# Evaluation of a novel type of reactor for the realization of continuous enzymatic hydrolysis of lignocellulosic materials.

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December 2019

**Abstract**

Nowadays there is a high consumption of plastics, fossil fuels and a wide range of chemicals, which generate a variety of impacts on the environment. There is a need to provide more sustainable pathways to generate products that can replace the fossil fuel-based industry. Biorefineries have a high potential to provide a variety of products from lignocellulosic materials. Residues of forestry and agriculture are available in great quantity and can be processed to obtain a wide range of products, such as biofuels, bioplastics and biochemicals. In this context, the project 'OSCYME' was launched in 2017 to test a new type of reactor, the Oscillatory Flow Bioreactor (OFB). The aim of this work was to continue previous work, this time testing the reactor in continuous mode to assess the enzymatic hydrolysis of pure α-cellulose. Tests were conducted in OFB batch mode and OFB continuous mode and compared to results of hydrolysis obtained in a stirred tank reactor (STR). The results show a great potential for the OFB continuous mode, yielding 14% more glucose equivalent than STR even when considering the maximum error. Compared to the STR, the OFB presents improvements in vessel size, solid loading processing and energy consumption, all leading to a reduction of costs. Nevertheless, a number of challenges remain, and further studies need to be conducted to better understand the mixing capacities and enzyme-substrate binding in the OFB.

**Keywords:** Lignocellulosic materials, enzymatic hydrolysis, oscillatory baffled reactor, cellulose, glucose production

## 1. Introduction.

Nowadays, most of the plastic, fuels as well as a wide range of chemicals that we consume are produced mainly from fossil fuels and petrochemical industry. These products are consumed vastly around the world, leading to major environmental issues. In addition to the environmental impacts, the demand for transportation fuels, chemicals and plastic for packaging will continue (and increase), which constitutes yet another problem considering that the sources of fossil fuels are finite [1,2]. Due to the aforementioned problems, there is a need to boost biorefineries in order to provide more sustainable solutions to fulfil the need and demand of transportation fuels, chemicals and plastics.

There is high interest on the biorefining of lignocellulosic materials, which are composed of cellulose, hemicelluloses and lignin [3]. These materials are the only foreseeable resource for sustainable carbon to support the rapidly rising demand for energy and chemicals [4]. They are available in abundance [1] and currently a large amount is discarded as waste, which could be given a second life. However, there are several challenges yet to be overcome to make this type of biorefinery attractive and competitive for commercialization. Nevertheless, there is interest in this area and ongoing research to make this type of industry more feasible.

In this context, the project ´´OSCYME´´ was launched in 2017 initiated by the Institute for Sustainable Technologies (AEE INTEC) in partnership with the University of Newcastle, Austrian Centre of Industrial Biotechnology and Möstl Anlagenbau GmbH A new type of reactor was developed with the intention to optimize the formation of fermentable sugar from enzymatic hydrolysis of ligno-cellulosic waste material. The designed bioreactor, OFB, potentially decreases the energy demand and the amount of enzyme required, while at the same time increasing the yields of obtained sugar, resulting in a reduction of costs.

This work is developed with the objective to continue the former work on the laboratory plant [5] and test the OFB in a different set-up, this time as a semi-continuous process, incorporating new elements to the system, such as a net flow pump. Hydrolysis tests, energy measurements and total solids were conducted and compared with OFB batch and STR results.

## 2. Fundamentals.

2.1 Lignocellulosic biomass.

In the last decades there has been an urgent need to develop more sustainable energy systems and industries based on renewable biomass, such as lignocellulosic biomass, due to the increase in demand for energy and the rapid depletion of fossil fuels [6]. These materials show great potential for the sustainable production of chemicals and fuels [1] and do not generate problems related to food competition.

The process to obtain glucose monomers from cellulose via hydrolysis is called saccharification. The glucose obtained can be further processed to produce biofuels such as bioethanol and biobutanol [7].Enzymes are needed for the biologic saccharification and have a high cost [8]., which makes the price per gallon of biofuel not competitive in today's market. In general, a number of steps need to be improved in order to make the lignocellulosic biorefinery more attractive commercially.

Lignocellulosic biomasses are composed in the greatest part by cellulose (35 – 50%), hemicellulose (20 – 35%), lignin (10-25%) and other components such as pectin, ashes, lipids and protein in smaller amounts [1]. Cellulose, hemicellulose and lignin are highly organized forming complex matrices [9]. The glucose in cellulose is present in the form of cellobiose, which is a disaccharide. Cellulose has a mixture of amorphous and crystalline regions, the amorphous region being easier to be decomposed by enzymes than the precisely arranged crystalline areas [10]. At the same time, it is surrounded by lignin and hemicellulose, which makes it hard to directly hydrolyse lignocellulose [11]. To facilitate the hydrolysis, lignocellulosic materials are pre-treated, to isolate the components and decrease the crystallization of the cellulose.

After the cellulose is obtained, it can be hydrolysed via chemical or biological paths. In this study, the biological path is used, since higher glucose yields can be obtained at lower energy costs and milder operating conditions.

