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1 Executive summary

The AFTERLIFE project proposes an innovative process for recovering and valorising relevant fractions from food industrial wastewater (WW). The AFTERLIFE process is able to separate the different components of value present in WW by means of technologies specifically designed for the purpose. These fractions will then be treated to obtain value-added biopolymers, Polyhydroxyalkanoates (PHAs). In addition to the value extracted from the solid fraction, the remaining outflow of the water will be ultrapure and ready for re-use. Finally, what remains as waste from the developed process is used as a useful raw material for biogas production through anaerobic fermentation. The project is funded by Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme (https://afterlife-project.eu).

As part of the project, nova-Institut explored the environmental sustainability of the process in order to guide the process design optimization. A first hotspot life cycle assessment (LCA) was carried out at an early stage of the development, assessing the lab scale process design. This study can be found in D7.1 “Hot spot LCA analysis for further optimization” (delivered on January the 31st, 2020). The present study, D7.2 “Final Life Cycle Assessment”, assessed the pilot scale operation. It is based on the latest information from upscaled experiments, thus showing interesting insights into how the sustainability of the process is affected by a larger scale operation. In contrast to the first study, which examined the AFTERLIFE processes developed for 4 different WW feedstocks, this study only examines the pilot scale process with the Jake WW (WW from the sweet and candies manufacturer Jake). This decision was taken because the experimental data describing the latter process is the one with the highest quality and the fewest data gaps, and therefore best reflects the pilot plant operations. Moreover, as far as PHA production is concerned, this production line showed the best results among the others. Two different PHA fermentation processes were assessed, pure and mixed culture fermentation. These systems were benchmarked against the starch-based PHA production process.

The analysis is based on the current developments of each work package and uses the mass and energy flows provided by the responsible project partners. The following key outcomes could be obtained:

- The mixed and the pure culture production systems show a significantly higher environmental impact than the starch-based reference system considered. This appears to be particularly true in the case of mixed culture system, which is a significantly more impactful process than the pure culture system.
- The main reason behind the major impact of the mixed culture system is mainly attributable to the high amount of fermentation medium components and pH control agents used in the PHA fermentation phase (sodium hydroxide, hydrochloric acid, ammonium chloride and potassium sulphate).
- In the pure culture system VFA fermentation and PHA purification are the main hotspots of the process, it is crucial to lessening the impact of the process by reducing the input of calcium carbonate and ethanol.

- Given that some uncertainty is present at the data inventory level the potential environmental impacts are to be considered informative and expected to become lower along the development path with increasing knowledge and decreasing uncertainty.

2 Introduction

In this study nova-Institut (from here on referred to as “nova”) explored the environmental sustainability of the developed AFTERLIFE technology. A first hotspot life cycle assessment (LCA) was carried out at an early stage of the development, assessing the lab scale design. This study can be found in D7.1 “Hot spot LCA analysis for further optimization” (delivered on January the 31st, 2020). The present study, D7.2 “Final Life Cycle Assessment”, assessed the pilot scale operation performed by BBEPP (Bio Based European Pilot Plant). This study only examines the pilot scale process with the Jake WW (WW from the sweet and candies manufacturer Jake) among the different tested WW streams, because the experimental data describing the Jake process is the one with the highest quality and the fewest data gaps, and therefore best reflects the pilot plant operations. Moreover, as far as PHA production is concerned, this production line showed the best results among the others. By means of LCA the main hotspots and impact contributors were examined, also comparing WW-based PHA in relation to starch-based PHA, which is today's most widely used technology for the production of this polymer, and is therefore considered as a valid reference system. Nova also explored the economic sustainability of the processes developed in the AFTERLIFE project, these studies can be found in D7.3 and D7.4.

The following sections describe the environmental assessment conducted as part of WP7 and are structured as follows:

- *LCA framework and methodology*
- *Goal and scope definition*
- *Life Cycle Inventory analysis*
- *Life Cycle Impact Assessment*
- *Conclusions*
- *Appendix*

3 Life Cycle Assessment framework

An increased awareness of the importance of environmental protection, as well as possible impacts associated with the manufacturing and consumption of products and services, has raised increasing interest in the development of methods to better understand, measure and diminish these impacts. Life Cycle Assessment (LCA) is a method to quantitatively assess (based on physical metrics) the potential environmental impact of a product or service throughout its entire life cycle by quantifying all inputs and outputs of material and energy flows and assessing how these flows affect the environment (Figure 1). It assesses environmental impacts such as climate change or eutrophication as well as the impacts on natural resources and/or human health.

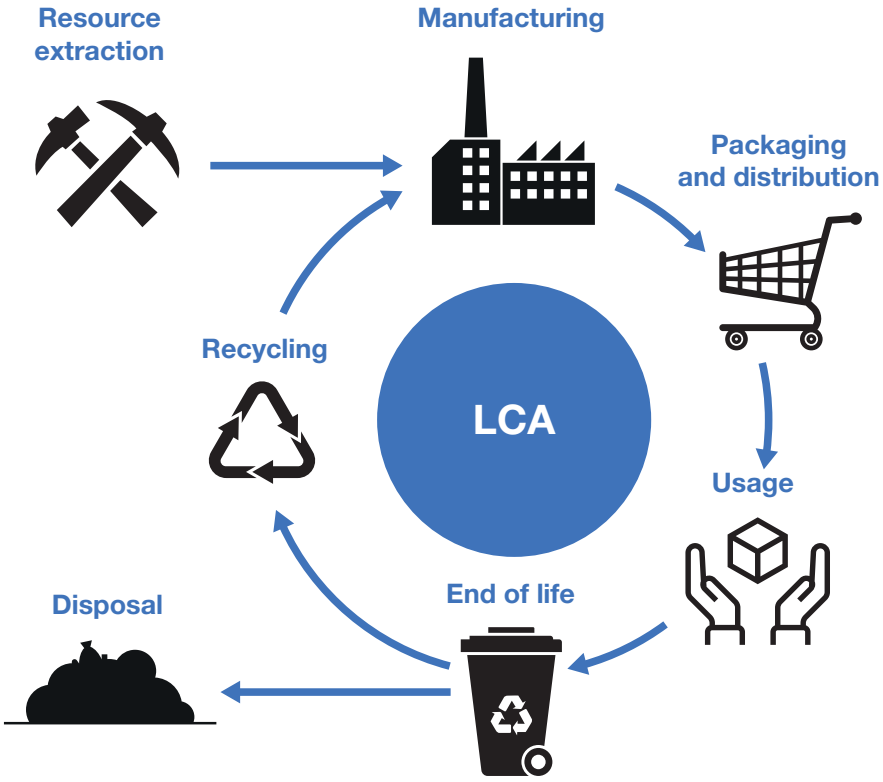


Figure 1 The life cycle model

LCA is an internationally standardized method laid out in ISO 14040:2006 and ISO 14044:2006. The strength of LCA is that it studies a whole product system. This avoids sub optimisation that may be the result if only a few processes are focused on. An LCA study consists in four different phases:

1. Goal and scope definition
2. Life Cycle Inventory analysis (LCI)
3. Life Cycle Impact Assessment (LCIA)
4. Interpretation of the results

In the goal and scope definition phase, the product to be studied and the purpose of the study are decided on. The functional unit to which the study refers is also defined. Many other choices related to the modelling are made during the goal and scope definition.

In the life cycle inventory analysis (LCI) phase, the system model is built according to the requirements of the goal and scope definition. The system model is a flow model of the system with certain types of system boundaries. The result is a mass and energy balance for the system.

