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Impact of Dispersing on the Production and Extraction of Bio-Based Basic Chemicals from Biogenic Secondary Waste

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***Abstract* – Biogenic waste from waste treatment plants, secondary waste, can be used to produce bio-based carboxylic acids, conventionally produced by chemical synthesis from petroleum-based feedstocks or by synthesis from natural oils. The cascading use of organic residues and wastes to produce bio-based products can contribute to the circular bioeconomy. In the process of biologically treating waste to produce bio-based carboxylic acids, microorganisms already present in the secondary waste use ethanol to convert short-chain into medium-chain carboxylic acids. The medium-chain carboxylic acids were separated from the secondary waste using in-situ extraction (liquid-liquid extraction). In previous studies, laboratory-scale bioreactors without a dispersing function were used. To optimise production and extraction of medium-chain carboxylic acids, the bioreactors were equipped with a device to disperse the extraction solvent in the secondary waste. By increasing the surface area between the phases, the ability to extract the medium-chain carboxylic acids was improved. The study aimed to evaluate the impact of this dispersing process. Production and extraction rates of static bioreactors without dispersing were compared to those of dynamic bioreactors with dispersing on a laboratory scale, using leachate from a composting plant as secondary waste. The results confirmed that dispersion has a positive effect on the process. Dispersing increased the reduction of the nutrient ethanol, the production of medium-chain carboxylic acids in the secondary waste and the extracted medium-chain carboxylic acids.**

***Keywords* – Bioeconomy; carboxylic acids; circular economy; composting plant; fermentative extraction; leachate; liquid-liquid extraction.**

1. INTRODUCTION

As fossil resources and raw materials are limited, the cascading use of organic waste as a resource for the production of new materials and products is becoming increasingly important in the future [1]. The use of raw materials including biomass, fossil fuels, metals and non-metallic minerals will double by 2060, as predicted by the Organisation for Economic Co-operation and Development [2], [3]. This implies that a transformation from a linear economy to a circular bioeconomy is inevitable. Stegmann *et al*. [4] defined the combination of circular economy and bioeconomy, circular bioeconomy, as follows: "The circular bioeconomy focuses on the sustainable, resource-efficient valorization of biomass in integrated, multi-output production chains (e.g., biorefineries) while also making use of residues and wastes and optimizing the value of biomass over time via cascading" (cf. [5]).

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Circular bioeconomy can be achieved by using unused secondary waste to produce bio-based basic chemicals, such as carboxylic acids. Secondary waste results from waste treatment processes, for example residues from recovery and disposal processes [6]. If these biogenic secondary wastes contain organic compounds with one or more carboxyl groups (-COOH), they can be used to produce higher-value medium-chain carboxylic acids [7]. Carboxylic acids are classified according to the number of carbon atoms: short-chain (1–3 carbon atoms), medium-chain (4–10 carbon atoms) and long-chain (>10 carbon atoms) [8]. Conventionally, carboxylic acids are produced by chemical synthesis from petroleum-based resources or by synthesis from natural oils such as coconut or palm kernel oil [9] (cf. [5]).

Specialised microorganisms are able to convert existing short-chain carboxylic acids into medium-chain carboxylic acids by using carbon atoms from the additive ethanol. The medium-chain carboxylic acids that are produced during the treatment process are separated from the secondary waste by an in-situ extraction process (liquid-liquid extraction) using an extraction solvent. After further treatment processes, these medium-chain carboxylic acids can be used as precursors for the production of lubricants and surfactants [10], [11].

This treatment process of secondary waste can be raised one level in the waste hierarchy by using secondary waste that would otherwise end up in a disposal process. Thus, the treatment process could be classified as material recovery in accordance with Article 4 number 1c) of the Waste Framework Directive (Directive 2008/98/EC) (cf. [5]).

The production of bio-based carboxylic acids from secondary waste has previously been analysed in static bioreactors. However, separation of medium-chain carboxylic acids from secondary waste has been limited by the interphase between extraction solvent and secondary waste due to usage of a non-polar liquid-liquid extraction solvent. To enhance the treatment process, it is necessary to increase the interphase surface for the transfer of carboxylic acids. Therefore, a suitable agitator should be used to disperse the extraction solvent in the secondary waste. It is essential that the stirrer construction avoids formation of emulsions, which would complicate separation of carboxylic acids (cf. [5]).

