Biological processing in oscillatory baffled reactors: operation, advantages and potential

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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 proved 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.

1. Introduction

Bioprocessing uses complete living cells or any of their components for the production of useful products ranging from high value pharmaceuticals [1] to low value fuels [2]. A growing interest in renewable technologies to replace traditional fossil-derived chemicals with, for example, biomass [3] 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 with traditional technologies. This is challenging, given the decades of optimization and intensification of chemical processes. In addition, biopharmaceuticals (e.g. trastuzumab) [4], nutraceuticals (e.g. astaxanthin) [5], CO2 capture [6] and protein refolding [7] use 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 owing 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, they 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 CO₂ but suffer from cell fragility [8].

Continuous technologies for bioprocessing and biopharmaceutical sectors have become more prevalent owing to their ability to reduce footprint, waste, cost and energy compared with batch technologies by, for example, removing down-time inherent in batch processing [9]. 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.

Oscillatory baffled reactors (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 maximize 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 [10].

2. Theory governing oscillatory baffled reactor design and operation

2.1. Design

2.1.1. The ‘standard’ design

A ‘standard’ OBR consists of a tube, generally 10–150 mm internal diameter (D), containing equally spaced orifice plates (baffles) with a reciprocating pump required for generation of oscillatory motion (figure 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.

2.1.2. Other designs

Although figure 1 shows the most common design, other OBR designs exist for scaling down (mesoscale) and up (e.g. ‘multi-orifice’, see §4.2). Mesoscale OBRs have a niche application for the rapid screening and characterization of reactions (e.g. biodiesel formation) [11]. These meso-reactors have a small diameter (approx. 5 mm) and subsequently volumes of a few millilitres [12]. Several baffle designs have been evaluated at the mesoscale: helical [13,14], smooth periodic constricted tube [15], central and integral [16].

2.2. Mixing through vortices

Unlike STRs and conventional tubular reactors that rely on stirring mechanisms and/or turbulent flow conditions for mixing [17,18], the OBR uses oscillations to produce vortices (figure 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.

Solutions of the Navier–Stokes equations have been used to calculate the flow patterns generated inside periodically constricted tubes [19] 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 [20] and are relevant...
to OBRs because orifice plates produce constricted regions, resulting in similar flow conditions when oscillated.

2.3. Geometrical parameters

The geometrical parameters, or physical dimensions, important for OBR design are summarized in Table 1 and figure 1b. In total, there are five parameters that need to be considered with the baffle spacing \( L \) and baffle open area \( \alpha \), defined as \( \frac{(D_o/D)^2}{D} \), being the most important.

The shape and length of vortex formation are defined by \( L \). Generating 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 \( D \) have been evaluated [22]. A spacing of 1.5\( D \) gave the most effective mixing over the greatest range of oscillation amplitudes \( (X_o) \), and so has been standardized and used for most subsequent work with OBRs.

The width of vortices formed within inter-baffle zones is defined by \( \alpha \), 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 \( 11 \leq \alpha \leq 51 \) per cent have 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’ [21]. Using 4 per cent sodium hydroxide as tracer, \( 20 \leq \alpha \leq 22 \) per cent was found to minimize the mixing time. Baffle thickness \( \delta \) was also evaluated in a 50 mm diameter OBR using thicknesses of 1–48 mm [21]. It was found that thicker baffles resulted in vortex deformation owing to an increased ‘cling time’ with the optimum thickness being identified as 2–3 mm.

![Figure 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.](image)

<table>
<thead>
<tr>
<th>parameter</th>
<th>symbol</th>
<th>optimal value</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>baffle thickness</td>
<td>( \delta )</td>
<td>2–3 mm</td>
<td>[21]</td>
</tr>
<tr>
<td>baffle spacing</td>
<td>( L )</td>
<td>1.5 ( D )</td>
<td></td>
</tr>
<tr>
<td>baffle open area</td>
<td>( \alpha )</td>
<td>20–22%</td>
<td>[21]</td>
</tr>
<tr>
<td>orifice diameter</td>
<td>( D_o )</td>
<td>0.45–0.50 ( D )</td>
<td>[21]</td>
</tr>
<tr>
<td>tube diameter</td>
<td>( D )</td>
<td>usually 10–150 mm</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

2.4. Operational parameters

2.4.1. Batch operation

Table 2 summarizes the dimensionless groups and dynamic parameters used in oscillatory flow.