A blend of enzymes is used, combining endoglucanases (endo-1,4-β-glucanase), cellobiohydrolases (exo-1,4-β-glucanases), and β-glucosidases [12] that work together to achieve the complete hydrolysis of cellulose. The group of enzymes that are currently used for the saccharification of cellulose are inhibited by the presence of glucose and cellobiose, which restricts the yield of glucose that can be obtained. [13]. The inhibitions mentioned, are known as product inhibition, which happens when the substrate binding capacity of the enzyme is decreased with a higher product concentration. This means that during the length of the reaction, as more glucose is being produced, the more the enzymes are inhibited. This affects the reaction rates and extends the duration of the reaction. The reactor design has an important role regarding the inhibitions, and by improving it, the conversion efficiency can be raised.

The main reactions in the enzymatic hydrolysis are the breakdown of cellulose by cellobiohydrolases (CBH) and endoglucanases (EG) into cellobiose (path 1, fig 1) followed by the catalytic cleave of cellobiose into glucose by β-glucosidases (ßGs) (path 2, fig 1).

Regardless of the numerous attempts by different combinations of pre-treatment and hydrolysis, in general the total sugar concentration obtained range of 30-80 g/L [12].

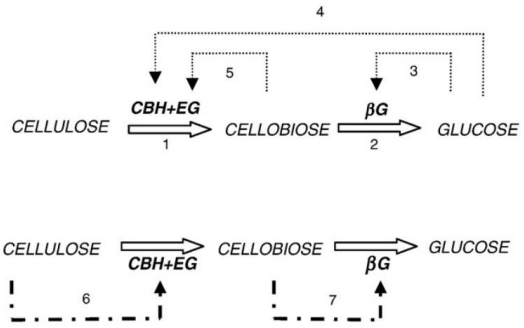



**Figure 1. Main reactions and inhibitions during enzymatic hydrolysis. Main reactions (1, 2), glucose inhibition (3, 4), cellobiose inhibition (5), substrate inhibition (6, 7).** [14].

Enzyme loadings are typically around 15 FPU/gram cellulose, needing a high amount of enzymes for the processing of cellulose adding considerable costs. To tackle this problem, there are two things that can be improved. On one hand, lowering the costs of the enzyme production and on the other, working on the development of mechanisms and technologies for the reaction to need a lower enzyme loading to obtain the desired glucose yields. The oscillatory flow bioreactor was designed with the purpose of improving the hydrolysis process and reducing the costs associated to it.

For the experiments, pure α-cellulose is used, obtained from sigma-aldrich and the enzyme blend Cellic Ctec2 for the hydrolysis experiments.

2.2 Reactors for the processing of lignocellulosic materials.

The lignocellulosic biomass is commonly processed in batch reactors. A batch process consists of a simple flowsheet employing standard unit operations, such as mixing and heating. A main characteristic of this mode is its flexibility since the configuration can be adapted to fit the needs. The most common type of reactor for this kind of process is a stirred tank reactor (STR), which is composed of a closed tank with a motor that is connected to an agitator, mixing the materials in the tank. Other types of reactors that can be used are tubular reactors.

A process which has a higher productivity than batch is a continuous process. In this case the material is carried as a flowing stream, usually with the reactants being fed continuously to the system while the products are being extracted.

2.2.1 Oscillatory flow bioreactor

In order to potentially overcome the challenges associated to the hydrolysis of lignocellulosic materials, a new type of reactor is needed, were a continuous mode can be implemented, removing the products to restrain the inhibition effects.

One of the innovative concepts for reactors is the continuous oscilattory baffled reactor (COBR). This type of reactor has a tubular shape, where a baffle is situated on the inside of the reactor and the fluid in it is submitted to an oscillation. The fluid oscillates and bumps into the baffle, where the mixing is induced by the formation of eddies. This allows the net flow to be uncoupled from mixing, allowing longer residence times which is good for long reactions. By controlling the oscillatory movement, the degree of mixing can be adjusted [15]. In order to avoid the sedimentation of the cellulosic material and to achieve suitable conversion rates, it is necessary to have a sufficient mixing that can ensure homogeneity in the solid-liquid suspension. A good mixing provides the enzymes with good access to the substrate and can also remove some of the reaction limiting concentration gradients. Additionally, using technologies that incorporate an oscillatory baffled reactor has proven to reduce the power density needed to enzymatically hydrolyse alpha cellulose to 94-99% for maximum conversion rates when compared to a stirred tank reactor [16].

A continuous oscillatory flow reactor concept (OFB), for effective hydrolysis of lignocellulosic materials was set-up in the early stages of the 'OSCYME' project. This OBR reactor was designed specifically for bioprocesses. Preliminary tests to evaluate the flow and mixing behaviour were conducted at the University of Newcastle in order to optimize the process conditions. This was followed by enzymatic tests performed at AEE INTEC with the OFB pilot plant [5]. Among the findings, it was identified that the best baffle was the helical baffle, as opposed to single and multiple orifices baffles, since it provided a good mixing quality when combined with the oscillatory movement.

In conventional tube reactors, turbulent flow regimes are usually induced by higher flow rates, this results in lower residence times which are conditioned by the length of the reactor. The desired turbulence can be achieved in an oscillatory baffled reactor (OBR) with the oscillation, providing a better mixing and since the net flow conditions can be disjoined a better residence time distribution can be reached in order to process long reactions. Additionally, the adjusting of the conditions for the oscillation parameters can set different mixing conditions [17]. In general, the chemical conversion of large amounts of materials is more economical and feasible in reactors that have a continuous regime in contrast with batch reactors [18].

