Biochemical Engineering Journal 113 (2016) 26–41

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Modelling succinic acid fermentation using a xylose based substrate

Chrysanthi Pateraki[a], Henrik Almqvist[b], Dimitris Ladakis[a], Gunnar Lidén[b], Apostolis A. Koutinas[a], Anestis Vlysidis[a,*]

[a] Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens, Greece
[b] Department of Chemical Engineering, Lund University, P.O. Box 124, 221 00 Lund, Sweden

ARTICLE INFO

Article history:
Received 19 January 2016
Received in revised form 12 May 2016
Accepted 6 June 2016
Available online 7 June 2016

Keywords:
Modelling
Fermentation
Substrate inhibition
Product inhibition
Actinobacillus succinogenes
Basfia succiniciproducens

ABSTRACT

This study focuses on the development of unstructured models, including both substrate and product inhibition, that predict the cultivation of *Actinobacillus succinogenes* and *Basfia succiniciproducens* on a mixture of C5 and C6 sugars, similar to the sugar composition contained in spent sulphite liquor, the liquid waste stream from the sulphite pulping process. The main sugar monomer contained in the medium was xylose (72.6%) with galactose (12.2%), glucose (10.9%), mannose (4.2%) and arabinose (0.1%) making up the remaining sugar content. The growth inhibition caused by metabolic products (succinic, lactic, acetic, formic and mixed acids) and initial mixed sugar concentration was determined. The highest obtained succinic acid yield, final concentration and productivity in fermentations carried out in Duran bottles were 0.76 g/g, 26.0 g/L and 0.66 g/L/h for *B. succiniciproducens* and 0.69 g/g, 27.4 g/L and 0.60 g/L/h for *A. succinogenes*, respectively (the units in yield calculations are referred to grams of succinic acid produced per gram of total sugars consumed). The kinetic parameters for both strains were estimated from experimental results. The obtained R^2 values for the fitted models were 0.96 for *A. succinogenes* and 0.94 for *B. succiniciproducens*. A sensitivity analysis on the obtained parameters showed that the maximum specific growth rates (μ_{max}) and the growth associated substrate consumption parameters (γ) are the most influential model parameters for both microorganisms. The model was validated by fermentations conducted in lab-scale bioreactors showing good agreement between experimental data and model simulations.

1. Introduction

Within the last decade, the interest in production of biochemicals from renewable resources has increased in both academia and industry as a result of an increased societal awareness of the need to shift to a less petroleum-dependent economy. A first target substrate has been organic waste, which can be valorized in biobased industries either directly or after simple pretreatment in microbial fermentations [1]. New bio-routes from lignocellulosic wastes have been investigated and very promising examples have been reported for the production of various bio-based chemicals and polymers, such as ethanol, succinic acid, and xylitol [1].

The traditional pulp and paper industry generates as side streams large amounts of lignin and other biomass derived compounds not retained in the paper. Depending on the process, significant amounts of sugar monomers such as pentoses (xylose and arabinose) and hexoses like glucose, galactose and mannose are generated in side streams [2,3]. Sulphite wood pulp mills generate large volumes of spent sulphite liquor (SSL) that mainly contains xylose as the predominant sugar. These sugars are destroyed in the currently applied recovery process of lignosulphonates from SSL, although they in fact constitute a first-class carbon source that could be used, either directly or after detoxification of SSL, for the production of various bio-based chemicals and polymers, such as ethanol, succinic acid and bacterial cellulose [1,3,4]. Apart from sugar monomers, SSL contains some toxic components, most important of which being several phenolic compounds such as gallic acid, ellagic acid and syringic acid [4] and lignosulphonates [5]. Alexandri et al. [4,5] have illustrated that untreated SSL shows a poor performance as substrate in microbial fermentations compared to treated SSL with solvent extraction for removing either phenolic compounds [4] or lignosulphonates [5].