The impact of dispersion on production and extraction of bio-based chemicals was analysed by comparing the results of static and dynamic bioreactors (cf. [5]).

2. Material and Methods

2.1. Materials

The secondary waste used in this experiment was leachate from composting plants, which already contains carboxylic acids from the composting process. It was assumed that bacterial cultures were already present in the leachate, so no additional cultures were necessary (cf. [5]).

The microorganisms use ethanol to elongate the carboxylic acids that are already present in the waste. An amount of 9 g/l Ethanol was added to the secondary waste, since Sarkar et al. [12] achieved the highest formation rates of caproic acid (C6) during chain elongation by adding this concentration (cf. [5]).

To separate the carboxylic acids produced in the process, methyl oleate (SysKem Chemie GmbH; fatty acid methyl ester C16 - C18) was used as an extraction solvent for an in-situ liquid-liquid extraction at a volume ratio of 1:10 (cf. [5]).

2.2. Experimental Setup

2.2.1. Bioreactor Setup

The aim of the analysis was to test the effect of the dispersion on the production and extraction of medium-chain carboxylic acids. Therefore, results from static and dynamic bioreactors were compared. The only difference between the two types of bioreactors was the dispersing function. Both types have a batch-operated system and an operating volume of 14 litres. To ensure the required anaerobic conditions, they are airtight. Furthermore, the bioreactors were operated in a temperature-controlled room. In-situ liquid-liquid extraction was used to separate the produced medium-chain carboxylic acids from the secondary waste (cf. [5]).

Sampling devices were installed in the bioreactors. A mixed sample of the secondary waste was generated by using two sampling devices: one in the upper area (sampling device secondary waste 1) and one in the lower area (sampling device secondary waste 2). A third sampling device was used to sample the extraction solvent, which was connected by a flexible tube to a floater (sampling device extraction solvent) in order to take samples at different filling levels, as the extraction solvent settles on the surface of the secondary waste due to its polarity. A valve in the lid of the bioreactor enables the produced biogas to be collected in a foil gas bag. The dynamic bioreactors were equipped with an agitator for dispersion, which includes a brushless DC motor (BPC Instruments AB) and a stirrer. The stirrer is a combination of a tailormade constructed and self-manufactured diagonal flat blade stirrer and an anchor stirrer [13], [14], with a rotation speed of approximately 70 RPM.

The described static bioreactor (left) and dynamic bioreactor (right) are illustrated in Fig. 1.

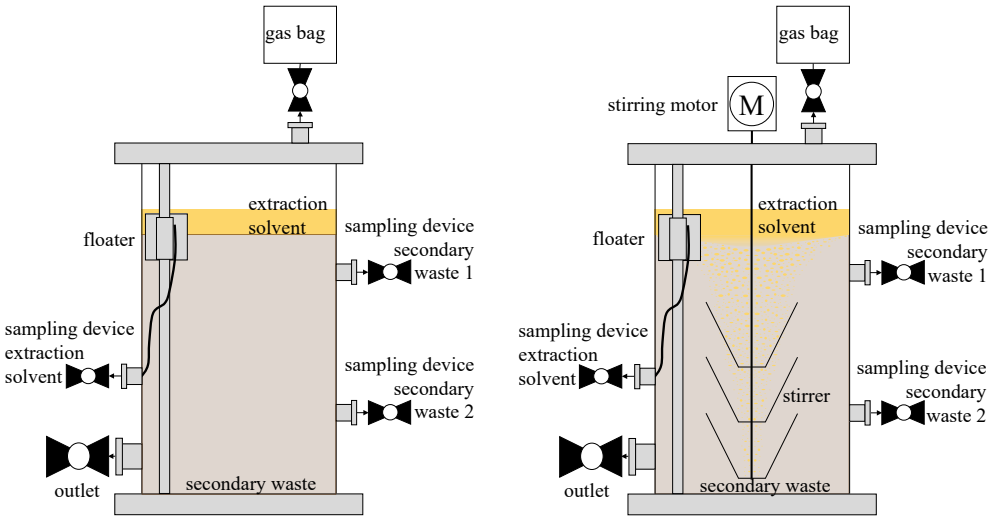

Fig. 1. Technical design of static bioreactor (left) and dynamic bioreactor (right) (cf. [5]).

