PHA production from residual streams
How the substrate composition influences the final product

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Centre of Expertise Biobased Economy

Agenda

- Part I – Production of PHA from residual streams
  - The WOW-project
  - Biopolymers: Polyhydroxyalkanoates (PHA)
  - Pilot-plant setup
  - PHA-production from residual streams of the food industry

- Part II – Extraction of PHA from dried biomass
  - The perspective of Downstream Process
  - Solvent selection & sustainability
  - PHA extraction optimization
  - PHA Extraction Method

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Wastewater as a resource

Project: **WOW!**

Wider business **O**pportunities for raw materials from **W**astewater

PHA

LIPIDS

CELLULOSE

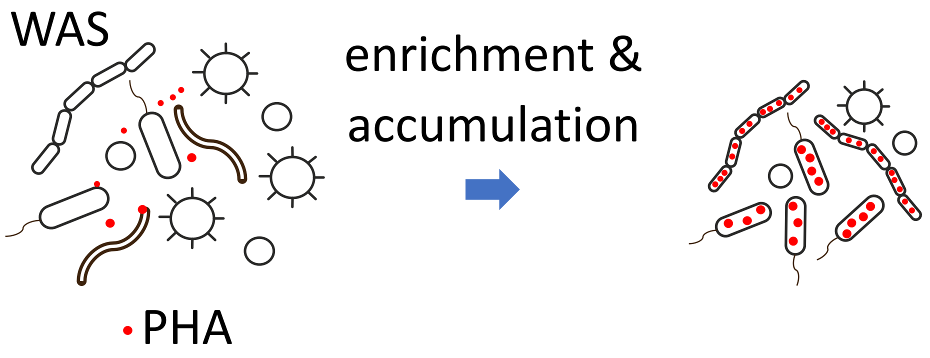
→ Production of polyhydroxyalkanoates (**PHA**) from primary sludge

→ Results

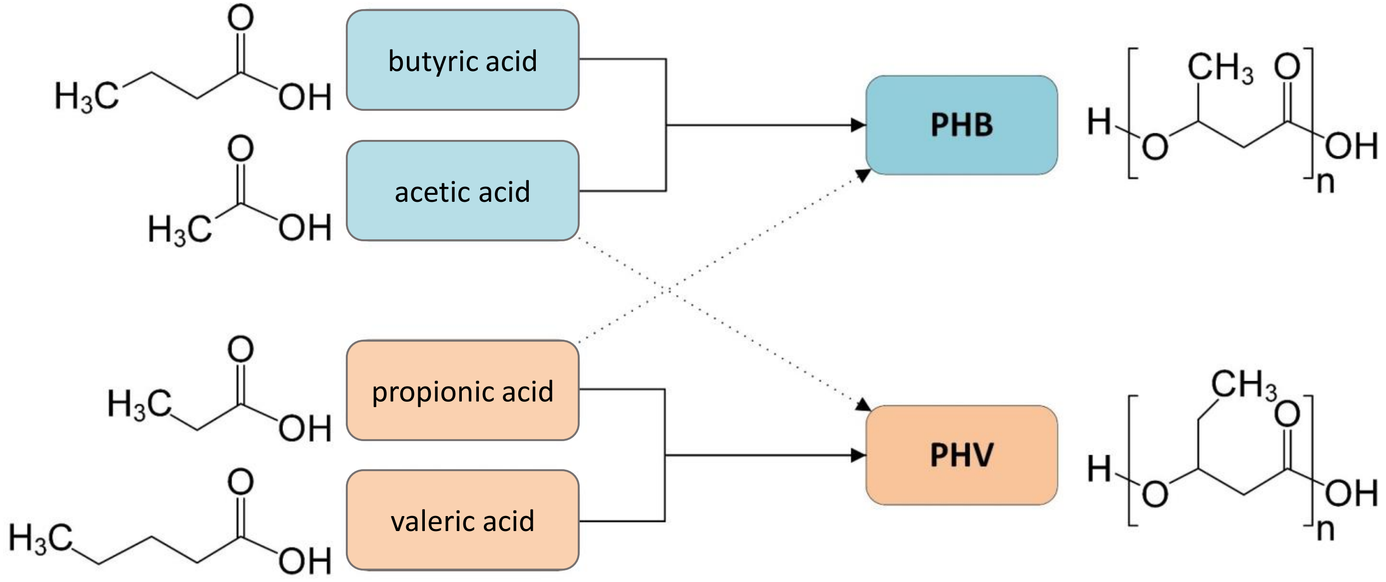
PHA - Polyhydroxyalkanoates

- Group of biodegradable polymers
- Microbiologic storage molecule

  PHA-producing bacteria are present in waste activated sludge (WAS)



- Volatile fatty acids (VFA) from fermentation of org. residual streams as substrate for enrichment & PHA-accumulation



Modified from: Uhrig, T. (2023): Eignung von kommunalen und industriellen Abwasserströmen für die PHA-Produktion und Ansätze zum Up-Scaling des Prozesses. Unpublished dissertation.

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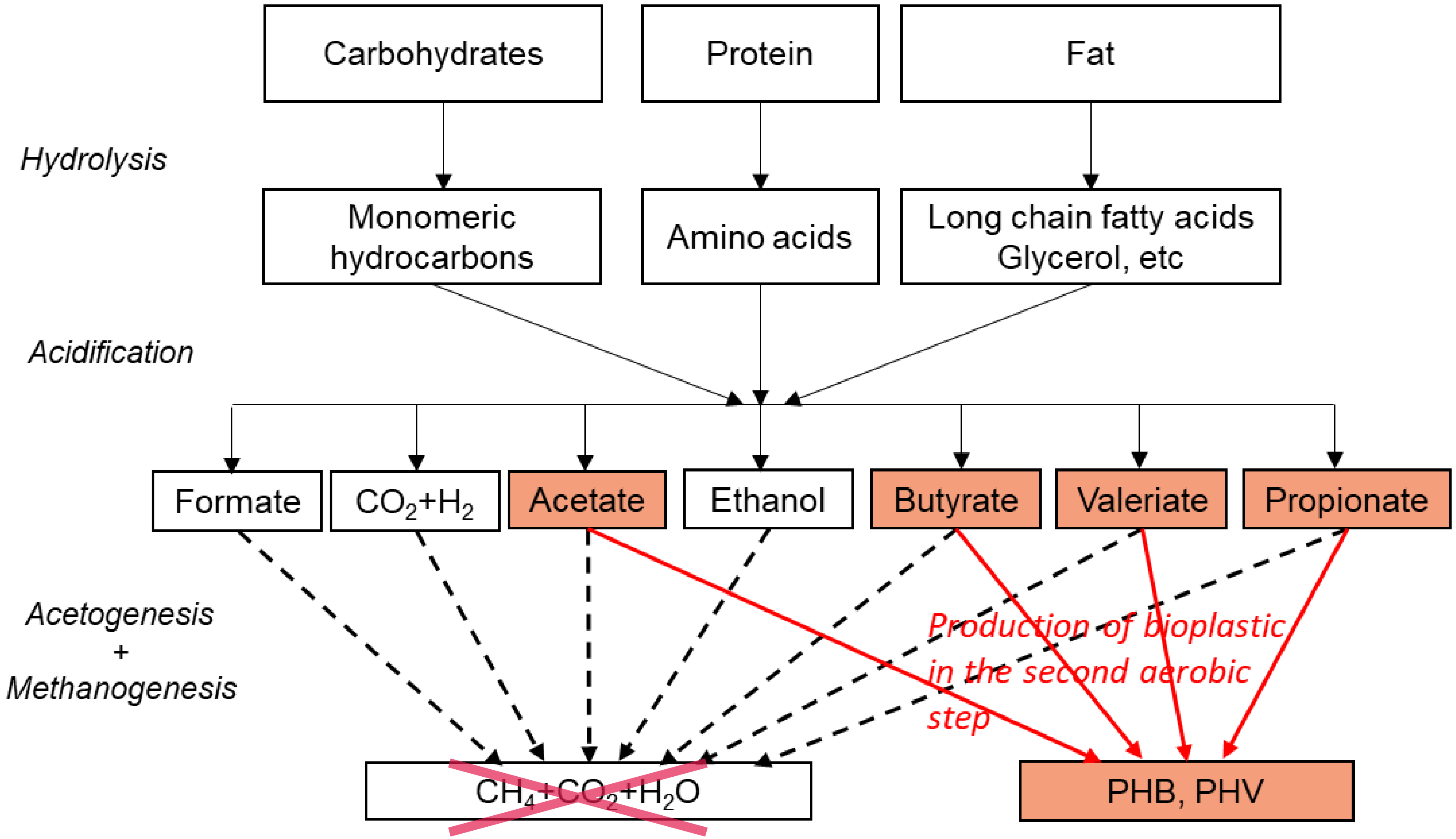
Where does the substrate for PHA-production come from?



- Anaerobic decomposition to VFA
- Methanogenesis is suppressed
- pH < 6
- Sludge age < 8d

Process of PHA production from sewage

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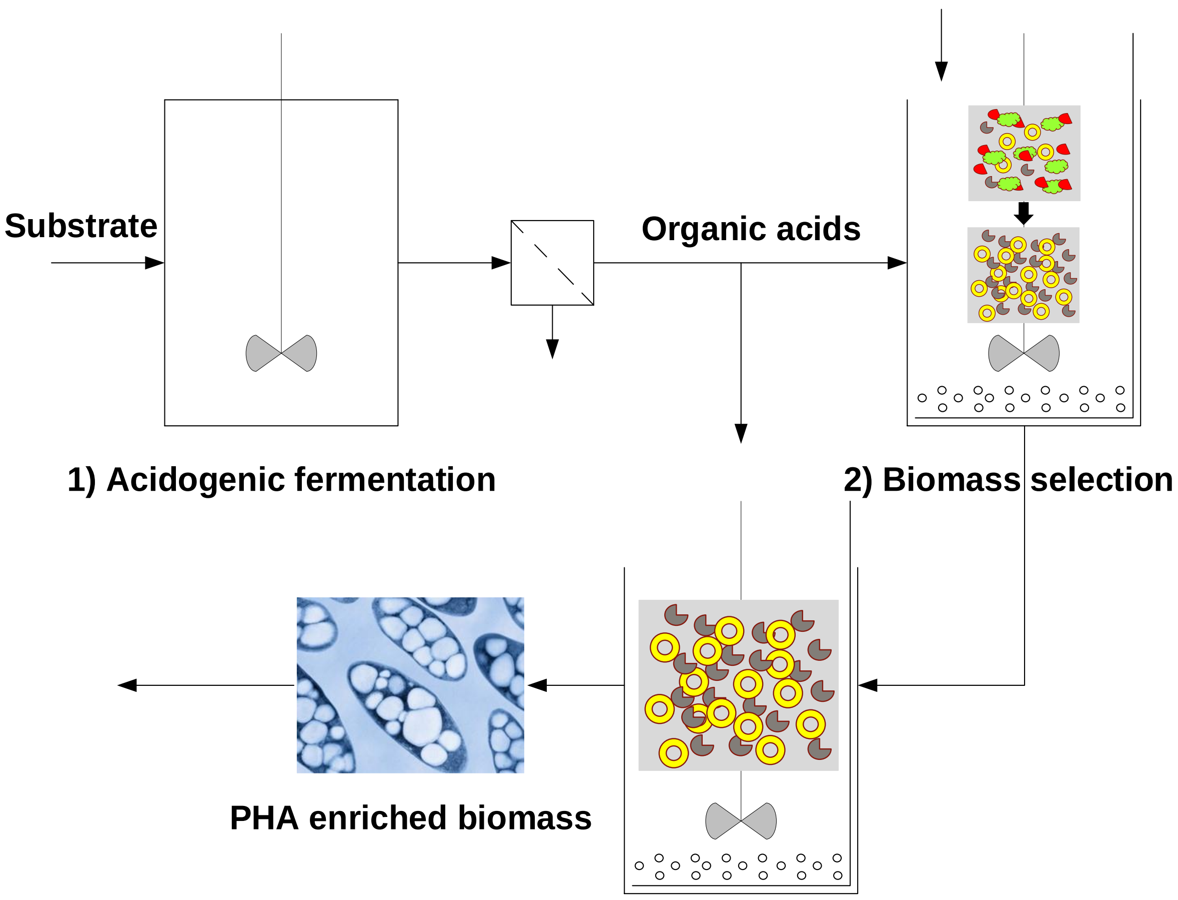

Uhrig et al. 2020

Pilot plant installation for PHA production
→ Video of the pilot

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Next step: WOW! Capitalisation - Widening the scope -

