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Production of succinic acid from *Basfia succiniciproducens* up to the pilot scale from *Arundo donax* hydrolysate

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HIGHLIGHTS

- *B. succiniciproducens* BPP7 has a yield of 0.75 mol/mol of succinic acid on *Arundo donax* hydrolysate.
- Titer, yield and productivity are conserved up to the pilot scale.
- Initial acetic acid concentration in the medium is crucial to improve productivity.
- *B. succiniciproducens* BPP7 adapts to up to 12 g/L acetic acid overcoming inhibition.

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ABSTRACT

In the present work the recently isolated strain *Basfia succiniciproducens* BPP7 was evaluated for the production of succinic acid up to the pilot fermentation scale in separate hydrolysis and fermentation experiments on *Arundo donax*, a non-food dedicated energy crop. An average concentration of about 17 g/L of succinic acid and a yield on consumed sugars of 0.75 mol/mol were obtained demonstrating strain potential for further process improvement. Small scale experiments indicated that the concentration of acetic acid in the medium is crucial to improve productivity; on the other hand, interestingly, short-term (24 h) adaptation to higher acetic acid concentrations, and strain recovery, were also observed.

1. Introduction

Succinic acid was recently indicated from the United States Department of Energy as one of the top value added platform chemicals from biomass with great commercial potential and technical feasibility (US department of energy). This four carbon dicarboxylic acid is employed in the food and pharmaceutical industry, and as a precursor for obtaining a variety of commodity and specialty chemicals (eg. Bio-plastics) (Choi et al., 2015) with an expected market size of over 700,000 tons/year by 2020. This prompted in the last years a race towards developing biotechnological production processes, and, in fact, several companies such as Myriant, Reverdia, BioAmber, and Succinity established bio-based production platforms by exploiting mainly genetically modified microorganisms for the conversion of purified sugars to succinic acid (Becker et al., 2015; Cok et al., 2014).

However, one of the key parameters asserting the sustainability of a biotechnological process is the type of raw materials used, that ideally allow the conversion of harvested waste, such as lignocellulosic biomasses, into value added chemicals. Arguably the biggest challenge and technical hurdle is in fact the required treatment of cellulosic and hemi-cellulosic materials into usable fermentation substrates, as these processes typically also lead to the release of toxic by-products in the medium such as organic acids, furfural, hydroxymethyl furfural (HMF), and other aromatic and phenolic compounds, that can inhibit cell growth.

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Several microorganisms that have succinic acid as end product of metabolic pathways, or use it as metabolic intermediate were evaluated as potential cell factories, and several metabolic engineering strategies were developed to reach high titers of succinic acid, suitable for allowing commercialization (Ahn et al., 2016).

Among natural producers, *Basfia succiniciproducens* recently demonstrated to be an appealing microorganism, although literature on this strain is still quite limited. Scholten et al. (2013) used the wild type to produce 14 g/L of succinic acid in anaerobic 0.3 L batch experiments with a yield on glucose of 0.75 g/g. Shifting to a mixture of glycerol and maltose as C sources an even higher yield of 1.2 g/g was reached on the 1 L scale (Scholten et al., 2013). By applying genetic engineering tools it was also demonstrated that the double knock out strain (Δ*pflD*Δ*ldhA*) resulted in an improved yield of 0.71 g/g on a minimal medium containing glucose and vitamins (Becker et al., 2013). Recently a yield of 0.69 g/g and a productivity of 0.43 g/L·h of succinic acid were obtained by growing *B. succiniciproducens* on corn stover in 0.5 L reactors, indicating indeed that this species is a promising candidate for succinic acid production also from lignocellulosic substrates (Salvachúa et al., 2016a).

