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Optically pure lactic acid production from softwood-derived mannose by *Pediococcus acidilactici*

Joana Campos [a], Jie Bao [b], Gunnar Lidén [a,*]

[a] Department of Chemical Engineering, Lund University, PO Box 124, 221 00, Lund, Sweden
[b] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, 200237, China

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ABSTRACT

Softwood is of interest as a renewable carbon source for production of lactic acid. Softwood hydrolysate contains a high content of mannose. Lactic acid production from mannose by two modified strains, L-lactic acid producing *Pediococcus acidilactici* TY112 and D-lactic acid producing ZP26, was investigated in the current work. The two strains efficiently converted mannose to L- and D-lactate isomers with an optical purity exceeding 99 %, although the mannose utilization rates were lower than the glucose utilization rates. The mannose conversion to L- and D-lactic acids by *P. acidilactici* was also confirmed in dilute spruce hemicellulose hydrolysate. The present study provides important knowledge on utilization of the spectrum of fermentable sugars in softwood for future production of chiral lactic acid from lignocellulose feedstocks.

1. Introduction

Polylactic acid (PLA) is a biodegradable, biobased and biocompatible polymer capable of substituting petroleum-based polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET) in the manufacture of fibers, foams, nonwoven fabrics, packaging, and films (Cubas-Cano et al., 2018; E4tech and Wur, 2015; Eş et al., 2018; Hatti-Kaul et al., 2018). PLA production requires high chiral purity monomers, D- and L-lactic acid, to obtain the high molecular weight PLA of poly-L-lactic acid (PLLA) or poly-D-lactic acid (PLDA). When a racemic mixture of L- and D-lactic acid is used for polymerization, the PLA material formed is amorphous and unstable (Djukić-Vuković et al., 2019). The current microbial lactic acid production is based on corn starch or sugar from sugar cane as substrates (Hatti-Kaul et al., 2018). This scenario is non-optimal if a larger fraction of the very high-volume production of petroleum-based polymers is to be replaced, due to the potential competition for food use of the starch or sugar feedstock available. Feedstock costs and availability are some of the challenges for the expansion of production of PLA and other biobased polymers. Among non-food substrates, lignocellulosic biomass, a readily available and abundant feedstock, is an interesting alternative (Cubas-Cano et al., 2018; Qiu et al., 2018). In regions like Scandinavia, Canada and Russia, softwood forest materials are widely available. In Sweden, about 70 % of the land area is covered by forest, the main part of which by the softwoods spruce (42 % of the forest) and pine (39 %) (Swedish Wood, 2011). Softwood is composed of a large fraction of carbohydrates, typically approximately 40 % glucans, 10 % mannans, 6% xylan, as well as minor fractions of arabinan and galactan (Frankó et al., 2015). While glucose and xylose, which are two of the major sugars present in hemicellulose from hardwoods and agricultural residues, have been investigated for lactic acid fermentation (Qiu et al., 2018,2017), softwood-derived mannose utilization by lactic acid bacteria has been less studied.

Pediococcus acidilactici is a typical lactic acid bacterium, with a high tolerance to environmental stresses like temperature, pH, and inhibitory compounds, which makes it an interesting microbe for industrial application (Porto et al., 2017). *P. acidilactici* DQ2 was found to efficiently use corn stover hydrolysate resulting in a high titer of lactic acid production (Zhao et al., 2013) with high temperature tolerance and tolerance to inhibitors e.g. 2-furaldehyde (furfural), 5-(hydroxymethyl) furfural (HMF), acetate and 4-hydroxybenzaldehyde. By knockout of the *ldhD* gene (encoding D-lactate dehydrogenase) or the *ldh* gene (encoding L-lactate dehydrogenase), the engineered *P. acidilactici* TY112 and *P. acidilactici* ZP26 were capable of producing optically pure L-lactic acid and optically pure D-lactate, respectively (Yi et al., 2016). In a recent comprehensive comparison of lactic production from many types of starch, as well as agricultural lignocellulosic feedstocks, using different production hosts (supplemental Table 1 in Djukić-Vuković et al. (2019)),

* Corresponding author.
E-mail address: gunnar.liden@chemeng.lth.se (G. Lidén).

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titers of lactic acid from a batch fermentation of corn stover reported for *P. acidilactici* (97 g/L) were among the highest reported for a lignocellulosic feedstock. The fact that the same host organism has been engineered to give either D- or L-lactic acid can give an advantage for industrial production, since the engineered strains will show similar behavior during cultivation.

The objective of the current work was to assess the feasibility of mannose utilization by engineered *P. acidilactici* strains for production of optically pure lactic acid. The conversion of mannose and glucose/mannose mixtures was examined in both shake flasks and well-controlled bioreactors on synthetic medium supplemented with the sugars and spruce hemicellulose hydrolysate, which contains the main part of the mannose content of the wood as well as inhibitors. The remaining solid fraction was not investigated in the current work. A preliminary assessment on mannose utilization for optically pure lactic acid production was conducted and interpreted in terms of the metabolic pathways used in the conversion.

2. Materials and methods

2.1. Microorganisms

The strains *Pediococcus acidilactici* TY112 and *Pediococcus acidilactici* ZP26 (Chinese General Microorganisms Collection Center (CGMCC), registration numbers 8664 and 8665, respectively) were used in the study. These strains are derived from the wild-type *P. acidilactici* DQ2 – isolated from corn stover slurry (Zhao et al., 2013) – and *ldh* or *ldhD* have been separately knocked-out resulting in strains producing either L-lactic acid (*P. acidilactici* TY112) or D-lactic acid (*P. acidilactici* ZP26) (Yi et al., 2016).

