LEUPHANA UNIVERSITY LÜNEBURG

DOCTORAL THESIS

# Material utilization of organic waste

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# *Abstract*

**Material utilization of organic waste**

by Jan Christoph PEINEMANN

As modern society progresses, waste treatment becomes a pressing issue. Not only are global waste amounts increasing, but there is also an unmet demand for sustainable materials (e.g. bioplastics). By identifying and developing processes, which efficiently treat waste while simultaneously generating sustainable materials, potentially both these issues might be alleviated. Following this line of thought, this dissertation focuses on procedures for treatment of the organic fraction of waste. Organic waste is a suitable starting material for microbial fermentation, where carbohydrates are converted to smaller molecules, such as ethanol, acetic acid, and lactic acid. Being the monomer of the thermoplastic poly-lactic acid, lactic acid is of particular interest with regard to bioplastics production and was selected as target compound for this dissertation.

Organic waste acted as substrate for non-sterile batch and continuous fermentations. Fermentations were initiated with inoculum of *Streptococcus* sp. or with indigenous consortium alone. During batch mode, concentration, yield, and productivity reached maximum values of 50 g $L^{-1}$, 63%, and 2.93 g $L^{-1}$ $h^{-1}$. During continuous operation at a dilution rate of 0.44 $d^{-1}$, concentration and yield were increased to 69 g $L^{-1}$ and 86%, respectively, while productivity was lowered to 1.27 g $L^{-1}$ $h^{-1}$. To fully exploit the nutrients present in organic waste, phosphate recovery was analyzed using seashells as adsorbent.

Furthermore, the pattern of the indigenous consortium was monitored. Evidently, a very efficient *Enterococcus* strain tended to dominate the indigenous consortium during fermentation. The isolation and cultivation of this consortium gave a very potent inoculum. In comparison to the non-inoculated fermentation of a different organic waste batch, addition of this inoculum lead to an improved fermentation performance. Lactic acid yield, concentration, and molar selectivity could be increased from 38% to 51%, 49 g $L^{-1}$ to 65 g $L^{-1}$, and 46% to 86%, respectively.

Eventually, fermentation process data was used to perform techno-economic analysis proposing a waste treatment plant with different catchment area sizes ranging from 50,000 to 1,000,000 people. Economically profitable scenarios for both batch and continuous operation could be identified for a community with as few as 100,000 inhabitants. With the experimental data, as well as techno-economic calculations presented in this dissertation, a profound contribution to sustainable waste treatment and material production was made.

# *Acknowledgements*

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# 1. Introduction

We live in a resource-limited world, where the demand for materials and energy is increasing (Keijer, Bakker, and Slootweg, 2019). In order to ease pressure on ecosystems, both political and research focus is directed towards utilization of waste for the production of materials and energy (Clark, 2019). In this regard, organic waste might be of particular interest (Sindhu et al., 2019). Containing both a carbon source (carbohydrates) and a nitrogen source (proteins), organic waste can act as ideal substrate for microbial fermentation.

Fermentation is a naturally occuring process, which is facilitated by microorganisms (Gram et al., 2002). Nowadays, most biotechnological processes rely on fermentative approaches. During so called non-sterile fermentations, the indigenous microbiata populating the susbtrate is steered into the desired direction with help of external parameters, such as temperature- and pH-control (Chen and Wan, 2017). Disadvantageously, the outcome of this process is difficult to predict since it is directly influenced by factors such as inhabiting microorganisms and the composition of the starting material (Dai et al., 2017). Common products of non-sterile fermentations are ethanol, acetic acid, and lactic acid (Strazzera et al., 2018).

Lactic acid is the monomer of poly-lactic acid, a promising thermoplastic biopolymer, which accommodates the demand for sustainable plastics (Oliveira et al., 2018). Sterile lactic acid fermentations have been carried out with various starting materials. Substrates like apple pomace (Gullon et al., 2008), coffee mucilage (Neu et al., 2016), jackfruit seeds (Nair et al., 2016), canteen leftovers (Zhang et al., 2017), bakery waste (Yang et al., 2015), and kitchen refuse (Tashiro et al., 2013) were subjected to batch fermentation. In these studies strains of *Lactococcus*, *Lactobacillus*, or *Streptococcus* facilitated fermentation processes.

Besides batch processing, fed-batch and continuous processing exist as alternative operational techniques. During fed-batch operation, medium is periodically fed to the fermented substrate. While this mode of operation often delivers good yields, it is complex to handle and difficult to scale up (Hewitt and Nienow, 2007, Rani and Rao, 1999). Due to these constraints, fed-batch operation was not considered in this study. Continuous fermentation, on the other hand, might offer better plant

capacity utilization, more thorough substrate conversion, and help mitigate product inhibition (Li et al., 2014, López-Gómez et al., 2019).

Previous studies have provided proof for the feasibility of continuous lactic acid fermentation utilizing either raffinated sugar in aqueous solution (Gao and Ho, 2013, Lee et al., 2014) or some type of hydrolysate (obtained from corn stover (Ahring et al., 2016), rice bran (Li et al., 2015), or cassava starch (Bomrungnok et al., 2012)). Yet, these substrates are of low viscosity and therewith easily pumpable. In comparison to hydrolysates, organic waste is of high viscosity, which in return aggravates continuous processing. Nonetheless, some attempts of continuously utilizing diluted organic waste exist. For example, biohydrogen was successfully produced in a continuously stirred tank reactor. Raw food waste was further diluted 1:3 with water and ground in order to facilitate pumping of the slurry into the reactor (Reungsang, Sreela-or, and Plangklang, 2013). A well-established process for continuous fermentation of organic matter is anaerobic digestion. For instance, anaerobic fermentation was carried out with a 10% solid content food waste slurry (Lee et al., 2010).

In summary, continuous fermentation of organic waste on the one hand, as well as continuous lactic acid fermentation on the other hand are feasible in principle. However, the combination of the two, which would be continuous lactic acid fermentation with undiluted organic waste as substrate has not been established thus far. Considering the benefits, direct and continuous non-sterile organic waste fermentation poses a promising topic. Therefore, a newly designed method for efficient and beneficial treatment of organic waste can truly alleviate stress on ecosystems by decreasing extraction of resources. In this context, nutrient recovery after fermentation plays an important role (Withers, 2019). As fermentation broth contains phosphate, which is a critical nutrient for agricultural food production (Mueller et al., 2012), phosphate recovery from fermentation broth was investigated, as well. It has been reported that seashells are capable of removing phosphate from aqeous solution (Namasivayam, Sakoda, and Suzuki, 2005). Transferring this knowledge to fermentation broths, a leap towards thorough substrate utilization and mindful use of resources might be made.

This dissertation aims at bringing together lactic acid production, non-sterile fermentation, and continuous operation. Food waste serves as exemplary organic waste. It is assumed that continuous fermentation more efficiently exploits food waste than batch fermentation. In the course of this thesis, this hypothesis will be validated. As both indigenous microbiota and inoculated species contribute to fermentation performance, strain monitoring becomes eminent and will be examined with microbial analyses. Moreover, techno-economic feasibility of the whole process will be assessed in view of up-scaling. Do profitable scenarios exist? What is the minimum plant size? On the bigger scale, this thesis might potentially lay the foundation for food waste treatment coupled with lactic acid production in a small city.

# 2. Objectives

Food waste is a highly abundant, yet complex and challenging resource. It consists of many components with varying shares. As food waste traces back to consumer diets, its composition will alternate each day. Nonetheless, with its generally high carbohydrate and protein content, food waste holds a lot of promise as fermentation substrate. In the same line, the precious composition demands the development of the most efficient and thorough utilization process. With this in mind, this dissertation sets the following objectives:

- to evaluate food waste as substrate for non-sterile fermentations
- to develop a continuous culture lactic acid fermentation for mechanically pretreated food waste
- to compare batch and continuous lactic acid fermentation processes
- to analyze the indigenous consortium inhabiting food waste
- to investigate possibilities of removing phosphate directly from fermentation broth
- to techno-economically asses an up-scaled fermentation process.

# 3. Articles

Research for this dissertation was conducted between June 2017 and November 2019 at the Institute of Sustainable and Environmental Chemistry at Leuphana University Lüneburg. Hitherto, seven articles have been successfully published in peer-reviewed journals.

1 Peinemann, J. C., & Pleissner, D. (2018). "Material utilization of organic residues" *Applied biochemistry and biotechnology*. https://doi.org/10.1007/s12010-017-2586-1

2 Pleissner, D., & Peinemann, J. C. (2020). "The Challenges of Using Organic Municipal Solid Waste as Source of Secondary Raw Materials" *Waste and Biomass Valorization*. https://doi.org/10.1007/s12649-018-0497-1

3 Peinemann, J. C., Krenz, L. M. M., & Pleissner, D. (2019). "Is seashell powder suitable for phosphate recovery from fermentation broth?" *New biotechnology*. https://doi.org/10.1016/j.nbt.2018.08.003

4 Pleissner, D., Zaman, T., & Peinemann, J. C. (2020). "The effect of organic acids and alcohols on precipitation of phosphate using calcined seashell powder" *Chemical Papers*. https://doi.org/10.1007/s11696-019-00966-9

5 Peinemann, J. C., Demichelis, F., Fiore, S., & Pleissner, D. (2019). "Techno-economic assessment of non-sterile batch and continuous production of lactic acid from food waste" *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2019.121631

6 Peinemann, J. C., & Pleissner, D. (2020). "Continuous pretreatment, hydrolysis, and fermentation of organic residues for the production of biochemicals" *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2019.122256

7 Peinemann, J. C., Rhee, C., Shin, S. G., & Pleissner, D. (2020). "Non-sterile fermentation of food waste with indigenous consortium and yeast–Effects on microbial community and product spectrum" *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2020.123175

# 4. Materials and Methods

## 4.1 Food waste fermentation

During the course of the dissertation, food waste was collected at three different time points from the Leuphana university canteen. Macroscopically, food waste included potatoes, pasta, rice, salad, gravy, vegetable, fish, and meat leftovers. Each collection (A, B, C) of food waste (FW) received a unique label: FWA (May 2017), FWB (April 2018), and FWC (June 2018). Directly after collection, the food waste was homogenized with a blender, pressed through a sieve (3 mm mesh size), and stored at -18 °C until further usage. Food waste was analyzed for carbohydrate, lipid, protein, as well as ash content, and dry matter.

Food waste fermentations with FWA and FWB were carried out non-sterile at pH 6 and 35 °C in a bioreactor. For each fermentation, 1 kg undiluted food waste was used. The indigenous consortium present within the food waste was tested by itself, with added external glucoamylase (0.1% (v/v)), with added inoculum of *Streptococcus* sp. strain (5% (v/v)) (Pleissner et al., 2017), or with both *Streptococcus* sp. and glucoamylase. External glucoamlyse addition facilitated starch hydrolysis to increase glucose concentration in the substrate. For continuous flow fermentations, the set-up was expanded by a 0.5 L storage container, which was refilled three times a day. By employing a peristaltic pump, the food waste was pumped from the storage container to the bioreactor, while another peristaltic pump withdrew the same amount at the bottom of the reactor. Initially, continuous flow fermentations were started as batch. After 24 hours of batch operation, flow was switched on (dilution rate between 0.39 $d^{-1}$ and 1.15 $d^{-1}$). Dilution rate $D$ is defined as:

$$D = \frac{\text{flow rate}}{\text{reactor volume}} = \frac{[\text{L} \cdot \text{d}^{-1}]}{[\text{L}]} = [\text{d}^{-1}]$$

Concentrations of glucose, fructose, sucrose, lactic acid, ethanol, and acetic acid were monitored by sampling regularly followed by HPLC analysis. The yield of lactic acid per gram of carbohydrates ($Y_{LA}$) was calculated by dividing the concentration of lactic acid ($LA_{final}$) by the sum of glucose (including starch), fructose, and sucrose

concentrations ($\sum \text{carbohydrates}_{initial}$) in the starting material.

$$Y_{LA}[\%] = \frac{\text{LA}_{final}}{\sum \text{carbohydrates}_{initial}} \cdot 100\%$$

Molar lactic acid selectivity $S_{LA}$ was calculated by dividing the molar concentration of lactic acid by the sum of all metabolites (lactic acid, ethanol, acetic acid).

$$S_{LA}[\%] = \frac{\text{LA}_{final}}{\sum \text{metabolites}_{final}} \cdot 100\%$$

## 4.2 Microbial community analysis

In order to analyze changes within the microbial community during fermentation, microbial strain identification was performed via 16S rRNA gene sequencing (data kindly provided by Chaeyoung Rhee and Seung Gu Shin, Gyeongnam National University of Science and Technology, Korea). Non-sterile fermentations were carried out with 400 mL FWC as substrate at pH 6 and 35 °C in a bioreactor. For starch hydrolysis, 0.1% (v/v) glucoamylase was added at the start of fermentation. Firstly, fermentation was conducted without inoculum addition. Thereafter, another batch was fermented adding a 5% (v/v) inoculum obtained from FWB. Inoculum was prepared by transferring 1 g of FWB to nutrient solution and subculturing subsequently. Strain analysis was conducted on the starting material at 0 h and the substrates after fermentation at 48 h, as well as the prepared inoculum. Additionally, concentrations of glucose, fructose, sucrose, lactic acid, ethanol, and acetic acid were monitored by sampling regularly followed by HPLC analysis.

## 4.3 Phosphate removal applying waste seashells

Phosphate is one of the most critical nutrients with respect to agricultural food production. Therefore, the potential of removing phosphate directly from lactic acid fermentation broth was tested applying waste seashells. Firstly, seashells were dried, ground, and sieved (< 1mm mesh size), then 4.8 g of the ground seashells were added to an Erlenmeyer flask, filled with 50 mL fermentation broth adjusted to pH 7. The broth was obtained from lactic acid fermentation of glucose and yeast extract with *Streptococcus* sp. After fermentation and prior to removal, concentrations of phosphate and lactic acid were determined as 132 mg $L^{-1}$ and 50 g $L^{-1}$, respectively. For comparison, aqeous solutions (pH 7) containing 132 mg $L^{-1}$ phosphate in demineralized water or 132 mg $L^{-1}$ phosphate alongside 50 g $L^{-1}$ lactic acid

in demineralized water were prepared and subjected to phosphate removal with 4.8 g seashell powder. The slurries were stirred magnetically for 2 hours at 140 rpm. During each run, phosphate concentration in solution was monitored. The seashell powder was characterized by X-ray diffraction and scanning electron microscopy (data kindly provided by Tasmia Zaman, Rajshahi University of Engineering and Technology, Bangladesh).

## 4.4 Techno-economic assessment

Experimentally generated process data from both batch and continuous flow fermentations were subsequently used for techno-economic assessment. Additionally, technical and economic factors from literature were used. Key figures of the economic assessment were: net present value, return of investment, and payback time (data kindly provided by Francesca Demichelis and Silivia Fiore, Politecnico di Torino, Italy). For this assessment, the fermentation plant lifetime was assumed to be 20 years.

The return of investment relates the annual net profit to the initial total investment:

$$\text{return of investment } [\%] = \frac{\text{annual net profit}}{\text{initial total investment}} \cdot 100\%$$

The net present value describes the profitablity of a plant by considering all cash flows during the plant lifetime. It is calculated as follows:

$$\text{net present value } [€] = \sum_{t=1}^{20} \frac{C_t}{(1+d)^t} - C_0$$

with $C_t$ as net cash flow at year $t$, $C_0$ as initial capital investment, and $d$ as discount rate rate on future cash flows. For this study, a discount rate of 5% was used. (With the discount rate, the investment in a plant is compared to an investment in an alternative venture with a fixed discount rate $d$. If the net present value is positive, the investment in the plant is more profitable than the compared venture.)

Finally, payback time $T$ is the time in years needed to restore the initial investment.

$$\sum_{t=1}^{T} \frac{C_t}{(1+d)^t} = C_0$$

The lower the payback time of a scenario, the faster economic profits are generated.

# 5. Results and Discussion

## 5.1 Food waste analyses of different collections

The overarching objective of this dissertation was the material utilization of food waste. However, prior to utilization, assessment of the organic substrate is necessary. Three batches (FWA, FWB, FWC) were collected at different time points throughout the course of the thesis. The major components protein, lipid, starch, free saccharides, and ash are found repetitively, yet, their individual shares vary from batch to batch (Table 5.1) (Pleissner and Peinemann, 2018). Protein content is as low as 13.6% (w/w) in FWA and as high as 19.1% (w/w) in FWB. The spread is even larger regarding lipid content, where FWA holds 21.5% (w/w), while lipids in FWC amount to 37.7% (w/w). The carbohydrate content (including starch) ranges from 34.3% (w/w) in FWB to 48.2% (w/w) in FWC to 59.8% (w/w) in FWA.

Carbohydrates in food waste can be subdivided into free saccharides and starch. Starch is a polysaccharide comprising glucose as monomer. In this study, the term free saccharides denominates the aggregate of sucrose (disaccharide of fructose and glucose), fructose, and glucose. Since microorganisms primarily metabolize free saccharides, starch hydrolysis is required in order to make the full carbohydrate content

TABLE 5.1: Composition of food waste based on dry weight from different collections.

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

accessible (Peinemann and Pleissner, 2018). As one mole of the C6-molecules glucose or fructose can be converted into two moles of lactic acid (C3-molecule), carbohydrate content stoichiometrically constrains the maximal lactic acid concentration obtainable from each individual batch. This makes the yield of lactic acid in respect to sugars present in the starting material ($Y_{LA}$) a relevant comparable figure.

## 5.2 Batch and continuous lactic acid fermentations

Both batch and continuous fermentations were carried out with food waste as substrate (Peinemann et al., 2019). As food waste was not sterilized, the indigenous consortium naturally populating the waste contributed wholly or partially to the fermentation performance. Fermentations were conducted without inoculum addition, as well as with inoculum of *Streptococcus* sp., a strain, which had previously been identified as efficient lactic acid producer (Pleissner et al., 2017). Starch hydrolysis was facilitated by external glucoamylase addition at the start of the experiment. Furthermore, by not supplementing glucoamylase, it was tested whether the indigenous consortium would be capable of hydrolyzing starch in food waste on its own accord.

In presence of *Streptococcus* sp., $Y_{LA}$ reached a total of merely 12% in FWA and a concentration of 26 g $L^{-1}$. FWB on the other hand gave 47% and 37 g $L^{-1}$ (Table 5.2). In this case, addition of *Streptococcus* did not affect the yield. Adding glucoamylase at the start increased $Y_{LA}$ for both FWA and FWB to 55% and 58% (concentration of 44 g $L^{-1}$ and 47 g $L^{-1}$) in the presence of *Streptococcus*. Utilizing merely the indigenous consortium on FWB and FWC, as well as glucoamylase, $Y_{LA}$ of 63% and 38% with respective concentrations of 50 g $L^{-1}$ and 49 g $L^{-1}$, were obtained (Table 5.2). This data indicates that neither the indigenous consortium nor *Streptococcus* sp. were able to hydrolyze starch during the 24 hour fermentation. Sucrose diminishes, however, underlining the ability of bacteria present to break the $\alpha$,$\beta$-1,2-glycosidic bond.

With respect to process efficiency and downstream processing, yield and concentration are important figures. Both figures are influenced by glucoamylase addition and subsequently increase when the enzyme is added (Table 5.2). Simultaneously, *Streptococcus* sp. does not exert a positive influence on yield and concentration as can be seen with data from FWB. This might be attributable to better adaption of the indigenous consortium towards the substrate. In a previous study where the same *Streptococcus* strain was used for the fermentation of sterilized food waste, 58 g $L^{-1}$ lactic acid, $Y_{LA}$ of 63%, and productivity of 2.08 g $L^{-1}$ $h^{-1}$ could be reached (Pleissner et al., 2017). Fermenting sterilized food waste with inoculated indigenous consortium, Tang et al. report $Y_{LA}$ of 58% and 68% with lactic acid concentrations of 33 g $L^{-1}$ and 28 g $L^{-1}$, alongside productivities of 0.28 g $L^{-1}$ $h^{-1}$ and 0.17 g $L^{-1}$ $h^{-1}$,

TABLE 5.2: Productivity [g $L^{-1}$ $h^{-1}$], concentration [g $L^{-1}$], and yield ($Y_{LA}$) [%] of different food waste fermentations carried out for 24 hours in batch mode without glucoamylase / with glucoamylase.

| | FWA, *Str.* sp. | FWB, *Str.* sp. | FWB, – |
|---|---|---|---|
| Productivity | 2.28 / 2.95 | 1.54 / 1.87 | 1.58 / 2.93 |
| Concentration | 26 / 44 | 37 / 47 | 38 / 50 |
| $Y_{LA}$ | 12 / 55 | 47 / 58 | 47 / 63 |

*Str.* sp.= *Streptococcus* sp.
– = no inoculum

respectively (Tang et al., 2016, Tang et al., 2017). Seeing these numbers, $Y_{LA}$ found in glucoamylase-supplemented fermentations of this dissertation compares very well to other publications. Additionally, productivity reported here is higher and given the usage of non-sterile food waste, this process is much simpler.

As a follow-up of batch experiments, continuous fermentations were conducted with FWB. For this experiment, a storage container and discharge tank were installed. The storage container was refilled with food waste three times a day, resulting in substrate residence times of up to 14 hours. Being operated at room temperature without thermal or pH-control, microbial growth in the storage container is to be expected. However, neither concentrations of free sugars nor lactic acid concentration were largely affected in comparison to the starting material (divergence after 14 hours < 5 g $L^{-1}$). Presumably, lactic acid generation and pH < 4 suppressed unwanted microbial activity.

Continuous flow fermentations were initiated uniformly to the batch fermentations for the first 24 hours. Thereafter food waste flow was started with a dilution rate of around 0.4 $d^{-1}$ (Figure 5.1A). In the first continuous run, glucoamylase was added every 24 hours to ensure sufficient glucose levels. During the first day, lactic acid concentration increased from 47 g $L^{-1}$ to 61 g $L^{-1}$. This trend was maintained until 148 h, where fermentation was terminated with a lactic acid concentration of 74 g $L^{-1}$, corresponding to $Y_{LA}$ of 93%. Looking at other metabolites, analysis unveiled an increase in acetic acid concentration from 5 g $L^{-1}$ to 7 g $L^{-1}$, while ethanol concentration remained below 1 g $L^{-1}$. Throughout the whole fermentation, 2270 g food waste was processed with an overall productivity of 1.27 g $L^{-1}$ $h^{-1}$.

In an attempt to simplify operation, continuous fermentation without glucoamylase was performed (Figure 5.1B). As before, lactic acid concentration increased during the first day, reaching 53 g $L^{-1}$. Thereafter, concentration seemed to be levelling at that concentration. With free sugars present at constantly low concentrations, dilution rate was expected to be below the growth rate of bacteria. In order to increase

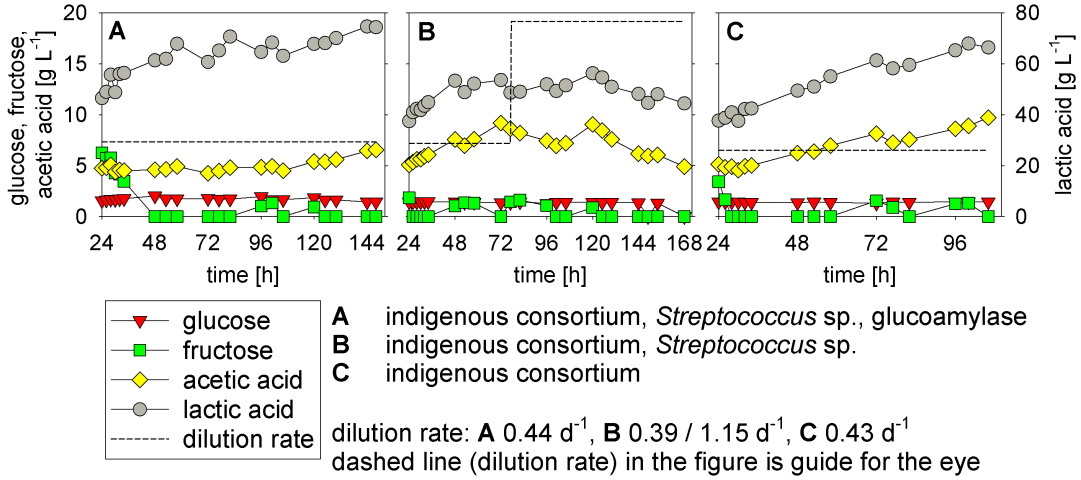


FIGURE 5.1: Continuous fermentation of FWB at given dilution rate.

productivity and efficiency, dilution rate was elevated to 1.15 $d^{-1}$ after 77 h. During the consecutive 48 hours, no dramatic change in concentration could be detected. From 119 h until the end of fermentation at 168 h, lactic acid concentration decreased to 45 g $L^{-1}$. Having reached a maximal $Y_{LA}$ of 70% at 120 h, final $Y_{LA}$ was only 56%.

Observing this decrease of concentration and yield, it is deduced that 1.15 $d^{-1}$ is too high of a dilution rate, resulting in the wash-out of cells. This hypothesis is further supported by examining the acetic acid concentration, which shows a trend similar to lactic acid concentration. For a sustainable lactic acid production, it is crucial to carefully choose a dilution rate, which does not lead to cell wash-out. Since productivity is the arithmetic product of concentration and dilution rate, high dilution rates should be favored. In this case, the optimal dilution rate lies between 0.4 $d^{-1}$ and 1.15 $d^{-1}$. Productivities calculated from said dilution rates give 0.91 g $L^{-1}$ $h^{-1}$ for the first and 2.39 g $L^{-1}$ $h^{-1}$ for the latter. In literature, only few cases of continuous lactic acid fermentation are reported. Utilizing easily pumpable hydrolysates, productivities range from 0.09 g $L^{-1}$ $h^{-1}$ with *Lactobacillus bulgaricus* on cheese whey (Bassi, Rohani, and MacDonald, 1991) to 13.8 g $L^{-1}$ $h^{-1}$ with *Bacillus coagulans* on corn stover hydrolysate (Ma et al., 2016). In the first case, sugar utilization amounts to merely 25%, while the latter attains 59%. Ahring et al., working with *Bacillus coagulans* and corn stover hydrolysate, report $Y_{LA}$ of 95% (Ahring et al., 2016). In their study, the hydrolysate is quite diluted and lactic acid reaches only 22 g $L^{-1}$, underlining the difficulty of finding a good balance between $Y_{LA}$ and concentration.

All previous examples utilize one specific inoculated strain. On the process side, an even simpler approach to continuous fermentation would be to utilize the indigenous consortium. This way, neither glucoamylase nor inoculum addition might be needed. In this respect, the performance of FWB was tested (Figure 5.1C). Lactic acid concentration of 38 g $L^{-1}$ at 24 h gradually increased to 65 g $L^{-1}$ after 96 h. For

the subsequent 10 hours, lactic acid concentration remained stable and fermentation was finalized thereafter. At the end, $Y_{LA}$ amounted to 79%, a yield that outperforms even glucoamylase supplemented batch fermentations, where maximal yields of 70% were attained.

The fact that sugar utilization during continuous fermentations exceeds batch fermentations is seen through all experimental runs. Just by changing the mode of operation from batch to continuous, sugar utilization is ameliorated. Microbial communities, such as the indigenous consortium inhabiting the food waste undergo compositional changes, when subjected to a set of conditions (Wu et al., 2018). As a result, microorganisms capable of hydrolyzing starch could gain larger shares. Problematically, glucose as hydrolysis product might not only be converted into lactic acid, but into other metabolites, as well. This is demonstrated by calculating the molar selectivity of one product (e.g. lactic acid) over all other detected fermentation products (lactic acid, acetic acid, ethanol). For all runs carried out with FWB, lactic acid selectivity ranged between 80% and 87%.

