Biological Processing in Oscillatory Baffled Reactors (OBRs)

A Thesis Submitted by

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Abstract

Bioprocessing involves using complete cells or any of their components for the manufacture of products such as pharmaceuticals, fuel, health products and precursor compounds for plastics. Bioprocessing can provide sustainable routes for the manufacture of products which are traditionally manufactured from fossil-derived chemicals. The stirred tank reactor (STR) is the prevalent fermenter/reaction vessel in industry due to its simplicity and cost. However; the basic design has not changed for centuries. This thesis describes the use of oscillatory baffled reactors (OBRs) for bioprocessing. Generally, the “niche application” of OBRs is in performing ‘long’ processes in plug flow conditions, so they should be suitable for many bioprocesses.

In this thesis, four research projects using OBRs are presented: modelling of plug flow and OBR design; enzymatic saccharification; microalgae culture; and anaerobic digestion (AD).

A robust method to maximise plug flow in various OBR designs is described. Second order, polynomial models (R^2=92.1% and 97.3%) were used to maximise plug flow at Ψ=1.9. The net flow rate (Q) was shown to affect the quality of plug flow which has implications for OBR design.

Enzymatic saccharification was conducted in reactors based on OBR and STR technology. The OBR required 94-99% less power to achieve the necessary mixing intensities to maximise glucose production.

Chlamydomonas reinhardtii was cultured in a modified OBR for use as a photobioreactor (PBR). Maximum growth rates were increased by 95% in the OBR compared to cultures conducted in T-flasks. A flotation effect was observed that suggests that a dual culture and harvest device for microalgae is possible.

Anaerobic digestion of dairy slurry and co-digestion with glycerol was conducted in digesters based on OBR and STR technology. The OBR achieved a maximum specific methane yield 28% higher than the STR. However, blockages occurred in the OBR and 89% less power was required for temperature control in the STR, predominantly due to differences in surface areas to volume ratios.

Overall, OBR technology was successfully used in three bioprocesses, with improvements demonstrated over traditional technologies such as STR and/or T-

flasks. Commercial systems based on OBR technology could be designed, provided that sufficient data is generated to overcome the risks associated with adoption of a novel technology such as OBRs.

Dedication

To Richard and Brenda.

Acknowledgements

I would like to acknowledge the help, input and support of the following people during the course of my EngD:

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Abbreviations and notation

Abbreviations

ABE	Acetone, butanol, ethanol
AD	Anaerobic digestion
API	Active pharmaceutical ingredient
CBU	Cellobiase unit
cDS	Centrifuged dairy slurry
COD	Chemical oxygen demand
CPI	Centre for Process Innovation
CSTR	Continuous stirred tank reactor
DI	De-ionised
DoE	Design of experiment
DS	Dairy slurry
EngD	Engineering doctorate
FBR	Fluidised bed reactor
FPU	Filter paper unit
GMO	Genetically modified organism
GMP	Good manufacturing practice
HRT	Hydraulic residence time
IP	Intellectual property
IU	International unit
LED	Light emitting diode
NREL	National Renewable Energy Laboratory
OBR	Oscillatory baffled reactor
OFAT	One factor at a time
OLR	Organic loading rate (kg COD/m^3 day)
OTR	Oxygen transfer rate
OUR	Oxygen uptake rate
PBR	Photobioreactor
PHA	Polyhydroxyalkanoate
PHB	Poly-β-hydroxybutyrate
PhD	Post honours doctorate
PIV	Particle image velocimetry
QbD	Quality by design
rpm	Revolutions per minute
rps	Revolutions per second
RTD	Residence time distribution
Rubisco	Ribulose-1,5-bisphoshate carboxylase
sCOD	Soluble chemical oxygen demand
SMY	Specific methane yield (m^3/kg VS_{added})
SRT	Solids (microorganism) retention time
STR	Stirred tank reactor
TIC	Technology Innovation Centre
TiS	Tanks-in-series
TS	Total solids (%)
TSB	Technology Strategy Board
UASB	Upflow anaerobic sludge blanket

VFA	Volatile fatty acids
VOA	Volatile organic acids
VS	Volatile solids (%)
vvm	Volume per volume per minute

Notation

A	Area under C(t) curve
C_D	Discharge coefficient (taken as 0.7)
C_i	Concentration at point i (M)
D	Tube diameter (m)
D_c	Dispersion coefficient (m^2/s)
D_e	Effective tube diameter (m)
D_o	Orifice diameter (m)
D_s	Diameter of the impeller (m)
D_v	Diameter of the vessel (m)
E	Exit age (s)
E_θ	Exit age normalised with time
f	Frequency (Hz)
I_c	Compensation irradiance point (W/m^2)
I_o	Incident light intensity (W/m^2)
I_x	Light penetration depth (cm) for I_c
k	Doubling time (days)
k_La	Volumetric mass transfer coefficient
L	Baffle spacing (m)
L_h	Liquid height in the vessel (m)
M	Number of inter-baffle zones
N	Impeller speed (rps)
N_B	Number of baffles per unit length (m)
N_t	Number of tanks-in-series
P/V	Power density (W/m^3)
P_o	Power number of the impeller
Q	Net flow rate (mL/min)
R^2	Coefficient of determination
Re_n	Net flow Reynolds number
Re_o	Oscillatory Reynolds number
St	Strouhal number
t_i	Time at point i (s)
u	Superficial fluid velocity (m/s)
X	Cell concentration (cells/mL)
X_o	Centre to peak amplitude (m)

Greek letters

ɣ	Shear rate (/s)
α	Baffle open area
α_a	Specific light absorption coefficient (cm^2/cell)
δ	Baffle thickness (mm)
η	Mixing efficiency

θ	Normalised time
μ	Fluid dynamic viscosity (Pa.s) / specific growth rate
μ_{max}	Maximum specific growth rate (/day)
ρ	Fluid density (kg/m^3)
ψ	Velocity ratio

Chapter 1: Introduction

1.1 Background

Oscillatory baffled reactors (OBRs) are novel, tubular devices that provide a platform for continuous process development under plug flow conditions (Stonestreet and van der Veeken, 1999). Reactor contents are oscillated via the action of a pump or piston relative to internal baffles spaced periodically along the reactor length. Vortex formation creates a novel mixing mechanism that is power-efficient (Abbott et al., 2014b, Jambi et al., 2013), uniform (Ikwebe, 2013), even under low shear (Ni et al., 2000) and enhances mass (Ni et al., 1995, Al-Abduly et al., 2014) and heat transfer (Mackley and Stonestreet, 1995). These attributes have proven successful for the development and intensification of continuous chemical processes, especially within crystallization (Ni and Liao, 2010, Chew et al., 2004). For example, the reaction time for an active pharmaceutical ingredient (API) was reduced from 10 hours in a traditional batch stirred tank reactor (STR) to 20 minutes in a continuous OBR (Ni, 2006). This demonstrates the great potential this technology exhibits for process intensification.

Numerous bioprocesses have been conducted in OBRs with mixed results. Glucose production from the enzymatic saccharification of cellulose was enhanced by only 7% in an OBR when compared to a shake flask (Ikwebe and Harvey, 2011), whereas the required time to reach a pullulan concentration of 12.1 g/L was reduced by 73% when compared to an STR (Gaidhani et al., 2005). These findings demonstrate that process intensification is not guaranteed by the use of enhanced mixing. However, great potential exists for the application of OBR technology to specific processes where mixing is the rate determining step. This thesis provides a comprehensive review of bioprocessing in OBRs (chapter 2) and summarises research findings from four distinct projects. The research covers plug flow modelling (chapter 3), the enzymatic saccharification of cellulose (chapter 4), the culture of microalgae (chapter 5) and anaerobic digestion (chapter 6).

1.2 The engineering doctorate (EngD)

The engineering doctorate (EngD) programme is distinct from a traditional PhD in that research is directed by a sponsor company in response to commercial needs. These programmes were developed in response to industry following

recommendations from the Parnaby report that concluded ‘PhDs lack industrial relevance’ and ‘…are too narrow and academic for the industry’s needs’ (Parnaby, 1990). The first EngD programmes began in 1992 and aimed to build on existing skills acquired at undergraduate and Master’s level while being distinct from, but complementary to, traditional PhDs.

In this project, the sponsor company was The Centre for Process Innovation (CPI). CPI is a technology innovation centre (TIC) that uses applied knowledge in science and engineering, combined with state of the art facilities, to enable clients to develop, prove, prototype and scale up the next generation of products and processes.

Central to the EngD philosophy is cross-disciplinary research conducted in an industrial environment, exposing students (known as research engineers) to business cultures of the workplace. Research consists of a series of linked projects brought together by an overarching theme to provide a contribution to knowledge. The overarching theme for research during this EngD was bioprocessing in oscillatory baffled reactors (OBRs).

1.3 Commercial motivation

The first published example of equipment similar to the ‘standard’ OBR (see §2.3.1.1) appeared in 1973 and described a high efficiency membrane oxygenator (Bellhouse et al., 1973). This was later patented for the specific application of blood oxygenation, as it requires enhanced mass transfer under non-turbulent flow conditions to minimise protein damage (Bellhouse, 1978). Subsequent research focused on aspects of the technology including flow patterns (Sobey, 1980, Brunold et al., 1989), axial dispersion (Howes and Mackley, 1990, Howes et al., 1991) and heat transfer (Mackley et al., 1990). The first biological application of OBR technology was published in 1992 and described the culture of a rapidly growing micro-organism (Harrison and Mackley, 1992). Significant research into bioprocessing in OBRs followed suggesting great potential for industry adoption of the technology (see §2.6).

CPI identified an intellectual property (IP) space for biological process applications of OBR technology. This was surprising given the amount of information available in the literature (see §2.6). Twelve patents associated with OBR technology with a diverse range of applications have been identified dating from 1978. These have been

divided into chemical and biological process applications and are summarised below in Tables 1.1 and 1.2, respectively.

Table 1.1: Summary of chemical related patent applications involving OBR technology.

Title	Summary	Reference
Method and apparatus for phase separated synthesis	Continuous polymerisation inside a multi-column, tubular vessel containing annular baffles for even mixing of fluid provided by oscillations.	(Ni, 2002)
Tubular oscillatory flow reactor with multiple conical ring baffles inside for reaction mixture of high solid content	Apparatus for solid particle suspension in a liquid. Can be operated continuously and prevents local accumulation of solids. Suitable for uncatalysed or catalysed processes of liquid-solid suspensions.	(Wu et al., 2008)
Apparatus and method for temperature controlled process	Apparatus allowing separate columns to be controlled at a specific temperature for local control. Can be operated continuously during a crystallisation process.	(Ni et al., 2009b)
Apparatus and method for applying oscillatory motion	An apparatus for applying oscillatory motion to a fluid inside a tubular vessel containing annular baffles whereby the oscillations are controlled by a predetermined waveform.	(Ni et al., 2009a)
Method and apparatus for fluid liquid reactions.	A continuous, semi-continuous or fed-batch apparatus for heterogeneous catalysis. The vessel contains orificed baffles and oscillations to maintain uniform mixing and efficient dispersion.	(Ni et al., 2010a)
Continuous process for producing toner using an oscillatory flow continuous reactor	Apparatus for continuous formation of emulsion aggregation toners. Resin, colorant and wax are introduced into the apparatus where they aggregate to form toner particles before recovery.	(Mang et al., 2012)
Device for inducing nucleation	Device for inducing crystal nucleation in a crystalliser (OBR). The device is significantly cheaper and more reliable than known ultrasound devices.	(Ni and Callahan, 2013)

Professor Xiongwei Ni from Heriot-Watt University and Ni-Tech Solutions is associated with half of all patents listed. The majority of his work is focused on chemical related applications, especially crystallisation (Ni and Liao, 2010), with only one patent overlapping the bioprocessing IP space: a general mixing apparatus (Ni et al., 2010b). CPI recognised that this competition could hinder commercial progress within chemical related applications, stimulating research into a different area i.e. bioprocessing.

Table 1.2: Summary of biological related patent applications involving OBR technology. Bold italics represent patents filed by CPI.

Title	Summary	Reference
Method for effecting heat or mass transfer	Method for effecting heat or mass transfer with a fluid (blood) inside a constricted tube oscillated at 0.83 to 3 Hz and another fluid (oxygen) diffusing through a membrane.	(Bellhouse, 1978)
The reduction of redox-sensitive substances by bacteria of the genus Alteromonas or Shewanella	Apparatus for bio-treatment of aqueous effluents using bacteria from the genus *Alteromonas* or *Shewanella*, or other facultative anaerobes. Includes continuous operation and addition of a red-ox mediator such as riboflavin.	(Loyd et al., 2010)
*Mixing apparatus and process	A continuous, semi-continuous or fed-batch mixing apparatus for achieving consistently mixed substances. Consists of a tubular vessel with a plurality of annular baffles. No oscillations are present; mixing achieved via unidirectional flow.	(Ni et al., 2010b)
Anaerobic process	Method for a controlled AD[1] process. Substrate, anaerobic organisms and a feed material are continuously fed into the apparatus under anaerobic conditions while oscillating relative to the vessel, increasing digestion rate.	(Cooper et al., 2009)
Continuous culture of anaerobic solvent-producing bacteria	Continuous anaerobic culture of *Clostridia* (a solvent producing bacterium) under approximately plug flow conditions. Bacteria reach their productive point before the end of the OBR and are recycled with solvents extracted.	(Cooper et al., 2011)

*General application [1]Anaerobic Digestion

Three of the five patents listed in Table 1.2 have been filed by CPI since 2009 and aim to protect biological related applications. Of the remaining two, one was filed in 1978, so has exceeded the 20 year protection term (Intellectual Property Office, 2011) and another is a general patent for a mixing device (Ni et al., 2010b).

The bioprocessing gap in the IP landscape enabled CPI to freely pursue and protect commercially relevant applications of OBR technology. Initial filing of two patents required experimental data in the selected areas of anaerobic digestion (AD) and solvent production. This EngD project aimed to provide the necessary data to support and develop bioprocessing in OBRs. Evaluation of the scientific literature and industry needs gave direction to the research, supporting that already given from evaluation of the IP landscape.

The initial project proposal required the following aims to be achieved:

'…1) to construct a laboratory prototype OBR suitable for continuous conversion of plant biomass using appropriate microorganisms; 2) to model and demonstrate enhanced operating characteristics of the reactor under conditions that approximate to plug flow; 3) to construct an economic model to compare cost and sustainability benefits of OBR technology versus conventional, completely-mixed, stirred-tank reactors (CSTRs); and 4) to deploy the reactor to demonstrate one or more process applications.'

The following chapters describe the route taken to achieve these aims.

1.4 Thesis structure

The thesis is broken down into seven chapters with chapters 1 and 7 being the introduction and conclusion, respectively. Chapters 2-6 are presented in a journal format and reproduced here in their published/submitted form. They are intended to be read in isolation or as a whole and therefore some repetition occurs. The end of each chapter provides insight into how the results were used to guide further research and links back to the original aims.

1.4.1 Chapter 2: Literature review

This review focuses on bioprocessing in OBRs and covers three main areas of the technology: operation, advantages and future potential. A critical assessment is provided to outline the main barriers facing industry adoption of OBRs, followed by several suggested strategies to overcome these. Chapter 2 forms a review article published by The Royal Society in a special edition of *Interface Focus* entitled *Biofuels, Science and Society* (Abbott et al., 2013). The objectives of conducting this review were to gain an understanding of the field, provide a literature review of bioprocessing in OBRs, and increase public awareness of CPI in relation to OBR technology.

1.4.2 Chapter 3: Modelling plug flow and OBR design

This research describes plug flow behaviour in a 'standard' design OBR installed at CPI. The chapter forms a research paper published in the *International Journal of Chemical Reactor Engineering* (Abbott et al., 2014a). Previous experiments conducted by CPI used pH vs. time plots generated from 'pulses' of acid and alkaline material to assess flow conditions. A more robust and accurate method was required

that used a theoretical model to quantify the degree of plug flow. The objectives of conducting this research were to model plug flow in an OBR; provide a simple and effective tool to rapidly quantify and maximise plug flow behaviour in OBRs of 'standard' and 'non-standard' designs; and discuss more general implications for OBR design.

1.4.3 Chapter 4: Enzymatic saccharification

This research describes batch comparisons between an OBR and STR for the enzymatic saccharification of cellulose. The chapter forms a research paper published in a special edition of *Chemical Engineering Research and Design* entitled *Green Processes and Eco Technologies* (Abbott et al., 2014b). Power density calculations enabled reactions to be conducted under comparable conditions. A direct comparison of reaction rates and energy requirements between OBR and STR designs is given to highlight any differences and potential advantages of either technology. This is followed by a simple economic assessment of the two technologies at industrial scale for a commercial process. The objectives of conducting this research were to compare an OBR with an STR for enzymatic saccharification as well as calculate minimum energy requirements for mixing to maximise conversion rates.

1.4.4 Chapter 5: Microalgae culture

This research describes the use of OBRs as photobioreactors (PBRs) for culture of *Chlamydomonas reinhardtii* under photoautotrophic conditions. The chapter forms a research paper published in *Chemical Engineering Science* (Abbott et al., 2015). *C. reinhardtii* has great potential for the production of biopharmaceuticals (Mayfield et al., 2007), especially anti-cancer immunotoxins (Tran et al., 2013). It has been reported, however, that *C. reinhardtii* cells are susceptible to shear stress (Gudin and Chaumont, 1991) so may require low shear culture conditions. The objectives of this research were to test the feasibility of OBRs for the culture of *C. reinhardtii*; determine how the mixing intensity affects the maximum growth rate; and compare to T-flasks run under comparable conditions.

1.4.5 Chapter 6: Anaerobic digestion

This research describes anaerobic digestion (AD) of cow slurry and its co-digestion with glycerol at pilot scale (40 L) in an OBR and an STR. Funding for the project was provided by an additional grant from The Technology Strategy Board (TSB) and aimed to test the hypothesis that low shear and well mixed conditions in an OBR could provide an environment conducive to mixed community floc formation that could improve biogas production (Schink and Stams, 2006). Objectives of conducting this research were to test the feasibility of OBRs for AD; determine how the agitation intensity and feed rate affect biogas production and composition; and compare overall performance to a traditional STR design run under comparable conditions.

1.5 Research objectives

The objectives of this research were to:

1) Compose a comprehensive literature review of bioprocessing in OBRs.
2) Demonstrate, model and maximise plug flow in an OBR using a DoE approach, and consider associated design implications.
3) Compare an OBR to a conventional STR for the enzymatic saccharification of cellulose.
4) Evaluate an OBR for the photoautotrophic culture of *C. reinhardtii*.
5) Compare a pilot scale OBR to a conventional digester for AD of dairy slurry and co-digestion with glycerol.

Chapter 2: Literature review

'Biological processing in oscillatory baffled reactors: operation advantages and potential.'

2.1 Abstract

The development of efficient and commercially viable bioprocesses is essential for reducing the need for fossil-derived products. Increasingly, pharmaceuticals, fuel, health products and precursor compounds for plastics are being synthesized using bioprocessing routes as opposed to more traditional chemical technologies. Production vessels or reactors are required for synthesis of crude product before downstream processing for extraction and purification. Reactors are operated either in discrete batches or, preferably, continuously in order to reduce waste, cost and energy. This review describes the oscillatory baffled reactor (OBR), which, generally, has a niche application in performing 'long' processes in plug flow conditions, and so should be suitable for various bioprocesses. We report findings to suggest that OBRs could increase reaction rates for specific bioprocesses owing to low shear, good global mixing and enhanced mass transfer compared with conventional reactors. By maintaining geometrical and dynamic conditions, the technology has been proven to be easily scaled up and operated continuously, allowing laboratory-scale results to be easily transferred to industrial-sized processes. This is the first comprehensive review of bioprocessing using OBRs. The barriers facing industrial adoption of the technology are discussed alongside some suggested strategies to overcome these barriers. OBR technology could prove to be a major aid in the development of commercially viable and sustainable bioprocesses, essential for moving towards a greener future.

2.2 Introduction

Bioprocessing uses complete living cells or any of their components for the production of useful products ranging from high value pharmaceuticals (Meyer et al., 2008a) to low value fuels (Shi et al., 2009). A growing interest in renewable technologies to replace traditional fossil derived chemicals with, for example, biomass (Lynd and Wang, 2003) has stimulated increased research and development targeting a range of bioprocesses. The aim is to develop bioprocesses based on renewable and organic feedstocks, with the constraint of maintaining or

decreasing current production costs compared to traditional technologies. This is challenging, given the decades of optimisation and intensification of chemical processes. In addition, biopharmaceuticals (e.g., Trastuzumab) (Barginear and Budman, 2009), nutraceuticals (e.g., astaxanthin) (Guerin et al., 2003), CO_2 capture (Chiu et al., 2008) and protein refolding (Lee et al., 2001) utilise bioprocessing routes.

The entire production pathway from feedstock to product requires many stages including: pre-treatment, production, extraction and purification. Traditional batch stirred tank reactors (STRs) and continuously stirred tank reactors (CSTRs) have existed for centuries and are still widely adopted throughout the chemical and bioprocessing sectors for production due to their simplicity. In essence, STRs and CSTRs are nothing more than large vessels mixed using a paddled shaft and, although suitable for many processes, lack specific characteristics essential for intensified and cost effective bioprocessing. For example, achieving good global mixing complemented with low shear is difficult in STRs: a combination essential for specific bioprocesses including the culture of microalgae that require mixing to provide illumination and CO_2 but suffer from cell fragility (Gudin and Chaumont, 1991).

Continuous technologies for bioprocessing and biopharmaceutical sectors have become more prevalent due to their ability to reduce footprint, waste, cost and energy compared to batch technologies by, for example, removing down time inherent in batch processing (Plumb, 2005). Once at steady state, a continuous process produces product with little variation in output; providing variable factors such as temperature, pH and feed constituents are kept constant. OBRs allow the development of continuous processes under plug flow conditions whereby biological components move continuously through the reactor with laminar flow. Plug flow in OBRs can, therefore, be viewed as batch culture with the time dimension replaced by reactor length. This enables bioprocesses containing cell cultures to be extracted at the outlet, with cells at any desired metabolic state to maximise product concentration. This includes cultures with zero or negative growth rates, such as those in the decline phase of batch culture: unachievable using traditional continuous chemostats in CSTRs that rely on net growth rates for dynamic stability (Voloshin et al., 2005).

2.3 Theory governing OBR design and operation

2.3.1 Design

2.3.1.1 The 'standard' design

A 'standard' OBR consists of a tube, generally 10–150 mm internal diameter (D), containing equally spaced orifice plates (Figure 2.1). Typically, a reciprocating pump or piston located at one end oscillates back and forth generating oscillatory flow. For continuous operation, a second pump is required to create net flow through the column. Uniform mixing at exceptionally low shear is provided by vortices that form as fluid is forced through each orifice plate. OBRs can act as either batch or continuous systems depending on whether a net flow of new material is being introduced to the reactor and product removed at an equal rate.

2.3.1.2 Other designs

Although Figure 2.1 shows the most common design, other OBR designs exist for scaling-down (meso-scale) and up (e.g., 'multi-orifice' see §2.5.2).

Meso-scale OBRs have a niche application for the rapid screening and characterisation of reactions (e.g., biodiesel formation) (Phan et al., 2011b). These meso-reactors have a small diameter (~5 mm) and subsequently volumes of a few millilitres (Phan et al., 2011a). Several baffle designs have been evaluated at the meso-scale: helical (Phan et al., 2011a, Phan and Harvey, 2012), smooth periodic constricted (SPC) tube (Reis et al., 2007), central and integral (Phan and Harvey, 2010).

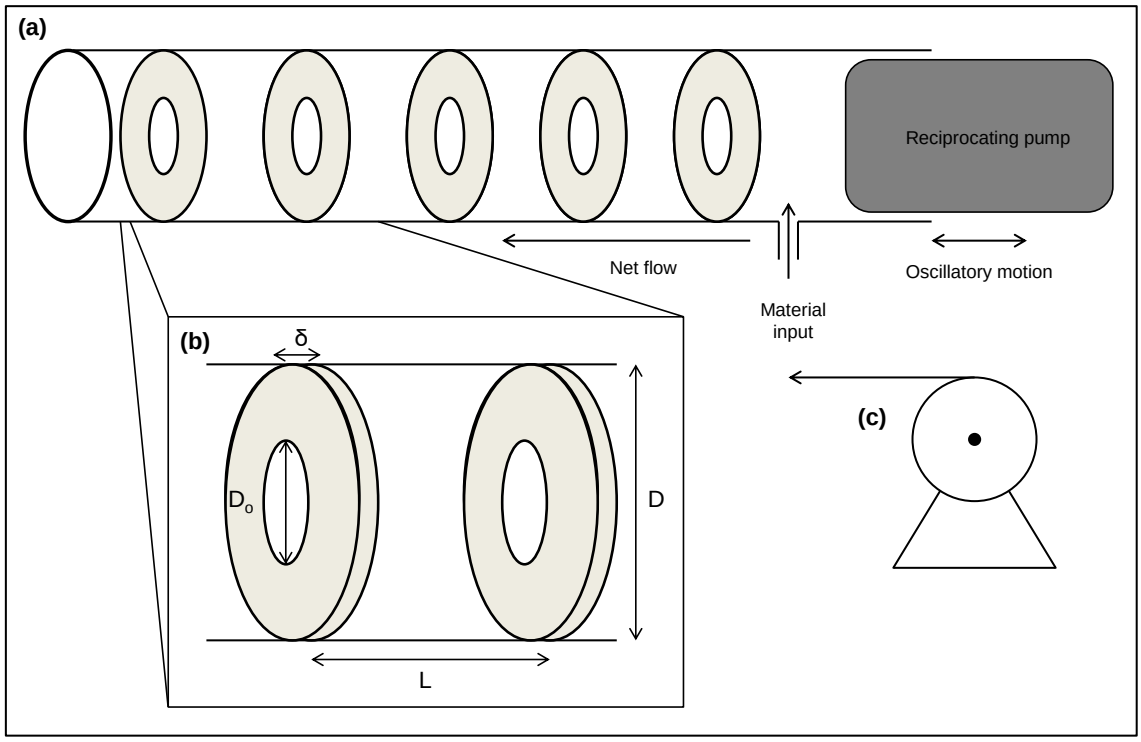


Figure 2.1: Standard OBR design. (a) Tube containing equally spaced orifice plates (baffles) with a reciprocating pump required for generation of oscillatory motion. (b) Geometrical parameters important for OBR design. (c) Continuous operation requires a 2nd pump to create net flow through the column.

2.3.2 Mixing through vortices

Unlike STRs and conventional tubular reactors which rely on stirring mechanisms and/or turbulent flow conditions for mixing (Rossi, 2001, van Vliet et al., 2005), the OBR uses oscillations to produce vortices (Figure 2.2). These form periodically along the entire length of the reactor, effectively causing each inter-baffle zone to act as a CSTR; the entire reactor therefore consists of a finite number of CSTRs connected in series. The key difference between a conventional tubular reactor and an OBR is that mixing intensity in the latter can be controlled, not by altering the flow rate, but instead by changing the oscillating conditions, impacting the size and frequency of vortex formation.

The Navier-Stokes equations have been used to calculate the flow patterns generated inside periodically constricted tubes (Sobey, 1980) and predict a two phase cycle for oscillatory flow: during acceleration vortices form behind constrictions in furrows, growing until flow reversal when they are forced into the mainstream flow and fade. These predictions were observed experimentally (Stephanoff et al., 1980) and are relevant to OBRs because orifice plates produce constricted regions, resulting in similar flow conditions when oscillated.

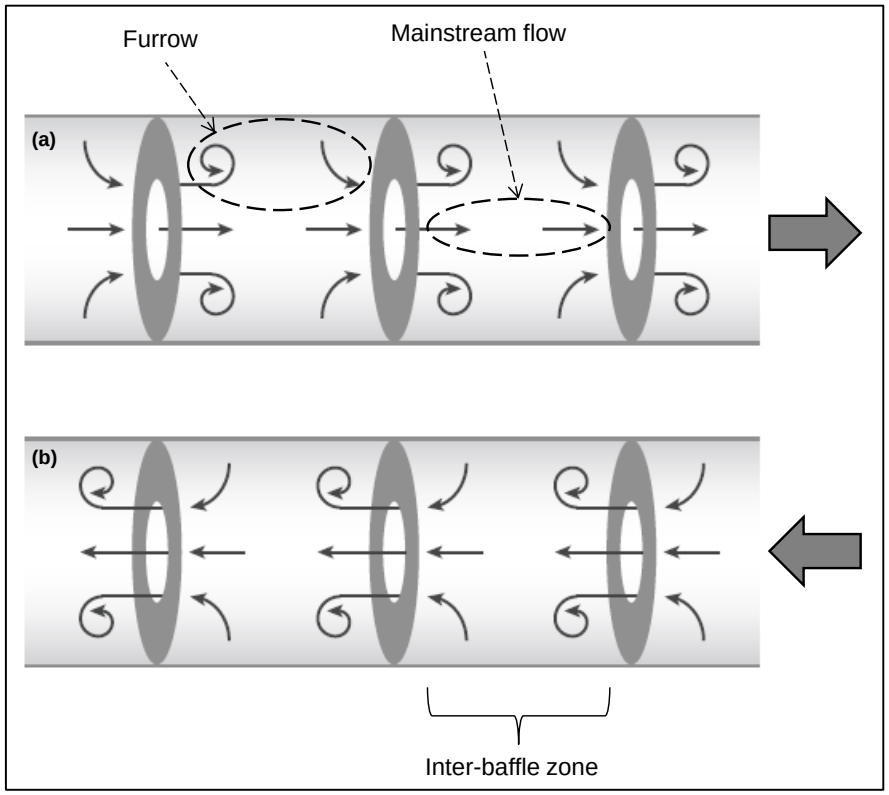


Figure 2.2: Vortex formation in an OBR created by oscillatory flow. Vortices form in the furrows during acceleration and are forced into the mainstream flow during flow reversal. (a) Back stroke. (b) Forward stroke.

2.3.3 Geometrical parameters

The geometrical parameters, or physical dimensions, important for OBR design are summarised in Table 2.1 and Figure 2.1b. In total there are five parameters which need to be considered with the baffle spacing (L) and baffle open area (α), defined as $(D_o/D)^2$, being the most important.

Table 2.1: Geometrical parameters important for OBR design.

Parameter	Symbol	Optimal value	Reference
Baffle thickness	δ	2-3 mm	(Ni et al., 1998)
Baffle spacing	L	1.5 D	(Brunold et al., 1989)
Baffle open area	α	20-22%	(Ni et al., 1998)
Orifice diameter	D_o	0.45 -0.50 D	(Ni et al., 1998)
Tube diameter	D	Usually 10-150 mm	N/A

The shape and length of vortex formation is defined by L. To generate uniform and effective mixing, vortices require adequate room to fully expand and spread throughout the inter-baffle zone. Suboptimal distances result in vortices colliding with neighbouring baffles before full expansion, leading to undesired axial dispersion when operating continuously. Superoptimal distances lead to vortices that do not

propagate through the full volume of the inter-baffle region, producing stagnant regions. The effects on mixing of altering the baffle spacing using distances of 1, 1.5 and 2 times D has been evaluated (Ni et al., 1998). A spacing of 1.5D gave the most effective mixing over the greatest range of oscillation amplitudes (X_o) so has been standardised and used for most subsequent work with OBRs.

The width of vortices formed within inter-baffle zones is defined by α, with larger values giving rise to narrow vortices and, consequently, poor mixing. By reducing the orifice diameter (D_o) fluid is constricted to a greater extent as it passes through each baffle resulting in wide vortex formation, generating effective mixing conditions. The effects on mixing for $11 \leq \alpha \leq 51$ % has been evaluated using the 'mixing time' defined as 'the time measured from the instant of tracer addition until the column contents has reached a specified degree of uniformity' (Brunold et al., 1989). Using 4% sodium hydroxide as tracer, $20 \leq \alpha \leq 22$% was found to minimise the mixing time. Baffle thickness (δ) was also evaluated in a 50 mm diameter OBR using thicknesses of 1-48 mm (Brunold et al., 1989). It was found that thicker baffles resulted in vortex deformation due to an increased 'cling time' with the optimum thickness being identified as 2-3 mm.

2.3.4 Operational parameters

2.3.4.1 Batch operation

Table 2.2 summarises the dimensionless groups and dynamic parameters used in oscillatory flow.

The oscillatory Reynolds number (Re_o) gives an indication of mixing intensity. Flow separation occurs when the boundary layer becomes detached from a surface and forms vortices, in this case the OBR contents detaching from the wall. For flow separation to occur in OBRs, Re_o must exceed 50-100 (Neves-Saraiva, 1998), as opposed to a net flow Reynolds number (Re_n) in conventional smooth walled, baffle-free tubular reactors of 2100 (Perry et al., 1997). An increase in Re_o can be achieved *in situ* by increasing the amplitude (X_o) or frequency (f) of oscillation, producing a wide range of mixing intensities from 'soft' ($50 \leq Re_o \leq 500$), where vortex formation occurs, to the most intense ($Re_o > 5000$) corresponding to mixed flow with the OBR acting as a STR (Harvey et al., 2001).

Table 2.2: Dimensionless groups and dynamic parameters required for OBR operation.

Parameter	Symbol	Equation	Description
Centre to peak amplitude of oscillation (m)	X_o	N/A	Half the fluid oscillation distance.
Frequency of oscillation (Hz)	f	N/A	Number of oscillations per second.
Volumetric flow rate (e.g. mL/min)	Q	N/A	Volume of material entering the OBR over a given time period.
Reynolds number of oscillation	Re_o	$(\rho.2\pi f.X_o.D) / \mu$	A measure of mixing intensity.
Strouhal number	St	$D / 4\pi.X_o$	A measure of effective vortex propagation.
Net flow Reynolds number	Re_n	$(\rho.D.u) / \mu$	A measure of the net flow.
Velocity ratio	ψ	Re_o / Re_n	The ratio of Re_o to Re_n.

The Strouhal number (St) is inversely proportional to X_o and measures vortex propagation (Brunold et al., 1989, Ni and Gough, 1997). Large St values are produced at small amplitudes giving poor vortex formation and vice versa. From studies available in the literature, the tested range for St is 0.01–9 (Ni et al., 1998, Dickens et al., 1989) however, the most common range used is 0.15–4 (Mackley and Stonestreet, 1995, Smith, 1999).

2.3.4.2 Continuous operation

When operating continuously, the Re_o should dominate, giving almost full reversal in flow, thereby creating vortices and generating effective mixing. The value of the velocity ratio (ψ) is a measure of the degree of plug flow achieved (Stonestreet and van der Veeken, 1999) and can be evaluated using the tanks-in-series (TiS) model for plug flow (Levenspiel, 1999).

The TiS model assumes flow conditions can be represented by a variable number of ideal CSTRs in series (N_t). As N_t increases, the predicted residence time distribution (RTD) curve during a pulse test approaches one which would be observed during perfect plug flow. RTD is the probability distribution that describes how long material could spend in a reactor. Mixed flow is characterised by an RTD with a peak followed

by a steady decline whereas plug flow is symmetrical about the mean residence time. Mixed and plug flow describe two extreme RTDs achievable but, in reality, the true flow condition lies somewhere in between. If N_t is infinite, then all molecules leave the reactor with identical residence times, a characteristic of perfect plug flow. Achieving approximations to plug flow is beneficial for processes that require precise residence times or removal of back mixing.

Experimental RTD profiles have been produced by injecting 3 M aqueous potassium chloride into a 1.3 L OBR with a net flow of deionised water under varying Re_o and Re_n (Stonestreet and van der Veeken, 1999). By comparing to theoretical RTD profiles, N_t was evaluated and plotted against ψ. The range of $2 \leq \psi \leq 4$ maximised N_t and generated optimal plug flow conditions however, useable degrees of plug flow can still be achieved using values of ψ between 2 and 10. Using values of ψ below 2 results in loss of the major design advantage of achieving plug flow in reduced length reactors for long residence time processes, whereas values of ψ above 10 lead to loss of plug flow.

2.4 Bioprocessing advantages

2.4.1 Overview

The OBR offers numerous advantages over conventional reactors that not only allow development of continuous bioprocesses, but can also enhance reaction rates and productivity during batch operation. Some of these advantages are unique to culturing living organisms that require key nutrients for maximum growth. For example, living cells that often require oxygen (e.g., yeast) or carbon dioxide (e.g., microalgae) to grow and can be shear sensitive because of their relative fragility and large size. Table 2.3 summarises these major advantages.

Table 2.3: Advantages provided by OBRs over conventional CSTRs and tubular reactors.

Advantage	Description	Benefit	References
Uniform mixing	The vortex cycle creates equal radial transfer across the tube producing uniform mixing patterns throughout the OBR, reducing heat and concentration gradients.	The removal of gradients can reduce the overall reaction time; e.g., up to 18-fold reduction for speciality chemicals.	(Ni, 2006)
Low and uniform shear	Low, uniform shear in the OBR compared to STRs makes the reactor more suitable for shear-sensitive organisms and large molecules.	Up to a 10-fold reduction in shear rates compared to STRs. Generates increased particle flotation.	(Ni et al., 2000, Anderson et al., 2009)
Increased mass transfer	Uniform bubble size and increased gas hold up produce enhanced mass transfer rates.	6-fold and 75% increases for oxygen transfer (k_La) into water and yeast culture, respectively.	(Hewgill et al., 1993, Ni et al., 1995)
Compact reactor design	The ability to generate long residence times, under plug flow, with reduced reactor lengths allows compact designs.	Up to a 600-fold decrease in reactor length compared to conventional tubular reactors.	(Stonestreet and Harvey, 2002)
Linear scale up	Maintaining St, Re_o and Re_n allows mixing intensity and flow conditions to be predicted in large volume OBRs using data from lab-scale experiments.	Lab-scale experiments can be scaled up by increasing OBR diameter or length while maintaining predictability.	(Smith, 1999, Smith and Mackley, 2006, Jian and Ni, 2005)

2.4.2 Reduced shear rate

Shear is an important factor for bioprocesses involving cells or large molecules, such as enzymes, which can be inhibited by high shear rates. The biological definition is given as 'the rate of change of velocity at which one layer of fluid flows over an adjacent parallel layer, often expressed in seconds^{-1}' (Biology-online, 2010). Particle image velocimetry (PIV) has been used to record the shear rate distribution in a 50 mm diameter OBR and a close correlation between Re_o and the mean shear rate (γ_{OBR}) was observed (Ni et al., 2000).

The average shear rate generated in STRs is proportional to the impeller speed (N), although this relationship differs according to various authors (Tanguy et al., 1996, Harnby et al., 1992, Nagata, 1975). A comparison is shown in Figure 2.3 using the lowest estimation of shear for a 2 L STR (Harnby et al., 1992), previously used for

comparison against a 50 mm OBR ($251 \leq Re_o \leq 4021$) (Ni et al., 2000). The average shear rate has been plotted against power density (P/V): a measure of energy being applied to a system, expressed in W/m^3.

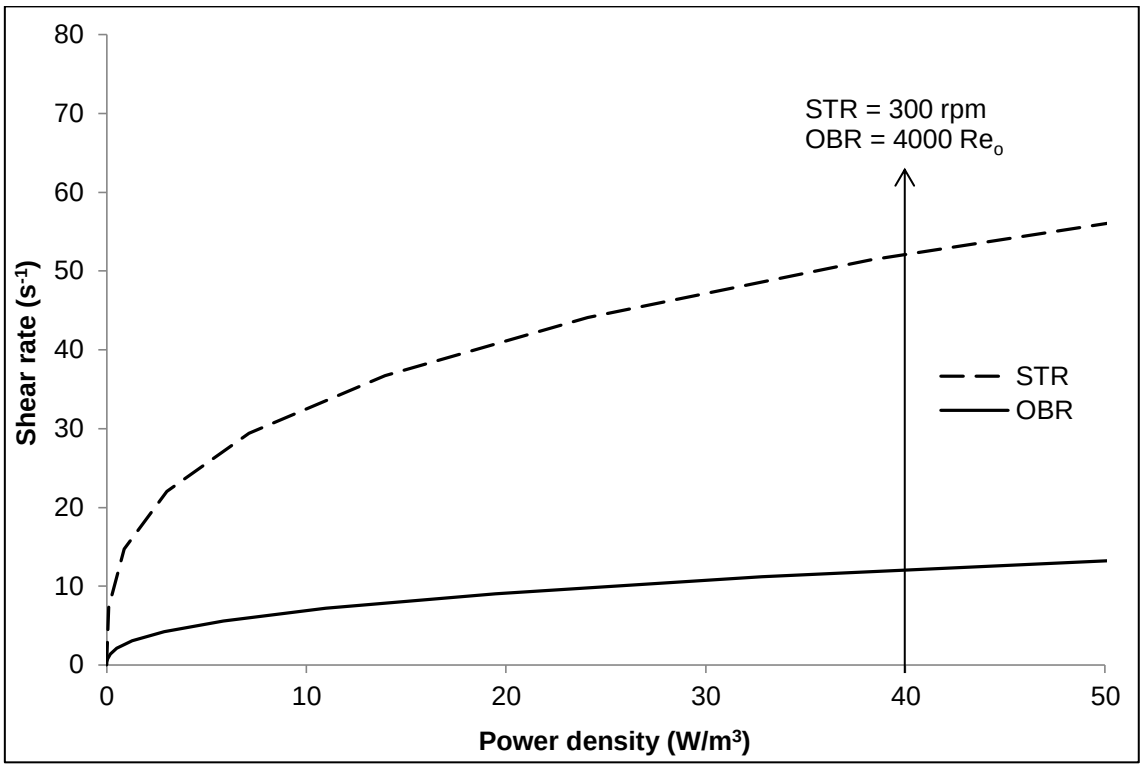


Figure 2.3: Theoretical average shear rates for a 50 mm OBR (solid) and 2 L STR (dashed) during unaerated operation at increasing power densities (Ni et al., 2000).

Figure 2.3 demonstrates that the average shear rate is much higher in STRs: at 40 W/m^3, the OBR shows a 5-fold reduction. Periodic shedding of vortices and vortex-vortex interactions provide uniform shear, controlled by the oscillation conditions. The flow patterns in OBRs are such that radial and axial flows are of similar magnitude. This leads to a more uniform shear field. In particular the high shear points around impellers in typical STRs are absent. High shear near the impeller in STRs can damage micro-organisms even if the experience is brief. Mammalian and insect cells have been reported to be shear-sensitive (van der Pol and Tramper, 1998, Tramper et al., 1986), as well as cellulase enzymes, which are important for saccharification reactions (Reese and Ryu, 1980, Kaya et al., 1996, Ganesh et al., 2000, Gunjikar et al., 2001), and microalgae (Gudin and Chaumont, 1991). The OBR offers a viable alternative for bioprocesses inhibited by high shear rates produced in STRs where a degree of mixing is required to achieve sufficient mass transfer.

Shear uniformity throughout a reactor benefits bioprocesses where larger particles, millimetres in size, are being used. For example, during flotation particles must be

suspended and sedimentation avoided. Flotation is a separation technique whereby material is ground into fine particles before being made hydrophobic by surfactant addition. Once suspended in an agitated tank sparged with air, the hydrophobic particles attach to rising air bubbles forming a surface froth to be concentrated and purified.

Fine quartz particles, 3–104 μm in size, rendered hydrophobic using dodecylamine were used to test the suitability of an OBR for use as a flotation device (Anderson et al., 2009). A 60% improvement in flotation for finer particles, <30 μm, and 30–40% for coarse particles up to 104 μm at much lower power densities compared to conventional flotation devices was reported. Compared to Rushton impeller agitated flotation devices that can have up to 30–40 times the energy dissipation close to the impeller compared to the bulk of the vessel (Koh and Schwarz, 2003), OBRs have been shown to have a more even distribution of shear (Ni et al., 2000), explaining the improvements in flotation. This strongly suggests that OBRs are suitable for performing bioprocesses containing biomass particles that need to be retained in suspension without sedimentation.

2.4.3 Enhanced mass transfer

Many bioprocesses utilise aerobic organisms to produce useful products such as polyhydroxyalkanoates (PHA), a precursor for bioplastics, synthesised by the aerobic bacterium, *Pseudomonas putida* (Troeger and Harvey, 2009). During culture of these organisms, air must be sparged into the reactor providing oxygen. In some cases, the oxygen uptake rate (OUR) becomes higher than the maximum achievable oxygen transfer rate (OTR) resulting in oxygen limitation. One method to overcome this is to sparge with pure oxygen but this creates additional safety issues and adds cost. However, OBRs offer an alternative solution to increasing OTRs with 6-fold increases in k_La for oxygen transfer into water reported compared to conventional STRs (Hewgill et al., 1993).

