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Techno-economic assessment of non-sterile batch and continuous production of lactic acid from food waste

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(Article begins on next page)

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Keywords: Simultaneous saccharification and fermentation; Continuous flow fermentations; Indigenous consortium; Viscous food waste; Economic and energy evaluation

Corresponding Author: Dr. Daniel Pleissner,

Corresponding Author's Institution: Leuphana University Lüneburg

First Author: Jan C Peinemann

Order of Authors: Jan C Peinemann; Francesca Demichelis; Silvia Fiore; Daniel Pleissner

Abstract: Non-sterile lactic acid (LA) fermentation of highly viscous food waste was demonstrated in batch and continuous flow fermentations. With Streptococcus sp., an indigenous consortium, and/or applied glucoamylase, food waste was fermented without addition of external carbon or nitrogen sources. Experimental results were used for economic and energy evaluations under consideration of different catchment area sizes from 50,000 to 1,000,000 inhabitants. During batch mode, addition of glucoamylase resulted in a titer (after 24 hours), yield, and productivity of 50 g L-1, 63%, and 2.93 g L-1 h-1, respectively. While titer and yield were enhanced, productivity was lower during continuous operation and 69 g L-1, 86%, and 1.27 g L-1 h-1 were obtained at a dilution rate of 0.44 d-1 when glucoamylase was added. Both batch and continuous flow fermentations were found economically profitable with food waste from 200,000 or more inhabitants.

LEUPHANA
UNIVERSITÄT LÜNEBURG

Leuphana Universität Lüneburg · Nachhaltige Chemie· 21335 Lüneburg

Prof. Christian Larroche
Editor
Bioresource Technology

Prof. Dr. Daniel Pleissner

Assistant Professor for Sustainable Chemistry with Focus on Resource Efficiency

Leuphana Universität Lüneburg
Institut für Nachhaltige Chemie
Universitätsallee 1
21335 Lüneburg

Fon 04131.677-1350
Fax 04131.677-2848
Mobil 0172.1638.754
daniel.pleissner@leuphana.de

www.leuphana.de

7. Juni 2019

Ref.: Cover Letter

Dear Prof. Larroche**,**
We have omitted Table 3 as suggested.

Many thanks for your kind attention. Please do not hesitate to contact me anytime if you need anything else from our side.

Yours sincerely,

Prof. Dr. Daniel Pleissner

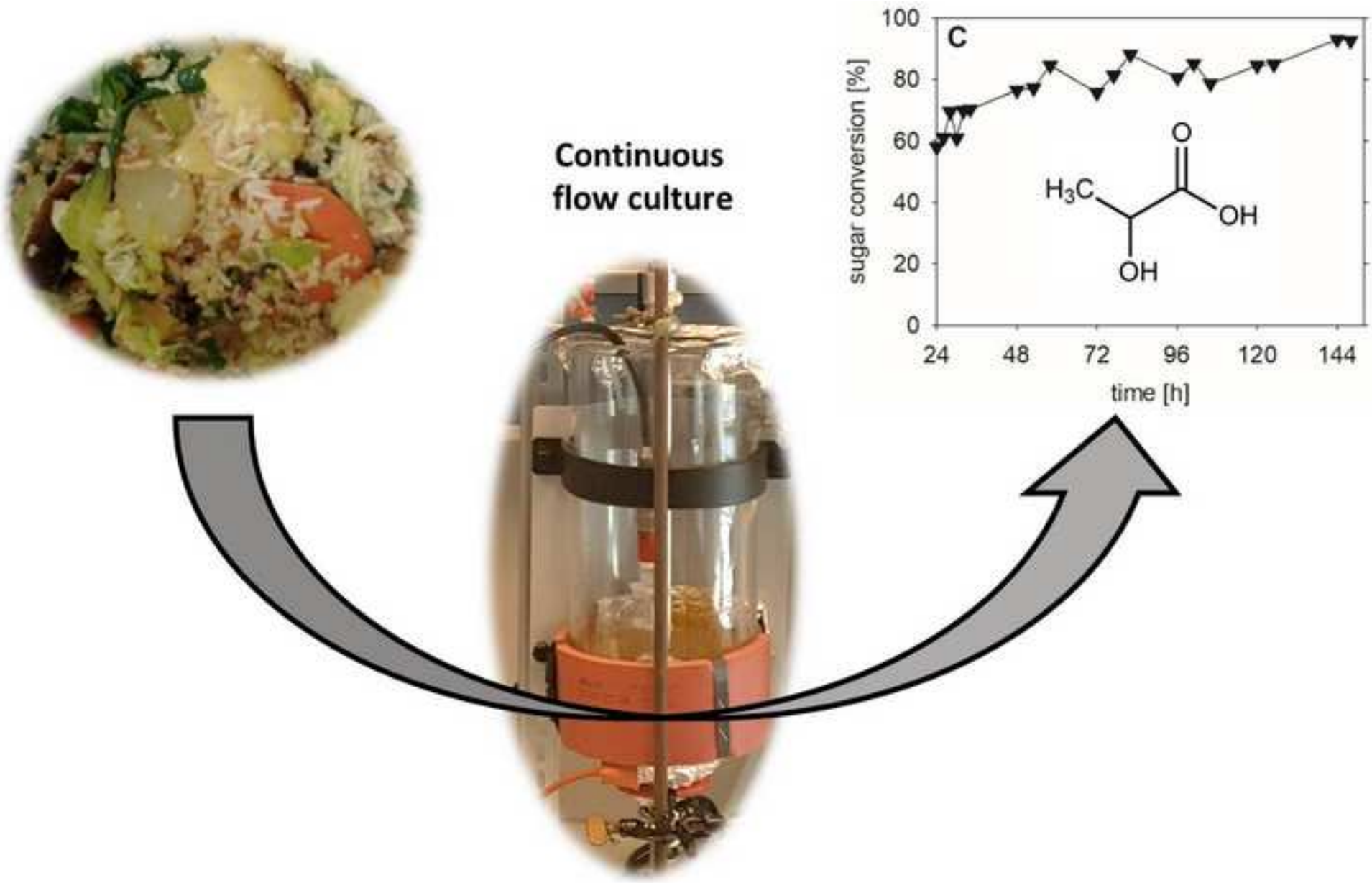
***Detailed Response to Reviewers**

Comment to Editor:

This manuscript is not a review. So, please incorporate to the text data from table 3 useful for discussion. The table itself can be submitted as supplementary material (take care to instructions)

Thanks for pointing this once more. We overlooked this comment. Table 3 has now been omitted and information is incorporated to the text.

Graphical Abstract (for review)



Highlights

- Highly viscous blended food waste was fermented to lactic acid without dilution
- Maximum lactic acid titer during batch operation was 50 g L^{-1}
- Continuous flow fermentation at 0.44 d^{-1} gave up to 74 g L^{-1} lactic acid
- Lactic acid selectivity was up to 91 % with indigenous culture
- Plants with minimum 200,000 inhabitants in catchment area are economically profitable

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Techno-economic assessment of non-sterile batch and continuous production of lactic acid from food waste

Jan Christoph Peinemann[a], Francesca Demichelis[b], Silvia Fiore[b], Daniel Pleissner[a]*

[a] Sustainable Chemistry (Resource Efficiency), Institute of Sustainable and Environmental Chemistry, Leuphana University of Lüneburg, Universitätsallee 1, C13.203, 21335 Lüneburg, Germany

[b] DIATI, Politecnico di Torino, Corso Duca Degli Abruzzi 24, 10129 Torino, Italy

*Corresponding author: Daniel Pleissner, Sustainable Chemistry (Resource Efficiency), Leuphana University of Lüneburg, Universitätsallee 1, C13.203, 21335 Lüneburg, Germany, E-mail: daniel.pleissner@leuphana.de, Tel: +49 4131 677 1350

Abstract

Non-sterile lactic acid (LA) fermentation of highly viscous food waste was demonstrated in batch and continuous flow fermentations. With *Streptococcus* sp., an indigenous consortium, and/or applied glucoamylase, food waste was fermented without addition of external carbon or nitrogen sources. Experimental results were used for economic and energy evaluations under consideration of different catchment area sizes from 50,000 to 1,000,000 inhabitants. During batch mode, addition of glucoamylase resulted in a titer (after 24 hours), yield, and productivity of 50 g L^{-1}, 63%, and 2.93 g L^{-1} h^{-1}, respectively. While titer and yield were enhanced, productivity was lower during continuous operation and 69 g L^{-1}, 86%, and 1.27 g L^{-1} h^{-1} were obtained at a dilution rate of 0.44 d^{-1} when glucoamylase was added. Both batch and continuous flow fermentations were found economically profitable with food waste from 200,000 or more inhabitants.