Within its microbial community, FWB seemingly held very efficient lactic acid producers, outcompeting microorganisms with metabolites other than lactic acid. When fermenting FWC with added glucoamylase and no inoculum, however, molar lactic acid selectivity dropped to 46%. Besides 49 g $L^{-1}$ lactic acid, 19 g $L^{-1}$ ethanol, and 13 g $L^{-1}$ acetic acid were produced. Such complex mixtures render downstream processing technologically demanding and expensive (Komesu et al., 2017). Furthermore, carbon efficiency decreases, as more carbon ends up in non-desired molecules. The diverse product spectrum traces back to the diverse microbial community populating the waste. Insight into the community can be gained with help of 16S rRNA gene sequencing. Microbial strain identification was conducted and respective shares of the strains were calculated.

## 5.3 Microbial composition of the indigenous consortium

Within FWC, primarily strains of the genus *Streptococcus* (49%) and *Lactobacillus* (11%) were found (Figure 5.2 FWC at start) citepPeinemann2020non. *Bombella*, *Lebetimonas*, *Mesoaciditoga*, *Pseudomonas*, and *Lactococcus* were detected with individual shares below 3%. 24% of the strains could not be classified. With glucose depletion, fermentation ceases after 48 hours. At this point, strain analysis reveals alterations in the composition under the given conditions. Dominating at the start, *Streptococcus* diminishes to nearly 1%, while *Leuconostoc* and *Lactobacillus* possess almost equal shares of 47% and 44%, respectively. *Enterococcus* contributes 3% to the total, *Pediococcus* holds 1% (Figure 5.2 FWC* 48 h). Being a heterofermentative strain, *Leuconostoc* is capable of fermenting carbohydrates to equal amounts of lactic acid,

ethanol, and carbon dioxide. As no yeast were found at the start or end of fermentation, the production of ethanol during fermentation should be attributed to such heterofermentative strains. Among species belonging to the genus *Lactobacillus*, on the other hand, both homofermentative and heterofermentative strains are found. *Enterococci* and *Pediococci* are homofermentative and should produce two moles of lactic acid from one mole glucose.

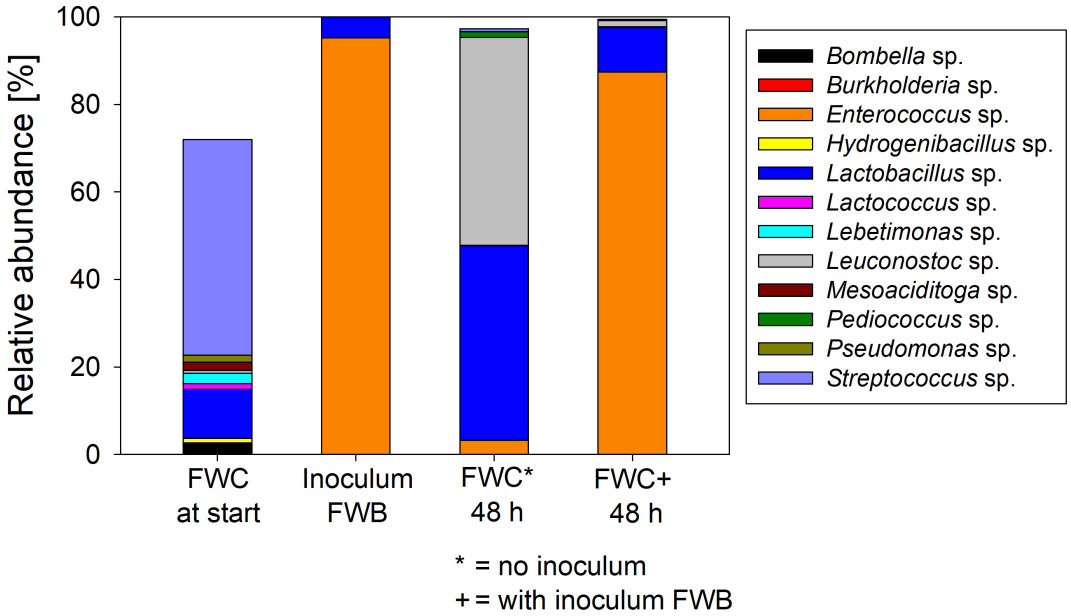


FIGURE 5.2: Strain analysis of different samples before and after fermentation.

As mentioned before, lactic acid producers on FWB were dominating fermentation (Figure 5.1 and 5.2). By combining a substrate rich in carbohydrates – such as FWC (5.1) – with an inoculum rich in efficient lactic acid bacteria, lactic acid selectivity and yield might be increased. For this purpose, a small portion of FWB was extracted and cultivated in a glucose / yeast extract solution. Microbial community almost exclusively comprised of *Enterococcus* (95%) and *Lactobacillus* (5%) (Figure 5.2 Inoculum FWB). Inoculating with this cultivated indigenous consortium from FWB, FWC was fermented again. After 48 hours, 65 g $L^{-1}$ lactic acid were produced alongside 7 g $L^{-1}$ acetic acid, while ethanol concentration was below detection limits. Accordingly, molar selectivity amounted to 86% (Table 5.3). Strain analysis unveiled the dominance of *Enterococcus* after 48 hours. 86% of the bacteria, belonged to said genus, whereas *Lactobacillus* represented most of the remainder with a share of 10% (Figure 5.2 FWC+ 48 h). Other studies report the dominance of *Enterococcus*, as well, however, this is often linked to elevated pH over 9 (Jang et al., 2015, Ma et al., 2019). Working with a model solution in non-sterile fermentation, an *Enterococcus* strain was utilized at pH 6, similar to this study, underlining both pH-tolerance and resilience of the strain in a non-sterile environment (Abdel-Rahman et al., 2013).

TABLE 5.3: Concentration [g $L^{-1}$], yield ($Y_{LA}$) [%], and molar selectivity [%] of two FWC fermentations after 48 hours in batch mode.

| | FWC* | FWC+ |
|---|---|---|
| Concentration | 49 | 65 |
| $Y_{LA}$ | 38 | 51 |
| Selectivity | 46 | 86 |

* = no inoculum

+ = with inoculum FWB

In a study on repeated batch fermentation of food waste utilizing a food waste inoculum, Tang et al. report the presence of a very diverse microbial community at the start (*Lactobacillus* and *Weissella* dominate followed by *Propionibacterium*, *Leuconostoc*, and *Acetobacter*). After 13 days of fermentation, however, more than 99% of the microbial community belongs to *Lactobacilli* (Tang et al., 2016). In a different exeriment, they observe the same shift from 43% *Lactobacillus* share to 98.5% 120 hours into the fermentation (Tang et al., 2017). Food waste fermentation with activated sludge (originating from a wastewater treatment plant) as inoculum gave a similar behavior (Liang et al., 2016). After 48 hours, *Lactobacilli* held the main share. In concert with these findings, this dissertation confirms the drastic shift occurring within the microbial community from many to one or two major strains populating the waste under the given conditions.

Knowing the performance of FWC fermented without inoculum addition and possessing effective lactic acid producers from a different source, lactic acid concentration, yield, and selectivity can be increased considerably (Table 5.3). High lactic acid selectivity is particularly favorable regarding downstream processing, as large quantities of other compounds (e.g. acetic acid, ethanol) tend to complicate isolation of lactic acid.

## 5.4 Phosphate removal from fermentation broth

In the sense of a thorough and mindful substrate utilization, nutrient recovery from fermentation broth should be considered. Amongst the nutrients naturally present in fermentation broth, phosphate plays an important role due to the option to use it as fertilizer in agriculture. For this application, separation from fermentation broth has to be carried out. Making use of a waste product from seafood industry, seashells might serve as adsorbent for phosphate (Namasivayam, Sakoda, and Suzuki, 2005, Millero et al., 2001, Chen et al., 2013).

X-ray diffraction measurements of the seashell powder used in this dissertation revealed the presence of calcite and aragonite citepPleissner2019SSP2. Both are carbonate minerals with the chemical formula $CaCO_3$, but different crystal lattices. Furthermore, electron microscopy scans of the seashell powder unveiled a coarse-grained surface with large surface area. Accordingly, removal of phosphate would be attributable to either precipitation as a calcium phosphate, such as hydroxylapatite ($Ca_5(PO_4)_3OH$) or adsorption onto the seashell powder surface.

To test the practicability of phosphate removal with fermentation broth as substrate, lactic acid fermentation was carried out with *Streptococcus* sp. in a solution containing glucose and yeast extract. After fermentation, phosphate concentration was 132 mg $L^{-1}$, lactic acid amounted to 50 g $L^{-1}$. By combining 50 mL fermentation broth and 4.8 g seashell powder, 28.3 mg $L^{-1}$ or 21.5% phosphate were removed from this solution within 120 minutes (Figure 5.3) citepPeinemann2019seashell1. This removal correlates to a phosphate loading on the seashell powder of 0.29 mg $g^{-1}$. Simultaneously, lactic acid concentration diminished to 44 g $L^{-1}$.

To put these values into perspective, trials with 4.8 g seashell powder in pure solutions were carried out, as well. From a solution containing 132 mg $L^{-1}$ phosphate in demineralized water, more than 77% phosphate could be removed, corresponding to a phosphate loading on the powder of 1.07 mg $g^{-1}$. Subsequently, removal capacity was tested on a solution containing both 132 mg $L^{-1}$ and 50 g $L^{-1}$ lactic acid in demineralized water. Attaining a loading of 0.67 mg $g^{-1}$, 4.8 g seashell powder removed 48% of the phosphate initially present in solution.

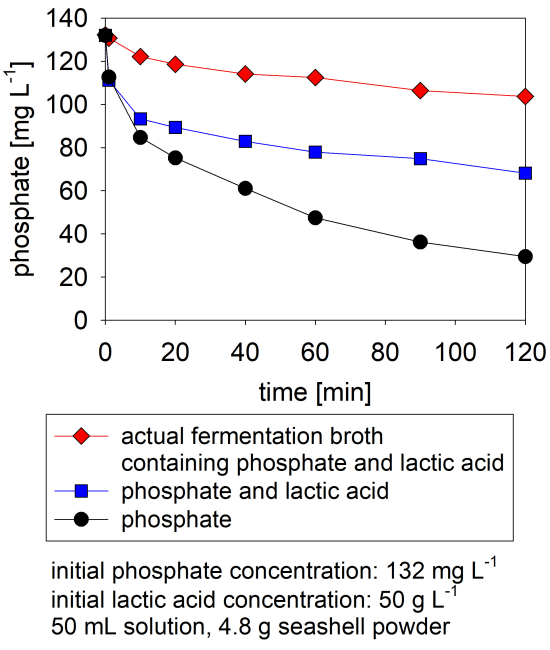


FIGURE 5.3: Phosphate removal from different solutions.

As a general trend, increased complexity of solution is directly linked to loss of removal capacity. Still, these experimental findings substantiate the feasibility of phosphate removal with seashell powder directly from fermentation broth. However, compared to other studies, the phosphate loadings obtained in this dissertation are relatively low. Studies with oyster shells, for example, report loadings of 1.96 mg $g^{-1}$ (Namasivayam, Sakoda, and Suzuki, 2005). In spite of that, commercially available ion-exchange resins outcompete these numbers with maximal loadings of 32.24 mg $g^{-1}$ and 40.23 mg $g^{-1}$ for Amberlite IRA-400 (Marshall and

Wartelle, 2004) and Dowex (Anirudhan and Senan, 2011), respectively.

In order to ascertain usability of seashells on a larger scale, a mass balance can be created on the basis of experimental data: Considering 1000 L fermentation broth containing 50 kg lactic acid and 132 g phosphate, 96 kg seashell powder could be added. After 120 minutes of application, phosphate in solution would still amount to 103 g. Requiring 96 kg seashell powder for the removal of only 29 g phosphate is disproportionate and uneconomical. Taking furthermore into consideration that lactic acid concentration decreases by 12% during application, the proposed process does not seem appropriate for industrial implementation.

## 5.5 Techno-economic assessment

Interrelating experimental results with real world application is a pivotal part of sustainable process development. Besides constructing mass balances, techno-economic assessment serves as a helpful tool for this task. Conclusory, results of aforementioned batch and continuous food waste fermentations were techno-economically evaluated (Peinemann et al., 2019). Scenarios were constructed combining experimental findings with tabulated literature data in a simulated plant. The profitability of such a plant was calculated, considering 50,000 to 1,000,000 people (as food waste producers) in the catchment area.

A measure of profitability is the net present value (Figure 5.4). All cash flows are summed up over the plant lifetime of 20 years. Profitability is given, when the net present value is positive. Overall, a set of scenarios, both batch and continuous, fulfilling this criterion could be identified. In general, with more people in the catchment area, higher profits are generated. The most profitable scenario was found for a continuously operated plant serving 1,000,000 inhabitants in the catchment area (Figure 5.4 7*). This scenario relates to continuous fermentation of FWB utilizing *Streptococcus* sp., indigenous consortium, and glucoamylase. With a return of investment of 176% and net present value of 51.4 million €, payback time amounted to only one year. Downsizing the plant, profitability was sustained with as few as 100,000 people living in the catchment area. Given these circumstances, net present value decreases to 0.9 million €. Being profitable with as few as 100,000 inhabitants in the catchment area, the process becomes attractive for smaller cities.

The view that lactic acid production from food waste can be carried out economically profitable is supported in other studies, as well. Taking food waste powder as substrate and performing hydrolysis and fermentation separately, Kwan et al. found a payback time of 5.1 years and a net present value of more than 200 million € after 20 years plant lifetime (Kwan, Hu, and Lin, 2018). Considering poly-lactic acid as final product, a different study proposes a food waste biorefinery with 7.8 years

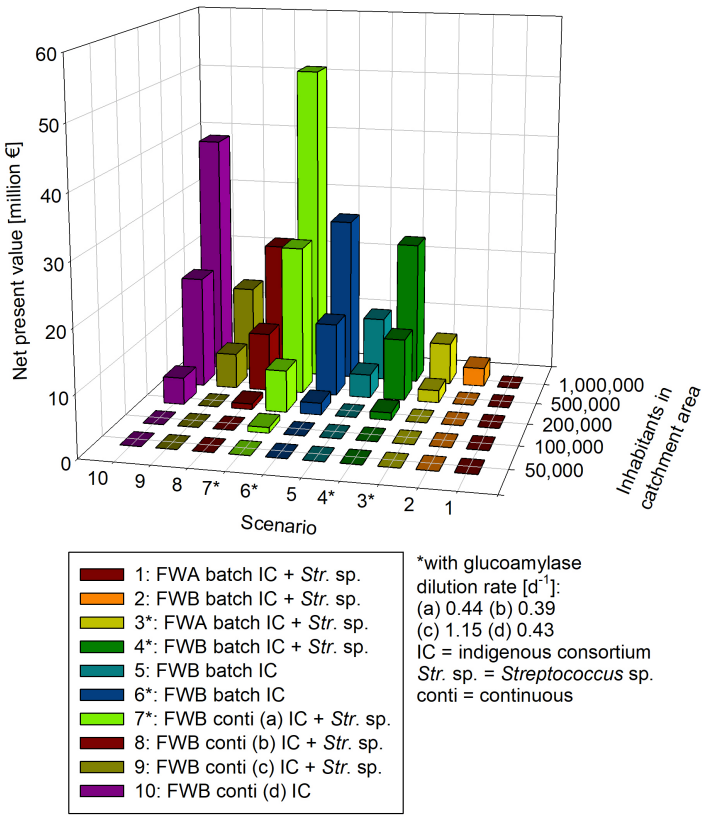


FIGURE 5.4: Net present value of food waste treatment plant after 20 years lifetime considering different scenarios.

payback time and 98% return of investment (Bastidas-Oyanedel and Schmidt, 2018). Conclusively, large scale production of lactic acid (also with food waste as substrate) is feasible in principle and a growing number of industrial lactic acid plants substantiates this finding (Kumar, Thakur, and Panesar, 2019).

# 6. Conclusion and Outlook

Material utilization of organic residues requires careful substrate analysis, sensible process operation, and thorough evaluation of obtained data from a chemical, biological, and economic perspective. This dissertation not only delivers the practical proof-of-concept of continuous food waste fermentation, but also demonstrates the monetary benefits accompanied with this approach. With lactic acid as target product, structural functionality found in the substrate can be conserved to a large extent, while simultaneously supplying a sustainable bulk chemical suitable for the production of poly-lactic acid.

In contrast to previous batch fermentation, this dissertation presents a way of continuously utilizing a prevalent waste source. Despite of its high viscosity, food waste slurry was successfully fermented with yields outperforming batch fermentation. Within the substrate, a very vital indigenous consortium was identified with *Enterococcus* sp. as dominant lactic acid producing strain. Through simulation and extrapolation of experimental findings, a profitable food waste treatment plant for a smaller city is proposed in the context of this thesis. As a novelty, process data from fermentation, techno-economic assessment, and microbial analysis are united to deliver profound insight into food waste fermentation from micro- to macroscale.

Future research should be directed towards utilization of substrates additionally containing lignocellulosic material, such as grass clippings, leaves, and woody matter. With this, the substrate scope could be broadened to include municipal organic waste in general, allowing a more widespread application. As waste treatment touches on a societal subject, outreach to consumers should be sought. Only in open dialogue, scientifically sound results can be implemented in every day practice. Conventional routes of organic waste treatment on industrial scale, such as biogas production or composting, could be extended by continuous processing towards lactic acid. A large issue affecting economic profitability remains the isolation of lactic acid from fermentation broth. The development of efficient downstream methods would really push forward fermentative utilization approaches. Research regarding the transfer from laboratory to industrial scale is needed to help bringing forth new or improved processes.

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1 Peinemann, J. C., & Pleissner, D. (2018). "Material utilization of organic residues" *Applied biochemistry and biotechnology*. https://doi.org/10.1007/s12010-017-2586-1

2 Pleissner, D., & Peinemann, J. C. (2020). "The Challenges of Using Organic Municipal Solid Waste as Source of Secondary Raw Materials" *Waste and Biomass Valorization*. https://doi.org/10.1007/s12649-018-0497-1

3 Peinemann, J. C., Krenz, L. M. M., & Pleissner, D. (2019). "Is seashell powder suitable for phosphate recovery from fermentation broth?" *New biotechnology*. https://doi.org/10.1016/j.nbt.2018.08.003

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# Material Utilization of Organic Residues

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**Abstract** Each year, 1.3 billion tons of food waste is generated globally. This waste traces back to industrial and agricultural producers, bakeries, restaurants, and households. Furthermore, lignocellulosic materials, including grass clippings, leaves, bushes, shrubs, and woods, appear in large amounts. Depending on the region, organic waste is either composted, burned directly, or converted into biogas. All of the options set aside the fact that organic residues are valuable resources containing carbohydrates, lipids, proteins, and phosphorus. Firstly, it is clear that avoidance of organic residues is imperative. However, the residues that accumulate nonetheless should be utilized by material means before energy production is targeted. This review presents different processes for the microbial utilization of organic residues towards compounds that are of great importance for the bioeconomy. The focus thereby is on the challenges coming along with downstream processing when the utilization of organic residues is carried out decentralized. Furthermore, a future process for producing lactic acid from organic residues is sketched.

**Keywords** Decentralized utilization · Downstream processing · Hydrolysis · Lactic acid fermentation

## Introduction

Organic residues are a valuable source of functionalized molecules, such as sugars, lipids, and proteins. In accordance with the composition of mixed food wastes collected from restaurants [1], the following shares of the components were assumed: 450–750 g/kg total carbohydrates (thereof 300–600 g/kg starch), 50–100 g/kg protein, 70–400 g/kg lipids, and around 2 g/kg phosphorus. Furthermore, enormous amounts of lignocellulosic material, including grass clippings, leaves, bushes, shrubs, and woods, is produced, which additionally can serve as a source of carbohydrates and other functionalized molecules. Due to the theoretical potential of

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organic residues as sources of functionalized molecules, more emphasis should be put on utilization rather than treatment.

Efficient organic material utilization can be achieved with the help of microbes. Carbohydrates and proteins are hydrolyzed to glucose and amino acids, respectively, and subsequently converted into value-added chemicals [2]. In technical terms, either simultaneous saccharification and fermentation (one-step approach) [3] or a separate saccharification and fermentation (two-step approach) [4] is feasible. As products, for instance, lactic acid [5], polyhydroxybutyrate [6], and succinic acid [7] can be obtained. The three compounds are used in the synthesis of bio-based plastics and materials and contribute directly to the reduced use of fossil resources in plastic production. Furthermore, nutrients, such as phosphorus, which is urgently needed to maintain and increase the biomass productivity, can be recovered from organic material and recycled to arable land [8, 9]. The recovery and reuse of phosphorus as fertilizer contribute to a mitigation of greenhouse gas emissions as $CO_2$ is assimilated during biomass production.

The goal should be that utilization processes are applied where large amounts of organic wastes appear, such as in buildings in urban areas. Thusly, a sustainable waste management system could be incorporated in urban environments [10]. The technical realization of a decentralized utilization process for organic residues depends on the determination of biological basic scenarios of fermentation processes. The first step is the upstream processing (e.g., hydrolysis) under consideration of the abundance and composition of organic residues. The second step is the investigation whether batch, fed-batch, or continuous fermentation processes are appropriate for utilization. The third step is the development of a fast and efficient downstream processing in order to separate and purify the wanted products.

After portraying different utilization strategies, a fermentation process including downstream processing is sketched. This conception is laid out to be particularly suitable for application in urban areas where large amounts of organic residues appear and are expected to contribute to the development of an alternative organic residue treatment process. It is hypothesized that lowering the complexity of a fermentation process and downstream processing is contributing to its technical implementation and development of efficient organic residue utilization. Various fermentation processes were simplified in the past already but downstream processing remains still complex and a challenge when utilization is carried out decentralized.

## Separate Saccharification and Fermentation

By means of hydrolysis, the organic matter is converted into a homogeneous hydrolysate. The carbohydrates like starch and in part also hemicellulose and cellulose, as well as the proteins, are broken down into monosaccharides (mainly glucose) and amino acids, respectively. Polyphosphate is degraded to phosphate. In order to utilize lignocellulosic feedstock, some types of pretreatment, e.g., acid hydrolysis, steam explosion, or organosolv prior to fermentation, are needed. Pretreatment disrupts the lignin structure and renders the carbohydrates enzyme accessible [11].

## Saccharification with Enzymes

Hydrolysis can be carried out with chemical or biological methods. Chemical methods usually require harsh conditions like higher temperatures and strong acids or bases, triggering the

formation of byproducts [12]. Biological methods work at moderate temperature levels and pH values with either enzymes or whole cells suitable for the hydrolysis [13]. Due to the complicated composition of organic residues, no single enzyme or microbe but a whole set was applied in previous studies in order to hydrolyze the entire sample. In the case of isolated enzymes, a mixture of amylases, hemicellulases, cellulases, and proteases qualifies for the task [14]. Working with food waste, Kim et al. facilitated an amyloglucosidase, an α-amylase, and a protease to obtain a nutrient-rich hydrolysate [15]. Focusing only on the glucose fraction of the food waste, Yan et al. applied an α-amylase and a glucoamylase for the saccharification [16]. In a similar sense, Pleissner et al. showed that seawater could be used as medium to hydrolyze bakery food waste with commercial glucoamylases [17]. Advantages of the application of pure enzymes are their high activity and accessibility, the fact that no nutrients are consumed, and good control over the enzyme mixture.

## Saccharification with Whole Cells

When using whole viable cells for the degradation of food waste, different consortia of microbes showed to be effective [18]. In a consortium analyzed by Fong [19], species of the genera *Flavobacterium*, *Pseudomonas*, *Micrococcus*, *Aeromonas*, *Xanthomonas*, *Vibrio*, and *Sphingomonas* could be identified. In a different study, food waste was hydrolyzed with the help of *Aspergillus awamori* and *Aspergillus oryzae*, the first producing mainly amylolytic enzymes and the latter mainly proteolytic enzymes [1, 20]. The two fungi can be applied either as solid mashes or as crude enzyme extracts [17].

The usage of consortiaproducing enzymes is favorable since enzymes are produced in situ under partial nutrient consumption. Once established, the microbial community remains stable. Such an approach requires basic research as the presence of inhibitors, and interferences with other organisms can affect the hydrolytic performance [21]. After the hydrolysate is obtained, a fermentation step is appended. This two-step method is useful, when the hydrolysis requires different conditions than the fermentation. Another reason for a two-step approach might be the versatility of the hydrolysate, making it possible for the producer to switch between fermenting organisms, regarding whichever value-added compound is currently economically feasible to generate. The variety of value-added compounds resulting from fermentation will be discussed in the next section.

Since the hydrolysate is rich in glucose, amino acids, lipids, and phosphate, it poses a potent basis for all kinds of microorganisms. As C5 or C6 sugars are the starting materials, the compounds to be produced range from being carbon free (hydrogen) to containing up to six carbon atoms (e.g., adipic acid) to even oils and fatty acids with more than 20 carbon atoms. Depending on the choice of target compound, the fermenting organism is selected.

## Fermentation of Hydrolysate

In a study with hydrolyzed tropical biomass waste, *Clostridium butyricum* was utilized to produce hydrogen [22]. A hydrolysate obtained from food waste was fermented with the help of the *Biohydrogenbacterium* R3, successfully producing hydrogen as well [23]. Besides its potential as energy source, hydrogen can be used materially, e.g., as coolant.

The yeast strain *Saccharomyces cerevisiae* is broadly used in the production of ethanol [24]. Although other organisms are capable of converting glucose to ethanol, *S. cerevisiae* is most

practical in industry. In order to accept substrates other than glucose, both *S. cerevisiae* and *Escherichia coli* have been genetically modified to work with xylose as well [25].

A C3 molecule that showed to be cumbersome to obtain is propionic acid. Under anaerobic conditions, propionibacteria are able to produce propionic acid; however, even low concentrations of the acid are inhibitory to further cell growth [26]. Means of both molecular and process engineering are used to increase the acid tolerance, yield, and productivity [27]. With cheese whey as substrate, an immobilized *Propionibacterium acidipropionici* mutant produced 135 g/L propionic acid. Fermentation was carried out in a fibrous bed bioreactor [28]. Furthermore, the wild-type strain *Lactobacillus collinoides* was found to produce both 1,3-propanediol and 3-hydroxypropionic acid, another C3 compound [29].

When targeting the material utilization of hydrolysates towards lactic acid, a variety of bacteria can be applied, including *Lactobacillus*, *Lactococcus*, and *Streptococcus* strains. Homofermentative strains like *Lactobacillus amylophilus* and *Lactococcus acidophilus* produce 2 mol lactic acid from 1 mol of glucose, while heterofermentative strains like *Lactobacillus brevis* and *Lactobacillus fermentum* generate 1 mol of lactic acid alongside acetic acid, ethanol, and $CO_2$ [30]. After enzymatic hydrolysis of wheat and rice brans, *Lactobacillus* sp. was used to produce 129 g/L DL-lactic acid without addition of nutrients [31]. A different group reported the utilization of food waste towards lactic acid with bacterial strains from *Bacillus coagulans* and *Bacillus subtilis* in a two-step approach. The *B. subtilis* strain generated 36.9 g/L lactic acid. Subsequently, the fermentation residue was used as plant growth promoter. It was revealed that both lactic acid productions were feasible and nitrogen, phosphorus, and potassium-rich compost could be attained [32]. In a study conducted on the co-fermentation of food waste and *Sophora flavescens* residues, the effect of different substrate ratios on the lactic acid production were investigated. The highest lactic acid concentration of 67.5 g/L was found at a ratio of 1.5:1. The fermentation was performed in one pot; however, prior to the addition of *Lactobacillus casei*, the organic matter was subjected alkaline pretreatment and enzymatic hydrolysis by added cellulases and amylases [33]. *L. casei* and *Lactobacillus delbruckii* were also used in the fermentation of peels from different sources including potato, sweet corn, green peas, and mango, which were hydrolyzed by steam explosion prior to fermentation. The highest concentration of 63.33 g/L lactic acid was reached with *L. casei* and mango peels [34].