The di-carboxylic acid succinic acid is a key building block for various commodity and niche chemicals, and has received

* Corresponding author.
E-mail addresses: paterakichr@aua.gr (C. Pateraki), henrik.almqvist@chemeng.lth.se (H. Almqvist), ladakisdimitris@gmail.com (D. Ladakis), gunnar.liden@chemeng.lth.se (G. Lidén), akoutinas@aua.gr (A.A. Koutinas), anestisvlysidis@yahoo.com (A. Vlysidis).

http://dx.doi.org/10.1016/j.bej.2016.06.011
1369-703X/

a lot of both academic and industrial attention. The global bio-based production of succinate in 2013 has been estimated to be around 38,000 t, which is equal to the amount derived from petrochemicals [6]. Its market may, however, expand considerably since high volume chemicals, such as polybutylene succinate (PBS), 1,4-butanediol (BDO), tetrahydrofurane (THF) and gamma-butyrolactone (GBL), can be derived from succinic acid and new biobased succinic acid production plants are planned in the coming years [6]. The predicted market growth is as high as 48%, with an estimated market size of 600,000 t per year by 2020 [6]. The current production cost difference between bio-based succinic acid and petroleum derived succinic acid has been estimated to be $ 0.44 per kg ($ 2.94 instead of $ 2.5) [6]. This is a relatively small difference, which may be overcome by a combination of bioprocess and strain improvements. Obviously, changing oil and sugar prices will have a large impact on the price balance as well.

A substantial number of studies have recently been published on the fermentative production of succinic acid by different microorganisms, either natural or genetically modified, from various carbon sources, either in pure form or present in waste streams [7,8]. Two of the most promising natural succinic acid producers are *Actinobacillus succinogenes* and *Basfia succiniciproducens*. The first one was introduced in 1999 by Guettler et al. [9] and has been studied extensively since then, whereas the latter appeared later in 2009. There are therefore still many aspects to be investigated regarding *B. succiniciproducens* [10,11].

The industrial importance of succinate production has triggered interest in development of models to simulate, control and optimise this bioprocess. Lin et al. [12] reported an unstructured model that predicts succinic acid production by *A. succinogenes* cultivated on glucose and wheat hydrolysates. Corona-Gonzales et al. [13] also reported succinic acid production from glucose with *A. succinogenes* ZT-130 and estimated kinetic parameters with glucose as carbon source. The succinic acid production from glycerol was simulated by Vlysidis et al. [14] again using *A. succinogenes*. Vlysidis et al. used a modified *Monod* equation and considered both substrate and product inhibition in order to predict the behavior of the most important variables at various initial glycerol concentrations [14]. A modified *Monod* expression was also proposed by Song et al. [15], who modelled the succinic acid bioprocess from glucose using *Mannheimia succiniciproducens*. The growth of this bacterium was strongly affected by the total organic acid concentration and it completely stopped when the concentration was above 17.23 g/L [15].

This study focusses on the development of simple unstructured models that predict process behavior and succinic acid production by *A. succinogenes* and *B. succiniciproducens* when they are cultivated on a mixture of C5 and C6 sugars, simulating the sugar content of SSL. A modified *Monod* model was used for this purpose, and parameter values were obtained by non-linear regression. A range of small-scale fermentation experiments were carried out, and since end-product inhibition is likely an important factor, microtiter plate based experiments at different initial concentrations of various organic acids were carried out to quantify these effects. To the best of our knowledge, this is the first time that an unstructured model was developed for the succinic acid fermentation process using a xylose-rich carbon source for these two microorganisms. Finally, the validation of the model was implemented in bioreactor cultures.

2. Materials and methods

2.1. Microorganisms and pre-culture medium

Actinobacillus succinogenes 130Z (DSM-22257) and *Basfia succiniciproducens* JF 4016 (DSM-22022) were purchased from the Leibniz Institute DSMZ – German collection of microorganisms and cell cultures. Both microorganisms were preserved in cryopreservation vials at −80 °C containing 50% of glycerol solution. Tryptic soya broth (TSB), 30 g/L, was used as pre-culture medium for inoculum preparation. The medium was placed in 250 mL Duran bottles and sterilized at 121 °C for 20 min. The pre-culture of the two strains was prepared by adding the content of a cryopreservation vial into the sterile conical flasks. Pre-cultures were then incubated at 37 °C, in an orbital shaker at 150 rpm for approximately 12–16 h.