Using an OBR in continuous mode has shown to have a higher degree of plug flow characteristics, using a helical baffle. This effect is beneficial for the mixing and is achieved by a combination of swirl flows and vortices [19]. The plug flow effect in an OBR is reached for Reynold numbers ranging from 50 to 800, which is strongly linked to the oscillatory amplitude which affects the flow characteristics. A plug flow is different from laminar flow and turbulent flow, and does not have a radial velocity gradient, which means every particle in the flow experiences the same residence time (fig 2).

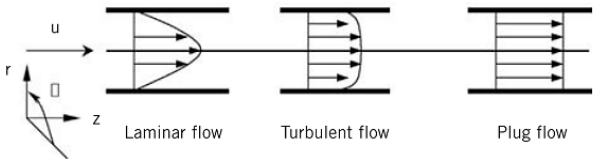



**Figure 2. Different types of flow and their explanatory illustration by** [15].

Additionally, the rounded shape of the baffle has proved to reduce the deposits of cellulose during the mixing operations in the preliminary experiments conducted in UNEW. The absence of flat exposed surfaces is the explanation for this.

The intensification potential of bioconversion processes is high, due to the fact that most of these processes take place in stirred tank reactors, often in batch mode. The limitations of STRs are well known and have been studied and identified in literature and, therefore, there is a lot of room for improvement.

The use of OBRs in continuous mode can potentially be the solution for these limitations, adding the oscillations and the baffle enhances the mixing while increasing the residence time, and has the flexibility to be configurated both in batch mode and in a more productive continuous mode, the being a better match for long reactions [20,21]. Furthermore, the mixing in the OBRs is not dependent on the net flow, which means that smaller reactor sizes are effective [22].

## 3. Experimental.

The Oscillatory Flow Bioreactor (OFB) was specifically designed within the ´´Oscyme´´ project with the goal of processing bio-slurries containing up to 18% w/v solid loadings of cellulosic materials. The concept is based on studies on OBR technology [20,22,23] and experimental studies have been conducted at the University of Newcastle [16,24] as well as the first experimental tests conducted in the pilot plant located at AEE INTEC. The aim of this work is to extend the testing series and set an appropriate layout for the plant in continuous mode, running tests to measure the glucose yield.

The oscillatory power depends on the set frequency and amplitude on the oscillatory pump, therefore, by regulating these parameters the overall mixing quality can be determined. This is the main feature of the OBR systems and it is an important characteristic that was incorporated

in the design of the OFB. The OFB pilot plant concept was designed for continuous enzymatic processing of lignocellulosic materials, using low net flows and at the same time providing a good mixing by introducing the plug flow behavior that was previously discussed. The plant is expected to achieve high mixing quality and homogeneity by using low energy inputs, with the possibility of integrating waste heat recovery to the heat exchanger.
The plant is composed of four jacketed OFB modules that are heatable and their positions can be adjusted in angle and inclination. A closed heating system was incorporated to maintain a constant temperature through the system. Additionally, a circular pump system is in charge of both the substrate feed and the product collection. The plant can be seen in figure 3.

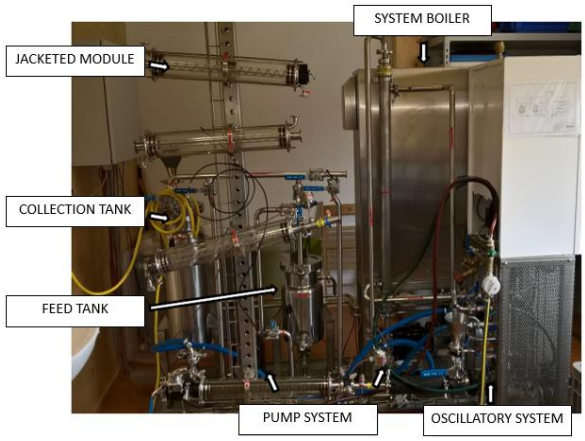



**Figure 3. OFB pilot plant with one module connected.**

During this study, only one jacketed OFB module was used. A first set of experiments was conducted using a batch set up, testing two different methods of enzyme addition and sampling in two areas of the reactor. The configuration of the system was then changed to fit a continuous mode set up connecting a net flow pump to the system and samples were taken to measure the glucose yield in the reactor. The OFB plant could be easily monitored and operated by a central software that was specifically designed for it. The main operating units such as the heating system and oscillatory pump, could be controlled and the power of the engines were able to be set precisely in percentage numbers. Temperature monitoring systems were installed to make sure the appropriate temperature was kept on the system. The system boiler and peristaltic pump could also be monitored and controlled through the software, being able to heat the water at the desired temperature and pump the flow through the system at the desired velocity respectively.
Frequencies associated to each engine power configuration were previously calculated by [5], the needed value could be easily entered in the software to obtain the desired operation frequency.

The plant was designed to be modular, and for the purpose of this study, two configurations were used; OFB batch and OFB continuous mode. Both configurations only used one jacketed module (M as seen in the batch configuration). The batch configuration was simpler, connecting the jacketed module in a vertical position to the oscillatory pump, as seen in figure 4, with the help of modular parts L and T.
For the continuous mode configuration, more modular parts were needed, in order to connect the jacketed module to the net flow pump creating a continuous flow through the system. The layout for the OFB continuous mode can be seen in figure 5.