Few fungi are reported to produce lactic acid, namely, species belonging to the genera *Mucor*, *Monilia*, and *Rhizopus*. Modifying *B. coagulans* genetically enabled the strain to convert xylose to L-lactic acid, thusly broadening the substrate scope for the lactic acid production [35].

Succinic acid is a C4 dicarboxylic acid and can be obtained by fermentation as well. Regardless the substrate sugar, phosphoenolpyruvate is formed as an intermediate. To this, intermediate $CO_2$ is added enzymatically and the resulting oxaloacetate is stepwise reduced to succinic acid [36]. Critical parameters are hence the availability of dissolved $CO_2$ and electron donors in the reaction mixture. Besides *E. coli* mutants [37], bacteria like *Anaerobiospirillum succiniciproducens* [38] and *Actinobacillus succinogenes* [39] are used in the fermentation.

Malic acid possesses an additional hydroxyl group in respect to succinic acid, creating an asymmetric carbon atom. Fermenting glucose with *Aspergillus flavus*, a high production of malic acid was observed; however, potential aflatoxin generation impedes utilization of this organism for food-grade chemicals [40]. In *Aspergillus niger*, toxin production does not occur, and malic acid is obtained in lower yields, though [41].

Other compounds microbial conversion can be aimed at include fumaric acid, 2,3-butanediol, butyric acid, and itaconic acid [42]. Fumaric acid is produced by *Rhizopus* strains with glucose or starch hydrolysates as substrate. With immobilized *R. oryzae*, up to 85 g/L fumaric acid could be accumulated [43]. Up-scaling, however, showed to be difficile due to the need to control subtle reaction parameters very carefully. During anaerobic growth of several enteric bacteria, 2,3-butanediol is formed. This, however, occurs alongside the formation of side-products like ethanol, acetate, lactate, formate, and succinate [44]. Butyric acid can be produced by a number of organisms. Since metabolic products of the butyric acid pathway are inhibitory, genetic engineering is needed to enhance the yield [45]. Starting from glucose, the C5 itaconic acid can be obtained, utilizing strains of *Aspergillus terreus* [46], *Pseudozyma antarctica* [47], or *Ustilago maydis* [48].

With help of microalgae, certain yeast, bacterial, or fungal strains, the production of oils and fatty acids is possible. By limiting the accessible nitrogen and supplying excess carbon (e.g., in the form of sugars), the growth rate of organisms and therewith the carbon assimilation rate are reduced. This leads in return to the organism initiating the lipid accumulation process. Herein, citric acid is an important intermediate which ultimately ends up in fatty acids like lysophosphatidic acid [49]. On a food waste hydrolysate, the microalgae *Schizochytrium mangrovei* and *Chlorella pyrenoidosa* were grown and the resulting biomass was analyzed. It was found to be rich in carbohydrates, lipids, proteins, and omega-3 fatty acid and is thusly a viable food or feed source [50].

Another group of compounds that is generated under nitrogen-deficient conditions are polyhydroxyalkanoates. Off the polyester backbone, differently sized alkyl resins are found. Intracellular, these polymers are synthesized for carbon and energy storage reasons. Organisms capable of producing polyhydroxyalkanoate include *Ralstonia eutropha* and *Burkholderia sacchari*. For the fermentation to yield a polymer with the desired properties, cellular regulation is crucial [51, 52].

Bacterial cellulose likens plant cellulose in the chemical structure, but differs regarding the physical and chemical properties. The model organism for the production of bacterial cellulose is *Acetobacter xylinum* [53]. This organism was applied on different types of bagasse hydrolysates. While the acidhydrolyzed substrate resulted in 1.09 g/L bacterial cellulose, the enzymatically hydrolyzed sample gave 0.42 g/L [54]. The cellulose is synthesized between the outer and the plasma membrane, with uridine diphosphate glucose supplying the monomers and the cellulose synthase linking the individual units [55].

All aforementioned processes contribute to the bioeconomy, but their implementation in order to utilize organic residues is due to complexity challenging. Processes can be simplified by combining saccharification and fermentation and are known as simultaneous saccharification and fermentation.

## Simultaneous Saccharification and Fermentation

Saccharification and fermentation can also be performed in one pot. A widespread example is the biogas production [56], which is widely used due to its simplicity, but does not allow an efficient utilization of organic waste. Different substrates have been investigated for their material utilization in so-called one-pot fermentations. For instance, pretreated rice straw, wheat straw, and sugarcane bagasse were used as substrate for *Aspergillus terreus* and *Klyuveromyces* sp. The first organism hydrolyzes the cellulose while the latter produces ethanol from released glucose. The maximal ethanol production of 23.23 g/L was attained

with rice straw after 60 h [57]. For the fermentation of potato waste, *Aspergillus niger* and *S. cerevisiae* were used as co-cultures in a biofilm reactor. After 72 h, an ethanol concentration of 37.93 g/L could be reached [58]. Nguyen et al. targeted ethanol production using agricultural biomass residues. Using pretreated substrate, 11.1 g/L ethanol could be obtained, while in the non-pretreated case, the final ethanol concentration was 2.5 g/L. In this example, saccharification and fermentation were carried out using a mixture of cellulose, β-glucosidase, and *S. cerevisiae* [59]. With help of *Clostridium beijerinckii*, food waste was fermented to yield butanol. At 129 g/L food waste, 19.7 g/L acetone-butanol-ethanol was produced. Even though a considerable share of glucose remained in solution, fermentation stopped at this concentration due to inhibition. By combining the fermentation with a vacuum stripping system, the same amount of food waste yielded 27.2 g/L acetone-butanol-ethanol [60]. Outcomes from a study dealing with the production of lactic acid from food waste revealed that simultaneous saccharification and fermentation can be simple enough to be implemented at locations where organic residues appear in large amounts [61].

## Fermentative Lactic Acid Production—a Case Study

Organic residues can also be treated in a different way, leading to the production of lactic acid. Specific residues have been evaluated in respect to their potential as feedstock in one-pot fermentation without preceding separate hydrolysis.

With coffee mucilage as substrate and *B. coagulans*, concentrations of up to 43.3 g/L lactic acid can be generated. While the addition of yeast extract as nitrogen source improved the productivity, the overall yield remained unaffected [62]. In a one-pot approach, jackfruit seed powder was subjected α-amylases and glucoamylases before *Streptococcus equinus* was added to perform the lactic acid fermentation. With 200 g/L jackfruit seed powder, a maximum lactic acid concentration of 109 g/L was reached [63]. By means of microaerobic digestion, food waste was fermented with indigenous cultures. After a certain time, the acid-rich phase containing 40 g/L lactic acid was separated from the residues by centrifugation. The lactic acid was purified by nanofiltration and electrodialysis, while the residues were further processed to yield biogas. During the fermentation, *Lactobacilli* were found to be the predominant culture. It was calculated that 1.6 mol of lactic acid/mol of hexoses present were produced [64]. Also, working with indigenous microbiota and food waste, the influence of pH, temperature, and organic loading rate was investigated. Optimal operating conditions were determined as pH 6 and 37 °C, giving a maximum lactic acid concentration of 32.8 g/L. In accordance with the aforementioned report, the microbes involved belonged almost exclusively to the *Lactobacilli* [65]. In a later report, Tang et al. discussed the effect of different inocula on the fermentation of food waste. Highest concentrations (28.4 g/L) were obtained for an inoculum from fresh food waste when the fermentation was carried out at pH 5 [66].

Furthermore, a *Streptococcus* sp. strain was recently investigated which is able to simultaneously hydrolyze and convert the recovered nutrients into lactic acid under microaerobic conditions [61, 67]. The application of this strain makes it unnecessary to carry out a hydrolysis prior to fermentation while yield, lactic acid titer, and productivity are comparable to conventionally carried out fermentations. Around 0.3 g lactic acid can directly be produced per gram of dry-mixed restaurant food waste.

Downstream processing is challenging when compounds have similar chemical and physical properties. For instance, after lactic acid fermentation, water and lactic acid/sodium lactate need to be separated. However, water and lactic acid as well as water and sodium lactate are

fully miscible. Thusly, no precipitation method without additional chemicals is applicable. Nevertheless, melting and boiling points of water (0 °C/100 °C), lactic acid (17 °C/122 °C), and sodium lactate (161 °C/–) differ significantly and can be utilized in separation strategies. The hygroscopicity and viscosity, however, strongly impede work-up.

There are processes already in practice for isolating lactic acid from the fermentation broth. The classical approach is to precipitate lactate with calcium hydroxide, filtering, and combining the calcium lactate with sulfuric acid, yielding lactic acid, and calcium sulfate [68]. This method produces large amounts of inorganic waste, and therefore, other processes are demanded. A promising alternative, for instance, is electrodialysis [69]. When put into practice on fermentation broth, downstream processing comprises five steps: filtration, softening, electrodialysis, ion exchange chromatography, and distillation. During filtration, filters with different pore sizes are applied, the last one being a nanofilter. Subsequent softening removes calcium and magnesium ions. To concentrate the lactic acid stream further, an electrodialysis step is appended. Since this method accumulates salt ions in general in a concentrate, it is followed by the more specific ion exchange chromatography. Thereafter, the purified fraction almost exclusively consists of lactic acid and water. The mixture is then evaporated by means of vacuum distillation. With water having a lower boiling point than lactic acid, the latter remains in the condenser as pure liquid. For the overall process, a lactic acid recovery of 38.2% has been reported [62]. Recently, an improved downstream method was published, utilizing anion exchange resins bearing tertiary amines on an acrylic matrix. After microfiltration, a chromatography step with said resins is appended. Using sulfuric acid as eluent, lactic acid can be obtained in high purity. Subsequent distillation allowed a lactic acid recovery of 90%. It was further shown that salt ions present in the fermentation broth could be recovered and reused in follow-up runs [70].

Besides electrodialysis, there are other means of purifying lactic acid. Working with an aqueous solution of lactic acid, mixed extractants showed promising results. When applying tri-n-octylamine and Aliquat 336 in decanol, up to 90% of the lactic acid could be extracted [71]. In a study with different organic acids in a model fermentation broth, it was shown that lactic acid is more difficult to extract than the other acids present (acetic, butyric, isobutyric, propionic, succinic acids). Best results were found with 2-pentanol (66% recovery) and 1-butanol (49% recovery) [72]. An aqueous two-phase extraction was performed with polymer-polymer conjugates consisting of ethylene oxide propylene oxide. The lactic acid partitioned mainly to the polymer-rich top phase, while Lactococcus cells were found in the bottom phase [73]. With functionalized silica compounds as extractants, recovery rates of 80% could be obtained [74].

By using ammonia water to adjust the pH during fermentation followed by membrane filtration and centrifugation to remove the cells and gross particles, ammonia lactate was obtained as 30 wt% fermentation broth. In a rectifying column with an excess of butanol and $FeCl_3$ as catalyst, the ammonia lactate was esterified to butyl lactate. The fraction containing butanol and butyl lactate was then submitted to distillation to remove butanol. Thereafter, butyl lactate was hydrolyzed to LA on an ion exchange resin and the butanol was distilled off. Yields for the individual steps ranged from 86 to 96% [75]. In a similar procedure, protonated lactic acid was esterified by reactive distillation with methanol and an ion exchange resin as catalyst. The methyl lactate was subsequently hydrolyzed with Amberlyst CSP-2 as catalyst, and the two liquids were separated by distillation [76]. Altering the classical approach with calcium lactate, sulfur dioxide, an industry by-product, was used to recover lactic acid. Sulfur dioxide

was introduced to the calcium lactate solution leading to the formation of solid calcium sulfite and protonated lactic acid in solution [77].

Besides treating the broth at the end of the fermentation, in situ product recovery can be applied. In this concept, lactic acid removal takes place while the fermentation is ongoing. Boonmee et al. used an anion exchange resin, which lead to the lactic acid concentration not exceeding 20 g/L in the broth. The total amount of lactate was 1.2-fold higher when resins was added in respect to the unmodified fermentation. Lactic acid was eluted off the resin with HCl, and the resin regenerated with NaOH [78]. In a different study, sweet cheese whey was fermented with *Lactobacillus bulgaricus* in a membrane bioreactor. After screening different membrane types, best results were found for a nanofiltration membrane, which retained 79% of the substrate sugar while 78% of the lactic acid could pass [79]. From an aqueous lactic acid solution, lactic acid could be extracted utilizing imidazolium-based ionic liquids in combination with tri-n-butyl phosphate. Tests confirmed that the extractant is non-toxic to *Lactobacillus rhamnosus*. The strain consumed glucose and produced lactate in the presence of the ionic liquid indicating its possible use as in situ extractant [80].

Downstream methods involving esterification or reactive extraction require additional chemicals, which should be avoided in view of green chemistry principles. It would be best to utilize the lactate directly as it is obtained from the fermentation broth. Spray drying has been used in the food industry for many years and offers a benign way of demoisturizing substances [81]. Generating lactate containing no or little water is beneficial in many ways when targeting a decentralized fermentation process with a centralized downstream processing. The lactate is storable, easily transportable, and no pH adjustment is needed succeeding fermentation. Furthermore, the moderate pH renders special non-corrosive equipment redundant. Collected lactate can then be further processed to lactic acid.

## Future Process for the Decentralized Production of Lactic Acid from Organic Residues

For the future, it would be desirable to establish a continuously run process with optimal yield that includes all steps from organic residues, such as food waste, to lactic acid carried out decentralized where organic residues appear and lactic acid is a wanted product. Only when considering and optimizing all steps in concert, competitive production of high-grade lactic acid will be possible. Homogenized food waste alongside water for dilution could be fed directly to a reactor operating under steady-state conditions. It was shown that solid to liquid ratios of up to 20% are feasible for lactic acid fermentation [61]. Depending on the residence time in the reactor and the composition of the waste, the amount of lactic acid produced will vary.

Using conventional downstream processing techniques, the waste/water-mixture can be filtrated through a hollow fiber module, which allows a continuous separation of fermentation broth and remaining solids [82]. Remaining solids including microbial biomass should be added again to the fermenter in order to increase the microbial biomass concentration and consequently to increase the volumetric productivity as well as the hydrolytic efficiency of microbes. The big advantage of the continuous microbial fermentative approach is that biomass, which is essential for the fermentation performance, regenerates itself and is not washed-out when an appropriate dilution rate is used. Once the lactic acid solution is separated from gross particles, further purification should be targeted. Applying filters with different pore sizes, the last one being a nanofilter, an aqueous lactic acid-water solution containing only monovalent ions is obtained. By lowering the pH value to 3.5 with HCl, it is ensured that only

protonated lactic acid is present. With help of ion exchange chromatography, the majority of remaining monovalent ions are removed [83] and as final step, vacuum distillation would yield lactic acid. Both water content and purity would qualify the lactic acid to be used for poly(lactic acid) production.

Due to their complexity, conventional downstream processing techniques for lactic acid separation and purification are inappropriate for decentralized utilization approaches. It is particularly challenging when the know-how in running those complex processes is not available at location, such as food processing industries. It should be admitted here that downstream processing is tailor made and optimized in accordance to the product. Nevertheless, solving the technical drawbacks of downstream processing may lead to efficient utilization processes. To date, there is no infrastructure implemented for the widespread decentralized production of lactic acid.

Overall, the following mass balance can be proposed: starting from 1000 kg food waste (335 kg starch, 148 kg proteins, 129 kg fat, 85 kg free sugars) in 4000 L water, 290 kg (58 g/L) [61] lactic acid could be produced via simultaneous saccharification and fermentation. Likewise, 330 kg (66 g/L) [67] lactic acid could be obtained with separate hydrolysis preceding fermentation. The broths could subsequently be centrifuged to remove cell biomass, filtrated, and treated with activated carbon to remove colorants. After concentrating the clear broths to 400 g/L lactic acid, ultrasonic-mediated extraction with ethyl acetate at a mixing ratio of 1:2 *v*/*v* (broth/ethyl acetate) could be performed. The organic solvent can then be removed under reduced pressure, before final purification is achieved via freeze drying at − 20 °C. Finally, 238 and 271 kg lactic acid with a purity of 98% could be attained from simultaneous and separate saccharification and fermentation, respectively [84].

Generally, the scale-up from lab scale to semi-industrial scale causes differences in the fermentation performance. For instance, gas transfer is influenced by several variables, such as physical properties of the fluid, operational conditions, filling volume, and geometry of the reactor. Reduced oxygen concentration can result in limited cell growth for large-scale fermentations [85]. Scale-up experiments comparing 5-, 50-, and 500-L reactors revealed that agitation was more difficult to ensure at high reactor volumes. High-medium viscosity can lead to an uneven distribution of the medium in the reactor and consequently to an incomplete utilization of the sugars present [86]. In general, heat and mass transfer rates can differ significantly from lab to industrial scale, requiring an optimized reactor and mixing design [87]. Contrarily to defined fermentation media, hydrolysates obtained from organic residues can be high in viscosity which additionally influences gas transfer and mixing. In order to utilize organic residues cost-efficiently, it is recommended to carry out continuous processes. This counterbalances possible drawbacks in productivity when scale and viscosity (solid loading), and consequently final product titer, are reduced. A further advantage is that impurities which may have a negative impact on fermentation performance are kept at low concentrations. A continuous process, however, requires advanced knowledge as control, and adaption to different substrates is necessary. Furthermore, substrate needs to be supplied and fermentation broth removed and purified continuously.

## Conclusions

In view of strengthening bioeconomy, efficient processes for the utilization of organic residues are demanded. Research has shown that a great variety of compounds can be

produced by biotechnological means. Compounds as structurally simple as hydrogen and as complex as polyhdroxyalkanoates can be attained and offer a great variety of possible products. A huge issue that remains unsolved, however, is the downstream processing of the reaction media towards industrially applicable chemicals. Exemplarily, the production of lactic acid from food waste including downstream processing was delineated. In the future, such a process could reduce the amount of organic waste in urban areas, decrease the dependency on fossil resources, and support the recycling of materials. Nevertheless, without innovative and simple downstream processes which operate continuously, the decentralized utilization will remain a future approach. Furthermore, the logistical requirements need to be created allowing a continuous supply in substrate and continuous downstream processing and product formation.

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**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflict of interest.

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ORIGINAL PAPER

# The Challenges of Using Organic Municipal Solid Waste as Source of Secondary Raw Materials

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**Abstract**
The diversity of molecules with different functionalizations allows targeting of various end products, such as biomaterials, biobased plasticizer, food additives and fertilizer. The heterogeneity of organic municipal solid waste (OMSW) streams, however, challenges the formulation of reliable statements regarding the share of functionalized molecules. The aim of this study was the assessment of OMSW as source of functionalized molecules when hydrolysis was carried out enzymatically, thermo-chemically as well as thermo-chemically and enzymatically. Results revealed that OMSW is only quantitatively assessable at carbohydrate, protein and lipid levels. This is due to a changing seasonal and spacial composition, and consequently different hydrolytic products. However, also the treatment had an impact on the quantity. Depending on the treatment 230–640 mg $g^{-1}$ carbohydrates, 150–250 mg $g^{-1}$ lipids and 80–200 mg $g^{-1}$ proteins were quantified in food waste and organic street waste. The intensity of treatment had an impact on the quality of sugars. When wastes were treated enzymatically glucose, fructose and sucrose were found. Using thermochemical treatment glucose can be the only product. Contrarily, lipid and fatty acid as well as protein contents seemed not affected by the treatment.

**Keywords** Municipal solid waste · Hydrolysis · Secondary raw materials · Characterization

**Abbreviations**

| | |
|---|---|
| FAN | Free amino nitrogen |
| HPLC | High performance liquid chromatography |
| Nd | Not detected |
| OMSW | Organic municipal solid waste |
| U | Enzyme units |
| Total-C | Total-carbon content |
| Total-N | Total-nitrogen content |

## Statement of Novelty

Nowadays, biotechnological and chemical processes are used for converting OMSW as a whole into biochemicals and energy-rich compounds. It is beneficial that such an approach does not require a separation of constituents beforehand. The potential of the organic material as source of functionalized molecules, such as sugars, amino acids and fatty acids, however, cannot be conserved. This, however, is needed to develop new and innovative utilization processes. The novel aspect of this study is the consideration and assessment of OMSW as direct source of functionalized molecules. An assessment can only be carried when sufficient data on the presence of functionalized molecules is available, which also allows a conclusion on the presence of functionalized molecules in different waste streams.

## Introduction

Sustainable chemistry investigates processes in order to apply resources efficiently and to achieve a holistic use in chemical and/or biotechnological processes [1]. Organic municipal solid waste (OMSW) is currently either composted or directly/indirectly energetically used. The indirect energetic use of organic materials is based on the conversion of highly functionalized molecules (Table 1) into methane and carbon dioxide. The conversion of sugars, for instance, into methane and carbon dioxide results in a loss of functionalization [2]. Furthermore, 50% of the carbon is lost as carbon dioxide [3]. Instead of using OMSW

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**Table 1** Composition of OMSW and by hydrolysis obtainable secondary raw materials

| Composition | Secondary raw materials (functionalized molecules) | Building blocks (selection) |
|---|---|---|
| Starch, hemicellulose, cellulose | Glucose, fructose, xylose, arabinose, lactose, sucrose | Lactic acid, propionic acid, succinic acid, fumaric acid, malic acid, furfurale |
| Proteins | Alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine | |
| Lipids | Glycerol, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid | |
| Lignin | Coumaryl alcohol, coniferyl alcohol, sinapyl alcohol, coumaryl aldehyde, coniferyl aldehyde, sinapyl aldehyde | |
| Polyphosphate | Phosphate | |

Secondary raw materials can be converted into building blocks and final products by chemical and biotechnological industries. Amino and fatty acids and phenols can be used directly as building blocks, while sugars require first fermentative and/or catalytic conversions

energetically, a direct use as source of highly functionalized molecules is indicated.

OMSW is heterogeneous and may consist of food and kitchen wastes, paper, coffee and tea residues, grass and green clippings. An average composition (w/w) of OMSW of 17.5% lipids, 17.7% proteins, 17.1% starch, 10.5% free sugars, 18.6% cellulose, 9.7% lignin and 8.6% hemicellulose [4] illustrates, that the easily hydrolysable parts, such as starch, lipids and proteins, may represent 62.8% of the waste matter. Recalcitrant parts, such as cellulose, hemicellulose and lignin may represent 36.9%. It is suggested that the utilization of organic matter should follow a cascading principle in order to develop a biobased society [5]. An option to utilize organic matter materially is its use as feedstock in biotechnological processes. Particularly in fermentative processes [6–10], the easily hydrolysable waste constituents can serve as substrates for microorganisms with or without hydrolysis carried out beforehand.

Another utilization approach might be the separation of present functionalized compounds (Table 1) as secondary raw materials directly from hydrolyzed organic matter, which can be converted into final products, such as biomaterials, biobased plasticizer, food additives and fertilizer [11]. It is crucial for the efficiency of OMSW-based utilization processes that the whole process is flexible and runs stable irrespective the waste applied [12]. It is hypothesized here that this might be achieved by carrying out a sequential hydrolysis of hydrolysable parts. The advantage is that functionalized molecules can be separated sequentially after every hydrolytic step and the inhibition of hydrolytic performance by waste constituents, such as the protection of carbohydrates by lipids [13], as well as products can be avoided. Nevertheless, the direct utilization of OMSW through the recovery of all functionalized molecules is challenging. The variable composition makes a continuous adaption of the pretreatment (hydrolytic approach) and separation necessary. Furthermore, there is no sufficient database regarding the presence of functionalized molecules in OMSW.

The investigation of hydrolytic processes and inventory of functionalized molecules, obtained by hydrolysis of OMSW, is expected to lead to highly wanted basics in order to develop more efficient utilization processes. Information regarding the effect of hydrolytic processes on the presence and recovery of functionalized molecules contributes to the development of technical processes which can be applied where organic residues appear, such as near food processing and agriculture industries as well as municipalities. It has never been more important to develop efficient utilization strategies of organic residues in order to cope with the challenges to cover the demand of resources in the future.

Therefore, the aim of this study was to carry out a characterization of functionalized molecules, such as sugars, long- and short chained fatty acids, proteins and amino acids, obtainable from OMSW using different hydrolytic treatments and an assessment of separation techniques in order to recycle those molecules back in the sense of a circular economy as secondary raw materials. For this purpose food waste and street waste have been collected and waste constituents sequentially or in one-batch approaches enzymatically, thermo-chemically or thermo-chemically as well as enzymatically hydrolyzed.

## Materials and Methods

### Organic Waste

Food waste (leftover food), made of potatoes, noodles, bread, meat, vegetables, was randomly collected from the canteen at Leuphana University of Lüneburg (Germany) at different times in May 2017 and April 2018. Directly after, the waste was blended and stored at − 18 °C until further usage.

Street waste was randomly collected from waste bins located in Lüneburg at different times in October and November 2017. Organic waste was mixed with inorganic material and the organic fraction (15–22%, w/w)

was separated by hand. The organic fraction was predominantly made of thrown away food, such as buns, sandwiches and sausage. After collection it was blended and stored at − 18 °C until further usage.

## Enzymes

All enzymes used were obtained from ASA Spezialenzyme GmbH (Wolfenbüttel, Germany). The activities of the different enzyme formulations reported by ASA Spezialenzyme GmbH were: > 30 U $mL^{-1}$ for cellulase (TXL); 1.200 U $mL^{-1}$ for glucoamylase (AN); > 300 U $mL^{-1}$ for exo-polygalacturonase, > 3000 U $mL^{-1}$ for endo-polygalacturonase and > 300 U $mL^{-1}$ for pectinase present in pectinase (L-40) formulation from *Aspergillus niger*; > 400,000 U $g^{-1}$ for xylanase and > 900 U $g^{-1}$ for cellulase present in xylanase (2) formulation; > 18,000 U $mL^{-1}$ (glyceryl tributyrate) and > 13,000 U $mL^{-1}$ (olive oil) for Lipase (FE-01). For the protease (S-02) formulation no activity was provided. Hydrolytic treatments were carried out at provided optimal temperature and pH conditions as shown below.

## Reference Compounds

To assess the complexity of organic materials, homogeneous reference compounds, such as starch powder, cellulose in form of paper tissue, protein powder and butter were hydrolyzed enzymatically or thermo-chemically as well as enzymatically. Reference compounds were chosen in order to illustrate the recalcitrant character and resistance against hydrolytic treatments. Focus was laid on digestibility and released monomers, such as glucose, fructose, sucrose, amino acids, fatty acids and phosphate. All hydrolyses were carried out as batch processes.

Paper tissue was collected from a tissue dispenser at a university restroom, cut into small pieces and 5 g mixed with 500 mL demineralized water. Thereafter, the suspension was added to a 1 L EloFerm bioreactor (Biotronix GmbH, Berlin, Germany). Temperature was set to 50 °C and pH to 4.5 before cellulase was added. Enzymatic hydrolysis was carried out for 24 h. Furthermore in a second approach, to 5 g of cut paper tissue 500 mL of 0.6% (v/v) sulfuric acid was added. The suspension was autoclaved for 15 min at 121 °C in a Schott flask and the resulting suspension enzymatically hydrolyzed as described before for 24 h. Samples were taken regularly.

