TÉCNICO LISBOA

Evaluation of Coflore technology for enzymatic carbohydrate oxidation

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Abstract: This project evaluates the potential of the Coflore Agitated Cell Reactor (ACR) as an alternative to batch or continuous stirred tank reactors (CSTRs) in series. An evaluation and comparison to ideal biocatalytic reactors was performed based on the quality of the data obtained in the study of an enzymatic reaction with focus on carbohydrate oxidase technology, more specifically in a glucose oxidation reaction.

Results showed that this reactor is inefficient for processes in the presence of foam loosing 70 to 90 % of the liquid volume. The ACR also demonstrated to have a liquid volume correspondent to 50 % of the reactor volume when added aeration and agitation, which decreases the expected production rates. However, it presented high oxygen transfer rates (OTR) comparable with large-scale oxygen supply rates and it's behavior was concluded to be close to 6 CSTRs in series both by a residence time distribution (RTD) study and by the construction of a MATLAB model based in the kinetic parameters. Therefore it was concluded that the ACR presents advantages for bioprocess development and for replacing processes that normally use multi-stage CSTRs.

Keywords: Agitated Cell Reactor (ACR); Glucose Oxidase; Antifoam; Residence Time Distribution (RTD); Oxygen Transfer Rate (OTR); Oxygen Transfer Coefficient (k_La).

1. Introduction

Nowadays, there are many opportunities to either replace existing chemical manufacturing reaction steps with biocatalytic process steps, or to enter new markets with entirely new products made via biocatalysts.[1] It is widely expected that the use of bioprocesses can contribute considerably to a more sustainable development.

Although, for future success in the biological manufacturing sectors the adoption of more efficient development strategies and manufacturing techniques is becoming essential. Process engineering solutions to achieve an efficiency increase can undergo by a different reactor selection based on cost, space, mass transfer, kinetics, heating and cooling, easiness of operation, operation mode and reusability of the catalyst. Therefore, opportunities to make changes in the reactor format are being studied to accommodate these special features.[2] Additionally modelling the process under development and thorough assessment helps to improve the understanding of the actual future production process as early and detailed as possible.[3]

Several variations of standard design reactors exist in the market but, in the recent years, also some novel reactor designs have been proposed. An example is the Coflore Agitated Cell Reactor (ACR) which is a dynamically mixed plug flow reactor designed so to take advantage of the benefits that continuous flow offers over batch processes. Such reactor is still in its infancy and has not yet been applied at large scale.

The Coflore Agitated Cell Reactor (ACR) is a multi-stage flow reactor that can be used for several different applications. This reactor, initially designed for lab development and small scale manufacturing, is described by Gasparini et al. [4] and Jones et al. [2] as having a good transfer performance and delivering good mixing.

The reactor system includes two main elements: a reactor block and a mixing platform. The reactor block of the ACR is a PTFE block with 10 equal sized holes interconnected by small channels where product flows from one cell to the next. Inside each cell, agitating rollers can be placed to promote mixing when the reactor body is subjected to lateral shaking. Agitating rollers vary in design and diameter to give the option of carrying different functions, such as offering flexible volume, ensure consistent mixing and accommodate fluids of varying viscosity or catalysts. [2] The mixing platform causes rollers to move and allows changing the intensity of agitation from 2 to 9 Hz in order to achieve from mild to vigorous shaking. An inlet of compressed air to the mixing platform allows its movement.

a) b)

Figure 1 - a) Coflore® ACR mounted in the shaking platform; b) Reactor block, Coflore® ACR. [5]

The main goal of this project is to evaluate the potential of the Coflore Agitated Cell Reactor (ACR) as an alternative to batch or continuous stirred tank reactors (CSTRs) in series. An evaluation and comparison to ideal biocatalytic reactors will be performed based on the quality of the data obtained in the study of an enzymatic reaction with focus on carbohydrate oxidase technology, more specifically in a glucose oxidation reaction.

In order to evaluate the ACR potential residence time distribution (RTD) and oxygen transference studies were performed. Moreover, the effect of foam presence and antifoam addition was also evaluated. Also a model construction of the process under development helped to improve the understanding of the actual future production process.

1.1 Process Principle

The glucose oxidase model system is chosen because it is a highly stable biocatalyst, which has been extensively studied and is easily accessible. Glucose oxidase is a key enzyme, which is potentially useful in food, pharmaceutical, biotechnology and flavoring industries. Additionally, it is being exploited commercially in biosensors for monitoring the glucose level in blood, as well as in fermentation broth for online estimation of residual glucose.[6,7]

Furthermore, this model system is highly specific for β-D-glucose and oxygen, which enables the study of the ACR's performance in the fulfillment of the reaction's oxygen requirement. Oxygen plays a central role as an electron acceptor in oxidase-based biocatalysis and it is required in stoichiometric quantities for a reaction. Therefore it is advantageous to measure oxygen concentrations in the reaction system and to collect kinetic data.

