POLITECNICO DI TORINO

Corso di Laurea Magistrale in Ingegneria per l'Ambiente e il Territorio

Assessment of the production of bioplastics from industrial wastewater from fish canning industry

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Marzo 2020

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ABSTRACT

At present, the necessity to contain the environmental impacts derived from the whole life cycle of plastics, from extraction of fossil resources to disposal, has led to a great attention towards bioplastics. Among them, Polyhydroxyalkanoates (PHAs), which are both biobased and biodegradable, could be considered promising and have a great potential in consumer goods applications. However, their high production cost makes their scaled-up manufacturing and market share still marginal.

This work provides an overview on the current state of the art of PHA production in order to identify well-established aspects, as well as future challenges that should be overcome to foster the replacement of plastic by biopolymers. To do so, a systematic review of Life Cycle Assessment (LCA) studies on PHAs production is performed with the aim to support key-LCA decisions. By this point, this thesis investigates from an environmental perspective an innovative potential pathway to produce PHA, i.e. using the wastewater derived from mussel processing industry as substrate. Both the pure and mixed culture production are assessed in order to investigate the most sustainable system. The analysis is focused on the fermentation step, even if the whole production process, including the extraction phase, is assessed. Moreover, the valorisation scenario is compared to the baseline scenario, i.e. the mussel processing wastewater treatment. The methodology used to carry out the analysis is the standard LCA methodology (ISO, 2006). The results show that mixed culture PHA production process results in better environmental performance than pure culture route and also exhibits lower environmental impacts compared to the baseline scenario.

1 ANALYSIS OF THE CONTEXT

1.1 PLASTICS: PRODUCTION, DEMAND AND WASTE DATA

A world without plastics seems to be now unimaginable. In just a few decades, plastics have radically changed economy and society, becoming an essential element in the modern life. Combining excellent functional properties, such as low weight, high strength, wide application range, easy processability on an industrial scale, with low cost, these materials are omnipresent and have outgrown most man-made materials (European Commission, 2019; Geyer et al., 2017).

1.1.1 GLOBAL AND EUROPEAN PLASTIC PRODUCTION

Commercial production of plastics started around 1950's, after the World War II, when the innovations in material field dictated by military needs to find substitutes for natural not available products, invade the civil world. Since this moment, plastics production has enjoyed exceptional growth and, between 1950 and 2015, an estimated 8.3 billion tonnes of plastics were produced, of which 6.3 billion tonnes are considered as waste (Geyer et al., 2017). In 2018 global plastics production reached 359 million tonnes (figure 1), with 61.8 million tonnes generated in Europe alone (PlasticsEurope— The Facts 2019). At the present rate of growth, worldwide plastics production is estimated to double within the next 20 years (Lebreton and Andrady, 2019).

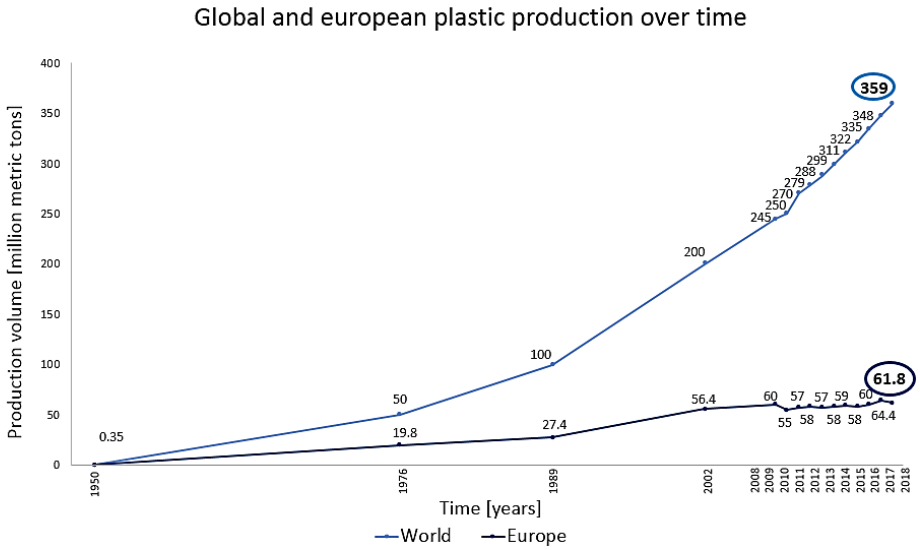


Figure 1 Plastic production from 1950 to 2018 in the world and in EU28+NO/CH. It includes: Thermoplastics, Polyurethanes, Thermosets, Elastomers, Adhesives, Coatings and Sealants and PP-Fibers. Not included: PET-fibers, PA-fibers and Polyacryl-fibers (data are taken from PlasticsEurope - The Facts 2019 and Statista 2019).

Currently, the distribution of global plastics production is heterogeneous, and it sees China as first producer in 2018 with the 30% of the total plastic produced (figure 2), followed by NAFTA[1] and Europe, with 18 and 17% respectively (PlasticsEurope— The Facts 2019).

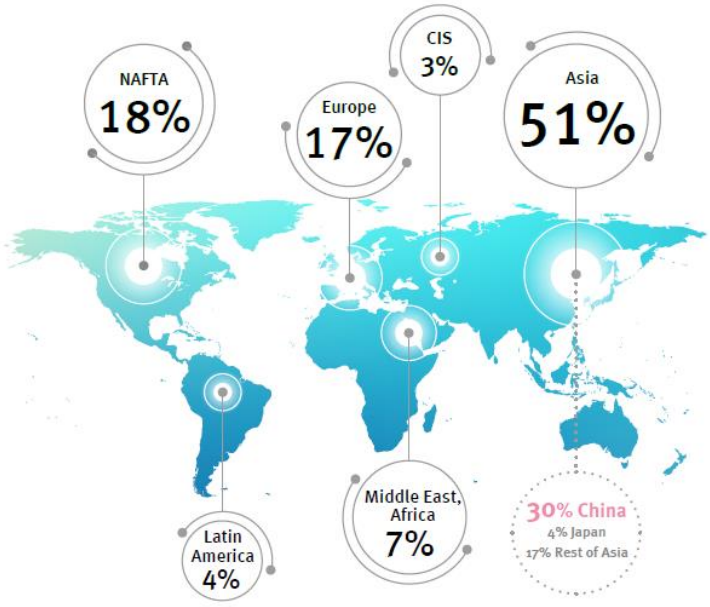


Figure 2 Distribution of global plastics production in 2018 (Plastic Europe— The Facts 2019)

Plastics production is part of the chemical industry that absorbs 7-9% of global oil supply, with 4-6% being used as raw material for plastics. Chemical industry globally represents EUR 3.36 trillion in sales, with a European share of 15.1% in 2016 (European Commission, 2019). The industry is fuelled by readily available and relatively cheap oil and has moved from Western Europe and USA to Asia, mainly China (figure 3).

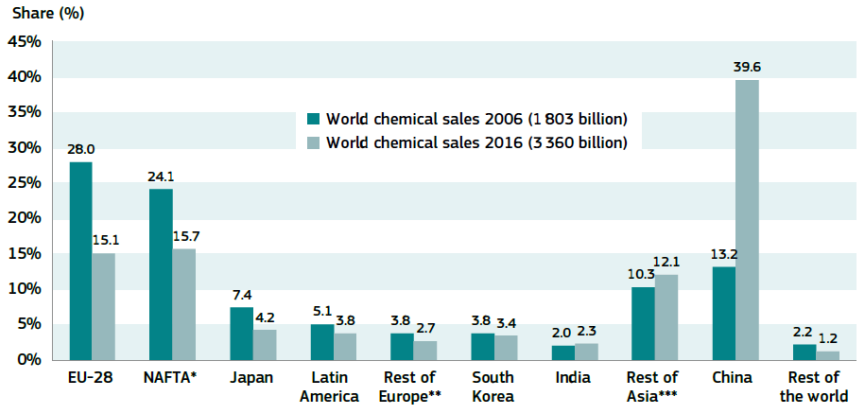


Figure 3 Overview geographical spread of global sales of the chemical industry in 2006 and 2016 (European Commission, 2019)

[1] North America Free Trade Agreement including United States, Canada and Mexico.

Within Europe, the plastic industry in one of the largest; it ranks 7th in industrial value added contribution. There are 60,000 companies that generate 1.6 million of job positions, creating a turnover of 360 billion euros in 2018 (PlasticsEurope — The Facts 2019).

However, several issues threaten the dependency on fossil fuels resources as raw materials, such as the depletion of no renewable resources, the geopolitical instabilities, the greenhouses gas emissions, spills and wastes. Considering also the forecast growth of consumer demand for plastics, the requirement of fossil fuel, energy, as well as the associated carbon emissions by the industry, will increase. According to Lebreton and Andrady (2019), by year 2050 plastics manufacturing and processing may account for as much as 20% of petroleum consumed globally and 15% of the annual carbon emissions budget. These issues suggest investigating and using new renewable resources for plastics production, and therefore to implement new industrial production system, based on bioeconomy and biotechnologies processes.

1.1.2 PLASTIC PACKAGING AND WASTE GENERATION

The largest application of plastics is packaging, currently representing 26% of the total volume of plastics used globally (European Commission, 2019). The growth of this application has been accelerated by a global shift from reusable to single-use containers; it is also linked with many advantages that the plastics packaging films offer over other type of material (e.g. transparency, water resistance, impermeability to gases and moisture, easily form adaptability etc.). In Europe packaging accounts for 39.9% of the total plastic converters demand, followed by building and construction (19.8%) and automotive industry (9.9%) (PlasticsEurope — The Facts 2019).

Approximately 42% of all non fiber plastics produced have been used for packaging, which is predominantly composed by PE, PP, and PET (Geyer et al.2017). These polymers are also the most commonly found plastics in the environment, especially in aquatic environments (Li et al., 2016).

Packaging products dominate the waste stream of plastics since they have a relatively short in-use phase. In fact, according to Geyer et al. (2017), most of the packaging plastics leave use the same year they are produced, whereas construction plastics leaving use were produced decades earlier. Plastics can be recycled or reprocessed into a secondary material at their end-of-life; however, at the end of this second (or third, fourth …) use plastic will finally be disposed. So, recycling delays, rather than avoids, final disposal. Alternatively, plastics can be destroyed thermally by incineration with possible energy recovery or they can be discarded and either contained in sanitary landfills or left uncontained in open dumps or in the natural environment (i.e. marine litter). Estimates about global plastic wastes produced between 1950 and 2015 says that this quantity amounted to 6300 Mt. Actually

approximately 12% of this waste amount have been incinerated and 9% have been recycled. Around 60% of all plastic ever produced were discarded and are accumulated in landfill or in the natural environment (Geyser et al., 2019). Focusing on Europe context, in 2018, 29.1 million tonnes of plastic waste were collected in the EU28+NO/CH in order to be treated. The different percentages for recycling, energy recovery and landfill are shown in figure 4 (a) (PlasticsEurope – The Facts 2019). It is interesting to note how European countries with landfill restrictions of recyclable and recoverable waste have, on average, higher recycling rates of plastic post-consumer waste (figure 4 b).

