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Modelling and experimental validation of continuous biocatalytic oxidation in two CSTRs in series

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ABSTRACT

The rate of oxygen transfer from gas to liquid phase is a critical process parameter for biocatalytic oxidations, due to the poor water-solubility of molecular oxygen and the low oxygen-affinity of many of the relevant enzymes, such as oxidases. In gas-liquid systems, mechanical mixing can be used to increase the interfacial area available for mass transfer and thereby increase $k_{L,a}$. As such, operation of these reactions in a continuous stirred tank reactor (CSTR) may allow for better performance in a readily scalable way. Even so, to achieve a high substrate conversion in a single reactor would require operation at high pressure, to improve the solubility of oxygen, as well as a high enzyme concentration. An alternative, more cost-effective, means of improving substrate conversion might be to operate a series of multiple CSTRs. As such, the oxidation of glucose to gluconic acid by glucose oxidase, coupled with catalase, was modelled in a series of identical well-mixed reactors. It was found that achieving full conversion would require an impractical number of reactors at atmospheric pressure. However, the overall conversion of the reaction could be doubled by simply using two CSTRs in series. Subsequently, experiments were carried out to validate this, and the results showed that the overall conversion was, in fact, tripled. This likely resulted from a higher $k_{L,a}$ in the second reactor, potentially caused by the change in media composition from the first reactor.

KEYWORDS: dissolved oxygen, continuous, biocatalysis, oxidation, CSTR
INTRODUCTION

Oxidation reactions find common use in industrial chemistry for the production of both low-value and high-value chemicals. In recent years, continuous operation of oxidation reactions has become very desirable in industry to capitalize upon improved safety and better heat and mass transfer rates.\textsuperscript{1-3} Furthermore, the use of enzyme-catalyzed (biocatalytic) oxidation is especially attractive due to exceptionally selective catalysis\textsuperscript{4, 5}, as well as to facilitate redox cascades\textsuperscript{6}. In particular, the oxidases have great potential for industrial implementation since they require no expensive cofactors and use molecular oxygen as the oxidant, which is inexpensive, abundant and non-toxic\textsuperscript{2}. However, the downside of this is that the effective transfer of gaseous oxygen into the liquid system during the reaction becomes essential, since the solubility of molecular oxygen in aqueous media (where the enzymes operate) is merely 0.26 mM in distilled water (at 25 °C and 1 atm, based on its Henry’s Law constant\textsuperscript{7}). Hence, continual replenishment of the oxygen in solution is required and additionally the driving force for gas-liquid mass transfer is very low. Coupled with this, based on data found in the BRENDA database\textsuperscript{8}, the affinity constants of oxidases towards dissolved oxygen (K\textsubscript{MO}) are often high relative to oxygen solubility\textsuperscript{9}, greatly limiting the effectiveness with which the available enzyme activity is used. Although, recent advances in protein engineering may potentially allow the affinity of oxidases towards oxygen to be improved, so as to enable more effective use of the enzyme at low dissolved oxygen concentrations, still, there are also a number of process strategies that can be used to improve the situation. For instance, the solubility of oxygen can be increased by operating at elevated pressures. Unfortunately, this also increases the cost and the safety risks of the process, and may not be practical in some industries. Alternatively, the rate of gas-liquid oxygen mass transfer can be improved by increasing the volumetric mass transfer coefficient (k\textsubscript{L}a) to enable operation closer
to the solubility limit of oxygen in the media. This can be achieved by increasing the surface-to-volume ratio between the gas and the liquid. Consequently, operation of oxidation reactions in microfluidic reactors can be advantageous.\textsuperscript{10-12} However, scale-out of these reactors to meet industrial production throughputs may have some practical limitations. At larger scales, surface-to-volume ratios can be improved by increasing the gas sparging rate into the reactor, though this may not be cost effective for reaction systems that are prone to stripping. Surface-to-volume ratios can also be increased by reducing bubble size. This can be achieved by using a sparger with smaller pores, but may necessitate a higher upstream gas pressure to overcome the resulting increase in capillary pressure.\textsuperscript{13} More commonly, mechanical mixing is used to break up large bubbles in the readily scalable stirred tank reactor configuration. Although these are frequently operated in batch mode, mass transfer can be further improved by shifting to continuous processing, thus eliminating downtime, which enables the use of smaller reactors (process intensification). As such, the continuous stirred tank reactor (CSTR) is a promising reactor configuration for oxygen dependent reactions.