Mass transfer of gases, in particular oxygen, into liquids is usually quantified using the k_La: ‘the volumetric mass transfer coefficient that describes the efficiency with which oxygen can be delivered to a bioreactor’ (Kane, 2012). Rate of mass transfer of gas is a function of the mass transfer coefficient of the specific gas in question and the surface area available for transfer into the bulk liquid medium (which itself is a

function of both bubble size and hold-up time). The k_La values for a 2 L STR and 50 mm diameter OBR during the fermentation of yeast cells, *Saccharomyces cerevisiae*, have been determined (Ni et al., 1995). A comparison of k_La against power density using this data is shown in Figure 2.4.

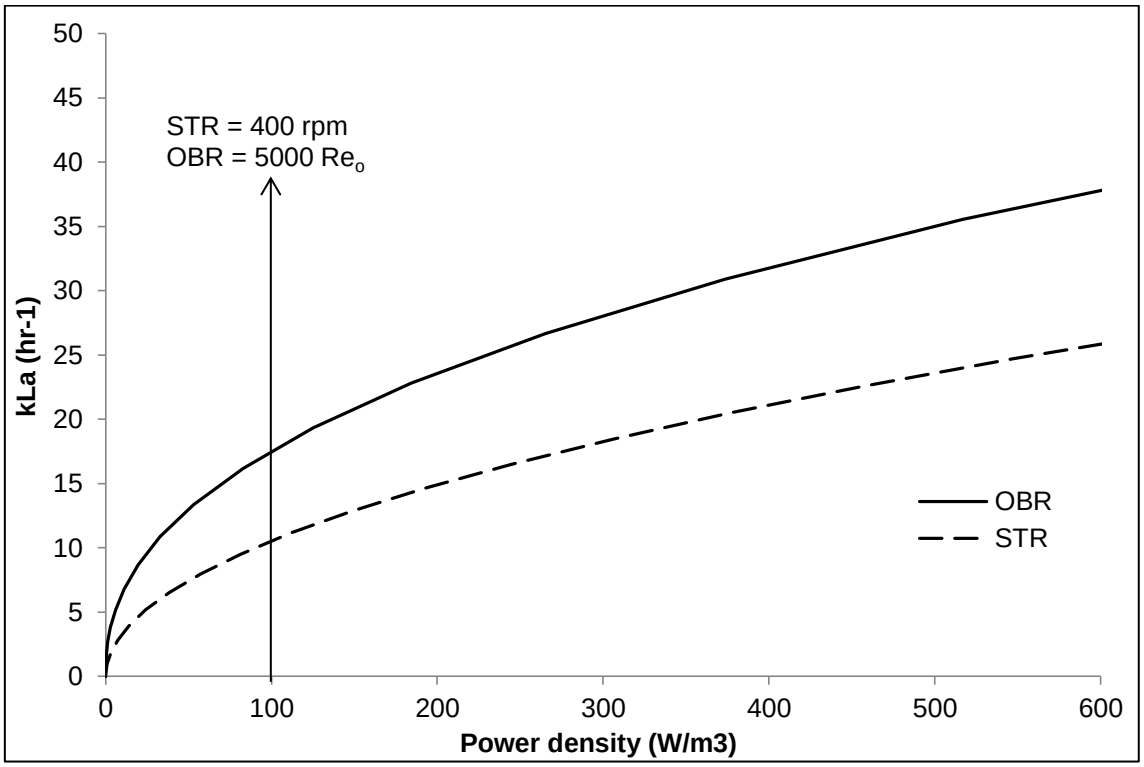


Figure 2.4: k_La against increasing power density for a 50 mm OBR (solid) and 2 L STR (dotted) at a constant aeration rate (Ni et al., 1995).

At a power density of 100 W/m^3 intense mixing conditions are produced in both reactor types. However, the k_La value produced in the OBR is approximately 75% higher than the STR: predominantly a result of enhanced gas hold-up time but also reduced bubble diameter (Oliveira and Ni, 2001). The unique fluid mechanics in OBRs produce a longer path length for individual gas bubbles, thereby increasing gas hold-up, by increasing each bubble's residence time. Vortex interaction with gas bubbles causes breakup producing a larger surface area for gas transfer. These characteristics allow OBRs to maintain sufficient oxygen transfer with good mixing at low shear. Achieving similar values for k_La in STRs would require reactor modification (Jenzsch et al., 2004), increased impeller speeds (producing increased shear rates) or a switch to pure oxygen.

2.4.4 Compact design for plug flow

Traditionally plug flow has been achieved on an industrial scale by either connecting a series of CSTRs together or using tubular reactors under turbulent flow conditions

(Ni, 2006). On a practical level, there is a limit to how many CSTRs can be connected in series or how long a reactor can be. In OBRs mixing intensity is decoupled from the flow rate allowing long residence time processes in reduced length reactors. For example, it has been calculated that for a flow rate of 2,381 L/hr and residence time of 4 hours, the required reactor lengths for an OBR and conventional tubular reactor are 1,213 and 757,894 m, respectively (Stonestreet and Harvey, 2002). The OBR offers an alternative process intensification methodology that provides a high degree of plug flow in a reactor with a much reduced length (Stonestreet and van der Veeken, 1999).

2.5 Scale up

2.5.1 Direct diameter increases

A promising aspect of OBR technology is the ability to scale up processes by maintaining geometric and dynamic similarity, allowing mixing and flow conditions produced at laboratory scale to be easily replicated for pilot and industrial scale processes. St, Re_o and Re_n are assumed to fully define the fluid dynamic conditions for a particular OBR geometry (Smith and Mackley, 2006). By keeping these parameters constant, an OBR with a diameter of, for example, 24 mm should behave the same as one with a diameter of 150 mm.

Axial dispersion coefficients (D_c) have been used to study the effects of tube diameter on the mixing and flow conditions of three OBRs with 24, 54 and 150 mm diameters (Smith and Mackley, 2006). An imperfect pulse technique was adopted and the pulse concentration measured at a minimum of two points downstream. By comparing the two RTD profiles, D_c was calculated using the dispersion model (Levenspiel, 1999). The model assumes that a diffusion-like process is occurring that is superimposed on to plug flow resulting in axial spreading of material. D_c represents the extent of spreading with large values equating to rapid spreading and small values to slow spreading, or closer approximations to true plug flow. Methylene blue was selected as the tracer due to its high optical density at low concentrations and measured using optical sensors placed at known distances from the site of injection.

Three distinct flow regimes were identified: for $Re_o<80$, D_c tends towards $5x10^{-4}$ m^2/s; for $80<Re_o<800$ axial dispersion was minimised with values for D_c as low as 10^{-4} m^2/s; and for $Re_o>800$, D_c increases approximately linearly with Re_o (Smith and

Mackley, 2006). Optimal interaction between the net flow and oscillatory mixing was identified as the reason for the minimum: at $80<Re_o<800$ the vortices created were optimal for radial redistribution of dye, thereby minimising axial dispersion (Smith, 1999). It was established that axial dispersion was not a function of D, indicating that fluid dynamic conditions could be maintained during scale up providing St, Re_o and Re_n were kept constant.

2.5.2 Multi-orifice design

During scale up of OBRs, a doubling of the reactor diameter must be complemented with a doubling in X_o to maintain St at a specified value. This results in X_o being fixed leaving only f as a variable operational parameter to control the Re_o. Table 2.4 summarises the values of X_o and f required to maintain St and Re_o at 1.0 and 500, respectively, during scale up from 25 to 150 mm diameter.

Table 2.4: Required operating conditions to maintain St and Re_o at 1.0 and 500, respectively.

Tube diameter (mm)	Required X_o (mm)	Required f (Hz)
25	1.99	1.6
50	3.98	0.4
150	11.94	0.04

At higher diameters, the frequency of oscillation must be extremely low which reduces the mixing intensity and the opportunity for improved mass transfer (Smith, 1999). To overcome this problem, a method of scale up involving a bundle of relatively small diameter OBRs operated in parallel, thereby removing the need for extremely low frequencies, has been proposed (Ni, 1994). However, this solution produces two other problems: how to maintain an equal distribution of flow to each separate tube; and generating equal oscillating conditions.

A different approach has been adopted whereby baffles containing multiple orifices are used with D being replaced by the effective tube diameter (D_e), calculated by dividing the total baffle area by the number of orifices. It was predicted that a 150 mm diameter OBR with internal baffles consisting of 37 orifices would behave in a similar way to a 24 mm diameter OBR (Smith and Mackley, 2006). This was demonstrated by recording similar axial dispersions at increasing Re_o, while maintaining Re_n at 107, in both 24 mm and multi-orifice 150 mm OBRs (Smith and Mackley, 2006). The major advantage of this design is that the same shear rates and intensity of mixing

achieved in a smaller diameter OBR can be maintained while greatly increasing the throughput of the process (per unit length of reactor). Multi-orifice designs are particularly attractive due to the ease of manufacture and ability to maintain fluid mechanics and axial dispersion, allowing experiments conducted at laboratory scale to be increased to industrial volume with predictability of axial dispersion and mixing intensity (Smith, 1999). Figure 2.5 depicts a multi-orifice baffle design, 100 mm in diameter that produces characteristics observed in a conventional 25 mm OBR.

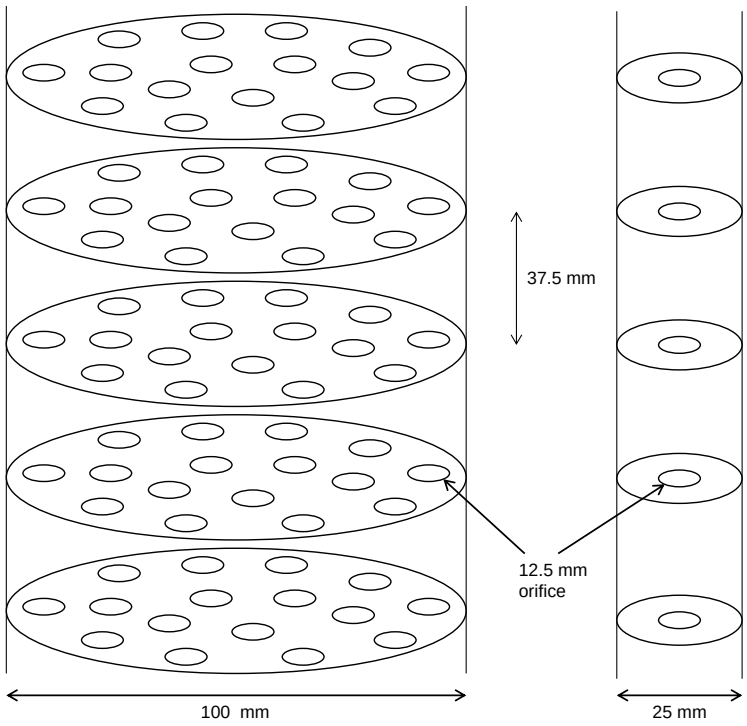


Figure 2.5: Multi-orifice baffle design creating the effect of 16 ‘standard design’ 25 mm diameter OBRs operated in parallel for a 100 mm diameter reactor (Smith, 1999).

2.6 Bioprocessing in OBRs

2.6.1 Overview

Typical chemical processes can take anywhere from fractions of a second to many hours in a conventional reactor. The process advantages of OBRs have been investigated and described for a range of applications including biofuel production (Harvey et al., 2003, Masngut et al., 2010), flotation (Anderson et al., 2009), butylation of phenylacetonitrile (Wilson et al., 2005), oil droplet breakage (Mignard et al., 2006, Mignard et al., 2004, Ni et al., 2002, Zhang et al., 1996), photo-oxidation (Gao et al., 2003, Fabiyi and Skelton, 1999), polymerisation (Ni et al., 1999) and extensive work on crystallisation (Brown and Ni, 2011, Ni et al., 2004, Ni, 2009, Lawton et al., 2009, Chew and Ristic, 2005, Ristic, 2007). However, this review aims

to target bioprocesses so chemical reactions will not be discussed in any further detail.

Tables 2.5-2.7 summarise the current bioprocesses conducted in OBRs available in the literature, all being performed in batch mode. They have been grouped into those processes that use a cellular component such as an enzyme (Table 2.5) and those using living cells, under both anaerobic (Table 2.6) and aerobic (Table 2.7) conditions.

2.6.2 Bioprocesses using cellular components

Three of the four bioprocesses described in Table 2.5 are related to protein refolding: a key unit operation when producing recombinant biopharmaceuticals from expression systems such as *Escherichia coli*. Most protein refolding operations are optimised with respect to the chemical environment (Lee et al., 2002) however, the mixing environment also impacts on refolding yield (Lee et al., 2001, Goldberg et al., 1991). The preferred protein refolding method at industrial scale remains direct dilution in STRs, mainly because of its simplicity and widespread use. However, as STRs are scaled from laboratory to industrial volumes, the mixing efficiency declines (Lee et al., 2002) impacting negatively on protein refolding yield.

Results summarised in Table 2.5 demonstrate that protein refolding can be performed in OBRs with comparable yields to STRs at laboratory scale. The lack of improvement does not suggest, however, that OBRs offer no additional benefit to this bioprocess. The major advantage on offer is a scalable mixing environment (Smith and Mackley, 2006), allowing yields obtained in the lab to be predictably replicated on an industrial scale: currently unachievable using STRs. As a result, higher yields are possible at large scale during protein refolding bioprocesses, reducing overall production costs.

Table 2.5: OBR bioprocesses using cellular components.

Bioprocess	Cellular component	Year	Conclusion	Process Type	Reference
Protein refolding	Hen egg white lysozyme	2001	OBRs are suitable refolding devices and comparable to STRs.	Non-cell culture	(Lee et al., 2001)
Protein renaturation	Hen egg white lysozyme	2002	Refolding yield in OBR comparable to STR. Uniform shear important for successful refolding.	Non-cell culture	(Lee et al., 2002)
Protein refolding	Chicken egg white lysozyme	2006	Prevention of aggregation enhances refolding during initial stages (0-4 mins).	Non-cell culture	(Reis, 2006)
Saccharification	Cellulase from *Trichoderma reesei*	2011	7% increase in glucose production after 48 hours compared to shake flask.	Enzymatic	(Ikwebe and Harvey, 2011)

Lignocellulosic materials are ubiquitous in nature with cellulose, the support molecule in plants, being the most abundant carbohydrate on Earth. It is possible to hydrolyse cellulose (saccharification) using various chemical or biological methods, thereby liberating the glucose monomers that constitute its structure, which can be fermented into a variety of useful chemicals including ethanol and lactic acid (Sarkar et al., 2012, Abdel-Rahman et al., 2011). During the enzymatic conversion of cellulose into monosaccharide, cellulase deactivation occurs. This deactivation of cellulases is caused by a number of process-dependent factors: shear inactivation (Reese and Ryu, 1980, Kaya et al., 1996, Ganesh et al., 2000, Gunjikar et al., 2001), sugar inhibition (Takagi, 1984, Xiao et al., 2004, Ye et al., 2012), ion strength (Kumakura, 1996), temperature (Demerdash and Attia, 1992) and formation of inert enzyme substrate complexes. A further factor involved in cellulose depolymerisation is the changing nature of the substrate over time; the easily hydrolysable amorphous regions are digested first leaving the recalcitrant crystalline regions (Gan et al., 2003). Saccharification has been conducted in a 25 mm diameter OBR using X_o and f values of 3 mm and 3 Hz, respectively (Ikwebe and Harvey, 2011). The substrate used was pure microcrystalline cellulose at a loading of 2.5% w/v. An enzyme loading of 40 filter paper units (FPU) per gram of cellulose and 10% β-glucosidase was used. The results of the study showed an increase in the glucose yield of 7%

after 48 hours compared to a shake flask run under the same conditions. The increase in glucose production in the OBR was attributed to a 'better mixed hydrolysis environment' (Ikwebe and Harvey, 2011). In other words, the uniform and effective mixing under low shear rates allowed the cellulase enzymes access to substrate, increasing glucose production. A further possible benefit is the reduced shear rates present in OBRs. One of the factors contributing to cellulase deactivation is shear which, if reduced, will result in cellulases retaining their activity for longer generating increased glucose. However, as the comparison was made with a shake flask, this requires further investigation.

2.6.3 Anaerobic bioprocesses

Table 2.6 lists the anaerobic bioprocesses previously conducted in an OBR.

Flocculation is a process by which small particles aggregate, with the aid of a polymer, to form flocs large enough to settle or be filtered: commonly used industrially for example in water and wastewater treatment (Ni et al., 2001). Traditionally, STRs known as stirred tank flocculators are used for this process to provide agitation essential for generating particle collisions. An OBR has been used as a flocculation device for the bacterium *Alcaligenes eutrophus* and compared to an STR, assessing the percentage flocculation at various operating conditions (Ni et al., 2001). Although 100% flocculation was not reached in the OBR, a comparison to another study (Whittington and George, 1992) demonstrated that, for similar starting bacterial concentrations, fuller flocculation was achieved in the OBR at much lower shear rates. Previous authors have commented on the non-homogenous nature of STRs that result in floc breakup near the impeller zone where shear rates can be 2 orders of magnitude higher than the average (Glasgow and Kim, 1986). The more even shear distribution in OBRs (Ni et al., 2000) allows flocculation to occur at much lower average shear providing an attractive, alternative flocculation device to STRs.

Table 2.6: OBR bioprocesses using anaerobic cell cultures. *Denotes patent pending

Bioprocess	Organism	Year	Conclusion	Cell type	Reference
Flocculation	*Alcaligenes eutrophus*	2001	Higher degree of flocculation compared to STR at lower polymer dose. St the dominant factor	Bacterial	(Ni et al., 2001)
*ABE fermentation	*Clostridium acetobutylicum*	2009	115% increase in solvent production in OBR from 0-0.78 Hz. 90% increase in butanol compared to STR	Bacterial	(Takriff et al., 2009, Masngut et al., 2010, Cooper et al., 2011)*
*Anaerobic process	Patent for culture of facultative / obligate anaerobes in OBRs	2010	Patent publication	Bacterial	(Cooper et al., 2009)*
Ethanol production	*Saccharomyces cerevisiae*	2011	9% increase in ethanol production after 48 hours compared to shake flask	Yeast	(Ikwebe and Harvey, 2011)

Acetone, butanol and ethanol (ABE) fermentation and bioethanol production have been extensively covered in a previous review (Masngut et al., 2010) so will not be discussed in any further detail. No data currently exists for anaerobic digestion (AD) using OBRs as the reference is to a patent pending (Cooper et al., 2009). The patent describes a system for using OBRs as a generic platform for culture of facultative and obligate anaerobes. Volatile fatty acids and methane were produced using microbial consortia from the rumen. The culture of gut fungi was also highlighted in the patent.

2.6.4 Aerobic bioprocesses

Table 2.7 lists the aerobic bioprocesses previously conducted in an OBR.

Alcaligenes eutrophus H16 has commercial interest for production of the biodegradable plastic, poly-β-hydroxybutyrate (PHB). This bacterium has been cultured in an OBR (Harrison and Mackley, 1992) with a maximum specific growth rate (μ_{max}) of 0.39 h^{-1} compared to 0.36 h^{-1} and 0.35 h^{-1} when using Erlenmeyer flasks at 10 and 40% working volumes, respectively. OBRs are, therefore, suitable for culturing rapidly growing, oxygen demanding microorganisms. The same paper alluded to the use of OBRs for culturing animal cells: an interesting proposal as they

are notoriously shear-sensitive (Chisti, 2001) with cell death being proportional to energy input so any scaled up reactor should minimise energy input (van der Pol and Tramper, 1998). Bioprocesses containing animal cells could greatly benefit from the low shear, high mass transfer environment produced in OBRs to minimise shear while maintaining sufficient OTRs.

The fruity, peach-like aroma compound, γ-decalactone, has applications in the food industry as flavouring and can be biologically produced from the yeast, *Yarrowia lipolytica*. Micro-reactors are important tools for rapidly screening and optimising bioprocesses (Reis et al., 2006b). A mesoscale OBR (D=4.4 mm) has been used to culture *Yarrowia lipolytica* for the production of γ-decalactone (Reis et al., 2006a). The processing time required to reach maximum γ-decalactone concentration in the OBR was 50% lower compared to traditional scaled down platforms: STRs and shake flasks. Enhanced mass transfer rates were reported as the reason for the observed reduction in processing time. The same mesoscale OBR was used to culture *Saccharomyces cerevisiae,* with an increase in biomass of 83%, using 93.6% less air, compared to a scaled down STR. The use of mesoscale OBRs for rapid screening and optimisation has two major advantages: precise control over mixing and mass transfer; and optimisations achieved at this scale can be predictably scaled up to industry volumes.

A. pullulans IMI 145194 was cultured in a 100 mm diameter OBR, with a working volume of 2.5 L, using fixed X_o and f values of 20 mm and 2 Hz, respectively. The aeration rate was optimised and then kept constant at 1.0 vvm (volume of air per unit volume of medium per minute). The results showed that production of the versatile biopolymer pullulan occurred during the exponential and stationary phases, reaching a concentration of ~11.7 g/L after 38 hours. These values were compared to STR data available in the literature (Madi, 1995), indicating that to reach a comparable pullulan concentration in 2 and 10 L STRs, the fermentation time must be increased to 96 and 144 hours, respectively. This equates to a reduction in the required processing times of 60% and 74% when using OBRs as opposed to STRs and highlights the problems encountered when scaling up STRs. The move from 2 L to 10 L using STRs has resulted in a 50% increase in the required processing time to reach similar product concentrations.

Table 2.7: OBR bioprocesses using aerobic cell cultures.

Bioprocess	Organism	Year	Conclusion	Cell Type	Reference
Animal cell culture	Unknown	1991	Feasible to culture animal cells in OBRs.	Mammalian	Cited in (Harrison and Mackley, 1992)
PHB production	*Alcaligenes eutrophus* H16	1992	Feasible to culture rapidly growing, O_2 demanding microorganisms in OBRs.	Bacterial	(Harrison and Mackley, 1992)
Yeast culture	*Saccharomyces cerevisiae*	1995	75% increase in OTR (k_La) compared to STR.	Yeast	(Ni et al., 1995)
Pullulan production	*Aureobasidium pullulans* IMI 145194	2005	Pullulan of 11.3 and 12.1 g/L takes 96 and 144 hours in STRs compared to 37-39 hours in OBR.	Fungal	(Gaidhani et al., 2005)
γ-decalactone production	*Yarrowia lipolytica* W29	2006	50% time reduction for maximum [γ-decalactone] compared to other scaled down reactors.	Yeast	(Reis et al., 2006a)
Yeast growth rate	*Saccharomyces cerevisiae*	2006	Biomass increases of 83 and 214% in OBR when compared to STR and shaken flask respectively	Yeast	(Reis et al., 2006b)
PHA production	*Pseudomonas putida*	2009	56% increase in biomass after 25 hours compared to STR	Bacterial	(Troeger and Harvey, 2009)

No reasons were given for possible causes of increased pullulan production but it seems likely that both effective oxygen transfer and low shear rates contribute. It is not entirely clear which of these factors is more important however, previous studies have shown that pullulan production increased at higher impeller speeds and, therefore, high oxygen transfer but also higher shear (Rho et al., 1988, McNeil and Kristiansen, 1987). These are contradicted by another study suggesting that low impeller speeds and low shear are optimal (Wecker and Onken, 1991). It has been demonstrated that an assisted airlift reactor designed for maximum oxygen transfer

and mixing produced a significant increase in pullulan production from *A. pullulans* (Gibbs and Seviour, 1992). These studies suggest that both high oxygen transfer and low shear are most effective for pullulan production. Both of these conditions are hard to achieve simultaneously in conventional STRs because any increase in the impeller speed for increased oxygen transfer is accompanied by an increase in shear. Figures 2.3 and 2.4 show, however, that an OBR can achieve both of these conditions which could explain the increased pullulan production observed, highlighting the potential of OBRs for this type of bioprocess.

2.7 Industrial implementation

The ultimate niche application for the technology would be in continuous bioprocessing under plug flow conditions, removing down-time inherent in batch processing and reducing plant footprint as a result of compact reactor design. However, in reaching this goal, several key barriers must be overcome in both the complex design of a large OBR and the conservative attitude prevalent in the bioprocessing industry.

2.7.1 Barriers

Table 2.8 highlights the key barriers facing adoption of OBR technology by the bioprocessing industry.

Bioprocesses typically require residence times in excess of 24 hours. Standard tubular plug flow reactors would need to be thousands of metres in length compared to the OBR's hundreds of metres. Nevertheless, over these distances transmission of oscillations, gas sparging and maintenance of a compact design remain problematic. There are also issues with fouling and the requirement to process large volumes, both relevant to any bioprocess utilising micro-organisms.

Table 2.8: Keys barriers facing adoption of OBR technology
Design
Lengths hundreds of metres required for fully continuous operation with residence times of hours to days
Pressure drop due to frictional losses will dampen oscillations
Gas bubbles during aerobic operation may dampen oscillations and disrupt plug flow
Multiple sparging and feeding points required down OBR length
Fouling of baffles and internal surfaces due to micro-organism adhesion
Large volumes required
Conservative nature of bioprocessing industry
Increased complexity of OBR technology compared to other bioreactor designs
No industrial scale OBRs dedicated to bioprocessing exist
Lack of data for industrial scale bioprocessing using OBRs

There are currently no industrial bioprocessing facilities using OBR technology so it has not yet been proven for an industrial process. The issue is that for industry to invest time and money developing an OBR capable of performing a continuous bioprocess with a residence time of hours or days, there must be solid evidence supporting the benefits of doing so. This evidence is currently based on smaller pilot and lab scale experiments in batch because even at reduced diameters, the length must still be hundreds of metres to support fully continuous operation: unlikely to occur in a university or small research company. Experimental evidence is therefore based on batch experiments (focusing on low shear and enhanced mass transfer) with advantages from continuous operation and predictable scale up being theoretical.

2.7.2 Recommended strategies

2.7.2.1 Design solutions

Obviously, a straight tube hundreds of metres in length would be impracticable. A compact reactor design can be maintained using a serpentine shape consisting of numerous short sections connected using baffled ‘u-bends’: a successful design used at lab scale. The benefit of this design is that it provides the opportunity to add numerous oscillators ensuring transmission of oscillations down the reactor which would otherwise diminish over the lengths required. Vertical orientation enables gas sparging at the base (and removal at the top) of each column for aerobic

bioprocesses. A major issue that needs addressing is how the flow conditions would be affected in those columns where the internal fluid is flowing against rising gas bubbles. Numerous oscillators and sparging points add complexity and possible contamination sites to the design and must be weighed against the benefits OBR technology would bring to the bioprocess.

Industrial scale bioprocesses require large volumes to be processed as product titres can be as low as 2 g/L (Meyer et al., 2008a). Current scale up using STRs is complex due to different mixing patterns occurring at scale. Conventional STRs are prone to impeller flooding, a phenomena characterised by gas rapidly flowing axially upwards passed the impeller with no radial discharge, and the formation of stagnant zones (Bombac and Zun, 2006, Rau et al., 1992). There are also other acknowledged limitations when using STRs including gas channelling, resulting in reduced gas dissolution, and poor bulk mixing (Rossi, 2001, Leib et al., 2001). This requires implementation of different scale up strategies depending on the specific bioprocess (Garcia-Ochoa and Gomez, 2009, Junker, 2004). By lengthening an OBR or increasing the diameter (see §2.5), volumes can be increased while maintaining the mixing environment. This allows large volumes to be processed either in batch or continuously under plug flow: the ultimate niche application of the technology not achievable in CSTRs.

It is possible that micro-organism adhesion to baffles and internal surfaces will present fouling issues, as occurs in other bioprocesses. Careful selection of construction material could mitigate this problem but it is likely that periodic cleaning will need to take place depending on the extent and rapidity of fouling.

2.7.2.2 Selecting a model bioprocess

The range of bioprocesses highlighted in Tables 2.5-2.7 demonstrate the variability in improvement witnessed from OBRs. It is imperative that each bioprocess is evaluated on a case-by-case basis to ensure the technology is being utilised to its full potential. The key operating advantages available from OBRs are low shear, enhanced mass transfer, scalability and continuous operation under plug flow. Selection of a bioprocess that benefits from at least one (and preferably more) of these is vital to ensuring the correct application of OBR technology.

However, proving the benefits of continuous operation and predictable scale up are difficult without development of long OBRs. This has resulted in all experiments being batch comparisons benefitting purely from low shear and enhanced mass transfer. Therefore, a shear sensitive organism (or component) requiring aerobic conditions will show the greatest improvements during batch operation (e.g., pullulan production (Gaidhani et al., 2005)). Several anaerobic bioprocesses have been conducted in OBRs and, with the exception of ABE production, have shown marginal improvements with the main justification for using the technology being scalability. In comparison, four aerobic bioprocesses witnessed a greater than 50% improvement providing greater incentive for industry to adopt OBRs (Troeger and Harvey, 2009, Gaidhani et al., 2005, Reis et al., 2006a, Reis et al., 2006b). It is difficult to predict those bioprocesses that will benefit from continuous operation and predictable scale up at this stage.

2.7.2.3 Open access facilities

Open access facilities provide equipment capable of testing various bioprocesses at scale. Large companies could utilise these facilities to gather data assessing the benefits of OBR technology for their specific bioprocess. Such facilities as The Centre for Process Innovation (CPI) on Teesside in the UK already house a number of OBRs available for industry-focused research. The next stage is to develop an OBR capable of fully continuous operation with at least a 24 hour residence time to generate solid data on the benefits of predictable scale up and continuous operation for specific bioprocesses.

2.8 Summary

OBR technology provides a novel production vessel for bioprocessing over a wide range of cellular components and microorganisms. The reactor has several advantages over conventional STRs: good mixing complemented with low shear; increased mass transfer rates; linear and predictable scale up; and continuous operation under plug flow conditions. As mixing intensity is controlled by oscillating conditions, long residence time processes required for biological reactions are possible in relatively short OBRs compared to conventional tubular reactors that rely on high flow rates to achieve mixing.

The advantages OBR technology could bring to bioprocessing are evident however, issues remain regarding the design and uptake of industrial scale OBRs. Reactors hundreds of metres in length will be required to realise the ultimate goal of using OBRs for continuous bioprocessing under plug flow conditions. Over these distances, it is likely that multiple oscillators and sparging points will be required and it is unclear as to how rising gas bubbles will interact with internal fluid, possibly disrupting plug flow. Open access facilities could prove essential in providing industry with OBRs capable of testing bioprocesses in a continuous fashion on large scale: currently not possible using small lab scale OBRs. Nevertheless, work to date has demonstrated the ability OBRs have to enhance product production during bioprocessing, moving closer towards developing viable replacement technologies based on sustainable, biological systems. More research is required to identify those bioprocesses that could be greatly intensified through OBR technology and funding provided to develop industrial scale systems, operated continuously. Table 2.9 summaries the findings of this review.

Table 2.9: Summary of literature review.

The OBR	Tubular reactor, containing orifice plates.
	Capable of batch or continuous operation.
	Reciprocating piston or pump creates oscillatory flow forming vortices for effective mixing.
Process advantages	Low and even distribution of shear compared to STRs.
	Increased mass transfer, specifically gases including O_2.
	Good global mixing.
	Enhanced reaction rates for specific bioprocesses.
	Bioprocesses inhibited by mass transfer, high shear and heterogeneity are likely to benefit more from OBRs.
Continuous operation	Plug flow achievable over a range of laminar flow rates.
	Compact plug flow design compared to conventional tubular reactors.
Scale up	Predictable scale up (for batch and continuous) from laboratory to industrial scale by maintaining geometric and dynamic similarity.

Chapter 3: Modelling plug flow and OBR design

'Rapid determination of the residence time distribution (RTD) function in an oscillatory baffled reactor (OBR) using a design of experiments (DoE) approach.'

3.1 Abstract

Residence time distribution (RTD) profiles were investigated in a standard oscillatory baffled reactor (OBR) as a function of oscillatory and bulk flow components using a design of experiments (DoE) approach. Two second order, polynomial models (R^2=92.1% and 97.3%) were fitted to N_t values estimated from concentration profiles and used to maximise plug flow conditions. The velocity ratio (Ψ) required to maximise plug flow was 1.9, agreeing well with the range previously identified by Stonestreet and van der Veeken (1999) (1.8<Ψ<2.0), suggesting the approach used here is valid. This method could be used to rapidly quantify and maximise plug flow in various OBR designs in a simple and robust manner which could prove valuable for the operation and design of continuous processes using OBR technology.

3.2 Introduction

Oscillatory baffled reactors (OBRs) have been investigated for the last two decades as novel systems for achieving efficient and uniform mixing at average shear rates up to one order of magnitude lower than standard stirred tank systems (STRs) (Ni et al., 2000). Mixing in OBRs occurs via fluid oscillation relative to equally spaced, low constriction orifice plates (baffles). Typically, a reciprocating pump or piston provides oscillatory motion of the fluid, forcing it through the baffles. This creates vortices behind each baffle along the entire OBR length, resulting in similar radial and axial flows that generate effective, uniform mixing, the intensity of which is precisely controlled by oscillation amplitude (X_o) and frequency (f).

Scale up of OBR technology on the basis of maintaining the same degree of axial dispersion has been achieved by maintaining the values of three dimensionless groups: the net flow Reynolds (Re_n), oscillatory Reynolds (Re_o) and Strouhal (St) numbers (Smith and Mackley, 2006, Smith, 1999). This simple scaling rule should allow optimisation studies conducted at laboratory scale to be easily transferred to pilot and manufacturing scales with a low degree of risk. Current STR technology suffers from a degree of unpredictability during scale up, caused by phenomena

including impeller flooding, stagnant zone formation, gas channelling and poor bulk mixing (Bombac and Zun, 2006, Rau et al., 1992, Rossi, 2001, Leib et al., 2001). Numerous scale up strategies that are highly process-dependent exist for STR technology, but no standardised strategy is guaranteed to replicate lab scale results at a larger scale (Garcia-Ochoa and Gomez, 2009, Junker, 2004).

Continuous processing is one method of process intensification as it allows increased production per unit reactor volume compared to conventional batch manufacturing (Anderson, 2012). OBR technology allows for the development of continuous processes that could reduce plant footprint and increase throughput (Stonestreet and Harvey, 2002). In continuous operation the reactor is often substantially smaller, thereby reducing the inventory of material in a hazardous state (although small feed vessels are usually still required). Continuous processing enables the exploration of novel manufacturing techniques of, for example, pharmaceuticals using reactions that utilise hazardous chemicals or are too exothermic for STRs (Trafton, 2012).

When converting a batch process to continuous in an OBR or any other form of batch reactor, the time dimension is effectively replaced by reactor length, provided axial dispersion is minimised. Plug flow describes the condition where no axial dispersion occurs (reactants have identical residence times) and, in practice, only approximations to plug flow are achievable. The velocity ratio (Ψ) is a dimensionless group associated with OBR operation based on oscillatory and bulk flow components. A range of $1.8<\Psi<2.0$ to maximise plug flow conditions was identified for ‘standard’ OBR designs (Stonestreet and van der Veeken, 1999). However, this range may not maximise plug flow for ‘non-standard’ OBR designs that use modified baffles, smaller diameters or other geometries (Phan and Harvey, 2011). For example, in meso-scale OBRs (4-5 mm diameters) with central baffles, plug flow was maximised for $4<\Psi<8$, and with integral baffles for $5<\Psi<10$ (Phan and Harvey, 2010).

An experimental design would be needed to identify the operating conditions required to maximise plug flow in ‘non-standard’ OBR designs, if plug flow was important for the process. A ‘one-factor-at-a-time’ (OFAT) approach involves the selection of numerous factors that are likely to affect the response (in this case, the degree of plug flow), and then changing the value of only one. A more efficient method is the factorial design of experiments (DoE) where the values of selected factors are

changed simultaneously (Fisher, 1926). The advantages of DoE over OFAT are the use of less time and resources as fewer runs are required. Interactions between factors can be estimated and experimental information is gathered from a larger region of factor space (Czitrom, 1999).

In this study, a simple, effective and robust method that is based on a DoE approach to rapidly assess and maximise plug flow conditions in a 'standard' OBR design is described, demonstrated and validated. Key operating conditions (factors) can be chosen to maximise plug flow (the response) in a methodical manner with the removal of extensive and time-consuming studies associated with an OFAT approach. A central composite design provides the framework to assess how relevant experimental factors and their possible interactions affect the RTD, which is modelled and quantified using the tanks-in-series model (TiS) for plug flow (Levenspiel, 1999).

3.3 Materials and methods

A horizontal 700 mL OBR operated continuously was used for this study. The design is such that both baffle spacing (L) of 1.5 times the reactor diameter (D) and baffle constriction ratio (α) of 0.25 are consistent with the 'standard' OBR design (Ni et al., 1998, Brunold et al., 1989). The baffle constriction ratio is defined as $(D_o/D)^2$ where, D_o is the baffle orifice diameter.

De-ionised (DI) water was used as bulk flow material, pumped through Norprene® tubing (Masterflex, size 36) by a peristaltic pump (Cole Palmer, Model 77200-62) at desired flow rates. A pulse dampener (Cole Palmer, HV-07596-20) was used to minimise oscillations caused by the pump that would interfere with oscillations in the OBR. Tracer pulses for each run consisted of 1 mL sodium hydroxide (2.5 M) injected over 3 seconds (Fisher Scientific, BP359-212). Two pH probes with response times of 10 s (Mettler Toledo, InPro 3250) were connected to digital displays (Mettler Toledo, M300) and measured pH at the exit (probe 2) and 50 mm from the injection point (probe 1). pH values were recorded every 10 seconds on a data logger (Eurotherm Chessel, 5180V). Each run began at the time of injection and ended once pH had returned to the starting value. A photograph of the apparatus used in this study is shown in Figure 3.1.

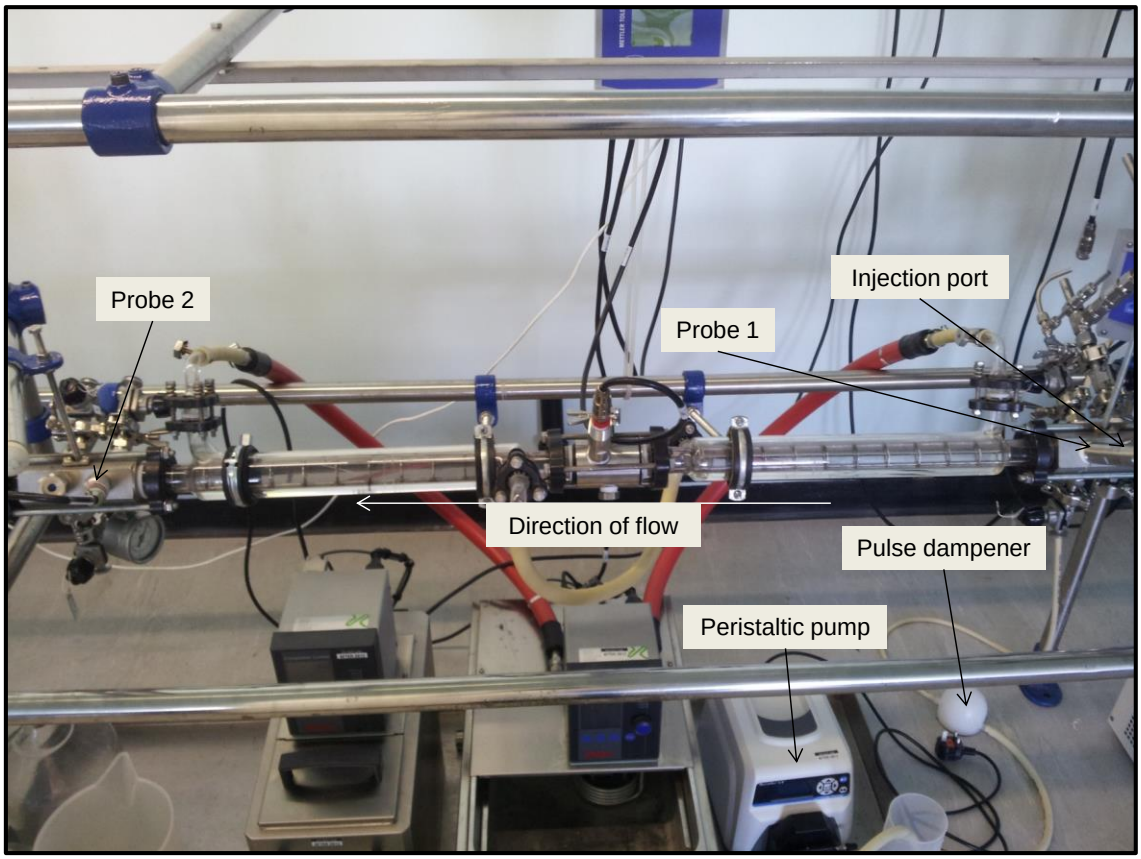


Figure 3.1: A photograph of the apparatus used for this study with the tracer injection port, pH probes, pulse dampener, peristaltic pump and direction of flow labelled.

3.4 Theory and calculation

3.4.1 Factor selection

DoE requires the selection of experimental factors that are systematically varied in order to determine their effect on a response variable. RTD profiles in OBRs are influenced by interactions between oscillatory and bulk flow components. The Re_o gives an indication of mixing intensity (oscillatory component) and the Re_n describes the net flow during continuous operation (bulk flow component), calculated using equations 3.1 and 3.2, respectively. The velocity ratio (Ψ) is Re_o divided by Re_n.

$$Re_o = \frac{\rho 2\pi f X_o D}{\mu} \qquad \text{Eq. 3.1}$$

$$Re_n = \frac{\rho D u}{\mu} \qquad \text{Eq. 3.2}$$

Where, ρ is the fluid density (kg/m^3), f and X_o, the frequency (Hz) and amplitude (m) of oscillation, respectively, D, the reactor diameter (m), μ, the fluid dynamic viscosity (Pa.s) and u, the superficial fluid velocity (m/s).

Fluid density and viscosity were kept constant (1000 kg/m^3 and 0.001 Pa.s, respectively) and the reactor diameter fixed at 25 mm, leaving X_o, f and u (or flow rate, *Q*) as the factors in the experimental design.

3.4.2 Experimental design

A basic full factorial design consists of *k* factors that are tested at *p* levels, for example, low and high (Fisher, 1926, Fisher, 1935, Box and Behnken, 1960). The number of runs required is p^k. For the three factors outlined above (X_o, f and Q) tested at two levels, 8 runs (2^3) would be required for a full two-level factorial. The limitation with this design is that second order models cannot be developed (Czitrom, 1999). This can be addressed by increasing the number of levels in the design to three, for example, however; the number of runs is substantially increased (3^3=27).

Central composite designs are able to build second order models without the need for a full three-level factorial (Box and Behnken, 1960). These designs consist of a full two-level factorial augmented with centre and axial points. The values of centre points are the median of each factor, with replication to estimate error. The values of axial points are +/- α_e from the centre points, where α_e is calculated by $(p^k)^{0.25}$. A central composite design was chosen for this study because it enabled a second order model to be developed and minimised the run number. The design was created using Minitab® (Minitab, 2007) with axial point ranges for X_o, f and Q of 0.6-3.6 mm; 0.47-2.29 Hz and 86-304 mL/min, respectively. The design consisted of 20 runs summarised in Table 3.1, with the factor space represented in Figure 3.2. Table 3.1 also shows the corresponding values of dimensionless groups associated with OBR operation. Re_n, Re_o and Ψ have been discussed (§3.4.1). The Strouhal number (St) is a measure of effective vortex propagation relative to the OBR diameter and is calculated using equation 3.3.

Strouhal number: $St = \frac{D}{4\pi X_o}$ Eq. 3.3

Table 3.1: A central composite experimental design to assess the effects of X_o, f and Q on the flow conditions in an OBR. The corresponding dimensionless groups associated with OBR operation (Re_n, Re_o, Ψ and St) are also shown. *Indicates the centre point replicated six times.