Residual streams of the food industry


Brewery

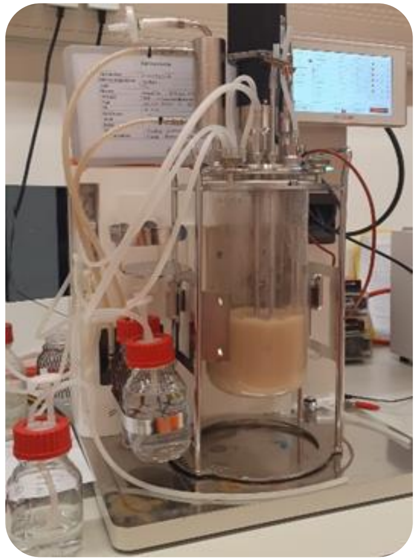

Condensed milk factory


Pizza factory


Starchy residual streams

Experiments at lab scale

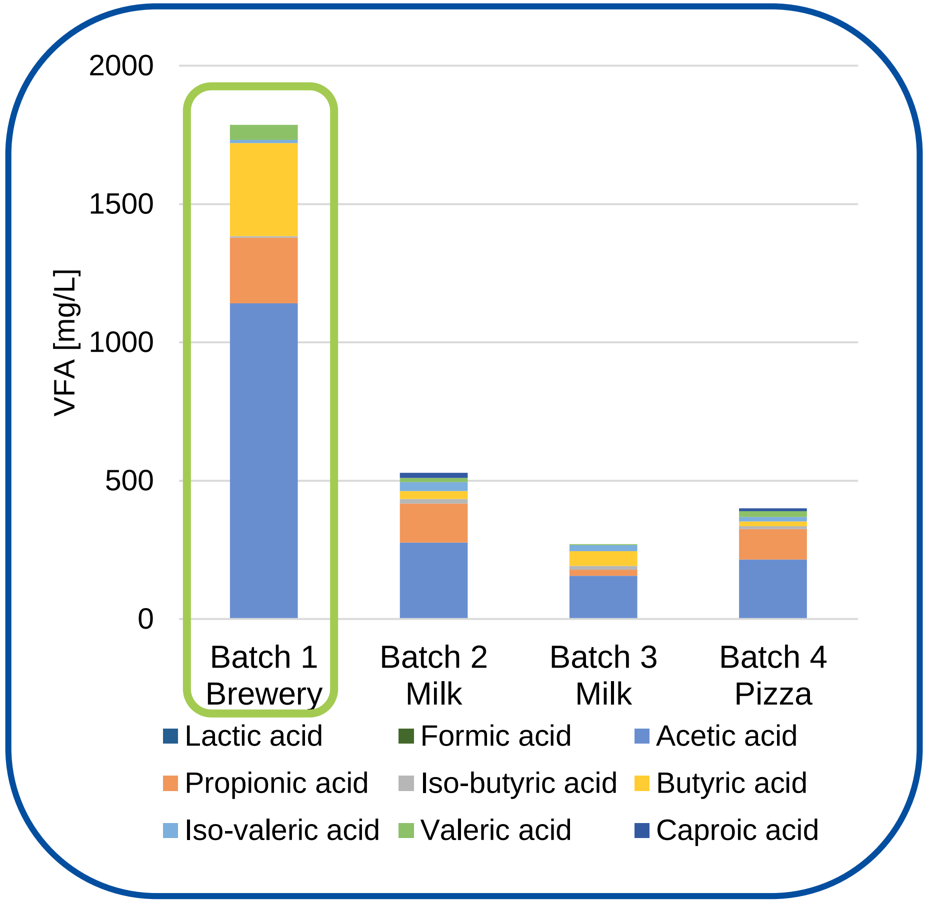

In Germany…


…& in the Netherlands

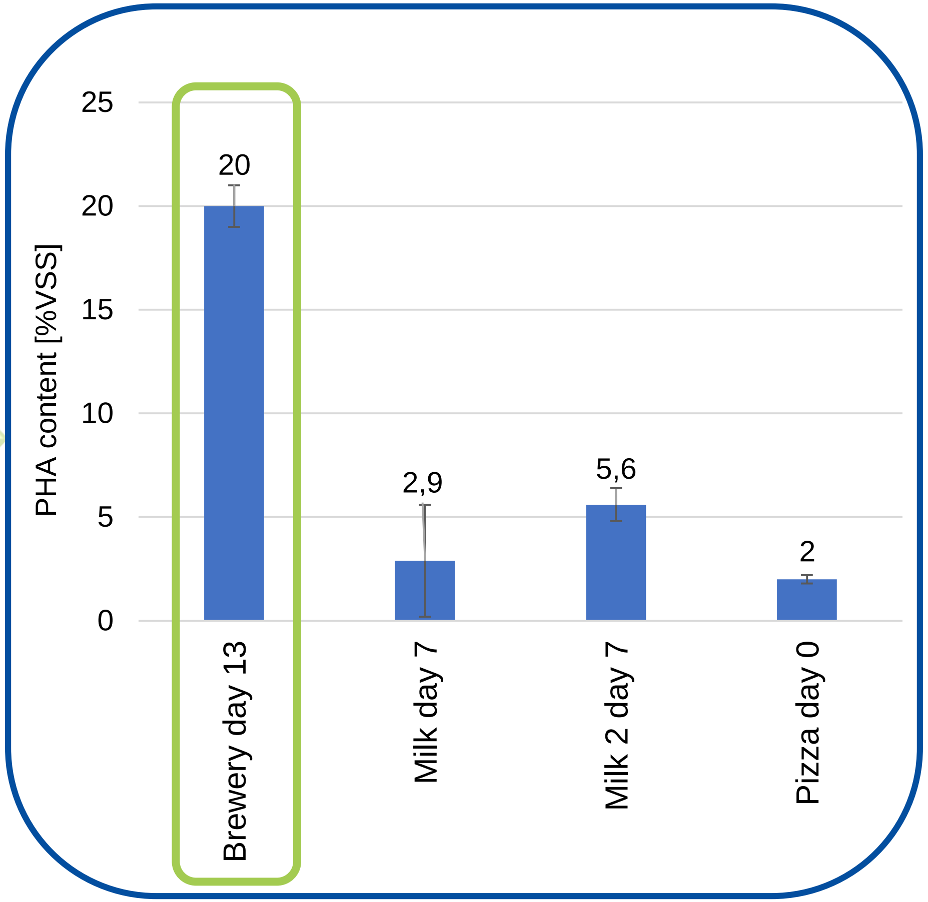
Lab-scale results:

Brewery's residual stream achieved the best results

VFA-concentration after acidification



PHA-content after accumulation

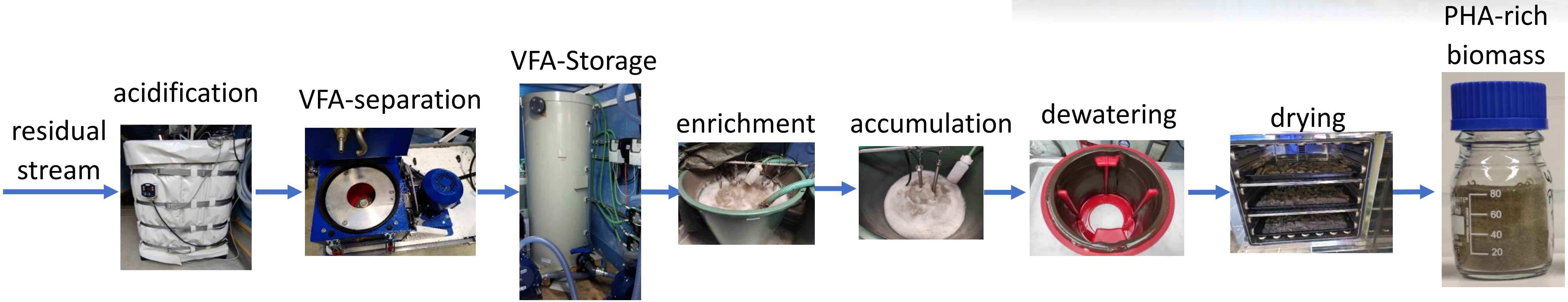


Laumeyer et al. (2022): Technical report on suitability for PHA production of residual streams of different food processing companies. Project report.

→ Results

Process of PHA production in pilot scale

- Operation: 07/2022 – 03/2023 with 2 different residual streams from the food industry
- 20 weekly acidification batches without pH control at 34 – 36°C
- 3 enrichment cycles of 2 – 4 weeks per residual stream
- 2 – 3 accumulations per week, no pH-control



Objectives of the pilot plant operation

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Research questions during the production of PHA from residual streams of the food industry

- Investigating different residual streams of the food industry for PHA-production at pilot scale
- Characterisation of the VFA-rich substrates
- Investigating the influence of the substrate composition on the PHA-production process
- Characterisation of the produced PHA

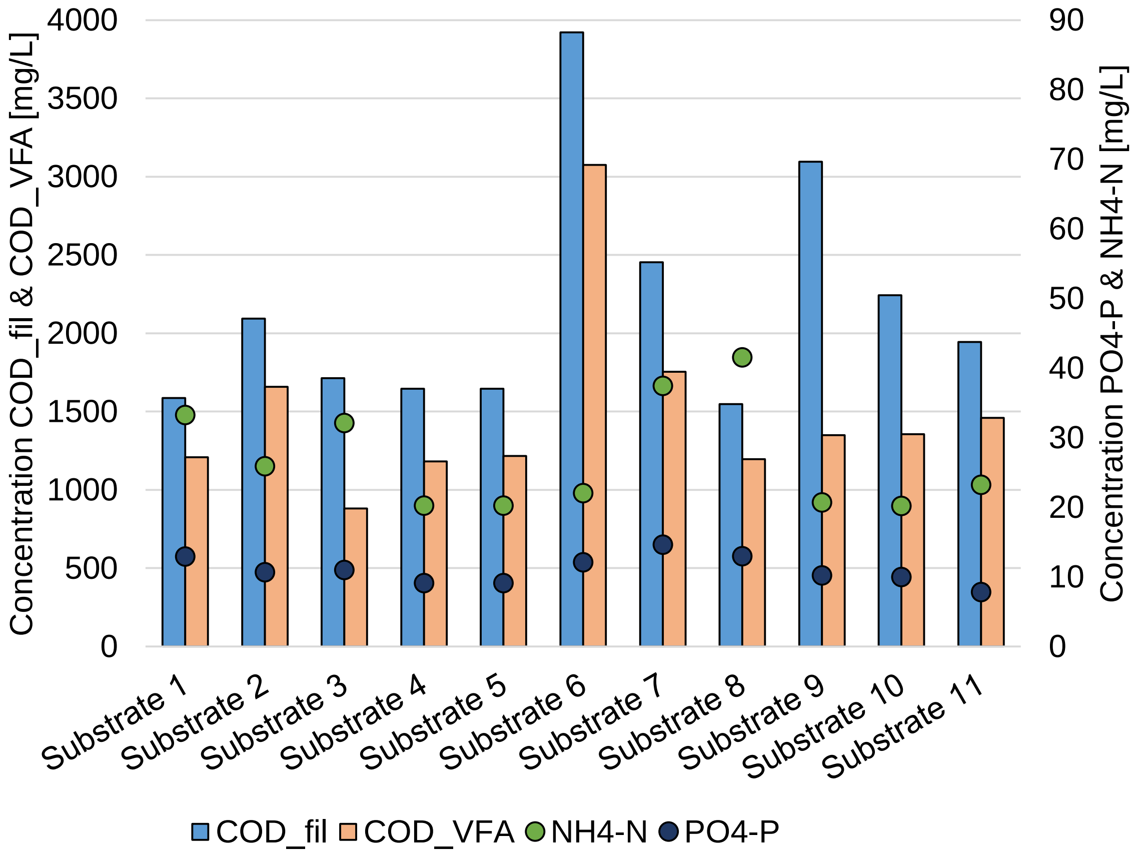
Properties of the residual streams

- Production all year around
  → composition of the residual stream rather stable

- Brewery has on-site treatment & a buffer tank

Produced substrate
- C:N:P–ratio*
  100 : 2.0±0.9 : 0.8±0.2 (n=11)
- COD_{VFA}/COD_{fil}: 0.69 ± 0.12

Brewery



*(COD_{VFA}:NH_4-N:PO_4-P)

