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Chemical Engineering Journal 65 (1997) 195–199

Chemical Engineering Journal

Enzymatic hydrolysis of wheat proteins Part 2: comparison of performance of batch-stirred and torus reactors [1]

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Received 12 March 1996; accepted 8 January 1997

Abstract

It was shown that limited enzymatic hydrolysis of wheat storage protein yielded hydrolysates with valuable functional properties for food uses. To develop an industrially efficient process to perform this hydrolysis, studies have been carried out in a torus reactor. In the investigation, limited hydrolysis of native gliadin by pepsin was carried out in a 2.1 l torus reactor, to study the effect of operating conditions on the degree of hydrolysis. The results are discussed and compared with those obtained in a batch-stirred reactor (part 1 of this series). The comparison of the performance of both types of reactor is made by using a criterion based on the power per unit volume and the concentration of peptide bonds cleaved during the hydrolysis process. Finally, a model of plug flow with recirculation is proposed to characterize the performance of the limited enzymatic hydrolysis of native gliadin in the torus reactor. © 1977 Elsevier Science S.A.

Keywords: Enzymatic hydrolysis; Torus reactor; Wheat proteins

1. Introduction

Very often, difficulties are encountered in a conventional stirred-tank reactor process, including insufficient heat transfer capacity and the deposition of polymer and micro-organism materials on to the reactor wall and impeller. To solve these problems, Sato et al. [1] proposed the torus reactor for the polymerization of olefins. It is characterized by a directed circulation, which can be achieved by a propeller. Furthermore, the torus reactor has potential application for enzymatic reactions and processing of highly viscous liquids [1,2].

The main advantages of the torus reactor are as follows: easy scale-up and design; as a result of the absence of dead volume, the deposition of polymer material on the reactor wall may be prevented under high Reynolds number operation [1,2]; and low power consumption.

The torus reactor was recently used by Tanaka and O'Shima [3] and Hosogai and Tanaka [4] for suspension polymerization of styrene. Laederach and Widmer [5] showed that mixing and biomass production were better in the torus reactor than in continuously stirred tank bioreactors. For the cultivation of *xanthomonas campestris*, Krebser et al. [6] have shown that xanthan production was greater in the torus reactor than in conventional stirred tanks.

The flow characteristics, such as the average circulation velocity, velocity distribution and pressure loss, were investigated for the first time by Sato et al. [1]. Recently, Tanaka et al. [7] and Belleville et al. [8] studied the mixing characteristics in the torus reactor. They proposed an empirical correlation for calculating the average velocity from the impeller speed.

In the present paper, we have studied the limited hydrolysis of a wheat storage protein, i.e. gliadin, by pepsin in a 2.1 l torus reactor. These results are discussed and compared with the results obtained in a 2.6 l batch-stirred reactor [9]. A plug flow model with recirculation has been proposed to characterize the performance of the torus reactor.

2. Experimental details

2.1. Description of the torus reactor

Fig. 1 shows of the experimental apparatus. Geometrically, the torus reactor is the same as that used by Tanaka et al. [7] and Belleville et al. [8]. It was constructed from four polyvinyl chloride (PVC) bends of inner diameter (D_t) 55 mm.

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[1] This work is a part of the Research Programme PROGEBIO: Bioengeneering West France Research Group.

PII S1385-8947(97)00003-X

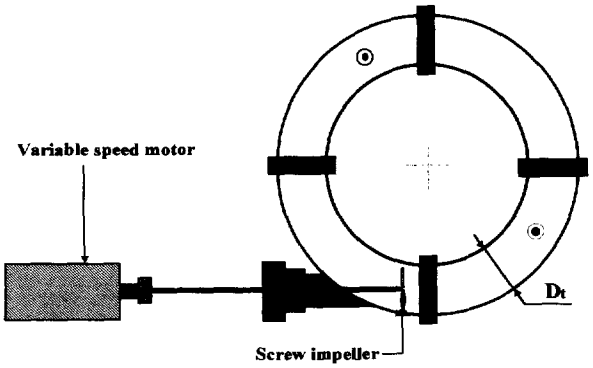

Fig. 1. Schematic diagram of the experimental apparatus.