The Life Cycle Impact Assessment (LCIA) aims to indicate the impacts of the environmental loads quantified in the inventory analysis by classifying the inventory parameters to the type of environmental impact that they contribute to and finally by calculating the relative contribution of the emissions and resources consumption to each type of environmental impact (characterization). Such calculations are based on scientific models of cause-effect chains in the natural system. Sometimes these results need to be interpreted and aggregated even further. This can be done in different ways, for instance with formalized and quantitative weighting procedures.

The last phase is the interpretation in which the findings of both, the inventory analysis and the impact assessment are evaluated, in relation to the defined goal and scope, in order to reach conclusions and recommendations. The relationships between these phases have been illustrated in Figure 2, which shows that an LCA study is a highly iterative process among the different phases. Four critical issues in LCA methodology determine the outcomes of an LCA study: the definition of the functional unit, system boundary issues in general and allocation of environmental burdens among product and co-products in particular, the type and quality of data used in the study and how the impact assessment is made.

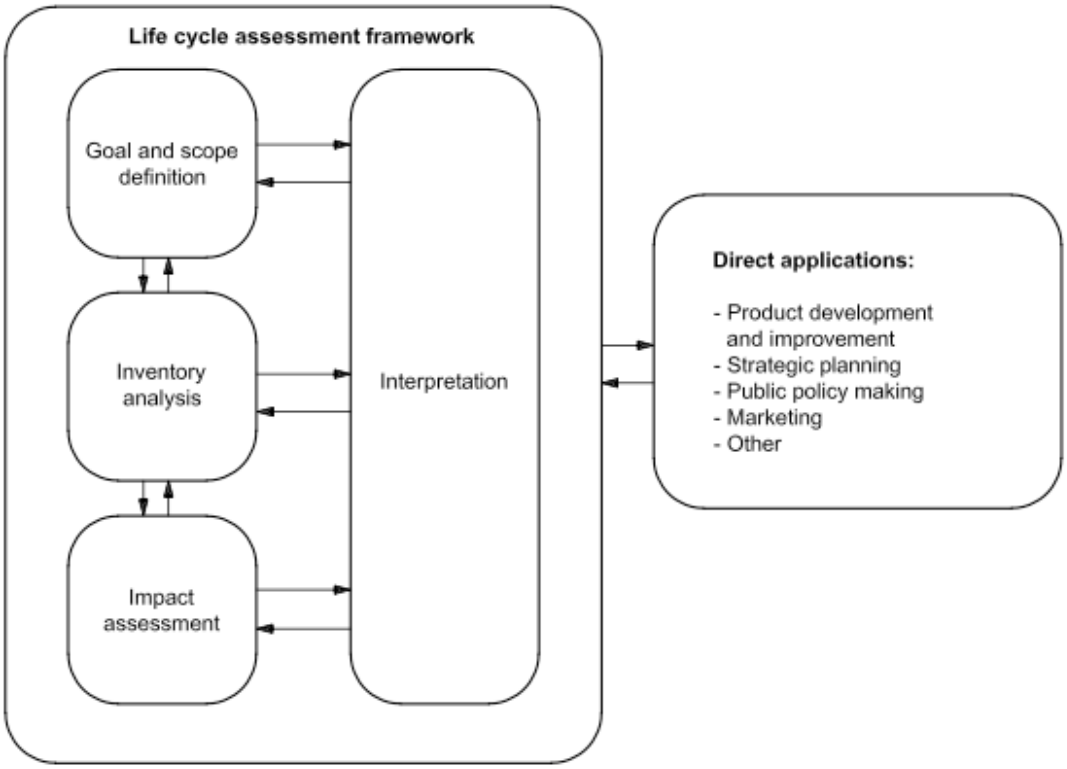


Figure 2 Stages of an LCA (ISO 14044 2006)

The results of an LCA can be used for revealing hotspots which can lead to identification of approaches to mitigate the impacts for the development of less harmful processes and products (product and process design and decision making). The LCA may also enable the comparison of different products

(benchmarking) and can support marketing and public policies, for instance, to support LCA-based eco-labelling. Another important application of LCA is that of learning, e.g., exploring the environmental properties of the product system studied and learning about relationships of the production system.

A final word: it must be noted that “positive” LCA results do not necessarily mean a process is sustainable. One limitation of the LCA is that the method is restricted to quantify only the ecological aspect of sustainability, thus, excluding from the assessment economic and social factors other than when used as basis for weighting. This report compiles the goal and scope, the inventory data as well as the LCA results along with their interpretation and corresponding recommendations. This study has been conducted widely according to the requirements of ISO 14040 and ISO 14044.

4 Goal and scope definition

This chapter describes the goal and scope together with the methodological framework of the LCA study. More precise, it comprises the objectives and intended application of the study, a general description of the product function and product system, the system boundaries together with the system function and functional unit as well as the methodological framework.

4.1 Goal

The goal of the study is assessing the environmental impacts of the AFTERLIFE process. The assessment focuses particularly on the identification of potential environmental hotspots to validate the designed pilot plant scale operation. Furthermore, the AFTERLIFE WW-based PHA was benchmarked against the conventionally produced starch-based PHA, in order to assess the environmental benefits and/or disadvantages.

4.2 Scope

4.2.1 Targeted audience

The results and inventory data of this LCA have a public dissemination level. Targeted audience are within the project, the project partners and externally all interested stakeholders. As the products are still under development and this LCA has not been independently verified, the results shall not be published in comparative assertions.

4.2.2 Geographical and time representativeness

At the current status of the project the goal of the study is to reflect the European situation. Hence, the corresponding background data, i.e. all materials and utilities are considered from datasets of production in Europe (RER) whenever available. Otherwise, global (GLO) production data are considered. Data reflects the current status of development on December 2021.

4.2.3 Function and functional unit

The functional unit in this assessment is defined as one kg of PHA polymer (namely poly(3-hydroxybutyrate) (P3HB)) with a purity of 99 %.

4.2.4 System boundaries and cut-off criteria

The assessment includes all production steps from *cradle-to-gate*, meaning that all production phases from Jake WW acquisition in the hypothetical AFTERLIFE factory to the final products are considered. As shown in Figure 3, the main production phases consist of: (1) VFA production, (2) PHA fermentation (mixed or pure culture systems), (3) PHA purification downstream processing, (4) water purification and (5) biogas production.

In this assessment, the Jake WW input is considered as a waste product of candy and sweet manufacture, therefore no impact was allocated on this stream. This methodological LCA approach, known as cut-off approach, can be employed in case a waste from another process is used as input in the considered system. Moreover, it was considered that the hypothetical AFTERLIFE production

facilities would be located close to the Jake factory, thus neglecting the possible WW transportation. Credit associated with energy production from the produced biogas and compost production from the produced digestate were given to the system, due to the avoided energy and compost generation. Clean water generated in step 4 is used in the PHA fermentation and purification phases. A detailed system description is provided in the LCI chapter.

