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Enzymatic Hydrolysis of Starch in a Fixed-Bed Pulsed-Flow Reactor

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ABSTRACT

One of the most important problems in the design and operation of fixed-bed biological reactors is the control of the process rate by mass-transfer limitations. In order to overcome this problem, a new technology, based on the use of pulsed reactors, was developed. A new type of pulsing device, giving a see-saw-type of disturbance, was assayed. To quantify the possible improvement obtained, we have chosen as an example the hydrolysis of concentrated starch solutions by glucoamylase (from *Aspergillus niger*) immobilized on chitin slabs. The reactor has an internal diameter of 50 mm and a bed height of 200 mm. Temperature was controlled at 25°C, and the working hydraulic retention times were from 0.29 to 1.8 h. The results revealed that pulsation helps to lessen the diffusional difficulties, since the maximum reaction velocity increased 10%. Additional improvements, up to 20% in some cases, are achieved by recycling a part of partially converted feed.

Index Entries: Pulsed flow; packed-bed; starch hydrolysis; immobilized glucoamylase; chitin.

NOMENCLATURE

τ	mean residence time	h
S_o, S	initial and final substrate concentration	g/L
$r = V$	hydrolysis rate	g/L·min
V_{max}	maximum reaction rate	g/L·min
K_s	Michaelis constant	g/L
R	recirculation rate = return flow/out flow	

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t	time	h
θ	normalized time	
D	axial dispersion coefficient	m^2/s
N	number of tanks in series	
C_θ	normalized tracer response to a pulse input	
μ_a	apparent viscosity	kg/m·s
$-dv/dr$	velocity gradient	s^{-1}

INTRODUCTION

The use of packed-bed reactors in biotechnology processes usually presents several drawbacks, among others an increase in mass-transfer resistances, defficient use of the equipment volume, because of which preferential paths and dead zones could arise in the reactor, an inter- and intraparticle accumulation of the gas produced, and a special difficulty in supplying gas (especially air) in aerobic processes. In order to overcome these problems, a new technology based on the use of pulsed reactors was developed with the objective of increasing productivity.

Pulsation is a technique commonly used in numerous chemical engineering processes, such as extraction and filtration. A high level of turbulence, as well as an increase in the interfacial area are achieved in L/L and S/L extractions (*1*) by using pulsation. The profitable effect of a pulsed flow on the filtration performance of an ultrafiltration membrane in tubular form has been proved by Finnigan and Howell (*2*).

The pulsation concept in biotechnological processes has been applied only in the last few years, especially in the pollution and production fields. In anaerobic wastewater treatment processes with biogas production, Hwang and Brauer (*3*) reached a conversion efficiency of about 85%. Etzold and Stadlbauer (*4*) developed a pulse-driven loop reactor, a pulsed anaerobic filter, and a pulsed anaerobic baffled reactor; data indicated in all cases that pulsed digesters favor degassing and avoid reactor clogging. In other experiments, Hamamci and Ryu (*5*) determined the effect of introducing oxygen pulsation to ethanol fermentation in a packed-bed column. In this same field of production, Ghommidh et al. (*6*) studied the production of acetic acid in a pulsed column packed with immobilized cells, reaching a very interesting productivity value of about 10.4 g/L·h.

The different pulsed reactors are characterized by their pulsing devices. Among these, we can mention

1. Pulsed perforated-plate columns, commonly used in liquid–liquid extraction industrial processes, which employ as the pulsing device a reciprocal pump. Sequential bubbles coalescence, and their redistribution through each pulsation cycle enhances the surface's renewal (*7*).

2. Packed-bed pulsed reactors, in which the pulsing device is a positive-displacement pump (*2*).
3. Air-pulsed columns, which are very well suited to aseptic operations, because they have no moving elements (*8*).
4. Pneumatic pulsation, which impels liquid to the reactor using a reserve of compressed air (*9*).

Most of these appliances provoke an increase in the axial dispersion within the packed bed. This fact, which may be inconsequential or even desired in many processes, can cause harmful results when a biological product-inhibited process takes place.

Because of the potential problem mentioned above, we studied new pulsing devices (currently being patented) that generate see-saw-type disturbances (instead of the sinusoidal waves obtained with the use of previous devices) which permits the maintenance of a desired plug flow model.

