World Journal of Microbiology & Biotechnology **15**: 115–117, 1999.

Short Communication

Continuous cultivation of the yeast *Candida utilis* at different dilution rates on pineapple cannery effluent

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Received in revised form 18 August 1998; accepted 19 August 1998

Keywords: *Candida utilis*, pineapple cannery effluent, continuous culture, steady-state

Summary

Candida utilis was grown on a pineapple cannery effluent in a chemostat at dilution rates ranging between 0.05 and 0.65 h^{-1} to establish optimal conditions for biomass production and chemical oxygen demand (COD) reduction. Sucrose, fructose and glucose were the main sugars in the effluent. Maximum value for cell yield coefficient and productivity were (0.686, g_x/g_s) and (2.96, g_x/l/h) at a dilution rate of 0.425 and 0.475 h^{-1}, respectively, while maximum COD reduction (98%) was attained at a dilution rate of 0.1 h^{-1}. The maintenance coefficient attained a value of (0.093, g_s/g_x/h). An increase in dilution rate produced a higher protein content of the biomass.

Introduction

Recently increasing attention has been given to the conversion of fruit and vegetable processing wastes into single cell protein (SCP) (Litchfield 1983). The recovery of a valuable by-products with simultaneous reduction of the organic load are the chief economic advantages of such processes. Batch culture study with *Candida utilis* gave good yield and reduced the chemical oxygen demand (COD) (Nigam 1998). The purpose of this study was to establish optimal conditions for the cultivation of *C. utilis* and COD reduction using chemostat culture.

Materials and Methods

Microorganism and culture conditions

Candida utilis NRRL Y-900 was maintained on yeast extract (0.5%), tryptic soy agar (4%) (Difco). A loopful of culture was transferred to 100 ml feed medium and grown at (30 °C, 200 rev/min) before it was used to inoculate the fermentor.

Substrate (Raw material)

The collection of cannery effluent and its processing was done by the methods described earlier (Nigam, 1998).

Feed medium

Effluent samples were supplemented with (g/l): $(NH_4)_2HPO_4$, 4; $MgSO_4 \cdot 7H_2O$, 0.6. Antifoam, 0.1 ml (DOW Corning, FG-10) was added. pH was adjusted to 4.5.

Fermentor and continuous cultivation

Cultivation was carried out in a 5-L Microferm fermentor (NBS Co. Inc., N.J., USA) with a 2 l working volume, equipped with a foam, temperature, pH and dissolved oxygen (DO) control systems at a stirring speed of 700 rev/min, an air flow rate of 4 v/v/m, and a temperature of 30 °C. The fermentor was inoculated with 100 ml culture broth and allowed to grow before the medium pump was started. Dilution rates ranging between 0.05 and 0.65 h^{-1} were evaluated. The culture was assumed to be in steady-state when several determination of optical density (OD) gave similar values, measured at time intervals of 1 h. Each dilution rate was examined with five exchanges of fermentor volume. Dissolved oxygen tension (DOT) in chemostat was never less than 60% of air saturation.

Analytical methods

Samples withdrawn at steady-state were centrifuged (10,000 × *g*, 15 min) and analysed for Kjeldahl nitrogen, COD, total solids and lipid by the standard methods. Total carbohydrate and reducing sugars were analysed by the anthrone and Somogyi methods, respectively. Individual sugars were analysed by high-performance

liquid chromatography (HPLC) (Van Zyl *et al.* 1988). Trehalose and glycogen (Grba *et al.* 1975), Lowry protein, phosphorus, DNA and RNA (Herbert *et al.* 1971), and amino acid composition (Simpson *et al.* 1976) of dry cells was determined. The cell pellet was washed and cell mass was determined after drying at 105 °C to constant weight.

Results and Discussion

Candida utilis can utilize various carbon sources for the production of SCP. Pineapple cannery effluent contains a high amount of carbohydrates (13.8–16.2 g/l) and its reducing sugar content varies (10.2–12.6 g/l), with a COD value of 15.8–16.6 g/l. This effluent therefore is a cheap and suitable waste material for single cell protein production.

Cultivation was carried out at dilution rates ranging from 0.05 to 0.65 h^{-1}. The maximum value for cell yield (0.686, g_x/g_s) and productivity (2.96, g_x/l/h) were achieved at a dilution rate of 0.425 and 0.475 h^{-1}, respectively which are comparable to published data (Blanch & Einsele 1973). The productivity and yield increased (4.9- and 1.81-fold), respectively over batch culture (Nigam 1998). The maximum values for sugar utilization and COD reduction were 89.6 and 98% at a dilution rate of 0.35 and 0.1 h^{-1}, respectively.

The protein content in biomass increased from 48.6 to 56.8% with increasing dilution rates (0.05 to 0.65 h^{-1}) (Fig. 1). This is probably due to an increase in dilution rate which provides larger amounts of nutrients and cells assimilate a greater quantity of nitrogen from the medium, and thereby synthesize a larger quantity of protein. The increase in protein content was proportional to the phosphorus content of the biomass. This is probably due to maintenance of constant nitrogen:phosphorus ratio which is characteristic of *Saccharomyces cerevisiae* (White 1954). The effect of growth rate on protein content has been studied widely and it has been found that protein content increases as the dilution rate increases (Paredes-Lopez *et al.* 1976). The decrease in trehalose and glycogen content were observed with increasing dilution rate (Figure 1), which are in agreement with published data (Kuenzi & Fiechter 1972). The difference in carbohydrate contents were attributed to the media composition, aeration, strain, specificity and growth phase (Polakis & Bartley 1966), though not much attention was paid to the relation between growth kinetics and reserve carbohydrate synthesis. The glycogen and trehalose are known to serve as endogenous carbon and energy source for budding, irrespective of generation time (Kuenzi & Fiechter 1969). Under excess exogenous substrate the budding proceeds without degradation of endogenous carbohydrates.