Keywords: Simultaneous saccharification and fermentation; Continuous flow fermentations; Indigenous consortium; Viscous food waste; Economic and energy evaluation

1. Introduction

Each year, up to 15 million tons of food waste appear in Germany. Large-scale consumers, like canteens, restaurants, and cafeterias contribute 1.8 million tons to the total sum (Scherhaufer et al., 2012). It is estimated that 48.5% of this could be avoided (Baier & Reinhard, 2007), leaving still roughly 0.9 million tons of food waste to be treated the most efficient way (Dugmore et al., 2017).

Macroscopically, food waste may contain potatoes, rice, noodles, vegetables, fish, and meat. This heterogeneous substrate can be prepared for treatment by thorough blending. Through this mechanical processing, a highly viscous solution is obtained. This solution primarily comprises of carbohydrates, proteins, and lipids. These molecules possess high functionality, emphasizing the imperative to conserve as much functionality as possible during utilization processes. In this sense, material valorization should be pursued prior to energetic usage (Ragauskas et al., 2006). Furthermore, economic considerations favor specialty chemicals over fuels (RedCorn et al., 2018). Combined utilization via lactic acid (LA) fermentation followed by anaerobic digestion leads to a profit of 42 Euro per ton of food waste treated. Exclusive methane production, on the other hand, would only give 5 Euro per ton of food waste (Bastidas-Oyanedel & Schmidt, 2018). With microbial fermentations, small chain organic molecules, such as succinic acid (Zhang et al., 2013), 2,3-butanediol (Dai et al., 2015), or LA (Esteban & Ladero, 2018; Pleissner et al., 2017), can be produced. Especially LA has gained prominence owing to its versatile applications e.g. as food preservative, additive in cosmetics, and platform chemical for the preparation of poly-LA (Jantasee et al., 2017).

Having highly viscous food waste as substrate, batch fermentation is the most common approach to produce LA. Besides batch processes, means of continuous flow fermentations exist. Continuous systems offer better plant capacity utilization (Eş et al., 2018). Continuous flow fermentations require substrate feed and simultaneous withdrawal of fermentation broth. Particularly, feed and withdrawal as well as mixing in the reactor are complicated when highly viscous substrates are applied. Compared to diluted food waste, processing of viscous food waste makes an addition of water unnecessary.

Apart from the mentioned operational parameters, sterilization is a major factor influencing fermentation. Often, substrates are autoclaved to avoid contamination with unwanted

microorganisms. This type of thermal pretreatment imposes compositional changes in the substrate. Polysaccharides are degraded to oligomers and monomers, enzymes are denatured, recalcitrant structures, like cell walls, are disrupted, and inhibitory byproducts might be formed (Brodeur et al., 2011). Besides these microscopic alterations, energy consumption drastically increases and challenges economic feasibility of the whole process (Moustogianni et al., 2015). The cost of sterilization represents 10-15% of fermentative operational cost and 5-10% of energy required to run the lactic acid production (including purification steps) (Demichelis et al., 2018). While the eradication of microbial activity in the substrate might be beneficial for investigating single organisms added after autoclavation, one charming feature of organic waste is squandered: The presence of a substrate-adapted indigenous consortium. Food waste is populated with an indigenous consortium (Probst et al., 2015; Tang et al., 2016; Wu et al., 2018) and avoiding sterilization keeps microbes viable. Furthermore, combining an indigenous substrate-adapted consortium and a cultivated bacterial strain could affect substrate conversion, productivity, and yields.

The present study introduces to a process for efficient conversion of food waste into LA without sterilization and utilizing the indigenous microbial population present on the substrate. For techno-economically assessment, collections of food waste containing undefined indigenous consortia were combined with *Streptococcus* sp., a strain that was found to efficiently produce LA from food waste (Demichelis et al., 2017; Pleissner et al., 2017). Based on these results, the performance of the indigenous consortium alone was analyzed as well. Furthermore, the impact of glucoamylase addition for starch degradation was investigated. Energy and economic evaluations of different process scenarios (Table 1) were carried out to determine the minimum plant size needed for profitable operation on the basis of batch and continuous process modes.

2. Materials and methods

2.1 Food waste

Three batches of food waste were collected from the Leuphana university canteen (one in May 2017, FWA, 4 kg, one in April 2018, FWB, 24 kg – and one in June 2018, FWC, 2 kg). Directly after, the waste was homogenized with a blender, pressed through a sieve (0.3 mm mesh size), and stored at -18 °C until further usage. Batches were analyzed in triplicate and employed separately.

2.2 Fermentation

All fermentations were carried out non-sterile with or without addition of a mesophilic *Streptococcus* sp. strain (Pleissner et al., 2017) and with or without glucoamylase (E.C. 3.2.1.20). In order to assess the replicability of the non-sterile biological approach, fermentations were carried out, if not otherwise stated, in duplicate. *Streptococcus* sp. was cultivated at 35 °C in 250 mL flasks containing 50 mL medium consisting of 5 g L^{-1} glucose and 2 g L^{-1} yeast extract. Every 48 hours, 1 mL of this solution was added to 50 mL freshly prepared medium to maintain a stable culture. As inoculum, the 48 hours old medium was used. Fermentations were conducted in a cylindrical glass vessel with a 1 L working volume. Temperature and pH were controlled automatically at 35 °C and pH 6. pH was regulated using 2 M NaOH or 2 M HCl. For stirring, an overhead stirrer (160 rpm, behr Labortechnik) with propeller was used.

Batch and continuous flow fermentations were carried out with 1 kg undiluted food waste (dry matter is shown in Table 2) using a 5% (v/v) *Streptococcus* sp. inoculum (60,000 cells μL^{-1}). No inoculum was added when fermentations were carried out with indigenous consortium. For continuous flow fermentations, the set-up was expanded by a 0.5 L storage container. The storage container was refilled with food waste three times a day. While the large food waste batch was stored in the freezer, small amounts were thawed and kept at 4 °C before filling the storage container. Through a peristaltic pump, a continuous stream of food waste was pumped from the storage container into the reactor, while another peristaltic pump withdrew the same amount at the bottom of the reactor. After 24 hours, batch fermentation was changed to continuous flow fermentation (dilution rates range between 0.39 and 1.15 d^{-1}, see Section 3.3 for details). For simultaneous saccharification and fermentation, 1 mL glucoamylase (E.C.3.2.1.20, 1,200 U mL^{-1}) was added at the start and subsequently every 24 hours to make glucose available from starch.

Fermentations were monitored by taking samples (5 mL) regularly and analyzing for glucose, fructose, sucrose, LA, ethanol, and acetic acid concentrations. After sampling, samples were microfiltrated (0.2 µm) and inactivated by heating at 90 °C for 10 min. After each continuous fermentation, dry matter, protein, and lipid contents were determined as described below.

2.3 Analytics

In order to determine the dry matter of food waste, an aliquot was weighed and dried at 105 °C until constant weight.

Ash content was quantified by heating 1 g dry food waste for 4 h at 550 °C in a muffle furnace and weighing the remainder.

Starch content was determined by enzymatically hydrolyzing food waste. To 800 mL of a 2.4% (w/w) food waste in demineralized water, 1 mL glucoamylase (E.C. 3.2.1.20) was added. After 24 h at 55 °C and pH 4.5, released glucose was measured.

Glucose, fructose, sucrose, LA, ethanol, and acetic acid concentrations were determined using HPLC (Shimadzu: LC-10AD pump, SIL-10AD auto-sampler, CTO-10AD oven, refractive index detector RID-20A, CBM-20A communication module): 10 µL of sample was injected in an Aminex HPX-87H column (300 mm × 7.8 mm) and eluted isocratically with 0.4 mL min^{-1} 5 mM H_2SO_4 at 27 °C. For each analyte, calibration curves were generated with pure solutions of known concentration.

Nitrogen content of food waste was measured with an elemental CN analyzer at 1150 °C (Elementaranalysator vario Max CN).