Even so, we have previously found that the rate of biocatalytic glucose oxidation in a CSTR remains predominantly limited by the dissolved oxygen (DO) concentration that can be achieved in the system.\textsuperscript{14} Increasing the partial pressure of oxygen in the feed gas (by using oxygen-enriched air) was found to result in higher rates but, at atmospheric pressure, the gains in oxygen solubility were simply too small to overcome this limitation, due to the comparatively low affinity of glucose oxidase towards oxygen ($K_{MO} = 0.51 \text{ mM}^{11}$). Moreover, the results suggested that the enzyme may become more susceptible to deactivation if directly exposed to gas with an oxygen composition above 80\%. Of course, reaction rates can also be increased through higher enzyme concentrations, but this creates a trade-off between reaction performance and effective use of the enzyme, which
may dramatically increase the cost contribution of the enzyme to the process. One means of overcoming this is by extending the useful life of the enzyme, for example by immobilization to help retain the biocatalyst or through the use of membranes. Both approaches also have limitations.\textsuperscript{9,10} Immobilization of glucose oxidase in a porous carrier introduces additional internal mass transfer limitations. It would also be necessary to co-immobilize catalase for hydrogen peroxide degradation, which would limit the overall oxidative activity, especially given that the loading of an immobilized catalyst into a stirred tank is restricted to about 10\% (v/v) to avoid breakage of the carrier particles by the impeller.\textsuperscript{15} Likewise, due to the difference in size between glucose oxidase (150 kDa) and bovine liver catalase (60 kDa), co-immobilization would be preferable for membrane retention, as a higher molecular weight cut-off enables higher flux through the membrane. If left soluble, the enzymes could form a concentration polarization layer at the membrane, within which diffusion of dissolved oxygen could again become limited. Nevertheless, if stability is a concern, as in the case of glucose oxidase exposed to oxygen-rich air, retention of a fixed amount of enzyme may prove deleterious. In such cases, the specific biocatalyst yield (mass product/mass biocatalyst) can instead be increased by feeding the enzyme leaving the reactor into a second reactor, where it can continue to produce product. In theory, if sufficient reactors were configured in series their behavior would begin to resemble a plug-flow reactor: a highly desirable reactor configuration for continuous operation.\textsuperscript{16}

Herein, biocatalytic glucose oxidation in two identical CSTRs in series is modelled and experimentally tested.

THEORY

Since glucose oxidase (GOx) produces hydrogen peroxide as a by-product, which is known to deactivate the oxidase\textsuperscript{17}, it is normally coupled with catalase to scavenge the hydrogen peroxide
and convert it into benign water and oxygen. The coupled GOx-catalase system, illustrated in Scheme 1, was modeled in MATLAB R2018a as a system of ordinary differential equations (ODEs). Equations 1 to 6, which represent the mole balances of glucose, dissolved oxygen, gluconic acid and hydrogen peroxide in the reactor (as well as at the outlet, since the reactor is well-mixed), respectively. This system of equations was solved using the ode45 function. The reaction rates of glucose and catalase were calculated based on the ping-pong bi-bi kinetic rate law\textsuperscript{11}, including competitive inhibition by hydrogen peroxide\textsuperscript{18}, and the Michaelis-Menten equation\textsuperscript{19}, respectively. Q represents the total volumetric flowrate (3 mL.min\textsuperscript{-1}) through the reactor.

