**Biotechnological production of succinic acid from lignocellulosic raw material.**

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**Highlights**

* *B.succiniciproducens* produced 37 g/L of succinic acid on *A. donax* hydrolysate.
* *A fermentation efficiency of about 75% was demonstrated on pilot scale FB experiments*.
* *Effective and theoretical optimized process performance was assessed via material flow analysis.*

**1. Introduction**

Due to its wide range of applications in the food, pharmaceutical and chemical fields, microbial synthesis of succinic acid is receiving growing attention, generating already relevant industrial results, as well as fueling constant research for improvements. In order to develop a sustainable process, a special focus is now set on the exploitation and conversion of lignocellulosic biomasses into platform chemicals.

**2. Methods**

Fermentation experiments were performed on a Biostat C (150 L total volume) with a working volume of 70-80 L (Sartorius Stedim; Melsungen, Germany). All fermentations were carried out at 37°C on MH medium supplemented with *A. donax* hydrolysate. The culture was sparged with CO2 at 0.1 vvm and agitation speed was set to 100-200 rpm and a constant pH of 6.5 was maintained. For the fed-batch phase a concentrated feeding solution containing sugars and yeast extract*,* was added to the broth in order to prolong growth. The feeding profiles provided addition of about 33-55 g/L of total sugars either pure or diluted in *A. donax* with a rate ranging from 0.2 to 1.2 g/L∙h. The global evaluation of process efficiency considering the conversion of cellulose and hemicellulose to monosaccharides and their fermentation to succinic acid was performed [2].We carried out material flow analysis (MFA) by using the STAN software (published by TU Wien, Institute for Water Quality, Resource and Waste Management) that is a freeware that follows the Austrian standard ÖNorm S 2096 (Material flow analysis – Application in waste management).Shake flask experiments were conducted in 0.1 L bottles filled with 0.1 L of medium at 37°C and 140 rpm, in a rotary shaker incubator (model Minitron, Infors, Bottmingen, Switzerland). Bottles were sealed with stainless steel headpiece caps and sterile venting filters to insufflate CO2, before starting the experiment and during growth. Experiments were conducted on standard MH medium supplemented with lignocellulosic hydrolysates as C source.

**3. Results and discussion**

In the present work we used *Basfia succiniciproducens* BPP7 in separated hydrolysis and fermentation experiments with *Arundo donax* as starting material. We developed batch and fed-batch strategies, the latter showing a maximal production of about 37 g/L of succinic acid a productivity of 0.9 g/L∙h on the pilot scale. Global mass balance calculations demonstrated a hydrolysis and fermentation efficiency of about 75%.

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|  | SA  (g/L) | LA+FA/SA  (g/g) | AA/SA (g/g) | SA/TA (g/g) | TA/SA  (g/g) | Fermentation efficiency  (%) |
| FB1 | 26.5 | 0.30 | 0.18 | 2.06 | 0.48 | 46.9 |
| FB2 | 21.0 | 0.36 | 0.22 | 1.77 | 0.56 | 67.0 |
| FB3 | 24.0 | 0.22 | 0.20 | 2.37 | 0.42 | 49.2 |
| FB4 | 37.0 | 0.29 | 0.17 | 2.16 | 0.46 | 75.4 |

Table 1-Fed-batch fermentations on pilot scale. SA, succinic acid; LA, lactic acid; FA, formic acid; AA, acetic acid; TA, LA+FA+AA.

A Material Flow Analysis was applied showing that, in the identified conditions 88.5 % of succinic acid per Kg of virgin biomass used, and 52% of product on the total generated output, were obtained. Considering an optimized process that uses *A. donax* as only C source, data indicated a potential yield of about 30% of product, and an unconverted residue of 31% mainly composed of lignin, a potentially valuable feedstock for bioenergy and biochemicals production.

**4. Conclusions**

The use of fed-batch strategies for the growth of *B. succiniciproducens* on *A. donax* improved the titer and productivity of succinic acid on a scale that is close to industrial application. Best experimental results were analysed by material flow analysis to evaluate another aspect of industrial development and assess the performance of the entire production process.

Currently, investigations on the possibility of using other lignocellulosic biomasses and microorganisms are in progress.

**References**

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