Protein content was calculated by multiplying the obtained nitrogen content with 5.7 (Merril & Watt, 1973).

Free amino nitrogen (FAN) was determined following the modified EBC-ninhydrin method. First, two reagents were prepared. For reagent A, 1 g $Na_2HPO_4*12H_2O$, 0.6 g KH_2PO_4, 0.05 g ninhydrin, and 0.03 g fructose were dissolved in 10 mL demineralized water. Reagent B contained 0.2 g KIO_3, 60 mL demineralized water, and 40 mL absolute ethanol. For analysis, 20 µL sample, 50 µL A, and 30 µL demineralized water were combined and heated at 90 °C for 5 min. Then 900 µL of B was added and absorption at 570 nm was measured. A calibration curve with glycine as standard was used as reference.

Lipid extraction and transmethylation were carried out as described elsewhere (Pleissner & Eriksen, 2012).

All food waste analyses were performed in triplicate and the mean values are shown in Section Results and discussion.

Yield of LA per gram of sugars (Y_S) was calculated by dividing the LA concentration by the sum of glucose (including starch), fructose, and sucrose concentrations present in the starting material. Yield of LA per gram of food waste (Y_{FW}) was calculated by dividing LA present in the reactor by dry matter of the respective food waste. Since LA formation is growth associated, productivity of batch cultures carried out with FWA and B was calculated with LA titer after 8 hours, while for the culture carried out with FWC the titer after 10 hours was used. Productivity of continuous cultures was calculated by multiplying titer and dilution rate of the whole run. Standard deviation of continuous cultures was calculated by taking the titers measured. Selectivity of LA was calculated by dividing LA concentration by the sum of all detected fermentation products (LA, acetic acid and ethanol).

2.4 Boundary conditions for the evaluation of process scale-up

Economic and energy evaluations were performed on the basis of various process data obtained in the current study. Process scenarios were simulated using SuperPro Designer® 8.0 to investigate technical factors (mass and energy balances) and economic factors (costs and incomes). Energy and economic evaluations of the scale-up to full-scale plant of the scenarios were performed with a conversion factor of 0.8 and costs evaluation carried out in accordance to the Chemical Engineering Plant Cost Index. To scale-up scenarios, a downstream processing was modeled (Figure 1) to reach an optical purity of L(+)-LA equal to 90%, as required by market criteria. Downstream process included centrifugation, microfiltration, ultrafiltration, electrodialysis, and concentration of LA by vacuum distillation (de Oliveira et al., 2018; Demichelis et al., 2017). For LA production in continuous flow fermentation (Figure 1B), storage and discharge reactors were further included before and after the vessel, respectively. The volumes of storage and discharge vessels were calculated according to the applied dilution factor and temperature control. In addition, pH control by adding NaOH was taken into account for these two vessels.

2.4.1 Economic evaluation

Economic analysis was performed considering 300 working days per year for batch configuration and 330 working days per year for continuous configuration using SuperPro Designer® 8.0. In both, batch and continuous LA productions, 90% of the volume of the

vessel was considered as working volume. As LA titer, the end value of the respective fermentations was used. In batch configuration, the time to fill, empty, and clean the fermenter was estimated to amount to 2 hours. A plant lifetime of 20 years was set (Demichelis et al., 2017; Pommeret et al., 2017).

Economic analysis consisted of: Capital and operational costs, revenues, and profitability. Capital costs considered fixed capital investment (*FCI*) for equipment purchase and working capital cost, which is 6.5% of *FCI* (Peters et al., 1968; Pommeret et al., 2017). The capital cost (expressed as Euro per unit) for the different plant sizes was adjusted according to Chemical Engineering Plant Cost Index. The cost of land was not taken into account. Increasing the size of the catchment area, the economic scale increased and the multiplicator factor belonged to the range of (0.8-1.7) (Peters et al., 1968; Pommeret et al., 2017).
A 5-years amortization with a 2% interest was adopted for the capital costs according to (Åkerberg & Zacchi, 2000; Demichelis et al., 2018) (Eq. 1):

$$A[Euro] = C_0 \cdot \frac{i \cdot (1+i)^n}{(1+i)^n - 1} \quad (1)$$

with A: Amortization cost, C_o: Initial capital cost, i: Interest, and n: Number of years considered for amortization.

Operational costs included food waste collection and transport, equipment maintenance as fuel, steam, process air, electricity, utilities, and labor. For the cost of FW collection, the economic calculation was not scaled with the multiplicator factor only, since it was based on the database of (Arpa, 2017) with the unit of measure Euro/metric ton (Euro t^{-1}). The steam was produced on site and the electricity was purchased from the grid.

According to market research of LA (90% optical purity and origin from renewable resources) in EU, the market value of LA was set to 1,600 Euro t^{-1} (Eurostat, 2018). Annual income was calculated as difference between revenue and amortization for the first five years and operational costs.

The profitability of the seven proposed scenarios was evaluated through: Return of interest (*ROI*, Eq. 2), net present value (*NPV*, Eq. 3), assuming a 20 years plant lifetime with 5%

discount on the future cash flows to the present value, and according to Euros paid (Eq. 4) and Euros gained (Eq. 5) per ton of food waste treated.

$$ROI\ [\%] = \frac{Annual\ net\ profit}{Initial\ total\ investment} \cdot 100 \quad (2)$$

NPV represents the scenario profitability for the plant lifetime considering a 5% discount on future cash flows to the present value (*NPV* > 0 means profitability).

$$NPV\ [Euro] = \sum_{t=1}^{T} \frac{C_t}{(1+d)^t} - C_0 \quad (3)$$

NPV was calculated by Eq. 3 with *T*: Plant lifetime, C_t: Net cash flow at time *t*, C_0: Initial capital investment, and *d*: Discount rate.

To conclude the economic profitability, assessment payback time, which is the time required to restore the investment cost, was calculated. Furthermore, P_{feed} and P_{net} were calculated, which refer to annual operational costs per ton of treated food waste (FW, Eq. 4), and Euro gained per ton of treated food waste (FW, Eq. 5), respectively.

$$P_{feed}\ [\frac{Euro}{t}] = \frac{annual\ operational\ cost}{annual\ FW\ treated} \quad (4)$$

$$P_{net}\ [\frac{Euro}{t}] = \frac{net\ profit\ after\ 5\ years\ of\ amortisation}{annual\ FW\ treated} \quad (5)$$

2.4.2 Energy evaluation

The energy balance, performed in thermodynamic equilibrium and steady state condition, was based on the following assumptions (Mehr et al., 2017):

- Atmospheric air consists of 79% (v/v) N_2 and 21% (v/v) O_2.
- Ideal gas law applies.
- Gas leaks of connecting pipes are insignificant.

The total system thermal load (Q_s) was calculated considering the seasonal temperature variations averaged on European temperature trend (IPCC, 2017) (Eq. 6):

$$Q_s[kW] = Q_{sub} + Q_{loss} + Q_p \quad (6)$$

$$Q_{sub}[kW] = m_{sub} \cdot c_p \cdot (T_{in} - T_{reac}) \quad (7)$$

with Q_{sub}: The thermal power required for heating the substrate from inlet temperature (5-26 °C, seasonal) to 35 °C (Eq. 7), m_{sub}: The substrate mass flow rate, T_{in} and T_{reac}: Inlet and fermenter temperatures, respectively, and c_p: The specific heat capacity (equal to the value of water, as food waste dry matter is 22-24%, w/w) as well as Q_{loss}: The heat loss by the fermenter reactor bulwark (Eq. 8):

$$Q_{loss}[kW] = U_{ug} \cdot A_{ug} \cdot (T_{reac} - T_{gr}) + U_{ext} \cdot A_{ext} \cdot (T_{reac} - T_{ext}) \quad (8)$$

with U_{ug} and U_{ext}: The coefficients of heat transfer for underground walls and non-ground walls, respectively, A_{ug} and A_{ext}: The areas of underground and partial walls, and roof, respectively, as well as T_{gr} and T_{ext}: The temperatures of the respective walls. Q_p represents heat loss by piping and it is calculated using Eq. 9, where $\%_p$ is equal to 5% (Mehr et al., 2017):

$$Q_p[kW] = \%_p \cdot (Q_{sub} + Q_{loss}) \quad (9)$$

3. Results and discussion

3.1 Characterization of food waste

The food wastes differed regarding their carbohydrate, lipid and protein contents (Table 2). These compositional differences between the collections are common since the leftovers trace back to alternating canteen menus. pH of all batches was around 4.