The steady-state kinetics of glucose oxidation by molecular oxygen obeys the Ping-Pong bi-bi mechanism, thus the initial rate data (v_0) is given by Equation (1) [8]:

$$v_0 = [E_0]k_{cat}\frac{[Glc][O_2]}{K_{m,O2}[Glc] + K_{m,Glc}[O_2] + [Glc][O_2]} \quad (1)$$

1.2 Residence Time Distribution (RTD)

Residence Time Distribution (RTD) was studied since the residence time characteristics of the reactor can be of significant importance and reveal useful information. In general, depending on the reaction order and on the formation of by-products a near plug flow response is most favorable, although in reality rarely achieved. Perfect plug flow characteristics imply that each fluid element passing through the reactor has the same residence time and therefore also the reaction time, which means that the entire reaction mixture is processed under identical conditions. A residence time distribution (RTD) that strongly deviates from plug flow,

resulting in a spread of the reaction mixture slug as it is passed through the reactor, can lead to a reduced conversion rate or selectivity. This deviation can be caused by channeling or recycling of fluid, or by creation of stagnant regions in the vessel.[9,10]

The two most common stimulus-response techniques to experimentally determine RTD are: pulse input and step input injection. In this project only the step input was carried.

1.2.1 RTDs in Ideal Reactors

Models are useful for representing flow in real vessels, for scale up, and for diagnosing poor flow. There are different kinds of models depending on whether flow is close to plug, mixed, or somewhere in between. For the study of fluid flow characteristics in continuous flow chemical reactors, two general ideal flow patterns can be used as comparisons: plug flow and perfect mixing. In real vessels, the residence time distribution (RTD) of any given continuous flow device lies somewhere between the PFR and the CSTR.[10]

The RTDs in plug flow reactors and ideal batch reactors are the simplest to consider. As referred before in these reactors all the atoms spend precisely the same amount of time within the reactors. The distribution function in this case is given by the Dirac delta function [9,11]:

$$E(t) = \delta(t - \bar{t}_m) \quad (2)$$

The use of tank-in-series model can also be used to describe nonideal reactors. This is a one-parameter model where it is possible to analyze the RTD to determine the number of ideal tanks, N, in series that will give approximately the same RTD as the nonideal reactor.

Equation (3) gives the distribution function for N reactors in series and can be used to describe the number of tanks in series that best fits the RDT data.

$$E(t) = \frac{t^{N-1}}{(N-1)!\,\bar{t}_{m,i}} e^{-t/\bar{t}_{m,i}} \quad (3)$$

1.3 Oxygen Transfer Rate (OTR)

Supply of oxygen to the enzymatic reaction can be a rate-limiting step due to poor solubility of oxygen in the liquid phase. The availability of oxygen in solution, which is determined by the oxygen transfer rate (OTR), is governed by the volumetric oxygen transfer coefficient (k_La) and the concentration gradient of oxygen in the liquid phase. In order to establish the aeration efficiency in the batch reactor and in the ACR, and to quantify the effects of the operating variables on the transfer of dissolved oxygen it was essential to determine the k_La. The mass balance for the dissolved oxygen can be establish as:

$$\frac{dC}{dt} = OTR - OUR \quad (4)$$

where dC/dt is the rate of oxygen accumulation in the liquid phase, OTR represents the oxygen transfer rate from the gas to the liquid and OUR is the oxygen uptake rate by the product formation.

The availability of oxygen in solution, which is determined by the OTR, is governed by the volumetric oxygen transfer coefficient (k_La) and the concentration gradient of oxygen in the aqueous phase, as demonstrated in Equation (5).

$$OTR = k_La.(C^* - C_L) = k_La.C^*.(1 - {}^{C}/_{C^*}) \quad (5)$$

where C* is the oxygen saturation concentration in the bulk liquid in equilibrium to the bulk gas phase and C_L is the dissolved oxygen concentration in the bulk liquid.[12]

2. ACR Model

To model and simulate the ACR its performance was approximated to a cascade of continuous stirred tank reactors and its transient state was studied. The equations that describe the transient profile of glucose consumption and gluconic acid production are presented below. Also the oxygen and enzyme profiles were included in the construction of the model since oxygen is being consumed and in transient state the concentration of enzyme changes with time.

