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48. PROCESS FOR THE PRODUCTION OF YEAST-LYTIC ENZYMES AND THE DISRUPTION OF WHOLE YEAST CELLS

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ABSTRACT

A process for the production of yeast-lytic enzymes and the disruption of whole yeast cells has been proposed. The main aim is to produce a high-quality protein for human consumption. The lytic enzyme is produced in a batch fermentation and appropriate reactors for the enzymatic yeast disruption are discussed. The release of protein from yeast cells follows a Michaelis-Menten type kinetics. A preliminary economic evaluation of such a process has been attempted. Although some aspects should be studied in more detail, the production cost obtained in this first approach seems to be economically competitive.

KEYWORDS

Yeast-lytic enzyme production; batch fermentation; disruption kinetics and reactors; protein production; process design; economic evaluation.

INTRODUCTION

Cell-lytic enzymes that have the ability to dissolve or break microbial cells have a great potential as an alternative to mechanical disruption methods. Once the cell has been broken it is possible to isolate the protein for food purposes. It is also possible to use the cell-lytic enzymes for the degradation of partially disrupted cells. The indigestibility of the wall is an important limitation to the use of microorganisms in human and animal foods, hence the use of a lytic enzyme preparation in a combined process of mechanical disruption and enzymatic degradation might be valuable in circumventing this problem (Asenjo and Dunnill, in press).

If the aim is to produce a human-grade Single-Cell Protein, the choice should be yeast; this is the only SCP that has been consumed by man in relatively large quantities. Therefore, in order to evaluate the potential of yeast-lytic enzymes for the production of a human-grade protein, a suitable enzyme has to be produced and appropriate disruption reactors have to be designed.

RESULTS AND DISCUSSION

Lytic Enzyme Production

The production of constitutive yeast-lytic enzymes by Cytophaga sp.(NCIB 9497) has

been studied in a 5 L and in a 1400 L fermenter (Asenjo, Dunnill and Lilly, in press). The enzyme production was found to be growth associated and the lysis of some Cytophaga cells during fermentation enhanced the enzyme titers in the supernatant. The yeast-lytic activity was measured as the rate of protein released in an incubation of whole yeast cells and enzyme and is expressed in Lytic Units, L.U. (Asenjo, Dunnill and Lilly, in press). The production of yeast-lytic enzymes by the Cytophaga 9497 strain was constitutive, which gives considerable flexibility in the selection of a growth medium and may well allow the use of cheap nutrients in order to produce inexpensive yeast-lytic enzymes. The lytic enzyme complex lysed whole yeast cells of brewers yeast (NCYC 1006) and of commercial baker's yeast.

Reactor Kinetics and Design

For the action of the lytic enzymes on whole brewer's yeast cells, the initial rates of protein release were a linear function of enzyme concentration. These rates followed an approximately hyperbolic pattern as a function of substrate concentration (whole yeast cells). Figure 1a. shows the rate of total protein released and Figure 1b. shows the rate of protein release minus the controls lacking enzyme. In both cases a relationship of the Michaels-Menten type could be used.