Run	X_o (mm)	f (Hz)	Q (mL/min)	Re_n	Re_o	ψ	St
1*	2.1	1.38	195	166	455	2.7	0.95
2	2.1	2.29	195	166	755	4.6	0.95
3*	2.1	1.38	195	166	455	2.7	0.95
4	3	1.92	260	221	905	4.1	0.66
5	3	1.92	130	111	905	8.2	0.66
6	3.6	1.38	195	166	783	4.7	0.55
7	1.2	1.92	260	221	362	1.6	1.66
8*	2.1	1.38	195	166	455	2.7	0.95
9	1.2	0.84	260	221	158	0.7	1.66
10	1.2	1.92	130	111	362	3.3	1.66
11	3	0.84	130	111	396	3.6	0.66
12	3	0.84	260	221	396	1.8	0.66
13	1.2	0.84	130	111	158	1.4	1.66
14	2.1	1.38	304	259	455	1.8	0.95
15*	2.1	1.38	195	166	455	2.7	0.95
16	0.6	1.38	195	166	127	0.8	3.32
17*	2.1	1.38	195	166	455	2.7	0.95
18	2.1	0.47	195	166	156	0.9	0.95
19	2.1	1.38	86	73	455	6.3	0.95
20*	2.1	1.38	195	166	455	2.7	0.95

The response for this study was the degree of plug flow, estimated in terms of a variable number (N_t) of continuously stirred tank reactors (CSTRs) in series (see §3.4.3). The values of N_t for each run were fitted to a second order, polynomial equation (Coward et al., 2013) in the form shown in equation 3.4 to model N_t in terms of X_o, f and Q. The model was used to select values for each factor that maximised plug flow. Each term is either linear, square or an interaction between factors and has an associated coefficient that generates the best fit (as determined by regression analysis) between the model and experimental values.

$$N_t = \beta_0 + \beta_1 X_o + \beta_2 f + \beta_3 Q + \beta_{11} X_o^2 + \beta_{22} f^2 + \beta_{33} Q^2 + \beta_{12} X_o f + \beta_{13} X_o Q + \beta_{23} fQ \quad \text{Eq. 3.4}$$

Where, N_t represents the degree of plug flow; β_0, a constant; X_o, f and Q, values of the experimental factors; β_1, β_2 and β_3, linear coefficients; β_{11}, β_{22} and β_{33}, square coefficients; and β_{12}, β_{13} and β_{23}, interaction coefficients.

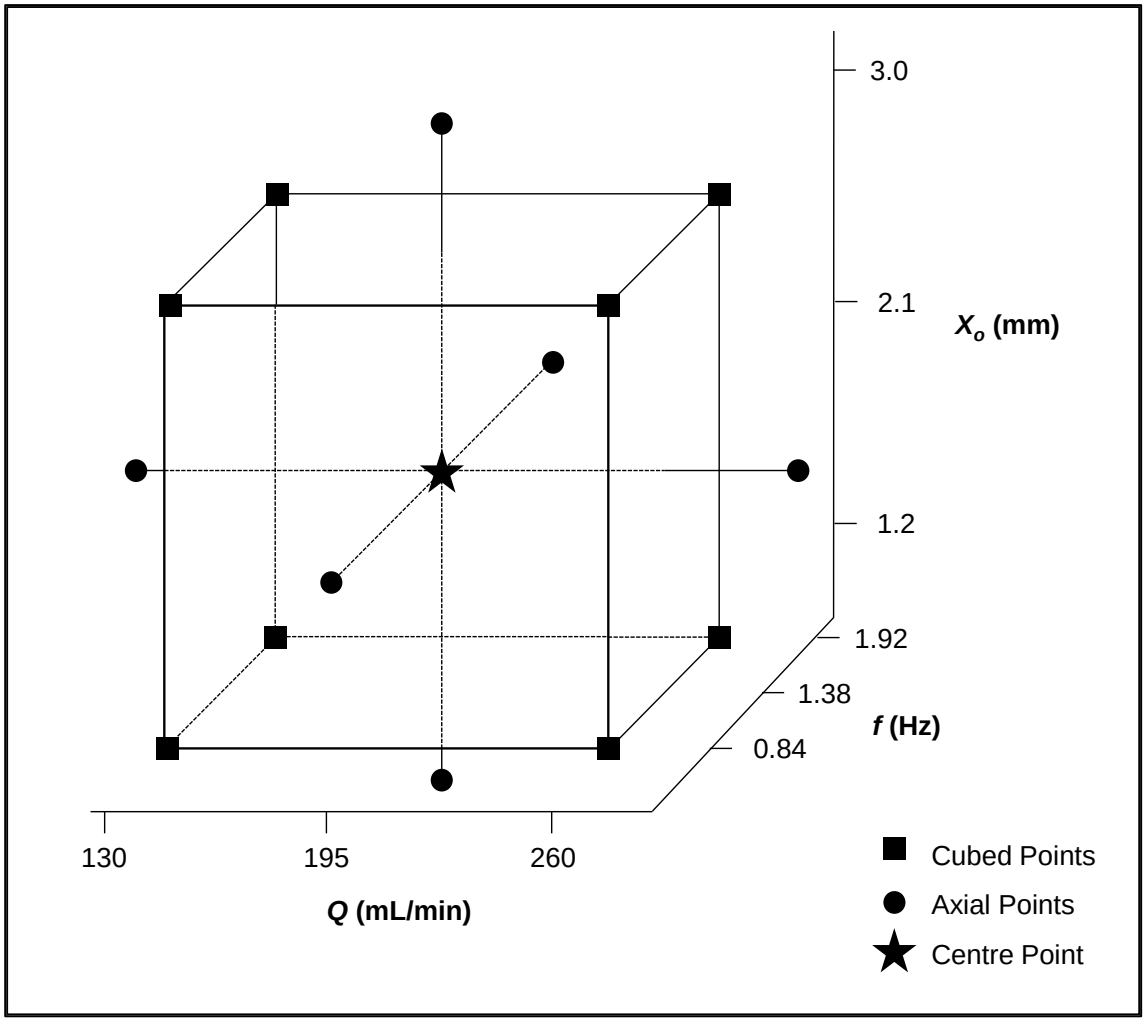


Figure 3.2: A representation of the factor space for the experimental design described in Table 3.1.

The significance of each term in equation 3.4 was determined by generated p-values which are the probabilities of obtaining the results if the null hypothesis is true (i.e. the term has no significant effect). A cut-off value of 0.05 was selected, meaning that terms with p-values above this were removed from the model. A backward, stepwise elimination technique was applied whereby significant terms ($p<0.05$) contribute to the model and insignificant terms are eliminated (Bosma et al., 2003). Coefficients were put into the polynomial model equation and the term with the highest p-value selectively removed. To establish model hierarchy, a linear term remained in the model when an interaction or square effect of that term was significant (Bosma et al., 2003). For example, Table 3.3 shows that the p-value for the X_o term is 0.684 (original model). However, this term remains in the model because the p-values for the X_o^2 and $X_o f$ terms are both below 0.05. The coefficient of determination (R^2) was used to evaluate the quality of fit for the final model.

3.4.3 Residence time distribution analysis

Normalised RTD profiles were created for each run using equations 3.5-3.10 from pH data gathered from probe 2 (Figure 3.1). The data was offset by 10 s to account for the probe response time. Concentration data was used to perform a mass balance of hydroxide ions between probes 1 and 2.

Hydroxide ion concentration (M): $[OH^-] = 10^{-(14-pH)}$ Eq. 3.5

Mean residence time (s): $t' = \frac{\Sigma t_i C_i}{\Sigma C_i}$ Eq. 3.6

Dimensionless time: $\theta = \frac{t_i}{t'}$ Eq. 3.7

Area under C(t) curve: $A = \Sigma C_i \Delta t$ Eq. 3.8

Residence time distribution: $E = \frac{C_i}{A}$ Eq. 3.9

In normalised form: $E_\theta = t'E$ Eq. 3.10

Where, t_i and C_i are the time and $[OH^-]$ (M) at time i, respectively, and Δt, the time interval between readings (10 s in this study).

The tanks-in-series (TiS) model for plug flow has been used to quantify flow conditions in OBRs in terms of a variable number (N_t) of continuous stirred tank reactors (CSTRs) in series (Stonestreet and van der Veeken, 1999, Dickens et al., 1989, Fitch and Ni, 2003, Phan and Harvey, 2010). Normalised RTD profiles using the TiS model are created using equation 3.11 (Levenspiel, 2012).

$$E_\theta = \frac{N_t(N_t\theta)^{N_t-1}e^{-N_t\theta}}{(N_t-1)!} \quad \text{Eq. 3.11}$$

Where, N_t represents a variable number of CSTRs in series.

Closer approximations to plug flow are achieved with increasing N_t. Perfect plug flow occurs when N_t is infinite (Levenspiel, 1999) although in practice $N_t \geq 10$ usually provides an adequate level of plug flow (represented in Figure 3.3b) (Phan and Harvey, 2010). Mixed flow occurs for lower values of N_t and the RTD profile for $N_t=2$ is shown in Figure 3.3a.

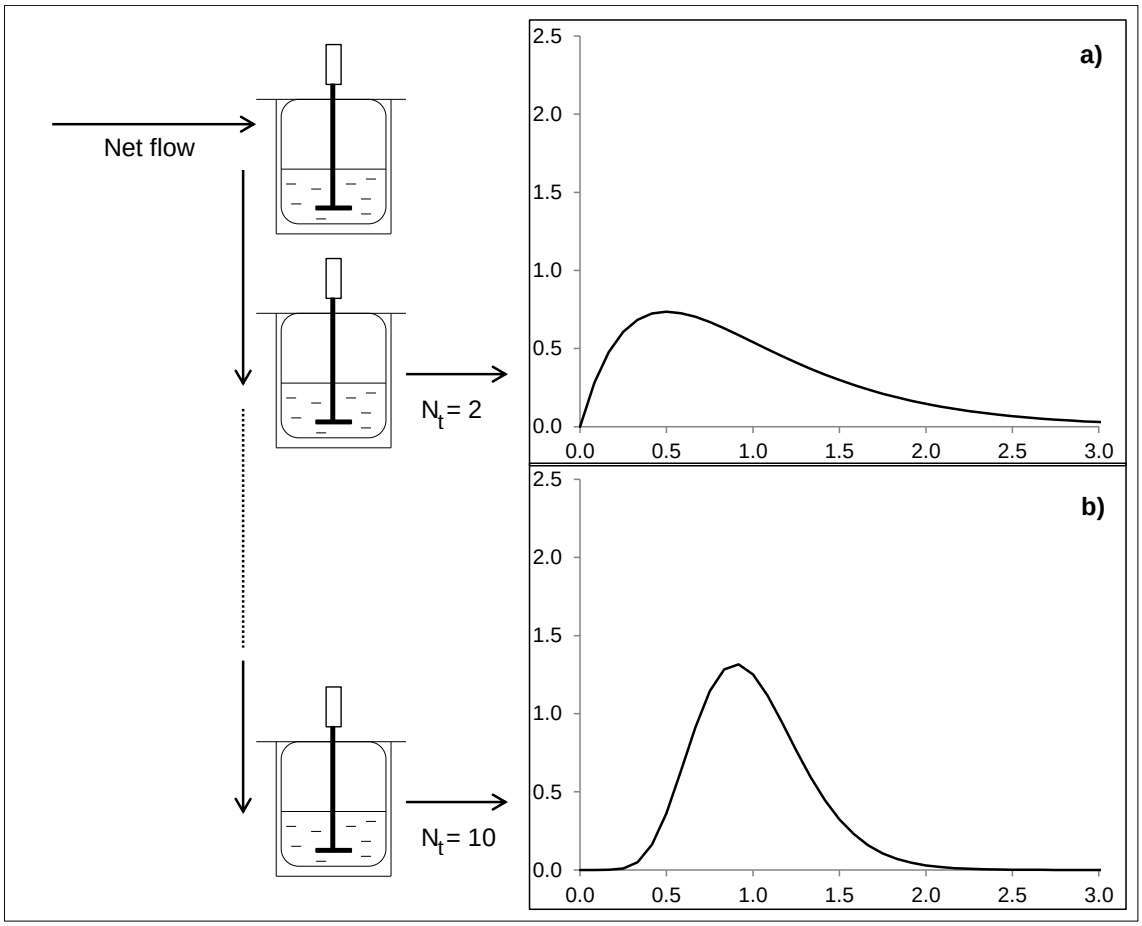


Figure 3.3: Theoretical RTD profiles for a) near mixed flow (N_t=2) and b) increased plug flow (N_t=10). The x-axis represents dimensionless time (θ) and the y-axis normalised exit age function (E_θ).

The response for this study was the degree of plug flow estimated in terms of N_t. One of the advantages of this method is that quantification of plug flow is expressed in terms of only one variable. Each inter-baffle zone can act as a discrete compartment during continuous operation of OBRs in the soft mixing regime (Re_o<5000) resulting in the column behaving as numerous CSTRs in series (Dickens et al., 1989, Stonestreet and van der Veeken, 1999). The experimental design uses a maximum Re_o of 905. Therefore the TiS model is appropriate as it approximates the mode of operation of this reactor in this regime.

Data from probe 2 was used to create normalised RTD profiles (experimental) for each run, which were visually compared in Excel (Microsoft, 2010) to normalised RTD profiles created from the TiS model (model). The value for N_t in the TiS model that gave the closest fit to the experimental RTD profile gave the response variable for that run (the degree of plug flow). Regression analysis was performed to determine the quality of fit (R^2) between the experimental and model RTD profiles.

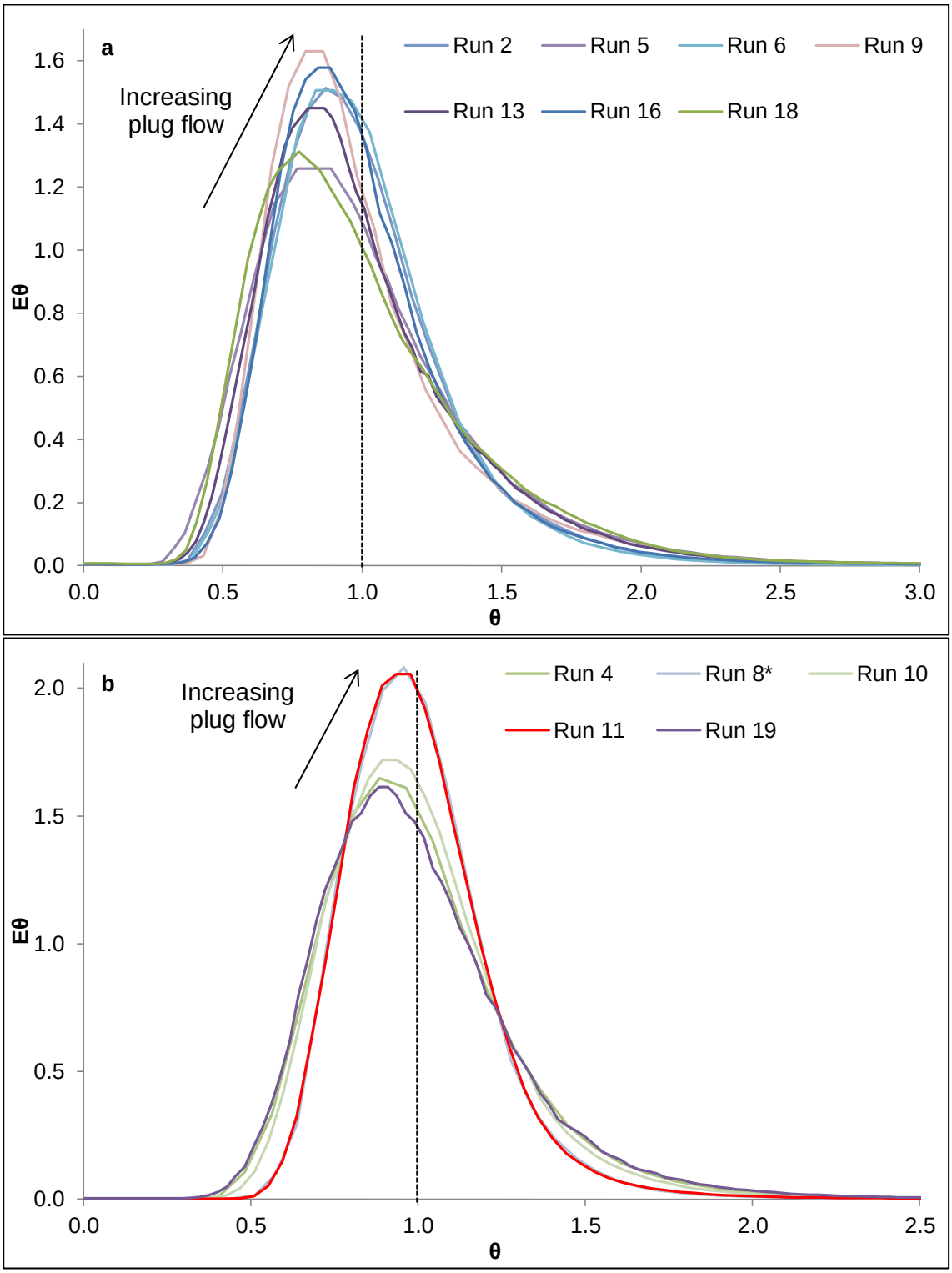
3.5 Results and discussion

3.5.1 Flow condition analysis

Tracer material was introduced into the OBR and its concentration measured over time at a specified location downstream from the site of injection (probe 2). Sodium hydroxide was used for this study, as the concentration is easily determined from pH measurements (equation 3.5). The RTD profiles created from probe 2 for the 20 runs in the experimental design are shown in Figure 3.4 a-d.

There is a clear difference in the RTD profiles in response to changes in the experimental factors (X_o, f and Q). This indicates that the factors affect the quality of plug flow. The profile becomes symmetrical about the mean residence time (t') corresponding to θ=1 as the flow conditions tend towards plug flow. Figure 3.3b clearly shows this symmetry. The profile peaks at θ=1 and becomes more symmetrical as the quality of plug flow increases, indicated by the arrows in Figure 3.4.

The level of plug flow achieved for each run was quantified by comparison to the TiS model which gave the response value for each run (N_t). These values are shown in Table 3.2. Plug flow was maximised for conditions described in run 14 (N_t=33) and minimised for run 5 (N_t=9). Changes in the values of selected factors within the space described in Figure 3.2 resulted in a response range of $9 \leq N_t \leq 33$. The result from run 14 suggests the OBR shown in Figure 3.1 can act as 33 CSTRs in series when appropriate values for X_o, f and Q are selected.


a
Increasing plug flow
Run 2
Run 5
Run 6
Run 9
Run 13
Run 16
Run 18
Eθ
θ
b
Increasing plug flow
Run 4
Run 8*
Run 10
Run 11
Run 19
Eθ
θ

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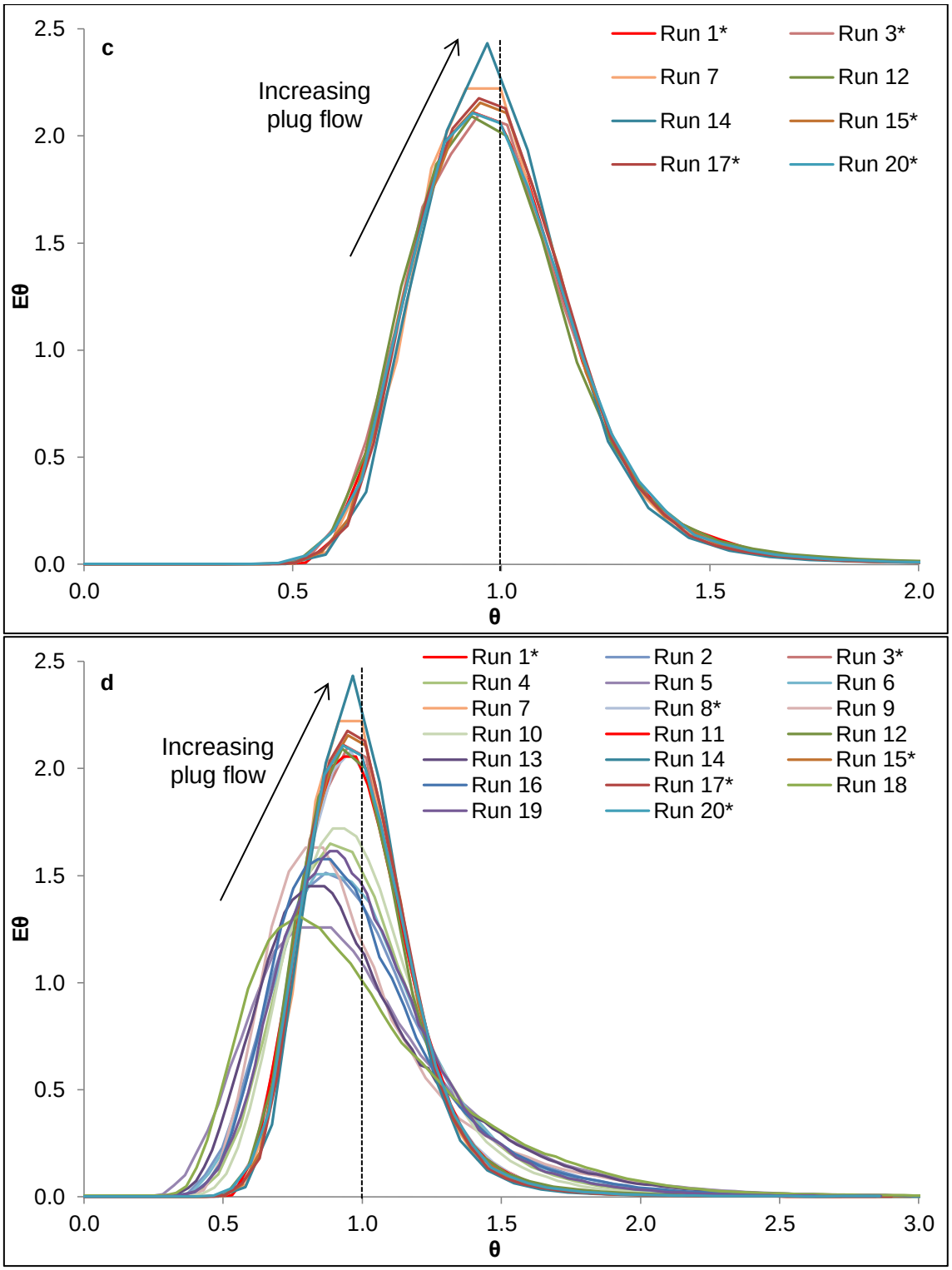

Figure 3.4: Experimental (probe 2) RTD profiles for runs outlined in Table 3.1 forming the experimental design. The vertical dashed line corresponds to the mean residence time (t') and * denotes replicated runs at the centre point. Figure (a) shows runs with N_t values of 9-13, (b), N_t values of 14-25, (c), N_t values of 26-33 and (d), all 20 runs in the experimental design.

Regression analysis was performed for each run (Minitab, 2007) to evaluate the model's fit. Coefficients of determination (R^2) were calculated for each run (shown in Table 3.2). The R^2 values are >95% except for runs 9 (88.5%), 13 (94.1%) and 18 (85.4%) where the N_t values were relatively low at 13, 10 and 12, respectively. Figure

3.5 shows the RTD profiles, and their associated model fit plots, for runs 17 and 18 which represent the best and poorest fits, respectively.

Table 3.2: The quality of plug flow (N_t), coefficients of determination (R^2), mixing efficiencies (η) and total hydroxide ions recorded at probes (Pb.) 1 and 2 for the 20 runs in the experimental design (Table 3.1). Bold represents poor fit (R^2<95%).

Run	N_t	R^2	η	Pb.1 (mmol)	Pb.2 (mmol)	Run	N_t	R^2	η	Pb.1 (mmol)	Pb.2 (mmol)
1*	26	99.1%	0.87	2.65	2.59	11	24	99.2%	0.80	2.62	2.53
2	13	98.0%	0.43	2.76	2.61	12	26	98.4%	0.87	2.52	2.43
3*	26	99.0%	0.87	2.69	2.56	**13**	**10**	**94.1%**	**0.33**	**2.38**	**2.27**
4	15	98.7%	0.50	2.54	2.41	14	33	99.2%	1.10	2.53	2.32
5	9	96.4%	0.30	2.55	2.48	15*	27	99.3%	0.90	2.60	2.48
6	13	98.8%	0.43	2.60	2.54	16	13	95.8%	0.43	2.62	2.37
7	30	99.0%	1.00	2.63	2.52	17*	28	99.4%	0.93	2.57	2.47
8*	25	99.2%	0.83	2.60	2.43	**18**	**12**	**85.4%**	**0.40**	**2.54**	**2.43**
9	**13**	**88.5%**	**0.43**	**2.50**	**2.36**	19	14	98.4%	0.47	2.51	2.38
10	17	99.0%	0.57	2.54	2.39	20*	26	99.3%	0.87	2.47	2.45

It is clear that the model accurately represents flow conditions in an OBR for relatively high N_t values (e.g. 28) as the experimental and model RTD profiles match. The model can lose accuracy for N_t<15 as indicated by the poorer fit when N_t=12 (Figure 3.5). However, a low N_t value does not necessarily correspond to a poor fit as indicated by the R^2 values for runs 2 (98.0%), 5 (96.4%), 6 (98.8%), 16 (95.8%) and 19 (98.4%) having N_t values of 13, 9, 13, 13 and 14, respectively.

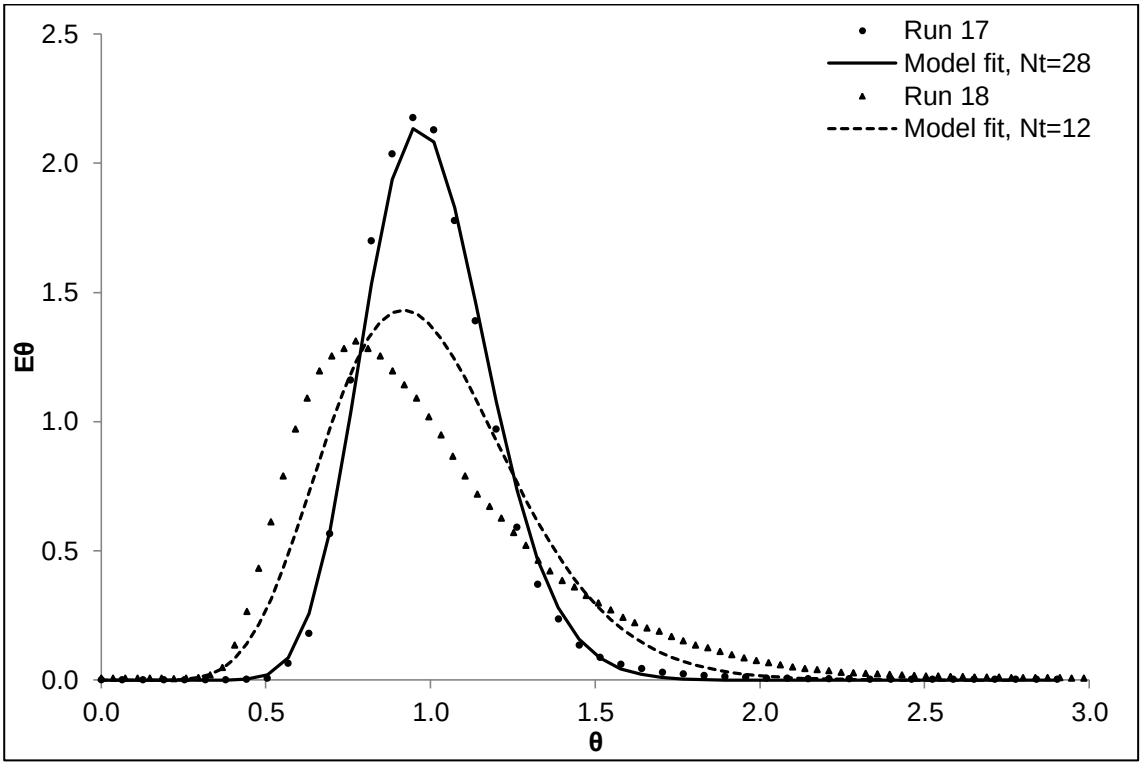

Figure 3.5: Experimental (markers) and model (lines) RTD profiles for runs 17 (circles, solid) and 18 (triangles, dashed) which represent the best and poorest fits observed in the study, respectively.

In theory the maximum value calculated for N_t cannot exceed the number of inter-baffle zones because under ideal conditions each can behave as one CSTR (Stonestreet and van der Veeken, 1999, Dickens et al., 1989, Phan and Harvey, 2010). The actual number of CSTRs in series (M) can therefore be directly related to the OBR length and corresponding number of inter-baffle zones. There are 30 inter-baffle zones forming the OBR shown in Figure 3.1, therefore M=30.

The mixing efficiency (η) can be used to estimate the performance of an OBR in relation to plug flow (Stonestreet and van der Veeken, 1999, Carberry, 1958) and is calculated using equation 3.12. A theoretical maximum value (η=1) occurs when N_t=M and the OBR is achieving the maximum level of plug flow possible.

Mixing efficiency: $\eta = N_t/M$ Eq. 3.12

Where, N_t is the calculated number of CSTRs in series and M, the actual number of CSTRs (equivalent to the number of inter-baffle zones for OBR operation).

The mixing efficiencies for the experimental runs are shown in Table 3.2. A maximum η was achieved for run 14 where η=1.10 (33/30) which is higher than the theoretical maximum value of 1. The apparatus that forms the OBR consists of two borosilicate glass columns coupled by stainless steel blocks that provide probe entry and sample ports (Figure 3.1). The column sections of the reactor contain 30 inter-baffle zones, however; this does not include three blocks connected at the centre and both ends. It is probable that these blocks act as mixed compartments which would increase the value of M and explain how N_t>M. Further work is needed to determine the effect each block has on the flow conditions and allow a precise value for M to be given.

Changes in the factor values (X_o, f and Q) within the experimental space produced a mixing efficiency range of $0.3 \leq \eta \leq 1.1$. Response values (N_t) were calculated assuming a perfect pulse technique as tracer (NaOH) was rapidly added over 3 seconds. Addition of an imperfect pulse would produce values for η below the theoretical maximum. The values for η obtained suggest the perfect pulse technique assumption is valid because the theoretical maximum value for η was achieved (exceeded for run 14).

3.5.2 Mass Balance Analysis

Data collected from probes 1 (entry) and 2 (exit) enabled a mass balance of hydroxide ions to be performed between the OBR entry and exit. Each 1 mL tracer pulse contained 2.50 mmol hydroxide ions (2.50/1000). The total amount of hydroxide ions recorded at probes 1 and 2 are shown in Table 3.2. The data for probe 1 shows a standard deviation of 3.31% from 2.50 mmol and a maximum deviation of +10.40% for run 2. The major source of error probably occurred from the volume of tracer injected, which for most runs was very close to the target of 1 mL. There is an average reduction of 4.66% for the total hydroxide ions between the OBR entry (probe 1) and exit (probe 2) points. >95% of the tracer passed through the OBR, which indicates that few stagnation points were present where tracer material could accumulate. The use of 2.5 M NaOH was therefore suitable.

3.5.3 Model Development

A second order model was developed in Minitab® to predict the quality of plug flow (N_t) that would be achieved with different values for the experimental factors (X_o, f and Q). The method used (see §3.4.2) resulted in seven terms for the final model, which are shown in Table 3.3. These consist of a constant, all three linear terms, two square terms (X_o^2 and f^2) and one interaction term ($X_o f$). Table 3.3 also shows the coefficient for each term and its associated p-value (original model).

The p-values for the X_o and f terms in the original model are both higher than the cut off for this study (0.05) however; both are required for hierarchy terms (X_o^2 and f^2, respectively) so must remain in the model. The p-values for the constant, square and interaction terms are all <0.05 which suggest they contribute significantly to the model. The R^2 (=92.1%) shows that 7.9% of the total variability cannot be explained by the model. Sources of this error could be slight variation in tracer addition (e.g. volume), as this was done manually so perfect replication is unlikely, and experimental RTDs being poorly modelled. Although the model produced a relatively high R^2 (=92.1%), a second model was developed after removal of runs where experimental RTDs were poorly modelled (R^2<95%). This second model resulted in eight terms and a higher R^2 (=97.3%), shown in Table 3.3 (outlier corrected). This indicates that some unknown variation in the original model was a result of a poor fit

between experimental RTDs and the TiS model. The outlier corrected model was deemed sufficiently accurate to predict factor values that maximise plug flow.

Table 3.3: Terms included in the original and outlier corrected models, their coefficients and associated p-values.

Original Model			Outlier Corrected		
Term	***Coefficient***	***p-value***	***Term***	***Coefficient***	***p-value***
Constant	-76.200	0.000	Constant	-63.500	0.000
X_o	40.300	0.684	X_o	41.850	0.730
f	68.200	0.974	f	62.760	0.961
Q	0.063	<0.000	Q	-0.020	<0.000
X_o^2	-5.258	<0.000	X_o^2	-5.656	<0.000
f^2	-14.889	<0.000	f^2	-16.420	<0.000
$X_o f$	-12.951	<0.000	$X_o f$	-12.950	<0.000
			fQ	0.050	0.018
Coefficient of determination (R^2)=92.1%			Coefficient of determination (R^2)=97.3%		

3.5.4 Selection of Factor Values to Maximise Plug Flow

Contour plots are useful for showing the effects that two experimental factors have on a response. Figure 3.6 shows contour plots for all three pairs of experimental factors used in this study and their relative effect on the quality of plug flow (N_t).

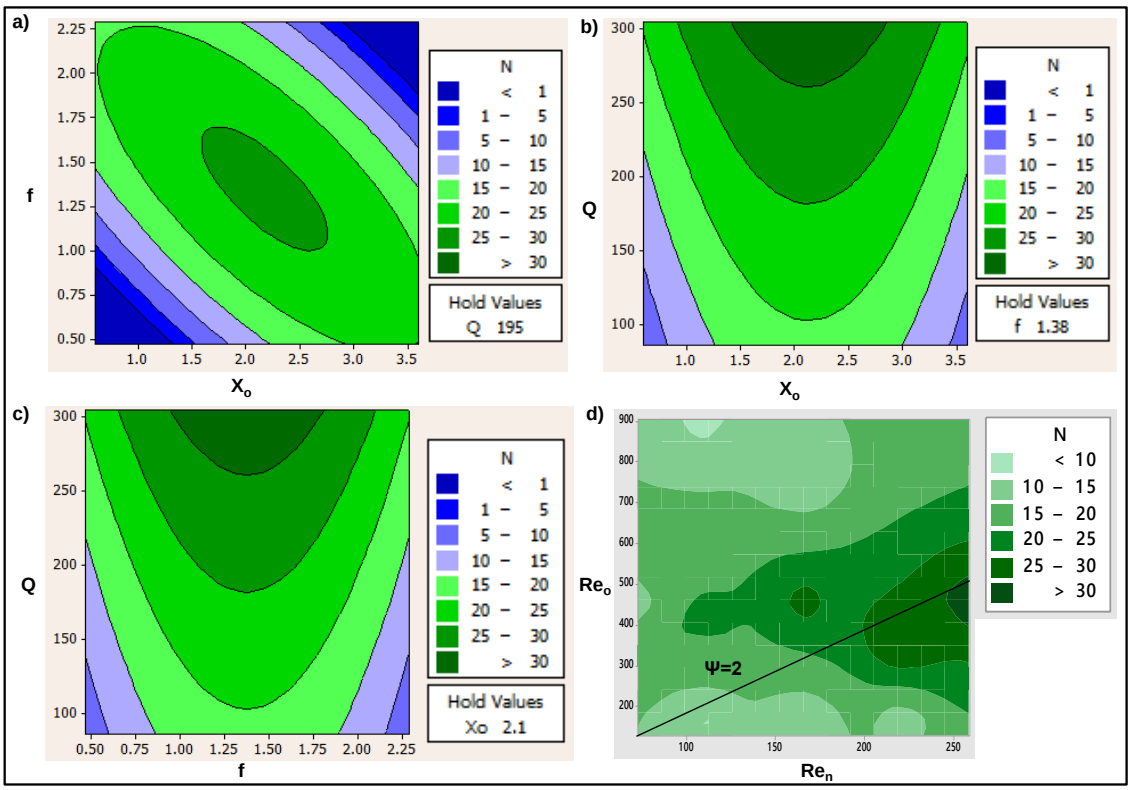

Figure 3.6: Contour plots for the effects a) f and X_o, b) Q and X_o, c) Q and f, and d) Re_o and Re_n have on plug flow (N_t) in a 'standard' OBR design. Hold values are defined as the centre point during the experimental design. The line in d) represents a velocity ratio (Ψ) of 2.

It is clear from Figure 3.6 that all three experimental factors in this study affect the quality of plug flow. A range to maximise plug flow exists for both X_o (1.5-3.0 mm) and f (1.00-1.75 Hz) indicated by the central region in Figure 3.6a (where N_t>30). Flow rate (Q) affects plug flow quality with an increased net flow producing closer approximations to plug flow, maximised for Q>260 mL/min (where N_t>30). The response optimisation tool in Minitab® was used to select values for X_o (=2.2 mm), f (=1.4 Hz) and Q (304 mL/min) that maximise plug flow conditions (target=40, lower=30, upper=50). These values produce a velocity ratio of 1.9, within the range (1.8<Ψ<2.0) stated by Stonestreet and van der Veeken (1999). The optimisation tool selected the highest available value for Q. It indicated that closer approximations to plug flow are achieved with increased Re_n, consistent with several studies (Stonestreet and van der Veeken, 1999, Phan and Harvey, 2010).

Figure 3.6d shows Re_o against Re_n. These dimensionless groups are functions of all three experimental factors. N_t increases with Re_n and is maximised for the highest Re_n when Ψ~2.0, consistent with the previous analysis. A plot showing Ψ against N_t over the factor range tested in the experimental design is shown in Figure 3.7. As Ψ is a function of Re_o and Re_n, which are themselves functions of X_o, f and Q, the trend shown in Figure 3.7 captures all known variation with the quality of plug flow (N_t).

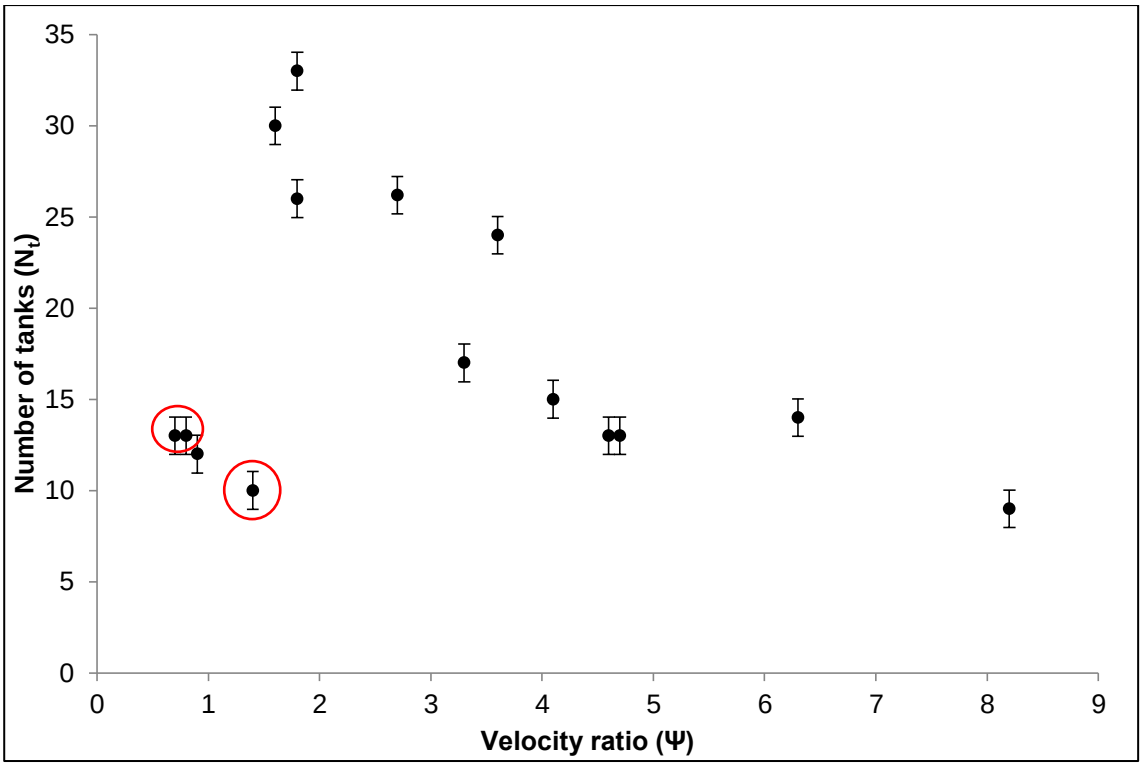

Figure 3.7: The dependency of plug flow quality (N_t) on the velocity ratio (Ψ). Error bars represent +/- one standard deviation calculated from six replicates at the centre point. Red circles indicate runs where the experimental RTD fit poorly with the model (R^2<95%).

The N_t values generated from the six replicated runs at the centre point have been averaged and used to calculate the error for Figure 3.7 (standard deviation=1.03). There is a clear maximum level of plug flow achieved within the range $1.6<\Psi<2.0$ which supports the factor values suggested from the model to maximise plug flow ($\Psi=1.9$). This range is almost identical to that stated in a previous study ($1.8<\Psi<2.0$) (Stonestreet and van der Veeken, 1999), which suggests the method described above is accurate and can be used with confidence to select factor values to maximise plug flow conditions. However, Figure 3.7 suggests an extremely rapid increase in the quality of plug flow with a minor change to the velocity ratio from 1.4 to 1.6 which seems unrealistic. This could be due to all N_t values being plotted, irrespective of how accurate the experimental data fit the TiS model.

Values circled in red in Figure 3.7 are runs 9, 13 and 18 which produced low N_t values (<14). The experimental RTDs resulting from these runs did not fit the TiS model as accurately as other runs, with R^2 values below 95%. With these runs removed from the analysis, the R^2 value for the final model is higher (=97.3%) and the rapid increase in the quality of plug flow is removed from Figure 3.7, producing a more gradual and realistic increase.

The third dimensionless group (St) has been plotted against N_t and shown in Figure 3.8 to support the conclusion that only velocity ratio shows a trend when plotted against the quality of plug flow. It is clear from Figure 3.8 that there is no obvious trend between the quality of plug flow (N_t) and the Strouhal number (St).

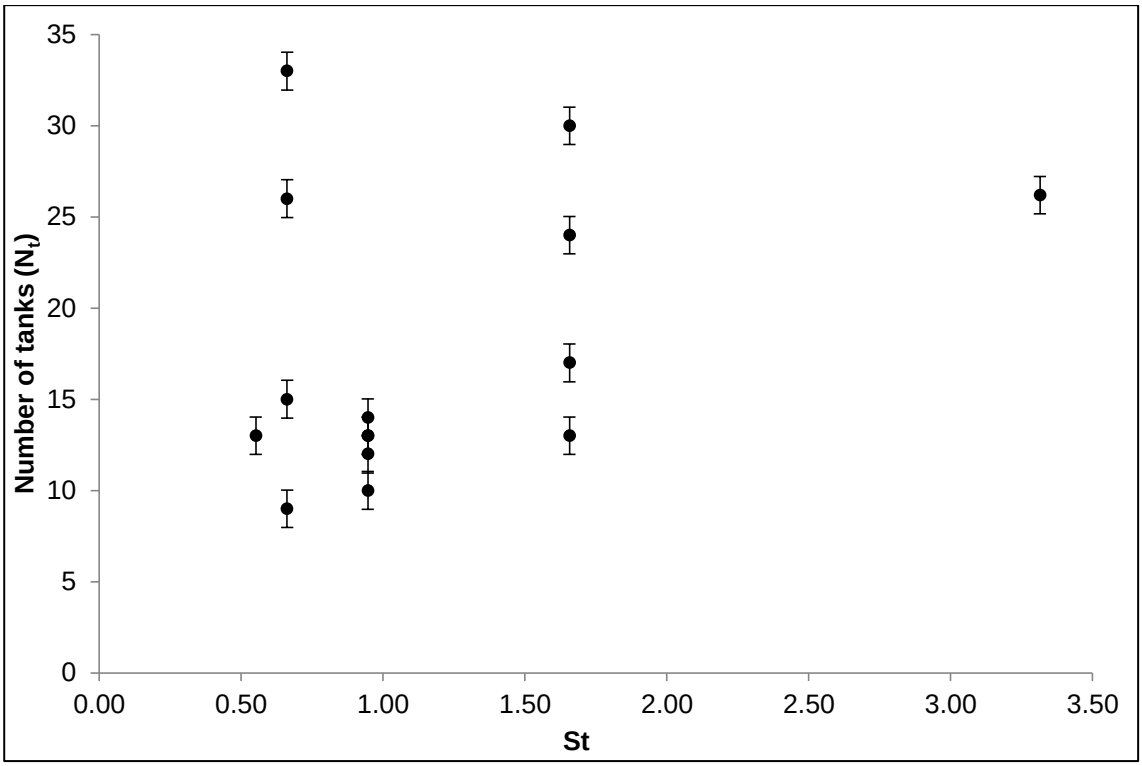

Figure 3. 8: The dependency of plug flow quality (N_t) on the Strouhal number (St).

3.5.5 Method Overview

The use of DoE in the form of a central composite experimental design has enabled a second order model to be developed that describes the relationship between the quality of plug flow (N_t) and three experimental factors (X_o, f and Q). The model was used to select factor values to maximise plug flow and the results agree with a previous study (Stonestreet and van der Veeken, 1999) where approximately 110 individual pulse tests were conducted and each factor assessed using a 'one factor at a time' (OFAT) approach. In this study the same conclusions were made using 20 runs which is an 82% reduction in the run number.

