (1988) '*In situ* extractive fermentation of acetone and butanol', *Biotechnology and Bioengineering*, 31: 135-143.
Salemme, L., Olivieri, G., Raganati, F., Salatinom, P. and Marzocchella, A. (2016) 'Analysis of the energy efficiency of some butanol recovery processes', *Chemical Engineering Transactions*, 49: 109-114.
Schügerl, K. and Hubbuch, J. (2005) 'Integrated bioprocesses', *Current Opinion in Microbiology*, 8: 294-300.
Setlhaku, M., Heitmann, S., Górak, A. and Wichmann, R. (2013) 'Investigation of gas stripping and pervaporation for improved feasibility of two-stage butanol production process', *Bioresource Technology*, 136: 102-108.
Shah, M. and Lee, Y. Y. (1994) 'Process improvement in acetone-butanol production from hardwood by simultaneous saccharification and extractive fermentation', *Applied Biochemistry and Biotechnology*, 45-46: 585-597.
Sheldon, R. A., Sanders, J. P. M. and Marinas, A. (2015) 'Sustainability metrics of chemicals from renewable biomass', *Catalysis Today*, 239: 1-2.
Shi, Z., Zhang, C., Chen, J. and Mao, Z. (2005) 'Performance evaluation of acetone-butanol continuous flash extractive fermentation process', *Bioprocess and Biosystems Engineering*, 27: 175-183.
Shin, C., Baer, Z. C., Chen, X. C., Ozcam, A. E., Clark, D. S. and Balsara, N. P. (2015) 'Block copolymer pervaporation membrane for in situ product removal during acetone-butanol-ethanol fermentation', *Journal of Membrane Science*, 484: 57-63.
Shukla, R., Kang, W. and Sirkar, K. K. (1988) 'Toxicity of organic solvents to *Clostridium acetobutylicum* for extractive ABE fermentation', *Applied Biochemistry and Biotechnology*, 18: 315-324.
Shukla, R., Kang, W. and Sirkar, K. K. (1989) 'Acetone-butanol-ethanol (ABE) production in a novel hollow fiber fermentor-extractor', *Biotechnology and Bioengineering*, 34: 1158-1166.
Staggs, K. W. and Nielsen, D. R. (2015) 'Improving n-butanol production in batch and semi-continuous processes through integrated product recovery', *Process Biochemistry*, 50: 1487-1498.
Stark, D. and von Stockar, U. (2003) '*In situ* product removal (ISPR) in whole cell biotechnology during the last twenty years', in von Stockar, U., Wielen, L. A. M., Bruggink, A., Cabral, J. M. S., Enfors, S. O., Fernandes, P., Jenne, M., Mauch, K., Prazeres, D. M. F., Reuss, M., Schmalzriedt, S., Stark, D., Stockar, U., Straathof, A. J. J. and Wielen, L. A. M. (eds.) *Process Integration in Biochemical Engineering*. Springer Berlin Heidelberg, pp. 149-175.

Stockhardt, J. S. and Hull, C. M. (1931) 'Vapor-liquid equilibria and boiling-point composition relations for systems n-butanol–water and isobutanol–water ', *Industrial & Engineering Chemistry*, 23: 1438-1440.

Tanaka, S., Tashiro, Y., Kobayashi, G., Ikegami, T., Negishi, H. and Sakaki, K. (2012) 'Membrane-assisted extractive butanol fermentation by *Clostridium saccharoperbutylacetonicum* N1-4 with 1-dodecanol as the extractant', *Bioresource Technology*, 116: 448-452.

Tashiro, Y., Yoshida, T., Noguchi, T. and Sonomoto, K. (2013) 'Recent advances and future prospects for increased butanol production by acetone-butanol-ethanol fermentation', *Engineering in Life Sciences*, 13: 432-445.

Towler, G. and Sinnott, R. (2013a) 'Chapter 3 - Utilities and Energy Efficient Design', in Towler, G. and Sinnott, R. (eds.) *Chemical Engineering Design (Second Edition)*. Boston: Butterworth-Heinemann, pp. 103-160.

