Oscillatory Flow Bioreactor (OFB) Applied in Enzymatic Hydrolysis at High Solid Loadings

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Within this study, an enzymatic hydrolysis process using α-cellulosic feedstock was performed in a specially designed plug-flow reactor, referred to as an Oscillatory Flow Bioreactor (OFB). The aims of this approach were to achieve intensification in terms of realising a more energy- and resource-efficient enzymatic hydrolysis, as well as to set the basis for continuous processes in such a reactor. The OFB performance was evaluated for high solid loadings of up to 15 %, and compared to the performance of a Stirred Tank Reactor (STR). Experimental results of the OFB operating at an oscillation frequency of 2 Hz and an oscillation amplitude of 10 mm exhibit better conversion efficiencies (+ 6.7 %) than the STR after 24 h, while requiring only 7 % of the STR power density (W m–3). Therefore, the OFB enables efficient, uniform mixing at lower power densities than STRs for applications with high solid loadings.

Keywords
oscillatory baffled reactor, biorefinery, enzymatic hydrolysis, lignocellulosic feedstock, reactor design

Introduction

The potential of biogenic resources for the processing of its valuable compounds such as cellulose, hemicellulose, and lignin is tremendous. Lignocellulosic waste serves as a feedstock for a wide range of value-added products in biobased industry, the food and pharmaceutical industries, and the pulp and paper sector. The abundance of lignocellulosic waste in agricultural and forest residues or food waste makes it a cost-effective and sustainable feedstock for the production of high-value chemicals and commodities.

However, there are numerous challenges inhibiting the development of an industrial enzymatic bioconversion process that can compete with fossil-based routes. An often prominent problem within the enzymatic saccharification step of cellulose and hemicellulose is that it is difficult to efficiently extract soluble sugars such as glucose or pentoses.

Regardless of enormous efforts over the past decades, the actual costs of this core step of any bioconversion process remain high due to two strongly intertwined factors: the large amount of enzyme needed for a particular conversion/time, and the rather slow reaction kinetics, resulting in inefficient process performance. Significant cost savings can be achieved by lower enzyme requirements and shorter hydrolysis time. Another major impediment in bioprocess engineering is accomplishing reactions at high solid loadings for further process intensification.

Usually, enzymatic hydrolysis is performed in batches in Stirred Tank Reactors (STR). However, the STR has a number of fundamental drawbacks from a reaction engineering viewpoint, despite its conceptual simplicity and widespread use in industrial processing. STRs suffer from inadequate mixing and a non-structured media flow, which may lead to problems in process monitoring and control.

Oscillatory Baffled Reactors (OBR) offer advantages such as eminent mixing and plug-flow properties, which result in consistent product quality in the chemical and process industries, as shown in previous studies. The optimization of oscillatory flow reactors has been studied for various processes such as separations, flocculations, and polymerizations. Significant enhancement of heat
and mass transfer has already been shown in several studies in the early nineties\textsuperscript{13,14}. In this study, the OBR concept is further refined for biobased applications at high solid loadings, resulting in what has been termed an Oscillatory Flow Bioreactor (OFB). Yet, there are few studies on the application of OBRs in bioprocesses. An overview on OBRs in biological processes is given by Abbott \textit{et al.}\textsuperscript{15}; reporting a 50 %–time reduction in an OBR cultivation of microorganisms compared to a parallel STR approach\textsuperscript{16}, such as the approach of simultaneous saccharification and fermentation as potential in the bioethanol production\textsuperscript{17}. A general core characteristic of OBRs is their ability to decouple flow velocity and residence time, enabling the treatment of continuous processes with extended residence times. Oscillations induce short-lived vortices and thus increase the turbulence of the process media times. Oscillations induce short-lived vortices and thus increase the turbulence of the process media.

The Oscillatory Flow Bioreactor was designed based on existing Oscillatory Baffled Reactor (OBR) studies, such as the study involving preliminary experiments for this study, at the University of Newcastle\textsuperscript{16,21,22,24}. The final plant concept was conducted. Different amplitudes were set for monitoring the power consumption of the operating units separately. Three frequency converters (VLT Midi Drive FC 280) were installed to ensure the correct power supply of the built-in three-phase asynchronous motors. Finally, the momentary electric power consumption, expressed in Watts, was calculated from the recorded data.

