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Fermentative lactic acid production from coffee pulp hydrolysate using *Bacillus coagulans* at laboratory and pilot scales

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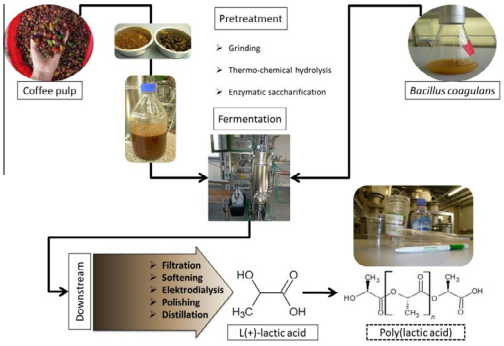
HIGHLIGHTS

- Hydrolysis of coffee pulp recovered 70–80% of available sugars.
- Lactic acid production from hydrolysate was investigated at 2 L and 50 L scales.
- At 50 L scale productivity was highest at 4.0 g L^{-1} h^{-1}.
- The yield was 0.78 g lactic acid per gram of sugar consumed.
- Downstream processing resulted in a 937 g L^{-1} optical pure L(+)-lactic acid solution.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the lignocellulosic residue coffee pulp was used as carbon source in fermentative L(+)-lactic acid production using *Bacillus coagulans*. After thermo-chemical treatment at 121 °C for 30 min in presence of 0.18 mol L^{-1} H_2SO_4 and following an enzymatic digestion using Accellerase 1500 carbon-rich hydrolysates were obtained. Two different coffee pulp materials with comparable biomass composition were used, but sugar concentrations in hydrolysates showed variations. The primary sugars were (g L^{-1}) glucose (20–30), xylose (15–25), sucrose (5–11) and arabinose (0.7–10). Fermentations were carried out at laboratory (2 L) and pilot (50 L) scales in presence of 10 g L^{-1} yeast extract. At pilot scale carbon utilization and lactic acid yield per gram of sugar consumed were 94.65% and 0.78 g g^{-1}, respectively. The productivity was 4.02 g L^{-1} h^{-1}. Downstream processing resulted in a pure formulation containing 937 g L^{-1} L(+)-lactic acid with an optical purity of 99.7%.

1. Introduction

The possibility to convert biomass constituents using microorganisms into value added products paves the way to a bio-based economy and helps to overcome the restriction and environmental issues of fossil-based resources. The change to a bio-based economy, however, requires the efficient use of all kinds of biomass and organic waste streams. Therefore an investigation of biomass as substrate and nutrient source in biotechnological processes is necessary.

In the past different biomasses have been tested for the production of platform chemicals which have the potential to substitute petroleum-based chemicals in chemical reactions. Predominant approaches were the conversion of biomass constituents using microorganisms in fermentative processes. Microorganisms can

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convert glucose and other sugars into organic chemicals (Koutinas et al., 2014). Nevertheless, basic studies on the availability of biomass derived compounds to microorganisms are urgently required in order to assess processes regarding productivities and yields. While starchy materials are easily available to microorganism, lignocellulosic materials are not and require pretreatment and hydrolysis (Pleissner and Venus, 2014). Pretreatment and hydrolysis are often carried out at high temperatures in presence of acids and mostly followed by an enzymatic treatment to improve the release of monosugars, such as glucose and xylose. Such treatments, however, may lead to the formation of inhibiting compounds, which negatively affect the performance of bioprocesses. Hence, not only the fermentative process, but also the upstream process requires attention. Finally, in order to obtain a ready-to-market product, downstream processing needs to be performed and studied. Particularly, complex substrates which contain high amounts of impurities can complicate downstream processes. For instance, downstream processing of lactic acid can be carried out by conventional processes, such as precipitation with H_2SO_4, which, however, generates a large amount of $CaSO_4$. Alternative technologies, such as electrodialysis with monopolar and bipolar membranes, may reduce the environmental impact of downstream processes and will be investigated in the present study for purification of lactic acid produced from coffee pulp hydrolysate.

Of particular interest for the bioeconomy regarding market volume and strong growth is lactic acid (Jong et al., 2011). In theory 2 mole lactic acid can be produced from 1 mole glucose which makes the fermentative utilization of biomass highly efficient. Lactic acid finds applications in the food and beverage, and in the pharmaceutical sectors as well as for the production of the biodegradable plastic poly(lactic acid) (Castillo Martinez et al., 2013; Jong et al., 2011).