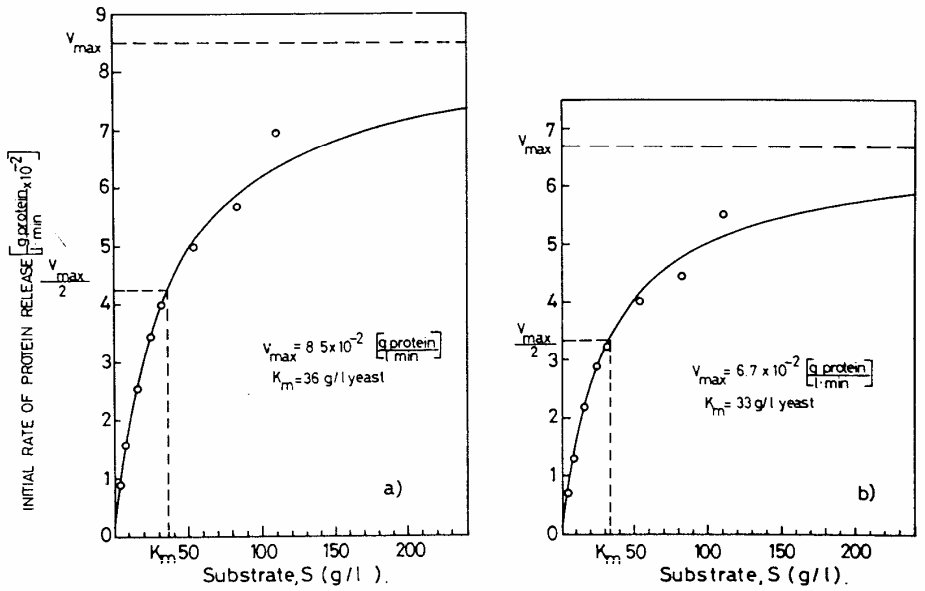

Figure 1.- Effect of yeast substrate concentration on rate of protein release from whole yeast cells.
a) Total initial rate in the presence of yeast-lytic enzyme
b) Initial rate in the presence of enzyme minus initial rate of protein released due to autolysis in the absence of enzyme.

Applying the known equations for enzyme reactors (Vieth and coworkers) to this kind of process, the conversion into free protein was evaluated. Figure 2 shows the conversion (x) as a function of the residence time (τ) for different substrate concentrations (S_o) for a CSTR or back-mix reactor and a PFR or continuous tubular reactor. The operational limit of the reactors lies between 160 and 213 g/L (the packed weight of the yeast was 240 g/L after centrifugation at 23,000 g for 30 min). With most kinetic models, when high conversions of substrate into product are sought, there is an advantage in using a tubular (PFR) or even a batch reactor instead of a CSTR.

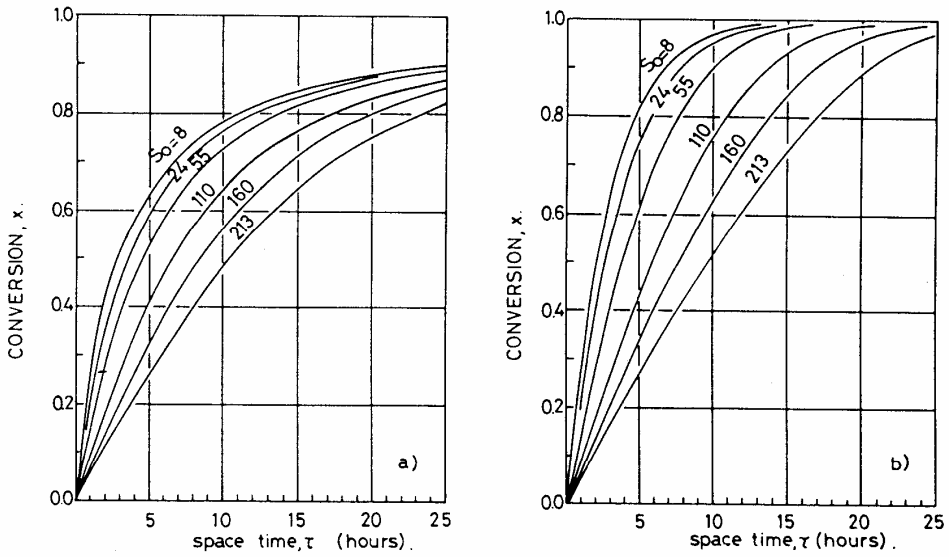

Figure 2. Residence Time τ as a function of conversion, x, and initial yeast substrate concentration, S_o (in g/L yeast) at an enzyme activity concentration of 7,900 LU/L.
a) CSTR enzyme reactor
b) PFR reactor.

The reaction time (or residence time) and the conversion into soluble protein will depend strongly on the susceptibility to lysis of the yeast cells and on the enzyme concentration in the reactor; therefore, great improvements can be obtained by using weaker yeast cells and by increasing the titers in the enzyme preparation. It was observed that the susceptibility to lysis of the yeast cells could be enhanced 2-3 times during growth with the addition of lactate to the yeast extract and glucose medium. Another observation was that cells became stronger with age, an effect that was monitored during growth. 5-hour grown cells in the exponential growth phase were 6-7 times more susceptible to lysis than 15 and 17 hour grown cells. Table 1 shows the reaction times that would be necessary to obtain conversions of 0.80, 0.90 and 0.95 of the total amount of protein available for release in the yeast cells by using a continuous tubular (ideal plug flow) reactor or a batch reactor, without considering the turnaround time. The yeast concentration (114 g/L dry weight) and the enzyme concentration of 4,100 LU/L correspond to the ones that have been used in the process design (Fig.3). Reaction times for higher enzyme titers are also shown in Table 1. It is evident that shorter reaction times are obtained under such conditions. These figures are optimistic because, if an enzyme reactor is operated at high substrate concentration and under conditions where most substrate is converted the rates of conversion become lower, and the theoretical model established by measuring the initial rates may be inappropriate for the final stages of reaction where product inhibition becomes important.

