1. ABSTRACT

Lactobacillus casei ssp. rhamnosus was used in this study. This strain was earlier known as *Lactobacillus delbrueckii* (NRRL B 445).

L. casei was first cultivated in batch culture under a controlled temperature 42 °C and a pH value of 6.0 at two different glucose concentrations of 95 and 150 g l^{-1}. In the batch culture, this organism grew logarithmically for 25 h with a maximum specific growth rate of 0.52 h^{-1}. Under the experimental conditions, the final concentrations of cell mass obtained were 6.1 and 10.0 g l^{-1} for glucose concentrations of 95 g l^{-1} and 150 g l^{-1} respectively. The final cellular yields (Yx/s) obtained in these two cultivations were 0.10 and 0.14 respectively.

The final lactic acid concentrations were 70 g l^{-1} and 86.7 g l^{-1} for glucose concentrations of 95 and 150 g l^{-1} respectively. The final product yield coefficients (Yp/s) in these two cultivations were 0.88 and 0.80 respectively. In the conventional batch mode of cultivation, the L-form of lactic acid was 95.3% and the D-form 4.7%.

The *L. casei* was then cultivated in a system with complete cell recycling in order to obtain information on how this cultivation technique affected the microorganism. Cultivation at two different glucose concentrations (25 and 50 g l^{-1}) were performed. Hollow fibre filter from Gambro, Sweden was used for separating the cells from the spent broth. The cell recycling was carried out for 135 h.

This cell recycling fermentation system allowed continuous removal of lactic acid, an inhibitory product, while retaining the cells completely in the fermenter. The final cell mass concentrations (on dry weight basis) were 61 g l^{-1} after 128 h of cell recycling and 86 g l^{-1} after 135 h of recycling for glucose concentrations of 25 and 50 g l^{-1} respectively.

When the feed with 25 g l^{-1} glucose was used, nearly all the glucose was utilized. With feed of glucose concentration of 50 g l^{-1}, only about 41 g $l^{-1}$of glucose were utilised. The maximum product yield (Yp/s) obtained in these two cultivations were 1.25 and 0.86 for glucose concentrations of 25 g l^{-1} and 50 g l^{-1} respectively.

In the cell recycling cultivation, the final levels of lactic acid produced were 27.2 and 31.1 g l^{-1} for glucose concentrations of 25 and 50 g l^{-1} respectively. The maximum volumetric productivities of lactic acid of the two feeds were 8.16 g l^{-1} h^{-1} and 10.0 g l^{-1} h^{-1} in the lower and higher glucose concentrations respectively. The cell recycling fermentation showed a clear increase in the proportion of D-lactic acid level when compared with the conventional batch fermentation. When 25 g l^{-1} glucose was used in the feed, the %D-lactic acid level increased to 13% whereas in 50 g l^{-1} glucose, the increase was up to 6-7% D-lactic acid. The permeate and cell homogenate were tested for racemase enzyme activity. No racemase could be detected in the permeate during the prolonged cell recycling cultivation. In addition no racemase was found in the cell homogenate.

To investigate whether the increase in percentage of D-lactic acid was connected to starvation of cells, *Lactobacillus casei* was cultivated in a batch culture with 25 g l^{-1} which underwent starvation for 200 h. An almost linear increase of percentage D-lactic acid was observed during this period from 4 to 18%. Hence the problem of increase in D-lactic acid deserves further attention.

The data collected from these two recycling experiments show that the continuous cultivation with complete cell recycling is an efficient way to obtain high cell density but this method can affect the product formation pattern of the microorganism.

The end product inhibition can also be reduced by the *in situ* removal of inhibitory fermentation products as they form. Extractive fermentation, in which an immiscible organic solvent is added to the fermenter in order to extract the inhibitory products, was applied to lactic acid fermentation system.

The extractant selected for this research was paraffin oil which, in addition to having satisfactory physical properties, is non-toxic and does not affect the fermentability of the broth. The extraction capacity of the paraffin oil for lactic acid was tested at different pH values and phase volume ratios. Maximum efficiency was obtained at pH 2 and phase volume ratio one. The distribution coefficient (K_D) of lactic acid in paraffin oil was found to be 0.15 when phase volume ratio was one and pH was 6.0.

Several liquid amine compounds such as Trioctylamine (TOA), Amberlite LA-2, Adogen 464 and Aliquat 336 were investigated in order to increase the extraction efficiency of paraffin oil. Addition of these compounds improved the K_D value of lactic acid in pure diluent

(paraffin oil). Among the compounds tested, Aliquat 336 performed best. It increased the K_D value of lactic acid in paraffin oil from 0.15 to 0.50. However, all of the amine compounds tested are highly toxic to the free cells of *L. casei* even at the concentration of 0.1% (v/v).