2.2.2. Operating Parameters

The experiments were performed in four static and four dynamic bioreactors. Two of the static and two of the dynamic bioreactors were used for double determination of carboxylic acid chain elongation with integrated liquid-liquid extraction (CE-LLE 1 and CE-LLE 2). Each of these bioreactors was filled with 12 litres of secondary waste, 1.2 litres of extraction

solvent and 9 g/L of ethanol. To evaluate the impact of integrated liquid-liquid extraction on secondary waste without chain elongation, one static and one dynamic bioreactor were used (LLE). Each of these bioreactors was filled with 12 litres of secondary waste and 1.2 litres of extraction solvent. One of the static and one of the dynamic bioreactors was filled with only 12 litres of secondary waste as a blind reactor. Table 1 summarises the contents of the different bioreactors. CE represents chain extension, LLE represents liquid-liquid extraction, S represents static, and D represents dynamic (cf. [5]).

In order to have an optimal pH range between pH 6.0 and pH 6.5 for the microorganisms and to inhibit the possible formation of methane, the pH of the secondary waste was adjusted to approximately 6.0 by using hydrochloric acid (Carl Roth GmbH + Co. KG, c = 2.87 mol/L, technical) before starting the experiment (cf. [5]).

Additionally, the headspace of the bioreactors was purged with inert gas (nitrogen) to displace any oxygen and ensure anaerobic conditions in the bioreactor. The bioreactors were tempered at 36 ± 1 °C for the experimental period of 14 days (cf. [5]).

TABLE 1. COMPOSITION OF THE CONTENTS OF THE BIOREACTORS

Bioreactor	Secondary waste	Extraction solvent	Ethanol
CE+LLE 1-S / CE+LLE 1-D	12 L	1.2 L	9 g/L
CE+LLE 2-S / CE+LLE 2-D	12 L	1.2 L	9 g/L
LLE-S / LLE-D	12 L	1.2 L	–
Blind-S / Blind-D	12 L	–	–

Note: CE = chain elongation, LLE = liquid-liquid extraction, S = static, D = dynamic

2.2.3. Technical Sampling of Secondary Waste and Extraction Solvent

The secondary waste and extraction solvent were sampled daily during the first seven days of the experiment, and on the eleventh and fourteenth day. To ensure an accurate sampling of the extraction solvent, the agitators of the dynamic bioreactors were switched off one hour before sampling to ensure that all the extraction solvent settles on the surface of the secondary waste (cf. [5]).

First, 12 mL of extraction solvent was discarded from the bioreactors conducting liquid-liquid extraction, then another 10 mL of extraction solvent was sampled. The samples of extraction solvent were stored at room temperature until analysis. Then, secondary waste from each bioreactor was sampled, starting from the upper sampling device (sampling device secondary waste 1). After discarding 15 mL of secondary waste, another sample of 20 mL of secondary waste was taken. The procedure was repeated for the secondary waste sample from the lower sampling point (sampling device secondary waste 2). The two secondary waste samples were combined to prepare a composite sample. The on-site analysis of the secondary waste samples (as described in section 2.3) was carried out immediately after sampling. After the on-site analysis, the secondary waste samples were stored at approx. minus 20 °C (cf. [5]).

2.3. Analytical Methods

The on-site analysis includes all parameters that were determined immediately after the sample was taken, such as pH, redox potential, and conductivity. For this purpose, specific probes were used to determine these parameters (Xylem Analytics Germany Sales GmbH & Co. KG, WTW pH electrode SenTix® 41, WTW redox electrode SenTix® ORP, and WTW conductivity measuring cells TetraCon® 325). The pH and redox potential in the secondary waste indicated the necessary conditions for chain elongation for microorganisms. The

concentration of ethanol in the secondary waste was also analysed by gas chromatography with flame ionization detection (GC-FID, Shimadzu Deutschland GmbH, GC 2025) equipped with a TG-WAXMS A capillary column (Thermo Scientific, length: 30 m, inner diameter: 0.32 mm, film thickness 0.5 µm). Helium was used as the carrier gas at a flow rate of 30.3 mL/min. The secondary waste sample was injected into a split injector with a volume of 0.5 µL and a split ratio of 1:10. The column oven programme was as follows: heating up to 60 °C for 2 minutes, at a rate of 20 °C per minute to 80 °C for 2 minutes, at a rate of 10 °C per minutes to 100 °C and at a rate of 50 °C per minute to 235 °C. The secondary waste samples were diluted with ultrapure water (0.05 µS/cm) in ratios of 1:10 and 1:20, and filtered by using a PTFE syringe filter with a pore size of 0.45 µm.