A complex biomass which has been underexploited is coffee pulp. Pulp surrounds the coffee bean and remains from coffee production. Annually 15 million tons of residues from coffee production appear worldwide, whereby 9.4 million tons are formed by coffee pulp (Bakker, 2013). Coffee residues are mainly used as fertilizer, livestock feed and compost (Murthy and Madhava Naidu, 2012). Coffee pulp may contain (w/w) proteins (9–11%), lipids (2–17%), cellulose (13–27%), tannins (4.5%), pectic matter (6.5%), reducing sugar (12.4%) and non-nitrogen extracts (57–63%) (Woiciechowski et al., 2000), and thus is an interesting feedstock for the biotechnological production of enzymes, organic acids and aroma compounds (Pandey et al., 2000). The composition and quality of different coffee pulp batches, however, can be different and also the regional availability needs to be considered when using this feedstock in biotechnological utilization processes.

The aim of this study was the hydrolysis of coffee pulp, use of hydrolysate in fermentations carried out at laboratory (2 L) and pilot scales (50 L) and downstream processing for pure L(+)-lactic acid formulation production. The outcomes of this study are expected to contribute to the further development of approaches for coffee residues valorization in regions where it appears in large amounts. Lactic acid from coffee residues has been produced earlier in mixed-acid fermentation (Woiciechowski et al., 1999). However, we used the thermophilic bacterium *Bacillus coagulans*, which was shown to efficiently convert sugars from hydrolyzed agricultural residues into only L(+)-lactic acid (Pleissner and Venus, 2014).

2. Material and methods

2.1. Coffee pulp

Coffee pulp was supplied by CENICAFÉ, the national coffee research institute in Colombia, as dried fruit pulp containing marginal amounts of coffee beans and coffee wood. Prior to hydrolysis the material was ground using a cutting mill (Grindomix GM 200, Retsch, Germany). The particle size was approximately 1 mm.

2.2. Microorganism

The thermophilic *B. coagulans* strain used in all fermentations was isolated from mulberries. The strain is available at the Leibniz Institute for Agricultural Engineering Potsdam-Bornim (Germany). Inocula for fermentations were grown in 250 mL conical flasks containing 60 mL of MRS broth (Merck, Germany) and 0.67 g Everzit Dol (Evers, Germany) dolomite as buffer. Cultivation was carried out at 52 °C and an initial pH of 6.0 for 10–16 h. The flasks were shaken at 100 rpm in an orbital shaker. Autoclavation of flasks containing MRS broth was carried out at 118 °C for 15 min.

2.3. Hydrolysis

Hydrolysis of 16.6% (w/v) coffee pulp suspension was first carried out at 121 °C for 30 min in presence of 0.18 mol L^{-1} H_2SO_4. Afterwards the pH was adjusted manually to 5 by adding 20% (w/w) NaOH. As a second step the chemically pretreated coffee pulp was enzymatically hydrolyzed using Accellerase 1500. Per gram of initially added coffee pulp 0.3 mL of Accellerase 1500 was added and the reaction carried out stirred (150 rpm) at 50 °C for 24 h in a 2 L BIOSTAT bioreactor (Sartorius AG, Germany).

Hydrolysis was evaluated for the total amount of sugar released after hydrolysis compared to the theoretically available sugars in the coffee pulp. The theoretical amount of sugar was calculated from the sugar content of the substrate and the cellulose and hemicellulose contents. For cellulose a suggested conversion factor of 0.9 was used according to Wyman et al. (2004). Since the structure of hemicellulose could not be determined an estimated conversion factor of 0.9 was used.

2.4. Fermentation

2.4.1. Laboratory scale fermentation

Fermentations were carried out in triplicate at 52 °C and pH 6 with coffee pulp hydrolysate in presence of 10 g L^{-1} yeast extract (Ohly KAT, Deutsche Hefewerke GmbH & Co. OHG, Germany). For all fermentations a 2 L BIOSTAT bioreactor (Sartorius AG, Germany) containing 1 L of coffee pulp hydrolysate was used. Stirring occurred at 400 rpm using a double Rushton turbine. Regulation of pH was carried out by adding 20% (w/w) NaOH. A 6% (v/v) inoculum was used in all fermentations. Samples were taken regularly for the analysis of sugar (glucose, xylose, arabinose and sucrose) and lactic acid concentrations. Samples were inactivated by heating at 95 °C for 20 min. After inactivation, samples were stored at −20 °C until used in analysis.