In the present paper the growth and productive capacity of the newly isolated *B. succiniciproducens* strain named BPP7 (Ventorino V. et al. Bio-based succinate production from *Arundo donax* hydrolysate with the new wild-type strain *Basfia succiniciproducens* BPP7. Under review) on enzymatically hydrolysed *Arundo donax* as carbon source was evaluated. *A. donax* is a perennial herbaceous dedicated crop previously reported as a promising energy crop for bioethanol production (Angelini et al., 2005). A preliminary characterization of the strain on *A. donax* at different concentrations of complex substrate (and its inherent potentially inhibitory compounds) was performed in 0.25 L bottles to monitor growth, sugar metabolism and succinic acid production. The effect of acetic acid and inhibitors was further studied in small scale experiments to deepen understanding of the metabolism of this rather uncharacterized host. Finally, batch experiments in anaerobic conditions were conducted up to the pilot scale on this complex although inexpensive feed-stock.

2. Materials and methods

2.1. Enzymatic hydrolysis of A. donax

The lignocellulosic biomass, derived from dedicated crops of *A. donax* and steam pretreated according to Garbero et al. (2010), was used for the production of fermentable monosaccharides, such as glucose and xylose, for subsequent fermentation experiments.

The bioconversion process was carried out in a Biostat 50 I bioreactor (B. Braun Biotech International, Germany) at 45 °C, 100 rpm for 72 h. The bioconversion mixture consisted of one liter of acetate buffer per kg of wet *A. donax* biomass with a final concentration of 100 mM, pH 5.2. After sterilization conducted for 1 h at 110 °C the mixture was cooled at 45 °C and the hydrolysis was performed by using the commercial enzymatic cocktail Novozymes NS22201, containing cellulase that hydrolyzes (1,4)-beta-D-glucosidic linkages in cellulose, and other beta glucans (Novozymes, Denmark).

140 U of the enzymatic cocktail were added to 1 g of wet biomass and the units, expressed as units of cellulase activity, were determined utilizing the soluble chromogenic substrate carboxymethyl cellulose-Remazolbrillant Blue R (Azo-CM-cellulose, Megazyme, Ireland), calculated from a standard curve constructed with known amounts of cellulase from *Tricoderma* sp. After bioconversion the mixture was sedimented for 12 h and the clarified hydrolysate was recovered with a peristaltic pump to remove the solid residue. The amounts of glucose and xylose released in the process, expressed as g/L, were determined as described in Section 2.4.

2.2. Shake flasks experiments

All cultivations were conducted in 0.25 L bottles filled with 0.25 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 CO_2 before starting the experiment and after 8, 24 and 36 h of growth. Experiments were conducted on standard MH medium containing per liter: 5 g yeast extract, 10 g soy peptone, 2 g $(NH_4)_2SO_4$, 0.2 g $CaCl_2$ H_2O, 0.2 g $MgCl_2$ $6H_2O$, 2 g NaCl, 3 g K_2HPO_4, 10 g $MgCO_3$, 1 mg Na_2S $9H_2O$, supplemented with glucose and/or xylose as C source.

Acetic acid toxicity tests were performed by adding glacial acetic acid 3, 5, 8 and 12 g/L to MH medium supplemented with both glucose and xylose as C-sources. Growth inhibition experiments were performed by supplementing a mixture of vanillin, HMF, furfural and 4-hydroxic benzoic acid at the concentration found in *A. donax* hydrolysate and ten times higher (vanillin 0.17 ± 0.02 g/L, HMF 0.55 ± 0.07 g/L, furfural 0.4 ± 0.06 g/L, and 4-hydroxic benzoic acid 0.27 ± 0.03 g/L). Glucose and/or xylose were heat sterilized separately and added to the medium before strain inoculation. Cell growth was monitored by removing $MgCO_3$ from the broth according to Becker et al. (2013) in order to avoid interference of this insoluble compound with biomass quantification. In particular the culture broth was diluted ten fold with 1 M HCl and immediately cell concentration was determined as optical density at 600 nm. Samples were withdrawn during the course of the experiment to analyze glucose and xylose consumption and organic acids production.

Small scale experiments on a medium containing MH salts, supplemented with yeast extract (5 g/L) and *A. donax* hydrolysate as carbon source diluted to 90, 50, 33 and 20% were conducted as previously described. Every bottle experiment was repeated at least three times. Data are reported as means ± standard deviations.