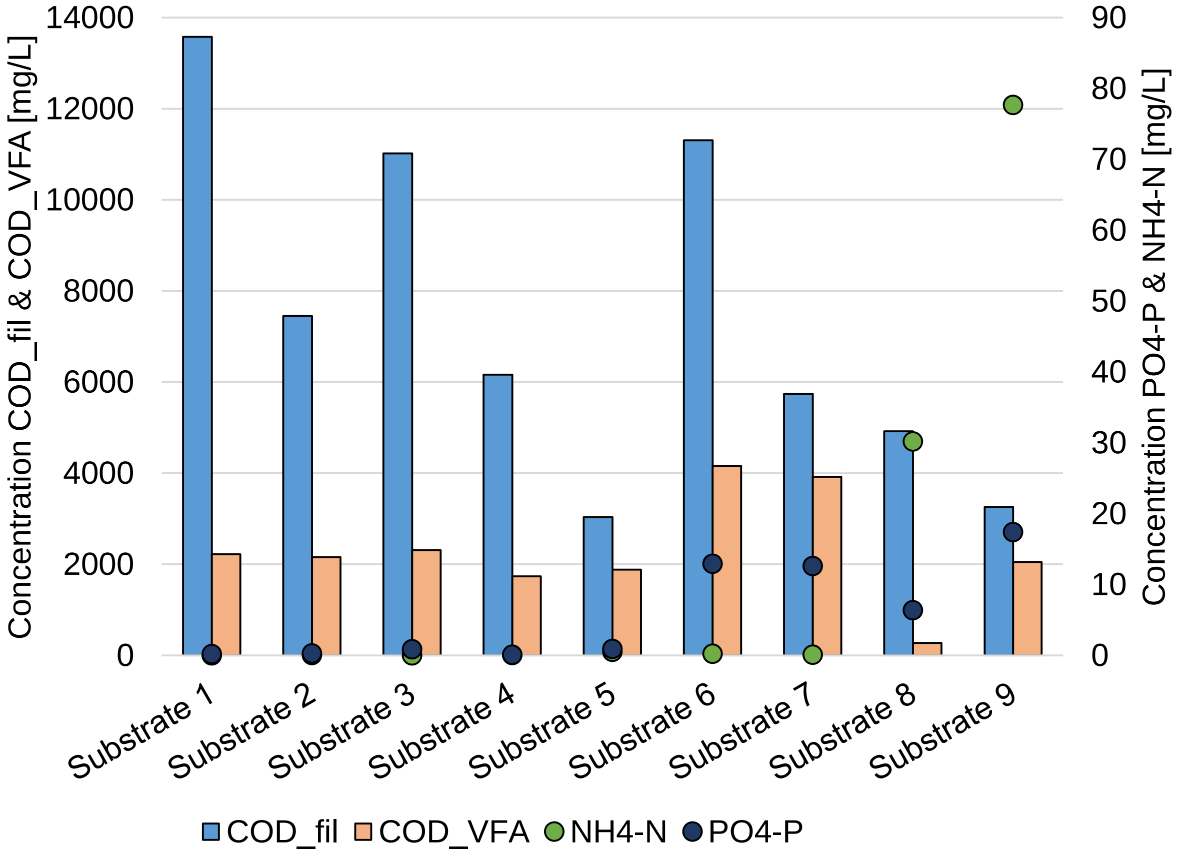
Properties of the residual streams

- Production during autumn, bottling year around
  → different residual streams
  → availability changes during the year

- Juice factory is connected to a municipal STP

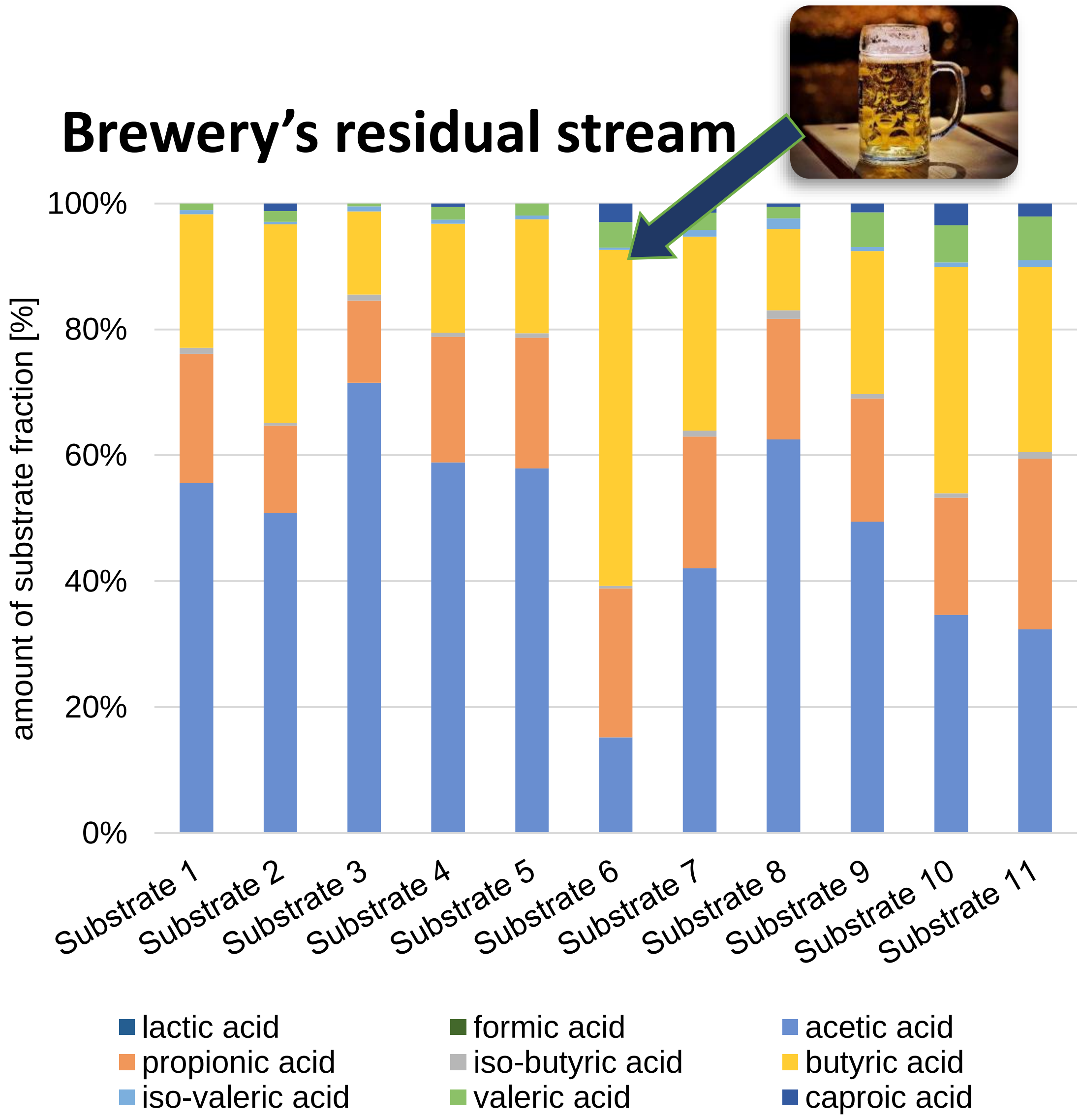
Produced substrate
C:N:P–ratio*
100 : 0.5±1.3 : 0.2 ±0.3 (n=8)
COD_{VFA}/COD_{fil}: 0.37 ± 0.23

Fruit juice company



*(COD_{VFA}:NH_4-N:PO_4-P)

VFA-composition after acidification



VFA	Molar fraction	Min-Max
acetic acid	0.48 ± 0.15	0.15 – 0.71
propionic acid	0.20 ± 0.04	0.13 – 0.27
iso-butyric acid	0.01 ± 0.00	0 – 0.01
butyric acid	0.26 ± 0.11	0.13 – 0.53
iso-valeric 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

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VFA-composition after acidification

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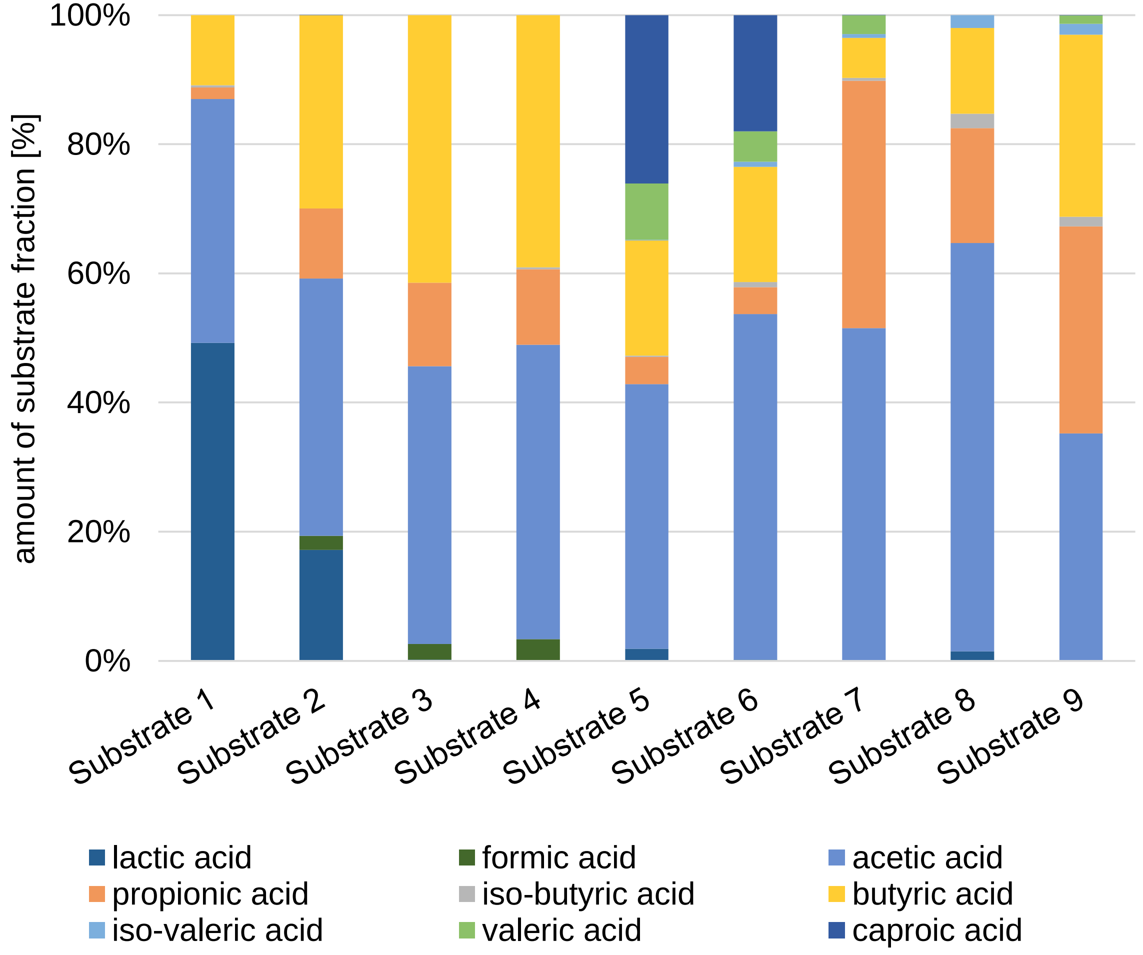
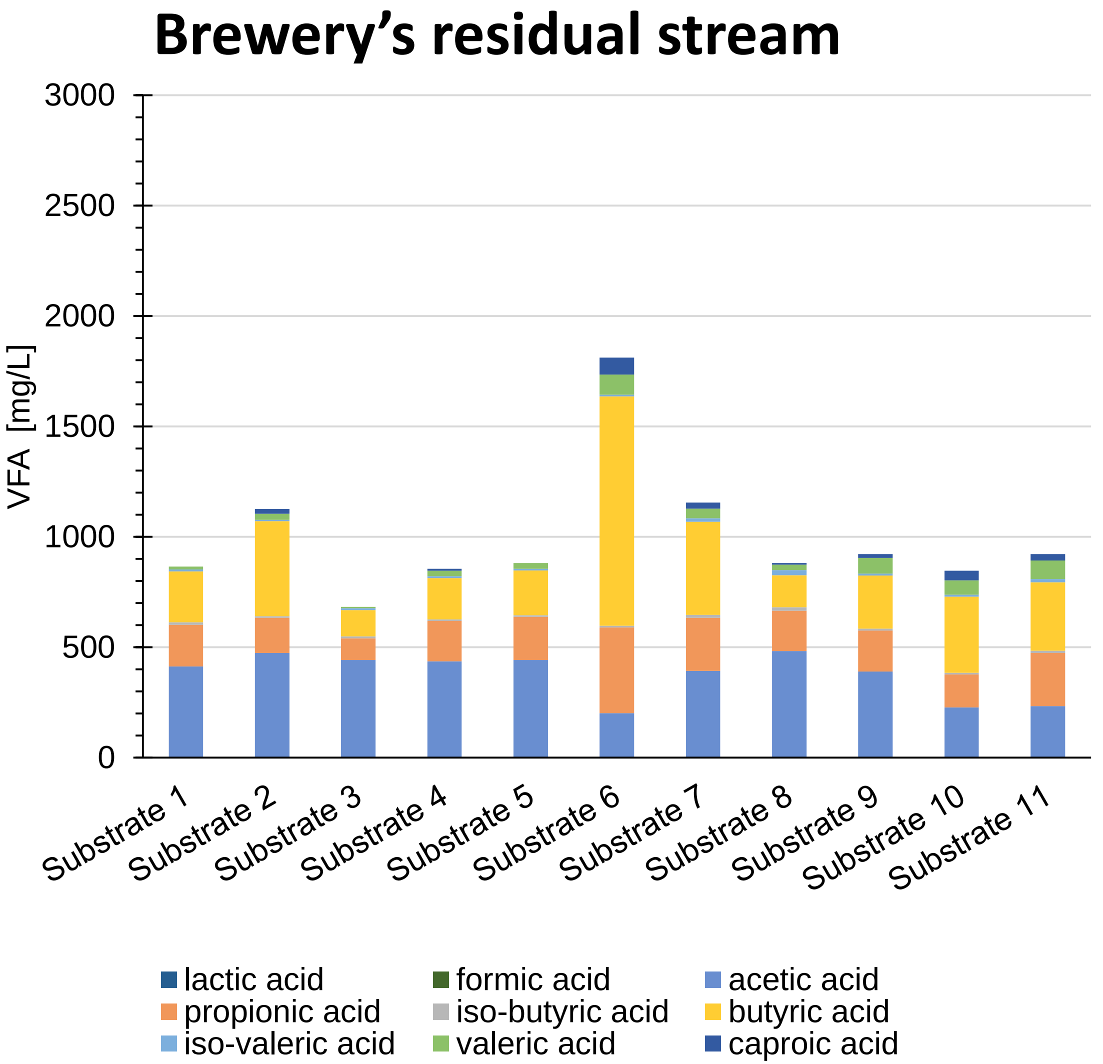
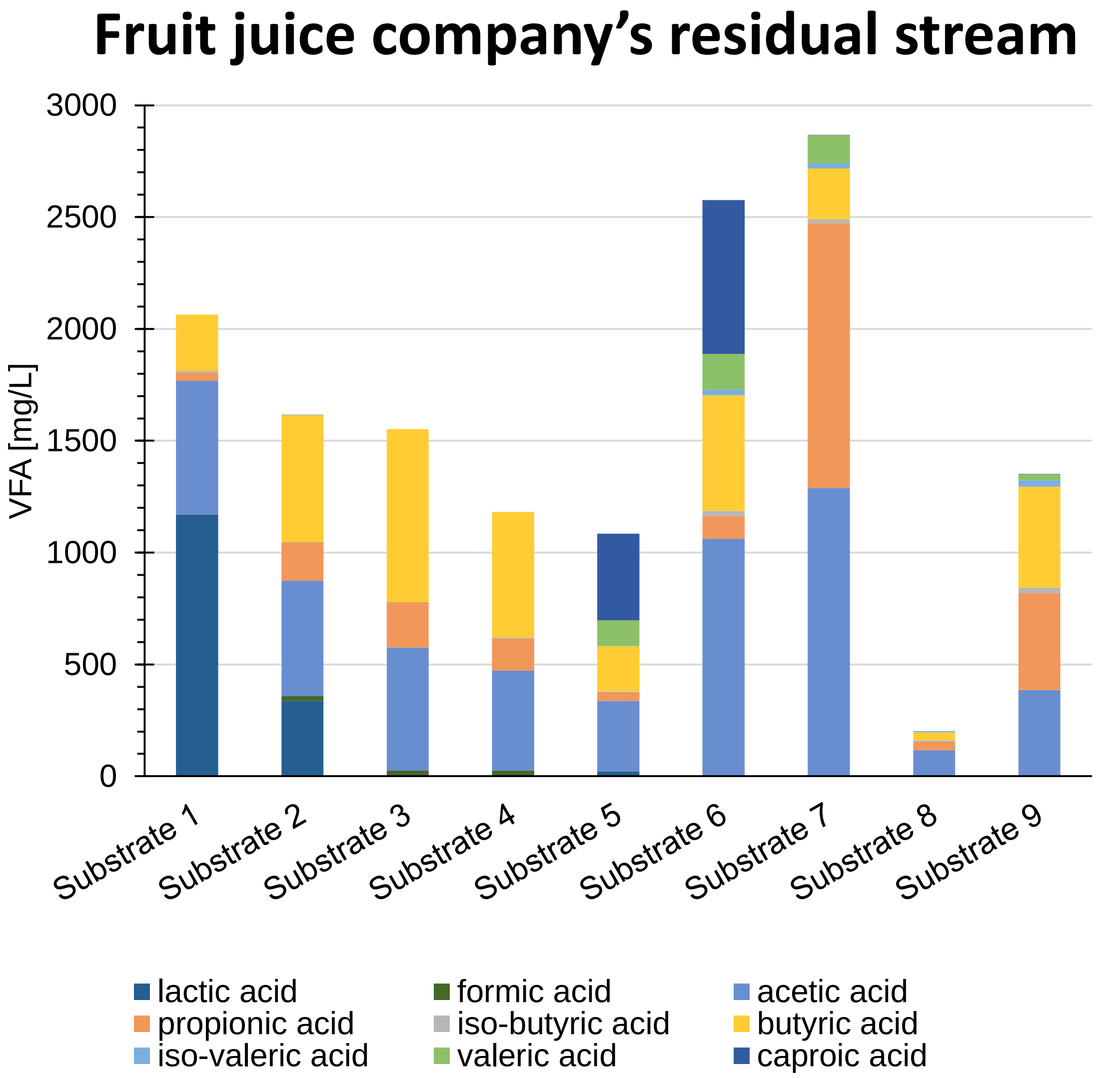
Fruit juice company's residual stream