Calibration of the adjustable engine power numbers and the corresponding oscillatory frequencies was conducted. Different amplitudes were set by deploying respective plate discs in the range of 2 mm to 10 mm.

A novel Oscillating Flow Bioreactor (OBF) has been developed for biogenic slurries to allow for a continuous enzymatic hydrolysis process using a plug-flow reactor with substantial positive effects on mixing at high solid loadings. The overall OBF design is based on existing Oscillatory Baffled Reactor (OBR) studies, such as the study involving preliminary experiments for this study, at the University of Newcastle\textsuperscript{16,21,22,24}. The final plant concept is shown in Fig. 1. The reactor was designed to decrease energy demand peaks and facilitate the potential integration of waste heat without the enlargement of heat exchanger surface at continuous processes.

The Oscillatory Flow Bioreactor was designed as a modular set-up – enabling batch and continuous modes with 1 to 4 500 mL reactors. The reactors were polymeric double-jacketed acrylic tubes with a 25 mm inner diameter. Helical baffles of 2 mm and 3 mm thickness were used. A pump with an oscillating piston was implemented to impose sinusoidal waveform oscillations in ranges of 0–10 Hz frequencies and 0–11 mm amplitudes. Temperature sensors were located in front of each module, as well as inside the tube. Enzyme addition and sampling ports were also placed in front of each module. Temperature was controlled automatically; the heating circuit was powered by a conventional heatboy (Heatboy Type E-Tech W 09).

All conversion tests were conducted in 50 mM sodium citrate buffer (citric acid monohydrate and tri-sodium citrate dihydrate, Merck Millipore) at pH 4.8, at 50 °C.Glucose formation was measured in the form of glucose equivalents by density assessment with DMA 35 (Anton Paar) whereas the samples were prefiltered with MN 615; 11 cm filters (MACHEREY-NAGEL). Conventional Dinitrosalicilic (DNS) assay was used for the evaluation of the glucose equivalents measured with the DMA device. Heat transfer coefficients were evaluated based on flow and temperature conditions of the heated double-wall reactor modules monitored with Flexim ultrasonic measurement equipment.

The switchboard installation included one main power meter (Neris – DVHS241-M) to capture the total plant power consumption accurately. In addition, seven bypass power meters (DDS 353) were set up for monitoring the power consumption of the operating units separately. Three frequency converters (VLT Midi Drive FC 280) were installed to ensure the correct power supply of the built-in three-phase asynchronous motors. Finally, the momentary electric power consumption, expressed in Watts, was calculated from the recorded data.

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STR reference tests were conducted in a conventional Dasgip stirred tank bioreactor (1.5 L fed-batch-pro\textsuperscript{®} bioreactor system) equipped with a six-bladed Rushton turbine impeller (DASGIP AG) with 500 mL working volume. Celic Ctec2 enzymes (Novozyme Corp) and pure α-cellulose (Sigma Aldrich) were used. An energy logger 4000 (Voltacraft) was applied for power measurements.

\textbf{OFB reactor design}

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All conversion tests were conducted in 50 mM sodium citrate buffer at pH 4.8, at 50 °C. Enzyme loading was at 10 Filter Paper Units (FPU); the experiments ran for 24 h hours and glucose formation was analysed with the conventional Dinitrosalicylic (DNS) assay.

The correlation between the STR and the OFB systems was based on the respective power density equations. The impeller speed of the STR was calculated to correspond to a frequency setting of a 2 mm and 3 mm helical baffle, respectively in the OFB system. The formula for the theoretical power input for mixing operations in STRs was as follows:

$$P = \frac{P_0 \rho N^3 D_b^4}{\pi D_{bi}^2 l / 4}$$

where $P_0$ is the power number of the impeller (4.6), $N$ is the impeller rotational speed (rps), $D_b$ is the impeller diameter (m), $D_{bi}$ is the vessel diameter (m), and $l$ is the height of the mixture in the reactor (m).