**Figure 4. OFB batch configuration**

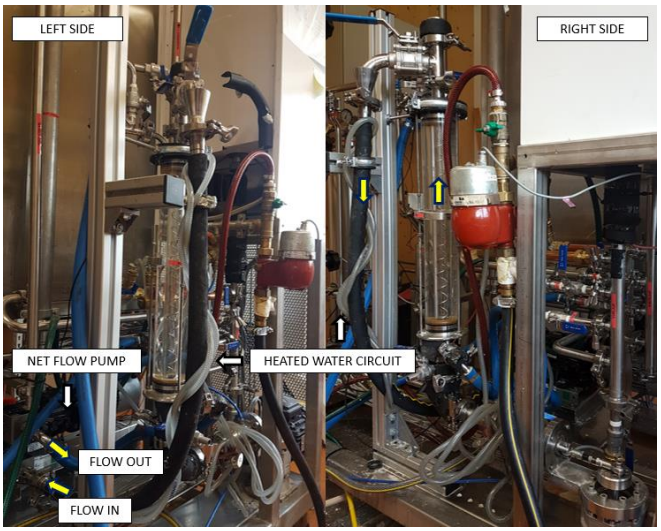



**Figure 5. OFB continuous configuration seen from the left and right sides.**

3.1 Methodology.

3.1.1 Experimental procedure.
To prepare for the experiments, 50mM citrate buffer was first prepared and stored in a fridge. The buffer was prepared with 21.94 g of citric acid monohydrate and 42,81 g of tri-sodium citrate dihydrate for a volume of 5 L. The chemicals were first dissolved in one litre of deionized

water with the help of a magnetic stirrer. Once 80% of the requested volume was reached, the pH – value was adjusted to 4,8 with 5M NaOH, then the last litre was poured into the container. Up to 10 L of buffer were stored in the fridge at a given time. In the same fridge, the enzyme bottles were stored to preserve them.

Once the desired system was set-up, the first thing was to switch on the water heating circuit to regulate the temperature in the jacketed module, setting the temperature in the software. The water jet for the oscillatory pump lubrication was then opened to enable the use of both the oscillatory pump and net flow pump. The oscillatory pump and the net flow pump desired engine power in percentage was entered in the system. While the system was heating up, the buffer was pre-heated to 50°C in the laboratory stirrer, stirring with a magnet to even out the temperature throughout the glass beaker. The cellulose was weighted and mixed with the buffer once it reached 50°C. The solid loading was defined as 11-12% for most of the experiments. This was the usual procedure followed for all experiments, both in batch and continuous mode. Experiments that lasted up to 45 hours were conducted, with focus on 24-hour experiments. To clean the system, once the experiment was concluded, the system was emptied with the oscillatory pump still running and net flow pump in case of a continuous mode. Then, the system was filled with water while the oscillation helped to remove the particles that remained in the system. Finally, the system was emptied and occasionally completely disassembled to thoroughly clean it. The heating and pumps were then shut down and the water flow for lubrication closed.

Ink experiments were conducted for the continuous mode at different solid concentrations, with two objectives: first, measuring the residence time of the particle and second, identifying the presence of plug flow behavior. The procedure consisted on the filling of the system with the desired concentration of cellulose diluted in water. Once the system was full and with both the oscillatory and net flow pump running, purple ink was added in one of the open parts of the system and a timer was set to start at that moment. Once the ink was visibly coming from the other end of the reactor, the timer was stopped, and that counted as the measured residence time of the particle. Additionally, the flow was measured at the different concentrations and the residence time was calculated based on the total volume in the system, which was 1100 ml.

A variety of experiments were conducted in batch and continuous mode. The method of enzyme addition is noted IA for inline addition, when the enzyme was added directly into the reactor, and PA, as in pre-addition, for when the enzyme was added outside the reactor, in the mixture of cellulose and buffer. In all experiments the oscillatory pump operated at 26% power, meaning 3,55 Hz of frequency, with an amplitude of 10 mm.

3.1.2 Sampling procedure and analysis

Samples for hydrolysis were usually taken during the first 6 or 7 hours of the experiment in an hourly basis, sometimes the first half an hour was measured as well. In longer experiments, samples were also taken at 18, 21, 24, 27 and 30 hours of experiment. In both configurations, the samples were extracted with a tube and a syringe from the top of the reactor at two heights, namely the bottom of the reactor, close to the 62cm of length of the module and in the middle of the reactor, at around 31cm. An additional port was added for the continuous mode, close to the outlet of the jacketed module M, the sample was then poured into a 50ml tube previously prepared with a filter paper, at times with the help of a small funnel. The filtrate was used to measure the amount of glucose using the densimeter. Between measurements, the densimeter was cleaned using room temperature buffer. The filter cakes were usually disposed of, except for the total solid measurements. The filtrate at room temperature was measured with the densimeter twice, registering the second value along with the temperature. If filtrate was left in the tube, it was disposed of in the biowaste container.