VFA	Molar fraction	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
iso-butyric acid	0.01 ± 0.01	0 – 0.02
butyric acid	0.23 ± 0.12	0.06 – 0.41
iso-valeric 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

VFA-composition after acidification





VFA-composition after acidification

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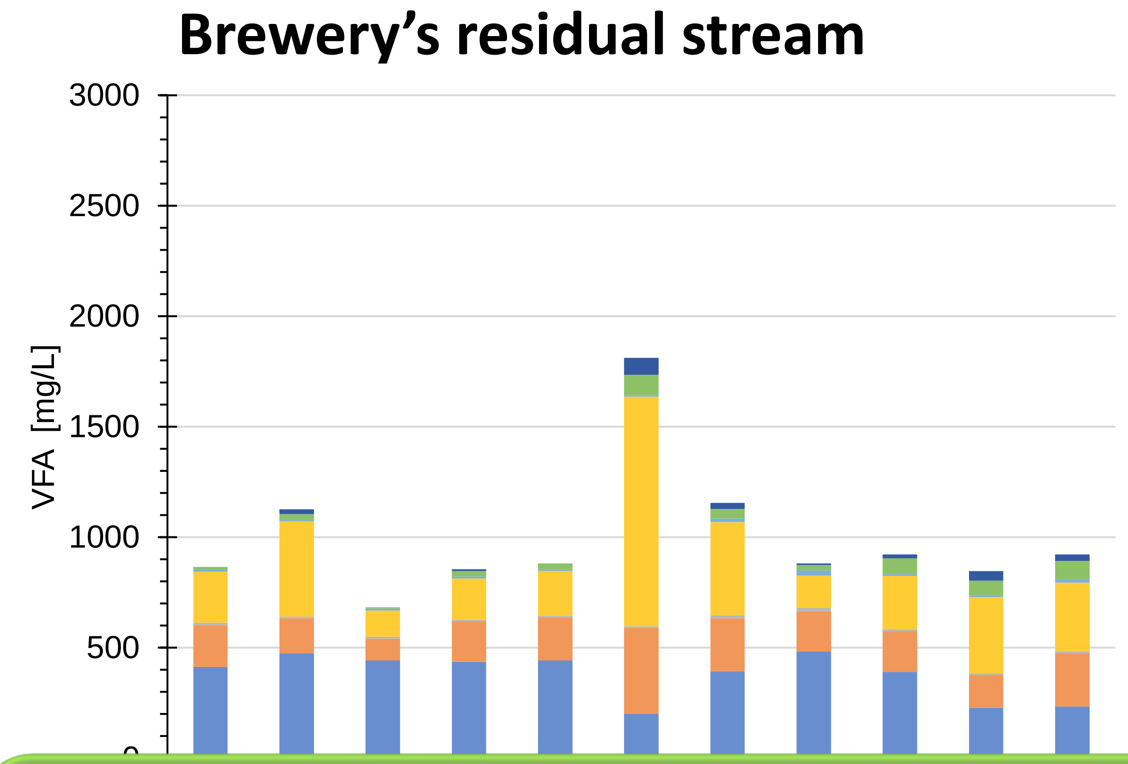
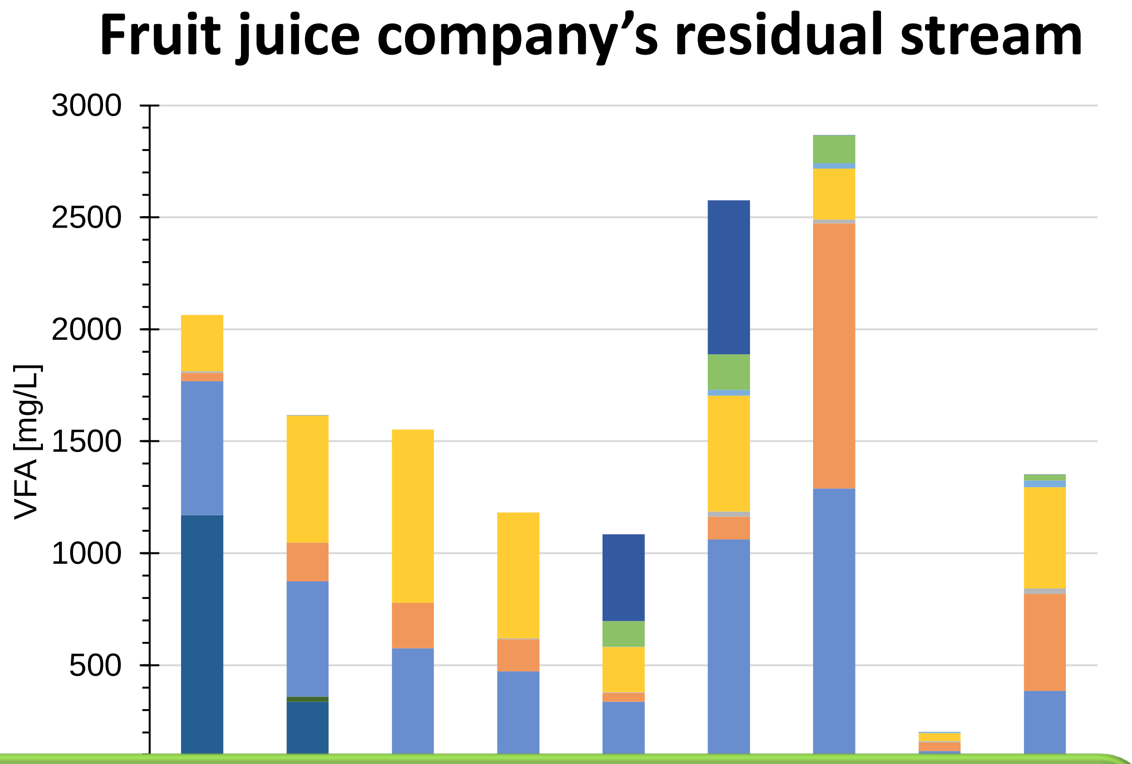
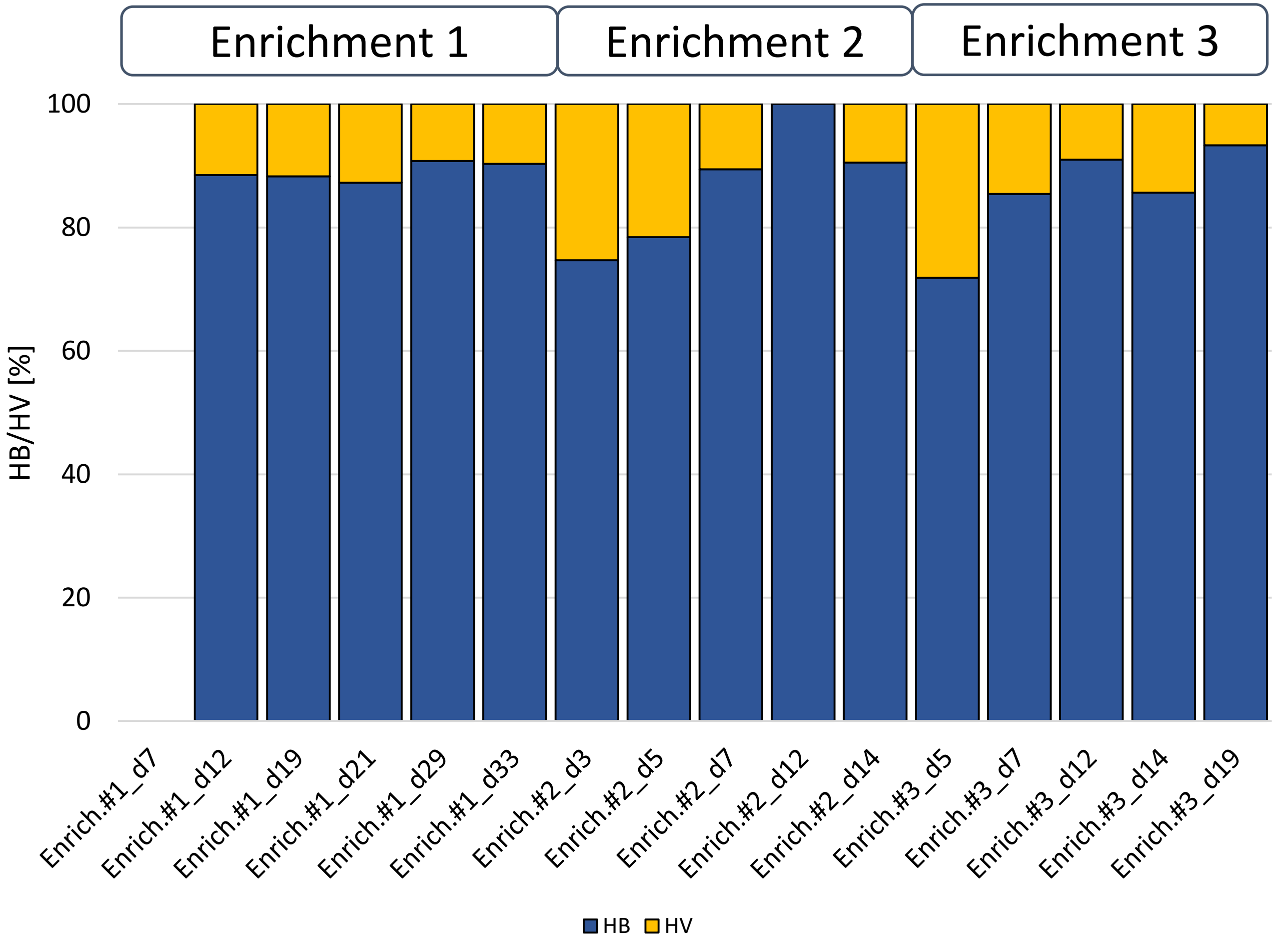