Towler, G. and Sinnott, R. (2013b) 'Chapter 7 - Capital cost estimating', in Towler, G. and Sinnott, R. (eds.) *Chemical Engineering Design (Second Edition)*. Boston: Butterworth-Heinemann, pp. 307-354.

Uyttebroek, M., Van Hecke, W. and Vanbroekhoven, K. (2015) 'Sustainability metrics of 1-butanol', *Catalysis Today*, 239: 7-10.

van der Ham, F., Witkamp, G. J., de Graauw, J. and van Rosmalen, G. M. (1998) 'Eutectic freeze crystallization: Application to process streams and waste water purification', *Chemical Engineering and Processing: Process Intensification*, 37: 207-213.

van der Merwe, A. B., Cheng, H., Görgens, J. F. and Knoetze, J. H. (2013) 'Comparison of energy efficiency and economics of process designs for biobutanol production from sugarcane molasses', *Fuel*, 105: 451-458.

Van Hecke, W., Hofmann, T. and De Wever, H. (2013) 'Pervaporative recovery of ABE during continuous cultivation: Enhancement of performance', *Bioresource Technology*, 129: 421-429.

Van Hecke, W., Kaur, G. and De Wever, H. (2014) 'Advances in in-situ product recovery (ISPR) in whole cell biotechnology during the last decade', *Biotechnology Advances*, 32: 1245-1255.

Van Hecke, W., Vandezande, P., Claes, S., Vangeel, S., Beckers, H., Diels, L. and De Wever, H. (2012) 'Integrated bioprocess for long-term continuous cultivation of *Clostridium acetobutylicum* coupled to pervaporation with PDMS composite membranes', *Bioresource Technology*, 111: 368-377.

Van Hecke, W., Vandezande, P., Dubreuil, M., Uyttebroek, M., Beckers, H. and De Wever, H. (2015) 'Biobutanol production from C5/C6 carbohydrates integrated with pervaporation: experimental results and conceptual plant design', *Journal of Industrial Microbiology & Biotechnology*, 43: 1-12.

Vane, L. M. and Alvarez, F. R. (2008) 'Membrane-assisted vapor stripping: energy efficient hybrid distillation–vapor permeation process for alcohol–water separation', *Journal of Chemical Technology & Biotechnology*, 83: 1275-1287.

Vane, L. M. and Alvarez, F. R. (2013) 'Hybrid vapor stripping–vapor permeation process for recovery and dehydration of 1-butanol and acetone/butanol/ethanol from dilute aqueous solutions. Part 1. Process Simulations', *Journal of Chemical Technology & Biotechnology*, 88: 1436-1447.

Vane, L. M., Alvarez, F. R., Rosenblum, L. and Govindaswamy, S. (2013) 'Hybrid vapor stripping-vapor permeation process for recovery and dehydration of 1-butanol and acetone/butanol/ethanol from dilute aqueous solutions. Part 2. Experimental