A 'standard' OBR design was used, so that conclusions could be validated against Stonestreet and van der Veeken (1999).

The method described in this study consists of:

1) Factor selection (X_o, f and Q).
2) Experimental design in the form of a central composite.
3) Data acquisition at the OBR exit.
4) Tracer pulse method and suggested material.
5) RTD profile generation from pH versus time data.

6) Quantification of plug flow by comparison to the TiS model.
7) Model development using the response values for each run (N_t).

There is no reason to suggest this method could not be applied to 'non-standard' OBR designs. Therefore, this method could provide a simple and robust way to rapidly quantify flow conditions and select factor values to maximise plug flow, which is often preferred for continuous operation when using OBR technology (Abbott et al., 2013).

3.5.6 Application to OBR Design

A major advantage of OBR technology is the ability to control mixing intensity independently of the net flow rate through manipulation of X_o and f. In conventional tubular reactors turbulent flow conditions (Re_n>2100) are required to achieve good radial mixing (van Vliet et al., 2005). This results in residence times for continuous operation using conventional tubular reactors being severely limited because extremely long, narrow reactors, that are often simply impractical to operate, are required to achieve long residence times (Harvey et al., 2001). OBR technology mitigates this problem by decoupling the achievement of plug flow from net flow allowing longer residence time processes in significantly shorter reactors.

The results from this study and previous work (Stonestreet and van der Veeken, 1999, Dickens et al., 1989, Reis et al., 2004) have demonstrated that net flow during continuous operation in OBRs affects the RTD. Mixing intensity is decoupled from net flow but the RTD is not. This fact must be taken into consideration when designing a continuous process, under plug flow conditions, based on OBR technology. For fixed OBR dimensions a lower limit exists for net flow below which plug flow conditions are lost and the OBR behaves as a mixed vessel. This limit for Re_n will put an upper limit on the residence time achievable for a specified OBR. To increase the residence time for a specified Re_o and maintain an adequate level of plug flow, the OBR length must be increased. Figure 3.9 shows 'standard' OBR design lengths required to achieve a 12 hour residence time for two mixing intensities (Re_o=1000 and 2000) and velocity ratios (Ψ=1.8 and 10).

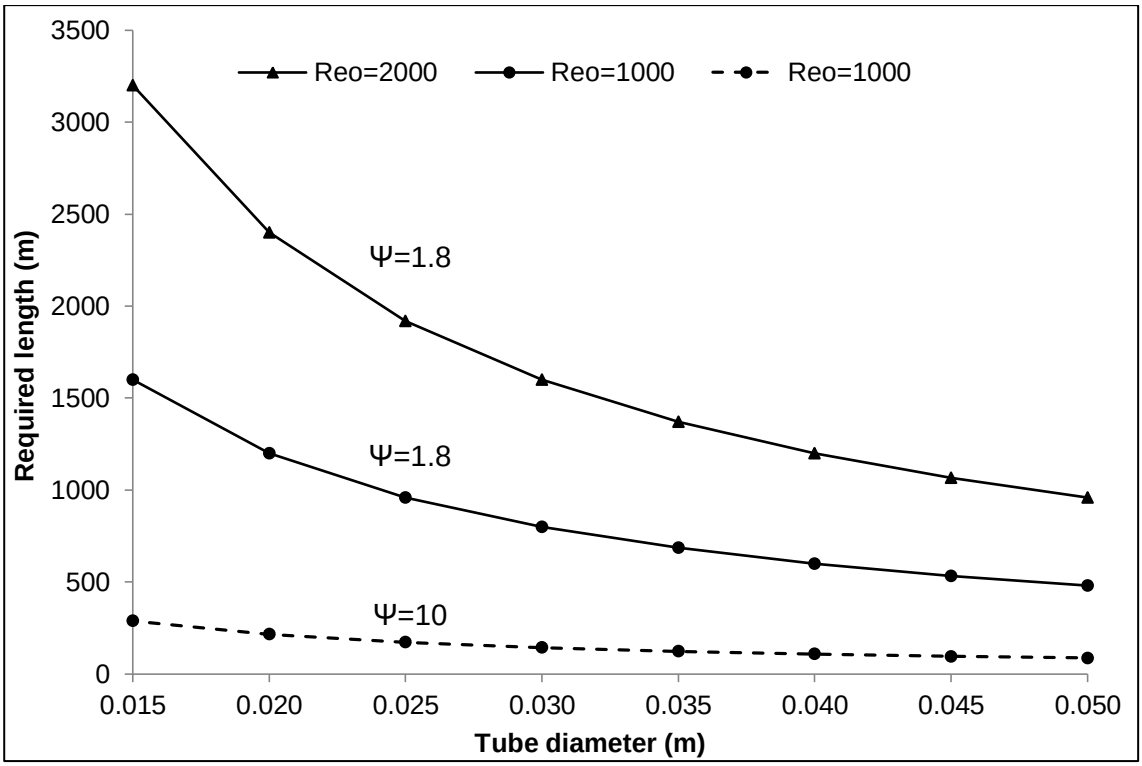

Figure 3.9: 'Standard' OBR design lengths required to maximise plug flow (Ψ=1.8) for a 12 hour residence time process with mixing intensities (Re_o) of 2000 (triangles) and 1000 (circles). The length for reduced plug flow conditions (Ψ=10) with a mixing intensity of 1000 is shown for comparison (circles, dashed).

Figure 3.9 shows that for a 25 mm diameter OBR, similar to one used in this study, a length of 1920 m would be required to maximise plug flow (Ψ=1.8) over 12 hours at Re_o=2000. This length is halved to 960 m if the required mixing intensity is also halved to Re_o=1000 and again reduced by a factor of ~5, to 173 m, if Ψ is increased from 1.8 to 10 (which may not produce the desired level of plug flow). This length may still be impractical and highlights the importance of establishing the required operating conditions (mixing intensity, residence time and flow conditions) for each process to ensure the resultant OBR is suitable. Note that this is just illustrative, as it is based upon a fixed diameter, which would usually be a degree of freedom in design. Hence, the methodical approach to designing processes based on OBR technology in Figure 3.10.

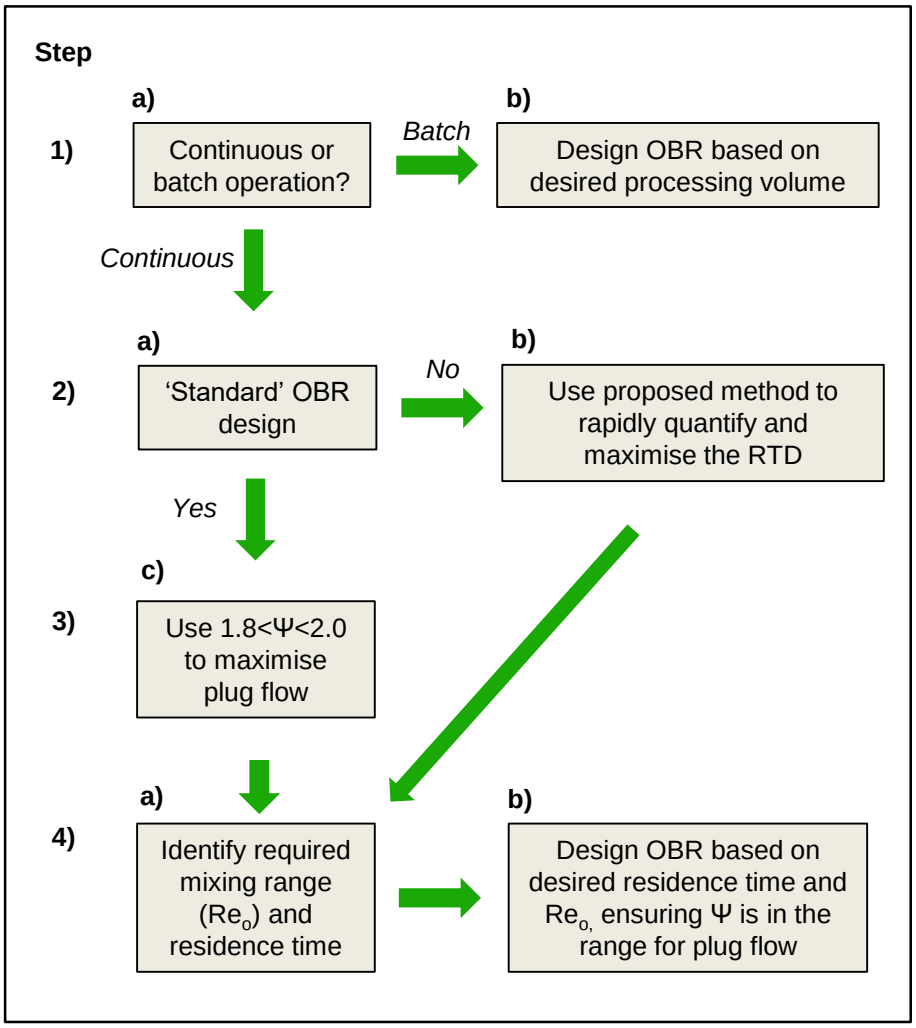

Figure 3.10: A methodical approach for designing batch or continuous processes based on OBR technology.

The method presented in this study allows step 2b (Figure 3.10) to be performed in a robust manner with minimal time required for extensive testing. There are several benefits to this method: fewer runs are required (up 82% fewer) compared with OFAT, saving both time and reagents; relevant experimental factors and their interactions are assessed; and a framework is provided that accurately describes the conditions to be tested.

Figure 3.10 demonstrates that operating OBRs continuously under plug flow conditions is more complex than batch operation, but is described as the niche application for this technology (Abbott et al., 2013).

3.6 Conclusions

A central composite experimental design was used to evaluate the effects three experimental factors (X_o, f and Q) have on the quality of plug flow achieved during continuous operation of a 'standard' OBR design by analysing RTD profiles. The

tanks-in-series model was shown to be a good representation of the flow conditions in the OBR, especially for relatively high N_t values (N_t>15) where R^2>95%. At relatively low N_t values (N_t<15), the model could lose some accuracy with the poorest fit occurring for N_t=12 (R^2=85.4%). Mass balances demonstrated that >95% of tracer material introduced passed through the reactor. The level of plug flow was quantified by comparison to the TiS model to give the response for each run (N_t). The N_t values were used to build a second order polynomial model which was shown to fit the experimental results well (R^2=92.1%). This fit was improved by removal of the outliers (R^2=97.3%). The optimisation tool in Minitab (Minitab, 2007) selected a Ψ (=1.9) to maximise plug flow in the range previously identified by Stonestreet and van der Veeken (1999) (1.8<Ψ<2.0). This suggests plug flow can be maximised using the method described in this study, which is simple and robust and could speed up successful design of continuous processes under plug flow conditions based on OBR technology.

3.7 Research implications

The following research project focused on enzymatic saccharification and used the same OBR as described above. One of the initial aims was to conduct saccharification in the OBR under plug flow conditions and compare to a traditional STR. However, as has been proven, an OBR 173 m in length would be required to achieve plug flow with the present design. The current OBR is ~1 m in length and therefore plug flow was not achievable over the 12-24 hour residence times required. Batch experiments were therefore required for the following study which aimed to evaluate the energy requirements to maximise glucose production from the enzymatic saccharification of cellulose.

Chapter 4: Enzymatic saccharification

'Reduced power consumption compared to a traditional stirred tank reactor (STR) for enzymatic saccharification of alpha-cellulose using oscillatory baffled reactor (OBR) technology.'

4.1 Abstract

Enzymatic saccharification of pure α-cellulose was conducted in oscillatory baffled (OBR) and stirred tank (STR) reactors over a range of mixing intensities requiring power densities (P/V) from 0-250 Watts per cubic metre (W/m^3). Both reactor designs produced similar saccharification conversion rates at zero mixing. Conversion increased with increasing mixing intensity. The maximum conversion rate occurred at an oscillatory Reynolds number (Re_o) of 600 in the OBR and at an impeller speed of 185-350 rpm in the STR. The OBR was able to achieve a maximum conversion rate at a much lower power density (2.36 W/m^3) than the STR (37.2-250 W/m^3). The OBR demonstrated a 94-99% decrease in the required power density to achieve maximum conversion rates and showed a 12% increase in glucose production after 24 hours at 2.36 W/m^3.

4.2 Introduction

The utilisation of lignocellulosic materials to aid replacing fossil based fuels and chemicals has received much attention over the past decade (Lynd and Wang, 2003, Sanders et al., 2012). Lignocellulosic materials are ubiquitous in nature with cellulose, the dominant structural polysaccharide in terrestrial plants, being the most abundant carbohydrate on Earth. It is possible to hydrolyse cellulose using chemical or biological methods, liberating the glucose monomers constituting its structure (saccharification), before fermenting into various useful chemicals including ethanol and lactic acid (Sarkar et al., 2012, Abdel-Rahman et al., 2011). Utilisation of crop wastes, including straws and stover, avoids conflict between human food and industrial use (Boddiger, 2007). An estimated worldwide production of lignocellulosic biomass from seven major crops including rice straw and corn stover is 1.5 Pg per year (Kim and Dale, 2004) offering great potential. However, there are numerous problems surrounding the creation of industrial processes using lignocellulosics that can economically compete with current chemical manufacturing routes based on fossil sources.

Plant materials have evolved support and protective properties in the form of lignocellulose, which comprises a mixture of cellulose, hemicellulose and lignin. The ratio of these three components varies with, for example, wheat straw containing 35-45% cellulose, 20-30% hemicellulose and 8-15% lignin (Saha and Cotta, 2006). In order to maximise chemical production from lignocellulosics, a pre-treatment step is required to delignify the material, exposing cellulose content making it more accessible for conversion into fermentable sugars. Detailed reviews are available describing current pre-treatment protocols (Min et al., 2011, Agbor et al., 2011, Li et al., 2010).

Enzymatic saccharification requires a cellulase enzyme system to convert cellulose to glucose. During this bioprocess the reaction rate diminishes with time due to cellulase inhibition and deactivation (Zhang et al., 2010). Reduced enzyme activity can be caused by a variety of process factors: shear inactivation (Kaya et al., 1996, Reese and Ryu, 1980, Ganesh et al., 2000, Gunjikar et al., 2001), sugar inhibition (Xiao et al., 2004, Takagi, 1984, Ye et al., 2012), ion strength (Kumakura, 1996), temperature (Demerdash and Attia, 1992) and formation of inert enzyme-substrate complexes. Cellulose properties change over time because easily hydrolysable amorphous regions are digested leaving recalcitrant crystalline regions (Gan et al., 2003). The combination of these factors results in a typical saccharification curve consisting of an initial rapid conversion rate lasting approximately 12 hours followed by an almost stationary phase lasting 2-3 days.

The aims of this study were to compare two reactor designs, the oscillatory baffled reactor (OBR) and conventional stirred tank reactor (STR), to identify any differences in the required power density for mixing and overall conversion rates of pure α-cellulose to glucose.

The OBR is a novel production vessel possessing several key advantages over conventional reactors, including uniform mixing at low shear (Ni et al., 2000); enhanced mass transfer (Ni et al., 1995, Hewgill et al., 1993); scalability using linear relationships (Smith, 1999, Smith and Mackley, 2006); and the possibility of continuous process development under plug flow conditions (Abbott et al., 2013). A ‘standard’ OBR consists of a tube, generally 10-150 mm internal diameter (D), containing equally spaced orifice plate baffles. A piston located at one end oscillates back and forth generating oscillatory flow required for vortex formation and mixing. A

detailed review of the operation and advantages associated with OBR technology in relation to bioprocessing is available (Abbott et al., 2013). A schematic describing the OBR used in this study is shown in Figure 4.1 and is consistent with the 'standard' design (Brunold et al., 1989, Stonestreet and Harvey, 2002).

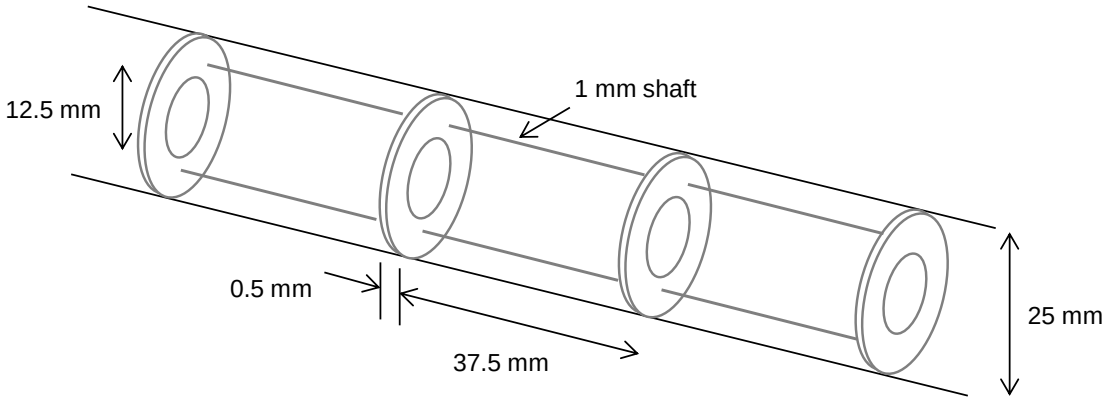

Figure 4.1: Schematic describing the OBR geometry used for this study.

Ikwebe and Harvey (2011) demonstrated that an OBR produced a 7% increase in glucose production after 48 hours compared to a shake flask during enzymatic saccharification. This increase in glucose production was attributed to a 'better mixed hydrolysis environment' (Ikwebe and Harvey, 2011). In other words uniform and effective mixing under low shear allowed cellulase enzymes improved access to their substrates, increasing the conversion rate and, therefore, glucose production. This study aimed to build on that research by making a direct comparison between OBRs and STRs for enzymatic conversion of cellulose to glucose. The low shear environment and efficient mixing in the OBR suggest that this reactor design is suitable for saccharification, as it involves shear-sensitive components, where energy usage and other process economic factors are critical.

4.3 Materials and methods

4.3.1 Reactor designs

A horizontal 700 mL batch OBR was constructed of two borosilicate glass columns (QVF, DPS25/500), coupled by stainless steel collars, containing two stainless steel shafts connecting equally spaced baffles (Figure 4.1). An oscillating piston was used to impose sinusoidal wave form oscillations with set frequency (0-3 Hz) and amplitude (0-6 mm) ranges.

The oscillatory Reynolds number (Re_o) provides an indication of mixing intensity in an OBR and is precisely controlled by the frequency (f) and amplitude (X_o) of oscillation (equation 4.1).

$$Reo = \frac{\rho 2\pi f X_o D}{\mu} \qquad \text{Eq. 4.1}$$

Where, ρ is the fluid density (kg/m^3), f and X_o the frequency (Hz) and amplitude (m) of oscillation, respectively, D the OBR diameter (m) and μ the dynamic fluid viscosity (Pa.s).

Temperature and pH were measured at three locations in the OBR using probes (Mettler Toledo, InPro 3250) inserted through the stainless steel collars and connected to digital displays (Mettler Toledo, M300). Temperature was controlled by a water bath (Huber) connected to glass jackets surrounding both columns. The apparatus used in this study is shown diagrammatically in Figure 4.2.

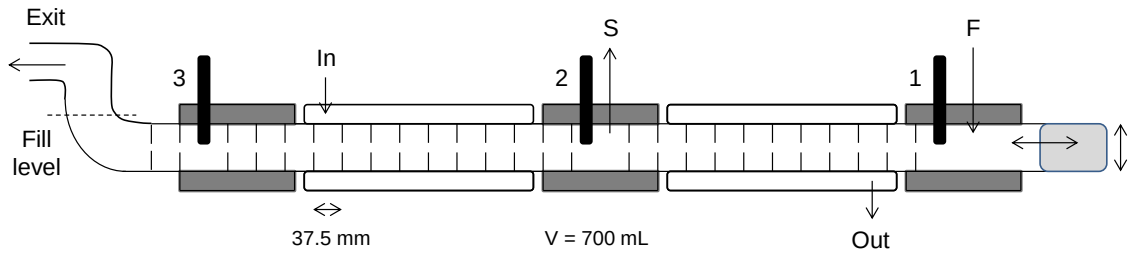

Figure 4.2: The OBR used during this study. 1, 2 and 3 are pH and temperature probes; F the fill port; S the sample port; and in / out the water jacket inlet and outlet, respectively. The OBR exit is open ended and raised above the main reactor body to prevent draining of contents during batch operation.

Three 1.6 L Univessel® double jacket STRs (working volumes of 0.4-1.0 L) connected to a Sartorius Biostat Q plus control system and operated in parallel at 700 mL were used in this study. A water bath (Frigomix® 1000) provided temperature control, and mixing was achieved using one 6-bladed impeller placed approximately 25 mm from the base with a power number (P_o) of 4.6. Figure 4.3 shows a diagram of the STRs used for the study with important dimensions labelled.

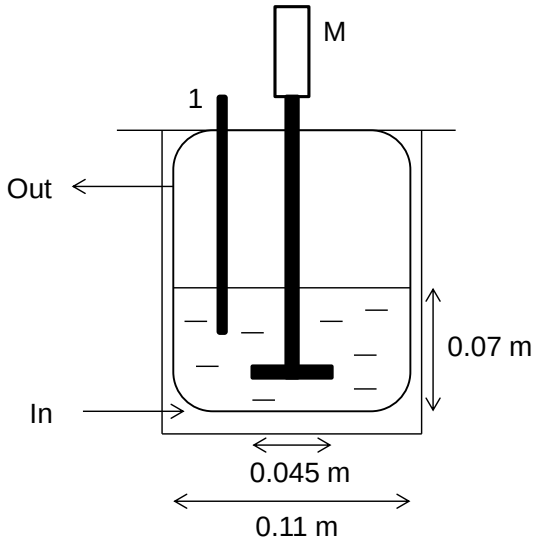

Figure 4.3: The STRs used during this study. M is the impeller motor; 1 the temperature probe; and in / out the water jacket inlet and outlet, respectively.

4.3.2 Enzyme system

Commercially available Celluclast 1.5 L (Sigma, C2730), containing exo- and endo-glucanases (cellulase) from *Trichoderma reesei*, and Novozyme 188 (Sigma, C6105), containing β-glucosidase (cellobiase) from *Aspergillus niger*, were used. Cellulase activity was measured in terms of filter paper units (FPU) defined as the amount of enzyme which produces 2.0 mg of glucose from 50 mg of Whatman No.1 filter paper in 1 h (Ghose, 1987). 50 mM citrate buffer was used to maintain pH at 4.8, formed by adding citric acid monohydrate (Sigma, C1909) and sodium hydroxide (Sigma, 221465) to de-ionised (DI) water according to the standard protocol (Adney and Baker, 1996). A reaction mixture containing 0.5 mL of diluted enzyme solution, 1.0 mL of 50 mM citrate buffer and 50 mg of Whatman No. 1 filter paper was incubated at 50^{o}C for 1 h. Cellobiase activity was measured in terms of cellobiase units (CBU) based on the international unit (IU) of enzyme activity. A reaction mixture containing 1.0 mL of diluted enzyme solution (in 50 mM citrate buffer) and 1.0 mL of 15 mM cellobiose (Sigma, 22150) was incubated at 50^{o}C for 30 minutes. Glucose concentrations were measured using a biochemical analyser (YSI Life Sciences, 2700 SELECT^{TM}) before calculating cellulase (FPU/mL) and cellobiase (CBU/mL) activities according to the standard protocols (Adney and Baker, 1996, Ghose, 1987). Activities were measured as 40 FPU/mL and 19 CBU/mL for Celluclast 1.5 L and 712 CBU/mL for Novozyme 188.

4.3.3 Conditions and procedure

Enzymatic saccharifications were conducted using 5.2% (w/v) α-cellulose (Sigma, 100953734) and 9.7 FPU/g α-cellulose supplemented with an excess of β-glucosidase at 14.6 CBU/g α-cellulose. Microbial growth was inhibited by adding the appropriate amount of 2% sodium azide according to the standard protocol (Selig et al., 2008). All runs were conducted at 50^{o}C and pH 4.8 with 1 mL samples taken at 0, 0.5, 1, 3, 6 and 24 hours. Mixing intensity was increased briefly before taking each sample to ensure homogenisation of reactor contents and provide the most representative sample. Each sample was centrifuged at 16,000 g for 2 minutes before removal of 250 µL supernatant stored at 4^{o}C for later glucose analysis.

Tap water was flushed through the OBR before each run to clean internal surfaces and remove any debris. High flow rates prevented air pocket formation at inter-baffle zones that could dampen oscillations. To fill the OBR α-cellulose, sodium azide and 50 mM citrate buffer, previously equilibrated to 50^{o}C, were homogenised in a 1 L measuring cylinder using a magnetic stirrer (ρ = 1000 kg/m^3 and μ = 0.001 Pa.s assumed for calculations). The appropriate amounts of Celluclast 1.5 L and Novozyme 188 were added before immediately filling the reactor with a peristaltic pump (Cole Palmer, Model 77200-62) through Norprene® tubing (Masterflex, size 36) before setting the oscillating conditions as described in Table 4.1.

Table 4.1: Mixing intensities and corresponding power densities (P/V) for the OBR (Re_o) and STR (Re) (numbers in brackets denote impeller speed (rpm).

Run	Reactor	Mixing	P/V (W/m^3)
1	OBR	0	0.00
2		300	0.30
3		400	0.71
4		600	2.36
5		1500	37.2
6		2827	250
7	STR	0 (0)	0.00
8		1250 (37)	0.30
9		1690 (50)	0.71
10		2530 (75)	2.36
11		6240 (185)	37.2
12		11800 (350)	250

The volume of each STR was maintained at 700 mL consistent with the OBR. Appropriate amounts of 50 mM citrate buffer, α-cellulose and sodium azide were added to each STR and homogenized to 50^{o}C. The impeller speed was set to 200 rpm before addition of Celluclast 1.5 L and Novozyme 188 to ensure a homogenous starting mixture. The impeller speed was then adjusted to give the desired mixing intensity as described in Table 4.1. Three runs were conducted in parallel (runs 7, 8 and 9 followed by runs 10, 11 and 12).

4.3.4 Calculations

The power density (P/V) was used to estimate power required to achieve mixing intensities outlined in Table 4.1 and is expressed in terms of power per unit volume in Watts per cubic metre (W/m^3). The power consumption for an unaerated reaction mixture in an STR is defined by equation 4.2 (Holland and Chapman, 1966).

$$\frac{P}{V} = \frac{P_o \rho N^3 D_s^5}{\frac{1}{4}.\pi D_v^2 L_h} \qquad \text{Eq. 4.2}$$

Where P_o is the power number of the impeller (4.6), N the impeller rotational speed (rps), D_s the impeller diameter (m), D_v the vessel diameter (m) and L_h the height of liquid in the reactor (m).

The power density for a ‘standard’ OBR design (Brunold et al., 1989, Stonestreet and Harvey, 2002) can be estimated using equation 4.3 (Hewgill et al., 1993).

$$\frac{P}{V} = \frac{2\rho N_B}{3\pi C_D^2} \frac{1-\alpha^2}{\alpha^2} X_o^3 (2\pi f)^3 \qquad \text{Eq. 4.3}$$

Where N_B is the number of baffles per unit length (m), C_D the discharge coefficient and α the reactor to orifice area ratio. The value of C_D was taken as 0.7, to be consistent with a previous study (Ni et al., 2000).

Error bars represent the average error of +/- 2.5% present from the YSI biochemical analyser calculated using a range of standard glucose solutions from 0-25 g/L.

4.4 Results and discussion

4.4.1 OBR saccharifications

Glucose concentration profiles for 6 runs conducted at different mixing intensities in the OBR are shown in Figure 4.4.

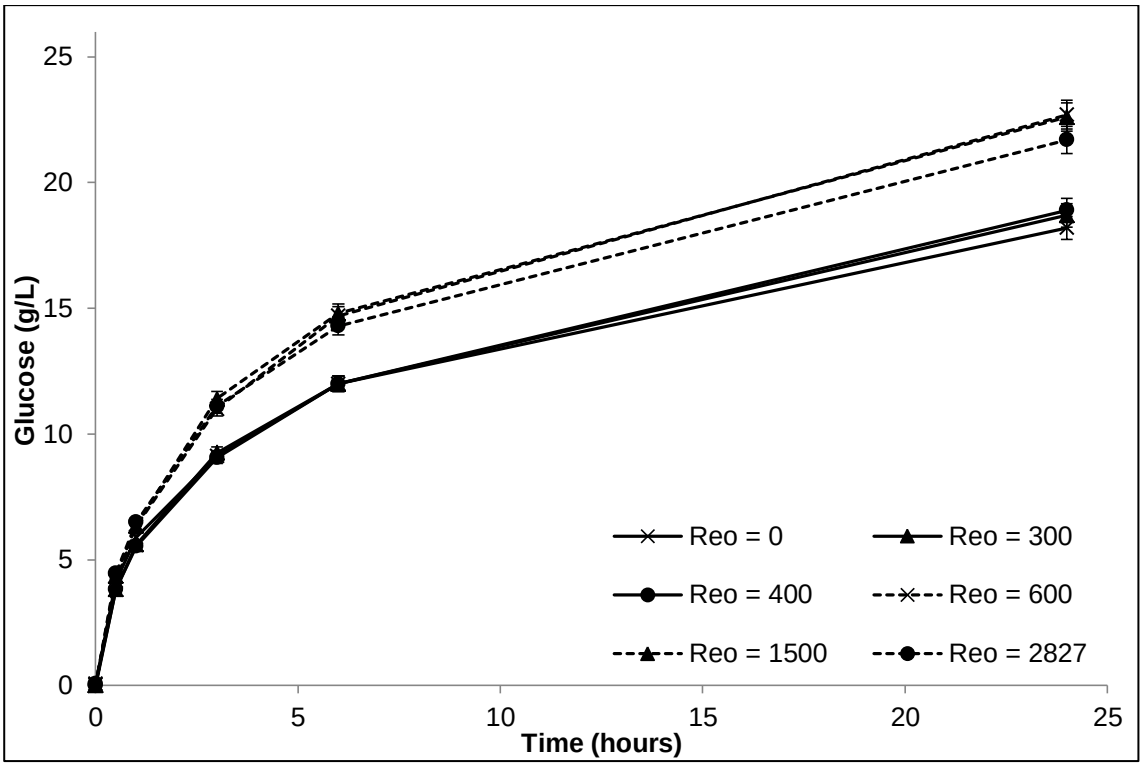

Figure 4.4: OBR saccharification runs showing glucose concentration over 24 hours.

At low mixing intensities, up to Re_o=400, no significant effect of mixing was observed i.e. the time profiles were indistinguishable from those at Re_o=0. Vortex formation and propagation were not achieved at these mixing intensities. This resulted in sedimentation of cellulose particles on the OBR wall (due to the horizontal orientation of the reactor), presumably inhibiting some exposure of cellulose to the enzyme.

The enzymatic conversion of cellulose to glucose still proceeded in low mixing conditions suggesting access to substrate was not completely prevented by reduced mixing intensity. Presumably the initial homogenisation spread enzyme proteins throughout the cellulose allowing local catalysis to occur. However, agitation has been shown to enhance adsorption of exo-glucanase on to cellulose increasing the conversion rate up to 500 rpm in an STR (Sakata et al., 1985). At low mixing intensities adsorption of exo-glucanase was reduced, slowing the conversion rate.

The opportunity of moving and adhering to fresh cellulose was possibly reduced due to sedimentation of material. This could have led to local conversion of amorphous cellulose to glucose leaving more recalcitrant crystalline regions that slow the conversion rate. Amorphous (and crystalline) regions not initially in contact with enzyme proteins remained isolated due to lack of mixing and mass transfer resulting in reduced glucose production at low mixing intensities.

Cellulase enzymes are susceptible to product inhibition from cellobiose and glucose (Ye et al., 2012, Takagi, 1984, Xiao et al., 2004). At low mixing intensities the transport of glucose away from regions containing cellulose and enzymes was reduced, possibly leading to localised glucose build-up and subsequent product inhibition, slowing the conversion rate. This inhibitory effect coupled with reduced adsorption of exo-glucanase and the possible increase in crystallinity reduced conversion by 20% after 24 hours in runs 1-3 compared to those runs with sufficient mixing to prevent sedimentation.

Conversion rate increased with increasing mixing intensity to achieve a maximum rate between 400 and 600 Re_o. The mixing intensity was sufficient to prevent sedimentation of cellulose thereby removing glucose concentration gradients, to minimise effects of product inhibition, and enhance exo-glucanase adsorption. Presumably, enzymes were constantly moving throughout the reactor so came into contact with easily hydrolysable amorphous regions of cellulose resulting in increased conversion rates. Conversion began to slow after approximately 6 hours due to amorphous regions being converted to glucose leaving only more recalcitrant crystalline regions. At a mixing intensity of 600 Re_o the conversion rate was no longer mass transfer limited so increasing the mixing intensity had no effect. A maximum conversion rate was achieved for 600 Re_o with any increase in mixing intensity beyond this having no impact.

4.4.2 STR saccharifications

Glucose concentration profiles for 6 runs conducted at different mixing intensities in STRs (at comparable power densities to the OBR) are shown in Figure 4.5.

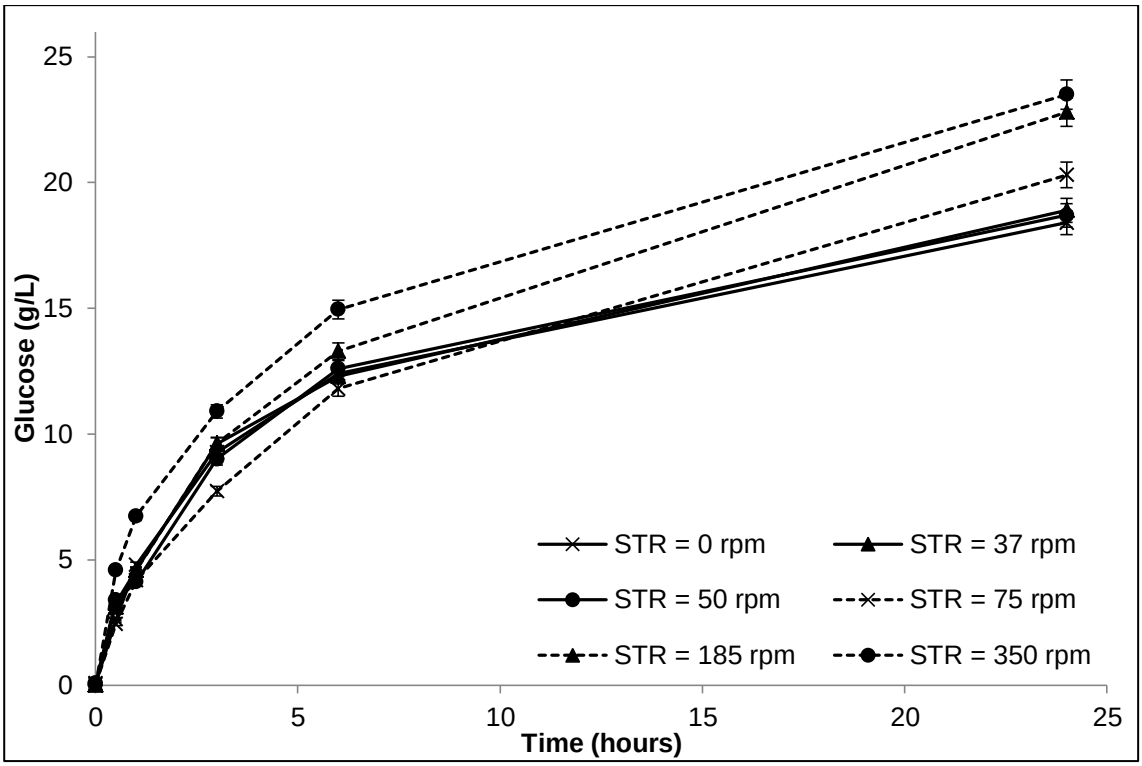

Figure 4.5: STR saccharification runs showing glucose concentration over 24 hours.

A similar trend to the OBR was seen. At lower mixing intensities the conversion rates were lower, but increase to a maximum with increasing mixing intensity. The product inhibition effect coupled with an increase in crystallinity caused by concentration gradients and minimal mass transfer could explain these lower conversion rates (see §4.4.1). The conversion rate only began to increase after a sufficient mixing intensity was reached to prevent sedimentation of material and enhance exo-glucanase adsorption. This began to occur at an impeller speed of 75 rpm, however a fully homogenous mixture did not appear to be achieved until 185 rpm (see Figure 4.7). At this mixing intensity the conversion rate was still below the maximum suggesting mass transfer was limiting at 185 rpm requiring an increase in mixing intensity to achieve maximum conversion rates.

The highest conversion rate was seen at maximum mixing intensity suggesting shear was not inactivating enzyme proteins at any point during this study for runs conducted in STRs. Previous studies have identified the shear-sensitive nature of cellulase enzymes in STRs (Gunjikar et al., 2001, Ganesh et al., 2000). These studies show an impeller speed of 1000 rpm (approx. 2 kW/m^3) is required to cause 10% inactivation (Gunjikar et al., 2001) and 500 rpm (approx. 600 W/m^3) to cause 3% inactivation in 5 hours (Ganesh et al., 2000). These impeller speeds are substantially

higher than those used in this study and are not required to achieve maximum conversion rates.

4.4.3 Comparison of designs

A similar trend was seen in both reactor designs. Increased mixing had a positive effect on conversion rates. In the OBR the mixing intensity required to maximise cellulose conversion was seen at 2.36 W/m^3. This should be compared to the STR, which exhibited maximum conversion at 250 W/m^3. Without mixing similar conversion rates were produced in both the OBR and STR as shown in Figure 4.6 (green). This suggests conditions were similar in both reactor designs with subsequent runs differing in only the mixing mechanism and intensity.

At 2.36 W/m^3 the resultant Re_o and impeller speed were 600 and 75 rpm, respectively. This power input was sufficient to produce maximum conversion in the OBR which was 12% higher after 24 hours and 25% higher after 6 hours than the STR. This result demonstrates the OBR is a more efficient mixing device able to prevent sedimentation of material with a much reduced power input. Photographs taken throughout the runs are presented in Figure 4.7 showing the extent of sedimentation for different power densities in both the OBR and STR.

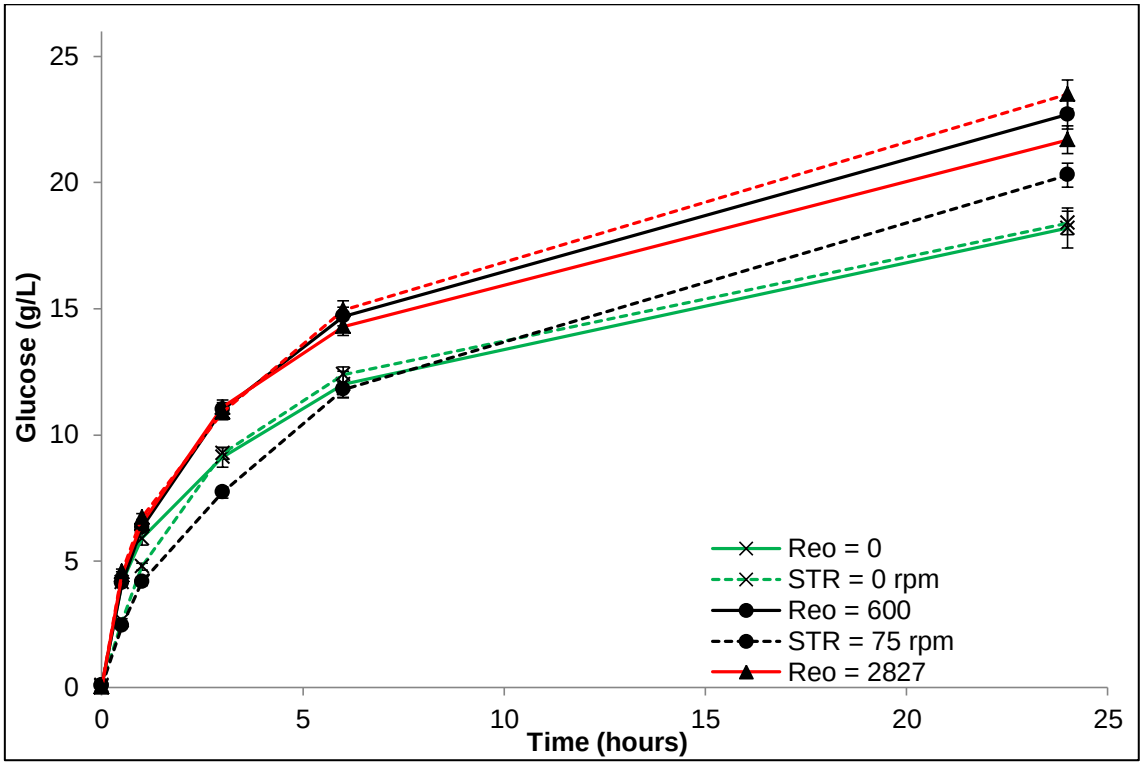

Figure 4.6: Comparison of glucose concentrations between OBR and STR designs over 24 hours for different mixing intensities requiring comparable power densities. Green = 0 W/m^3, black = 2.36 W/m^3, red = 250 W/m^3.

Figure 4.7c shows the extent of sedimentation in the STR for a power density of 2.36 W/m^3, explaining the reduced conversion rate compared with the OBR, which was able to generate a fully homogenous mixture at this power density (3 in Figure 4.7). The maximum conversion rate in the OBR (2.36 W/m^3) was most similar to that seen in the STR for a power density of 250 W/m^3, although after 24 hours a power density of 37.2 W/m^3 in the STR produced a similar glucose concentration (22.8 g/L) to the OBR at 2.36 W/m^3 (22.7 g/L). This is a reduction of between 94-99% for the required power input in the OBR compared to the STR to achieve the maximum rate of cellulose conversion to glucose.

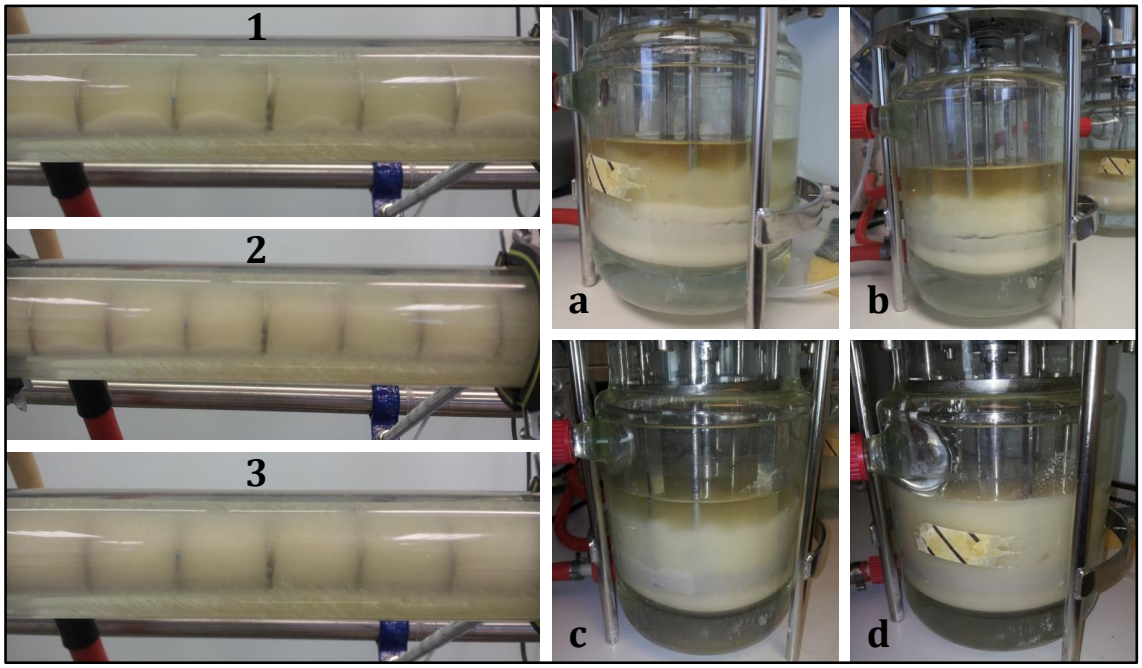

Figure 4.7: Photographs taken during different saccharification runs in the OBR (left) and STR (right). 1) and a) = 0.30 W/m^3, 2) and b) = 0.71 W/m^3, 3) and c) = 2.36 W/m^3, d) = 37.2 W/m^3.