2.2. Seed culture

P. acidilactici strains were grown on a simplified Man-Rogosa-Sharp (MRS) medium containing 10.0 g of peptone, 10.0 g of yeast extract, 2.0 g of ammonium citrate dibasic, 5.0 g of sodium acetate, 0.58 g of magnesium sulfate heptahydrate, 2.0 g of dipotassium phosphate, 0.25 g of manganese sulfate monohydrate in 1 L demineralized water. Solutions with 250 $g{\cdot}L^{-1}$ glucose and mannose were prepared in demineralized water, autoclaved, and supplemented to the desired sugar concentration to the simplified MRS medium in sterile conditions. Petri dish agar was prepared with a MRS Agar formulation from Merck. All the media and water used were autoclaved at 121 °C for 20 min.

One single colony from each strain was inoculated into 50 mL of simplified MRS medium supplemented with 10 $g{\cdot}L^{-1}$ glucose in a 250 mL shake flask. It was grown at 42 °C, 150 rpm for 12 h. These culture broths were frozen in 1.5 mL stocks at -80 °C in 15 % glycerol. One stock vial was inoculated and grown at the same conditions for each activation and seed culture.

2.3. Softwood pretreatment

Spruce material, *Picea abies*, kindly provided by a local sawmill, was partially debarked and chipped to pieces between 2 and 10 mm. The chips had a dry matter content of 50 % and were stored at 4 °C until further use. The spruce chips were impregnated with 2.5 % w/w SO_2, based on the water content of the softwood, in tightly sealed plastic containers for 20 min at room temperature. Afterwards the mixture was left to evaporate for 30 min and then subjected to steam pretreatment. Steam pretreatment was performed at 210 °C for 5 min in a 10 L reactor (Process- & Industriteknik AB, Kristianstad, Sweden) (Palmqvist et al., 1996), as described previously by Frankó et al. (Frankó et al., 2019). The liquid fraction, primarily containing hydrolysed hemicellulose, was separated from the solid fraction on a filter press (HP25 M, Fischer Maschinenfabrik GmbH) (Nielsen et al., 2020), with a yield of 52 % (w/w) on the steam pretreated feedstock total weight. This material is here referred to as "hydrolysate" and was stored at 4 °C until further use. Its composition was analyzed, and the main compounds are listed in Table 1.

2.4. Shake flask experiments using defined carbon source and softwood hydrolysate

The cultures were carried out in 50 mL flasks. The medium contained the simplified MRS medium ingredients as described above, replacing 20 % of the volume of demineralized water for softwood hydrolysate. The pH of the medium was adjusted to 6.5 with NaOH 2 M and sterilized by filtration with 0.2 μm syringe filters. The medium was inoculated with 10 % (v/v) inoculum for both strains, in duplicates, at 42 °C, 150 rpm for 12 h.

Cultivations were made in a 300 mL E-flasks, containing 50 mL medium at 10 % (v/v) of inoculation, at 42 °C, 150 rpm for 12 h. The flasks were equipped with rubber stoppers with glycerol traps to minimize oxygenation. Either 20 $g{\cdot}L^{-1}$ glucose, 20 $g{\cdot}L^{-1}$ mannose or a mixture of 10 $g{\cdot}L^{-1}$ glucose and 10 $g{\cdot}L^{-1}$ mannose was used as carbon sources. The samples were withdrawn every two hours for the measurement of the cell growth, pH, sugar consumption, and lactic acid production. To enable growth to be followed over the entire cultivation time of 24 h, two flasks for each strain and growth condition were prepared as follows. A first shake flask was inoculated early in the day to collect representative samples of the first 12 h. At 12 h, another flask was inoculated, but its sampling only started 12 h after, giving samples of the time interval between 12 and 24 h of cellular growth. This method led to double sampling at 12 h. All experiments were made in duplicates.

2.5. Bioreactor experiments using defined medium and softwood hydrolysate

Cultivations were made in 2 L Biostat CT bioreactors (B. Braun International, Melsungen, Germany) with a working volume of 1.0 L at 42 °C, stirring rate 100 rpm with no aeration. pH was maintained at 5.5 by automatic titration using a 2 M NaOH solution. Experiments were made in duplicates. All materials and solutions were sterilized at 121 °C for 20 min. The sugar solution was autoclaved separately and added to the reactor according to the desired concentration. Cultivations were carried out using either 40 $g{\cdot}L^{-1}$ glucose, 40 $g{\cdot}L^{-1}$ mannose or a mixture of 20 $g{\cdot}L^{-1}$ glucose and 20 $g{\cdot}L^{-1}$ mannose. The whole content (47 mL) of the seed culture was centrifuged for 15 min at 5,300×g, resuspended in 10 mL of simplified MRS medium and added to the bioreactor in sterile conditions. Samples were regularly withdrawn for the measurement of the cell growth, pH, sugar consumption, and lactic acid production. The last sample of the bioreactors grown only on mannose was subjected to hydrolysis by amyloglucosidase to possibly indicate the presence of EPS, as described below.

One cultivation was made for each strain in which 5 mL (0.5 % v/v) amyloglucosidase from *Aspergillus niger* (≥ 260 $U{\cdot}mL^{-1}$, aqueous solution) (Sigma-Aldrich) was added. This has previously been reported to prevent cell flocculation for these strains (Qiu et al., 2018). The enzyme

Table 1
Monomeric sugar and inhibitor profile in the hydrolysate.

