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YEAST BIOMASS PRODUCTION FROM ACID WHEY PERMEATE

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SUMMARY

The yield of *Kluyveromyces marxianus* Y-113 grown on sulphuric acid casein whey permeate was decreased at high lactose or ethanol concentrations and slightly increased with high agitation and supplementation of the permeate with nutrients. The maximum specific growth rate was most influenced by the stirrer speed, ethanol concentration and nutrient supplementation.

INTRODUCTION

The disposal of whey arising from the manufacture of cheese or casein presents a major challenge for the world's dairy industry. Whey is a dilute solution comprising approximately 6.5% solids by weight of which the major components are lactose, proteins and minerals. Protein can be recovered in an insoluble form as lactalbumin following heat treatment and centrifugation or as soluble whey protein concentrate, typically using ultrafiltration (Marshall, 1982). However utilization of the remaining lactose-rich fraction has always been difficult. In New Zealand about half of the whey produced is further processed to generate whey powders, lactose and ethanol (New Zealand Dairy Board, 1986). Since 1980 four whey distilleries have been established to supply the entire local market and limited export markets for both industrial and potable ethanol (Mawson, 1987).

At the New Zealand Distillery Co. Ltd. plant in Edgecumbe *Kluyveromyces marxianus* Y-113 is used in the production of over 2 x 10^6 litres of absolute ethanol p.a. from sulphuric acid casein whey permeate. The yeast is propagated at the distillery in a six-stage batch process in which the cell yield rarely exceeds 0.3 g/g lactose. The work reported here examined the effect of

several fermentation parameters, namely stirrer speed, lactose concentration, nutrient supplementation and ethanol concentration on the batch production of yeast biomass from acid whey permeate.

MATERIALS AND METHODS

Organism. *Kluyveromyces marxianus* Y-113 was maintained on LYGP agar slopes at 4°C. The medium composition was (per litre distilled water); lactose 3g, yeast extract (Difco) 5g, glucose 10g, peptone (Difco) 3g, agar 12g.

Inoculum Preparation. The medium used in inoculum preparation comprised (per litre distilled water); lactose 20g, yeast extract 5g, peptone 5g, $(NH_4)_2SO_4$ 5g, K_2HPO_4 5g. Broth cultures (2 x 10 ml) were inoculated from a slope and incubated for 24 hr at 28°C. These cultures were transferred to 100 ml broth and incubated (24 hr, 28°C) prior to inoculation of the fermenter.

Fermentation Conditions. The yeast was grown in a 2 litre bench-top fermenter constructed by the Biotechnology Department, Massey University. The working volume was 1.2 litres and the temperature and aeration rate were set at 32°C and 1 vvm for all runs. Sulphuric acid casein whey permeate supplied by Bay Milk Products Limited, Edgecumbe was used in this study. The permeate was removed aseptically from a sample port after a pasteurizer and stored in pre-sterilized containers at 4°C. Prior to an experiment the glass fermenter pot was sterilized (121°C, 15 mins) and allowed to cool. Permeate and other solutions required were added and the fermenter raised to the desired temperature with low agitation (150 rpm). The yeast inoculum was then added and the stirrer speed and aeration rate set. Experimental conditions for each run are summarized in Table 1. Antifoam (Bevaloid 5901; Bevaloid Chemicals, Levin, N.Z.) was added manually as required. Broth samples were withdrawn by pipette at regular intervals during the 24 hr fermentation period.

Analytical Methods. Yeast cells were harvested by centrifugation, washed twice with distilled water and the dry weight determined after drying overnight at 105°C. Lactose was determined using the phenol-sulphuric acid method (Dubois *et al.*, 1956). Ethanol was measured by gas-liquid chromatography using a Pye-Unicam PU 4500 system. A 1.8m x 2mm i.d. glass column packed with 23% Carbowax 1500 on Chromosorb W (A.O.A.C., 1984) was operated with an F.I.D. detector under the following conditions: column temperature, 80°C, injector and detector temperature, 130°C; carrier gas (N_2) flowrate, 15 ml/min.