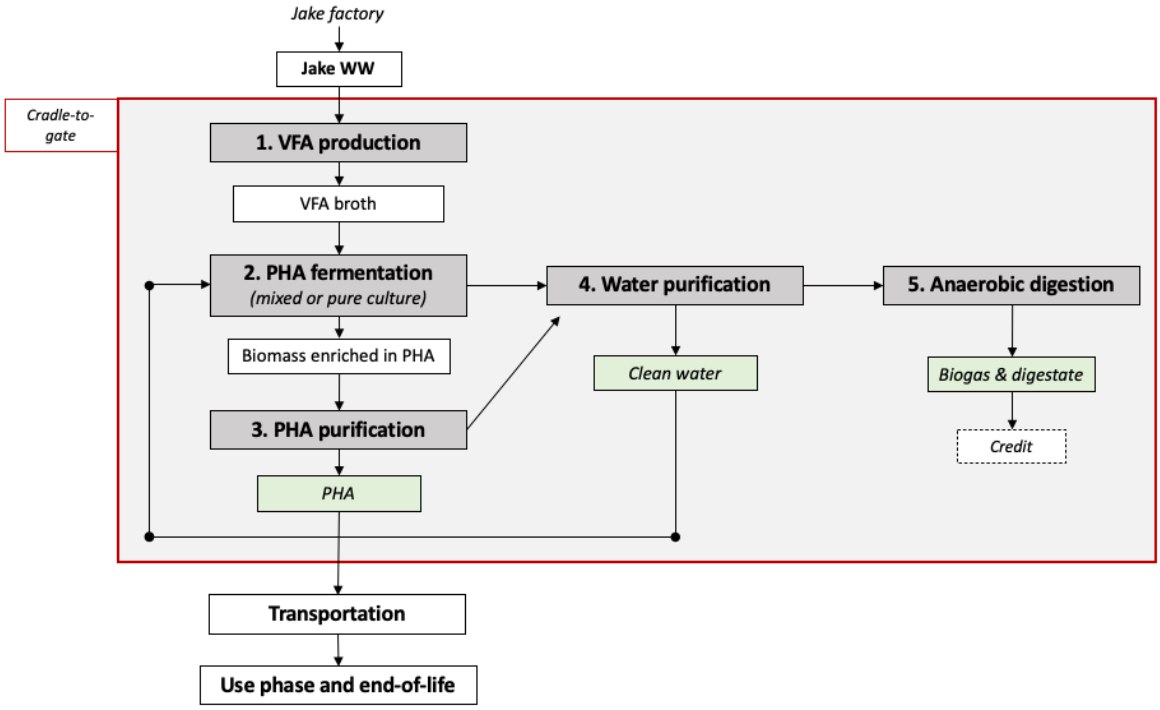


Figure 3: Jake WW system boundaries and main production phases. System boundaries are marked in red.

With regards to the reference system, starch-based PHA, this system was studied on the basis of the available scientific literature and then modelled on the mass and energy balance model reported by Harding et al. (2007), which turned out to be the study most in line with the needs of this investigation. The collected data was used to evaluate the environmental impact of the reference system, linking them to specific ecological impacts through the LCA software.

The reference system is examined as a closed system (or black box model), which means that all process inputs and outputs are aggregated into a single LCI and are not separated for each individual process step. The modelled system includes consumption of raw materials and energy in the starch-based PHA production but also in the background activities. Also in this case, the system boundaries were drawn from cradle-to-gate. In order to better depict the differences between the evaluated and reference production process, the main processing phases of the latter are shown in Figure 4 . For more detailed information on the reference system please see Appendix 1. Since the AFTERLIFE and reference PHA polymer obtained are assumed to follow equivalent utilization and disposal steps, the use phase and end-of-life of the product were not considered in this LCA study.

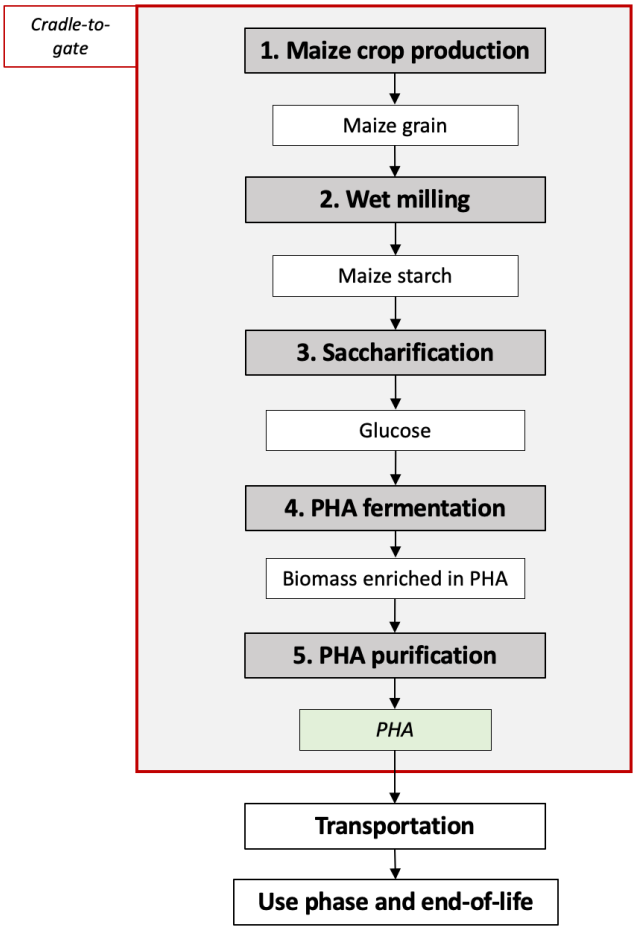


Figure 4: Starch-based PHA system boundaries and main production phases. System boundaries are marked in red.

4.2.5 LCIA impact categories

The inventory data of the AFTERLIFE process, was aggregated and linked to environmental impacts (e.g., global Warming). This is done during the Life Cycle Impact Assessment (LCIA) phase by means of impact category models. In this study the scientifically robust and internationally recognized EF 3.0 (Environmental Footprint), containing the environmental characterisation factors for various impact categories have been applied. The following impact categories have been assessed:

- Climate Change: emissions of greenhouse gases that cause climate change (for example CO2, CH4, N2O, CFC, CO...). It is given in kg CO2 eq./1 kg PHA.
- Acidification: Acidification is mainly caused by air emissions of NH3, NO2 and SOX, expressed in mol H+ eq /1 kg PHA.
- Eutrophication, aquatic freshwater: includes all impacts due to excessive levels of nutrients released in the aquatic freshwater environment. It is given in kg P eq./ 1 kg PHA.
- Eutrophication, terrestrial: includes all impacts due to excessive levels of macro- nutrients in the environment caused by emissions of nutrients to soil. It is given in mol N eq./ 1 kg PHA.

- Eutrophication, aquatic marine: it covers all impacts associated to excessive levels of nutrients released in the aquatic marine environment. It is given in kg N eq./ 1 kg PHA.
- Ecotoxicity, freshwater: it estimates potentially affected fraction of species integrated over time and the volume of the freshwater compartment, per unit of mass of the chemical emitted. It is measured in CTUe (Comparative Toxic Unit equivalent)/ 1 kg PHA.
- Land use: it is based on the soil quality index, which is based on the result of the aggregation, performed by JRC, of the four indicators provided by LANCA model (biotic production, erosion resistance, mechanical filtration and groundwater replenishment). It is a dimensionless index originated from the aggregation of the four above mentioned indicators.
- Water use: it is built on the user deprivation potential (deprivation weighted water consumption), based on the AWARE model (Available WAter REmaining) by United Nations Environmental Programme (UNEP). It is measured as kg world eq. Deprived.
- Resource use, fossils: quantification of the energy demand (in MJ/1 kg PHA) of overall fossil resources (direct and indirect).
- Resource use, minerals and metals: depletion of minerals based on concentration reserves and rate of de-accumulation. Expressed as kg Sb eq. depleted/1 kg PHA.