$$\frac{d[Glc]_n}{dt} = \frac{[Glc]_{n-1} - [Glc]_n}{\tau} - r \quad (6)$$

$$\frac{d[GA]_n}{dt} = \frac{[GA]_{n-1} - [GA]_n}{\tau} + r \quad (7)$$

$$\frac{d[O_{2,L}]_n}{dt} = k_L a.\left([O_2]_{sat} - [O_{2,L}]_n\right) - 0.5.r + \frac{[O_2]_{n-1} - [O_2]_n}{\tau} \quad (8)$$

$$\frac{d[O_2]_n}{dt} = -k_L a.\left([O_2]_{sat} - [O_{2,L}]_n\right) + \frac{[O_2]_{n-1} - [O_2]_n}{\tau} \quad (9)$$

$$\frac{d[E]_n}{dt} = \frac{[E]_{n-1} - [E]_n}{\tau} \quad (10)$$

$$r = \frac{k_{cat}.[E]_n.[Glc]_n.[O_{2,L}]_n}{[Glc]_n.[O_{2,L}]_n + K_{m,Glc}.[O_{2,L}]_n + K_{m,O2}.[Glc]_n} \quad (11)$$

where [Glc], [GA], [E] and $[O_2]$ are the glucose, gluconic acid, glucose oxidase and oxygen concentrations, respectively, n is the number of CSTRs, τ is the residence time per CSTR, k_{cat} and K_m are the estimated kinetic parameters, and k_La is the estimated oxygen transfer coefficient. $[O_2]_{sat}$ corresponds to the oxygen saturation concentration in the bulk liquid in equilibrium to the bulk gas phase and $[O_{2,L}]$ to the dissolved oxygen concentration in the bulk liquid.

3. Materials and Methods

Gluzyme Mono, commercial catalyst form of glucose oxidase was obtained from Novozymes (Denmark) and catalase from Sigma Aldrich (Denmark). An aqueous solution was prepared of both enzymes.

D(+)-Glucose and D-gluconic acid from Sigma Aldrich (Denmark) were used as reagent and reference compound. Phosphoric acid, 85 % purity, from Sigma Aldrich (Denmark) was used as mobile phase. Dipotassium hydrogen phosphate and potassium dihydrogen phosphate from Sigma Aldrich (Denmark) were used to prepare phosphate solution used as solvent for all the experiments. Sodium hydroxide from Sigma Aldrich (Denmark) was used to stop the enzymatic reaction. Antifoam 204 from Sigma Aldrich (Denmark) was used to decrease the formation of foam.

The first set of experiments was carried out in a 250 mL MiniBio Reactor combined with a bio controller software, *my*-Control, from Applikon® Biotechnology (Delft, Netherlands). The reactor setup is equipped with two Rushton turbines as impellers, an aeration assembly, a sleeve diaphragm pH sensor and dissolved oxygen (DO) and temperature sensors. *my*-Control software was used to measure the pH and the stirrer speed and control the temperature values.

The second set of experiments was carried out in a 100 mL Coflore™ Agitated Cell Reactor (ACR) from AM Technology (Cheshire, England). The reactor setup is equipped with 10 high shear agitators, a mid-infrared temperature probe in cell 6 and DO sensors in cells 3, 5, 8 and 10. Additionally the setup also includes a pump responsible to pump the reagents into the reactor and a water bath (Julabo, Germany) for temperature control. Glucose and enzyme solutions were fed using a T piece.

All the samples were analyzed using high performance liquid chromatography (HPLC). A Dionex Ultimate 3000 series HPLC system equipped with a Phenomenex column with 5 µm sized amine particles (Luna 5u NH2 100A), operated at 40 ºC and 140 bar, was used to separate the glucose and the gluconic acid. The mobile phase consisted of a 20 mM H_3PO_4 solution, flowing at 1 mL/min. The eluted compounds were detected by an ultraviolet (UV) multiple wavelength detector and a Refract Max 520 refractive index (RI) detector. The UV detector at a wavelength of 205 nm was used to quantify gluconic acid concentrations and the RI detector was used to determine the concentration of glucose.

4. Results

The first part of this project consisted in the model development in a batch reactor of a glucose oxidation reaction. The second part consisted in the study and evaluation of the ACR.

4.1 Batch - Kinetic Study

Reaction kinetics has to be found under relevant process conditions; therefore the glucose oxidase kinetics was studied under the conditions selected previously (30 °C and pH 7.0). The effect of substrates

concentration on the kinetics of GOx was studied and determined using the initial rates method. In this method initial glucose and oxygen concentrations are practically constants and the reverse reactions after the binding of the substrates can be neglected.

