Valorisation of the organic fraction of Municipal Solid Waste: Improving succinic acid production efficiency by immobilized continuous fermentation

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Introduction

Global waste generation keeps increasing over the years and requires innovative solutions to minimize their environmental impact. Municipal solid waste (MSW) generation is continuously increasing worldwide. The composition of MSW varies among EU countries consisting mainly of organic material (25 - 48%), paper (16 - 51%), plastics (0.8 - 23.7%), glass/ceramic (4.5 - 16%), metals (2 - 8.8%) and textiles and other components (3.1 - 32.2%) (Eurostat, 2020). The management of the organic fraction of municipal solid waste (OFMSW) poses an important challenge around the world. Waste management methods such as landfilling or incineration are not sustainable routes. Novel approaches that convert waste into valuable products or energy are more promising than traditional waste management methods (Matsakas *et al,*. 2017). The utilisation of renewable resources for the production of bio-based products is essential in order to develop sustainable bioprocesses and biorefineries. MSW could be used for the biotechnological production of succinic acid, one of the most important platform chemicals in the bio-economy era.

The main objective of this study was the valorisation of OFMSW for the biotechnological production of succinic acid using *Actinobacillus succinogenes*, a natural succinic acid producing strain. The OFMSW was treated with commercial hydrolytic enzymes. OFMSW hydrolysate has been used as fermentation feedstock for the production of succinic acid by *A. succinogenes* via batch, fed-batch and continuous fermentations. The effect of total carbon source concentration was evaluated in batch fermentations using both OFMSW hydrolysate and synthetic medium. The effect of yeast extract, corn steep liquor and different $MgCO_3$ concentrations on succinic acid production efficiency were investigated in fed-batch fermentations. Continuous fermentations in a cell free system or with immobilised bacterial cells were carried out in order to improve succinic acid fermentation efficiency using glucose and OFMSW hydrolysate as carbon source.

Material and methods

The bacterial strain employed for succinic acid production was *Actinobacillus succinogenes* 130Z (DSM - 22257). Inoculum preparation was carried out in Erlenmeyer flasks in tryptic soya broth (TSB). Inoculum preparation was carried out in an orbital shaker for 16-18 h at 37 ºC and 180 rpm agitation. Batch, fed-batch and continuous fermentations were carried out in 1 L bench-top bioreactor (Labfors 4, Infors HT) with 0.5 L working volume. Fermentation pH was controlled at 6.7 with 5 M NaOH. Inoculum size was 10% (v/v). The temperature and agitation were maintained at 37 °C and 100 rpm throughout fermentation. Continuous supply of CO_2 gas at a flow rate of 0.5 vvm was applied.

The effect of initial sugar concentration (30, 50, 80 g/L) was evaluated in batch fermentations using OFMSW hydrolysate and synthetic medium which contained commercial carbon sources in a similar ratio as the one contained on average in OFMSW hydrolysate. The effect of yeast extract (5 g/L), corn steep liquor (5 g/L) and $MgCO_3$ concentrations (5, 10, 20 g/L) was evaluated in fed-batch fermentations. Continuous fermentation was carried out in a cell free system with glucose as carbon source until 900 h at three different dilution rates (0.02, 0.04, 0.08 h^{-1}), while OFMSW hydrolysate was used as feeding solution from 900 h until 2400 h at six dilution rates (0.02, 0.04, 0.05, 0.06, 0.08, 0.1 h^{-1}). Continuous fermentation was also carried out with immobilised cells using both glucose and OFMSW hydrolysate. In all continuous fermentations, the culture was initially operated in batch mode for 24 h and then continuous fermentation was initiated. The medium at the beginning of continuous fermentation contained 30 g/L glucose, 10 g/L yeast extract, 5g/L $MgCO_3$ and minerals. The continuous fermentations were initially conducted at a dilution rate of 0.02 h^{-1} in order to increase bacterial cell concentration, create wall growth and biofilm formation and stabilise bioreactor operation. When OFMSW hydrolysate was used as feeding medium, yeast extract was added into the OFMSW hydrolysate to adjust the free amino nitrogen (FAN) concentration at around 500 mg/L. In all continuous fermentations, each dilution rate lasted for approximately 7-8 hydraulic retention times (HRT).

C6 and C5 sugars and organic acids were analysed by high-pressure liquid chromatography (HPLC) with refractive index (RI) detector and an HPX-87H column. The phosphorus concentration was determined using a method developed by Harland and Harland (1980) and FAN concentration was assayed according to the ninhydrin colorimetric method (Lie, 1973).

Results and discussion

The total carbon source concentration in OFMSW hydrolysate was around 90 g/L containing mainly glucose (84.5%). OFMSW hydrolysate also contained fructose, xylose, glycerol, sucrose, galactose, arabinose and mannose. FAN and inorganic phosphorus concentration was 638.7 mg/L and 100.6 mg/L, respectively. The addition of $MgCO_3$ improved succinic acid production. The highest succinic acid concentration (34.8 g/L) was achieved when 20 g/L of $MgCO_3$ concentration was added with yield and productivity of 0.60 g/g and 0.79 g/L/h respectively. Yeast extract supplementation resulted in higher succinic acid concentration (34.3 g/L) with productivity of 0.75 g/L/h, while corn steep liquor supplementation led to succinic acid concentration of 28.7 g/L. However, the supplementation of OFMSW hydrolysate with 5 g/L yeast extract resulted in the highest by-product to succinic acid ration among all fed-batch fermentations. Continuous fermentations in a cell free system was carried out at different dilution rates using glucose based medium and OFMSW hydrolysate. The productivity achieved using glucose-based synthetic medium and OFMSW hydrolysate was 1.7 g/L/h and 1.4 g/L/h, respectively, at dilution rate of 0.08 h^{-1} (Figure 1). Immobilisation of the culture under continuous operation resulted in higher productivities at all dilution rates. The productivity increased 32.8 % in glucose-based medium where succinic acid concentration was 28.3 g/L, while when OFMSW hydrolysate was used as feeding solution the productivity increased 22.8 % with succinic acid concentration of 26.2 g/L at dilution rate of 0.08 h^{-1}.

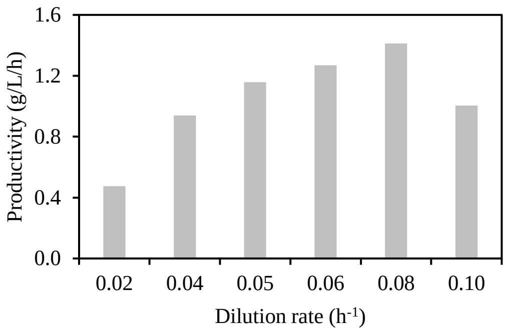

Figure 1: Productivity achieved during continuous fermentation in a cell free system using OFMSW hydrolysate

Conclusions

The OFMSW hydrolysate can be considered as a promising feedstock for the biotechnological production of succinic acid. Using the OFMSW hydrolysate as substrate, efficient succinic acid production has been achieved in batch, fed-batch and continuous fermentations. The use of immobilized cells in continuous cultures could further improve the biotechnological production of succinic acid.

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