These impacts include the impacts that shall be investigated by default according to the ILCD Handbook (European Commission JRC, 2010). Moreover, considering the current political and societal discussion with regard to climate change, and the reduced use of fossil resources, they are of significant relevance. These impact categories are midpoint impacts and are determined through aggregation of data on emissions to potential impacts in various categories. In the case of the Climate change impact category, for instance, it is measured in terms of CO2 equivalents and is contributed to by a number of air-borne emissions. Carbon dioxide itself is a contributor, as is carbon monoxide (CO) and methane (CH4). The impact factor weight assigned to these chemicals depends on their impact on global warming relative to the impact of CO2 emissions, i.e., CH4 has a higher impact than CO2 by a factor of 25.

5 Life Cycle Inventory analysis

The Life Cycle Inventory (LCI) consists of detailed tracking of all flows into and out of the product system, including raw resources or materials, energy by type, water, and emissions to air, water and land by specific substance as well as wastes occurring in each process step. The in- and outputs of all necessary processes were collected during the data collection phase from project partners and literature.

5.1 Sources of Life Cycle Inventory data

This information was obtained by several data exchange rounds along the project development with several consortium partners. Among all, the main partners involved in data gathering were: BBEPP for pilot plant mass balance, IDENER for pilot plant energy balance data, NID and CSIC for mixed and pure culture fermentation media components and INNOVEN for anaerobic fermentation information. **Foreground data** for wastewater to PHA processes were provided by the responsible project partners throughout bilateral email and, conference calls. Further data of each process step were gathered through an excel data collection sheet, which was sent to the involved project partners. For **background processes** (e.g. feedstocks, materials, utilities and waste treatment), data were used from the Ecoinvent inventory database. This database is internationally recognized, both from a qualitative (completeness of data, quality of validation process) as well as from a quantitative perspective (scope of included processes). Background production data from Ecoinvent were kept as local (Europe, RER) as possible. When no local processes (RER) were available global data (GLO) were used as a reasonable alternative.

5.2 System description and inventory data

5.2.1 AFTERLIFE process

The process starts with converting Jake WW into VFA (volatile fatty acids). As mentioned, no transportation from the WW production point to the AFTERLIFE process facility is considered. In a first step WW is equalized with Calcium carbonate ($CaCO_3$) and mixed for further processing. The equalized medium is fermented to produce VFA by using anaerobic cultures. The fermentation liquid is further purified via ceramic microfiltration which separates solid from liquids. The solid fraction is sent to the anaerobic digester for biogas production, while the purified liquid containing VFAs is sent to the PHA production.

Two different fermentation techniques were tested in the pilot plant and therefore also analysed separately in this study: mixed and pure culture system. The differences between these systems, shown in Table 1, are in the fermentation medium components and pH control agents used and also in the respective amounts, the amount of VFA used per fermentation cycle and the system outputs. In both fermentations the water input, necessary for the dilution of the nutrients, comes from the clean water generated at the end of the water purification phase in step 4.2. In this way both systems do not need to acquire additional water sources but can merely reuse the water they produce themselves. A threshold of 1% on a mass basis was chosen, below which fermentation components were considered as no relevant. All fermentation nutrient amounts reported in Table 1 are on a dry

matter basis. The mixed broth enriched in PHA produced undergoes the PHA purification phase, while the supernatant broth is sent to the water purification step.
In the PHA purification phase, first the fermentation broth is transferred into a decanter in which two different fractions are obtained, the supernatant and the sediment. The first one is sent to the water purification phase, while the sediment continues the purification cascade. The next PHA recovery step consists of digestion with sodium dodecyl sulphate, dilution and homogenization, obtaining the homogenate. The homogenate is now processed via ceramic microfiltration; the retentate obtained continues the recovery phase while the filtrate is sent to the water purification step. The retentate from microfiltration is sent to digestion with H_2SO_4 at the end of which, similar to the previous process, the resulting retentate continues the recovery phase while the filtrate is sent to the water purification step. The next step is the last PHA recovery step to obtain 99% pure PHA, by means of ethanol wash and drying. It was assumed that 99% of the ethanol used in the process can be recycled via distillation, however the distillation burden was not included in the model due to data gaps. It is important to note that the PHA obtained from the mixed culture fermentation broth is less than that obtained from the pure culture system, in the former case 3 kg while in the latter 4.1 kg is obtained.

In the next phase all waste water streams generated along the processing cascade are sent to the water purification phase, in order to obtain pure water out of the AFTERLIFE system. As mentioned, this water is looped back into the system.

The last process phase is the biogas generation step, in which all solid streams from previous steps are turned into valuable energy and digestate. It is important to appreciate that the energy obtained from the mixed culture fermentation line is less than that obtained from the pure culture system, in the former case 177 MJ while in the latter 198 MJ is obtained as reported by the partners. The anaerobic digestion is also generating digestate as a side product, a valuable nutrient-rich substance that can be used as a compost fertiliser and soil enricher.

The Ecoinvent datasets used in the assessment are listed in Appendix 2.

Table 1: Jake mixed and pure culture systems LCI.

1. VFA production					
1.1 Anaerobic Fermentation to VFA					
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount**	**Comments**	
TOT Jake WW	IN	kg	1245,00	zero burden as it is a waste	
Agrodigestate		kg	22	neglected	
CaCO3	IN	kg	40		
Electricity	IN	kWh	0,04	Mixing	
Electricity	IN	kWh	1,77	Pumping	
Fermented VFA broth	OUT	kg	1045	next step	
WW to municipal treatment	OUT	kg	262,00	Own calculation for mass balance purposes	
1.2 Ceramic microfiltration 0.2 µm					
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount**	**Comments**	
Fermented VFA broth	IN	kg	1045		
Electricity	IN	kWh	0,26		
Retentate VFA	OUT	kg	200	to biogas production step	
Filtrate VFA	OUT	kg	800	next step	
WW to municipal treatment	OUT	kg	45,00	Own calculation for mass balance purposes	
2. PHA fermentation - Mixed and Pure Culture					
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount (Mixed Culture)**	**Amount (Pure Culture)**	**Comments**

Filtrate VFA	IN	kg	800	1244,3	
Water for dilution		kg	4956	897	This water input is supplied with the RO water generated at the end of the AFTERLIFE process
NH4Cl	IN	kg	66,50952	0,89	
K2PO4	IN	kg	26,96064	1,34	
(NH4)2SO4	IN	kg	//	8,07300000	
MgSO4.7H2O	IN	kg	5,9472	//	
HCl	IN	kg	90,447	//	
NaOH	IN	kg	99,12	17,94	
Electricity	IN	kWh	0,01	0,01	Mixing
Electricity	IN	kWh	1,77	1,77	Pumping
Electricity	IN	kWh	1,35	1,35	Aeration
Mixed broth enriched in PHA	OUT	kg	1475	2500	
Broth supernatant (to filtration cascade)	OUT	kg	4425	//	to water purification step
3. PHA purification and processing					
3.1 Decanter					
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount**	**Comments**	
Mixed broth enriched in PHA	IN	kg	1475		