Figure 4.6 demonstrates that both reactor designs were capable of achieving similar conversion rates with optimal power input for mixing (2.36 W/m^3 for the OBR and 250 W/m^3 for the STR). Once at this level, the rate was no longer mass transfer limited, so increasing the mixing intensity had no effect. The large difference in required power input to achieve maximum conversion suggests the OBR is more suitable for performing saccharification reactions where running costs are critical. It is probable that most processes involving cellulose saccharification will be large scale, particularly ethanol production (Humbird et al., 2011), where running costs tend to have more of an impact on the process economics.

4.5 Conclusions

Enzymatic saccharification of pure α-cellulose was mass transfer limited in both OBR and STR designs with no or minimal mixing. Concentration gradients of glucose and enzymes presumably increased product inhibition and substrate crystallinity, reducing the overall rate compared to well-mixed conditions. The maximum conversion rate in the OBR was seen at a relatively low power density (2.36 W/m^3). To achieve a similar conversion rate in the STR an impeller speed of 350 rpm (250 W/m^3) was required, although 185 rpm (37.2 W/m^3) was sufficient to produce similar glucose concentrations after 24 hours. No evidence of shear inactivation was observed for STR runs due to a relatively low impeller speed compared to previous studies (Gunjikar et al., 2001, Ganesh et al., 2000). A comparison of the power density required to achieve maximum conversion rates shows a reduction of 94-99% in the OBR (2.36 W/m^3) compared to the STR (37.2-250 W/m^3).

This study has shown that OBRs are suitable for performing enzymatic saccharification reactions in a power-efficient manner compared to conventional STRs. The development of continuous enzymatic saccharification using OBR technology could further enhance process economics. Results from this study provide essential data on power input to allow an economic assessment of industrial scale enzymatic saccharification processes based on both OBR (batch and continuous) and STR (batch) designs.

Scale up of STR technology can be difficult with numerous strategies available, for example, by maintaining geometric similarity and constant power density (Junker, 2004). OBR technology, in theory, can be easily scaled using a combination of linear relationships and multi-orifice baffles (Smith and Mackley, 2006) potentially enabling lab scale results to be transferred to industrial processes. More research needs to be done to prove this in practice however; this possible benefit offers great potential over the conventional STR.

4.6 Economic assessment

The study has proven that in theory OBR technology can be operated with significantly less power requirements for mixing compared to STR technology for enzymatic saccharification. However a commercial process at industrial scale which converts biomass to platform chemicals consists of numerous unit operations other

than saccharification. A simple economic assessment was required to evaluate the commercial benefits that would be realised from the use of OBR technology as the saccharification reactor. The following assessment was developed based on a commercial process for conversion of lignocellulosic biomass to ethanol produced by the National Renewable Energy Laboratory (NREL) (Humbird et al., 2011). Only brief details are provided as the report available in the literature provides extensive information. It must be noted that the comparative values are important and not the absolute values which will change significantly based on the stated assumptions.

4.6.1 Commercial enzymatic saccharification

Conversion of corn stover to ethanol requires a facility with the unit operations outlined in Figure 4.8. Corn stover is delivered to the facility where it is stored before entering the pretreatment stage. Sulphuric acid pretreatment has been selected to convert the xylan component to its constituent 5-carbon monomer units (xylose) as well as delignify and expose the material. The addition of ammonia and water are required after pretreatment to raise the pH to ~4.8 before enzymatic saccharification begins. A genetically modified bacterium (*Zymomonas mobilis*) is added to the mixture after saccharification to ferment the sugar monomers to ethanol which is then distilled and stored for sale and/or use. Solids and liquids produced through the distillation process are used to generate electricity through a turbogenerator and for process water after treatment, respectively. Total processing time is 160 hours which consists of feed handling (36 hours), pretreatment (2 hours), saccharification (84 hours), fermentation (36 hours) and distillation (2 hours).

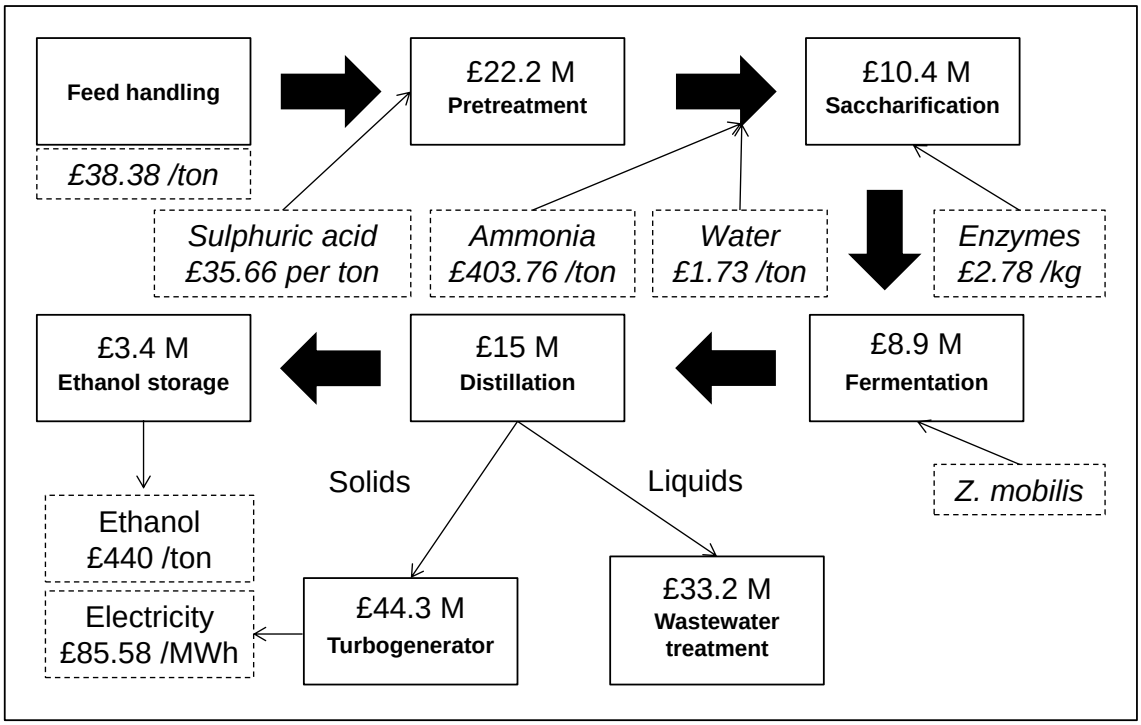

Figure 4.8: Unit operations required for a commercial process to convert corn stover to ethanol. Capital costs (solid), operating costs (dashed, italics) and revenue (dashed) are shown.

Table 4.2 shows the assumed composition of corn stover entering the facility, and the reactions and their assumed conversions for the pretreatment and saccharification stages.

Capital costs were taken directly from the NREL report (Humbird et al., 2011) and the operational costs used were as follows: corn stover £38.36 per ton, sulphuric acid £35.66 per ton, ammonia £403.70 per ton, process water £1.73 per ton, cellulase system £2.78 per kg protein and electricity £85.58 per MWh. It was assumed that saccharification reactors based on OBR and STR technologies would cost the same at £10.4 M; that the OBR could process 30% solids which is the concentration used by NREL; and the energy requirements for mixing were constant upon scale up. The model also assumed treatment of 2000 ton corn stover per day.

Table 4.2: Corn stover composition and the reactions and their assumed conversions for the pretreatment and saccharification stages.

Corn stover		**Pretreatment**	
Glucan	35.05%	*Reaction*	*Conversion (%)*
Xylan	19.53%	(Glucan)n + n H_2O -> n Glucose	9.9
Lignin	15.76%	(Glucan)n + n H_2O -> n Glucose Oligomer	0.3
Ash	4.93%	(Glucan)n + n HMF -> 2n H_2O	0.3
Acetate	1.81%	Sucrose -> HMF + Glucose + 2 H_2O	100.0
Protein	3.10%	(Xylan)n + n H_2O -> n Xylose	90.0
Extractives	14.65%	(Xylan)n + m H2O -> m Xylose Oligomer	2.4
Arabinan	2.38%	(Xylan)n -> n Furfural + 2n H_2O	5.0
Galactan	1.43%	Acetate -> Acetic Acid	100.0
Mannan	0.60%	(Lignin)n -> n Soluble Lignin	5.0
Sucrose	0.77%	**Saccharification**	
Glucose and xylose conversion to ethanol during fermentation was assumed to be 95 and 85%, respectively.		*Reaction*	*Conversion (%)*
		(Glucan)n -> n Glucose Oligomer	4.0
		(Glucan)n + 1/2n H_2O -> 1/2n Cellobiose	1.2
		(Glucan)n + n H_2O -> n Glucose	90.0
		Cellobiose + H_2O -> 2 Glucose	100.0

4.6.2 Results

Reactions and their assumed conversions defined in Table 4.2 were used to determine the amount of material after each stage of the process. Figure 4.9 shows the amounts of glucan (cellulose), xylan, glucose, xylose, ethanol and CO_2 present in the raw corn stover and after pretreatment, saccharification and fermentation. Other components were omitted due their concentrations being negligible. Figure 4.9 shows that pretreatment converts the majority of xylan to xylose; saccharification, the majority of glucan to glucose; and fermentation converts these sugars to ethanol and CO_2.

The profit generated per year based on the operational costs and revenue generated was calculated for both scenarios. A P/V of 2.36 W/m^3 is required to maximise glucose production in OBRs and 40-250 W/m^3 in STRs. This equates to a profit for the process based on OBR technology of ~£61 M per year compared to that based on STR technology of £54-60 M per year. The process based on OBR technology could therefore potentially increase profits by 2-14% compared to processes based on STR technology. However, this is a very simplistic assessment and makes various assumptions that significantly increase the risk of developing saccharification processes based on OBRs. These assumptions are 1) the capital cost is equal to that

of STRs; 2) OBRs could process 30% solids; and 3) power requirements remain constant upon scale up.

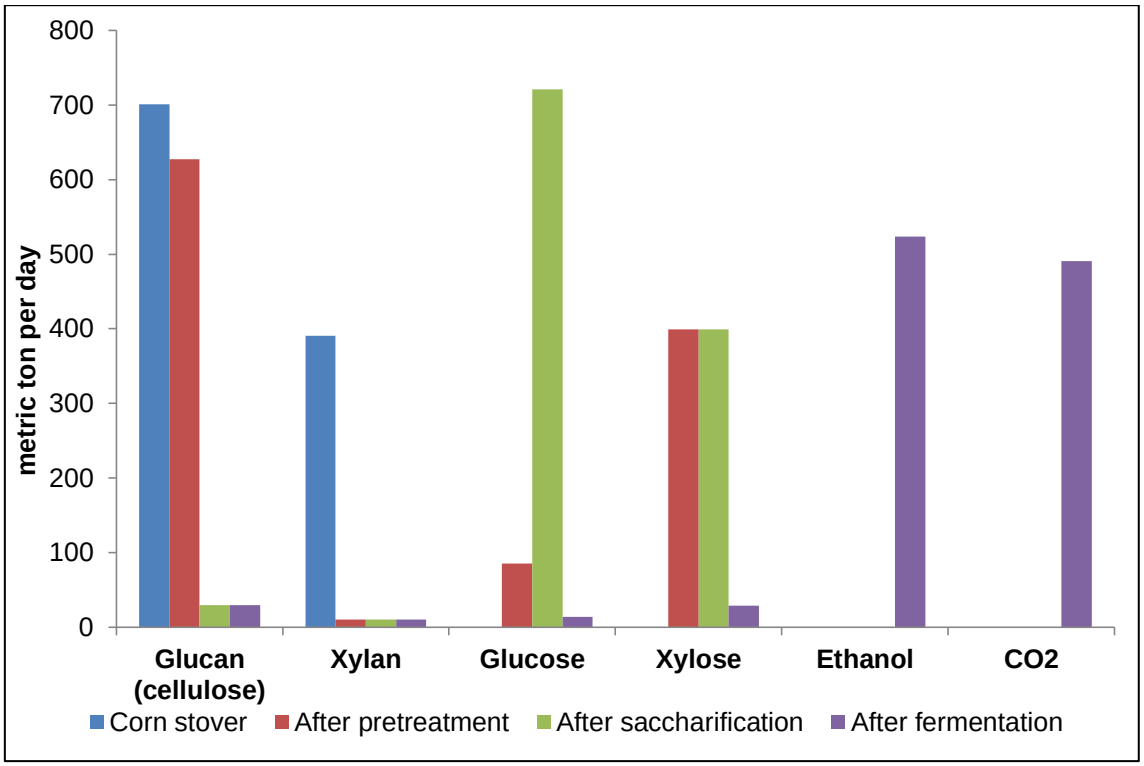

Figure 4.9: The mass of material at each stage of the process.

This assessment has highlighted that power requirements for mixing are significantly less than other operational and capital costs associated with a commercial process at industrial scale for the conversion of biomass to ethanol. The 94-99% reduction in power in OBRs compared to STRs for saccharification therefore results in only marginal improvements in profit if the assumptions are correct. The risks of developing OBR technology outweigh the possible benefits making commercialisation of OBRs for saccharification less attractive. These results motivated a change in the research direction to another bioprocess which could be commercially more attractive.

4.6.3 Bioprocess selection

The following two bioprocesses were selected for possible development using OBR technology based on their commercial and scientific potential. They were presented to managers at CPI to help with their decision making for future research.

4.6.4 Heterologous protein production using Pichia pastoris

The methylotrophic yeast *P. pastoris* has been developed into a successful expression system for heterologous protein production over the last two decades. Its increased popularity is due to several factors: ease of genetic manipulation; similarity to *Saccharomyces cerevisiae,* the most well characterised experimental system in biology; its ability to produce foreign proteins at high levels both intra and extracellularly; presence of post translational modification machinery; and its commercial availability (Cereghino and Cregg, 2000). The major advantage over *S. cerevisiae* is *Pichia's* preference for respiratory growth, removing risks of ethanol production that can 'pickle' the culture.

Hundreds of heterologous proteins have been successfully produced using *P. pastoris* ranging from viral to human. 5% of expression systems requested by Lonza customers were *P. pastoris* with a total of 10% requesting *Pichia* sp. (Meyer et al., 2008b). The therapeutic nature of heterologous proteins make them very high value products, and developing a continuous bioprocess using OBR technology could increase production efficiency and allow improved process monitoring, in line with new quality by design (QbD) initiatives.

The *Pichia* fermentation process guidelines, supplied by Invitrogen and available online (Invitrogen Co., 2002), provide operational and equipment information required for successful heterologous protein production. There are three phases for production of a protein using a transgene linked to the *AOX*1 promoter (transcribing alcohol oxidase required for the first step in methanol metabolism).

1) Glycerol batch: starting at 40 g/L glycerol for 18-24 hours producing 90-150 g/L wet cells.
2) Glycerol fed-batch: 50% w/v glycerol fed over 4 hours producing 180-220 g/L wet cells.
3) Methanol fed-batch: 100% methanol at 3.6 mL/hr/L fermentation volume stimulating protein production and producing 350-450 g/L wet cells.

The protocol emphasises the importance of maintaining a dissolved oxygen concentration above 20%. This is achieved by supplementing with pure oxygen at 0.1-0.3 vvm or cutting the glycerol/methanol feed to slow cell growth, reducing oxygen demand. A method for retro-fitting a standard STR to avoid pure oxygen

supplementation in an attempt to overcome limitation to productivity has been described (Jenzsch et al., 2004), demonstrating that current reactors are often incapable of reaching required oxygen transfer rates (OTR) for this bioprocess.

OBRs have been reported to have a 6-fold increase in oxygen transfer (k_La) into water (Hewgill et al., 1993) and a 75% increase into a yeast culture of *S. cerevisiae* (Ni et al., 1995). These studies highlight the suitability for using OBRs to culture *P. pastoris* as oxygen limitation may be overcome removing the need for a pure oxygen supply. The increased OTR could also allow increased growth rates, reducing the overall processing time while maintaining productivity. A further advantage is that plug flow conditions are achievable during continuous operation. It would be possible to select the optimal time to start methanol feeding without inducing protein production upstream of the injection site. This is not possible using an STR because methanol will rapidly mix throughout the entire reactor volume requiring the start of another batch.

4.6.5 Astaxanthin production using Haematococcus pluvialis

Astaxanthin is a keto-carotenoid used mainly in aquaculture but has an increasing demand for use in dietary supplements (Guerin et al., 2003). This red pigment is responsible for the colouration of salmon and certain crustaceans so is essential for farming of these organisms. Recent studies have shown that astaxanthin could have many health promoting effects in the prevention and treatment of diseases including cancers, chronic inflammatory diseases, diabetes and cardio-vascular diseases (Yuan et al., 2011).

The market is dominated by synthetic astaxanthin with 95% being derived from chemical sources (Lorenz and Cysewski, 2000). Growing demand for natural sources of this pigment has stimulated production using *H. pluvialis* which can contain up to 3% dry weight astaxanthin (Lorenz and Cysewski, 2000). Four global leaders dominate the market for natural astaxanthin production from *H. pluvialis*: Valensa (USA), Algatechnologies (Israel), Cyanotech (Hawaii) and Astareal (Japan, Scandanavia) (Heller, 2009). The value of astaxanthin has been estimated to be $2500 per kg but could increase for biological sources which, unlike chemical routes, do not contain a mix of isomers (Del Campo et al., 2007). The mixture of isomers

prevents synthetic astaxanthin being accepted for human consumption, except indirectly through aquaculture.

Production of natural astaxanthin by *H. pluvialis* occurs in two stages:

1) During exponential growth, green vegetative cells are produced until a relatively high cell density is reached.
2) The cells are subjected to stressful conditions e.g. increased light intensity or salt concentrations stimulating them to encyst and produce astaxanthin.

Figure 4.10 shows the contrast between cells during phase 1 and 2. During phase 1, the cells exist in biflagellate form with the polysaccharide link between the flagella and cell wall acting as a fragility zone (Gudin and Chaumont, 1991). If this area is damaged, the cells encyst, entering stationary growth resulting in lower cell concentrations and, therefore lower astaxanthin production. A semi-continuous, 2 phase technique achieved maximum astaxanthin concentrations at higher initial cell concentrations (Fabregas et al., 2001). Reaching high cell concentrations in phase 1 produces enhanced astaxanthin production. Cell fragility in phase 1 requires low shear environments for maximum growth complemented with good mixing to achieve sufficient overall illumination which is difficult to achieve in conventional tubular photobioreactors (PBRs) which require turbulent flow for mixing. The alternative solution is to use open raceways.

This bioprocess has been identified as a candidate for OBRs because it contains a stage requiring low shear rates with good mixing. Conventional tubular PBRs require turbulent flow conditions to overcome light limitation which can result in reduced growth caused by high shear rates. Open ponds are used by some companies culturing *H. pluvialis* but these are difficult to monitor, control and are prone to contamination which is undesirable for products aimed at human consumption. The OBR could offer an alternative platform for *H. pluvialis* culturing under continuous and controlled conditions with improvement in astaxanthin production.

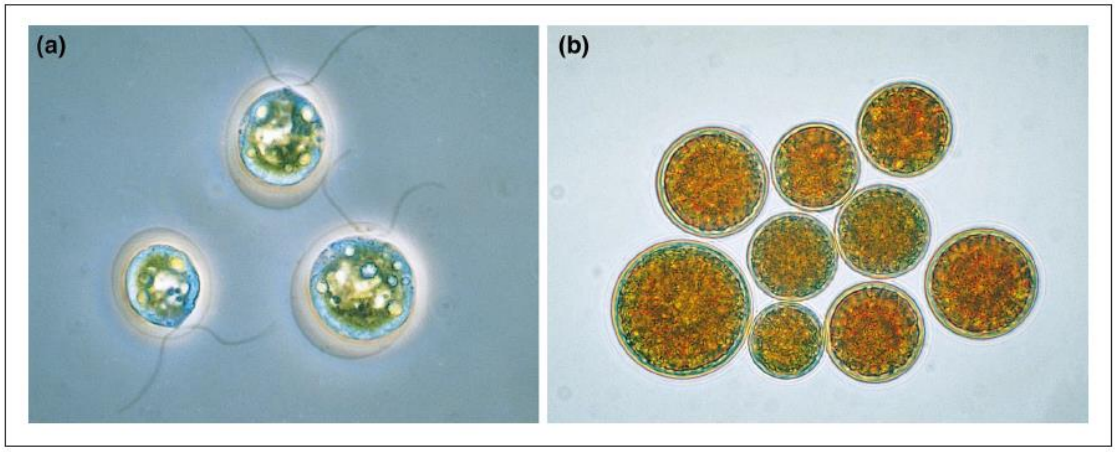

Figure 4.10: a) Vegetative, actively growing *H. pluvialis* cells and b) *H. pluvialis* haematocysts that have accumulated astaxanthin as a result of nutrient starvation and sunlight. 400 x magnification.

There is evidence in the literature to suggest OBRs could intensify microalgae cultures. Static mixers have been used, similar to baffles in an OBR, to enhance mass transfer characteristics in tubular PBRs (Ugwu et al., 2002). The results indicate higher k_La values and increased biomass production from *Chlorella sorokinina*. Although similar to an OBR, this design still relies on turbulent flow for mixing. OBRs are capable of achieving good mass transfer, independent of net flow, at low shear so appear to be an ideal candidate for developing continuous microalgae bioprocesses, especially shear sensitive ones such as *H. pluvialis*.

4.6.6 Summary

Both *P. pastoris* and *H. pluvialis* are viable candidates for study using OBRs. These bioprocesses have commercial applications already in use and the OBR could provide a platform for developing continuous processes that increase production rates and allow better process control. Microalgae in particular seem promising as no published literature currently exists whereas several studies are reported on the effects of yeast culture in OBRs (Ni et al., 1995, Reis et al., 2006b). There is currently an upward trend into research using microalgae for a range of applications from low value products such as biodiesel to therapeutic compounds. Table 4.3 summarises the advantages and disadvantages of each process.

Table 4.3: Advantages and disadvantages of proposed bioprocesses in an OBR.

	Advantages	Disadvantages	Possible gains due to OBR
Pichia pastoris	Relatively easy protocol	Other methods available to overcome oxygen demand	Better oxygen transfer rates
	Widely used expression system		Removes need for pure oxygen
	Used for high value products	Yeast studies exist in literature	Improved growth rates
Haematococcus pluvialis	Already commercially used	Light adds complexity to process	Low shear
	Possibility of applying to microalgae in general		Improved illumination due to efficient mixing
	Market growth for astaxanthin	Likely to foul reactor wall	Increased growth in stage 1
	No currently published data for microalgae in OBRs		Increased astaxanthin production

4.6.7 Conclusions

The above-mentioned bioprocesses were evaluated by managers at CPI. Their decision was to focus on the development of microalgae cultures using OBR technology. Modifications to the OBR were required to convert it to a photobioreactor (PBR) and *Chlamydomonas reinhardtii* was selected as the organism due to its fragility and potential for biopharmaceutical production.

Chapter 5: Microalgae culture

'Liquid culture of microalgae in a photobioreactor (PBR) based on oscillatory baffled reactor (OBR) technology – A feasibility study.'

5.1 Abstract

Chlamydomonas reinhardtii (CCAP 11/32C) cells were grown in liquid culture under photoautotrophic conditions using a photobioreactor (PBR) based on oscillatory baffled reactor (OBR) technology. A flotation effect was observed when using a porous gas sparger which resulted in accumulation of microalgae at the top of the column. Linear growth was achieved with a different sparger, designed to produce larger, faster rising gas bubbles. Changes in the mixing intensity had no effect on the maximum growth rate of 0.130 OD_{750}/day (+/- 0.010) achieved which was 95% higher than that achieved in T-flasks of 0.067 OD_{750}/day (+/- 0.011) under comparable conditions. The increase in growth rate achieved in the OBR was probably a result of increased gas transfer, and exponential growth was not achieved probably due to the relatively low light intensity used of 78 µmol/m^2s (+/- 20). The results demonstrate the feasibility of OBR technology for use as PBRs with the potential for the dual culture and harvest of microalgal biomass through manipulation of the bubble diameter. This could greatly improve bioprocess economics for microalgae culture.

5.2 Introduction

Microalgae form a diverse group of prokaryotic and eukaryotic photosynthetic organisms (Li et al., 2008b) that can be photosynthetically more efficient than land plants (Chisti, 2010). The simple structure of microalgae enables rapid photoautotrophic growth in either open or closed systems from basic components, namely carbon dioxide (CO_2), light, water and trace elements (Pragya et al., 2013). Great potential exists to develop bioprocesses based on microalgae that require low cost raw materials and would reduce dependency on fossil based resources. These potential bioprocesses could serve as production systems for a variety of low to high value, sustainable products that include biomass (Sialve et al., 2009), biofuels (Brennan and Owende, 2010, Pittman et al., 2011), pigments, such as astaxanthin (Phan et al., 2011b), and therapeutic proteins (Mayfield et al., 2007). However, there are several barriers to the development of economic bioprocesses for production of sustainable products based on microalgae. These include light penetration (Lee,

1999), oxygen removal (Richmond et al., 1993), CO_2 addition (Eriksen, 2008), contamination (Chen et al., 2011) and harvesting (Coward et al., 2013).

There is an exponential decrease in light penetration associated with increased microalgae cell concentrations (Lee, 1999). The culture depth at which light intensity becomes too low to support net photosynthesis is referred to as the irradiance compensation point (I_c) and differs for each species of microalgae (Nag-Jong et al., 2002, Sorokin and Krauss, 1958). The I_c is reached at relatively low depths (<100 mm) for microalgae cultures due to the efficiency of light absorbing pigments (Nag-Jong et al., 2002). This prevents high cell densities from being obtained for deep cultures where long light path lengths are present. Low cell concentrations require extremely large volumes to be de-watered to obtain relatively low quantities of product. For example, lipid concentrations of between 0.3 to 5 g/L are typical for biodiesel production (Li et al., 2008a, Wang et al., 2008) even though cells contain 50 to 70% lipid per dry weight (Chisti, 2007).

Photosynthesis is the process by which light energy is used to fix CO_2 into carbohydrate molecules. The principal carboxylating enzyme in this process is ribulose-1,5-bisphoshate carboxylase (Rubisco) which can also utilise oxygen (O_2) for photorespiration at high O_2 concentrations. It was reported that microalgae growth could be severely inhibited by accumulation of photosynthetically generated O_2, especially in closed systems (Weissman et al., 1988). Sufficient mixing to promote turbulence and/or a dedicated gas exchange unit is required in microalgae cultivation systems to ensure net photosynthesis and remove inhibitory effects from O_2 build-up (Wang et al., 2012).

Cultivation of microalgae currently occurs in either open or closed systems. An open system typically consists of a pond or raceway with a paddle wheel to provide some agitation (da Rosa et al., 2011). Most industrial microalgae cultivation, for example astaxanthin production in Hawaii, is currently based on open systems due to their low capital and operational costs (Milledge, 2011). However, an open system can only sustain low cell concentrations because growth is limited to the surface where photosynthesis is not light limited. Furthermore, little control is provided over species population or general contamination (Wang et al., 2012). In comparison, closed systems enable greater process control which includes temperature, mass transfer,

pH and microorganism population. Closed systems are therefore able to generate higher cell concentrations but suffer from increased capital costs (Wang et al., 2012).

Regulatory requirements must be considered for any industrial production system, especially those products designed for human consumption such as therapeutics (Manuell et al., 2007) and nutraceuticals (Bishop and Zubeck, 2012). In the case of therapeutic protein production in microalgae, use of genetically modified organisms (GMOs) is unavoidable. The European Parliament has issued several directives associated with the use of GMOs, notably two concerned with their contained use (Directive 2009/41/EC, 2009) and controlled release (Directive 2001/18/EC, 2001). Manufacture of active pharmaceutical ingredients (APIs), such as therapeutic proteins, must comply with good manufacturing practice (GMP) guidelines. These guidelines include stringent process controls defined by '…process parameters that could affect the critical quality attributes of the API' (CPMP/ICH/4106/00, 2000). The use of closed systems would almost certainly be required to comply with the regulations surrounding use of GMOs and the guidelines for manufacture of APIs.

Photobioreactors (PBRs) are closed systems for microalgae cultivation and there are currently three main design groups available (Carvalho et al., 2011). These designs are tubular (Molina et al., 2001), flat panel (Issarapayup et al., 2009) or fermenter-type (Carvalho et al., 2006). Tubular and flat-panel PBRs are designed for the efficient harvest of sunlight and are therefore based on the principle of high area to volume ratios. Fermenter-type PBRs require artificial illumination so are less popular due to additional power requirements (Carvalho et al., 2011). The use of tubular or flat panel PBRs for the development of bioprocesses based on microalgae cultivation, especially therapeutic protein production, has the following advantages:

1) Good control of process parameters which include temperature, pH, mixing, and mass transfer. This enables higher cell concentrations to be obtained thereby reducing harvesting requirements.
2) The use of a closed system reduces contamination risk and greatly improves process control. This allows compliance with the necessary regulations and guidelines associated with GMO use and API manufacture.
3) A high area to volume ratio is conductive to the efficient harvest of sunlight. This can reduce power requirements associated with artificial illumination.

Although microalgae can potentially be used to produce many products, one exiting area is protein production. Numerous studies have reported the successful use of the microalga *Chlamydomonas reinhardtii* for production of therapeutic proteins (Mayfield et al., 2007, Muto et al., 2009, Rosales-Mendoza et al., 2012, Tran et al., 2009, Mayfield, 2013). Of particular interest are synthetic fusion proteins that combine the characteristics from different molecules to provide an enhanced physiological response (Mayfield, 2013). For example, immunotoxins combine protein portions that deliver a toxin to cells displaying a specific antigen (Goldenberg and Sharkey, 2012). A major hurdle to production of these immunotoxins is high cost from both the lack of a suitable expression system and their production in cultivation systems (Mayfield, 2013). Bacterial expression systems lack the protein chaperones responsible for the successful folding of complex proteins required for biological function (Baneyx, 1999). Mammalian (Wurm, 2004) and yeast expression (Cereghino and Cregg, 2000) systems can produce functional and complex proteins however; these immunotoxins are lethal to eukaryotic cells (Tran et al., 2013).

C. reinhardtii cells can be used to produce immunotoxins due to the presence of a large chloroplast (40-70% cell volume) that contains its own protein translation apparatus (Harris, 2008, Mayfield, 2013). Immunotoxins produced in the chloroplast are sequestered there and so are unable to target and inhibit the eukaryotic translational system which would otherwise cause cell death. An immunotoxin containing a single chain antibody (scFv) that targets the B-cell antigen CD22, fused to a ribosome inactivating protein (gelonin) was successfully expressed in *C. reinhardtii,* supporting its use as an expression system for immunotoxins (Tran et al., 2013). The development of economically viable bioprocesses based on this technology requires a suitable cultivation system that is both cost effective and able to comply with the relevant regulations and guidelines. This will most likely require use of a PBR of either a tubular or flat-panel design to provide a closed system and the potential to operate with minimal cost.

Of the three PBR designs discussed, tubular reactors have had the greatest success. The world's largest, closed microalgae cultivation system consists of approx. 500 km of glass tubes with a total volume of 600 m^3 (Schenk et al., 2008, Algomed, 2014). An important design consideration for PBRs is mixing which is necessary to prevent settlement of cells and fouling, ensure uniform exposure to light and nutrients,

prevent temperature gradients and enhance gas exchange (Wang et al., 2012). Mixing can be achieved by aeration, net flow, mechanical agitation or a combination of these, however; some microalgae cells are sensitive to hydrodynamic stress which leads to a reduction in growth and metabolic activity (Suh and Lee, 2003, Vunjak-Novakovic et al., 2005). Cell fragility has been reported as a key problem for the mass cultivation of microalgae in closed systems (Gudin and Chaumont, 1991). In particular, the photosynthetic activity (PA) of *C. reinhardtii* cells cultivated in a fermenter-type PBR decreased by approx. 15% with an increase in the impeller tip speed from 126 to 589 cm/s (Leupold et al., 2013).

The oscillatory baffled reactor (OBR) is a novel, tubular device that has demonstrated several key advantages over conventional tubular and fermenter-type reactors. A 'standard' OBR design consists of a tube, generally 10 to 150 mm internal diameter (D), with a piston located at one end to generate oscillatory motion of internal material. Vortices form down the length of the OBR as material is forced through periodically spaced orifice plates (baffles). The advantages of this agitation type include uniform mixing at low shear (Ni et al., 2000), enhanced mass (Hewgill et al., 1993, Ni et al., 1995) and heat (Mackley and Stonestreet, 1995) transfer, the possibility of linear scale-up (Smith and Mackley, 2006, Smith, 1999) and power efficient agitation (Abbott et al., 2014b, Jambi et al., 2013). The mixing intensity is also decoupled from the net flow rate which enables plug flow to be achieved in substantially shorter reactors compared to conventional tubular designs where high net flows are required for turbulence (Stonestreet and van der Veeken, 1999, van Vliet et al., 2005, Stonestreet and Harvey, 2002, Abbott et al., 2014a). Numerous bioprocesses have been developed using OBR technology with mixed success (Abbott et al., 2013). This ranges from a 7% increase in glucose production for the enzymatic saccharification of cellulose (Ikwebe and Harvey, 2011) to a 56% increase in biomass for the culture of the aerobic bacterium *Pseudomonas putida* (Troeger and Harvey, 2009). These studies and the advantages offered suggest potential for the use of OBR technology as a PBR for the cultivation of microalgae.

This study presents results from the cultivation of *C. reinhardtii* cells in a lab scale (700 mL) OBR, modified for use as a PBR. There is no reported use of OBR technology for microalgae cultivation so this study provides essential information that may enable the development of economically viable cultivation systems for

production of various sustainable products, especially therapeutic proteins. There are four objectives:

1) Test the feasibility of OBR technology for use as a PBR in microalgae cultivation, specifically *C. reinhardtii*.
2) Determine the effects of two different sparger designs.
3) Compare the maximum growth rates achieved in the OBR to control cultures conducted in T-flasks.
4) Quantify the effect mixing intensity in the OBR has on the maximum growth rate achieved.

5.3 Materials and methods

5.3.1 Reactor operation and design

The mixing intensity for an OBR is controlled by adjustments to the amplitude and frequency of oscillation. The oscillatory Reynolds (Re_o) and Strouhal (St) numbers are dimensionless groups used to measure the mixing intensity and degree of vortex propagation, respectively. These are defined by equations 5.1 and 5.2, below.

$$Re_o = \frac{\rho 2\pi f X_o D}{\mu} \qquad \text{Eq. 5.1}$$

$$St = \frac{D}{4\pi X_o} \qquad \text{Eq. 5.2}$$

Where, ρ is the fluid density (kg/m^3), f and X_o, the frequency (Hz) and centre-to-peak amplitude (m) of oscillation, respectively, D, the tube diameter (m) and μ, the dynamic fluid viscosity (Pa s). ρ and μ are assumed to be that of water for the remainder of this study (1000 kg/m3 and 0.001 Pa s, respectively). The net flow Reynolds number (Re_n) is a measure of net flow through the reactor but is not relevant for batch operation.

The geometrical parameters associated with OBR design are the baffle thickness (δ), spacing (L), orifice diameter (D_o) and open area (α), where, $\alpha = (D_o/D)^2$. A ‘standard’ OBR design is such that L = 1.5D and α = 22%. More detail on this geometry can be found elsewhere (Ni et al., 1998).

A ‘standard’, vertical 700 mL batch OBR shown in Figure 1 was constructed from two 0.5 m (D=25 mm), jacketed, borosilicate glass columns (QVF, PS25/500) coupled

directly together and containing two stainless steel shafts that connected equally spaced baffles (L=37.5 mm, D_o=12.5 mm). Two stainless steel collars were fixed to each end of the resulting 1.0 m column to which a capsule filter (Pall, Novasip^{TM}) and vent line at the top, and a four-way cross piece (QVF, PX15) via a reducer (QVF, PR25/15) at the bottom were connected. The oscillating piston and feed lines were connected to the horizontal ends of the cross piece (D=15 mm) via peristaltic and stainless steel tubing, respectively. Two stainless steel shafts connected smaller, equally spaced baffles (L=22.5 mm, D_o=7.5 mm) in the cross piece in horizontal and vertical directions. A steam line connected a steam generator to the oscillating pump housing. Three sanitary diaphragm valves (Gemu, type 601) were placed on the feed, vent and steam lines. Temperature and pH were measured by a probe (Mettler Toledo, InPro 3250) inserted through the bottom steel collar and connected to a digital display (Mettler Toledo, M300). Temperature was controlled by a water bath (Huber) connected to the jackets surrounding both columns. Pressure was monitored with two pressure gauges (Parker, G63-102BG) placed before and after the filter.

5.3.2 Light setup

Two 0.55 m LED strip lights were connected to form one 1.10 m light bank and attached vertically to the OBR so the diodes were ~100 mm from the column. The top strip emitted warm white light (Leyton Lighting, SL-LED-550-WW) and the bottom, cool white light (Leyton Lighting, SL-LED-550). A second, identical bank was inverted and attached in a similar manner so it faced the column at ~90 degrees from the first bank. The light intensity was measured using a photoradiometer (Delta Ohm, HD2102.2) at 12 separate locations on the OBR surface, with an average intensity of 78 µmol/m^{2}s (+/- 20). Figure 5.1 shows a diagrammatical representation of the reactor setup.

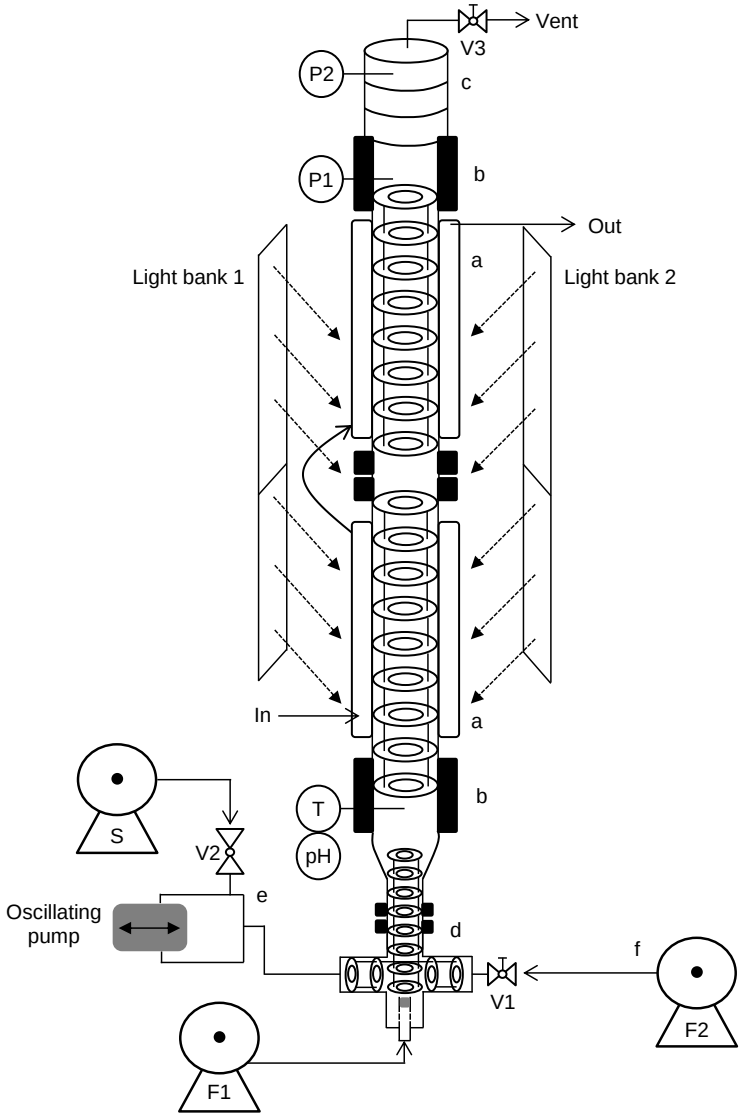

Figure 5.1: A diagrammatical representation of the OBR setup used for this study. a are the glass columns, b, the steel collars, c, the filter, d, the four-way cross piece, e, the oscillating line, f, the feed line, P1 and P2, pressure gauges, F1 and F2, the air and feed peristaltic pumps, respectively, T/pH, the temperature and pH probe, S, the steam generator, In/Out, the water jacket inlet and outlet, respectively and V1, V2 and V3, diaphragm valves on the feed, steam and vent lines, respectively.

5.3.3 Sparger design

A section of peristaltic tubing (Masterflex, size 15), the end of which was plugged with a solid, plastic rod and the first 20 mm punctured approx. 15 times with a hypodermic needle, was used as the gas sparger for all cultures and designed to produce large, fast-rising bubbles. The sparger was inserted through the vertical end of the cross piece and a peristaltic pump (Cole Palmer, Model 77200-62) used to pump air through at 35 mL/min (0.05 vvm). A porous gas sparger (Mott, type 6400) designed to produce small, slow-rising bubbles to maximise mass transfer was tested. However, the porous sparger was not suitable for microalgae culture in the OBR due to generation of a flotation effect (see §5.4.1). Figure 5.2a shows a

photograph of the porous sparger tested and Figure 5.2b, the sparger used for all cultures.

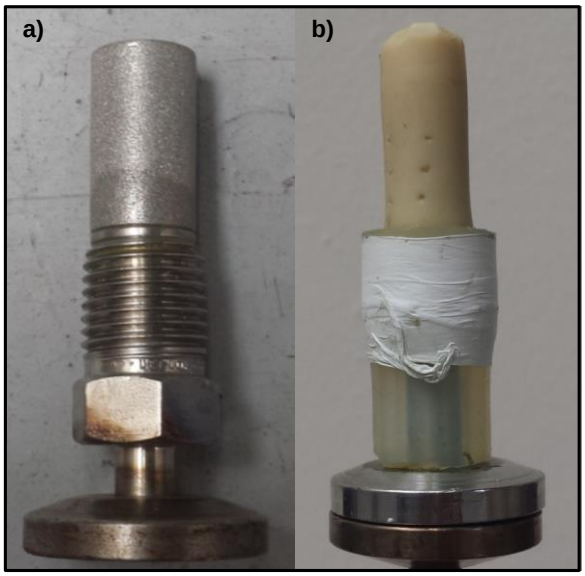

Figure 5.2: The a) porous gas sparger tested and b) punctured peristaltic tubing used as the sparger for all cultures in this study.

5.3.4 Culture conditions and procedure

Chlamydomonas reinhardtii CCAP 11/32C was ordered from the Culture Collection of Algae and Protozoa in two, 10 mL tubes containing liquid culture. Two streak plates were made from each tube on potato dextrose (PDA) and tryptone soya agar (TSA) and left to incubate at 28^{o}C (+/-1) in a cooled incubator (LMS, model 600) illuminated with strip lights (Sylvania, FHO39W/865) that emitted in the colour temperature of 600k. Healthy growth was observed with no contamination after 7 days. A 50mL T-flask (Greiner, Cellstar® T-25) containing 30 mL of Sueoka's high salt (HS) medium (Sueoka, 1960) was inoculated with one colony from a TSA plate. The T-flask was placed inside the cooled incubator at a location where the light intensity was ~80 µmol/m^{2}s and on a shaker set to 40 rpm (IKA®, KS260 Basic). The culture was grown for 7 days and used as inoculum for experiments in the OBR. Table 5.1 shows the compounds and their relative amounts required to make the three solutions used in the medium preparation, full instructions of which are available online (*Chlamydomonas* connection, 2011).

The OBR was steam sterilised before each run. Valves on the steam and vent lines were opened to allow steam to flow through the system. The vent line valve was slowly closed to increase the internal pressure to approx. 0.12 MPa (1.2 barg) creating a temperature above 120^{o}C which was held for 15 minutes. Condensate was

periodically released through the feed line valve. The steam generator was turned off, the steam and feed line valves closed and the vent line opened. The system was left to cool to room temperature. 1 L of medium was autoclaved in a Duran bottle and left to cool in the incubator before 30 mL was transferred to a fresh T-flask. The 7 day old culture was used to inoculate the Duran bottle and new flask to a target starting optical density at 750 nm (OD_{750}) of 0.05. Inoculated medium in the Duran bottle was pumped through the feed line and into the OBR to a volume of 700 mL via a peristaltic pump. The newly inoculated flask was placed on the shaker in the incubator to act as a control and inoculum for the next run. Temperature was controlled at 28^{o}C (+/-1) and the starting pH at 7.0, as recommended in the *C. reinhardtii* guidelines (Invitrogen, 2012). Growth was monitored with a spectrophotometer (Biochrom, WPAS800) by measuring the OD_{750} (Harris, 2008).

Table 5.1: Compounds and their relative amounts used to make the solutions required for Sueoka's HS medium.