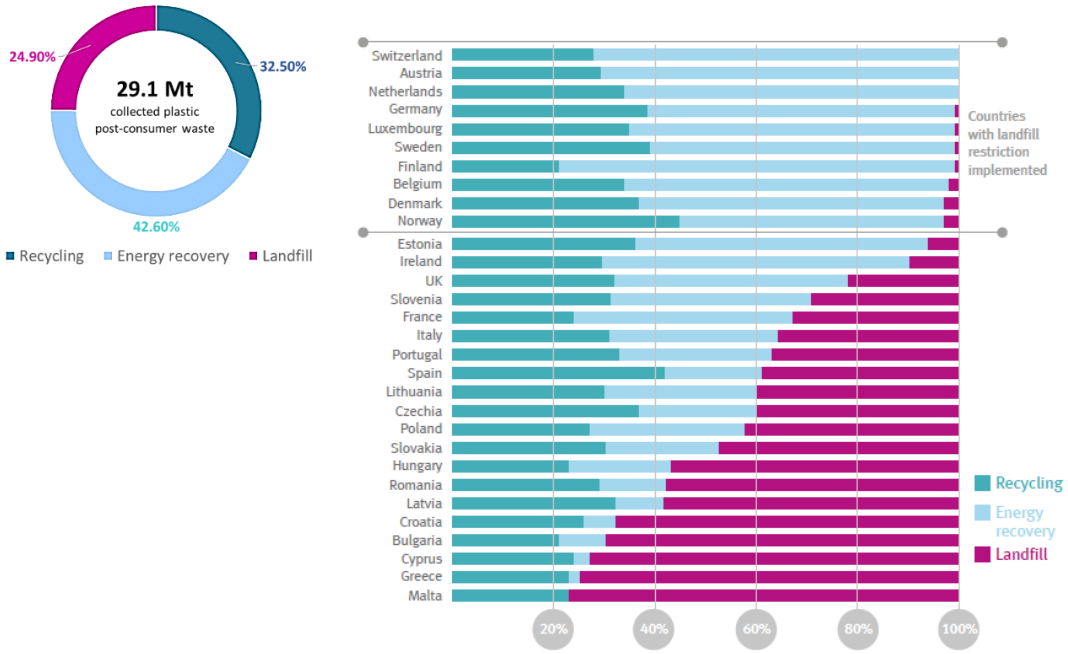


Figure 4 (a) On the left, percentage amount of different European plastic postconsumer waste treatments in 2018. (b) On the right, plastic post-consumer waste rates of recycling, energy recovery and landfill per European country in 2018. (PlasticsEurope – The facts, 2019)

1.1.3 PLASTIC WASTE: DATA AND POLLUTION EFFECTS

After the use, if plastic waste is not properly managed, it will accumulate in natural environment. Here, the same properties, such as stability and material persistence, that have made plastics very valuable and versatile in several applications, represent at the end-of-life of plastics products the main environmental trouble. None of the commonly used polymers are indeed biodegradable and they will persist in the environment for up to century (Li et al., 2016). The mismanaged plastic waste (MPW) is heterogeneous between the various countries (figure 5). For example, Asia, hosting 60% of the global population, was in 2015 the leading generating region of plastic waste with 82 Mt, followed by Europe (31 Mt) and Northern America (29 Mt). Latin America (including the Caribbean) and Africa each produced 19 Mt of plastic waste while Oceania generated about 0.9 Mt. However, considering the

unsound waste disposal, Africa results to have the highest rate of unsound waste disposal with an average of 88.5%, despite the low levels of resin production. The unfair practice of importing waste, especially e-waste, from developed nations, is to a large part responsible for this problem in Africa for example. (Lebreton and Andrady, 2019).

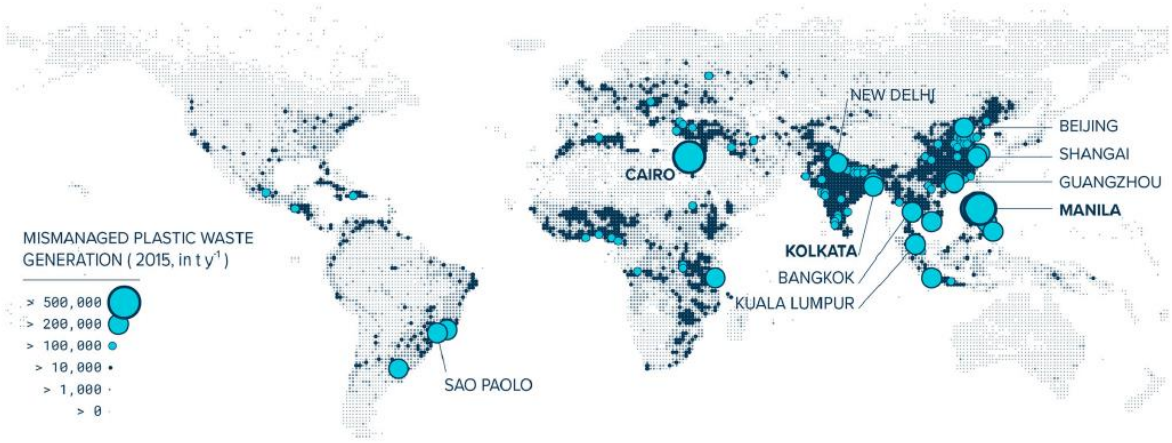


Figure 5 Global mismanaged plastic waste (MPW) generation in 2015. The 10 largest producing urban centres are labelled on the map with Manila, Cairo and Kolkata as the leading agglomerations (Lebreton and Andrady, 2019)

The question that arises now is what is the fate of this huge amount of plastic litter?

Initially, most contamination by plastics originates from terrestrial areas, specifically from uncovered landfill sites, untreated sewage, wind-blown debris, vehicle's tyres, plastic bags and boxes. These generally start as macroplastic (size >5 mm) and then they tend to become brittle, break down into small particles that are more capable of moving around the ecosystems (Waring et al., 2018). Plastic buried within covered landfill sites will remain there for many decades, posing potential problems for the future. On the other hand, a certain amount of plastic- estimated to be in the order of 10 Mt/y (Billard and Boucher, 2019)- and called leakage, flow into waterways and, ultimately, into the oceans. Ocean contamination by plastics is nowadays a big environmental concern, since plastics represent the majority of marine litter on the ocean surface, on beaches and on the sea bottom (European Commission, 2019). The sources of the plastic debris present in marine environment are land-based for the 80% and ocean-based for the remaining 20% (Li et al., 2016). Marine-based source of plastic litter comes from shipping, oil and gas platforms and fishing (discarded nets) (Waring et al. 2018). On the other side, main land-based sources have been found in: densely populated or industrialised areas, plastic bag usage, solid waste disposal; coastal recreational activities and land-based sources in northern South China Sea; raw manufacturing materials transported onto beaches following accidental spillage during handling and other processes; wastewater effluent and refuse site leachate. The ways

by which plastics are transported from their land-based sources to the marine environment are watercourses and sewage systems. In addition, extreme weather events increase the transfer of land-based debris to sea (Li et al., 2016).

Although the fraction of plastic waste entering the ocean may vary between locations, numerous studies on the abundance of plastics debris, especially in the oceans, have established that plastic pollution is pervasive with even the remotest locations affected (Figure 6). While concentrations vary locally, plastic debris has been found in the Arctic, the Antarctic, uninhabited islands and the deep sea (European Commission, 2019).

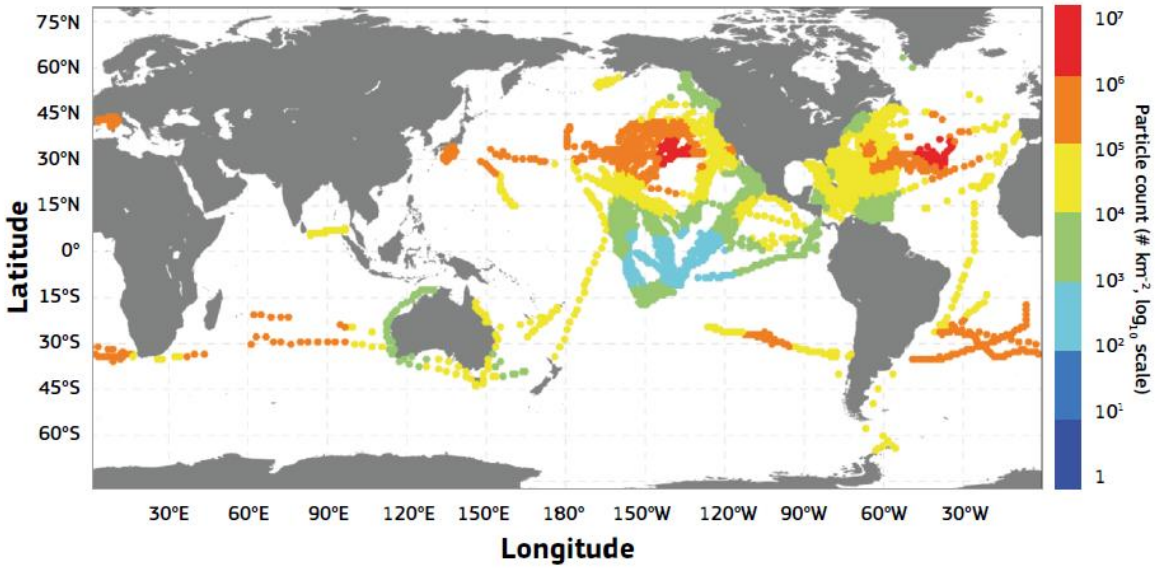


Figure 6 Overview of plastic pollution around the globe (European Commission, 2019)

In contrast to the ubiquity of plastic pollution, its impacts on biota and ecosystems are far from clear and there are knowledge gaps on long-term ecological consequences of plastics pollution (European Commission, 2019). The effects of plastics contamination on biota and ecosystems can vary according to the different size of plastic debris: plastics can be encountered as larger wastes called macroplastics, which usually enter the marine environment in their manufactured sizes, small particulates called microplastic (size between 5 mm and 100 nm) and nanoplastic (size between 100 nm – 1 nm)[2]. Microplastics break down into two types: primary microplastics, that are directly released into the environment in the form of small particles, and secondary microplastics. (Billard and Boucher, 2019). It was found that one of the most significant sources of microplastics in the marine environment was sewage polluted by fibres from washing clothes. Moreover, there was a positive relationship between

[2] There is no consistency on size classification of plastics debris and the dimension range can vary between various authors. The size classification reported here is in accordance to Waring et al., 2018.

the abundance of microplastics and human population density. Secondary microplastics derive from the degradation of larger plastics items into smaller fragments once exposed to UV radiation either under direct sunlight or in seawater (Li et al., 2016).

In the light of the analysis of the context related to plastics, two main future challenges emerge:

- the dependency on non-renewable fossil resources and the related issues - such as climate change, natural resource scarcity and environmental pollution- demand a transition to an economic system where materials, chemicals and energy are derived from renewable biological resources, such as plant and animal sources. These concepts are under the umbrella of the bioeconomy's policies, which therefore involves strategies for the replacement of fossil-based plastics with bio-based plastics.
- the quantitative data about mismanagement plastic waste and the relative critical impacts on biota and natural environment, point out the necessity to shift from an omnipresent linear economy and persistent plastics pollution to a circular economy, where the plastics materials should be substituted with biodegradable plastics.

