Sixth International Conference on Fuzzy Systems and

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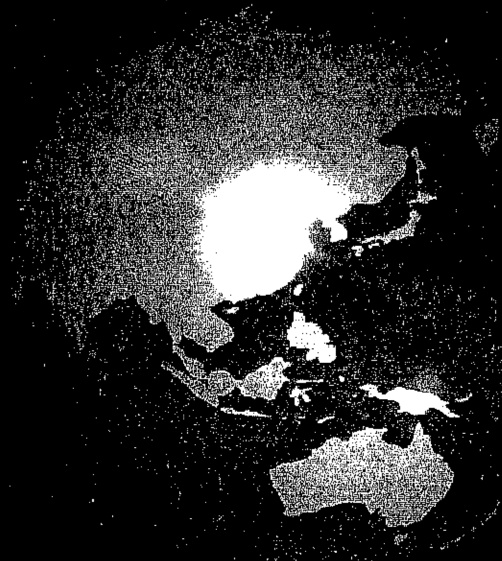

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Edited by Yixin Chen, Hepu Deng, Degan Zhang, and Yingyuan Xiao

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Optimization of Bioreactors Performances in the Production of Fructose from Inulin with Immobilised Inulinase

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Abstract

Fructose is a monosaccharide present in most fruits, honey and in some vegetables, such as onion and chicory As a sweetener, fructose is 1.5 times sweeter than sucrose and it is the sweetest among natural sugars; in sweetening operations lower quantities of fructose can be used instead of sucrose. In this paper a study of enzymatic production of fructose from inulin by immobilized inulinase hydrolysis is presented. A kinetic study of the reaction both for the native and the immobilized enzyme has been carried out. Reaction kinetics and enzyme deactivation rates predictions were coupled in a comprehensive model able to predict reaction performances in a batch and in a continuous bioreactor.

1. Introduction

Fructose is a monosaccharide which molecular formula is $C_6H_{12}O_6$ and it may have a cyclic or a linear form.

As a result, in sweetening operations lower quantities of fructose can be used instead of sucrose [1,2]. Fructose is also more performing than sucrose as a food ingredient, because its high igroscopicity prevents foods from drying and from mould formation, lowering water activity.

Regarding consumers health, fructose is often compared to sucrose. Some authors report that the latter is cariogenic, contributes to atherosclerosis, leads to obesity due to its high caloric content and is unfit for diabetics [3], whereas the former does not display these drawbacks and, on the contrary, can have beneficial effects on iron absorption in children [4]. In this regard, it is worthy noticing that glycemic index1 for glucose is 100, for sucrose is 86 and for fructose is 23 [5], confirming the data on the effects of these sugars on diabetics. Some data about negative effects of fructose consumption upon obesity exist, but they are controversial and not clearly proven.

Fructose is one of the most widespread sugars in the world, especially in the form of HFCSs (High Fructose Corn Syrups), which are mixtures of sugars, mainly fructose and glucose, obtained from corn with an estimated production of 11 million tons/year in 2000.

The most used process for fructose production is an enzyme-based process with starch as primal substrate, [6], where three main steps constitute the process:

- starch liquefaction
- saccharification (hydrolysis of starch into glucose)
- isomerization of glucose into fructose

Although very successful, the traditional process exhibits two main drawbacks: i) glucose isomerization is a reversible reaction and maximal fructose concentration at equilibrium is 50% of total sugars; ii) chromatographic separation to reach high purity is very costly.

Based on these considerations, an alternative process is being studied in the last decades overcoming the above said disadvantages: the production of fructose from its natural polymer inulin. Inulin hydrolysis is an irreversible reaction that can bring 95% fructose in one stage, without further separations.

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This process can be carried out either enzymatically or by acidic catalysis; however, the latter results in bad fructose quality and has become of little interest for industrial applications.

In this paper a study of enzymatic production of fructose from inulin by immobilized inulinase hydrolysis is presented.

