Influence of the Dilution Rate on the Bioproductivity of Lactose-Utilizing Yeasts: Fuzzy Logic Modeling

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The studied problem is of commercial interest because whey, the cultivation substrate, is a waste by-product from the transformation of milk into cheese and casein. Investigations on the influence of the dilution rate (D) on the bioproductivity of lactose-utilizing yeasts were carried out with two model strains – the oxidative strain *Candida blankii 35* and the fermentative strain *Candida pseudotropicalis 11*. The increase of D led to the different changes in productivity. The best synthesizing ability of both continuously cultivated strains is established at $D = 0.4$ [h-1] despite the different type of metabolism. The oxidative strain *C. blankii 35* is more effective in comparison with the fermentative strain *C. pseudotropicalis 11* because of its ability to synthesize 1.5 fold higher biomass and protein yields. These experimental facts were proved also by simulative research with a Fuzzy Knowledge-Based System (FKBS) developed for modeling the influence of D on several process variables.

Key words: Lactose-Utilizing Yeasts, Protein-Synthesizing Ability, Fuzzy Logic Modeling

Introduction

The aim of the present work was to study the protein yields, protein-synthesizing ability and bioproductivity of oxidative and fermentative lactose-utilizing yeasts at different dilution rates under conditions of carbon limitation. The usual nutrient substrate whey is a waste by-product from the transformation of milk in the cheese and casein industry. It contains a high concentration of lactose, low level of proteins, N-components and vitamins. The conversion of whey to single-cell protein by yeast cultures of *Candida intermedia*, *C. pseudotropicalis*, *C. valida*, *C. kefyr*, *Kluyveromyces fragilis*, *Trichosporon cutaneum* was investigated (Bayer and Meyrath, 1979; Ben-Hassan *et al.*, 1992; Blakebrough and Moresi, 1981; Carlotti *et al.*, 1991; Moresi *et al.*, 1990; Sandhu and Waraich, 1983).

The possibility of yeast strains for better utilizing of essential C-substrate in whey-lactose and biomass production has a great importance. A detailed study of microbial population behavior in dependence of the investigated factor becomes possible using continuous cultivation (Baserga, 1981; Bayer and Meyrath, 1979; Bayer, 1983; Katranushkova *et al.*, 1997; Postma *et al.*, 1988; Weusthuis *et al.*, 1994).

In our previous investigations we studied the influence of the dilution rates (D) on the activity of some enzymes of nitrogen metabolism in lactose-utilizing yeasts *C. blankii 35* and *C. pseudotropicalis 11* (Katranushkova *et al.*, 1999).

There are problems of expensive and long time-consuming biotechnological experiments, uncertain and incomplete bioprocesses information, and to summarize gathered expert knowledge and experimental records concerning real-time control on the influence of D on bioproductivity of lactose-utilizing yeasts. Accordingly, a Fuzzy Knowledge-Based System (FKBS) was developed to obtain the missing information about the observed variables in the real-time process control.

Materials and Methods

Microorganisms

The strains were obtained from the collection of the Institute of Microbiology, Bulgarian Academy of Sciences – *Candida blankii 35* with oxidative

Table I. Dilution rate influence on growth and productivity of *C. blankii 35*.

Dilution rate D [h^{-1}]	Residual lactose concentration [gl^{-1}]	Yield of biomass Y_X [gl^{-1}]	Biomass X [%]	Lactose-utilising rate q [h^{-1}]	Productivity P [$gl^{-1}h^{-1}$]
0.1	0.0	6.39	63.9	0.15	0.64
0.2	0.0	6.40	64.0	0.31	1.28
0.3	0.0	6.00	60.0	0.50	1.80
0.4	2.1	5.78	57.8	0.55	2.31
0.5	5.1	4.34	43.4	0.56	2.17

type of metabolism and *Candida pseudotropicalis 11* with a fermentative type of metabolism.

Nutrient medium

The nutrient medium contained (g/l): lactose – 10.0; $(NH_4)_2SO_4$ – 3.0; $MgSO_4$ – 0.7; NaCL – 0.5; KH_2PO_4 – 1.0; K_2HPO_4 – 0.1; $CaCl_2$ (50% solution) – 0.2 ml, biotin – 40 mg/l.

Cultivation

The strains were maintained on malt extract agar slants. All experiments were carried out in a bioreactor containing 1.5 l working volume at 38 °C, pH 4.0–4.2, aeration 1.5 l/min.l and agitation speed of 600 rpm. The strains were cultivated in a chemostat under carbon limitation at five dilution rates D = 0.1; 0.2; 0.3; 0.4 and 0.5 h^{-1}.