validation with simple mixtures and actual fermentation broth', *Journal of Chemical Technology & Biotechnology*, 88: 1448-1458.
Viegas, R. M. C., Rodríguez, M., Luque, S., Alvarez, J. R., Coelhoso, I. M. and Crespo, J. P. S. G. (1998) 'Mass transfer correlations in membrane extraction: Analysis of Wilson-plot methodology', *Journal of Membrane Science*, 145: 129-142.
Visioli, L., Enzweiler, H., Kuhn, R., Schwaab, M. and Mazutti, M. (2013) 'Technological prospection for biobutanol production', *Recent Patents on Engineering*, 7: 115-124.
Wang, Y., Ho, S.-H., Yen, H.-W., Nagarajan, D., Ren, N.-Q., Li, S., Hu, Z., Lee, D.-J., Kondo, A. and Chang, J.-S. (2017) 'Current advances on fermentative biobutanol production using third generation feedstock', *Biotechnology Advances*.
Wayman, M. and Parekh, R. (1987) 'Production of acetone-butanol by extractive fermentation using dibutylphthalate as extractant', *Journal of Fermentation Technology*, 65: 295-300.
Weilnhammer, C. and Blass, E. (1994) 'Continuous fermentation with product recovery by *in-situ* extraction', *Chemical Engineering & Technology*, 17: 365-373.
Weizmann, C., Bergman, E., Sulzbacher, M. and Pariser, E. (1948) 'Studies in seltective extraction and adsorption III. The adsorption of acetone, butylalcohol and 2:3 butanediol from dilute solution', *Journal of the Society of Chemical Industry*, 67: 225-227.
Werpy, T., Petersen, G., Aden, A., Bozell, J., Holladay, J., White, J., Manheim, A., Eliot, D., Lasure, L. and Jones, S. (2004) *Top value added chemicals from biomass - Vol. 1: Results of screening for potential candidates from sugars and synthetic gas*. Washington, DC.
Wiehn, M., Staggs, K., Wang, Y. and Nielsen, D. R. (2014) '*In situ* butanol recovery from *Clostridium acetobutylicum* fermentations by expanded bed adsorption', *Biotechnology Progress*, 30: 68-78.
Wijmans, J. G. and Baker, R. W. (1995) 'The solution-diffusion model: a review', *Journal of Membrane Science*, 107: 1-21.
Willke, T. and Vorlop, K. D. (2004) 'Industrial bioconversion of renewable resources as an alternative to conventional chemistry', *Appl Microbiol Biotechnol*, 66: 131-142.
Wilson, R. (2011) *Cellulosic n-butanol*. Available at: http://www.tappi.org/content/Events/11BIOPRO/20.2Wilson.pdf (Last Accessed: 14/01/2014).
Wollan, D. (2008) *Alcohol Reduction in Beverages*. United States Patent and Trademark Office WO2005121306A1. [Online]. Available at: https://www.google.co.uk/patents/US20080272041.
Woodley, J. M., Bisschops, M., Straathof, A. J. J. and Ottens, M. (2008) 'Future directions for *in-situ* product removal (ISPR)', *Journal of Chemical Technology & Biotechnology*, 83: 121-123.
Wooley, R. J. and Putsche, V. (1996) *Development of an ASPEN PLUS physical property database for biofuels components* (MP-425-20685) (MP-425-20685). NREL.
Wu, H., Chen, X.-P., Liu, G.-P., Jiang, M., Guo, T., Jin, W.-Q., Wei, P. and Zhu, D.-W. (2012) 'Acetone–butanol–ethanol (ABE) fermentation using *Clostridium acetobutylicum* XY16 and *in situ* recovery by PDMS/ceramic composite membrane', *Bioprocess and Biosystems Engineering*, 35: 1057-1065.
Wu, Z. and Yang, S.-T. (2003) 'Extractive fermentation for butyric acid production from glucose by *Clostridium tyrobutyricum*', *Biotechnology and Bioengineering*, 82: 93-102.

Xue, C., Du, G.-Q., Chen, L.-J., Ren, J.-G., Sun, J.-X., Bai, F.-W. and Yang, S.-T. (2014a) 'A carbon nanotube filled polydimethylsiloxane hybrid membrane for enhanced butanol recovery', *Scientific Reports*, 4: 5925.

Xue, C., Du, G.-Q., Sun, J.-X., Chen, L.-J., Gao, S.-S., Yu, M.-L., Yang, S.-T. and Bai, F.-W. (2014b) 'Characterization of gas stripping and its integration with acetone–butanol–ethanol fermentation for high-efficient butanol production and recovery', *Biochemical Engineering Journal*, 83: 55-61.

Xue, C., Du, G. Q., Chen, L. J., Ren, J. G. and Bai, F. W. (2014c) 'Evaluation of asymmetric polydimethylsiloxane-polyvinylidene fluoride composite membrane and incorporated with acetone-butanol-ethanol fermentation for butanol recovery', *Journal of Biotechnology*, 188: 158-165.