The total mean length of the torus reactor was 884 mm, which corresponds to a volume of 2.1 l.

Stirring was achieved using a marine screw impeller of diameter (d_i) 53 mm, driven by a variable-speed motor (IKA-WERK-RW20-DZM).

2.2. Experimental procedure

For this work, the procedure used for the preparation of the substrate and enzyme was the same as that used previously [9]. The enzymatic reaction in the torus reactor was performed for two native gliadin concentrations, i.e. 100 and 200 g l^{-1}, with an enzyme-to-substrate ratio of 1/100 and for a range of impeller speed varying between 250 and 1200 rev min^{-1}, corresponding to an impeller Reynolds number ($Re_m = \rho N d_i^2/\mu$) of 11 700–56 200. The hydrolysis was carried out during 420 min at room temperature (20–25 °C). The reaction was quantitatively followed using a trinitrobenzenesulfonic (TNBS) method described previously [9].

3. Results

3.1. Effect of native gliadin concentration on the hydrolysis reaction

The results of this investigation are shown in Fig. 2, and indicate that the concentration of free amino-acid group ($-NH_2$) obtained by hydrolysis, after 420 min at 250 rev min^{-1}, was proportional to the native gliadin concentration and the corresponding degree of hydrolysis (DH) remained practically constant (DH = 0.96%), as was the case for the batch-stirred reactor. Therefore, the degree of hydrolysis was the same for the two protein concentrations, even if the initial viscosities of the protein suspension were very different [9]. This result is very interesting from an industrial application point of view. When compared on the basis of impeller speed, the efficiency of the torus reactor appears lower than that of the batch-stirred reactor. However, because the geometrical characteristics of the two reactors are completely different, this comparison has no practical meaning. The results obtained for the torus and for the stirred reactors will be compared later in this paper, using a more relevant criterion.

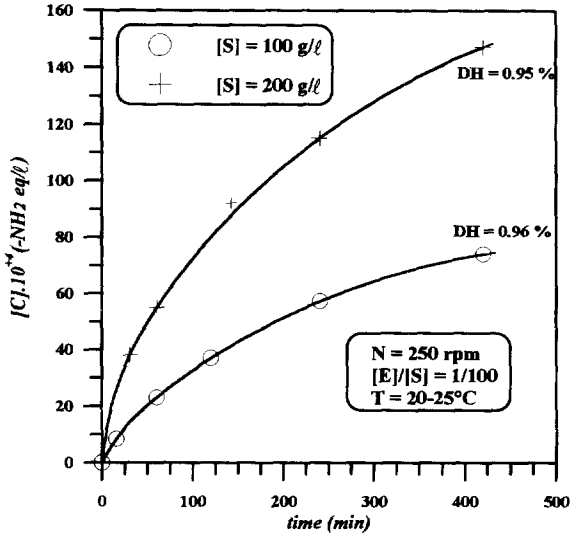

Fig. 2. Effect of the native gliadin concentration on the enzymatic reaction in a torus reactor.

3.2. Effect of impeller speed on the hydrolysis reaction

It can be seen from Fig. 3 that the concentration of free amino-acid groups obtained after 420 min of hydrolysis was proportional to the impeller speed, but the degree of hydrolysis, for a given stirring speed, remained lower than that in the batch-stirred reactor. However, no decrease was observed at high impeller speeds, in contrast to what happens in the batch-stirred reactor, where the evolution of the concentration of free amino-acid group was affected by foaming. This difference can be attributed to the closed geometry of the torus reactor and the absence of air, which prevents the formation of foam in the reactor. This is one of the advantages of the torus reactor for this type of reaction.