Hutner's Trace Elements		**Salt Solution**		**Phosphate Solution**	
Compound	*Amount (g/L)*	*Compound*	*Amount (g/L)*	*Compound*	*Amount (g/L)*
$ZnSO_4.7H_2O$	220	NH_4Cl	100	KH_2PO_4	144
H_3BO_3	57	$MgSO_4.7H_2O$	4	K_2HPO_4	288
$MnCl_2.4H_2O$	101.2	$CaCl_2.2H_2O$	2		
$CoCl_2.6H_2O$	32.2	**Sueoka's High Salt Medium**			
$CuSO_4.5H_2O$	31.4	All solutions were made in distilled water. For the final medium, 5 mL salt and phosphate solutions and 1 mL Hutner's trace elements (Hutner et al., 1950) were added per litre of distilled water.			
$(NH_4)_6Mo_7O_{24}.4H_2O$	22				
$FeSO_4.7H_2O$	99.8				
$Na_2EDTA.2H_2O$	200				

5.3.5 Experimental design

The study aimed to assess, firstly, the feasibility to culture *C. reinhardtii* in an OBR and secondly, the effect mixing intensity had on the maximum growth rate (OD_{750}/day). The experimental design consisted of 8 separate runs in the OBR at duplicated Re_o values of 0, 300, 1000 and 2827 corresponding to no, light, moderate and intense mixing conditions. Runs in the OBR lasted for ~3 days and were conducted in parallel to a control that consisted of a T-flask in the incubator. The starting pH and OD_{750}, temperature and light intensity were kept constant in the OBR and control flasks at 7.0, 0.05, 28^{o}C (+/-1) and 80 µmol/m^{2}s (+/-20), respectively. OD_{750}/day was determined by identification of the steepest part of the curve for OD_{750} against time (over at least three data points). The maximum specific growth rate (µmax) was not used because cultures appeared not to achieve exponential growth

at any time. Table 5.2 summarises the mixing intensity used for each of the 8 runs in the study.

Table 5.2: The run order and corresponding mixing intensity (Re_o).

Run	Re_o	Run	Re_o	Run	Re_o
1	1000	4	300	7	2827
2	2827	5	0	8	0
3	1000	6	300		

5.4 Results and discussion

5.4.1 Flotation effects

Preliminary test cultures (results not shown) were setup in the OBR and aerated through the porous gas sparger (Figure 5.2a) with the aim to maximise gas transfer. Smaller bubbles increase the surface area available for gas transfer (Mott Corporation, 2014), which in the case of microalgae cultivation improves the transfer of CO_2 into and O_2 out of the culture. This provides Rubisco with sufficient CO_2 to fix into carbohydrate and remove inhibition caused by O_2 build up, ultimately driving net photosynthesis and maximising cellular growth. However, growth did not occur in these test cultures in the OBR over a period of several days. The OD_{750} remained close to the starting value before decreasing to zero. In comparison, the control test cultures in T-flasks demonstrated constant and linear growth which indicated that conditions in the OBR were not conducive to growth of *C. reinhardtii* cells (results not shown).

A systematic approach was used to identify the reason(s) for lack of growth in the OBR. The light system, medium, pH and temperature were all verified and shown to support photoautotrophic growth in T-flasks. The aeration system was tested by introducing into the OBR a higher cell density culture (OD_{750} approx. 0.4) that was grown in a Duran bottle. There was an obvious accumulation of cells at the top of the column after 3 days which can be seen in Figure 5.3. This accumulation was not observed in the test cultures due to much lower starting cell concentrations. Figure 5.3 clearly shows the accumulation of *C. reinhardtii* cells at the top of the column. This suggests the presence of a flotation effect whereby small particles accumulate at the top of the column due to adherence to rising air bubbles.

Anderson et al. (2009) demonstrated that OBR technology could be used as a flotation device which was tested with a quartz-amine system, with addition of a

frothing agent. The flotation rate constant was improved by up to 60% compared to a standard flotation column for fine particles (<30 µm). This improvement was attributed to a combination of an even distribution of shear and oscillatory motion of reactor contents (Anderson et al., 2009).

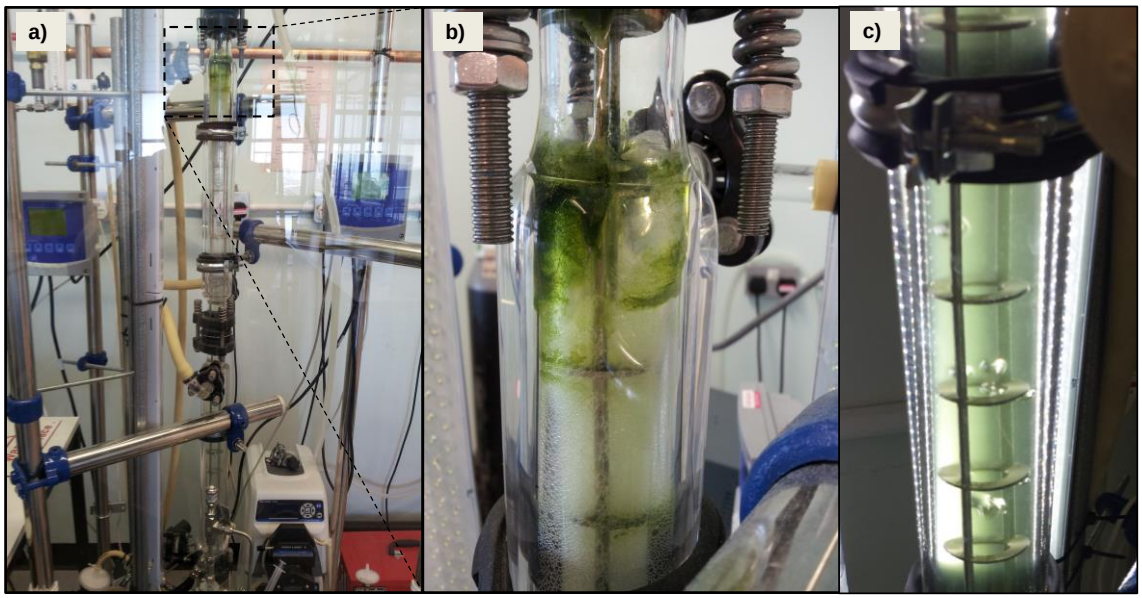

Figure 5.3: Photographs showing a) and b) *C. reinhardtii* accumulation at the top of the OBR during test cultures caused by flotation effects generated when using the porous gas sparger (Figure 5.2a) and c) removal of the flotation effect by use of a different sparger used for this study (Figure 5.2b).

A promising technology for the harvest of microalgae biomass is foam flotation systems. The efficacy of a foam harvester that combined dispersed air flotation with foam fractionation to allow harvesting, concentration, and physical separation of particles in suspension has been demonstrated. A foam flotation system for the harvest of microalgae biomass was used to demonstrate that variables which increased foam residence time produced the greatest concentration factors. In addition, the surfactant cetyl trimethylammonium bromide (CTB) was required to enhance foam flotation and stability (Coward et al., 2013).

Wild-type *C. reinhardtii* cells are approx. 10 µm in diameter (Harris, 2001). The observed accumulation of cells in Figure 5.3 indicates that small bubble diameters generated from the porous gas sparger combined with oscillatory motion, even shear distribution and the presence of 10 µm particles (cells) produced a flotation effect. This result is not surprising given previous studies that demonstrate OBR technology for use as a flotation device (Anderson et al., 2009) and the design of foam flotation systems (Coward et al., 2013).

The test cultures using the porous gas sparger demonstrated a flotation effect without addition of a frothing agent or surfactant suggesting OBR technology could be used to simultaneously culture and harvest microalgae cells through control of the bubble diameter. However, to achieve growth and culture microalgae using an OBR, the sparger was changed for this study to a design which generates larger, faster rising bubbles (Figure 5.2b) in order to remove the flotation effect.

5.4.2 Microalgae growth

Eight cultures of *C. reinhardtii* were setup in the OBR according to Table 5.2, in parallel to control cultures setup in T-flasks. Growth curves obtained for the OBR and control cultures are shown in Figures 5.4 and 5.5, respectively.

Figure 5.4 shows a lag period of approx. 24 hours for each of the growth curves obtained in the OBR. This lag phase is followed by a period of steady growth until the end of the experiment at approx. 72 hours. Cultures in the OBR were inoculated to a target starting OD_{750} of approx. 0.05 with *C. reinhardtii* cells grown in T-flasks. The conditions in the OBR were different to those in the T-flask in which inoculum was prepared. The presence of a lag phase in the OBR can be explained by the cells requiring a period in which to adjust to the new conditions as they moved from the inoculum environment (T-flask) to the OBR (Becker, 1995). This lag phase is not present in control cultures (Figure 5.5) because there were no changes in the environmental conditions between the inoculum and experimental cultures i.e. both were in T-flasks.

A common measure used to analyse growth of microorganisms is the specific growth rate (μ) or maximum specific growth rate (μ_{max}) which indicates the amount of biomass produced per biomass in the culture per unit of time. μ can also be calculated in terms of the doubling time (k) and is constant over the log (exponential) phase of growth which occurs when there are no environmental limitations present..

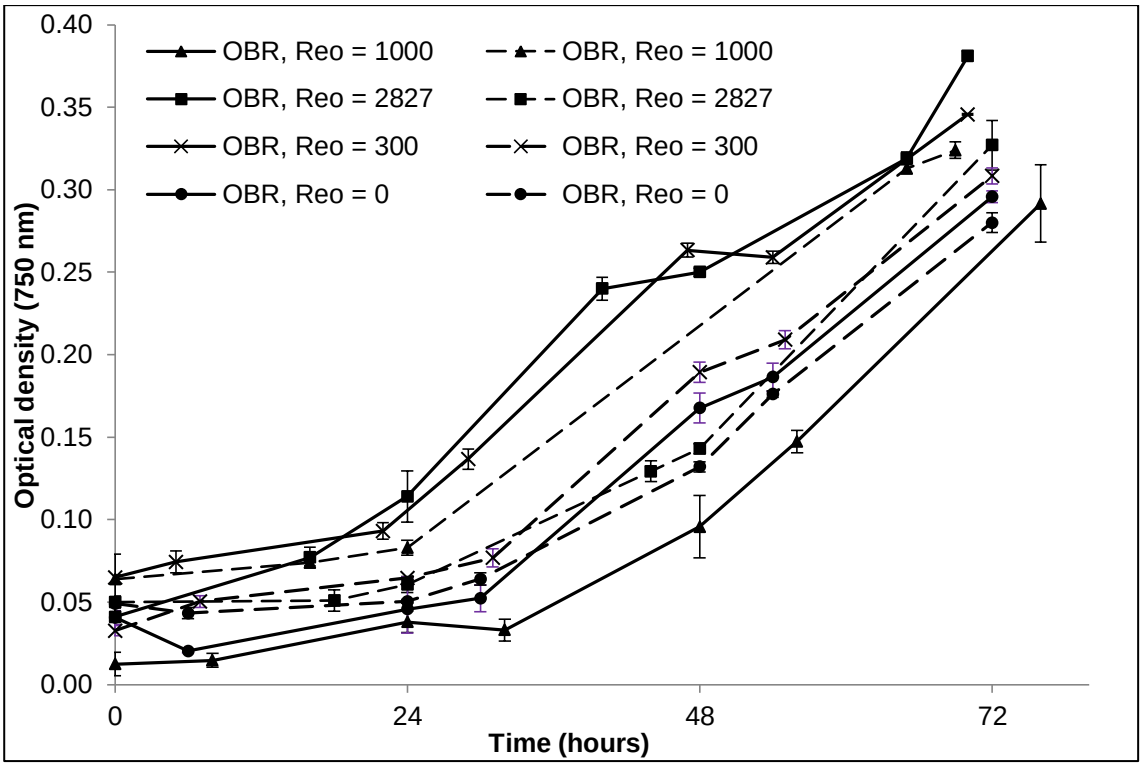

Figure 5.4: Cell concentration measured as optical density (OD_{750}) for the eight *C. reinhardtii* cultures in the OBR, conducted at mixing intensities identified in Table 5.2. Dashed and dotted lines represent duplicated cultures under identical conditions.

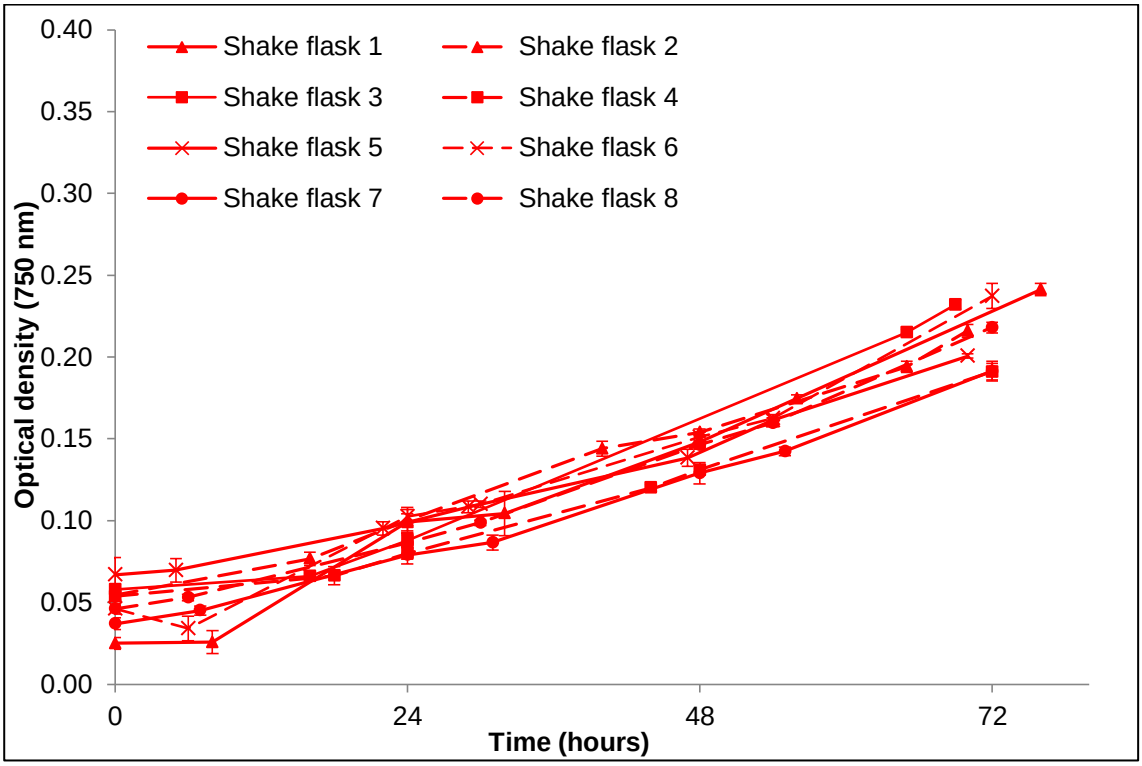

Figure 5.5: Cell concentration measured as optical density (OD_{750}) for the corresponding eight *C. reinhardtii* control cultures, all conducted under identical conditions.

It is imperative that μ is reserved to measure only exponential growth. μ can be calculated for linear growth (Devgoswami et al., 2011), however; the resultant value is almost meaningless and even miss-leading because it suggests exponential

growth when only linear growth is present. The use of μ values calculated from linear growth can result in the over prediction of potential biomass production which can be detrimental to researchers designing cultivation systems. Figures 5.4 and 5.5 show linear growth which suggests the cultures are limited in some way and therefore the use of μ is not appropriate. Therefore, growth has been analysed in terms of the growth rate which indicates the amount of biomass produced per unit of time. This can be given as OD_{750} per day (OD_{750}/day) as optical density at 750 nm is directly correlated to biomass concentration (Harris, 2008).

Figure 5.6 shows the maximum growth rates obtained in the eight control cultures and each of the duplicated OBR cultures at the four different mixing intensities summarised in Table 5.2.

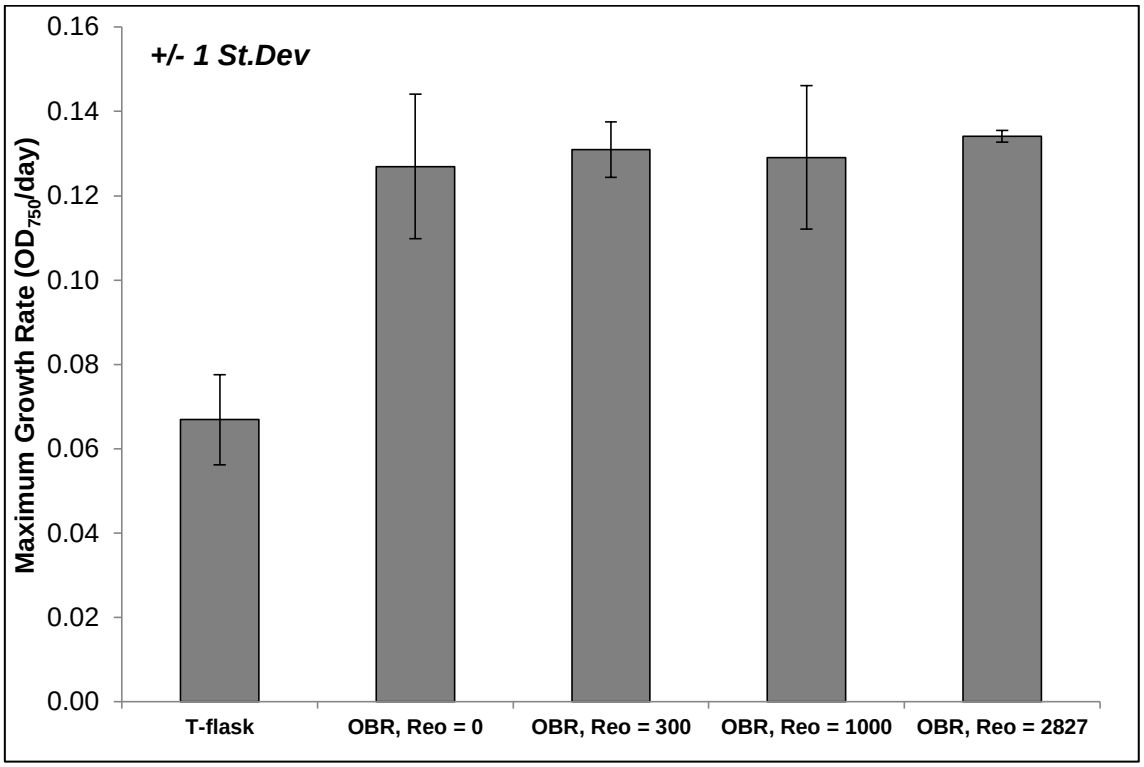

Figure 5.6: The maximum growth rates (OD_{750}/day) achieved for all eight control cultures (T-flask) and at each mixing intensity in the OBR. Errors bars represent +/- one standard deviation between the average maximum growth rates of eight control cultures and duplicate cultures in the OBR.

The average maximum growth rate achieved in control cultures was 0.067 (+/- 0.011) OD_{750}/day. This growth rate was approximately constant and linear in all control cultures over the 72 hours recorded. The presence of linear growth in these cultures suggests that growth is limited in some way. It is probable that the control cultures were CO_2 and/or light limited because no dedicated sparger was used and the light intensity was relatively low at approx. 80 μmol/m^2s (the light intensity in the OBR was

similar at approx. 78 µmol/m^2s). In general, photosynthesis is saturated at 100 to 500 µmol/m^2s and photoinhibition does not occur until irradiances over 1000 µmol/m^2s (Acién Fernández et al., 2013). However, to more accurately determine the limiting factor(s), further work is required.

The average maximum growth rates achieved in the OBR at Re_o values of 0, 300, 1000 and 2827 were 0.127 (+/- 0.017), 0.131 (+/- 0.007), 0.129 (+/- 0.017) and 0.134 (+/- 0.001) OD_{750}/day, respectively. The maximum growth rates achieved in the OBR were significantly higher compared to those obtained in T-flasks which indicate that the conditions in the OBR were more conducive to *C. reinhardtii* growth.

There was no significant difference between the maximum growth rates achieved in the OBR at different mixing intensities which indicates that the mixing intensity had no effect on the growth rate under these conditions. No negative effect on the growth rate was observed even at intense mixing conditions of Re_o = 2827, in contrast to impeller driven systems (Leupold et al., 2013). This indicates that intense mixing can occur in the OBR without impacting *C. reinhardtii* cells, a result of the low and even shear distribution generated in the OBR. This may be beneficial in cultivation systems with much higher cell concentrations where enhanced gas exchange is required.

The growth rates achieved in the OBR were combined to give an average value because the mixing intensity was shown to have no significant effect. The average maximum growth rates achieved in the control and OBR cultures were 0.067 (+/- 0.011) and 0.130 (+/- 0.010) OD_{750}/day, respectively. The OBR was able to generate a 95% increase in the maximum growth rate for *C. reinhardtii* cultures compared to control cultures under comparable conditions. The main differences between the control and OBR cultures were the presence of mixing and sparging in the OBR. As noted before, the mixing intensity was shown to have no significant effect on the growth rate under these conditions in the OBR. Therefore, the increase in growth observed in the OBR compared to the T-flask is most likely due to enhanced gas transfer provided by the sparging device. However, linear growth was observed in all cultures which suggests limitation.

5.4.3 Light limitation

The compensation irradiance point (I_c) is the depth at which light intensity becomes too low to support net photosynthesis. The culture depth (I_x) at which I_c occurs is defined by equation 5.3 (Nag-Jong et al., 2002).

$$I_x = \frac{\mathrm{Log}(\frac{I_0}{I_c})}{\alpha . X} \qquad \text{Eq. 5.3}$$

Where, I_x is the light penetration depth at which I_c occurs (cm), I_0, the incident light intensity (W/m^2), I_c, the compensation irradiance point (W/m^2), α, the specific light absorption coefficient (cm^2/cell) and X, the cell concentration (cells/mL).

OD_{750} was correlated to the cell number (cells/mL) using a Hawksley (improved Neubauer) double cell haemocytometer. 1.0 OD_{750} was shown to correlate to 2.57 x 10^6 cells/mL. α was calculated as 6.13 x 10^{-8} cm^2/cell, in close agreement with a previous study where α was reported as 4.33 x 10^{-8} cm^2/cell for *Chlorella kessleri* (Lee, 1999), and I_0 was determined as 21.8 W/m^2. I_c for *C. reinhardtii* has been calculated at 60 foot candles or 1.91 W/m^2 (Sorokin and Krauss, 1958). Figure 5.7 shows on log axes the calculated I_x values for a range of *C. reinhardtii* cell concentrations and I_0 values from 5 to 2000 W/m^2.

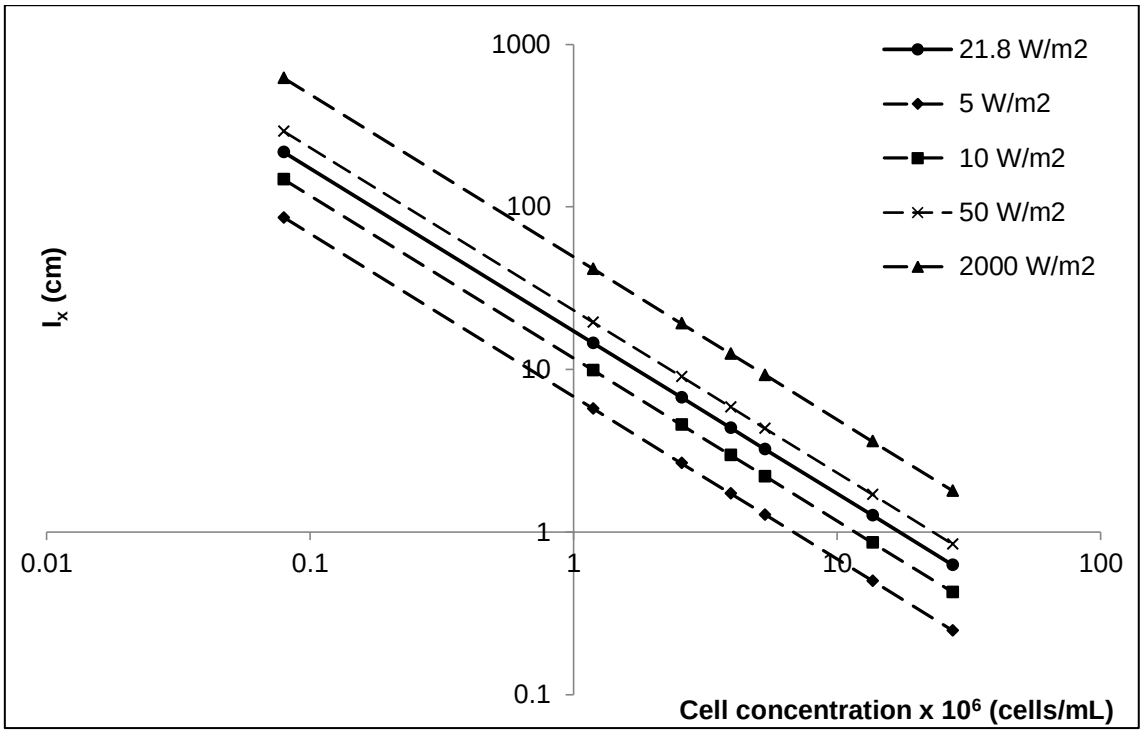

Figure 5.7: I_x against *C. reinhardtii* cell concentration (cells/mL) for five different light intensities (I_0) from 5 to 2000 W/m^2. The I_0 used in this study was 21.8 W/m^2.

The highest OD_{750} achieved in this study was approx. 0.40 which correlates to a cell concentration of approx. 1.0 x 10^6 cells/mL. This corresponds to the y-axis in Figure 5.7 which indicates that at this cell concentration and I_0 (21.8 $W.m^2$), the light intensity only becomes too low to support net photosynthesis at approx. 10 cm. The internal diameter of the OBR used in this study was 2.5 cm therefore I_x was not reached and the cells were not light limited. However, although light intensity was sufficient to support net photosynthesis, it appears to have been insufficient to support maximum growth, hence linear growth was observed.

These results suggest that growth was both CO_2 and light limited in T-flasks. Dedicated sparging in the OBR removed CO_2 limitation thereby increasing the average maximum growth rate by 95%, however; it appears that the relatively low I_0 could not support maximum cellular growth resulting in linear growth in the OBR.

5.5 Conclusions and future work

This study has demonstrated for the first time the feasibility of using OBR technology as a cultivation system for microalgae, and specifically *C. reinhardtii*. The tubular design results in a large surface area to volume ratio providing efficient light harvesting which could reduce operational costs associated with the cultivation of microalgae. Furthermore, OBR technology offers a closed system which enables compliance with the necessary regulations and guidelines associated with GMO use and API manufacture.

An unexpected result was a flotation effect without the need for addition of a frothing agent or surfactant. The potential exists to develop a dual culture and harvesting device through control of the bubble diameter. This could greatly improve process economics for production of microalgae derived products by removal of the de-watering step which is currently a major obstacle in this area.

The OBR demonstrated a 95% increase in the average maximum growth rate compared to control cultures in T-flasks. However, linear growth was still observed in the OBR so further work is required to achieve exponential growth and higher cell concentrations where the efficient mixing and enhanced gas transfer provided by this technology may confer even greater benefits to the culture of microalgae.

5.6 Research implications

The results generated from this study demonstrated that OBR technology could be modified into PBRs suitable for the photoautotrophic growth of microalgae cultures. Work was planned to conduct a follow on study that aimed to grow higher cell density cultures and operate the OBR as a dual culture and harvest device for microalgae. This could greatly improve process economics due to removal/reduction of the expensive and time consuming dewatering step present in these systems. In addition, the efficient mixing under low shear could improve growth rates and cell densities obtained if conducted over longer time periods; mixing had actually been shown to have no significant effect on growth rates - probably a result of the low cell densities and light intensities used.

CPI were successful with a funding bid to InnovateUK (formerly TSB) which I had helped draft in 2012. Circumstances required someone with OBR expertise to take technical lead on the 12 month project which aimed to test a pilot scale OBR for anaerobic digestion (AD). Results from the study would inform CPI with regards to the commercial potential of this application for OBR technology as well as help with their intellectual property (IP) management in terms of a patent application (Cooper et al., 2009). Research into microalgae was therefore postponed and focus was given to AD which forms the final study in this thesis.

Chapter 6: Anaerobic digestion

'Anaerobic co-digestion of dairy slurry and glycerol: A pilot (40 L) study to compare digesters based on novel oscillatory baffled (OBR) and conventional stirred tank (STR) reactor technologies.'

6.1. Abstract

Anaerobic digestion (AD) of dairy slurry (DS) and co-digestion with glycerol was conducted in two pilot scale (40 L) digesters based on oscillatory baffled (OBR) and stirred tank (STR) reactor technologies. The OBR was unable to process raw DS due to particulate accumulation and blockages in the 'u-bends'. Centrifuged DS (cDS) with reduced solids content was used for the majority of the study to prevent the formation of blockages in the OBR. The OBR with continuous agitation produced 43% more biogas compared to the STR with intermittent agitation for digestion of cDS. Addition of 1.4% glycerol to cDS resulted in destabilisation in the STR which required a reduction in the feed rate and continuous agitation to prevent complete process collapse. The OBR recovered from the shock load of glycerol addition due to the presence of a 'buffer zone' near the feed inlet which enabled maintenance of a higher feed rate. Both digesters showed ~270% increase in methane production with addition of 1.4% glycerol to cDS. Methane yields were maximised in the OBR for $3220<Re_o<6440$ and the STR for ~80 rpm which corresponded to power densities (P/Vs) in Watts per cubic meter (W/m^3) of $23<P/V<190$ and ~20, respectively. The STR showed signs of destabilisation at P/V=150 W/m^3 which suggested floc disruption due to increased shear rates near the impeller. The optimum organic loading rate (OLR) to maximise the specific methane yield (SMY) was ~4.3 kg COD/m^3 day in both digesters. This OLR produced maximum SMYs of 0.51 and 0.40 $m^3/kg\ VS_{added}$ in the OBR and STR, respectively. SMYs were similar in both digesters for the majority of conditions tested. Variation in feed quality throughout the long study period caused fluctuations in the SMY and added an additional uncontrolled variable. Power consumption required for temperature control was 89% less in the STR compared to the OBR as a result of an 82% reduction in the surface area available for heat loss. The actual power consumption for agitation was significantly higher in both digesters compared to theoretical values which indicated the difficulty of achieving the calculated efficiencies as well as the need for adequate equipment specification. These results demonstrate the potential of using OBR technology for

AD, however; the design needs to be carefully considered before commercial adoption occurs.

6.2 Introduction

6.2.1 Anaerobic digestion

Anaerobic digestion (AD) is a fermentation process by which mixed communities of microorganisms grow and breakdown biodegradable material in the complete absence of oxygen. Material suitable for AD can be derived from a variety of sources which include but are not limited to forestry and agricultural crops and residues (Sawatdeenarunat et al., 2014), sewage (Astals et al., 2013), algae (Ward et al., 2014) municipal solid (Cecchi et al., 1991, Chen et al., 1990) and industrial (Meyer and Edwards, 2014) wastes. Fermentation occurs in the second of four main stages of AD and methane is the final product of the reaction pathway: the key processes are hydrolysis, acidogenesis, acetogenesis and methanogenesis.

Proteins, fats and carbohydrate molecules such as cellulose and starch are initially hydrolysed to amino acids, long chain fatty acids and sugars. These are converted to volatile fatty acids (VFA) in acidogenesis, predominantly lactic, propionic, butyric and valeric acids. Mixed communities of microorganisms metabolise the VFAs produced to generate acetic acid, carbon dioxide (CO_2) and hydrogen which are then metabolised by methanogenic microorganisms to produce methane (Abbasi et al., 2011). The resultant biogas produced through AD comprises 40 to 70% methane as well as carbon dioxide and other trace gases, and can be used as a sustainable fuel.

6.2.2 Environmental considerations and sustainable/renewable energy

The EU energy directive requires that 20% of the bloc's final energy consumption should be produced from renewable sources by 2020, with the UK given a target of 15% (European Parliament - Council of the European Union, 2009). In addition, the UK's climate change act requires greenhouse gas emission reductions of 80% by 2050 compared to 1990 levels (UK Parliament, 2008). There were 213 AD plants in the UK in August 2011 treating wet sewage, food and agricultural waste that represented 151 MW of electricity generating capacity (Allen and Wentworth, 2011). In comparison, Germany had approximately 6,800 AD plants. The use of AD in combination with other renewable energy technologies could help the UK reduce

greenhouse gas emissions and meet the above-mentioned targets, a major factor contributing to the UK government's commitment to an increase in energy production from AD (Department for Environment Food and Rural Affairs, 2011).

Biodiesel is another renewable energy technology which could contribute to reductions in greenhouse gas emissions and has been produced at industrial scales in Europe by transesterifying lipid feedstocks with alcohol (Astals et al., 2011). Over 8,000,000 tonnes of biodiesel were produced in Europe in 2011 (European Biodiesel Board, 2011) with the main by-product being crude glycerol. The current market demand for glycerol is unable to absorb the increase in supply from biodiesel production (Johnson and Taconi, 2007). Furthermore, treatment of crude glycerol is prohibitively expensive for many plants (Pachauri and He, 2006) and few direct uses exist (Pagliaro and Rossi, 2008). This has led to the disposal of crude glycerol as waste in many regions.

6.2.3 Co-digestion

One potential source of biodegradable material for biogas production through AD is animal slurry and specifically dairy slurry. However, typical methane yields obtainable from the AD of dairy slurry are relatively low and range from 10 to 20 m^3/tonne slurry (Angelidaki and Ellegaard, 2003). This low methane yield can render farm-scale plants uneconomical where significant capital costs are required (Cavinato et al., 2010). Nevertheless, dairy slurry is an excellent carrier material for more concentrated wastes due to its high water content, buffering capacity and nutrient content, all required for optimal microorganism growth (Angelidaki and Ellegaard, 2003).

Crude glycerol produced from biodiesel production contains predominantly glycerol, alcohol, water and small quantities of other materials including heavy metals (Astals et al., 2012). The precise composition of crude glycerol depends on the substrate and process utilised for biodiesel production. Glycerol consists of carbon, hydrogen and oxygen ($C_3H_8O_3$) and is therefore classed as a carbohydrate which can be used to increase the carbon content available to microorganisms during AD. However, the optimum carbon to nitrogen (C/N) ratio for AD is 20-30. Elevated C/N ratios lead to rapid nitrogen consumption by methanogens to meet their protein requirements,

followed by a repression of biogas production (Abbasi et al., 2011). The amount of glycerol added must therefore maintain a suitable C/N ratio.

Co-digestion involves the combination of biodegradable material from different sources. Biogas production through AD has been demonstrated for the co-digestion of dairy slurry supplemented with crude glycerol from biodiesel production. Methane yields of 0.299 (Castrillón et al., 2011) and 0.590 (Castrillón et al., 2013) m^3 methane/kg volatile solids (VS) have been achieved when supplementing dairy slurry with 6% crude glycerol. In comparison, AD of pure dairy slurry produced methane yields of 0.15 to 0.19 m^3 methane/kg VS (Castrillón et al., 2011, Amon et al., 2007). Glycerol supplementation therefore increased methane yields by up to 293% which greatly improves the economics of farm-scale AD plants. This could promote and enable development of AD plants which would not only increase production of sustainable energy in the form of biogas, but also provide a use for the increased amounts of crude glycerol from biodiesel production.

6.2.4 Digester design and agitation

Simple digester designs for AD typically consist of a large vessel with a feed inlet, digestate outlet and biogas collector. The hydraulic residence time (HRT) can be used to estimate the average time feed spends in the digester and is derived from the digester volume and volumetric flow rate, as shown in equation 6.1.

$$\text{HRT (days)} = \frac{V}{Q} \qquad \text{Eq. 6.1}$$

Where, V is the digester volume (L) and Q, the volmetric flow rate (L/day).

These simple digesters are common throughout India, China and the developing world as well as in the UK within the agricultural sector due to their simplicity, however; they are poorly agitated and require long HRTs of up to 50 days (Abbasi et al., 2011). This must be reduced in order to increase biogas productivity and improve process economics without impacting overall conversion. Research over the past few decades has aimed to achieve decreased HRTs while maintaining high solids retention times (SRTs), where ‘solids’ refers to microorganisms. This results in low feed to microorganism (F/M) ratios which increase digestion rates and biogas productivity. Common methods employed to retain microorganisms include 1) formation of sludge aggregates e.g. upflow anaerobic sludge blanket (UASB)

digesters, 2) use of films for microorganism adhesion e.g. fluidised bed reactors (FBR) and 3) retention of sludge aggregates between packing material e.g. downflow anaerobic filters (Rajeshwari et al., 2000).

Agitation is important during AD to prevent the formation of a floating layer of solids and to encourage distribution of enzymes and microorganisms (Chapman, 1989, Parkin and Owen, 1986, Lema et al., 1991). However, numerous studies exist that report conflicting effects of agitation during AD.

A floating layer of solids was observed in a non-agitated digester which exhibited a higher methane yield than a continuously agitated digester. This was attributed to a longer SRT (Chen et al., 1990). Switching from continuous to intermittent agitation (2 min agitation/hour) produced significantly more biogas during AD of a liquid waste stream which was attributed to increased bioflocculation and solids retention (Dague et al., 1970). Operational problems were experienced with a high total solids loading caused by accumulation of a scum layer of cellulosic fibre at the surface which interfered with mechanical agitators (Stroot et al., 2001). Satisfactory performance at low agitation was observed in one study that concluded low agitation was preferable due to reduced energy requirements (Rivard et al., 1990).

Little or no agitation may improve high solids AD by providing an immobile environment for microorganisms. However, agitation is required to mix in feed and form new spatial associations among different microbial populations (Lettinga, 1981, Stroot et al., 2001). This is particularly relevant for co-digestion where a concentrated feed is added which must be properly mixed throughout the digester material to prevent formation of concentration gradients. Agitation is also required to ensure single cells do not remain isolated and surrounded by their own progeny which results in reduced kinetic effectiveness (Schink and Stams, 2006). However, rapid agitation can disrupt floc structures and disturb syntrophic relationships between microorganisms (Whitmore et al., 1987). In vigorously agitated systems, spacial associations can be continuously disrupted causing instability. During AD of the organic fraction of municipal solid waste, continuous agitation was detrimental to the process as a result of instability which stabilised when agitation intensities were reduced (Stroot et al., 2001).

Evidence from the literature therefore suggests that agitation is required to generate homogeneity within the digester and promote syntrophic relationships between microorganisms. However, the agitation intensity must not be so high as to disrupt floc formation and also be achieved in an energy efficient manner.

6.2.5 Oscillatory baffled reactor (OBR) technology

Oscillatory baffled reactors (OBRs) are novel, tubular devices that have demonstrated several key advantages over conventional tubular and stirred tank reactors (STRs). A 'standard' OBR design consists of a tube, generally 10-150 mm internal diameter (D), with a piston located at one end to generate oscillatory motion of internal material. Vortices form down the length of the OBR as material is forced through periodically spaced orifice plates (baffles). Agitation intensity is controlled by adjustments to the amplitude (X_o) and frequency (f) of oscillation. The oscillatory Reynolds (Re_o) and Strouhal (St) numbers are dimensionless groups used to measure the level of agitation and degree of vortex propagation, respectively. These are defined by equations 6.2 and 6.3, respectively.

$$Re_o = \frac{\rho 2\pi f X_o D}{\mu} \qquad \text{Eq. 6.2}$$

$$St = \frac{D}{4\pi X_o} \qquad \text{Eq. 6.3}$$

Where, ρ is the fluid density (kg/m^3), f and X_o, the frequency (Hz) and centre-to-peak amplitude (m) of oscillation, respectively, D, the tube diameter (m) and μ, the dynamic fluid viscosity (Pa s).

The net flow Reynolds number (Re_n) which is defined by equation 6.4 is a dimensionless group used to measure net flow and is relevant for continuous operation when using OBR technology.

$$Re_n = \frac{\rho D u}{\mu} \qquad \text{Eq. 6.4}$$

Where, u is the superficial fluid velocity (m/s).

The geometrical parameters associated with OBR design are the baffle thickness (δ), spacing (L), orifice diameter (D_o) and open area (α), where, $\alpha = (D_o/D)^2$. A 'standard' OBR design is such that L = 1.5D and α = 22%. More detail on this geometry can be found elsewhere (Ni et al., 1998, Brunold et al., 1989).

Agitation performed in this manner confers several key advantages to OBR technology, which include uniform mixing at low shear (Ni et al., 2000), enhanced mass (Hewgill et al., 1993, Ni et al., 1995) and heat (Mackley and Stonestreet, 1995) transfer, the possibility of linear scale up (Smith and Mackley, 2006, Smith, 1999) and power efficient agitation (Abbott et al., 2014b, Jambi et al., 2013). Furthermore, agitation intensity is decoupled from the net flow rate which can enable development of processes under plug flow conditions in substantially shorter reactors compared to conventional tubular designs where high net flows are required for turbulence (van Vliet et al., 2005, Stonestreet and van der Veeken, 1999, Stonestreet and Harvey, 2002). However, long reactor lengths are still required to achieve plug flow for long residence time processes, such as AD (Abbott et al., 2014a).

These advantages (specifically low shear and power efficiency) could provide a level of agitation that generates homogeneity to promote syntrophic relationships between microorganisms without floc disruption. Uniform and efficient agitation would also ensure concentrated feeds such as glycerol were dispersed throughout the digester material thereby preventing the formation of concentration gradients. These advantages conferred by OBR technology could increase digestion rates and therefore increase biogas productivities to result in process intensification of AD. Numerous bioprocesses in OBRs have been reported in the literature, which demonstrate the potential of this technology (Abbott et al., 2013). These include a 90% increase in solvent concentration compared to an STR for an anaerobic process (Masngut et al., 2006).

The present study is directed towards AD of dairy slurry and co-digestion with glycerol and aims to 1) test the feasibility of a novel digester design based on OBR technology; 2) compare its overall performance to a more conventional digester based on STR technology and 3) determine the effects of agitation and HRT on biogas production and quality in both digester designs. Co-digestion with glycerol was chosen to improve biogas production thereby providing an economically attractive process. STR technology was used as a comparison because of its wide application and the ability to accurately control agitation intensities. Both digesters were designed at pilot scale (40 L) and operated in a semi-continuous mode.

6.3 Materials and Methods

6.3.1 Digester designs

The novel digester based on OBR technology consisted of a jacketed stainless steel tube ~20 m in length and 50 mm internal diameter (D) with a working volume of ~40 L. 11 vertical columns were connected via circular bends to give 6 ‘n’ bends at the top and 5 ‘u’ bends at the bottom forming a serpentine shape. Gas-liquid separators (Spirax-Sarco) were connected at the top of each ‘n’ bend to allow gas to escape while retaining digester material. Drain valves (Arita, 1000 WOG) at the bottom of each ‘u’ bend allowed digestate samples to be taken if required.

A 3-way valve connected to an air operated, pneumatic actuator (Valbia, 52) was connected to the digester inlet and to the top and bottom of a 50 L feed tank. Feed was continuously pumped (Watson Marlow, 620U) from the bottom to the top of the feed tank (0.2 L/min) and periodically diverted into the digester via control of the 3-way valve (red lion). Digestate exited the digester at the opposite end to the inlet. Probes connected to controllers (Walchem, WDP410-52NU) were inserted through columns 2, 3, 5, 7, 9 and 11 to monitor and record pH and temperature. A water bath (Grant, GR150/R4) and motor (AO Smith) were used for temperature control (35.8^{o}C +/- 2.1) by pumping water through the jacketed column. An oscillating piston was connected to the digester before the feed inlet. Gas from each separator was combined into one line and passed through catch and bubble pots (Duran, GL45), a coalescing filter (micrafilter, MG102-2564) and a flow meter (Aalborg, GFM17) before being vented. Figure 6.1 shows a diagrammatical representation of the digester setup based on OBR technology.