Similar to the paper tissue hydrolysis, in a first approach, starch or protein powder was hydrolyzed using glucoamylase at 55 °C and pH 4.5 or using protease at 60 °C and pH 3.0 for 24 h, respectively. The solid-to-liquid ratio was 5.4% (w/w) for starch powder and 1.4% (w/w) for protein powder due to solubility issues. In a second approach, both powders were first thermo-chemically treated in presence of 0.6% (v/v) sulfuric acid and autoclaved for 15 min at 121 °C in a Schott flask. The solid-to-liquid ratio was again 5.4% (w/w) for starch powder and 1.4% (w/w) for protein powder. Afterwards, enzymatic hydrolysis was carried out as described above. Samples were taken regularly.

In order to assess the impact of complexity of organic waste on hydrolytic performance, organic waste was simulated by mixing 65% (w/w) starch powder, 20% (w/w) butter and 15% (w/w) protein powder. The mixture (20 g dry weight) was resuspended in 780 g demineralized water. First for lipid hydrolysis, temperature was adjusted to 40 °C, pH set to 7.5, and 1 mL lipase was added. After 4 h, temperature was increased to 55 °C, pH set to 4.5, and saccharide hydrolysis was initiated by adding 1 mL of glucoamylase. After 4 h, temperature was increased to 60 °C, pH set to 3.0 and 1 mL protease was added for protein hydrolysis. Hydrolysis was stopped after 24 h in total. Samples were taken regularly. In a second approach, artificial organic waste was first thermo-chemically treated in presence of 0.6% (v/v) sulfuric acid and autoclaved for 15 min at 121 °C in a Schott flask, and followed by sequential enzymatic hydrolysis as described above.

## Organic Waste

### Free Organic Molecules

The liquid phase of food waste was first investigated for the presence of organic molecules by centrifuging 1 mL of blended food waste (solid-to-liquid ratio 24%, w/w) at 19,000×*g* for 5 min. Obtained solution was subjected to HPLC.

Blended street waste of 0.1 g (dry weight) was resuspended in 1 mL demineralized water and vortexed for 5 min. Afterwards the suspension was centrifuged at 19,000×*g* for 5 min. Obtained solution was subjected to HPLC.

### Sequential Hydrolysis of Waste Constituents

Enzymatic hydrolysis of food wastes 1 and 2 was carried out at a 2.4% (w/w) solid-to-liquid ratio in a 1 L EloFerm bioreactor (Biotronix GmbH, Berlin, Germany). The individual components were hydrolyzed sequentially in order to ensure an optimal performance of hydrolytic enzymes as described for the artificial waste in "Reference Compounds" section. For food waste 1 a 24 h reaction time was considered for each enzyme. Due to the fast reaction, reaction time was shortened to 4 h for food waste 2.

In a second approach, food waste 2 was first thermo-chemically treated in presence of 0.6% (v/v) sulfuric acid and autoclaved for 15 min at 121 °C in a Schott flask, and followed by sequential enzymatic hydrolysis as described for the artificial waste in "Reference Compounds" section.

### Separate Hydrolysis of Waste Constituents

The characterization of food waste 2 and street wastes regarding dry matter, ash, total carbon (C)- and nitrogen (N)-contents has been carried out separately as described in "Analytics" section.

As a tough treatment and for comparison, street wastes were thermo-chemically hydrolyzed by resuspending ca. 0.1 g dry material in 3 mL 2.5 M $H_2SO_4$ and autoclavation for 15 min at 121 °C. Released sugar monomers were determined by HPLC.

## Analytics

Analysis of total carbon—(C-content) and nitrogen—(N-content), water, ash, carbohydrate and lipid contents was carried out for both wastes and performed in triplicate or single measurements. Mean value and standard deviation are presented for triplicate measurements in "Results and Discussion" section.

In order to determine the dry matter, aliquots were weighed and dried at 105 °C in a compartment dryer (ST 5028, Heraeus, Hanau, Germany) until constant weight.

Ash content was quantified by heating 1 g dry waste for 4 h at 550 °C in a muffle furnace (Muffle furnace LT 5/12, Nabertherm, Bremen, Germany) and weighing the remainder.

Glucose, fructose and sucrose were analyzed with HPLC (LC-10AD pump, SIL-10AD auto-sampler, CTO-10AD oven, CBM-20A communication module, Shimadzu, Kyoto, Japan): 10 µL of sample was injected in an Aminex (Bio-Rad, Hercules, California, USA) HPX-87H column (300 mm × 7.8 mm) and eluted isocratically with 0.4 mL $min^{-1}$ of 5 mM $H_2SO_4$ at 27 °C. Detection was carried out by a refractive index detector (RID-20A, Shimadzu, Kyoto, Japan) at 40 °C. For each analyte, calibration curves were generated with pure solutions of known concentration.

Total C- and N-contents were measured with an elemental CN analyzer at 1150 °C (Elementaranalysator vario Max CN; Elementar Analysensysteme GmbH, Hanau, Germany). Protein content was estimated by multiplying the N-content with 5.6 [14].

Lipid extraction was carried out by adding 5 mL $CH_3OH$/$CHCl_3$ (2:1, v/v) to 0.1 g dry waste or butter containing tridecanoic and nonadecanoic acids as internal standards and shaking for 24 h. After centrifugation, the supernatant was decanted and stored at − 18 °C. To the pellet 5 mL $CH_3OH$/$CHCl_3$ (1:1, v/v) was added, shaking continued for another 24 h and the suspension was centrifuged. Both supernatants were combined and 2 mL demineralized water was added to remove non-lipid components. The organic phase was collected, evaporated at room temperature under nitrogen flow and the mass of the crude oil extract was measured.

The individual fatty acids were converted to their respective fatty acid methyl esters by dissolving the crude oil extract in 0.2 mL $CHCl_3$, 2 mL $CH_3OH$ and 0.1 mL concentrated hydrochloric acid. The solution was heated at 100 °C for 1 h. After cool down, 2 mL hexane and 2 mL demineralized water were added, the solution was shaken and after phase separation the hexane phase isolated. 1 µL of the hexane phase was injected for GC/EI–MS analysis (Trace 1310 gas chromatograph interfaced with a single-quadrupole ISQ, Thermo Scientific, Waltham, Massachusetts, USA). As column, a Select FAME fused silica capillary column (50 m × 0.25 mm ID, 0.25 µm film thickness, Agilent Technologies, Waldbronn, Germany) was used with helium as carrier gas. Ionization was conducted with an electron energy of 70 eV, the temperature of the ion source was set to 250 °C. Scans were recorded over the range of m/z 60–400.

Release of amino acids was measured as free amino nitrogen (FAN) in supernatants obtained from proteolytic treatment of protein powder and waste using a modified version of the EBC-ninhydrin method [15]. First, two reagents were prepared. For reagent A, 1 g $Na_2HPO_4 \cdot 12H_2O$, 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 (Ultrospec III, Pharmacia, Uppsala, Sweden) was measured. A calibration curve with glycine as standard was used as reference.

Determination of amino acids after proteolytic treatment of food waste and protein powder was carried out using the conversion of amino acids into corresponding alpha-hydroxy acids [16, 17]. Alpha-hydroxy acids were analyzed using HPLC as described above. Peaks were identified by combining retention time with reference compounds.

Phosphate concentration was determined photometrically via generation of molybdenum blue. At first, four separate solutions were prepared: (I) sulfuric acid (2.5 M), (II) potassium antimonyl tartrate solution (1.3715 g K(SbO)$C_4H_4O_6 \cdot 1/2H_2O$ in 500 mL demineralized water), (III) ammonium molybdate solution (20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL demineralized water) and (IV) ascorbic acid solution (1.76 g ascorbic acid in 100 mL demineralized water). Molybdenum reagent (V) was prepared by combining 2.5 mL (I), 0.25 mL (II), 0.75 mL (III) and 1.5 mL (IV). Sample (100 µL), 900 µL demineralized water, 10 µL (III) and 160 µL (V) were mixed. After incubating at 60 °C for 15 min, absorption was measured at 880 nm (Ultrospec III, Pharmacia, Uppsala, Sweden). In the case of fermentation broth, 40 µL of sample was taken and 960 µL demineralized water was added.

## Results and Discussion

### Reference Compounds

It can be seen from the results shown in Table 2 that an intensive treatment and hydrolysis result in higher contents of constituents and yields of hydrolytic products, respectively. When paper tissue was treated enzymatically no glucose was detected. However, when the same paper was first thermo-chemically treated followed by enzymatic hydrolysis then 139 mg glucose per g paper was released. Additional di- and oligosaccharides were detected but not identified. Starch has a less recalcitrant structure than cellulose and was applied as powder rather than a fiber, and thus 451 mg glucose could be released per g when hydrolysis was carried out enzymatically. With a precedent thermo-chemical treatment, the yield could be increased to 780 mg $g^{-1}$. Furthermore, the release of FAN increased by a factor of around 7 when protein powder was pretreated prior to enzymatic hydrolysis (Table 2). Nevertheless, the release of carbohydrates, FAN and phosphate from artificial waste shown in Table 2 was similar or did only slightly increase when thermo-chemical treatment was applied beforehand.

The fatty acid profile of butter was dominated by oleic and stearic acid with contents of about 635.9 mg $g^{-1}$ and 300.2 mg $g^{-1}$, respectively. Fatty acids present at contents of 1.4 mg $g^{-1}$, 17.8 mg $g^{-1}$ and 44.0 mg $g^{-1}$ were dodecanoic, myristic and palmitic acids, respectively (Table 3). The glycerol content of butter was estimated at 100 mg $g^{-1}$. The total lipid content was 100% (w/w).

### Free Organic Molecules

Regarding the separation of functionalized molecules, it was first investigated whether functionalized molecules can be directly obtained from wastes by separation of liquid and solid phases. After washing of street wastes and analyzing the resulting washing water with HPLC no considerable

**Table 2** Constituents in reference substances and artificial waste using different hydrolytic treatments

| Constituent (mg $g^{-1}$) | Paper[a] | Paper[b] | Protein powder[a] | Protein powder[b] | Starch powder[a] | Starch powder[b] | Artificial waste[a] | Artificial waste[b] | Butter[c,e] |
|---|---|---|---|---|---|---|---|---|---|
| Carbohydrates (glucose/fructose/sucrose) | Nd | 139.3[d] | – | – | 450.8[d] | 779.3[d] | 203.2/18.6/25.9 | 205.7/77.6/38.5 | – |
| FAN | – | – | 0.6 | 4.5 | - | – | 3.9[e] | 2.9[e] | – |
| Phosphate | – | – | – | – | – | – | 1.3[e] | 1.9[e] | – |
| Glycerol | – | – | – | – | – | – | ~20 | ~20 | ~100 |

The contents/yields are based on total weight of reference substances or artificial waste

Nd: not detected; –: not analyzed

[a]Hydrolysis was carried out enzymatically

[b]Hydrolysis was carried out first thermo-chemically and second enzymatically

[c]Hydrolysis was carried out chemically as part of the transesterification step for fatty acid quantification

[d]Only glucose was identified

[e]Lipid content was considered 100% (w/w)

**Table 3** Fatty acid contents based on total weight of lipids detected in waste materials

| Fatty acids (mg $g^{-1}$) | Food waste 1 | Food waste 2 | Street waste 1 | Street waste 2 | Butter |
|---|---|---|---|---|---|
| Dodecanoic acid | Nd | Nd | 3.2 | 16.4 | 1.4 |
| Myristic acid | Traces | 38.5 ± 0.2 | 4.9 | 15.7 | 17.8 |
| Palmitic acid | 288.3 ± 64.8 | 339.3 ± 9.1 | 384.9 | 311.2 | 44.0 |
| Palmitoleic acid | Nd | Nd | 0.3 | 1.6 | Traces |
| Stearic acid | 46.1 ± 9.6 | 54.4 ± 0.4 | 51.9 | 75.2 | 300.2 |
| Oleic acid | 364.9 ± 76.4 | 430.7 ± 2.8 | 435.9 | 393.2 | 635.9 |
| Linoleic acid | 106.8 ± 17.0 | 127.5 ± 6.8 | 109.4 | 174.8 | Traces |
| Alpha-linolenic acid | Traces | 8.7 ± 6.0 | 7.0 | 10.3 | Traces |
| Eicosanoic acid | 0.7 ± 0.2 | 0.9 ± 0.1 | 2.4 | 1.5 | Traces |

*Nd* not detected

amounts of detectable compounds were found. The supernatant of food waste fraction did reveal concentrations for glucose, fructose, sucrose and lactic acid of 25.3 g $L^{-1}$, 13.8 g $L^{-1}$, 11.2 g $L^{-1}$ and 7.2 g $L^{-1}$, respectively, at a solid-to-liquid ratio of 24% (w/w).

## Sequential and Separate Hydrolysis of Organic Waste

Street and food wastes were randomly collected at the same location, but at different times. All waste materials predominantly consisted of carbohydrate, lipid and protein, which made it an easily hydrolysable material.

While chemical composition of all wastes was similar, the quantity varied due to different hydrolytic treatments. The biggest deviation of all materials was found for the dry matter (Table 4). The lowest dry matter of 217 mg $g^{-1}$ had food waste 2, while the highest dry matter of 461 mg $g^{-1}$ was found in street waste 1. Ash content (Table 4) was around 62 mg $g^{-1}$ in both food wastes and street waste 2 and 92 mg $g^{-1}$ in street waste 1. Even though no phosphatases were applied, 5.2 mg phosphate was recovered per g of food waste 2.

When materials were enzymatically or thermo-chemically as well as enzymatically treated, carbohydrate content was 200–300 mg $g^{-1}$ in food waste 2 and in both street wastes, while food waste 1 contained around 600 mg $g^{-1}$. Fructose and sucrose yields differed between all wastes. The pure thermo-chemical hydrolysis with 2.5 M $H_2SO_4$ resulted in glucose yields of 417 mg $g^{-1}$ and 568 mg $g^{-1}$ in street waste 1 and 2, respectively. There was neither fructose nor sucrose detectable after chemical treatment.

Food waste 1 and street waste 2 had a similar C-content of about 495 mg $g^{-1}$ (Table 4). The C-content of food waste 2 and street waste 1 was 467 mg $g^{-1}$ and 482 mg $g^{-1}$, respectively. N-content ranged from 14 mg $g^{-1}$ in street waste 1, around 24 mg $g^{-1}$ in food waste 1 and 2, and 36 mg $g^{-1}$ in street waste 2. Correspondingly, also the protein contents ranged from 79 to 204 mg $g^{-1}$. All amino acids present in the hydrolysate of food waste 1 could not successfully be separated and identified. Clearly separated and identified were asparagine, valine, lysine, cysteine and tryptophan. Additionally present, but not clearly identified owing to very similar retention times, might be serine, threonine, glutamic acid, phenylalanine, asparagine, glycine, alanine, proline, tyrosine, leucine and isoleucine (not shown). Because of difficulties to separately detect all amino acids only food waste 1 was investigated.

The lipid content was between 215 and 252 mg $g^{-1}$ in food wastes 1 and 2, respectively, and 171 mg $g^{-1}$ and 158 mg $g^{-1}$ in street wastes 1 and 2, respectively. The glycerol content was estimated at 10% (w/w) of the lipid content. The fatty acid profiles for food and street wastes were similar. The contents of fatty acids based on the total weight of lipids, however, slightly differed (Table 3).

**Table 4** Constituents in organic waste materials based on dry weight

| Constituent (mg $g^{-1}$) | Food waste 1[b] | Food waste 2[c] | Food waste 2[b] | Street waste 1[c] | Street waste 1[e] | Street waste 2[c] | Street waste 2[e] |
|---|---|---|---|---|---|---|---|
| Total-C | 495.5 ± 0.7 | 466.9 | | 481.6 ± 8.8 | | 495.0 ± 2.7 | |
| Total-N | 23.8 ± 0.2 | 24.9 | | 14.1 ± 0.5 | | 36.4 ± 2.2 | |
| Carbohydrates (glucose/fructose/sucrose) | 604.3/21.5/19.3 | 290.6/65.3/18.9 | 223.3/66.7/35.9 | 227.9/29.8/10.6[d] | 417.3[f] | 218.1/6.2/Nd[d] | 567.6[f] |
| Lipid | 214.7 ± 12.8 | 251.5 ± 24.6 | | 170.6 ± 59.3 | | 157.5 ± 17.1 | |
| Glycerol | ~ 22 | ~ 25 | | ~ 17 | | ~ 16 | |
| Protein[a] | 133.3 ± 1.1 | 139.4 | | 78.9 ± 2.8 | | 203.8 ± 12.3 | |
| Dry matter | 242.5 ± 0.4 | 216.9 ± 0.1 | | 461.0 ± 44.2 | | 302.0 ± 88.7 | |
| Ash | 60.6 ± 0.6 | 64.5 ± 0.5 | | 91.8 ± 6.9 | | 61.8 ± 19.0 | |
| Phosphate | – | 5.2 | | – | – | – | – |

The contents/yields are based on total weight of reference substances or artificial waste

Nd: not detected; –: not analyzed

[a]Protein content was estimated by multiplying the N-content with 5.6 [14]

[b]Waste material was sequentially hydrolyzed

[c]Waste material was separately hydrolyzed

[d]Carbohydrates hydrolyzed first thermo-chemically and second enzymatically

[e]Carbohydrates hydrolyzed thermo-chemically using 2.5 M $H_2SO_4$

[f]Only glucose was detected

## Composition, Treatment and Functionalized Molecules

The composition of organic waste varies not only between origins, but also due to metabolic activities of microbial consortia, nutritional habits, season and temperature [4, 18, 19]. The same origin may provide organic waste with the same composition of major constituents, the quantity of each constituent, however, can vary and consequently an adaption of quantification methods might be necessary. Food wastes 1 and 2 used in this study, for instance, were collected at the same location, the glucose yield after sequential and separate enzymatic digestion, however, differed by a factor of 2, while the protein and lipid contents were similar (Table 4). In daily routine work it is rather challenging to discriminate between differences in composition due to different waste materials or due to different quantification procedures.

With regard to changing composition and most likely metabolic activity, an assessment of functionalized molecules present in organic material is a tilt at windmills. In this study, which was carried out at lab scale and where materials as well as samples were stored in a refrigerator or freezer, it was difficult to deduce whether differences in quantities should be ascribed to different composition or treatment. It seems more appropriate to estimate from the main constituents: carbohydrate, protein and lipid, which monomers and quantities may appear during storage by hydrolysis and conversion by indigenous consortia. Microbial consortia are active when temperature increases in spring and summer, and convert major constituents, such as carbohydrates, proteins and lipids, into monomers and metabolites. The supernatant of food waste investigated in this study contained significant concentrations of glucose, fructose, sucrose and lactic acid. It is not unusual that food contains free sugars and organic acids, however, the high concentrations found may indicate an active indigenous microbial consortium. Contrarily, street waste was collected in winter when temperature was between 0 and 5 °C, and neither free sugars nor organic acids were detected in supernatants. An active microbial consortium results in a continuous change of composition, which complicates not only the quantification, but also the utilization in the sense of a direct use of secondary raw materials. Organic waste serves as substrate in biomethane and biohydrogen production and methods have been developed to estimate its bioaccessible fraction [4, 18, 19]. A change of the bioaccessible fractions, such as cellulose, hemicellulose, starch, protein and lipids, significantly influences the productivity of those processes, but also the presence of functionalized molecules.

Determining poly- and oligomers in organic waste predominantly bases on degradation towards their monomers. For instance the quantification of starch is based on the chemical or enzymatic degradation and analysis of released glucose [20]. The performance of enzymes, however, is influenced by microscopic phenomena. Lipids may cover carbohydrates and proteins, and prevent them from being hydrolyzed [13]. Another aspect is solubility, since only solubilized undergo hydrolysis [21].

A method which is applicable for the hydrolysis of different materials and to increase yields of all studied hydrolytic products is the thermo-chemical treatment at 121 °C for 15 min and 0.6% (v/v) $H_2SO_4$ (Table 2). While the application of diluted $H_2SO_4$ makes more carbohydrates available to enzymes, and thus favors the hydrolysis [22], the application of concentrated $H_2SO_4$ does result in side-reactions. When street wastes 1 and 2 were sequentially hydrolyzed enzymatically the released products were glucose, sucrose and fructose. The glucose yield was around 220 mg $g^{-1}$ (Table 4). When both wastes were thermo-chemically treated with 2.5 M $H_2SO_4$, the glucose yield was between 400 and 600 mg $g^{-1}$. Furthermore, the treatment caused a complete hydrolysis of sucrose to fructose and glucose, and apparently a conversion of fructose into furfural [23, 24], which may further complicate the separation of functionalized molecules.

Different treatments did not only result in different sugar yields, but also in different FAN yields (Table 2). When protein powder was digested first thermo-chemically and afterwards with protease, a seven times higher FAN yield was found compared with the pure enzymatic hydrolysis. In the case of protein powder it was observed that powder was not totally solubilized and clumps were formed which were not completely bioaccessible. Surprisingly, this difference was not found when artificial wastes (Table 2) or food waste 2 (not shown) was treated with or without heat and acid, which may indicate that processed proteins are better water soluble than unprocessed ones.

Investigating the composition of food waste and OMSW is crucial to various research questions. Most study published aim on an understanding of the effect of waste composition on product formation. The essential question thereby is how fast do organic wastes degrade and provide compounds, which can easily be converted into products of interest under given conditions ([25–32], Table 5). One utilization process, which is predominantly under investigation, is anaerobic digestion. It is of interest to the novelty of the present study that the majority of published research focuses on the use of organic waste as substrate in anaerobic digestion. Only one of the studies shown in Table 5 considered the direct use of waste constituents as feedstock in chemical processes. In this study, Li et al. aimed on a use of fatty acids as functionalized molecules in biodiesel production [33].

Even though the quantification of composition of different waste streams shown in Table 5 has been carried out differently to the methods used in the present study, the composition is comparable. This indicates that waste

**Table 5** Quantification of composition of different organic waste streams and motivation (aim) for quantification

| Substrate | C-content (%) | N-content (%) | Cellulose (%) | Ligno-cellulose (%) | Hemicel-lulose (%) | Carbohydrate (%) | Protein (%) | Lipid (%) | Aim | Refs. |
|---|---|---|---|---|---|---|---|---|---|---|
| Food waste from a university canteen | 49.4 | 3.5 | – | – | – | 59.0 | 18.1 | 18.0 | Improving anaerobic digestion | [25] |
| Food wastes from different canteen | 45.5–51.5 | 2.6–5.3 | – | – | – | 3.1–11.0 | 3.6–7.5 | 2.9–10.2 | Studying the effect of composition on anaerobic digestion | [28] |
| Mixed flower and vegetable wastes | 68.6 | 3.8 | 16.1 | 12.4 | 21.4 | – | 13.8 | 2.7 | Improving hydrolysis for anaerobic digestion | [32] |
| Food waste from a canteen | 51.0 | 4.0 | – | – | – | – | – | – | Understanding enzyme activities in anaerobic digester | [26] |
| Organic municipal solid waste | 47.7 | 3.1 | – | – | – | – | – | – | Enhancement of waste degradation | [31] |
| Organic municipal solid waste | – | – | – | 20.2 | – | 58.6[a] | 8.3 | 6.5 | Production of ethanol from organic municipal solid waste | [30] |
| Kitchen waste | 46.1 | 3.2 | – | – | – | – | – | – | Changes in fatty acid composition by thermal treatment | [33] |
| Kitchen waste[b] | 46.1 | 3.2 | – | – | – | 11.8 | 2.5 | 3.5 | Investigating organic degradation during anaerobic degradation | [29] |
| Food waste from canteens[b] | 47.6 | 3.2 | – | – | – | 8.9 | 4.8 | 5.4 | Investigation the effect of composition on anaerobic digestion | [27] |

[a]Based on starch

[b]Based on wet weight

streams have a similar composition in common, but hydrolytic products can differ. In order to consider OMSW as a source of functionalized molecules, it is considered here to carry out the characterization using a three levels differentiation scheme (Fig. 1). The totality of organic matter is thereby considered level 1, level 2 stands for carbohydrates, proteins and lipids. The monomers obtainable are glucose, various amino acids, glycerol and fatty acids, respectively, and stand for level 3. Nevertheless, it should be admitted here that level 3 is unpredictable and underlies a continuous change.

Level 3 is associated with a certain unpredictability due to compounds originating from side reactions, metabolic products or not completely hydrolyzed materials. Yet, based on level 2, it might be possible to carry out a "superficial inventory" with an estimation of level 3, such as glycerol from lipids. With starch as starting material, one would expect that only glucose appears after hydrolysis. However, when hydrolysis was carried out firstly thermochemically and secondly enzymatically, 0.8 g glucose per g starch powder was obtained (Table 2), which indicates that a certain amount of the initially applied starch is still present as starch, oligosaccharides or hydrolysis byproducts. An unpredictable fraction is also remaining when protein powder was hydrolyzed. Despite the significant increase in FAN yield after first thermo-chemical and second enzymatic hydrolysis compared with pure enzymatic hydrolysis, there might still be a certain fraction remaining as oligopeptides and/or protein.

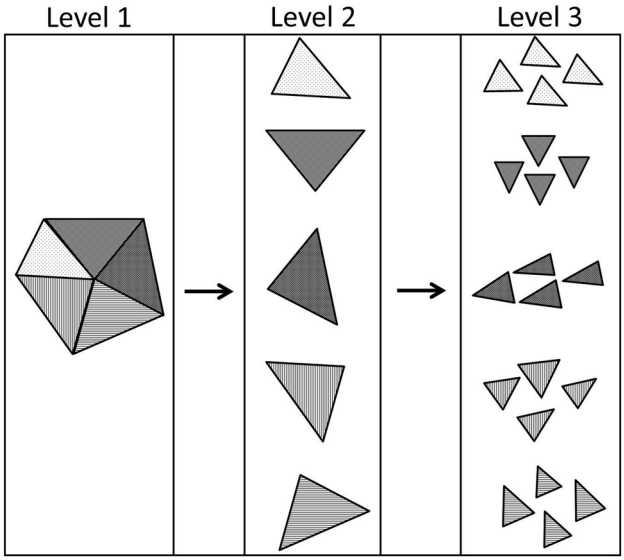


**Fig. 1** Levels of characterization. Level 1 stands for the organic content, level 2 represents the organic constituents, such as starch, protein and lipids, and level 3 the monomers obtainable from organic constituents

## Practical Implications

### Separation of Functionalized Molecules

The complex composition of organic waste challenges a complete utilization [13]. Particularly when biological methods are applied, strategies need to be carefully designed in order to make use of the whole potential. The separation of functionalized molecules and development of tailor made direct conversion strategies, for instance catalytic approaches, for each stream may contribute to efficient and complete utilization of organic waste [34]. However, as discussed above, the heterogeneous and continuously changing composition makes a detailed characterization rather impossible. Therefore, it is recommended to only consider the quantification of carbohydrates, proteins and lipids in organic waste, and theoretical estimation of obtainable functionalized molecules after hydrolysis (Fig. 1).