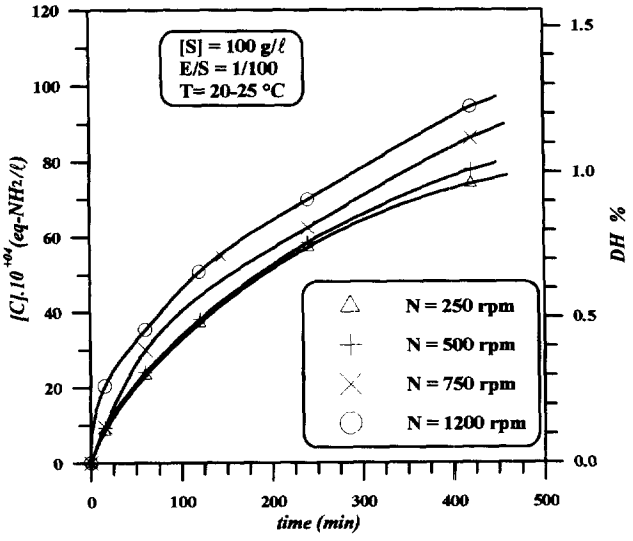

Fig. 3. Effect of the impeller speed on the enzymatic reaction in a torus reactor.

3.3. Performance comparison of batch-stirred and torus reactors

The aim of this study was to compare the performance of batch-stirred and torus reactors, by performing the hydrolysis reaction of native gliadin. In general, the performance comparison in the reactors is made on the basis of process yield against stirring speed conditions. When the geometrical characteristics of the reactors are completely different, the comparison is based on the power consumption and the production rate. Laederach and Widmer [5] have shown the advantages of torus reactors in terms of the power per unit volume. Krebser et al. [6] have compared the performance of the torus reactor and that of a continuously stirred tank reactor on the basis of power consumption for xanthan production.

In the present work, the performance comparison was made on the basis of the power per unit volume ($P = N_P \rho N^3 d_1^5 / V$) and the concentration h of free amino-acid groups obtained or peptide bonds cleaved after 7 h of hydrolysis. This criterion was the same as that used previously, particularly by Ni and Mackley [10], to compare the performance of pulsatile flow and stirred-tank reactors.

The performance comparison was realized with different impeller speeds, varying between 250 and 1200 rev min^{-1}. The fluid density ρ that corresponded to the native gliadin concentration of 100 g l^{-1} was 1020 kg m^{-3}, while the power number N_p that corresponded to a Rushton turbine in the batch-stirred tank was 5.2 [11]. The power number that corresponds to a marine screw in a torus geometry was determined from the power consumption, obtained by the determination in previous work [12,13] of the pressure drops in torus reactors with volumes of 5.2 and 9.2 l.

Fig. 4 shows that, for Reynolds number values between 2×10^4 and 10^5, the power number can be considered as being a constant equal to 0.18. Therefore, the value $N_p = 0.18$ was used for the determination of the power consumption in the torus reactors studied.

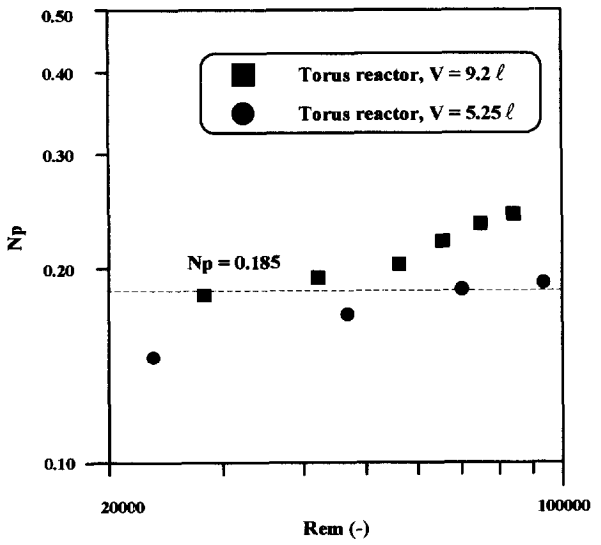

Fig. 4. Relationship between the power number N_p and mixing Reynolds number Re_m.