2.2. Fermentation experiments

Different fermentation experiments were carried out in this study in order to study the behavior of the two microorganisms under different conditions. Initially, experiments with different initial organic acid concentration were conducted (see Section 2.1.1) followed by fermentations with different initial substrate concentration (see Section 2.2.2). Finally, fermentations were also implemented in a scaled up system for validation purposes (see Section 2.2.3). All chemicals used in this study were of analytical grade and were purchased from Sigma-Aldrich.

2.2.1. Experimental runs in microplates

Experimental runs evaluating the inhibition of sugar, the product and the by-products were conducted in a microplate reader (Thermo Labsystems Microtiter 96 Plate). For the product and by-product inhibition experiments, the fermentation medium contained per liter: xylose, 10 g; yeast extract, 5 g; $NaHCO_3$, 10 g; $NaH_2PO_4{\cdot}H_2O$, 1.16 g; Na_2HPO_4, 0.31 g; NaCl, 1 g; $MgCl_2{\cdot}6H_2O$, 0.2 g; $CaCl_2{\cdot}2H_2O$, 0.2 g. Different organic acids were added individually in different initial concentrations. Succinic acid (SA), formic acid (FA), acetic acid (AA) and lactic acid (LA) were added in 0–60, 0–20, 0–40 and 0–60 g/L, respectively. Also, mixed organic acids were added in different initial concentrations ranging from 0 to 37 g/L in a ratio (g/g) of SA:FA:AA:LA equal to 1:0.45:0.59:0 for *A. succinogenes* and 1:0.12:0.29:0.22 for *B. succiniciproducens*. For the substrate inhibition experiments, different initial sugar concentration of 10–220 g/L were used with sugar composition of 72.6% xylose, 12.2% galactose, 10.9% glucose, 4.2% mannose, and 0.1% arabinose. This ratio of sugars was chosen as it is the one observed in the SSL represented in this study. The SSL considered is produced by the Sniace Group located in Cantabria, Spain.

For both substrate and product inhibition experiments, initial pH was adjusted to 7 with 0.1 M of NaOH or 7% of HCl in all concentrated solutions before reaching the final volume. All solutions were steamed sterilized at 121 °C for 20 min separately and microplates were sterilized for 1 h under UV light exposure. Inoculum size for both microorganisms was 10% (v/v) in a 200 μL total fermentation volume. To avoid evaporation and to maintain anaerobic conditions 50 μL of mineral oil was added after inoculation. Incubation was carried out at 37 °C and shaking mode was set at 960 rpm for 5 s every 10 min. Experimental values were the average of six replicates. Growth was followed by measuring optical density at 620 nm at 20 min intervals.

2.2.2. Experimental runs in anaerobic Duran bottles

Fermentations in 0.5 L Duran bottles with working volume of 0.25 L were conducted using various concentrations of a mixed sugar solution contained 72.6% of xylose, 12.2% of galactose, 10.9% of glucose, 4.2% of mannose, and 0.1% of arabinose. The fermentation broth was enriched with 5 g/L yeast extract, 1.16 g/L $NaH_2PO_4{\cdot}H_2O$, 0.31 g/L Na_2HPO_4, 1 g/L NaCl, 0.2 g/L $MgCl_2{\cdot}6H_2O$, 0.2 g/L $CaCl_2{\cdot}2H_2O$ and a few drops of antifoam. An equal amount to the total sugar concentration of $MgCO_3$ was also added in each experiment in order to maintain pH at levels of 6.4 – 7.0. The fermentation medium was sterilized at 121 °C for 15 min. Sugar