A solid hydrophobic polymeric resin was then investigated. The incorporation of Bonopore in paraffin oil (diluent) increased the K_D value of lactic acid in paraffin oil to 0.72. In addition, the Bonopore is non-toxic to the organism investigated.

So paraffin oil and Bonopore appeared to be the only compounds tested that did not stop the growth of *L. casei*. Hence these two compounds were used in the extractive fermentation studies.

In batch extractive fermentation studies with paraffin oil, the product yield coefficient obtained (Yp/s) was greater than the control: 0.94 g/g compared to 0.82 g/g. But in the continuous extractive fermentation with paraffin oil, the product yield coefficient (Yp/s) obtained was lower (0.64 g/g) than the conventional batch fermentation.

Bonopore is the polymer giving good adsorption pattern and no deleterious effects were detected with paraffin oil in repeated batch culture of*L. casei*. In this study about 152 g glucose was utilized in 67 h whereas in the control 90 g glucose was utilized in 70 h. The production of lactic acid and cell growth (OD) followed similar pattern. The product yield (Yp/s) in repeated batch extractive fermentation with Bonopore was 0.67 which is higher than in the control (0.50).

Ethanol fermentation is another important aspect to study in a nation like Sri Lanka where there are no oil resources. The palmyrah fruit pulp

deserves great attention as a source of energy for ethanol fermentation. The pulp from palmyrah fruit has been traditionally extracted by hand. This manual extraction is a strenuous and time consuming process and hence it limits the utilization of the fruit pulp. Hence a mechanical extractor was developed for our specification.

A mechanical palmyrah fruit pulp extractor was constructed which had square blades fitted at an angle to the vertical shaft through rods. The shaft was connected with a 0.75 horse power motor. A gear box was used to regulate the speed of the shaft. Using this extractor, the manual and mechanical extraction of pulp from palmyrah fruit were compared. The rate and cost for manual extraction of fruit pulp were 11.9 min $fruit^{-1}$ and SLR 1.52 $fruit^{-1}$ while for mechanical extraction were 0.48 min $fruit^{-1}$ and SLR 0.54 $fruit^{-1}$ respectively. Eventhough there is 2% difference in the brix content of the pulp obtained by both processes, the soluble solids separated per fruit is the same (0.08 kg. $fruit^{-1}$) in both processes.

A *Saccharomyces* yeast strain (Y_7) was used in the bioconversion of palmyrah fruit pulp sugar to ethanol. The optimum pH and temperature of this organism for ethanol production were 4.5 and 35 °C respectively. No pH control appeared necessary for the ethanolic fermentation of palmyrah fruit pulp. Supplements of salt mixture consisting of 1.0 (g/l), $(NH_4)_2SO_4$, $MgSO_4$ and KH_2PO_4 into the pulp have a pronounced effect on cell mass and ethanol fermentation. The fruit pulp containing 90 g l^{-1} fermentable sugar produced 43 g l^{-1} of ethanol in 20 h when the fruit pulp (with nutrient) was fermented at ambient temperature (28-32 °C) using 10%, (v/v) log phase yeast (Y_7) inoculum containing 1 $x10^7$ cells ml^{-1}.

Under such conditions, about 93% of pulp sugar was converted to ethanol. A study on the use of palmyrah palm sap (toddy) cultures as an inoculum in the production of ethanol from palmyrah fruit pulp is also presented. When toddy was used as an inoculum about 87% of the fruit pulp sugar was converted to ethanol.

The alcohol from the fermented fruit pulp was recovered by simple distillation. The fermented fruit pulp with 4.3%, (w/v) alcohol was distilled in a Drum-still distillation unit. The percentage recovery was 91.

In addition to palmyrah fruit pulp, corn flour hydrolysate also could be used for ethanol production. The optimal conditions for the enzymatic hydrolysis of corn flour was also studied.

The optimal α-amylase concentration for liquefaction was found to be 0.2% (v/v) when 40%, (w/w) corn flour was treated with thermostable α-amylase (Termamyl) at pH 7.0 and at 90-95 °C for 1 h. When liquefaction was followed by saccharification with glucoamylase at pH 4.5 and 40 °C for 24 h, the dextrose equivalent was increased to 87. When saccharification by glucoamylase was supplemented with commercially available enzymes like cellulases, proteases and pectinases, no improvement in the rate of filtration was observed. However, the application of these β-glucanases and proteases together with glucoamylase reduced the dry weight of the undigested solid matter from 28.6 to 16.0%, (w/w).

The treatment of corn flour hydrolysate with the activated charcoal ($50\ g\ l^{-1}$) improved the rate of filtration to 63 ml min^{-1}. The filtrate obtained after the treatment with charcoal was clear and the dextrose equivalent was 87.5.