2.4.2. Pilot scale fermentation

Pilot scale fermentation was carried out in a 72 L BIOSTAT UD bioreactor (B-Braun Biotech, Germany) containing 45 L of coffee pulp hydrolysate and 10 g L^{-1} yeast extract. Fermentation was carried out at 52 °C and pH 6. Due to limitations in coffee pulp, fermentation was carried out only once. Stirring occurred at 400 rpm using a double Rushton turbine. Regulation of pH was carried out by adding 20% (w/w) NaOH. A 5% (v/v) inoculum was used. The inoculum was grown for 17 h in a 5 L fermentation vessel containing 2 L of medium consisting of 66 g L^{-1} dextrose monohydrate and 15 g L^{-1} yeast extract inoculated with 120 mL MRS culture (see Section 2.2). Samples were taken regularly and treated as described in Section 2.4.1. After fermentation, culture broth was inactivated at 85 °C for 30 min and stored at −20 °C until used in downstream processing.

2.5. Downstream processing

Downstream processing included micro- and nanofiltrations, softening, mono- and bipolar electrodialysis, anion- and cation-exchange chromatographies, and distillation. The methods are explained in detail by Neu and coworkers (Neu et al., 2016).

2.6. Analytics

Total number of cells was determined using a THOMA cell chamber (Glaswarenfabrik Karl Hecht GmbH & Co KG, Germany) and number of living cells was determined as colony forming units counted on a plate containing Nutrient Agar (Merck, Germany) after 24 h of incubation at 52 °C.

To determine the dry matter of coffee pulp a certain amount was weighed and dried at 105 °C until constant weight. Afterwards, a certain amount of dried coffee pulp was combusted at 550 °C for 5 h in a muffle furnace. The weight of remaining ash was subtracted from the dry matter in order to obtain the organic fraction of dry matter.

Lactic acid and sugar concentrations in fermentation samples and coffee pulp hydrolysate were analyzed by high performance liquid chromatography (HPLC, DIONEX, USA): 10 µL of sample volume was added on a Eurokat H column (300 mm × 8 mm × 10 µm, Knauer, Germany) and eluted isocratically with 0.8 mL min^{-1} of 5 mM H_2SO_4. Detection was carried out by a refractive index detector (RI-71, SHODEX, Japan). Each analysis was carried out in duplicate and peak areas and retention times were compared to analyses of known concentrations of pure lactic acid, glucose, xylose, arabinose and sucrose. The mean concentration of two analyses is presented.

Cat- and anion concentrations in fermentation samples and pulp hydrolysates were analyzed by ion chromatography (DIONEX, USA). For quantification of cations, 25 µL of sample volume was added on an IonPac CS 16 column (250 mm × 4 µm, DIONEX, USA) and eluted isocratically with 1.0 mL min^{-1} of 30 mM CH_3SO_3-H at 40 °C. For quantification of anions, 25 µL of sample volume was added on an IonPac AS9-HC column (250 mm × 4 µm, DIONEX, USA) and eluted isocratically with 1.2 mL min^{-1} of 9 mM Na_2CO_3 at room temperature. Detection of cat- and anions was carried out by a conductivity cell. Each analysis was carried in duplicate and peak areas were compared to analyses of known concentrations of salt-solutions consisting of cat- and anions of interest. The mean concentration of two analyses is presented.

The ratio of the optical isomers in the lactic acid formulation was checked using HPLC (KNAUER, Germany) coupled with a Chiralpak®MA(+) column (DAICEL, Japan, 50 mm × 4.6 mm × 3 µm) and an ultraviolet detector. The mobile phase was 2 mM $CuSO_4$ and the flow rate 0.8 mL min^{-1}.

Fat analysis was performed by means of ANKOM Technology (USA) according to the ANKOM Technology Method 2, 01-30-09: Determination of Oil/Fat Utilizing High Temperature Solvent Extraction (ANKOM, 2009).

Analyses of hemicellulose, cellulose and lignin contents were carried out using an ANKOM2000 fiber analyzer (ANKOM, 2014).

Sugar content determination was carried out by cold water extraction. To 3–5 g of dried coffee pulp 50 mL of demineralized water was added and the mixture shaken for 30 min. Afterward 2 mL of a 30% (w/w) $ZnSO_4$ solution and 2 mL of a 15% (w/w) $C_6N_6FeK_4$ solution were added. After shaking, the mixture was filtrated and the clear filtrate analyzed by HPLC as described above.

Kjeldahl-nitrogen (Kjeldahl-N) content of mucilage was determined according to the DIN-EN-25663 standard method.