\[
\frac{dC_G}{dt} = \frac{Q}{V} (C_{G,F} - C_G) - v_{GOx} \tag{1}
\]

\[
\frac{dC_O}{dt} = \frac{Q}{V} (C_{O,F} - C_O) - v_{GOx} + \frac{1}{2} v_{Cat} + k_{L,a} (C_O^* - C_O) \tag{2}
\]

\[
\frac{dC_{GA}}{dt} = \frac{Q}{V} (C_{GA,F} - C_{GA}) + v_{GOx} \tag{3}
\]

\[
\frac{dC_{HP}}{dt} = \frac{Q}{V} (C_{HP,F} - C_{HP}) + v_{GOx} - v_{Cat} \tag{4}
\]

\[
v_{GOx} = \frac{k_{cat,GOx} C_{GOx} C_G C_O}{K_{MG} C_O + K_{MO} C_G \left(1 + \frac{C_{HP}}{K_i}\right) + C_G C_O} \tag{5}
\]

\[
v_{CAT} = \frac{k_{cat,CAT} C_{CAT} C_{HP}}{K_{MHP} + C_{HP}} \tag{6}
\]
Scheme 1. GOx-catalyzed conversion of D-glucose to D-glucono-δ-lactone, which spontaneously hydrolyzes to form gluconic acid. Catalase is used to decompose the potentially harmful hydrogen peroxide by-product of GOx, resulting in the recovery of some oxygen in solution. Reproduced with permission from reference 14. (Copyright 2020 Org. Process Res. Dev.)

MATERIALS AND METHODS

Glucose oxidase (GOx, Novozym® 28166) used in the experiments was kindly donated by Novozymes A/S, Denmark. All other chemicals and catalase were purchased from Sigma-Aldrich, Denmark. Reactions were carried out in an identical pair of 150 mL (liquid volume) my-Control stirred tank reactors (Applikon Biotechnology B.V., Netherlands), illustrated in Figure 1, connected in series as shown in Figure 2. An Ismatec Reglo Independent Channel Control peristaltic pump (Cole-Parmer, USA) was used to supply the first reactor with an enzyme feed (2 g.L⁻¹ GOx, 2 g.L⁻¹ bovine liver catalase, 100 mM pH 7 potassium phosphate buffer) and an independent substrate feed (2 M glucose, 100 mM pH 7 potassium phosphate buffer), each at a rate of 1.5 mL.min⁻¹, as well as pump the mixed reactor contents out of each vessel at a rate of 3 mL.min⁻¹. Therefore, overall feed concentrations into the reactor were 1 M glucose and 1 g.L⁻¹ GOx and catalase with a dilution rate of 1.2 h⁻¹. These same concentrations were used to initiate the reaction, although only the first reactor contained glucose at the start of each experiment.
Catalase was supplied to the reaction as a means of removing hydrogen peroxide, a by-product of the oxidation reaction, to avoid deactivation of the GOx. The activity of an enzyme is measured in Units (U), defined as the amount of enzyme required to convert 1 µmol of substrate per minute. According to the manufacturer specifications, the activity of the GOx and catalase were 6.5 U.mg_{pure enzyme}^{-1} and 2000 U.mg_{protein}^{-1}, respectively, at 25°C and pH 7. However, the overall activity of the lyophilized catalase formulation may be lower, since its protein content is only specified to be ≥ 60%. Even so, this suggests that the catalase concentration required to consume all of the hydrogen peroxide produced by GOx should be fairly low in comparison to the concentration of GOx in the reactor, which would help to reduce costs. But, as the affinity of GOx towards hydrogen peroxide has been found to be similar to its affinity towards dissolved oxygen (DO), it may already become severely inhibited at hydrogen peroxide concentrations as low as 0.51 mM. In contrast, the affinity constant of bovine liver catalase towards hydrogen peroxide has been found to be 35 mM, indicating that its reaction rate would be significantly reduced at hydrogen peroxide concentrations low enough to avoid inhibition of GOx. This means that, opting for a catalase concentration such that U_{GOx} = U_{CAT}, according to the 1:1 stoichiometry of the reaction, would likely require significant hydrogen peroxide accumulation to occur before the catalase reaction rate could balance that of GOx. Instead, to ensure that hydrogen peroxide concentrations during experiments could be minimized as far as possible, a large excess of catalase was employed (U_{CAT} > 100U_{GOx}), by supplying each reaction with equal concentrations of catalase and GOx.