The composition of *A.donax* hydrolysate used in the work is the following: glucose 25–37 g/L, Xylose 13–22 g/L, acetic acid 6–10 g/L, HMF 40–70 mg/L, furfural 20–50 mg/L, 4-hydroxibenzoic acid 10–30 mg/L, vanillin 10–30 mg/L.

2.3. Fermentation experiments

Fermentation experiments were performed on a Biostat CT bioreactor (3 L total volume) with a working volume of 2.5 L and on a Biostat D (150 L total volume), working volume of 70 L (Sartorius Stedim; Melsungen, Germany). The seed culture was inoculated with the *B. succiniciproducens* BPP7 working cell bank and grown for 16 ± 1 h in 0.25 L bottles on MH medium with glucose as C-source before performing the main culture in the Biostat CT reactor on *A. donax* based medium. The same procedure was used for larger scale experiments however in this case preculture on Biostat CT was run for 16 ± 1 h on MH medium before inoculating the biostat D fermenter. All main fermentations were carried out at 37 °C on glucose-free MH medium supplemented with 30–50% *A. donax* hydrolysate and run for 48 h. The culture was sparged with CO_2 at 0.5 and 0.1 vvm in the 3 L and 150 L reactors respectively, and agitation speed was set to 100–200 rpm. A constant pH of 6.5 was maintained via automated addition of 30% v/v NH_4OH and 30% v/v H_2SO_4. For the duration of all cultivations 10–50 mL samples were withdrawn from the reactors at regular time intervals for the determination of dry cell weight, substrate consumption and extracellular metabolites production.

2.4. HPLC quantification of sugars, organic acids and inhibitors

Broth samples were collected every two hours during cultivations to follow biomass formation (if possible), substrate consumption and product formation. The supernatants obtained after centrifugation were ultrafiltered on 3KDa centricon devices (Millipore, Bedford, MA, USA) at 5000×*g* and the flow through was analysed for the determination of glucose, xylose and acids produced during growth by HPLC (UHPLC Dionex Ultimate 3000; Thermofisher) on a Alltech IOA-2000 column (150 mm × 6.5 mm ID). Analyses were performed at 40 °C with 0.1% v/v sulphuric acid in water as mobile phase at a flow rate of 0.6 ml/min. Detection was performed via UV absorbance at 200 nm and refraction index (Shodex RI-101 detector, Max auto step 5, 1 s, Temperature 32 °C, Rise time 1 s, Polarity plus, Record Range 512 μRIU, Integrator Range 500 μRIU/UV).

The concentration of potentially inhibitory compounds such as furfural, HMF, 4-hydroxic benzoic acid and vanillin in the *A. donax* hydrolysates was quantified after sample centrifugation to remove solid residues with the protocol developed within this research.

Runs were executed in gradient mode on a Macherey-Nagel Ec 250/4.6 Nucleodur 100-5 C18ec (4.6 × 250 mm, 5 μm) column. The mobile phase consisted of CH_3COOH/H_2O 0.2:99.8 v/v (A) and CH_3OH/ CH_3CN 1:1 v/v (B). Gradient elution was applied as follows: 0–17 min 10% B (1.0 ml/min), 17–18 min 30% B (1.0 ml/min), 18–20 min 50% B (1.0 ml/min), 20–30 min 70% B (0.8 ml/min), 30–35 min 80% B (0.8 ml/min), 35–40 min 80% B (0.6 ml/min) 35–45 min 10% B (1.0 ml/min) and hold on 10% B 5 min for equilibration. Separations were performed at 25 °C, the analytes were detected over the UV spectrum at 280 nm. Peak areas were evaluated through the Thermofisher Chromeleon Software. Standard solutions in the range of 0.0040–0.25 g/L were used to test the linearity, sensitivity and reproducibility of the analytical method. The standard solutions were injected (10 μL) twice and the averaged areas were plotted versus the amount of injected sample to obtain the calibration curves.