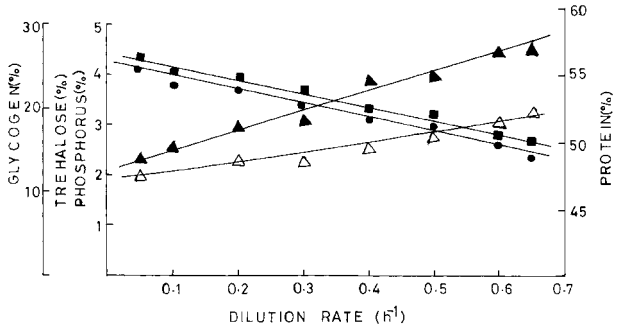

Figure 1. Effect of dilution rate on biomass composition of *Candida utilis* growing in chemostat culture. Results are expressed in dry weight basis. The pH was controlled at 4.5 ± 0.1, temperature at 30 °C, and agitation speed at 700 rev/min. (●): trehalose; (■): glycogen; (△): phosphorus; (▲): protein.

The main constituent of yeast SCP is protein (55.3%), which is quite high and suggests that this yeast could be a suitable cattle feed supplement. Nucleic acid content (7.9%) was comparable to the reported values for another strain of *C. utilis* (Alroy & Tannenbaum 1973). The amino acid composition compares favorably in quality to soybean protein concentrate (Nigam 1998).

The maintenance coefficient (m) was determined from the plot of specific rate of sugar uptake (q_p, g_s/g_x/h) and dilution rate (D, h^{-1}), which gives a straight line with a slope $1/Y_{x/s}$ and intercept m (plot not shown here), which shows that this relationship was valid only up to dilution rate of 0.45 h^{-1}. The value of maintenance coefficient (0.093, g_s/g_x/h) is comparable to published data (Abbott & Clamen 1973; Paredes-Lopez *et al.* 1976). From an economic point of view, it is important to use microorganisms having a low maintenance coefficient for biomass production.

The selection of a dilution rate will depend upon the purpose for which the effluent used. For maximum productivity, a dilution rate (0.475 h^{-1}) was found suitable. At this dilution rate, COD reduction was 78%. If the objective is effective COD reduction, a dilution rate of 0.1 h^{-1} should be suitable at which COD reduction is 98%. An optimum for effective biomass cultivation and COD reduction would be a dilution rate of 0.425 h^{-1} where cell yield and productivity are (0.686, g_x/g_s) and (2.82, g_x/l/h) and COD reduction is (85%).

Further studies will investigate the problems associated with achieving higher productivity and high cell density for economic yeast production.

Acknowledgements

Author thanks the Director for providing facilities to carry out this work.

References

Abbott, B.J. & Clamen, A. 1973 The relationship of substrate, growth rate, and maintenance coefficient to single cell protein production. *Biotechnology and Bioengineering* **15**, 117–127.

Alroy, Y. & Tannenbaum, S.R. 1973 The influence of environmental conditions on the macromolecular composition of *Candida utilis*. *Biotechnology and Bioengineering* **15**, 239–256.

Blanch, H.W. & Einsele, A. 1973 The kinetics of yeast growth on pure hydrocarbons. *Biotechnology and Bioengineering* **15**, 861–877.

Grba, S., Oura, E. & Suomalainen, H. 1975 On the formation on glycogen and trehalose in baker's yeast. *European Journal of Applied Microbiology* **2**, 29–37.

Herbert, D., Phipps, P.J. & Strange, R.E. 1971 Chemical analysis of microbial cells. In *Methods in Microbiology*, Vol. 5B, eds. Norris, J.R. & Ribbons, D.W. pp. 209–344. London: Academic Press.

Kuenzi, M. & Fiechter, A. 1969 Change in carbohydrate composition and trehalose activity during the budding cycle of *Saccharomyces cerevisiae*. *Archive of Microbiology* **64**, 396–407.

Kuenzi, M. & Fiechter, A. 1972 Regulation of carbohydrate composition of *Saccharomyces cerevisiae* under growth limitation. *Archive of Microbiology* **84**, 254–265.

Litchfield, J.H. 1983 Single cell protein. *Science* **219**, 740–746.

Nigam, J.N. 1998 Single cell protein from pineapple cannery effluent. *World Journal of Microbiology and Biotechnology* **15**, 115–117.

Paredes-Lopez, O., Camargo-Rubio, E & Ornelas-Vale, A. 1976 Influence of specific growth rate on biomass yield, productivity, and composition of *Candida utilis* in batch and continuous culture. *Applied and Environmental Microbiology* **31**, 487–491.

Polakis, E & Bartley, W. 1966 Change in dry weight, protein, deoxyribonucleic acid and reserve and structural carbohydrate during aerobic growth cycle of yeast. *Biochemical Journal* **98**, 883–887.

Simpson, R.J., Neuberger, M.R. & Liu, T.Y. 1976 Complete amino acid analysis of protein from a single hydrolysate. *Journal of Biological Chemistry* **252**, 1936–1940.

Van Zyl, C., Prior, B.A. & Du Preez, J.C. 1988 Production of ethanol from sugar cane begasse hemicellulose hydrolysate by *Pichia stipitis*. *Applied Biochemistry and Biotechnology* **19**, 357–369.

White, J. 1954 *Yeast Technology*. pp. 13–26, Chapman and Hall, London.