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Technical report on pilot scale testing of PHA production and extraction from industrial residual streams

Authors: Laumeyer, Cora[1]; Andrade Leal, Mithyzi[2]; Zimmer, Julia[1]; de Best, Jappe[2]; Steinmetz, Heidrun[1]

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[1] RPTU University of Kaiserslautern-Landau; Department of Resource Efficient Wastewater Technology

[2]Avans University of Applied Sciences, MNEXT

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COD	Chemical oxygen demand
COD_{sol}	Soluble chemical oxygen demand
COD_{tot}	Total chemical oxygen demand
COD_{VFA}	Chemical oxygen demand of the volatile fatty acids
dm	Dry matter
DMC	Dimethyl Carbonate
DSC	Differential Scanning Calorimetry
DSP	Downstream processing
FID	Flame ionisation detector
GC	Gas-chromatography
HB	Hydroxybutyrate
HH	Hydroxyhexanoate
HRT	Hydraulic retention time
HV	Hydroxyvalerate
IC	Ion-chromatography
NH_4-N	Ammonia nitrogen
OLR	Organic loading rate
PO_4-P	Ortho-phosphate phosphorus
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHH	Polyhydroxyhexanoate
PHV	Polyhydroxyvalerate
Tc	Crystallization temperature
Tg	Glass transition temperature
TGA	Thermogravimetric analysis
Tm	Melting point temperature
TNb	Total nitrogen, bound
TS	Total solids

TSS	Total suspended solids
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids

1. Introduction

It is known that sewage contains valuable resources that can be used as raw materials for biobased products. As described by Uhrig et al. (2022), this potential has hardly been exploited in its full potential in North-West-Europe. With the aim of a more sustainable and circular economy, these valuable materials shall be recovered, preventing CO_2-emissions and saving natural resources. During the first period of the Interreg North-West Europe project WOW! – Wider business Opportunities for raw materials from Wastewater (sewage), three different value chains for the recovery of carbon from municipal sewage have been explored. (1) the production of biodiesel, (2) the production of bio-oil, biochar and acetic acid and (3) the production of PHA (bioplastic). After the successful first phase of the project, the follow-up Interreg North-West Europe project WOW! Capitalisation widened the scope of the resource recovery for two of the value chains, switching from using municipal sewage to using sewage from the food industry in the case of PHA production.

One of the main activities in this project was the identification of suitable residual streams from the food industry for the production of polyhydroxyalkanoates (PHA). After a first screening of several different residual streams from the food industry presented in a previous report (Laumeyer et al. 2022), the PHA pilot plant used in the first phase of the project (Uhrig et al., 2022) was now operated with two different promising residual streams from the food industry for PHA production. The main objective was to produce enough PHA for the 3D-printing of demonstration products. This report focuses on the production of PHA from residual streams of the food industry and the extraction of the biopolymer from the dried biomass.

2. Operational objectives of the PHA-pilot at University Kaiserslautern-Landau (RPTU)

The focus of the pilot plant operation lied within the use of residual streams of the food industry and to obtain knowledge about the interrelation of the initial substrate's composition and its influence on (1) the composition of the VFA-rich substrate obtained by acidification, (2) on the PHA composition and (3) on the PHA content. Furthermore, material aid flows should be limited to ensure a sustainable production process and to minimize operational costs. Moreover, insights on operational strategies for a consistent PHA-production using residual streams from the food industry shall be deduced.

3. Objective of the PHA extraction at Avans University of Applied Sciences

The PHA produced by two promising residual streams were extracted using a green solvent for the purpose of application in 3D printing. The different materials were tested for composition, purity and characteristics based on industrial needs. Besides, the mass balance, extraction yield and observations were addressed to discuss the extracted material's characteristics and processability.

4. Materials and methods

The following chapter comprises the materials and methods that were applied during the pilot plant operation at the RPTU University Kaiserslautern-Landau, Germany (Chapter 4.1) as well as of the PHA extraction in the laboratories of Avans University of Applied Sciences in Breda, Netherlands (Chapter 4.2).

4.1 PHA-pilot installation and experimental focus

The pilot plant operation consisted of 7 process stages, as described by Uhrig et al. (2022) with minor adjustments:

1. Anaerobic acidogenic fermentation (V=1.000 L) of the residual stream to produce a VFA-rich substrate
2. Centrifuge for solid-liquid-separation of the VFA-rich soluble substrate and the suspended solids (anaerobic sludge)
3. VFA-storage tank (V=1.100 L) to feed the following stages for PHA-production
4. Biomass enrichment tank (V=510 L) for selecting biomass with high PHA-production capacities
5. PHA-accumulation tank (V=510 L) for producing biomass with a high intracellular PHA-content.
6. Centrifuge for dewatering the PHA enriched biomass.
7. Drying cabinet for drying the biomass at 80°C

The process stages 1 – 3 (acidification of the residual streams, solid/liquid-separation and storage) are described in chapter 4.1.1. Chapter 4.1.2 comprises the process stages 4 – 7 (biomass enrichment, PHA-accumulation, dewatering and drying of the PHA-rich biomass). The analytical methods applied are described in chapter 4.1.3.

4.1.1 Acidification of the residual streams, solid/liquid-separation and VFA-storage tank

After initial substrate screenings performed both at Avans and at RPTU in the beginning of 2022 described in Laumeyer et al. (2022), a total of two different residual streams were chosen for pilot plant operation, namely sewage of a brewery and a residual stream from a fruit juice factory.

The anaerobic acidogenic fermentation was operated as a batch reactor with a working volume of 1 m^3, filled with 100 L anaerobic sludge from the brewery's UASB as inoculum and 900 L of residual stream.

During the pilot operation a total of 20 acidification batches were successfully performed with two different residual streams. Hereof 11 acidifications were performed with the residual stream of a brewery and 9 batches were acidified using the residual stream of a fruit juice factory.

The reaction temperature was set to 35.5°C and maintained with a heating sleeve (PiT). The residual stream and inoculum were circulated within the reactor using two aquarium pumps (TUNZE Turbelle nanostream 6045) at a flow rate of ca. 4,500 L/h. The pH was monitored but not controlled during the acidification process. However, in the beginning of the process the pH was adjusted around 5.0 – 5.5 to prevent methane formation, using either HCl (35 %, standard of purity > 99 %, Carl Roth®) or NaOH depending on the substrate's initial pH. To prevent the contamination of the surrounding air with any occurring digester gases (H_2S, CH_4, etc.), off-gas was directed to a gas washer. Additionally, the exhaust air system consisting of a radial ventilator (C 30/4 T ATEX; Vortice) was still mounted in the pilot container, since it has been formerly operated using primary sludge with a minor formation of H_2S and used to prevent an H_2S accumulation in and around the pilot.

There were three different operation stages during the experiments:

(1) During the first weeks of the pilot plant operation with brewery sewage the reactor was operated as a semi-batch reactor, by withdrawing 90 % of the volume after a retention time of six days for an additional prevention of methanogenesis, while the remaining volume was used as inoculum for the next batch. After that, each acidification of the brewery's residual stream was inoculated with 10 % anaerobic sludge from the brewery's UASB.

(2) During the first five weeks of the operation with fruit juice sewage, the anaerobic fermentation was performed without an inoculum, while keeping the retention time at six days. During this operational phase a solid-liquid-separation was not necessary, as the solid content was negligible.
(3) Due to low VFA-conversion rates within the fruit juice sewage without an inoculum, the process was changed and operated as batch process as described in (1). The only difference being the full batch operation, using new inoculum from the brewery's UASB for every fermentation batch.

For solid-liquid-separation, the reactor was emptied using a submergible pump connected to a basket centrifuge (ZS21 EUR; Eurotec Innovation) with a 3.5 L basket and 2070 g to separate the VFA-rich liquid from the solids. The VFA-rich substrate was collected in the storage tank (V = 1,100 L), with a retention time of ca. seven days. The storage tank was connected to two peristaltic pumps (Verderflex Rapide R8, Verder) to feed the enrichment and PHA accumulation tanks. Uhrig et al. (2022) showed in their previous work, that the VFA concentration in the storage tank was stable and no loss of VFA occurred during the storage.

The experimental focus of the acidification of the different residual streams was to examine the composition of the substrates for the PHA enrichment and to gain an insight into the influence of the initial substrate components on the final VFA concentration and composition. Especially due to changing loads depending on the current production step within the food industry (processing, cleaning, packaging, etc.). As stated by Uhrig et al. (2022) the stability of the VFA composition as well as of the VFA concentration and the nutrient composition are key parameters when aiming for an upscaling of the process.

4.1.2 Biomass enrichment, PHA-accumulation, dewatering and drying

The biomass enrichment was operated with a working volume of 200 – 370 L, depending on the operation phase, filled with 150 – 185 L excess sludge from the brewery's sewage treatment plant. Due to logistical reasons the second fruit juice enrichment was inoculated with excess sludge from the sewage treatment plant Kaiserslautern.

Both the enrichment and accumulation reactors were equipped with a compressor unit (LA-120A, Nitto Kohki) and a fine-bubble membrane plate diffuser (OXYFLEX®; Supratec) for both aeration and mixing. Due to the high airflow an additional mixing unit was not necessary. The compressors were operated at full capacity to prevent an oxygen limitation during all reaction states, especially during the feast-phase and PHA accumulation (airflow up to 120 L/min). The pH was monitored within both reactors but not controlled and the reactors did not comprise a temperature control unit. However, during the winter months, especially in December, the heating in the pilot container was turned on, to prevent a temperature drop below 10°C within the reactors.