Xue, C., Liu, F., Xu, M., Tang, I. C., Zhao, J., Bai, F. and Yang, S.-T. (2016a) 'Butanol production in acetone-butanol-ethanol fermentation with in situ product recovery by adsorption', *Bioresource Technology*, 219: 158-168.

Xue, C., Liu, F., Xu, M., Zhao, J., Chen, L., Ren, J., Bai, F. and Yang, S.-T. (2016b) 'A novel in situ gas stripping-pervaporation process integrated with acetone-butanol-ethanol fermentation for hyper n-butanol production', *Biotechnology and Bioengineering*, 113: 120-129.

Xue, C., Yang, D., Du, G., Chen, L., Ren, J. and Bai, F. (2015) 'Evaluation of hydrophobic micro-zeolite-mixed matrix membrane and integrated with acetone–butanol–ethanol fermentation for enhanced butanol production', *Biotechnology for Biofuels*, 8: 1-9.

Xue, C., Zhao, J.-B., Chen, L.-J., Bai, F.-W., Yang, S.-T. and Sun, J.-X. (2014d) 'Integrated butanol recovery for an advanced biofuel: Current state and prospects', *Applied Microbiology and Biotechnology*, 98: 1-12.

Xue, C., Zhao, J., Liu, F., Lu, C., Yang, S. T. and Bai, F. W. (2013a) 'Two-stage in situ gas stripping for enhanced butanol fermentation and energy-saving product recovery', *Bioresoure Technology*, 135: 396-402.

Xue, C., Zhao, J., Lu, C., Yang, S. T., Bai, F. and Tang, I. C. (2012) 'High-titer n-butanol production by *Clostridium acetobutylicum* JB200 in fed-batch fermentation with intermittent gas stripping', *Biotechnology and Bioengineering*, 109: 2746-2756.

Xue, C., Zhao, X.-Q., Liu, C.-G., Chen, L.-J. and Bai, F.-W. (2013b) 'Prospective and development of butanol as an advanced biofuel', *Biotechnology Advances*, 31: 1575-1584.

Yang, X., Tsai, G.-J. and Tsao, G. T. (1994) 'Enhancement of *in situ* adsorption on the acetone-butanol fermentation by *Clostridium acetobutylicum*', *Separations Technology*, 4: 81-92.

Yang, X. and Tsao, G. T. (1995) 'Enhanced acetone-butanol fermentation using repeated fed-batch operation coupled with cell recycle by membrane and simultaneous removal of inhibitory products by adsorption', *Biotechnology and Bioengineering*, 47: 444-450.

Yen, H.-W. and Wang, Y.-C. (2013) 'The enhancement of butanol production by *in situ* butanol removal using biodiesel extraction in the fermentation of ABE (acetone–butanol–ethanol)', *Bioresource Technology*, 145: 224-228.

Zakrzewska-Trznadel, G. (2013) 'Advances in membrane technologies for the treatment of liquid radioactive waste', *Desalination*, 321: 119-130.

Zigová, J., Šturdík, E., Vandák, D. and Schlosser, Š. (1999) 'Butyric acid production by *Clostridium butyricum* with integrated extraction and pertraction', *Process Biochemistry*, 34: 835-843.

Zverlov, V., Berezina, O., Velikodvorskaya, G. and Schwarz, W. (2006) 'Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed

agricultural waste for biorefinery', *Applied Microbiology and Biotechnology*, 71: 587-597.

Appendix A. Checklist for data to be included with ISPR results

- Duration of ISPR as well as the total fermentation duration (h).
- Volume of fermentation broth (L).
- Product concentrations in the fermentation broth (g L^{-1}).
- The volume of the recovered phase (L). E.g. the volume of condensate collected during gas stripping.
- Volume or mass of any separating agent used (L or g). E.g. the volume of extractant used during LLE or perstraction.
- Concentration of the products (including by-products such as acetic and butyric acid) in the recovered phase (g L^{-1}). E.g. for LLE the concentration of products in the extractant.
- Total amount of substrate consumed (g).
- Total amount of product produced (fermentation broth + recovered) (g).
- Fermentation productivity (g ABE L^{-1} h^{-1}).
- Fermentation yield (g ABE g^{-1} substrate).
- Substrate utilisation rate (g substrate L^{-1} h^{-1}).