The international and European strategies adopted to foster circular and bio-based economy are described next.

1.2 STRATEGIES FOR A CIRCULAR AND BIOECONOMY

1.2.1 BIOECONOMY

Bioeconomy is the production, utilization and conservation of biological resources, including related knowledge, science, technology and innovation, to provide information, products, processes and services across all economic sectors aiming towards a sustainable economy (Global Bioeconomy Summit, 2018). In other words, bioeconomy is a term for a politically desired transition from fossil feedstock to renewable resources that requires a systematic change of the entire energy and chemical industry (Dietrich, 2016).

A supportive and cohesive policy framework is considered a key issue for the development of the bioeconomy, and the market success of biobased polymers will depend on their inclusion into these frameworks (Dietrich, 2016). The bio-based economy first emerged as a policy concept within the Organization for Economic Co-Operation and Development (OECD) at the start of the 21st century. It linked advances in biotechnology to innovation and "green growth" via the use of renewable biological resources and innovative bioprocesses in industrial scale and biotechnologies, to produce sustainable products (OECD, 2001). In 2009, a landmark publication of the OECD named 'The Bioeconomy to 2030: Design a Policy Agenda', provides a broad-based analysis of future developments in the three sectors

where biotechnology has the greatest potential impact: agriculture, health and industry. It also explores the implications of developments in these sectors for the economy and society over the next two decades and develops a policy agenda. After the introduction of a vision for bioeconomy policies by the OECD, numerous countries were moving towards the implementation of national bioeconomy strategies. Today the promotion of a bioeconomy has been placed on the political agenda of more than fifty countries (FAO, 2019) (figure 7).

Figure 7 Bioeconomy policies around the world (OECD, Policy Initiatives for Health and the Bioeconomy, 2019)

The US government released its National Bioeconomy Blueprint in 2012. It has two stated purposes: to lay out strategic objectives that will help to realise the full potential of the US bioeconomy and to highlight early achievements toward those objectives. It envisages “a previously unimaginable future” in which two of the categories of new materials are: “ready to burn” liquid fuels produced directly from CO_2; and biodegradable plastics made not from oil but from renewable biomass (OECD, 2014).

Also in 2012 the European Commission first published a European bioeconomy strategy and Action Plan in a report entitled “Innovating for Sustainable Growth: a Bioeconomy for Europe” (European Commission, 2012), further updated in 2018 (EC, 2018). The updated version of this document identifies five objectives: ensuring food and nutrition security, managing natural resources sustainably, reducing dependence on non-renewable resources, mitigating and adapting to climate change, and creating jobs and strengthening Europe competitiveness. The update Bioeconomy Strategy of 2018 keeps the same objectives, but aiming at accelerating the European bioeconomy so as to maximise its contribution towards the 2030 Agenda and its Sustainable Development Goals (SDGs), as well as the Paris Agreement (EC, 2019).

Biobased plastics made from renewable resources such as crops or from waste streams, such as the residues from food processing, can play a crucial rule in the transition to a bio-based economy; likewise the mentioned strategies are fundamental to support the efficiency improvement of the technological production process of biobased polymers, that is actually one of the main bottlenecks of bioplastics production.

1.2.2 CIRCULAR ECONOMY

The model of Circular Economy predicts that the value of products, materials and resources is maintained in the economy for as long as possible, and the generation of waste minimised. This model is an essential contribution to the EU's efforts to develop a sustainable, low carbon, resource efficient and competitive economy (EC, 2015). In this model, products are designed to be reused or recycled, thereby becoming a feedstock for a subsequent process instead of waste (Dietrich, 2016).

In 2015, the European Commission adopted an ambitious Circular Economy Action Plan (EC, 2015) that establishes a concrete programme of action, with measures covering the whole cycle of products: from production and consumption to waste management, the market for secondary raw materials and a revised legislative proposal on waste. The proposed actions will contribute to "closing the loop" of product lifecycles through greater recycling and re-use and bring benefits for both the environment and the economy. One of the priority areas considered in the Action Plan regards plastics and the reduction of marine litter, according to the 2030 SDG which comprise a target to prevent and significantly decrease marine pollution of all kinds, including marine litter. In the context of the Circular Economy Action Plan and with the aim to protect the environment from the plastic contamination, in 2018 the EC published its ambitious Strategy for Plastics (EC, 2018), followed by Directive (EU) 2019/904 on the single use plastic ban. The European Strategy for Plastics involves the development of different measures to decrease the impact of plastic on the environment. Among these, there is the search for alternative feedstocks for plastic production, including bio-based feedstocks, if it is demonstrated that they result in genuine environmental benefits compared to the non-renewable alternatives in a life cycle perspective, being Life Cycle Assessment (LCA) the appropriate tool to do so.

This policy framework will make a tangible contribution to reaching the 2030 Sustainable Development Goals, among which there are recycling of 65% of municipal waste, 75% of packaging waste, reduction to a maximum 10% of all waste to landfills and economic incentives for producers of greener products, e.g. of packaging (Dietrich et al., 2016). Moreover, it will give a great impulse to the R&D for the development of biodegradable polymers whose life cycle shows low environmental impacts.

1.3 BIOPLASTICS: STATE OF ART

The term bioplastics is referred of a whole family of materials with different properties and applications. According to the European Bioplastics (European Bioplastics, 2019), a plastic material is defined as bioplastic if it is either bio-based, biodegradable, or features both properties (figure 8).

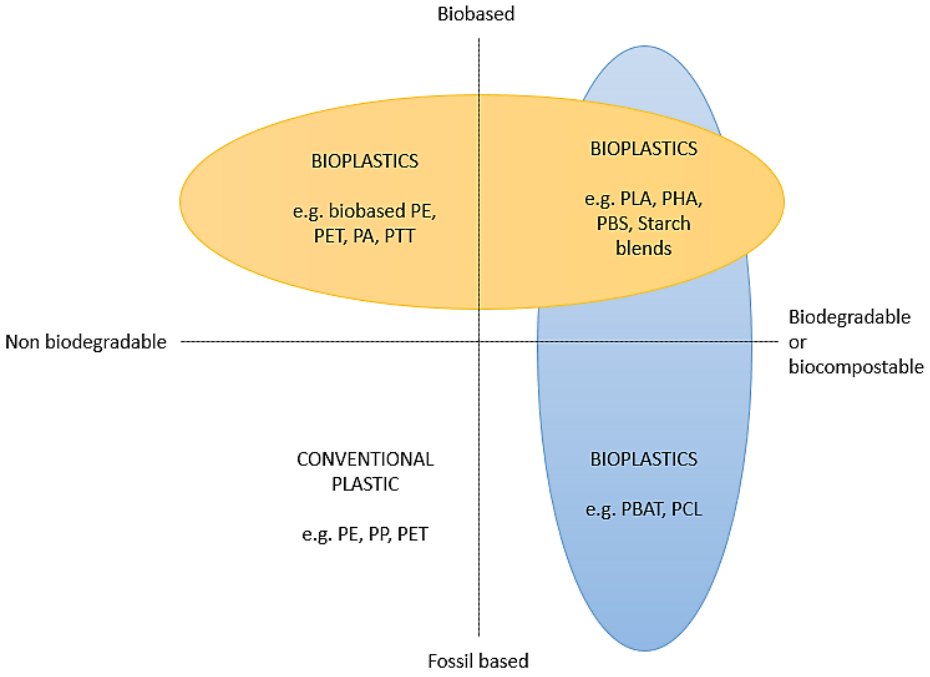


Figure 8 Bioplastic classification. PE, polyethylene; PET, polyethylene terephthalate; PA, polyamide; PTT, polytrimethylene terephtalate; PP, polypropylene; PLA, polylactic acid; PHA, polyhydroxyalkanoate; PBS, polybutylene succinate; PBAT, polybutyrate adipate terephthalate; PCL, polycaprolactone (adapted by European Bioplastics, 2019).

The advantage of bio-based plastics over conventional plastics is the reduction of the dependency on no renewable fossil resources and the reduction of the carbon footprint, according to the results of some performed life cycle analysis (European Bioplastic, 2018). Biodegradable plastics could have the great advantage to decompose in natural environment, despite the petrochemical counterparts, soothing the issue about the mismanagement plastic waste accumulation in natural environment.

At present the global market of the plastics based on renewable feedstock represent less than 1 % of the current total volume of plastics commercially offered annually. Bio-based or biodegradable plastics currently have a global production capacity of only 4 Mt (Geyer et al., 2017). The global market for bioplastics is predicted to grow continuously over the next years. According to the latest market data compiled by European Bioplastics in collaboration with the nova-Institute, global production capacities of bioplastics are predicted to grow from around 2.11 million tonnes in 2018 to approximately 2.62 million tonnes by 2023 (Figure 9).

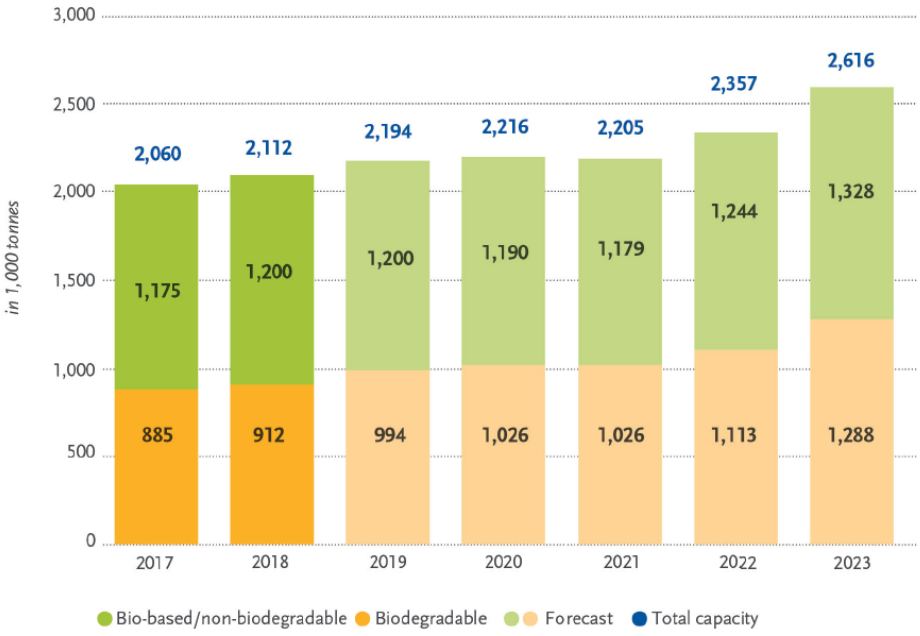


Figure 9 Global production capacities for bioplastic 2018-2023 (European Bioplastics, 2018)

The global distribution of bioplastics production and regional capacity development in 2018 is shown in figure 10**.** Asia is the major production hub, with the 55% of the global bioplastics produced in 2018. Regions such as Asia, the USA, and Latin America are implementing close-to-market measures to attract investment and production hubs to promote faster market development (European bioplastics, 2018). Around one fifth of the global bioplastics production capacity is in Europe. Here, the large-scale capital-intensity and decades-long optimisation of the petrochemical industry have made and still make it difficult to scale up the production of new materials that do not fit into the existing infrastructure (EC, 2019). However, the expected growth until 2023 will be supported by recently adopted policies in several European Member States. In particular, the first aim of The European Bioeconomy Strategy and its Action Plan is to *strengthen and scale-up the bio-based sectors, unlock investments and markets* (EC, 2018) and it is expected to support the young bioplastics market.

