Valorization of By-Product Streams from the Pulp and Paper Industry for Succinic Acid Production

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ABSTRACT

Spent sulphite liquor (SSL) is a by-product stream generated by the pulp and paper industry employing the sulfite pulping process. SSL contains high quantities of lignosulphonates and various C5 and C6 monosaccharides. The monosaccharides contained in SSL should be utilized for the production of value-added chemicals preferably before the separation of lignosulphonates. Succinic acid is an important platform molecule for the future sustainable chemical industry. Two of the most promising microorganisms (*Actinobacillus succinogenes* and *Basfia succiniciproducens*) for the production of succinic acid were evaluated in batch fermentations under anaerobic conditions. Process optimization, including both fermentation and downstream separation stages, will be conducted in future studies in order to enhance succinic acid production.

Keywords: succinic acid production, bacterial fermentation, spent sulfite liquor

1 INTRODUCTION

Succinic acid (SA) is a 4C molecule, which is currently used in the pharmaceutical industry, as a feed or flavor additive, a surfactant and detergent. The key objective, though, is to expand its current market outlets to commodity chemical production via its utilisation as a building block for the production of a wide range of value-added products, one of which is polybutylsuccinate (PBS); a polymer used in packaging films [1]. The development of integrated biorefineries by restructuring conventional industrial plants, such as the pulp and paper industry, is a central target for achieving sustainable production of chemicals and materials. The utilisation of waste streams derived from traditional industrial sectors for the production of succinic acid leads to the improvement of the economic viability of conventional industrial plants [2].

Although succinic acid is currently produced via petrochemical processing, it is envisaged that fermentative succinic acid production will be established in the future chemical industry. There are several bacteria that produce succinic acid as the main product from various C5 and C6 monosaccharides, namely *Bacteroides ruminicola*, *Bacteroides amylophilus*, *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Basfia succiniciproducens*, *Mannheimia succiniciproducens* and *Escherichia coli*.

Spent Sulfite Liquor (SSL), a waste stream generated from the pulp and paper industry, contains a mixture of sugars resulted from the degradation of hemicellulose contained in wood. Apart from sugars, this by-product also has a very high concentration of lignosulphonates (>400 g/L) as well as other components that can act as inhibitors suppressing bacterial growth, such as acetic acid, methanol and furfural [3]. Lignosulphonates are primarily used as plasticizers for the production of concrete, whereas sugars are currently destroyed during lignosulphonate precipitation after the addition of excess calcium or sodium hydroxide.

In this study, two of the most promising succinic acid producers have been evaluated regarding their ability to consume C5 (xylose and arabinose) and C6 (glucose, galactose and mannose) sugars that are present in SSL. Both strains were also evaluated in preliminary fermentations regarding their tolerance on high concentrations of extracted lignosulphonates (LS) and industrially derived SSL streams.

2 MATERIALS AND METHODS

Both bacterial strains, *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. Bacterial inocula were prepared in Duran bottles within 10-12 h at 37 °C and 170 rpm with media that contained 30 g/L Tryptic Soya Broth (TSB).

Fermentations were carried in 0.5 L Duran bottles containing 0.25 L of synthetic medium with the following composition: 5 g/L yeast extract, 10-35 g/L $MgCO_3$ (depending on the initial sugar concentration), 1.16 g/L $Na_2H_2PO_4 \cdot H_2O$, 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 antifoam. Fermentations were carried out under the following conditions: a) around 10 g/L of single sugar concentration including mannose, xylose, galactose, glycose or arabinose (in the case of xylose, fermentations were also carried out with 20 and 30 g/L initial concentrations); b) 26 - 36 g/L mixed sugar concentrations containing 67% of xylose, 11.5% of galactose, 9% of mannose, 8.5% of glucose and 4% of arabinose; and c) various concentrations of SSL or extracted LS (36 - 73 g-LS/L) with a similar mixed sugar concentration used in the experiment with commercial

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sugars (b). SSL and LS were provided by Green Source SA (Spain). All experiments were carried out under continuous sparging of CO_2 (0.5 vvm) at 37 °C and 170 rpm agitation. Fermentation media were sterilised at 121°C for 20 minutes prior to fermentation. An inoculum of 10 % (v/v) was used in each fermentation.