3. Results and discussion

3.1. Performance of *B. succiniciproducens* BPP7 on *A. donax* hydrolysate

A first set of experiments was executed with the goal of identifying conditions that would allow optimal growth of *B. succiniciproducens* on *A. donax*. Different concentrations of the hydrolysate were tested in small scale bottle experiments to analyse glucose and xylose consumption, and succinic acid production (Fig. 1). During hydrolytic pretreatments lignocellulosic biomasses normally liberate various sugars and inhibitory compounds such as acetic acid, and phenolic and furan derivatives. Experiments were followed for 48 h to determine initial growth, consumption and production rates, as longer process duration would already heavily impact process economics, and therefore render commercialization much more challenging. As can be seen in Fig. 1 sugar consumption and product formation were clearly delayed in the presence of 90% *A. donax* hydrolysate. In this condition a rather long lag phase was observed before the re-activation of metabolism, and, only 30% of the available sugar was consumed during the first 48 h of growth; this resulted in the lowest productivity that was equal to 0.11 ± 0.01 g/L·h. It should be noted that only with *A. donax* hydrolysate concentrations of 20% all measureable glucose and xylose was consumed within the first 48 h of cultivation, whereas at 33% only glucose was depleted, and on all other concentrations the cultures still contained significant amounts of detectable sugars that had not yet been converted. The highest final titers of succinic acid, of about 7.5–8 g/L, were achieved on 33 and 50% *A.donax* hydrolysate, respectively, and under these conditions also the sugar consumption and succinic acid production rates were quite similar and higher compared to those obtained in the other conditions. Furthermore, on average a 50% higher volumetric productivity (r_{succP}) was found compared to that obtained on 90% *A. donax* (Table 1). It is also interesting to notice that the presence of 90% *A. donax* in the medium had a major impact on the process during the first 24 h of experiment, as demonstrated not only by the reduced sugar consumption but also by the significantly lower yield of succinic acid on the total carbon consumed $Y_{Csucc/C(gluc + xyl)24\ h}$. However, considering the entire process duration ($Y_{Csucc/C(gluc + xyl)48h}$) yields on all diluted *A. donax* media were almost comparable (Table 1). In order to develop an economically sustainable process, the final titer of target molecule and the process time/productivity are nevertheless crucial aspects. In this respect best results were obtained by using the media containing 50 and 33% *A. donax* hydrolysates.

3.2. Resistance of *B. succiniciproducens* BPP7 to acetic acid and potentially inhibitory compounds

It has previously been reported that various hosts engineered to ferment xylose for the production of ethanol were severely affected by the presence of inhibitors in the medium that are released during biomass pretreatment (Palmqvist and Hahn-Hagerdal, 2000; Sonderegger et al., 2004; Takahashi et al., 1999; Klinke et al., 2004). *A. donax* hydrolysates were previously reported to contain acetic acid, that is a product of hemicellulose deacetylation, and also furfural and HMF (Aska et al., 2012). In order to understand the causes of the different performance of *B. succiniciproducens* BPP7 on different dilutions of *A. donax* hydrolysate, 0.25 L experiments were conducted on semi defined MH medium containing