While the separation of lipids from all other waste constituents is relatively simple due to lower density, the separation of carbohydrates from organic acids and other constituents is not. For instance, Chen et al. studied the separation of glucose, arabinose and xylose from lignocellulosic hydrolysates using cation exchange resin [35]. Using the resin Amberlite IRP69 ($Ca^{+}$) they obtained high-purity xylose (88%) from hydrolysate and high-purity arabinose (92%) from a synthetic solution. The hydrolysate contained cellobiose, glucose, arabinose and xylose, short organic acids as well as phenolic compounds. Contrarily, the synthetic solution was less complex and contained only glucose, arabinose and xylose. The separation of functionalized molecules is particularly difficult when structure, size and charge are similar. This, for instance, applies for xylose and glucose. Therefore, Morthensen et al. first converted glucose into gluconate and second applied nanofiltration to separate both [36]. Using a pH of 9.5, 25 °C and 4 bar a throughput of 18.7 L $m^{-2}$ $h^{-1}$ and separation factor of 34 for xylose were obtained. Nanofiltration was also applied by Lyu et al. who separated glucose, monophenols and cyclopentenones as well as acetic acid from hydrolysates of lignocellulosic biomass [37]. They used three nanofiltration modules with different molecular cut-offs in a row in order to achieve the sequential separation. Malmali et al. have also studied nanofiltration for a separation of acetic acid and furfural from biomass hydrolysates. Even though the separation did work, the authors claimed that it is essential to select the right membrane and operation conditions [38]. The question however is, what is the right membrane and operation condition when the composition is continuously changing?

A separation of monosaccharides, organic acids and phenols from hydrolysates of lignocellulosic biomass has

been carried out by Chen et al. using the anion and cation exchange resins Amberlyst A21 and Amberlite IR-120, respectively [39]. Using A21 glucose and acetic acid could be separated at purities of 87% and 98%, respectively. The resin IR-120 resulted in a separation of acetic acid and phenol, and purities of 80% and 90%, respectively, were obtained. Even using a real biomass hydrolysate from pine branch the recovery of monosaccharide and organic acid streams were 80% and 88%, respectively. For separating acetic acid and lactic acid in the organic acid stream it was suggested to apply membrane filtration [39].

Due to the promising bioactive properties of peptides obtained after protein hydrolysis, effort has been put on the separation of bioactive molecules from complex mixture. For this purpose electrodialysis with ultrafiltration has been studied for the separation of peptides and charged functionalized molecules like amino acids. According to Suwal et al. this is basically a batch process with one or more filtration membranes stacked into an electrodialytic cell [40]. The separation performance depends on number of membranes, pore size and material as well as pH and electric strength [40]. Electrodialysis with ultrafiltration was successfully tested for the separation of peptides and amino acids from marine protein sources, such as snow-crab by-product hydrolysate [40, 41]. An application of this method for the separation of peptides and amino acids from organic waste hydrolysates is therefore possible and promising approach to upcycle organic waste streams.

The ongoing progress in the field of separation technology may allow the complete and selective separation of functionalized molecules in hydrolysates from organic waste in the future. Nevertheless, due to the continuous changing composition either caused by origin or microbial activities, the applied separation techniques need to be fast, highly flexible and easily adjustable to different waste streams.

### Future Work and Strategies

The policy objective of the German government gives an outlook to future research work. The German government aims in its bioeconomy strategy on a complete utilization of all components of biological resources in order to create an independence from fossil raw material suppliers. In order to reach this goal, the development of different conversion processes for primary and secondary refining as well as production of target molecules for relevant industries [42]. Thus, the chance of realization of a direct utilization of OMSW is considered as good when technical drawbacks regarding the separation of molecules are overcome. The chance of realization can further be improved when relevant industries, which apply the recovered secondary raw materials, are involved in the development of new utilization approaches. However, more solid data is needed to proof the predictability regarding quantity and quality of functionalized molecules in OMSW.

The results of the present study revealed that the presence of functionalized molecules changes due to a changing seasonal and spacial composition as well as treatment. Thus, one needs to consider that the composition underlies local differences and data regarding the composition may not be transferable directly. However, a long-term investigation period of several years may result in a data basis which can be transferred to other localities for estimating the presence of functionalized molecules.

## Conclusions

From the results of this study it can be concluded that assessment of functionalized molecules in hydrolyzed OMSW has its limitation. Due to the heterogeneous and by time and location changing composition it seems rather impossible to provide a reliable detailed list of functionalized molecules. The question is finding the level of detail until which a characterization makes sense. This level is most likely the quantification of carbohydrates, proteins and lipids in waste material. Based on this level the possibly present functionalized molecules can theoretically be estimated in hydrolysates. Despite the challenges experienced, the potential of organic waste as a source of functionalized molecules is high. It is expected that more attention will be paid to the potential of organic waste in the future beyond its use as substrate in anaerobic digestion, composting or incineration. The matter, however, is finding the right separation technology, which is flexible enough to separate molecules from hydrolysates of varying composition obtained from different OMSW-streams.

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# Is seashell powder suitable for phosphate recovery from fermentation broth?

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ABSTRACT

This communication elaborates on the use of seashell powder (SP) for the removal of phosphate from lactic acid-containing fermentation broth. Despite extensive past research regarding the application of SP for phosphate removal from wastewater, no information is available for solutions containing various organic compounds. In order to fill this knowledge gap, tests were performed with pure phosphate solution (PPS) and PPS containing 0.83 M of three alcohols, ethanol, propanol or 1,2-propanediol, or 0.83 M of three organic acids, acetic, propionic or lactic acid. Furthermore, a real fermentation broth (RFB) obtained from the fermentative production of lactic acid from food waste was tested. Using 4.8 g SP, more than 95% of phosphate, present at an initial concentration of 50 mg $L^{-1}$, could be removed from PPS and PPS containing alcohols after 120 min. The presence of organic acids reduced the removal capacity of SP and only 55%–73% of the phosphate initially present was removed. The presence of lactic acid also substantially affected the removal of phosphate from RFB when 132 mg $L^{-1}$ phosphate was initially present: after 120 min, only 28.6 mg $L^{-1}$ of phosphate had been removed. The results indicate the use of SP for phosphate removal from fermentation broth, contributing to multi-component utilization of fermentation broth. However, the effects of respective fermentation products on removal capacity should first be tested.

## Introduction

Phosphorus is a key element needed to maintain productivity and fertility of arable land [1,2]. With global phosphorus deposits depleting and degrading in quality, phosphorus management becomes a major issue in politics, society, and science [3]. Phosphorus is predicted to become one of the most critical elements in the near future [4,5]. With a foreseeable decline in the amount of mined phosphorus, its price will increase, making recovery more cost-competitive and economically attractive [6]. Furthermore, avoiding the release of phosphate into the environment prevents eutrophication of water bodies.

Industrially, fermentations are often supplemented with yeast extract. Considering a large scale 30 $m^3$ reactor [7] with 10 g $L^{-1}$ yeast extract [8], the phosphate content would amount to approximately 11 kg. To a large extent, phosphate is not taken up by the microorganisms and is found in the broth after fermentation [9]. It would be most desirable for the bioeconomy and associated biomass production to have a process for the recovery of phosphate from these streams.

Fermentations are carried out on an industrial scale for the production of e.g. acetic acid, lactic acid, and ethanol [10–13]. Of these, lactic acid has developed into a model compound for the bioeconomy [14,15] due to its versatile use as a food additive, as an accessory agent in pharmaceutical and cosmetic products or for poly(lactic acid) formation [10,16–18]. With help of lactic acid-forming bacteria, such as *Streptococcus* sp. or *Bacillus coagulans*, even complex substrates including canteen waste and agricultural residues can be converted into lactic acid [19–21]. After fermentation, the phosphate concentration can be as low as 50 mg $L^{-1}$. Nevertheless, recovery of phosphate is still worthwhile, though challenging. Usually, downstream processing aims to create a stream rich in the organic target compound [22], while minerals are diluted in the waste stream. Therefore, it would be best to integrate a recovery step directly after the fermentation is completed.

Phosphate recovery from solution can be conducted by adsorption on some kind of base or precipitation with help of metal ions. Precipitation is carried out with magnesium [23], calcium [24,25], iron [26], or aluminum ions [27]. The metal salts are added to the solution and the insoluble phosphate salts are collected. In the case of magnesium salts, magnesium ammonium phosphate (struvite) is produced. Calcium salts yield calcium hydroxyl phosphate (apatite) and other phosphates [28]. For both struvite and apatite, their direct use as fertilizers is possible [29–31]. Iron and aluminum salts are disfavored for the precipitation, since phosphate binds strongly and once bound,

*Abbreviations:* HPLC, high performance liquid chromatography; PPS, pure phosphate solution; RFB, real fermentation broth; SP, seashell powder

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release is difficult to achieve. Furthermore, the addition of metal salts may affect fermentations when recycled water streams are used.

It has been found that seashell powder (SP) possesses the ability to remove phosphate from solution [32–34]. Seashells mainly consist of calcium carbonate and remain as waste streams from the aquaculture industry. With removed phosphate, apatite is formed. Thus, the aim of this study is to review cost-efficient methods for phosphate removal from solutions. It first provides an overview on the applicability of SP for phosphate removal and is thereafter supported by experiments to assess the effect of organic compounds on removal capacity, an aspect missing when it comes to an assessment of applicability of seashells for phosphate removal from complex liquid waste streams. Experiments were carried out using pure phosphate solution (PPS) and PPS including ethanol, propanol or 1,2-propanediol, or acetic, propionic or lactic acids. Finally, real fermentation broth (RFB) from the microbial conversion of food waste constituents into lactic acid was subjected to phosphate removal. In particular, connecting organic residue utilization and phosphate recovery paves the way for use of a holistic material as feedstock for fermentations and sources of phosphate.

### *Seashells for phosphate removal: state-of-the-art*

There are many natural materials, such as sand, shell-sand, limestone, pumice, seashells, and tree bark, available to remove phosphate from solution. Ballantine and Tanner reviewed different materials for their phosphate removal potential, availability, costs and the possibility for reuse [35]. Based on a 10 score scale they evaluated seashells as a 7. This promising evaluation is due to the high availability and low costs of seashells, beneficial reuse and medium capacity to remove phosphate. The capacity of seashell for phosphate removal is attributable to its chemical composition and particularly the high CaO content. In mussel and oyster shells, CaO is the predominant constituent with 95.7% (w/w) and 98.2%, respectively. Other constituents are $SiO_2$, $Al_2O_3$, MgO, $Fe_2O_3$, $K_2O$ and $Na_2O$ [36,37]. It has been suggested that phosphate removal is based on the binding to positively charged calcium and precipitation of calcium phosphates as well as adsorption of phosphate on the surface of the adsorbent [38]. The latter is based on a high porosity and surface area, e.g. crushed oyster shells with a particle size of 0.3–0.6 mm showed a porosity of 58.3% and a surface area of 7,960,000 $m^2\,m^{-3}$ [39].

In previous studies, several kinetic models, such as a pseudo-second-order model, an Elovich model for chemisorption and an intra-particle diffusion model, have been applied to describe the adsorption of phosphate on SP [40]. It was found that the pseudo-second-order model best fit the dynamic adsorption behavior and that intra-particle diffusion was not the only rate-controlling step. From the results the authors concluded that the three mentioned kinetic models may operate simultaneously and that “other kinetic mechanism may also play a role” [40]. It is difficult to characterize a natural-based material and it is even more difficult to investigate meaningful data, such as adsorption and diffusion kinetics, when the actual composition in unknown. Nevertheless, the reported capacity of SP to remove phosphate makes it a valuable material.

Nguyen et al. reviewed the phosphate removal capacity of unmodified biosorbents, including oyster and scallop shells, and commercially available adsorbents, including Amberlite IRA-400 and Dowex [41]. Using oyster shell powder, a phosphate loading of 1.96 mg $g^{-1}$ could be obtained in a 50 mg $L^{-1}$ phosphate solution [33]. Scallop shells showed a maximum phosphate loading of 23 mg $g^{-1}$ [42]. Working with 200 mg mussel shell powder in 10 mL 1.5 g $L^{-1}$ phosphate solution, loadings of around 50 mg $g^{-1}$ were reported [43]. The removal capacities of commercially available adsorbents depend on the material. The ion-exchange resins, Amberlite IRA-400 and Dowex, showed a maximum adsorption capacity of 32.24 mg $g^{-1}$ [44] and 40.23 mg $g^{-1}$ [45], respectively. Elsewhere, alkali-treated calcium-silicate composites could be loaded with 105 mg $g^{-1}$ phosphate after 24 h in 500 mg $L^{-1}$ phosphate solution [24]. Thus, commercially available adsorbents show a better removal capacity, but the cost-efficiency of natural adsorbents, such as seashells, is an asset.

Considering the studies dealing with the removal of phosphate from artificial liquid streams, it would be reasonable to transfer the experience gained to real liquid waste streams. The majority of studies dealing with the removal of phosphate have been carried out using synthetic defined media simulating wastewater streams. Wastewater streams and fermentation streams, however, are complex, and thus the capacity for phosphate removal may change substantially. One of the few studies dealing with real municipal wastewater was published by Liu et al. [46]. When wastewater was treated with oyster shell powder at neutral pH, phosphate removal of on average 23.2% was obtained. It should be noted that the initial concentration of phosphate of 1.7 mg $L^{-1}$ was very low and probably insufficient to initiate precipitation. Consequently, when the pH was increased to 9–10, favorable for the precipitation of calcium phosphate, removal increased to 79.9% and 90.6% on average [46].

Testing of SP for phosphate removal from fermentation broth is currently missing from literature studies. Equally, it is necessary to assess the impact of various organic constituents on phosphate removal.

## Materials and methods

### *Seashell*

Seashells (*Mytilus edulis*, *Cerastoderma edule*, *Barnea candida*) were collected at the Baltic Sea. Organic material was removed and shells were placed into boiling water for 30 min and dried in an oven at 60 °C for 24 h. Thereafter, SP was produced by grinding shells in a ball mill for 3 min and sieved with < 1 mm mesh particle size.

### *Removal experiments*

Phosphate removal was investigated with PPS, PPS containing ethanol, propanol or 1,2-propanediol, or acetic acid, propionic acid or lactic acid, and RFB containing phosphate and lactic acid in batch mode. All experiments were performed in triplicate and mean values and standard deviations determined. Temperature and pH values were not controlled and ranged between 20 °C and 26 °C, and 8.0 and 8.9, respectively. During experiments, solutions were stirred magnetically at 140 rpm. Experiments lasted for 120 min and samples of 1 mL aliquots were taken regularly.

PPS with 50 mg $L^{-1}$ phosphate was prepared by adding 0.0726 g $NaH_2PO_4 \times H_2O$ to 1 L demineralized water. In order to investigate the effect of organic compounds on phosphate removal, PPS was supplemented with 0.83 M ethanol, propanol or 1,2-propanediol, or acetic acid, propionic acid or lactic acid. 0.6, 1.2, 2.4, or 4.8 g SP was placed in a 100 mL Erlenmeyer flask, and 50 mL of the respective phosphate solution was added. The amounts of the organic compounds were selected in order to provide reasonably high and comparable concentrations for the investigation of the effect on phosphate removal capacity.

Phosphate removal was further tested on RFB containing 132 mg $L^{-1}$ phosphate and 50 g $L^{-1}$ lactic acid using 4.8 g SP. Furthermore for comparison, removal from PPS containing 132 mg $L^{-1}$ phosphate and from PPS containing 132 mg $L^{-1}$ phosphate as well as 50 g $L^{-1}$ lactic acid was investigated.

### *Lactic acid fermentation and recovery of fermentation broth*

For lactic acid fermentation, a mesophilic *Streptococcus* sp. strain [20] was used. Inoculum preparation was carried out at 35 °C for 24 h at an initial pH 7 in 250 mL flasks containing 10 *g* $L^{-1}$ glucose and 10 g $L^{-1}$ yeast extract. In the fermentation with 10% (w/w) solid-to-liquid ratio of food waste, a 10% (v/v) inoculum was used. 60 g $L^{-1}$ glucose was added to enable a lactic acid yield close to 50 g $L^{-1}$ and 10 g $L^{-1}$

yeast extract were added to supply sufficient nutrients. Fermentation was carried out at 35 °C and pH 7 in a 1 L EloFerm bioreactor (Biotronix GmbH, Germany). Constant pH was accomplished by automatically adding 2.0 M NaOH or 1.7 M HCl. After fermentation the broth was inactivated at 121 °C for 20 min and centrifuged to separate liquid, solids, and oils. Lactic acid concentration in the liquid supernatant was 50 g $L^{-1}$ and phosphate concentration was 132 mg $L^{-1}$. The solution was stored at -20 °C until use. The RFB was used in the removal experiments similar to the procedure described above.

*Analytics*

Phosphate concentration was determined photometrically via generation of molybdenum blue. Four separate solutions were prepared: (I) sulfuric acid (2.5 M), (II) potassium antimonyl tartrate solution (1.3715 g $K(SbO)C_4H_4O_6 \times 1/2\ H_2O$ in 500 mL demineralized water), (III) ammonium molybdate solution (20 g $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$ in 500 mL demineralized water), and (IV) ascorbic acid solution (1.76 g ascorbic acid in 100 mL demineralized water). Molybdenum reagent (V) was prepared by combining 2.5 mL (I), 0.25 mL (II), 0.75 mL (III), and 1.5 mL (IV). Sample (100 µL), 900 µL demineralized water, 10 µL (III), and 160 µL (V) were mixed. After incubating at 60 °C for 15 min, absorption was measured at 880 nm. In the case of fermentation broth, 40 µL of sample was taken and 960 µL demineralized water was added.

Ethanol, propanol, 1,2-propandiol, acetic acid, propionic acid and lactic acid as well as sugar concentrations (glucose, sucrose, fructose) were analyzed by HPLC (Shimadzu: LC-10AD pump, SIL-10AD autosampler, CTO-10AD oven, CBM-20 A communication module): 10 µL of sample was injected onto an Aminex HPX-87H column (300 mm × 7.8 mm) and eluted isocratically with 0.4 mL $min^{-1}$ of 5 mM $H_2SO_4$ at 27 °C. Detection was carried out by a refractive index detector (Shimadzu, RID-20 A) at 40 °C. For each analyte, calibration curves were generated with pure solutions of known concentration.

*Statistical analysis*

SigmaPlot was used to carry out one way analysis of variance and to determine the statistical differences of phosphate removal between repeated determinations. Differences in median values were regarded as statistically significant when $P < 0.05$.

## Results and discussion

*Phosphate removal from pure phosphate solution and pure phosphate solutions with organic compounds*

In line with the recent development of processes dealing with the removal of phosphate from wastewater streams, the recovery directly from fermentation broth was investigated. In contrast to wastewater streams, fermentation broths contain small organic molecules, such as alcohols and organic acids, at high concentrations. It is expected that those constituents could have an impact on the removal of phosphate.

From Fig. 1 it is seen that phosphate removal increased with increasing amount of SP. With 4.8 g SP and 50 mL PPS, > 95% of the phosphate could be removed within 120 min. By reducing the amount of SP to 2.4, 1.2, and 0.6 g, the removal was 91%, 74%, and 57%, respectively (Fig. 1). The removal of phosphate was not negatively affected when ethanol, propanol or 1,2-propanediol were added at a concentration of 0.83 M to PPS. In all experiments the removal was similar to PPS and > 95% was removed with 4.8 g SP (Fig. 1). Ethanol and 1,2-propanediol even improved the phosphate removal when 0.6 g or 1.2 g SP was applied for both alcohols, around 20% more phosphate was removed when 0.6 g SP was applied and 10% more when 1.2 g SP was applied compared with PPS. However, when acetic, propionic or lactic acids were added to PPS at a concentration of 0.83 M, phosphate removal was negatively affected. With acetic or propionic acid, removal decreased from around 70% with 4.8 g SP to 25% with 0.6 g. When lactic acid was present, the removal was only 56% with 4.8 g SP and further decreased to 10% with 0.6 g (Fig. 1). Dividing the removed mass by the initial mass of phosphate shows that the specific loading is low (Fig. 1): in all experiments using 4.8 g SP the specific loading was 0.5-0.35 mg $g^{-1}$. There was no significant difference between repetitions. It should, however, be noted that the specific loading and removal capacity can significantly be improved when SP undergoes calcination at 700 °C as shown for snail and clam shells [47].

The concentrations of ethanol and propanol were slightly affected and 86% and 80%, respectively, were found after treatment with SP, probably due to evaporation during phosphate removal, while the concentration of the 1,2-propanediol was not affected, being less volatile than ethanol and propanol. Up to 10% of the initial concentration of organic acids was lost after SP treatment. Working with uncharacterized waste streams is always challenging. Even though an analysis of the elemental composition of the seashell mixture from *M. edulis*, *C. edule* and *B. candida* used was not carried out, CaO is expected to be the predominant constituent, as reported earlier [36,37]. The organic acids tested have one functional carboxyl group. The final pH value in all experiments was 8.2–8.7, so that the carboxyl group was deprotonated. Since the capacity of SP for phosphate removal decreased significantly and their initial concentrations were reduced by 10%, the organic acids appeared to interact with SP. This may have occurred either by complexing with alkaline earth metals or by binding to positively charged calcium sites [48]. Either could result in a reduction of the concentration of organic acids. The former is most likely to be the case for insoluble calcium lactate.

*Phosphate removal from real fermentation broth*

Due to the complexity of food waste as substrate, the phosphate concentration is difficult to assess. 132 mg $L^{-1}$ was found in the broth after fermentation rather than the 50 mg $L^{-1}$ as used above. In order to compare the removal efficiency of 132 mg $L^{-1}$ phosphate either from PPS, from PPS containing 50 g $L^{-1}$ lactic acid and from RFB containing 50 g $L^{-1}$ lactic acid, further experiments were performed. In contrast to PPS and PPS with organic compounds, which were clear solutions, RFB was brown and contained leftover sugars from the fermentation. The removal of phosphate from PPS and PPS with lactic acid was 102.5 and 63.8 mg $L^{-1}$, corresponding to 77.7 and 48.3%, respectively, after 120 min with 4.8 g SP (Fig. 2). In comparison with the results shown in Fig. 1, it is clear that a higher initial phosphate concentration results in a higher removal and consequently higher loading of SP. Using the same amount of SP, 28.3 mg $L^{-1}$ (21.5%) phosphate was removed from RFB after 120 min (Fig. 2). This result suggests that the complex composition of RFB regarding sugars and other constituents can further hinder phosphate removal.

Nevertheless, the application of seashells for phosphate removal from fermentation broths seems possible. However, small molecules such as organic acids, usually present at concentrations of 50 g $L^{-1}$ and other broth constituents negatively affect the removal efficiency. Therefore, preliminary tests are necessary to assess the effect of fermentation constituents. This finding is also relevant for the removal of phosphate from wastewater.

*Applicability of seashells for phosphate removal from fermentation broths*

Generally, at sites where seashells are available in larger amounts, this process could be applied to add value to wasted seashells and to recover phosphate from fermentation broths. Considering the experimental findings for an application of powdered seashells for phosphate removal after lactic acid fermentation, the following mass balance can be assembled (Fig. 3): to 1000 L fermentation broth containing 50 kg lactic acid and 132 g phosphate, 96 kg SP could be added. After 120 min, phosphate in solution would be reduced to 103 g. The

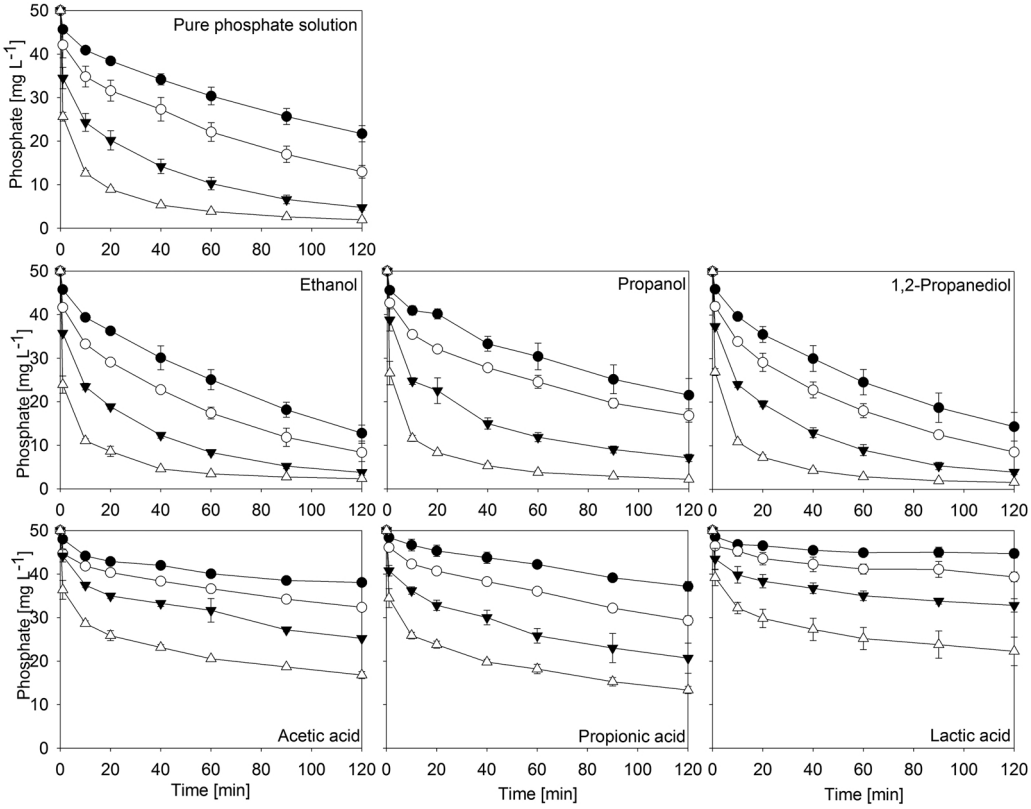


**Fig. 1.** Phosphate removal from pure phosphate solution and phosphate solutions containing 0.8 M ethanol, propanol, 1,2-propandiol, acetic acid, propionic acid or lactic acid using 0.6 g (closed circles), 1.2 g (open circles), 2.4 g (closed triangles) or 4.8 g (open triangles) of seashell powder.

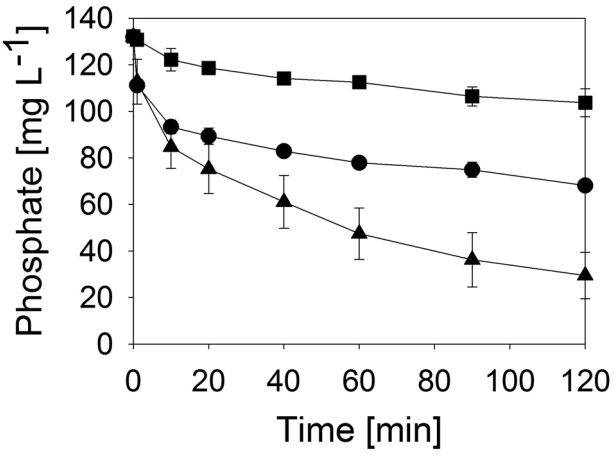


**Fig. 2.** Phosphate removal from a pure phosphate solution containing 132 mg $L^{-1}$ phosphate (full triangles), a 132 mg $L^{-1}$ phosphate solution containing 50 g $L^{-1}$ lactic (full circles), and a real fermentation broth containing 132 mg $L^{-1}$ phosphate and 50 g $L^{-1}$ lactic acid (closed squares) carried out in batch mode using 4.8 g seashell powder.

application of 96 kg SP for the removal of 29 g phosphate is unfavorable. Repeated phosphate removal with new unloaded SP might be an option; this will lead to further removal of phosphate, but also to higher amounts of seashell that need to be processed and a loss of 6 kg lactic acid after each run. Where lactic acid exists as precipitated calcium lactate, the addition of sulfuric acid may result in the formation lactic acid and calcium sulfate, and phosphate might be converted simultaneously into phosphoric acid. SP, however, cannot be reused for phosphate removal after such a treatment.