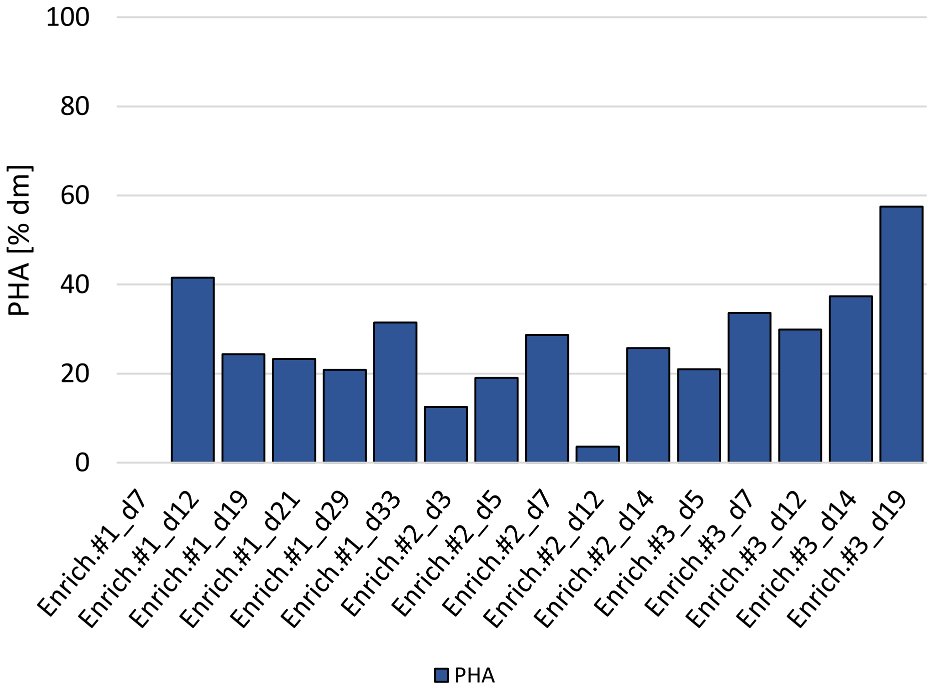
- **Different residual streams** result in **substrates** with a **different VFA composition**
- Not only differences between the substrates of the 2 industries, but **also between batches** of the same residual stream
- Residual stream of the **fruit juice company** resulted in **higher COD_{VFA}**, but **lower ratio of COD_{VFA}/COD_{fil}**

