Succinic acid fermentation by *Actinobacillus succinogenes*: continuous production

Mariateresa Ferone, Francesca Raganati, Alessia Ercole, Giuseppe Olivieri, Piero Salatino, Antonio Marzocchella*

Dipartimento di Ingegneria Chimica, dei Materiali e della Produzione Industriale - Università degli Studi di Napoli Federico II, P.le V. Tecchio 80, 80125 Napoli – Italy

**Corresponding author: antonio.marzocchella@unina.it*

Highlights

- Succinic acid production was carried out by biofilm of *A. succinogenes*
- The process was carried out in a biofilm packed bed reactor with Tygon rings as carrier
- High productivity, yield and conversion degree achieved with GAX medium
- The effect of HMF and furfural on *A. succinogenes* biofilm was investigated

1. Introduction

Succinic acid (SA) - a four carbon dicarboxylic acid - is a very interesting bicarboxylic acid that can be produced by fermentation of renewable resources. SA is currently used in the food industry - as a pH regulator and as a flavoring agent - in the pharmaceutical industry - as additive for the preparation of drugs - in the agricultural food, and as ion chelator and surfactants[1]. A potential microbial platform to produce SA is *Actinobacillus succinogenes*: it can produce SA at high productivity, yield and concentration during mixed-acid, batch fermentation, by using a variety of carbon source[2].
The present contribution regards the continuous production of SA by a biofilm of *Actinobacillus succinogenes* in a packed bed reactor. The performance of the reactor was assessed under a wide range of dilution rate and by feeding the reactor with a stream bearing a mixture of glucose, arabinose and xylose. The effect of two putative fermentation inhibitors was also investigated.

2. Methods

Microorganism and media - *Actinobacillus succinogenes* DSM 22257 was supplied by DSMZ. Stock cultures were reactivated according to the supplier procedure and stored at -80 °C. The thawed cells were inoculated in 15 mL Hungate tubes containing 12 mL of containing Brain Hearth Infusion broth (BHI) and were grown under anaerobic conditions for 24 h at 37 °C. The fermentation medium consisted of: 5 g/L Yeast Extract, 1 g/L NaCl, 0.3 g/L Na_2HPO_4, 1.4 g/L NaH_2PO_4,1.5 g/L K_2HPO_4, 0.2 g/L $MgCl_2 \cdot 6H_2O$, 0.23 g/L $CaCl_2 \cdot 2H_2O$.
Apparatus and operating conditions - The apparatus consisted of a fixed bed reactor, liquid pumps, a heating apparatus, a system for pH control and on-line diagnostics. The reactor was at a 166-mL glass lined bottle (5 cm ID, 8,5 cm high) jacketed for the heat exchange. The bed was made of Tygon rings. The liquid phase volume in the reactor (the reaction volume) was set by tuning the level of the overflow duct. Carbon dioxide was sparged at the reactor bottom to support anaerobic conditions and to provide the CO_2 for the succinic acid production pathway. The system for pH control consisted of a pH-meter, a peristaltic pump, a vessel with NaOH 0.3 M solution, and a pH controller.
The reactor with the carriers was sterilized in autoclave at 121 °C for 20 min. The gas stream was sterilized by filtration. The sterile medium was fed at the bottom of the reactor by means of a peristaltic pump. 34.4 g of Tygon rings were used as carrier to prepare a 4.5 cm high packed bed. The volume of the reactor was set at 40 mL by means of the overflow duct. No chemical was used to assist cell immobilization on the selected carrier. The carbon source was a mixture of glucose, arabinose and xylose. The reactor was operated continuously at

volumetric flow rate of the feeding ranging between 0.02 and 0.06 L/h.

Analytical methods - Cell density was measured as optical absorbance at 660 nm (OD660) by using a spectrophotometer (Cary- 50 Varian). The concentration of soluble species was measured in the liquid phase after spinning down the cells by centrifugation (13000g, 10min). Sugar and organic acid concentrations were measured by means of a high-performance liquid chromatography (HPLC) (HP1250 working station system - Agilent Technologies, USA) equipped with a cation-exclusion column (Aminex HPX-87H; 300 mm×7.8 mm, 9 µm; Bio-Rad Chemical Division, Richmond, CA). Analytes were detected by UV absorbance (Agilent Technologies, G1315D) and refractive index (Agilent Technologies, G1362A). H_2SO_4 5mM was used as mobile phase at 0.6 mL/min flow rate at room temperature. The injection volume was 20 µL.

3. Results and discussion

The PBR performances were evaluated by feeding the bioreactor with a synthetic lignocellulosic hydrolysate (inhibitor free) containing glucose, arabinose and xylose. The total sugars concentration in the synthetic medium was set to 80 g/L and the mass ratio between the sugars was 55:15:30 for the GAX mixture [3]. Succinic acid concentration decreased with the dilution rate; the maximum SA concentration was 20.5 g/L at D=0.7 h^{-1} (see Figure 1). The succinic acid productivity was quite stable at all the dilution rates investigated: about 15 g/L*h. The concentration of succinic acid produced reduced when inhibitors were supplemented at the feeding. In particular, the concentration of produced SA reduced of about 5.6% in presence of furfural (0.28 g/L) and of about 10.8% in presence of HMF (1 g/L), when compared to the inhibitor-free GAX medium at same operating conditions.

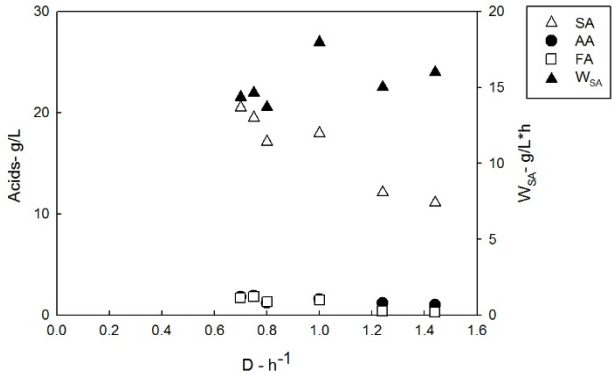

Figure 1. Data measured during the PBR operation as a function of the dilution rate. Feeding: GAX medium,.

4. Conclusions

The effects of the dilution rate (D) on the performance of a biofilm packed bed reactor (PBR) of *A. succinogenes* were investigated. Succinic acid concentration, productivity and sugar(s) conversion decreased with D. HMF was characterized by a high inhibition effect on succinic acid production compared to furfural.

References

[1] Zeikus, J. G.; Jain, M. K.; Elankovan, P. *Appl. Microbiol. Biotechnol.* **1999**, *51* (5), 545–552.
[2] Guettler, M. V; Rumler, D.; Jainf, M. K. *Int. J. Syst. Bacteriol.* **1999**, *49*, 207–216.
[3] Li, J.; Zheng, X.-Y.; Fang, X.-J.; Liu, S.-W.; Chen, K.-Q.; Jiang, M.; Wei, P.; Ouyang, P.-K. *Bioresour. Technol.* **2011**, *102* (10), 6147–6152.

Keywords

Actiniobacillus succinogenes, biorefinery, biofilm, lignocellulose