For all samples collected during the experiments, the carboxylic acids in the secondary waste and in the extraction solvent were analysed after the experiments.

Carboxylic acids in secondary waste were analysed using high-pressure liquid chromatography (HPLC, Shimadzu Deutschland GmbH, 20A series) with a diode array detector (DAD, Shimadzu Deutschland GmbH, SPD-M20A) at 210 nm. A ZORBAX SB-Aq column (Agilent Technologies Inc., length: 150 mm, inner diameter: 3.0 mm, particle size: 3.5 µm) was used with a security guard cartridge AQ C18 (Phenomenex Ltd. Deutschland, length: 4 mm, inner diameter: 2.0 mm) at a column oven temperature of 40 °C. Solvent A, consisting of 0.2 % orthophosphoric acid (Carl Roth GmbH + Co. KG, 1 mol/L – 3N, volumetric standard solution) in ultrapure water (0.05 µS/cm), and solvent B, acetonitrile (Carl Roth GmbH + Co. KG, ROTISOLV® HPLC Gradient Grade), were used as mobile phases. The gradient was run at a flow rate of 0.8 L/min, starting with 100 % solvent A for 0–1.5 min, followed by 35 % solvent A for up to 14 min, 5 % solvent A for up to 15 min, 5 % solvent A for up to 16 min and 100 % solvent A for up to 20 min. The injection volume was 5 µL. The secondary waste samples were diluted with an acetonitrile-ultrapure water mixture (ratio 1:1) and filtered using a PTFE syringe filter with a pore size of 0.45 µm. Dilution ratios were 1:10 and 1:100.

Carboxylic acids in the extraction solvent were analysed using gas chromatography with flame ionization detection (GC-FID, Shimadzu Deutschland GmbH, GC 2025) equipped with a TG-WAXMS A capillary column (Thermo Fisher Scientific Inc., length: 30 m, inner diameter: 0.32 mm, film thickness 0.5 µm). Helium was used as the carrier gas at a flow rate of 29.1 mL/min. The extraction solvent sample was injected into a split injector with a volume of 0.5 µL and a split ratio of 1:10. The column oven programme was as follows: heating to 80 °C for 2 minutes, at a rate of 10 °C per minute to 180 °C and at a rate of 5 °C per minute to 235 °C for 10 minutes. The extraction solvent samples were filtered using a PTFE syringe filter with a pore size of 0.45 µm and then diluted 1:10 with n-hexane (Carl Roth GmbH + Co. KG, ROTISOLV® Pestilyse® plus ≥99 %) [15].

3. Results and Discussion

3.1. On-Site Analysis of Secondary Waste

The temperature of secondary waste in the reactors was continuously measured throughout the experiment. The temperature reached 34.4 °C approximately 24 hours after the start of the experiment and reached the required temperature of 36 °C between the fourth and fifth day of the experiment. (cf. [5]).

The changes in pH in the static and dynamic bioreactors over the experimental period are shown in Fig. 2 and Fig. 3. The pH was initially adjusted to approx. 6.0 before the start of the experiments, whereby both the static and the dynamic bioreactors started with a pH of approx.

6.65. It is assumed that this increase is based on buffering effect of the secondary waste. Throughout the experimental period, the pH decreased in both types of bioreactors which may indicate that the elongation process has taken place. At the end of the experiment, the pH inside the bioreactors with chain elongation and extraction (CE-LLE) was approx. 5.9 for the static bioreactors and approx. 6.0 for the dynamic bioreactors. Therefore, the pH inside the static bioreactors with chain elongation and extraction was slightly lower than that of the dynamic bioreactors. The pH of the material inside the reference bioreactors (LLE and blind) changed in a similar way in the static as well as in the dynamic bioreactors and reached a pH of around 6.2 at the end of the experimental period (cf. [5]).