The enrichment stage was operated in a 12 h feast and famine regime. Every 12 h a VFA feed was pumped into the reactor from the VFA-storage tank using a peristaltic pump (Verderflex Rapide R8, Verder), introducing the feast-phase. During the first enrichment batch of the residual stream from the brewery, the VFA concentration of the feed determined the hydraulic retention time (HRT), as no water for dilution was added into the process to limit the external material flows. Due to the large variations within the VFA concentration of the substrate, the operation was adjusted to allow a consistent HRT while maintaining the desired organic loading rate (OLR) and tap water was used for dilution (pumped with Oxylift 2; Jung Pumpen). In the first batch, the pilot plant was operated as previously described by Uhrig et al. (2022), implementing a sedimentation phase before every second VFA feed of ca. 1 h to retain biomass. Therefore, after 12 h either the mixed liquor or the supernatant was pumped out (Oxylift 2; Jung Pumpen). Due to a loss of solids, the operational process was changed after the first enrichment batch, adding a sedimentation phase before every VFA feed. From then on, the HRT was set to 2 days with a constant OLR of 0.5 g COD_{VFA}/(L·d).

During the experiments with the residual stream from the brewery, no nutrients were added. However, during the first batch of enrichment using the residual stream from the fruit juice industry, a nutrient shortage prevented biomass growth within the enrichment reactor. Therefore, from the second enrichment batch onwards, nutrients in form of NH_4Cl and KH_2PO_4 were added to the dilution water, to match the need of the bacteria and provide a nutrient ratio of C:N:P of 100:5:1.

During the pilot operation a total of 6 biomass enrichments were successfully performed with two different residual streams. Hereof 3 enrichments with the acidified brewery substrate and 3 enrichments were performed using the acidified fruit juice substrate.

To meet the needs of the project's deliverable, two to three times a week the mixed liquor of the sedimented biomass was used for PHA accumulation. The accumulation was operated as a fed-batch reactor, as described by Uhrig et al. (2022), adding a VFA feed every 30 min over 24 h to the reactor (Verderflex Rapide R8; Verder). Every feed consisted of ca. 100 mg $COD_{VFA}/L_{starting\ volume}$. The 24 h duration of the accumulation was chosen for a better workability and to allow a comparison with the results of the laboratory experiments. At the end of the accumulation phase, the untreated mixed liquor was pumped with a submergible pump connected to a basket centrifuge (ZS21 EUR; Eurotec Innovation) with a 3.5 L basket and 2070 g to separate the PHA-rich biomass from the supernatant. The dewatered biomass was dried at 80°C for at least 48 h in a drying cabinet (U40; Memmert).

The experimental focus of the biomass enrichment and PHA accumulation was to study the influence of the variable VFA concentration on the process operation and to examine the influence of the changing VFA composition on (1) the amount of PHA produced and (2) the composition of the PHA. To operate the process as sustainable as possible, the pilot was operated without temperature- or pH control, and nutrients were only added to the enrichment stage when necessary. To gain more knowledge for a process understanding, samples for DNA analyses from the enrichment tank were taken regularly.

4.1.3 Analytical Methods applied at University Kaiserslautern-Landau (RPTU)

The analytical methods are in line with the previously applied methods of (Uhrig et al., 2022) and are specified in the following paragraphs for completeness.

4.1.3.1 Standard urban water management parameters

Standard urban water management parameters were measured either according to standard methods or with Hach cuvette tests. Table 4-1 summarizes the applied methods.

Table 4-1: Applied methods for determining standard urban water management parameters

Parameter	Method	Remarks
COD_{TOT}	Photometric quick test	Hach cuvettes; LCK 514
COD_{SOL}	Photometric quick test	Hach cuvettes; LCK 514
TOC	DIN EN 1484 (08/1997)	-
DOC	DIN EN 1484 (08/1997)	-
TN_b	DIN EN 12260	-
NH_4-N	Photometric quick test	Hach cuvettes; LCK 303 or 304
PO_4-P	Photometric quick test	Hach cuvettes; LCK 350
TSS and VSS	DIN 38 409- H 2-2	Black ribbon filter; 589/1, D = 100 mm, Fa. Whatman
TS	DIN EN 15934	-
VS	DIN EN 15935	-

To remove solids for the analyses of COD_{sol}, NH_4-N and PO_4-P samples were centrifuged for 10 min at 4500 g (SORWALL LYNX 600 380/400 V, Thermo Fisher Scientific™) and two-step filtrated through a folded filter (595½, D = 185 mm; Whatman) and a syringe filter (0.45 µm, Minisart, regenerated cellulose, D = 25 mm; Sartorius).

4.1.3.2 Volatile fatty acids

Before analysis, samples were prepared by centrifugation for 10 min at 4500 g (SORWALL LYNX 600 380/400 V, Thermo Fisher Scientific™) and a two-step filtration through a folded filter (595½, D = 185 mm; Whatman) and a syringe filter (0.45 µm, Minisart, regenerated cellulose, D = 25 mm; Sartorius). Afterwards, the pH of the samples was adjusted to a value of < 2 with sulfuric acid (95-97 %, EMSURE®, Merck KGaA) and they were subsequently frozen and stored at -20°C until further procession (van Loosdrecht et al. 2016). The measurement of the VFA concentration and -composition was done by ion chromatography (930 Compact IC Flex with Metrosep Organic Acids - 250/7.8 cation exchange column; Metrohm). The samples were analysed for formic-, lactic-, acetic-, propionic-, butyric-, iso-butyric-, valeric-, iso-valeric- and caproic-acid.

4.1.3.3 PHA

For evaluation of the PHA production, grab samples (3 x 45 mL per reactor) were taken directly after each accumulation batch, acidified with sulfuric acid (95-97 %, EMSURE®, Merck KGaA) to a pH < 2, to inactivate the microorganisms and prevent PHA degradation, and centrifuged at 4,500 g for 10 minutes (SORWALL

LYNX 600 380/400 V, Thermo Fisher Scientific™). The supernatant was discharged and the samples were washed with deionized water, before a second centrifugation. This washing step was repeated twice to remove the sulfuric acid and therefore prevent the subsequent analytical equipment from any damage. After the third and final washing, the supernatant was discarded and the samples were frozen at -20°C until freeze-dried (VaCo 5, ZIRBUS technology GmbH). PHA-content and polymer composition were determined at least in duplicates according to (Braunegg, Sonnleitner & Lafferty 1978) with some minor changes. Approximately 5 mg of the sample was weighed into a 9 mL centrifuge DURAN®-glass tube (16 x 100 mm, conical bottom, PTFE coated GL 18 cap, Rettberg) and mixed with 1 mL of a methanol solution (LiChrosolv®, Merck KGaA) containing 5 % sulfuric acid, 0.8 mL of chloroform (>99 %, Fisher Scientific) and 0.2 ml of benzoic acid (99.5 %, Acros Organics) dissolved in chloroform (5 mg/mL) as an internal standard. Subsequently, the samples were heated in a thermo shaker (MHR 23, Hettich) for 6 h at 100°C with continuous shaking at 450 rpm. After cooling to room temperature, 1 mL of 1 % NaCl solution (>99.5 %, Carl Roth GmbH + Co. KG, in ultrapure water) was added and samples were first shaken to promote phase separation and then centrifuged at 4,500 g for 5 min (Hermle Z 206). This was followed by extraction of the lower solvent phase using a 1 mL-syringe. The solvent phase was measured using a GC-FID (Agilent 8860 design; Agilent Technologies™) with a HP 5 column (30 m x 0.32 mm, 0.2 µm), an injection volume of 0.5 µL, a flow rate of 20 mL/min helium, a split ratio of 1:50 with nitrogen. The Detector temperature was set to 250°C with a heating rate of 10°C/min after an initial temperature holding phase of 60°C over 4 min. Evaluation was based on calibration using a PHBV standard (poly(3-hydroxybutyric aicd-co-3-hydroxyvaleric acid), 8 % PHV-content; Sigma-Aldrich Co.) as well as an PHBH standard (Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), 15.2 % PHH-content; Sigma-Aldrich Co.) and an internal benzoic acid standard (see above).

4.1.3.4 DNA

To gain more insight on the PHA production process and possible starting points for a process optimization, samples of the PHA-producing microbial community were taken regularly from the enrichment reactor of the pilot plant. As described in Uhrig et al. (2022), the samples were subsequently frozen and stored at -20°C. Before further processing, these samples were thawed, centrifuged for 10 min at 4,000 g (Varifuge 3.0, Sorvall LYNX 6000; Thermo Fischer) and the supernatant was discarded. Afterwards, total genomic DNA was extracted from each sample with the DNeasy PowerSoil Kit (QIAGEN). For this, the manufacturer's protocol was followed with one exception; instead of 0.25 g 1 ml of sample was used. After verifying the quality and quantity of extracted DNA by nanodrop measurement (Nanodrop™ 2000, Thermo Fisher Scientific™; Waltham), samples were sent to an external company for PCR (“polymerase chain reaction”) amplification and sequencing of the V3-V4 region of the bacterial 16S-rDNA via Illumina Miseq (Illumina Inc.). After quality control of the raw sequences and bioinformatic analysis using the DADA2 workflow (Callahan et al. 2016), the ASVs ("amplicon sequence variants") were taxonomically assigned using the Greengenes database (DeSantis et al. 2006). The following biostatistical analysis was done with RStudio (Version 4.1.0, RStudio Team, Boston, Massachusetts, USA).

4.2 PHA extraction and experimental focus

4.2.1 Sieving biomass

The received biomass from residual streams from Brewery and Fruit Juice was first sieved to determine the particle size distribution. A horizontal vibrating sieve shaker Retsch AS 200 comprising of six sieves with opening sizes of 1,000, 250, 180, 90, 63, 32 µm and a bottom pan (< 32 µm) was used.

A representative weighed sample from each material was poured into the top sieve with the largest screen opening size and 15-minute sieve shaking time and amplitude 3.0 mm/g was applied.

4.2.2 PHA extraction

A reflux extraction method was applied to the PHA-rich biomass accordingly to the protocol described in Reis et. al (2020).