3.2 Batch fermentation

With the food waste collections, non-sterile batch fermentations were carried out. Since the production of LA was targeted, both FWA and FWB were inoculated with 5% (v/v) *Streptococcus* sp. to have a significant share of LA producing bacteria. However, owing to the non-sterility of the waste material, even with inoculum, the present indigenous consortium additionally contributes to the fermentation performance. Since FWA and FWB were treated

the same way, this repetition is considered to reveal replicability even when different batches of food waste are used.

3.2.1 Indigenous consortium and *Streptococcus* sp.

With the indigenous consortium, *Streptococcus* sp. and FWA, a LA concentration of 26 g L^{-1} could be reached during the course of 24 hours (Figure 2A). From 1 gram of food waste, 0.11 g LA was produced. This corresponds to a yield of 12% in respect to the total amount of sugars, including starch.

In order to increase yields in the next set of experiments, glucoamylase (0.1%, v/v) was added to improve fermentation performance. Starch hydrolysis enhanced glucose concentration from 19 g L^{-1} at the start to 45 g L^{-1} after 4 hours, boosted productivity and a LA titer of 44 g L^{-1} LA could be detected after 24 hours (Figure 2B), corresponding to Y_S of 55% and Y_{FW} of 18%. However, a feature that is unseen in all other fermentations was the high ethanol concentration of 18 g L^{-1} found after 24 hours. This concentration indicates the presence of ethanol producing organisms. Furthermore, 10 g L^{-1} fructose was left after 24 hours. FAN was consumed during the first 8 hours of fermentation irrespective the addition of glucoamylase and more than 200 mg L^{-1} remained (Figure 2C).

In continuation, fermentations with FWB were performed. Although the total amount of sugars was lower in FWB, batch fermentation with *Streptococcus* sp. yielded a LA concentration of 37 g L^{-1} (Figure 2D). Based on the sugar concentration in the starting material prior to addition of inoculum, Y_S was 47%, while Y_{FW} was 17%. The obtained yields in combination with free sugar concentrations below 5 g L^{-1} indicate that unused carbohydrates are likely to be present in the form of starch. Equally, neither *Streptococcus* sp. nor the indigenous consortium seems to be able to hydrolyze starch.

As with FWA, simultaneous saccharification and fermentation was carried out with FWB by adding glucoamylase at the start of the fermentation. Here, ethanol generation remained unproblematic. After 24 hours, ethanol concentration was below 5 g L^{-1}. LA titer, on the other hand, reached 47 g L^{-1} corresponding to Y_S of 58% and Y_{FW} of 21% (Figure 2E). With respect to substrate utilization towards LA, these figures showed a favorable product spectrum. Free sugars were almost depleted, undesired acids and alcohols were found at low concentrations, and LA was the main fermentation product (84 % selectivity). The FAN concentration in the

fermentation carried out with glucoamylase decreased from almost 400 mg L^{-1} to 200 mg L^{-1} (Figure 2F). Contrarily, the FAN concentration in the fermentation carried out without even increased to 600 mg L^{-1}.

Even though the repetition of experiments was carried out with different food waste batches, the results show the same trend (Figure 2A-C). As the aforementioned runs were performed with non-autoclaved substrate, the added strain might not have been the only LA producer. In order to get further insight into the performance of the indigenous consortium present in FWB, batch experiments were conducted without addition of *Streptococcus* sp. As repetition, a batch experiment with FWC without the addition of *Streptococcus* sp., but with glucoamylase was performed (Figure 2G-K).

3.2.2 Indigenous consortium

As before, batch fermentation was done with and without addition of glucoamylase to FWB. With selectivities of 84% and 91%, LA showed to be the main fermentation product in both cases (Figure 2G and H). In the absence of glucoamylase, 38 g L^{-1} LA, 5 g L^{-1} acetic acid, and 2 g L^{-1} ethanol were produced with Y_S of 47% and Y_{FW} of 17%. All free sugars were efficiently converted into LA (Figure 2G). There was no indication for starch hydrolysis. The addition of glucoamylase resulted in an increase of LA titer to 50 g L^{-1} after 24 hours (Figure 2H) and Y_S as well as Y_{FW} were 63% and 21%, respectively. As observed before, FAN was not limited and around 100 mg L^{-1} and 400 mg L^{-1} remained in the fermentation carried out with and without glucoamylase, respectively (Figure 2I).

The repetition of the batch fermentation with FWC and addition of glucoamylase is shown in Figure 2J and K. Contrarily to the fermentation with FWB, the concentration of glucose increased during the first 6 hours to almost 80 g L^{-1} due to a hydrolysis of starch and was almost consumed within the following 38 hours. Even though the final titer after 48 hours was with 65 g L^{-1} higher than in the batch with FWB, productivity, yield, and selectivity were similar. Due to food waste scarcity the repetition was limited to the fermentation with glucoamylase. However, the outcome confirms the finding from batch fermentation with *Streptococcus* sp.

Regardless the substrate and treatment, concentration of acetic acid was around 5 g L^{-1} after 24 hours. All seven batch fermentations revealed that glucose is metabolized first and only

thereafter fructose is taken up. As a measure of amino acids, FAN was monitored throughout the fermentations (Figure 2C, F, I, and K). Microorganisms crucially depend on the availability of essential amino acids to synthesize proteins needed in their metabolism. Some microorganisms are able to excrete proteases in order to degrade proteins to amino acids or peptide fragments. This feature, however, is also dependent on sufficient growth conditions like temperature, pH, and presence of nutrients (Ozturkoglu-Budak et al., 2016). Consequently, there should be a subtle balance between uptake and hydrolysis. Even though FAN is quantifiable, bioaccessibility might not directly correlate with these figures. As reported in literature, nitrogen is often the limiting factor of fermentations (Pleissner et al., 2017). In all batch fermentations, FAN values are consistently above 100 mg L^{-1}.

In previous studies, the *Streptococcus* sp. strain was used for the conversion of diluted and autoclaved food waste (Demichelis et al., 2017; Pleissner et al., 2017). The authors reported that obtained LA titers are only explainable when the strain hydrolyzes starch. This hydrolytic activity, however, was not observed in the present experiment. Looking at productivities found in this study, a clear trend can be seen. Firstly, productivity is higher when fermentation is supplemented with glucoamylase. In the case of FWA it increases from 2.28 g L^{-1} h^{-1} to 2.95 g L^{-1} h^{-1}. With FWB, productivity of 1.54 g L^{-1} h^{-1} is observed when *Streptococcus* sp. is added and 1.58 g L^{-1} h^{-1} with indigenous consortium alone. These values raise to 1.87 g L^{-1} h^{-1} and 2.93 g L^{-1} h^{-1}, respectively. Secondly, the fermentation with only indigenous consortium can give slightly higher productivities than the fermentations carried out with *Streptococcus* sp.

Differences in performance between FWA, FWB, and FWC presumably originate from different starting concentrations of free sugars and differences in indigenous communities. The fact that the pure indigenous consortium outperforms the fermentation with added *Streptococcus* sp. might be attributable to the better adaption of the indigenous consortium towards the substrate.

3.2.3 Comparison with other food waste fermentations

LA bacteria are often applied for food conservation, and thus their presence and the formation of LA is not surprising. The present study did not further investigate the composition of the consortium. Tang et al. followed a similar approach and investigated the conversion of food waste from a university canteen into LA using an indigenous consortium. They found a LA

productivity of 0.17 g L^{-1} h^{-1} and 0.28 g L^{-1} h^{-1}, which is 5 to 10 times below the one found in the present study (Tang et al., 2016; Tang et al., 2017). They further found that *Lactobacillus* was the dominant species in the indigenous consortium and responsible for the LA formation. It is assumed that *Lactobaccillus* may also be the dominant species in the waste materials used in the present study. Other groups report productivities of up to 3.06 g L^{-1} h^{-1} with an inoculum obtained from wastewater treatment sludge (RedCorn & Engelberth, 2016). Working with selected *Lactobacillus* strains on food waste, yields of 70% (Wang et al., 2012) and 60% (Kim et al., 2003) with productivities of 1.01 g L^{-1} h^{-1} and 1.13 g L^{-1} h^{-1}, respectively, are published. These values further reveal that the presented yields and productivities of this study compare well to other studies done on food waste fermentation.