The formula for estimations of the power density, expressed in Watts per cubic metre (W m$^{-3}$), in OBR systems is given as follows:

$$P = \frac{2 \rho N_h l - \alpha - X_0 (2 \pi f)^3}{3 \pi \eta D_b C_D}$$

where $\rho$ is the density of the fluid (kg m$^{-3}$), $N_h$ is the number of baffles per metre, $C_D$ is the discharge coefficient and $\alpha$ is the baffle orifice area to the tube area ratio. $X_0$ represents the oscillation amplitude (m), and $\omega$ is the angular oscillation frequency (rad s$^{-1}$). In conformity with other studies, the value of $C_D$ was defined to be 0.7, and $\alpha$ was given with 0.71 (2 mm baffle) and 0.58 (3 mm baffle), respectively, and $N_h$ amounted to 26.67. Table 1 shows the calculated relation of STR and OFB systems.

### Table 1 – Impeller speed setting of STR and corresponding frequency setting of OFB

| Impeller speed STR | 2 mm helical baffle [Hz] OFB | 3 mm helical baffle [Hz] OFB |
|--------------------|-----------------------------|-----------------------------|
| 60 rpm             | 0.91                        | 0.72                        |
| 120 rpm            | 1.82                        | 1.44                        |
| 250 rpm            | 3.78                        | 3.00                        |
| 500 rpm            | 7.56                        | 6.00                        |

**Enzymatic hydrolysis**

The enzymatic saccharifications were conducted with pure α-cellulose at 9 %, 12 % or 15 % (w/v) mixed with the corresponding amount of 50 mM sodium citrate buffer. For the preparation of 500 mL buffer, 2.194 g citric acid monohydrate and 4.281 g tri-sodium citrate dihydrate were diluted in distilled water, and the pH was adjusted to 4.8 with 5 M NaOH.

Enzyme loadings of 8 FPU or 10 FPU of Cellic CTec2 cellulase enzyme blend per gram cellulose...
were used. All conversions were examined at a controlled temperature inside the reactor of 50 °C ± 0.8. Samples were taken at different positions along the reactor length (top, middle, bottom, or as indicated) at sample volumes of minimum 5 mL, with a specially adjusted syringe at 0 h, 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 12 h, and 24 h. The remaining particles were removed by filtration of the samples. Glucose concentration was calculated based on the density measurement from the DMA 35 density meter. To validate this concentration measurement method, several samples of known concentration were analysed using the DNS standard method accordingly. If the sugar concentration analysis was not conducted immediately after taking the samples, then the samples were stored at 4 °C to minimise further conversion reactions. Consistent results proved sufficient accuracy and reproducibility of the DMA measurement, as depicted in Table 3.

For the determination of cellulose and glucose gradients along the reactor length, 10 – 20 mL samples were taken at different distances from the oscillatory piston throughout the reactor length.

The double-jacketed OFB module and the α-cellulose – buffer slurry were preheated to 50 °C separately before filling the reactor. Respective oscillation conditions were set and the reaction was started with the addition of the enzyme blend to the bioslurry. Uniform particle distribution at all tested concentrations was observed, and neither air pocket nor dead zone formation occurred. Defined conditions (15 % SL; α-cellulose, 50 °C, 24 h, OFB: 2.05 Hz, 10 mm amplitude, 3 mm baffle, STR: 500 rpm) were referenced to lab-scale STR- experiments in terms of glucose formation after 24 h.

Concentration measurement

Within this study, an alternative method for the measurement of the produced sugar concentration throughout the experiment was developed. Usually, the DNS standard method is applied for glucose concentration measurement; however, in this study, density measurements were related to the concentration, which showed sufficient accuracy and reproducibility for this experimental set-up. The samples were filtered, cooled to room temperature, and measured twice with the DMA 35 density meter. The temperature dependence of the DMA device was determined, and a correction factor was included in all subsequent measurements. The measurement technique is based on the fact that the quantity of dissolved sugars of a solution defines its density. The density showed a strong dependence on temperature and pressure, a temperature coefficient of 0.0003 g cm⁻³ K⁻¹, valid for most aqueous solutions up to 20 % was set. Thus, a calibration curve was generated at four glucose concentrations (0, 10, 20, 50, 100 mg mL⁻¹ in 50 mM sodium citrate buffer).