Appendix B. Overall Mass Transfer Coefficient Calculation

Starting from equation (5.14) in section 5.3.3:

$$V_A \frac{dC_{iA}^b}{dt} = K_{ov,A} A \left(C_{iA}^b - C_{iA}^*\right) \quad \textbf{(5.14)}$$

Integrating this equation at boundary conditions, $t = 0, C_{iA} = C_{iA,0}$:

$$\int_{C_{iA,0}}^{C_{iA}} \frac{1}{C_{iA} - C_{iA}^*} dC_{iA} = \int_0^t -\frac{K_{ov,A} A}{V_A} dt \quad \textbf{B.1}$$

$$\ln(C_{iA} - C_{iA}^*) - \ln\left(C_{iA,0} - C_{iA}^*\right) = -\frac{K_{ov,A} A t}{V_A} \quad \textbf{B.2}$$

$$\ln(C_{iA} - C_{iA}^*) = \ln\left(C_{iA,0} - C_{iA}^*\right) - \frac{K_{ov,A} A t}{V_A} \quad \textbf{B.3}$$

Or

$$\ln\left(\frac{C_{iA} - C_{iA}^*}{C_{iA,0} - C_{iA}^*}\right) = -\frac{K_{ov,A} A t}{V_A} \quad \textbf{B.4}$$

The mass transfer coefficient can be found from a plot of $\ln(C_{iA} - C_{iA}^*)$ or $\ln\left(\frac{C_{iA} - C_{iA}^*}{C_{iA,0} - C_{iA}^*}\right)$ vs. t.

This expression can also be derived of the extractant phase based mass transfer coefficient $K_{ov,E}$. Using a mass balance across the membrane, the transfer can be described as:

$$N_{iT} = N_{iA,0} - N_{iA} = N_{iE} - N_{iE,0} \quad \textbf{B.5}$$

Assuming each phase has a constant volume:

$$\frac{N_{iA,0} - N_{iA}}{V_A} = C_{iA,0} - C_{iA} \quad \textbf{B.6}$$

$$\frac{N_{iE} - N_{iE,0}}{V_E} = C_{iE} - C_{iE,0} \quad \textbf{B.7}$$

Therefore:

$$N_{iT} = \left(C_{iA,0} - C_{iA}\right) V_A = \left(C_{iE} - C_{iE,0}\right) V_E \quad \textbf{B.8}$$

Based on this principle that the change in moles in the aqueous phase is the same as the change of moles of i in the extractant, equation (5.14) can written as equation (B.9) to represent the overall mass transfer based on the extractant phase.

$$V_E \frac{dC_{iE}}{dt} = K_{ov,E} A (C_{iE}^* - C_{iE}^b) \quad \textbf{B.9}$$

Following the same process as for the aqueous phase, results in:

$$\ln(C_{iE}^* - C_{iE}) = \ln\left(C_{iE}^* - C_{iE,0}\right) - \frac{K_{ov,E} A t}{V_E} \quad \textbf{B.10}$$

$$\ln\left(\frac{C_{iE}^{*}-C_{iE}}{C_{iE}^{*}-C_{iE,0}}\right)=-\frac{K_{ov}At}{V_E} \quad \textbf{B.11}$$

In theory the starting concentration of i in the extractant should be 0 $\left(C_{iE,0}=0\right)$, therefore:

$$\ln(C_{iE}^{*}-C_{iE})=\ln(C_{iE}^{*})-\frac{K_{ov,E}At}{V_E} \quad \textbf{B.12}$$

$$\ln\left(1-\frac{C_{iE}}{C_{iE}^{*}}\right)=-\frac{K_{ov,E}At}{V_E} \quad \textbf{B.13}$$

The mass transfer coefficient can be found from a plot of $\ln(C_{iE}^{*}-C_{iE})$ or $\ln\left(1-\frac{C_{iE}}{C_{iE}^{*}}\right)$ vs. t.