GOx presented a bi-substrate (glucose and oxygen) mechanism. However, in the first part of the experiment only the effect of the glucose was considered, and in the second part only the effect of the oxygen. Equation (1) considers both subtracts, although when one of the subtracts is kept constant it is possible to simplify the equation. In the first set of experiments the oxygen concentration was kept constant using a high specific air flow rate (595.6 mL/min) and in the second set glucose was in excess (20 g/L). Thus the reaction can be described in each case as a Michaelis-Menten kinetic model.

The kinetic parameters were estimated using a nonlinear regression technique. The best fit was obtained by means of numerical optimization procedure where the sum of squared residuals (SSR) was minimized by varying the model parameters.

Table 1 - Kinetic parameters and Michaelis-Menten model for GOx at 30 °C and pH 7,0.

	Glucose	Oxygen
Kinetic Parameters	$K_{m,Glc}$ (mol/L) 1.71×10^{-2}	$K_{m,O2}$ (mol/L) 1.72×10^{-4}
	$V_{max,Glc}$ (mol/L.min) 2.09×10^{-4}	$V_{max,O2}$ (mol/L.min) 2.78×10^{-4}
	$k_{cat,Glc}$ (mol/g_{enz}.min) 4.19×10^{-3}	$k_{cat,O2}$ (mol/g_{enz}.min) 5.56×10^{-3}
Correlation Coefficient	0,989	0,993
Model	$v_0 = \frac{V_{max,Glc}\cdot[Glc]}{K_{m,Glc}+[Glc]}$	$v_0 = \frac{V_{max,O2}\cdot[O_2]}{K_{m,O2}+[O_2]}$

4.2 Batch - Evaluation of antifoam's presence

In order to establish the aeration efficiency in the batch reactor and to quantify the effect of the antifoam agent addition on the transfer of dissolved oxygen it was essential to determine the volumetric coefficient of oxygen transfer (k_La).

To estimate the values of k_La a dynamic method was applied that measures the oxygen consumption in the production of gluconic acid. The values of OUR and k_La are shown in Table 2.

Table 2 - Values of Oxygen Uptake Rated (OUR) and k_La obtained by a dynamic model. C* corresponds to the oxygen saturation in water and C/C* to the DO concentration measured by the oxygen probe when the steady state is achieved.

	1st experiment	2nd experiment (antifoam)
OUR (mol/L.min)	5.00×10^{-6}	3.00×10^{-5}
C* (mol/L)	2.38×10^{-4}	2.38×10^{-4}
C/C*	0.98	0.68
k_La (min^{-1})	1.05	0.395

The rate of oxygen transfer in the aqueous phase from the gaseous phase is influenced by several physical and chemical factors that change either the value of k_La or the concentration gradient of oxygen, which is the major driving force for oxygen transfer. In this project only the influence of antifoam agent presence was studied. It was found that contrary to what appeared in Figure 2, antifoam addition at low concentrations markedly decreases the k_La despite it increases the oxygen uptake rate. Figure 2 shows a percentage of DO around 3% that could mislead to the conclusion that the presence of antifoam does not have an effect on the oxygen transfer. Although as stated by Morão et al. [13] antifoam addition at low concentrations markedly decreases the gas-liquid volumetric mass transfer coefficient, k_La. Therefore it is likely that the measurements done by the oxygen probe for low concentrations of dissolved oxygen concentrations, like in Figure 2, are not accurate to enable the study of the influence of an antifoam agent.

Therefore it is possible to assume that also in the ACR the antifoam will reduce the oxygen transfer rate.

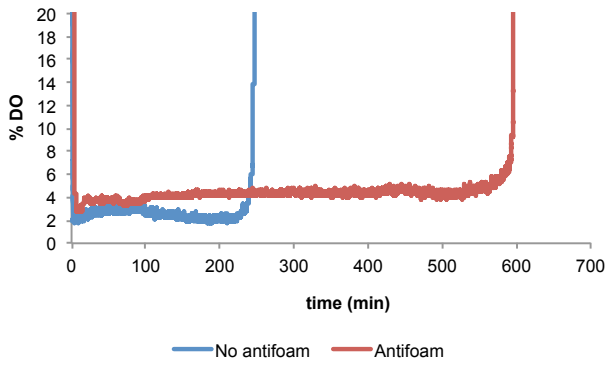

Figure 2 - Evolution of dissolved oxygen in the liquid-phase with the progression of a glucose oxidation reaction in the absence and presence of antifoam under standard conditions in a batch reactor.