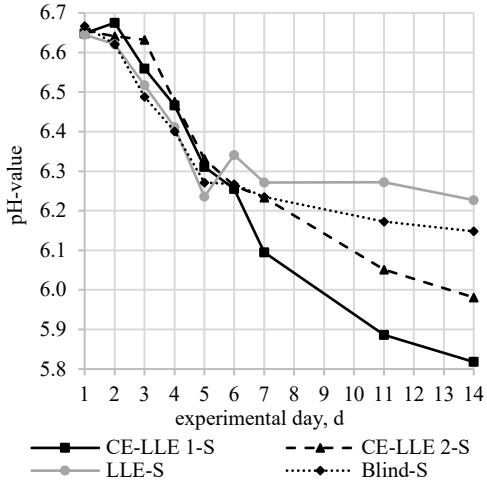

Fig. 2. pH value in static bioreactors (cf. [5]).

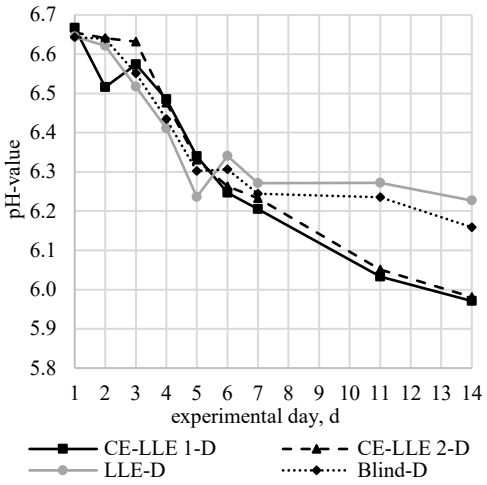

Fig. 3. pH value in dynamic bioreactors (cf. [5]).

It was not possible to use the redox potential to determine whether the milieu in the used secondary waste was anaerobic or aerobic. The high conductivity values which were measured may be the reason for this, according to the manufacturer of the redox potential measurement technology (Xylem Analytics Germany Sales GmbH & Co. KG). The conductivity values range between 32.6 °mS/cm and 35.8 °mS/cm (cf. [5]).

Fig. 4 and Fig. 5 show the reduction of the ethanol added in the static and dynamic bioreactors over the duration of the experiment. At the start of the experiments, 9000 mg/l of ethanol was fed to each of the CE-LLE bioreactors. The reference bioreactors (LLE and blind) did not receive any additional ethanol. It can be seen that both the static and the dynamic reference bioreactors contain about 1000 mg/L ethanol without addition of ethanol. During the experimental period, this concentration was decreased until the fourth day of the experiment. In the first few days of the experiment, a decrease of approximately 1000 mg/L ethanol was observed in the static and dynamic bioreactors with the addition of ethanol. The reduction of ethanol continued until experiment day four. A rapid reduction of ethanol follows. In the experiment, the concentration of ethanol in the dynamic bioreactors with chain elongation and liquid-liquid extraction was increased to 1000 mg/L on the seventh day. In comparison, the concentration of ethanol in the static bioreactors with chain elongation and liquid-liquid extraction was 2.5 times higher than in the dynamic bioreactor. The levels of ethanol were significantly reduced in the dynamic bioreactor with chain elongation and extraction, which led to concentrations of ethanol that could not be measured on day eleven of the experiment. In comparison, one of the static bioreactors with chain elongation and

liquid-liquid extraction contained about 1000 mg/L ethanol on day 14 of the experiment. In the second static bioreactor with chain elongation and extraction there was also no detectable concentration of ethanol on the 14th experimental day (cf. [5]).

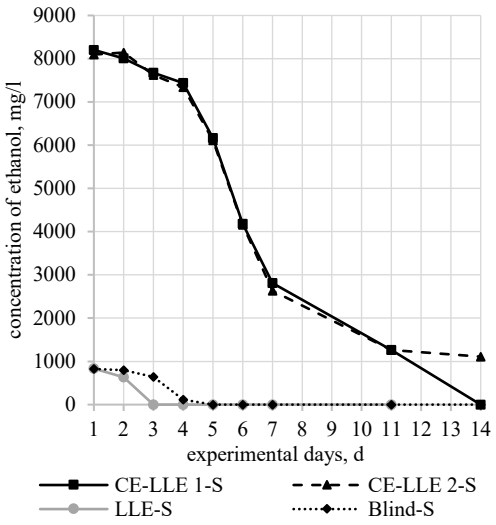

Fig. 4. Reduction of ethanol in secondary waste in static bioreactors [5].