A kinetic study of the reaction and a study on enzyme thermal deactivation through time have been preliminarly carried out.

Reaction kinetics and enzyme deactivation rates predictions were coupled in a comprehensive model able to predict reaction performances in a batch bioreactor where commercial native enzymes has been used. The enzyme was, then, covalently immobilized on supports of industrial interest to gain biocatalysts for industrial applications and a theoretical mathematical model, based on transport phenomena equations couplet with kinetic analysis, was developed in order to predict and optimize performances of different bioreactors configurations.

A comparative analysis of bioreactors performances will be presented and discussed in the paper.

2. Experimental

First of all the study went deep into the enzyme knowledge. Experimentation was devoted to obtaining activity and stability data. These data were computed in order to achieve a kinetic equation to describe the reaction rate for an assigned quantity of active enzyme and a deactivation equation to predict the quantity of active enzyme during time at different conditions. The two equations were coupled together to reach a complete model able to predict the reaction performance even during enzyme temperature dependent deactivation

2.1. Materials and Methods

Reactants

Inulin derived from chicory (Sigma–Aldrich, Italy) was used as a substrate. Its degree of polymerization (DP) was quantified by inulin complete acidic hydrolysis. Based on the hypothesis that each inulin molecule contains one glucose residue, the DP was defined as the number of fructose residues (F) per glucose residue (G) and evaluated as the ratio F/G after complete hydrolysis. The DP of inulin was found to equal 28, corresponding to a molecular weight of 4700 Da.

Enzyme and immobilization supports

The enzyme consisted of a commercial liquid mixture (r = 1.13 g/mL) of exo- and endo-inulinases (Fructozyme LTM) kindly provided by Novozymes A/S (Denmark): its activity was measured under the following defined conditions: T = 60 8C, inulin initial concentration S0 = 10 g/L, pH 5.0, and reaction time t = 20 min. The activity of Fructozyme LTM was found to equal 5500 U/g, with U defined as micromoles of fructose produced after 1 min of reaction under the standard conditions listed above.

Immobilization carriers were Sepabeads®, methacrylic polymers produced and commercialized by Resindion S.r.l. (Mitsubishi Chem. Corp, Milano, Italy), supplied with a water content of about 70% w/w.

Inulinase was covalently immobilized on a class of chemically homogeneous methacrylic carriers, Sepabeads, presenting amino or epoxy reacting group, Sepabeads® EC-HA and Sepabeads® EC-EP respectively. Immobilisation, for both supports, proceeds through covalent bonds formation between lysines on the surface and the functional groups on the polymer. Immobilisation on epoxy supports proceeds through direct condensation of lysines and oxyrane groups on the polymer, whereas immobilisation on amino supports proceeds with previous pre-activation of the support by glutaraldehyde [7].

All chemicals used for acetate buffer preparation in distilled water (acetic acid, sodium acetate trihydrate) were reagent grade.

Analytical methods

Sugar analysis was carried out by HPLC. All liquids were HPLC grade. The mobile phase was acetonitrile/water 75/25% (v/v) and was pumped at a flow rate of 1 mL/ min. The column was: Alltima amino 100 A 5 m 250 mm _ 4.6 mm, Alltèch. Detection of the sugars relied on refractive index (RI 930 Jasco).

Batch experiments

Batch tests were run in a system (Applikon, The Netherlands) consisting of: a 1.5 l glass vessel, a six-blade impeller driven by an electric motor (Stirrer Motor Assembly P100) controlled by a stirrer controller (P100, ADI 1032), pH and temperature sensors, a jacket for temperature control by means of a Bio Controller (ADI 1030).

The reaction volume was 500 ml, pH was kept to 5.0 by means of an acetate buffer and its values were constantly measured and all other variables were varied as follows: S0=10, 40 g/l, T=40, 50°C, E0=1, 4 gwet. The reuse cycle of the enzyme was performed after 28 h of operation and after filtering and washing the biocatalyst with distilled water.