The reactor was sparged at 1 vvm (volume gas per volume reaction liquid per minute) with 80% (v/v) oxygen, to avoid significant enzyme deactivation, and was agitated at 1000 rpm to ensure well-mixed conditions. The effluent gas (so called ‘off-gas’) from the reactor was passed
through a condenser to avoid evaporation. The temperature and pH of the reactor contents were controlled at 25°C and pH 7, to match the conditions at which kinetic parameters for GOx were available. The reactors were operated as pH-stat, whereby PI controllers regulated the addition of 5 M NaOH to the media to maintain the desired pH, based on measurements from a pH probe in each reactor. Foam formation in the reactors was controlled by manual dropwise addition of Antifoam 204.

**Figure 1.** Single CSTR setup for continuous biocatalytic oxidation of glucose to gluconic acid. Two flights of Rushton impellers were used for mixing. Due to the small liquid volume (150 mL) and number of internals, no additional baffles were included. Gas was sparged through a sintered frit (stainless steel) with a pore size of 15 µm (Applikon Biotechnology B.V., Netherlands). Reproduced with permission from reference 14. (Copyright 2020 Org. Process Res. Dev.)
O₂ saturation (%) in each reactor was monitored and logged using robust optical oxygen probes (Pyroscience AT GmbH, Germany). The probes were calibrated by saturating the reaction media in each reactor, prior to initiation of the reaction by enzyme addition, with nitrogen to achieve 0% O₂ saturation and separately, pure oxygen to achieve 100% O₂ saturation. The percentage of O₂ saturation was converted to dissolved oxygen concentration (mM) using the Henry’s Law constant of oxygen in water (1.2x10⁻⁵ mol.m⁻³.Pa⁻¹). During operation, samples (950 µL) were taken from the reactor at regular intervals, using 50 µL of 5 M H₂SO₄ to quench the reactions. These samples were then analyzed by HPLC on an Aminex HPX-87H column (Bio-Rad Laboratories, Inc., USA), at 20°C with a mobile phase flowrate of 0.6 mL.min⁻¹ of 5 mM H₂SO₄. Refractive Index (RI) and 205 nm Ultraviolet (UV) spectra were used to determine the glucose and gluconic acid concentrations, respectively. Each reaction was monitored for 6 hours (>7 residence times in each reactor), to ensure that steady-state operation was achieved. The k₅a in each reactor was measured to be approximately 100 h⁻¹ using the dynamic gassing-in method in distilled water, whereby the
first order response of the dissolved oxygen (DO or CO) to a step change in feed gas, from nitrogen to oxygen, is monitored and linearized (Equation 7).

\[
\ln \left( \frac{C_{O}^* - C_{O,t=0}}{C_{O}^* - C_{O}} \right) = k_{La} \cdot t
\]