	Concentration in the hydrolysate ($g{\cdot}L^{-1}$)
Arabinose	6.1
Xylose	9.2
Galactose	7.8
Glucose	24.1
Mannose	29.4
Acetic Acid	6.8
Formic Acid	1.4
Levulinic Acid	1.0
HMF	3.4
Furfural	1.1

solution was filtrated through a 0.2 μm pore sterile syringe filter into the reactor, before inoculation.

Cultivations were carried out in the same conditions as mentioned above for the defined medium. However, instead of using refined sugars as carbon source, 20 % of the total volume was softwood hydrolysate (see Section 2.3). The addition of the complex carbon source led to an increase in the optical density (OD) of the medium, that was subtracted to the values obtained for each sample. The experiments with hydrolysate were made in duplicates.

2.6. Enzymatic hydrolysis

Enzymatic hydrolysis was performed with amyloglucosidase from *Aspergillus niger* ($\geq$ 260 U mL^{-1}, aqueous solution, from Sigma-Aldrich) at 1% (v/v). The supernatant and biomass re-suspended in NaCl 0.9 % (w/v) were submitted to 1 h enzymatic hydrolysis on a rocking platform (VWR) at 50 rpm, inside an incubator (Kuhner LT-X) at 42 °C. These were obtained by centrifugation for 15 min at 5300×g of the last samples of a bioreactor cultivation, for each strain, and made in duplicate. The biomass was washed with NaCl 0.9 % and afterwards with demineralized water, to be resuspended in NaCl 0.9 % again in order to decrease cytolysis. Samples were collected before the addition of enzyme and after the incubation period to quantify mannose, glucose and lactic acid. This last one provides information on any biomass sugar consumption for the production of acid. The sugar content of the enzyme cocktail was quantified by HPLC and subtracted to the sugar content of each sample.

2.7. Analyses

Optical density was measured at 600 nm using a spectrophotometer (VWR, V-1200) and the samples diluted with 0.9 % NaCl to get an OD reading below 0.5. Dry cell weight (DCW) (g/L) was determined at the end of each shake flask experiment and in some samples in the bioreactor fermentations, in duplicates. DCW was determined by centrifuging samples at 12,000×g for 3 min. The precipitated cells were washed once with 0.9 % NaCl solution and once with demineralized water, both followed by centrifugation. The cells were then resuspended in demineralized water before being transferred to pre-dried, pre-weighed 5 mL glass test tubes. The test tubes were dried in a convection oven (Termaks, Bergen, Norway) at 105 °C for 24 h after which they were placed in a desiccator to cool before weighing. An OD_{600} of 1.0 corresponded to a DCW of 0.62 $g{\cdot}L^{-1}$.

Samples were centrifuged at 12,000×g for 3 min, filtered with 0.2 μm syringe filters and stored at −20 °C before being analyzed for monosaccharides and organic acids on an HPLC (Waters, Milford, MA, USA). The HPLC system consisted of an isocratic solvent pump (Waters 1515), a thermostated autosampler (Waters 717 plus), a column oven (Waters Column Heater Module), a UV detector (Waters 2487) and an RI detector (Waters 2410). Monosaccharides were analyzed on a Shodex SP0810 (Showa Denko America, Inc., New York, NY, USA) column at 80 °C with 1.0 $mL{\cdot}min^{-1}$ flow rate of distilled water as mobile phase. Organic acids, hydroxymethilfurfural (HMF) and furfural were analyzed on an Aminex HPX-87H (BIO-RAD, Hercules, CA, USA) column at 60 °C with 0.6 $mL{\cdot}min^{-1}$ flow rate of 0.005 M sulphuric acid in demineralized water as mobile phase. Lactic acid isomers titers were analyzed on a Supelco Astec CLC-D (Merck, Darmstadt, Germany) column at room temperature with 1.0 $mL{\cdot}min^{-1}$ flow rate of 5 mM copper sulphate in demineralized water as mobile phase.

2.8. Calculations

Volumetric glucose and mannose consumption rates ($g{\cdot}L^{-1}\ h^{-1}$) in batch shake flasks cultures were calculated from measured concentrations after 12 h of cultivation. In the bioreactors studies, volumetric consumption rates were calculated at 24 h, in $g{\cdot}L^{-1}\ h^{-1}$, or at full consumption in the case of it being depleted before the 24 h interval. Lactic acid production yields were calculated with total lactic acid production by total sugar consumption, in $g{\cdot}g^{-1}$.

3. Results and discussion

3.1. Evaluation of mannose utilization for optically pure lactic acid production

The conversion of mannose was tested in defined sugar medium in shake flask cultivations of the two strains of *P. acidilactici*, TY112 and ZP26 (Fig. 1). Both strains were able to consume mannose as pure mannose or mixed with glucose. Lactic acid yields were similar for the two strains and the lactic acid isomers were obtained at very high purity (>99 %). The consumption rates of mannose were similar for both strains, but slightly lower than that of glucose consumption. The lactic acid generation rates from mannose were also slightly lower than that from glucose for both strains. Previous studies on some *Pediococcus* species, other than *P. acidilactici*, have shown ability of these to produce lactic acid from mannose (Porto et al., 2017). This study confirmed that the engineered *P. acidilactici* strains were capable of utilizing mannose for production of optically pure lactic acid.