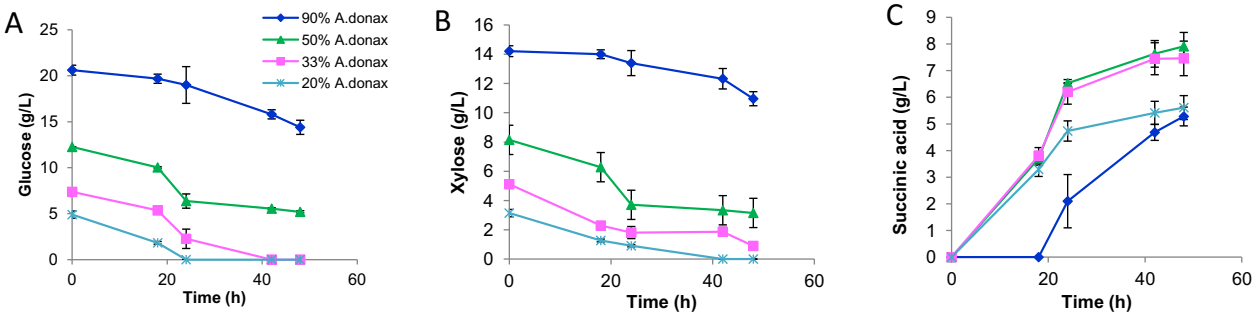

Fig. 1. Performance of *B. succiniciproducens* BPP7 on *A. donax* hydrolysate. Rates for glucose (A) and xylose (B) consumption and succinic acid (C) production in 0.25 L bottle experiments. Different concentrations of *A. donax* hydrolysate (90–50-33–20%) were supplemented with salts and yeast extract. Experiments lasted 48 h and were performed in triplicate.

Table 1
Performance of *B. succiniciproducens* BPP7 on *A. donax* hydrolysate at different concentrations. Y indicates the carbon moles of succinic acid produced on the total carbon moles of glucose and xylose consumed. $r_{succ24h}$ and r_{succP} indicate the volumetric production rate after 24 h, and over the whole process duration, respectively.

A. donax (%)	$Y_{Csucc/C(gluc+xyl)}$ 24h (mol/mol)	$Y_{Csucc/C(gluc+xyl)}$ 48h (mol/mol)	$r_{succ24h}$ (g/L·h)	r_{succP} (g/L·h)	[Acetic acid][i] (g/L)
90	0.35 ± 0.10	0.58 ± 0.05	0.09 ± 0.04	0.11 ± 0.01	8 ± 0.2
50	0.65 ± 0.06	0.70 ± 0.07	0.27 ± 0.00	0.16 ± 0.01	5 ± 0.2
33	0.76 ± 0.14	0.67 ± 0.02	0.26 ± 0.02	0.17 ± 0.02	3 ± 0.1
20	0.69 ± 0.05	0.73 ± 0.06	0.20 ± 0.02	0.13 ± 0.01	2 ± 0.2

[i] indicates the initial concentration of acetic acid in the medium.

glucose and xylose in the same ratios found in the hydrolysate. Two types of experiments were performed, one in which the medium was supplemented with acetic acid, and the other with known hydrolysis-derived inhibitory molecules, more specifically furfural, HMF, vanillin and 4-hydroxic benzoic acid. The final concentration of the latter components in the medium was applied equal and 10-fold higher compared to that found in the hydrolysate reported in Section 2.2.

In these experiments sugar consumption, biomass and succinic acid formation were followed for 53–55 h with starting concentrations of 0, 3, 5 8 and 12 g/L acetic acid (Fig. 2). These concentrations were chosen based on the acetic acid concentrations found in the different dilutions of *A. donax* hydrolysate (see Table 1), and should therefore elucidate if the difference in the cultures with the different concentrations of this complex material are due to the acetic acid concentrations. As shown in the figure (Fig. 2) it seems that in the presence of up to 5 g/L of acetic acid in the medium growth and sugar consumption rates were only slightly affected at the beginning of the experiment; higher concentrations of acid instead had a severe impact on the analyzed parameters in the first 24 h of growth, which is indeed very similar to the results on the *A. donax* hydrolysate, as described above. Moreover, succinic acid production was fully blocked in the presence of 12 g/L of acetic acid in the medium showing a $Y_{Csucc/C(gluc + xyl)}$ at 24 h equal to zero (Table 2). Interestingly, however, challenge with higher concentrations of acetic acid (8–12 g/L) did not decrease the overall process yields (Table 2). These results are very similar to that obtained on diluted *A.donax* containing from 2 to 8 g/L of initial acetic acid, and confirm the ability of *B. succiniciproducens* BPP7 to adapt to relatively high acetic acid concentrations within about 24 h and then almost fully recover its metabolic performance.