Appendix C. Flat Sheet Membrane Perstraction System Design

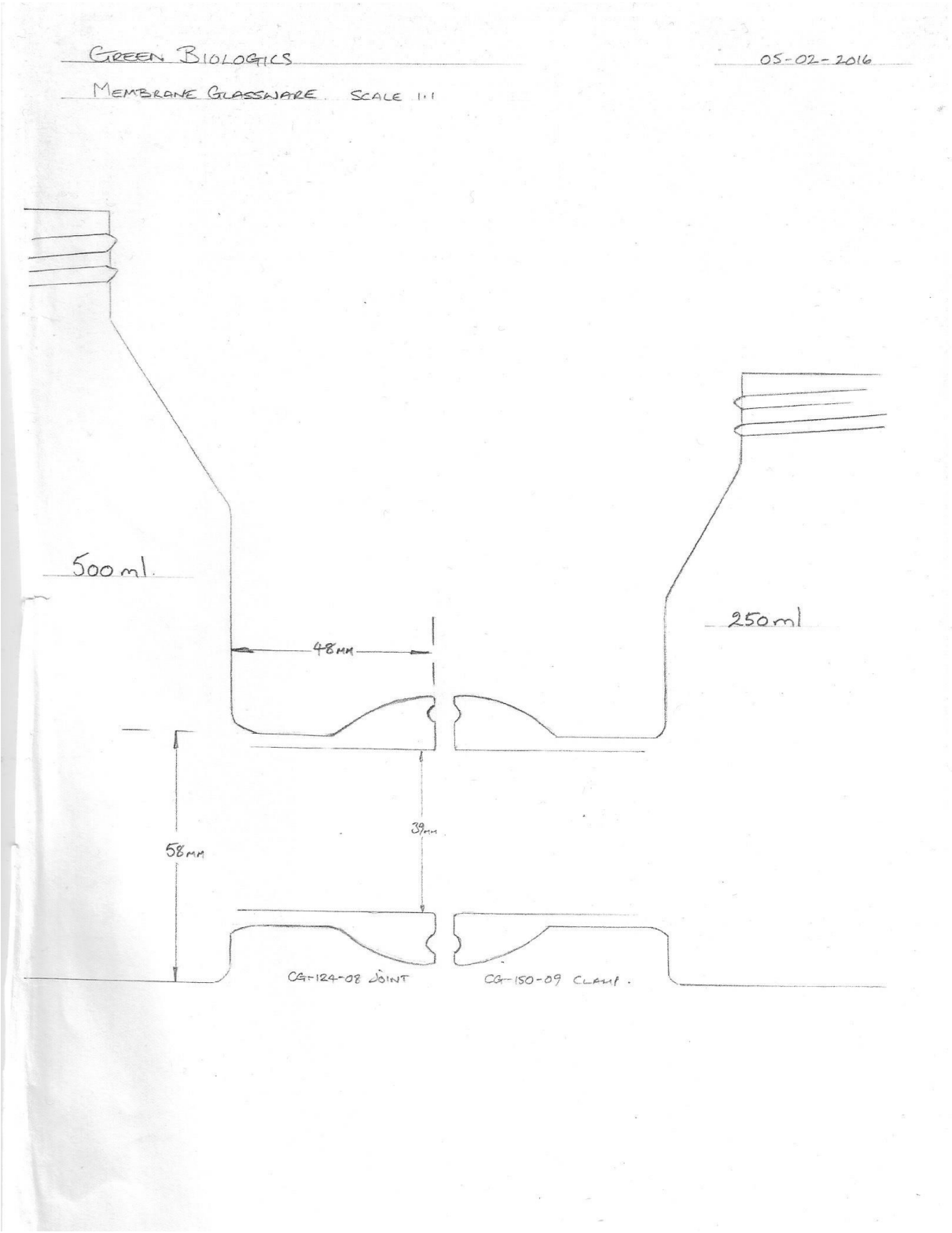


Figure C.1: Technical drawing of flat sheet perstraction system.