4.3 ACR - RTD's determination

By a method of step input the effect of several operating parameters (aeration, agitation, foam) in the $\bar{t}_m$ of glucose inside the ACR was studied. Different patterns of glucose concentration distribution were measured in the outlet of the ACR analyzing the influence of aeration and agitation on the $\bar{t}_m$.

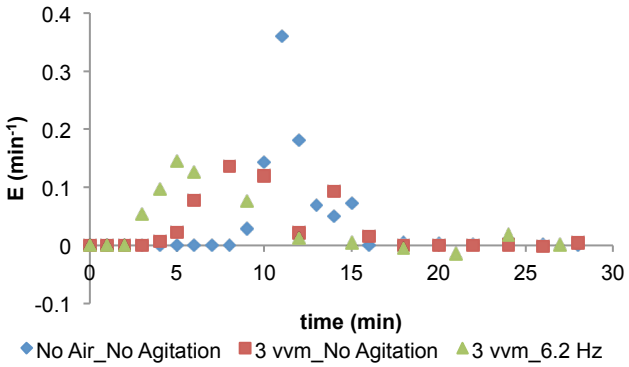

Figure 3 - ACR's residence time distribution function (E(t)) in the presence/absence of aeration and agitation, using glucose as a tracer.

It was concluded that the addition of air flow and agitation to the reactor decrease significantly $\bar{t}_m$. The addition of air decreased the volume of each cell due to the space occupied by the bubbles of air, moreover when added the agitation there is a better mixing of the air inside the cells which increase their residence time inside the cells decreasing even more the liquid volume of the reactor.

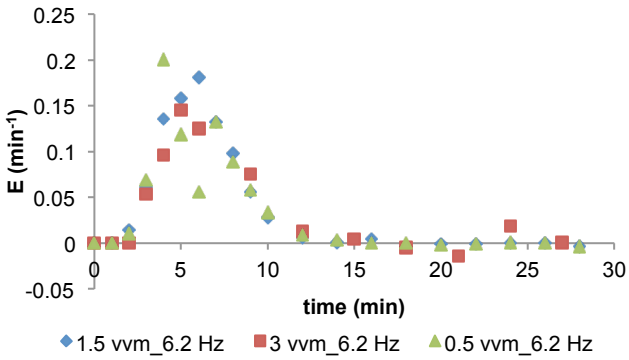

Figure 4 - ACR's residence time distribution function (E(t)) in the presence of different air flow rates, using glucose as a tracer.

Figure 4 shows that although the introduction of aeration in the ACR affects considerably the $\bar{t}_m$ the variation of the air flow rate (3.0 vvm, 1.5 vvm and 0.5 vvm) is not significant for a change in the reactor outline profile.

Table 3 - Mean residence time ($\bar{t}_m$, min), liquid volume (V_L, mL) and air volume (V_{air}, mL) of the ACR dependent on the air flow and agitation.

	No air + No agitation	3 vvm + No agitation	3 vvm + 6.2 Hz	1.5 vvm + 6.2 Hz	0.5 vvm + 6.2 Hz
$\bar{t}_m$ (min)	10.8	9.2	6.0	5.7	5.5
V_L (mL)	103.3	88.3	56.9	54.7	52.4
V_{air} (mL)	0.0	7.3	38.7	40.9	43.2
% V_L	108.2	92.4	59.6	57.2	54.8
% V_{air}	0	7.6	40.5	42.8	45.2

It was observed that the enzyme solution (GOx + Catalase) produces foam during the reaction; therefore it was essential to study its influence in the reactor liquid volume (V_L). Once again it was used a step input method but this time the reactor was filled with water and at time t=0 a flow of gluconic acid and enzyme solution was started. It is observed that the foam occupies 75 % to 95 % of the reactor volume.

4.3.1 Comparison to RTDs in Ideal Reactors

The ACR experimental data is compared by an approximation to ideal reactors models. Later in this project a glucose oxidation reaction in the presence and absence of foam will be studied, therefore the RTD experiments chosen to design the model were the ones that have similar operating parameters to the future experiments. Hence an experiment with glucose as a tracer, an aeration of 0.5 vvm and agitation of 6.2 Hz and an experiment with gluconic acid as a tracer, an aeration of 0.25 vvm and agitation of 6.2 Hz were chosen.

Figure 5 and **Figure 6** show a comparison between the experimental data, a tank-in-series model and a plug flow reactor (PFR) model.