Supernatant	OUT	kg	1393	to water purification step
Sediment	OUT	kg	82	next step
3.2 Digestion with SDS, dilution and homogenization				
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount**	**Comments**
Sediment	IN	kg	82	
Sodium dodecyl sulphate (SDS)	IN	kg	1	
decarbonised Water		kg	82	This water input is supplied with the RO water generated at the end of the AFTERLIFE process
Electricity	IN	kWh	0,034	mixing
Homogenate	OUT	kg	164	next step
3.3 Ceramic microfiltration (0.45) with diafiltration				
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount**	**Comments**
Homogenate	IN	kg	164	
RO water		kg	327	This water input is supplied with the RO water generated at the end of the AFTERLIFE process
Electricity	IN	kWh	0,26	*(in pure culture system it is 0,31)*
Filtrate + diafiltrate (to step 4.9)	OUT	kg	232	to water purification step
Retentate	OUT	kg	259	next step
3.4 Digestion with H2SO4 and microfiltration				
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount**	**Comments**
Feed	IN	kg	259	
H2SO4 (96%)	IN	kg	38	
RO water for dilution		kg	676	This water input is supplied with the RO water generated at the end of the AFTERLIFE process
NaOH (30%)	IN	kg	0,3	

RO water for diafiltration		kg	402	This water input is supplied with the RO water generated at the end of the AFTERLIFE process	
Electricity	IN	kWh	0,034		
Heat	IN	MJ	7,49		
Filtrate + diafiltrate	OUT	kg	1173	to water purification step	
Retentate	OUT	kg	201	next step	
WW to municipal treatment	OUT	kg	1,30	Own calculation assuming for mass balance purposes	
3.5 Ethanol wash and drying					
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount (Mixed Culture)**	**Amount (Pure Culture)**	**Comments**
Feed	IN	kg	201	same as mixed	
EtOH	IN	kg	40,22	same as mixed	Assuming 99% of ethanol is recycled.
RO water for EtOH washout		kg	302	same as mixed	This water input is supplied with the RO water generated at the end of the AFTERLIFE process
Electricity	IN	kWh	0,26	same as mixed	pumping
Steam	IN	MJ	20,12	same as mixed	Drying phase
Wash water	OUT	kg	400	same as mixed	to water purification step
PHA	OUT	kg	3,00	4,10	DM 100%
4. Water Purification					
4.1 Ceramic microfiltration 0.2 µm					
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount (Mixed Culture)**	**Amount (Pure Culture)**	**Comments**

All water streams from previous steps	IN	kg	7623	3198	
Electricity	IN	kWh	0,26	0,31	
filtrate	OUT	kg	6861	2878	next step
retentate	OUT	kg	762	320	to biogas production step
4.2 RO filtration					
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount (Mixed Culture)**	**Amount (Pure Culture)**	**Comments**
Filtrate 4.9	IN	kg	6861	2878	
Electricity	IN	kWh	0,31	0,37	
filtrate (Final clean RO water)	OUT	kg	6175	2590	Looped in PHA fermentation and purification
retentate	OUT	kg	686	288	to biogas production step
5. Biogas production					
Material/energy	**IN/OUT**	**Dimensional unit**	**Amount (Mixed Culture)**	**Amount (Pure Culture)**	**Comments**
All biogas streams from previous steps	IN	kg	1648	808	
Electricity	IN	kWh	1,95	2,08	
Heat	IN	MJ	125,82	140,59	
Energy produced by Biogas burning	OUT	MJ	177	198	Energy credit
Digestate	OUT	kg	1318,70	646,10	Compost credit

5.2.2 Reference system, starch-based PHA

Starch-based PHA production process was modelled based on the mass and energy balance data reported by Harding et al. (2007). As shown in Figure 4 the process feedstock is maize, from which starch and then glucose is produced. The reference system is examined as a black box model, which means that all process inputs and outputs are aggregated into a single LCI and are not separated for each individual process step, as shown in Table 2.

Table 2: LCI of reference system.

Material/energy	IN/OUT	Dimensional unit	Amount	Comments
Decarbonated Water	IN	kg	78,30	
Glucose	IN	kg	1,81	
Magnesium sulphate	IN	kg	0,0209	
Hydrogen peroxide	IN	kg	0,0529	
Potassium sulphate	IN	kg	0,0186	
Sulfuric acid	IN	kg	0,0030	
Phosphoric acid	IN	kg	0,0081	
Ammonium sulphate	IN	kg	0,0148	
Calcium chloride	IN	kg	0,0023	
Sodium sulphate	IN	kg	0,0030	
Zinc monosulphate	IN	kg	0,0012	
Manganese sulphate	IN	kg	0,0009	
Iron sulphate	IN	kg	0,0008	
Copper sulphate	IN	kg	0,0001	
Sodium phosphate	IN	kg	0,0001	
Enzymes	IN	kg	0,0024	Needed in the fermentation
Non-ionic surfactant	IN	kg	0,0004	
Electricity	IN	kWh	1,71	
Heat (Steam)	IN	kg	4,9	
Starch-based PHA	OUT	kg	1	99% purity
WW to municipal treatment	OUT	kg	65,00	
Biowaste generated	OUT	kg	0,42	

5.2.3 Use of proxies

When necessary to fill data gaps, approximations based on estimates were considered and where no information was available for example for certain chemical substances proxies were used. In this study the only proxy used was potassium sulphate, to represent the burden of potassium phosphate, used in the fermentation medium of the evaluated and reference systems.

5.2.4 Assumptions

Here below the main assumptions taken in this LCA study are shown:

- Afterlife facility is close to the Jake factory, so WW transportation can be neglected.
- When Jake WW enters the VFA fermentation phase it has the right processing temperature (37 Celsius), therefore it does not need to be further heated or cooled.
- An ethanol recovery of 99% in the PHA-recovery was assumed based on BBEPP, by that only the lost amount (1%) was considered in the assessment.
- The infrastructure for example reactor, facility or other equipment needed for the foreground AFTERLIFE process was neglected, as the focus of this assessment are the environmental hotspots of the process itself.
- Electricity is supplied by medium voltage grid based on the average transformation technology and the average electricity loss during transmission in EU.

5.2.5 Data quality assessment and limitations

Since LCA is a tool founded on quantification, uncertainty is present at the data inventory level. Incorrect estimations or modelling assumptions, outdated data and data gaps are sources of uncertainty. A qualitative analysis of the uncertainty of the inventory data was carried out, to validate the LCIA results. Indications on the quality of data include the evaluation of the reliability and completeness of the data itself, combined with the evaluation of the representativeness (temporal, geographical and technological) of the processes used to model it. The inventory data quality assessment is assessed in Table 3 according to (Weidema and Wesnæs 1996). The indicators are explained in Appendix 3.

Overall uncertainty is present at inventory level, technical complications were encountered in the design of pilot plant operation by BBEPP. Moreover, the data gathered are not entirely experimental but also estimates, especially with regards to the energy balance which comes from the mathematical model developed by IDENER. Based on that, the AFTERLIFE LCI scores relatively bad in completeness, which is a measure of the representativeness of the data. All in all, the quality of the inventory is in line with the low TRL of the production process examined.

Table 3: Data quality assessment of AFTERLIFE and reference processes.
Data Source: 1 primary (from experiments), 2 secondary (LCI databases), 3 tertiary (literature/estimates).
Indicator score: 1-2 very good to good, 3- fair, 4-5, poor to very poor.

Source	*Reliability*	*Completeness*	*Temporal correlation*	*Geographical correlation*	*Further technological correlation*
AFTERLIFE process					
1,3	2	4	1	1	2
Reference system					
3	1	2	4	2	2
Background data (Ecoinvent datasets)					
2	2	2	2	2	2

6 LCIA Results

The result and discussion are the phase of the LCA aimed at understanding and evaluating the magnitude and significance of the potential environmental impacts of the AFTERLIFE process. The following sections present the study results with first a detailed look at the Jake WW line main sources of ecological burden and then benchmarking the AFTERLIFE environmental performance against the reference system considered.