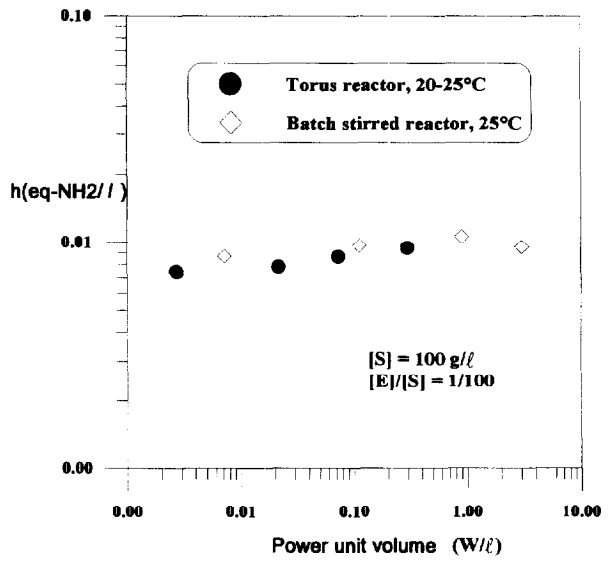

Fig. 5. Performance comparison of batch-stirred and torus reactors by enzymatic hydrolysis of gliadin.

Fig. 5 characterizes the variation in the concentration of peptide bonds cleaved after 7 h of hydrolysis against the power per unit volume in the batch-stirred and torus reactors. The experimental results obtained show that, for a given power per unit volume, the concentration h of peptide bonds cleaved in the torus reactor appears to be slightly lower than that in the batch-stirred reactor. However, the difference is insignificant, given the low degree of hydrolysis (DH = 0.75%–1.5%) obtained after 7 h of reaction and the quasi-independence of the degree of hydrolysis from the mixing conditions. Moreover, this comparison was not realized with the same temperature conditions in both reactors. It will be interesting to compare the performance of the torus and the batch-stirred reactors with other enzymatic or chemical reactions, such as the acetylation of pea isolates used recently to evaluate the performance of the torus microreactor [14].

It can also be seen from Fig. 5 that the evolution of the concentration of peptide bonds cleaved in both types of reactor increases with power consumption. Above a certain power per unit volume—corresponding to the high rotational impeller speed—in the batch-stirred reactor, the concentration of peptide bonds cleaved decreases with the power per unit volume. This difference is probably more important for a high stirring speed, for which the hydrolysis reaction in the batch-stirred reactor is affected by the formation of foam [9].

3.4. Modelling the limited enzymatic hydrolysis of native gliadin in the torus reactor

To estimate the performance of the torus reactor, the enzymatic hydrolysis of native gliadin was characterized by a model of a plug flow reactor with recirculation [15]. The proposed model is the consequence of previous work by Belleville et al. [8] devoted to the study of the mixing char-

acteristics in the torus reactor. The model of dispersed plug flow with recirculation has been used to characterize the torus reactor. The results obtained have shown that the axial dispersion is negligible in the torus reactor. Then, the torus reactor can be assimilated to a plug flow reactor.

3.4.1. Presentation of the model (plug flow reactor with recirculation)

The above-mentioned model can be expressed in terms of the following mass balance equation for Michaelis–Menten kinetics:

$$K_m \ln\left(\frac{h_0}{h_0 - h}\right) + h = V_{max}\frac{V}{Q} \quad (1)$$

or

$$h_0 DH - K_m \ln(1 - DH) = V_{max}\tau \quad (2)$$

where $V/Q = \tau$ is the mean residence time in the reactor, K_m is the apparent kinetic constant, V_{max} is the maximum reaction rate, h_0 is the total number of peptide bonds in a given protein and $DH = h/h_0$ is the degree of hydrolysis.

For an initial substrate concentration S_0 that corresponds to the total number of peptide bonds in a given protein, the degree of hydrolysis obtained after a complete circulation in the torus reactor is determined using Eq. (2). In the case of a plug flow reactor with recirculation, the mean residence time τ in the reactor is equivalent to the mean circulation time t_c, and the inlet substrate concentration in the second circulation is equivalent to the precedent outlet substrate concentration.

The conditions of the initial substrate concentration and the enzyme/substrate ratio applied in the model were as described in Section 2. The model parameters K_m, V_{max} and t_c used in this model were obtained in part 1 of this series [9] and in previous work [8].