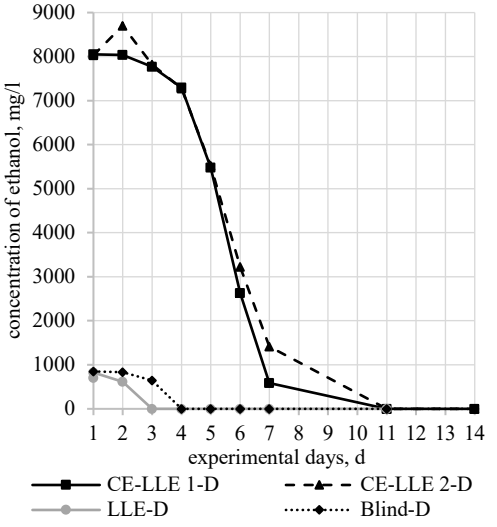

Fig. 5. Reduction of ethanol in secondary waste in dynamic bioreactors [5].

3.2. Impact of Dispersing on Production of Carboxylic Acids

The impact of dispersing on the production of medium-chained carboxylic acids was evaluated by taking the average of the double determinations and determine their relative change between the dynamic and static bioreactors. Fig. 6 summarises the relative changes for acetic acid (C2), propionic acid (C3), butyric acid (C4), valeric acid (C5), caproic acid (C6), enanthic acid (C7) and caprylic acid (C8) on the second, fourth and sixth day of the experiment. The positive results indicated the presence of more carboxylic acids in the secondary waste of the dynamic bioreactor, while the negative results indicated the presence of more carboxylic acids in the secondary waste of the static bioreactor.

Overall, it is noticeable that the dispersion has a positive impact on the production of carboxylic acids. The treatment process of secondary waste led to fluctuations of carboxylic acid concentrations during experiment duration. However, there was a significant trend towards an increase in carboxylic acids in the secondary waste treated by the dynamic bioreactor. On the second day of the experiment, the dynamic bioreactor contained approx. 6 % less acetic acid (C2) and a slight decrease of 0.5 % for enanthic acid (C7). By the fourth day, more carboxylic acids were produced and the dynamic bioreactor contained between 18 % more acetic acid (C2) and 36 % more enanthic acid (C7). By the sixth day of the experiment, the relative change in the dynamic bioreactor was decreasing. The secondary waste of the dynamic bioreactor contained 11 % more acetic acid (C2) but also 11 % less propionic acid (C3). This could be related to the fact that the short-chain carboxylic acids have already been converted to medium-chain carboxylic acids in the dynamic bioreactor, and thus transferred to the extraction solvent (see chapter 3.3). On the sixth day of the experiment, the secondary waste of the dynamic bioreactor contained up to 12 % more butyric

acid (C4), up to 7 % more valeric acid (C5), up to 19 % more caproic acid (C6), up to 26 % more enanthic acid, and up to 30 % more caprylic acid (C8).

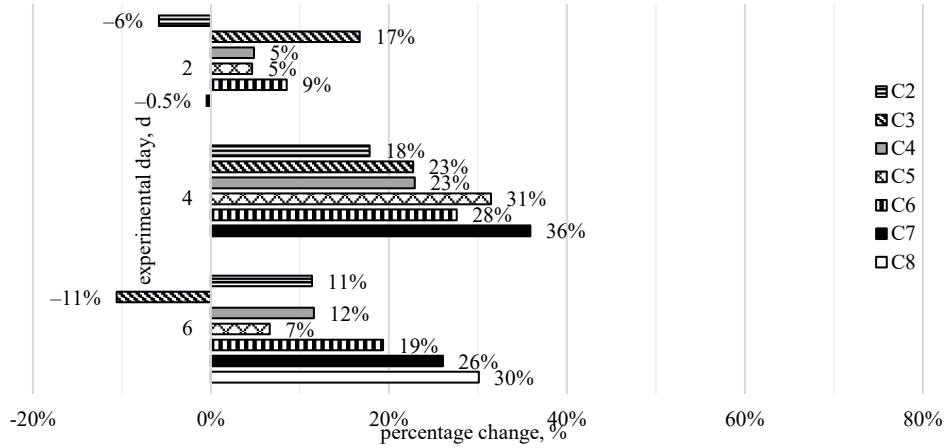

Fig. 6. Relative change of carboxylic acids in secondary waste from dynamic bioreactor to static bioreactor.