PHA-Accumulation results

Brewery’s residual stream

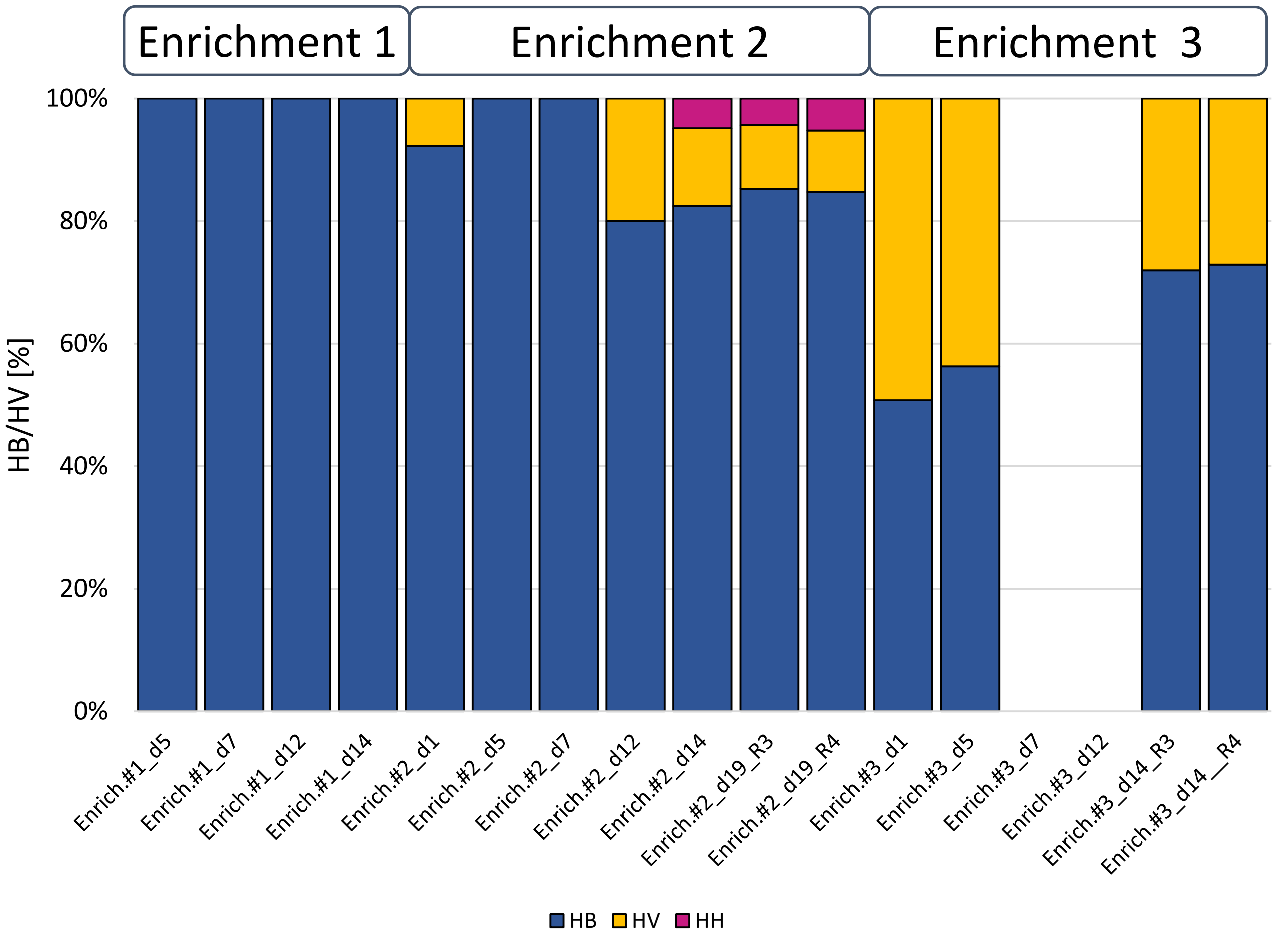


PHA	Fraction of PHA	Min-Max
HB	0.81 ± 0.22	0.0 – 1.00
HV	0.13 ± 0.8	0.0 – 0.30

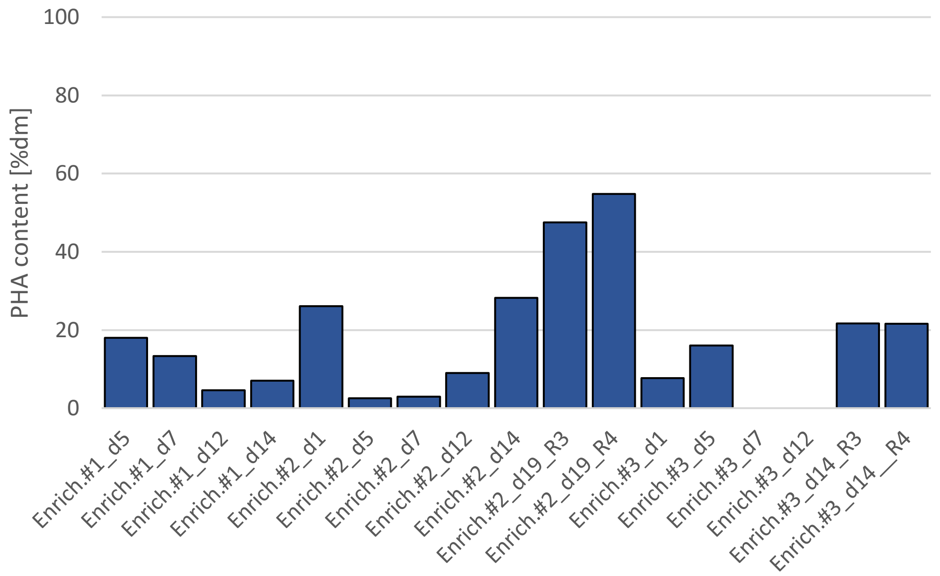


PHA-Accumulation results

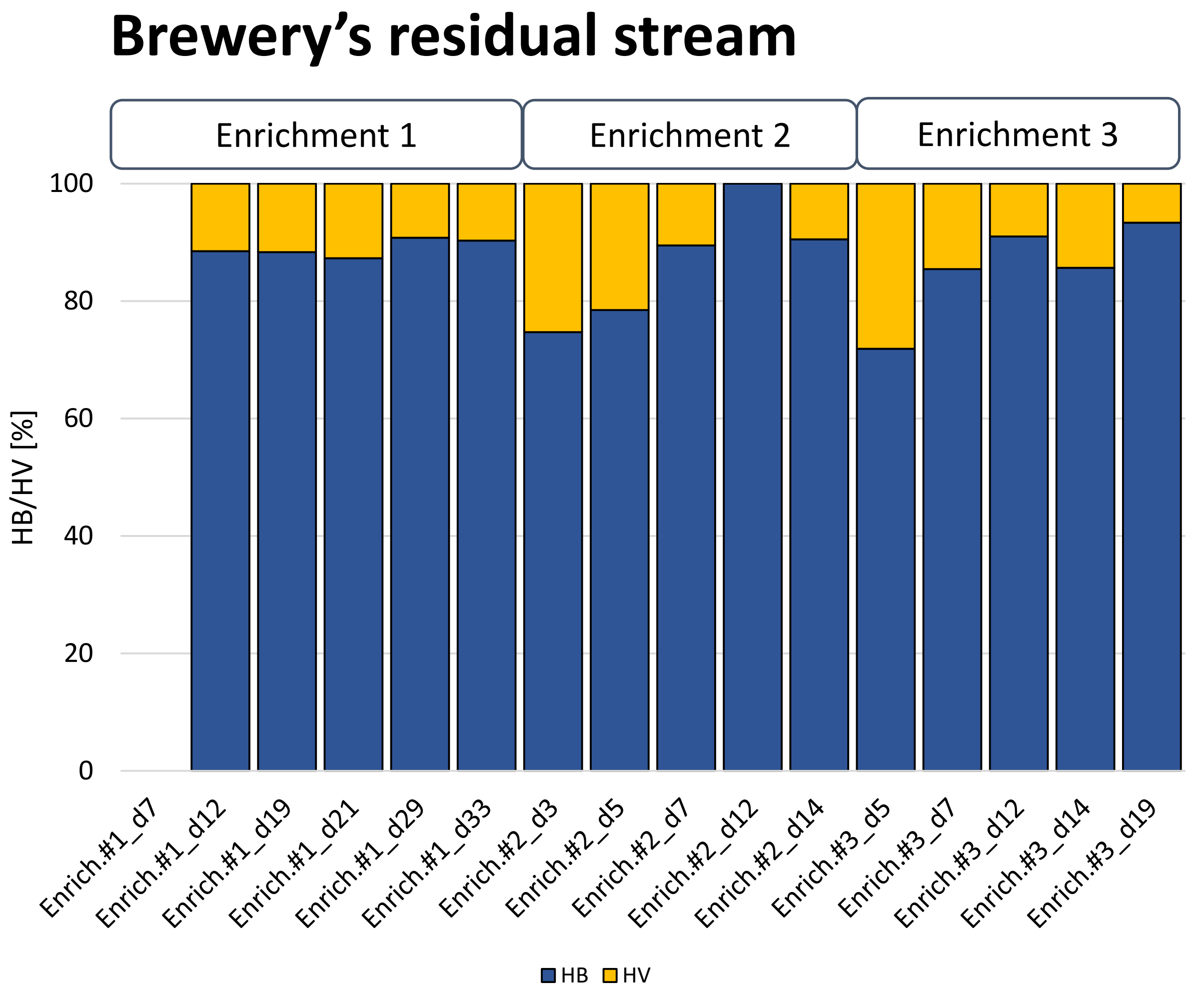
Fruit juice company's residual stream

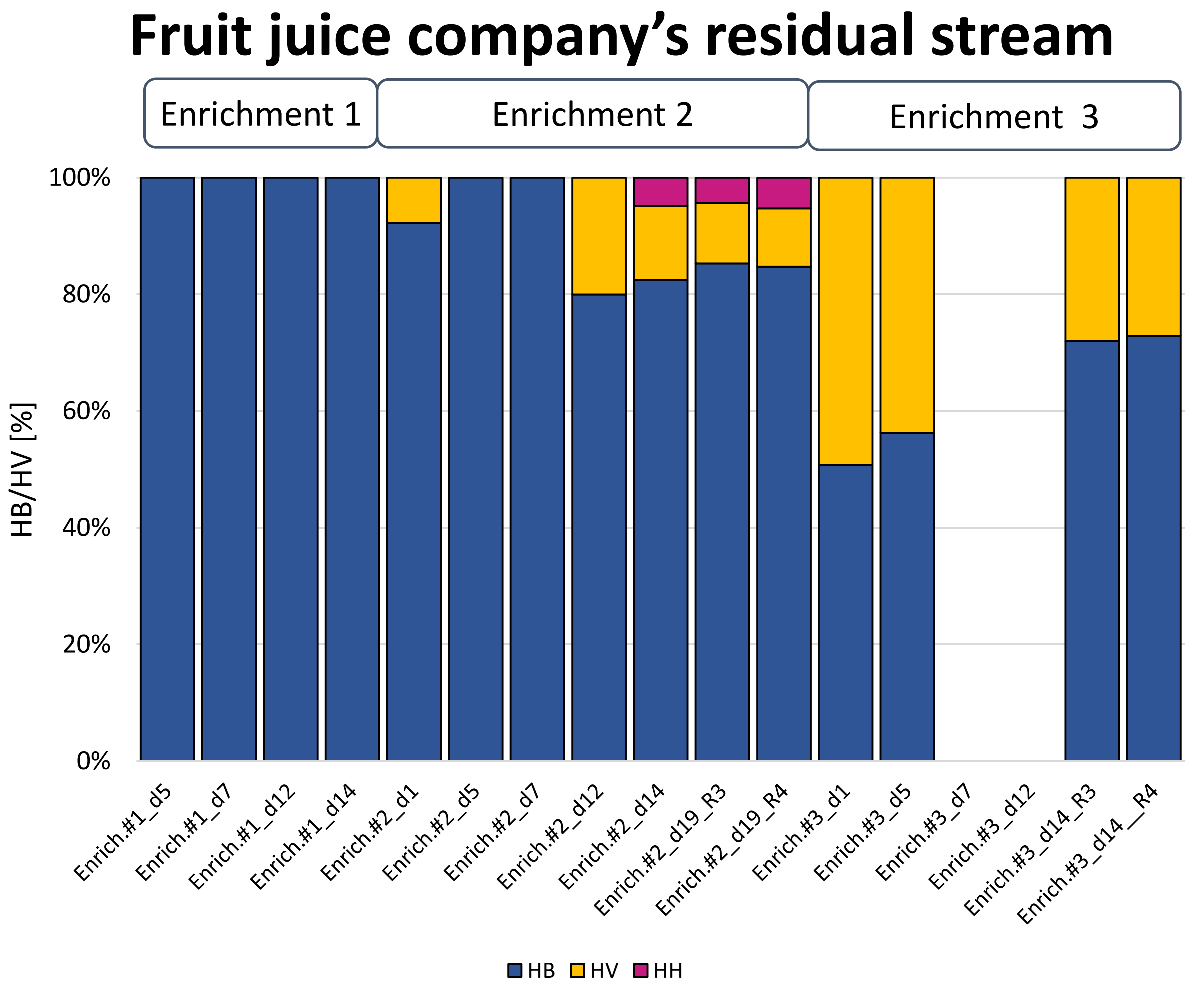


PHA	Fraction of PHA	Min-Max
HB	0.79 ± 0.27	0.0 – 1.00
HV	0.13 ± 0.16	0.0 – 0.49
HH	1.2 ± 2.2	0.0 – 5.3



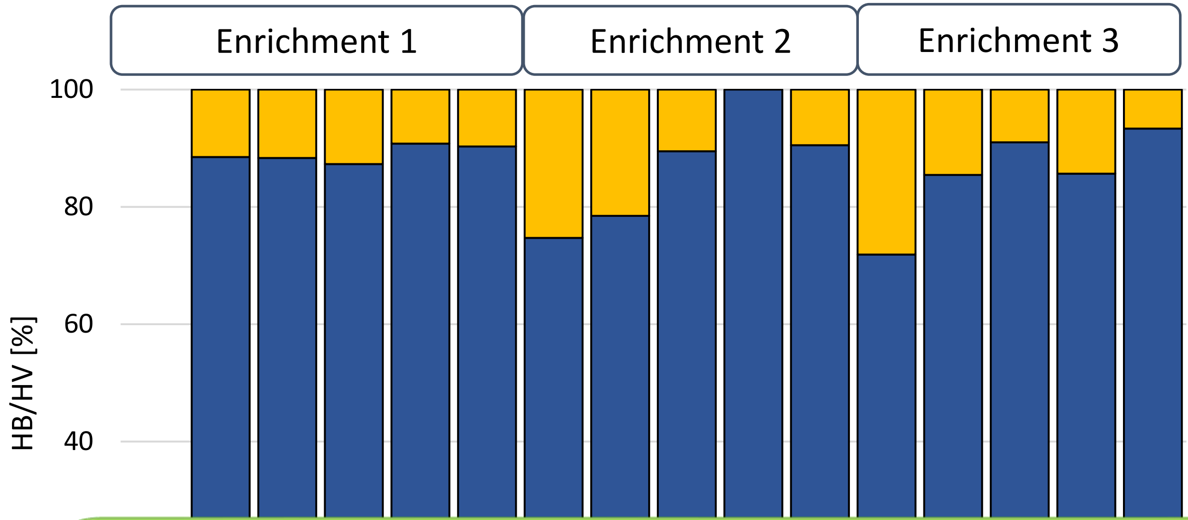
PHA-Accumulation results



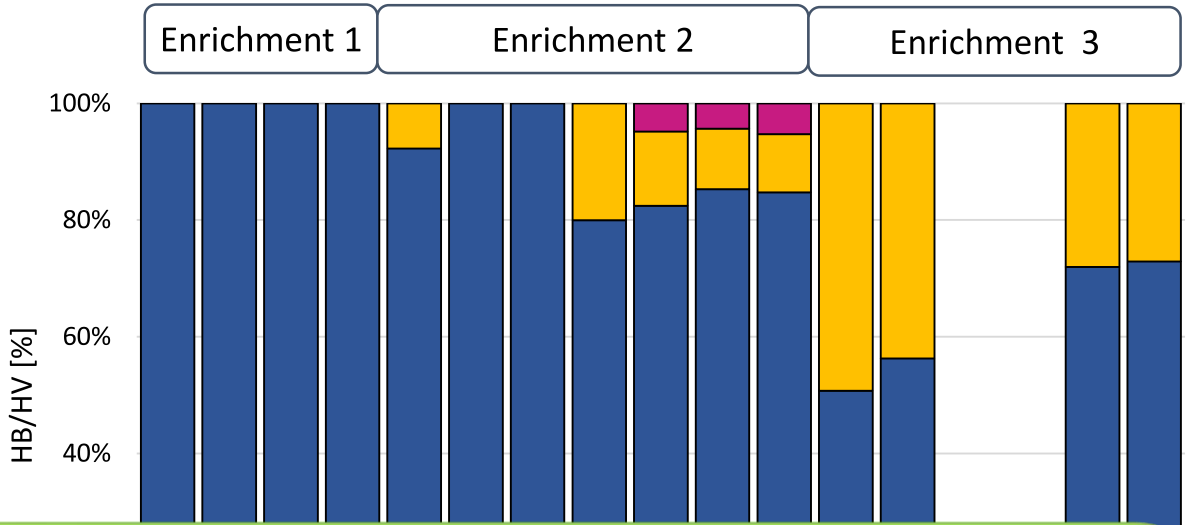


PHA-Accumulation results

Brewery's residual stream

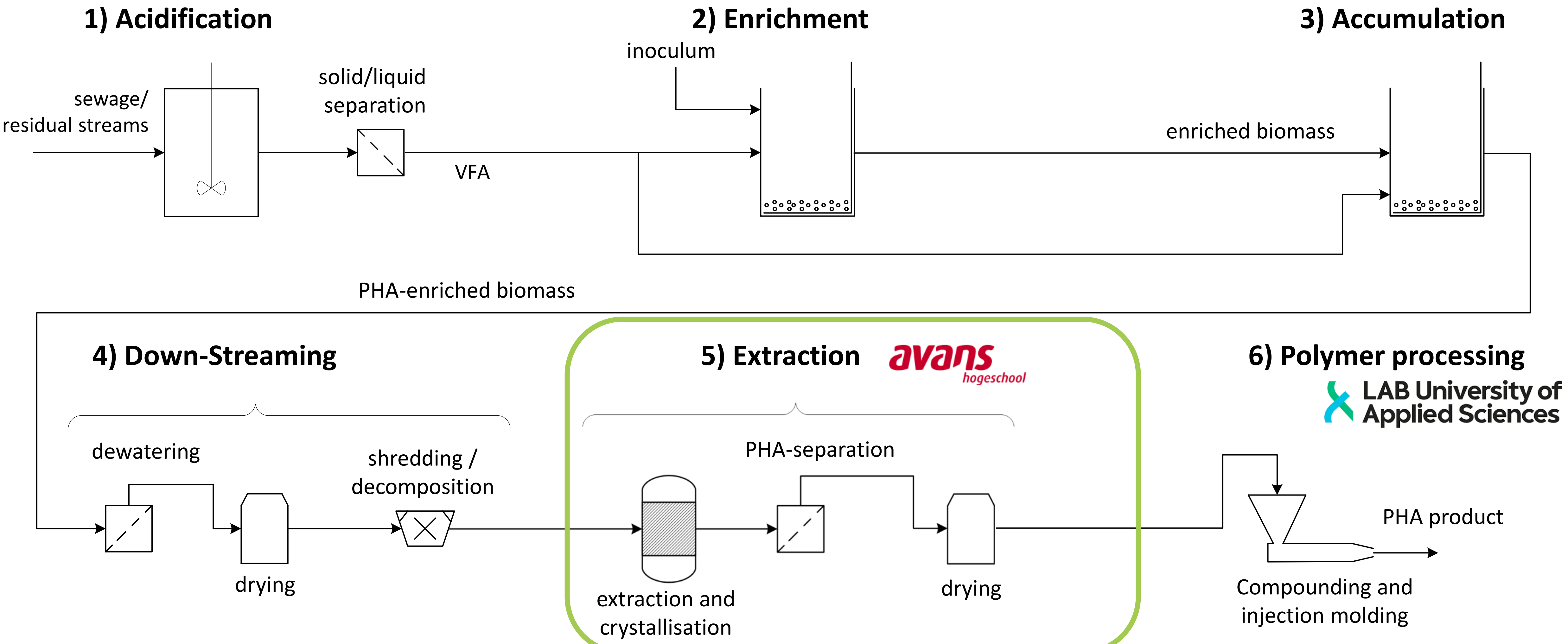


Fruit juice company's residual stream



- **PHA mainly** consists of **HB** for **both residual streams**
- **Different VFA composition** causes **differences in the PHA composition**
- Substrate rich in **caproic acid** causes **hydroxyhexanoate (HH) production**
- **PHA** produced from **brewery** have a **more stable composition** compared to the residual stream of the fruit juice company
- **Future objective:** adjust HB/HV composition to the needs of the plastic industry

PHA-production – production chain



Modified from: Uhrig, T. (2023): Eignung von kommunalen und industriellen Abwasserströmen für die PHA-Produktion und Ansätze zum Up-Scaling des Prozesses. Unpublished dissertation.

