CONTINUOUS PROCESS FOR THE PRODUCTION OF A GENERIC FERMENTATION MEDIA BASED ON SAGO STARCH

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Introduction

The mold such as *Aspergillus awamori*, *Aspergillus niger* and *Aspergillus flavus* are able to grow in cooked starch by secreting amylolytic enzymes (amylase and glucoamylase) at 30°C. As a result, glucose is accumulated in the culture broth. Limitation of nitrogen supply will inhibit growth, so that glucose produces is not consumes for further growth. The activity of this mold can be exploited in continuous hydrolysis of cooked starch at room temperature for the production of fermentable sugars (Arbakariya et al. 1990). The out flow from the continuously operated bioreactor, once sterilized, is an ideal fermentation medium. In addition, the biomass produces during the process can also be used as nitrogen source in the formulation of the fermentation media. The resulting process could provide the first stage of a fermentation plant for the production of a range of final products. In this project, the possibility of using amylolytic producing mold and genetically engineered yeast in hydrolysis of starches for the production of fermentable media (a mixture of fermentable sugars and nitrogen source) was studied using two modes of fermenter operation, batch and continuous.

Materials and Methods

The industrial strain of filamentous fungus, *Aspergillus awamori*, was used in all experiments. The fungus was used in industry for glucoamylase production. Potato dextrose agar (PDA) slants incubated at 30EC for at least 5 days were used for spore production. To obtain a spore suspension, a sterile 0.001% (v/v) aqueous solution of Tween 80 was added aseptically to an agar slant with dense sporulation and shaken by hand for a short time. A 2 l stirred tank fermenter was used in all experiments. The fermenter was equipped with pH and DOT measurement systems. The cell concentration was determined by filtering the sample of fermentation broth on the filter paper (Whatman no. 1) and drying in an oven at 95°C for more than 24 h before weighing. Glucoamylase activity was determined using 2 ml of culture supernatant with 18 ml of 60 mM maltose as substrate (pH 4.4 using acetate buffer) incubated at 40°C. The glucose produced from the reaction was determined using a glucose (Trinder) 100 (Sigma Diagnostics, USA).

Results and Discussion

In batch process, glucose was produced during active growth from the hydrolysis of starch by the action of amylolytic enzymes produced by the fungus. Substantially high glucoamylase activity was detected during growth of the fungus. Glucose concentration in the culture reached maximum after about 40 h and 25 h, with a value of 25.4 g/l and 28.6 g/l for sago and potato starches, respectively. The concentration of biomass obtained at a time where glucose concentration was maximum was higher for hydrolysis of sago starch (12.3 g/l) compared to potato starch (3.8 g/l). The glucoamylase activity during active growth was also lower during growth of the fungus in sago starch compared to potato starch. The maximum glucose produced was increased with increasing starch concentration. However, the maximum cell concentration was only increased up to starch concentration of 80 g/l. The maximum cell concentration obtained in 80 and 100 g/l sago starch was more or less the same. At starch concentration higher than 100 g/l, the fungus did not grow well due to high viscosity of the solution which reduced oxygen transfer rate and mixing efficiency (data not shown). The yield of biomass based on starch consumed was not significantly different at a range of starch concentration studied. On the other hand, the yield of glucose produced was higher at high starch concentrations (>60 g/l). The overall productivity, which is calculated as the maximum glucose produced divided by the time taken to reach maximum glucose concentration, was increased almost linearly with concentration of sago starch. Continuous culture experiments were conducted to investigate the relationship between dilution rate (D) and glucose production. Typical time courses of sago and potato starches hydrolysis by *A. awamori* in continuous process, including the initial batch periods and transition to continuous operation are investigates. In both cases, biomass, glucoamylase and glucose concentrations reached their steady-state values after about 32 residence time. The pH value was constant at 2.5, throughout the continuous fermentation. Both processes, continuous hydrolysis of sago and potato starches, were still in steady-state even after about 400 h of operation. The time courses of liquefaction and saccharification processes during hydrolysis of 300 g/l sago starch, which was the optimum concentration for enzymatic hydrolysis of sago starch show that batch liquefaction and saccharification processes took about 7 and 50 h, respectively. This means that a total time required to complete the enzymatic hydrolysis of sago starch to glucose was about 57 h. The final concentration of glucose obtained in enzymatic hydrolysis was 255 g/l, which gave an overall activity and yield of 4.47 g/l.h and 0.75 g/g, respectively.

Conclusions

Single-step biological hydrolysis of sago and potato starches using *A. awamori* to produce glucose has been successfully carried out in batch and continuous processes at 30 °C. Although the yield and overall productivity obtained by the biological hydrolysis were lower compared to enzymatic hydrolysis, the process can be carried out at lower temperature than that required for enzymatic hydrolysis. This means that the operating cost to control the temperature at high levels during the hydrolysis can be reduced significantly.

References

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