In shake flasks using a mixture of mannose and glucose, lactic acid accumulated continuously until the complete consumption of glucose. Flocculation of the cell mass occurred from 6 h of cultivation, likely as a result of the presence of EPS in the medium as well as attached to the cell mass, in agreement with the previous knowledge of exopolysaccharide formation by *Pediococcus* (Song et al., 2013; Yasutake et al., 2016).

Shake flask cultivations were unable to maintain a constant pH value during the lactic acid production and in order to exclude the influence of pH on the fermentation, mannose conversion by the two *P. acidilactici* strains was also studied in bioreactors with the pH value controlled at 5.5 (Fig. 2). When both mannose and glucose were used as carbon sources, the uptake rates in the bioreactor (Table 2) were in most cases notably higher than those obtained in the shake flasks. In pure mannose cultivation, the carbon uptake rate increased for both strains from approximately 0.5 $g{\cdot}L^{-1}\ h^{-1}$ in the shake flasks to 0.9 $g{\cdot}L^{-1}\ h^{-1}$ in the bioreactors. Comparatively with the bioreactors grown with the mixture, mannose uptake rate was closer to the values obtained in the shake flasks with the single sugar (0.5 $g{\cdot}L^{-1}\ h^{-1}$), while glucose was consumed at a much higher rate (approximately 1.8 $g{\cdot}L^{-1}\ h^{-1}$). Mannose and glucose were co-consumed in the mixed sugar medium, in which glucose showed a faster and stable consumption rate until complete exhaustion whereas the mannose consumption rate decreased after the cell growth ceased. The maximum cell mass concentrations, 2.5 $g{\cdot}L^{-1}$ for *P. acidilactici* TY112 and 2.1 $g{\cdot}L^{-1}$ for *P. acidilactici* ZP26, were obtained in the glucose/mannose mixture.

Based on the annotated genome of *P. acidilactici* ZPA017 (Liu and Ji, 2016), a putative metabolic pathway for mannose conversion in *P. acidilactici* TY112 and *P. acidilactici* ZP26 was proposed (Fig. 3). Identified and tentatively annotated sequences support that glucose and mannose are both actively transported into the cell by the phosphotransferase systems (PTS) and phosphorylated as the first step for their incorporation in the Embden-Meyerhof-Parnas (EMP) pathway. From pyruvate, both lactic acid isomers can be formed by isomer-specific lactate dehydrogenases. There are identified genes encoding PTS specific for glucose transport, and in addition genes for both mannose-specific and non-specific PTS (for fructose and sorbose) have been suggested (Liu and Ji, 2016). The regulation of the transport systems is not known for the *P. acidilactici* strains in the present study. The lower consumption rate of mannose than glucose in the studied strains of *P. acidilactici* could be due either directly to activity of the PTS system, or the capacity of the isomerase step.