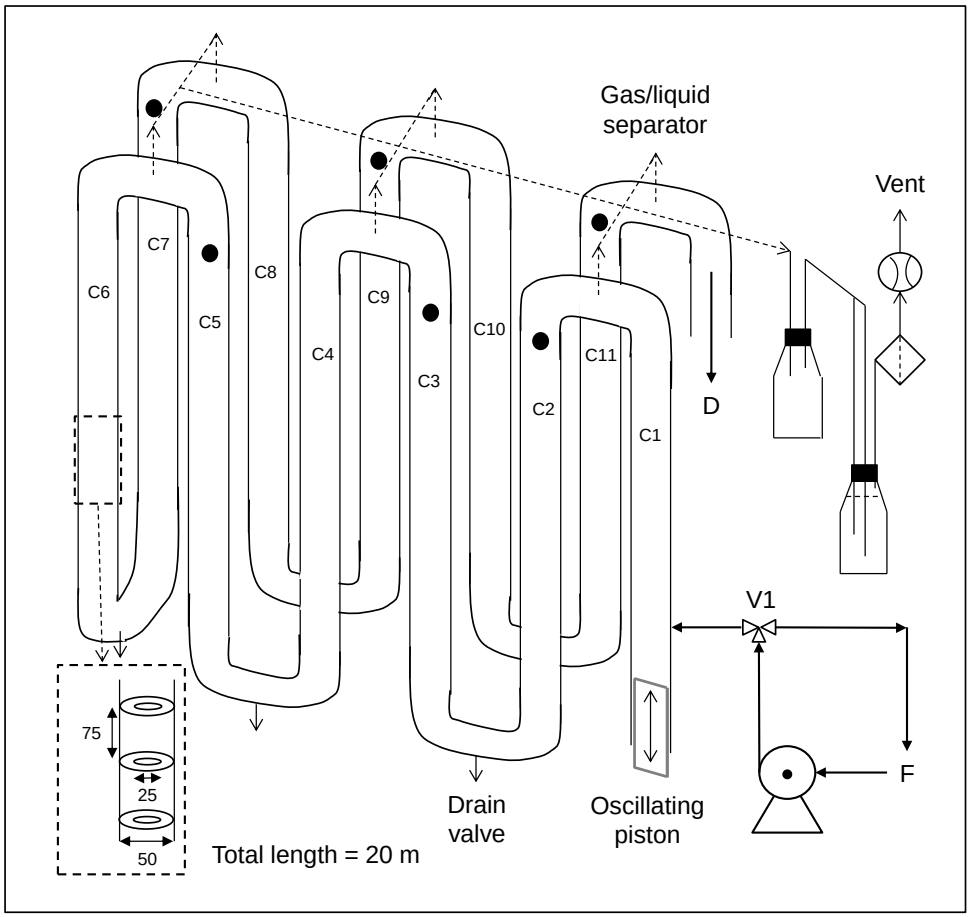

Figure 6.1: The digester setup based on OBR technology. Units in mm unless stated. V1 represents the 3-way valve, F, the feed tank and D, the digestate exit. The gas lines (dashed), probes (black circles), filter (diamond), flow meter (circle), bubble/catch pots and columns (C1-C11) are also shown.

The more conventional digester based on STR technology consisted of a stainless steel vessel 320 mm in diameter (D_v), also with a working volume of ~40 L when operated at a liquid height (L_h) of 490 mm. Agitation was achieved by three 120 mm diameter (D_s), 6-blade disc impellers connected to a motor, each with a power number (P_o) of 4.1 (Nienow and Miles, 1971). A probe (Mettler Toledo, InPro3250) connected to a controller (Mettler Toledo, M300) was inserted through the digester wall to monitor pH and temperature. A water bath (Grant, GR150/R4) was used for temperature control (35.6^{o}C +/- 0.5) by pumping water through an internal coil.

A peristaltic pump (Cole-Parmer) with two heads (easy-load II, 77200-62) was connected between the digester and 1) a 50 L stainless steel feed tank and 2) a collection vessel. The two lines (Masterflex, size 36) were setup to pump in opposite directions. The pump was programmed to periodically and simultaneously pump 1) new feed from the feed tank into the digester and 2) digestate from the digester into a collection vessel via a tundish. The top of the digester was sealed except for a tundish for digestate removal and two gas lines. Gas from one gas line was diverted

through a bubble pot (Duran, GL45), a coalescing filter (micrafilter, MG102-2564) and a flow meter (Aalborg, GFM17) before being vented. The other gas line acted as an overpressure release. Figure 6.2 shows a diagrammatical representation of the digester setup based on STR technology.

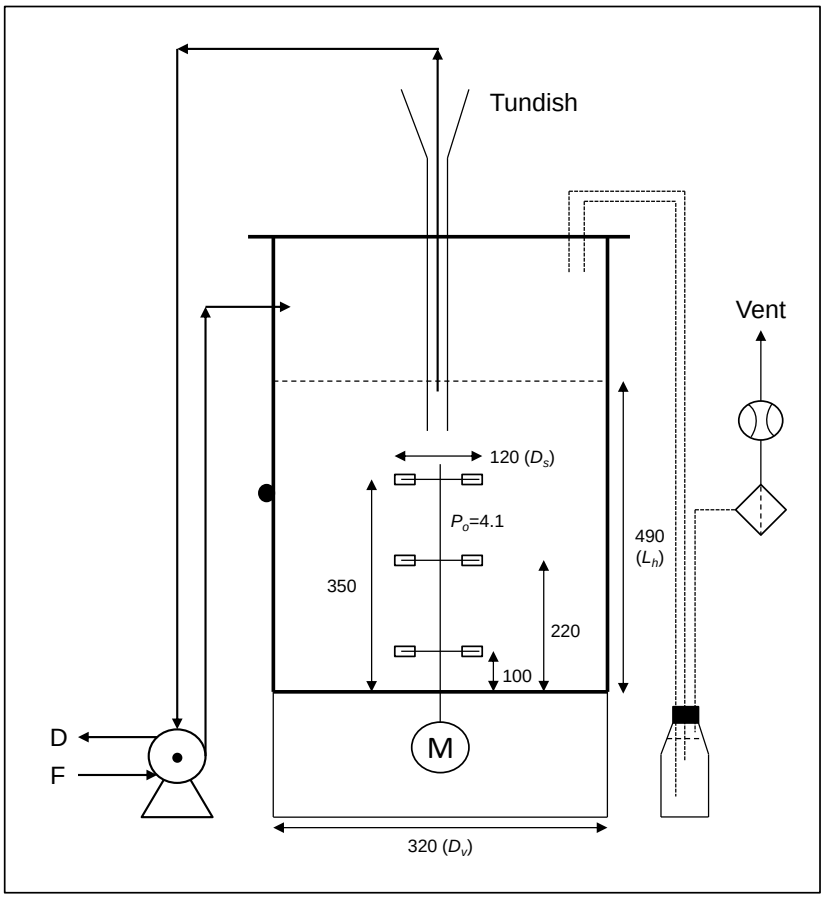

Figure 6.2: The digester setup based on STR technology. Units in mm. D represents the digestate exit, F, the feed tank and M, the impeller motor. The gas lines (dashed), probe (black circle), filter (diamond), flow meter (circle) and bubble pot are also shown.

Material was agitated in both feed tanks with a paddle impeller connected to a motor (Stuart Stirrer, SS30). Flow meters connected to both digester gas lines recorded the flow rate every minute. The gas flow data along with the pH and temperature data from the STR digester were logged on a data logger (dataTaker, DT80) and displayed on a laptop (IBM ThinkPad, R40) with appropriate software (DeLogger).

6.3.2 Digestate and gas analyses

Digestate was collected daily (where possible) from the exit lines of both digesters. Hach-Lange cuvette tests were used to determine the chemical oxygen demand (COD), soluble COD (sCOD), ammonium and organic acid (OA) concentrations. The cuvette test for OA specifically measured acetate, propanoate and both isomers of

butanoate. Instructions on how to use the cuvette tests are available online from the manufacturer (Hach-Lange, 2014) with brief details provided below.

Digestate was initially passed through a small, 1-2mm sieve to remove large particulates for all cuvette tests. COD concentrations were determined by pipetting the appropriate digestate volume into the cuvette (Hach Lange, LCK914). Ammonium concentrations were determined by diluting the digestate by 20 with distilled water and pipetting the appropriate volume into the cuvette (Hach Lange, LCK303). sCOD and OA concentrations were determined by centrifuging (Heraeus, Pico 21) 1 mL digestate at 10,000 g for 10 minutes, passing the supernatant through a 0.45 µm filter (Whatman, GD/X), diluting the filtrate by 4 with distilled water and pipetting the appropriate volumes into the cuvette for sCOD (Hach Lange, LCK014) or OA (Hach Lange, LCK365). All cuvettes were heated appropriately in a thermostat (Hach Lange, LT200) and left to cool before reading in a spectrophotometer (Hach Lange, DR3900). COD, ammonium, sCOD and OA concentrations were determined with errors of +/- 0.90, 0.08, 0.40 and 0.19 g/L, respectively.

A titrimetric method was used to determine total volatile organic acid (FOS) and inorganic carbonate (TAC) concentrations. Digestate was passed through the small 1-2mm sieve before syringing 30 mL into a glass beaker, adding 120 mL distilled water and placing on the automatic titrator (SI Analytics, TitroLine 6000). FOS and TAC concentrations were determined with an error of +/- 0.05 g/L.

Total solids (TS) were determined by adding ~30 mL digestate into pre-weighed ceramic crucibles, weighing on a lab balance (Sartorius, CPA3245), placing in an oven (memmert) at 100^{o}C for at least 24 hours, leaving to cool in a desiccator (Duran, DN300) and then weighing. Volatile solids (VS) were then determined by placing the crucibles in a chamber furnace (Carbolite) for at least 2 hours at 550^{o}C, before leaving to cool in a desiccator and reweighing. TS and VS were determined from recorded masses using equations 6.5 and 6.6, respectively, with VS expressed here as a percentage of the digestate and not a percentage of TS.

$$TS\ (\%) = \frac{M_o - M_c}{M_t - M_c} \times 100 \qquad \text{Eq. 6.5}$$

$$VS\ (\%) = \frac{M_o - M_f}{M_t - M_c} \times 100 \qquad \text{Eq. 6.6}$$

Where, M is mass (g) and the subscript letters c, t, o and f represent the crucible conditions: empty, with digestate (full), post-oven and post-furnace, respectively.

Gas samples were taken (daily where possible) from the gas lines on both digesters. Plastic, 3-way valves connected to the digester gas lines were opened and left for ~1 minute to prime the sample line with gas before collecting ~5 mL gas in a plastic syringe. A gas chromatograph (Shimadzu, GC-2014) with a 20 m long, 0.32 mm internal diameter mesh column (Hayesep, 60/80) and a thermal conductivity sensor (DTCD) set at 250^{o}C was used to determine methane (+/- 1.79%), carbon dioxide (+/- 2.97%), oxygen (+/- 9.26%), nitrogen (4.97%) and hydrogen (+/- 2.06%) concentrations using an argon gas carrier at 23 mL/min. A pulsed flame photometric detector (PFPD) set at 100^{o}C simultaneously determined hydrogen sulphide (+/- 14.91%) concentrations. The temperature profile was: hold at 45^{o}C for 4.5 min; ramp at 40^{o}C per min to 175^{o}C; and hold for 45 s.

6.3.3 Experimental design, calibration and calculations

Digesters were initially seeded with onsite dairy slurry digestate to ensure appropriate microorganisms were present for biogas production. Manual feeding of fresh dairy slurry in 2 L amounts occurred daily (where possible) to produce a HRT of ~20 days (conditions 0, Table 6.1). This continued for 20 days after which extensive blockages were seen in the bends of the OBR. In addition, gas flow data were unreliable due to variable times at which the digesters were fed. Dairy slurry was centrifuged to remove large particulates for the remainder of the study to prevent blockages. The feed regime was automated (see §6.3.1) to remove the variable feed times and generate more reliable data. Table 6.1 summarises the conditions used throughout the study.

Table 6.1: The conditions used throughout the study. Dairy slurry (DS), centrifuged dairy slurry (cDS), glycerol (G) and revolutions per minute (rpm). Time (days) represents the time in the study at which the step change occurred.

Condition	Feed material	HRT (days)		Agitation		Time (days)
		OBR	*STR*	*OBR (Re_o)*	*STR (rpm)*	
0	DS	20	20	1610	40 (2/30 mins)	N/A
1	cDS	10	10	1610	40 (2/30 mins)	0
2	cDS + G (1.4%)	10	20	1610	40	48.0
3	cDS + G (1.4%)	10	10	1610	40	69.0
4	cDS + G (1.4%)	10	10	3220	80	83.0
5	cDS + G (1.4%)	10	10	6440	160	92.0
6	cDS + G (1.4%)	6.7	6.7	3220	80	97.0
7	cDS + G (1.4%)	5	5	3220	80	105.0
8	cDS + G (1.4%)	4	4	3220	80	118.2
9	cDS + G (1.4%)	4	4	3220	80 (2/30 mins)	125.1

Conditions 0 and 1 aimed to test the feasibility of a novel digester design based on OBR technology for digestion of dairy slurry. The remaining conditions aimed to compare the digesters and determine the effects of agitation and HRT on biogas production and quality for co-digestion of dairy slurry and glycerol. Pure glycerol (70%) was added to dairy slurry at 2% v/v to give a total glycerol concentration of 1.4% for conditions 2 to 9. This relatively low pure glycerol concentration was used compared to 6% crude glycerol in previous work (Castrillón et al., 2013) to ensure appropriate C/N ratios, which should be 25-30:1 for optimal AD.

Gas flow data were used to calculate gas volumes produced over time for each digester. Flow rates were input into Excel and multiplied by the interval between readings (1 min) to give the volume produced each minute. This method was calibrated by sequentially connecting the gas lines from each digester to a tube bank in order to capture the evolved gas and directly measure the actual volume produced over time. The actual and calculated volumes were plotted against each other over a 21 day period. A linear relationship (R^2=0.99) was observed for both digesters with a coefficient of 0.57 required to convert the calculated volumes to the actual volumes. This coefficient was used for the remainder of the study to determine gas volumes and plot cumulative production over time for both digesters.

Power densities (P/V) are a measure of the amount of power per unit volume required to achieve agitation in vessels and can be expressed in Watts per cubic metre (W/m^3). P/V can be estimated for an unaerated reaction mixture in an STR using equation 6.7 (Holland and Chapman, 1966).

$$\frac{P}{V} = \frac{P_o \rho N^3 D_s^5}{\frac{1}{4}.\pi D_v^2 L_h} \quad \text{Eq. 6.7}$$

Where, P_o is the power number of the impeller (4.1), N, the impeller rotational speed (rps), D_s, the impeller diameter (m), D_v, the vessel diameter (m) and L_h, the height of liquid in the reactor (m).

P/V can be estimated for a 'standard' OBR design such as that used in this study using equation 6.8 (Hewgill et al., 1993).

$$\frac{P}{V} = \frac{2\rho N_B}{3\pi C_D^2} \frac{1-\alpha^2}{\alpha^2} X_o^3 (2\pi f)^3 \quad \text{Eq. 6.8}$$

Where, N_B is the number of baffles per unit length (m), C_D, the discharge coefficient and α, the reactor to orifice area ratio. The value of C_D was taken as 0.7, to be consistent with a previous study (Ni et al., 2000).

6.4 Results and discussion

6.4.1 Flow conditions

OBR technology has the potential to generate plug flow conditions when operated continuously, provided the oscillatory (Re_o) and bulk flow (Re_n) components result in a suitable velocity ratio (Ψ), where $\Psi = Re_o/Re_n$. A range of $1.8 < \Psi < 2.0$ maximises plug flow in conventional OBRs (Abbott et al., 2014a, Stonestreet and van der Veeken, 1999), however; $\Psi < 10$ has been reported as sufficient for practical applications (Phan and Harvey, 2010).

The OBR was required to operate in a semi-continuous mode for the present study where 2 L of fresh material was fed every 4.8-24 hours to give HRTs of 4-20 days. Achieving plug flow could be beneficial to AD because it would enable the four process stages to be separated along the reactor length, effectively replacing the time dimension associated with batch manufacturing. Variables such as pH and temperature could then be controlled down the OBR length to optimise the conditions for each stage in the process. For example, maintaining a slightly lower pH for hydrolysis. This optimisation could increase the reaction rates associated with AD thereby increasing productivity and biogas production (Cooper et al., 2009).

A simple method based on previous work (Abbott et al., 2014a) was developed to determine the mixing times for a range of mixing intensities ($1003 < Re_o < 10{,}000$) in

the OBR. The mixing time is defined as 'the time taken for the reactor to reach a specified degree of uniformity' (Ni et al., 1998) and was determined by recording the time taken for pH to reach similar levels (+/- 0.1) at each probe down the OBR length. 50 mL of 2.5 M sodium hydroxide was injected into the OBR contents (water) at the base of column 10. The mixing times were recorded for batch operation (i.e. Re_n=0) which were plotted against Re_o and shown in Figure 6.3.

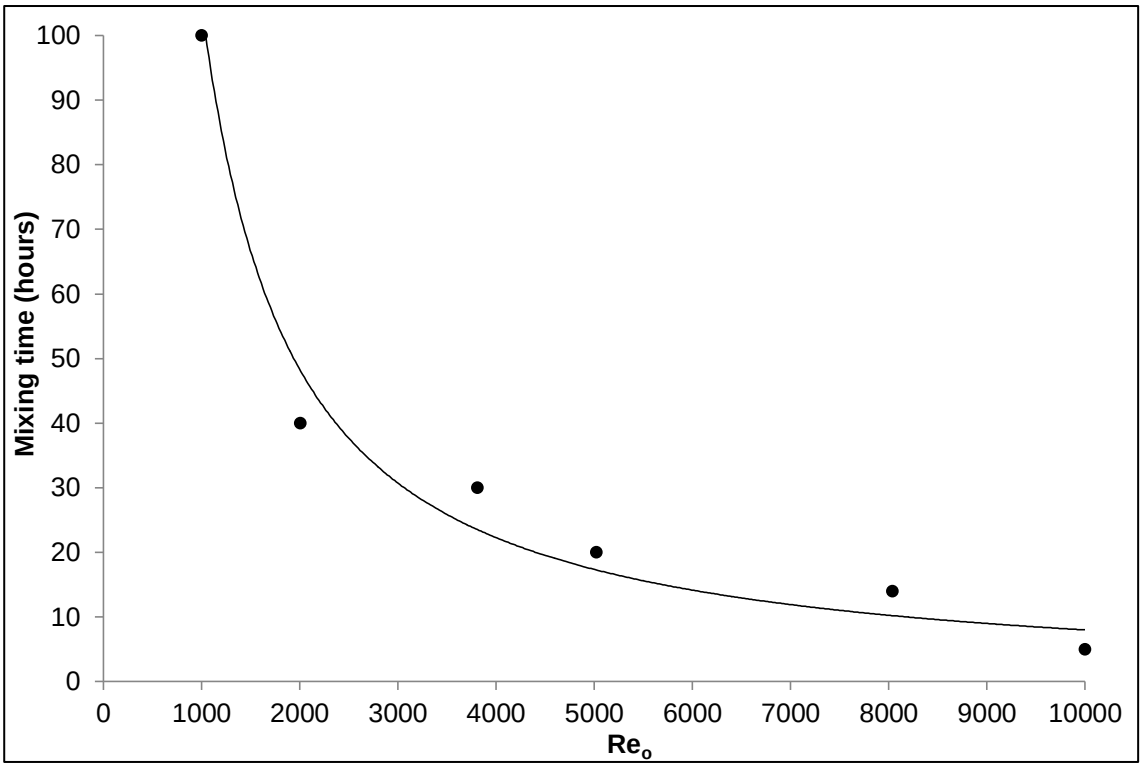

Figure 6.3: The mixing time (hours) plotted against mixing intensity (Re_o) for batch operation of the OBR used in this study.

Figure 6.3 shows that the mixing time decreases exponentially as the mixing intensity increases which occurs for other systems (Bonvillani et al., 2006). Increased agitation results in more rapid dispersion of material throughout the OBR which reduces the time taken to reach uniformity. The mixing time increases with reduced Re_o due to lack of vortex formation and effective propagation. The mixing time was shown to be 40 hours at Re_o=2006 which represents a relatively moderate level of agitation. AD requires HRTs of 4-50 days, however; these results indicate that the contents are fully mixed within 2 days for moderate levels of agitation (Re_o=2006). This suggests that the process stages would not be separated when operated semi-continuously to achieve the required HRTs for AD.

These results are not surprising given that the range $695<\Psi<3400$ is required to achieve HRTs of 4-50 days, assuming the net flow is continuous. This range is

orders of magnitude higher than required to achieve practical levels of plug flow and indicates that the oscillatory component dominates over the bulk flow component. The OBR was therefore predicted to behave as a mixed vessel for the remainder of the study, although a higher concentration of feed would be expected near the feed inlet. OBR lengths (with the same design used in this study) of 1380-6900 m would be required to achieve plug flow (Ψ=10) for HRTs of 4-20 days. These lengths are almost certainly impractical to build and operate but would allow separation of the AD process, however; such levels of plug flow may not be required to achieve separation given there are only four stages present in the process.

6.4.2 Digestion of dairy slurry

The digesters were seeded with digestate from on-site digesters before manually feeding fresh dairy slurry at 2 L per day (conditions 0). The initial indications were positive with both digesters producing biogas which could be seen in the bubble pots. However, oscillation transmission had markedly reduced in the OBR after ~10 days caused by a build-up of particulates in the 'u-bends'. An attempt was made to overcome the blockages with increased agitation (Re_o=10,000) which proved unsuccessful after several days. Data (not shown) were also very unreliable and variable due to sporadic feed times dependent on personnel presence in the facility. Blockages were manually removed and both digesters re-seeded with digestate.

Dairy slurry was centrifuged to remove larger particulates and minimise blockages in the OBR. Blockages indicate that the OBR design used in this study is more suitable for liquid feed stocks and that a different design is required to process feeds containing particulates, such as fresh dairy slurry. The feed regime was automated as described (see §6.3.1) to remove the variability associated with feed times and enable the generation of more reliable and consistent data.

Fresh, centrifuged dairy slurry (2 L) was fed into the digesters every 12 hours to give a HRT of 10 days (conditions 1). Both digesters stabilised after 10 days which was expected because a full digester volume had passed through, replacing most of the digestate used as seed material. Biogas production remained stable in both digesters over 11 days with cumulative volumes shown in Figure 6.4.

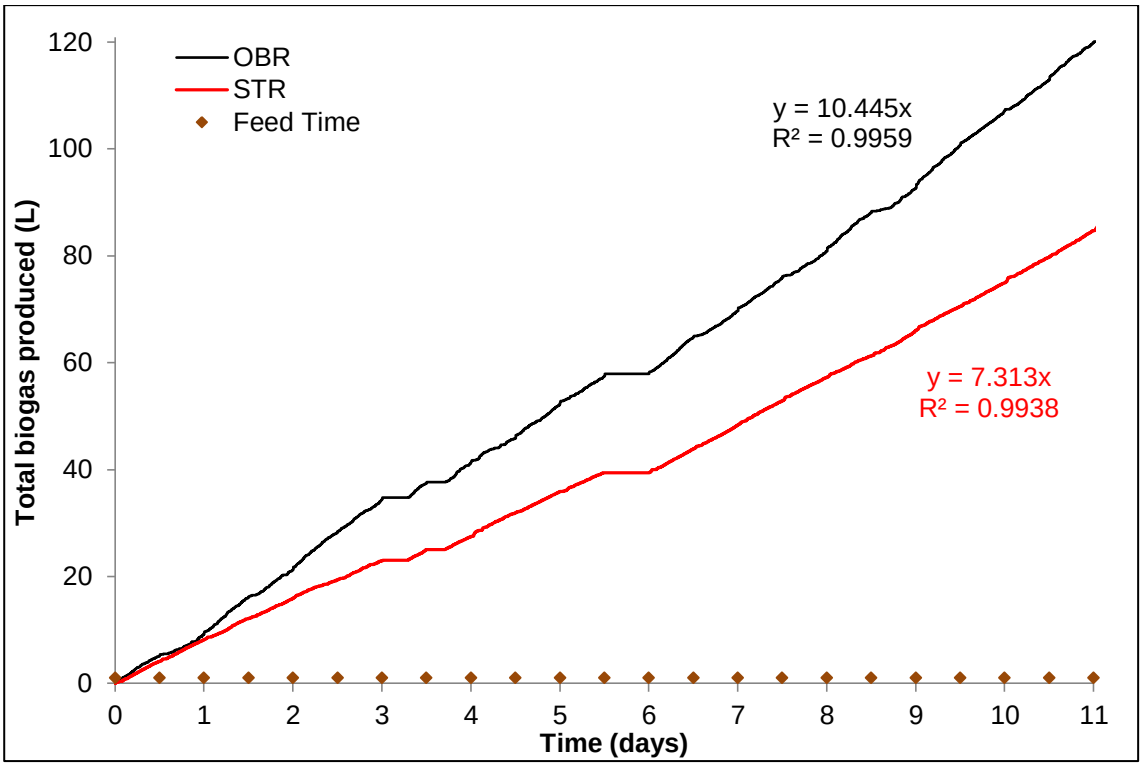

Figure 6.4: Cumulative biogas volumes produced by the digesters based on OBR (black) and STR (red) technologies. Brown diamonds represent feed times where 2 L of fresh dairy slurry was pumped into the digesters and 2 L digestate simultaneously removed. Linear models are shown for comparison.

Digestion of centrifuged dairy slurry resulted in biogas productions in the OBR and STR of 10.4 and 7.3 L/day, respectively. The results shown in Figure 6.4 indicate that the OBR produced 43% more biogas compared to the STR. Methane contents (averaged over 3 samples) of biogas produced from the OBR and STR were 78.9% (+/- 1.4) and 82.0% (+/- 2.3), respectively, which indicates that differences in biogas quality were insignificant between the two digester designs. Digestate and feed samples were analysed for numerous compounds/parameters which are summarised in Figures 6.5 and 6.6. These are average values from numerous samples taken over the 11 day period shown in Figure 6.4.

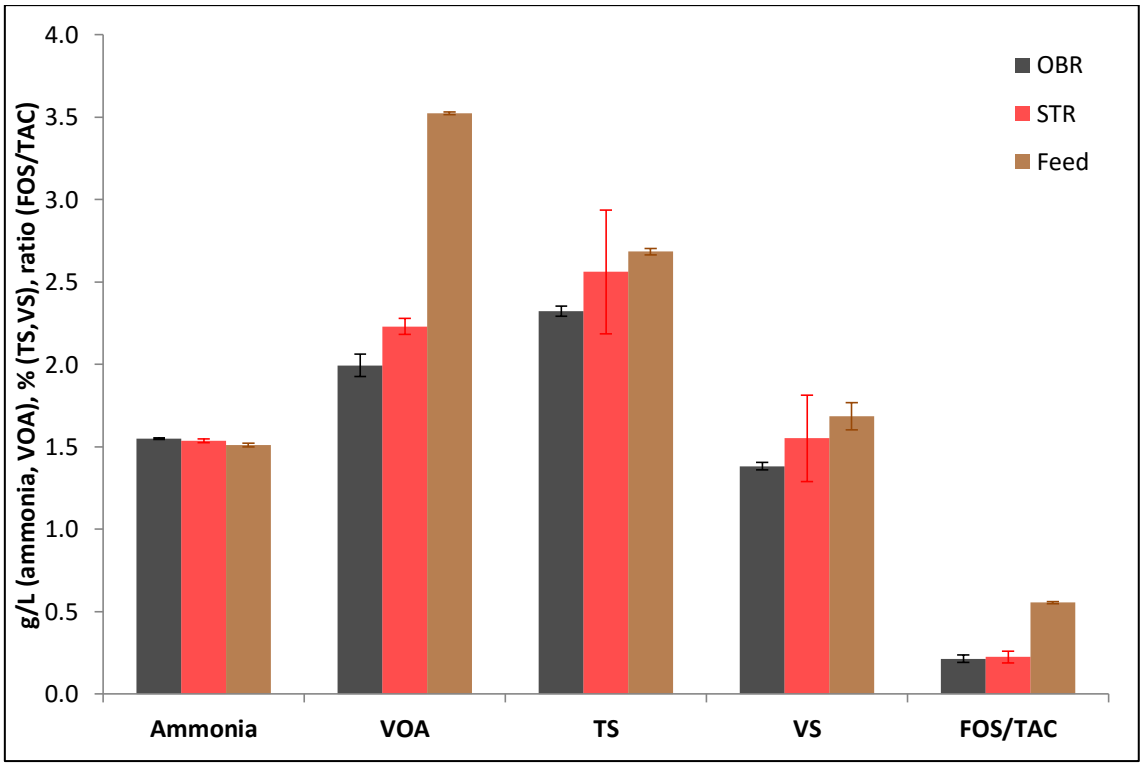

Figure 6.5: Ammonia, volatile organic acids (VOA), total solids (TS), volatile solids (VS) and the FOS/TAC ratios for the OBR (black), STR (red) and feed (brown). Error bars represent +/- 1 standard deviation from at least 3 measurements.

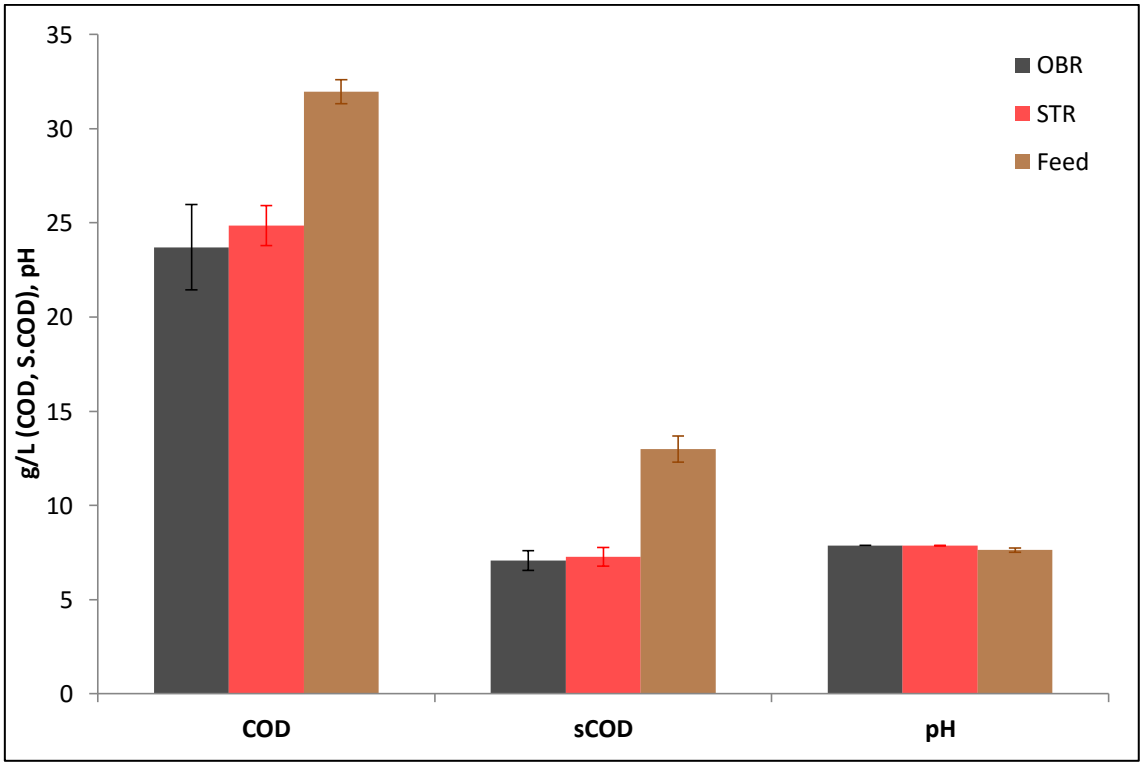

Figure 6.6: Chemical oxygen demand (COD), soluble COD (sCOD) and pH for the OBR (black), STR (red) and feed (brown). Error bars represent +/- 1 standard deviation from at least 3 measurements.

Differences in ammonia, TS, VS, FOS/TAC, COD, sCOD and pH were insignificant between the two digester designs with average values of 1.5 g/L, 2.4%, 1.4%, 0.2, 24.3 g/L, 7.1 g/L and 7.9, respectively. The FOS/TAC ratio has long been recognised

as a guide value for assessing fermentation processes. It enables process problems extending as far as the imminent inversion of the digester biology to be detected at an early stage, so that countermeasures can be initiated (Lange, 2007). Each digester has its own optimal FOS/TAC ratio at which point it can be considered 'healthy'. Values of 0.2 suggest that both digesters are able to cope with this rate of nutrient addition and are behaving in a similar manner. The OBR produced lower values for TS and VS compared with the STR possibly due to either increased conversion or small particles settling in the OBR, however; this difference is statistically insignificant. The concentration of VOAs was lower in the OBR than the STR with values of 2.0 and 2.2 g/L, respectively. This reduction of 9% suggests the OBR is utilising a greater proportion of nutrients and converting it to biogas thereby increasing the volumes produced. Concentrations of COD, sCOD and VOAs in the feed were significantly higher compared to digestate from both digesters. This is expected because fresh feed is rich in complex organic compounds which serve as a nutrient source for biogas production. Their concentrations reduce in the digesters as the reactions proceed and biogas is produced.

These results suggest that OBRs operated with continuous agitation are capable of intensifying the AD process compared to standard technologies based on conventional STR designs operated with intermittent agitation. The OBR demonstrated a 43% increase in biogas production coupled with a 9% decrease in the concentration of VOAs compared to the STR. Analysis of compounds and parameters showed that both digesters were stable. However, agitation in the OBR was continuous whereas the STR was agitated for 2 minutes every half hour. The reason for increased biogas production in the OBR could be a result of the mixing type, some separation of the process stages or purely a result of continuous agitation, and should be identified to enable development of the technology.

6.4.3 Glycerol addition

Glycerol was added to the feed after 42.5 days at a total concentration of 1.4% to provide a readily available carbon source, increase biogas productivity and accentuate any differences between the digesters. Addition occurred without restarting the digesters thereby subjecting the process to a 'step change' in feed composition. The agitation intensities and feed rates were maintained at the levels described in conditions 1 with a HRT of 10 days, continuous agitation in the OBR and

intermittent agitation in the STR. The performance of both digesters was determined principally by methane production rates. Figure 6.7 shows the cumulative methane volumes calculated from biogas composition and volume data produced over the entire study for both digesters.

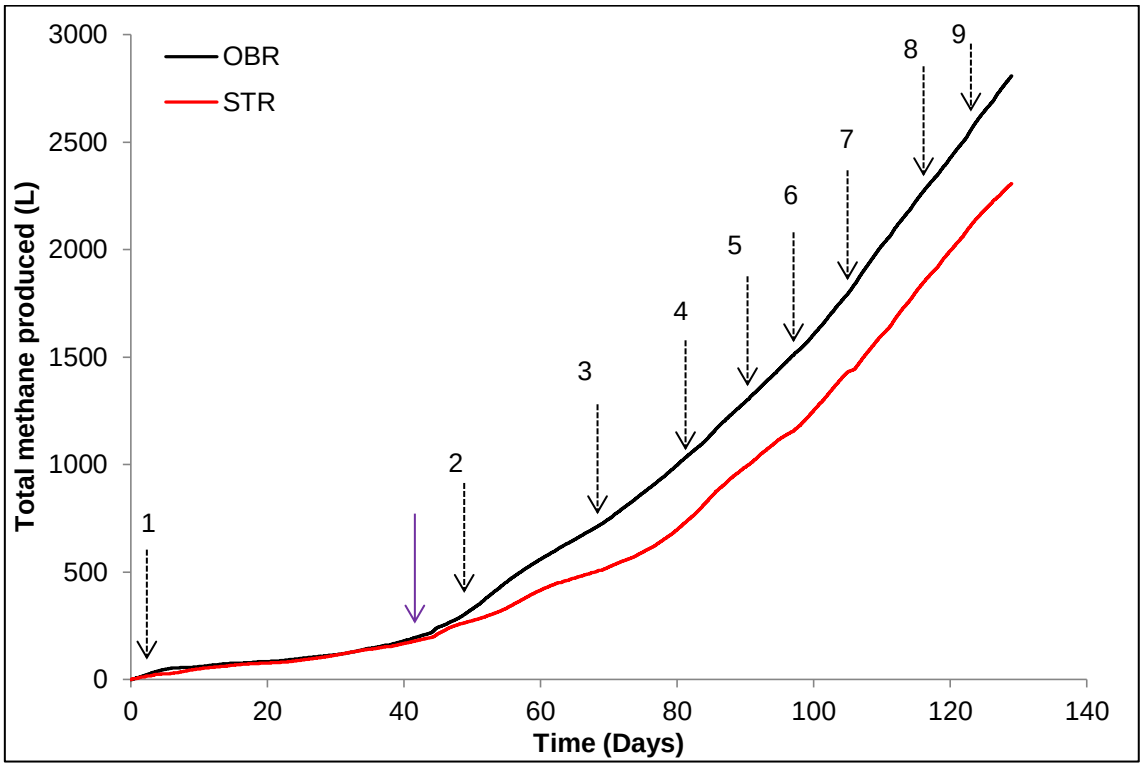

Figure 6.7: Cumulative methane volumes produced by the digesters based on OBR (black) and STR (red) technologies over the entire study. Dotted arrows represent step changes in agitation intensity or feed rate outlined in Table 6.1. The purple arrow indicates 1.4% glycerol addition.

There was an increase of ~270% in methane production in both digesters ~3 days after glycerol addition which is consistent with previous studies (Robra et al., 2010, Castrillón et al., 2011). However, methane production decreased significantly in the STR with continued addition of glycerol at 1.4% whereas the OBR maintained increased levels of methane production. This suggests the STR destabilised due to the rapid increase in nutrient availability caused by glycerol. Figures 6.8 and 6.9 show the pH and FOS/TAC ratios, respectively, for both digesters over the entire study.

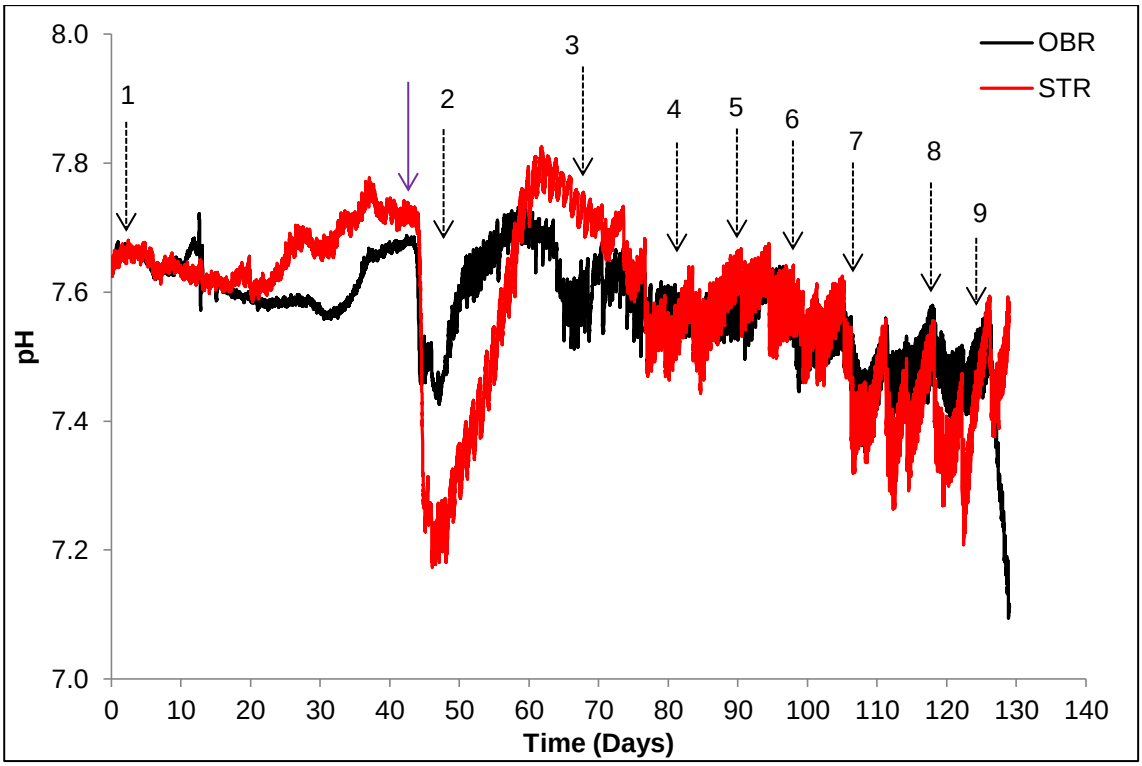

Figure 6.8: Average pH for the digesters based on OBR (black) and STR (red) technologies over the entire study. Dotted arrows represent step changes in agitation intensity or feed rate outlined in Table 6.1. The purple arrow indicates 1.4% glycerol addition.

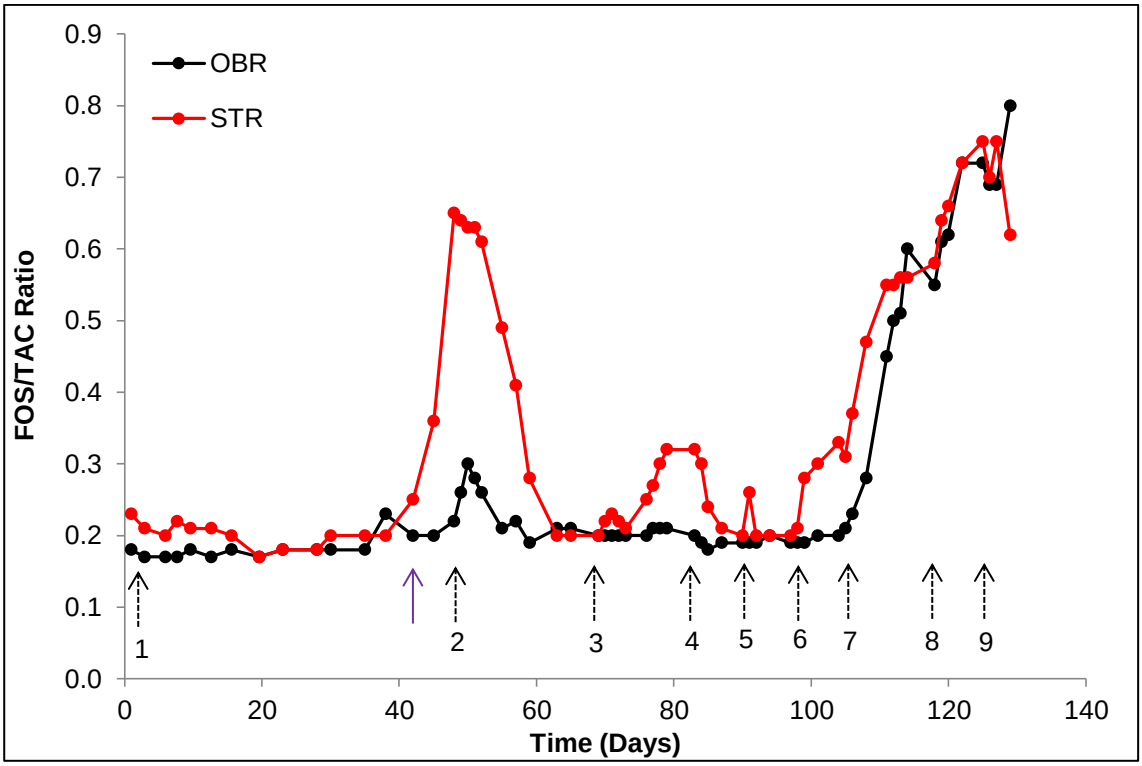

Figure 6.9: FOS/TAC ratios for the digesters based on OBR (black) and STR (red) technologies over the entire study. Dotted arrows represent step changes in agitation intensity or feed rate outlined in Table 6.1. The purple arrow indicates 1.4% glycerol addition.

The pH decreased significantly in both digesters shortly after glycerol addition. Rapid conversion of glycerol to VOAs occurs during the initial hydrolysis and acidogenesis stages of AD. Increases in acid concentrations in the digester contents results in a

decrease in pH, shown in Figure 6.8 for both digesters. This also increases the FOS/TAC ratio, as shown in Figure 6.9, as more acids are present. High FOS/TAC ratios indicate reduced buffering capacity and the imminent destabilisation of the process. A continued reduction in pH results in complete process collapse due to unsuitable conditions for the correct consortia of microorganisms to grow and metabolise, especially those responsible for methanogenesis.

Figures 6.8 and 6.9 show severe decreases in pH and increases in FOS/TAC ratios, respectively, for the STR compared to the OBR after glycerol addition. A reduction in the feed rate to 2 L/day coupled with continuous agitation was required in the STR to prevent complete process collapse. The process slowly recovered over a period of 10 days in the STR at this reduced feed rate until steady state was reached (conditions 2). It was then possible to increase the feed rate to the initial value of 4 L/day in the STR (conditions 3) without process destabilisation. However, the OBR demonstrated an enhanced capacity to cope with the shock load of increased nutrients as it was able to recover at the continued, higher feed rate of 4 L/day.

Any acids produced in the STR are rapidly mixed throughout the entire contents, thereby immediately affecting all microorganisms. The tubular nature of the OBR provides a ‘buffer zone’ near the feed inlet where acids produced through hydrolysis and acidogenesis accumulate. The mixing time studies showed that components would be fully mixed within at least 40 hours, however; it is likely that a large proportion of the acids would be converted by acetogenesis and methanogenesis before this time. This results in reduced acid exposure to microorganisms further down the OBR which could minimise the inhibitory effect caused by a decrease in pH. Figure 6.10 shows the average pH down the OBR length for the period of dairy slurry and co-digestion with glycerol.