Appendix D. Oleyl Alcohol Chromatograms

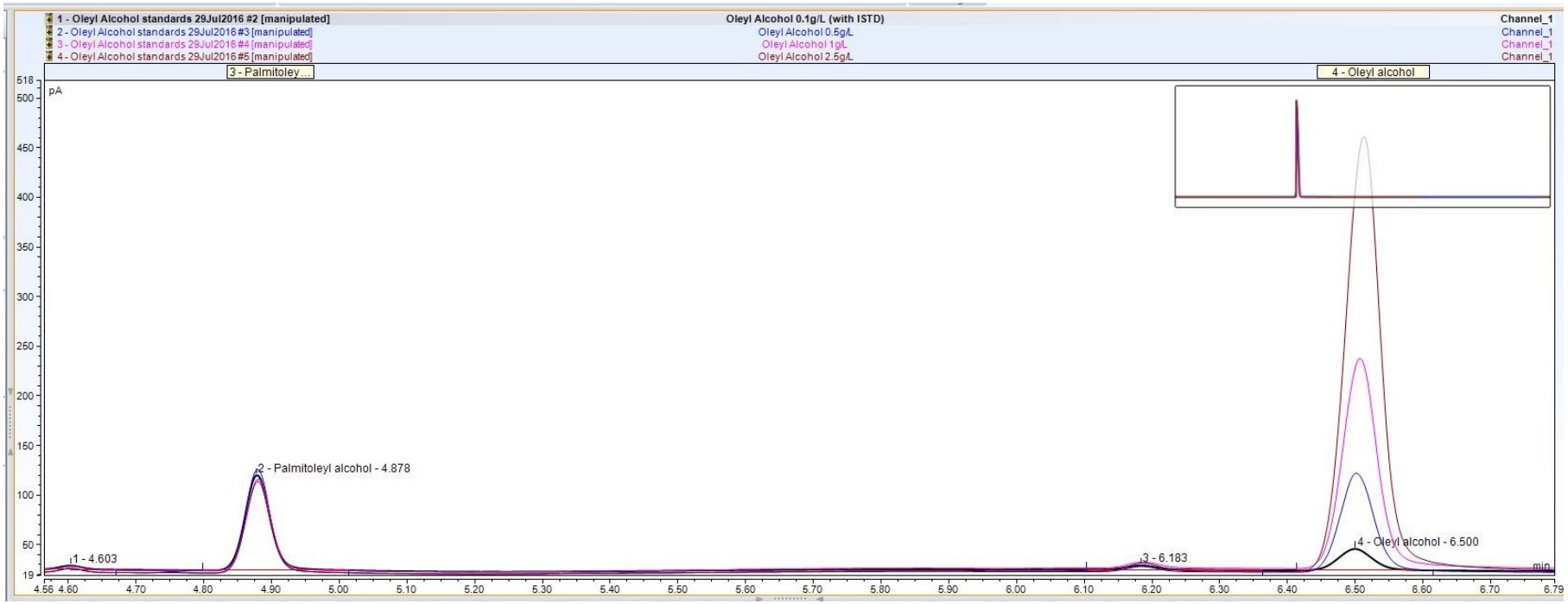


Figure D.1: Chromatogram of known oleyl alcohol concentrations, black=0.1 g L^{-1}, blue=0.5 g L^{-1}, pink=1 g L^{-1}, brown=2.5 g L^{-1}.