Figure 10 Global production capacity by region (European Bioplastics, 2018)

1.3.1 BIOPLASTICS PRODUCTION ACCORDING TO THE MAIN APPLICATIONS AND TYPE OF BIOPOLYMER

As for conventional plastic, packaging remains the largest field of application for bioplastics with almost 65% (1.2 million tonnes) of the total bioplastics market in 2018. Other applications are catering products, consumer electronics, automotive, agriculture/horticulture and toys to textiles (European Bioplastics, 2018). According to the type of biopolymer, the leading biobased non-biodegradable plastics are biobased PET (polyethylene terephthalate), biobased PA (polyamides) and biobased PE (polyethylene). Among the bio-based biodegradable polymers, starch blends are the most produced in 2018. Recently two innovative biopolymers, PLA (polylactic acid) and PHAs (polyhydroxyalkanoates), are the main drivers of the research and production in the field of bio-based, biodegradable plastics (figure 11).

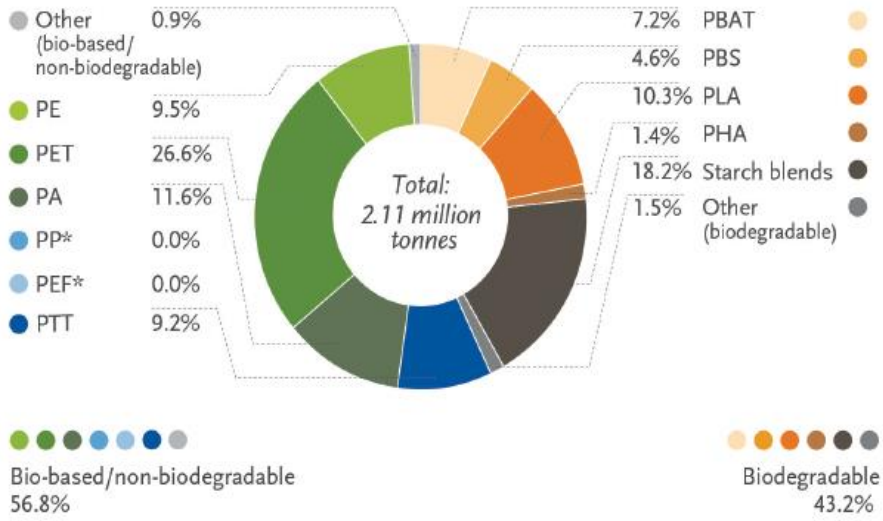


Figure 11 Global production capacities of bioplastics 2018 (by material type) (European bioplastics, 2018)

1.3.2 BIOPLASTICS FROM DIFFERENT FEEDSTOCKS

The biggest challenge of the bio-based plastics is the replacement of the fossil fuels as raw materials with renewable sources, in a way that does not lead to irreversible depletion of natural resources or other negative externalities.

The possible feedstocks from which bioplastics can be produced are: biomass, carbon dioxide (CO_2), methane (CH_4) derived from biological processes, waste streams.

At present, bioplastics are mostly produced from biomass, since these plants are designed to produce the highest yields and withstand pests and demanding weather conditions (EC, 2019). The biomass used for biopolymer production may be distinguished on the basis of the origin and the composition. Regarding to the origin, a biomass can be a first generation biomass (crop cultivated ad hoc, such as sugar cane, sugar beet and whey) or a second generation biomass, that is to say by products-biomass, such as plants that are not eligible for food or feed production or the organic fraction of the municipal solid waste. On the basis of their composition, the biomass can be carbohydrate starchy biomass, such as corn or sugar cane; ligno-cellulosic biomass (as agricultural waste or organic fraction of municipal solid waste) and lipid biomass, like manure and animal wastes.

Issues related to the environmental impacts of the agricultural phase for the production of the feedstock, such as competition of land use between biomass for plastics production and food, water consume, fertilisers and pesticides, forestry practices, threat the use of biomass as feedstock. Currently the production of bio-based plastics utilises 1.4 million hectares of land, which is approximately 0.02 % of the global agricultural area totalling 4.9 billion hectares. (European Commission, 2019). But if the demand for industrial bio-based products and energy from biomass continues to grow, this could lead to an expansion of global arable land at the expense of other agriculture or natural ecosystems. Therefore, there is an emerging interest for the transition from first to second generation feedstock, and to the use of by-products and waste streams as substrate.

1.3.3 BIOPLASTICS' END-OF-LIFE

At the end-of-life, bioplastics are suitable for a broad range of options with the overwhelming part of the volumes of bioplastics produced today already being recycled alongside their conventional counterparts where separate recycling streams for certain material types exist (e.g. bio-based PE in the PE-stream or bio-based PET in the PET stream). Innovative materials such as PEF and PLA can also be mechanically recycled but still face the hurdles of low market shares.

An additional waste treatment option, feasible only for biodegradable polymers, is composting. Biodegradable products can be treated together with organic waste in industrial composting plants or anaerobic digestion plants and are thus diverted from landfills and turned into biogas or valuable compost.

If bioplastics can no longer be reused or recycled, they can be used to produce renewable energy (Euopean Bioplastics, 2018). Natural cellulose fibre and starch have relatively lower gross calorific values (GCV) than coal but are similar to wood, and thus still have considerable value for incineration. In addition, the production of fibre and starch materials consumes significantly less energy in the first place, and thus contributes positively to the overall energy balance in the life cycle (Coles et al., 2011).

1.3.4 POLYHYDROXYALKANOATES (PHAS)

Among the various types of biopolymers, a promising candidate has been seen in polyhydroxyalkanoates (PHAs), being recognized as completely biosynthetic and biodegradable with zero toxic waste, and completely recyclable into organic waste (Chanprateep, 2010). The eco-friendly nature and flexible modulation properties of PHAs have put their research on a pedestal (Arumugam, 2019).

PHAs are homo- or heteropolyesters produced and intracellularly stored by numerous types of microorganisms. It is known that there are more than 300 types of microorganisms able to synthesize and accumulate PAHs under nitrogen limiting condition along with excess carbon source. This class of natural esters exhibits high variability by encompassing more than 150 monomer types that provide different properties and functionalities. The first PHA, poly(3-hydroxybutyrate) (P3HB), was discovered in Bacillus megaterium, by the French scientist Lemoigne (1926). Surprisingly, this material presented the structure and properties of thermoplastic polyester. Depending on the monomer's chemical structure, PHAs properties span a wide range, including materials that resemble polypropylene and others that are elastomeric (Williams, 1999). Among them, PHBs are considered strong candidates for bioplastic production as they have very similar properties to synthetic polymers (Harding et al., 2007). In terms of molecular weight, brittleness, stiffness, melting point, and glass transition temperature, the PHB homopolymer is comparable to some of the more common petrochemical-derived thermoplastics, offering good resistance to moisture and aroma barrier properties as shown in table 1 (Grothe, 1999; Bugnicourt et al., 2014).

Table 1 Properties of polypropylene and poly-β-hydroxybutyric acid (PHB) (derived from Harding et al., 2007)

	Polypropylene	**PHB**
Density [kg/m^3]	900-910	1250
Melting point [°C]	176	45-180 P(3HB) = 180
Tensile strenght [MPa]	38	13-40
Shrinkage [%]		1-3
Elongation [%]	400	5-680
Young's modulus	17000	350-1000
Glass-transition temperature [°C]	-10	15 P(3HB) = 4
Service temperature [°C]		-30 to 120
Specific heat (20-80 °C) [kJ/kgK]	1.9	
Thermal conductivity (20-150°C) [kW/mK]	0.42-0.61	

One of the main advantages of PHAs is their biodegradability. They are efficiently degraded in the environment because many microorganisms in soils are able to secrete PHB depolymerases, enzymes that hydrolyse the ester bonds of a polymer into water-soluble monomers and oligomers; and microorganisms then metabolize these degradation products into water and CO_2 (Torreiro, 2017; Chanprateep, 2010). Biodegradation depends on the properties of the polymer (composition, degree of crystallinity, molecular weight) and environmental conditions (pH, temperature, microbial activity, humidity, colonized surface) (Bugnicourt et al., 2014). According to Rostkowski (2012) PHAs resins in soil, sludge, and seawater, will degrade rapidly, with aerobic mineralization to carbon dioxide and anaerobic biodegradation to biogas. In methanogenic bioreactors both PHAs and biocomposites containing PHAs rapidly degrade. They are, however, stable like paper in ambient conditions or in the absence of high concentrations of microorganisms (Shogren, 2019). A study found, depending on the conditions, they may degrade in a period of time between 45 to 56 days (Queiroz et al., 2009).

PHAs have had a multidimensional evolution in different fields for various applications. Once established their identity as natural, biocompatible, not toxic polymers, several uses and applications have been developed such as medical implants, cosmetics, healthy food additives, textile industry, scaffold material in tissue engineering, drug carriers. Owing to their comparable properties to synthetic polymers, several companies have been interested in their use for packaging application, such as mainly for use as shopping bags, containers and paper coatings (Chen, 2009; Zinn, 2010).

The production of PHAs happens via bacterial fermentation of sugars, fatty acids and waste streams, and applying particular culture conditions. Typical used substrates used as carbon source includes

agricultural crops (sugar cane, corn starch and corn stover, vegetable oils such as soybean and rapesees oils, genetically modified corn), biogas and waste streams containing complex organic substrates, like wastewaters from food industries to sewage sludge (Mannina et al., 2019). Therefore, in this context, resource recovery from wastewater treatment processes can have a role in the plastic's circular economy (Mannina et al., 2019).

Figure 12 shows several possible production pathways for PHAs that result from the conversion of different biomass feedstock. After fermentation, the produced PHA polymer must be extracted from the microbial intercellular organelles and purified. This extraction is typically performed by solvent extraction, but other technologies have been proposed to increase extraction efficiency such as enzymatic, mechanical and chemical cell disruption and supercritical extraction (Cristóbal et al., 2016).