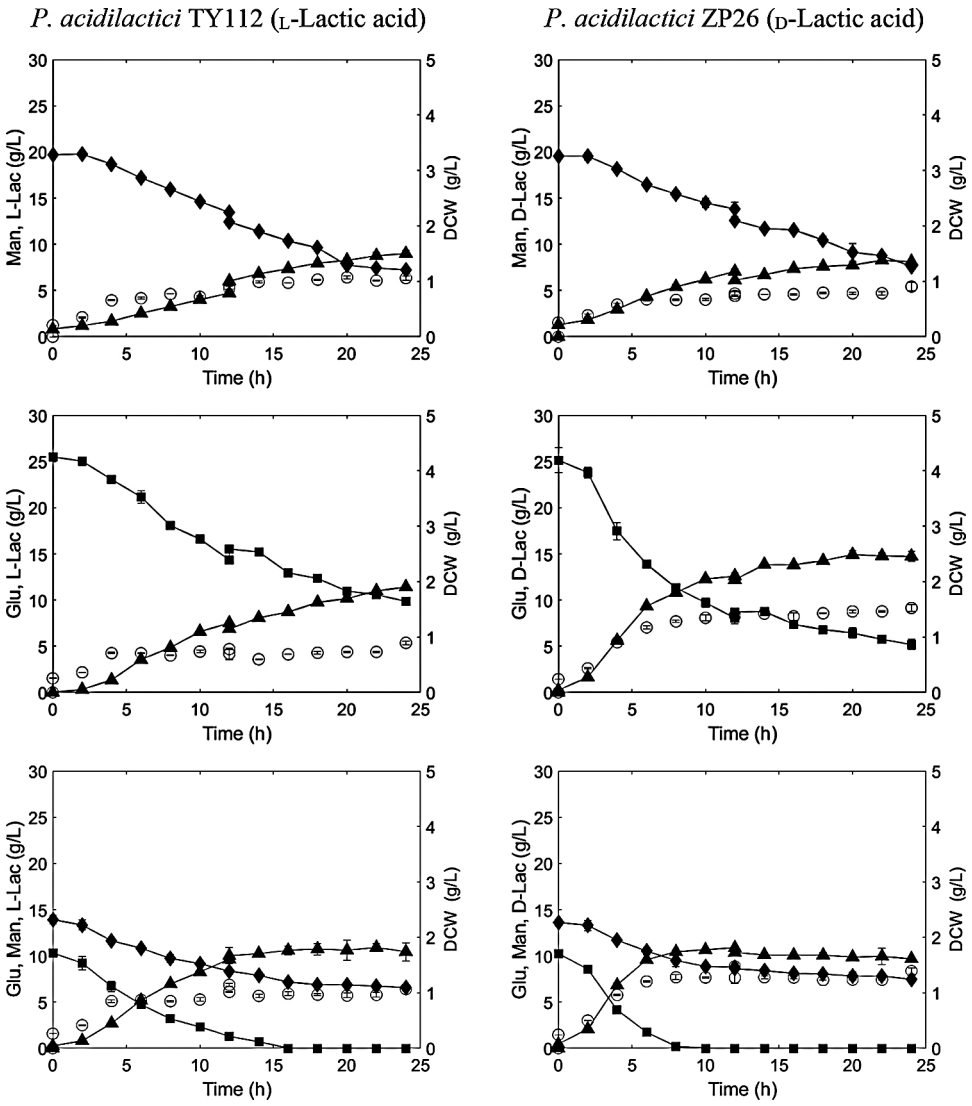

Fig. 1. Batch growth in shake flasks of *P. acidilactici* TY112 and *P. acidilactici* ZP26 in simplified MRS medium with glucose, mannose and an equal mixture of glucose and mannose. DCW (○), mannose (⧫), glucose (■), lactic acid (▲).

3.2. Flocculation of P. acidilactici during mannose conversion to optically pure lactic acid

As mentioned above, determination of biomass concentration was a challenge in both shake flask and bioreactor experiments because of the formation of agglomerates. The biomass agglomerates where larger and had a bigger impact on the DCW and OD determination in the bioreactor experiments, where growth was seen also on the impeller blades and shaft. In a previous study on the same strains of *P. acidilactici* (Liu et al., 2015; Qiu et al., 2018, 2017), amyloglucosidase was added to decrease sugar polymerization that might lead to these agglomerates. Also in the current work, it was confirmed that the variation in biomass determination could be decreased by this method. There was no visible formation of the previously observed agglomerates and the reproducibility of OD and biomass dry weight determinations was improved (Fig. 4). The biomass concentration was also slightly higher than the ones in the reactors without enzyme, but sugar or lactate concentrations were not markedly affected.

In the present work, a test was made using amyloglucosidase (Qiu et al., 2018, 2017) on the final sample from mannose cultivations in bioreactors. Enzymatic hydrolysis of the supernatant resulted in 0.4 $g{\cdot}L^{-1}$ of glucose and 1.51 $g{\cdot}L^{-1}$ of mannose for *P. acidilactici* TY112, and in 0.5 $g{\cdot}L^{-1}$ of glucose and 1.69 $g{\cdot}L^{-1}$ of mannose for *P. acidilactici* ZP26. Together with the difficulty of filtration of the broth for sample treatment, these data confirm that a fraction of the consumed sugars is directed to the production of EPS. Glucose-6-phosphate isomerase can catalyze reversible isomerization, leading to partial conversion of the mannose content to glucose, for it to be possibly assembled into a heteropolymer. To test if the agglomeration of biomass originated from an EPS coating of the biomass, enzymatic hydrolysis of the washed biomass was made. No monomeric sugars were detected in the biomass resuspension solution prior to the addition of the enzyme cocktail. After 1 h, there was an increase on the mannose content that corresponded to up to 30 % of the dry mass content submitted to the hydrolysis.

For the understanding of the influence of the carbon source in the EPS formation, and its influence in the cultivation and lactic acid formation, a more detailed investigation is necessary. There is a wide range of EPSs produced by lactic acid bacteria, which can have many applications and have very complex levels of regulation (Song et al., 2013; Zhou et al., 2019). There are previous reports on exopolysaccharide formation by *Pediococcus* (Panthavee et al., 2017; Song et al., 2013; Yasutake et al., 2016), and it is possible that the flocculation is caused by EPS both attached to the cell wall and excreted to the culture broth. The *P. acidilactici* strain ZPA017, has been reported to have the sequences for