The Reynolds number of oscillation \( (Re_o) \) 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 [23], as opposed to a net flow Reynolds number \( (Re_n) \) in conventional tubular reactors of 2100 [24]. An increase in \( Re_o \) can be achieved \textit{in situ} by increasing the amplitude \( (X_o) \) or frequency \( (f) \) of oscillation, producing a wide range of mixing intensities from ‘soft’ \( (50 \leq Re_n \leq 500) \), where vortex formation occurs, to the most intense \( (Re_n > 5000) \) corresponding to mixed flow with the OBR acting as a STR [24].

The Strouhal number \( (St) \) is inversely proportional to \( X_o \) and measures vortex propagation [21,25]. 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 [22,26]; however, the most common range used is 0.15–4 [27–29].

2.4.2. Continuous operation

When operating continuously, \( Re_n \) should dominate, giving almost full reversal in flow, thereby creating vortices and generating effective mixing. The value of the velocity ratio \( (\psi) \) is a measure of the degree of plug flow achieved [30] and can be evaluated using the tanks-in-series (TIS) model for plug flow [31].

The TIS model assumes that flow conditions can be represented by a variable number of ideal CSTRs in series \( (N_i) \). As \( N_i \) increases, the predicted residence time distribution (RTD) curve during a pulse test approaches one that would be observed during perfect plug flow. RTD is the probability distribution that describes how long material 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 characterized 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 \( N_i \) 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 deionized water under varying Reo and Ren [30]. By comparing with theoretical RTD profiles, Nt was evaluated and plotted against c. The range of 2/c20 maximized Nt and generated optimal plug flow conditions; however, usable degrees of plug flow can still be achieved using values of c between 2 and 10. Using values of c 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 c above 10 lead to loss of plug flow.

### 3. Bioprocessing advantages

#### 3.1. 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 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 3 summarizes these major advantages.

#### 3.2. 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$^{-1}$’ [39]. Particle image velocimetry has been used to record the shear rate distribution in a 50 mm diameter OBR, and a close correlation between Reo and the mean shear rate ($\gamma_{OBR}$) was observed [33].

The average shear rate generated in STRs is proportional to the impeller speed (N), although this relationship differs

### Table 2. Dimensionless groups and dynamic parameters required for oscillatory baffled reactor operation.

<table>
<thead>
<tr>
<th>parameter</th>
<th>symbol</th>
<th>equation</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>centre to peak amplitude of oscillation (m)</td>
<td>$X_0$</td>
<td>n.a.</td>
<td>half the fluid oscillation distance</td>
</tr>
<tr>
<td>frequency of oscillation (Hz)</td>
<td>f</td>
<td>n.a.</td>
<td>number of oscillations per second</td>
</tr>
<tr>
<td>volumetric flow rate (e.g. ml min$^{-1}$)</td>
<td>Q</td>
<td>n.a.</td>
<td>volume of material entering the OBR over a given time period</td>
</tr>
<tr>
<td>Reynolds number of oscillation</td>
<td>Reo</td>
<td>($\mu.2\pi f X_0 D$)/$\mu$</td>
<td>a measure of mixing intensity</td>
</tr>
<tr>
<td>Strouhal number</td>
<td>St</td>
<td>$D/4\pi X_0$</td>
<td>a measure of effective vortex propagation</td>
</tr>
<tr>
<td>net flow Reynolds number</td>
<td>Ren</td>
<td>($\mu.D ho$)/$\mu$</td>
<td>a measure of the net flow</td>
</tr>
<tr>
<td>velocity ratio</td>
<td>$\psi$</td>
<td>$Re_o/Re_n$</td>
<td>the ratio of $Re_o$ to $Re_n$</td>
</tr>
</tbody>
</table>