Bearing in mind that higher amounts of SP result in higher removal rates, it is suggested that the amount of SP be adjusted according to the concentration of phosphate present in fermentation broth. An option to improve the phosphate removal capacity is calcination of SP [37]. However, further studies are needed in order to investigate the behavior of calcinated SP regarding the simultaneous removal of phosphate and organic compounds.

## Conclusions

Even though many studies are available dealing with the investigation of phosphate removal from defined solutions using SP, little information is available when it comes to real liquid waste streams containing various small organic molecules and macromolecules at high concentrations. It was shown here that SP removes phosphate from fermentation broth and thus a simultaneous utilization of waste streams, such as seashells and food waste, is possible. However, the results also revealed that fermentation broth constituents and in particular organic acids can significantly affect the phosphate removal capacity of SP. In contrast, alcohols did not affect the removal capacity and ethanol as well as 1,2-propanediol even resulted in an increase. In order to remove high amounts of phosphate, large quantities of SP are needed.

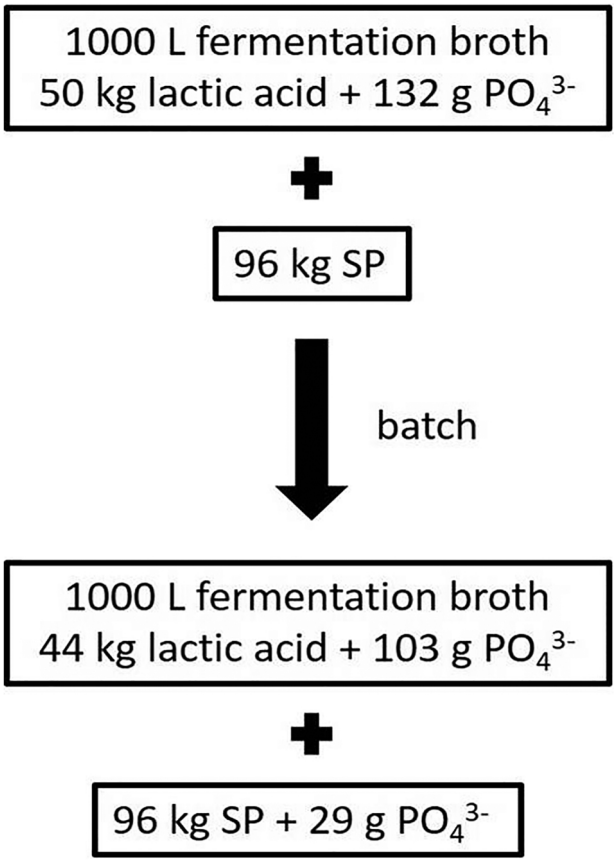


**Fig. 3.** Mass balance of phosphate removal from fermentation broth.

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ORIGINAL PAPER

# The effect of organic acids and alcohols on precipitation of phosphate using calcined seashell powder

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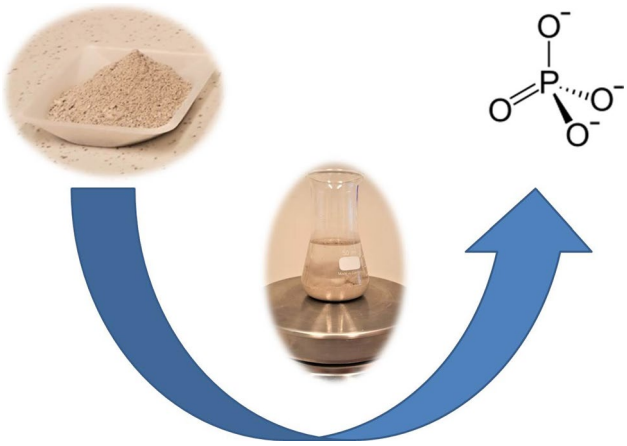
## Abstract

This study elaborates on the use of calcined seashell powder (SP) for the precipitation of phosphate from solutions containing high concentrations of organic compounds. Precipitation of phosphate was carried out in pure phosphate solution containing 0.1, 0.5 or 1 g $L^{-1}$ phosphate or pure phosphate solutions containing ethanol, propanol, propionic acid or lactic acid. The concentration of each organic compound was 1 M and the amount of calcined SP added to each batch was 2 g $L^{-1}$. This amount of SP was sufficient to remove 0.5 g $L^{-1}$ phosphate. Interestingly, at 1 g $L^{-1}$ phosphate and in presence of propanol, propionic acid or lactic acid the precipitation was finished within 10 min. Contrarily, 120 min was needed in water or ethanol. In 1 M lactic acid and 0.1 or 0.5 g $L^{-1}$ phosphate no or inhibited phosphate removal, respectively, was observed. The outcomes of this study revealed that organic acids and alcohols can have a positive or negative effect on the precipitation of phosphate. The effect is not only dependent on the organic compound, but also on the concentration of phosphate.

## Graphic abstract




**Keywords** Phosphate minerals · Resource recovery · Waste utilization · Bioeconomy

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## Introduction

The successful transformation to a biobased economy is strongly dependent on the availability of phosphate (Mueller et al. 2012; Ringeval et al. 2017). Phosphorous is essential as fertilizer to produce biomass at industrial scale as it forms the backbone of DNA and is involved in the phosphorylation of enzymes. Furthermore, the anhydride bonds between

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phosphate in adenosine triphosphate serve as energy source for biochemical reactions. The increasing demand of phosphate to foster the production of biomass will result in the decline of natural phosphate deposits in the near future (Mayer et al. 2016) and more attention needs to be laid on the recovery of phosphate from liquid waste streams.

One of the most investigated processes for phosphate removal from liquid waste streams is the precipitation with metal ions, such as magnesium (Le Corre et al. 2009), calcium (Jiang et al. 2017; Roques et al. 1991), iron (Wilfert et al. 2015) or aluminum (Guaya et al. 2016). Those ions form insoluble phosphate minerals. To provide the required concentration of ions for precipitation, calcium oxide (CaO), for instance, can be added to the liquid stream. The hydration of calcium oxide leads to the formation of calcium hydroxide ($Ca(OH)_2$) (Eq. 1, Minakshi et al. 2019). Calcium hydroxide dissociates into $Ca^{2+}$ and $2OH^-$ (Eq. 2, Tangboriboon et al. 2012), and in presence of $PO_4^{3-}$, insoluble phosphate minerals, such as hydroxylapatite ($Ca_5(PO_4)_3OH$), are formed under alkaline conditions (Eq. 3, (Ferguson et al. 1973)). The reactions are given below:

$$CaO + 3H_2O \Rightarrow Ca(OH)_2 + 2H_2O, \quad (1)$$

$$Ca(OH)_2 \Rightarrow Ca^{2+} + 2OH^-, \quad (2)$$

$$5Ca^{2+} + OH^- + 3PO_4^{3-} \Rightarrow Ca_5\left(PO_4\right)_3OH. \quad (3)$$

Other species of phosphate minerals are brushite ($Ca[PO_3(OH)] \times 2H_2O$), octacalcium phosphate ($Ca_8H_2(PO_4)_6 \times 5H_2O$), monetite ($CaHPO_4$) or amorphous calcium phosphate (Karunanithi et al. 2016).

Even though simple phosphate recovery strategies, such as precipitation with calcium oxide, have been developed for waste water treatment, these strategies may not work for liquid containing high concentrations or organic compounds. The increasing application of biotechnological processes will result in increased volumes of liquid streams containing high concentrations of organic compounds that need to be managed in the future. These liquid streams are usually sent to downstream processing where the envisaged products, such as biomass and/or organic compounds, are separated and purified. The recovery of phosphate, however, is often neglected due to the strong dilution and unpredictable effects of organic compounds on the removal. For instance, citric acid was found to bind to the active sites of phosphate minerals precipitation seeds, which resulted in an inhibition of precipitation, while acetic acid had a negligible effect (van der Houwen and Valsami-Jones 2001). Their experiments have been carried out at concentrations of 1 mM, which is substantially below the concentration usually found in liquid streams from biotechnological processes (López-Garzón and Straathof 2014), and thus it remains unknown how high concentration of organic compounds affect the precipitation of phosphate using calcium oxide.

The aim of this study was to investigate a phosphate precipitation using calcium oxide from liquid waste streams containing organic compounds at high concentrations. To include the concept of circular bioeconomy, a side product from aquaculture, seashell, was used as source of calcium oxide after calcination. The precipitation process was studied in water, in presence of organic acids, namely lactic acid and propionic acid, and in presence of alcohols, namely ethanol and propanol, at concentrations of 1 M to reveal their effect on phosphate removal. Organic compounds have been chosen due to their relevance as products from biotechnological processes carried out at large scale.

## Materials and methods

### Seashell

Seashells from the blue mussel *Mytilus edulis* were collected at the Baltic Sea. Organic material was removed, and shells were put in boiling water for 30 min and dried in an oven at 60 °C for 24 h. Thereafter, seashell powder (SP) was produced by grinding shells in a ball mill for 3 min and sieved with < 1 mm mesh particle size.

### Calcination

Calcination of SP was carried out at 800 °C under air atmosphere and a keeping time for 2 h in a muffle furnace (Nabertherm LT9/11/B410, Germany). Heating rate was 10 °C per min. Cooling occurred unregulated overnight. Calcined SP was stored in a bottle under air atmosphere.

### X-ray diffraction

X-ray diffraction measurements of untreated and calcined SP for phase identification were performed using Cu-Kα radiation at 1.54060 Å (Bruker AXS D8 Advance, Germany) at room temperature. The diffraction was done at 40 kV and 40 mA using Bragg angles from 20 to 70° (scan speed of $0.01° \ s^{-1}$). No preparation of samples was carried out prior to measurements.

### Scanning electron microscopy

Morphological characterization was done using a scanning electron microscope (ZEISS-EVO 18, UK). Samples were first washed with ethanol and dried to avoid agglomeration and second coated with gold for 120 s using a Quorum Sputter Coater to prevent sample charging. The chamber was vacuumed at 0.4–0.6 mbar. The applied voltage was 20 kV.

### Phosphate precipitation

Phosphate precipitation was investigated in batch mode with pure phosphate solution containing 0.1, 0.5 or 1.0 g $L^{-1}$ phosphate by adding 0.1452, 0.726 or 1.452 g $NaH_2PO_4 \times H_2O$ to 1 L demineralized water. To investigate the effect of organic compounds on phosphate removal, pure phosphate solution was supplemented with 1 M ethanol, propanol, or propionic acid or lactic acid. The pH was initially adjusted to 7. Changes in pH during the experiments are shown in Results section. In experiments, 0.1 g calcined SP was put in a 100 mL Erlenmeyer flask containing 50 mL of the respective phosphate solution (2 g $L^{-1}$ calcined SP). All experiments were performed in triplicate and mean values as well as standard deviations are presented. Temperature and pH were not controlled and ranged between 20 and 26 °C, and 7.0 and 12 (maximum detection was pH 12), respectively. During experiments, solutions were stirred with magnetic stirring at 140 rpm and pH was recorded continuously using the EloFerm bioreactor (Biotronix GmbH, Germany) control system. Experiments lasted for 120 min and samples of 1 mL aliquots were taken regularly, filtered through a 0.2 µm filter membrane and stored frozen until used for analytics.

### Analytics

Phosphate concentration was determined photometrically via generation of molybdenum blue. At first, four separate solutions were prepared: (1) sulfuric acid (2.5 M), (2) potassium antimonyl tartrate solution [1.3715 g $K(SbO)C_4H_4O_6 \times 1/2\ H_2O$ in 500 mL demineralized water], (3) ammonium molybdate solution [20 g $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$ in 500 mL demineralized water] and (4) ascorbic acid solution (1.76 g ascorbic acid in 100 mL demineralized water). Molybdenum reagent (5) was prepared by combining 2.5 mL (1), 0.25 mL (2), 0.75 mL (3) and 1.5 mL (4). Sample (100 µL), 900 µL demineralized water, 10 µL (3) and 160 µL (5) were mixed. After incubating at 60 °C for 15 min, absorption was measured at 880 nm.

Ethanol, propanol, propionic acid and lactic acid were analyzed with high-performance liquid chromatography (Shimadzu: LC-10AD pump, SIL-10AD auto-sampler, CTO-10AD oven, 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 $min^{-1}$ of 5 mM $H_2SO_4$ at 27 °C. Detection was carried out by a refractive index detector (Shimadzu, RID-20A) at 40 °C. For each analyte, calibration curves were generated with pure solutions of known concentration.

## Results

### Characterization of seashell powder

Figure 1 depicts the room temperature X-ray diffraction patterns of calcined SP (sample A) and untreated SP (sample B). Two calcium carbonate species namely calcite and aragonite were present in SP. After calcination aragonite disappeared and calcium oxide was formed. Nevertheless, calcite was still found after calcination. The surface of SP was rather undefined, while after calcination the surface was uniform (Fig. 2).

### Phosphate precipitation

Precipitation of phosphate was carried out using calcined SP added to pure phosphate solution or pure phosphate solution containing 1 M ethanol, propanol, propionic acid or lactic acid.

#### Pure phosphate solution

In Fig. 3a and b is shown the changes in phosphate concentration and pH, respectively, when 2 g $L^{-1}$ calcined SP was added to water containing 0.1, 0.5 or 1 g $L^{-1}$ phosphate. In presence of 0.1 g $L^{-1}$ phosphate, the phosphate concentration decreased immediately to 0.03 g $L^{-1}$ within 1 min and further to almost 0 g $L^{-1}$. When 0.5 g $L^{-1}$ phosphate was applied, the concentration decreased to 0.08 g $L^{-1}$

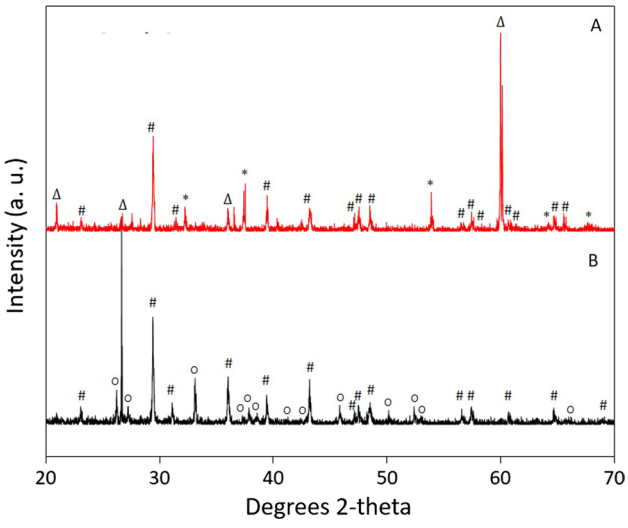


**Fig. 1** X-ray diffraction patterns of calcined SP (**a**) and untreated SP (**b**). The identified two polymorphs of calcium carbonate are calcite (hash) and aragonite (circle). After calcination (sample **a**) calcium oxide (asterisk) was detected. Furthermore, free carbon (delta) was present in sample **a**

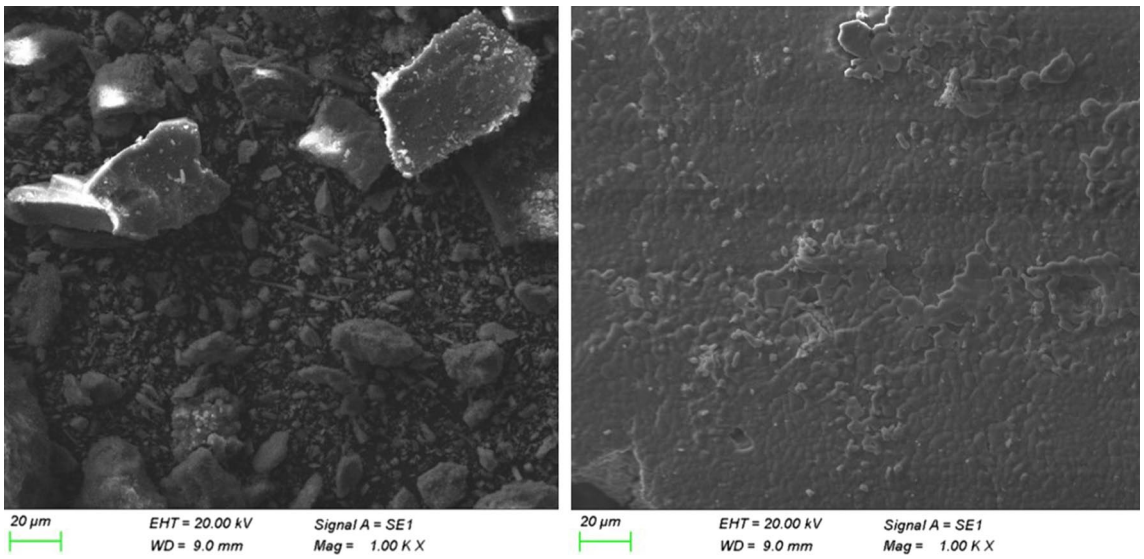


**Fig. 2** Scanning electron microscopic picture of untreated SP (left) and calcined SP (right)

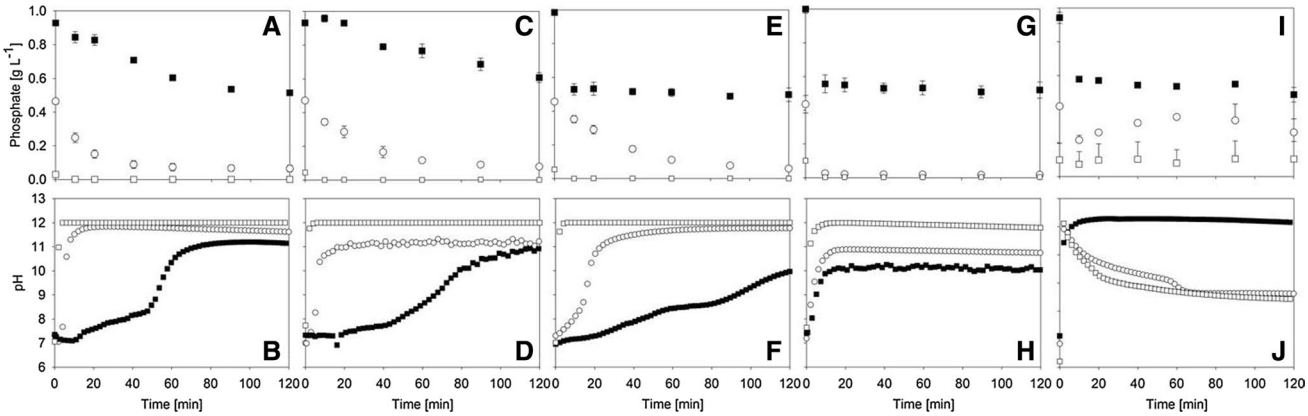


**Fig. 3** Precipitation of phosphate after calcined SP addition to a solution containing 0.1 (open square), 0.5 (open circle) or 1 (closed square) g $L^{-1}$ phosphate in presence of water (**a**), 1 M ethanol (**c**), 1 M propanol (**e**), 1 M propionic acid (**g**) or 1 M lactic acid (**i**). The respective pH profiles are shown in panels **b**, **d**, **f**, **h** and **j**, respectively

within 60 min and leveled off afterwards. A phosphate concentration of 1 g $L^{-1}$ did result in a linear decrease to almost 0.5 g $L^{-1}$ within 120 min (Fig. 3a). From 0.1, 0.5 or 1 g $L^{-1}$ phosphate, 100%, 85% or 45% was removed, respectively.

The different phosphate concentrations did influence the removal of phosphate and the pH (Fig. 3b). When 0.1 g $L^{-1}$ phosphate was added, the pH increased from 7 to 12 within 4 min and remained at 12 (or probably above) during the experiment. At 0.5 g $L^{-1}$ phosphate, the pH reached a value of 12 (or probably above) after 20 min and decreased to 11.7 after 120 min. Contrarily, the profile with 1 g $L^{-1}$ showed a slow initial increase to 8.4 after 50 min, followed by a quick increase to 10.7 within 10 min. Afterwards the pH leveled-off at pH 11.2 (Fig. 3b).

## Pure phosphate solution containing ethanol or propanol

The removal of phosphate from a solution containing 1 M ethanol was similar to the removal in water (Fig. 3c). In presence of 1 M ethanol and 0.1, 0.5 or 1 g $L^{-1}$ phosphate, 100%, 83% or 35% of the initial phosphate concentration was removed, respectively. Also the pH profile was similar to the tests found with water (Fig. 3b, d). While in the experiment with 0.1 g $L^{-1}$ phosphate the pH quickly increased to 12 (or probably above) and leveled-off afterwards, a slower increase to pH 11 within 20 min was found for 0.5 g $L^{-1}$. Using 1 g $L^{-1}$ phosphate, the pH increased from 7 to 11 in 120 min.

When 1 M propanol and 0.1, 0.5 or 1 g $L^{-1}$ phosphate were present, the removal was 100%, 87% or 55%,

respectively. The removal pattern for 0.1 and 0.5 g $L^{-1}$ was similar to experiments in water or 1 M ethanol. However, when 1 g $L^{-1}$ phosphate and 1 M propanol were applied, the phosphate concentration dropped to about 0.5 g $L^{-1}$ within 10 min and remained at this concentration (Fig. 3e). Also for propanol a slower increase in pH with increasing phosphate concentration was measured and the pH of the experiment with 1 g $L^{-1}$ reached only pH 10 after 120 min (Fig. 3f).

#### Pure phosphate solution containing propionic or lactic acid

The presence of 1 M propionic acid resulted in a drop of the phosphate concentration within 10 min and leveled-off at around 0.5, 0.02 and 0 g $L^{-1}$, when 1, 0.5 or 0.1 g $L^{-1}$ phosphate was initial used, respectively (Fig. 3g). The pH increased in presence of 0.1, 0.5 and 1 g $L^{-1}$ phosphate to 12 (or probably above), 10.9 and 10.2, respectively, within 10 min (Fig. 3h).

A similar drop of phosphate concentration from 1 g $L^{-1}$ to around 0.5 g $L^{-1}$ and a similar increase in pH from 7 to 11.2 within 10 min were found when 1 M lactic acid was present (Figs. 3i, j). However, the pattern was different when 0.1 or 0.5 g $L^{-1}$ phosphate was used. At 0.5 g $L^{-1}$ the concentration decreased to 0.2 g $L^{-1}$ within 10 min and increased afterwards again to around 0.3 g $L^{-1}$ (Fig. 3i). The pH first quickly increased to 11 and decreased after to around 9 within 120 min (Fig. 3j). The same pH pattern was observed for the experiment with 0.1 g $L^{-1}$ phosphate, however, no removal was detected (Fig. 3i, j).

In presence of 1 M propionic acid and 0.1, 0.5 or 1 g $L^{-1}$ phosphate, 100%, 95% or 48% of the initial phosphate concentration was removed, respectively, and 0%, 38% or 48%, respectively, when 1 M lactic acid was used.

## Discussion

The aim of this study was to make use of a side-product from aquaculture, seashell, by investigating its application as reagent for phosphate precipitation after calcination. Calcium carbonate has been present as calcite, as also shown in eggshells (Minakshi et al. 2018), and aragonite. During calcination, aragonite has been converted into calcium oxide and carbon dioxide (Tangboriboon et al. 2012). However, calcination did not result in a complete conversion of calcite (Fig. 1), which may indicate that a higher temperature is necessary (Tangboriboon et al. 2012). The surface, however, was more uniform after calcination (Fig. 2). Due to the co-presence of calcium carbonate and calcium oxide, one may expect that phosphate removal is based on adsorption and precipitation (please see reaction Eqs. 1–3). Owing to the removal of only small amounts of phosphate by adsorption (Peinemann et al. 2019), precipitation most likely exceeded adsorption on SP particles in this study.

Phosphate removal by precipitation can be applied even when a concentration of 10 mg $L^{-1}$ or less phosphate is present (Seckler et al. 1998). The efficacy of phosphate precipitation, however, can be affected by waste water or effluent constituents. In the present study the concentration of phosphate was 0.1, 0.5 or 1 g $L^{-1}$, or 0.001, 0.005 and 0.01 mol $L^{-1}$, respectively. The concentrations of organic compounds and calcined SP were similar in all experiments. On molar level, the ratio of calcium to phosphorous was 16.7, 3.1 or 1.6, respectively. Irrespective of the concentration of phosphorous used, calcium was added in excess. The higher the calcium to phosphorous ratio, the more effective and efficient is the precipitation of phosphate (Hosni et al. 2008).

The general pattern of phosphate precipitation follows an initial period where a slow precipitation takes place, followed by fast precipitation period and, in case reactants reach equilibrium, a period with slow precipitation (Ferguson et al. 1973). This general behavior can be linked to the growth of crystal nuclei (Ferguson et al. 1973). The different periods might be visible in Fig. 3a and c for the eluents water and ethanol, respectively, containing 1 g $L^{-1}$ phosphate. The initial phase took place between 0 and 20 min, followed by fast precipitation between 20 and 90 min, and a slow precipitation afterwards. This general pattern, however, cannot be seen for the eluents: Propanol, propionic acid and lactic acid containing 1 g $L^{-1}$ phosphate (Fig. 3e, g, i). Here, in less than 10 min phosphate dropped to around 0.5 g $L^{-1}$ and leveled-off afterwards. Considering crystal growth as essential for the precipitation of calcium phosphate (Ferguson et al. 1973), one may conclude that the three eluents and the used concentration of 1 M substantially affected the formation of nuclei. This effect, however, seems to depend on phosphate concentration, as it was only observed in presence of 1 g $L^{-1}$ phosphate.

It has been reported that citrate adsorbs at crystallization seeds, inhibit the growth of crystals and consequently precipitation (van der Houwen and Valsami-Jones 2001). It further inhibits the transformation in stable calcium phosphate species (Sharma et al. 1992). Acetate, however, did not affect precipitation (van der Houwen and Valsami-Jones 2001). Lactic acid is known to act as chelating agent, which favors a precipitation and this effect has been utilized to prepare hydroxyapatite whiskers (Ioku 1998). That propanol and propionic acid have the same effect on the precipitation of calcium phosphate as lactic acid was not expected prior to experiments. The possibility to apply this approach even to low phosphate concentration (100 mg $L^{-1}$ and probably below) may allow the efficient recovery of phosphate from propanol or propionic acid containing fermentation broths or waste waters.

Not only has the calcium to phosphorous ratio an impact on which calcium phosphate species is formed, but also the pH (Dorozhkin 2016). In the present study, experiments were pH uncontrolled, but started at a pH of 7. At pH 7, dihydrogen phosphate and hydrogen phosphate are present in equimolar amounts. With increasing pH, hydrogen phosphate (pH 10) and phosphate (pH 12) dominate. Except for the experiments carried out with lactic acid, the pH ramped to 12 (or probably above) when 0.1 g $L^{-1}$ phosphate, or to pH 10–11 when 0.5 or 1 g $L^{-1}$ was used. Generally, the pH increased slower in water, ethanol or propanol than in propionic acid or lactic acid. The slower increase in pH might be attributable to the elevated buffer capacity at higher phosphate concentration, the formation of calcium phosphate species taking up hydroxide ions and the slower dissolving of calcium hydroxide. This may explain the shape of pH curves shown in Fig. 3b, d, f, h and j. Due to the present phosphate species at pH 12 and above, octacalcium phosphate, calcium-deficient hydroxyapatite and/or hydroxyapatite are most likely the calcium phosphate species formed, while at pH 7–12 anhydrous monocalcium phosphate and monocalcium phosphate monohydrate might be present (Dorozhkin 2016).