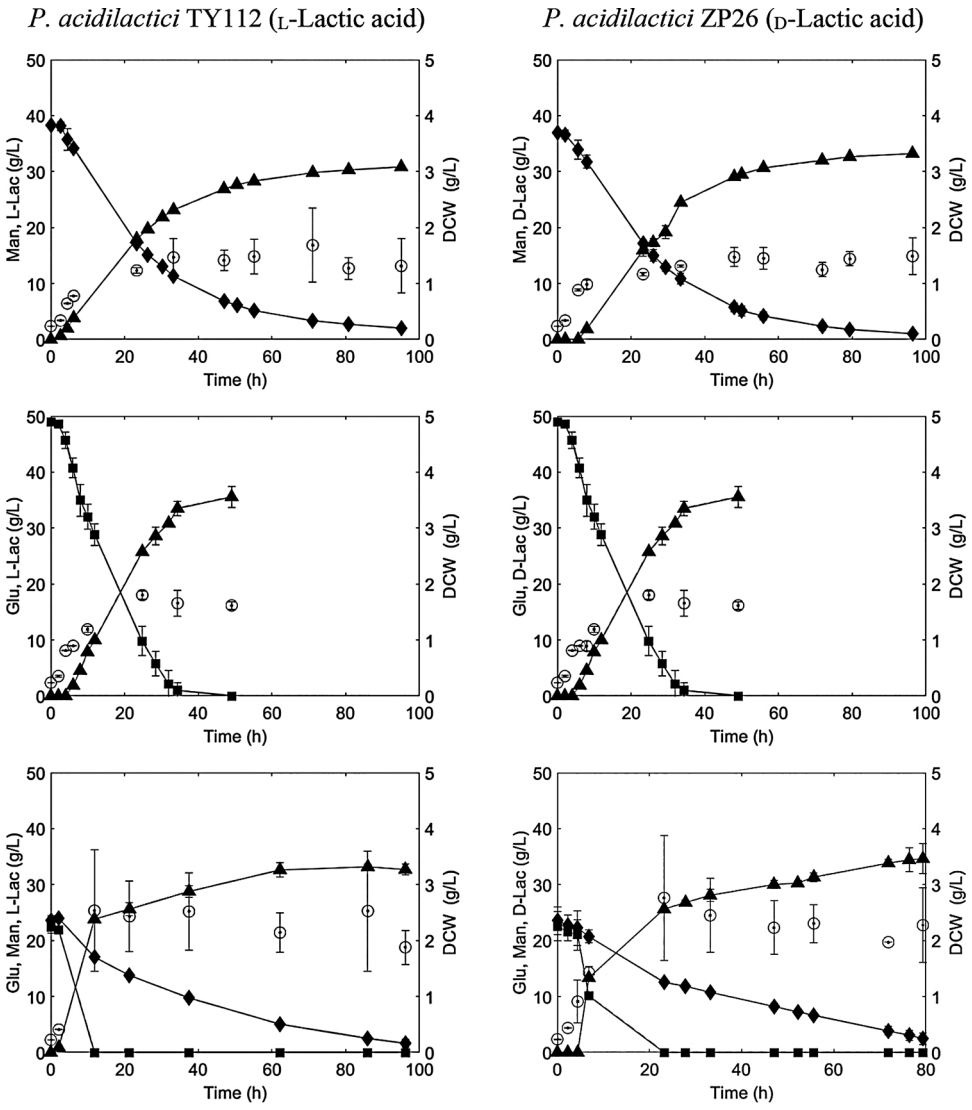

Fig. 2. Batch growth in 1 L bioreactors of *P. acidilactici* TY112 and *P. acidilactici* ZP26 in simplified MRS medium with glucose, mannose and an equal mixture of glucose and mannose. DCW (○), mannose (◆), glucose (■), lactic acid (▲).

Table 2
Sugar uptake rates and lactic acid yields from batch growth in bioreactor with simplified MRS medium with glucose, mannose and an equal mixture of glucose and mannose.

		r_{man} ($g \cdot L^{-1} h^{-1}$)	r_{glu} ($g \cdot L^{-1} h^{-1}$)	$Y_{P/S}$ ($g g^{-1}$)
TY112 (L-Lac)	Mannose	0.90	–	0.85
	Glucose	–	1.56	0.75
	Glucose + Mannose	1.89	0.46	0.74
ZP26 (D-Lac)	Mannose	0.84	–	0.93
	Glucose	–	1.39	0.73
	Glucose + Mannose	1.74	0.47	0.78

EPS-forming enzymes in its genetic material (Liu and Ji, 2016).

3.3. Bioreactor fermentations with softwood hydrolysate

After the characterization of mannose conversion in synthetic medium, the ability of the strains to convert mannose in a spruce hemicellulose hydrolysate was examined. Softwood hydrolysates obtained through steam pretreatment are known to contain certain inhibitors, e.g. acetic acid, furfural, HMF (Frankó et al., 2019), and the microorganism used must be sufficiently robust to tolerate the medium. The growth profile in a medium containing different percentages of hydrolysate (10 %, 20 % and 50 %) was evaluated for 72 h in preliminary shake flask experiments (data not shown). In these preliminary experiments, the complex medium was supplemented with refined sugars to eliminate the influence of sugar concentration in the cultivation. The growth observed in the hydrolysate supplied media was always lower than the control. At 50 % of hydrolysate there was no growth, but there was growth reaching approximately the same maximum OD for both 10 % and 20 %. Based on these results, it was decided to make bioreactor cultivations with 20 % of the total volume of softwood hydrolysate (i.e. a 5X dilution).

The consumption pattern of the two main sugars in the hydrolysate follows that of these sugars in defined media experiments (Fig. 5). Glucose is consumed first, together with some consumption of mannose. The time for consumption of mannose is considerably longer than that of glucose, which takes longer to be exhausted because of its lower rate of consumption when in comparison with cultivations in defined media. Galactose, present in low amounts, is also consumed when the glucose uptake is almost complete, after approximately 5 h of cultivation (data not shown). As expected, xylose concentration is unchanged throughout