RESULTS AND DISCUSSION

High substrate conversions and product yields are attractive for both the production of simple commodity chemicals, where low profit margins demand efficient substrate use, and complex fine chemicals, where residual substrates from one reaction in a multistep sequence may inhibit those downstream or else require separating. High conversions also help to simplify downstream purification of the final product\textsuperscript{22}. But, while the well-mixed nature of CSTRs allows for increased mass transfer rates, it also ensures that the product stream leaving the reactor will always contain some substrate, since the contents of the reactor are homogenous\textsuperscript{23}. The conversion in the reactor and, subsequently, the concentration of substrate in the product stream are determined by the amount of catalyst as well as the residence time. However, since the rate of a biocatalytic reaction is heavily dependent on substrate concentration, achieving higher conversions requires large amounts of enzyme to counter the decrease in reaction rate due to substrate depletion. Equations 1 to 6 were solved in MATLAB for a variety of feed gas compositions (% O\textsubscript{2}) and operating pressures, with a feed glucose concentration of 1 M. Figure 3 shows the resulting relationships between GOx concentration and steady-state glucose conversion. Also shown are the results under the hypothetical assumption that oxygen could be made available in such high excess (C\textsubscript{O} \gg K_{MO}) that the reaction would effectively be oxygen-independent (Equation 8).
\[ v_{GOx} \approx \frac{k_{cat,GOx}C_{GOx}C_G}{K_{MG} + C_G} \]  

(8)

**Figure 3.** Glucose oxidase concentration required to achieve desired conversion of a 1 M glucose feed stream in a single CSTR, with different operating pressures and feed gas compositions (\(k_{La} = 100 \text{ h}^{-1}, \tau = 50 \text{ min}\)). Reproduced with permission from reference 21. (Copyright 2020 R.M. Lindeque).

There exists an upper limit to the glucose conversion that can be achieved in a single CSTR by increasing the enzyme concentration, and this limit is proportional to the oxygen partial pressure in the feed gas. The reason for this is that an increase in enzyme concentration reduces the dissolved oxygen concentration in the reactor, as shown in Figure 4. Since the solubility of oxygen in water is much lower than that of glucose, relative to the corresponding affinity constants of GOx towards each, oxygen is generally the dominant rate-limiting substrate. This means that the concentration of dissolved oxygen in the bulk media is a critical factor. For optimal reaction rates,
the dissolved oxygen concentration should be as close as possible to the saturation oxygen concentration of the media. However, this drastically reduces the driving force for mass transfer of oxygen from the gas phase into the liquid phase. Therefore, the steady-state concentration of dissolved oxygen in the bulk medium is determined by an equilibrium between the rate at which oxygen is transferred into the medium from the gas phase, the rate at which dissolved oxygen is consumed by GOx and the rate at which dissolved oxygen is produced by catalase (although this rate is tied to that of GOx). Furthermore, high enzyme concentrations make ineffective use of the catalyst ($v_{GOx} \ll k_{cat}.C_{GOx}$), as illustrated in Figure 5, thereby increasing operating costs. In contrast, increasing the $k_{La}$ of the reactor increases the oxygen transfer rate, irrespective of the driving force for mass transfer. This results in the bulk concentration of dissolved oxygen being higher, as shown in Figure 4, allowing higher reaction rates at low enzyme concentrations.
Figure 4. Simulated results from Equations 1 to 6, showing the effect of GOx concentration and \( k_{L,a} \) on the dissolved oxygen concentration (mM) in the bulk medium at steady state in a single CSTR with a feed stream of 1M glucose and a residence time of 50 min, using air as the feed gas.
Figure 5. Reaction rate in single CSTR versus maximum rate as GOx concentration increases. Higher GOx concentrations result in less effective use due to substrate depletion ($k_{L,a} = 100 \text{ h}^{-1}$, $\tau = 50 \text{ min}$). Reproduced with permission from reference 21. (Copyright 2020 R.M. Lindeque)

Nevertheless, to achieve near-complete glucose conversion, the residence time in the reactor would have to be so long that the process begins to resemble batch operation. In theory, a maximum conversion of approximately 94% could be achieved with a residence time of 50 min and a $k_{L,a}$ of 100 h$^{-1}$, provided the medium can be completely saturated with dissolved oxygen. However, as Figure 3 shows, a pressure increase of more than 25-fold would be required to sufficiently increase the oxygen solubility to reach this upper limit, which significantly raises the safety risk of the process and necessitates additional capital investment. Regardless, even with a high GOx concentration and operating pressure, complete conversion could not be achieved, as the reaction inevitably becomes limited by low glucose concentrations. Therefore, as expected, if near-complete conversion is desired, it would be inefficient to operate an oxygen-dependent biocatalytic reaction in a single CSTR.

