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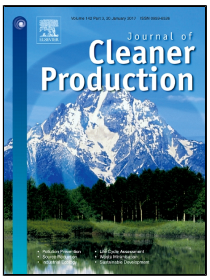

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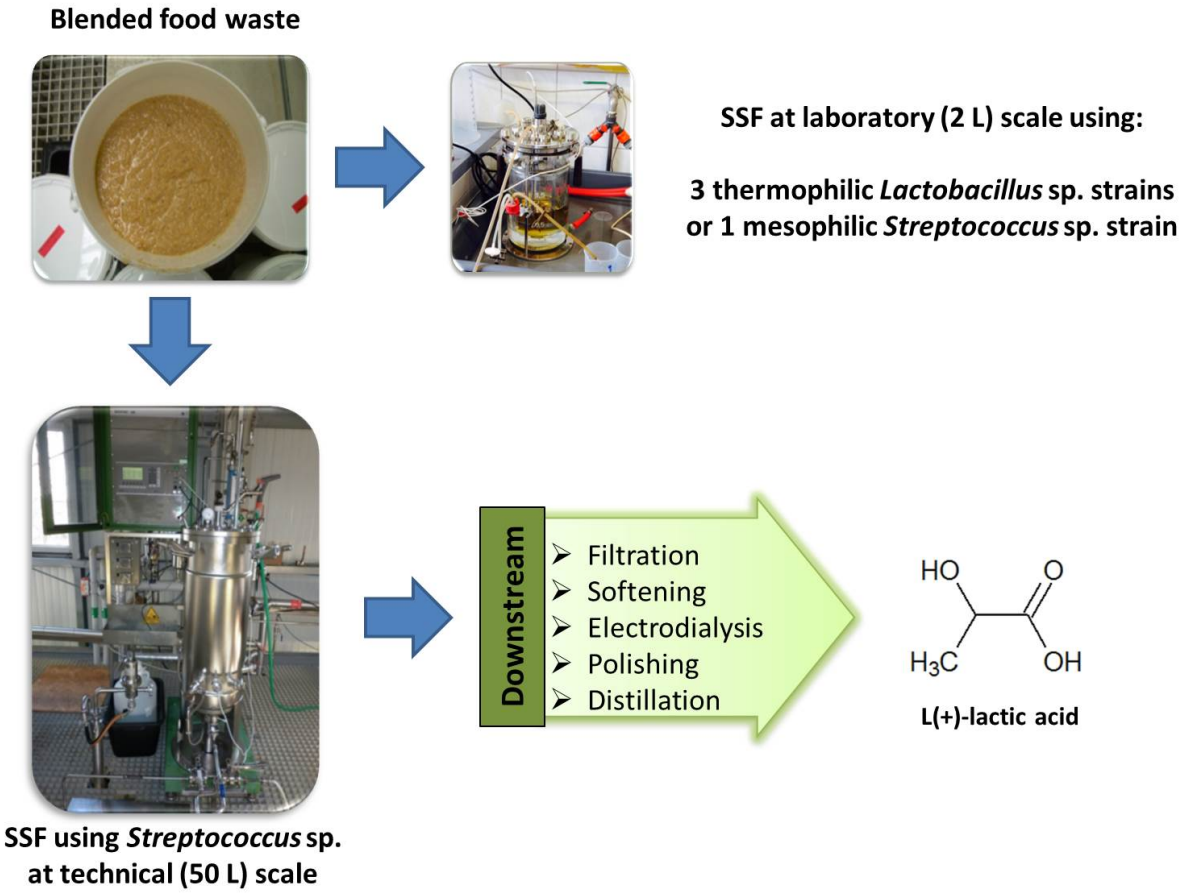

- Simultaneous saccharification and fermentation of food waste was carried out
- An isolated *Streptococcus* sp. strain converted food waste efficiently into lactic acid
- Max. productivity was 2.16 g L^{-1} h^{-1} and yield was 0.81 g g^{-1} of available sugars
- Increase in solid-to-liquid ratio resulted in increased lactic acid titer
- A solid-to-liquid ratio of 20% (w/w) gave 58 g L^{-1} lactic acid
- Downstream processing resulted in a 702 g L^{-1} optical pure L(+)-lactic acid solution

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Direct production of lactic acid based on simultaneous saccharification and fermentation of mixed restaurant food waste

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Abstract

This study introduces to a one-step process for the fermentative production of L(+)-lactic acid from mixed restaurant food waste. Food waste was used as carbon and nitrogen source in simultaneous saccharification and fermentation (SSF) using *Lactobacillus* sp. or *Streptococcus* sp. strains for L(+)-lactic acid production. Waste consisted of (w/w) 33.5% starch, 14.8% proteins, 12.9% fat and 8.5% free sugars. *Lactobacillus* sp. strains showed a productivity of 0.27-0.53 g L^{-1} h^{-1} and a yield of 0.07-0.14 g g^{-1} of theoretically available sugars, while *Streptococcus* sp. more efficiently degraded the food waste material and produced lactic acid at a maximum rate of 2.16 g L^{-1} h^{-1} and a yield of 0.81 g g^{-1}. For SSF, no enzymes were added or other hydrolytic treatments were carried out. Outcomes revealed a linear relationship between lactic acid concentration and solid-to-liquid ratio when *Streptococcus* sp. was applied. Statistically, from a 20% (w/w) dry food waste blend 52.4 g L^{-1} lactic acid can be produced. Experimentally, 58 g L^{-1} was achieved in presence of 20% (w/w), which was the highest solid-to-liquid ratio that could be treated using the equipment applied. Irrespective if SSF was performed at laboratory or technical scale, or under non-sterile conditions, *Streptococcus* sp. efficiently liquefied food waste and converted the released nutrients directly into lactic acid without considerable production of other organic acids, such as acetic acid. Downstream processing including micro- and nanofiltration, electrodialysis, chromatography and distillation gave a pure 702 g L^{-1} L(+)-lactic acid formulation.

Keywords: Food waste, Larger scale fermentation, Lactic acid, Downstream processing

1. Introduction

Enormous amounts of food is wasted globally which creates pressure on finding appropriate processes that allow a management without harming the environment and utilization in order to recover parts of the resources initially spent on food production and processing. In Germany, 18 million tons of food waste is produced annually. While 8 million tons cannot be avoided, 10 million tons are avoidable by changing predominantly consumers` behavior. If one considers that the production of 10 million t of food in Germany occupies 2.6 million ha of arable land and creates 21.8 million t of CO_2 eq. (WWF, 2015), the development of processes to make the best out of food waste is of serious relevance.

Food waste may consist, depending on the source, of meat, noodles, potatoes, vegetables, fruits, bread and cake. Processes of food waste utilization are mainly biotechnology based (Koutinas et al., 2014) and include first a hydrolysis using commercial enzymes, such as amylases and proteases, or microorganism, such as *Aspergillus awamori* and *A. oryzae,* with the ability to secrete hydrolytic enzymes (Pleissner et al., 2014a; Pleissner et al., 2014b). Hydrolysis results in the production of a hydrolysate which is rich in sugar monomers, such as glucose and fructose, free amino nitrogen (FAN), such as amino acids, and phosphate. The hydrolysate has been used as nutrient source for the production of microalgal biomass, a source of polyunsaturated long chain fatty acids (Pleissner et al., 2013; Pleissner et al., 2015a), for the production of short fatty acids, such as succinic acid (Leung et al., 2012) and lactic acid (Kwan et al., 2016; Pleissner et al., 2015a), energy-rich compounds in form of hydrogen (Han et al., 2016) and biogas (Zhang et al., 2007), and biomaterials in form of polyhydroxybutyrate (Pleissner et al., 2014b).

Even though the hydrolysis of food waste can be done relatively fast using commercial enzymes, the costs of enzymes, associated process steps and equipment needs to be considered when assessing the techno-economic feasibility (Kwan et al., 2015). Generally, utilization processes of food waste should be as simple as possible in order to foster its economic feasibility and technical realization at locations where food waste appears in amounts, such as urban areas (Pleissner, 2016).