6.1 AFTERLIFE mixed and pure culture process environmental hotspots

The results shown in Table 4 reveal the environmental impact of the mixed and pure culture systems explored. It is important to note that in all impact categories explored the pure culture system is significantly less environmentally harmful than the mixed system, 77% to 93% less environmentally damaging than the mixed system, depending on the impact category considered.

Table 4: LCIA results of the mixed and pure culture systems investigated. The yellow column indicates the percentage difference between the two systems, showing how much less impactful pure culture is than mixed one.

Impact category	***Unit***	**Mixed culture**	**Pure culture**	**Δ (Pure/mixed-1)**
Climate change	*kg CO2 eq*	148,99	33,10	-78%
Acidification	*mol H+ eq*	0,88	0,16	-82%
Eutrophication, freshwater	*kg P eq*	0,065	0,013	-80%
Eutrophication, marine	*kg N eq*	0,26	0,039	-85%
Eutrophication, terrestrial	*mol N eq*	1,49	0,10	-93%
Ecotoxicity, freshwater	*CTUe*	14198,53	1172,75	-92%
Land use	*Pt*	668,77	143,37	-79%
Water use	*m3 depriv.*	109,98	12,62	-89%
Resource use, fossils	*MJ*	1806,75	333,86	-82%
Resource use, minerals and metals	*kg Sb eq*	0,0032	0,00073	-77%

The impact categories showing the greatest relative decrease in impact are terrestrial and marine eutrophication, freshwater ecotoxicity and water use. The marked difference in impact between the two processes is mainly attributable to the lower amount of fermentation medium components and pH control agents used in the pure culture compared to the other system. In fact, as shown in the LCI chapter (see Table 1), the mixed culture PHA fermentation step requires about 13 times more fermentation nutrients and about 14 times more pH control agents (overall and in dry matter terms) than the pure culture system per kg of PHA produced. It is therefore the high impact of mixed culture PHA fermentation that appears to be the main cause behind the significant difference in impact compared to the pure culture system. In fact, the other process phases (VFA fermentation, PHA purification, water purification and biogas production) show much smaller differences between the two processes than the PHA fermentation phase. The fact that the mixed culture system has a lower

final PHA yield than the pure culture system (3 vs. 4.1 kg PHA) certainly also plays a role in the higher overall impact of the former.

In order to better highlight the distinctions between the two processes, Table 5 and Table 6, show the ecological impacts for each processing step investigated. It was chosen to focus on 4 impact categories that best reflect the variation between processes, to view the full results please see Appendix 4.

Table 5: Mixed culture process environmental results divided per processing phase.

Impact category	***Unit***	***Total***	**1. VFA fermentation**	**2. PHA fermentation**	**3. PHA purification DSP**	**4. Water Purification**	**5. Biogas production**
Climate change	kg CO2 eq	*148,99*	20,04	109,31	20,55	0,08	-0,98
Eutrophication, terrestrial	mol N eq	*1,49*	0,09	1,31	0,21	0,0006	-0,13
Ecotoxicity, freshwater	CTU e	*14198,53*	274,70	13349,57	589,17	0,85	-15,77
Resource use, fossils	MJ	*1806,75*	183,23	1487,16	668,49	1,63	-533,75

Table 6: Pure culture process environmental results divided per processing phase.

Impact category	***Unit***	***Total***	**1. VFA fermentation**	**2. PHA fermentation**	**3. PHA purification DSP**	**4. Water Purification**	**5. Biogas production**
Climate change	kg CO2 eq	*33,10*	13,46	5,36	15,04	0,03	-0,80
Eutrophication, terrestrial	mol N eq	*0,10*	0,06	0,08	0,16	0,0003	-0,20
Ecotoxicity, freshwater	CTUe	*1172,75*	184,47	569,91	431,16	0,37	-13,14
Resource use, fossils	MJ	*333,86*	123,04	75,95	489,24	0,70	-355,08

It is evident from observing Table 5 and Table 6 that, as mentioned above, the main difference in terms of impact between the two processes lies in the PHA fermentation phase (the 2nd process step). In fact, the impact of mixed PHA fermentation is about 20 times greater than the pure culture system, due to the greater supply of nutrients and pH control agents. The main contributors behind the relatively high impact of mixed culture fermentation are sodium hydroxide, hydrochloric acid, ammonium chloride and potassium sulphate. These 4 components are responsible for about 70% of the overall impact of the mixed culture system across the explored impact categories, thus proving to be the main sources of environmental impact of the mixed system studied. In the case of the pure culture system, the main

sources of impact of PHA fermentation are sodium hydroxide and ammonium sulphate. These are responsible for 64% and 26% of the overall impact of PHA pure culture fermentation, respectively.

The other process phases (VFA fermentation, PHA purification, water purification and biogas production), while again more impactful in the case of mixed culture, show much smaller differences between the two processes than the just examined PHA fermentation phase. The associated impact in VFA fermentation of the mixed culture system is about 1.5 larger than that of the pure culture. In both cases the main source of impact is the calcium carbonate used for VFA equalisation. The fact that the burden is higher in the first case has to do with the fact that, as discussed, the final PHA yield is lower than in the pure culture system. Similarly, the greater impact of the mixed culture system in the PHA purification (main contributors are ethanol and sulfuric acid) and water purification (main contributor is electricity) phases has to do with the differences in productivity of the two systems. The production of biogas and digestate, and thus the credit derived that reduces the environmental impact of the two processes overall, is greater in the case of mixed culture. This is associated with the higher energy and digestate production obtained in the case of mixed culture.

Overall, in the mixed culture system PHA fermentation is the main hotspot, thus requiring a crucial mitigation intervention by decreasing the input of sodium hydroxide, hydrochloric acid, ammonium chloride and potassium sulphate, or finding alternative inputs with a better environmental impact profile. On the other hand, in the pure culture system VFA fermentation and PHA purification are the main hotspots of the process. In this case it is crucial to mitigate the impact of the process by reducing in particular the input of calcium carbonate and ethanol respectively or finding alternative products with a lower environmental burden. In addition, increasing the PHA yield of the processes (e.g. by improving the PHA purification efficiency) would certainly reduce the overall ecological impact in both cases examined. Energy use (electricity and heat) does not appear to be among the critical impact points of the systems studied. The use of electricity has a greater impact than the use of heat. In the case of the mixed culture system, the use of electricity accounts (on average among the impact categories) for about only 0.5% of the total impact categories of the process, while the use of heat only accounts for 0.15%. In the case of the pure culture system, electricity amounts to about 1.7% of the overall average impact across the various impact categories, while heat use accounts to about 0.8%.

6.2 Benchmark against starch-based PHA

Looking at the results of the environmental impact profile of starch-based PHA, shown in Table 7, it is evident that both mixed and the pure culture production system show a significantly higher environmental impact than the reference system considered (especially the mixed culture system).

Table 7: LCIA results of the AFTERLIFE mixed and pure production processes and starch-based PHA reference system (in the grey column). The yellow columns show the percentage difference between the AFTERLIFE and reference systems, indicating how much less impactful the reference system is than the AFTERLIFE mixed and pure culture routes.