The glucose in the filtrate was measured using a densimeter. This is possible due to the fact that the amount of dissolved sugars defines the density of the solution, as shown by [25]. For this purpose, a portable density meter was the instrument of choice to measure the density of the filtrates. In order to calculate the glucose amount in the filtrate, the first step was to calibrate the buffer that was used for the experiment with different concentrations of glucose (0, 10, 20, 50 and 100 mg/ml) and generate a calibration curve. Then, using equation 1, the sugar mass concentration was calculated.

$$\mathbb{C} = (\rho - \rho_{buffer})/s \quad \textbf{(1)}$$

Where $\mathbb{C}$ is Sugar mass concentration [mg/ml], ρ the liquid density of the sample [g/cm$^3$], $\rho_{buffer}$ is the buffer density [g/cm$^3$] and *s* the slope of calibration curve.

For the STR experiments conducted at ACIB, the measurement technique for the glucose concentration was a DNS based assay, which is often the chosen method for unspecific sugar analysis. In this method the aromatic compound 3,5-Dinitrosalicylic acid interacts in a strong manner with reducing sugars or other reducing agents resulting in 3-amino-5-nitrosalicylic acid. This product is known for its ability to absorb light at 540 nm and therefore spread for a wide range of applications.

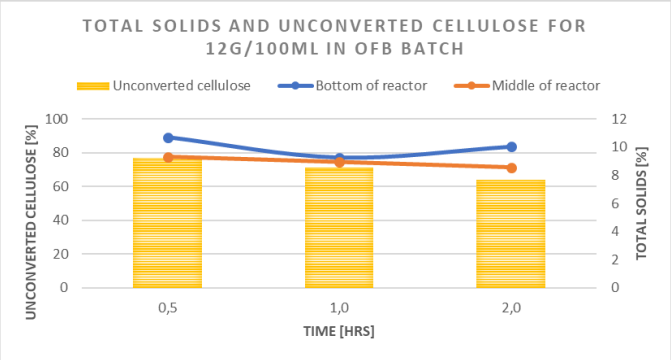
An error between DNS and DMA measurements was identified, as 14% variation for DMA in relation to DNS. This could be explained by the measurement of cellobiose, which has a higher density than glucose. Recalculating the s value for a calibration done with 50% glucose and 50% cellobiose, will result in a steeper calibration line and a lower concentration of glucose measured. Energy tests and total solids test were also conducted for both

configurations. The error of the DMA measurement tool is ± 1,08 mg/ml considered for all results.

## 4. Results and Discussion

### 4.1 Batch Configuration

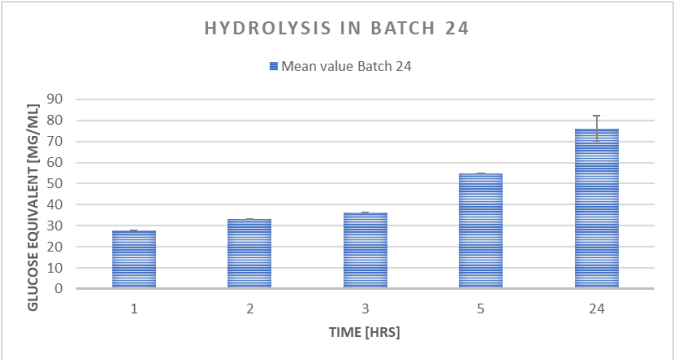
The total solids were measured for a solid loading of 11%, taking samples in two locations of the reactor: middle and bottom of the reactor, at 31 cm and 62 cm along the reactor respectively. The results are seen in graph 1. As seen in the graph, the total solids are higher at the bottom of the reactor than at the middle. It must be noted that the reactor is placed in a vertical position, forming a 90° angle between the oscillatory pump and the reactor. The latter could have an influence in the solid's distribution by gravity effects. There seems to be a lower mixing in the bottom of the reactor which might cause an accumulation of particles in this area. More detailed studies on mixing would be needed to explain this particle behavior.




**Graph 1. Total solids in OFB batch configuration.**

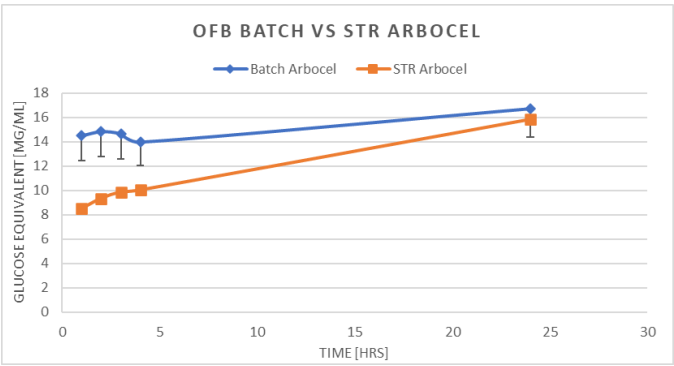
The total solids are expected to diminish with time, as the solid cellulose reacts with the enzymes and transforms into liquid glucose. This is observed in the middle of the reactor. The solid unconverted cellulose diminishes with time as well, as it converts into glucose.

As for the hydrolysis tests, it was found that adding the enzyme directly into the reactor showed a 7,13% improvement in relation to other methods. This enzyme addition method was then adopted for all subsequent experiments. The 24-hour hydrolysis in batch yielded a maximum glucose equivalent of 82,9 ± 1,08 mg/ml, as seen in graph 2. The error bars correspond to standard deviation. Two batches of cellulose were used in this set of experiments, namely Original and Partner, with the partner cellulose yielding considerably lower glucose equivalent concentrations, 17,8% lower than the Original cellulose glucose yield. This could be a result in part of the different particle sizes, but further studies are needed to understand the influence of the particle size of the cellulose in the enzymatic hydrolysis.