Each concentration was prepared and tested twice. The pure citrate buffer gave density values of 1.0065 – 1.0067 g cm⁻³. For all further calculations, the average 1.0066 g cm⁻³ was used. Using the sum of least squares method and the available data, the following regression was derived:

\[
\text{Density} = 0.000370 \cdot \text{conc.} + 1.0066 \quad (4)
\]

Table 2 – Oscillatory Reynolds numbers for hydrolysed and unhydrolysed samples

| Frequency [Hz] | Re₀ unhydrolysed (12 % SL) | Re₀ hydrolysed (12 % SL) |
|---------------|----------------------------|-------------------------|
| 1.5           | 0.02                       | 200.31                  |
| 2.05          | 0.03                       | 273.75                  |
| 3.55          | 0.06                       | 474.06                  |

Results and discussion

Flow characteristics in the OFB reactor

The OFB system was typically operated between 1.5 – 3.5 Hz. Applying eq. 1, the resulting oscillatory Reynolds numbers Re₀ were largely dependent on the state of hydrolysis and the respective viscosity of the medium. The calculated Reynolds numbers of the OFB system with 12 % solid loading ranged from 0.02 to 474, as presented in Table 2, whereas the unhydrolysed slurry showed significantly lower Reynolds values, a factor of 8296 over the time course of 6 hours, than the corresponding hydrolysed sample. Full turbulence in OBRs is achieved at Reynold numbers above 2000. Therefore, there is still huge potential for improvement regarding mixing quality, taking into account that, e.g., doubling the length of amplitude would bring doubled Reynolds number.

Influence of physical feedstock parameters on the power requirement

The basic reactor set-up consisting of one module in batch mode was chosen for the first series of tests. Energy input measurements of the oscillatory piston on different media such as air, water, 5 %, 18 % α-cellulose, 50 % and 100 % glycerol concentration were conducted.

Fig. 2 pictures the oscillatory power requirement as the relative power increase on air measurements for various fluids at a fixed oscillation amplitude of 6 mm. The resulting oscillatory power requirement is not significantly correlated to the viscosity of the feed media, allowing the treatment of viscous slurries at low energy intensity. Within
this test series, it could be shown that neither density nor viscosity had a direct measurable influence on the oscillatory power requirement. Even when comparing water and 18% cellulose slurry, only minor deviations on the power requirement for the oscillation were measured, whereas the oscillation frequency had an obvious and direct influence on power requirement in any media, indicating that the motor characteristics were most decisive for the measurement results. The mixing results proved that frequencies > 2 Hz would be sufficient for mixing, meaning that the power requirement of the OFB can be minimised by optimising the required frequency at around 2 Hz. Oscillatory power gave a linear trend referring to an average power consumption measured, as shown in Fig. 3.

The effect of the reactor length is depicted in Fig. 4, whereas it can be clearly seen that doubling the reactor length only resulted in modest increases in power requirement. When operating at 3.5 Hz, a power requirement increase of 2% was observed, and at 5 Hz a 16% increase was observed. These numbers give a further indication that the engine power of the oscillatory pump itself overlaps smaller influences on the system, which therefore cannot be monitored in a precise manner.
In Fig. 5, the recorded power data based on minutes is shown as the mean power consumptions during time periods of 60 minutes, and for the overall operating time of 6 hours. Due to strong drops in overall viscosity (particle size), a decrease in the power consumptions over time was expected and confirmed. The slope of the correlating function reveals an overall decrease in power consumption of \(-1.59\) W per hour, resulting in a mean drop of \(-9.52\) W within 6 hours, which is related to the overall viscosity drop within the conversion reaction. On average, decrease in power consumption of \(-8.3\) % within 6 hours was recorded. The power consumption at 1.5 Hz was stable over the entire duration of the experiment. This can likely be explained with a lowered degree in solids distribution (homogeneity) in the reactor, since sufficient homogeneity was identified at 2.05 Hz and above. Hence, the sedimented solids are expected to accumulate in front of the piston, which might result in a constant resistance to the moving piston overpowering the influence of the viscosity.
The absolute power consumption in the given system is very high. Since the idling system consumes high amounts of electricity for a 1.1 kW – engine, the influence of the process media on the overall energy demand is certainly quite small and therefore difficult to detect within this system. Further on, external influences such as the operating temperature (± 9–18 W, 24 h) or the extent of lubrication (±5.41 W) were identified to potentially overpower certain effects caused by alternating the process media.