Our main objectives, then, are to study the behavior of such a pulsed-flow packed-bed reactor and to compare the results against those obtained from the packed-bed without pulsation.

In this first study, the hydrolysis of medium-concentrated starch solutions by immobilized glucoamylase was the chosen process, because the solution's viscosity could provoke undesireable diffusional limitations.

MATERIALS AND METHODS

Enzyme

Glucoamylase NOVO (NOVO Industries, Denmark; AMG 300 L, from *Aspergillus niger*) was selected because of its commercial importance in the amylolytic industries and its low α-amylase content (because of its origin). The original enzymatic preparation shows an activity of 300 NOVO U/mL (1 NOVO U = amount of enzyme that produces 1 μmol maltose/min). Our solution was prepared by diluting (1:1000) the enzymatic preparation with distilled water.

Substrate and Support

Soluble starch without any pretreatment (Panreac Chemical Products, Spain) was used as substrate. The enzyme support was chitin slabs (Sigma) because of its low price and its porous structure, which seems useful for processing a substrate of high viscosity.

Immobilization

The immobilization technique was simple adsorption. Most of the enzyme was immobilized on only the external surfaces of the chitin slabs, so only external mass-transfer resistance needs be taken into account.

Stability

To evaluate the stability of the immobilized enzyme, a small fixed-bed column was employed, continuously fed with 150 mL/h of a 50 g/L starch solution. Samples were analyzed daily to quantify reducing sugars.

Viscosity Measurements

To determine the viscosity of starch solutions, a Brookfield (Brookfield Engineering Lab, Inc., Massachusetts, USA) Syncro-Lectric LVT rotary viscometer with an UL adapter, was used at 25°C.

RTD Determinations

The hydrodynamic behavior of the packed-bed reactor with and without pulsation has been evaluated by means of residence time distribution (RTD) experiments, which make possible calculating the mean residence time and, therefore, the flow model of the reactor. A stimulus–response technique was employed, using as tracer LiCl (which is not adsorbed by the support and is also inert against the substrate and products). Once a steady state was achieved, 1 mL of the tracer was injected at the reactor entrance; samples were taken at periodic intervals in the reactor outlet. Li^+ concentration was determined by atomic absorption spectophotometry (Perkin-Elmer ICP 15500). Numerical treatment of data to obtain a number of characteristic parameters was carried out by means of a computer program developed by our group (*10,11*).

Batch-Hydrolysis Experiments

Batch experiments were carried out in 250 mL glass flasks at 25°C in static or shaking (150 rpm) operation. In previous experiments we had proved that a linear relationship between hydrolysis rate and time is kept during the period of time considered (10 min). Accordingly, a kinetic analysis of data based on an initial rates method was followed. The working method consisted of mixing 1 vol of the enzymatic solution with 2 vol of 0.15*M* acetate buffer, pH 4.4, and 3 vol of the substrate solution (1-mL samples). The reaction was stopped after 10 min by adding an equivalent volume of dinitrosalycilic acid (DNS) reagent, which allowed us to determine the released reducing sugars according to Bernfeld method (*12*).

Packed-Bed Reactor

Hydrolysis of starch was studied in a glass-upflow packed-bed reactor with and without pulsed flow. Starch solutions of 50, 100, and 150 g/L were used as substrate. The column had an internal diameter of 50 mm and a 200 mm bed height. Flow adapters at the bottom of the column were

filled with glass balls (4.5 mm in diameter) in order to minimize dead volume in the reactor and to improve the liquid distribution. Temperature was controlled at 25°C by means of a circulating water bath coupled to the reactor jacket.

Packed-Bed Reactor with Recycle

The experimental device and operation were similar to those used for the preceding reactor. Several recycling ratios (0, 1, 2, 3, 4, and 5) were employed, and the substrate was a 100 g/L starch solution.

Calculations

Determination of kinetic parameters from batch experimental data was performed by means of a nonlinear optimization program based on a Nelder and Mead method that considers as objective function the sum of the square differences between the experimental and calculated values (*13*). A dynamic-simulation program was used in the case of packed-bed reactors.