Reaction was quenched by separating the enzyme from the reacting mixture by means of a 90 μm filter and samples were immediately analyzed.

2.2. Native enzyme

A grid of experimental results was obtained in the ranges 40-60°C and 3-60 g/l, for temperature and substrate concentration respectively. A reaction scheme and its simplifications, has been proposed and it has been possible to follow the progress of inulin hydrolysis by monitoring fructose production and not inulin consumption itself. The reaction scheme is reported in [7] where a series-parallel mechanism has been hypothised in order to formulate the kinetic model as of native, as of immobilized enzyme.

2.2. Immobilised enzyme

One of the most attractive perspectives when studying an enzymatic reaction is to design a continuous process without losing the biocatalyst within the product stream. To do that the two enzymatic preparations, EC-HA and EC-EP, were assayed in a BBR where fructose production was monitored overall the reaction up to complete reaction.

The immobilization process is highly efficient since the enzyme maintains its initial activity after immobilization

Using both immobilised preparations the reaction was complete in 28 hours and inf Fig. 1 their performances are shown. Despite of a lower activity (ability to catalyze the reaction in the first stages), the epoxydic preparation showed a better performance with respect to the amminic one, when observed on a long time scale.

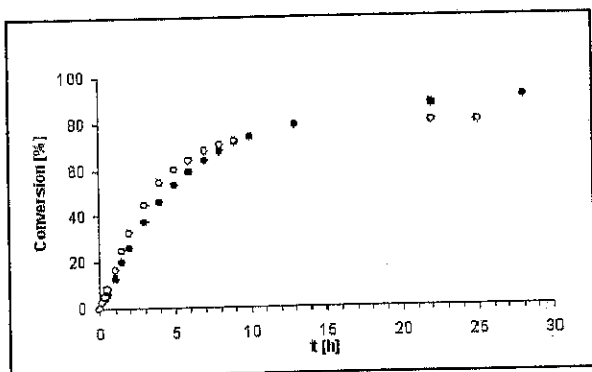

Figure 1. Conversion of inulin catalysed by Fructozyme L immobilized on EC-EP (Black circles) and EC-HA (Empty circles). Conditions: T = 50°C, So = 10g/l.

Concerning their stability, test runs have been effected in order to estimate kinetic rate and deactivation during time.

3. Theoretical

3.1. Modeling of Batch Bioreactor, BBR

A predictive model has been formulated in order to simulate the behaviour of a batch bioreactor in which free inulinase will be used as biocatalyst for the inulin hydrolysis. To do that a preliminary kinetic analysis coupled with a deactivation enzyme study have been carried out in order to formulate a complete kinetic model able to describe the long term bioreactor performances.

The BBR mass balance has been formulated in terms of disappearance of the substrate, inulin, indicated with S, following a kinetic rate Vr, as the following eq. 1

$$-\frac{dS}{dt} = V_r \qquad (1)$$

with the initial condition $t=0$, $S=S_0$.

In the eq. 1 it need the kinetic rate equation that has been found with the construction of the kinetic model following the classical pattern of kinetic data formulation and analysis.

Initial velocity tests were run at any temperature and substrate concentration value, in order to prevent reaction rates from being influenced by enzyme deactivation.

A Michaelis-Menten model was proposed even though some of its fundamental hypotheses were not satisfied; like: there is not a unique substrate, but a mixture of polymers; there is not a unique enzyme, but a mixture of enzymes; the conversion of inulin to fructose is not due to a single stage reaction, but to a series-parallel mechanism, [8].

Nevertheless, it was used as a mathematical relationship between the rate of reaction and the substrate concentration, relying on the "condensed" reaction mechanism, on the extreme system dilution (water is a reactant, but is in excess) and on the consideration of inulin not as a mixture of oligomers and polymers, but as single molecule characterized by a degree of polymerization (DP) equal to the mean DP.