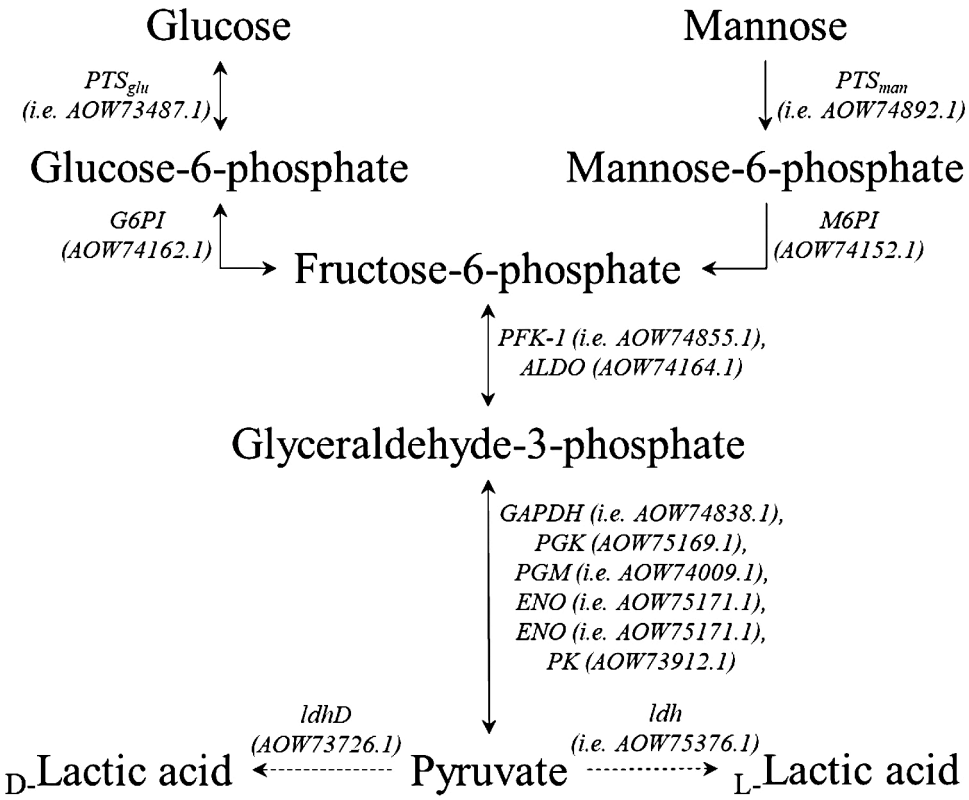

Fig. 3. Putative glucose and mannose metabolism for D- and L-lactic acid production. The putative enzymes have been selected from the annotated genome for *P. acidilactici* ZPA017 (Liu and Ji, 2016). Each enzyme has one example for the protein identification number.

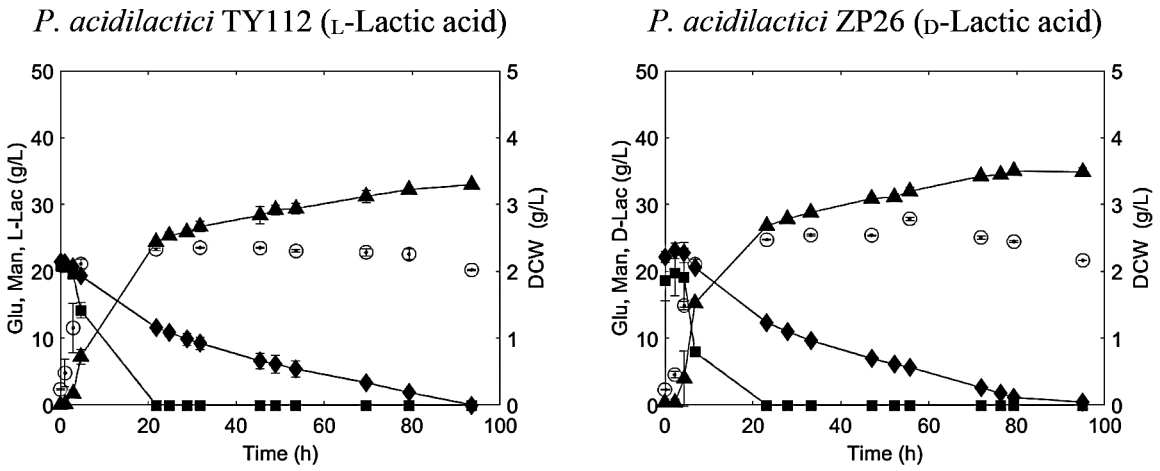

Fig. 4. Batch growth in 1 L bioreactors of *P. acidilactici* TY112 and *P. acidilactici* ZP26 in simplified MRS medium with an equal mixture of glucose and mannose, supplemented with 0.5 % (v/v) of amyloglucosidase. DCW (○), mannose (◆), glucose (■), lactic acid (▲).

the cultivation since it is not assimilated by either of the present strains, as demonstrated by works of Qiu et al. (Qiu et al., 2018, 2017). *P. acidilactici* TY112 grew to a maximum of 1.5 $g{\cdot}L^{-1}$ of dry biomass while producing close to 14 $g{\cdot}L^{-1}$ of L-lactic acid. *P. acidilactici* ZP26 grew to a maximum of 1.7 $g{\cdot}L^{-1}$ of dry biomass while producing close to 12 $g{\cdot}L^{-1}$ of D-lactic acid. These cultivations confirm the potential for the use of these strains for production of optically pure lactic acid from softwood hydrolysate. These data are coherent with those obtained for the defined media experiments; however, the specific conversion rates were decreased approximately by half. This is probably explained by the presence of degradation products from softwood hydrolysis, which act as inhibitors for cell metabolism. The influence of the concentration of possible inhibitory compounds, such as furfural, HMF, acetic acid, formic acid and vanillin, has previously been evaluated for *P. acidilactici* TY112, ZP26 and DQ2 (parent-strain for TY112 and ZP26) by cultivation on defined media supplemented with one of the compounds mentioned above in each trial (Yi et al., 2016; Zhao et al., 2013). Furfural, HMF, syringaldehyde, vanillin (Yi et al., 2016) and formic acid (Zhao et al., 2013) showed to decrease cell growth overall. Yi et al. (2016) also quantified the variation in productivity of lactic acid. There was a general decrease in productivity with the increase in inhibitor concentration. Productivity was only affected by furfural and HMF at higher concentrations (8 $g{\cdot}L^{-1}$). However, *P. acidilactici* becomes less resistant when cultivated in hydrolysate, in the presence of a mixture of inhibitors

