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TOWER FERMENTATION OF WHEY PERMEATE  
AND  
SUCROSE-ENRICHED WHEY PERMEATE TO ETHANOL

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## ABSTRACT

Tower fermentation of sulphuric acid whey permeate using *Kluyveromyces marxianus* Y42 has been investigated. The tower fermenter used was 0.025 m in diameter and 2.69 m high. The straight section of the tower was 2.37 m. The total tower volume was 2.9 litres and the separator section made up 1.6 litres of the total volume. The operating temperature was 30°C. The optimum medium feed rate was observed at a superficial liquid velocity of 0.24 mm/s. It was found that a tower height of only 0.82 m was required, excluding the separator section, and the corresponding residence time was 1 hour. An exit ethanol concentration of 16 g/l was produced at a productivity of 16 g/lh from 45 g/l lactose in the whey permeate feed (94% utilization). This was an ethanol yield of 71% on lactose utilized. If the separator section were considered, the ethanol productivity was 5 g/lh and the exit ethanol concentration was 19 g/l, while the overall retention time was 3.7 hours. The cell concentration inside the tower varied between 10 and 100 g/l dried weight (54 and 350 g/l wet weight) being greatest at the bottom of the tower.

*K. marxianus* was found to be inhibited by a high level of ethanol in the growth medium and unable to ferment completely a high concentration of lactose when tested in 10 litre-scale-batch fermentation. Further tests in the presence of sucrose and lactose found that this yeast exhibited diauxic behaviour by utilizing sucrose before lactose. This behaviour generally resulted in incomplete lactose utilization in the tower. In the screening for a flocculent lactose-fermenting yeast, the yeast strain *K. marxianus* was found to be the only flocculent yeast, but it was only moderately flocculent. Further investigation found that it had good flocculence when grown in media which support good growth, and poor flocculence when grown in acidic media and in media which do not support good growth. A subculture of this yeast strain showed moderate flocculence when grown in whey permeate.

Tower fermentation of whey permeate enriched with molasses by mixed culture of *Saccharomyces cerevisiae* CFCC39 and *K. marxianus* Y42 was found to be difficult. The difficulty arose because of incomplete lactose utilization even at a very low feed rate (up to 0.14 mm/s) and incompatible flocculation properties of the two yeast species employed. Blockage of the separator and gas slug formation were caused by the very flocculent yeast mass of *S. cerevisiae* CFCC39. This caused

*K. marxianus* to be slowly washed out of the tower fermenter. Sucrose was completely utilized at the bottom of the tower fermenter, while lactose utilization was slow and incomplete. The incomplete lactose utilization has been attributed to the diauxic behaviour of *K. marxianus*, ethanol inhibition and molasses inhibition (probably due to its reaction with whey permeate during autoclaving).

Results of tower fermentation of cane molasses have also been given for characterization of the tower fermenter used.

Experiments to isolate an ethanol tolerant *K. marxianus* using a serial subculture in a medium containing increasing ethanol concentrations were performed. The isolate obtained could tolerate up to 50 g/l ethanol. It could ferment lactose in whey permeate to produce ethanol at a faster rate than the parent strain and other lactose-fermenting yeast tested. The isolate was found to be stable. It was not used in the tower fermenter as it was non-flocculent.

An attempt was made to isolate a sucrose-negative *K. marxianus*. This was only partially successful. The mutant did not grow on sucrose agar but reverted to the wild type when grown in liquid medium containing both sucrose and lactose.

An experiment to isolate a diauxie-negative *K. marxianus* strain using D-glucosamine as a glucose analogue was also described. This was unsuccessful because *K. marxianus* was able to grow on lactose in presence of the analogue.

TO MY KIWI AND THAI PARENTS

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## LIST OF ABBREVIATIONS

### PREFIX

$\Delta$  change in concentration, g/l or %

### SUBSCRIPTS

a average  
E effective  
i condition at a particular tower height or section  
l lactose  
o overall  
r residence time  
s sucrose or superficial  
t total sugar  
u substrate utilization

### NOTATIONS

A aluminium sulphate,  $\text{Al}_2(\text{SO}_4)_3$   
AFEB attached-film-expanded-bed fermenter  
B broth  
B 95% confidence interval uncertainty  
Ca calcium sulphate,  $\text{CaSO}_4$   
CB yeast cleaning buffer ( $\text{CaSO}_4$  wash)  
CP *Candida pseudotropicalis*  
CC39 *Saccharomyces cerevisiae* CFCC39  
CSTR continuous stirred tank reactor  
D dilution rate  
DGA D-glucosamine  
DW cell dried weight, g/l DW  
E ethanol concentration, g/l  
E' volumetric rate of ethanol production, g/lh  
EF extremely flocculent  
F membrane filtration (0.45  $\mu\text{m}$ )  
FM flocculating medium (acetate buffer)  
G glucose  
H, H<sub>E</sub> tower height, effective tower height, mm or m  
H\* average tower height  
KL *Kluyveromyces lactis*  
KM *Kluyveromyces marxianus*

KMY42 *K.marxianus* Y42

LVE	limiting volumetric efficiency
M	malt extract broth
M*	malt extract broth (Oxoid)
Ma	maltose
MBN	modified Burn's number
MBN*	non-standard modified Burn's number
Me	malt extract powder medium
MF	moderately flocculent
Mo	molasses
Ms	malt extract syrup (Maltexo)
P	whey permeate
P	percentage uncertainty
Pe	peptone
P4.6	whey permeate with no pH adjustment
q	specific rate of substrate utilization, g/gh
Q	volumetric flow rate, ml
r	(Linear regression) correlation coefficient
R	rough
S	substrate concentration, g/l
s'	volumetric rate of substrate utilization, g/lh
SC	<i>Saccharomyces cerevisiae</i>
SC146	<i>S.cerevisiae</i> FT146 (AWRI 350)
SG <sub>e</sub>	exit specific gravity
SM	spent malt extract broth
S <sub>u</sub>	substrate utilization, %
T <sub>r</sub>	residence time, h
T <sub>r</sub> *	average residence time, h
T <sub>ro</sub>	overall residence time, h
TS	subcultured from the tower fermenter
V <sub>E</sub>	effective tower volume, ml
V <sub>i</sub>	volume of a section of the tower fermenter, ml
V <sub>s</sub>	superficial liquid velocity, mm/s
VF	very flocculent
WF	weakly flocculent
X	total cell number or cell weight, cell/ml or g/l
X <sub>a</sub>	average cell concentration, g/l
X <sub>KM</sub>	<i>K.marxianus</i> cell number or weight, cell/ml or g/l

Y      yield coefficient, yield of ethanol on substrate utilized, %  
         yeast extract

10      100 g/l whey permeate solution

44,46   ratio of lactose to sucrose of 40:40 g/l and 40:60 g/l

5      pH 5.0

#### GREEK NOTATIONS

$\mu$       specific growth rate, g/gh

$\nu$       specific rate of ethanol production, g/gh

$\phi$       diameter