Impact category	***Unit***	**Mixed culture**	**Pure culture**	***Starch-based PHA***	**Δ (Reference/Mixed -1)**	**Δ (Reference/Pure -1)**
Climate change	*kg CO2 eq*	148,99	33,10	*4,66*	-97%	-86%
Acidification	*mol H+ eq*	0,88	0,16	*0,04*	-96%	-75%
Eutrophication, freshwater	*kg P eq*	0,065	0,013	*0,0017*	-97%	-87%
Eutrophication, marine	*kg N eq*	0,26	0,039	*0,013*	-95%	-67%
Eutrophication, terrestrial	*mol N eq*	1,49	0,10	*0,12*	-92%	21%
Ecotoxicity, freshwater	*CTUe*	14198,53	1172,75	*162,07*	-99%	-86%
Land use	*Pt*	668,77	143,37	*76,27*	-89%	-47%
Water use	*m3 depriv.*	109,98	12,62	*3,28*	-97%	-74%
Resource use, fossils	*MJ*	1806,75	333,86	*66,29*	-96%	-80%
Resource use, minerals and metals	*kg Sb eq*	0,0032	0,00073	*0,00012*	-96%	-84%

Among the various impact categories explored, the reference system is between 89% and 99% less ecologically harmful than the mixed culture system. When compared instead to the pure culture system, except for the terrestrial eutrophication impact category, the reference system is between 47% and 87% less ecologically harmful among the impact categories explored. In the case of the terrestrial eutrophication impact category, the pure culture system shows the only positive result of this benchmark assessment, showing a 21% lower impact than the starch-based PHA production process. This is attributed to the glucose production process in the reference system, which is in fact, not only in this impact category, the main source of environmental impact of starch-based PHA, followed by the use of steam and electricity. The results of this benchmark show that substantial optimisation of the AFTERLIFE process is still needed, in particular for the mixed culture system, in order to become more ecologically advantageous than starch-based PHA.

7 Conclusions

The environmental burden associated with the pilot plant scale process for recovering and valorising relevant fractions from Jake WW developed in the AFTERLIFE project was assessed via the LCA methodology. Several impact categories were explored, thus providing a comprehensive picture of the ecological impact of the mixed and pure culture production systems. The identification of hotspots can lead to the identification of approaches to mitigate the impacts.

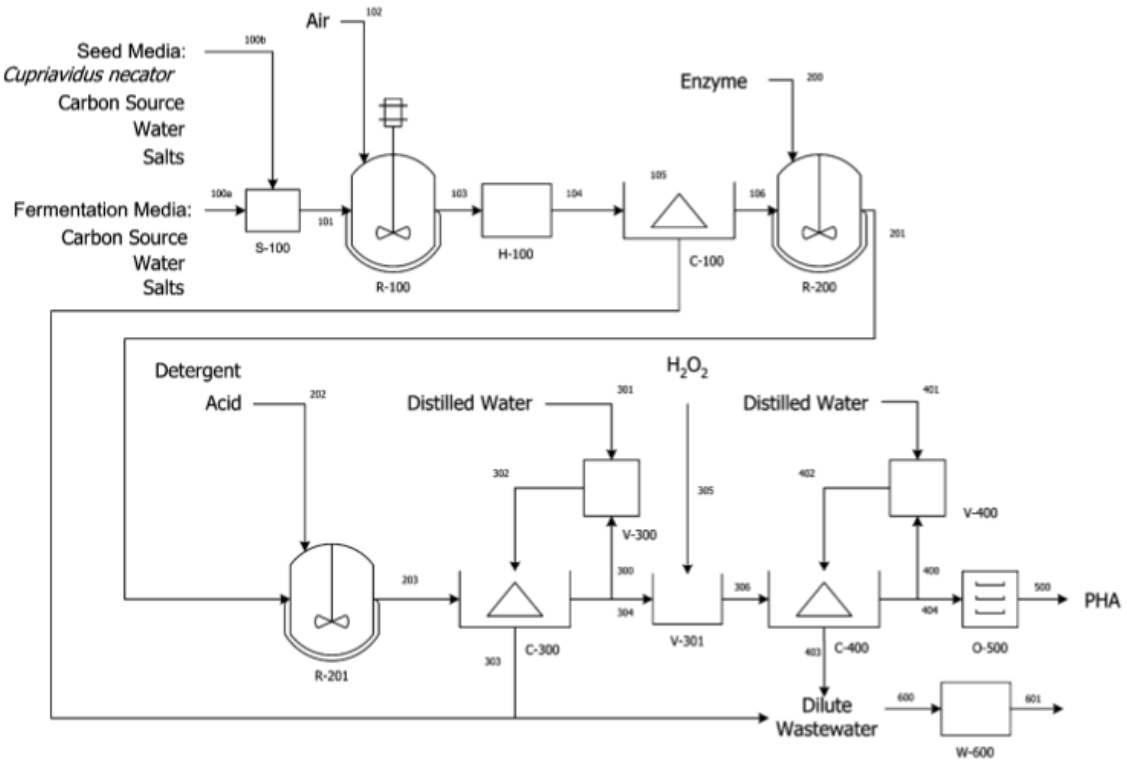
It was shown that both mixed and the pure culture production systems show a significantly higher environmental impact than the starch-based reference system considered. This appears to be particularly true in the case of mixed culture fermentation, which is a significantly more impactful process than the pure culture system. The results of the benchmark analysis shows that substantial optimisation of the AFTERLIFE process is still needed, in particular for the mixed culture system, in order to become more ecologically advantageous than starch-based PHA.

The main reason behind the major impact of the mixed culture system is mainly attributable to the high amount of fermentation medium components and pH control agents used in the PHA fermentation phase. In fact, the impact of mixed culture PHA fermentation is about 20 times greater than the pure culture PHA fermentation, the main contributors behind the relatively high impact of mixed culture PHA fermentation are sodium hydroxide, hydrochloric acid, ammonium chloride and potassium sulphate. These components were proven to be responsible for about 70% of the overall impact of the mixed culture system across the explored impact categories. In the case of the pure culture system, the main sources of impact of PHA fermentation have been identified being sodium hydroxide and ammonium sulphate. The other process phases (VFA fermentation, PHA purification, water purification and biogas production) show much smaller differences between the two processes than the PHA fermentation phase. The fact that the mixed culture system has a lower final PHA yield than the pure culture system (3 vs. 4.1 kg PHA) certainly also plays a role in the higher overall impact of the former. Therefore, the main interventions recommended to mitigate the ecological impact of the mixed culture system processes are decreasing the input of sodium hydroxide, hydrochloric acid, ammonium chloride and potassium sulphate or finding alternative inputs with a better ecological footprint. On the other hand, being in the pure culture system VFA fermentation and PHA purification the main hotspots of the process, it is crucial to lessening the impact of the process by reducing the input of calcium carbonate and ethanol. In addition, increasing the PHA yield of the processes (e.g. by improving the PHA purification efficiency) would certainly reduce the overall ecological impact in both cases examined.

The LCAs carried out in this study take place during the experimental and modelling stage of development. Given that some uncertainty is present at the data inventory level the potential environmental impacts are to be considered informative and expected to become lower along the development path with increasing knowledge and decreasing uncertainty.

8 Appendix

Appendix 1: Starch-based PHA process description and flow diagram, taken by Harding et al. (2007). The carbon source considered in the assessment is glucose from maize starch.