RESULTS AND DISCUSSION

Immobilization of Glucoamylase

Optimal immobilization conditions enabling a higher specific activity were fixed. Several enzyme/support ratios (in this case glucoamylase/chitin) were assayed. To evaluate the possible benefit of adding glutaraldehyde, about which a great number of controversies (*14,15*) exist, a series of experiments were carried out, mixing the enzyme with different ratios of glutaraldehyde at a given support weight. Activity retention decreases with the addition of glutaraldehyde, perhaps because of the existence of residual unsaturated polyaldehydes accompanying it.

Several experiments varying enzyme concentration for the same chitin weight were done to evaluate how this ratio influences the retention capacity of the enzymatic activity of bioparticles.

A zone of maximum activity for a glucoamylase/chitin range of 1.6–2.8 ([g/L] protein/g chitin) is achieved, 2.2 being the chosen value for the following experiments (Fig. 1).

Stability

After 5 d, activity levels off at 70% of the initial value and remains at that (reduced) level for a long period. In order to avoid the interference of a stability effect in data analysis, data from experiments in packed-bed reactors were taken after this activity level was reached.

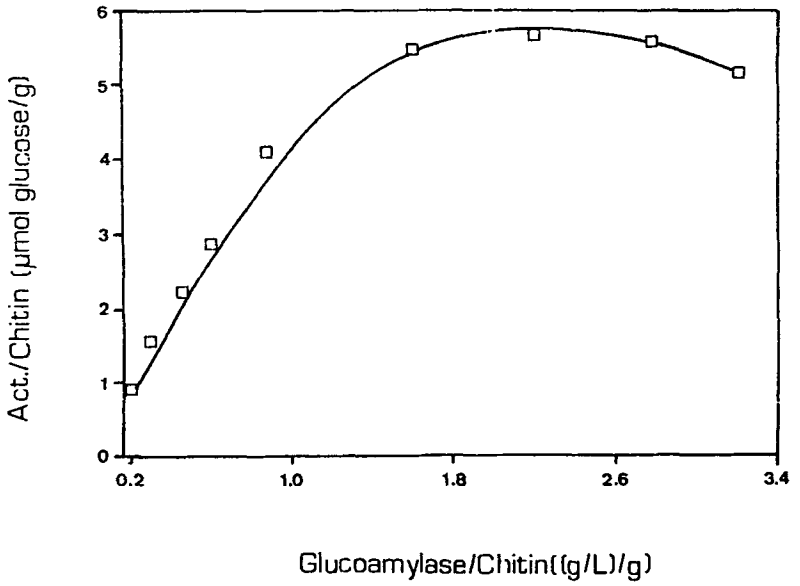

Fig. 1. Retained activity vs enzyme/support ratio.

Mass-Transfer Effects

Once a rheological study (Fig. 2) at rotation speed between 1.5 and 60 rpm was accomplished, a pseudoplastic behavior of 20–150 g/L starch solution was observed. Data were well fitted to the Ostwald de Waele model.

To evaluate the effect of possible external diffusional resistances, kinetic parameters from batch experiments for immobilized enzyme with and without agitation (150 rpm) were calculated, using an optimization program that makes possible direct fitting of data to a kinetic model. The V_{max} values were 0.15 and 0.16 g/L·min and K_s values were 8.34 and 7.88 g/L for the systems without and with agitation, respectively. These facts indicate that resistance to mass transfer is not very important, although agitation did slightly increase the hydrolysis rate.

Hydrodynamic Behavior

The hydrodynamic characterization of the two packed-bed reactor configurations was carried out by applying two different models. In operation without recycle, an axial dispersion model was employed, because its behavior was similar to that of ideal plug flow. The axial dispersion coefficient, D, is the parameter being determined.

However, for a reactor with recycle, a model with tanks in series was used, because the behavior is closer to that of a Continuous Stirred Tank Reactor (CSTR). The reactor model could be represented by a series of various ideal CSTRs of equal size; the parameter to be determined is the number of tanks, N.