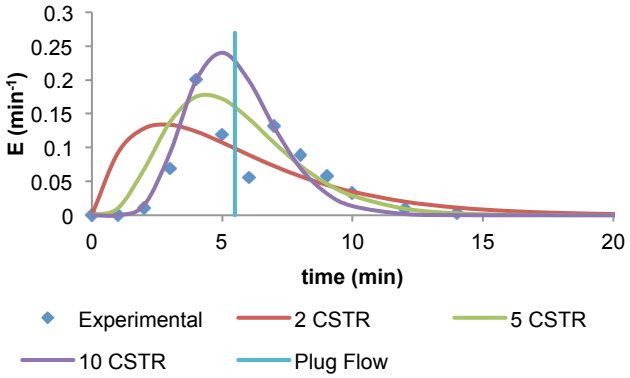

Figure 5 - Experimental profile of the residence time distribution function (E(t)), using glucose as a tracer, 0.5 vvm of air and 6.2 Hz of agitation. Tank-in-series model for a setup with 2, 5 and 10 CSTRs and a Plug Flow model are also represented.

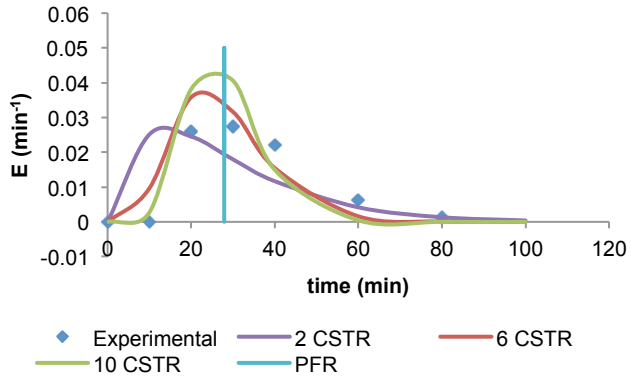

Figure 6 - Experimental profile of the residence time distribution function (E(t)), using gluconic acid as a tracer, in the presence of enzyme solution, 0.25 vvm of air and 6.2 Hz of agitation. Tank-in-series model for a setup with 2, 6 and 10 CSTRs and a Plug Flow model are also represented.

The RTD were analyzed to determine the number of ideal tanks, N, in series that give approximately the same RTD as the non-ideal reactor. It was found that 5 and 6 are the number of reactors that minimizes the sum of the squares between the experimental RTD and the RTD tank-in-series model for the experiment done in the absence (glucose as a tracer) and in the presence of foam (gluconic acid as a tracer and presence of enzyme solution), respectively. The experimental data was also compared with a plug flow reactor model where all the particles inside the reactor take exactly the same time to leave it.

4.4 ACR – Oxygen Transfer Study

In order to evaluate, model and improve the performance of the ACR it was necessary to quantify the oxygen transfer. Due to the high quantity of foam it was not possible to make measurements of dissolved oxygen concentrations in reactions without antifoam, therefore all the oxygen transfer studies in the ACR were made in the presence of an antifoam agent.

Figure 7 shows the percentage of dissolved oxygen in cell 5 for different residence times. As expected with higher residence times the reaction rate is slower and so there is higher concentrations of dissolved oxygen. The dissolved oxygen concentration for a residence time of 35 min is considered to be lower than expected and should be repeated.

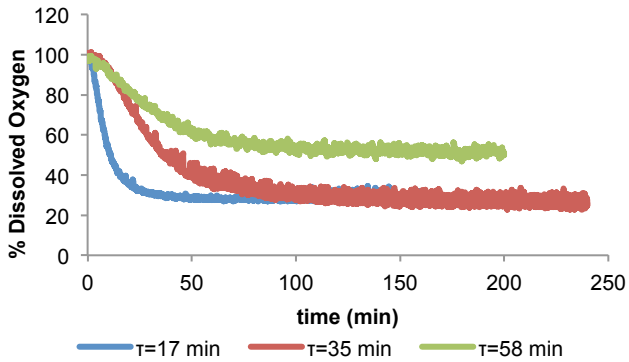

Figure 7 - Percentage of dissolved oxygen (DO) during glucose oxidation reactions with different residence times in the ACR.

Figure 8 shows the percentage of dissolved oxygen in different cells along the reactor for the same operating conditions.