Kinetic data at each temperature were fitted according to Lineweaver-Burk representation to determine k2 and Km depending on temperature. The functional dependence of such kinetic parameters from temperature was found by data fitting through Arrhenius equation, [9] which is appropriate for many enzyme catalyzed reactions in the limited ranges of interest of these processes [10], and the values for the activation energy and the pre-logarithmic constant were found.

The complete predictive model has been built by coupling a deactivation model and a kinetic model, in

order to determine long time scale performances of the reaction under study.
The deactivation model, [9], relating enzyme activity to temperature and time, has been obtained by interpolating data of enzyme residual activity vs. time according to a first order dependence, as described in the eq. 2:

$$V_r = \frac{k_2(T)e_0 \exp[k_d(T)t]S}{K_m(T)+S} \qquad (2)$$

In the eq. 2 the kinetic constant k_2, K_m have been calculated with the initial velocity tests as function of the temperature T:

$$k_2(T) = \exp\left(\ln k_{20} - \frac{E_{a2}}{RT}\right)$$

$$K_m(T) = \exp\left(\ln K_{m0} - \frac{E_{km}}{RT}\right)$$

The deactivation constant k_d will be estimated with the desctivation tests carried out at different temperatures:

$$k_d(T) = \exp\left(\ln k_{d0} - \frac{E_{ad}}{RT}\right)$$

Model validation was achieved by comparison with experimental data; the results are presented in terms of conversion x, depending of the initial substrate concentration So:

$$x = \frac{S_0 - S}{S_0}$$

The validation of the model has been obtained by comparison of batch theoretical predictions with experimental data at different temperatures and substrate concentrations. In Fig. 2 the model validation is presented for one of the treated cases.

Optimization has been undertaken to find the optimal temperature T_{opt} as the temperature which allows the batch reaction (with other operating conditions set) to occur in the least time, Fig. 3. It has been found that this optimum is related to the enzyme amount. Because the deactivation, a higher amount of enzyme permits low reaction times, and higher operating temperatures equal the maximum enzyme temperature T_{max} of 60 °C. When low amount of enzyme is choose, it need to operate at lower temperature, to reduce the effect of deactivation.

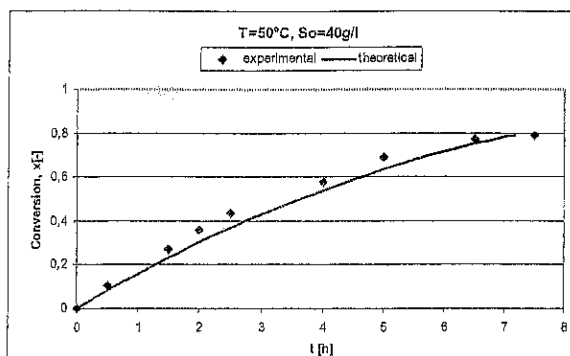

Figure 2. Experimental data vs predicted values, T=50°C and So=40 g/l.

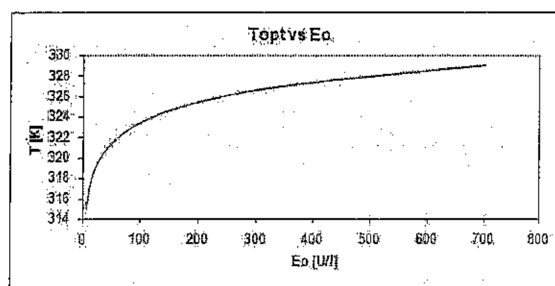

Figure 3. Optimization of BBR

As consequence the further analysis with immobilized biocatalyst has been carried out at 50°C, very close to the optimal temperature.