The PHA was extracted from the biomass via reflux by adding of dimethyl carbonate (DMC) as a solvent to a 1 L glass reactor with mechanical stirring connected to a cooling column following the scheme shown in Figure 4-1. For each 300 g of biomass loaded in the reactor, 800 mL of solvent was added, meaning 0.375 g/mL biomass to solvent ratio for each cycle of extraction. The extraction was done in 4 cycles for each batch of biomass added to the reactor. The overall biomass to solvent ratios was 0.10 g/mL. The glass reactor was heated up to the boiling point of the solvent (90°C for the DMC) for 30 min each cycle. After the extraction, a vacuum filtration with Whatman™ paper filters (Whatman 595 1/2 150mm, 4-7µm) was performed to separate the biomass from the solution. A rotary evaporator (Buchi Rotavapor R-300) was used to recover the solvent and to separate the PHA. The obtained PHA was left to dry overnight inside the fume hood and then weighed.

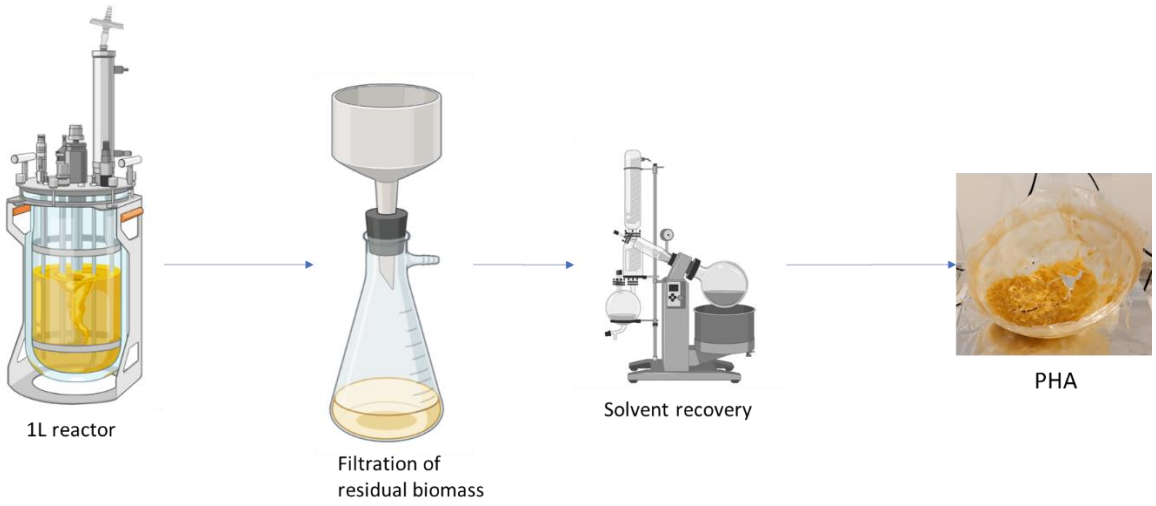


Figure 4-1: Scheme for extraction of WOW-PHA using DMC.

The extraction time of 300 g PHA-rich biomass was 4 h following immediately by a filtration step to obtain the PHA-solvent mixture. The amount of biomass and the limitation of the glassware apparatus increased the overall operation time. For each batch of extraction, the evaporation of the solvent consumed 30 minutes to obtain the dried PHA polymer until the highest possible vacuum of 30 mbar. The overall operation time for each batch extraction was 6 h 30 min, including a cleaning step.

4.2.3 Analytical Methods applied at Avans

4.2.3.1 Thermal Gravimetric Analysis (TGA)

Samples of produced biomass were analysed in duplicate for PHA, moisture and ash content, and the extracted PHA samples were analysed for purity with TGA (TGA 500Q, TA instruments). A mass of around 10 mg was placed into platinum pans and a ramp mode of 5°C per minute until 600°C was set under nitrogen atmosphere, with flow rates of 40 mL/min on the balance, 60 mL/min on the sample. The mass of PHA in each sample was determined as the mass loss in the temperature range between 230°C and 270°C. A commercial sample of PHBV was used to establish this range.

4.2.3.2 Differential Scanning Calorimetry (DSC)

The extracted PHA samples were further analysed for glass transition temperature (Tg), melting temperature (Tm), crystallization temperature (Tc), and crystallization behaviour a DSC (DSC Q20, TA instruments). A mass of around 2 mg was placed into platinum trays and a heating rate of 20°C/min until 200 C followed by a cooling rate of 10°C/min down to -50°C, and a heating rate of 20°C/min up to a temperature of 200°C.

Crystallization determination was calculated using the equation below according Lorini et al. (2021).

$$X_c = \frac{100 \cdot \Delta H_m}{\Delta H_m^0 \cdot purity}$$

Where ΔH_m is the melting enthalpy obtained from the DSC heating ramp and ΔH_m^0= 146 J/g is the enthalpy of fusion of 100% crystalline PHB sample.

4.2.3.3 Gas chromatography (GC)

Different batches of biomass were received according to the days of accumulation. The GC analysis was used to determine the PHA content in the biomass, 3HB and 3HV monomer composition and purity of the WOW-PHA extracts together with TGA analysis for comparison. The method followed is described in section 4.1.3.3 above. The biomass samples were mixed and the average PHA content value was calculated using the equation below:

$$\frac{g_{PHA}}{g_{TS}} = \frac{\sum(mass\ of\ biomass\ per\ batch) \cdot (PHA\%TS\ per\ batch)}{\sum mass\ of\ the\ combined\ biomass}$$

The PHA content determination in the biomass by GC presented 0.021 gPHA/gTS as the highest deviation among the different days of analysis.

5. Results and Discussion

5.1 Pilot scale experiments at University of Kaiserslautern-Landau (RPTU)

In the following chapter the results of the pilot scale experiments that were conducted at the University of Kaiserslautern-Landau (RPTU) will be presented and discussed. Subchapters will focus on the different stages of the process as well as on the composition of the microbial population.

5.1.1 Acidification of residual streams

The focus of the evaluation of the acidification lies on the VFA composition, which influences the PHA-properties, the overall VFA yield and the achievable VFA concentration, which determines the PHA-production potential. Furthermore, the nutrient composition was assessed, focusing on ortho-phosphate (PO_4-P) and ammonium-nitrogen (NH_4-N). The results are discussed both individually for each residual stream as well as in comparison for the two residual streams.

Brewery's residual stream and the composition of the derived substrate

As depicted in Table 5-1, the initial COD_{tot} of the mixture of brewery sewage with 10 % anaerobic digested sludge as inoculum amounted to 4.0 ± 1.5 g $COD_{tot, in}$/L, with a minimum concentration of 2.3 g $COD_{tot, in}$/L and a maximum of 6.9 g $COD_{tot, in}$/L. The maximum was caused by an operational failure that caused the sewage to consist of beer. The resulting COD_{VFA} amounted to 1.7 ± 0.8 g COD_{VFA}/L. Again, the maximum concentration was achieved with the initial substrate consisting of beer and amounted to 4.1 g COD_{VFA}/L. When comparing the inflow COD to the produced COD_{VFA}, the VFA yield can be calculated amounting to 0.41 ± 0.09 $COD_{VFA,out}/COD_{tot, in}$. This VFA yield is twice as high in comparison to the VFA yield that was achieved using primary sludge (PS), which resulted in a VFA yield of 0.19 ± 0.07 $COD_{VFA,out}/COD_{tot, in}$ (Uhrig et al. 2022). However, the overall achieved VFA concentration was significantly higher using PS as the produced feedstock contained 7.5 ± 2.3 g COD_{VFA}/L.

Table 5-1: Brewery: Initial COD concentration of the input of the acidification ($COD_{tot,in}$) and the composition of the acidified residual stream of the brewery

Parameter	Unit	Mean ± Std. dev.	Min / Max		n
$COD_{tot, in}$	**g/L**	4.0 ± 1.5	2.3	6.9	11
$COD_{VFA, out}$	**g/L**	1.7 ± 0.8	0.9	4.1	12
VFA yield ($COD_{VFA, out}$/ $COD_{tot, in}$)	**-**	0.41 ± 0.09	0.27	0.60	10
$COD_{sol, out}/COD_{tot, in}$	**-**	0.59 ± 0.12	0.34	0.79	10
$COD_{VFA, out}/COD_{tot, out}$	**-**	0.52 ± 0.18	0.14	0.75	12
$COD_{VFA, out}/COD_{sol, out}$	**-**	0.71 ± 0.12	0.35	0.82	12
$COD_{sol, out}/COD_{tot, out}$	**-**	0.73 ± 0.22	0.22	0.93	12
TNb	**mg/L**	151 ± 182	32	685	12
NH_4-N	**mg/L**	25 ± 10	13	46	12
PO_4-P	**mg/L**	10 ± 2	6	14	12
COD_{VFA} : NH_4-N : PO_4-P	**-**	100 : 1.8 (±1.0) : 0.7 (±0.3)			12
COD_{sol} : NH_4-N : PO_4-P	**-**	100 : 1.2 (±0.7) : 0.5 (±0.2)			12

Fruit juice company's residual stream and the composition of the derived substrate

In case of the fruit juice factory's residual stream, there were two different residual streams that were used during the experiments due to the seasonal fruit juice production campaign. The first residual stream comprised of water that was used to transport the fruits within the facility (*transportation residual stream*, substrate 1-4, 6) and the second residual stream consisted of sewage that was generated during the cleaning and bottling process (*general sewage*, batches 5, 7-9). In Table 5-2 the composition of the fruit juice residual stream is shown. The initial COD_{tot} at the inflow of the anaerobic fermentation process step accounted to 13.8 ± 8.7 g $COD_{tot, in}$/L, with a minimum concentration of 3.2 g $COD_{tot, in}$/L and a maximum of 32.9 g $COD_{tot, in}$/L. The resulting COD_{VFA} amounted to 2.3 ± 1.1 g COD_{VFA}/L. As previously described, the first batch acidifications were performed without an inoculum, however, the fraction of the COD_{VFA} of the COD_{sol} amounted to 31 ± 16 %, which showed that an enhancement of the VFA concentration was possible. Therefore, the acidifications were inoculated with anaerobic sludge from the brewery's UASB. Hereby, an increase of the fraction of the COD_{VFA} of the COD_{sol} was achieved, amounting to 56 ± 14 %. Showing, that the VFA yield was significantly increased by using an inoculum. However, as two different residual streams from the fruit juice company were investigated, it cannot be said that the improvement is solely by the use of an inoculum or if the composition of the general sewage had better start conditions for the VFA-production.