Fig. 6 shows two different baffle thicknesses used in 24-hour experiments at 15 % solid loading. Baffles with thicknesses of 2 mm and 3 mm were tested, whereas, a reduced inner open diameter at the 3 mm baffle only showed a minor effect on the glucose concentration; at higher frequencies this effect became nearly negligible. Therefore, when analysing the power consumption, hardly any effect of baffle thickness could be observed.

**Homogeneity of mixing along the reactor length**

The glucose concentration distribution along the reactor length is shown in Fig. 7. 12 % α-cellulose slurries were enzymatically converted under the following conditions: 6 h reaction time, 10 mm amplitude; 3 mm baffle thickness, 0.5 m reactor length, and a horizontal configuration. At the end of the experiment, samples were taken at different distances from the oscillation piston along the reactor length, and glucose concentrations were measured. Optimal mixing could be achieved at oscillation frequencies ≥ 2.5 Hz, and sufficient mixing was observed at frequencies above 2 Hz. At 1.5 Hz, differences (max. 9 %) in concentration along the reactor length (0.5 m) could be measured.

**Influence of various solid loadings on product formation**

The glucose concentrations for 6-hour experiments with solid loadings of 9, 12, and 15 % were compared (Fig. 8). Experiments were conducted in a 500-mL horizontal OFB set-up at 50 °C and 10 FPU enzymes. The series of experiments was conducted with a 10 mm oscillation amplitude and 2 mm helical baffles. The graph shows one replicate trial at each condition (dashed and solid line). Very consistent glucose formation can be observed with steadily increasing concentrations at higher solid loadings after 6 h.

Additionally, no substantial effect was observed of the angle of the reactor tested in a range of 0° to 90°, from a completely horizontal to completely vertical configuration (Fig. 9). However, a slight upward inclination (< 10°) of the OFB module helped to remove interfering air inclusions, but the shortest reactor design in a vertical position was preferred for this test series to facilitate handling.

For the evaluation of the influence of the amount of enzyme added, a test series at 3.7 Hz oscillation frequency and 2 mm baffle was conducted yielding a 9 % decrease in glucose formation when the enzyme added was decreased from 10 FPU to 8 FPU, shown in Fig. 10. In a further series of experiments, additional parameters, such as lower fre-
Fig. 7 – Glucose concentration distribution along the reactor length (0 at oscillator piston) (α-cellulose, 6 h, 10 mm amplitude, 3 mm baffle, 10 FPU, 50 °C)

Fig. 8 – Glucose concentration at various solid loadings; 9, 12, 15 % α-cellulose (α-cellulose, 10 mm amplitude, 2 mm baffle, 10 FPU, 50 °C, 6 h)
quency (2.1 Hz) and 3 mm baffle, were optimised yielding an only 6 % decrease in glucose formation while using 20 % less FPU of enzyme blend.

**Enzymatic hydrolysis in OFB compared to STR after 24 h**

Finally, the OFB and STR enzymatic hydrolysis results were compared. To assure comparability between DMA and DNS measurements, both measurement techniques were applied to identical samples; only minor deviations were observed (see Table 3). Table 4 shows the OFB and STR hydrolysis results for various operating conditions.