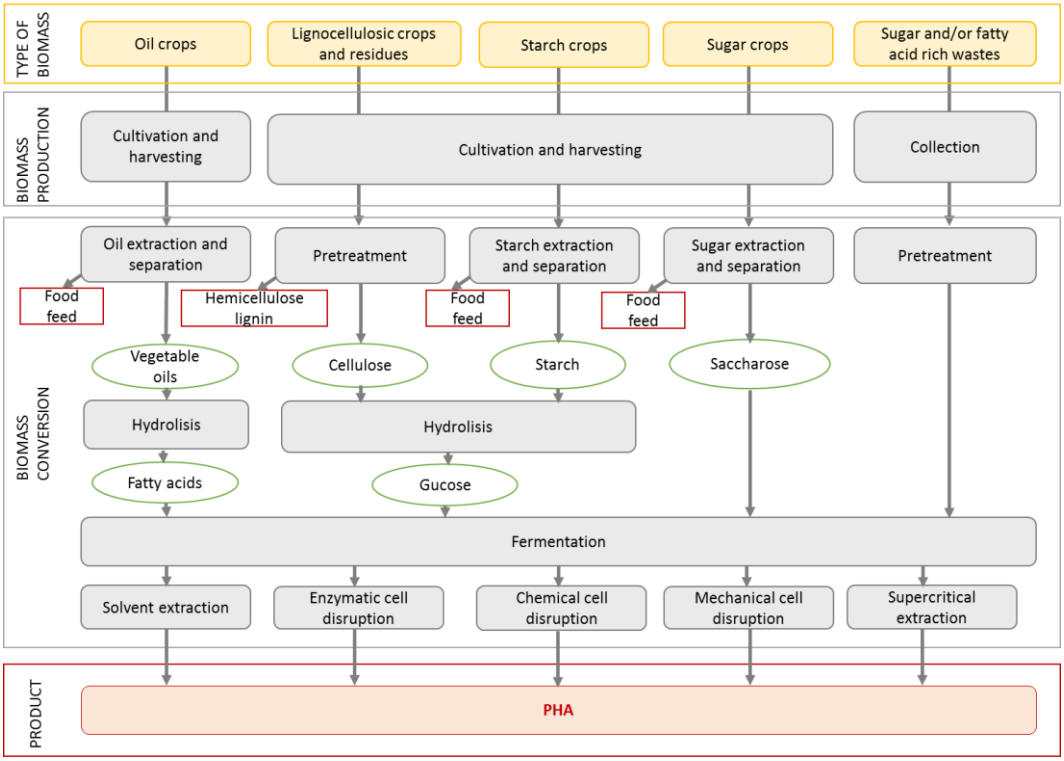


Figure 12 Flowchart of the polyhydroxyalkanoates production process (adapted by Cristóbal et al., 2016)

The production of PHA using pure culture happens in two-stage batch production process, with an inoculation of bacteria introduced into a sterile solution. Sterilisation is a highly energy intensive process so using mixed culture, where does requirements are not needed, are gaining attention as an interesting alternative. So, mixed-culture production can be cost-competitive because it can utilise complex, inexpensive feedstocks as substrates, without the sterilisation required by pure cultures (Heimersson, 2014).

Currently PHA production at large scale is still limited by its high production cost compared with conventional fossil-fuel based plastics. In fact, the current PHA price, depending on polymer composition, ranges from 2.2 to 5.0 €/kg that is at least three times higher than the major fossil-fuel based polymers which typically cost less than 1.0 €/kg (Gholami et al., 2016). Despite the high production costs, the convenience in the use of PHA should be evaluated taking into account the missed environmental costs related to their use in substitution of traditional plastics. Indeed, the use of PHA avoid plastic pollution (they are biodegradable) and accomplishing the need for the environmentally responsible use of resources. Among the most important factors in the overall production cost of PHA, the use of pure or genetically modified cultures, the cost of the raw materials used as precursors and the recovery methods employed are the most important ones (Mannina, 2019). However, prices have been dropping over past 20–30 years due to improved PHA production efficiencies as well as a focus on cheap raw materials from agro-industrial residues. This trend is expected to continue as much research and development effort has been focused on PHA's in both academic and industrial sectors (Shogren, 2019).

1.4 LIFE CYCLE ASSESSMENT

LCA is a compilation and evaluation of the inputs and outputs and the potential environmental impacts of a product system throughout its life cycle (Curran, 2016). It considers all the aspects of resource use and environmental releases associated with a system, as defined by the function provided by a product (for simplicity, the word 'product' is used although the life-cycle concept applies equally well to processes and activities). Specifically, LCA is a holistic view of environmental interactions that covers a range of activities, from the extraction of raw materials from the Earth and the production and distribution of energy, through the use, and reuse, and final disposal of a product (Curran, 2008).
LCA is a tool intended to support the choice of different (technological) options for fulfilling a certain function by compiling and evaluating the environmental consequences of these options, thereby helping decision makers (Curran, 2016).

1.4.1 LCA METHODOLOGY

The LCA methodology has been standardized under the ISO 14040 (ISO, 2006) and 14044 (ISO, 2006) that provide the methodological framework based on four phases (figure 13): goal and scope definition, life cycle inventory analysis (LCI), life cycle impact assessment (LCIA) and life cycle interpretation.

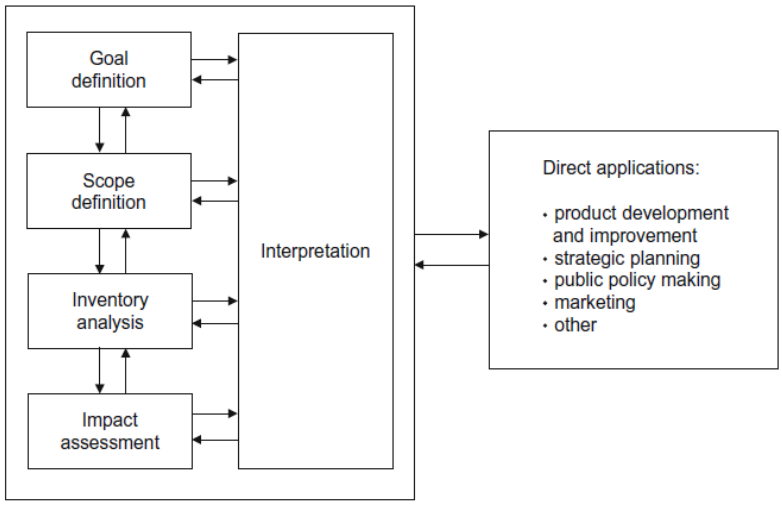


Figure 13 Phases of LCA (ISO 2006)

The goal and scope definition phase establishes the goal of the study, the functional unit, the system boundaries, the reference flow, the product system(s) under study, and the breadth and depth of the study in relation to the goal.

First, the goal of the study is clearly stated and justified. Its definition and intended use will guide the practitioner in setting the scope and boundaries for the analysis. It is also crucial for directing future data collection efforts - the inventory analysis phase. Another important requirement of goal and scope phase is the definition of functional unit, which describes the primary function fulfilled by a (product) system and quantifies how much of this function is to be considered in the intended LCA study. It is used as a basis for selecting one or more alternative (product) systems that might provide these function(s). The functional unit enables different systems to be treated as functionally equivalent and allows reference flows throughout the system to be calculated. On the basis of the functional unit, a number of alternative product systems may be declared functionally equivalent and reference flows will be determined for these systems. The reference flow is a measure of the needed outputs from processes in a given (product) system that are required to fulfil the function expressed by the functional unit. The system boundaries determine which unit processes – that are the elementary operations- are to be included in the LCA study (Curran, 2016).

The second phase, life cycle inventory analysis, consists in data collection of the material and natural resource inputs and the outputs to the environment. These data are determined, first qualitatively, and then quantitatively. The basis of the inventory analysis is the unit process to which associates the flows that cross the boundaries and the relative environmental interventions (e.g. extracted resources, emissions). The next step concerns drawing the flow diagram of the system studied. It constitutes the basis for the whole analysis and identifies all relevant processes of the product system with their

interconnections. The functional unit delivered by the system is the central element; starting from here, the processes ramify ‘‘upstream’’ up to the different resources used, and ‘‘downstream’’ to the different ways of waste management involved. In scaling the process data to the actual quantities needed, the problem of multiple processes and allocation frequently comes up. The problem lies in processes that are part of more than one product system, the so- called ‘‘multifunctional processes.’’ How the environmental impact of these processes should be allocated to the different product systems involved need to be defined. Allocation is often done based on the relative mass, energy content, or economic value of the coproducts.

Inventory analysis continues to be the most time-consuming phase of an LCA due to the lack of readily available data. Data collection is a core issue in LCA because a large amount of data is needed to model each unit process of the flow diagram. Proper evaluation of data quality is an important step in every LCA since it has a large influence on results (Curran, 2016).

The third phase (LCIA) aims at describing the environmental consequences of the list of materials and consumed energy quantities determined in the environmental analysis. The impact assessment is achieved “translating” the environmental loads from the inventory results into environmental impacts, such as acidification, ozone depletion, human health effects, etc. Several steps may be distinguished:

- selection of impact categories, which should be conducted during the initial goal and scope definition phase to guide the LCI data collection process. A difference must be made between midpoint and endpoint categories. Impact modelling can occur either at a midpoint within the cause-effect chain or at an endpoint. The more common midpoint approach has the advantage that it includes fewer debatable assumptions and accommodates less-established facts; the endpoint approach has the advantage of providing more intuitive metrics (like loss of crops instead of kg CO_2 equivalents);
- classification- assignment of inventory results to their respective impact categories;
- characterization: it is a quantitative step where the size of the environmental impacts of each input and output within the product system are calculated per each impact category and converted into indicators that represent the corresponding potential impacts on the environment;
- normalization: it consists in relating the characterization results to a reference value in order to gain a better understanding of the magnitude for each impact category;
- grouping: sorting and possibly ranking of the indicators. Examples of suitable group headings are *global/regional/local impacts and impacts with high / medium / low priority.* This can be useful for the analysis and the presentation of results;
- weighting, which is the aggregation of characterization results across impact categories;

- data quality analysis. It includes sensitivity analysis among other things in order to obtain a better understanding of the reliability of the LCIA results.

  The first three steps are mandatory, while the others are optional (Baumann and Tillman, 2004; Curran, 2016).

The last phase of LCA is the Life Cycle Interpretation. In this phase the results of the other phases are considered together and analysed in the light of the uncertainties of the applied data and the assumptions that have been made and documented throughout the study. The outcome of the interpretation should be conclusions or recommendations that respect the intentions of the goal definition and the restrictions that this imposes on the study through the scope definition and take into account the appropriateness of the functional unit and system boundaries. The interpretation should present the conclusions of the LCA in an understandable way and help the users of the study appraise their robustness and potential weaknesses.

The interpretation proceeds through three steps: the significant issues (key processes and assumptions, most important elementary flows) from the other phases of the LCA are identified; these issues are evaluated with regard to their influence on overall results of the LCA and the completeness and consistency with which they have been handled in the study; the results of the evaluation are used in the formulation of conclusions and recommendations from the study (Hauschild et al., 2018).

1.4.2 LCA APPLIED TO BIOPOLYMERS

Being an emerging technology, the production of biopolymers faces many challenges (e.g. technological improvement, process optimization) to become cost competitive over conventional alternatives. Moreover, all the stages of the production process, the use and the end-of-life phase of the resulting products have to be assessed under an environmental point of view for checking that bioplastics really lead to environmental benefits compared to the petrochemical counterparts. When referring to a new product or a new manufacturing process, routine questions asked of researches by funding bodies are “How much energy will this save?” and “By how much will this cut greenhouse gas emissions?” (Cooper and Gutowski, 2018). The answer is provided by LCA as it provides the best framework for assessing the potential environmental impacts of products currently available. The European Union's Research and Innovation funding programme for 2007-2013 and Horizon 2020 (2014-onwards) calls explicitly require addressing environmental aspects from a life cycle perspective within the innovative products development process (EC, 2019).