Analyses

Yeast cells were harvested by centrifugation, washed twice with distilled water and the dry weight determined after drying overnight at 105 °C. The residual lactose concentration (RLC) in the supernatant was measured using the phenol-sulfuric acid method (Dubois *et al.*, 1956). The protein content of the cells was calculated by Kjeldahl's method (N × 6.25). The RNA content was determined by the methods of Schmidt and Tannhauser (1945) and Schneider (1945). The protein-synthesizing ability (A, [%]) was calculated as an indicator for the state of the cell by using data about protein content (PR, [%]), RNA content (RNA, [%]), and dilution rate (D, [h^{-1}]) according the equation:

$$A = \frac{PR}{RNA} D \quad .$$

Results and Discussion

During the growth of model strain *C. blankii 35* no residual lactose was left when D increased from 0.1 to 0.3 h^{-1}. At D = 0.5 h^{-1} the culture did utilize half of the lactose of the culture medium (Table I). The biomass yield reached 60–64% at lower dilution rates. The biomass decreased with the increase of D and at 0.5 h^{-1} it was about 67% from the biomass obtained at 0.2 h^{-1}. The substrate utilization rate increased and correlated with the increase of D, but it was not sufficient for the complete lactose assimilation at 0.4 and 0.5 h^{-1}. The productivity of the system improved with increasing D and at 0.4 h^{-1} was 3.6 fold higger vsm that at 0.1 h^{-1}. The most suitable dilution rate was 0.3 h^{-1}, when lactose was assimilated completely and productivity increased 3-fold in comparison with that at 0.1 h^{-1}.

The dilution rate did not influence markedly the protein content of the cells of *C. blankii 35* (Table II). The decrease of biomass yield led to a reduced protein yield and was about 1.5 fold in comparison with that at 0.3 h^{-1}. RNA content remained constant and was augmented significantly at 0.5 h^{-1}, but the protein content did not increase. The protein-synthesizing ability increased with the in-

Table II. Dilution rate influence on protein and protein-synthesizing ability of *C. blankii 35*.

Dilution rate D [h^{-1}]	Protein PR [%]	Ribonucleic acid RNA [%]	Protein yield Y_{pr} [%]	Protein-synthesizing ability A [%]
0.1	43.9	8.17	28.05	0.54
0.2	43.7	7.96	27.96	1.09
0.3	43.5	9.58	26.10	1.36
0.4	43.7	8.46	25.25	2.07
0.5	40.8	12.20	17.70	1.67

Table III. Dilution rate influence on growth and productivity of *C. pseudotropicalis 11*.

Dilution rate D [h^{-1}]	Residual lactose concentration [gl^{-1}]	Yield of biomass Y_x [gl^{-1}]	Biomass X (%)	Lactose-utilising rate q [h^{-1}]	Productivity P [$gl^{-1}h^{-1}$]
0.1	0.0	4.64	46.4	0.22	0.46
0.2	0.6	4.19	41.9	0.45	0.84
0.3	1.6	4.04	40.4	0.62	1.21
0.4	3.8	3.71	37.1	0.67	1.48
0.5	7.0	3.21	32.1	0.47	1.61

crease of *D*. This is likely due mainly to augmentation of the growth rate and activation of metabolite processes in the cells. Probably the higher protein-synthesizing ability was due the increase of ribosomes (Baserga, 1981).

The biomass content decreased with the change of *D* during growth of *C. pseudotropicalis 11* under carbon limitation (Table III). The biomass yield was reduced 1.4 fold with increase of *D* from 0.1 to 0.5 h^{-1} despite the substrate utilization rate increased. The productivity of the system improved with increasing *D*. A considerable quantity of substrate was not assimilated at the higher dilution rate and that is why $D = 0.2$ and $D = 0.3$ h^{-1} were more effective.

A tendency to augmentation of the RNA content was found with increase of *D* and that led to increase of the protein content (Table IV). A decrease of the protein yield due to the biomass yield reduction was observed. *C. pseudotropicalis 11* showed a high variability of RNA synthesis with the change of *D*, which led to intensive activity of the ribosomal system. The protein-synthesizing ability of the cells increased with growth of *D* and reached the maximal value at 0.4 h^{-1}.

The substrate-utilizing rate and bioproductivity of the both strains increased with an increase of *D* to 0.4 h^{-1}, but biomass yield decreased under carbon limitation. The oxidative strain *C. blankii 35* was more effective compared with the fermentative strain *C. pseudotropicalis 11* because of its ability to synthesize a 1.5 fold higher biomass and protein.

Table IV. Dilution rate influence on protein and protein-synthesizing ability of *C. pseudotropicalis 11*.

Dilution rate D [h^{-1}]	Protein PR (%)	Ribonucleic acid RNA (%)	Protein yield Y_{pr} (%)	Protein-synthesizing ability A (%)
0.1	40.9	5.86	19.00	0.70
0.2	43.1	6.47	18.06	1.33
0.3	45.5	7.53	18.38	1.81
0.4	47.6	9.43	17.65	2.02
0.5	47.1	13.50	15.11	1.74

Fuzzy Knowledge Based System (FKBS)

This FKBS was developed to investigate the dilution rate influence on observed process variables as well as on the protein-synthesizing ability A, productivity P, yield of protein Y_{PR} and lactose-utilizing rate q. From a mathematical point of view the studied problem is a non-linear mapping problem.