**Graph 2. Hydrolysis in OFB batch for 24-hours.**

Additionally, OFB batch experiments were conducted with another type of lignocellulosic material, Arbocel. This material has 99,5% cellulose and is mixed with other components such as lignin and hemicellulose. It has a bigger particle size and unlike the white pure cellulose, it has a light brown colour. Results of the Arbocel experiment in OFB batch compared to STR are shown in graph 3.




**Graph 3.OFB batch vs STR hydrolysis experiments with Arbocel,**

Experiments with Arbocel yielded a low amount of glucose, with a maximum achieved in OFB batch of just 16,9 ± 1,08 mg/ml. It can be noticed in graph 3, that in the beginning of the reaction, the yield of the OFB batch is considerably higher, even when considering the maximum error, it surpasses the glucose concentration obtained by the STR. But as time approaches the 24 hours, the STR increases more rapidly, yielding a higher amount of glucose compared to OFB batch when considering the maximum error.

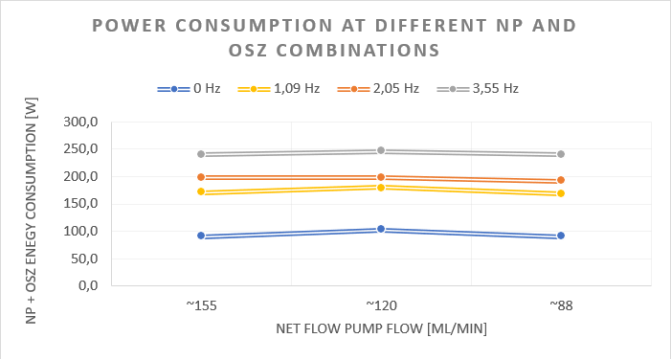
Hydrolysis in the STR seem to increase faster in the first hours compared to the OFB batch, in both cases, cellulose and Arbocel experiments.

### 4.2 Continuous configuration

In a first set of experiments, the flow patterns were assessed. First, measuring the flow in ml/min for different solid loadings, in order to find the maximum solid loading the determined layout could process without clogging the

system. A solid loading between 11-12% was determined to be used for the experiments in this configuration. Additionally, ink was added to the cellulose- buffer mixture, in an attempt to observe the behavior of the flow and identify the plug flow that could be observed for batch mode in the previous work by [5]. The expected plug flow was not observed, with the ink mixing through the cellulose quite rapidly, having a measured residence time in the system of 2-3 min, as opposed to the calculated residence time which was approximately 6,3 min. This means that the incorporation of the continuous flow changes the properties of the mixing, but in order to gain a deeper understanding of the difference in the mixing characteristics between OFB configurations, more research would be needed to understand the particle behavior inside the reactor.

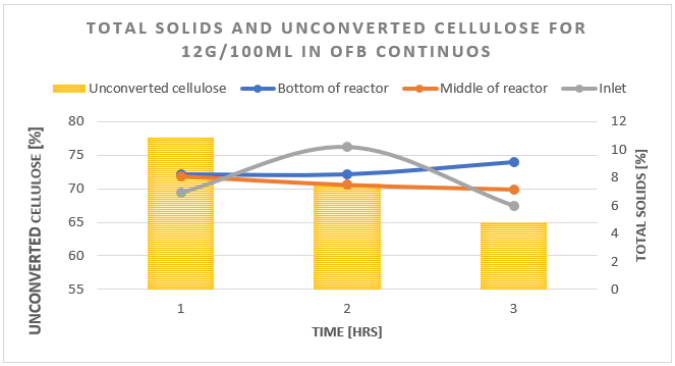
Regarding the power measurements, several tests were conducted combining different engine powers for the net flow pump and oscillatory pump in the system filled with water. Each net flow pump engine percentage resulted in a different flow; 100% engine giving a ~155 ml/min flow, 75% engine power giving a ~120 ml/min and 50% engine power resulting in ~88 ml/min. The results of the power tests are seen in graph 4.




**Graph 4. Power consumption at different net flow pump and oscillatory pump configurations for the continuous mode system filled with water.**

The main observation from this set of experiments is the fact that the frequency determines the power consumption on the oscillatory pump and represents the biggest variation. As the net flow pump has a constant consumption which is nearly the same for each power percentage configuration. Additionally, it was found that the solid loadings did not influence the power consumption of the net flow pump, at least in short periods, such as 16 min. Longer experiments would need to be run in order to confirm this.