This problem can potentially be overcome by instead operating multiple CSTRs in series, at least until the enzyme becomes completely inactivated. Figure 6 shows how the overall glucose conversion ($X_n$) in a series of $n$ identical CSTRs increases under different operating conditions. Although the enzyme is used more effectively at a relatively low enzyme concentration (0.1 g.L$^{-1}$), achieving near-complete conversion would require an impractical number of reactors (>10), even if the reaction were fully saturated with oxygen. By increasing the enzyme concentration 10-fold, the number of reactors required to reach near-complete conversion can be reduced to 8 at atmospheric pressure and further down to 3 at significantly higher pressures. However, in all cases, the factor by which the overall conversion is improved with the addition of subsequent reactors
\( \frac{X_n}{X_{n-1}} \) begins to diminish after the second reactor in the series. For this reason, it would appear to be most practical to simply optimize a dual-reactor configuration to achieve the highest conversion with the least amount of enzyme possible, since this yields the greatest benefit from connecting CSTRs in series without making the system overly complicated or costly to operate.

**Figure 6.** Results from simulation of up to 10 identical CSTRs at two different GOx concentrations (Left: 0.1 g.L\(^{-1}\), Right: 1.0 g.L\(^{-1}\)) and a variety of feed gas compositions and operating pressures. The upper panels show how the overall glucose conversion \( X_n \) with a given number of reactors
(n) changes under different operating conditions and the lower panels shows the factor by which overall conversion improves with addition of each new reactor in the series \( (X_n/X_{n-1}) \). Adapted with permission from reference 21. (Copyright 2020 R.M. Lindeque)

To test this, experiments were carried out in a dual CSTR set-up. The results of the experiments, shown in Figure 7, indicate that the glucose concentrations, particularly those of the second reactor, were subject to some experimental measurement variability. This is likely due to small differences in the calibrated flowrates into and out of the first reactor. Nevertheless, since the system is predominantly oxygen-limited, these differences produced little variation in the gluconic acid concentrations. The greatest variability between experiments was observed in the DO concentrations in the second reactor, which is an unfortunate consequence of controlling the foam formation with an antifoaming agent\(^{14}\), as well as the sensitivity of the oxygen probes (when using an oxygen-rich feed gas). These effects were less pronounced in the first reactor, where DO concentrations were significantly lower. The reactors reached steady-state after approximately 1 hour (1.2 residence times).
Interestingly, the overall glucose conversion after the second reactor was increased by a factor of 3.2, which is significantly higher than the model predicts (Figure 6). Specifically, the results suggest that the $k_{L}$a in the second reactor was higher than that in the first. This could be a result of controlling foam formation, which was more prevalent in the first reactor, since antifoaming agents are known to reduce $k_{L}$a$^{24}$. However, since the reactors are connected in series, antifoam added to