An example of a food waste utilization process is the anaerobic degradation for biogas production. This process is based on disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis, and considered to be simple enough for decentralized approaches and even for an integration in urban environments (Curry & Pillay, 2012). However, the anaerobic degradation does not allow the use of the whole potential of food waste as functionalized molecules and carbon are wasted. Contrarily, the decentralized realization of material use of food waste, such as the fermentative production of pure organic acid formulations to be used as feedstocks by chemical industry, allows a more efficient utilization. However, the implementation is challenging as those processes usually require upstream and downstream processing. In order to minimize the number of process steps and to provide the basis of a process which allows an efficient use of carbon, the purpose of this study was the development of an approach for the direct conversion of food waste into lactic acid using simultaneous saccharification and fermentation (SSF). This approach is supposed to be an advantage to the most recently reported approaches considering a separated food waste hydrolysis and lactic acid fermentation (Kitpreechavanich et al., 2016; Kwan et al., 2016). SSF is defined here as an approach where degradation of organic matter by secreted or added enzymes, and consumption of released carbon and nitrogen compounds occur simultaneously. Lactic acid was chosen as product due to its various applications in the cosmetic,

pharmaceutical, food and chemical sectors, and for the synthesis of poly(lactic acid) as well as its high market potential (Castillo Martinez et al., 2013; Jong et al., 2011). For this purpose, three thermophilic *Lactobacillus* sp. strains and one mesophilic *Streptococcus* sp. strain, all isolated from various substrates at the Leibniz Institute of Agricultural Engineering and Bioeconomy Potsdam and shown in preliminary flask studies to degrade organic material and to produce L(+)-lactic acid, were tested. Furthermore, different solid-to-liquid ratios of food waste were tested at laboratory scale (2 L) in order to identify its effect on lactic acid production. SSF has further been carried out at technical scale (50 L) and under non-sterile conditions in order to investigate the process at larger scale and real conditions. Finally, downstream processing, including filtration, electrodialysis, ion-exchange and distillation, was carried out for pure L(+)-lactic acid formation. This study introduces to a simple process for lactic acid production from food waste without hydrolysis prior to fermentation which allows a more efficient utilization of waste organic matter compared to the conventionally carried out anaerobic degradation.

2. Material and methods

2.1 Food waste

Food waste containing noodles, potatoes, vegetables, rice, fruits, meat and sauce was collected daily from the canteen located at the Leibniz Institute for Agricultural Engineering and Bioeconomy Potsdam for a period of 15 days in July 2015. Immediately after collection, the wasted food was homogenized using a kitchen blender and the blend stored at -20°C until used in experiments. All food waste blends were pooled and homogenized.

2.2 Microorganisms

Three thermophilic *Lactobacillus* sp. strains: A28a, A59 and A211 isolated from straw hydrolysate, rye corn and rye biomass, respectively, and one mesophilic *Streptococcus* sp. strain: A620 (internal labels) isolated from tapioca starch were employed in experiments. Classification was carried out by the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). All strains were cultured in 300 mL flasks, containing 60 mL of MRS broth (Merck, Germany) and 0.67 g Everzit Dol (Evers, Germany) dolomite as buffer. Autoclavation of flasks containing MRS broth was carried out at 118°C for 15 min. Thermophilic strains were incubated at 52°C for 14-16 h, while the mesophilic strain was incubated at 35°C for 24 h. The initial pH in all flasks was 6. Flasks were shaken at 100 rpm in an orbital shaker.

2.3 Fermentation

2.3.1 Laboratory scale SSF

For all laboratory SSF a 2 L BIOSTAT bioreactor (Sartorius AG, Germany) containing 1 L of blended food waste was used. The blended food waste was autoclaved at 118°C for 15 min. SSF was carried out at 35°C and 52°C for the mesophilic and thermophilic strains, respectively, and at pH 6. Stirring occurred at 200 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. For strain comparison, SSF was carried out using blended food waste with a solid-to-liquid ratio of 10% (w/w). Furthermore, SSF using the mesophilic *Streptococcus* sp. strain A620 was investigated in duplicate at solid-to-liquid ratios of 5, 10, 15 and 20% (w/w). Solid-to-liquid ratio was adjusted by adding demineralized water to the food waste blend. Finally, SSF was investigated in duplicate under non-sterile conditions at a solid-to-liquid ratio of 20% (w/w) using *Streptococcus* sp. strain A620. Samples were taken regularly for the analysis of sugar (glucose, fructose and sucrose), lactic and acetic acids

149 concentrations. Samples were inactivated by heating at 95°C for 20 min. After inactivation,
150 samples were stored at -20°C until used in analysis. Mean values are presented for all
151 fermentations carried out in duplicate.
152
153 **2.3.2 Technical scale SSF**
154 Technical scale SSF using *Streptococcus* sp. strain A620 was carried out in a 72 L BIOSTAT
155 UD bioreactor (B-Braun Biotech, Germany) containing 40 kg of sterilized and blended food
156 waste with a solid-to-liquid ratio of 20% (w/w). Fermentation was carried out at 35°C and pH
157 6. Stirring occurred at 400 rpm using a double Rushton turbine. Regulation of pH was carried
158 out by adding 20% (w/w) NaOH. A 5% (v/v) inoculum was used. The inoculum was grown
159 for 17 h in a 5 L fermentation vessel containing 2 L of medium consisting of 66 g L^{-1} dextrose
160 monohydrate and 15 g L^{-1} yeast extract inoculated with 120 mL MRS culture (see Section
161 2.2). Samples were taken regularly and treated as described in Section 2.3.1. After
162 fermentation, culture broth was inactivated at 85°C for 30 min and stored at -20°C until used
163 in downstream processing.
164
165 **2.4 Downstream processing**
166 Downstream processing included micro- and nanofiltrations, softening, mono- and bipolar
167 electrodialyses, purification through anion- and cation-exchange resins, and distillation. The
168 methods are explained in detail in (Neu et al., 2016).
169
170 **2.5 Analytics**
171 Total number of cells was determined using a THOMA cell chamber (Glaswarenfabrik Karl
172 Hecht GmbH & Co KG, Germany) and number of living cells was determined as colony
173 forming units counted on a plate containing Nutrient Agar (Merck, Germany) after 24 h of

incubation at 52°C for the thermophilic *Lactobacillus* sp. strains and 35°C for the mesophilic *Streptococcus* sp. strain.

To determine the dry matter of blended food waste, a certain amount was weighed and dried at 105°C until constant weight. Afterwards a certain amount of dried blended food waste was weighed and 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 were analyzed by high performance liquid chromatography (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, fructose and sucrose.

Cat- and anion concentrations in fermentation samples 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_3H at 40ºC. 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 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).

Sugar content determination was carried out by cold water extraction. To 3-5 g of dried blended food waste 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.

The theoretical amount of sugar was calculated from the sugar content of the blended food waste and the starch content. A conversion factor of 1.111 g glucose per g starch (obtained by dividing the molar mass of glucose by the molar mass of one starch unit, 180.16 g mol^{-1} / 162.16 g mol^{-1}) was used.

Kjeldahl-nitrogen (Kjeldahl-N) content of blended food waste was determined according to the DIN-EN-25663 standard method. Protein content was calculated by multiplying the Kjeldahl-N content with 5.7 (Leung et al., 2012).

Free amino nitrogen (FAN) concentration was measured using the ninhydrin reaction method described earlier (Lie, 1973). Glycine was used as standard.

2.6 Statistical analysis

In order to measure the statistical difference of lactic acid production of those fermentations carried out in duplicate using *Streptococcus* sp. strain A620 and different solid-to-liquid ratios, and under sterile and non-sterile conditions a t-test was performed in SigmaPlot. Statistically significant difference in median values was accepted for $P < 0.05$.