### Table 3. Advantages provided by OBRs over conventional CSTRs and tubular reactors.

<table>
<thead>
<tr>
<th>advantage</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>uniform mixing</td>
<td>the vortex cycle creates equal radial transfer across the tube producing uniform mixing patterns throughout the OBR, reducing heat and concentration gradients</td>
</tr>
<tr>
<td>low and uniform shear</td>
<td>low, uniform shear in the OBR compared to STRs makes the reactor more suitable for shear-sensitive organisms and large molecules</td>
</tr>
<tr>
<td>increased mass transfer</td>
<td>uniform bubble size and increased gas hold up produce enhanced mass transfer rates</td>
</tr>
<tr>
<td>compact reactor design</td>
<td>the ability to generate long residence times, under plug flow, with reduced reactor lengths allows compact designs</td>
</tr>
<tr>
<td>linear scale-up</td>
<td>maintaining St, Reo and Ren, allows mixing intensity and flow conditions to be predicted in large volume OBRs using data from laboratory-scale experiments</td>
</tr>
</tbody>
</table>

Experimental RTD profiles have been produced by injecting 3 M aqueous potassium chloride into a 1.3 l OBR with a net flow of deionized water under varying Reo and Ren [30]. By comparing with theoretical RTD profiles, Nt was evaluated and plotted against $\psi$. The range of $2 \leq \psi \leq 4$ maximized Nt and generated optimal plug flow conditions; however, usable degrees of plug flow can still be achieved using values of $\psi$ between 2 and 10. Using values of $\psi$ 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 $\psi$ above 10 lead to loss of plug flow.
according to various authors [40–42]. A comparison is shown in figure 3 using the lowest estimation of shear for a 2 l STR [41], previously used for comparison against a 50 mm OBR (251 ≤ Reo ≤ 4021) [33]. 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 3 demonstrates that the average shear rate is much higher in STRs: at 40 W m⁻³, the OBR shows a fivefold 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 [43,44], as well as cellulase enzymes, which are important for saccharification reactions [45–48], and microalgae [8]. 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 [34]. A 60 per cent improvement in flotation for finer particles, less than 30 μm, and 30–40% for coarse particles up to 104 μm at much lower power densities compared with conventional flotation devices were reported. Compared with Rushton impeller agitated flotation devices that can have up to 30–40 times the energy dissipation close to the impeller compared with the bulk of the vessel [49], OBRs have been shown to have a more even distribution of shear [33], 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.

### 3.3. Enhanced mass transfer

Many bioprocesses use aerobic organisms to produce useful products such as polyhydroxyalkanoates (PHA), precursors for bioplastics, synthesized by the aerobic bacterium *Pseudomonas putida* [50]. During culture of these organisms, air must be sparged into the reactor providing oxygen. In some cases, the oxygen uptake rate 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 sixfold increases in kₐO₂ for oxygen transfer into water reported compared with conventional STRs [35].

Mass transfer of gases, in particular oxygen, into liquids is usually quantified using kₐO₂: ‘the volumetric mass transfer coefficient that describes the efficiency with which oxygen can be delivered to a bioreactor’ [51]. 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 kₐO₂ value for a 21 STR and 50 mm diameter OBR during the fermentation of yeast cells, *Saccharomyces cerevisiae*, have been determined [28]. A comparison of kₐO₂ against power density using these data is shown in figure 4. At a power density of 100 W m⁻³, intense mixing conditions are produced in both reactor types. However, the kₐO₂ values produced in the OBR is approximately 75 per cent higher than the STR: predominantly a result of enhanced gas hold-up time but also reduced bubble diameter [52]. 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 break-up 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 kₐO₂ in STRs would require reactor modification [53], increased impeller speeds (producing increased shear rates) or a switch to pure oxygen.

### 3.4. 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 [32].
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 2381 l h\(^{-1}\) and residence time of 4 h, the required reactor lengths for an OBR and conventional tubular reactor are 1213 and 757 894 m, respectively [36]. The OBR offers an alternative process intensification methodology that provides a high degree of plug flow in a reactor with a much reduced length [30].

4. Scale-up

4.1. Direct diameter increases
A promising aspect of OBR technology is the ability to scale-up processes by maintaining geometrical 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 [37]. 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 [37]. 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 [31]. 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 owing 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 indentified: for \(Re_n < 80\), \(D_c\) tends towards \(5 \times 10^{-7} \text{ m}^2\text{s}^{-1}\); for \(80 < Re_n < 800\) axial dispersion was minimized with values for \(D_c\) as low as \(10^{-7} \text{ m}^2\text{s}^{-1}\); and for \(Re_n > 800\), \(D_c\) increases approximately linearly with \(Re_n\) [37]. Optimal interaction between the net flow and oscillatory mixing was identified as the reason for the minimum: at \(80 < Re_n < 800\), the vortices created were optimal for radial redistribution of dye, thereby minimizing axial dispersion [29]. It was established that axial dispersion was not a function of \(D_c\), indicating that fluid dynamic conditions could be maintained during scale-up providing \(St, Re_o\), and \(Re_n\) were kept constant.