**Figure 7.** Glucose and gluconic acid concentration, as well as oxygen saturation profiles in Reactor 1 (■) and Reactor 2 (▲), operated in series, with 1 g.L$^{-1}$ glucose oxidase and catalase, as well as 80% O$_2$ as feed gas. Error bars represent standard error of the mean. Reproduced with permission from reference 21. (Copyright 2020 R.M. Lindeque)
the first reactor would also affect the second. Instead, this difference likely stems from the fact that the two reactions in the two CSTRs are not in fact identical, as was assumed in the model, in that their media compositions differ, which has previously been found to have a significant effect on the $k_{L a}^{14}$. This could imply that $k_{L a}$ may be positively affected by the presence of gluconic acid and/or negatively affected by glucose. It is unlikely that oxygen accumulated in the second reactor, and not the first, because the reaction became glucose-limited. Figure 3 shows that, even if the reaction were oxygen-independent, glucose would only begin to limit reaction rates above a conversion of roughly 70%, corresponding in these experiments to a glucose concentration of 300 mM. Therefore, a higher $k_{L a}$ in the second reactor would appear to have been the cause of the higher dissolved oxygen concentration. This warrants further investigation into the dependence of $k_{L a}$ on media composition, especially since the net effect could be beneficial or detrimental depending on the specific species involved in a particular reaction. Another interesting aspect of the increase in dissolved oxygen concentration in the second reactor is that it suggests the enzyme is used more effectively in the second reactor than in the first. This is confirmed by the total oxygen consumption rates of Reactors 1 and 2 being 236 and 343 mmol.L$^{-1}$h$^{-1}$ respectively, at steady state, based on the amount of gluconic acid produced within each reactor. This is in contrast to what happens in a series of CSTRs when operating with a single-substrate enzyme. In such cases, the concentration of the rate-limiting substrate decreases in each subsequent reactor and so, while the conversion still increases with more reactors, the enzyme is used less effectively. Therefore, in a gas-liquid system there is an additional degree of freedom in the design of each reactor, which benefits the optimization of a dual reactor configuration. Moreover, the specific biocatalyst yield (mass product/mass enzyme) from the two-reactor system was calculated to be $95 \text{ g}_{GA}\text{ g}_{GOx}^{-1}$, 1.7-
fold higher than experiments done in a single reactor.\textsuperscript{14} Both of these improvements help to reduce the overall cost contribution of the enzyme.

CONCLUSIONS

The maximum conversion that can be achieved in a CSTR is always limited by substrate depletion if the reaction rate is dependent on substrate concentration. For biocatalytic glucose oxidation, the low water-solubility of oxygen relative to the $K_M$ of GOx drastically limits the conversion that is possible in a single CSTR without significant pressurization and high enzyme concentrations, both of which increase the cost of the process. An alternative means of improving conversion is to connect multiple CSTRs in series. Given enough reactors, it would even be possible to approximate plug-flow behavior. Since the glucose concentration is reduced in each subsequent reactor of the series, the reaction becomes less oxygen-limited and, eventually, glucose would become the limiting substrate. However, a basic model of the system shows that, while shifting from one to two CSTRs can double overall conversion, the gains in conversion from subsequent reactors quickly diminish, while the added capital and operating costs would remain the same. Thus, it may be more practical and economical to operate two CSTRs in series, followed by optimization of the operating conditions in each reactor. In fact, results from glucose oxidation experiments show that the overall conversion was actually tripled by addition of a second reactor, where the reaction was less oxygen-limited. Improved conversion and more effective use of the enzyme also helped to increase its specific yield and thereby reduce its overall cost contribution to the process.
NOMENCLATURE

- $k_{La}$: Volumetric mass transfer coefficient, $h^{-1}$
- $C_i$: Concentration of species $i$ (G = Glucose, O = Dissolved oxygen, GA = Gluconic acid, HP = Hydrogen peroxide, F = Feed stream), mol.L$^{-1}$
- $Q$: Reactor flow rate, L.h$^{-1}$
- $V$: Reactor working volume, L
- $v_j$: Reaction rate of enzyme $j$ (GOx = Glucose oxidase, CAT = Catalase), mol.L$^{-1}$.h$^{-1}$
- $k_{cat,j}$: Rate constant of enzyme $j$, mol.mg$^{-1}$.h$^{-1}$
- $K_{Mi}$: Enzyme affinity constant towards species $i$, mol.L$^{-1}$
- $K_I$: GOx inhibition constant, mol.L$^{-1}$
- $C_{O^*}$: Saturation concentration of oxygen according to Henry’s Law, mol.L$^{-1}$
- $\tau$: Reactor residence time, h
- $n$: Number of CSTRs in series
- $\chi_n$: Overall glucose conversion a series of $n$ CSTRs

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