The behavior of pH when experiments were carried out in presence of 1 M lactic acid and 0.1 or 0.5 g $L^{-1}$ phosphate is not in line with the findings in presence of water, alcohols or propionic acid, irrespective the phosphate concentration used (Fig. 3b, d, f, h and j). When 0.1 or 0.5 g $L^{-1}$ phosphate was used, the pH ramped to 12 (or probably above) and decreased afterwards to around 9, phosphate was not completely or not removed (Fig. 3i). Since the pH increased to 12 it is likely that calcium oxide was converted to calcium hydroxide. Hydroxyapatite was identified as the first precipitate appearing (Ferreira et al. 2003). The formation of hydroxyapatite results in a consumption of hydroxide-ions and consequently in a decreasing pH. Calcium-ions may react with lactic acid to calcium lactate and consequently calcium-ions are not available for the precipitation of phosphate, which may explain the reduced removal. This behavior seems to depend on phosphate concentration and at 1 g $L^{-1}$ the formation of hydroxyapatite might be faster than the formation of calcium lactate.

## Conclusion

This study revealed that calcined SP is applicable for the removal of phosphate from solutions containing water, 1 M ethanol, propanol or propionic acid, and 0.1, 0.5 or 1 g $L^{-1}$ phosphate. For lactic acid, however, inhibited removal was shown, which may be linked to side reactions of calcium ions and lactic acid at phosphate concentrations of 0.1 and 0.5 g $L^{-1}$. For the successfully tested solutions, 0.5 g phosphate can be removed using 2 g calcined SP. This simple phosphate precipitation process can potentially be applied to recover phosphate from liquid waste streams containing high concentrations of organic compounds.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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

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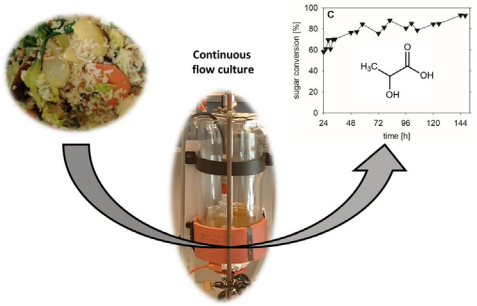
## GRAPHICAL ABSTRACT

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## 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 h), 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.

## 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 and 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

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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 and 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 and 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 h, 1 mL of this solution was added to 50 mL freshly prepared medium to maintain a stable culture. As inoculum, the 48 h 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 $\mu L^{-1}$). 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 h, 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, 1200 U $mL^{-1}$) was added at the start and subsequently every 24 h 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

**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 |

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 and 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 and 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 h, while for the culture carried out with FWC the titer after 10 h 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 (Fig. 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;

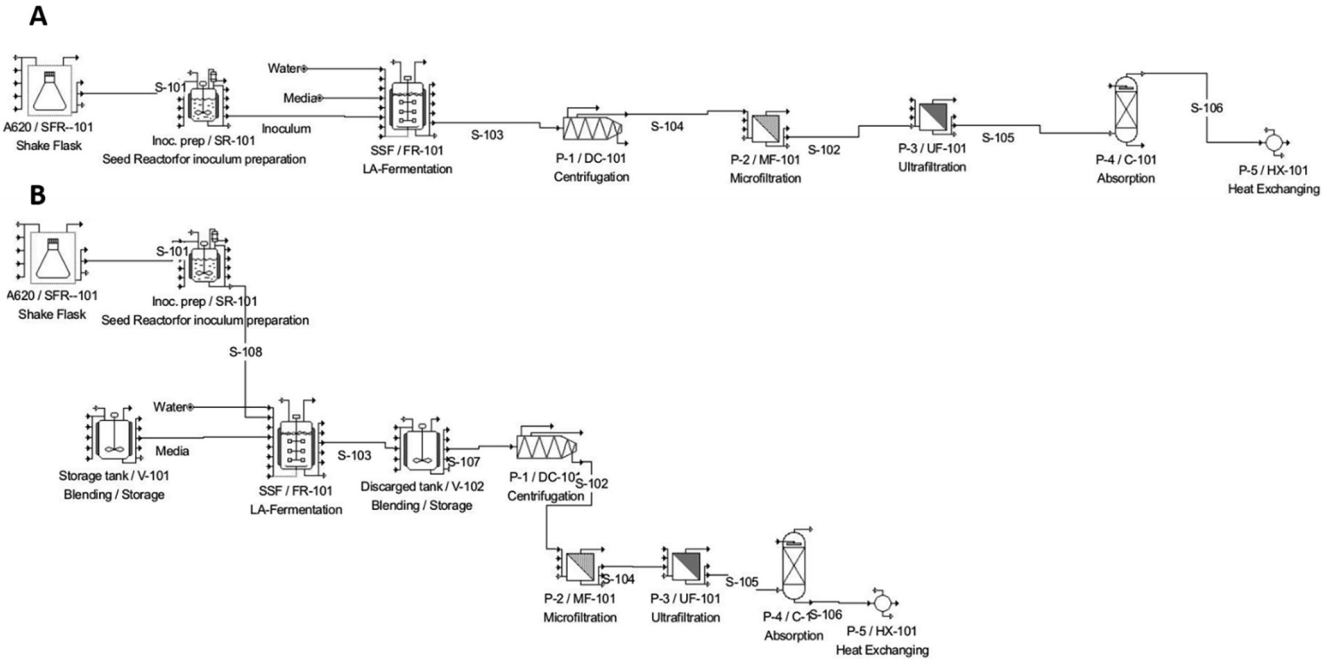


**Fig. 1.** Process scheme of batch (A) and continuous (B) flow fermentations.

Demichelis et al., 2017). For LA production in continuous flow fermentation (Fig. 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 h. 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 and Zacchi, 2000; Demichelis et al., 2018) (Eq. (1)):

$$A\,[Euro] = C_0 \bullet \frac{i \bullet (1+i)^n}{(1+i)^n - 1} \tag{1}$$

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 1600 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.

$$ROI\,[\%] = \frac{Annual\ \ net\ \ profit}{Initial\ \ total\ \ investment} \bullet 100 \tag{2}$$

*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).

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

*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.

$$P_{feed}\left[\frac{Euro}{t}\right] = \frac{annual\ \ operational\ \ cost}{annual\ \ FW\ \ treated} \tag{4}$$

$$P_{net}\left[\frac{Euro}{t}\right] = \frac{net\ \ profit\ \ after\ \ 5\ \ years\ \ of\ \ amortisation}{annual\ \ FW\ \ treated} \tag{5}$$

#### 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)):

$$Q_s\,[kW] = Q_{sub} + Q_{loss} + Q_p \tag{6}$$

$$Q_{sub}\,[kW] = m_{sub} \bullet c_p \bullet (T_{in} - T_{reac}) \tag{7}$$

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)):

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

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):

$$Q_p\,[kW] = \%_p \bullet (Q_{sub} + Q_{loss} \tag{9}$$

## 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 h

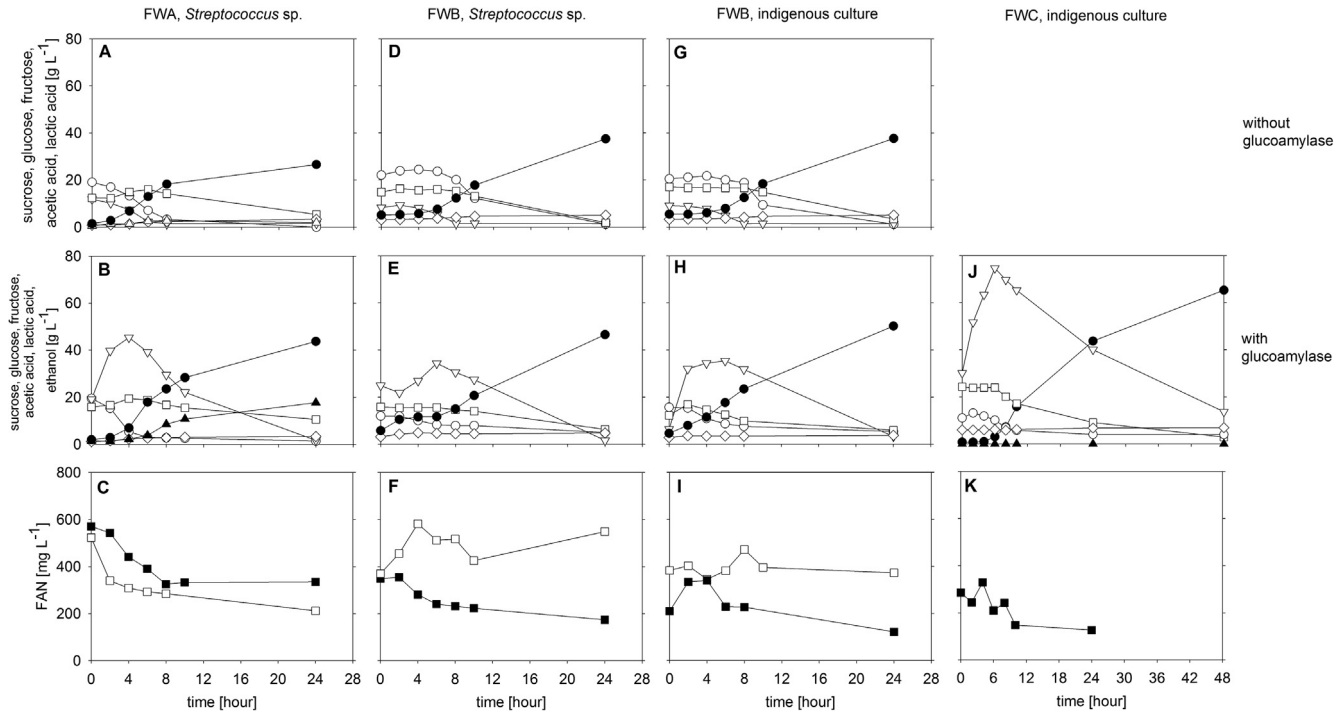


**Fig. 2.** Concentration of glucose (open triangle), fructose (open square), sucrose (open circle), acetic acid (open diamond), lactic acid (closed circle), ethanol (closed triangle) of non-sterile batch fermentations of food wastes A (FWA), B (FWB) and C (FWC) carried out with *Streptococcus* sp. (A–F) 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.

(Fig. 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 h, boosted productivity and a LA titer of 44 g $L^{-1}$ LA could be detected after 24 h (Fig. 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 h. This concentration indicates the presence of ethanol producing organisms. Furthermore, 10 g $L^{-1}$ fructose was left after 24 h. FAN was consumed during the first 8 h of fermentation irrespective the addition of glucoamylase and more than 200 mg $L^{-1}$ remained (Fig. 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 $L^{-1}$ (Fig. 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 h, 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% (Fig. 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 $L^{-1}$ (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 h (Fig. 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 (Fig. 2I).

The repetition of the batch fermentation with FWC and addition of glucoamylase is shown in Fig. 2J and K. Contrarily to the fermentation with FWB, the concentration of glucose increased during the first 6 h to almost 80 g $L^{-1}$ due to a hydrolysis of starch and was almost consumed within the following 38 h. Even though the final titer after 48 h 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 h. 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 (Fig. 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 5–10 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 and 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 h 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 h 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 h for FWA and FWB or 48 h for FWC (Fig. 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 h, glucoamylase was added to maintain sufficient glucose levels. Within the first day, LA titer rose from 47 g $L^{-1}$ to 61 g $L^{-1}$ (Fig. 3A). This trend was maintained at lower pace and after 148 h 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, 2270 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 h (Fig. 3D). For the consecutive 29 h, 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 h. For another 48 h, fermentation performance was seemingly unaffected, at most a slight increase in titer could be detected. However, starting from 119 h, LA concentration decreased to below 45 g $L^{-1}$ after 168 h total run time. FAN ranged between 380 mg $L^{-1}$ and 760 mg $L^{-1}$ (Fig. 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 (Fig. 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 $L^{-1}$ (Fig. 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-

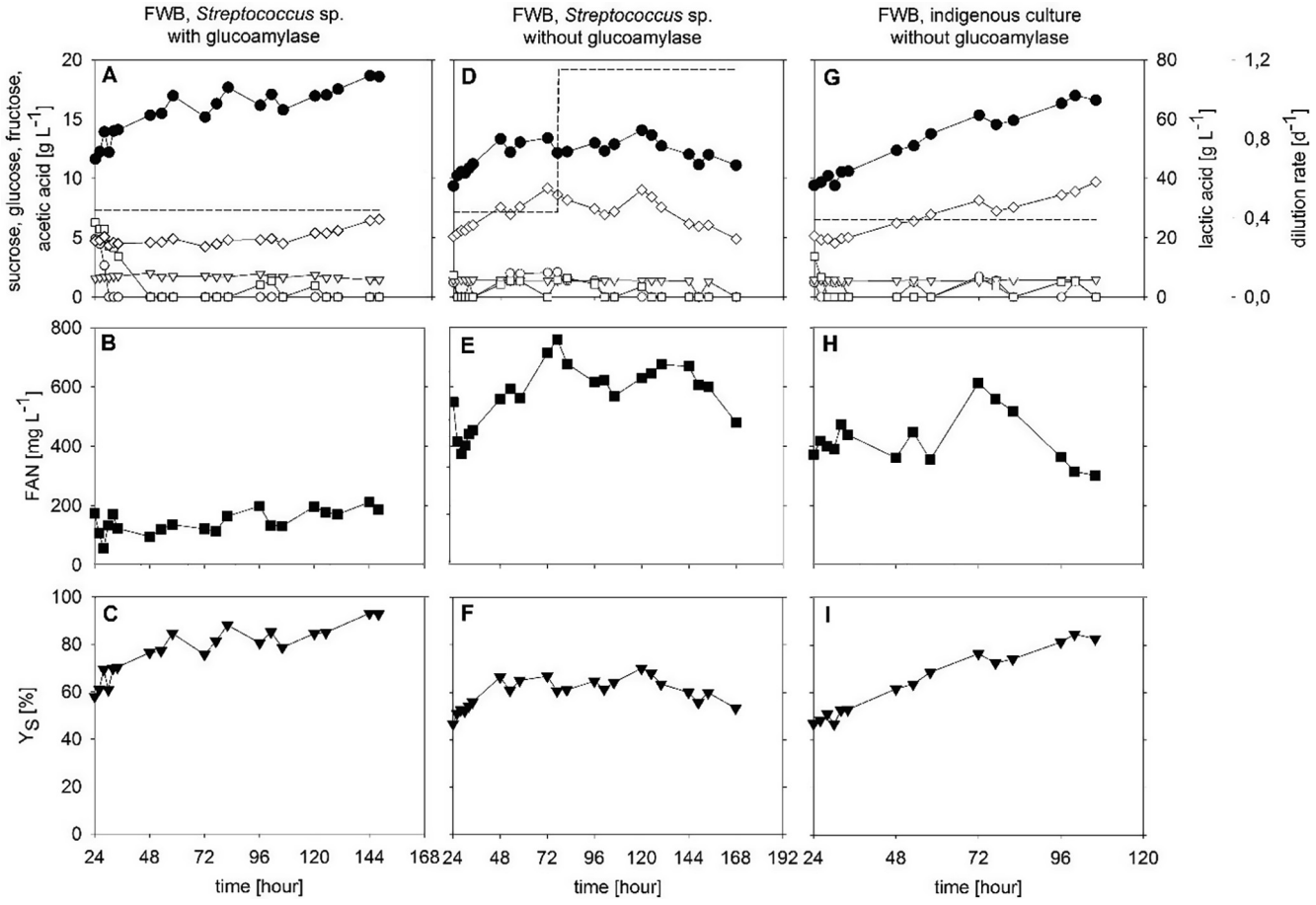


**Fig. 3.** Concentration of glucose (open triangle), fructose (open square), sucrose (open circle), acetic acid (open diamond), and lactic acid (closed circle), dilution rate (dashed line), free amino nitrogen (FAN, closed square), and lactic acid yield with respect to theoretically available sugars ($Y_S$, closed triangle) of non-sterile continuous flow fermentations of food waste B (FWB) started after 24 h 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).

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 h and 77 h) 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 h (Fig. 3G). Since the subsequent 10 h did result in a change in lactic acid concentration, fermentation was finalized after 106 h.

While showing no clear trend between 24 and 58 h, FAN exceeded 600 $mg\,L^{-1}$ after 72 h and dropped to 300 $mg\,L^{-1}$ nearly linearly thereafter (Fig. 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 (Fig. 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 (Fig. 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 and Xia, 2006), or corn steep liquor (Wee and 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.

### 3.5. 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 and 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.

### 3.6. 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.

**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 | 50 k | 100 k | 200 k | 500 k | 1 M |
|---|---|---|---|---|---|---|
| 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 |

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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.121631.

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Review

# Continuous pretreatment, hydrolysis, and fermentation of organic residues for the production of biochemicals

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ABSTRACT

Agricultural residues pose a valuable resource. Through microbial fermentations, a variety of products can be obtained, ranging from fuels to platform chemicals. Depending on the nature of the organic residue, pretreatment and hydrolysis are needed prior to fermentation in order to release fermentable sugars. Continuous set-ups are common for the production of methane or ethanol from lignocellulosic biomass, however, this does not apply for the fermentative generation of biochemicals, an approach that conserves chemical functionality present in biomass. Certainly, continuous set-ups could beneficially contribute to bioeconomy by providing techniques allowing the production of biochemicals in a sustainable and efficient way. This review summarizes research conducted on the continuous pretreatment, hydrolysis, and fermentation of lignocellulosic biomass, and particularly towards the production of the biobased molecules: Succinic and lactic acid.

## 1. Introduction

Processing of biomass is usually associated with the formation of organic residues. For instance, the production of crop and food as well as feed results in the generation of organic residues (Salihoglu et al., 2018). Organic residues appear at all stages from growing to processing of food and feed ingredients and finally consumption (Salihoglu et al., 2018). Since agricultural processes are subject to natural cycles of seeding, growing, and harvesting, the amount of residues accompanied to farming grows and decreases periodically. However, food and feed preparation takes place continuously throughout the year, and thus residue streams from preparation can be found continuously at household scale or at larger scale from industrial food and feed producers as well as canteens, restaurants, and bakeries. The continuous formation of residues may favor a continuous utilization over batch processes in order to avoid the storage of amounts of residues.

Although of heterogeneous origin, organic residues mainly comprise of carbohydrates, proteins, lipids, lignin, and minerals (ash) (Fu et al., 2019). These constituents pose a precious substrate for microbial fermentations (Koutinas et al., 2014). An obstacle towards direct fermentation, however, is the presence of oligo- and polysaccharides, like starch, hemicellulose, and cellulose, which most bacteria are unable to directly metabolize. Therefore, a hydrolysis step must be included prior to fermentation (Modenbach and Nokes, 2013). Furthermore, residues containing lignocellulose need to be thermo-chemically pretreated in order to qualify for complete hydrolysis (Hsu, 2018).

Carbohydrate-rich substrates with low fractions of lignocellulose are often used via anaerobic digestion. This process combines hydrolysis and fermentation carried out in one pot with biogas production (Zhao et al., 2019). Through the concerted action of various microbial consortia, starch is broken down to glucose and subsequently converted to a mixture of mostly carbon dioxide and methane. Anaerobic digestion is a robust process, which can continuously deal with a large number of different substrates. However, when aiming for utilization of lignocellulosic-rich residues, a thermo-chemical pretreatment is required.

Downstream processing of biogas, as the major product from anaerobic digestion, is relatively simple since biogas separates from the liquid fermentation broth. After upgrading, the product gas can be stored and used for energetic purposes (Santos-Clotas et al., 2019). Even though the whole process from anaerobic digestion to final biogas is relatively simple and implementable at small scale in rural areas, a drawback associated with this approach is the complete degradation of molecular functionalities that are present in the substrate. Hydroxyl, carboxyl, and other moieties of the carbon backbone are removed to almost exclusively yield carbon in its highest oxidation state (carbon dioxide) and the opposite fully reduced carbon (methane). By

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prioritizing material utilization of organic substrates over energetic utilization, functionalities could be partially conserved (Ragauskas et al., 2006). In view of a cascading utilization strategy, the products with chemical functionality might be separated from fermentation broth and the latter might still be a suitable substrate for anaerobic digestion (Demichelis et al., 2017).

While biogas production resembles a continuous fermentation, fermentations targeting the aforementioned molecules are often carried out in batch mode (Peinemann and Pleissner, 2018). In batch fermentations, substrate and microorganisms are combined and after depletion of fermentable molecules, downstream processing is conducted. Conditions in the reactor change over time with regard to concentration of metabolites and cell densities, which affects productivity. Conversely, continuous fermentations are operated at steady state. Addition of substrate and withdrawal of broth are in equilibrium, metabolite concentrations and cell densities are constant, and productivity can be, depending on the dilution rate, high. Therefore, it would be most desirable to possess a process with the aim of producing biochemicals, which is as simple as biogas production and runs continuously.

In order to achieve a throughout continuous conversion of organic residues to biochemicals as simple as the production of biogas, four steps need to be carried out continuously: Pretreatment, hydrolysis, fermentation, and downstream processing. This review is based on the hypothesis that processes for the throughout continuous conversion – from residue to product – are available and applicable to various organic residues including lignocellulosic materials and various products. In order to assess the opportunity to establish a simple material utilization, process steps (pretreatment, hydrolysis, fermentation, and downstream processing, separately or a combination of them (Fig. 1), will be critically reviewed with regard to different substrates and techniques. Wherever possible, linkages between steps are shown. Due to the economic relevance and chemical functionality focus has been laid on the biochemicals: Lactic acid and succinic acid (Pleissner et al., 2019). Conclusively, a scheme for the throughout continuous production of lactic acid, including continuous pretreatment, hydrolysis, fermentation, and downstream processing, as an example is constructed and combined on the basis of a mass balance.

## 2. Pretreatment

When considering lignocellulosic materials as substrate for fermentation, pretreatment is an inevitable step. The majority of pretreatment techniques are designed for batch operation (Alvira et al., 2010). However, continuous pretreatment methods are emerging and pose promising alternatives to existing batch techniques. In contrast to batch operation, which is carried out stationary, all continuous methods require some type of conveying and transporting. Nonetheless, the aim of both batch and continuous pretreatment is the same: Breaking down the structures that make up biological materials, removing lignin, reducing cellulose crystallinity, and facilitating subsequent hydrolysis (Fatma et al., 2018).

Depending on the substrate, a suitable pretreatment must be chosen. For substrates containing low amounts of lignocellulose, simple physical pretreatment may suffice. For domestic food waste, a rotary shear shredder was used to continuously homogenize food waste (Banks et al., 2011). Furthermore, grinding and milling are possible techniques that might continuously disintegrate food waste (Izumi et al., 2010). After mechanical treatment, the substrate is directly hydrolysable (Peinemann et al., 2019). In this manner, wet disk milling was applied to prepare rice straw for enzymatic hydrolysis. Rice straw and water were mixed at a ratio of 1:20 and passed through the wet disk mill several times. Glucose and xylose yields were determined as 79% (w/w) and 42% (w/w) after pretreatment and hydrolysis, respectively (Hideno et al., 2009). In another study, wet disk milling was used for the pretreatment of sugarcane straw. During the course of 20 continuous cycles, enzymatic digestibility steadily increased. While enzymatic hydrolysis of native sugarcane straw gave 24% (w/w) glucose and 14% (w/w) xylose, after 20 cycles of wet disk milling, 68% (w/w) glucose and 45% (w/w) xylose could be recovered (da Silva et al., 2010).

In the presence of higher shares of lignocellulose, physical pretreatment is combined with chemical methods. Working with corn stover, alkali pretreatment was combined with microwave irradiation in a continuously fed reactor. Corn stover was mixed with sodium hydroxide solution and conveyed through a microwave oven. Of the glucose present in the starting material, 63% (w/w) could be released enzymatically after said pretreatment. In respect to unpretreated corn stover, glucose yield could be enhanced more than fourfold (Peng et al., 2014). In a pilot scale reactor, wheat straw was pretreated hydrothermally. After being soaked at 80 °C for 5–10 min, excess water was removed and the straw fibers were passed through the reactor. The effect of different residence times (6, 9, and 12 min) and temperatures (185 °C, 195 °C, and 205 °C) was tested. Throughout the whole process, there was a counter current flow of process water, which was discharged at the soaking stage. Accordingly, the liquid fraction contains

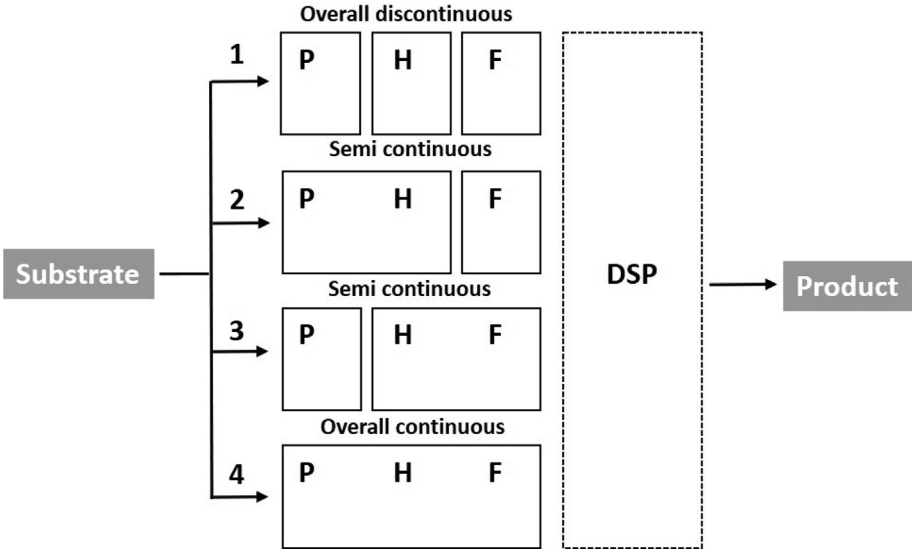


**Fig. 1.** Approaches to utilize organic residues by either (1) discontinuous pretreatment (P), hydrolysis (H) and fermentation (F), (2 and 3) combination of continuous and discontinuous processes (PH F, P HF), and (4) fully continuous process (PHF). DSP stands for downstream processing.

hemicellulose, minerals, and degradation products. The fiber fraction, which leaves the reactor after a final pressing operation, comprises most of the cellulose, some hemicellulose, and lignin and has a dry matter content of 25–40% (w/w). Over 90% recovery of cellulose was found in the fiber fraction when pretreatment was carried out at 195 °C combined with residence times of 9–12 min. Even though, hydrolysis was performed with cellulases and xylanases, no yields of sugars were reported. During subsequent ethanol fermentation, however, up to 93% of the theoretical ethanol yield were obtained, indicating sufficient enzyme accessibility (Petersen et al., 2009).

