Preliminary studies on ethanol production by simultaneous saccharification and fermentation

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Ethanol production by simultaneous saccharification and fermentation is a cost effective and less time consuming method. Preliminary studies were made to optimize the conditions for liquefaction and saccharification. Soluble starch (16%, w/w) at pH 7.0 was liquefied by α-amylase (0.225KNUml^{-1}) at 90°C. After 140 min of liquefaction, the hydrolysate obtained contained 82gl^{-1} reducing sugar with a DE value of 48.6. To the liquefied starch at pH 4.0, glucoamylase (0.4AGUml^{-1}) was added for saccharification and incubated at 60°C. After 3h from the addition of glucoamylase, 148gl^{-1} reducing sugar was produced with the DE of 88.0. *Saccharomyces cerevisiae* (Fermipan, The Netherlands) was selected for this study. As the relative price of unit weight of wheat flour is cheaper and readily available, it was used in the preliminary studies. The constituents of wheat flour were analyzed and it contained 69.7% (w/w) starch and 8.1% (w/w) protein. The pH of the medium was maintained at 5.0 and incubation was carried out at 30°C with shaking at 100rpm. The sterile medium containing 16% (w/w) liquefied wheat starch (reducing sugar 50gl^{-1} and total sugar 105gl^{-1}) supplemented with yeast extract (2.3gl^{-1}) and peptone (5.0gl^{-1}), 0.4AGUml^{-1} glucoamylase was added and inoculated with *S.cerevisiae*. The biomass and ethanol produced at 28h were 2.3x10^{8}cells ml^{-1}and 35.0gl^{-1} respectively. As the ethanol production efficiency (64.9%) in the medium was not satisfactory, to improve the saccharification, different amounts of glucoamylase (332, 664 and 996µl) was added to the liquefied starch containing fermentation medium. The biomass obtained at 28h was highest (2.41x10^{8} cells ml^{-1}) in 332µl glucoamylase added medium while ethanol production was highest (39.5gl^{-1}) in 664 and 996µl glucoamylase added medium. The glucoamylase concentration of 664µl was selected for further studies because above this concentration there was no increase in alcohol production and sugar utilization. To increase the ethanol production, different concentrations of liquefied wheat starch (with 100, 150, 200 and 250gl^{-1} of total sugar) was taken and the biomass obtained (3.51x10^{8} cells ml^{-1}) and alcohol produced (82.1gl^{-1}) were highest in 250gl^{-1} total sugar containing medium at 36h. The total sugar concentration of 250gl^{-1} and 664µl of glucoamylase for saccharification were selected as optimum amount of carbon source and enzyme concentration for simultaneous saccharification and fermentation to obtain an ethanol yield of 0.5 at 36h.