Table 1. Reaction Time for Continuous Tubular (Plug Flow) and Batch Reactor Susceptibility of yeast cells to lysis = 0.60 g/L (Asenjo, 1978). Yeast concentration = 114 g/L (dry weight).

Lytic enzyme activity concentration (LU/L)	4,100	7,900	15,000
Conversion	Reaction time (hours)		
0.80	8.5	4.4	2.4
0.90	10.6	5.5	2.9
0.95	12.1	6.3	3.4

Process Design

The preliminary design of a process has been made to include the production of yeast and yeast-lytic enzymes, the enzymatic disruption of whole yeast cells and the separation of nucleic acids for the production of a protein isolate. The process flow sheet is shown in Figure 3. The nucleic acid reduction by means of an alkali treatment and later an acid neutralization was based on that by Viikari and Linko (1976) where a 93% protein yield was obtained by using $Ca(OH)_2$. The equipment is dimensioned on the basis of a 100 m^3 (working volume) continuous yeast production fermenter. A dilution rate of 0.20 h^{-1}, lower than the maximum specific growth rate obtained for the yeast in a batch fermentation, has been used. After fermentation the cell suspension is concentrated in a continuous centrifuge. For the cell slurry obtained from this stage a value of 150-200 g/L is normally accepted (Burrows, 1970).

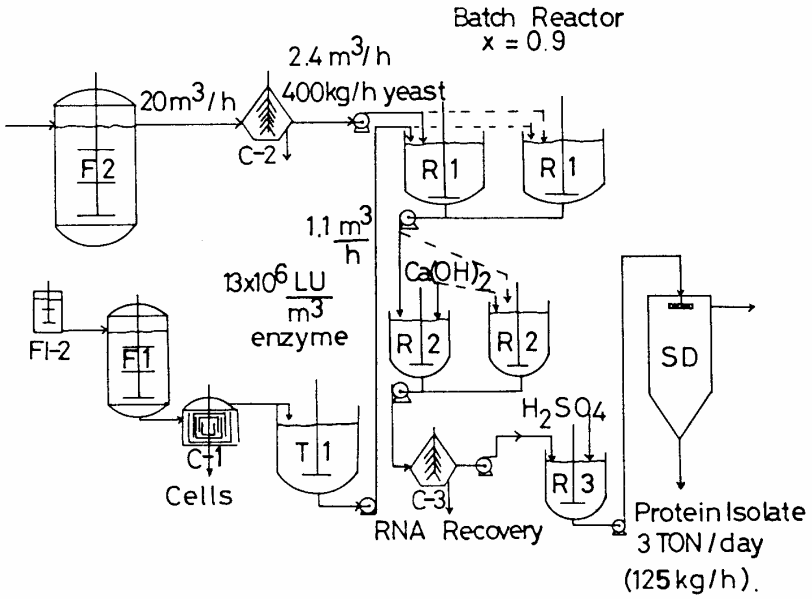

Figure 3.- Flow sheet of the process (Equipment characteristics in Table 2). Reactors for Yeast Disruption (R-1) (x = 0.9; 114 kg/m^3 yeast; 4,100 LU/L enzyme; ideal reaction time = 10.6 h; vol ideal = 37 m^3).

The enzyme is produced in a batch fermentation that lasts 30 hours; 6 hours have been allowed for harvest, medium preparation, sterilization and inoculation. After harvesting, the cells are separated in a continuous centrifuge and the crude supernatant is stored in a cooled holding tank. The enzyme is then pumped to the enzyme reactor. There are two identical reactors; one operates while the other is being charged or discharged. A 90% conversion has been allowed for.

Once the yeast cells have been disrupted the nucleic acids are separated by alkali

treatment with $Ca(OH)_2$ 0.04N at 80°C for 60 min (Viikari and Linko, 1976) where again 2 batch reactors that operate alternately, are used. A 93% protein yield has been reported for such a process, which results in 125 kg/hr (3 TON/day) pure protein. This value has been obtained by considering a 39% protein content in yeast (Harrison, 1967). Many authors quote higher protein contents, which would result in a higher production. The precipitated protein is separated as a slurry in a continuous centrifuge and the $Ca(OH)_2$ remaining in the slurry is neutralized with H_2SO_4. Finally the protein is spray dried. This plant would produce approximately 1000 TON/year of protein.