Even though food wastes were not stored frozen for the same time, it is of particular interest that the indigenous consortia of the independently selected wastes showed similar performances regarding LA production. As found in earlier studies, storage can result in a shift in the indigenous consortium, but "the presence of microorganisms may be more important than their relative abundance in retaining an active microbial community" (Yu et al., 2015). This underlines the suitability of the presented approach to be implemented as simple food waste conversion process without extensive process supervision. This also underlines, that a control of the indigenous consortium is not necessary in order to achieve an efficient conversion. It should, however, be admitted, that the ubiquitous presence of LA producing bacteria in various food waste streams favors this simple utilization approach. When focusing on other biobased molecules than LA, a knockout or steering of indigenous consortia might be necessary.

3.3 Continuous flow fermentation

FWB was continuously fermented with the indigenous consortium and *Streptococcus* sp. or with the indigenous consortium alone, and both, with and without sequential glucoamylase addition. After an initial 24 hours batch phase, flow was started and dilution rate kept at around 0.4 d^{-1}. Even though the storage container was refilled regularly, residence times of up to 14 hours were unavoidable. Since no thermal control was used, container and substrate rested at room temperature. Under these conditions, bacteria are generally active. However, concentrations of free sugars were only slightly lower than in the starting material, while the concentration of LA was slightly higher. It is believed that the generation of LA and a pH below 4 led to suppression of unwanted microbial growth.

3.3.1 Indigenous consortium and *Streptococcus* sp.

In batch experiments it was observed that free sugars were nearly depleted and LA production came to a halt after 24 hours for FWA and FWB or 48 hours for FWC (Figure 2). Addition of glucoamylase to fermentation broth at that time resulted in a release of glucose and resumed LA production. Conclusively, viable cells were still present, but unable to process starch. In the first continuous run with *Streptococcus* sp. and indigenous consortium, every 24 hours, glucoamylase was added to maintain sufficient glucose levels. Within the first day, LA titer rose from 47 g L^{-1} to 61 g L^{-1} (Figure 3A). This trend was maintained at lower pace and after 148 hours 74 g L^{-1} was reached. Simultaneously, acetic acid concentration went up from 5 g L^{-1} to 7 g L^{-1} and ethanol was present below 1 g L^{-1}. Looking at FAN, no dramatic changes were noted. Levels between 100 mg L^{-1} and 200 mg L^{-1} were sustained throughout the whole fermentation process. Considering the entire run, 2,270 g food waste was processed continuously at a dilution rate of 0.44 d^{-1} with an overall LA productivity of 1.27 g L^{-1} h^{-1}.

The addition of enzyme complicates operation and might increase process costs (Lam et al., 2014). Considering catchment areas from 200,000 to 1,000,000 inhabitants, the cost of enzymes ranges from 1-7% of operational fermentative cost (Demichelis et al., 2018). Therefore fermentation of *Streptococcus* sp. and indigenous consortium without glucoamylase addition was tested. With a dilution rate of 0.43 d^{-1}, LA concentration went from 37 g L^{-1} to 53 g L^{-1} within 24 hours (Figure 3D). For the consecutive 29 hours, titer seemed to be stabilizing around 50 g L^{-1}. Free sugars were detected at consistently low concentrations, showing that microbial activity kept up with addition of new substrate and washing out of cells remained uncritical. As a result of this consideration and in an attempt to increase productivity, dilution rate was increased to 1.15 d^{-1} after 77 hours. For another 48 hours, fermentation performance was seemingly unaffected, at most a slight increase in titer could be detected. However, starting from 119 hours, LA concentration decreased to below 45 g L^{-1} after 168 hours total run time. FAN ranged between 380 mg L^{-1} and 760 mg L^{-1} (Figure 3E). At low dilution rate, FAN was increasing, whereas high dilution rate led to a decrease.

With indigenous consortium, *Streptococcus* sp., and glucoamylase, 7 g L^{-1} acetic acid were obtained (Figure 3A). Without separate addition of glucoamylase, acetic acid leveled off at 9 g L^{-1} with 0.43 d^{-1} dilution rate. Elevating dilution rate to 1.15 d^{-1}, acetic acid remained at 9 g L^{-1} before steadily decreasing to 5 g L^{-1} (Figure 3D). Seeing that lactic and acetic acid

concentrations fall in concert, this behavior is most likely attributed to a high dilution rate and consequently washing-out.

Increasing the dilution rate from 0.44 to 1.15 d^{-1} resulted in an increase of productivity from 0.91 g L^{-1} h^{-1} (between 48 hours and 77 hours) to 2.39 g L^{-1} h^{-1} for the remainder time. However, it should be admitted that this dilution rate would also result in a wash-out of cells. In order to obtain a stable LA production, dilution rate must be kept within certain boundaries. Ideal dilution rate is a subtle substrate- and process-specific parameter. As dilution rate is linked to productivity, process efficiency relies on finding optimal values. The aim should be to work at highest dilution rates, which still allow steady state. Accordingly, the optimal dilution rate for this continuous flow fermentation would range between 0.43 d^{-1} and 1.15 d^{-1}.

3.3.2 Indigenous consortium

Finally, continuous flow fermentation with an indigenous consortium was investigated at a dilution rate of 0.39 d^{-1}. Starting from 38 g L^{-1}, concentration of LA steadily ramped up to 65 g L^{-1} after 96 hours (Figure 3G). Since the subsequent 10 hours did result in a change in lactic acid concentration, fermentation was finalized after 106 hours.

While showing no clear trend between 24 and 58 hours, FAN exceeded 600 mg L^{-1} after 72 hours and dropped to 300 mg L^{-1} nearly linearly thereafter (Figure 3H). Certain conditions might favor excretion of proteases, causing the increase, followed by intensified uptake. For the continuous part, productivity amounted to 1.02 g L^{-1} h^{-1}.

3.3.3 Composition of remaining solids

Composition of starting material is given in Table 2. After performing continuous flow fermentations with FWB, lipid content of remaining solids was measured. The lipid share of the dry matter decreased in all cases. Final lipid content was 22-23%. The composition of lipids changed only marginally. Regardless the experiment, nearly 50% oleic acid was present, followed by palmitic acid (~ 25%), and stearic acid (~20%). At the end of the respective continuous flow fermentations, total nitrogen content was determined. The protein content, calculated therefrom changed from 19.1% in the starting material to 16.6%, 18.0%, and 17.9% for the fermentation carried out with indigenous consortium, *Streptococcus* sp. with and without glucoamylase, and indigenous consortium without glucoamylase (Figure 3).

3.4 Comparison of batch and continuous fermentation

As a general feature, sugar conversion to LA could be enhanced by changing the mode of operation from batch to continuous flow fermentation (Figure 3C, F, and I). This occurred without addition of any external nutrients other than what was already contained in the feed substrate. Sugar conversion peaked when applying an indigenous consortium, *Streptococcus* sp., and glucoamylase. Of all sugars, including starch, 86% was converted to LA. Such high conversion might be ascribed to optimal nutrient availability, a stable culture in exponential growth, and improved adaption to the substrate. Without glucoamylase, the indigenous consortium and *Streptococcus* sp. less efficiently utilize the substrate and sugar conversion never exceeds 70%.

Sugar conversions of 79% could be attained in fermentation relying on the indigenous consortium even without glucoamylase. While being subjected to a set of conditions, bacterial communities might be subject to compositional changes. Therewith, organisms that are able to produce glucoamylases could gain larger shares within the indigenous consortium and contribute to more efficient substrate utilization.

Looking into literature, continuous LA fermentations are performed with pre-hydrolyzed natural substrates. These hydrolysates obtained from corn stover (Ahring et al., 2016; Ma et al., 2016), corn cob (Shen & Xia, 2006), or corn steep liquor (Wee & Ryu, 2009), are of low viscosity and allow easy pumping and stirring. This finds expression in much higher dilution rates (up to 4.01 d^{-1} (Ahring et al., 2016)) and productivities (up to 13.8 d^{-1} (Ma et al., 2016)) in comparison with the present study. It is noteworthy, that the indigenous consortium reaches LA yields in respect to theoretically available sugars of up to 79%, a value that is competitive with respect to the single cultures used in other studies.