Reasons for FKBS implementation are as follows. In common practice the biochemical analyses are time- and human effort-consuming, which does not allow their application in real-time control systems. Moreover, biotechnological processes are non-linear and non-stationary complex systems with a lot of often unknown interconnections and mutual impacts between bioprocess variables. To cope with such a task a fuzzy modeling technique was implemented for its excellent approximation abilities to process uncertain and incomplete information (Vassileva *et al.*, 2002).

The studied problem is related to more than one lactose-utilizing yeast strains. To summarize own and published expert knowledge and data, the Data Base and Knowledge Base were designed as basic components of FKBS. A proper algorithm (inference engine) is implemented to achieve the desired information about the modeled process variables (A, P, Y_{PR}, q), comprised in 16 fuzzy models with high accuracy. FKBS has an open structure or it can be expanded with new facts.

Interested readers should contact the corresponding author for mathematical details.

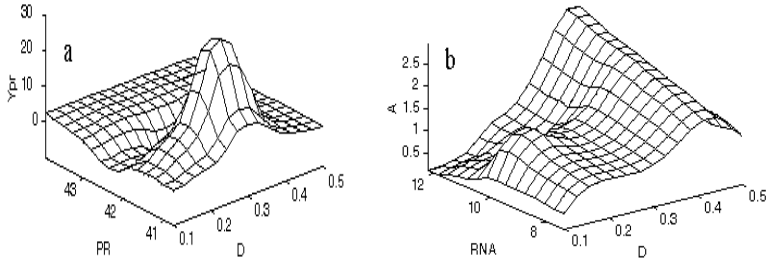

Fig. 1. Optimal model surface for *C. blankii 35* yield of protein, Y_{Pr} and protein-synthesizing ability, A, as functions of a. dilution rate *D* and protein Pr content; b. dilution rate *D* and RNA.

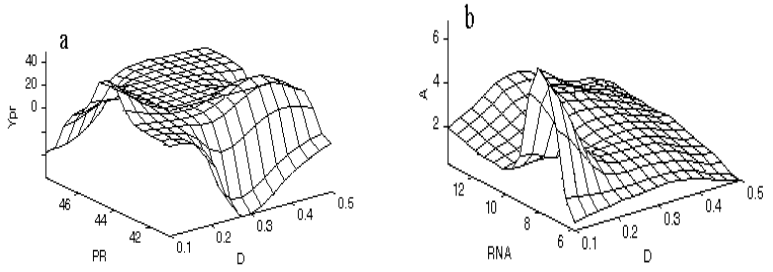

Fig. 2. Optimal model surface for *C. pseudotropicalis 11* yield of protein, Y_{Pr} and protein-synthesizing ability, A, as functions of a. dilution rate *D* and protein PR; b. dilution rate *D* and RNA.

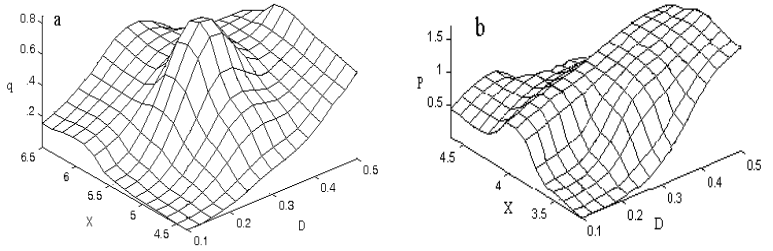

Fig. 3. a. Lactose-utilizing rate, *q*, of *C. blankii 35* dependence on dilution rate *D* and biomass X. b. Productivity *P* of *C. pseudotropicalis 11* dependance on dilution rate *D* and biomass X.

Simulation Results and Conclusions

Most important results are shown in the following simulation experiments with the developed FKBS. For example, as is shown from the 3D-optimal surfaces, the protein-synthesizing ability of *C. blankii 35* increase of protein and RNA content kept constant values with the increase of the growth rate to 0.4 h^{-1} at carbon limitation (Fig. 1). It was also found that the protein-synthesizing ability, protein and nucleic acid contents in *C. pseudotropicalis 11* cells increased with increasing *D* to 0.4 h^{-1} (Fig. 2). The bioproductivity and substrate utilization rate of both strains increased, the biomass yield decreased with increasing *D* to 0.4 h^{-1} (Fig. 3). The oxidative strain *C. blankii 35* was more effective

compared with the fermentative strain *C. pseudotropicalis 11* because of its ability to synthesize a 1.5 fold higher biomass and protein yield.

FKBS implementation helps to answer different questions concerning the missing information about the studied strains with high accuracy. The model accuracy was successfully tested by simulative research under control conditions of D = 0.1, 0.2, 0.3, 0.4, 0.5 h^{-1} and corresponding values of the other input variables as well as biomass X, protein PR, ribonucleic acid RNA.

In conclusion, recent trends in biotechnology show an increasing requirement for new analysis strategies that could be applied to facilitate monitoring and control of microbial growth, upstream and downstream processes. New efficient software tools are intelligent knowledge-based systems, which include excellent approximation quality, to process uncertain linguistic and numeric bioprocess information with a higher speed through parallel computing.

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