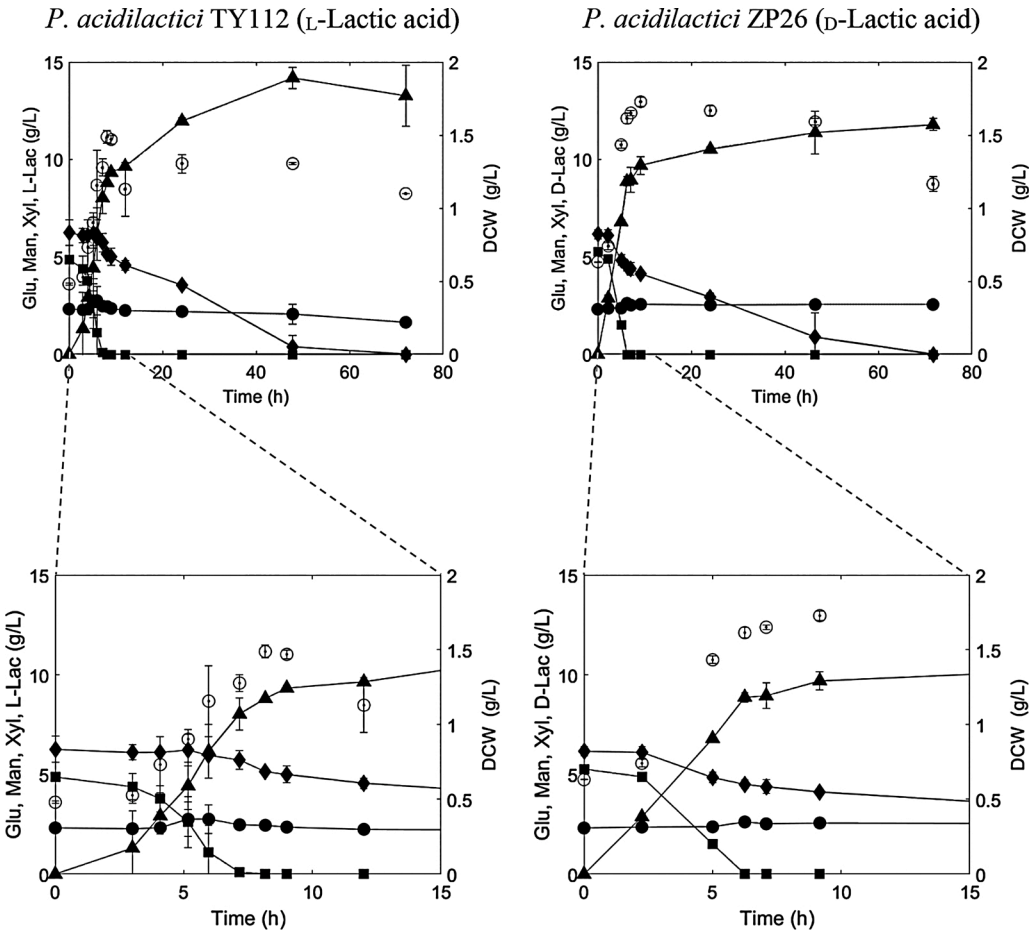

Fig. 5. Batch growth in 1 L bioreactors of *P. acidilactici* TY112 and *P. acidilactici* ZP26 in simplified MRS medium with 20 % (v/v) of softwood hydrolysate. A detailed image of the first 15 h of cultivation is presented below each graphical representation. DCW (○), mannose (♦), glucose (■), xylose (●), lactic acid (▲).

(Liu et al., 2013; Yi et al., 2016). Studies on simultaneous saccharification and fermentation (SSF) of corn stover showed that lactic acid production increased after 5–10 days of cultivation, when the concentration of furans and phenolic aldehydes had been decreased by detoxification (Yi et al., 2016). The possible adaptation of both strains to the hostile environment in this study will be explored in future work. Djukić-Vuković et al. (2019) compared the SSF performance of *P. acidilactici* in conversion of corn stover reported by Qiu et al. (2018) with other lactic acid producing microbes using other substrates and fermentation mode in terms of product concentration and productivity. *P. acidilactici* showed in that comparison both a high final concentration of lactic acid in the end of a batch SSF (130.8 $g{\cdot}L^{-1}$), and a high productivity (1.82 $g{\cdot}L^{-1}\ h^{-1}$). Qiu et al. have demonstrated the possibility of engineering *P. acidilactici* for the conversion of *p*-benzoquinone, a lignin-derived inhibitor, into a less toxic hydroquinone (Qiu et al., 2020). It may be of interest to also explore more genetic engineering opportunities to build robust strains of *P. acidilactici* for the production of optically pure lactic acid from lignocellulosics.

4. Conclusions

P. acidilactici TY112, a $_L$-lactic acid producer, and P. *acidilactici* ZP26, a $_D$-lactic acid producer, can use mannose as carbon source with yields close to 90 %. Mannose has a lower consumption rate than glucose. Interestingly, the sugars were found to be co-consumed. The strains showed flocculation, most likely due to the formation of excreted polysaccharides, some of which seemed to remain attached to the surface of the cells. This study serves as foundation for the research on optimization of production of optically pure lactic acid from softwood hydrolysates, which shown to be a promising carbon source.

Author contributions

Joana Campos: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Jie Bao:** Conceptualization, Writing – review & editing, Funding acquisition. **Gunnar Lidén:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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