In the second set of experiments addition of 1X (mock *A. donax*) and 10X potential inhibitors was evaluated. Growth, sugar consumption and succinic acid production rates were not altered in mock *A. donax*. A lower growth rate was observed in the first 4 h of growth in the presence of 10X inhibitors; this was also accompanied by 41 and 44% lower glucose consumption and succinic acid production rates over the first 8 h of growth. However, overall succinic acid yield and productivity were not affected (data not shown). These results also suggest that the concentration of inhibitors found in 90% *A. donax* is not responsible for the reduced $r_{succ24h}$.

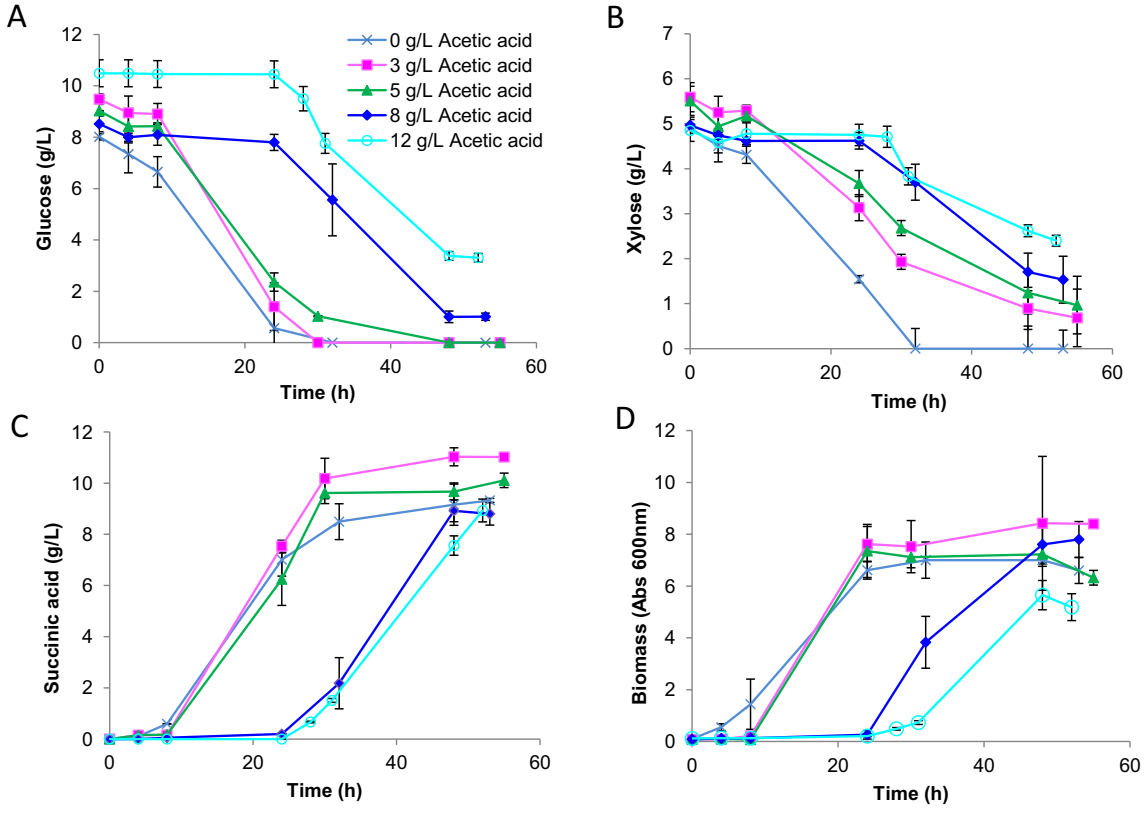

Fig. 2. Effect of increasing concentrations of acetic acidon growthof *B. succiniciproducens* BPP7. Rates for glucose (A) and xylose (B) consumption and succinic acid (C) and biomass (D) production in 0.25 L bottle experiments on semi defined MH medium. Data are mean ± S.D. values of three independent experiments.