3. Results

3.1 Strain comparison

From the culture collection at the Leibniz Institute for Agricultural Research and Bioeconomy Potsdam four bacterial strains, *Lactobacillus* sp. with the internal labels: A28a, A59 and A211, and *Streptococcus* sp. with the internal label A620, all identified to degrade organic material in preliminary flask experiments, were chosen and investigated for their ability to degrade wasted food material in SSF and to form lactic acid from the released nutrients. The dry matter and organic dry matter of blended food waste were 18.1% and 93.2% (w/w), respectively. It consisted of (w/w) 33.5% starch, 14.8% proteins, 12.9% fat and 8.5% free sugars. The composition of food waste is known to be highly variable, but German food usually contains potatoes and noodles, and thus the predominant fraction is most likely starch. Lactic acid bacteria require not only carbon to form lactic acid, but also nitrogen. It has been shown that lactic acid formation by *L. helveticus* is growth associated (Amrane & Prigent, 1998). Therefore, nitrogen sources are essential in order to keep cells growing and forming lactic acid. In the present study nitrogen was supplied in form of proteins and FAN, and carbon in form of starch and free sugars.

In Figure 1 is shown SSFs of blended food waste with a solid-to-liquid ratio of 10% (w/w). All four strains produced lactic acid, however, different concentrations, yields and productivities were obtained. Comparison of productivity usually bases on exponential growth phase. In the carried out SSFs, however, strains did not show a clear distinguishable exponential growth phase. Therefore, the calculation of productivity is based on the whole fermentation duration of 28 h. In all fermentations free sugars in form of glucose, fructose and sucrose were found. The concentration of free sugars ranged from 5 to 17 g L^{-1} (Figure 1). The variation in sugar concentration is caused by the complexity of the food waste material and the autoclavation prior to SSF. The oscillating sugar concentrations during fermentations are most likely caused by different activities of bacterial strains regarding enzymatic degradation of organic matter.

Strain A28a produced 7.4 g L^{-1} lactic acid within 28 h resulting in a productivity of 0.26 g L^{-1} h^{-1} (Figure 1A and Table 1). The yield was 0.07 g per g dry food waste. Based on starch content and theoretically obtainable sugars, yields were 0.22 and 0.14 g g^{-1}, respectively. The strains A59 and A211 showed a slightly better performance than strain A28a (Figure 1B and C, Table 1). However, a lactic acid concentration of 10-15 g L^{-1} was still low and one may conclude that only the free sugars were converted, but no starch. This is an interesting finding since it is known that bacteria from the genus *Lactobacillus* are able to produce extracellular amylases in order to make starch as carbon source available (Champ et al., 1983). However, it might be assumed that the presence of sugars, such as glucose and fructose, even at low concentrations inhibits the secretion of extracellular amylases. Other explanations might be that the three strains do not convert starch into reducing sugars or that secreted enzymes show a reduced activity at the applied pH (Guyot et al., 2000).

The *Streptococcus* sp. strain A620 behaved differently compared to the *Lactobacillus* sp. strains. While in *Lactobacillus* sp. SSF the lactic acid concentration level off after 10 h, a steadily increasing lactic acid concentration was found in the SSF shown in Figure 1D using *Streptococcus* sp. strain A620. After 28 h almost 37 g L^{-1} lactic acid was produced (Figure 1D). Productivity reached 1.32 g L^{-1} h^{-1} and yields based on dry food waste material, starch and theoretically obtainable sugars were 0.37, 1.10 and 0.67 g g^{-1}, respectively (Table 1). Hence, *Streptococcus* sp. not only converted free sugars, but also starch. *Streptococcus* sp. most likely secreted extracellular amylases to degrade starch as this has been reported for the strain *S. bovis* JB1 in presence of potato starch (Freer, 1993).

It should, however, also be admitted here that acetic acid has been formed and concentrations between 2 and 3 g L^{-1} were detected in all fermentation broths (Figure 1). Even when the acetic acid concentration was rather low compared to lactic acid, its formation may complicate downstream processing and an extra separation step, such as simulated moving bed (Lee et al., 2004), might be necessary when the target is the production of a pure lactic acid formulation. Nevertheless, due to the performance shown regarding conversion of food waste into lactic acid, further investigations were carried out with *Streptococcus* sp. strain A620.

3.2 SSF carried out at different solid-to-liquid ratios

With a higher solid-to-liquid ratio more food waste and consequently more starch and carbon sources are present for lactic acid formation. Therefore, it was hypothesized that the concentration of lactic acid is dependent on the solid-to-liquid ratio. Due to the shown performance of *Streptococcus* sp. strain A620, SSFs have been carried out at (w/w) 5%, 10%,

15% and 20% (Figure 2). It is obvious from Figure 2A, D, G and J that the lactic acid concentration increased with increasing solid-to-liquid ratio. No statistical difference was measured between repeatedly carried out fermentations ($P > 0.05$). A regression analysis revealed that lactic acid concentration increased linearly with increasing solid-to-liquid ratio (Figure 3). Due to a high viscosity, food waste suspensions with a solid-to-liquid ratio above 20% (w/w) could not be appropriately mixed and were therefore not investigated. Nevertheless, a solid-to-liquid ratio of 20% (w/w) was sufficient to produce 58 g L^{-1} lactic acid (Figures 2J and 3). The high concentrations of free glucose, fructose and sucrose additionally contributed to this high product formation (Figure 2). Generally, free sugar concentration was dependent on solid-to-liquid ratio applied. The majority of sugars used by *Streptococcus* sp. for the formation of lactic acid, however, came obviously from starch as the concentration of free sugar was not sufficient to reach the lactic acid concentrations obtained. Productivity and yield of fermentations carried out at different solid-to-liquid ratios are shown in Table 2. At 20% (w/w), productivity and yield were 2.08 g L h^{-1} and 0.63 g per g of theoretically obtainable sugars, respectively. At 5% (w/w) the potential of food waste as source of nutrients was fully exploited within 28 h and yields per g of dry food waste, starch and theoretically obtainable sugars were 0.39, 1.15 and 0.81 g, respectively. The obtained results can be compared to a recently published study of food waste hydrolysis and utilization of hydrolysate in lactic acid fermentation (Kwan et al., 2016). Kwan et al. (2016) first recovered 85% of available sugars from mixed food and bakery wastes by fungal hydrolysis and afterwards converted the sugars recovered at a yield 0f 0.94 g g^{-1} using *L. casei* Shirota into lactic acid. Hence the overall yield was 0.80 g g^{-1} which is near identical to the yield of 0.81g g^{-1} obtained in this study. The overall yield per g of dry food waste of 0.39 g (Table 2) obtained in this study was higher than the 0.23-0.27 g obtained by Kwan et al. at a comparable mixed food waste composition (Kwan et al., 2016). The productivity of 2.61 g L^{-1}

h^{-1} found by Kwan et al., however, was higher than the productivity obtained here. It is of particular interest for the development of decentralized processes that the hydrolysis of the substrate can be skipped and it can directly efficiently converted into lactic acid. The food waste blend in the present study had a solid-to-liquid ratio of 20% (w/w), but depending on the source of food waste and its composition the ratio may change. Therefore, further investigations with better stirring equipment are recommended in order to identify the impact of a higher solid content on *Streptococcus* sp. SSFs.

The FAN concentration was not affected to the same extent by the solid-to-liquid ratio as the concentration of free sugars. Even though the FAN concentration increased from 179 to 350 mg L^{-1} with an increase in the solid-to-liquid ratio from 5 to 10% (w/w), no further rise was observed at higher solid-to-liquid ratios. Remarkable was the constant number of living and total cells (Figure 2C, F, I and L). No sufficient data were collected to calculate the exponential growth rate, but growth was obviously fast in all cultures during the first 2 to 5 h and levelled off afterwards. This was also the period were FAN was consumed. Interestingly, the number of total cells and the number of living cells in all fermentations did not decrease after growth stopped. Contrarily, in previously reported studies of our group carried out with *Bacillus coagulans*, number of living cells decreased after growth stopped predominantly due to nitrogen limitation (Neu et al., 2016; Pleissner et al., 2016a). This may indicate that in the present study sufficient nitrogen was available to keep a predominant fraction of cells alive which causes a continuous production of lactic acid by further degradation of food waste (Figures 2 and 4).