The pH for digestion of dairy slurry was similar at all locations down the OBR at 7.5-7.8. However, with glycerol addition, the pH at probe 1 was significantly lower (7.4) compared to the rest of the digester (7.6-7.8), except for probe 6 (7.4). This is consistent with the hypothesis that VOAs are rapidly formed near the feed inlet and then converted by the AD process before being mixed down the OBR. The reduction in pH at probe 6 is difficult to explain and needs further work to identify the reason. These results strongly suggest that digesters based on OBR technology are able to

cope with shock loads in nutrient availability due to a ‘buffer zone’ effect even without the presence of plug flow.

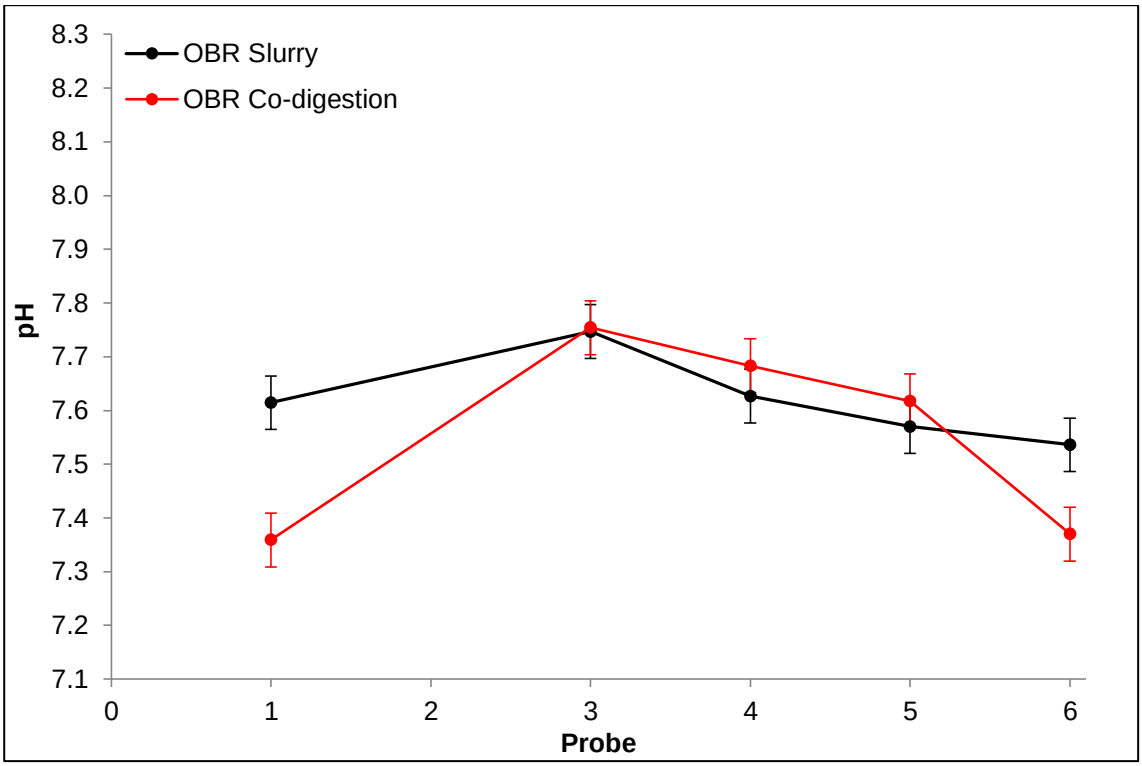

Figure 6.10: The average pH down the OBR digester length for dairy slurry (black) and glycerol addition (red). Data from probe 2 was not used due to a technical fault.

6.4.4 Digester performance, agitation intensity and feed rate

Agitation intensity or feed rate was changed in both digesters which were then left to stabilise at the new conditions, identified by maintenance of steady state in terms of methane production. The performance of each digester was then determined before implementing another step change in either the agitation intensity or feed rate. Previous analyses have described digester performance in terms of total biogas (Figure 6.4) or methane (Figure 6.7) production, however; more appropriate criteria are the yields of methane produced. Yield analyses were performed in terms of the volume of methane produced per 1) L feed added and 2) kg VS added, with average values calculated over a period of steady state methane production and shown for each of the conditions in Figures 6.11 and 6.12, respectively.

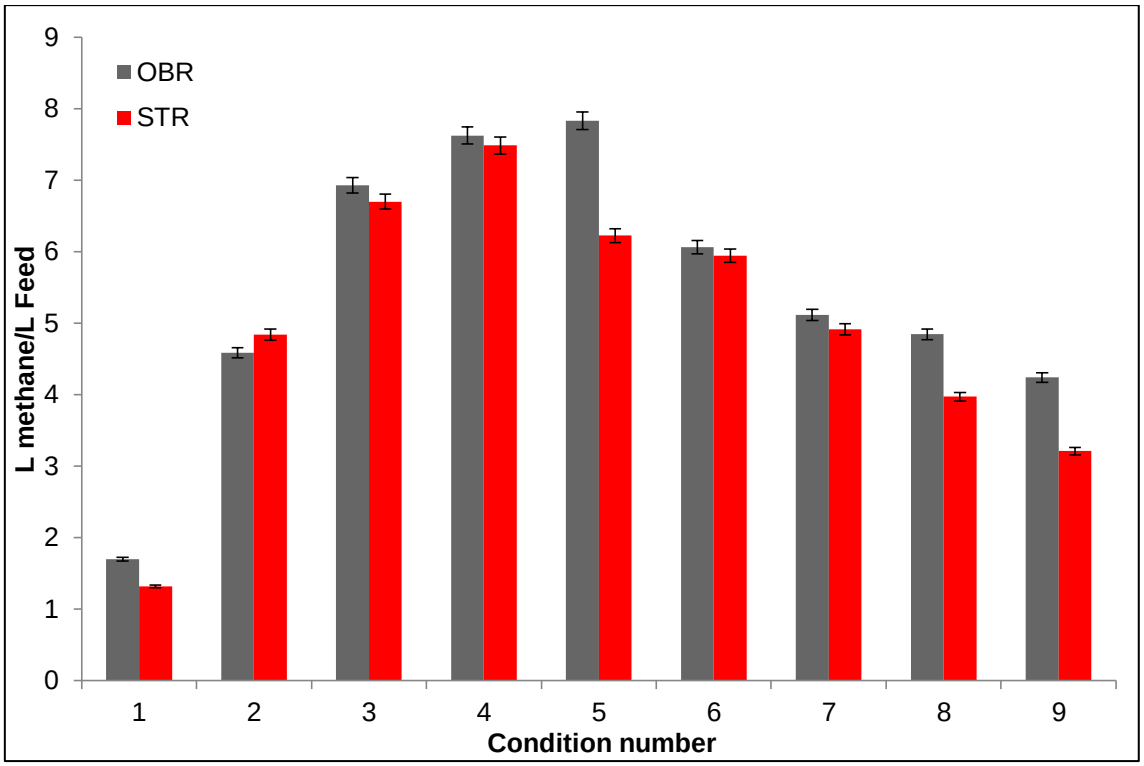

Figure 6.11: Methane yields for each condition in the OBR (black) and STR (red) in terms of L methane produced per L feed added to the digesters. Error bars represent +/- 1 standard deviation from the measurement with the highest error (gas composition).

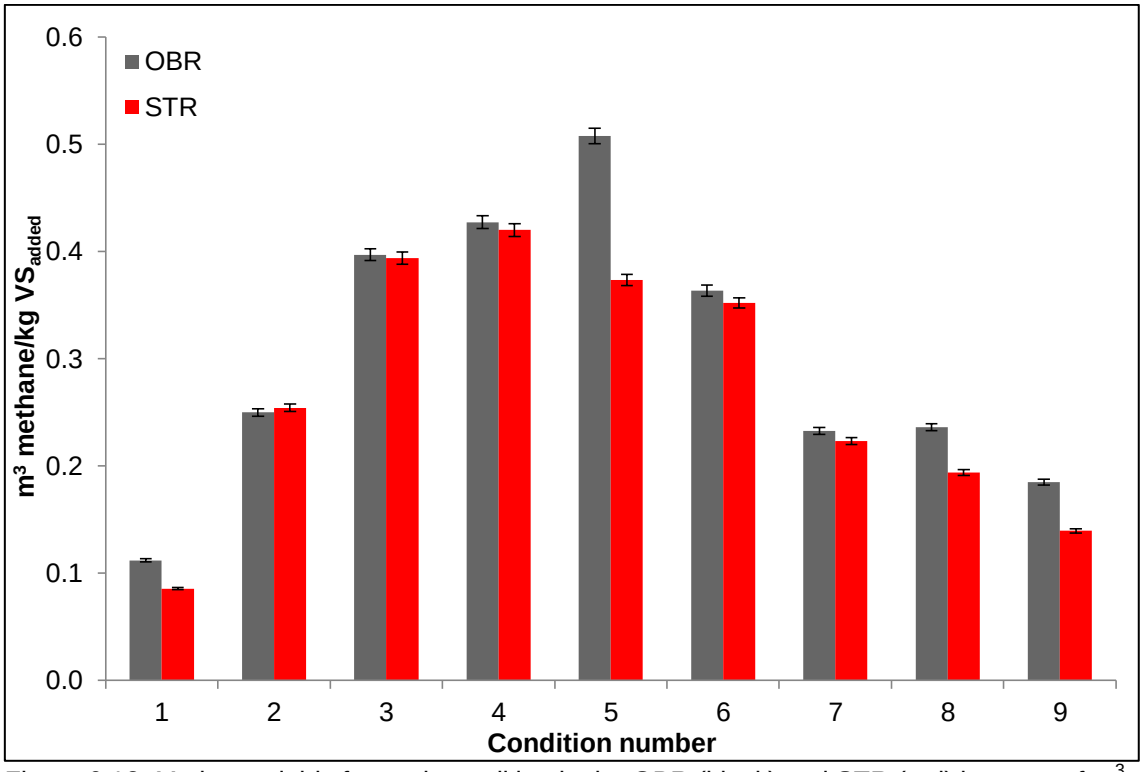

Figure 6.12: Methane yields for each condition in the OBR (black) and STR (red) in terms of m^3 methane produced per kg VS added to the digesters. Error bars represent +/- 1 standard deviation from the measurement with the highest error (gas composition).

Methane yields were also calculated for kg COD removed, however; the results were variable and significantly different from the other yield analyses performed and therefore not used.

The subsequent comparisons between conditions and digesters consider both methane analyses. Figures 6.11 and 6.12 show increased methane yield from the OBR compared to the STR for conditions 1 which is consistent with the previous analysis (see §6.4.2). Both digesters were continuously agitated for conditions 2 with double the feed rate in the OBR (4 L/day) to that of the STR (2 L/day). However, the differences in methane yield for both digesters was statistically insignificant which indicates that the OBR was able to utilise feed as efficiently as the STR at a much reduced HRT of 10 days.

The feed rate was increased to 4 L/day in the STR for conditions 3 and maintained in the OBR at those described in conditions 2. Both digesters produced similar methane yields with continuous agitation and feed rates of 4 L/day (HRT=10 days) which indicates that both technologies perform in a similar manner at these conditions and suggests that the increase in biogas production from the OBR, seen for conditions 1, was caused by continuous agitation. It must be noted that there was a significant increase in methane yield observed in the OBR between conditions 2 and 3 which was unexpected because the conditions were identical. This increase may be explained by changes in feed quality caused by inter-batch variability, which is supported by the fact that the STR and OBR performed similarly under conditions 3.

Fresh slurry was delivered to the facility on a weekly basis with no guarantee that the quality, in terms of nutrient content or availability, would be consistent. There was a long period of ~3 weeks between conditions 2 and 3 (Figure 6.7), as the STR recovered from the shock load caused by glycerol addition. The results suggest that feed quality changed significantly over this long period resulting in changes in methane yields. Therefore, reliable comparisons cannot be made between conditions over long time periods due to potential changes in feed quality which is difficult to control over the long study time required. However, maintenance of consistent feed quality could be achieved in future by sourcing a large volume of slurry sufficient for the entire study period and chilling to prevent degradation, if practical to do so.

Agitation intensities were increased in both digesters for conditions 4, which increased the methane yields by 9% and 10% for the OBR and STR, respectively, compared to conditions 3. This suggests that increased agitation intensities increased reaction rates through more efficient mixing and therefore feed conversion. The difference in methane yield between the OBR and STR for conditions 4 was

insignificant which indicates similar performance in both digesters for these conditions. Agitation intensities were increased again for conditions 5, at which point the STR showed signs of process destabilisation with a decreased methane yield, whereas the OBR maintained a similar methane yield compared to conditions 4. There was a significant increase in the methane yield in terms of VS added for the OBR but no significant change in terms of feed volume. VS in the feed was 1.77% and 1.54% for conditions 4 and 5, respectively, which results in a higher yield for conditions 5 compared to conditions 4 because similar methane volumes were being produced in the OBR. This indicates a change in the feed composition which significantly impacts on the yield obtained.

These results indicate that continuous agitation increases methane yields and is beneficial to AD processes. This could be caused by removal of concentration gradients throughout the digester contents and/or movement of microorganisms to encourage floc formation and syntrophic relationships, thereby increasing kinetic effectiveness (Schink and Stams, 2006). However, relatively moderate levels of agitation (160 rpm) in digesters based on STR technology result in process destabilisation with decreased methane yields. This could be caused by the generation of high shear environments that disrupt floc formation and reduce kinetic effectiveness, which is inhibitory to the process. Increased agitation intensities in the OBR were not inhibitory to the AD process because of the lower average shear (Ni et al., 2000) environments generated that are less likely to disrupt floc formation. A balance between continuous agitation and intensity is therefore required to maximise methane yields from AD processes.

Agitation intensities were reduced to the median values in both digesters (Re_o=3220 and 80 rpm) before periodically increasing the feed rate to 6 (conditions 6), 8 (conditions 7) and finally 10 (conditions 8) L/day to give HRTs of 6.7, 5 and 4 days, respectively. Figures 6.11 and 6.12 show a sequential reduction in methane yields in the OBR from conditions 5 through 7 and in the STR from conditions 4 through 8. This suggests that the increased feed rates provided insufficient time for optimum conversion of feed components to biogas which results in reduced methane yields. Differences in methane yields between the digesters for conditions 6 and 7 were also insignificant which indicates that both digesters were behaving in a similar manner with regards to methane yield.

The OBR demonstrated a 24% increase in methane yield compared to the STR at the highest feed rate (10 L/day) tested for conditions 8. However, a decrease in yield was observed in the OBR from conditions 8 to 9 which could be the result of microbial wash out caused by the consistently high feed rate. Wash out occurs when the rate of microbial growth is less than the feed rate which results in a higher F/M ratio and lower conversion of nutrients to biogas.

Agitation was set to intermittent in the STR whilst continuous agitation was maintained in the OBR for conditions 9. The results show that the OBR demonstrated a 30% increase in methane yields compared to the STR which is consistent with the hypothesis that continuous agitation (of the non-shear type) is required to maximise methane yields. The STR did not show any signs of process destabilisation at the high feed rate of 10 L/day with intermittent agitation; in fact the FOS/TAC ratio started to recover at condition 9, following an increase from condition 6 through to 9 due to increasing feed rate with constant mixing. This indicates that continuous agitation in an STR is not required to prevent process destabilisation once the digester has adapted to changes in feed quality. Therefore, the presence of a 'buffer zone' in the OBR is the aspect which enables this digester type to cope with shock loads in nutrient availability such as glycerol addition.

The pH dropped significantly in the OBR near the end of the study which suggests process destabilisation. Possible removal or reduction of the 'buffer zone' by the high feed rate could expose microorganisms near the end of the OBR to conditions at the feed inlet i.e. higher acid concentrations and lower pH. Further work is required to identify the highest feed rates achievable in both digester types before process destabilisation occurs.

The results generated from changes in agitation and feed rate demonstrate the following: 1) continuous agitation is required to maximise methane yield from AD processes; 2) relatively moderate agitation intensities (160 rpm) are inhibitory to AD for digesters based on STR technology; 3) presence of a 'buffer zone' in digesters based on OBR technology enable the process to cope with shock loads in nutrient availability; 4) This 'buffer zone' is generated without the presence of plug flow; and 5) HRTs of 10 days gave maximum methane yields in both digester types for the blend of feed used.

6.4.5 Optimum conditions

Sample analyses were used to determine the optimum conditions required to maximise the methane yield. The previous results demonstrate that continuous agitation is required to maximise the methane yield and increases in the intensity can improve methane yields but can also become inhibitory in STRs. Therefore, optimum continuous agitation intensities of ~3200<Re_o<6400 and 80 rpm in the OBR and STR, respectively, are required to maximise methane yields from this feed blend.

The organic loading rate (OLR) can be used to determine the amount of digestible organic solids (VS) or organic compounds (COD) being added to a digester and can be expressed as either kg of COD or VS added per m^3 of the digester per day. Figure 6.13 shows specific methane yields (SMYs) (m^3 methane/kg VS_{added}) produced at different OLRs for co-digestion with glycerol in both digesters (conditions 2-9).

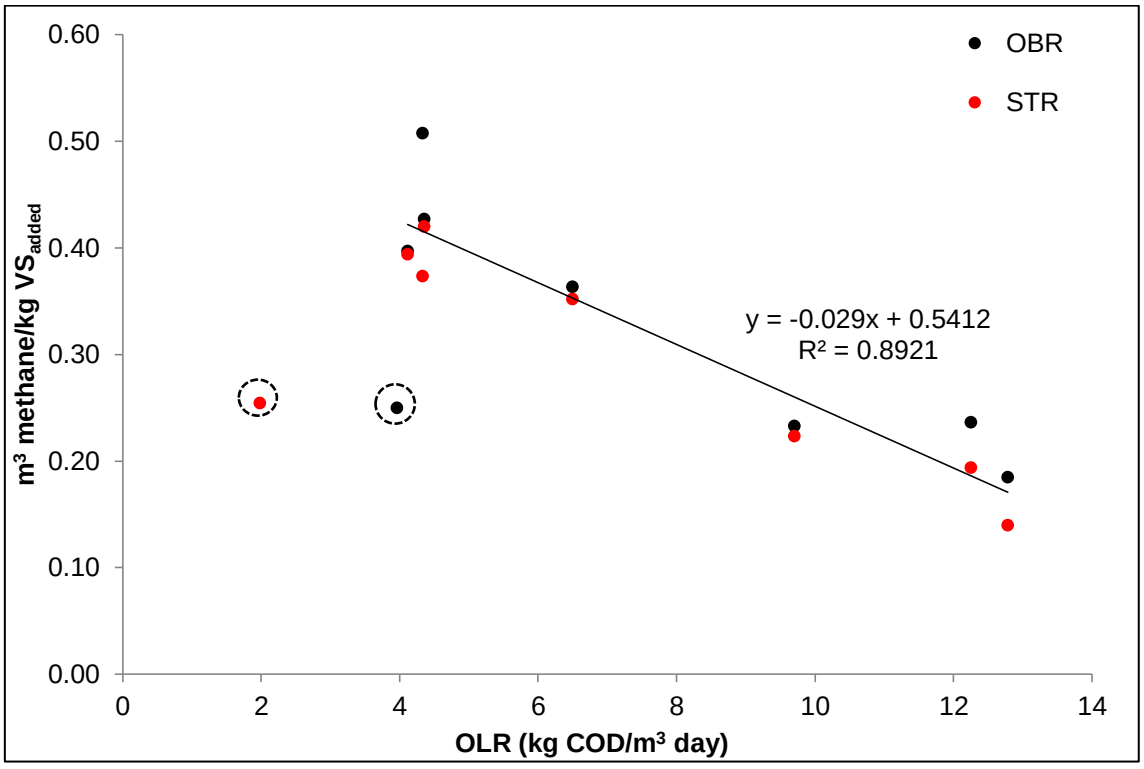

Figure 6.13: Specific methane yields plotted against the organic loading rates (OLRs) for the OBR (black) and STR (red) digesters during conditions 2-9 in both digesters. Black, dotted circles represent results achieved for conditions 2 in both digesters.

The OLR to maximise methane yields in both digester designs was shown to be ~4.3 kg COD/m^3 day. Increases in the OLR above 4.3 kg COD/m^3 day was shown to result in decreased SMYs in both digester designs which indicate the processes are performing less efficiently with regards to nutrient conversion to methane. The dotted, black circles in Figure 6.14 represent results obtained for conditions 2. The lower

SMYs observed for conditions 2 could be a result of reduced feed quality and a lower OLR, especially for the STR which was operated at half the feed rate of the OBR. The trend fitted to Figure 6.13 shows that SMY increases with reduced OLR. Further work is required to determine the optimum OLR as it is reasonable to assume that the SMY will begin to decrease with extremely low OLRs. Table 6.2 shows the maximum SMYs and associated OLRs obtained for digestion of dairy slurry and co-digestion with glycerol for this study and those available in the literature. Castrillón (2013) and Robra (2010) used STR type digesters, with no dedicated stirring device used by Robra (2010). Amon (2007) did not specify the digester deign used.

Table 6.2: Maximum specific methane yields (SMYs) (m^3/kg VS_{added}) obtained for AD of dairy slurry (DS) and co-digestion with glycerol and associated organic loading rates (OLRs) (kg COD/m^3 day).

Digester or study	*SMY (DS)*	*Glycerol*	*SMY(Co-digestion)*	*OLR (Co-digestion)*
	m^3/kg VS_{added}	% v/v	m^3/kg VS_{added}	kg COD/m^3 day
OBR	0.11	1.4	0.51	4.30
STR	0.09	1.4	0.40	4.30
(Robra et al., 2010)	-	5.8-8.7	0.58	-
(Castrillón et al., 2013)	-	3.0	0.59	6.44
(Amon et al., 2007)	0.17	-	-	-

The maximum SMYs obtained for digestion of dairy slurry in this study were 0.11 and 0.09 m^3/kg VS_{added} for the OBR and STR, respectively, compared to 0.17 m^3/kg VS_{added} in the literature (Amon et al., 2007). The maximum SMYs obtained for co-digestion with glycerol were 0.51 and 0.40 m^3/kg VS_{added} for the OBR and STR, respectively, compared to ~0.59 m^3/kg VS_{added} in the literature (Robra et al., 2010, Castrillón et al., 2013). It is difficult to make direct comparisons between studies because of the large number of additional variables which include glycerol concentration, digester design, feed quality/blend, process control and set-up. Nevertheless, the values generated in this study and those in the literature are somewhat similar and suggest the results from this study are reliable. The OBR was able to generate a SMY 28% higher than the STR over the entire study period, however; this increase cannot be generalised between the two digester designs. Similar SMYs were obtained in both digesters for conditions 2, 3, 4, 6 and 7 with the OBR outperforming the STR for conditions 5, 8 and 9. The STR had destabilised for conditions 5 due to high shear agitation and agitation was intermittent for conditions 9. Therefore, the SMYs were shown to be similar in both digesters for the majority of conditions tested in this study.

6.4.6 Power consumption

The two main objectives of AD are to produce biogas which can be used as a sustainable energy source and treat waste streams by reducing its organic matter content to acceptable levels. Digestate that meets the standards set out in the Publicly Available Specification (PAS) 110 is regarded as fully recovered and no longer considered as a waste material, which can be sold and/or used as a 'bio-fertiliser' (WRAP, 2014). Commercial AD systems can therefore generate profit from the sale of biogas and bio-fertiliser as well as the provision of a service to treat waste material. However, these products/services are high volume, low value which requires a strong focus on capital and operating costs to enhance the commercial potential of the endeavour. A major operating cost associated with AD is power consumption of the facility, particularly for agitation and temperature control.

Theoretical power densities (P/Vs) were calculated for the STR and OBR using the relationships defined in equations 7 and 8, respectively, for agitation intensities. The estimated P/Vs and agitation parameters required for each of the conditions in both digesters during the study are summarised in Table 6.3.

Table 6.3: Theoretical power density (P/V) requirements (W/m^3) to achieve the agitation intensities in this study for both digester designs. Intermittent (int.) agitation.

Condition	*OBR*			*STR*	*P/V (W/m^3)*	
	X_o (mm)	**f (Hz)**	**Re_o**	**rpm**	**OBR**	**STR**
0	10.3	0.5	1610	40 (int.)	3.0	0.2
1	10.3	0.5	1610	40 (int.)	3.0	0.2
2	10.3	0.5	1610	40	3.0	2.0
3	10.3	0.5	1610	40	3.0	2.0
4	20.5	0.5	3220	80	23	18
5	20.5	1.0	6440	160	190	150
6	20.5	0.5	3220	80	23	18
7	20.5	0.5	3220	80	23	18
8	20.5	0.5	3220	80	23	18
9	20.5	0.5	3220	80 (int.)	23	1.2

The P/Vs described in Table 3 indicate that the digesters were subjected to similar levels of power input for the period of the study focused on the effects of agitation intensity and feed rate (conditions 3-8). Figure 6.14 shows the methane yields obtained from both digesters at the three P/Vs tested.

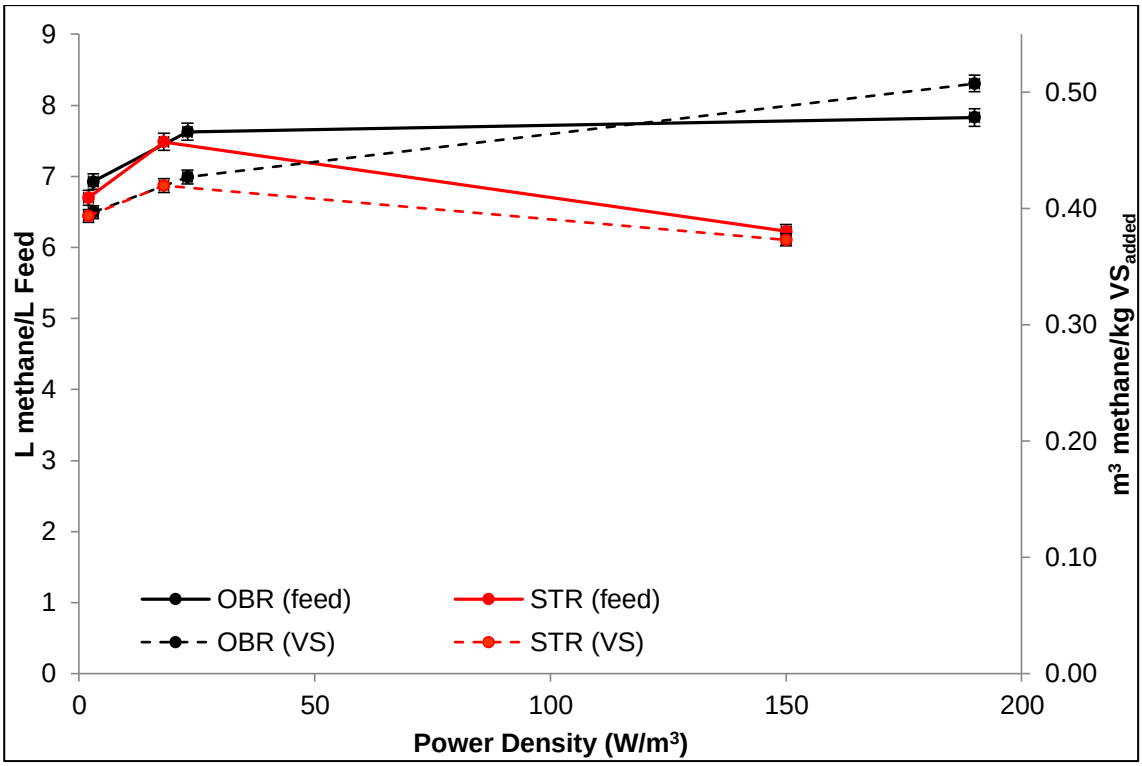

Figure 6.14: Methane yields in terms of feed volume (solid) and VS added (dotted) achieved at increasing power densities (W/m^3) for the OBR (black) and STR (red). Error bars represent +/- 1 standard deviation from the measurement with the highest error (gas composition).

Both digesters achieved similar methane yields at lower P/Vs which were maximised for ~20 W/m^3. The OBR maintained this yield at the highest agitation intensity tested which required ~190 W/m^3, however; the STR demonstrated a significant reduction in the yield at a significantly lower P/V of ~150 W/m^3. This reduction in the yield suggests initiation of process destabilisation and is consistent with previous work which has shown that OBR technology is able to generate lower average shear environments compared to STR technology (Ni et al., 2000). The lower shear environment is conducive to floc formation, which enhances syntrophic relationships and kinetic effectiveness (Schink and Stams, 2006). High shear environments generated in the STR near the impeller disrupt floc formation which results in process destabilisation and a reduction in methane yields. OBRs are therefore able to provide a significantly larger agitation intensity range for AD processes without inhibiting methane production.

The previous analysis was done with theoretical P/V calculations. However, the actual power consumption required for agitation was measured with an energy monitor (efergy, engage hub 1.1). For conditions 3, agitation in the OBR and STR required 1822 and 17 W/m^3, respectively. In comparison, the theoretical P/V requirements were significantly less than those actually used, especially for the OBR.

Equipment is never 100% energy efficient as losses are produced through heat and sound, for example. Therefore, the theoretical power consumption calculated is the absolute minimum which is never achieved in practice. Power consumption in the OBR was orders of magnitude higher than those calculated and used in the STR, which was unexpected because OBR technology is cited as being energy efficient (Abbott et al., 2014b, Jambi et al., 2013). However, the oscillating pump used was oversized for the equipment, so was operated at only 3% of its maximum output. This exacerbates energy losses as a significant amount of energy is required to merely keep the pump on stand-by. This highlights the need to ensure equipment meets specification requirements, thereby minimising energy consumption.

Another major source of power consumption associated with AD facilties is temperature control. Many facilities operate in mesophilic (20-45^{o}C) or thermophilic (49-57^{o}C) temperature ranges to maximise biogas production, which requires (especially in the UK) heating apparatus. An energy monitor (efergy, engage hub 1.1) was used to directly measure the power consumption of the temperature control units used for both digesters. The results showed that the OBR and STR required 44 and 5 kWh/day, respectively, to maintain the set point temperature (~36^{o}C). This translates as 89% less power consumption for the STR compared to the OBR.

The tubular design and in this instance, the material used (stainless steel), of the OBR resulted in a much greater heat loss compared to the STR. At 20 m in length, the OBR has a surface area of ~3.1 m^{2} compared to the STR at ~0.57 m^{2}. Both digesters have a volume of 40 L equating to surface area to volume (SA:V) ratios of 77.5 and 14.3 m^{-1} for the OBR and STR, respectively; a reduction of 82% in the STR compared to the OBR. The OBR also requires a larger pump to circulate water around the jacketed columns. These aspects of the OBR result in significantly increased power consumptions required for temperature control, which is undesirable for commercial facilties. This could be mitigated through digester lagging to reduce temperature loss; increasing the OBR diameter to reduce SA:V ratios; and/or housing the entire OBR unit in a closed vessel which is maintained at the desired temperature. However, it appears difficult to achieve similar power consumption requirements for temperature control to those digesters based on STR technology without significant design changes to the present OBR.

6.4.7 Design considerations

A major advantage of OBR technology is the ability to scale up in a linear and more predictable manner (Smith and Mackley, 2006, Smith, 1999) compared to STR technologies where numerous scale up methodologies exist (Junker, 2004). Smith (1999) was able to demonstrate that multi-orifice baffles could be used to maintain the conditions achieved in small diameter OBRs (10-100 mm diameter) in those with much larger diameters (>150 mm) by simulating the effect of numerous OBRs operated in parallel, another scale up methodology (Ni, 1994). By adopting the multi-orifice baffle scale up approach, the SA:V ratio of OBRs could be significantly reduced thereby decreasing the power consumption required for temperature control. Furthermore, it is likely that OBRs with increased diameters could process feed with a relatively high particulate content due to the removal of constricted regions formed by ‘u-bends’. Figure 6.15 shows the design of a 200 mm diameter OBR which could be operated as 16 individual 50 mm diameter OBRs in parallel.

OBRs scaled with multi-orifice baffles could be designed to have diameters approaching those of comparable STRs which would result in equal SA:V ratios and remove the power consumption issue identified for temperature control. This would enable replication of the agitation environment at larger scale, however; the OBR would lose its ‘buffer zone’ and become sensitive to ‘shock’ changes in feed composition. Plug flow would also not be possible, which prevents separation of the process stages and subsequent optimisation to maximise methane production. A balance therefore exists between power consumption and simple design on one hand; and process separation through plug flow and a ‘buffer zone’ on the other for digesters based on OBR technology. The OBR used in this study has achieved and sometimes exceeded methane yields obtained from a more conventional STR and was able to cope with shock changes in feed composition making it more robust. However, although the current OBR showed potential for the AD process, changes to the design are required to reduce power consumption and simplify the design before digesters based on the technology are commercially viable.

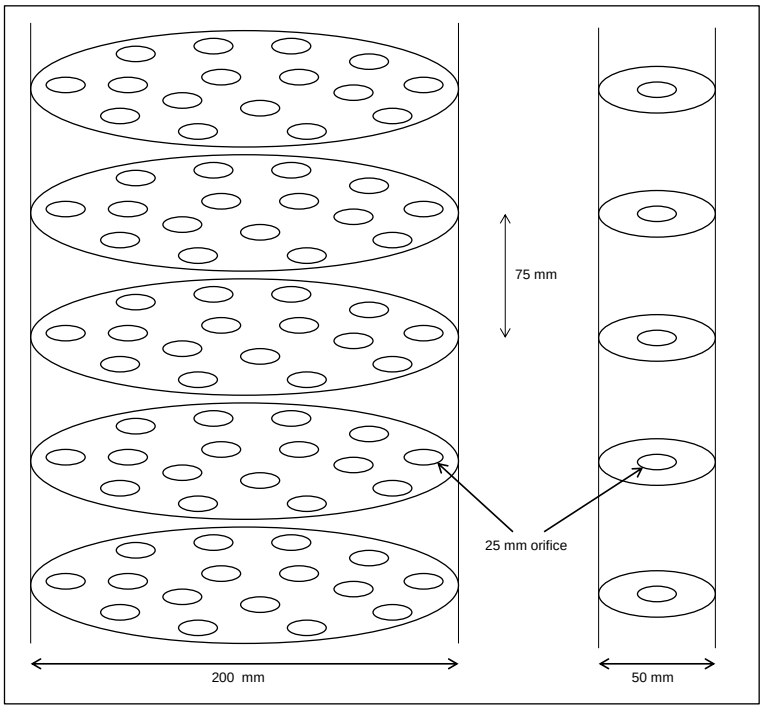

Figure 6.15: Multi-orifice baffle design creating the effect of 16 'standard design' OBRs operated in parallel for a 200 mm diameter reactor. Reproduced (Abbott et al., 2013).

6.5 Conclusions and future work

This study has compared the performance of two digester designs based on OBR and STR technologies for AD of dairy slurry and co-digestion with glycerol. Blockages demonstrated that feed with a particulate content was not suitable for this OBR design, which required centrifugation of slurry to prevent further blockages. Biogas production was enhanced by 43% in the OBR with continuous agitation compared to the STR with intermittent agitation. Destabilisation occurred in the STR with the addition of 1.4% glycerol to the feed which required a reduction in the feed rate and continuous agitation to prevent complete process collapse. The OBR was able to cope with a shock change in feed composition due a 'buffer zone' created by the tubular design. Co-digestion with glycerol enhanced methane production by ~270% in both digesters compared to AD of dairy slurry. Maximum SMYs of 0.51 and 0.40 m^3/kg VS_{added} were achieved at theoretical P/Vs of ~190 and 20 W/m^3 in the OBR and STR, respectively. At P/V=150 W/m^3 the STR showed a significant reduction in the SMY, which indicates process destabilisation at moderate agitation intensities. The optimum OLR in both digesters was shown to be ~4.3 kg COD/m^3 day which generated 0.51 and 0.40 m^3/kg VS_{added} for the OBR and STR, respectively, compared to 6.44 kg COD/m^3 day and ~0.59 m^3/kg VS_{added} in the literature (Castrillón et al., 2013). Theoretical power consumption calculations for

agitation were shown to be significantly less than those measured, probably due to equipment inefficiencies. These measurements also highlighted the importance of adequate equipment specification to minimise power consumption. A value of 89% less power consumption for temperature control was measured for the STR compared to the OBR. This difference was probably caused by the STR having 82% less SA:V ratio compared to the OBR, which would reduce heat loss.

These results demonstrate for the first time that OBR technology is capable of being used for AD and can equal or exceed the performance in terms of SMY of digesters based on STR technology. OBRs offer a platform for the development of processes under plug flow conditions, which for AD could enable separation and optimisation of the four process stages. This is difficult to achieve with conventional STR and vessel based digesters so offers a unique aspect which needs further development to determine the extent to which OBR technology could intensify the AD process and enhance uptake of commercial AD plants. Furthermore, the baffle plates required for OBR operation provide a large, internal surface area suitable for microorganism immobilisation which, if achieved, could significantly increase the SRT and generate low feed to microorganism (F/M) ratios that increase digestion rates and methane production.

Chapter 7: Conclusions and future work

7.1 Conclusions

OBR technology provides an alternative reactor design to conventional STR, tubular and/or flat panel technologies. It could be used to intensify a wide range of processes. This could be achieved through development of continuous processes under plug flow conditions to increase throughput per reactor volume and reduce plant footprint (Stonestreet and van der Veeken, 1999, Abbott et al., 2014a, Stonestreet and Harvey, 2002); generation of intimate mixing under low shear combined with enhanced mass and heat transfer to increase reaction rates (Ni et al., 2000, Mackley and Stonestreet, 1995, Ni et al., 1995); and reductions in power consumption required for mixing to improve overall process economics (Abbott et al., 2014b, Jambi et al., 2013). This chapter presents the main findings of four research projects, followed by suggestions for future research to continue development of OBR technology towards commercial applications.

7.2 Modelling plug flow

A central composite experimental design was used to evaluate the effects of amplitude (X_o), frequency (f) and net flow (Q) on the quality of plug flow achieved during continuous operation of a 'standard' OBR design by analysing residence time distribution profiles (RTD). The following were key findings:

- The tanks-in-series (TiS) model was shown to be a good representation of the flow conditions in an OBR.
- Mass balances demonstrated that >95% of tracer material introduced passed through the reactor indicating little stagnation.
- A second order polynomial model (R^2=92.1%) was developed to predict the quality of plug flow from three variable factors (X_o, f and Q).
- Plug flow was maximised for Ψ=1.9 which is in the range previously identified by Stonestreet and van der Veeken (1999) (1.8<Ψ<2.0).
- Generation of plug flow is not entirely decoupled from the mixing intensity. Hence OBRs can still be "long" if plug flow is desired over a long residence time. However, they are still orders of magnitude shorter than conventional plug flow designs.

The final point above is important for development of commercial processes based on OBR technology because it demonstrates the need for consideration of OBR design in relation to process requirements. For example, not all OBRs can achieve plug flow for all processes, especially those with long residence times (>24 hours) such as many bioprocesses (e.g. enzymatic saccharification).

7.3 Enzymatic saccharification

Enzymatic saccharification of pure α-cellulose was conducted using OBR and conventional STR technologies over a range of mixing intensities, generating the following key findings:

- Reaction rates were mass transfer limited in both reactor designs at conditions of no or minimal mixing.
- The maximum conversion rate in the OBR was observed at a relatively low power density (2.36 W/m^3) compared to the STR (37.2-250 W/m^3).
- No evidence of shear inactivation was observed for STR runs due to a relatively low impeller speed compared to previous studies (Gunjikar et al., 2001, Ganesh et al., 2000).
- A comparison of the theoretical power densities required to achieve maximum conversion rates shows a reduction of 94-99% in the OBR (2.36 W/m^3) compared to the STR (37.2-250 W/m^3).
- OBR technology could potentially increase profits by 2-14% compared to enzymatic saccharification processes based on STR technology.

The study demonstrated that OBRs are suitable for performing enzymatic saccharification reactions in a power-efficient manner compared to conventional STRs. However, a simple economic assessment with numerous assumptions suggested that the overall improvement would be 2-14% for a full scale process (2000 ton corn stover/day). This level of improvement is relatively low compared to the high risk associated with the design and manufacture of OBRs suitable for a full scale process. Commercial adoption of OBR technology is likely to require greater improvements that outweigh the risks associated with novel technologies.

7.4 Microalgae culture

Chlamydomonas reinhardtii was grown in a modified OBR to test the technology for use as a photobioreactor (PBR). The following were key findings:

- A flotation effect was observed without the need for addition of a frothing agent or surfactant.
- The OBR demonstrated a 95% increase in the average maximum growth rate compared to control cultures in T-flasks.
- Mixing intensity in the OBR had no effect on the maximum growth rate achieved, even with no mixing.
- Linear growth was observed in all cultures which indicates limitation.

The study demonstrated that OBR technology could be used for the liquid culture of microalgae. The tubular design is conducive to efficient harvest of sunlight and a closed system enables compliance with the necessary regulations and guidelines associated with GMO use and API manufacture. However, the mixing intensity was shown to have no effect on the growth rate achieved which suggests agitation caused by gas rising through the column is sufficient under these conditions. The main finding was a flotation effect which could enable development of a more economic process for the dual culture and harvest of microalgae cells.

7.5 Anaerobic digestion

This study compared the performance of two digester designs based on OBR and STR technologies for anaerobic digestion (AD) of dairy slurry and co-digestion with glycerol. The following were key findings:

- Feed with a particulate content was not suitable for this OBR design.
- Biogas production was enhanced by 43% in the OBR with continuous agitation compared to the STR with intermittent agitation.
- Destabilisation occurred in the STR with the addition of 1.4% glycerol to the feed which required a reduction in the feed rate and continuous agitation to prevent complete process collapse.
- The OBR was able to cope with a shock change in feed composition due a ‘buffer zone’ created by the tubular design.

- Co-digestion with glycerol enhanced methane production by ~270% in both digesters compared to AD of dairy slurry.
- Maximum specific methane yields (SMYs) of 0.51 and 0.40 m^3/kg VS_{added} were achieved at theoretical P/Vs of ~190 and 20 W/m^3 in the OBR and STR, respectively.
- At P/V=150 W/m^3 the STR showed a significant reduction in the SMY, which indicates process destabilisation at moderate agitation intensities.
- The optimum organic loading rate (OLR) in both digesters was shown to be ~4.3 kg COD/m^3 day which generated 0.51 and 0.40 m^3/kg VS_{added} for the OBR and STR, respectively, compared to 6.44 kg COD/m^3 day and ~0.59 m^3/kg VS_{added} in the literature (Castrillón et al., 2013).
- 89% less power consumption was required for temperature control in the STR compared to the OBR due to a reduction of 82% in the SA:V ratio which reduces heat loss.

These results demonstrate that OBR technology is capable of being used for AD and can equal or exceed the performance in terms of SMY of digesters based on STR technology. However, there are design issues with digesters based on OBR technology which include an increased SA:V ratio for heat loss; and potential blockages in ‘u-bends’ when using feed with a moderate particulate content.

7.6 Future work

This thesis has included a series of projects which have demonstrated the successful application of OBR technology to three distinct bioprocesses: enzymatic saccharification, microalgae culture and anaerobic digestion. The OBR was able to match or exceed process performance compared to more traditional technologies (i.e. STRs and/or T-flasks) for all three bioprocesses. This included a reduction in power requirements for mixing of 94-99% compared to an STR to maximise glucose production during enzymatic saccharification; a 95% increase in the maximum growth rate achieved compared to T-flasks for cultures of *C. reinhardtii*; and a 28% increase in the maximum specific methane yield obtained compared to an STR for co-digestion of dairy slurry and glycerol.

It is therefore clear that OBR technology offers a viable alternative to traditional technologies with the potential of further process intensification. If STR and OBR

technologies had been developed at similar times in history, then it is probable many commercial processes would use OBRs today due to the benefits on offer. However, the reality is that OBR technology is in its infancy with only ~30 years of development compared to STR technology which has been used for centuries. To justify the use of OBRs in place of STRs, the risks of adopting this novel technology must be far outweighed by the improvements. Further work is therefore required to demonstrate OBRs at pilot and industrial scales for applications that greatly benefit from the technology. Two possible applications identified in this thesis are the development of a dual culture and harvest device for microalgae culture; and intensification of AD through increased concentrated feed components and/or utilisation of the baffle surface for microorganism immobilisation. The research presented in this thesis provides results which further demonstrate the utility of OBR technology, especially for bioprocess related applications, and move a step closer to realising this potential in commercial systems which utilise the technology.

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