3.4 Energy evaluation

Energy evaluation was performed to support economic evaluation in order to investigate the plant size, which is economically profitable for the considered scenarios. The thermal yearly load value was calculated considering seasonal variation. The energy load calculation was based on the vessel volume. Consequently, Scenarios 1-4 had the same trend since the vessel volume, process mode, and running time were identical. Generally, in winter and fall, the energy requirement was higher than in spring and summer for all scenarios.

Energy thermal load is made up of two items: Thermal energy to heat the substrate and energy loss. For all scenarios the item with the highest energy request was thermal power required for heating the substrate. It ranged between 86-90% of the total energy required, which is in accordance to earlier findings (Aghbashlo et al., 2018; Alzate et al., 2018). The evaluation of energy evidenced two key points: 1) Batch fermentation required higher energy than continuous flow fermentation and 2) continuous flow fermentation carried out at higher dilution rates required less energy than that fermentation carried out at lower dilution rate (Scenarios 6.1-6.2FWB). The explanation of these two key points is the volume of the vessel. Batch fermentation vessel had a 30% higher volume than continuous flow fermentation vessel, which means a higher energy demand for heating the substrate and energy loss. Dilution rate played a strategic role in this aspect, since a higher dilution resulted in a higher productivity, a smaller vessel volume was needed to produce the same amount of LA (Bruno et al., 2018) and consequently energy demand and loss were reduced. The most beneficial aspect of the present study, however, is that compared to an early study (Demichelis et al., 2017), 18-20% of energy can be saved by skipping the autoclavation of substrate prior to fermentation.

3.5 Economic evaluation

Economic evaluation was carried out under consideration of the energy evaluation to define the minimum plant size needed for economic profitability by comparing two different feed configurations, batch and continuous, and employing different food waste compositions. To determine the minimum plant size, the required amount of food waste was referred to the number of inhabitants in different catchment areas assuming that in EU, 477 kg of food waste per year and capita was produced (Eurostat, 2018) and 30% (w/w) is organic (Tchobanoglous et al., 1993). Data on economic profitability of the scenarios regarding plant size is provided in Table 3.

It was assumed that a higher LA yield means less waste production, as more organic material is converted into LA. Moreover, a continuous flow fermentation requires smaller vessel volume than batch fermentation (Gu et al., 2018). This enhancement of technical performance results in an enhancement of economic income (de Oliveira et al., 2018). For all the analyzed scenarios (Table 1), it was considered that downstream processing contributes by more than 55% to operational costs (Aghbashlo et al., 2018; de Oliveira et al., 2018; Su et al., 2013) and the more complex the process, the more inhabitants are needed to counterbalance the costs.

Among the proposed batch fermentations, the most economically profitable were Scenarios 2FWB and 4FWB, which consider the application of glucoamylase (Table 3). For the two scenarios more than 20% of the investment can be returned annually at a catchment area of 200,000 inhabitants. This economic profitability is due to the addition of glucoamylase, which increases sugar utilization and LA productivity, and consequently the economic revenues (Abdel-Rahman et al., 2016). While it was expected that a higher productivity significantly increases economic profitability, the found impact of food waste composition on economy was not. FWA has a higher carbohydrate content that FWB (Table 2). However, scenario 2FWA, which is based on the same treatment as 2FWB, but on FWA, requires a catchment area of 500,000 inhabitants to achieve an annually investment return of 25%.

Economic profitability trend of LA production in batch fermentations (Table 3) is in agreement with earlier findings (Demichelis et al., 2017). The higher the return of investment the fewer years are needed for payback and the higher the net present value (Table 3). Furthermore, the larger the catchment area the more profit can be made with a certain scenario. Scenarios applied in a rather small catchment area (<200,000 inhabitants) can have payback times of more than 20 years. Compared to earlier finding (Demichelis et al., 2017), the present study revealed two economic and environmental benefits: Avoidance of water for the dilution of food waste and avoidance of autoclavation for the sterilization of waste material. Both benefits contribute to resource efficiency as less input in form of water and energy is needed to carry out the process.

Continuous flow fermentations using an indigenous consortium, *Streptococcus* sp. and glucoamylase reached higher technical and economic profits than corresponding batch fermentations. Scenarios 2FWB and 5FWB had the same process characteristics (presence of indigenous consortium, inoculation with *Streptococcus* sp., and addition of glucoamylase), but were carried out as batch and continuous flow fermentations, respectively (Table 1). Nevertheless, scenario 5FWB had a higher yield per gram food waste (28% vs 22%) and a smaller vessel volume (-4%).

The assumed operation period of 330 days per year benefits the economy of the scenarios carried out as continuous flow fermentations (Table 3). For scenarios 5FWB and 7FWB, 49.2% and 36.7% of the investment costs can be returned annually already at a catchment area

of 200,000 inhabitants, respectively, which is significantly higher than the return found for batch fermentations (Table 3). From all tested scenarios, scenario 5FWB (Table 1) showed best economic values due to high LA yields. The implementation of scenario 5FWB would only need 100,000 inhabitants in the catchment area to achieve a return of investment of 20.5%. Such a small catchment area makes the process interesting for small cities and rural areas. Nevertheless, from the productivities and yields found and the corresponding economic evaluation (Table 3), it is obvious that a certain threshold for LA titer must be overcome to allow for profitable operation.

The fundamental difference between Scenarios 6.1FWB and 6.2FWB was the dilution rate, equal to 0.39 and 1.15 d^{-1}, respectively. A higher dilution rate corresponds to higher productivity and consequently a smaller vessel volume is needed. The disadvantage, however, is that the substrate was not completely converted (Zhou et al., 2018). In fact, Scenario 6.1FWB reached a yield of 23% (Y_{FW}), while for Scenario 6.2FWB the yield was 21%. This reduced yield (-8%) resulted in an increase of waste production by 2%.

Both in batch and continuous flow fermentations, the addition of *Streptococcus* sp. to non-sterile food waste did not positively affect the LA production. From an economic perspective, inoculum addition represents a further operational cost item, without providing any benefits. Glucoamylase addition exhibited benefits in terms of LA yield enhancement, but it represented 42% and 58-62% of operational cost items for batch and continuous flow fermentations (in accordance to dilution rate), respectively. Nevertheless, the addition was highly beneficial to the economy (Table 3) and decreased the amount of organic waste streams to be treated after fermentations. Currently, at industrial scale, food waste management consists mainly of anaerobic digestion and/or composting processes. However, according to biorefinery principle (Task 42), Circular Economy EU policies, and Sustainable Development Goals of Agenda 2030, it is recommended to consider first the production of platform chemicals and later the formation of compost and bioenergy. The focus of only one product may not be beneficial to the economic feasibility and may result in organic waste streams that need to be treated. Thus, integration of different valorization strategies can significantly affect process performance, waste generation and economy.

In the present study, waste production had a significant impact on economic profitability. For all the proposed scenarios, wastes represented 70-83% of the fed food waste. Waste from LA

production can further undergo valorization in anaerobic digestion or biofuel production (Demichelis et al., 2017; Mandegari et al., 2018). Integration of energy production has three benefits: Energy generation to support LA production, reduction of waste, and a second product (energy) that may contribute to economic profitability and probably reduces the dependency on LA market price.

4. Conclusions

While productivity was higher during batch operation, both LA titer and yield were superior in continuous mode. This shows that continuous fermentation offers a more thorough and comprehensive utilization of substrate. Furthermore, economic evaluation revealed that profitability was reached at a population size of 200,000 inhabitants in the catchment area without the necessity to sterilize and inoculate the substrate with a potent LA producer. With these findings, an efficient way for food waste treatment is presented that might foster bioeconomy even in smaller cities and rural areas.

Supplementary information

E-supplementary data of this work can be found in online version of the paper.

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Tables

Table 1. Overview of scenarios carried out with food waste A (FWA), B (FWB), and C (FWC) with indigenous consortium (IC), *Streptococcus* sp. (*Str*. sp.) and glucoamylase.