3.3 SSF under non-sterile conditions

348 The previous experiments were carried out under sterile conditions in order to systematically
349 investigate SSF. However, autoclavation is energy intensive and processes running at
350 industrial scale are hardly economically feasible (Li et al., 2014). Therefore, SSF was carried
351 out under non-sterile condition at a solid-to-liquid ratio of 20% (w/w). There was obviously
352 no significant difference in lactic acid production ($P > 0.05$), productivity and yields
353 compared to sterile SSF (Figures 2 and 4, and Tables 2 and 3). Lactic acid concentration
354 increased within 28 h to 55 g L^{-1} (Figure 4A). Free glucose, fructose and sucrose were
355 detected at concentrations of 1.8 g L^{-1}, 6.3 g L^{-1} and 9.3 g L^{-1}, respectively, and except
356 sucrose completely consumed for lactic acid production. Of particular interest is that the
357 acetic acid concentration remained despite non-sterile conditions below 2 g L^{-1}.
358
359 **3.4 SSF carried out at technical scale**
360 In order to create the basis for a scale-up of food waste valorization processes, SSF has been
361 investigated at technical scale using *Streptococcus* sp. strain A620 and a solid-to-liquid ratio
362 of 20% (w/w, not shown). The concentration of lactic acid reached 60.5 g L^{-1} within 28 h
363 resulting in a productivity of 2.16 g L^{-1} h^{-1}. Yields of lactic acid per g of dry food waste,
364 starch and sugars theoretically obtainable sugars were 0.25, 0.75 and 0.64 g, respectively, and
365 highly comparable to the observations made at laboratory scale (Tables 2 and 3).
366
367 **3.5 Direct production of lactic acid from organic matter**
368 Some bacterial strains used for direct lactic acid formation are unable to secrete hydrolytic
369 enzymes which are necessary for the degradation of organic matter. Therefore, degradation of
370 organic material mostly based on added enzymes, such as α-amylase, glucoamylase,
371 commercially available enzyme formulations SAN Super 240L, Fructozyme L, Celluclast
372 1.5L, Novozyme 188 and Cellic CTec2, or using a crude enzyme extract from *A. niger* (Table

4). The application of specific enzyme formulations is cost-intensive but contributes to the degradation of recalcitrant structures, such as cellulose, hardwood, paper sludge, Jerusalem artichoke powder and corn stover. When delignified and pulverized hardwood pulp was treated with Cellic CTec2 and the released sugars directly converted into lactic acid using *L. planatarum* a yield of 0.88 g per g of sugars available was obtained at a productivity of 2.3 g L^{-1} h^{-1} (Hama et al., 2015). The degradation of cellulose (Yáñez et al., 2003) and paper sludge (Marques et al., 2008) was carried using Celluclast and Novozyme. The released sugars were converted into lactic acid by *L. coryniformis* and *L. rhamnosus* and yields of 0.89 g per g substrate and 0.97 g per g available sugars, respectively, were obtained. The productivity of *L. coryniformis*, however, was six times above the productivity of *L. rhamnosus* (Table 4). Hu et al. hydrolyzed NaOH pretreated and untreated corn stover using Cellic CTec2 and achieved lactic acid productivities of 1.6 and 1.9 g L^{-1} h^{-1} with *B. coagulans* and *L. pentosus*, respectively, at a similar yield in fed-batch cultures (Hu et al., 2016; Hu et al., 2015).

For less recalcitrant materials the application of enzyme formulations does not necessarily result in better degradation and consequently better lactic acid yields and productivities (Table 4). The strain *Geobacillus stearothermophilus* is able to secret extracellular amylases to degrade starch (Smerilli et al., 2015). This ability was used to convert raw potato starch directly into lactic acid. Yield and productivity were 0.66 g per g starch and 1.8 g L^{-1} h^{-1} (Table 4). Contrarily, the degradation of potato slurry using α-amylase and direct conversion of sugars into lactic acid using *L. plantarum* was less productive (Table 4) (Anuradha et al., 1999). In another process, food waste was simultaneously treated with SAN Super240L and nutrients used by the strain *L. delbrueckii* to produce lactic acid at a yield of 0.66 g per g available sugars and a productivity of 0.7 g L^{-1} h^{-1} (Kim et al., 2003). The productivity, however, is far below the productivity obtained in this study performed without additional

enzymes (Tables 1-3). It should be admitted here, that productivity and yield are influenced by the presence of other nutrients and thus, the right strain needs to be identified in order to directly degrade and convert a certain organic matter under certain conditions.

It was expected prior to the fermentation carried out under non-sterile conditions that microbial contaminations may cause a production of other organic acids than lactic acid. Tang et al., for instance, investigated the conversion of food waste into lactic acid using an indigenous microbial community in 10 L fermentation reactors and once-a-day feeding (Tang et al., 2016). The indigenous microbial community was present in raw food waste and consisted predominantly of *Lactobacillus*. They studied the effects of pH, temperature and solid-to-liquid rate. In their study, beside a high concentration of lactic acid (around 40 g L^{-1}) also acetic, propionic and butyric acid at around 10 g L^{-1} were found. However, this was not the case in the non-sterile fermentation shown in Figure 4. The fact that food waste was immediately brought to the laboratory certainly contributes to that result. Nevertheless, it may also be concluded that *Streptococcus* sp. outcompeted a possibly present indigenous microbial community.

It can be seen from the values shown in Tables 2 and 3 that the performance of SSF using *Streptococcus* sp. strain A620 was better than most of the processes listed as references in Table 4. The fact that no sterilization and hydrolysis are needed make SSF for lactic acid production a simple process that can be implemented relatively fast at locations where food waste appears in large amounts, such as densely populated urban areas and food industries. The simplicity of the fermentation process is comparable to the process of anaerobic degradation for biogas production, but the conversion of carbon into lactic acid is more efficient as no CO_2 is produced by microbial activity. Nevertheless, even though upstream

processing can be omitted downstream processing needs still to be carried out in order to produce pure lactic acid formulations.

3.6 Downstream processing

No remaining free sugars and acetic acid were present in the fermentation broth which certainly eases downstream processing. Nevertheless, advanced techniques were still needed to separate impurities and salts introduced by the food waste, and acids and base used for pH regulation. Downstream processing steps were selected in order to remove undissolved substances (micro- and nanofiltration), separate an- and cations (softening, mono- and bipolar electrodialyses) and concentrate lactic acid (distillation). In Figure 5 is shown the concentrations of salt ions and lactic acid during the downstream processing. In the 48 L of fermentation broth obtained from technical scale SSF the majority of ions was made of sodium, potassium and chloride with concentrations of 16.1 g L^{-1}, 1.1 g L^{-1} and 3.6 g L^{-1}, respectively. The lactic acid concentration was 60.5 g L^{-1}. After the fermentation broth was micro- and nanofiltrated, the majority of ions was made of 12.8 g L^{-1} sodium, 0.9 g L^{-1} potassium and 3.0 g L^{-1} chloride. The lactic acid concentration decreased due to dilution to 45.1 g L^{-1}. In order to concentrate lactic acid and to separate it from salts, mono- and bipolar electrodialysis has been carried out. After electrodialysis the lactic acid concentration increased to 171 g L^{-1}. The concentration of sodium, potassium and chloride was with 2.7 g L^{-1}, 0.3 g L^{-1} and 11.6 g L^{-1}, respectively, still high. Hence, anion- and cation-exchange was carried out which decreased the concentration of all salt ions to less than 0.01 g L^{-1}. However, due to a strong dilution the lactic acid concentration decreased by 70% to 54.1 g L^{-1}. Therefore, as a final step the water was evaporated in order to concentrate lactic acid. The final L(+)-lactic acid formulation had a volume of 1.6 L and a concentration of 702 g L^{-1}, and thus 38% of the initial lactic acid could be recovered from fermentation broth. The fact that

448 only 38% of lactic acid was recovered is certainly a drawback of the presented downstream
449 processing and further research is needed to avoid the loss of 62% of the product.
450 Nevertheless, it is of interest that conventional downstream techniques can be applied even
451 when a complex nutrient source, such as food waste, was used in fermentations. Pleissner et
452 al. used the same downstream processing technique but included an ion-exchange
453 chromatography after microfiltration carried out using the resin Amberlite FPA 53 und 12.5
454 mM H_2SO_4 as eluent (Pleissner et al., 2016b). By this approach 90% of the initial lactic acid
455 was recovered. Additionally carried out mono- and bipolar electrodialysis enabled a recovery
456 of additives in form of NaOH and HCl used during fermentation and downstream processing.
457 A recovery rate of 90% is comparable to state-of-the-art method which is based on
458 precipitation of calcium lactate and recovery of lactic acid by adding H_2SO_4 (Min et al.,
459 2011). This method, however, generates amounts of $CaSO_4$, while the technology used here
460 even allows a recycling of water in subsequently carried out fermentation (Pleissner et al.,
461 2016b).
462
463 The optical purity of the obtained formulation was 99.7%. Inkinen et al. reviewed the quality
464 requirements of lactic acid formulation used in poly(lactic acid) synthesis and stated that the
465 impurities should be below 0.05 mol % (Inkinen et al., 2011). Chloride-ions are the major
466 source of impurities in the formulation obtained here. The concentration found was 5 g L^{-1},
467 and thus below 0.05 mol %.
468
469 **3.5. Mass balance**
470 A mass balance from food waste to pure lactic acid is shown in Figure 6 in order to illustrate
471 the experimental findings. The mass balance starts with a theoretical amount of 1,000 kg dry
472 food waste which consists of 335 kg starch, 148 kg proteins, 129 kg fat and 85 kg free sugars.