The growth of the microorganisms was determined by measuring the optical density (OD) at 660 nm (U-2000 Hitachi). To eliminate any excess of $MgCO_3$, each sample was treated with 7 % (v/v) HCl solution. Fermentation samples and SSL were analysed with HPLC using an Aminex HPX-87H column (300mm x 7.8 mm) with an RI detector in order to monitor the concentration of acids (succinic, formic, acetic and lactic acid) and sugars. The temperature of the column was 45 °C and the mobile phase was a 5 mM H_2SO_4 solution with 0.6 mL/min flow rate. Lignosulphonate content of the LS and SSL was measured by the cited method [4].

3 RESULTS

3.1 Single sugar fermentations

Single sugar fermentations with around 10 g/L initial sugar concentration were carried out to assess the ability of *A. succinogenes* and *B. succinicciproducens* to consume different C5 and C6 sugars (Tables 1 and 2). Both microorganisms were able to produce succinic acid as the main product and lactic, formic and acetic acids as by-products. *B. succiniciproducens* was able to consume all five sugars, while *A. succinogenes* did not produce any succinic acid when galactose was used as substrate. *A. succinogenes* achieved higher final succinic acid concentration than *B. succiniciproducens* only when glucose was used as carbon source (4.94 and 4.29 g-SA/L, respectively). In all other cases (xylose, arabinose, mannose and galactose), *B. succiniciproducens* produced higher concentrations of succinic acid (Tables 1 and 2). Moreover, sugar consumption rates were higher in *B. succiniciproducens* fermentations when single sugars were used, apart from glucose in which case *A. succinogenes* demonstrated a higher glucose consumption rate (results not shown). The highest succinic acid production yield for *B. succiniciproducens* was obtained with arabinose (0.62 g-SA/g-sugar consumed), while the lowest was achieved with galactose (0.36 g-SA/g-sugar consumed). On the other hand, *A. succinogenes* achieved the highest yield with glucose (0.61 g-SA/g-sugar consumed) and the lowest with mannose (0.40 g-SA/g-sugar consumed).

Due to the fact that xylose is the main monosaccharide contained in the SSL, fermentations with higher initial concentration of xylose were carried out for both microorganisms. When 20.45 and 28.81 g/L of initial xylose concentration was used, *B. succiniciproducens* produced SA concentrations of 9.67 and 11.52 g-SA/L, respectively, leading to a SA yield of 0.47 g-SA/g-xylose in both cases. In the case of *A. succinogenes*, fermentations on 18.63 g/L and 26.15 g/L intial xylose concentrations resulted in 8.01 and 14.05 g-SA/L, respectively, with SA yields of 0.45 and 0.54 g-SA/g-xylose, respectively.

Sugar	Initial Sugar (g/L)	Residual Sugar (g/L)	Final SA (g/L)	SA yield g-SA/g-Sugar
Xylose	8.37	0.01	3.74	0.45
Xylose	18.63	0.93	8.01	0.45
Xylose	26.15	0.00	14.05	0.54
Glucose	8.15	0.02	4.94	0.61
Galactose	9.10	9.10	0.0	-
Mannose	9.67	0.34	3.73	0.40
Arabinose	8.88	3.15	2.70	0.47

Table 1 Sugar consumption and succinic acid production in single sugar fermentations for *A. succinogenes*.

Sugar	Initial Sugar (g/L)	Residual sugar (g/L)	Final SA (g/L)	SA yield g-SA/g-Sugar
Xylose	9.68	0.03	4.87	0.50
Xylose	20.45	0.01	9.67	0.47
Xylose	28.81	4.43	11.52	0.47
Glucose	9.00	0.02	4.29	0.48
Galactose	9.08	0.00	3.27	0.36
Mannose	8.18	0.00	4.45	0.54
Arabinose	8.52	0.00	5.29	0.62

Table 2 Sugar consumption and succinic acid production in single sugar fermentations for *B. succiniciproducens*.

3.2 Mixed sugar fermentations

A set of fermentations was also carried out using various mixed sugar concentrations with a sugar proportion similar to the one contained in SSL (Figure 1). *B. succiniciproducens* consumed completely all sugars up to an intial sugar concentration of 35.5 g/L. However, *A. succinogenes* did not consume all sugars, as galactose was not consumed by this strain. *B. succiniciproducens* achieved higher succinic acid yield and productivity than *A. succinogenes*. When 27.3 g/L initial sugar concentration was used, *A. succinogenes* produced 11.8 g/L succinic acid with a yield of 0.51 g-SA/g-sugars (the residual sugar concentration was 4 g/L) and a productivity of 0.46 g-SA/L/h. In comparison, *B. succiniciproducens* produced