Process Economics

The capital costs and the annual operating costs have been estimated using the tables and outlines given by Grethlein (1978), Perry and Chilton (1973), Peters and Timmerhaus (1968) and Vilbrandt and Dryden (1959). The index of Marshall & Stevens was used for estimating the cost of the equipment (Chem.Eng.); a value of 620.8, that corresponds to 1979, was used. Current quotations made by Alfa-Laval and Westfalia, Santiago, Chile, were used for the centrifuges.

Table 2. Capital Cost Estimates for the Process of Production of Protein by the Enzymatic Degradation of Whole Yeast Cells. Plant Capacity: 1000 TON/year.

Item	Process Equipment (all stainless steel) Unit Specification	Cost in US.$
Enzyme Production		
FI-1 Preinoculum Fermenter (not shown in Fig.3)	25 litre	20,000
FI-2 Inoculum Fermenter	1 m^3	50,000
F -1 Batch Production Fermenter	40 m^3	125,000
C -1 Continuous Centrifuge for cell separation	15 m^3/h	40,000
T -1 Enzyme Holding Tank	40 m^3	45,000
Yeast Production		
F -2 Continuous Fermenter	100 m^3	120,000
C -2 Continuous Centrifuge for yeast concentration	20 m^3/h	40,000
Protein Production		
R -1 2 Yeast Disruption Reactors	40 m^3 each	100,000
R -2 2 Alkali Treatment Reactors	5 m^3 each	36,000
C -3 Continuous Centrifuge for RNA separation	5 m^3/h	30,000
R -3 Neutralization Reactor	1 m^3	8,000
S-D Spray Drier	10 ft diameter; 500 L/h	160,000
TOTAL PROCESS EQUIPMENT COST (PEC)		774,000
Estimated Cost of Fixed Capital (FC) = 4.74 x PEC		3,669,000
ESTIMATED TOTAL CAPITAL COSTS (CC) = FC+WC (Working Capital) = 5.5 x PEC		4,257,000

Table 3. Estimation of Annual Operating Cost. (350 days of Plant Operation per year)

Operating Labour (OL)	149,800
3 men per shift, 4 shifts per 7 day week = 12 men x 6 dollar/h x 40 x 52	
Labour Related Expenses (0.95 x OL)	142,300
(Supervision 0.15 x OL; Payroll Overhead 0.15 x OL; Laboratory 0.15 x OL; Plant Overhead 0.5 x OL).	
Capital Related Expenses (0.24 x FC)	880,600
(Depreciation 0.10 x FC; Interest 0.06 x FC; Maintenance 0.06 x FC; Insurance 0.01 x FC; Plant Supplies 0.01 x FC).	
Materials Cost (10% of Total Product Cost)*	130,300
(Fermentation Medium, Water, $Ca(OH)_2$, others)	

(table continued overleaf)

TOTAL ANNUAL OPERATING COSTS	1,303,000

Annual protein production is approx. 1000 Ton; hence, production cost of protein = 1.30 dollars/kg protein.

*93,000 kg of enzyme production medium per year (20 g/L medium concentration, no special medium needed due to enzyme being constitutive); 6,000 TON medium for yeast production per year. A low medium value (US.$0.010-0.015/kg) that assumes that the yeast is grown in a waste stream of low value has been used. This is a central assumption in most microbial protein processes.

If waste yeast from a brewery is used the capital cost is reduced by 21% and the final cost of the product would be of ca. 1.12 dollars/kg protein. A cost of raw material for the yeast has to be added in this case.

The operations of yeast and enzyme production and yeast disruption were tried on a laboratory and pilot plant scale. The final stages of nucleic acid separation and spray drying were designed using accepted reference data. The production of a higher quality protein (e.g. $CaSO_4$ reduction) should be studied in more detail. The production cost obtained for such a product, in this first approach, seems to be economically competitive. Improvement in the process design could be obtained by futher studies of the enzyme production (higher titers, continuous fermentation) and of the yeast degradation reactors (reaction mechanism, rate controlling step, studies at high substrate concentration and high conversion yields).

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