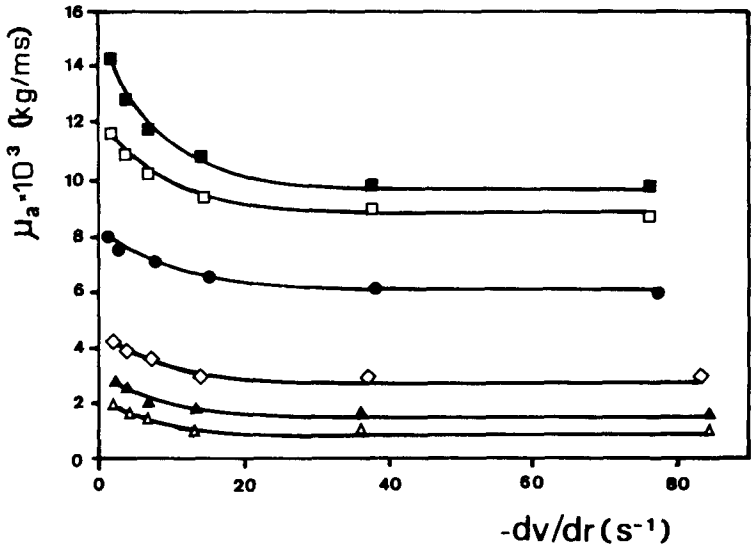

Fig. 2. Apparent viscosity (μ_a) of starch solutions (△, 20; ▲, 50; ◇, 80; ●, 100; □, 130; ■, 150 g/L) vs velocity gradient ($-dv/dr$) at 25°C.

A low value for D indicates a narrower concordance with a plug flow reactor (PFR), and a greater number of tanks in series, N, denotes an approximation to a stirred tank reactor.

Figures 3a and b show the results of an experiment in a fixed-bed reactor without recycle, with and without pulsation, so as the curve generated by the dispersion model. Coincidence between theoretical and experimental residence times proves that LiCl was a suitable tracer. In Fig. 4, values of D for two starch solutions (50 and 150 g/L) in both reactors, with and without pulsation, are shown. D declines as starch concentration and flow rates increase. In any case, pulsing flow allows axial dispersion coefficients to remain at a lesser value. Since values for D are very low, equations corresponding to an ideal PFR model can be correctly used to represent the process.

Results of operation at recycling ratios of 0, 3, and 5 (with and without pulsation) can be seen in Fig. 5. As could be foreseen, as the recycling ratio increases, the reactor is more homogeneous, its behavior being very close to an ideal CSTR at a recycling ratio ≥ 5.

Enzymatic Hydrolysis

The main objective of this work consists of using pulsing reactors to improve the efficiency of processes that present diffusional problems. Starch hydrolysis was selected (although its diffusional limitations are moderated) because of its industrial importance. The use of high starch concentrations (much higher than K_s), and the low conversions obtained allow the maintenance of conditions at which the reaction could be considered as a pseudoorder zero.

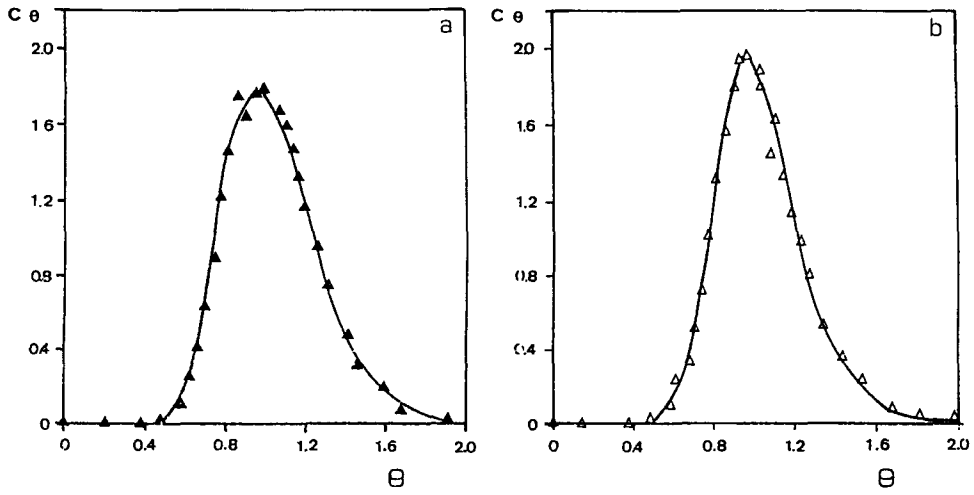

Fig. 3. Experimental (symbols) and calculated (solid line) RTD curve for a packed-bed reactor without recycle operating at $\tau = 1.1$ h; (a) without pulsation, (b) with pulsation.