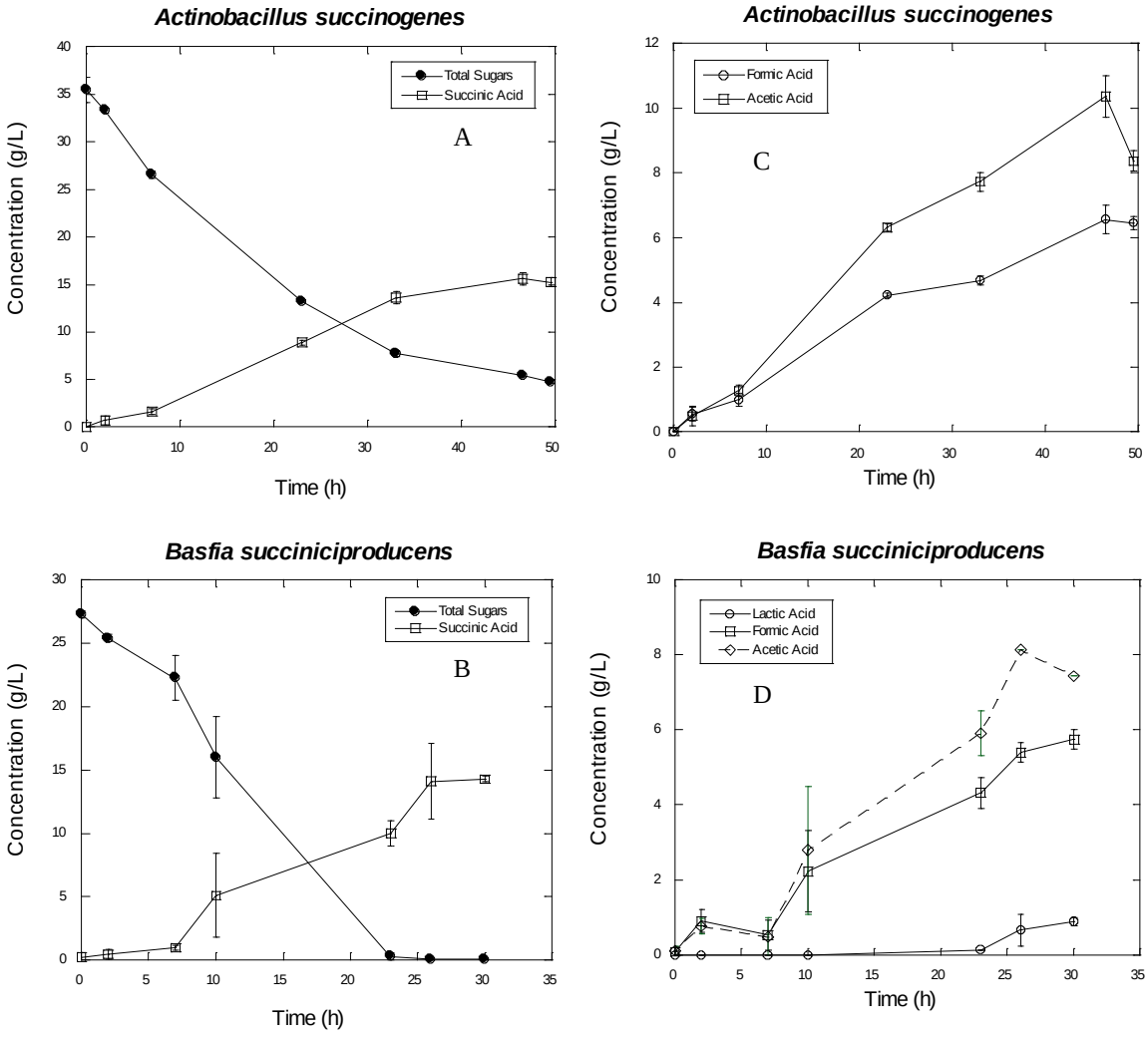

Figure 1: Fermentation Profiles for *A. succinogenes* (A and C) and *B. succiniciproducens* (B and D) in synthetic media with mixed sugars.

14.1 g/L succinic acid with a yield and productivity of 0.55 g-SA/g and 0.47 g-SA/L/h, respectively, when 27.3 g/L of initial sugar concentration was used. In 35.5 g/L initial sugar concentration, *B. succiniciproducens* produced 17.73 g-SA/L succinic acid with 0.5 g-SA/g yield and 0.37 g-SA/L/h productivity, while *A. succinogenes* produced 15.6 g/L succinic acid with 0.5 g-SA/g yield and 0.34 g-SA/L/h productivity. Both microorganisms produced acetic and formic acid, while lactic acid was only produced by *B. succiniciproducens*. However, total by-product formation was lower in the case of *B. succiniciproducens*.