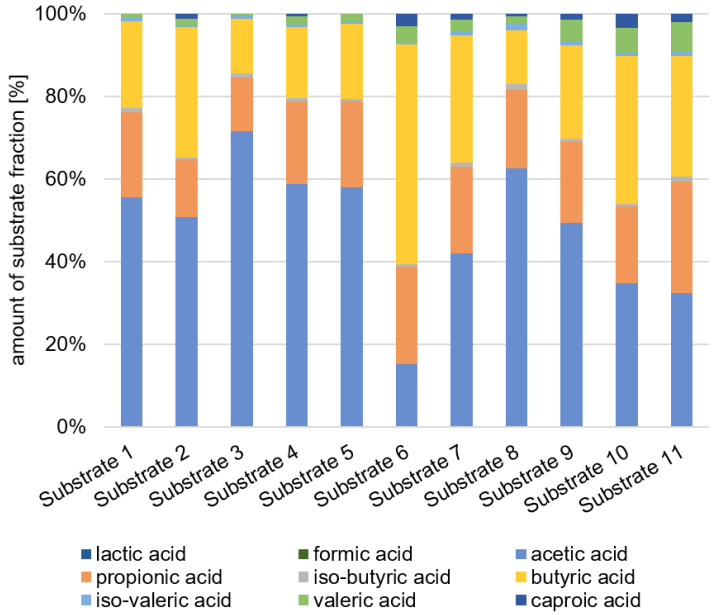
The maximum concentration of 4.2 g COD_{VFA}/L was achieved by fermenting one of the first batches of the "transportation residual stream" with an inoculum of 10 % anaerobic sludge from the brewery. The VFA yield of the residual streams of the fruit juice factory were comparable to the results of the PS with a VFA yield of 0.20 ± 0.11 $COD_{VFA,out}$/$COD_{tot, in}$.

Table 5-2: Fruit juice company: Initial COD concentration of the input of the acidification ($COD_{tot,in}$) and the composition of the acidified residual stream of the fruit juice company

Parameter	Unit	Mean ± Std. dev.	Min / Max		n
$COD_{tot, in}$	**g/L**	13.8 ± 8.7	3.2	32.9	9
$COD_{VFA, out}$	**g/L**	2.3 ± 1.1	0.3	4.2	9
VFA yield ($COD_{VFA, out}$/ $COD_{tot, in}$)	**-**	0.20 ± 0.11	0.06	0.46	9
$COD_{sol, out}$/$COD_{tot, in}$	**-**	0.73 ± 0.38	0.10	1.55	9
$COD_{VFA, out}$/$COD_{tot, out}$	**-**	0.21 ± 0.13	0.01	0.53	9
$COD_{VFA, out}$/$COD_{sol, out}$	**-**	0.37 ± 0.21	0.05	0.68	9
$COD_{sol, out}$/$COD_{tot, out}$	**-**	0.63 ± 0.33	0.17	0.96	9
TNb	**mg/L**	891 ± 1042	38	2732	9
NH_4-N	**mg/L**	10 ± 26	0.02	78	8
PO_4-P	**mg/L**	6 ± 6	0.06	17	9
COD_{VFA} : NH_4-N : PO_4-P	**-**	100 : 0.5 (±1.3) : 0.2 (±0.3)			8
COD_{sol} : NH_4-N : PO_4-P	**-**	100 : 0.3 (±0.8) : 0.1 (±0.2)			9

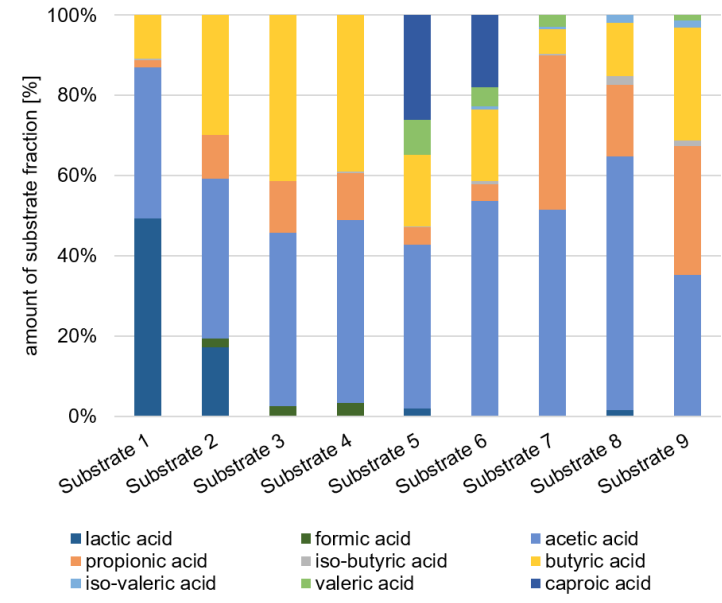
VFA composition and concentration

Figure 5-1 shows the VFA composition and the range of each volatile fatty acid of the acidification batches of the brewery's residual stream and Figure 5-2 shows the same for the batches of acidified fruit juice sewage. Since lactic acid and formic acid were present in the first two batches of the acidified residual stream of the fruit juice factory, they have been added in the table and graphs showing the VFA composition, even though they are not part of this group of chemicals. VFAs with an even number of carbon atoms (acetic, butyric, isobutyric, caproic acid) tend to be processed to PHB and VFAs with an odd number (propionic and valeric acid) to PHV (Albuquerque et al. 2007, Lemos et al. 2006). Hence, the stability of the VFA composition immediately affects the PHA composition, showing the importance of the initial residual stream that is being used to produce the VFA-rich substrate for PHA production.



Volatile fatty acid	Mean ± Std. dev.	Min Max
Lactic acid	–	–
Formic acid	–	–
Acetic acid	0.48 ± 0.15	0.15 0.71
Propionic acid	0.20 ± 0.04	0.13 0.27
Isobutyric acid	0.01 ± 0.00	0 0.01
Butyric acid	0.26 ± 0.11	0.13 0.53
Isovaleric acid	0.01 ± 0.00	0 0.02
Valeric acid	0.03 ± 0.02	0 0.07
Caproic acid	0.01 ± 0.01	0 0.03

Figure 5-1: VFA composition of the acidified residual stream of a brewery (batches: n = 11)



Volatile fatty acid	Mean ± Std. dev.	Min Max
Lactic acid	0.08 ± 0.16	0 0.49
Formic acid	0.01 ± 0.01	0 0.03
Acetic acid	0.46 ± 0.09	0.35 0.63
Propionic acid	0.15 ± 0.13	0.02 0.38
Isobutyric acid	0.01 ± 0.01	0 0.02
Butyric acid	0.23 ± 0.12	0.06 0.41
Isovaleric acid	0.01 ± 0.01	0 0.02
Valeric acid	0.02 ± 0.03	0 0.09
Caproic acid	0.05 ± 0.10	0 0.26

Figure 5-2: VFA composition of the acidified residual stream of a fruit juice factory (batches: n = 9)

Comparison of the two residual streams

In comparison the two residual streams showed differences both in the inflow COD_{tot} concentration as well as in the VFA yield. While the acidification of the brewery's residual stream showed a higher VFA yield compared to the fruit juice sewage batches, the initial COD concentration of the brewery sewage was not as high as in the fruit juice company's sewage. Therefore, the resulting VFA concentration of the two residual streams is still in a comparable range with 1.7 ± 0.8 g COD_{VFA}/L for the brewery and 2.3 ± 1.1 g COD_{VFA}/L

for the fruit juice sewage. When comparing the results of the residual streams with the VFA-rich substrate produced during the first phase of the WOW! Project using PS, the acidification of PS shows a significantly higher VFA concentration amounting to7.5 ± 2.3 g COD_{VFA}/L.

In the substrate derived from the brewery sewage, the given C-molar ratio of 76±5 % to 24±5 % of even to odd carbon atoms resembles the results of the screening experiments (81 to 19 %) (Laumeyer et al. 2022). In case of the fruit juice factory's residual stream, a similar ratio of even to odd VFA's was achieved (74±13 % to 26±13 %). Therefore, for both residual streams, the majority of the produced PHA is expected to be PHB with only a minor amount of PHV produced, indicating possible applications of the produced biopolymer.

For the brewery's sewage, the obtained VFA composition and concentration in COD equivalents is rather stable, as depicted in Figure 5-3 The brewery's batch *substrate 6* shows the influence in both the VFA composition (Figure 5-1) as well as the resulting VFA concentration (Figure 5-2), in case of operational differences. In this case, due to an operational failure at the brewery, the initial residual stream was beer instead of sewage.

As previously described, in case of the fruit juice factory's residual stream, there were two different residual streams that were used during the experiments. The first residual stream comprised of water that was used to transport the fruits within the facility (substrate 1-4, 6) and the second residual stream consisted of sewage that was generated during the cleaning and bottling process (batches 5, 7-9). This might explain the differences between the amount of VFAs that were produced during the acidification process and also the change in the VFA composition. While the batches 5 and 6 resulted in increased amounts of caproic acid, batches 7-9 achieved an increased amount of propionic acid. Batch 8 resulted in a low amount of VFAs due to an operational failure within the start-up phase of the acidification.

These results can be used for a first indication of which applications of the produced biopolymers are possible. Moreover, regarding the individual substrates it is also important to ensure that the PHA composition stays stable in order to meet market needs for stable product properties.