Feeding configuration	Scenarios	FWA	FWB	FWC
Batch	Scenario 1	IC + *Str*. sp.	IC + *Str*. sp.	/
	Scenario 2	IC + *Str*. sp. + glucoamylase	IC + *Str*. sp. + glucoamylase	/
	Scenario 3	/	IC	/
	Scenario 4	/	IC + glucoamylase	IC + glucoamylase
Continuous	Scenario 5	/	IC + *Str*. sp. + glucoamylase	/
	Scenario 6.1	/	IC + *Str*. sp. (0.39 d^{-1})	/
	Scenario 6.2	/	IC + Str. sp. (1.15 d^{-1})	/
	Scenario 7	/	IC	/

Table 2. Composition of food waste based on dry weight from the different collections. Food waste A (FWA) was collected in May 2017, food waste B (FWB) was collected in April 2018 and food waste C (FWC) was collected in June 2018.

Constituent	FWA [%, w/w]	FWB [%, w/w]	FWC [%, w/w]
Dry matter	24.3 ± 0.1	21.7 ± 0.1	24.9 ± 0.4
Protein	13.6 ± 0.2	19.1 ± 0.2	18.6 ± 0.1
Lipid	21.5 ± 1.3	25.2 ± 2.5	37.7 ± 3.4
Starch	30.7 ± 0.1	15.2 ± 0.1	29.3 ± 0.1
Free saccharides	29.1 ± 1.0	19.1 ± 1.0	18.9 ± 1.0
Ash	6.1 ± 0.1	6.5 ± 0.1	3.9 ± 0.9

Table 3. Results of the economic assessment: Scenarios representing different fermentation conditions (Table 1) are linked to catchment areas of different sizes (expressed as number of inhabitants served) of the prospective plant. Presented economic indicators are: Euro gained per ton of treated food waste (P_{net}), annual operational costs per ton of treated food waste (P_{feed}), return on investment (*ROI*), net present value (*NPV*) and payback time (C). Payback times of 20 years or longer are uneconomical, since plant lifetime is estimated at 20 years.

	Inhabitant	50k	100k	200k	500k	1M
Scenario 1FWA	P_{net}	<0	<0	<0	<0	<0
	P_{feed}	<0	<0	<0	<0	<0
	ROI	<0	<0	<0	<0	<0
	NPV	<0	<0	<0	<0	<0
	Payback	>20	>20	>20	>20	>20
Scenario 1FWB	P_{net}	<0	<0	1.7	5.5	27.4
	P_{feed}	<0	<0	27.6	59.6	119.4
	ROI	<0	<0	3.4	23.7	42.3
	NPV	<0	<0	<0	<0	3.0
	Payback	>20	>20	>20	<0	6.0
Scenario 2FWA	P_{net}	<0	<0	2.1	5.9	5.8
	P_{feed}	<0	<0	29.0	63.1	126.5
	ROI	<0	<0	4.0	25.0	44.8
	NPV	<0	<0	<0	2.0	6.8
	Payback	>20	>20	>20	10.0	5.0
Scenario 2FWB	P_{net}	<0	5.0	11.3	15.1	14.9
	P_{feed}	<0	9.9	8.0	6.9	6.9
	ROI	<0	5.0	22.0	64.6	116.1
	NPV	<0	<0	1.2	1.0	2.3
	Payback	>20	>20	12.0	3.0	2.0
Scenario 3FWB	P_{net}	<0	<0	4.0	7.8	7.
	P_{feed}	<0	<0	27.1	58.2	116.7
	ROI	<0	<0	7.8	33.4	60.0
	NPV	<0	<0	<0	3,8	10,3
	Payback	>20	>20	>20	7,0	3,0
Scenario 4FWB	P_{net}	0.5	6.8	13.1	16.9	16.9
	P_{feed}	11,8	9,9	8.0	6.8	6.9
	ROI	0,2	6,8	25,6	72,3	130,2
	NPV	<0	<0	1,9	1,2	2,7
	Payback	>20	>20	10	3	1
Scenario 5FWB	P_{net}	11.6	9.7	7.8	6.6	6.6
	P_{feed}	15.2	21.5	27.8	31.6	31.6

	ROI	7.1	20.5	49,2	110.6	175.7
	NPV	<0	0.9	6.8	24.3	513.8
	Payback	>20	>20	5	2	1
Scenario 6.1FWB	P_{net}	<0	2.5	11.3	15.1	15.0
	P_{feed}	<0	16.5	26.7	57.3	114.8
	ROI	<0	4.7	19.9	52.4	82.9
	NPV	<0	<0	0.9	9.5	21.8
	Payback	>20	>20	6	1	1
Scenario 6.2FWA	P_{net}	<0	<0	6.3	10.1	10.0
	P_{feed}	<0	<0	27.0	57.9	116.1
	ROI	<0	<0	12.0	41.4	71.6
	NPV	<0	<0	<0	5.7	1.4
	Payback	>20	>20	>20	6	3
Scenario 7 FWB	P_{net}	2.1	7.4	21.1	24.9	24.8
	P_{feed}	9.7	16.3	26.2	56.0	112.3
	ROI	3.9	13.9	36.7	84.3	132.0
	NPV	<0	<0	44	18.1	39.1
	Payback	>20	>20	>20	2	1

20

Figure captions

Figure 1. Process scheme of batch (A) and continuous (B) flow fermentations.

Figure 2. Concentration of glucose (open triangle), fructose (open square), sucrose (open circle), acetic acid (open diamond), lactic acid (closed circle), ethanol (closed triangle) of non-sterile batch fermentations of food wastes A (FWA), B (FWB) and C (FWC) carried out with *Streptococcus* sp. (A-F) and indigenous consortium alone (G-K). Fermentations were performed either without addition of glucoamylase (A, D, and G) or with addition of glucoamylase (B, E, H, and J). Corresponding concentration of free amino nitrogen (FAN) is shown in (C, F, I, and K). FAN concentrations in fermentations carried out with glucoamylase are illustrated as closed square, and those concentrations in fermentations carried out without glucoamylase as open square.

Figure 3. Concentration of glucose (open triangle), fructose (open square), sucrose (open circle), acetic acid (open diamond), and lactic acid (closed circle), dilution rate (dashed line), free amino nitrogen (FAN, closed square), and lactic acid yield with respect to theoretically available sugars (Y_S, closed triangle) of non-sterile continuous flow fermentations of food waste B (FWB) started after 24 hours of batch operation and carried out with *Streptococcus* sp. and glucoamylase (A-C), *Streptococcus* sp. without glucoamylase (D-F), and indigenous consortium without glucoamylase (G-I).

.