4.2. 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 \(Re_n\). Table 4 summarizes the values of \(X_o\) and \(f\) required to maintain \(St\) and \(Re_n\) at 1.0 and 500, respectively, during scale-up from 25 to 150 mm diameter.

At higher diameters, the frequency of oscillation must be extremely low, which reduces the mixing intensity and the opportunity for improved mass transfer [29]. 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 [54]. 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 [37]. This was demonstrated by recording similar axial dispersions at increasing \(Re_n\) while maintaining \(Re_o\) at 107, in both 24 mm and multi-orifice 150 mm OBRs [37]. 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 owing 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 [29]. Figure 5 depicts a multi-orifice baffle design (100 mm in diameter) that produces characteristics observed in a conventional 25 mm OBR.

**Figure 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 [29].

**Table 4.** Required operating conditions to maintain \(St\) and \(Re_o\) at 1.0 and 500, respectively.

<table>
<thead>
<tr>
<th>tube diameter (mm)</th>
<th>required (X_o) (mm)</th>
<th>required (f) (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1.99</td>
<td>1.6</td>
</tr>
<tr>
<td>50</td>
<td>3.98</td>
<td>0.4</td>
</tr>
<tr>
<td>150</td>
<td>11.94</td>
<td>0.04</td>
</tr>
</tbody>
</table>

During scale-up of 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 2381 l h\(^{-1}\) and residence time of 4 h, the required reactor lengths for an OBR and conventional tubular reactor are 1213 and 757 894 m, respectively [36]. The OBR offers an alternative process intensification methodology that provides a high degree of plug flow in a reactor with a much reduced length [30].

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At higher diameters, the frequency of oscillation must be extremely low, which reduces the mixing intensity and the opportunity for improved mass transfer [29]. 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 [54]. However, this solution produces two other problems: how to maintain an equal distribution of flow to each separate tube, and generating equal oscillating conditions.

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5. Bioprocessing in oscillatory baffled reactors

5.1. 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 [55,56], flotation [34], butylation of phenylacetonitrile [57], oil droplet breakage [58–60], photo-oxidation [61,62], polymerization [63] and extensive work on crystallization [64–71]. However, this review aims to target bioprocesses; so chemical reactions will not be discussed in any further detail.

Tables 5–7 summarize 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 5) and those using living cells, under both anaerobic (table 6) and aerobic (table 7) conditions.

5.2. Bioprocesses using cellular components

Three of the four bioprocesses described in table 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 optimized with respect to the chemical environment [72]; however, the mixing environment also impacts on refolding yield [7,83]. 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 [7], impacting negatively on protein refolding yield.

The table demonstrates that protein refolding can be performed in OBRs with yields comparable 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 [37], allowing yields obtained in the laboratory 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.

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 [84,85]. During the enzymatic conversion of cellulose into monosaccharide, cellulase deactivation occurs. This deactivation of cellulases is caused by a number of process-dependent factors:

