LONG TERM REPEATED BATCH FERMENTATION OF HYDROLYZED SAGO STARCH FOR ETHANOL PRODUCTION

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Introduction. Numerous challenges that lingers and must be overcome in ethanol fermentation (EF) are: cost of raw materials, microorganism's resistant to recycling and high ethanol concentration, and high production rate (Amorin *et al*., 2011). Ethanol from sugar cane had overcome almost all these challenges, but the ethanol concentration is still considered low (Voegele, 2009). In contrast, ethanol from corn is obtained at high concentration but the process is time consuming and the yeasts are generally not recycled (Voegele, 2009). Both processes depend on the types of raw materials used. Alternative substrates such as lignocellulosic materials are the focus for many researchers. Similarly, sago palm growing in tropical countries in Asia has proven to be a good substrate for EF. Sago palm produces large quantity of starch as high as 200-250 kg/three of starch in its trunk (Flach, 1997). The use of hydrolyzed sago starch (HSS) as the substrate for EF using repeated batch fermentation (RBF) is reported here. The main aim was to maintain enduring EF with high production rate.

Methods. HSS was produced using the enzymes Termamyl SC and Amylo-glucosidase (Novozymes) for liquefaction and saccharification, respectively. For EF the yeast *Saccharomyces cereviciae* CSI-1 was used. RBF was performed by harvesting the cells by centrifugation at 3,000 rpm for 5 min. EF was performed in 3L jar fermenter at a 2L working volume. OD, pH, and CO_2 production were monitored on line. The temperature was controlled at 33 °C without pH control. Ethanol and residual glucose were determined by enzymatic method. Initial and final CFU/mL as well as dry cell weight (DCW) were determined for each fermentation cycle. Commercial glucose (CG) was used as substrate control to compare it with HSS, both at level of 250 g/L. Yeast extract was the nitrogen source (5 g/L).

Results. Sago starch is an excellent substrate for EF when compared with commercial glucose. HSS produced a brown broth compared to the clear solution from CG. However, this condition did not affect the productivity of the EF and the RBF process operated for 50 cycles at a high production rate as shown in Table 1. The trend of biomass production during these 50 cycles was unwavering and as a result an average 17.5 g/l was enough to produce ethanol during the whole process, hence rendering a stable production of ethanol, as high as 14.2 % (v/v) (Table 1). The biomass was maintained metabolically active by adding only 5 g/L of yeast extract from the first to the last fermentation. Therefore, the cells grew up for two or three hours only and thereafter, the ethanol was produced for maintenance purposes but not for growing. The concentration of CG and HSS was fixed at 250 g/L to assess the high concentration of ethanol and to avoid variations of ethanol concentration. CG and HSS gave the same theoretical yield, but the specific productivity was higher for CG since less biomass produced more ethanol. The total ethanol produced in the 50 cycles was 11.5 kg. The productivity of the system was very similar and this suggested that HSS is effective as substrate as GC does for EF. HSS could overcome the problem of low concentration of ethanol since it produces a broth with very low concentration of solids (0.3-0.5 g/L). This characteristic makes possible to increase the concentration of sugar (glucose) up to 250 g/L or even up to 300 g/L. As reported, high concentration of solids in broth affect the performance of the fermentation because cell recycling becomes difficult (Voegele, 2009)

Table 1. Kinetics of EF in RBF mode.

Parameter	CG	HSS
Total sago used (kg)	20	25
[a]Total EtOH (kg)	9.2	11.5
EtOH (%v/v)	14.5±1.5	14.2±2.3
Residual Glucose (kg)	0.1±0.01	0.12±0.03
DCW (g/L)	15.2±3.0	17.5±4.1
Volumetric productivity (g/Lh)	4.8±0.2	4.7.0±0.1
Specific productivity (g/gh)	7.5±2.9	6.4±0.8
Theoretical yield $Y_{p/s}$	0.46±0.01	0.46±0.02

Conclusions. *Saccharomyces cereviciae* CSI- 1 is able to endure for at least 50 fermentation cycles to produce 11.5 kg of ethanol under RBF mode. HSS was effective as the substrate for ethanol production. Cell recycling and high ethanol concentration were achieved.

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