1. ABSTRACT

Hydrolysis of starch for the production of glucose is a two step process involves liquefaction and saccharification. Liquefaction consumes high energy and the whole process requires more than 24 h for completion. Hence, a method for complete hydrolysis of starch to glucose with a view to save energy and time was studied. In early studies, waxy maize starch was liquefied by α-amylase and saccharified by glucoamylase. Our studies showed that a mixture of α-amylase and glucoamylase, referred to as the enzyme mixture had, a synergistic effect on the hydrolysis of the starch. The optimum ratio of glucoamylase to α-amylase was 1.8 AGU/1 KNU and complete hydrolysis was observed in 3 h under optimized conditions.

The common industrial process for the production of glucose is based on the purified starch. During the processing of starch from cereals and tubers, about 20-30% of the total starch content is lost. To avoid the starch loss, a direct method was developed to process the starch in corn flour to glucose by the enzyme mixture. Under optimized conditions starch in corn flour was hydrolysed by 3 h. The time taken for filtration of the hydrolysate was increased by the change in pH from 5.0 to 4.5. Charcoal was better than cation- and anion-exchange resins for the removal of pigments, amino acids and proteins from the hydrolysate. When 1600 g (16%, W/W) and 4000 g (40%, W/W) of corn flour suspended in 0.025 M acetate buffer (pH 5.0) was hydrolysed, the glucose yield was 76% and 50.2% respectively, whereas the hydrolysis of wet processed corn grain (10%, W/W) yielded 41.1% glucose. In these cases the glucose yield was calculated based on the total starch content in corn.

For continuous glucose production by extractive bioconversion, an aqueous two-phase system was selected. An aqueous two-phase system consisting of crude dextran (3%, W/W) and 5% (W/W) polyethylene glycol (20 M) was observed to have a minimum settling time for starch (10%, W/W). Hence the above two-phase system was used for the hydrolysis of starch suspension (10%, W/W) by the enzyme mixture. The optimum conditions for the activity of the enzyme mixture were same for both the buffer medium and the aqueous two-phase system, but the activity of the enzyme mixture in the aqueous two-phase system was elevated. When the starch (10%, W/W) was hydrolysed in a mixer-settler enzyme reactor containing the aqueous two-phase system, a continuous stream of 11.6% (W/W) glucose was obtained for 8 days. Under similar experimental conditions when 30% (W/W) starch suspension was used, the reactor could be run only for 4 days.

Another aqueous two-phase system for simultaneous saccharification and fermentation was selected, which consisted of 10% (W/W) purified polyethylene glycol (20 M) and 18% (W/W) liquefied starch. The activity of glucoamylase in this aqueous two-phase system was higher than that in the buffer medium and was 80% of its original activity in presence of 10% ethanol.

To have the *Saccharomyces cerevisiae* in an aqueous two-phase system containing polyethylene glycol, the effect of polyethylene glycol of varying molecular weights and concentrations on the growth and fermentation was studied under optimized growth conditions in shake flasks. Growth was reduced and the ethanol yield was increased in presence of polyethylene glycol. Maximum effect on growth and ethanol yield in shake flasks was observed in 5% (W/W) polyethylene glycol (20 M). However the growth was delayed when the experiment was repeated in fermentor.

As an alternative to the aqueous two-phase system, immobilized *Saccharomyces cerevisiae* was tried for ethanol production. *Saccharomyces cerevisiae* entrapped in calcium alginate continuously produced ethanol for 10 days under optimized conditions and the process was terminated by the 14th day due to the distruption of the alginate beads.

Lactic acid is an industrially important product, which could be obtained by glucose fermentation. Thus a continuous glucose fermentation process was carried out to produce lactic acid using calcium alginate entrapped *Lactobacillus delbreuckii* for 32 days under optimized conditions.

Fructose is another bioconvesion product of glucose which has a commercial value. Hence glucose produced in the mixer-settler enzyme reactor was continuously isomerized to fructose for 7 days by immobilized glucose isomerase after optimizing the conditions for continuous operation.