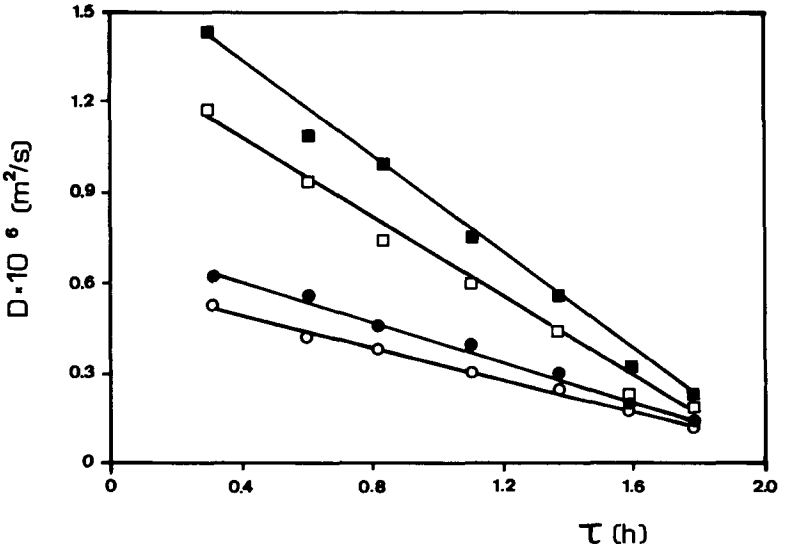

Fig. 4. Axial dispersion coefficient (D) vs residence time (τ) for 50 g/L (○) and 150 g/L (□). Solid and void symbols correspond to reactors without and with pulsation, respectively.

Fixed-Bed Reactor Without Recycling

In a first series of experiments, a comparison of the efficiency for fixed-bed reactors with and without pulsation was done. Reactors were operated with 50, 100, and 150 g/L starch solutions at hydraulic retention times between 0.29 and 1.8 h. At these operating conditions, the behavior of this equipment corresponds to an ideal PFR, the mathematical model of which is

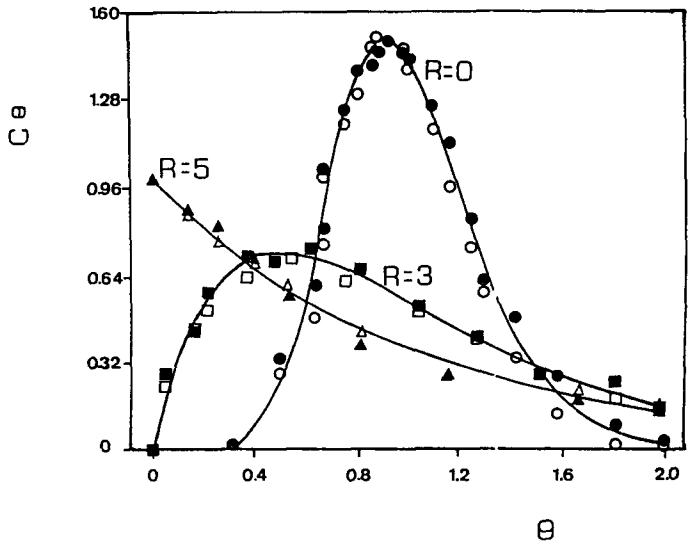

Fig. 5. RTD curves for a packed-bed reactor with recycle operating at different recycle ratios, *R*. Solid and void symbols correspond to reactors without and with pulsation, respectively.

$$\tau = \int_{S_0}^{S} dS / -r \tag{1}$$

The hydrolysis rate, as was determined in batch experiments, is expressed as

$$r = (V_{max} \cdot S) / (K_s + S) \tag{2}$$

Combining Eqs. 1 and 2 results in the following expression:

$$(V_{max} / K_s)\, \tau = -\ln (S / S_0) + (S_0 - S) / K_s \tag{3}$$

Determination of kinetic parameters was carried out by means of a dynamic-simulation program. Although the obtained parameters (Table 1) seemed to be lower than those from batch processes, it must be taken into account that experiments were performed when the activity of the enzyme was approx 70% of the initial level.