Application of LCA has great potential to drive the development of emerging technologies with improved environmental performance by identifying environmental hotspots and comparing with existing alternatives (Moni et al., 2019). However, the conventional LCA is a methodology that is well

suited to evaluate established technologies. Performing LCAs of emerging technology systems poses challenges because relevant observations are lacking with regards to the projected final system, projected unit process data, etc. (Cucurachi et al., 2018). First the inventory stage is more complex since there is no real production system to investigate: all the LCA studies reviewed, except one for some data (Kim and Dale, 2008), are based on lab- or pilot-scale, and process data are only available at these scales or computer simulated (e.g. Akiyama et al., 2003; Harding et al., 2007), and not at observed full-market scales. Second, for comparative studies, the definition of functional unit gets complicated since the new product or process might have unique properties that make the comparison with present products difficult and less straightforward. Third, manufacture of products or the processes themselves can, in some cases, be expected to start several years ahead, and assumptions on surrounding systems (e.g., energy supply; marginal or average, as well as what production system to assume) will be required (Hospido et al., 2010).

1.5 GOAL AND OUTLINE OF THIS THESIS

The goal of this thesis is to provide an overview on the current state of the art of bioplastic production. In particular, a key aspect of this work is to ascertain whether its environmental performance is better than the conventional production processes. In line with the life cycle thinking promoted by the European policy framework and objectives by 2030, the methodology used for this evaluation is the Life Cycle Assessment. Through this tool application, the hot spots and the technological gaps as well as the environmental opportunities of the bioplastic production process emerge. Therefore this thesis wants to collect information and data from the previous literature to deduce the background on LCA on bioplastic production; from this starting point, its purpose is individuating which are the well-established aspects as well as the future challenges that the researchers have to face in order to make the replacement of plastics by biopolymers possible. Particular attention is reserved to investigate the different feedstock that can be used as substrate for biopolymers production in order to minimize the environmental impacts and maximize the process optimization. In parallel, another element on which this work wants to dwell is understanding which are the opportunities that LCA methodology offers in the evaluation of an emerging product, and which are, on the other hand, its limits.

These objectives are tackled according to the following outline:

- In **chapter 2** a literature review on LCA studies on PHAs production is performed. This chapter has a great importance as it will provide a perspective on the knowledge level and the main results achieved until now on biopolymers life cycle aspects (e.g. the hot-spots of the

production process, the advantages obtained using certain feedstock as substrate, the awareness on their long-term impacts, etc.). It will allow clarifying the directions towards which the future researches and the engineering projects should move in order to reach a more sustainable production, to increase the diffusion on the market and to reduce the environmental burdens.

- In **chapter 3** the goal and scope of the LCA study of the PHA production from mussel processing wastewater performed in this work is defined.
- **Chapter 4** presents the LCA application to the pure culture fermentation process.
- In **chapter 5** the LCA is applied to the mixed culture fermentation process.
- **Chapter 6** is dedicated to perform a comparative analysis, integrating the LCA performed in chapter 4 and 5 with the LCA of the downstream process aimed to the PHA isolation. The aim of the comparison is to evaluate pro and cons of each available option, i.e. full pure and mixed PHA production. In this section the valorisation scenario is also compared to the baseline option: the mussel processing wastewater treatment.
- A brief conclusion (**chapter 7**) will resume the main results obtained from the present work and define research questions for future outlook.

2 REVIEW OF LCA STUDIES ON PHA PRODUCTION

2.1 METHODOLOGY

For the review of the literature, the search engine Google Scholar was used being the focus studies on LCA of PHAs production and end-of-life. The following keyword combinations were applied: life cycle assessment of PHA (PHB) production, LCA of bio-based polymers, sustainability of green plastics, mixed-culture PHA production assessment, review of LCA on PHA production. Some references taken from others review papers were useful to individuate the LCA studies of interest, such as the review works performed by Patel et al. (2005), Álvarez-Chávez et al. (2012), Hottle et al. (2013), Yates & Barlow (2013), Heimersson et al. (2014), Dietrich et al. (2017), Spierling et al. (2018).

2.2 LITERATURE REVIEW

Within the LCA literature review, twenty-four suitable studies have been identified. All the papers which supply information on the environmental sustainability of the production and disposal of PHA/PHB following the LCA methodology were selected for the review, including also assessment performed by using the sustainable process index (SPI) (Koller et al., 2013), a member of the ecological footprint family, and the waste reduction algorithm (WAR) (Leong et al., 2017). Both studies referred to LCA of PHAs as biopolymer and PHAs-based consumer products were included, while LCA analysis of bio-based composites made of PHAs together with other type of fibre were excluded, since not purely PHAs were evaluated. The time period of publication of these papers goes from 1998 to 2018 (table 2).

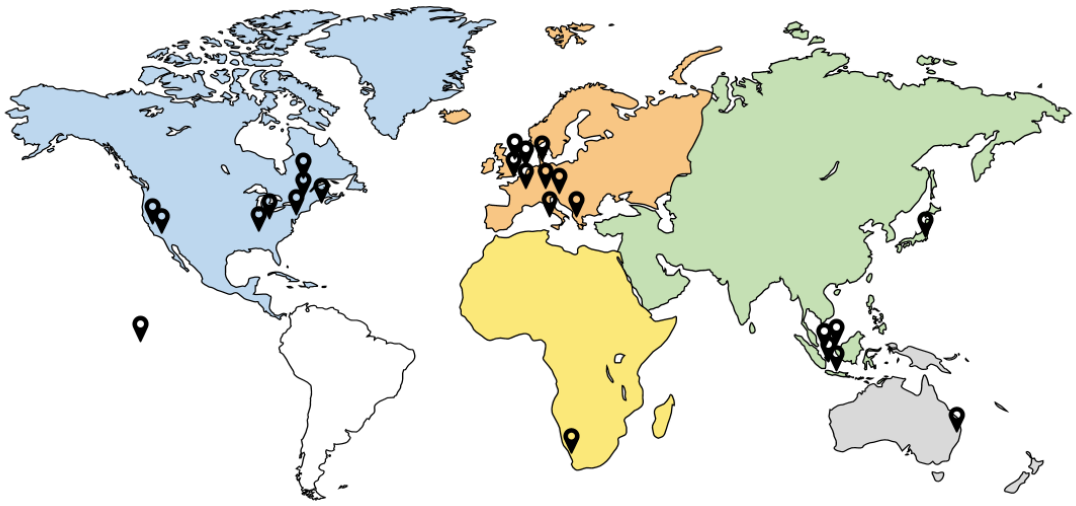
Table 2 Papers reviewed in chronological order

YEAR	AUTHORS
1998	Heyde M.
1999	Gerngross T. U.
2001	Kurdikar D. et al. [a]
2003	Akiyama M. et al.
2005	Kim S. & Dale B. E.
2006	Patel M. et al.
2007	Harding K. G. et al.; Pietrini M. et al. [b]; Hermann B. G. et al. [c]; Gurieff N. & Lant P.
2008	Yu J. & Chen L. X. L.; Kim S. & Dale B. E.
2009	Zhong ZW
2010	Tabone M. D. et al.; Khoo H. H. et al. (Part 1); Khoo H. H. & Tan R. (Part 2)
2011	Hermann B. G. et al. [d]
2012	Kendall A.; Rostkowski K. H. et al.
2013	Koller M. et al.

2015	Fernández-Dacosta C. et al.
2016	Righi S. et al.
2017	Leong Y. K. et al.
2018	Kookos I. K. et al.

[a] Gengross T. U. co-author, [b, c, d] Patel M. K. co-author

The geographical distribution of the studies is rather wide, including countries in U.S., Europe, South Africa, Asia and Oceania, although there a clear concentration in Europe (9 references) and U.S. (9 references) (figure 14).



USA	EUROPE	AFRICA	ASIA
Gengross T. U., New Hampshire, 1999	Hyde M., Germany, 1998	Harding K. G. et al., South Africa, 2007	Akyiama M. et al., Japan, 2003
Gerngross T. U. and Slater, New Hampshire, 2000	Patel M. K. et al., The Netherlands, 2006		Zhong ZW et al., Singapore, 2009
Kurdikar D. et al., Massachusetts 2001	Hermann B. G. et al., The Netherlands, 2007		Khoo H. H. et al, Part 1, Singapore, 2010
Kim S. and Dale B. E., Michigan, 2005	Pietrini M. et al., Italy, 2007		Khoo H. H. and Tan R. B. H., Part 2, Singapore, 2010
Yu J. and Chen L. X. L., Hawaii, 2008	Hermann B. G. et al., The Netherlands, 2010	OCEANIA	Leong. Y. K. et al., Malaysia, 2017
Kim S. and Dale B. E., Michigan, 2008	Koller M. et al., Austria, 2013	Gurieff N. and Lant P., Australia, 2007	
Tabone M. D. et al, Pennsylvania, 2010	Dacosta C. F. et al., The Netherlands, 2015		
Kendall A., California, 2012	Righi S. et al., Denmark, 2016		
Rostkowski K. H. et al., California, 2012	Kookos I. K. et al., Greece, 2018		

Figure 14 Global distribution of LCA studies on PHA

Being the first references from 1998, it can be assumed that the interest in the production of polymers based on alternative raw materials emerged when the feedstock (i.e. oil) for conventional plastics was affected by price increase and concerns about its scarcity. Consequently, also the researches began to critically assess the sustainability of these innovative materials under a life cycle perspective (e.g. Heyde 1998, Gengross 1999). PHAs industrial production started with the Imperial Chemical Industries in response to the oil crises of the 1970s. Since then, other companies began to produce bioplastic, but it turned out to cost substantially more than its fossil fuel–based counterparts and offered no performance advantages other than biodegradability (Gerngross and Slater, 2000). The earlier LCA

studies led to the conclusion that the replacement of conventional polymers with PHAs did not represent an advantageous alternative in terms of non-renewable energy use and greenhouse gases (GHGs) emissions reduction. For example, Heyde (1998) found that, regarding a cradle-to-grave study, the energy requirements for PHB production could be higher than those necessary for HDPE and PS. Also Gerngross (1999) reported that, considering a cradle-to-gate analysis, PHA production do not offer any opportunities for emissions reduction. Kurdikar et al. (2001), considering a cradle-to-gate system, concluded that plant-based PHA production does not provide GHGs reduction over PE, unless replacing the use of fossil fuels sources with an integrated system wherein the energy for polymer processing is provided by renewable biomass material, like corn stover. These disadvantages led to the closure of many PHAs related projects in some companies (Chen, 2009). The increase of oil prices to over US$ 100 per barrel at the beginning of 2003 led to a revived industrial interest for PHAs. Since then, new plants opened in China, the US, Italy and Brazil (Dietrich et al., 2016). Although the cost of petroleum has been drawn down by the financial tsunami in late 2008, PHAs as a bioplastic has been considered as useful for reducing CO_2 emissions (Chen, 2009) and some researchers have continued to investigate them in order to make their production cost competitive. Moreover, consumers awareness regarding the environmental impacts of fossil fuels oriented the market demand towards bioplastics (Chen 2009; Keshavarz & Roi 2010).

Another element that might be behind the growing number of LCA studies are the national and international strategies on circular and bioeconomy (seen in chapter 1- paragraph 2). So, the European Commission concluded its “Communication on Integrated Product Policy” (CEC 2003) stating that LCA provides the best framework to assess the potential environmental impacts of products currently available as Life Cycle Thinking is widely accepted as a guiding principle for designing the EU Product Policy Framework promoting a Circular Economy approach (EEB-European Environmental Bureau, 2018). In 2018 the EC, in its *Strategy for Plastic in a Circular Economy*, mentioned its commitment to develop Life Cycle Assessment to understand the environmental impacts of alternative feedstock used in bioplastic production and to identify the conditions under which the use of biodegradable or compostable plastics is beneficial (EC, 2018).

