1. ABSTRACT

In Sri Lanka, potable ethanol is mainly produced by distilling the naturally fermented coconut and palmyrah sap. Preliminary studies were carried out with palmyrah fruit pulp and palmyrah molasses as feed stocks for ethanol fermentation. The ethanol production efficiency in palmyrah fruit pulp supplemented with sucrose and distillery spent wash was 74.4 and 66.5% with palmyrah toddy mixed culture and bakers yeast respectively. The ethanol production efficiency of 75.3 and 68.5% was shown by palmyrah toddy mixed culture and bakers yeast culture respectively in molasses supplemented with spent wash medium. In industrial scale (5000l) fermentation of molasses (20brix) supplemented with $(NH_4)_2SO_4$ (10.0g l^{-1}), 70.0 (72h) and 65.0gl^{-1} (90h) ethanol was produced by palmyrah toddy mixed culture and bakers yeast culture respectively. Reduction in fermentation time from 72 to 65h with increased ethanol production from 72.0 to 80.0gl^{-1} was observed in cell recycle operation. In this studies the temperature (reached 42°C at 36h) induced cell death (90%) was observed. Thus the necessity towards a search of thermotolerant organism/s for the fermentation in hot climate was emphasized. The screening for the best wild - type organisms was undertaken as the first step. Among the yeast strains collected from different sources, thermotolerant strains with the capacity to withstand 45°C for 15h were found in samples collected from compost heap and distillery environment. Three colonies isolated from the distillery environment and a colony from compost heap showed ethanol yield of 0.4g ethanol / g glucose used and the ethanol produced at 48h was 40gl^{-1} ethanol for the colonies isolated from the distillery environment and for the colony from the compost heap was 30gl^{-1} ethanol. Therefore three colonies from the distillery environment were selected for further studies and named p1, p2 and p3. The screened strains p1, p2 and p3 retained 100% viability for 5, 3 and 2h respectively at 45°C in glucose (100gl^{-1}) - PYN medium (peptone, 3.5; yeast extract, 3.0gl^{-1}; $MgSO_4.7H_2O$, 1.0gl^{-1}; KH_2PO_4, 2.0 gl^{-1}; and $(NH_4)_2SO_4$, 1.0 gl^{-1}). The strains p1, p2 and p3 produced 38, 36 and 35gl^{-1}ethanol in glucose (100gl^{-1}) - PYN medium. Log phase cells (18h) of p1, p2 and p3 (obtained from p1, p2 and p3 respectively) cultures were subjected to fifteen temperature treatment cycles (at 50°C each for 3h).

Thermotolerance of thermally adapted strains pt1, pt2 and pt3 were 100, 30 and 20% viability at 50°C for 30mín. Thermal adaptation programme was very effective for the strain p1. The improvement achieved by thermal adaptation (strain pt1) was 100% viability at 55°C for 30min as against complete cell death under the same condition by parent strain p1. The UV and EMS treatments did not improve the thermotolerance or ethanol production. Morphological and culture characteristics of the strain confirmed it as *Saccharomyces cerevisiae*. Therefore the yeast was designated as *Saccharomyces cerevisiae* S1. Significant increase in thermotolerance of *Saccharomyces cerevisiae* S1 was achieved by soy flour (100% viability for 86h at 40°C) supplementation to glucose ($100gl^{-1}$) - PYN medium, whereas in unsupplemented glucose ($100gl^{-1}$) - PYN medium the *Saccharomyces cerevisiae* S1 retained 100% viability for 72h at 40°C. When *Saccharomyces cerevisiae* S1 was heat treated at 58°C for 5min, viable cells were observed for heat shocked (from 36 to 45°C for 15min) as against complete cell death for *Saccharomyces cerevisiae* S1 grown without heat shock. TCA soluble anthrone positive carbohydrate accumulation along with thermotolerance was observed in heat shocked cells. Ethanol shock ($200gl^{-1}$for 30min) increased the TCA soluble anthrone positive carbohydrate content of growing cells of *Saccharomyces cerevisiae* S1 by 44%. The optimum culture conditions for the maximum growth of *Saccharomyces cerevisiae* S1 were pH 5.0 and 36°C and for ethanol fermentation were pH 5.0 and 40°C. Osmotolerance was measured as viability at 48h with the increasing amounts of added sorbitol to the glucose ($100gl^{-1}$) - PYN medium. The viability with sorbitol ($300gl^{-1}$) was 38% at 48h and was improved to 60% by soy flour supplementation. Ethanol tolerance was measured as viability at 48h with added ethanol to the medium. At 40°C *Saccharomyces cerevisiae* S1 retained 70% viability with $100gl^{-1}$added ethanol to glucose ($100gl^{-1}$) - PYN medium at 48h and it was improved to 80% by soy flour supplementation. At sorbitol ($200gl^{-1}$) and ethanol ($50gl^{-1}$), 46% viability was retained by *Saccharomyces cerevisiae* S1 at 48h and it was improved to 80% by the addition of soy flour to the medium. In glucose ($300gl^{-1}$) - PYN medium the ethanol production efficiency and glucose utilization at 40°C were 53.5 and 58.3% respectively. The glucose ($300gl^{-1}$) - PYN medium supplemented with soy flour ($18gl^{-1}$) showed 97.2% ethanol production efficiency and 100% glucose utilization. The cell recycle batch fermentation of