S-100 – Steam sterilisation
R-100 – Microbial Growth Reactor
H-100 – High Pressure Homogeniser
C-100 – Centrifugation
R-200 – Enzyme Washing Reactor
R-201 – Detergent Washing Reactor
C-300 – Centrigugation
V-300 – PHB Re-suspension
V-301 – Hydroxide Addition
C-400 – Centrifugation
V-400 – PHB Re-suspension
O -500 – Spray Drying
W-600 – Wastewater treatment

The seed (Cupriavidus necator, sucrose, (NH4)2 SO4, K2HPO4, NaHPO4, MgSO4.7H2O, FeSO4.7H2 O and trace salts) and fermentation media (glucose, H3PO4, (NH4)2SO4 , K2SO4, MgSO4.7H2O, trace salts and antifoam) are prepared and sterilized in a continuous system at 139 ◦C (S-100). The medium is added to an aerated semi-batch reactor and agitated with a stirrer. Following batch production of biomass, a glucose feed is initiated. With the onset of phosphate limitation, PHB accumulates. The total reaction time is 80h, producing 1417 kg biomass (71% PHB) at a concentration of 12.4% biomass.

After PHB growth and accumulation, downstream processing is performed in batch. Cells are disrupted in a high-pressure homogeniser H-100 (70 MPa). Solids are then removed by centrifugation (C-100; 10,000 × g for 20 min) and sent for further purification. The solid PHB is re-suspended with the alkaline serine protease, Optimase L660, to digest the non-polymeric cell matter. The temperature is maintained at 70 ◦C and pH controlled at 8.0 with potassium hydroxide in a stirred tank reactor (R-200) for 2 h. PHB is further processed by treatment with a non-ionic detergent (Synperonic NP8) in a stirred tank reactor (70 ◦C and pH of 7.0) (R-210) for 2 h. Additional product purification is achieved in repeated cycles of dilution with water and centrifuge action (C-300/V-300), followed by hydrogen peroxide treatment (V-301) and a final water washing and centrifuge cycles (C- 400/V-400). The purified PHB is ultimately spray dried (O-500) from a moisture content of 25 wt% to below 200 ppm.

Appendix 2: Ecoinvent dataset used in the LCA.

Material/energy flow	**Dataset**
Electricity	Electricity, high voltage {Europe without Switzerland}| market group for | Cut-off, U
Heat	Heat, district or industrial, natural gas {RER}| market group for | Cut-off, U
Calcium Carbonated	Calcium carbonate, precipitated {RER}| market for calcium carbonate, precipitated | Cut-off, U
Ammonium chloride	Ammonium chloride {GLO}| market for | Cut-off, U
Potassium sulphate	potassium sulphate {RER}| potassium sulphate production | Cut-off, U
Magnesium sulphate	Magnesium sulphate {GLO}| market for | Cut-off, U
Hydrochloric acid	Hydrochloric acid, without water, in 30% solution state {RER}| market for | Cut-off, U
Sodium hydroxide	Sodium hydroxide, without water, in 50% solution state {GLO}| market for | Cut-off, U
Ammonium sulphate	ammonium sulphate {RER}| market for ammonium sulphate | Cut-off, U
Sodium dodecyl sulphate (SDS)	Alkyl sulphate (C12-14) {GLO}| market for alkyl sulphate (C12-14) | Cut-off, U
Sulfuric acid	Sulfuric acid {GLO}| market for | Cut-off, U
Ethanol	Ethanol, without water, in 99.7% solution state, from ethylene {RER}| ethylene hydration | Cut-off, U
Biogas *(avoided production)*	Methane, 96% by volume, from biogas, low pressure, at user {GLO}| market for | Cut-off, U
Digestate/compost *(avoided production)*	peat {RER}| market for peat | Cut-off, U

Appendix 3: Indicator of Inventory data quality assessment adapted from (Weidema and Wesnæs 1996)

Indicator score	1	2	3	4	5
Reliability	Verified data based on measurements	Verified data partly based on assumptions or non-verified data based on measurements	Non-verified data partly based on assumptions	Qualified estimate (e.g. by industrial expert)	Non-qualified estimate
Completeness	Representative data from a sufficient sample of sites over an adequate period to even out normal fluctuations	Representative data from a smaller number of sites over adequate periods	Representative data from an adequate number of sites over shorter periods	Representative data from a smaller number of sites and shorter periods or incomplete data from an adequate number of sites and periods	Representativeness unknown or incomplete data from a smaller number of sites and/or over shorter periods
Temporal correlation	Less than 3 years difference to year of study	Less than 6 years difference	Less than 10 years difference	Less than 15 years difference	Age of data unknown or more than 15 years difference
Geographic correlation	Data from study area	Average data from larger area that includes the studied area	Data from areas with similar production conditions	Data from areas with slightly similar production conditions	Data from unknown areas or areas with very different production conditions
Further technological correlation	Data from studied businesses, processes and materials	Data from studied processes and materials from different businesses	Data on studied processes and materials from a different technology	Data on related processes or materials with the same technology	Data on related processes or materials with different technology

Appendix 4: Mixed and pure culture processes environmental full results divided per processing phase.

MIXED CULTURE							
Impact category	***Unit***	**Total**	**1. VFA fermentation**	**2. PHA fermentation**	**3. PHA purification DSP**	**4. Water Purification**	**5. Biogas production**
Climate change	*kg CO2 eq*	148,991	20,042	109,306	20,546	0,078	-0,982
Acidification	*mol H+ eq*	0,881	0,046	0,690	0,175	0,000	-0,031
Eutrophication, freshwater	*kg P eq*	0,065	0,005	0,050	0,010	0,000	0,000
Eutrophication, marine	*kg N eq*	0,257	0,012	0,217	0,031	0,000	-0,002
Eutrophication, terrestrial	*mol N eq*	1,491	0,095	1,315	0,214	0,001	-0,134
Ecotoxicity, freshwater	*CTUe*	14198,528	274,698	13349,571	589,173	0,853	-15,767
Land use	*Pt*	668,766	56,642	499,454	119,052	0,226	-6,607
Water use	*m3 depriv.*	109,979	-1,232	99,929	12,566	0,016	-1,300
Resource use, fossils	*MJ*	1806,750	183,233	1487,157	668,487	1,625	-533,752
Resource use, minerals and metals	*kg Sb eq*	3,16E-03	2,72E-04	2,34E-03	5,59E-04	1,80E-07	-7,27E-06
PURE CULTURE							
Impact category	***Unit***	**Total**	**1. VFA fermentation**	**2. PHA fermentation**	**3. PHA purification DSP**	**4. Water Purification**	**5. Biogas production**
Climate change	*kg CO2 eq*	33,096	13,459	5,362	15,039	0,034	-0,798
Acidification	*mol H+ eq*	0,156	0,031	0,041	0,128	0,000	-0,045
Eutrophication, freshwater	*kg P eq*	0,013	0,004	0,003	0,007	0,000	0,000
Eutrophication, marine	*kg N eq*	0,039	0,008	0,011	0,022	0,000	-0,002
Eutrophication, terrestrial	*mol N eq*	0,103	0,064	0,078	0,157	0,000	-0,195
Ecotoxicity, freshwater	*CTUe*	1172,754	184,466	569,906	431,157	0,368	-13,143
Land use	*Pt*	143,369	38,036	23,277	87,126	0,098	-5,168
Water use	*m3 depriv.*	12,624	-0,827	5,126	9,196	0,007	-0,878
Resource use, fossils	*MJ*	333,856	123,045	75,945	489,241	0,702	-355,077
Resource use, minerals and metals	*kg Sb eq*	7,32E-04	1,83E-04	1,46E-04	4,09E-04	7,75E-08	-5,61E-06

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