2.7. Statistical analysis

Kruskal-Wallis One Way Analysis of Variance on Ranks was carried out in SigmaPlot and used to measure the statistical difference of lactic acid production between fermentations carried out at laboratory scale. Statistically significant difference in median values was accepted for $P < 0.05$.

3. Results and discussion

3.1. Hydrolysis

Coffee pulp had a dry matter and organic dry matter of (w/w) 86.8 ± 6.3% and 90.7 ± 3.0%, respectively. The contents of cellulose, hemicelluloses and lignin were (w/w) 14.7 ± 1.6%, 10.2 ± 0.4% and 10.1 ± 3.7%, respectively. The fat content was 1.3% (w/w). Coffee pulp was not only rich in lignocellulose, but also in Kjeldahl-N and sugars. In average the contents of Kjeldahl-N and sugar were (w/w) 14.3 ± 1.9% and 14.8 ± 5.3%, respectively. In order to use the hemicellulose and cellulose fractions as carbon sources in lactic acid fermentation first a thermo-chemical treatment and second an enzymatic hydrolysis were carried out. This approach was found to make between 72.8% and 89.1% of the theoretically present sugars available as carbon sources in fermentation. When 16.6% (w/v) coffee pulp was hydrolyzed a sugar concentration of around 60 g L^{-1} was obtained. In earlier studies, the hydrolysis of coffee pulp and use of hydrolysate in lactic acid fermentation have already been investigated in order to increase the amount of accessible sugar and to efficiently utilize coffee pulp (Urbaneja et al., 1996; Woiciechowski et al., 2000). Hydrolysis parameters, such as acid concentration and treatment time were varied and highest total sugar concentration obtained was 16.84 g L^{-1} including 6.31 g L^{-1} glucose when 0.2 mol L^{-1} H_2SO_4 was added to coffee pulp and the mixture heated at 100 °C for 240 min (Urbaneja et al., 1996). Initial concentration of coffee pulp was not provided, the recovery rate, however, was 64% of total sugar. Woiciechowski et al. obtained a sugar-rich hydrolysate from coffee husk after thermal treatment at 121 °C for 15 min and enzymatic hydrolysis (Woiciechowski et al., 2000). It might be considered to grind the material in order to decrease particle size and increase external surface areas (Taherzadeh and Karimi, 2008). In general smaller particles have a higher surface-to-volume ratio making them more accessible to enzymes and other chemicals as well as bacteria. In the present study the hydrolysis of 16.6% (w/v) coffee pulp at 121 °C for 30 min in presence of 0.18 mol L^{-1} H_2SO_4 and additional enzymatic treatment resulted in a recovery of 72.8%–89.1% of the total sugar. It might be considered to skip the addition of acid in order to minimize the amount of salt ions and to ease downstream processing. Furthermore, from an economic point of you, the application of enzymes is most likely a drawback and additional research in form of a techno-economic study is required to assess the feasibility of upstream processing in coffee pulp based lactic acid fermentation processes.

Hydrolysates obtained contained glucose, xylose, sucrose and arabinose. The concentration of sugars was dependent on the coffee pulp material used. Hydrolysates used for fermentations shown in Fig. 1A and C, containing around 20 g L^{-1} glucose, 15 g L^{-1} xylose, between 6 and 11 g L^{-1} sucrose and between 6 and 10 g L^{-1} arabinose, were produced from a different batch of coffee pulp than the hydrolysates used in fermentations shown in Figs. 1E and 2A, which contained 25.7 g L^{-1} glucose, 31.0 g L^{-1} xylose, 4.8 g L^{-1} sucrose and 0.7 g L^{-1} arabinose. Despite the difference in sugar concentrations between hydrolysates the composition of coffee pulp was comparable. This finding illustrates the importance of studies dealing with the investigation of biomasses. Even though same