SSF is carried out with *Streptococcus* sp. which converts the waste into lactic acid at yield of 0.39 kg kg^{-1}. This results in the production of 390 kg lactic acid. After downstream processing 148.2 kg of lactic acid is recovered in form of a pure formulation. However, 241.8 kg of lactic acid is lost which clearly shows the drawback and further research potential of the used downstream processing. In average, 78.3% of the initial dry weight of food waste was saccharified and converted into lactic acid by SSF. Therefore, SSF of food waste offers an interesting opportunity to significantly reduce the amount of waste that needs to be treated or disposed and to create value from waste. The remaining solids (21.7 %) consist of bacterial biomass and particularly fat which may serve as feedstock in material utilization approaches, such as the production of plasticizer and detergents (Pleissner et al., 2015b).

4. Conclusions

Lactic acid was produced from blended food waste in SSF at laboratory and technical scales. *Lactobacillus* sp. strains did not show an efficient conversion of food waste material into lactic acid. *Streptococcus* sp., however, liquefied the material and produced lactic acid. Maximum productivity of 2.16 g L^{-1} h^{-1} was achieved at technical scale, while the highest yield of 0.81 g g^{-1} of theoretically present sugars was obtained in fermentations carried at a solid-to-liquid ratio of 5% (w/w). From a 20% (w/w) food waste blend 58 g L^{-1} lactic acid was produced. Due to a linear relationship between solid-to-liquid ratio and lactic acid titer much higher concentrations can be obtained when higher solid-to-liquid ratios are treated with appropriate equipment. Irrespective of the scales and if SSF was carried out under sterile or non-sterile conditions, *Streptococcus* sp. directly converted food waste into lactic acid without considerable production of other acids.

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621 **Figure captions**

622 **Figure 1.** Strain comparison. Change of glucose (closed circle), fructose (open triangle),
623 sucrose (open square), acetic acid (open star) and lactic acid (open circle) concentrations
624 during SSF using *Lactobacillus* sp. strains A28a (A), A59 (B) or A211 (C), or using
625 *Streptococcus* sp. strain A620 (D) at a solid-to-liquid ratio of 10% (w/w).

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627 **Figure 2.** Influence of solid-to-liquid ratio. Change of glucose (closed circle), fructose (open
628 triangle), sucrose (open square), FAN (closed triangle), acetic acid (open star) and lactic acid
629 (open circle) concentrations during SSF using *Streptococcus* sp. strain A620 carried out at a
630 solid-to-liquid ratio (w/w) of 5% (A and B), 10% (D and E), 15% (G and H) or 20% (J and
631 K). The corresponding total number of cells (dashed line) and number of living cells (solid
632 line) are shown in C, F, I and L.

633

634 **Figure 3.** Relationship between lactic acid titer and solid-to-liquid ratio (line was forced to
635 zero).

636

637 **Figure 4.** SSF under non-sterile conditions. Change of glucose (closed circle), fructose (open
638 triangle), sucrose (open square), FAN (closed triangle), acetic acid (open star) and lactic acid
639 (open circle) concentrations during SSF using *Streptococcus* sp. strain A620 carried out at a
640 solid-to-liquid ratio of 20% (w/w, A and B) under non-sterile conditions. The corresponding
641 total number of cells (dashed line) and number of living cells (solid line) are shown in C.

642

643 **Figure 5.** Downstream processing. Ions and lactic acid concentrations during different
644 downstream processing steps (ED = electrodialysis).

645

646 **Figure 6.** Mass balance from food waste to lactic acid (*downstream processing was not
647 optimized). All figures are based on dry weight.
648

649 **Table 1.** Lactic acid productivity within 28 h of cultivation time (P), yield of lactic acid per g
650 of dry food waste (Y_{FW}), per g of starch (Y_{ST}) and per g of sugars theoretically present (Y_{SU})
651 of SSFs carried out at laboratory scale using different strains.
652

Strain	P [g L^{-1} h^{-1}]	Y_{FW} [g g^{-1}]	Y_{ST} [g g^{-1}]	Y_{SU} [g g^{-1}]
Lactobacillus sp. strain A28a	0.27	0.07	0.22	0.14
Lactobacillus sp. strain A59	0.53	0.14	0.43	0.29
Lactobacillus sp. strain A211	0.37	0.14	0.41	0.24
Streptococcus sp. strain A620	1.32	0.37	1.10	0.67

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667 **Table 2.** Lactic acid productivity within 28 h of cultivation time (P), yields of lactic acid per g
668 of dry food waste (Y_{FW}), per g of starch (Y_{ST}) and per g of sugars theoretically present (Y_{SU})
669 of SSFs carried out at laboratory scale at different solid-to-liquid ratios using *Streptococcus*
670 sp. strain A620.
671

Solid-to-liquid ratio [%, w/w]	P [g L^{-1} h^{-1}]	Y_{FW} [g g^{-1}]	Y_{ST} [g g^{-1}]	Y_{SU} [g g^{-1}]
5	0.69	0.39	1.15	0.81
10	1.25	0.35	1.04	0.73
15	1.67	0.31	0.94	0.67
20	2.08	0.29	0.88	0.63

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683 **Table 3.** Lactic acid productivity within 28 h of cultivation time (P), yields of lactic acid per g
684 of dry food waste (Y_{FW}), per g of starch (Y_{ST}) and per g of sugars theoretically present (Y_{SU})
685 of SSFs carried out at laboratory scale under non-sterile conditions or at technical scale under
686 sterile conditions using *Streptococcus* sp. strain A620 and a solid-to-liquid ratio of 20%
687 (w/w).
688

Batch	P [g L^{-1} h^{-1}]	Y_{FW} [g g^{-1}]	Y_{ST} [g g^{-1}]	Y_{SU} [g g^{-1}]
Non-sterile conditions	2.12	0.27	0.79	0.58
Technical scale	2.16	0.25	0.75	0.64