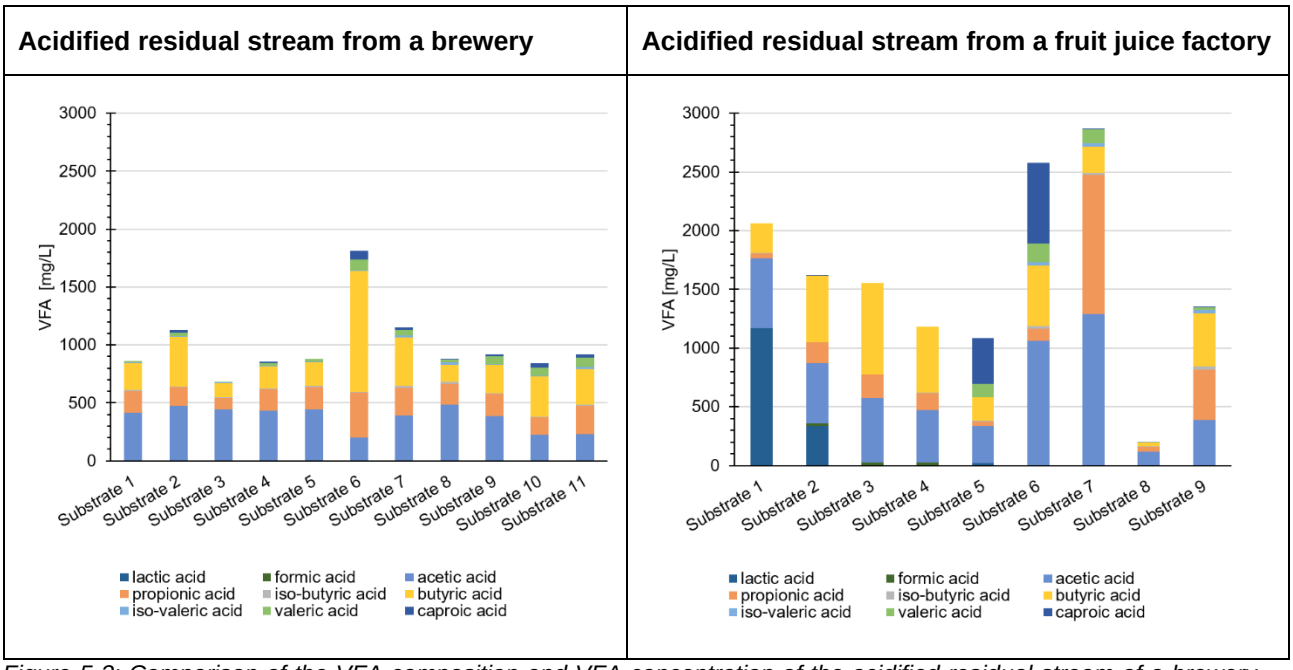


Figure 5-3: Comparison of the VFA composition and VFA concentration of the acidified residual stream of a brewery (batches n = 11) and a juice factory (batches: n = 9).

The nutrient concentration of the two examined residual streams showed a significant difference regarding the relation of the COD_{VFA} to the nutrients in form of the ammonium-nitrogen (NH_4-N) and ortho-phosphate-phosphorus (PO_4-P). The relation of COD_{VFA} : NH_4-N : PO_4-P amounted in the substrate derived from the brewery's residual stream to an average of 100 : 1.8 (±1.0) : 0.7 (±0.3) and for the fruit juice company's residual stream to 100 : 0.5 (±1.3) : 0.2 (±0.3). Even though the average ratio of the brewery derived substrate was not exactly as favoured by the bacteria (100 : 5 : 1), the amount of nutrients allowed an enrichment of PHA accumulating bacteria. However, in case of the fruit juice company's residual stream, the nutrient-ratio was too low, which made a supply of NH_4-N and PO_4-P necessary for the enrichment step.

Interestingly, the TNb concentration of the feedstock derived from the brewery's residual stream is with 151 ± 182 mg TNb/L lower when compared to the substrate derived from the fruit juice company, which amounted to an average of 891 ± 1042 mg TNb/L. The amount of the TNb concentration increased significantly when the acidification was started using the inoculum from the brewery's UASB.

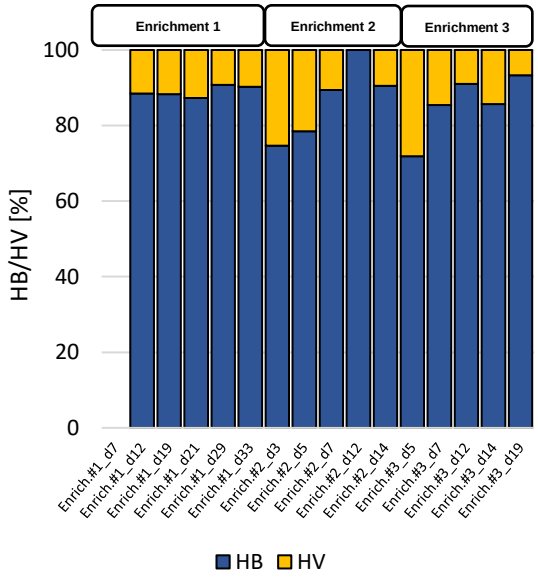
5.1.2 PHA production

The substrate produced by anaerobic fermentation of the residual streams of the brewery and the fruit juice company were used as feedstock for the PHA production process, comprising of an enrichment and an accumulation step. For each of the residual streams, three enrichments batches were performed with 24 h-accumulations being performed at different days of the process, as depicted in Table 5-3. The first enrichment batch that was performed using the feedstock derived from the brewery was operated for 33 days. As the results on day twelve showed the highest PHA concentration with 41.5 % with regards to the dry matter, the consecutive enrichments were operated for a shorter period of time. The amount of accumulations that were performed were predefined by the VFA concentration of the substrate. The aim was to perform at least one accumulation per week. Feedstocks with higher VFA concentrations allowed the execution of up to three accumulations per week. In total, a number of 17 accumulations were performed with the brewery's residual stream and also 17 accumulations were performed with the fruit juice factory's residual stream. Some of the accumulations did not result in the production of any PHA due to too low concentrations of VFA in the feedstock or operational failures such as power outages resulting in a process disruption. For the brewery derived PHA a total of two accumulations did not yield any PHA: the first accumulation of the first enrichment and the accumulation on day 12 of the second enrichment. Overall, the average PHA produced by the brewery's derived feedstock amounted to 21 ± 11 % PHA/dm. The first enrichment of the fruit juice factory's residual stream showed a loss of total solids during the operation, due the low nutrient concentration. Therefore, for the following enrichment processes, nutrients in form of NH_4Cl and KH_2PO_4 were added to the dilution water, to match the need of the bacteria and provide a nutrient ratio of C:N:P of 100:5:1.

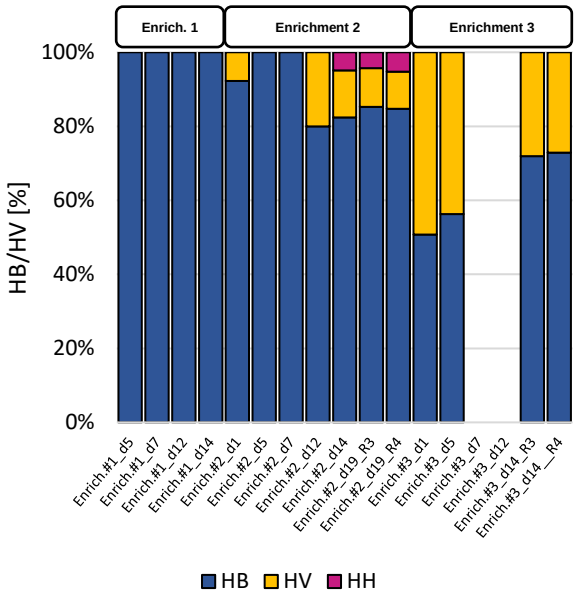
As written and depicted in Table 5-3, the last days of the second and third enrichment using the fruit juice derived feedstock were performed as duplicates by operating the enrichment reactor also as an accumulation reactor. The results that were obtained with these duplicates show a similar PHA composition in the second enrichment, with day 19 of the second enrichment presenting a ratio of HB : HV : HH of 82.4 : 12.7 : 4.9 (enrichment reactor, R3) and 84.7 : 10.0 : 5.3 (accumulation reactor, R4). On day 14 of the third enrichment a ratio of HB : HV of 71.9 : 28.1 (R3) and 72.9 : 27.1 (R4) was achieved. The results also show the influence of the initial substrate composition on the PHA composition, which is shown clearly in the accumulation results achieved with the batches number 5 and 6 of the fruit juice factory's residual stream – here the increased concentration of caproic acid resulted in the synthesis of hydroxyhexanoate (HH) on day 14 and 19 of the second enrichment process.

Table 5-3: Overview of the accumulations performed with feedstocks derived from a brewery and a fruit juice factory and the resulting PHA composition

PHA produced by brewery derived feedstock		**PHA produced by fruit juice factory derived feedstock**	
Enrichment number	**Days of accumulations**	**Enrichment number**	**Days of accumulations**
1	7, 12, 19, 21, 26, 29, 33	1	5, 7, 12, 14
2	3, 5, 7, 12, 14	2	1, 5, 7, 12, 14, 19*
3	5, 7, 12, 14, 19	3	1, 5, 7, 12, 14*



* accumulations performed as duplicate in the enrichment and the accumulation reactor



The HB/HV-ratio of the brewery-derived PHA amounted to 80 ± 21 % HB to 14 ± 8 % HV, which is relatable to the average composition of VFAs with a number of even to odd carbon atoms of 76 ± 5 % to 24 ± 5 %. For the fruit juice-derived PHA the HB/HV/HH-ratio amounted to 78 ± 26 % HB to 15 ± 17 % HV to 1 ± 2 % HH, which is also in line with the composition of the fruit juice factory's derived residual stream, with a ratio of even to odd VFA's of 74 ± 13 % to 26 ± 13 %.