The nominal oxygen transfer capacity of the fermenter at the two stirrer speeds employed was determined using the sulphite-oxidation method of Cooper *et al.* (1944).

Data Analysis. The simple logistic equation (De Witt, 1943; Edwards and Wilke, 1968) was fitted to the dry weight and lactose data by non-linear regression analysis using the GENSTAT V package (Version 4.04B; Lawes Agricultural Trust, 1984). An adequate fit, as judged by the average relative error (Edwards and Wilke, 1968), was achieved in all runs.

RESULTS AND DISCUSSION

Fermentation parameters for each experiment are summarized in Table 2 and data for a typical run (run 4) are shown in Figure 1 together with the regression curves fitted to the biomass and lactose data. Growth proceeded rapidly in all runs after a short lag of 1-2 hr. Lactose consumption averaged 98% over the 24 hr period and, as shown for run 4 (Figure 1), the fermentation was essentially complete after 14 hr. Similar behaviour was observed in the other experiments.

Ethanol derived from the inoculum was initially present in all cultures at an average concentration of 0.14 vol%. During the course of the fermentation this increased to a maximum value after 10-12 hr before decreasing again. The highest ethanol concentrations (0.5 - 0.7 vol %) were observed in those runs with the higher initial lactose concentration (runs 1, 2 and 5).

In the first four experiments the effect of stirrer speed and initial lactose concentration on the maximum specific growth rate (μ_{max}) and overall yield coefficient (Y_{XS}) of *K. marxianus* Y-113 were evaluated. The estimated μ_{max} (Table 2) was similar for each run at the same stirrer speed. At a given lactose concentration, decreasing the stirrer speed from 800 rpm to 600 rpm reduced μ_{max} by about 10% and a similar reduction in the biomass yield was also observed. However Y_{XS} increased by nearly 50% when the lactose concentration was reduced to approximately 17 g/l by dilution of the permeate (runs 3 and 4). The estimates of μ_{max} and Y_{XS} obtained are similar to values reported for batch growth of other *Kluyveromyces* strains on sweet wheys (Burgess 1977, Michel *et al.* 1987, Willets and Ugalde 1987) although the yield coefficients are significantly lower than in comparable experiments of Moresi *et al.* (1980).

The oxygen transfer capacity (OTC) of the fermenter was estimated as 130 and 70 mM O_2/1hr at stirrer speeds of 800 and 600 rpm

respectively. If these values were also characteristic of the whey fermentations conducted it is clear that decreasing the OTC by approximately 50% through lowering the stirrer speed had far less effect on the culture than reducing the lactose concentration by the same factor. Moresi *et al.* (1980) identified an "oxygen transfer coefficient factor", strongly dependent on the stirrer speed, and a "lactose inhibition factor", mainly characterized by lactose concentration, as the significant parameters influencing the yield of a *K. fragilis* strain growing on cheese whey medium. The absence of a significant oxygen transfer effect in this work may indicate a difference in the physiology of the strain studied or that the oxygen transfer rates measured by the sulphite-oxidation method were not achieved during the fermentation. This could result from the addition of the antifoaming agent, which is also added to propagation vessels in the commercial process.

In run 5 the yeast was inoculated into permeate supplemented with salts and yeast extract added at the optimum concentrations reported by Wasserman *et al.* (1958). Only a slight increase in the yield coefficient was observed which was consistent with the performance of the plant propagation vessels (The New Zealand Distillery Co. Ltd., unpublished data). The reason for the decrease in μ_{max} compared with run 1 was unclear.

Ethanol was added to the culture at 1.0 vol% in run 6. The biomass yield was decreased by 35 % from that achieved in run 1 where ethanol was not added. The estimated μ_{max} was decreased by only 12% however and was comparable to the value achieved in run 5. The observed reduction in yield was similar to that noted by Vienne and Von Stockar (1985) and confirmed the low ethanol tolerance of *Kluyveromyces* strains.