glucose ($300gl^{-1}$) - PYN supplemented with soy flour ($18gl^{-1}$) produced 120 (36h), 142 (20h) and $118gl^{-1}$ (20h) ethanol in first three batches. Since the objective of the project is high glucose fermentation at high temperature, the effect of temperature on the fermentation of different amounts of glucose was studied. The ethanol produced in glucose ($200gl^{-1}$) - PYN and glucose ($200gl^{-1}$) - PYN supplemented with soy flour ($18gl^{-1}$) were 75 & 95, 38 & 73 and 19 & 32 respectively at 40, 43 and 45°C. At 43°C the ethanol produced in glucose ($300gl^{-1}$) - PYN and glucose ($300gl^{-1}$) - PYN supplemented with soy flour ($18gl^{-1}$) was 28 and $80gl^{-1}$. The fermentation at 45°C was sluggish and has produced only 18 and $30gl^{-1}$ ethanol at 48h. The fermentation of glucose ($400gl^{-1}$) - PYN medium supplemented with soy flour was 78 (48h) and $25gl^{-1}$ (48h) at 43 and 45°C respectively. The ethanol produced in distillery spent wash supplemented with $100gl^{-1}$ glucose was $46gl^{-1}$with 90% ethanol production efficiency and the results indicated that the predefined PYN medium can be replaced with spent wash for the fermentation of glucose ($100gl^{-1}$). The yeast extract ($10gl^{-1}$) supplemented spent wash having glucose ($200gl^{-1}$) produced $91gl^{-1}$ ethanol as against $66.3gl^{-1}$ in spent wash - glucose ($200gl^{-1}$) medium. Neutrase hydrolysed spent wash - glucose ($200gl^{-1}$) produced 73.4 gl^{-1} ethanol as against $63.4gl^{-1}$ ethanol in inactivated Neutrase added spent wash glucose ($200gl^{-1}$) medium. The ethanol produced was 100 and $143gl^{-1}$with free and agar immobilized *Saccharomyces cerevisiae* S1 respectively in glucose ($300gl^{-1}$)-PYN medium at 40°C. The immobilized *Saccharomyces cerevisiae* S1 produced $139.0 \pm 2.0gl^{-1}$ethanol upto 30 repeated batch operations. The free cell suspension of *Saccharomyces cerevisiae* S1 produced 100 and $58gl^{-1}$ ethanol at 1st and 2nd batch operations at 40°C in glucose ($300gl^{-1}$)-PYN medium. Glucose ($300gl^{-1}$)-PYN medium supplemented with soy flour ($18gl^{-1}$) was the optimized medium for the free cells suspension of *Saccharomyces cerevisiae* S1 at 40°C and showed 97.2% ethanol production efficiency with complete glucose utilization. The soy flour supplementation has increased thermotolerance, ethanol tolerance and osmotolerance of *Saccharomyces cerevisiae* S1 and also has fulfilled the nutritional demands along with PYN medium for the fermentation of glucose ($300gl^{-1}$) at 40°C. The agar immobilized *Saccharomyces cerevisiae* S1 showed 91.3% ethanol production efficiency for 30 repeated batch operations.