<table>
<thead>
<tr>
<th>bioprocess</th>
<th>cellular component</th>
<th>year</th>
<th>conclusion</th>
<th>process type</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>protein refolding</td>
<td>hen egg white lysozyme</td>
<td>2001</td>
<td>OBRs are suitable refolding devices and comparable to STRs</td>
<td>non-cell culture</td>
<td>[7]</td>
</tr>
<tr>
<td>protein renaturation</td>
<td>hen egg white lysozyme</td>
<td>2002</td>
<td>refolding yield in OBR comparable to STR. Uniform shear important for successful refolding</td>
<td>non-cell culture</td>
<td>[72]</td>
</tr>
<tr>
<td>protein refolding</td>
<td>chicken egg white lysozyme</td>
<td>2006</td>
<td>prevention of aggregation enhances refolding during initial stages (0–4 min)</td>
<td>non-cell culture</td>
<td>[73]</td>
</tr>
<tr>
<td>saccharification</td>
<td>cellulase from Trichoderma reesei</td>
<td>2011</td>
<td>7% increase in glucose production after 48 h compared to shake flask</td>
<td>enzymatic</td>
<td>[74]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>bioprocess</th>
<th>organism</th>
<th>year</th>
<th>conclusion</th>
<th>cell type</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>flocculation</td>
<td>Alcaligenes eutrophus</td>
<td>2001</td>
<td>higher degree of flocculation compared to STR at lower polymer dose. St the dominant factor</td>
<td>bacterial</td>
<td>[75]</td>
</tr>
<tr>
<td>Acetone, butanol and ethanol fermentation</td>
<td>Clostridium acetobutylicum</td>
<td>2009</td>
<td>115% increase in solvent production in OBR from 0—I 0.78 Hz. 90% increase in butanol compared with STR</td>
<td>bacterial</td>
<td>[56,76,77]*</td>
</tr>
<tr>
<td>anaerobic process</td>
<td>patent for culture of facultative/obligate anaerobes in OBRs</td>
<td>2010</td>
<td>patent publication</td>
<td>bacterial</td>
<td>[78]*</td>
</tr>
<tr>
<td>ethanol production</td>
<td>Saccharomyces cerevisiae</td>
<td>2011</td>
<td>9% increase in ethanol production after 48 h compared with shake flask</td>
<td>yeast</td>
<td>[74]</td>
</tr>
</tbody>
</table>
 shearing [45–48], sugar inhibition [86], ion strength [87], temperature [88] and formation of inert enzyme substrate complexes. A further factor involved in cellulose depolymerization is the changing nature of the substrate over time; the easily hydrolysable amorphous regions are digested first leaving the recalcitrant crystalline regions [89]. Saccharification has been conducted in a 25 mm diameter OBR using Xo, and f values of 3 mm and 3 Hz, respectively [74]. The substrate used was pure microcrystalline cellulose at a loading of 2.5% w/v. An enzyme loading of 40 filter paper units per gram of cellulose and 10 per cent β-glucosidase was used. The results of the study showed an increase in the glucose yield of 7% per cent after 48 h compared with a shake flask run under the same conditions. The increase in glucose production in the OBR was attributed to a ‘better mixed hydrolysis environment’ [74]. 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 [45–48] 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.

5.3. Anaerobic bioprocesses

Table 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 [75]. 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 with an STR, assessing the percentage flocculation at various operating conditions [75]. Although 100 per cent flocculation was not reached in the OBR, a comparison with another study [90] 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-homogeneous nature of STRs that results in floc break-up near the impeller zone where shear rates can be two orders of magnitude higher than the average [91]. The more even shear distribution in OBRs [33] allows flocculation to occur at much lower average shear providing an attractive, alternative flocculation device to STRs [75].

Acetone, butanol and ethanol (ABE) fermentation and bioethanol production have been extensively covered in a previous review [56] and so will not be discussed in any further detail. No data currently exist for anaerobic digestion using OBRs as the reference is to a patent pending [78]. 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 [78].

5.4. Aerobic bioprocesses

Table 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 [79] with a maximum specific growth rate (μmax) of 0.39 h⁻¹ compared with 0.36 h⁻¹ and 0.35 h⁻¹ when using Erlenmeyer flasks at 10 per cent and 40 per cent working volumes, respectively. OBRs are, therefore, suitable for culturing rapidly growing, oxygen demanding micro-organisms. The same paper...
alluded to the use of OBRs for culturing animal cells: an interesting proposal as they are notoriously shear-sensitive [92] with cell death being proportional to energy input; so any scaled-up reactor should minimize energy input [43]. Bioprocesses containing animal cells could greatly benefit from the low shear, high mass transfer environment produced in OBRs to minimize shear while maintaining sufficient OTRs.