Table 2
Acetic acid toxicity tests. Experiments were conducted on MH standard medium containing glucose and xylose, supplemented with acetic acid. The experiments lasted 53–55 h. Y indicates the carbon moles of succinic acid produced on the total carbon moles of consumed glucose and xylose. $r_{succ24h}$ indicates the volumetric production rate after 24 h of growth.

Acetic acid (g/L)	$Y_{Csucc/C(gluc+xyl)24h}$ (mol/mol)	$Y_{Csucc/C(gluc+xyl)53/55h}$ (mol/mol)	$r_{succ24h}$ (g/L·h)
0	0.75 ± 0.19	0.77 ± 0.02	0.292 ± 0.026
3	0.71 ± 0.02	0.85 ± 0.03	0.313 ± 0.009
5	0.77 ± 0.14	0.78 ± 0.05	0.260 ± 0.004
8	0.19 ± 0.03	0.84 ± 0.07	0.008 ± 0.001
12	0	0.95 ± 0.14	0

In both conditions, mock and 10X, furfural disappeared after 24 h of growth, whereas a residue of HMF, about 7.5 and 15% respectively, was found in the treated cultures at the end of the experiments (48 h). This result is in accordance with the recent finding that, as *Actinobacillus succinogenes,* also *B. succiniciproducens* CCUG 57335 has the ability to detoxify furfural and HMF present in lignocellulosic biomasses (Salvachúa et al., 2016a,b) by reducing them to the corresponding alcohols. Also vanillin decreased throughout growth, in fact 45 and 50% of the initial concentrations were found in the mock and 10X broths, respectively, after 48 h. This could imply the ability of *B. succiniciproducens* to convert vanillin into vanillyl alcohol, as observed for *S. cerevisiae* (Ishida et al., 2016). The concentrations of 4-hydroxibenzoic acid remained constant throughout the experiments, most likely indicating no metabolic activity around this compound.

Overall, our results demonstrate that the concentration of acetic acid initially present in the growth medium is critical to increase process efficiency and that in order to have higher productivities lower initial concentrations of 3 to 5 g/L of acetic acid in the medium are necessary. This might also be useful and interesting to simplify recovery and purification procedures.

3.3. Performance of *B. succiniciproducens* BPP7 on *A. donax* hydrolysates in batch fermentations on the 3 L and 150 L scales

The key question for every biotechnological production process during its development is its scalability to larger scales, and this was the goal of yet another set of experiments.

Based on data reported in the previous section, 2 to 3-fold diluted *A. donax* hydrolysates were used for controlled batch fermentation experiments with pH correction to maintain a set-point of 6.5 and with constant sparging of CO_2. Experiments were initially performed on 3 L reactors and as shown in Fig. 3 glucose was completely exhausted after 48 h of growth, indicating a faster consumption rate compared to that obtained in small scale bottle experiments. Xylose consumption was slower, compared to glucose, resulting in a residual concentration of about 2 g/L at the end of the process. Succinic acid reached on average a final titer of about 17 g/L and acetic, formic and lactic acid were the major organic acid by products, whereas no ethanol was secreted in the medium (data not shown). The process was subsequently also scaled to a 150 L reactor ensuing highly similar results, and thereby also demonstrating fermentation reproducibility over different scales (Fig. 3). Overall from 0.25 L bottle experiments to pilot-scale fermentations a slight improvement of the yield of succinic acid on total carbon consumed up to 0.75 (mol/mol) was obtained and, furthermore, it is very interesting to notice that a 100% improvement of the volumetric productivity was achieved in these well controlled batch fermentations reaching a value that was equal to 0.35 g/L·h. This enhancement could be attributed to two factors: the control of pH and/or the constant sparging of CO_2.