An increase in the maximum hydrolysis rate (V_{max}) and a slight reduction in K_s are noted when a pulsing reactor is used. These effects are also observed for the kinetic study in a batch system, when shaking allows the diffusional problems of the process to be minimized. This is an outstanding fact, because it proves that pulsation originates an action similar to that of agitation in a batch system.

Fixed-Bed Reactor with Recycling

From the previous experiments it can be observed that pulsation exerts a noticeable, although not very important, effect. With the aim of determining the mechanical effect of pulsation on the effectiveness of the system, a series of experiments working at rising recycling ratios were realized. This allows us to increase the inlet flow rate, keeping the same operating hydraulic retention time. For this purpose a hydrolysis of 100 g/L starch

Table 1
Kinetic Parameters of the Hydrolysis of Starch Operating in Packed-Bed Reactor Without Recycle, and in Batch Process

Packed-bed reactor S(g/L)	without pulsation V_{max}	K_s	with pulsation V_{max}	K_s
50	0.099	12.44	0.110	12.42
100	0.097	13.02	0.123	12.50
150	0.102	13.36	0.144	12.53
Batch S(g/L)	static V_{max}	K_s	shaking V_{max}	K_s
0 to 150	0.150	8.34	0.161	7.88

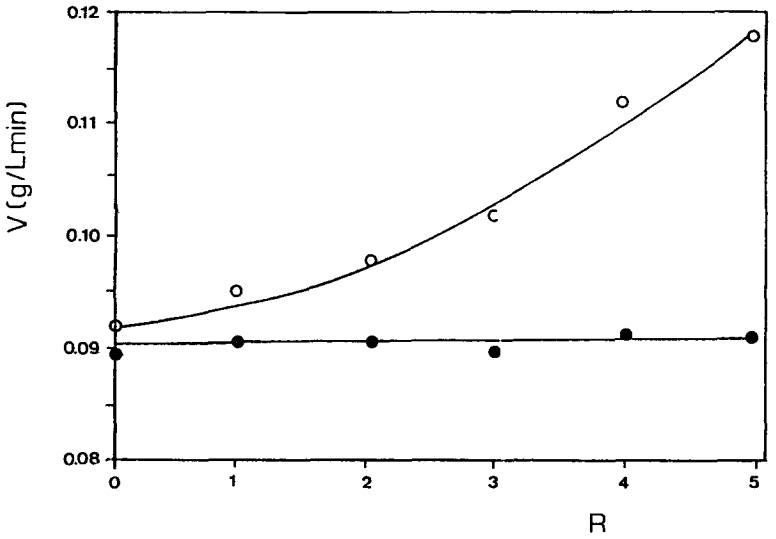

Fig. 6. Reaction rate vs recycle ratio, ○, with pulsation; ●, without pulsation.

solution, working at a residence time of 0.46 h and at recycling ratios of 1, 2, 3, 4, and 5, was carried out in fixed-bed reactors. As demonstrated previously (*16*), the inhibition by product is of less importance, so recycling the outlet stream will not affect the process negatively.

In Fig. 6 a comparison between the obtained reaction rates vs recycling ratios is shown. The observed increase (>20%) is not attributable to a change of the system flow model, because the operating conditions are such that they assure that reaction occurs following a pseudo-first-order behavior. In this way, the effect of pulsation can be clearly characterized, divorcing it from the possible effectiveness difference attributable to flow model employed (CSTR, PFR).

CONCLUSIONS

Pulsation in packed-bed reactors can be realized by using a special pulsing device without introducing significant deviations in the plug flow model. This fact is particularly interesting for processes inhibited by product, in which a higher degree of homogenization provokes a negative action. Starch hydrolysis by immobilized glucoamylase is partially controlled by the mass-transfer rate. When this process was carried out in an upflow fixed-bed reactor, the hydrolysis rate improved by 10% when using the new pulsation device. This profitable effect rose with the flow rate, so mechanical energy transferred to the solution is higher. In this sense, improvements up to 20% are achieved if part of the effluent is recycled. The effect of pulsation will likely be more important when applied to systems with higher diffusional limitations.

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