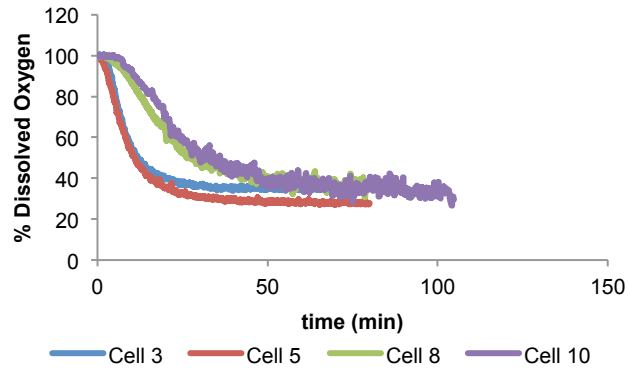

Figure 8 - Percentage of dissolved oxygen (DO) during glucose oxidation reactions in cells 3, 5, 8 and 10 of the ACR with a residence time of 17 min.

If the interfacial mass transfer rate was larger that the oxygen consumption by the reaction it was expected that the dissolved oxygen concentration would increase along the reactor. Though cell 3 shows a higher concentration of DO than cell 5 since the consume of oxygen is higher in cell 3 than in cell 5 but cell 3 is also closer to the air inlet (cell 1) having a higher availability of oxygen. In cell 8 and 10 the rate

of oxygen consumption decreases significantly showing higher DO concentrations.

Assuming a constant percentage of DO along the ACR it was possible to calculate a mean k_La with a sample variation of 0.50 % and with a value equal to 6.75 min^{-1}.

4.5 ACR - Reaction Profile in Presence/ Absence of Antifoam

In order to detect if there was any change in the reaction rate when added the antifoam a kinetic study based on the gluconic acid's production was made. **Figure 9** shows the percentage of conversion in the ACR dependent on the residence time inside the reactor for reactions done in the presence and absence of antifoam.

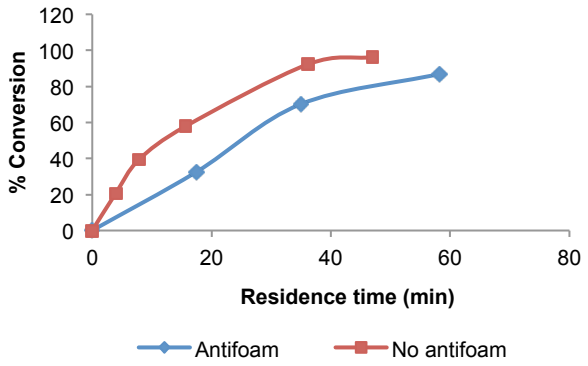

Figure 9 - Conversion's percentage variation with residence time in the ACR in the presence and absence of antifoam.

As expected the profile of the reaction done in the presence of antifoam has a lower reaction rate than when done in its absence. Although the decrease in the reaction rate is most likely due to the decrease of the oxygen transfer rate in the presence of the antifoam it cannot be stated since it was not possible to do a more detailed study of the profile of the oxygen concentration along the reactor in the absence of antifoam.

4.6 Model Validation

The ACR model was implemented in MATLAB, as described in Section 2, and fitted to the experimental data by varying the number of CSTRs in series. The minimum sum of square residuals (SSR) for different residence time (17.5, 34.9 and 58.2 min) and number of CSTRs in series model was calculated to evaluate which model fits better the experimental data. It was concluded by this study that the number of CSTRs that better fit the experimental data is 5.

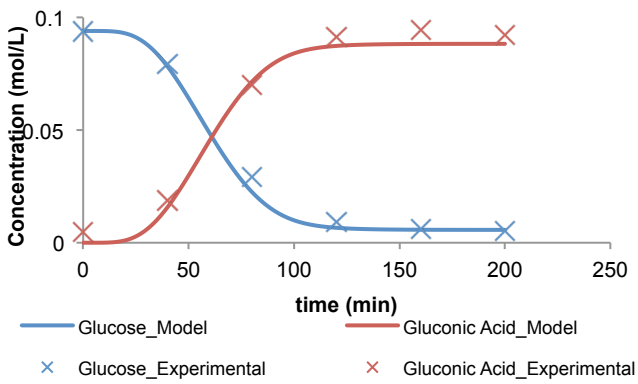

Figure 10 - Experimental data and fitted 5 CSTRs in series model for glucose consumption and gluconic acid production during the transient state and in the presence of antifoam in the Agitated Cell Reactor for a residence time of 58.2 min.

4.7 Batch versus ACR

In this project the efficiency of the glucose oxidation reaction performed in a batch and in an ACR reactor was compared.

Figure 11 shows the percentage of conversion in an ACR and in a batch with the same enzyme and glucose concentrations in the inlet. It is clearly observed an increase of the reaction rate in the ACR when compared with the batch reactor. With a k_La of 0.395 min^{-1} and 6.75 min^{-1} for the batch and the ACR respectively in the presence of antifoam, it can be concluded that the ACR has a higher aeration efficiency, which leads to the higher rates of gluconic acid production.