3.3. Fermentations in Lignosulfonates (LS) and Spent Sulfite Liquor (SSL)

Preliminary fermentations with industrially derived LS were conducted in order to evaluate the inhibition in bacterial cell growth and succinic acid production (Table 3). Different initial LS concentrations were used together with mixed sugars and the synthetic medium described in Section 2. The LS content of the crude lignosulphonates was 61.7 % g-LS/g-Dry Matter. Two LS concentrations were employed. Low LS concentration (36.1 g/L) resulted in similar results as in mixed sugars fermentations in the case of *B. succiniciproducens* that produced a high final SA concentration (17.4 g-SA/L). However, the SA concentration (9.03 g/L) produced by *A. succinogenes* was lower than the one achieved (15.6 g/L) in mixed sugar fermentations. In high LS concentrations (72.2 g/L), the inhibition was increased and the succinic acid production decreased to 5.23 g-SA/L in the case of *A. succinogenes* and 3.83 g-SA/L in the case of *B. succiniciproducens*. In the fermentations carried out with industrially extracted LS, SA production yields were higher when *B. succiniciproducens* was used (Table 3). At high LS concentration, *B. succiniciproducens* consumed only 31% of the initial sugars and *A. succinogenes* consumed 37% of the initial sugars.

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Substrate	LS conc. (g-LS/L)	*Actinobacillus succinogenes*				*Basfia succiniciproducens*			
		Initial sugars (g/L)	Residual sugars (g/L)	Final SA concentration (g/L)	Yield (g/g)	Initial sugars (g/L)	Residual sugars (g/L)	Final SA concentration (g/L)	Yield (g/g)
Crude (extracted) LS	36.1	31.22	7.56	9.03	0.38	34.71	3.24	17.40	0.55
	72.2	33.77	21.33	5.23	0.43	32.00	22.68	3.83	0.44
SSL (dilution 1:10)	45.9	28.25	7.19	9.49	0.45	26.73	2.92	6.86	0.29
SSL (dilution 1:7)	65.5	25.72	12.56	6.05	0.46	27.90	19.95	5.31	0.67

Table 3 Fermentation results for *A. succinogenes* and *B. succiniciproducens* cultivated in industrially derived LS and SSL

Preliminary fermentations with industrially derived SSL were also carried out and results are presented in Table 3. The industrially derived SSL contained 458.8 g/L of LS and 176.4 g/L of total sugars. Fermentations were conducted by diluting the initial SSL seven and ten times so as to acquire a LS concentration of 65.5 g-LS/L and 45.9 g-LS/L, respectively. Addition of sugars was carried out so as to acquire an initial total sugar concentration of around 25-30 g/L. The proportion of monosaccharides in the SSL was similar to the proportion used in mixed (commercial) sugar fermentations. From the obtained results, in the fermentations carried out with *A. succinogenes* the final SA concentrations achieved were 6.05 and 9.49 g-SA/L at seven and ten times SSL dilution, respectively. In the case of fermentations carried out with *B. succiniciproducens* with the same SSL dilutions, the final SA concentrations achieved were 5.31 and 6.86 g-SA/L, respectively. *A. succinogenes* demonstrated slightly higher tolerance than *B. succiniciproducens* to inhibitors contained in the SSL.

4 CONCLUSIONS

A. succinogenes and *B. succiniciproducens* are two promising natural SA producing microorganisms that can consume C5 and C6 sugars contained in the SSL for the production of succinic acid. In experiments conducted with synthetic media (without inhibitors) and with low LS concentration, *Basfia succiniciproducens* was a more efficient succinic acid producer than *A. succinogenes*. In high LS concentration (72.2 g/L), LS inhibition is significant in both microorganisms. Moreover, in the case of industrially derived SSL, *A. succinogenes* shows better tolerance towards SSL and produced slightly higher amounts of succinate. It should be mentioned that SSL contain many types of inhibitors including LS, acetic acid, furfural, HMF and methanol among others. The inhibition effect of single inhibitors as well as the synergestic effect of many inhibitors should be a subject of future investigation. The preliminary fermentation results presented in this study indicate that pre-treatment of SSL prior to SA fermentation maybe necessary in order to enhance SA production. Furthermore, adaptation of the microorganisms in the SSL or separation of LS prior to fermentation (e.g. via ultrafiltration) could improve succinic acid production.

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