Production of ethanol by simultaneous saccharification and fermentation: Endogenous proteins as nitrogen source from locally available starch based carbon sources

Sarmili Thurairetnam*, Senthuran,A. and Vasanthy Arasaratnam****

**Dept. of Agric. Chemistry, Faculty of Agriculture, University of Jaffna, Sri Lanka*
***Dept. of Biochemistry, Faculty of Medicine, University of Jaffna, Sri Lanka*

This paper describes the utilization of locally available starch sources for ethanol production by simultaneous saccharification and fermentation. Normally peptone and yeast extract are added as nitrogen sources to the fermentation medium to improve the ethanol production efficiency and growth of *Saccharomyces cerevisiae. Saccharomyces cerevisiae* (Fermipan, The Netherlands) was selected for this study. To avoid the addition of exogenous nitrogen source, the proteins present in the carbon sources were hydrolysed using Neutrase ($0.5AUml^{-1}$)-an endoprotease at pH 7.0 and 45°C. Starch in the carbon sources were liquefied with α-amylase ($0.225KNUml^{-1}$) at pH 7.0 for 4h. All the media used in the study contained $250gl^{-1}$ initial total sugar and $110gl^{-1}$ reducing sugar. For saccharification, glucoamylase ($0.8AGUml^{-1}$) was used and the pH of the fermentation medium was maintained at 5.0 and incubation was carried out at 30°C. Preliminary study was carried out with wheat flour. Wheat flour contained 8.1% (w/w) total protein and 0.1% (w/w) soluble protein. To the liquefied wheat starch (pH 7.0), Neutrase ($10.0mll^{-1}$) was added and incubated at 45°C for 4h. This wheat protein hydrolysed liquefied starch-containing medium was used for yeast cultivation without yeast extract and peptone supplementation and control medium contained liquefied starch with $2.3gl^{-1}$ yeast extract and $5.0gl^{-1}$ peptone. Biomass obtained and ethanol produced at 36h were $2.7x10^8$ & $2.8x10^8$ cells ml^{-1} and 82.1 & $90.8gl^{-1}$ in control medium and Test medium respectively. These results indicated that the endogenous protein in wheat flour hydrolysed by Neutrase could be utilised instead of exogenous nitrogen source for the ethanol production. To improve the protein hydrolysis, liquefied wheat starch was treated with different concentrations of Neutrase (2.5, 5.0, 7.5 and $10.0mll^{-1}$). At 36h the biomass obtained ($1.92x10^8$ $cellsml^{-1}$) and ethanol produced ($89.7gl^{-1}$) were highest in the wheat protein hydrolysed by $10.0mll^{-1}$ Neutrase. As wheat is not a local product, different starch based material such as rice, corn, manioc and soybean were selected. To the above-liquefied starch based carbon sources yeast extract ($2.3gl^{-1}$) and peptone ($5.0gl^{-1}$) were supplemented. At 56h biomass ($3.7x10^8$ and $3.5x10^8$cells ml^{-1} respectively) obtained was highest in manioc and soybean containing media (98.6 and $95.3gl^{-1}$ respectively). Proteins in all these liquefied starch based carbon sources were hydrolysed with Neutrase ($10.0mll^{-1}$). At 56h the biomass produced was highest in rice flour followed by manioc and soybean containing media ($4.4x10^8$, $3.7x10^8$ and $3.5x10^8$ cells ml^{-1} respectively) and ethanol produced was highest in corn flour followed by rice and soybean (105.0, 96.6 and $92.0gl^{-1}$ respectively). As the protein content in corn is 12% (w/w) and manioc is 1.3% (w/w), corn and manioc hydrolysates were mixed in different ratios (100:0, 75:25, 50:50, 25:75 and 0:100) and simultaneous saccharification and fermentation were carried out. The results indicated that the highest amount of biomass and ethanol were produced in corn flour containing medium at 56h. From the results it can be concluded that among the locally available starch based carbon sources corn flour (in suspension 38%, w/w) could be used after liquefaction (with $0.225KNUml^{-1}$

Termamyl) and endogenous protein hydrolysis (with 0.5AUml^{-1} Neutrase) for simultaneous saccharification (0.8AGUml^{-1} glucoamylase) and fermentation by *S.cerevisiae*. The simultaneous saccharification and fermentation have not only had reduced the inhibition of sugar in fermentation by *S.cerevisiae* but also reduced the total time required for liquefaction, saccharification and fermentation from 85h to 67h..