In Fig. 11, the expected shape of a typical cellulose hydrolysis curve in terms of the initial rapid conversion phase and the subsequent slower phase is shown. This phenomenon is frequently related to the breakdown of the mainly amorphous sections of the cellulose in the start of the bioconversion, followed by the more recalcitrant and crystalline part.
Both set-ups were run at 15 % solid loading with pure α-cellulose and 10 FPU of enzymatic blend in batch mode for 24 hours. The STR stirrer speed was set to 500 rpm and in the OFB set up a frequency of 2.1 Hz was chosen. According to eq. 2 and eq. 3, this would mean a lower power density of 85.8 W m\(^{-3}\) compared to a 1259.5 W m\(^{-3}\) input; however, there is a strong indication that a better approximation for this OFB set-up has to be developed in further studies, as discussed previously. The increase in the formed sugar was measured via glucose concentration increase by the DMA measurement (OFB) and

![Graph showing glucose equivalent concentration over time](image)

**Fig. 11** – Comparison of enzymatic hydrolysis of STR and OFB (15 % SL; α-cellulose, 50 °C, 24 h, OFB: 2.05 Hz, 10 mm amplitude, 3 mm baffle, STR: 500 rpm)

### Table 3 – Comparison of sugar concentrations measured by DMA and DNS assay

| Sample [Exp. No.] | Density No. 1 [g cm\(^{-3}\)] | Density No. 2 [g cm\(^{-3}\)] | Mean density [g cm\(^{-3}\)] | Conc. DMA method [g L\(^{-1}\)] | Conc. DNS method [g L\(^{-1}\)] | Deviation [g L\(^{-1}\)] | Deviation [%] |
|-------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|-----------------|---------------|
| B_16              | 1.0202                        | 1.0206                        | 1.0204                        | 37.3                           | 38.11                          | -0.81           | -2.13         |
| B_17              | 1.0267                        | 1.027                         | 1.0269                        | 54.73                          | 52.76                          | 1.97            | 3.73          |
| B_18              | 1.0214                        | 1.0215                        | 1.0215                        | 40.14                          | 40.11                          | 0.02            | 0.05          |
| B_19              | 1.0232                        | 1.0235                        | 1.0234                        | 45.27                          | 44.22                          | 1.05            | 2.37          |

### Table 4 – Overview of experimental data: STR – OFB system comparison

| % SL | Stirring speed [rpm] | Power density [W m\(^{-3}\)] | Sugar formation [g L\(^{-1}\)] | Frequency [Hz] | Power density [W m\(^{-3}\)] | Sugar formation [g L\(^{-1}\)] | Other parameter |
|------|----------------------|-------------------------------|--------------------------------|----------------|-------------------------------|--------------------------------|-----------------|
| 12 % SL | 60                   | 1.43                          | 65.54                          | 1.5            | 19.45                         | 69.19                          | 3 mm B.         |
|       | 120                  | 11.46                         | 66.73                          | 2.05           | 49.64                         | 70.54                          | 2 mm B.         |
|       | 250                  | 103.64                        | 66.29                          | 2.05           | 49.93                         | 82.16                          | 3 mm B.         |
| 15 % SL | 500                  | 1042.23                       | 77                             | 2.05           | 49.93                         | 77.3                           | 3 mm B./ 8 FPU g\(^{-1}\) |

Both set-ups were run at 15 % solid loading with pure α-cellulose and 10 FPU of enzymatic blend in batch mode for 24 hours. The STR stirrer speed was set to 500 rpm and in the OFB set up a frequency of 2.1 Hz was chosen. According to eq. 2 and eq. 3, this would mean a lower power density of 85.8 W m\(^{-3}\) compared to a 1259.5 W m\(^{-3}\) input; however, there is a strong indication that a better approximation for this OFB set-up has to be developed in further studies, as discussed previously. The increase in the formed sugar was measured via glucose concentration increase by the DMA measurement (OFB) and
DNS assay (OFB and STR). The deviation between the DNS and DMA method ranged from 0.02 to 1.97 g L⁻¹. This refers to a maximum deviation percentage of 3.7 %.