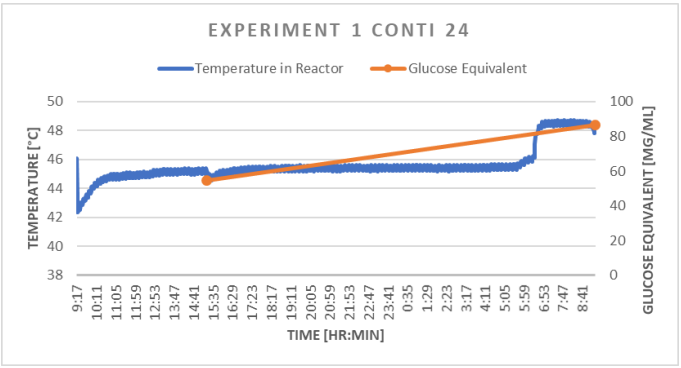
As for the total solids, similar results as those obtained in the OFB batch configuration were seen, with a similar solids distribution among the middle of the reactor and the bottom of the reactor. An additional sampling port was added, close to the top of the jacketed module, were the mixed slurry poured into the net flow pump. This sampling port was called Inlet (graph 5). The solid loading was 11%. The middle of the reactor presents the expected results, with the total solids diminishing with the time. The bottom of the reactor presents accumulations and the Inlet varies greatly with time. The Inlet presented accumulation of cellulose in the upper walls of the modular part in certain periods, which was pushed down at moments by the incoming flow from the jacketed module, which resulted in this total solid measurement. As the hydrolysis goes by, the available unconverted cellulose diminishes.




**Graph 5. Total solids distribution in 3 ports of the OFB continuous mode configuration, at a 11% solid loading.**

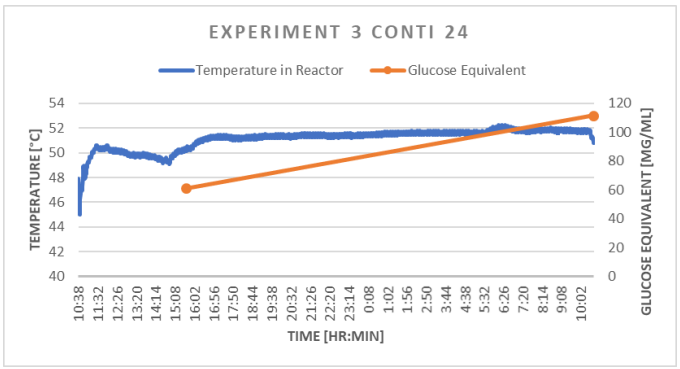
As in the OFB batch, accumulation of particles or lower mixing capacities could explain the higher total solids in the bottom of the reactor, were there was a 90° bend connecting the oscillatory pump with the reactor. More tests should be run to better understand these results.

For the hydrolysis tests, experiments were run for 24 hours (Conti 24 set), using the same type of cellulose and enzyme quantity. The enzyme was added 50% in modular part M and 50% in the pipe connecting to the net flow pump for the first 2 experiments (50/50). For the second 2 experiments, the enzyme was added just in modular part M. Hydrolysis results for experiment 1 and 3 are shown in graphs 6 and 7, with the correspondent temperature profile.




**Graph 6. OFB continuous hydrolysis experiment for 24 hours, enzyme (50/50).**

The temperature in this experiment was in average 45,6°C. As recommend by the enzyme manufacturers, the ideal temperature of operation for the enzyme blend is between 45-50°C. The maximum yield in this experiment was 86,6 ± 1,08 mg/ml.

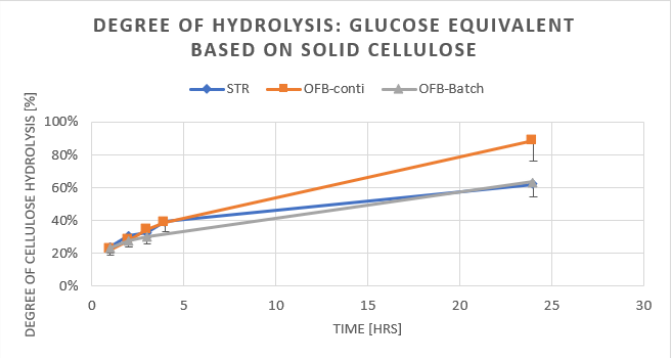



**Graph 7. OFB continuous hydrolysis experiment for 24 hours IA.**

The results are much higher for experiments 3 and 4, with experiment 3 yielding 111,6 ± 1,08 mg/ml and having an average temperature of 51,1 °C inside the reactor. These temperatures were measured inside modular part M, which was better insulated and heated than the rest of the system. This could explain the need of a temperature higher than the ideal 50°C, as in the rest of the reactor system there are heat losses, since modular parts are not perfectly insulated. The different enzyme addition applied could have also impacted the results, this should be studied in future work.

4.3 Comparison of hydrolysis results in the different types of processes.

A comparison between the three processes used along the study is crucial to gain a better perspective of the use of the OFB. First, it is important to note that for the OFB-Conti experiment, the solid loading matches that of the STR, both with 12%. The difference lies in the cellulose type, the STR experiment was done with Original cellulose while the OFB-conti was done with a blend of Original cellulose and Partner cellulose, due to lack of enough Original cellulose. In the blend, 83% corresponded to Original cellulose and the remaining 17% to Partner cellulose. All experiments used the same enzyme series with an activity of 144 FPU. The degree of cellulose hydrolysis achieved for the three processes can be observed in graph 8.