The analysis of the papers selected will cover different elements, as detailed in the coming subsections, trying to cover the main aspects of decision when defining an LCA to be able to identify common aspects or tendencies as well as open or unresolved questions.

2.2.1 MOTIVATION OF THE STUDIES

According to the summary presented in table 3, the main reason which has encouraged the researchers to perform LCA is evaluating the competitiveness of PHAs compared to their petrochemical counterparts, especially PS, PE, PP and PET. This is true except for Zhong et al., (2009), Hermann et al. (2011), Kendall (2010) and Rostkowski (2012), that are mainly focused on the comparison between the use of different bio-based materials as substrate. For example, Zhong et al. (2009) perform LCA to identify the environmental impacts related to the use of three different feedstocks: glucose from con grains, cheese whey and genetically engineered plants. Hermann et al. (2011) use LCA to evaluate the end-of-life phase, stage that may strongly influence the conclusions but it is often neglected because of the lack of data, and compare home and industrial composting, anaerobic digestion and incineration for PHAs and other biodegradable materials as paper, cellulose, starch, PLA, starch/polycaprolactone, PBAT. Kendall (2012) wants to examine the production of PHB from two different feedstocks: the cellulosic fraction or organic residuals from material recovery facilities and a dedicated feedstock as corn. Likewise, Rostkowski (2012) uses LCA to evaluate PHB production from methane or from cultivated feedstock (again corn). Akiyama et al. (2003), Kim and Dale (2005), Kookos et al. (2018) apply LCA both to compare the environmental burdens of PHA production from different renewable feedstocks and to assess the sustainability of the PHA production over the conventional plastics. Besides the comparison with the petrochemical counterpart, Gurieff and Lant (2007) uses LCA to evaluate the best option for industrial wastewater recovery, assuming two possible alternatives: PHA production or biogas production. Righi et al. (2016) and Leong et al. (2017) apply LCA to compare different processes for PHAs extraction/purification, being this life step challenging to reduce the cost and the environmental impacts of the PHA production.

Table 3 Motivation of the selected LCA studies

SOURCE	MOTIVATION OF THE STUDY
Hyde 1998	To compare the environmental impacts between products based on PHB, PS and HDPE.
Gerngross 1999	To evaluate the sustainability of the PHA manufacturing process, comparing it with PS.
Kurdikar et al., 2001	To compare the global warming potential of the production of PHA and of PE.
Akiyama et al., 2003	To compare the production costs, LCI of energy consumption and CO_2 emissions of P(3HB) from glucose and [P(3HB-co-5mol% 3HHx)] from soybean oil. The values of bio-based polymers are compared also with those of petrochemical polymers (HDPE, LDPE, PP, PS, b-PET: bottle grade PET).
Kim and Dale, 2005	To investigate the environmental performance of PHA obtained from corn and corn stover, comparing them with those of PS.
Patel at al 2006	To assess the environmental effects of substituting bio-based chemicals for petrochemicals. PHAs are compared to HDPE.
Harding et al., 2007	To clarify the environmental advantages of PHB over the petroleum-based plastics, specifically PP and PE.

Hermann et al., 2007	To analyse the environmental performance of producing ten bulk chemicals, including PHAs, from biomass, considering current and future (2030) technology. To compare it with bulk petrochemicals (HDPE is the benchmark for PHA).
Pietrini et al., 2007	To predict the environmental benefits that could be reached by the replacement of conventional petrochemical polymers with PHB for the production of two specific products: CRT monitor housing (conventionally made of HIPS: high impact polystyrene) and an internal car panel (conventionally made of PP-GF: glass-fibers-filled polypropylene).
Gurieff and Lant, 2007	To evaluate the recovery of industrial wastewater for mixed-culture PHA production or for biogas production. Comparison between production of mixed-culture PHA, pure-culture PHA and HDPE.
Yu and Chen, 2008	To estimate the GWP and NREU of the coproduction of PHA bioplastics in cellulosic ethanol biorefineries. To compare these indicators with those of petrochemical counterparts (PS, LDPE, PP, PET) and with other biopolymers (PLA, PHA from glucose, PHA from vegetable oils).
Kim and Dale, 2008	To estimate the environmental performance of PHB derived from corn grain using site specific process information.
Zhong et al., 2009	To identify the environmental impact of three PHA manufacturing processes, using three different feedstocks.
Khoo et al., 2010 Part 1	To compare the environmental performance of the production of carrier bag made of PP or of PHA.
Khoo and Tan, 2010 **Part 2**	To investigate the end-of-life options for conventional PP and bio-based carrier bag, considering three end-of life scenarios: landfill, incineration and composting.
Hermann et al. 2010	To approximate carbon and energy footprints of waste treatment phase of the PHA based product and to find out what the best waste treatment option for biodegradable material is, by modelling home and industrial composting, anaerobic digestion and incineration.
Tabone et al., 2010	To compare adherence to green design principles in currently available plastics to the life cycle environmental impacts of each plastic production. A case study of 12 polymers is presented, among which two are PHAs. The others polymers are PET, HDPE, LDPE, PP, PC, PVC, GPPS, two PHA, B-PET.
Kendall, 2012	To evaluate the environmental and energy performance of a potential production pathway for PHB from a waste stream (the cellulosic fraction of organic residuals), and to assess the consequences of diverting this waste material from landfills to biopolymer production. To compare the production of PHB from two different feedstock, a waste flow and a purpose-grown crop such as corn.
Rostkowski et al., 2012	To anticipate the environmental impacts of PHB production from waste biogas by extrapolation from laboratory scale studies.
Koller et al., 2013	To identify the ecological “hot spots” of the PHA production process. To compare the sustainability of PHA biopolymers from whey with fossil polymers (PS, PET, PP, PE). To compare the use of whey for PHA production or for whey powder production.
Dacosta et al., 2015	To perform a techno-economic and environmental performance of the industrial production of PHB from wastewater for identifying bottlenecks and best opportunities to scale-up the process prior to industrial implementation.
Righi et al., 2016	To assess the environmental performances of the novel protocol proposed by Samorì et al. (2015) for the extraction of PHAs with dimethyl carbonate (DMC) from microbial slurry and from dried biomass and compare them with the environmental impacts of extraction process based on the use of 1,2-dichloroethane (DCE).
Leong et al., 2017	To estimate the economic and environmental feasibility for the industrial-scale PHA production using aqueous two-phase extraction (ATPE) as primary recovery step, and compare it with other purification and recovery strategy which does not include ATPE step.
Kookos et al., 2018	To compare the environmental impacts of producing PHB from soybean oil or from sucrose derived by sugarcane with the impacts related to HDPE and corn-based PHB.

2.2.2 KEY LCA-RELATED DECISIONS OF THE SELECTED STUDIES

Table 4 summarizes, in chronological order, the selection of key elements for an LCA study:

- ✓ Related to the PHA production system under analysis: type of substrate and type of culture
- ✓ Related to the goal and scope definition phase: functional unit, system boundaries, inclusion/exclusion of end-of-life stage
- ✓ Related to the inventory phase: origin of primary and secondary data
- ✓ Related to the impact assessment phase: impact categories evaluated and LCIA method selected to do so

Table 4 Chronological summary of characteristics of reviewed LCA studies on PHA production

SOURCE	SUBSTRATE	TYPE OF CULTURE	F.U.	SYSTEM BOUNDARIES[3]	END OF LIFE (EoL)	ORIGIN OF PRIMARY DATA	ORIGIN OF SECONDARY DATA	IMPACT CATEGORIES EVALUATED[4]
Hyde 1998	Sugar beet Starch Methane	Pure	1 kg PHB (not clearly stated)	Cradle-to-grave	Conventional waste management (collection together with residual waste, 70% landfill + 30% incineration) // 100% incineration // separate collection of biodegradables and composting	Literature	Literature	Midpoint level GWP, NREU
Gerngross 1999	Corn	Pure	1 kg PHA	Cradle-to-gate	Not included	Calculations and estimates based on US Department of Energy (DOE) and United States Department of Agriculture (USDA); literature	Literature	Midpoint level NREU
Kurdikar et al., 2001	Corn stover from genetically modified plant	Pure	1 kg PHA	Cradle-to-gate	Not included (but discussed qualitatively)	Monsanto's data and assumptions; literature; Air Chief, EPA 1997; Ontario Ministry of Agriculture, Food and Rural Affairs, OMAFRA	Economic Research Service (ERS) of the United States Department of Agriculture-USDA; Ecobalance's DEAM™ database	Midpoint level GWP
Akiyama et al., 2003	Soybean Corn	Pure	5000 tons PHA	Cradle-to-gate	Not included	USDA and DOE; literature; own calculations; computer simulation using SuperPro Designer v4.5	Own calculations; literature; computer simulation using SuperPro Designer v4.5	Midpoint level GWP, NREU
Kim and Dale, 2005	Corn Corn stover	Pure	1 kg PHA	Cradle-to-gate	Not included	Literature; National Agricultural Statistics Service; Economic research service; National Oceanic &Atmospheric Administration; Natural Resources Conservation Service; Office of Industrial Technologies; International fertilizer Industry Association; DEAM™ LCA Database	Literature; DEAM LCA database DAYCENT model; ECAR (East Central Area Reliability Coordination Agreement), MAIN (Mid-America Interconnected Network), and MAPP (Mid–Continent Area Power Pool)	Midpoint level GWP, EP, AP, OFP
Patel at al. 2006	Maize starch Maize stover Sugarcane	Pure	1 ton PHA	Both cradle-to-gate and cradle-to-grave	Incineration with or without energy recovery and anaerobic digestion are evaluated	Data from companies and research institutes (A&F); data from pilot plant or industrial facilities, provided by BREW partners	Literature	Midpoint level GWP, NREU, REU, LU
Harding et al., 2007	Sugar cane	Pure	1 ton PHB	Cradle-to-gate	Not included (but discussed qualitatively)	Laboratory study scale data (referring to the PhD thesis of STL Harrison, Cambridge University, 1990).; literature; Aspen Plus model	Literature	Midpoint level GWP, NREU, EP, AP, AD, ODP, OFP, TETP, FAETP, MAETP, HTP
Hermann et al., 2007	Corn starch Corn stover Sugar cane	Pure	1 ton PHA	Cradle-to-grave	Post-consumer waste management is considered	Literature and own mass balance based on literature. Industrial data for comparison provided by A&F	Literature and own calculation based on literature	Midpoint level GWP, NREU, LU
Pietrini et al., 2007	Sugar cane Corn	Pure	Consumer products: CRT monitor housing and internal car panel	Cradle-to-cradle[5]	Municipal solid waste incineration with energy recovery is evaluated	Literature; calculations and estimations based on literature	Literature; calculations and estimations based on literature	Midpoint level GWP, NREU