3.3. Impact of Dispersing on the Extraction of Carboxylic Acids

The impact dispersing on the extraction of the medium-chained carboxylic acids can be visualised by taking the average of the double determinations and presenting their relative change between the dynamic and static bioreactors. Fig. 7 summarises relative changes for butyric acid (C4), valeric acid (C5), caproic acid (C6), enanthic acid (C7) and caprylic acid (C8) for the second, fourth and sixth day of the experiment. Positive results indicate that more carboxylic acids were enriched in the extraction solvent of the dynamic bioreactor. Negative results indicate that more carboxylic acids were extracted in the extraction solvent of the static bioreactor. Furthermore. the results indicate that all carboxylic acids increase in the extraction solvent over the experiment period. Up to 15 % of butyric acid, 16 % of valeric acid, 21 % of caproic acid, 44 % of enanthic acid and 65 % of caprylic acid have been extracted on the sixth day of the experiment (cf. [5]).

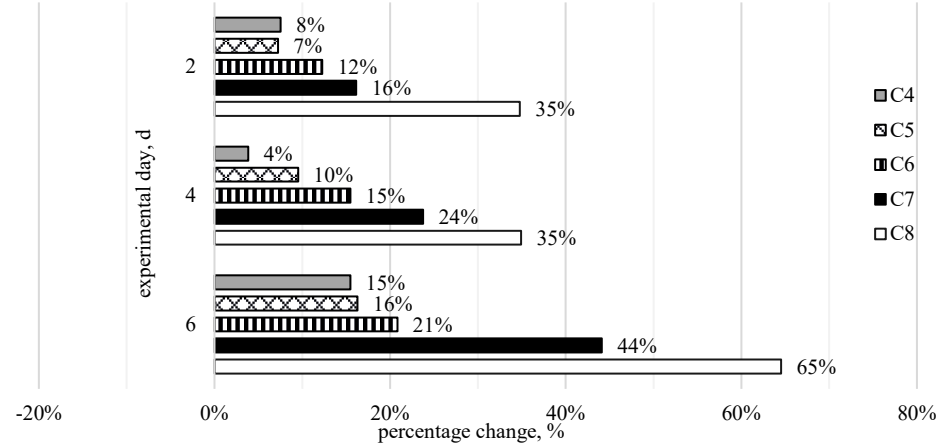

Fig. 7. Relative change of carboxylic acids in extraction solvent from dynamic bioreactor to static bioreactor (cf. [5]).

4. CONCLUSION

The impact of dispersion on the production and extraction of carboxylic acids in the leachate from a composting plant was evaluated by comparison of the results of the biological treatment process in bioreactors without dispersion (static bioreactor) with those in bioreactors with dispersion (dynamic bioreactor) (cf. [5]).

Based on the operating parameters, the experiments were successful. The microorganisms reduced the added ethanol over the experiment period and the required operating parameters, such as pH value and temperature, were achieved.

The results indicated that dispersion has a positive effect on the production of medium-chain carboxylic acids. Compared to non-dispersing bioreactors, dispersing bioreactors also showed higher extraction rates. As the number of carbon atoms in the carboxylic acids increased, more carboxylic acids were extracted in the dynamic bioreactors. For example, extraction of caproic acid (C6) increased by approximately 21 % and extraction of caprylic acid (C8) increased by approx. 65 %. Furthermore, the ethanol in the dynamic, dispersed bioreactors was completely reduced three experimental days earlier, resulting in a shorter experimental period (cf. [5]).

It should be noted that the results of the analysis are a snapshot and only valid at this exact time of sampling. In addition, it should be pointed out that further interaction within the bioreactor cannot be excluded and were not considered in the analyses.

In conclusion, it was verified that dispersion had a positive effect on production and extraction and thus the optimisation of the bioreactor was successful. Therefore, the optimised bioreactor will be used for further studies.

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