**Graph 8. Degree of hydrolysis for the cellulose in OFB-Batch, OFB-Continuous mode and STR.**

It is evident that the OFB-Conti results are superior when compared to both STR and OFB-Batch, achieving a degree of cellulose hydrolysis of 88%, being 26% greater than that achieved by the STR, which means that even when considering the 14% error, the OFB-Conti still yields a higher amount of glucose. The improvement in degree of conversion considering the maximum error is 14% higher for OFB-Conti versus the STR process. Even when considering the presence of cellobiose in the solution and considering it for the DMA based calculations, taking the s value associated to 50% cellobiose and 50% glucose, the improvement in the glucose yield is of 10,9%.

The increase happens after the 4 first hours, as seen in the graph, for the first hours the degree of conversion is very close for both methods. For this reason, it would be interesting to conduct further studies on mixing behavior and enzyme-substrate binding, in order to understand the fundamental reason for which the OFB-Conti process improves with time in such a dramatic way. It is apparent that the OFB´s better mixing coupled with a constant flow provided by the net flow pump, increases the enzyme-substrate binding, therefore improving the reaction between enzyme and cellulose and in this way, achieving a greater conversion. This becomes evident when comparing the OFB-Batch results against the OFB-Conti results, the same reactor is used, with the parameters set at equivalent quantities, but with the crucial difference of a constant flow, which makes a remarkable difference for the OFB-Conti results obtaining 24% more conversion.

## 5. Challenges and future work.

The oscillatory flow bioreactor laboratory plant has proven to work without any problems in batch mode, as also found by [5]. This time, the laboratory plant was also tested in continuous mode, finding a layout that was assembled and tried out with different solid loadings.

This study showed a significant variation between the results, with the DMA density measurement-based glucose calculation resulting in a glucose concentration that reached a 14% of error, giving higher concentrations than the DNS. This error was taken into account for all

hydrolysis results that compare the STR hydrolysis of cellulose with the OFB. In addition to this, it was found that the presence of cellobiose should be considered for the DMA measurements, as it has a higher density than glucose and when using the calibration of the buffer with glucose, the cellobiose presence is not considered. Therefore, for future work, the ratio of cellobiose and glucose should be identified in order to properly calculate a calibration line for the buffer. Additionally, HPLC tests should be conducted to precisely identify the presence of the different sugars in the filtrate.

The use of another lignocellulosic material was attempted. Arbocel, a plant-based material which has a bigger particle size than pure α-cellulose, composed by 99,5% cellulose, with the rest being lignin and hemicellulose, was tested in batch mode. The experiment was run for 24 hours, with the same amount of enzyme as that used for the cellulose experiments. The results in contrast with the STR process, show that the increase in the glucose yield for the first hours is smaller, but by the end of the reaction, the yields, once again, are nearly the same. This time batch results are slightly greater, achieving 1,1 mg/ml more than the STR mode. Although, when the maximum error of 14% was considered, the STR would perform better than OFB in batch mode.

For the continuous mode experiments, the chosen layout, seen in figure 5, showed good results for solid loading up to 12%, with the potential of treating higher solid loadings. This was concluded after running the parametric tests. A difference that came to light during these set of experiments, was the flow behavior observed in the ink experiments. Unlike batch ink experiments, continuous mode did not present the plug flow behavior described by [15]. The ink was mixing at a rapid time, spreading all along the reactor, showing a light purple colour when mixed with the cellulose. This made the calculated residence time seem much greater than the measured residence time, since the ink was quickly transported along the system, taking just 2 to 3 minutes to appear on the other end of the continuous OFB system, which corresponded to half of the calculated residence time. This could be a result of the incorporation of the net flow pump. The continuous flow that run through the system, which seemed to have an immediate effect on the mixing characteristics of the slurry contained in the system, no longer presenting the plug flow behavior. Further studies would be needed to identify the reasons behind the mixing differences between batch mode and continuous mode. These results could be crucial to understand what type of mixing enhances the enzymatic hydrolysis.

The solid distribution both in batch mode and continuous mode showed the same patterns, a better solid distribution in the middle of the reactor and a possible accumulation of particles due to a lower degree of mixing or the 90° bend in the system in the bottom of the reactor. It is also possible that the vertical position of the reactor has an effect on the distribution of the particles, further particle distribution tests should be conducted to better understand the reason for the difference along the reactor.

For the hydrolysis results, in the 24 hours experiment set Conti 24, the influence of the temperature is easily observed. It appears that a temperature close to 51 °C inside the modular part M has a positive impact in the hydrolysis results, even with the recommended temperature of the enzyme blend being in the range of 45-50°C. This higher temperature needed can be due to the losses along the system, in modular parts that are not perfectly insulated and possess a lower heat exchange with the heating pipes than that inside the double tubular modular part M. This increase in temperature might have secured an overall optimal temperature of 50°C along the system, but studies on the heat transfer along the system would be needed to prove this hypothesis.

Overall, the OFB continuous mode process showed a superior performance when compared to OFB batch configuration experiments and STR. The OFB continuous mode is superior and yielded a degree of cellulose hydrolysis of 88%, as opposed to the STR method which only achieved 62% degree of conversion in the same amount of time, 24 hours. These results show that even when considering the error of 14%, the performance of the OFB in continuous mode is far superior, having 14% more of conversion. Furthermore, with the estimation of a 50% glucose and 50% cellobiose in the filtrate, a new k was calculated and with it a more realistic glucose yield. As a result, the OFB continuous mode showed a 10,9% increase in glucose yield when considering the cellobiose calibration compared to the STR.

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