In conclusion it can be said that both residual stream-derived PHAs mainly consist of HB. However, the PHA derived from the brewery's residual stream is more stable than the one derived from the fruit juice factory. This is in line with the results of the feedstock that was produced, where the brewery also showed a more stable VFA composition compared to the fruit juice factory's residual stream.

5.1.3 Biological composition

Biological samples were analysed for two enrichment batches (Brewery Enrichment 2 and 3) using the feedstock derived from the brewery and one additional enrichment batch using the substrate derived from the fruit juice company (Fruit Juice Enrichment 2). The 40 most abundant genera across all samples are depicted in Figure 5-4. Taxa which could not be assigned to genus level were assigned to the last possible level.

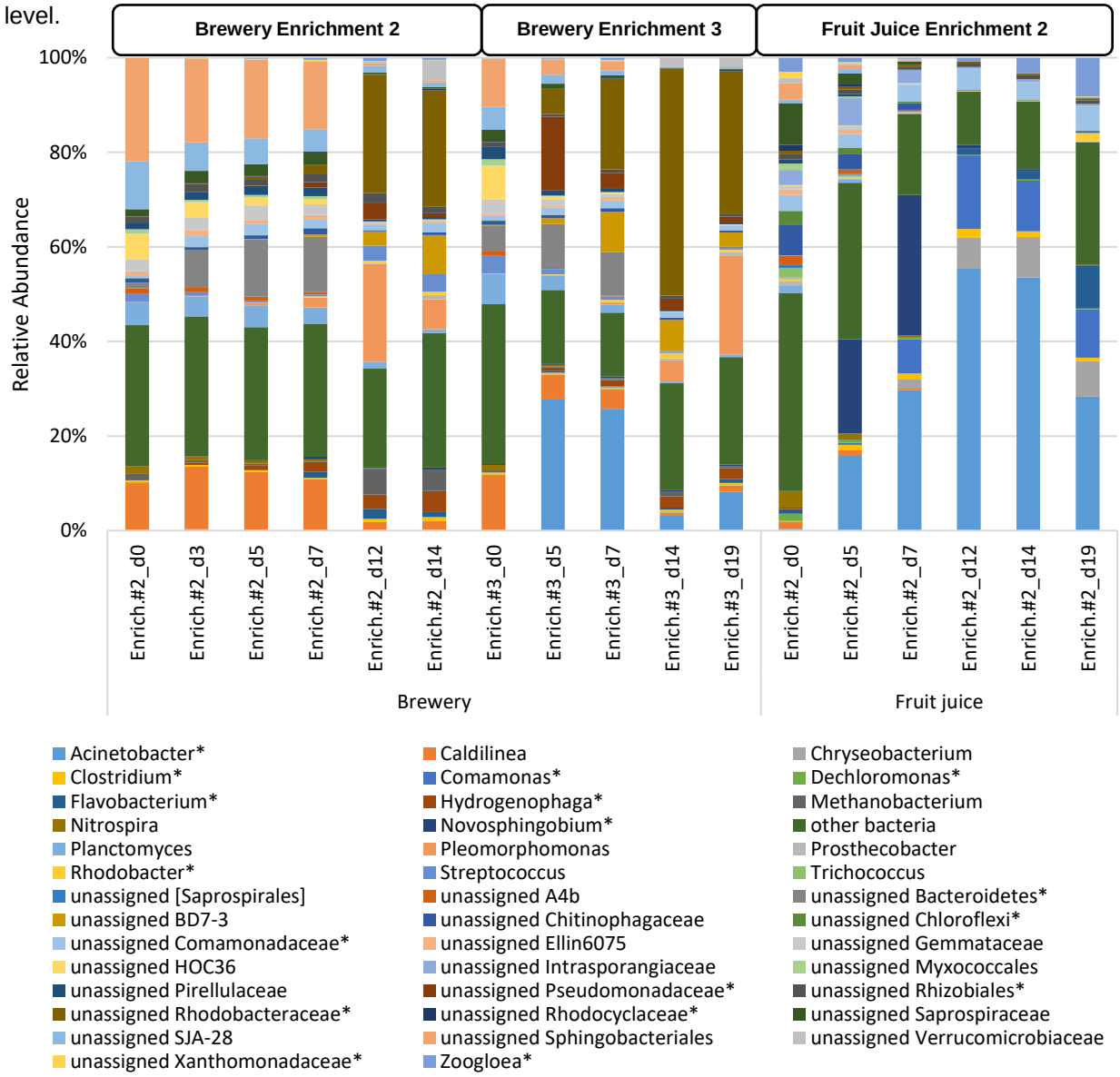


Figure 5-4: Microbial community composition of different enrichment batches using a brewery and fruit juice derived feedstock. Asterix indicate taxa with the potential ability to produce PHA.

A shift in microbial community composition during all three enrichment experiments over time could be observed. Furthermore, different organisms were dominating the systems, where in both the brewery enrichment experiments the composition was closer to each other as compared to the fruit juice enrichment batch, which is likely to be a result of the different feedstock as well as the different inoculum used. The most

dominant taxa in the enrichment batch number 2 of the brewery experiments were unassigned Sphingobacteriales (11.9 ± 8.3 %), *Caldilinea* (8.3 ± 4.75 %), unassigned Bacteroidetes (5.5 ± 5.2 %) and unassigned Rhodobacteraceae (8.7 ± 11.4 %). The two first mentioned taxa are not known to include organisms with the ability to produce PHA and their relative abundance was constantly decreasing from 21.8 to 0.5 % (Sphingobacteriales) and 10.2 to 1.8 % (*Caldilinea*) from day 1 to day 14 of the enrichment process, while some unassigned members of the Bacteroidetes and Rhodobacteraceae groups, which include potential PHA producers, increased in their relative abundance from 1.2 to 12.1 % from day 1 to 7 (Bacteroidetes) and from 0 to 24.7 % from day 1 to 14 (Rhodobacteraceae). In the third brewery enrichment batch among the most abundant taxa also were *Caldiliinea* (4.6 ± 4 %), unassigned Bacteroidetes (4.9 ± 4 %), unassigned Rhodobacteracea (20.5 ± 17.3 %) and *Acinetobacter* (13 ± 11.6 %), another group with the potential to produce PHA. *Caldilinea* experienced a further reduction in relative abundance from 11.9 to 1.2 % during the enrichment period. The relative abundance of unassigned Bacteroidetes and *Acinetobacter* initially increased from day 1 to 7 from 5.3 to 9.3 % (unassigned Bacteroidetes) and from 0 to 25.8 % (*Acinetobacter*) but then decreased until the end of the enrichment batch. In their place as PHA producers the group of unassigned Rhodobacteraceae dominated the system at the end of the enrichment (47.9 and 30 % on day 14 and 19, respectively). Organisms with zero abundance in the beginning of the experiment could potentially have been introduced in the system via the feedstock. The enrichment experiment with the fruit juice derived feedstock comprised of a completely different (initial) microbial composition with *Acinetobacter* (30.5 ± 19.5 %), *Comamonas* (7.4 ± 5.6 %) and *Novosphingobium* (8.5 ± 11.9 %) being the overall most abundant taxa, where all three genera include potential PHA producing organisms.

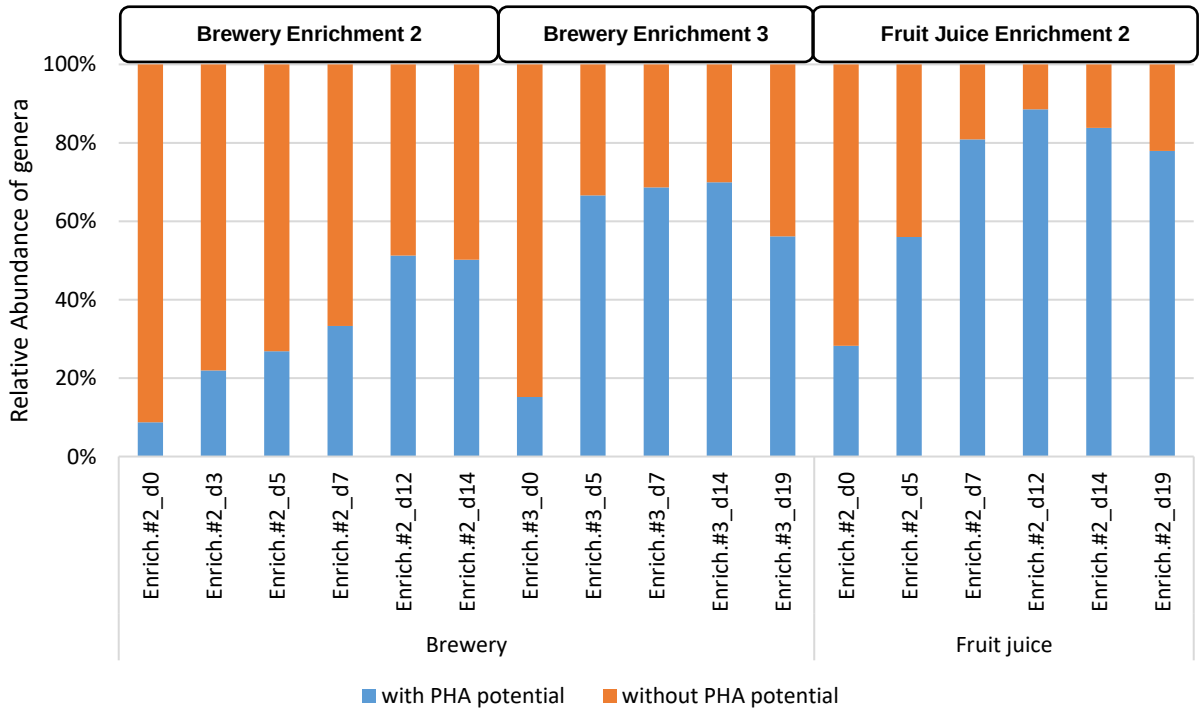


Figure 5-5: Relative abundance of microorganisms with and without the ability to produce PHA in the different enrichment batches using a brewery and fruit juice derived feedstock.