The perspective of Downstream Process (DSP)

- 14,000 tons PHA per million PE (based in 30 g-activated sludge dry solids per PE per year).[1]
- Opportunities
- Production quality control → targeted application

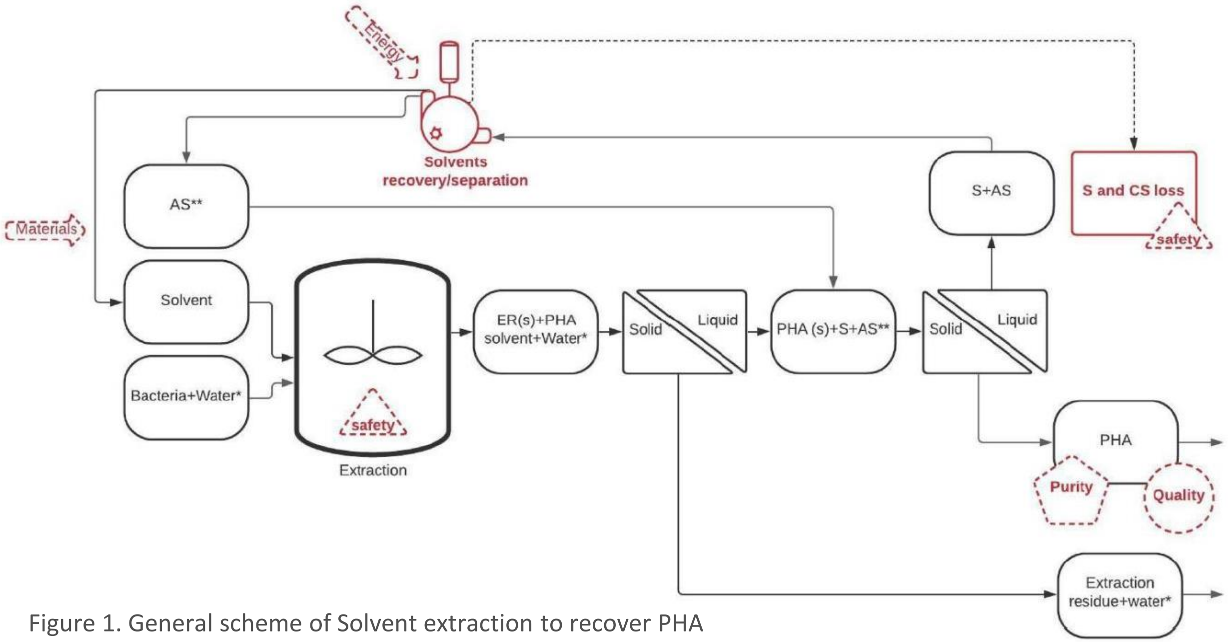


Figure 1. General scheme of Solvent extraction to recover PHA
Molenveld, K., Post, W., Ferreira, S. F., de Sévaux, G., & Hartstra, M. (2022). Paving the way for biobased materials : a roadmap for the market introduction of PHAs. (Report / Wageningen Food & Biobased Research; No. 2240). Wageningen Food & Biobased Research. https://doi.org/10.18174/561676

[1]R. Pei, A. Estevez-Alonso, L. Ortiz-seco, M.C.M. van Loosdrecht, R. Kleerebezem, A. Werker, Exploring the limits of polyhydroxyalkanoate production by municipal activated sludge, Environ. Sci. Technol. 56 (2022) 11729–11738, https://doi.org/10.1021/acs.est.2c03043.

The perspective of Downstream Process (DSP)

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- 2 main recovery methods: solvent and cellular lysis (disruption of the cell membranes)

Table 1. Comparative extraction methods

	Chemicals used	Advantages	Disadvantages
Extraction with solvent	Halogenated solvents, alcohols, esters, carbonates, ketones	High quality PHA and solvent recovery	Expensive and not environmental friendly
Cellular lysis	Oxidants, acids, alkalis, surfactants, enzymes	Can use wet biomass, low cost, better environmental performance	Lower quality PHA, not suitable for MMC*, recovery of additives is difficult

*MMC – Mixed microbial culture

Molenveld, K., Post, W., Ferreira, S. F., de Sévaux, G., & Hartstra, M. (2022). Paving the way for biobased materials : a roadmap for the market introduction of PHAs. (Report / Wageningen Food & Biobased Research; No. 2240). Wageningen Food & Biobased Research. https://doi.org/10.18174/561676

- When applying solvent extraction → process design.
- Investments in application developments require **stable supplies of specific and reproducible PHA grades** with well-defined polymer property specifications. Such specifications are tied to both the **monomer composition** and its distribution, as well as the **molecular weight distribution (MW)[2]**.

[2]Werker, A.; Pei, R.; Kim, K.; Moretto, G.; Estevez-Alonso, A.; Vermeer, C.; Arcos-Hernandez, M.; Dijkstra, J.; de Vries, E. Thermal pre-processing before extraction of polyhydroxyalkanoates for molecular weight quality control. Polym. Degrad. Stab. 2023, 10.1016/j.polymdegradstab.2023.110277

Solvent selection & sustainability

- Until recently, mainly organic solvents (chloroform, dichloromethane, acetone, ethyl acetate, 1-butanol) were used.
- Criteria:
  1. Safety, health, and environment
  2. PHBV solubility potential
  3. Solvent recovery from residual biomass
  4. Solvent regeneration
  5. Costs

Solvent	Solubility (g/L)
Chloroform	98
MEK	59
Cyclohexanone	56
Ethyl Acetate	51
THF	47
Acetone	43
DMC	41
Toluene	34
Anisole	31
MIBK	24
Butyl Acetate	21
Xylene	14
Methanol	12
DIBK	8
Ethanol	5
1-Propanol	2
2-Propanol	2
2-Ethylhexanol	2
Acetonitrile	2
1-Butanol	1
2-Butanol	1
Water	1
1-Pentanol	<1
Pent/Hept	<1

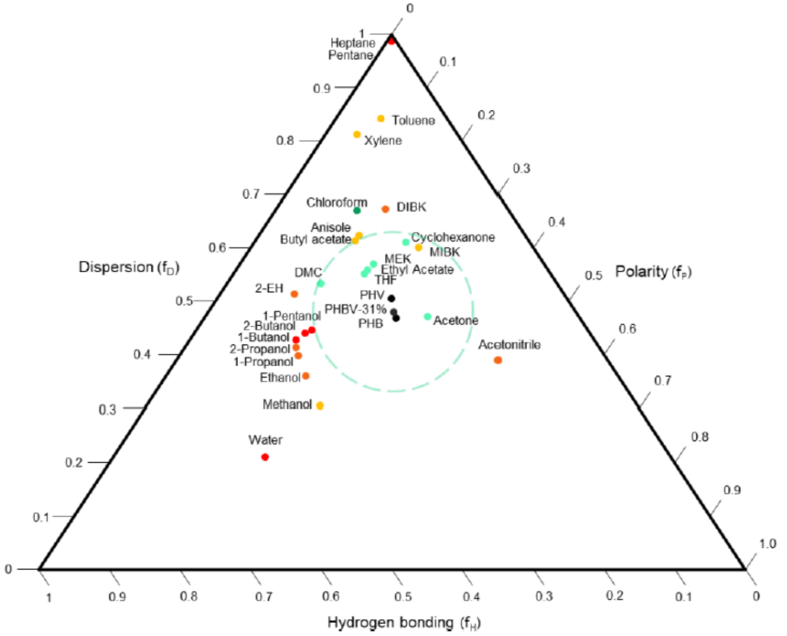

Figure 2. Solubility ternary graph of PHBV-31% test with different solvents at Delft University of Technology. Chloroform is the only exception outside the so-called solubility window.

Vermeer, Chris M. and Nielsen, Maaike and Eckhardt, Vincent and Hortensius, Matthijs and Tamis, Jelmer and Picken, Stephen J. and Meesters, Gabrie M. H. and Kleerebezem, Robbert, Systematic Solvent Screening and Selection for Polyhydroxyalkanoates (Phbv) Recovery from Biomass. http://dx.doi.org/10.2139/ssrn.4122159

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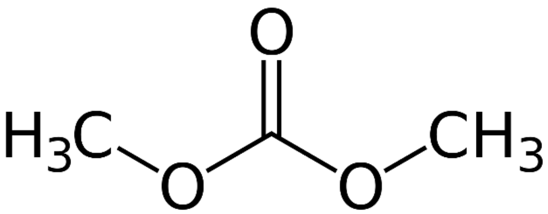
Solvent selection & sustainability

- Non-chlorinated solvents, when sufficiently heated, will diffuse into the PHA matrix, break up crystallinity and dissolve the resulting amorphous polymer mass into solution.

- Research group Biobased Resources & Energy developed a green extraction method using **dimethyl carbonate (DMC)**
  - low boiling point
  - low cost
  - non-toxic
  - can be reused
  - when it enters the environment, it will be hydrolyzed into harmless products
  - Recently studies show that DMC has less reactivity to PHBV[3].



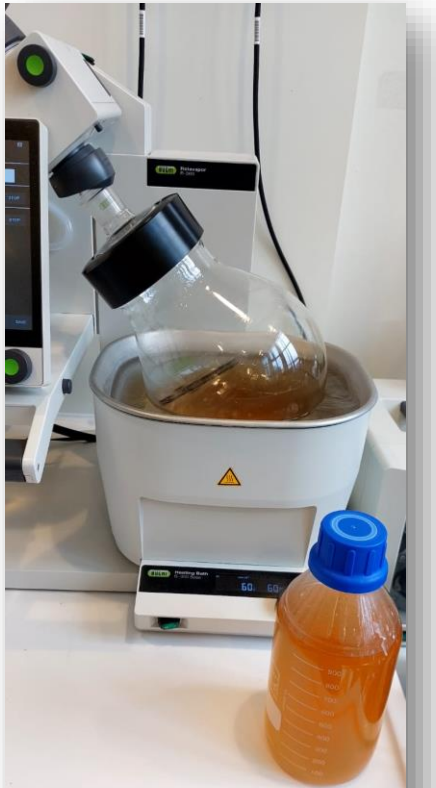


Figure 3. Filtered DMC solution containing extracted PHA. Production from the University of Kaiserslautern-Landau (RPTU)

[3] Vermeer, Chris M. and Nielsen, Maaike and Eckhardt, Vincent and Hortensius, Matthijs and Tamis, Jelmer and Picken, Stephen J. and Meesters, Gabrie M. H. and Kleerebezem, Robbert, Systematic Solvent Screening and Selection for Polyhydroxyalkanoates (Phbv) Recovery from Biomass. http://dx.doi.org/10.2139/ssrn.4122159

PHA extraction optimization

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Looking at several parameters such as:

- Yield
- Extraction time
- Purity
- Solvent use

A very **small variance**, with a maximum 2%, of PHA yield was observed for different **extraction times or biomass to solvent ratios**.

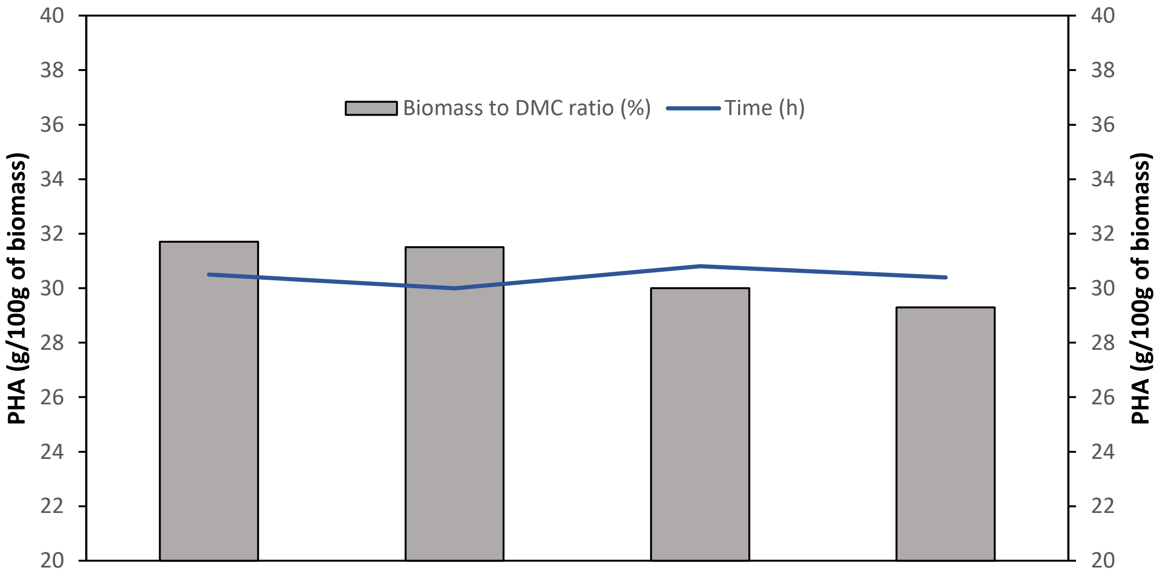

Figure 4. PHA yield influenced by extraction time and biomass to solvent ratio.