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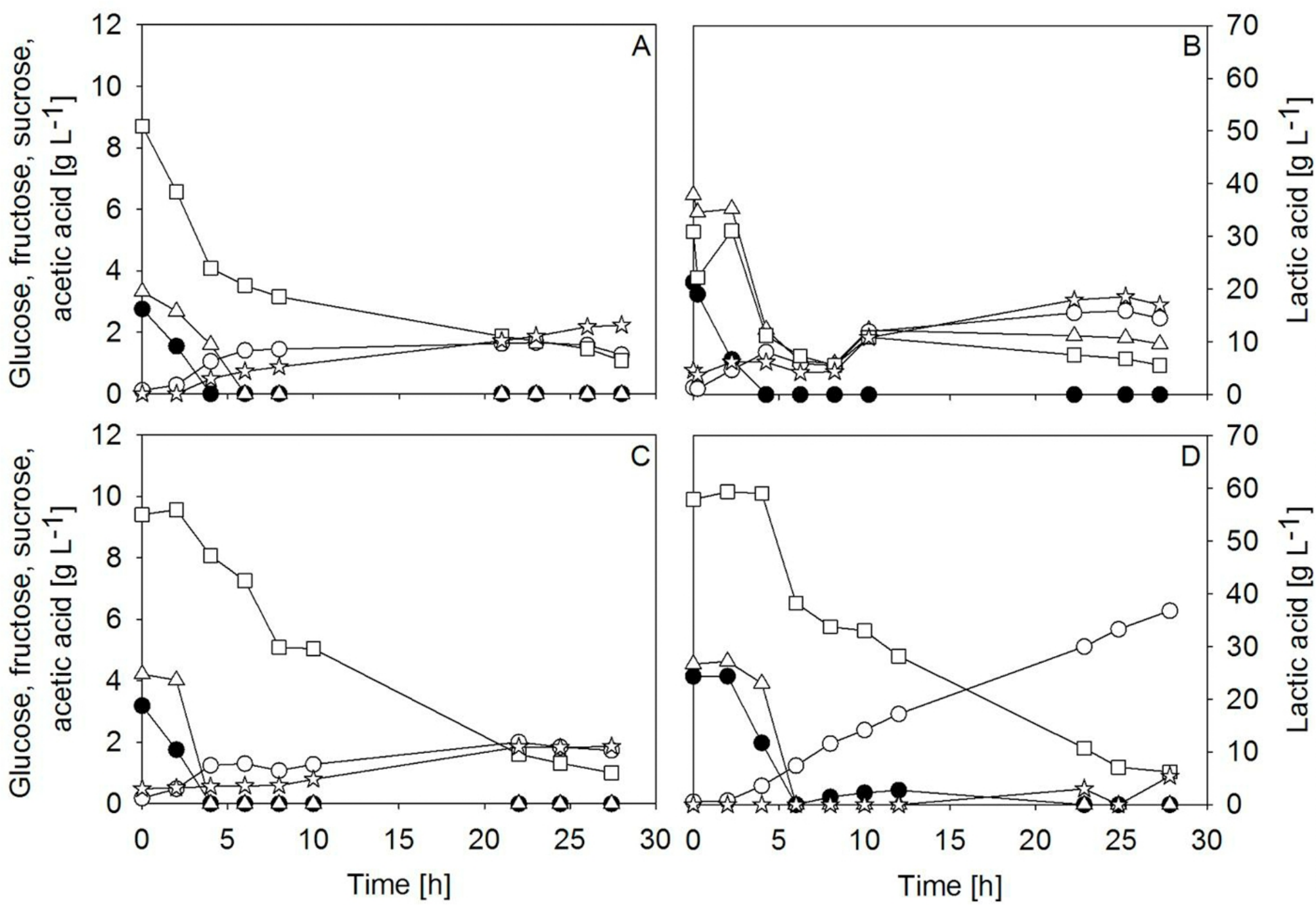
Table 4. Overview of lactic acid productivity (P), yields of lactic acid per g of dry substrate (Y_{SB}), per g of starch (Y_{ST}) and per g of sugars (Y_{SU}) of SSFs carried out using different substrates and microorganisms.

Substrate	Strain	P [g L^{-1} h^{-1}]	Y_{SB} [g g^{-1}]	Y_{ST} [g g^{-1}]	Y_{SU} [g g^{-1}]	Ref.
Food waste[a]	*L. rhamnosus*	0.9	0.45	-	-	(Wang et al., 2009)
Food waste	*Indigenous microbiota*	0.3	0.46	-	-	(Tang et al., 2016)
Food waste[b]	*L. delbrueckii*	0.7	-	-	0.75	(Kim et al., 2003)
Raw potato starch	*G. stearothermophilus*	1.8	-	0.66	-	(Smerilli et al., 2015)
Potato slurry[c]	*L. plantarum*	1.2	0.7	-	-	(Anuradha et al., 1999)
Corn stover[d]	*L. pentosus*	1.9	0.66	-	-	(Hu et al., 2016)
Corn stover[d]	*B. coagulans*	1.6	0.68	-	-	(Hu et al., 2015)

Jerusalem artichoke powder[e]	*B. coagulans*	2.5	-	-	0.96	(Wang et al., 2013)
Recycled paper sludge[f]	*L. rhamnosus*	2.9	-	-	0.97	(Marques et al., 2008)
Filter paper[g]	*L. coryniformis*	0.5	0.89	-	-	(Yáñez et al., 2003)
Hardwood[h]	*L. plantarum*	2.3	-	-	0.88	(Hama et al., 2015)

[a]Glucoamylase was produced using *A. niger* and directly applied to hydrolyze food waste, [b]Food waste was hydrolyzed using SAN Super 240L and fermentation was carried out in presence of yeast extract, [c]Hydrolysis was carried out using α-amylase, [d]Carried out as fed-batch process using Cellic CTec2, [e]Substrate was hydrolyzed using Fructozyme L and released reducing sugars converted to lactic acid in a fed-batch culture, [f]Paper was hydrolyzed using Celluclast 1.5 L and Novozym 188, [g]Filter paper was hydrolyzed using Celluclast and Novozym, lactic acid was produced as D(-)-isomer, [h]Pulverized pulp (delignified) was hydrolyzed with Cellic CTec2.