Figure 5-5 shows the cumulative relative abundance of the microbial taxa with and without the potential to produce PHA for all three enrichment batches. An overall increase in relative abundance of potential PHA

producers over time in all enrichment batches could be observed with 51.3 % (Brewery #2), 69.9 % (Brewery #3) and 88.5 % (Fruit juice #2) being the maximum reached values. However, a slight decrease after day 12 or 14 could be recorded in all experiments, which is in accordance with the observation that the maximum PHA content could be reached on day 12 (chapter 5.1.2). In conclusion, a successful selection of microorganisms with the potential to produce PHA could be achieved over time and within all three enrichment experiments, where the highest relative abundance of potential PHA producers could be reached in the enrichment experiment using fruit juice as a substrate.

5.2 PHA-extraction experiments at Avans University of Applied Sciences

5.2.1 Sieving of the PHA-rich biomass

All PHA-rich biomass from the pilot scale experiments at University of Kaiserslautern-Landau (RPTU) was mixed. The aim was to have a homogeneous mixture for the PHA extraction.

The initial attempts to extract the PHA from the PHA-rich biomass resulted in a settling time exceeding 2 hours due to the small particles being present in the biomass. This long settling time caused the solvent to cool down and the PHA to precipitate onto the biomass. This precipitation led to the formation of agglomerations with the residual biomass, making it considerably more challenging to dissolve the PHA back into the solvent. To reduce the settling time and solve the precipitation problem, it was decided to remove the smaller particle sizes from the PHA-rich biomass. For this, a particle size distribution was made for both brewery biomass and fruit juice biomass. See Figure 5-6 and Figure 5-7.

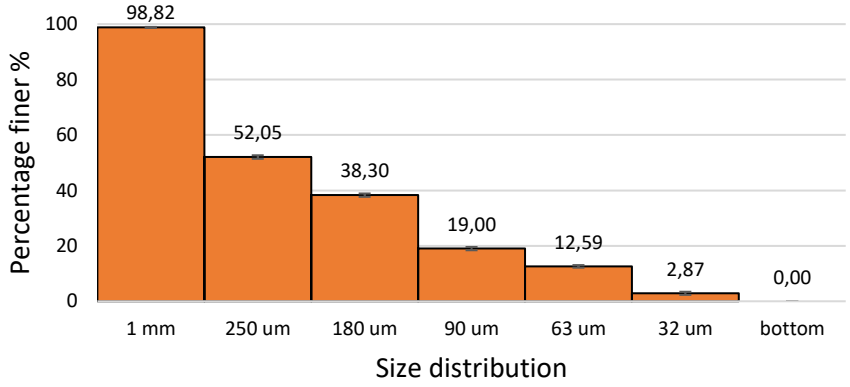


Figure 5-6: Size distribution PHA-rich biomass from Brewery VFA production

Based on the size distribution, settling times, and small scale tests on PHA extraction, it was decided that PHA biomass above 63 µm for brewery biomass and 180 µm for fruit juice biomass would be used for the PHA extraction. Thus, around 13 % of the brewery biomass and 26 % of the received fruit juice biomass was not used for extraction of PHA (Figure 5-6 and Figure 5-7).

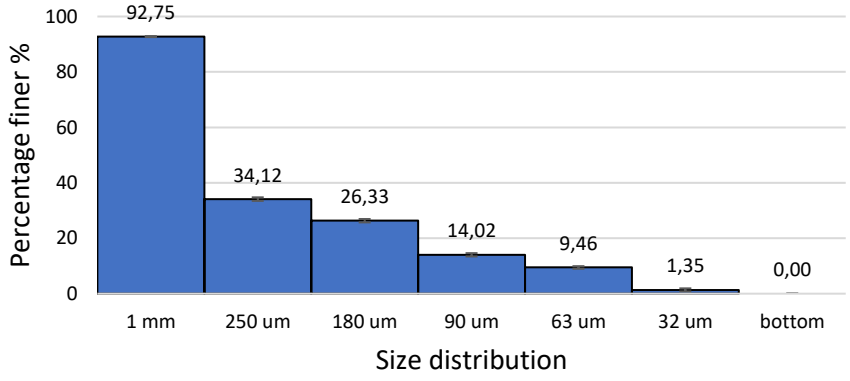


Figure 5-7: Size distribution PHA-rich biomass from Fruit Juice VFA production

5.2.2 WOW-PHA extraction mass balance

The amount of PHA-rich biomass used for the extractions was less than the quantities received from RPTU (1,466.78 g from Brewery production and 3,373.27 g from Fruit Juice production) since the biomass was separated through sieving (see Chapter 5.2.1), and part was spared for future analysis. The mass balances of the extractions are shown in Table 5-4. In total 257 g of PHA was extracted from brewery biomass and 497 g of PHA from fruit juice biomass. The materials differ not only in colour but also in their visual texture. Brewery-PHA had a flexible texture and was collected from the evaporator vessel mostly as a thick film. Figure 5-8 shows brewery biomass and the noticeable red colour of the extracted solution that eventually also ends up in the extracted material as shown in Figure 5-9.

For Fruit-PHA the material presented a brittle form, being collected most of the times as a bright yellow pieces, or sometimes powder, as shown in Figure 5-10. The polymer also retained more water than Brewery-PHA. This was observed by the texture and colour changing during the drying period in the fume hood. The Brewery-PHA dried completely within about 1-2 days after the evaporation inside the fume hood, while this time increased for Fruit-PHA by one week with additional anhydrous nitrogen flow to accelerate the drying process. Other drying processes were not considered to prevent structural modifications prior to polymer processing.

Figure 5-8: Glass reactor in settling phase after a cycle of extraction.

Table 5-4: Mass balance for PHA extraction of PHA-rich brewery biomass and fruit juice biomass

	Brewery biomass	**Fruit juice biomass**
Date	January 2023	January 2023
Method	Semi-continuous	Semi-continuous
Total mass [g]	1,307.10	2,685.40
Average PHA content [g PHA/ g TS]	20.44 %	19.39 %
Theoretical PHA, gTS	257.10	497.37
PHA like material extracted [g]	204.83	521.42*
Moisture content, %	3.41	12.2
Purity [g pure PHA/g dried extracted PHA]	76.8 %	81.1 %
Extraction yield [g PHA material/100 g intercellular PHA]	**76.9 % ± 8.2**	**93.2 % ± 7.2**

**The amount of material is higher than the theoretical PHA mass due to the impurities, remain solvent and moisture content in the extracted material.*

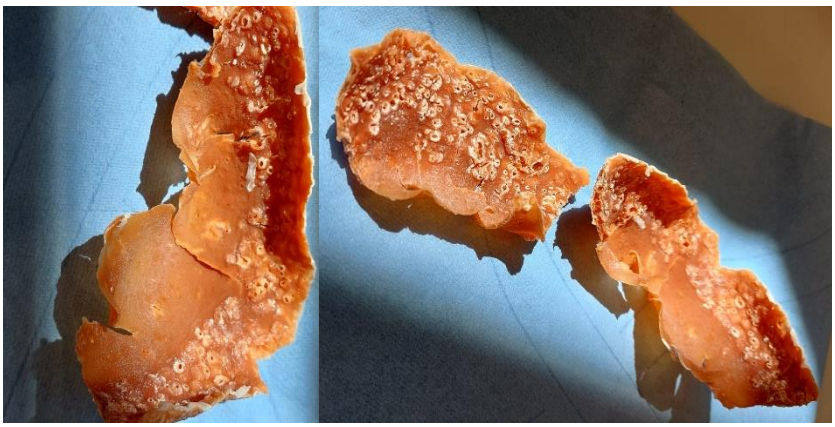


Figure 5-9: Extracted Brewery-PHA

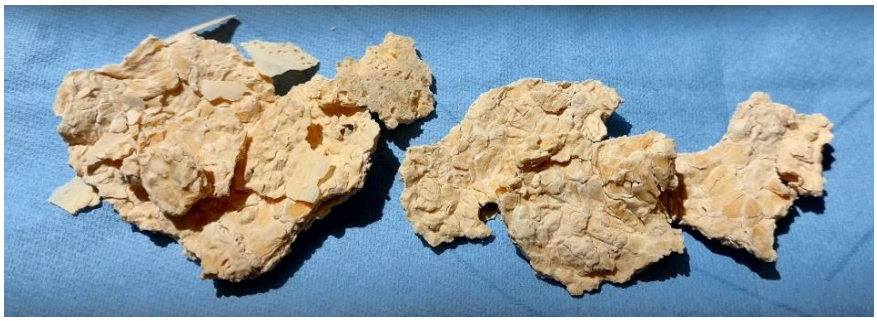


Figure 5-10: Extracted Fruit-PHA

The yield of extraction takes into account the whole material extracted (including impurities). Fruit-PHA extraction obtained a higher extraction yield (93%) and purity (81%) compared to the brewery-PHA. The reasons for this difference will be discussed in paragraph 5.2.4. The overall PHA recovery for both residual streams amounts to 69.7%. This recovery is calculated based on the amount of pure polymer extracted divided by the theoretical amount of the polymer inside the bacteria cells. The PHA recovery of 69.7% is lower than the PHA recovery reported by Samorì et al. (2015) for extraction with DMC. However, Samorì et al. used synthetic feed for PHA enrichment and accumulation which means that, in contrast to the fruit-PHA and brewery-PHA, the extraction is not (negatively) influenced by impurities in the feed.