Overall the biomass yields obtained were comparable to those achieved in the commercial propagation plant and the results support the importance of low lactose concentration in promoting optimal growth of *Kluyveromyces* species in whey solutions. However lowering the lactose concentration in batch culture does not increase the total amount of biomass formed. For the case of a propagation plant providing yeast inoculum for subsequent ethanol fermentation a fed-batch system employing a constant feed rate may offer the best solution. Such a system would appear to satisfy the demand for simplicity and flexibility in operation coupled with high biomass yield (Reed, 1982).

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Table 1. Summary of Experimental Conditions

Run	Lactose concentration (g/l)	Stirrer speed (rpm)	Additives
1	35.4	800	-
2	36.4	600	-
3	17.0[a]	800	-
4	16.7[a]	600	-
5	37.1	800	nutrients[b]
6	44.6	800	ethanol[c]

a 550 ml permeate and 550 ml distilled water added.
b permeate supplemented with 5 g/l $(NH_4)_2SO_4$, 5 g/l K_2HPO_4 and 1 g/l yeast extract.
c permeate supplemented with 1.0 vol % ethanol.

Table 2. Growth of *K. marxianus* Y-113 on sulphuric acid casein whey permeate.

Run	Lactose consumed[a] (%)	Biomass formed[a] (g/l)	Y_{xs} (g/g lactose)	μ_{max}[b] (hr^{-1})
1	98.3	8.13	0.23	0.50
2	97.3	7.57	0.21	0.44
3	97.6	5.59	0.34	0.53
4	98.2	5.08	0.31	0.48
5	98.4	9.46	0.26	0.43
6	98.2	6.67	0.15	0.44

a values after 24 hr.
b from logistic equation fitted to biomass data.

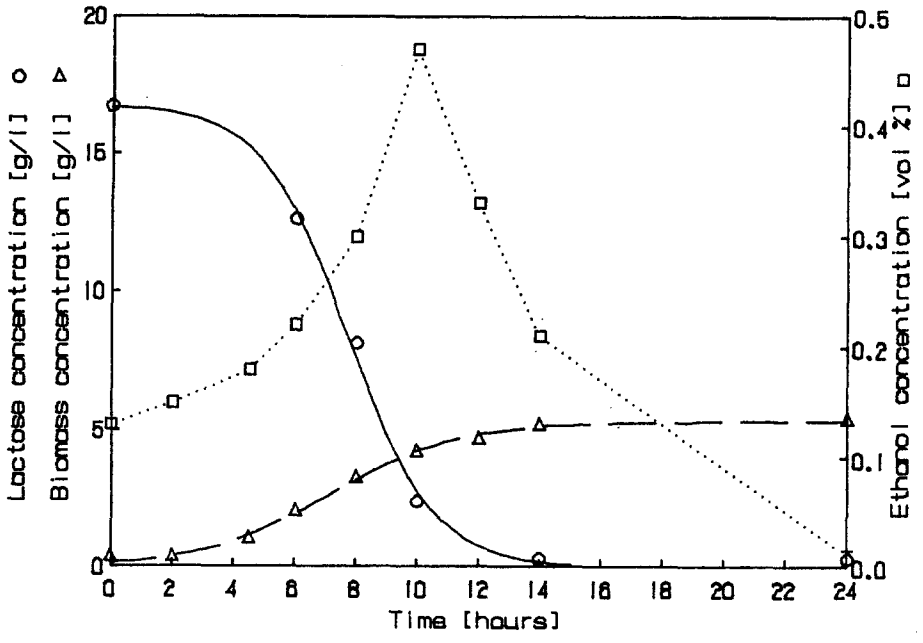

Figure 1. Growth curve for *K. marxianus* Y-113 on sulphuric acid casein whey permeate - run 4; 16.7 g/l lactose, 600 rpm. Curves fitted to dry weight and lactose data by non-linear regression (see text).