A
B
C
D
Glucose, fructose, sucrose, acetic acid [g L-1]
Lactic acid [g L-1]
Time [h]
0
2
4
6
8
10
12
0
10
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30
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50
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70
0
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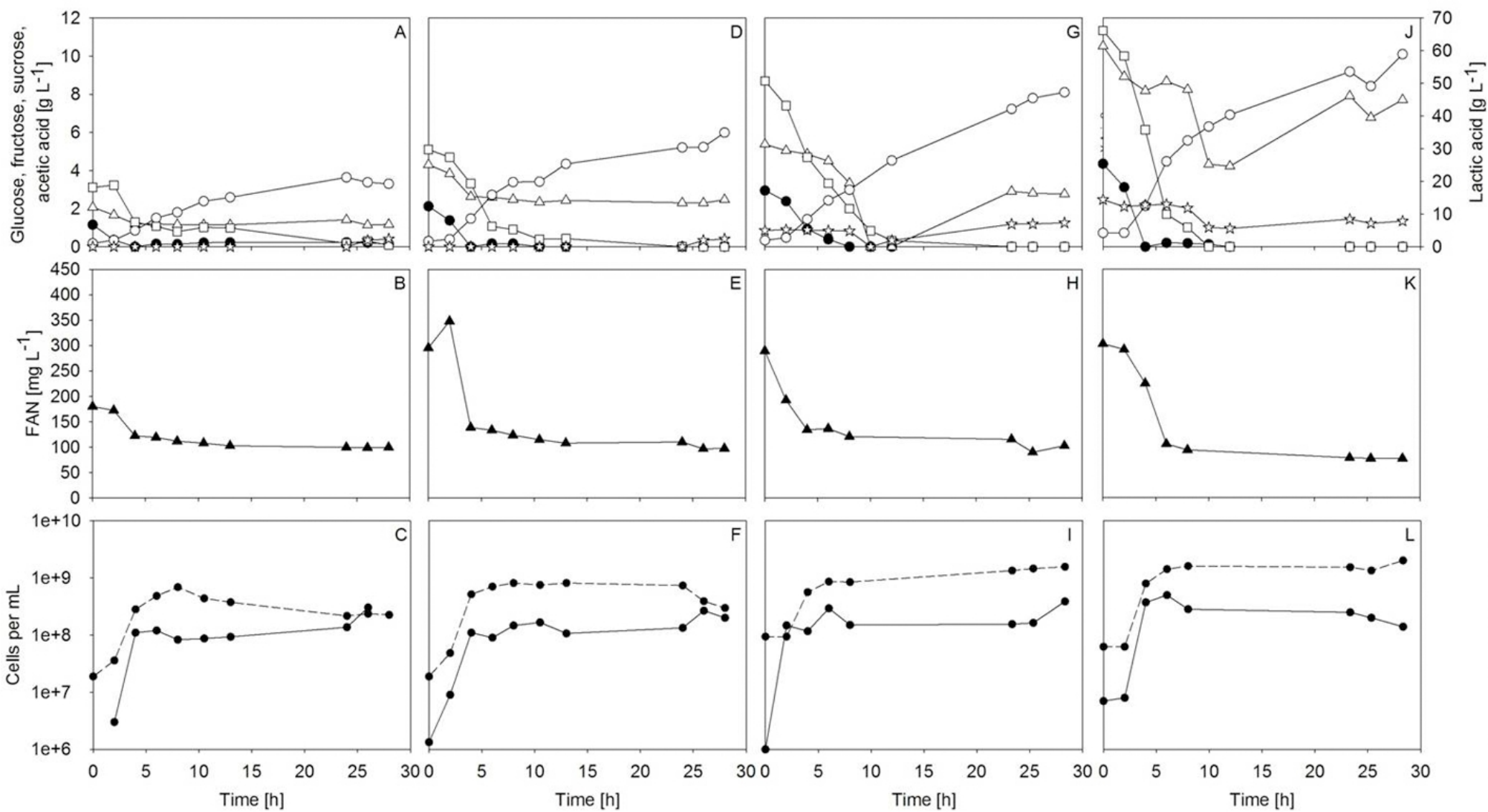
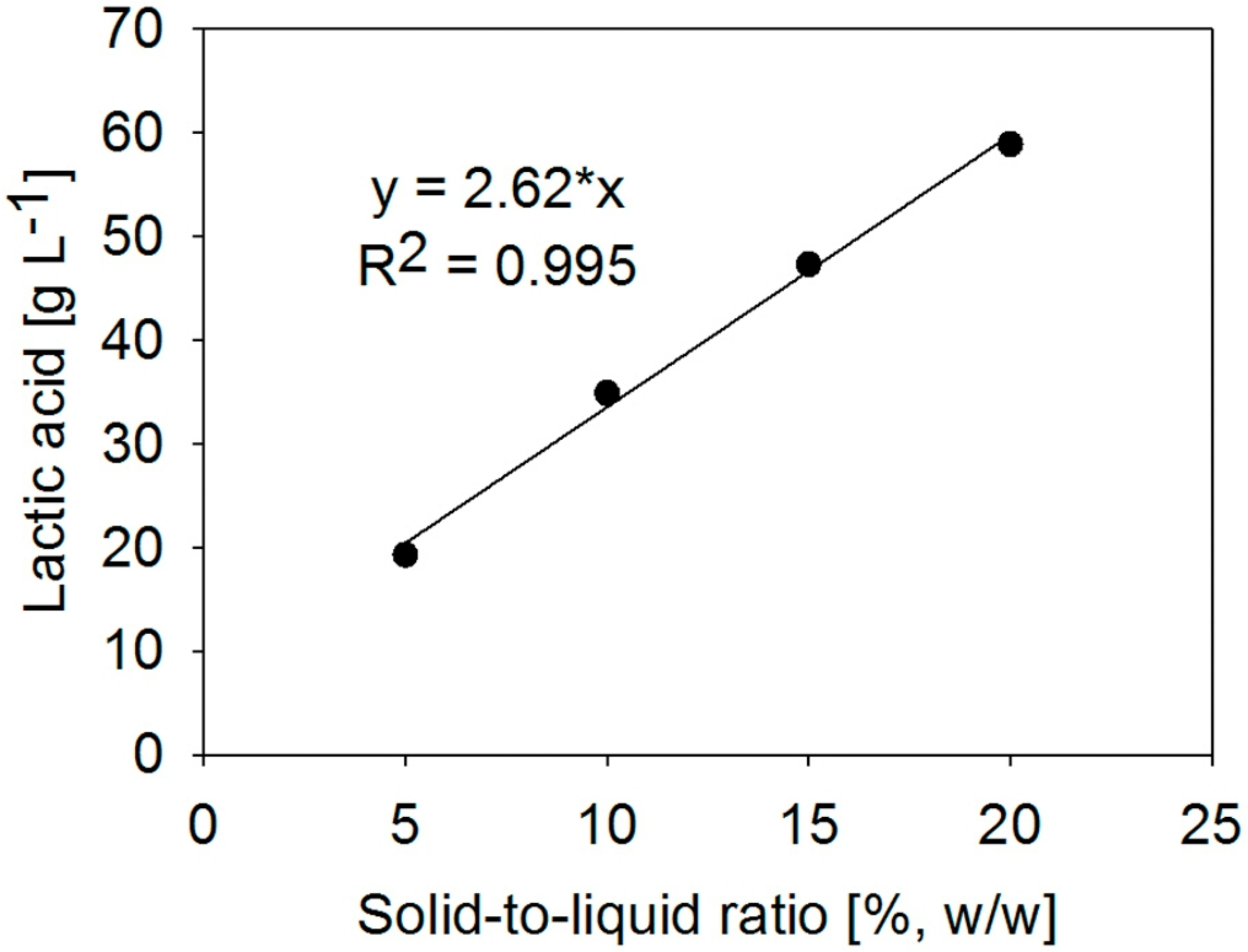
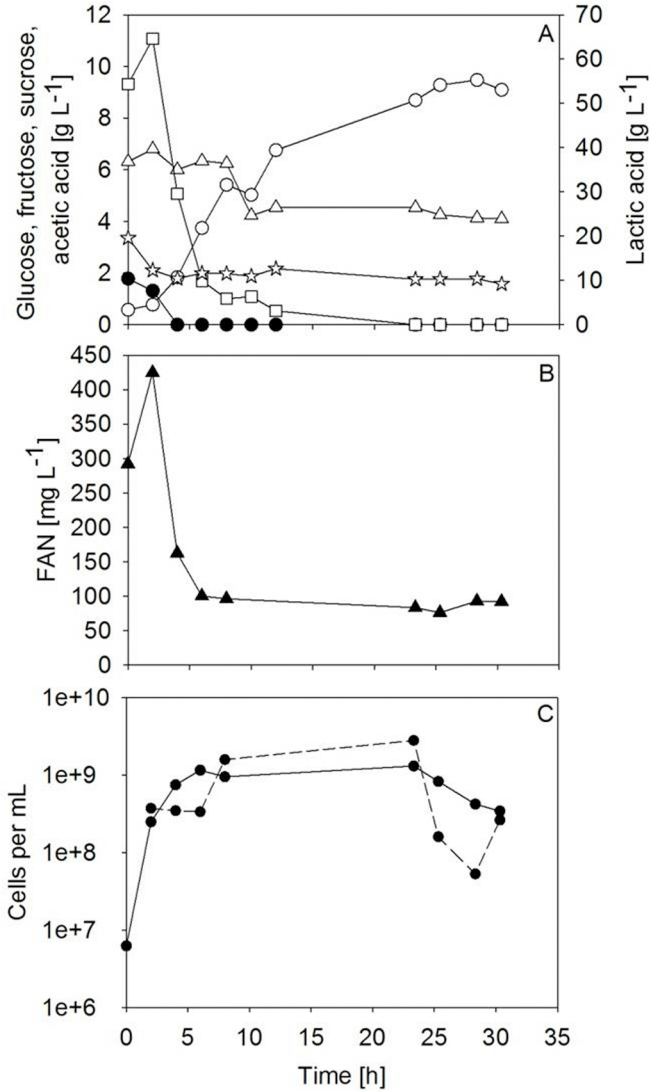
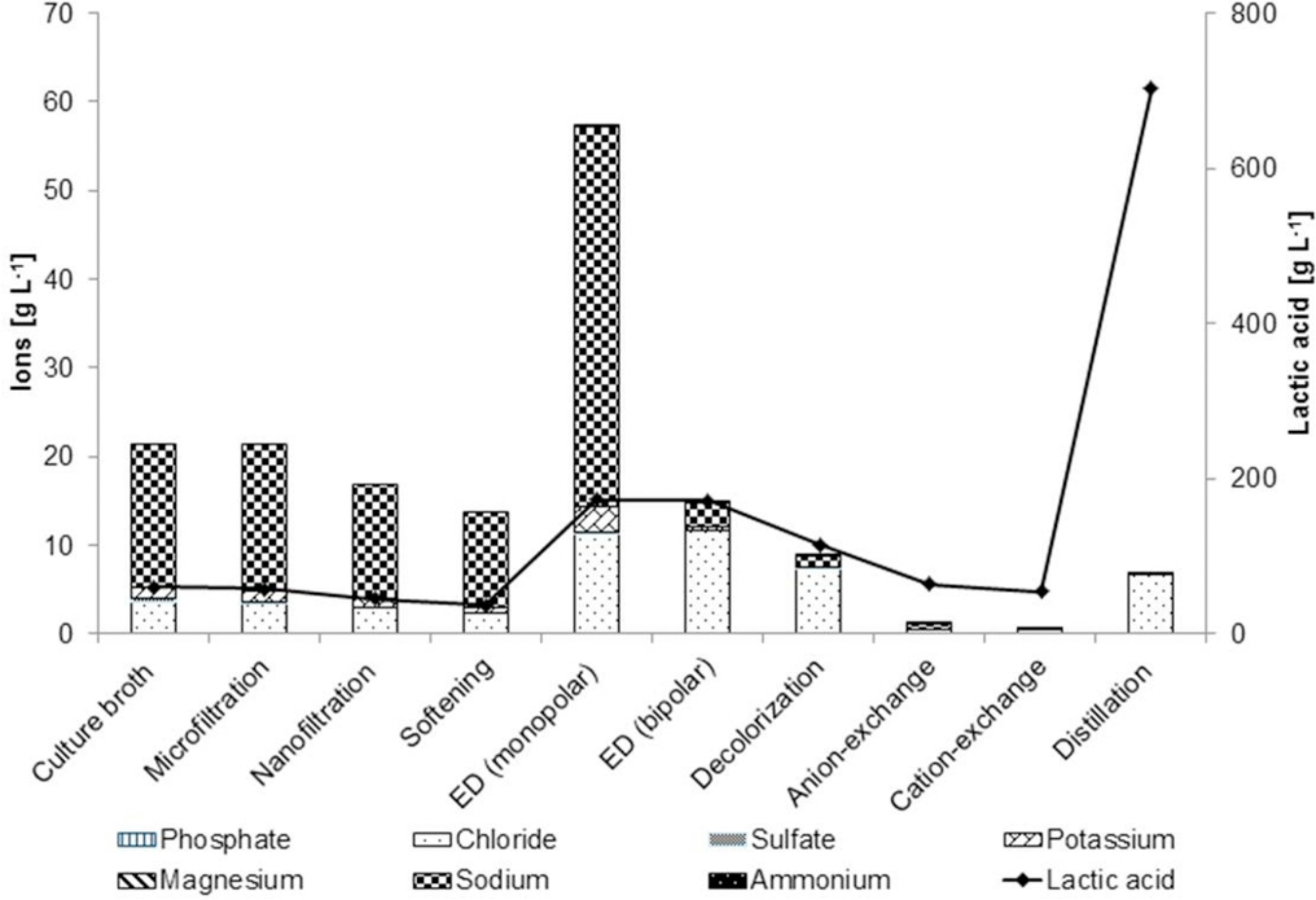
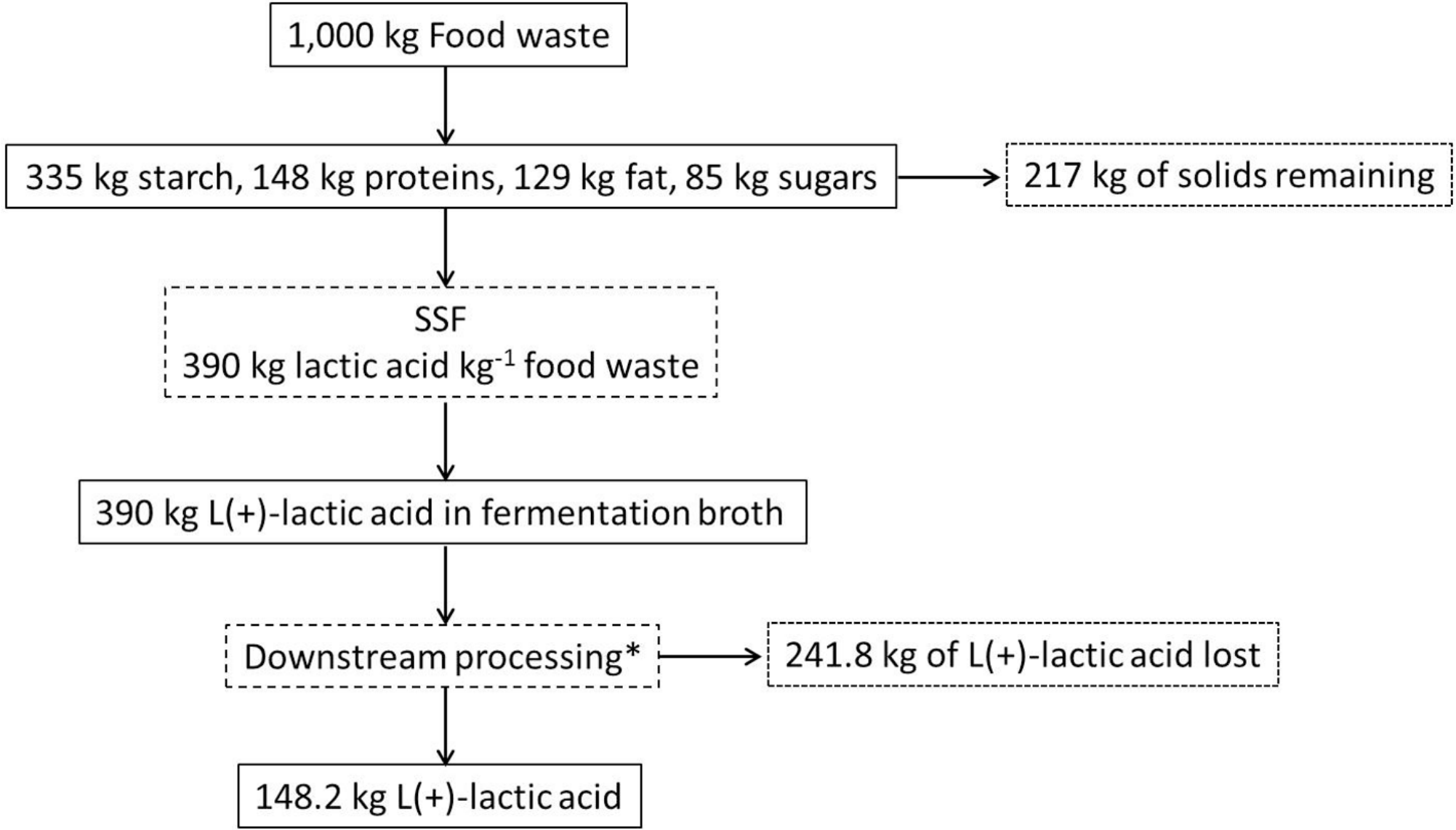