Figure 1

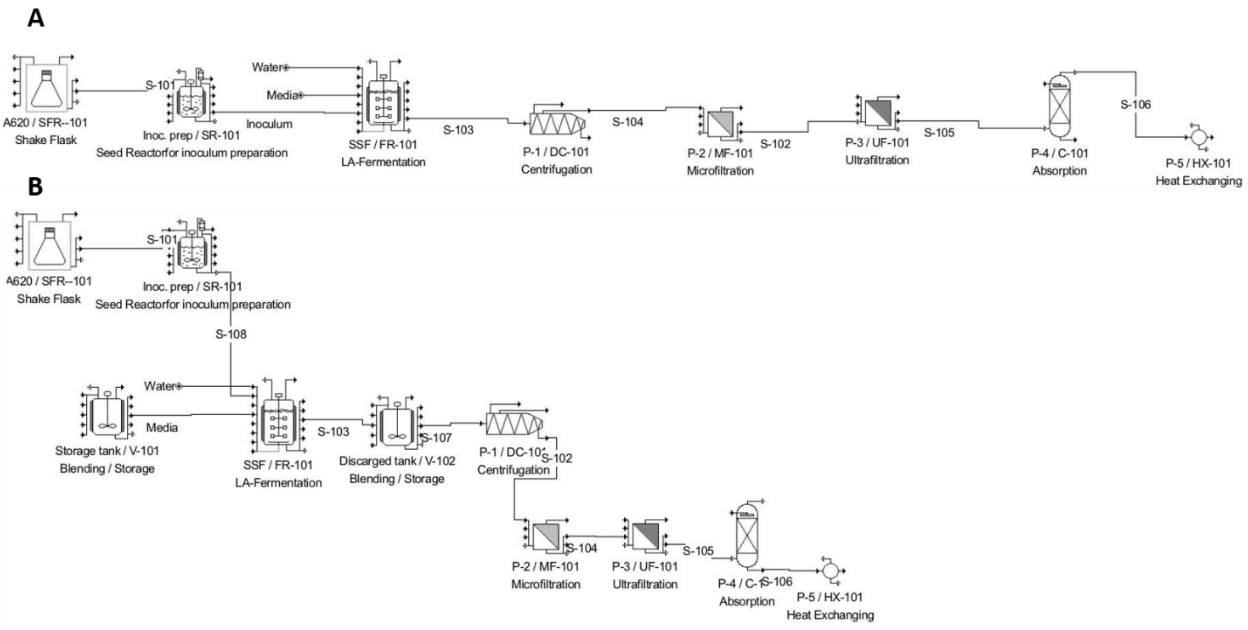

A
A620 / SFR--101
Shake Flask
S-101
Inoc. prep / SR-101
Seed Reactorfor inoculum preparation
Inoculum
Water
Media
SSF / FR-101
LA-Fermentation
S-103
P-1 / DC-101
Centrifugation
S-104
P-2 / MF-101
Microfiltration
S-102
P-3 / UF-101
Ultrafiltration
S-105
P-4 / C-101
Absorption
S-106
P-5 / HX-101
Heat Exchanging
B
A620 / SFR--101
Shake Flask
S-101
Inoc. prep / SR-101
Seed Reactorfor inoculum preparation
S-108
Water
Media
Storage tank / V-101
Blending / Storage
SSF / FR-101
LA-Fermentation
S-103
Discarged tank / V-102
Blending / Storage
S-107
P-1 / DC-101
Centrifugation
S-102
P-2 / MF-101
Microfiltration
S-104
P-3 / UF-101
Ultrafiltration
S-105
P-4 / C-101
Absorption
S-106
P-5 / HX-101
Heat Exchanging

Figure 2

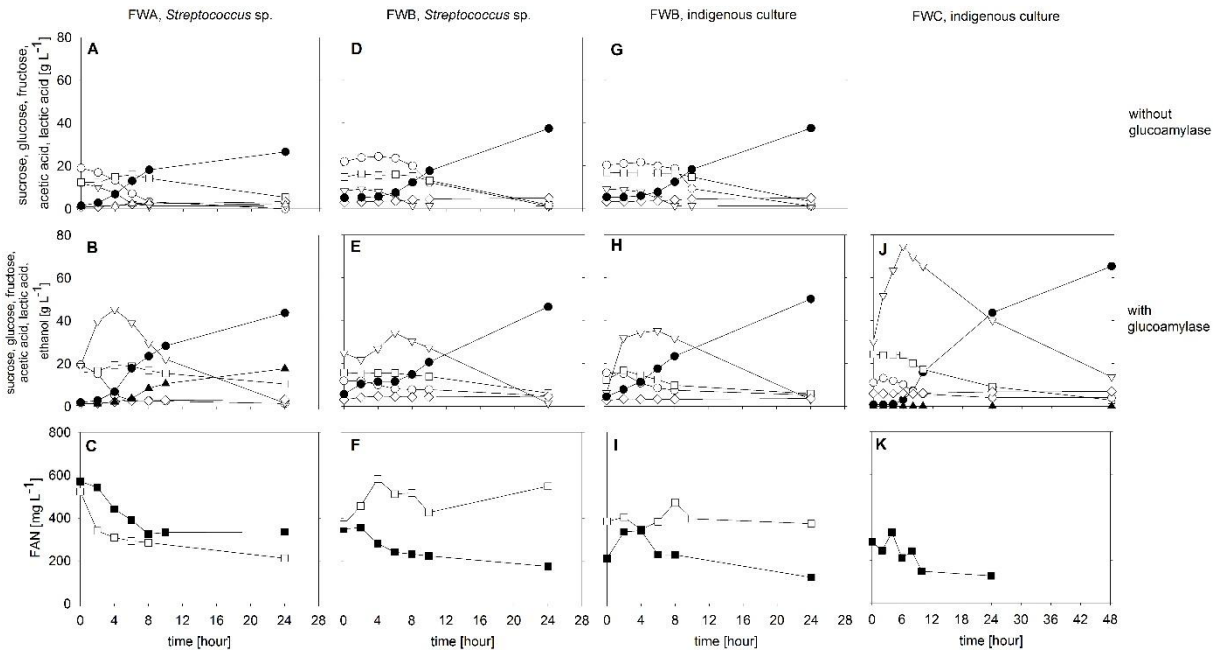
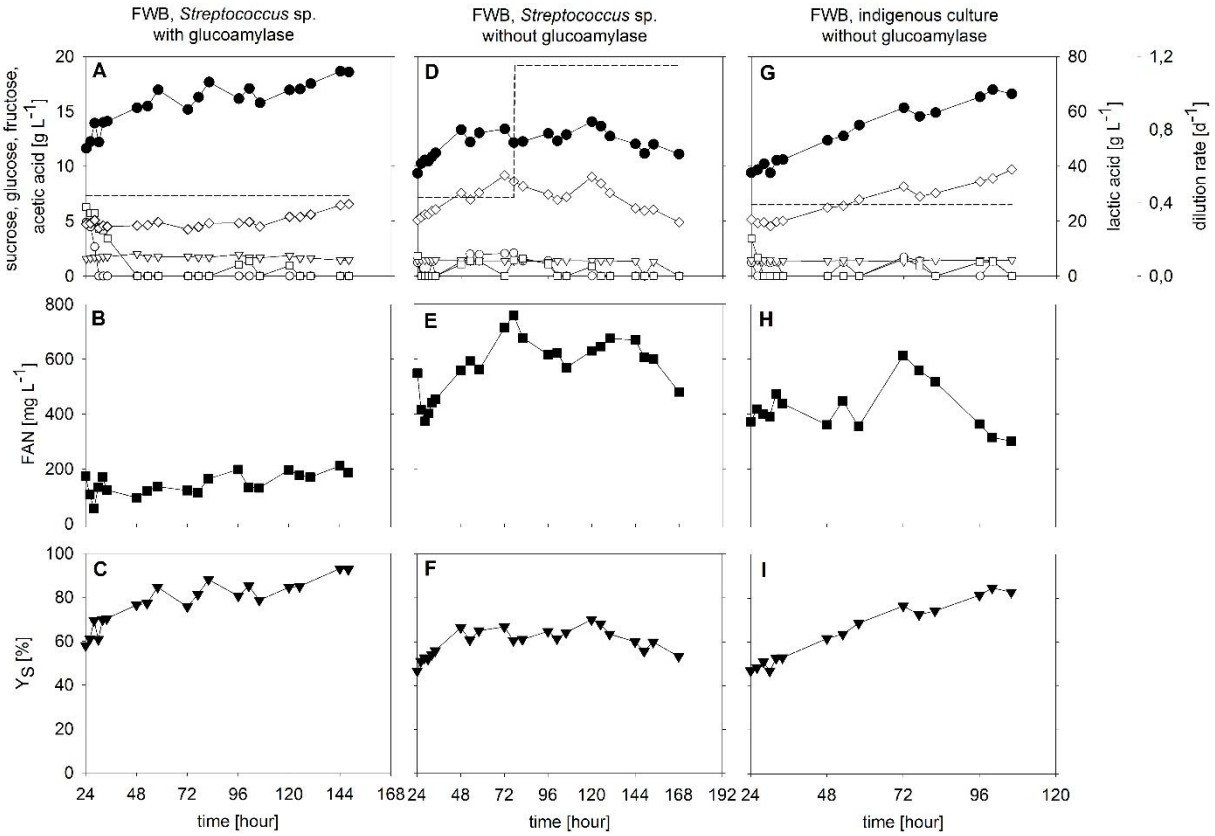

FWA, Streptococcus sp.
FWB, Streptococcus sp.
FWB, indigenous culture
FWC, indigenous culture
without glucoamylase
with glucoamylase
sucrose, glucose, fructose, acetic acid, lactic acid [g L-1]
sucrose, glucose, fructose, acetic acid, lactic acid, ethanol [g L-1]
FAN [mg L-1]
time [hour]

Figure 3


FWB, Streptococcus sp. with glucoamylase
FWB, Streptococcus sp. without glucoamylase
FWB, indigenous culture without glucoamylase
A
B
C
D
E
F
G
H
I
sucrose, glucose, fructose, acetic acid [g L-1]
FAN [mg L-1]
YS [%]
lactic acid [g L-1]
dilution rate [d-1]
time [hour]
time [hour]
time [hour]

Electronic Annex

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