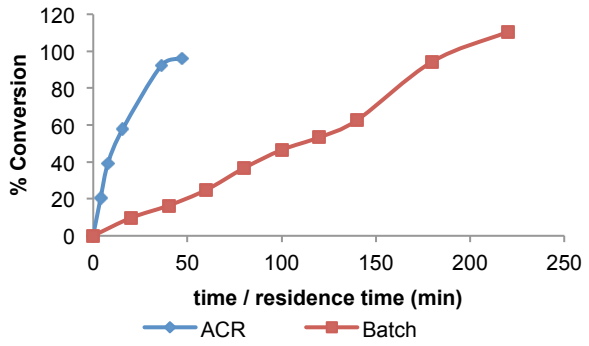

Figure 11 - Percentage of conversion variation with time in the batch reactor and with residence time in the ACR.

Figure 12 also shows the percentage of conversion in an ACR and in a batch but in the presence of an antifoam agent. As expected and observed before in this project the rate of conversion decreases with the presence of this agent.

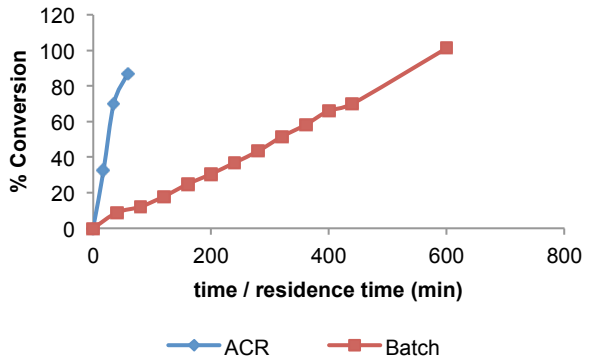

Figure 12 - Percentage of conversion variation with time in the batch reactor and with residence time in the ACR in the presence of antifoam.

5. Future Applications

Chemical and biological industries are nowadays concerned not only with the capability of designing and operating innovative reactors but also in the development of new and more efficient reaction technologies in order to become cost and product quality competitive and to meet environmental aspects. In industrial processes design and optimization of scale-up is one of the most basic issues and its study is essential to ensure that the optimal physiological conditions identified in the small-scale studies are maintained at larger scales. [14] AM Technology developed the Agitated Tube Reactor (ATR) as an industrial alternative to the ACR. The ATR employs the same mixing principle as the lab scale Coflore ACR and has a maximum capacity of 10 L, although there are still not many studies in this reactor. [15]

The ACR has demonstrated favorable oxygen transfer rates when compared with a batch reactor at a lab scale. Though, when scaling-up it is likely that the batch reactor will achieve close results to the ACR due to the increase of the mean bubble residence time and of the absorbed oxygen fraction by the liquid phase. Thus, it is not likely that the ACR is a good substitute for the batch reactor when thinking to go to bigger scales.

Fine chemicals are used mainly as starting materials for specialty chemicals, particularly pharmaceuticals, biopharmaceuticals and agrochemicals. They are produced in limited volumes and at relatively high prices, meaning that normally they are produced in small-scale plants. Considering the results obtained in this project for the lab scale ACR, it appears that the production of fine chemicals is a good asset for this reactor. The higher conversion rates observed will contribute to faster productions.

The behavior of the ACR was approximated to 5/6 CSTRs in series, therefore it seems promising to substitute setups composed by multi CSTRs. Despite the advantages of multi-stage CSTRs they are relatively uncommon due to its assembly and performance complexity. These systems present difficulties in altering the volumetric profile as in maintaining the same heat transfer rate in all the reactors. In addition they are responsible for large product losses in the start and shut-down of the system that is overcome by the addition of pumps between stages, yet this adds complexity and costs to the setup. Thereby the ACR it is a good substitute for processes at lab scale that normally use multi-stage CSTRs since it is easier to start and shut-down, it has good heating transfer and it is easier to perform.

6. Conclusions

Overall it can be concluded that this reactor inefficient for processes in the presence of foam and has a small

liquid volume, which decreases the expected production rates. However, it presents an advantage for oxygen dependent processes due to the high oxygen transfer rates observed comparable with large-scale oxygen supply rates. Therefore the ACR it is very efficient for bioprocess development. It also presents advantages in the replacement of processes that normally use multi-stage CSTRs since it has a similar behavior but it is much easier to implement.

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