A
B
C
D
E
F
G
H
I
J
K
L
Glucose, fructose, sucrose, acetic acid [g L-1]
Lactic acid [g L-1]
FAN [mg L-1]
Cells per mL
Time [h]
Time [h]
Time [h]
Time [h]


70
60
50
40
30
20
10
0
Lactic acid [g L-1]
y = 2.62*x
R2 = 0.995
0
5
10
15
20
25
Solid-to-liquid ratio [%, w/w]


A
Glucose, fructose, sucrose, acetic acid [g L-1]
Lactic acid [g L-1]
B
FAN [mg L-1]
C
Cells per mL
Time [h]


Ions [g L-1]
Lactic acid [g L-1]
0
10
20
30
40
50
60
70
0
200
400
600
800
Culture broth
Microfiltration
Nanofiltration
Softening
ED (monopolar)
ED (bipolar)
Decolorization
Anion-exchange
Cation-exchange
Distillation
Phosphate
Chloride
Sulfate
Potassium
Magnesium
Sodium
Ammonium
Lactic acid


1,000 kg Food waste
335 kg starch, 148 kg proteins, 129 kg fat, 85 kg sugars
217 kg of solids remaining
SSF
390 kg lactic acid kg-1 food waste
390 kg L(+)-lactic acid in fermentation broth
Downstream processing*
241.8 kg of L(+)-lactic acid lost
148.2 kg L(+)-lactic acid