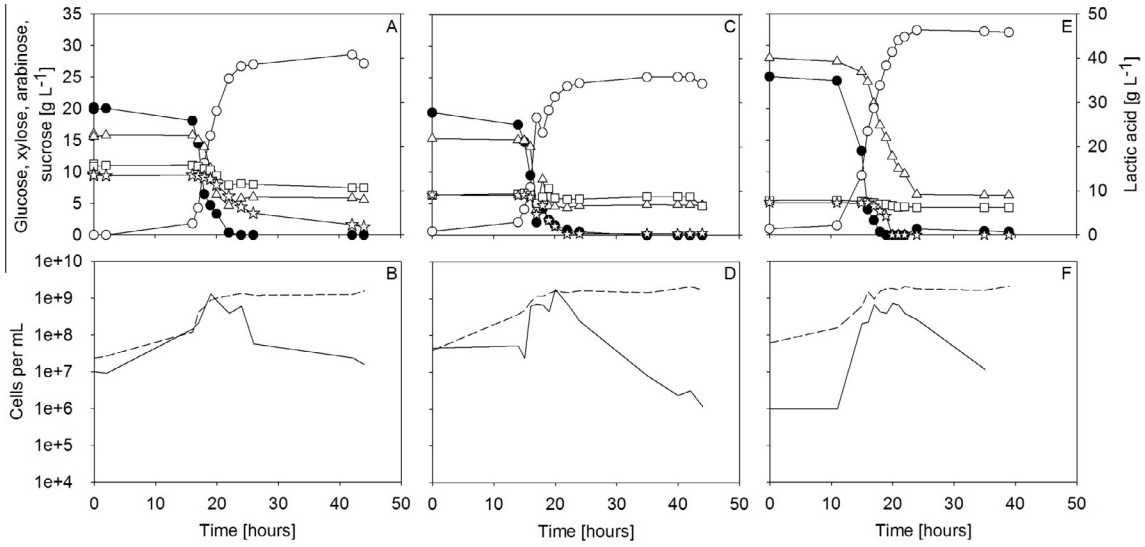

Fig. 1. Fermentation at laboratory scale. A, C, E: *B. coagulans* lactic acid (open circle) fermentation carried out in coffee pulp hydrolysate containing glucose (closed circle), xylose (open triangle), arabinose (open star) and sucrose (open square) as carbon sources. B, D, F: Total number of cells (dashed line) and number of living cells (solid line) during fermentation.

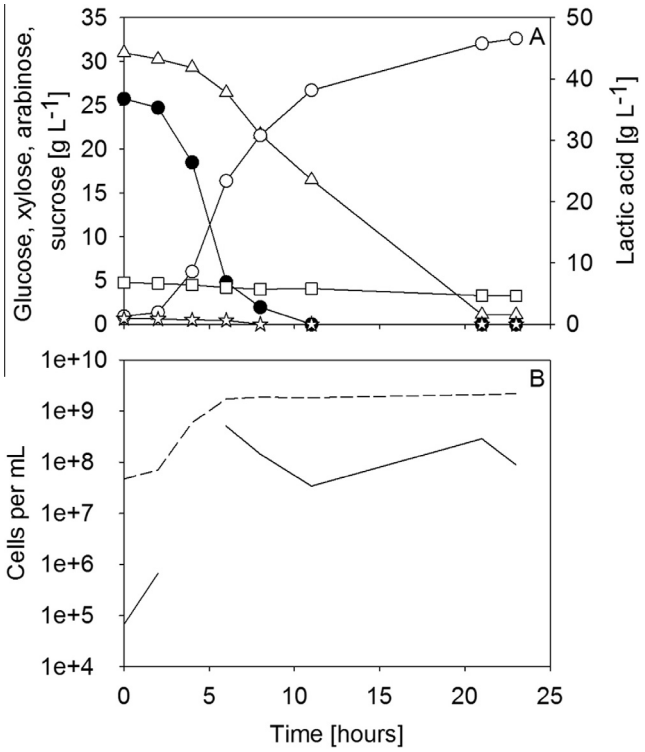

Fig. 2. Fermentation at pilot scale. A: *B. coagulans* lactic acid (open circle) fermentation carried out in coffee pulp hydrolysate containing glucose (closed circle), xylose (open triangle), arabinose (open star) and sucrose (open square) as carbon sources. B: Total number of cells (dashed line) and number of living cells (solid line) during fermentation.

biomasses are used as substrates in fermentations, quality, for example, may lead to different sugar concentrations in hydrolysates and this is challenging industrial scale productions.