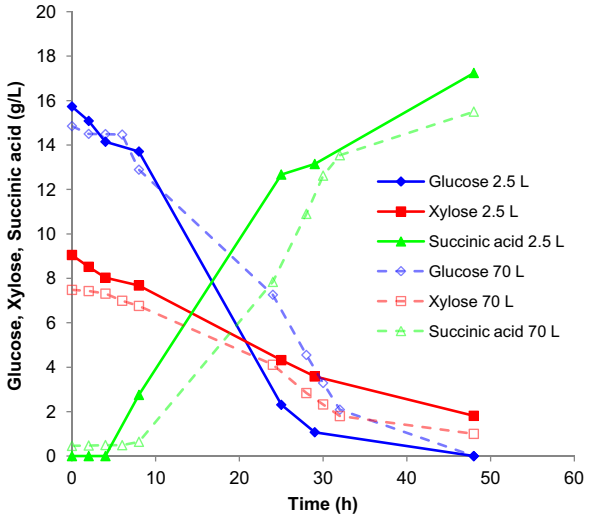

Fig. 3. Production of succinic acid from *B. succiniciproducens* growing in batch on 3 and 150 L scales. Fermentation profiles indicate glucose and xylose consumption and succinic acid production on *A. donax* hydrolysate during 48 h. The legend indicates the working volume on the two scales. Experiments on the 3 L scale were performed in triplicate with a deviation below 10%.

Although it is emerging as an extremely attractive host, only few studies report the use of *B. succiniciproducens* for the production of succinic acid, and they are mainly based on the use of purified sugars (Becker et al., 2013; Kuhnert et al., 2010; Scholten and Dägele, 2008; Scholten et al., 2009). It is however important to develop processes that use non-edible organic raw materials to develop economically viable biotechnological processes and in this respect the performance of other microorganisms was evaluated. In particular, *Mannheimia succiniciproducens* MBEL55E produced 13.5 g/L and 16 g/L of succinic acid by growing either on a whey based medium supplemented with corn steep liquor, or on oak wood hydrolysate, in 1 L batch fermentations (Lee et al., 2003; Kim et al., 2004). Process efficiency was further improved by using fed-batch fermentations. *Anaerobiospirillum succiniciproducens* growing on whey under anaerobic conditions reached 34.7 g/L of succinic acid with a yield of 0.91 g/g (Lee et al., 2003, 2008; Samuelov et al., 1999). *Actinobacillus succinogenes* synthesized 53.2 g/L of target product from corn straw hydrolysates in fed-batch fermentations at a rate of 1.21 g/L·h (Zheng et al., 2009).

The same strain produced 19 g/L of succinic acid and showed an even higher productivity of 1.67 g/L·h growing on carob pods in fed-batch processes (Carvalho et al., 2016).

Salvachúa et al. (2016b) for the first time described the growth of *B. succiniciproducens* CCUG57335 on a lignocellulosic food-crop, corn stover in particular. Although 30 g/L of succinic acid and a yield of 0.69 g/g were achieved controlled batch experiments were only performed by the authors on the 0.5 L scale.

The successful scaling of the process up to the pilot scale is herein reported, which is a critical milestone for the development of any biotechnological production process, and an important step towards industrial applications. As demonstrated this scale-up also brings a slight yield improvement even by using a dedicated non-food crop (*A. donax*) for growing the new isolate *B. succiniciproducens* BPP7.

4. Conclusion

Succinic acid production from *B. succiniciproducens* BPP7 on *A. donax* was evaluated for the first time. Testing known potentially

inhibitory molecules showed that acetic acid is significantly impacting process performance and that a low initial concentration is key to decrease process time. However, even with higher acetic acid concentrations a "short-term" (24 h) adaptation mechanism, that results in comparable succinic acid yields, was observed. Finally, scalability from laboratory to pilot-scale of an optimized batch process was demonstrated.

A. donax based fedbatch processes could next be developed to reach industrially sustainable results.

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DC and CS conceived the work; DC wrote the manuscript; OA and SD performed bottle and fermentation experiments; LL and IF perfomed *A. donax* hydrolysis; RF developed the HPLC method for the analysis of inhibitory compounds; OP provided the strain; CF provided the protocol for hydrolysis.

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