3.2. Modeling of Batch Bioreactor with Immobilized Enzyme, BBRIE

The batch bioreactor with immobilized enzyme has been modeled taking into account the kinetic rate found with a preliminary kinetic analysis carried out on both the biocatalysts, EC-HA and EC-EP.
The BBRIE is operated in kinetic control, with negligible transport resistance (Damkoler number, Da lower then 1).
Kinetic rate has been formulated as the eq. 2, at the fixed optimal temperature of 50°C.
In Tab. 1 the kinetic constant are summarised.

Table 1. Kinetic parameters for the native and immobilized enzyme.

Enzyme	k_2 $g_{substrate}/g_{enzyme}$ min	K_m $g_{substrate}/L$	k_d min^{-1}
Native	0,585	34,1	0,0022
EC-HA	0,442	48,38	0,0037
EC-EP	0,387	47,50	0,0030

Mass balance on the BBRIE has been formulated and the validation of the model with experimental results has been done.

3.3. Modeling of Continuous Bioreactor with Immobilized Enzyme, CBRIE

By using the kinetic rate the mass balance has been done and the eq. 3 has been formulated:

$$\frac{dS}{dt}=\frac{(S_0-S)}{\tau}-\frac{k_2\cdot e_0\cdot(1-\varepsilon)\cdot\rho_p\cdot S\cdot e^{-k_d t}}{K_m+S} \qquad (3)$$

in which ε is the void fraction in the CBR, τ the residence time (ratio between the volume of the CBR and the inlet flow rate) and ρ_p is the biocatalyst density equal to 1,1 g/mL.

The eq 3, with the initial conditions (t=0, S=So), has been numerically solved in order to estimate the performances as conversion x, at different residence time in the bioreactor, τ.

In Figs. 4 and 5 the research of an optimum value of τ as function of enzyme loading is reported.

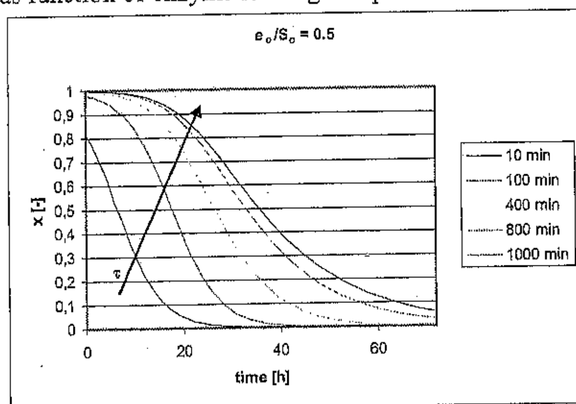

Figure 4. Prediction of performance in terms of conversion degree at different residence time τ.

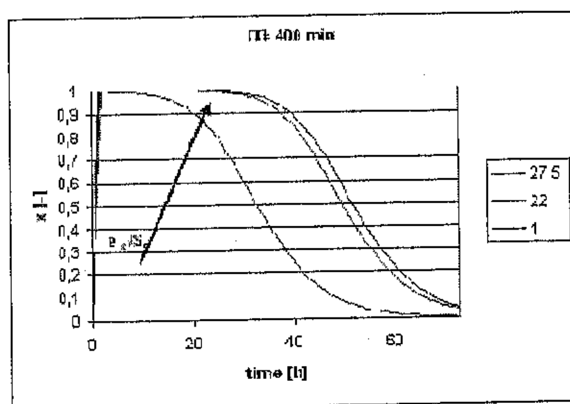

Figure 5. Prediction of performance in terms of conversion degree at different eo/So.

It will be found that for a kinetic time of 110 min, an optimum value of residence time τ= 400 min permits to achieve high conversion with low effect of enzyme deactivation.

4. Conclusions

The experimental study carried out to the bioreactors and the mathematical models permit to predict the optimal performances in order to the production of enzyme from inulin hydrolysis: in the CBRIE the optimal condition is related to a residence time of 400 min. The process might be improved and further optimized to be of interest for industrial production. The evaluation of intrinsic kinetics of immobilized enzyme permit also to estimate the possibility to use them also in packed an fluidized bed bioreactor.

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