3.2. Laboratory scale fermentation

Fermentations at laboratory scale were carried out in triplicate (Fig. 1). As mentioned above, the hydrolysates used in fermentations shown in Fig. 1A, C and E were produced from different coffee pulp batches which resulted in different sugar concentrations. Irrespective of the coffee pulp material used, *B. coagulans* used the recovered sugars for growth and lactic acid production. In all fermentations glucose was the preferred carbon source, followed by xylose and arabinose. Sucrose was obviously not the preferred carbon source. Contrarily to glucose and arabinose, xylose was not depleted and approximately 5 g L^{-1} remained in the medium. Even when the hydrolysates were different in terms of sugar concentrations, growth rate, productivity during exponential growth phase, total sugar consumption and yield of lactic acid per gram of sugars consumed were comparable (Table 1). Statistical investigation in lactic acid production revealed no significant difference ($P = 0.113$). The lag-phase was 10–15 h but afterwards growth proceeded at a rate of 0.29 h^{-1} for around 10 h. During this phase the highest fraction of viable cells was determined (Fig. 1B, D and F) and lactic acid was formed at an average productivity of 3.6 g L^{-1} h^{-1}. Interestingly, the number of viable cells in the fermentation shown in Fig. 1E and F (0.4×10^{12} cells L^{-1}) was only one-third of the number found in the other two laboratory scale fermentations (Fig. 1A and B, and C and D). The total number of cells, however, was equal at about 1.6×10^{12} cells L^{-1}. In all three fermentations in average 83.4% of sugars determined was consumed. The remaining 16.6% was formed by leftovers of xylose and sucrose. The yield of lactic acid was 0.54 g g^{-1}. After 40–44 h, when the lactic acid concentration did not further increase, the fermentations were stopped. Even though the three fermentations were comparable, it should be admitted here that the fermentation shown in Fig. 1E had slightly better performance, which also was reflected by the final lactic acid concentration. At the end of fermentation period 40 and 35 g L^{-1} (Fig. 1A and C), and 48 g L^{-1} (Fig. 1E) of lactic acid were obtained. The optical purity of L(+)-lactic acid was 99.5% (Table 1).

3.3. Pilot scale fermentation

Pilot scale fermentation was carried out in presence of 25.7 g L^{-1} glucose, 31.0 g L^{-1} xylose, 4.8 g L^{-1} sucrose and 0.7 g L^{-1} arabinose (Fig. 2). Interestingly, when fermentation was carried out at pilot scale the lag-phase was only 5 h long. After this short period lactic acid concentration increased within 23 h to

Table 1
Overview of growth rate (μ), lactic acid productivity during exponential growth phase (P), consumption of free sugars (C), yield of lactic acid per g of free sugars (Y) and optical purity of lactic acid produced ($OP_{L/D}$) of the batch fermentations carried out at laboratory and pilot scales.

Batch	μ [h^{-1}]	P [$g\,L^{-1}\,h^{-1}$]	C [%]	Y [$g\,g^{-1}$]	$OP_{L/D}$ [%]
Laboratory scale	0.29 ± 0.05	3.57 ± 0.87	83.43 ± 3.66	0.54 ± 0.04	99.50 ± 0.20
Pilot scale	0.54	4.02	94.65	0.78	99.50

45.3 $g\,L^{-1}$ under consumption of 94.65% of carbon sources supplied. This also indicates that *B. coagulans* utilized pentoses and hexoses. The yield of lactic acid was 0.78 g per gram of carbon source consumed (Table 1). The lactic acid productivity during exponential growth was 4.02 $g\,L^{-1}\,h^{-1}$. Sucrose was basically not used as carbon source. The total number of cells was 2.1×10^{12} cells L^{-1} and the number of viable cells during exponential phase, where growth occurred at a rate of 0.54 h^{-1}, was only 0.5×10^{12} cells L^{-1}. Fermentation was stopped after 23 h and the culture broth was used for downstream processing.

Sugars other than glucose originate mainly from the hemicellulose fraction of lignocellulosic materials. Dominant sugars in hemicellulose are xylose and mannose, but could also contain galactose, glucose, arabinose and small amounts of rhamnose, glucuronic acid, methyl glucuronic acid and galacturonic acid (Taherzadeh and Karimi, 2008). Analysis of sugars in hydrolysates used in this study revealed the presence of glucose, xylose, arabinose and sucrose. As described above the concentration of sugars in the two hydrolysates varied (Figs. 1 and 2). This aspect is certainly the main challenging part when using complex substrates in biotechnological processes at industrial scale and implies the importance of testing various batches of the same material as fermentation feedstock. The predominant sugar in the hydrolysate of fermentations shown in Fig. 1A and C was glucose (20 $g\,L^{-1}$). In contrast, the predominant sugar in fermentations shown in Figs. 1E and 2A was xylose (31 $g\,L^{-1}$). The variation in composition may explain the differences in growth rate and yield (Table 1). The productivity, however, was comparable. At laboratory scale productivity and yield were 3.57 g L-1 h^{-1} and 0.54 $g\,g^{-1}$, respectively. At pilot scale, however, productivity and yield were 4.02 $g\,L^{-1}\,h^{-1}$ and 0.78 $g\,g^{-1}$, respectively (Fig. 2 and Table 1). A yield of 0.78 $g\,g^{-1}$ is comparable to the findings of earlier using *B. coagulans* and paper sludge or cellulose as carbon sources (Budhavaram and Fan, 2009; Ou et al., 2010). The difference in performance between laboratory and pilot scales is explainable by the fact that in the latter almost all carbon sources were consumed, while 16% of the carbon sources supplied remained at laboratory scale. The better performance of *B. coagulans* regarding lactic acid formation at pilot scale might be due to better mixing and eventually better homogenization, but this effect requires further investigation.