5.2.3 PHA composition and characteristics

Table 5-5 gives an overview of the monomer composition (**Ø, %**) of the extracted Brewery-PHA and Fruit-PHA resulting from GC analysis. It shows that the HH content of the Fruit-PHA is considerably higher than for the brewery-PHA. A higher HH content means decrease in crystallinity since the 3-HH medium-length chain acts as short branches of the main 3-HB chains. The randomness of aleatory 3-HH chains reduces crystallinity and broadens the melt peak. PHBH copolymers with low 3-HH content experience physical aging characterized by increase in modulus and strength and reduction of ductile properties such as elongation at break, toughness and impact strength (Ivorra-Martinez et al. 2020).

Table 5-5: PHA monomer composition and characteristics

	Ø HB [%]	Ø HV [%]	Ø HH [%]	Crystallinity (%)	Tm^1 °C	Tm^2 (°C)	ΔHm (J/g)
Brewery	81.8	17.5	0.7	18.45	116.4	135.5	21.2
Fruit Juice	79.8	15.3	4.9	11.59	94.6	114.9	13.8

Table 5-5 also shows the DSC results for crystallinity, melting temperature (Tm,°C) and enthalpy of fusion (ΔHm, J/g) for Brewery-PHA and Fruit-PHA. Figure 5-11 displays a DSC second heating ramp curve for a Brewery-PHA and Fruit-PHA sample. The results exhibit discernible differences. The Brewery-PHA sample exhibits elevated values for the glass transition temperature, crystallization temperature, and melting temperature compared to the Fruit-PHA. Also, the crystallinity percentage is higher. These differences will highly influence the possibilities for polymer processing as discussed in deliverable D2.3 of WOW! Capitalisation. Establishing a link between these (differences in) properties and the composition and properties of different residual streams can give a better understanding of the applicability and market potential of PHA.

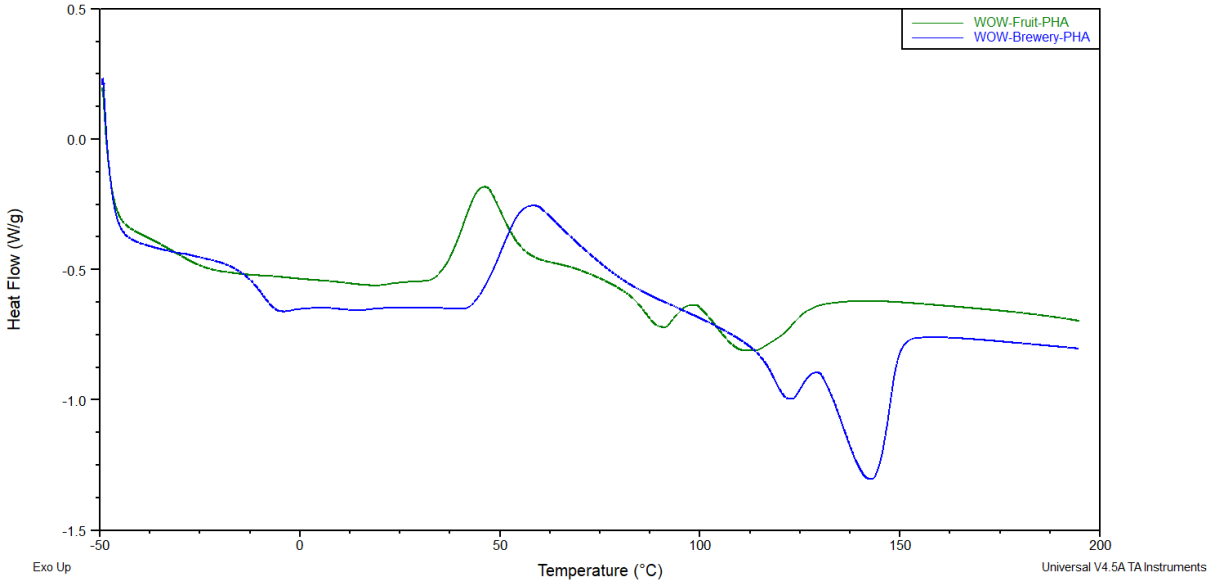


Figure 5-11: DSC curve of representative extracted WOW-PHA's overlay

5.2.4 PHA purity and extraction yield

The extracted PHA has a purity of 77 % and 81 % for brewery and fruit juice respectively (see Figure 5-12). According to de Souza Reis et al. (2020), the purity of extracted PHA using DMC is around 91.2 % using synthetic feed for PHA production. As expected, results for PHA purity in processes that use residual streams as feedstock for PHA production are lower. The composition of the residual stream will directly influence the solvent extraction due to a change in the solubility range and therefore the final product. A purification step either in the VFA production step (membrane separation, gas stripping, electrodialysis, etc) or in the downstream processing (anti-solvent or crystallization) of the PHA-rich biomass could increase PHA purity.

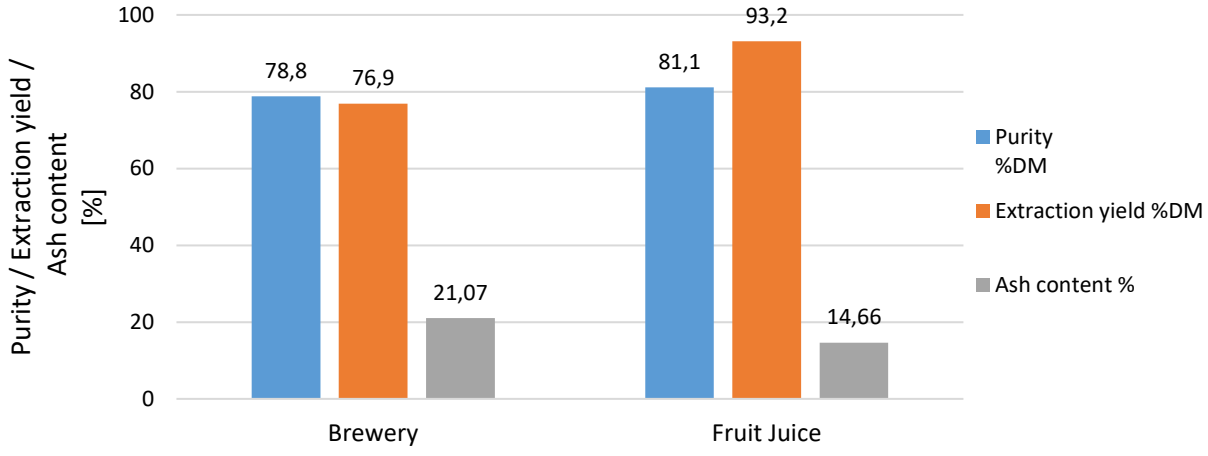


Figure 5-12: Results of the extraction of PHA from dried PHA-rich biomass using DMC

There is no clear correlation between the ash content and the extraction yield. A reasonable explanation for the difference between the extraction yields of the brewery and fruit juice PHA-rich biomass are the characteristics of the produced PHA as discussed in paragraph 5.2.3. According to Werker et al. (2023), lower melting temperatures and enthalpies are an indication for the ability to extraction at lower temperatures given average crystallinity with increased average 3HV co-monomer content. Thus, with a lower PHA crystallinity, a lower temperature of extraction is needed. The DSC results (table 5.5) show both lower melting temperatures and a lower crystallinity for Fruit-PHA. This indicates that at the extraction temperature of 90°C that was used, extraction of fruit-PHA will be more efficient than extraction of brewery-PHA.

6. Summary and conclusion

The operation of the PHA pilot with residual streams from two different food industries showed that it is possible to recover carbon in form of PHA, a biodegradable biopolymer. The results of the pilot show that the different residual streams result in substrates with a different VFA composition. Furthermore, the differences are not only between the two industries but also between the individual batches of residuals streams from the same source. The two industrial residual streams from the food industry comprised the sewage from a brewery and to different residual streams from a fruit juice company. The results showed that the residual stream of the fruit juice company resulted in higher COD_{VFA}, but lower ratio of COD_{VFA}/COD_{fil}.

It was possible to produce PHA from both of the two residual streams from the food industry with the PHA mainly consisting of HB. As discussed before, the substrates comprised of differing concentrations and ratios of VFAs, which had an immediate effect on the PHA composition when used as a feedstock. The brewery's residual stream had a more stable VFA concentration and also composition during the eleven performed acidification batches, which also resulted in a more stable composition of the produced PHA in comparison to the feedstock derived from the fruit juice company. In the case of two batches of the fruit juice company, it was observed that substrates rich in caproic acid cause the production of hydroxyhexanoate (HH), which is considered a medium chain-length PHA.

The deduction of this observation is the need of a process technique that allows for a stable composition of the substrate for PHA production or in the development of alternative process setups to adjust the PHA composition to the needs of the plastic industry.

In this study, the determination of particle size distribution proved crucial due to operational parameter constraints. Given the biomass quantity and the standard laboratory glassware typically employed in batch experiments involving 1-2 g of material, the particle size of the PHA-rich biomass significantly influenced the duration of downstream processing operations.

To summarize, we have provided a detailed account of the mass balance for our extractions, resulting in a total of 726.26 grams of WOW-PHA extracted from different biomass sources. This amount does not exclude moisture and impurities and it was all sent to be processed in Finland. It is important to note that these materials not only varied in colour but also in visual texture. The Brewery-PHA exhibited a flexible texture, often appearing as a thick film in the evaporator vessel. For Fruit-PHA, the material had a brittle form, often collected as bright yellow pieces or even as a powder. It retained more water compared to Brewery-PHA, evident from the changing texture and colour during the drying process in the fume hood

The WOW-Fruit-PHA had overall better results for purity, extraction yield and recovery. A reasonable explanation for the difference in extraction yield between the Brewery and Fruit Juice processes lies in the dissolution temperature of the PHA in DMC. There appears to be no direct correlation between ash content and extraction yield, suggesting that ash content is not a limiting factor.

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