The enzymatic hydrolysis showed higher conversions (OFB = 82.16 mg mL⁻¹ corresponds to 6.7 % higher concentration than at STR setting) in the OFB system, and the power density of the OFB was only at 7 % that of the STR.

Conclusions

Within this study, a modular Oscillatory Flow Bioreactor (OFB) for bioconversions at high solid loadings was designed and built. The modular set-up including four independently employable reactor modules with 360° adjustable angles was found to deliver very reliable results. Furthermore, a method for accurate glucose concentration determination was developed using a DMA density meter, and validated using DNS assays. The effects of amplitude, frequency, reactor angle, reactor length, sampling method, and enzyme addition (FPUs) on the system energy requirement and the overall conversion were studied.

The evaluation of the results was mainly based on achieved glucose concentrations and energy input. Power densities were initially calculated via eq. 2 to relate the STR power input to the OFB power input. Throughout the study, the measurement results showed that the measured power requirement for the oscillation \( W_{osc} \) was much less dependent on factors such as oscillation amplitude, medium density, and reactor length than was expected. The evaluation of the experiments points out that the motor in the set-up is too large to enable precise measurement of influential factors. The results indicate the need for a new correlation to be developed on the power requirement for OFB systems with helical baffles on the one hand, and a more harmonised system set-up in terms of engine power and power density measurement on the other hand.

The main aim of this study was to conduct the enzymatic hydrolysis of cellulosic material at high solid loadings, which was achieved with the OFB system, allowing the handling of high viscous slurries, and resulting in equal or better conversions compared to state-of-the-art reactors. The frequency shows a direct influence on the power requirement in all experiments, whereas in terms of mixing, an optimal frequency of around 2 Hz was determined because it generated sufficient turbulence for high conversions and minimal shear stress for the activity of the enzyme.

The scale-up ability of the OFB plant design was practically tested by comparing the use of one single reactor module and the use of more modules in series.

Qualitative and quantitative analysis of the glucose concentration distribution along the reactor module showed excellent results at oscillation frequencies above 2.1 Hz at high solid loadings (15 % α-cellulose). The effect of the angle of inclination of the reactor were insignificant.

The objective of ultimately applying less enzyme was tested at ratios of 10 and 8 FPU. A 20 % decrease in enzyme use only decreased glucose formation by 6 %.

Finally, in the 24-hour hydrolysis experiments, conversion rates of the OFB system were higher than those of the STR. This proves that the OFB system is a promising reactor that can achieve comparable or slightly better results than an STR in batch processes. The OFB also has potential to enable continuous processing when superimposing the oscillatory motion over a very slow net flow.

This study is ongoing; the primary objective is running future semi-batch and continuous mode experiments with different kinds of lignocellulosic media, and, if possible, at higher solid loadings and for at least 24 h. Additionally, further improvement in enzyme dosing and sampling will be investigated in the design optimisation steps.

Nomenclature

- \( a \) – Baffle orifice area to tube area ratio
- \( C_D \) – Discharge coefficient
- \( D_v \) – Vessel diameter, m
- DNS – Dinitrosalicylic assay
- \( D_s \) – Impeller diameter, m
- FPU – Filter paper units
- \( f \) – Oscillation frequency, Hz
- \( f_{geo} \) – Function of geometry
- \( f_{\rho} \) – Function of density
- \( l \) – Height of mixture in STR, m
- \( N_b \) – Number of baffles per meter
- \( N \) – Impeller rotational speed, rps
- OBR – Oscillatory Baffled Reactor
- OFB – Oscillatory Flow Bioreactor
- \( P \) – Power, W
- \( P_\omega \) – Power number of the impeller
- \( Re_n \) – Net flow Reynolds number
- \( Re_O \) – Oscillatory Reynolds number
- RPS – Impeller rotational speed
- \( \rho \) – Density of fluid, kg m⁻³
- STR – Stirred Tank Reactor
- \( \Omega \) – Angular oscillation frequency, rad s⁻¹
- \( X_o \) – Oscillation amplitude, m
- \( \mu \) – Fluid density, kg m⁻¹ s⁻¹
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