3.4. Downstream processing

Beside fibers coming from the coffee pulp material the culture broth was rich in salt ions. Salt ions are introduced by the substrate and to a larger extent by additives used to regulate pH. The downstream processing had thus been designed in order to first separate fibers and salt ions and second to concentrate lactic acid. Fig. 3 shows the concentrations of salt ions and lactic acid during the downstream processing including micro- and nanofiltrations, softening, mono- and bipolar electrodialysis, decolorization, anion- and cation-exchange chromatographies, and distillation. The majority of ions in culture broth were sodium and potassium with concentrations of 10.8 $g\,L^{-1}$ and 0.8 $g\,L^{-1}$, respectively. The lactic acid concentration was 49.4 $g\,L^{-1}$. After micro- and nanofiltrations 9.2 $g\,L^{-1}$ sodium, 2.2 $g\,L^{-1}$ potassium, 3.9 $g\,L^{-1}$ chloride (Fig. 3)

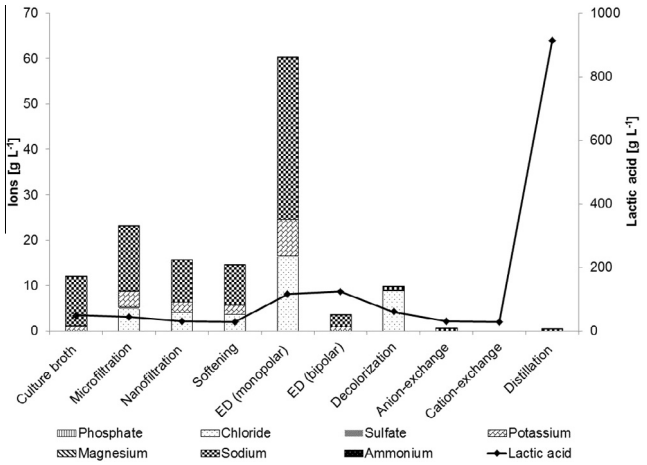

Fig. 3. Downstream processing. Ions and lactic acid concentrations during different downstream processing steps (V = volume, ED = electrodialysis).

were detected in the permeate stream. Since only the permeate streams were used, lactic acid present in retentate stream was lost and the concentration decreased to 30.8 $g\,L^{-1}$. Monopolar electrodialysis created a concentrated salt stream containing 35.6 $g\,L^{-1}$ sodium, 7.9 $g\,L^{-1}$ potassium and 16.3 $g\,L^{-1}$ chloride. This salt-rich fraction was further purified by bipolar electrodialysis, which resulted in a stream containing 2.5 $g\,L^{-1}$ sodium and 0.7 $g\,L^{-1}$ potassium. The other ions were present at concentrations below 0.2 $g\,L^{-1}$. Electrodialysis resulted also in a concentration of lactic acid to 123 $g\,L^{-1}$. In order to remove anions from lactic acid, anion-exchange has been carried out resulting in a stream containing only 0.5 $g\,L^{-1}$ sodium. Sodium was further removed by cation-exchange chromatography. Due to dilution, the lactic acid concentration decreased to 28.2 $g\,L^{-1}$. Thus a last concentration step, carried out by water evaporation under vacuum, was performed resulting in a final lactic acid formulation of 0.8 L with a concentration of 937 $g\,L^{-1}$. The concentration step also resulted in an increase in salt ions concentration and 0.19 $g\,L^{-1}$ chloride, 0.09 $g\,L^{-1}$ sodium and 0.03 $g\,L^{-1}$ sulfate were determined. The optical purity of L(+)-lactic acid was 99.7%.