Table 2. Comparative extraction results for the different solvents

Solvent	PHA content (wt.%)	Purity (%)
Dimethyl carbonate	31.7 ± 0.2	91.2 ± 0.1
Chloroform	37.5 ± 0.2	82.5 ± 3.3
Dichloromethane	39.0 ± 0.2	86.4 ± 3.7

PHA with **higher purity** was extracted with DMC

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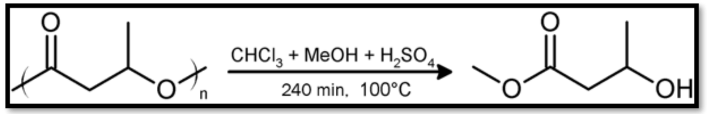
PHA Extraction Method

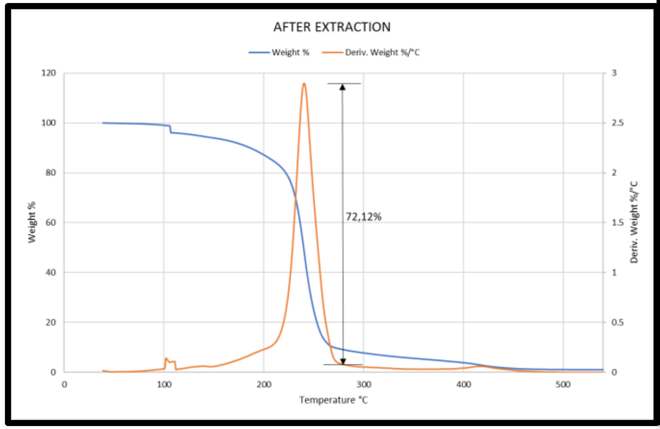
Extraction
- DMC
- Biomass/DMC ratio 10%

Determination of purity
- TGA

PHA monomer composition
- Acidic methanolysis
- GC-FID



Thermogravimetric Analysis



Gas Chromatography

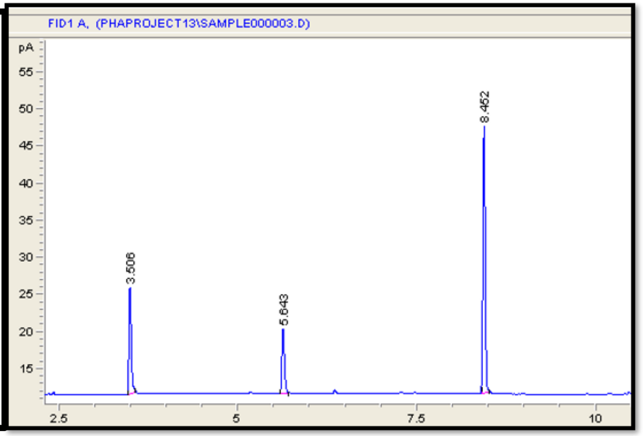
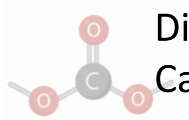
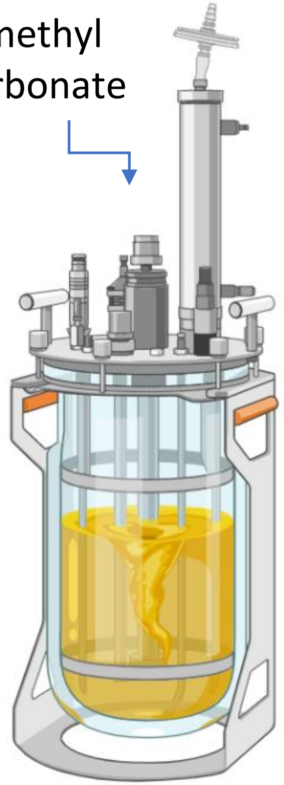


Figure 5. TGA and GC-FID response graph for PHA-rich biomass

Lab-scale Set-up for extraction PHA-biomass



Dimethyl Carbonate

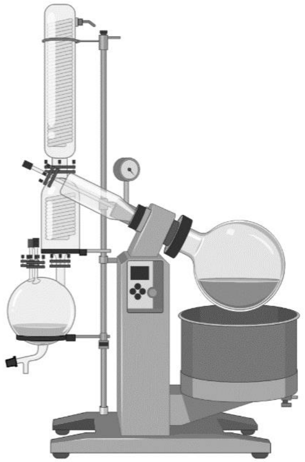


1L reactor
Loading based on theoretical extraction concentration 20 – 50 mg PHA/ml

Filtration of residual biomass

- Work at boiling point → increase solubility

  Load capacity up to 300 g.



Solvent recovery

PHA

PHA-rich biomass

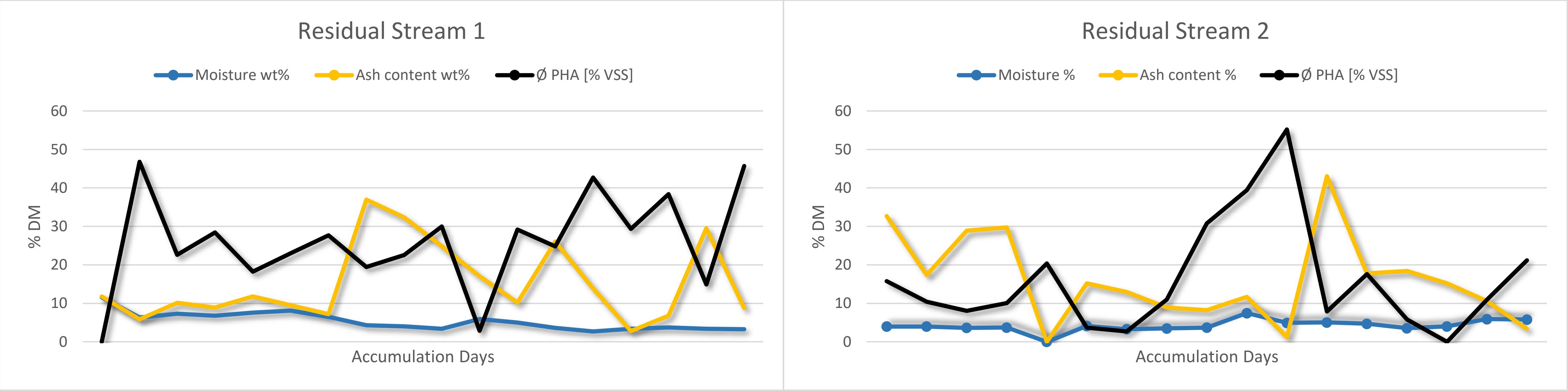
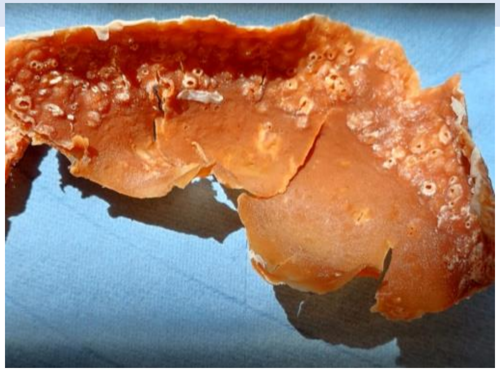


Figure 6. PHA-rich biomass from different residual streams received at Avans to be extracted

- The substrate acidification step, VFA stream composition, and feed strategy are some of the parameters that can influence the DSP.
- Ashes might influence the PHA content in the biomass but it is not a limiting factor
- The impurities present in the biomass affect the solubility range of the solvent

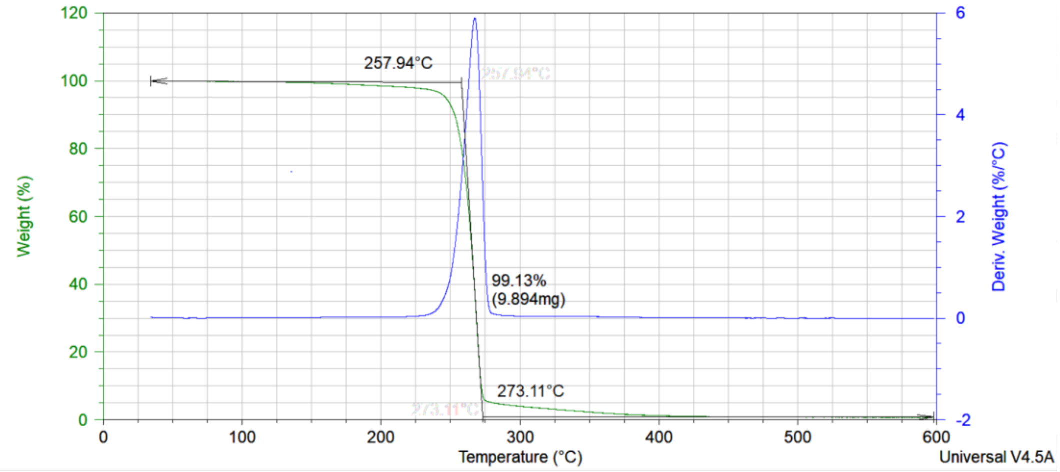
Extraction results

	Residual Stream 1	Residual Stream 2
Moisture, wt%	3,85	4,48
Ash content, wt%	23,68	14,66
PHA content, DM%	20,15	19,39
Purity, DM%	72%	-
Extraction yield (g PHA material like/100 g intercellular PHA)	84%	-



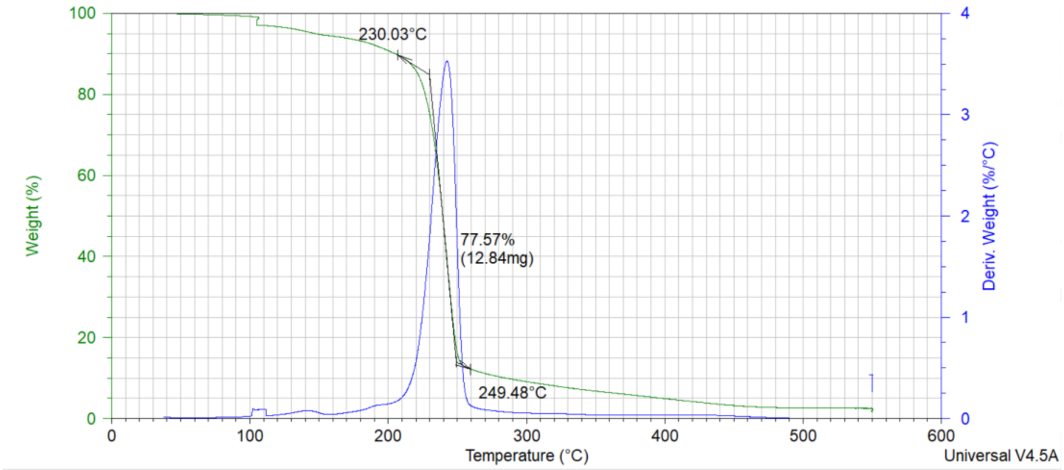
- ➢Extraction of Residual Stream 1 shower higher viscosity although both biomass has averaged similar PHA composition.
- ➢Solvent viscosity changes due to the temperature, the dissolved polymer concentration, and its average molecular mass (not evaluated in this work).

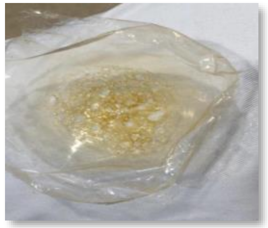
Purity

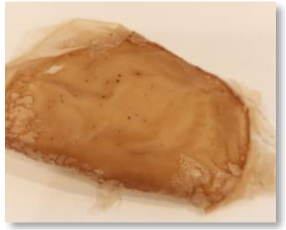


Thermal degradation of the polymer around 250°C.

- Influenced by the contaminants present in the biomass that could not be removed in the filtration step.









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SEVERN TRENT

KEEP IN CONTACT

Thank you for your attention!

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