The experimental results showed that the degree of hydrolysis achieved after 7 h in the torus reactor is very weak (about 1%). This degree of hydrolysis corresponds to the chosen experimental conditions to carry out the limited hydrolysis by the pepsin. To apply the proposed model, it was essential to know the maximum degree of hydrolysis obtained by limited hydrolysis in extreme conditions. In this case, an experimental degree of hydrolysis of 1.8% was chosen. This value of the degree of hydrolysis corresponds to that obtained previously by Masson [16], after 24 h at 50 °C.

For two stirrer speeds (250 and 500 rev min^{-1}), Fig. 6 shows the variation of the calculated (lines) and experimental (symbols) conversion degree, i.e. $X = 100 \times DH/1.8$, against dimensionless time (t/t_c). Under different process conditions, the fitted curves shown in Fig. 6 are in good agreement for a low hydrolysis time $(t/t_c < 2000)$. Above this value, the difference between the calculated and experimental values increases. This difference can be caused by different factors, such as uncertainty in the determination of the kinetic constants K_m and V_{max}.

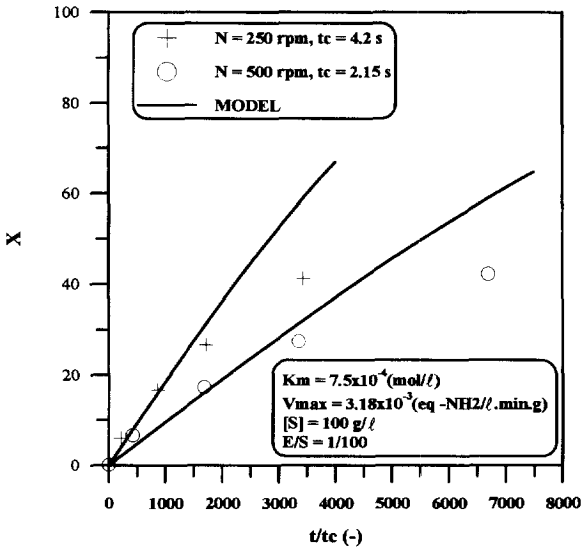

Fig. 6. Modelling the limited enzymatic hydrolysis of native gliadin in the torus reactor.

It can be seen in Fig. 6 that the kinetic parameters were probably not constant during the reaction, which could explain the difference observed when the degree of hydrolysis increases and, therefore, the structure of the substrate changes. The chosen degree of hydrolysis imposed on the model could also be the cause of the difference between the calculated and experimental values. Finally, it will more interesting to validate the aforementioned model with other complete reactions and with less complex substrates than the one used in this investigation.

4. Conclusions

The study of the enzymatic hydrolysis of native gliadin was performed in a 2.1 l torus reactor. The results obtained showed the following.

(1) The concentration of the free amino-acid group ($-NH_2$) is proportional to the substrate concentration. These results confirm those obtained in the batch-stirred reactor.

(2) Over a large range of stirrer speeds, the degree of hydrolysis is not affected by the formation of foam in the reaction medium, and remains proportional to the impeller speed.

(3) As a result of the limited hydrolysis of the proteins, the performance comparison between the stirred and torus batch reactors is not meaningful.

(4) The proposed model of plug flow with recirculation could be used to estimate the performance of the torus reactor.

Acknowledgements

This work is part of the research programme PROGEBIO of the Bioengineering West France Research Group.

Appendix A. Notation

[E]	enzyme concentration
[S]	substrate concentration
d_1	diameter of the impeller
DH	degree of hydrolysis
D_t	inner diameter of the torus reactor
h	number of peptide bonds cleaved during the hydrolysis process per kg protein
h_0	total number of peptide bonds in given protein per kg protein
K_m	apparent kinetic constant
N	impeller speed
N_p	power number
P	power per unit volume
t_c	mean circulation time
TNBS	trinitrobenzenesulfonic acid
V	volume of the reactor
V_{max}	maximum reaction rate
X	degree of conversion

Greek letters

μ	dynamic viscosity
ρ	density of fluid
τ	mean residence time

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