Particularly water-splitting electrodialysis has been shown here and in earlier studies to convert sodium lactate into lactic acid (Kim and Moon, 2001). The final formulation consisted beside lactic acid only 0.2 $g\,L^{-1}$ chloride, 0.09 $g\,L^{-1}$ sodium, 0.08 $g\,L^{-1}$ phosphate and 0.03 $g\,L^{-1}$ sulfate, and thus the concentration of salts was much lower than concentrations found by Neu et al. who purified lactic acid from coffee mucilage fermentation using the same downstream processing techniques (Neu et al., 2016). This is of particular interest when lactic acid is used in poly(lactic acid) polymerization reactions where salt impurities can negatively affect the reaction rate (Kucharczyk et al., 2011, 2013). The recovery rate of lactic acid, however, was only 23% which is even lower than the rate of 38.2% found by Neu and coworkers (Neu et al., 2016). It should be admitted here that the purpose of this study was to investigate if lactic acid can be purified using filtration, softening,

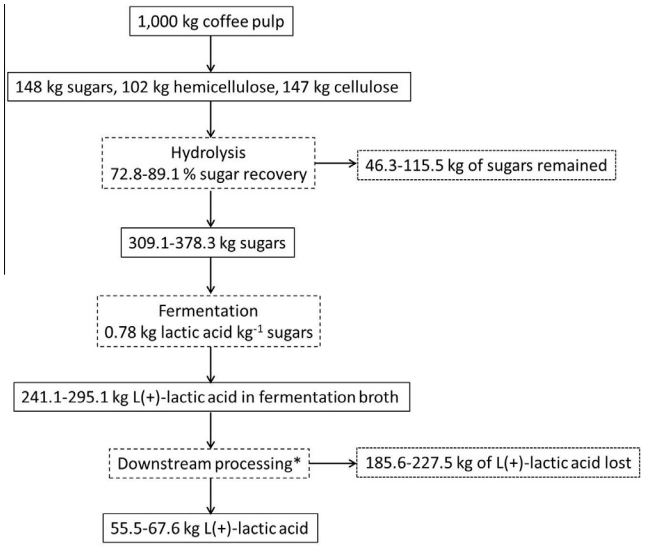

Fig. 4. Mass balance from coffee pulp to lactic acid (*downstream processing was not optimized). All figures are based on dry weight.

electrodialysis, an- and cation chromatographies, and distillation from a coffee pulp-based culture broth. Further research on optimizing downstream processing is therefore needed to reach the almost 100% reported by Wee et al. who recovered lactic acid using repeated batch electrodialysis (Wee et al., 2005). Another example was recently presented by Pleissner et al. who recovered 90% of lactic acid from fermentation broth constituents using the Amberlite resin FPA 53 and 12.5 mM H_2SO_4 as eluent (Pleissner et al., 2016). Both fermentations, however, were based upon a semi-defined medium, which may ease downstream and purification steps compared to complex media, such as coffee pulp hydrolysates.

3.5. Mass balance

In Fig. 4 a mass balance is shown to illustrate the experimental findings. The mass balance starts with a theoretical amount of 1000 kg dry coffee pulp containing 148 kg sugars, 147 kg cellulose and 102 kg hemicellulose. After hydrolysis 72.8–89.1% of the theoretically available sugars can be recovered which results in a hydrolysate containing 309.1–378.3 kg sugars in form of glucose, xylose, arabinose and sucrose. Even though 46.3–115.5 kg sugars remained, the presented hydrolytic approach is a promising way to reduce the amount of coffee pulp by around 40%.

The lactic acid yield at pilot scale was 0.78 g g^{-1}, and thus from the amount of sugars recovered 241.1–295.1 kg lactic acid can theoretically be produced under consumption of 94.65% of sugars supplied. The major drawback of this process from coffee pulp to lactic acid is the downstream processing. Around 77% (185.6–227.5 kg) of lactic acid is lost during filtration, electrodialysis and chromatography. Finally, based on the experimental findings 55.5–67.6 kg of pure lactic acid can be obtained from 1000 kg coffee pulp. However, with an optimized downstream processing and recovery of most of the lactic acid between 200 and 300 kg can be obtained from 1000 kg dry coffee pulp.

4. Conclusions

Lactic acid was produced from coffee pulp hydrolysate using *B. coagulans* at laboratory and pilot scales. Irrespective of the scales, *B. coagulans* converted the sugars obtained (glucose, xylose and arabinose), but only little of sucrose. *B. coagulans*, however, did grow faster and converted sugars more efficiently in lactic acid at pilot scale. Investigation of downstream processing revealed that optically pure lactic acid can be obtained from coffee pulp-based hydrolysate using conventional techniques. In order to reduce the cost of nitrogen sources, it may be considered mixing coffee pulp hydrolysate with other coffee production residues, such as coffee mucilage.

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