The fruity, peach-like aroma compound γ-decalactone has applications in the food industry as a favouring agent and can be biologically produced from the yeast Yarrowia lipolytica. Micro-reactors are important tools for rapidly screening and optimizing bioprocesses [81]. A mesoscale OBR (D = 4.4 mm) has been used to culture Y. lipolytica for the production of γ-decalactone [81]. The processing time required to reach maximum γ-decalactone concentration in the OBR was 50 per cent lower compared with 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 S. cerevisiae, with an increase in biomass of 83 per cent, using 93.6 per cent less air, compared with a scaled-down STR [82]. The use of mesoscale OBRs for rapid screening and optimization has two major advantages: precise control over mixing and mass transfer; and optimizations achieved at this scale can be predictably scaled up to industry volumes. *Aureobasidium pullulans* IMI 145194 was cultured in a 100 mm diameter OBR, with a working volume of 2.5 l, using fixed $X_c$ and $f$ values of 20 mm and 2 Hz, respectively. The aeration rate was optimized 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 approximately 11.7 g l$^{-1}$ after 38 h. These values were compared with STR data available in the literature [93], indicating that to reach a comparable pullulan concentration in 2 and 10 l STRs, the fermentation time must be increased to 96 and 144 h, respectively. This equates to a reduction in the required processing times of 60 per cent and 74 per cent when using OBRs as opposed to STRs and highlights the problems encountered when scaling up STRs. The move from 2 to 10 l using STRs has resulted in a 50 per cent increase in the required processing time to reach similar product concentrations.

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 [94,95]. These are contradicted by another study suggesting that low impeller speeds and low shear are optimal [96]. 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* [97]. 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 3 and 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.

### 6. 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, the authors are aware that 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.

#### 6.1. Barriers

Table 8 highlights the key barriers facing adoption of OBR technology by the bioprocessing industry.

<table>
<thead>
<tr>
<th>Design</th>
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<tbody>
<tr>
<td>lengths hundreds of metres required for fully continuous operation</td>
<td></td>
</tr>
<tr>
<td>with residence times of hours to days</td>
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<tr>
<td>pressure drop due to frictional losses will dampen oscillations</td>
<td></td>
</tr>
<tr>
<td>gas bubbles during aerobic operation may dampen oscillations and</td>
<td></td>
</tr>
<tr>
<td>disrupt plug flow</td>
<td></td>
</tr>
<tr>
<td>multiple sparging and feeding points required down OBR length</td>
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</tr>
<tr>
<td>fouling of baffles and internal surfaces due to micro-organism adhesion</td>
<td></td>
</tr>
<tr>
<td>large volumes required</td>
<td></td>
</tr>
<tr>
<td>conservative nature of bioprocessing industry</td>
<td></td>
</tr>
<tr>
<td>increased complexity of OBR technology compared with other bioreactor designs</td>
<td></td>
</tr>
<tr>
<td>no industrial-scale OBRs dedicated to bioprocessing exist</td>
<td></td>
</tr>
<tr>
<td>lack of data for industrial-scale bioprocessing using OBRs</td>
<td></td>
</tr>
</tbody>
</table>

Bioprocesses typically require residence times in excess of 24 h. Standard tubular plug flow reactors would need to be thousands of metres in length compared with 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 using micro-organisms.

There are currently no industrial bioprocessing facilities using OBR technology; so it has not yet been proved at this scale. 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 laboratory-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.

6.2. Recommended strategies

6.2.1. 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 laboratory 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$^{-1}$ [1]. Current scale-up using STRs is complex owing to different mixing patterns occurring at scale. Conventional STRs are prone to impeller flooding, a phenomenon characterized by gas rapidly flowing axially upwards past the impeller with no radial discharge, and the formation of stagnant zones [98,99]. There are also other acknowledged limitations when using STRs including gas channelling, resulting in reduced gas dissolution and poor bulk mixing [17,100]. This requires implementation of different scale-up strategies depending on the specific bioprocess [101,102]. By lengthening an OBR or increasing the diameter (see §§4.1 and 4.2), 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.

6.2.2. Selecting a model bioprocess

The range of bioprocesses highlighted in tables 5–7 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 used 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, we have already stated that proving the benefits of continuous operation and predictable scale-up is 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 [80]). 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 per cent improvement providing greater incentive for industry to adopt OBRs [50,80–82]. It is difficult to predict those bioprocesses that will benefit from continuous operation and predictable scale-up at this stage.

6.2.3. Open access facilities

Open access facilities provide equipment capable of testing various bioprocesses at scale. Large companies could use these facilities to gather data assessing the benefits of OBR technology for their specific bioprocess. Such facilities as the Centre for Process Innovation 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 h residence time to generate solid data on the benefits of predictable scale-up and continuous operation for specific bioprocesses.

7. Summary

OBR technology provides a novel production vessel for bioprocessing over a wide range of cellular components and
micro-organisms. 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 with 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 realize 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 laboratory-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 9 summarizes the findings of this review.

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