Cheng et al. (2019) presented the continuous hydrothermal pretreatment of sorghum bagasse combined with disk refining (Cheng et al., 2019). Yields of 82.6% (w/w) and 70.8% (w/w) for glucose and xylose, respectively, were obtained after 10 min at 180 °C and involving disk milling. In a continuously operated steam-pretreatment plant, woodchips were processed at a flow rate of 39 kg $h^{-1}$. Woodchips were coated with sulfuric acid or sulfur dioxide at different loadings and the effect of treatment temperature and coating agent was investigated. After pretreatment, solids and liquids were separated by centrifugation. When striving for highest glucose content in the liquid fraction and optimal enzymatic digestibility of the solid fraction, the authors suggest coating with sulfur dioxide and a temperature of 215 °C applied for 7 min. Under these conditions, more than 30 g $L^{-1}$ glucose were found in the liquid fraction, while up to 88% (w/w) of the theoretically available glucan in the solid fraction could be hydrolyzed to glucose (Wang et al., 2018). Steam explosion was also carried out continuously with wheat straw. Wheat straw was mixed with 0.5% (w/w) sulfuric acid at a ratio of 1:15 and fed through a single disk refiner before being subjected to steam explosion. Of the different testing conditions, steam temperature of 178 °C corresponding to 9.5 bar pressure gave best results when retention time was 6 min. More than half of the glucose could be released after enzymatic hydrolysis of solids, while 46% of the initial xylose was present in the pretreatment liquor (Fang et al., 2011).

In a different study, highly diluted rye straw (1%, w/w) was treated in a continuous flow reactor at temperatures between 197 °C and 257 °C at a pressure of 100 bar. Biomass solubilization reached values of up to 60% (w/w). However, this value refers to dissolved organic carbon after treatment in comparison to total organic carbon at the start of the experiment. Thusly, glucose recovery after hydrolysis of this solution is uncertain (Rogalinski et al., 2008).

Extrusion has shown to be a promising pretreatment technique for the preparation of lignocellulosic material as it simultaneously imposes heating, mixing, and shearing on the substrate. Studies were conducted both with and without addition of chemicals other than water. Screw speed and geometry, barrel temperature, and moisture content are important factors influencing pretreatment performance. Using a single-screw extruder, corn stover was pretreated varying temperature and screw speed. Dry matter of the substrate was adjusted to 21% (w/w) prior to feeding in to the extruder. Of the chosen conditions, medium screw speed of 75 rpm and high temperature of 125 °C were found to deliver best results. Glucose, 75% (w/w), and xylose, 49% (w/w), could be recovered with this pretreatment after subsequent hydrolysis (Karunanithy and Muthukumarappan, 2010b). The substrate scope was then extended to switch grass and prairie cord grass and extruder parameters were evaluated. For switch grass optimal conditions were determined as 50 °C, 50 rpm, and 15% (w/w) moisture content, while for prairie cord grass 50 °C, 50 rpm, and 25% (w/w) were sufficient. In the first case 45% (w/w) and in the latter case 61% (w/w) glucose could be recovered (Karunanithy and Muthukumarappan, 2010a).

Successful pretreatment was also achieved, changing the substrate from grass to pine wood chips. Three different temperatures, moisture contents, and screw speeds were tested in the single-screw extruder and evaluated with regard to sugar recovery. With 180 °C, 25% moisture (w/w, wet basis), and 150 rpm, 66% (w/w) of both, cellulose and hemicellulose, could be recovered (Karunanithy et al., 2012). Single screw extrusion coupled with alkaline pretreatment was shown with ground *Miscanthus* as substrate. At 140 °C, 8 min residence time, 15 g $min^{-1}$ biomass feed, and 120 mL $min^{-1}$ 0.5 M NaOH input, 89% (w/w) of the cellulose could be recovered. Subsequent enzymatic hydrolysis gave a hydrolysate with 32.8 g $L^{-1}$ glucose corresponding to a yield of over 90% (w/w) (Cha et al., 2015).

Elsewhere, ground soybean hulls were pretreated in a twin-screw extruder. In order to facilitate flow, the hulls were mixed with up to 20% (w/w) corn starch prior to pretreatment and water content of the blends was adjusted to levels between 20% and 40% (w/w, wet basis). Cellulose to glucose conversion peaked at 95% (w/w) when adjusting screw speed to 350 rpm and water content to 40% (w/w). Utilizing ethylene glycol as fibrillating aid with otherwise similar conditions resulted in much lower glucose yields (Yoo et al., 2011).

With a twin-screw extruder, wood flour mixed with either ethylene glycol, dimethyl sulfoxide, or glycol was pretreated. The additives were chosen as they are known to have good affinity with cellulose and prevent reaggregation of liberated fibrils. Of the tested temperatures and additives, best cellulose to glucose conversion of 62% (w/w) was attained with ethylene glycol at 40 °C. In respect to unpretreated wood flour, conversion could be increased six-fold. Prior to enzymatic hydrolysis, additives were removed by extraction (Lee et al., 2009).

By expanding a twin-screw extruder with feeders and discharge pumps, sequential treatment of biomass within one extruder is possible. In the first section of an extruder, corn stover and water were combined and heated to 210 °C. Through autohydrolysis, hemicellulose was depolymerized. At the end of the first section, so-called first-stage liquor was withdrawn, which contained up to 65% (w/w) of the xylose present in the feed. In the second section, temperature is increased to 220 °C and aqueous sodium hydroxide is added to delignify the substrate. At the end of the extruder, second-stage liquid is collected which contains most of the lignin in a low molecular weight form. The remaining solid fraction mostly comprises of cellulose with around 2% (w/w) residual lignin. After hydrolysis, 80% (w/w) glucose could be recovered therefrom (Kadam et al., 2008). Elsewhere, screw extrusion was coupled with steam explosion. Along the extruder, the substrate was compressed and heated so that a pressure of 1.5 MPa and 150 °C were reached at the die. The latter had a 1 mm ring slit through which the substrate was expanded and steam explosion occurred. Crushed eucalyptus was watered down to a moisture content of 50% (w/w) and used as substrate. After treatment, the fibers were examined regarding morphology and chemical structure. Even though, enzymatic hydrolysis is performed, no definite recovery rates are stated (Liang et al., 2016).

Except for one presented technique, where cellulose is collected as wet solid, aqueous slurries of oligosaccharides are obtained after pretreatment. These pretreatment liquors could directly be used for subsequent hydrolysis and especially microbial fermentation. However, in some cases the presence of inhibitors might affect further process performance. While high shares of lignin are known to decrease enzymatic activity, inhibitors like furanic or phenylic compounds could cease microbial activity altogether (Jönsson and Martín, 2016). For this reason, pretreatment liquors must be examined carefully and possibly detoxified prior to hydrolysis and fermentation (Canilha et al., 2012).

In a continuously operated twin-screw extruder, pretreatment and enzymatic hydrolysis was coupled and tested on several lignocellulosic substrates. In the first module of the extruder, alkaline pretreatment was carried out with NaOH solution. Thereafter, $H_3PO_4$ was used to neutralize the substrate. In the final stage, an enzyme preparation was added to release glucose from cellulose. The slurry was subsequently diluted with water and transferred to a stirred tank reactor. Retention time in the stirred tank reactor was between 16 h and 24 h. Highest glucose yield of 84% (w/w) was reported for sweet corn as substrate. In this case, the flow rate was maintained at 33 kg $h^{-1}$, screwing speed was 200 rpm, and the highest temperature in the extruder was 100 °C with pressures of up to 1.7 bar (Vandenbossche et al., 2016).

## 3. Hydrolysis

In the preceding section, enzymatic hydrolysis is considered an analytical parameter showing the highest possible sugar recovery related to each pretreatment. In the overall continuous scheme, hydrolysis would have to be carried out continuously, too. Through hydrolysis, a versatile carbon-rich hydrolysate is generated, which provides carbon sources to fermenting organisms (Abdel-Rahman et al., 2011; Akhtar et al., 2014).

Cellulose hydrolysis requires three different enzymes. Endoglucanases hydrolyze internal bonds of the cellulose polymer, releasing oligomeric sections. With help of exoglucanases, these oligomers are then cleaved to yield cellobiose. Finally, the reaction of cellobiose to two glucose molecules is catalyzed by β-glucosidases. In order to fully hydrolyze cellulose to glucose, a mixture of all three is needed. Especially cellulolytic enzymes acquired from the fungus *Trichoderma reesei* are often used (Rana et al., 2014). Unfortunately, both glucose and cellobiose are inhibitory to cellulases, thusly impeding generation of glucose-rich hydrolysates in one pot (Andrić et al., 2010). This problem could be tackled by removing glucose from the hydrolysate while retaining enzymes and polysaccharides (e.g., by means of membrane filtration). Another option is to work with diluted solutions, which are concentrated after hydrolysis.

With pretreated corn stover, continuous enzymatic hydrolysis was carried out. To a stirred tank reactor, biomass slurry and enzymes with buffer were continuously fed. Reactor volume was kept constant by withdrawing hydrolysate at the top of the desired fill level. Hydrolysate was also pumped in a loop passing through a tubular ultrafiltration membrane, from which a stream of clarified sugar permeate was collected at a set flow rate. Due to cake building and fouling, transmembrane pressure increased over time. For this reason, two membranes were installed in parallel, flow was switched between them periodically, and the offline membrane was cleaned. Based on experimental results, a model is presented, where two reactors are operated in sequence. With a permeate flow rate of 5.7 mL $min^{-1}$, an enzyme loading of 20 mg $g^{-1}$ biomass, the outlet glucose concentration is predicted to amount to 28 g $L^{-1}$ at a maximal productivity of 3.8 g $L^{-1}$ $h^{-1}$ (Stickel et al., 2018).

Instead of separating a membrane module, placement at the bottom of the reactor is also feasible. Experiments with such a submerged membrane reactor have been conducted utilizing pure cellulose as substrate. Advantageous is the redundancy of an external loop. Deposition of substrate and enzyme is reduced by rapid mixing, thusly hindering fouling and cake formation. Permeate withdrawal and flow rate of buffered biomass and enzyme feed were kept constant so that the volume of the solution remained unaltered throughout the experiment. At a permeate flow of 2 mL $min^{-1}$, cellulose concentration of 25 g $L^{-1}$ in the feed, and 10 mg $L^{-1}$ enzyme, glucose concentration of around 20 g $L^{-1}$ could be maintained at steady-state. The authors point out that adaption of this process for actual pretreated biomass would require careful lignin removal prior to hydrolysis and intermittent discharge of accumulated solids from the reactor (Malmali et al., 2015).

For the purpose of reducing enzyme load, countercurrent saccharification of pretreated corn stover was investigated. A set of 16 bottles was filled with diluted pretreated biomass (100 g $L^{-1}$) and every 48 h, solids and liquids were separated by centrifugation. While liquids where transferred from bottle 16 to bottle 1, solids where transferred countercurrently. Enzymes at a loading of 2 mg $g^{-1}$ biomass were added in bottle 4. In the final liquid fraction, sugar concentrations amounted to 55 g $L^{-1}$ glucose and 20 g $L^{-1}$ xylose, corresponding to yields of 67% (w/w) and 53% (w/w), respectively (Lonkar et al., 2017).

It was demonstrated that purified cellulose could be continuously hydrolyzed in a tubular membrane reactor. During a batch start-up phase, enzymes and pre-hydrolyzed cellulose were allowed to attach to the membrane surface. After reaching a glucose conversion of 50%, 2.5% (w/v) cellulose solution was fed to the reactor while permeate was withdrawn through the porous membrane-covered tube. With one load of enzymes and a flux of 6.6 L $m^{-2}$ $h^{-1}$, 50% (w/w) glucose conversion could be maintained for another 25 h after batch operation (Bélafi-Bakó et al., 2006).

## 4. Fermentation

Having obtained a hydrolysate with high concentrations of carbon-sources, fermentation to a great number of products is feasible. Depending on the fermenting organism, sugars present in the hydrolysate, even xylose, mannose or arabinose, could be utilized (Nogué and Karhumaa, 2015). The possibility to release sugars from lignocellulosic sources has resulted in continuous processes for the production of 2nd generation biogas and/or ethanol (Brethauer and Wyman, 2010; Huang et al., 2017). Similarly, sugars can also be used to continuously produce 2nd generation materials and chemicals, as shown in the following section.

### 4.1. Lactic acid

Lactic acid is a versatile chemical with application as food additive, pharmaceutical agent or commodity chemical for the production of polylactic acid. Three different *Lactobacillus* species were used to ferment hydrolyzed steam exploded crushed corn cobs. As the hydrolysate was poor in nutrients, yeast extract and minerals were added as supplement prior to fermentation. The reactor consisted of a continuous flow glass vessel equipped with a separate loop incorporating an ultrafiltration module. The module allowed lactic acid and sugars to pass through while cells and biomass were recycled to the reactor. With 120 g $L^{-1}$ glucose in the hydrolysate feed, up to 90 g $L^{-1}$ lactic acid were found in the permeate at a dilution rate of 0.2 $h^{-1}$ (Melzoch et al., 1997). The amount of yeast extract needed to supply nitrogen was reduced in a different study by replacing part of it with corn steep liquor. Wood chip hydrolysate served as carbon source for a *Lactobacillus* species. Continuous fermentation was carried out both with and without an ultrafiltration module for separation of metabolites and cells. While lactic acid yield and substrate conversion were highest for low dilution rates, productivity peaked at 6.7 g $L^{-1}$ $h^{-1}$ with 0.16 $h^{-1}$ dilution rate when cell recycle was included. Although lactic acid yield was comparable without cell recycling, substrate conversion was substantially lower, reaching 36% (w/w) compared to 59% (w/w) in the aforementioned case (Wee and Ryu, 2009).

*Bacillus coagulans*, a thermophilic microorganism was used in the lactic acid fermentation of corn stover hydrolysate. Yeast extract was added to the hydrolysate as nitrogen source. Cell recycling was facilitated with a hollow fiber module. After a batch start-up phase, dilution rates from 0.05 $h^{-1}$ to 0.30 $h^{-1}$ were tested. Mixed sugar concentration in the hydrolysate feed was adjusted to 100 g $L^{-1}$ and during continuous fermentation at 0.15 $h^{-1}$ dilution rate, both glucose and xylose were almost completely metabolized to lactic acid giving a yield of 91% (w/w), productivity of 13.8 g $L^{-1}$ $h^{-1}$, and titer of 92 g $L^{-1}$ (Ma et al., 2016). In another study with *B. coagulans*, wheat straw hydrolysate was utilized as carbon source. Again, yeast extract and minerals were added to the solution. In the continuous reactor set-up, a microfiltration module was included to retain cells. During the 17 h of continuous operation at a dilution rate of 0.127 $h^{-1}$, productivity and titer were 9.2 g $L^{-1}$ $h^{-1}$ and 72.8 g $L^{-1}$. This number corresponds to a carbohydrate utilization of 75% (w/w). The authors report that switching from artificial sugar mixtures, which were used in the start-up phase, to the actual hydrolysate resulted in clogging of the membranes due to higher viscosity. This obstacle was overcome by using five membranes in parallel and increasing the internal diameter of the modules from 1.5 mm to 3 mm (Van Hecke et al., 2017).

Pretreated and hydrolyzed corn cob residues, wheat bran hydrolysate, yeast extract, and minerals were used as medium in a continuous fermentation with a *Lactobacillus delbrueckii* strain. The cells were

immobilized in gel beads first and thereafter added to a column reactor. The medium flowed from the bottom to the top of the reactor with dilution rates ranging from 0.08 $h^{-1}$ to 0.20 $h^{-1}$. The optimal balance of dilution rate, productivity, and yield is reported as 0.13 $h^{-1}$, 5.746 g $L^{-1}$ $h^{-1}$, and 92% (w/w) (Shen and Xia, 2006). It was shown, that corn stover hydrolysate could also be fermented with corn steep liquor as single supplement utilizing *B. coagulans*. At a dilution rate of 0.167 $h^{-1}$, productivity amounted to 3.69 g $L^{-1}$ $h^{-1}$ (Ahring et al., 2016). Another possible nitrogen supplement for fermentations is lees. In combination with hydrolyzed vine shoots, two waste streams from vine industry could be employed for lactic acid production. At a dilution rate of 0.058 $h^{-1}$, a concentration of 21.8 g $L^{-1}$ lactic acid could be maintained in the reactor with *Lactobacillus pentosus* as fermenting organism. Accordingly, productivity was 1.26 g $L^{-1}$ $h^{-1}$. Yield in respect to both xylose and glucose was 70% (w/w). Part of this mediocre conversion must be attributed to the generation of a significant share of acetic acid, which was present in the broth at 9.8 g $L^{-1}$ (Bustos et al., 2007).

Peinemann et al. (2019) recently presented a simultaneous hydrolysis of food waste and lactic acid fermentation approach (Peinemann et al., 2019). The focus was on a continuous fermentation of undiluted and non-sterile canteen food waste with the aim to establish a process, which is as simple as anaerobic digestion. Their work revealed that due to the high viscosity and the presence of particles and fibers, pumping of the food waste slurry is a tedious task especially at high solids loading. Nonetheless, food waste was mechanically pretreated and used without further dilution for lactic acid fermentation. Hydrolysis was carried out by addition of amylase to the fermenter. Continuous operation was carried out for more than 148 h giving conversions of sugars (including starch) to lactic acid of more than 93% (w/w) (Peinemann et al., 2019).

Classical downstream processing techniques for the purification of organic acids and in particular shown for lactic acid are: Filtration, ultrafiltration, mono- and bipolar electrodialysis, cation- and anion exchange chromatography, and distillation (Olszewska-Widdrat et al., 2019). Those techniques can be carried continuously allowing the purification of a continuous appearing fermentation broth. Other studies describe the use of solvents or ionic liquids to separate organic acids from fermentation broths (Li et al., 2016). A continuous extraction of lactic acid from fermentation broths was shown using ethyl acetate under ultrasonication (Hu et al., 2017). Using this approach an overall yield of 82–84% (w/w) and a purity of around 98% were achieved. Solvent extraction seems therefore superior in terms of process steps compared to the techniques used by Olszewska-Widdrat et al. (2019).

### 4.2. Succinic acid

Succinic acid is a dicarboxylic acid with large potential as platform chemical (e.g., for the production of plastics). Hydrolysate obtained from deacetylated dilute acid pretreated corn stover was used alongside corn steep liquor as nitrogen source and carbon dioxide aeration in a continuous set-up. Cells of *Actinobacillus succinogenes* were immobilized on polypropylene and fermented the xylose enriched hydrolysate at maximum productivities of 1.77 g $L^{-1}$ $h^{-1}$ with a titer of 39.6 g $L^{-1}$ and 78% (w/w) sugar conversion. It was observed that concentrations of the fermentation inhibitors furfural and hydroxymethyl furfural were reduced during the course of continuous fermentations. This was interpreted as a result of strain adaption leading to tolerance and ultimately utilization as carbon source (Bradfield et al., 2015). Also applying an immobilized *A. succinogenes* strain, succinic acid was produced. Oil palm frond hydrolysate served as carbon supplier, while tryptone and minerals were added as nutrient source. Using the oil palm frond hydrolysate bagasse 0.69 g $g^{-1}$ succinic acid could be produced. Succinic acid titer peaked at 33.9 g $L^{-1}$ when dilution rate was set to 0.03 $h^{-1}$, the lowest used throughout the experiments. On the other hand, productivity amounted to 6.58 g $L^{-1}$ $h^{-1}$ with a titer of 17.5 g $l^{-1}$ when dilution rate was 0.4 $h^{-1}$ (Luthfi et al., 2019). Succinic acid production was also targeted with wood chip hydrolysate as substrate. As nitrogen source, yeast extract was added. Addition of $Na_2CO_3$ ensured high $CO_2$ concentration in the medium. Despite the organism *Mannheimia succiniproducens* fermenting xylose alongside glucose, yields of only 55% (w/w) were reached when setting dilution rate to 0.4 $h^{-1}$. Under these conditions, succinic acid concentration was 7.98 g $L^{-1}$ and productivity 3.19 g $L^{-1}$ $h^{-1}$. The authors report that this productivity was 2.72-fold what was obtained in batch fermentations (Kim et al., 2004).

A potentially continuously operating process for the separation of succinic acid from fermentation broth has been presented by Pateraki et al. (2019). This approach is based on membrane electrolysis where succinic acid precipitates at the anode and can be separated. This attempt, however, has not been tested in continuous mode (Pateraki et al., 2019). Generally, the process for succinic acid separation is similar to the separation of lactic acid. Following filtration and electrodialysis, succinic acid can be extracted using commercially extractants. Proschka et al. (2018) used a 3-step reactive extraction and the extractants Cyanex 923 and TOA. At a pH of 2 the efficiency was 100% and 51% for Cyanex 923 and TOA, respectively. By decreasing pH to 2 and using Cyanex 923 the authors achieved a complete separation of succinic acid from acetic and lactic acids, and glycerol. The continuous extraction might be carried out with solvents like t-butanol, 1-propanol, 2-propanol, and ethanol. The extraction, however, depends on various parameters, such as pH and salt concentration. Finding the appropriate conditions and using 1-propanol up to 72% of succinic acid can recovered (Pratiwi et al., 2015).

## 5. Continuous pretreatment, hydrolysis, fermentation, and downstream processing – proposed scheme

In view of the presented studies, a scheme for the conversion of corn stover to lactic acid is proposed and a mass balance is prepared (see Fig. 2). Corn stover, as lignocellulosic material, requires pretreatment and hydrolysis in order to make nutrients available for microorganisms.

Using a twin-screw extruder, as proposed by Kadam et al. (2008), 85% (w/w) of the cellulose present in corn stover could be recovered after treatment as wet solid. Besides cellulose, a stream rich in xylose and one rich in lignin is obtained. Subsequently, cellulose could be diluted to 25 g $L^{-1}$ and fed to a submerged membrane reactor alongside 10 mg $L^{-1}$ enzyme preparation containing α-cellulase and CTec2 enzyme. At a dilution rate of 0.1 $h^{-1}$, permeate with 20 g $L^{-1}$ glucose could be withdrawn (Malmali et al., 2015). By concentrating the hydrolysate to about 2-fold the glucose content, the solution would closely resemble what was used for fermentation with *B. coagulans* for lactic acid production.

Considering the set-up of Ahring et al. (2016), supplementation with 2% (w/w) corn steep liquor would ensure sufficient nitrogen supply. Furthermore, pH of 6.0, temperature of 50 °C, and a dilution rate of 0.167 $h^{-1}$ would deliver optimal results. In the end, a titer of around 38 g $L^{-1}$ could be maintained with acetic acid amounting to 5 g $L^{-1}$. Following this route, 100 t dry corn stover would yield around 22 t lactic acid. This number refers to a fermentation broth with 38 g $L^{-1}$ lactic acid.

When aiming at pure lactic acid solution, downstream processing must be conducted. It was shown that yields of 82% (w/w) are feasible by applying continuous ultrasonic-mediated solvent extraction (Hu et al., 2017). In this case, 18 t lactic acid with a purity of 98% would be generated. In respect to 100 t starting material with 35 t cellulose, 0.18 t lactic acid $t^{-1}$ biomass or 0.51 t lactic acid $t^{-1}$ cellulose could be produced continuously. It must be mentioned that the suggested scheme is of theoretical nature and has not been proven experimentally. It is imaginable that scale-up of the operation leads to compositional changes in the streams which in return might complicate fermentation.

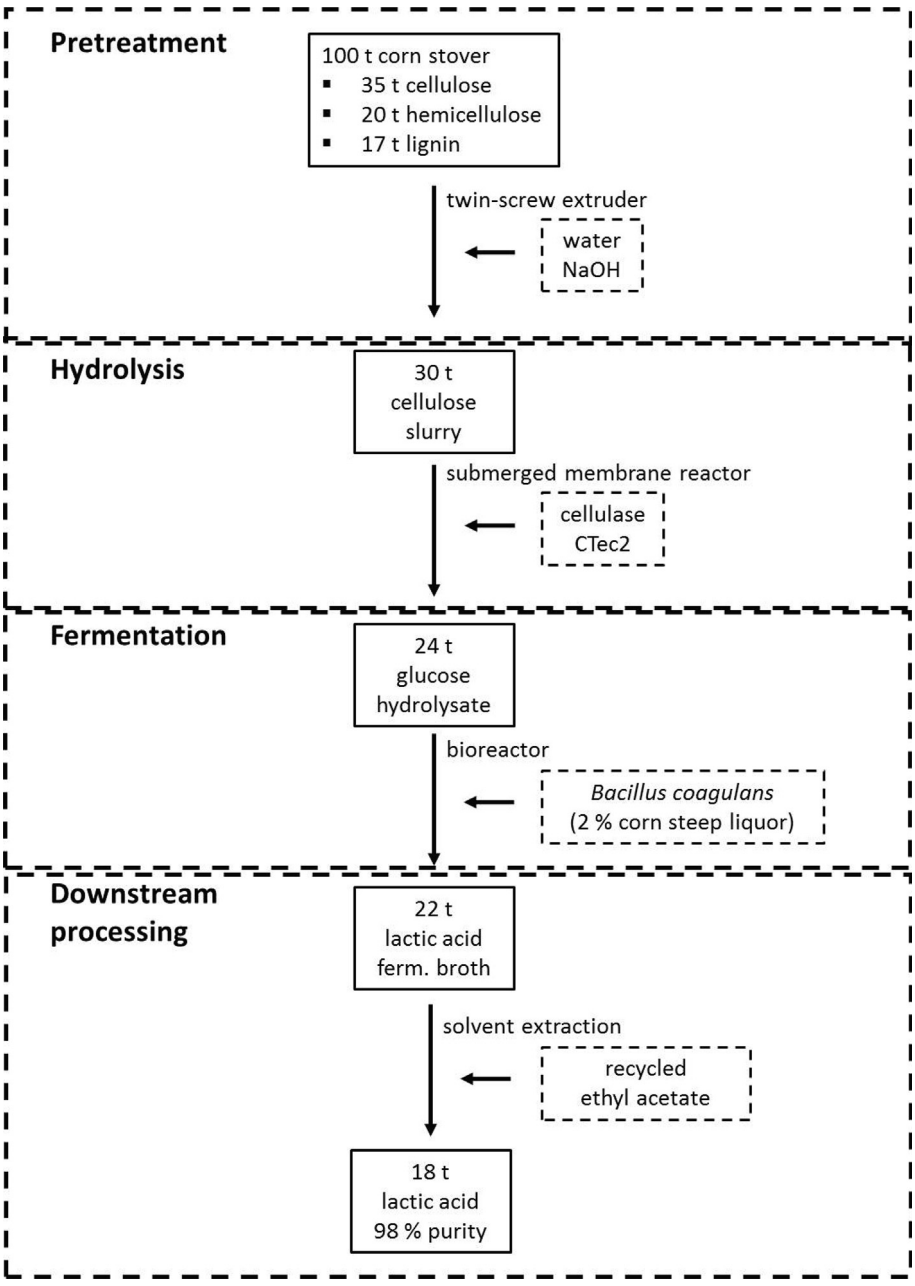


**Fig. 2.** Mass balance of a throughout continuous process for the production of lactic acid from corn stover.

Additionally, an entire continuous process would require feed rates of the individual steps – pretreatment, hydrolysis, and fermentation – to be adjusted to each other. This could not be factored in during this consideration as studies were performed separately. It should also be admitted that the proposed scheme would theoretically also work to produce succinic acid. The fermentation part and particularly environmental conditions and nutrient concentrations, however, must be adjusted to the requirements of microbes used for succinic acid production.

## 6. Conclusion

In this review it was presented how organic residues could be utilized materially in a continuous manner via pretreatment, hydrolysis, and fermentation as well as downstream processing. Exemplarily, the continuous conversion of corn stover to lactic acid was shown. It is of interest that a throughout continuous process from pretreatment of substrate to pure product is only available for a limited number of biochemicals and substrates. More continuous processes should be developed to further develop utilization approaches. Bioeconomy could largely benefit from possessing such continuous treatment techniques in order to produce biochemicals in a sustainable way.

## Declaration of Competing Interest

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

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