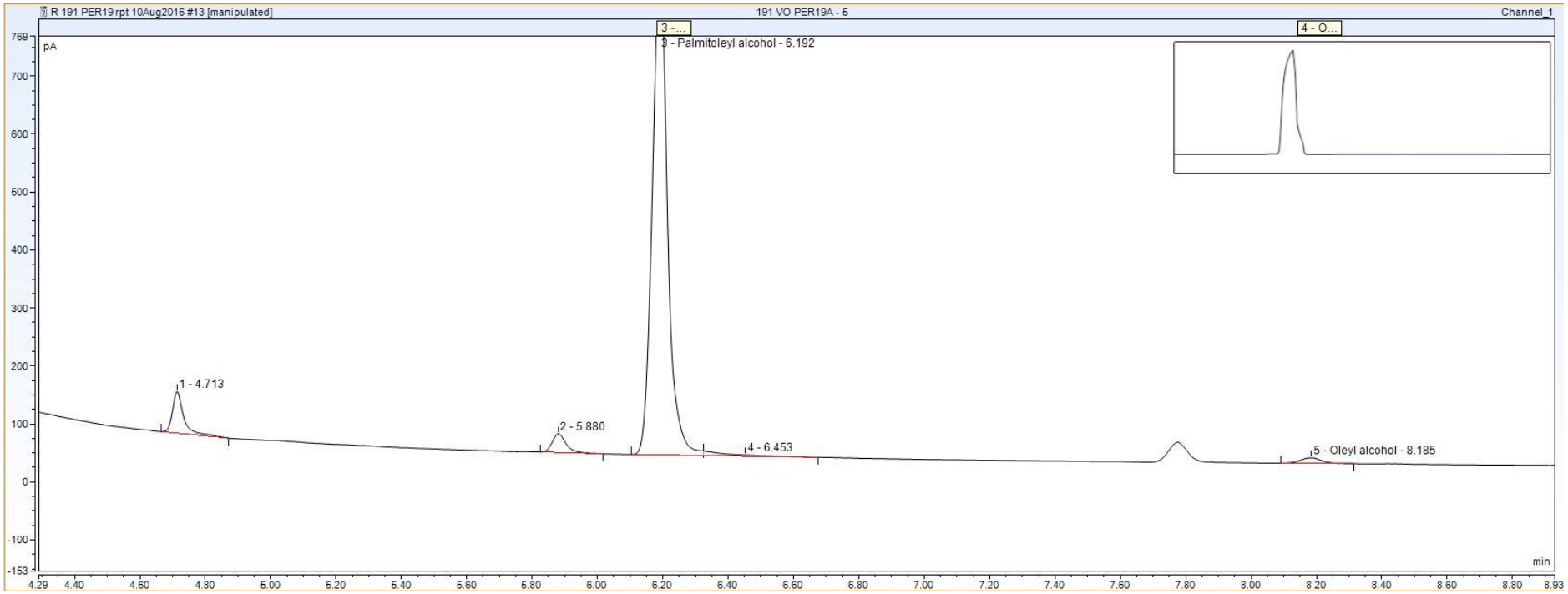


Figure D.2: Chromatogram showing trace amounts of oleyl alcohol from sample taken during perstraction experiments with silicone tubing.

Appendix E. Estimating Membrane Area

The membrane area was estimated based on the following assumptions:

- Fermentation volume = 100,000 L (100 m^3). This was selected as it is similar to the fermentor sizes used for production, as the largest fermentor size for the ABE fermentation, in the USA, was 189,250L (Dürre, 1998).
- Butanol productivity = 1 g L^{-1} h^{-1}, an easily attainable productivity for fed-batch ABE fermentations with perstraction (Table 3.6) and other ISPR methods.
- Butanol flux (g h^{-1} m^{-2}) was calculated using equation (5.13), which translates to:

$$J_b = \frac{V\left(C_{b,0}^b - C_{b,t}^b\right)}{A.t} \quad \textbf{(F.1)}$$

The membrane area (m^2) was then estimated as follows:

$$Production\ rate\ (gh^{-1}) = Butanol\ productivity \times V_F \quad \textbf{(F.2)}$$

Where:

V_F is fermentation volume (L)

$$A = \frac{Production\ rate}{J_b} \quad \textbf{(F.3)}$$