Gurieff and Lant, 2007	Wastewater from food industry	Mixed	1 kg COD in the feed	Gate-to-gate	Not included	All the environmental figures are taken from Australian based source (local utility companies, the Australian Greenhouse Office); literature	Literature	Midpoint level GWP
Yu and Chen, 2008	Black syrup	Pure	1 kg PHA	Gate-to-gate	Not included	Literature; computer simulation (data of a simulated ethanol plant that are based on laboratory and pilot-plant results)	Data from Agricultural Resource Management Survey (ARMS); Literature; average performance of chemical industry in the U.S.	Midpoint level GWP, NREU
Kim and Dale, 2008	Corn grain	Pure	1 kg PHA	Cradle-to-gate	Treatment in a compost facility in assessed	Telles facility data; literature; LCA database; DAYCENT model simulation	Literature; DAYCENT model simulation	Midpoint level GWP, NREU
Zhong et al., 2009	Corn Whey Transgenic corn	Pure	1 kg PHA (not clearly stated)	Cradle-to-gate	Not included	Literature; GaBi 4.0. database	Literature	End-point level Ecosystem quality, human health, supply of resources
Khoo et al., 2010 Part 1	Corn	Pure	Consumer product: standard carrier bag	Cradle-to-gate	Not included	Literature	Literature	Midpoint level GWP, AP, OFP
Khoo and Tan 2010 Part 2	Corn	Pure	Consumer product: standard carrier bag	Gate-to-cradle	Three disposal scenarios are assessed: i), landfill, ii) incineration and iii) composting	Data from Singapore electricity mix; literature	Not specified	Midpoint level GWP, AP, LU, OFP
Hermann et al., 2010	Not specified	Pure	1 kg PHA	Gate-to-cradle[6]	Four different treatment options are evaluated: -home composting; -industrial composting; -anaerobic digestion; -incineration	Literature, experiments and analogies with materials for which significance experience has been made	Literature	Midpoint level GWP, NREU
Tabone et al., 2010	Corn grain Corn stover	Pure	1 L polymer contained in pellets (prior to product molding).	Cradle-to-gate	Not included	Literature	Literature	Midpoint level GWP, NREU, EP, AP, ODP, OFP, ETP, CHHH, NCHHH, respiratory effects
Kendall, 2012	Cellulosic fraction of organic residuals	Pure	1 kg PHB	Gate-to-gate	Not included	Literature; EPA's LandGem model to simulate landfill gas generation; Ecoinvent v2.0 database; GaBi Professional Database	Literature	Midpoint level GWP, Primary energy, EP, AP, OFP
Rostkowski et al., 2012	Waste biogas	Pure	1 kg PHB	Gate-to-gate	Not included	Literature; Western Electricity Coordinating Council (WECC) according to eGrid (2005). Experimental data and system parametrization using literature values and stechiometric calculations. Estimates, when data are unavailable	Ecoinvent database	Midpoint level GWP, EP, AP, ODP, OFP, ETP, CHHH, NCHHH, respiratory effects
Koller et al, 2013	Industrial surplus whey	Pure	Not clearly defined	Gate-to-gate	Not included	Data provided by the project partners based on bioreactor experiments on 300 L scale	Literature	Not defined

Dacosta et al., 2015	Wastewater from paper mill and food industry	Mixed	1 kg PHB	Gate-to-gate	Not included	Laboratory and pilot scale data and literature data integrated with process modelling in ASPEN Plus software	EcoInvent 2.2	Midpoint level GWP, NREU
Righi et al., 2016	Undefined	Both pure and mixed	1 kg PHB	Gate-to-gate (extraction step only)	Not included	Scale-up of laboratory data; Gabi Professional Database; Ecoinvent Database; estimates; literature	Ecoinvent database	Midpoint level GWP, OFP, FAETP
Leong et al., 2017	Glycerol	Pure	1 kg PHA (not clearly stated)	Gate-to-gate	Not included	Laboratory data; literature	WAR database	Midpoint level GWP, AP, ODP, OFP, TETP, ATP, HTPI, HTPE
Kookos et al., 2018	Soybean oil Sugarcane	Pure	1 kg PHB	Cradle-to-gate	Not included	Literature; Gabi Professional database; Ecoinvent	Literature	Midpoint level GWP, NREU, EP, AP

[3]*Cradle-to-grave* includes feedstock production, substrate pre-treatment, PHA production and extraction, and PHA EoL (collection and disposal). *Cradle-to-gate* excludes the PHA EoL and *Gate-to-gate* also excludes the feedstock production (for example when the substrate is a second-generation crop (an agricultural residue that has not been cultivated ad hoc) or a waste stream). *Gate-to-cradle* includes collection, transportation and waste treatment phases. Note that the final product manufacture and its use are always excluded from the LCA scope.

[4] GWP: global warming potential; NREU: no renewable energy use; REU: renewable energy use; EP: eutrophication potential; AP: acidification potential; LU: land use; AD: abiotic depletion; OFP: ozone formation potential; ODP: ozone depletion potential; ETP: ecotoxicity potential; TETP: terrestrial ecotoxicity potential; ATP: aquatic toxicity potential; FAETP: fresh aquatic ecotoxicity potential; MAETP: marine aquatic ecotoxicity potential; HTP: human toxicity potential; CHHH: carcinogenic human health hazards; NCHHH: noncarcinogenic human health hazards; HTPI: human toxicity potential by ingestion; HTPE: human toxicity potential by either inhalation or dermal exposure.

[5] Use phase is considered here within the system boundaries, being significant in terms of fossil fuel consumption in the case of the internal car panel LCA.

[6] Actually in this study the collection and transportation of the wastes is excluded so that only the waste treatment phase is assessed.

The overview (table 4) shows that there are differences but also similarities across the studies, which will further be discussed in the following.

PHA production system under analysis: type of substrate and type of culture

The choice of the feedstock can widely influence the environmental impacts associated to the PHA production process. Two groups of feedstocks can be differentiated: cultivated feedstocks or residual streams.

Among the former, the main substrate considered is glucose from corn and C_5/C_6 sugars from lignocellulosic feedstock as corn stover. Indeed, corn is easy to cultivate in Europe and U.S. thanks to the favourable climate conditions and, particularly relating to the second-generation corn (so agricultural waste derived from food crops) it is readily available at large scale. Fermentable sugars from lignocellulosics such as woody biomass are considered a key component of a bio-based economy because they are foreseen to provide fermentation feedstocks at low price also in moderate climate due to the wide availability of lignocellulosics biomass in the form of agricultural waste (e.g. maize stover) (Patel aet al., 2006). Kurdikar et al. (2001) and Zhong et al. (2009) consider transgenic corn, which affects the PHA production process as the biopolymer grows directly in the plant (Gerngross and Slater, 2000). However, Zhong et al. (2009) reported worse environmental performance for PHA production from transgenic corn than from glucose from corn or whey. Another possibility is using dextrose from sugar cane; it would be the preferred feedstock because it requires the lowest amount of NREU and leads to the lowest release of GHG emissions: the cradle-to-gate assessment performed by Kookos et al. (2018) shows that PHAs production from sugarcane leads to environmental credits (so negative values of GWP and NREU) due to the practice of burning the bagasse (residues from milling) to generate electricity which can displace fossil-derived electricity and to the fact that the agricultural production requires virtually no input of fossil fuel energy. However, for climatic reasons this crop is not cultivable in Europe or U.S. (Patel et al., 2006). So a possible option would be to import fermentable sugars from sugar cane from tropical countries, like Brazil, but "*this is nor the interest of European farmers which raises the question about its implementability in view of the hitherto great importance of agricultural policy and its subsidy in the EU. As an alternative, European chemical companies could build their production plants in tropical countries with ample sugar cane production. This, however, raises questions about the limits of such a strategy in view of the availability of land in the longer term (including its demand for food and feed), about the impacts on natural biodiversity and also about social sustainability in view of the low wages paid to workers on sugar cane plantation and the dismal working conditions"* (Patel et al., 2006). PHAs production from soybean oil (Akiyama et al. 2003, Kookos et al. 2018), sugar beet (Heyde 1998) and whey (Zhong et al., 2009) have been also assessed from an LCA perspective, finding that soybean oil is a promising crop offering environmental benefits compared to corn grain, while whey shows similar environmental impacts than corn. Sugar beet as feedstock has not had a great consideration among the references considered, being evaluated

only by the earliest study of Heyde (1998). About that Patel et al. (2006) states that producing fermentable sugar by sugar beet has higher production cost compared to dextrose from starch for example.

Residual streams (e.g. industrial wastewater, industrial surplus material whey which constitutes a waste from the cheese industry, …) have been raised as competitive option to the agricultural substrates for PHAs production, as they are valorised while being treated. Moreover, they are inexpensive substrates (Heimersson, 2014) whose use eliminates the need for a purpose-grown feedstock. For example, Kendall (2012) found that producing PHB from corn requires approximately twice the energy and generates double GHGs emissions than PHB from the cellulosic fraction of organic residuals from material recovery facility (MRF). MRF residuals are the remaining waste stream once recoverable materials are removed from the incoming municipal solid waste and they are often dominated by organic material of which cellulose useful for PHAs production constitutes approximately 47% (Kendall, 2012).

Regarding the type of culture for the fermentation, pure culture has been extensively considered so far and only two references (Gurieff and Lant 2007 and Dacosta et al. 2015) assess PHA production by mixed cultures. PHA production through pure bacteria culture uses selected strains and *ad hoc* designed growth media. Because of the costs of culture maintenance, substrate formulation and both substrate and reactor sterilization, this type of culture is a key factor affecting the overall cost of PHA production process (Mannina et al., 2019). As alternative, the use of mixed microbial cultures (MMCs) will help to reduce the production costs of PHAs, since MMCs do not require sterile conditions and have a wider metabolic potential than single strains, making easier the use of low-cost feedstocks, such as industrial waste effluents (Villano et al., 2014). Being to the best of our knowledge, the unique references that compares both pure and mixed cultures, Gurieff and Lant (2007) and Dacosta et al. (2015) demonstrate that a system optimization of the downstream processes is necessary to reduce the NREU of PHA mixed culture production that are currently higher compared to the ones from PHA production from agricultural crops.

Goal and scope definition phase: functional unit, system boundaries, inclusion/exclusion of end-of-life stage

A mass-based functional unit (FU) is the most common approach, being normally 1 kg or 1 ton of PHA (or specifically PHB) the preferred option. Similarly, Akiyama et al. (2003) simulates a large-scale fermentative production and base the calculations on the annual quantity of polymer produced, i.e. 5000 tons. Being an exception, Tabone et al. (2010) considers volume-based FU, 1 litre of polymer contained in pellets (prior to product molding), as this study compares twelve polymers with different physical properties for each plastic (e.g., density and modulus) and therefore different mass required for the same final plastic product (e.g., gift cards, bottles, and cups). On the same logic, Pietrini et al. (2007) define two specific products, a CRT monitor housing of 17'' for a desktop computer with a weight of 2.2 kg and internal panels of an average car with an own weight of 20 kg, as FU. The same approached is applied by Khoo et al. (2010a) and Khoo and Tan (2010b) that choose an standard carrier bag with a carrying capacity of 20 kg as FU. Finally, using a treatment

perspective instead of a production one, Gurieff and Lant (2007) defines the FU at the feedstock wastewater (1 kg of COD).

When looking at the scope of the LCA performed, the majority of the studies (10/24) are classified as *cradle-to-gate*. When feedstock is a crop, this means to include all the agricultural inputs and related emissions which represents an environmental disadvantage in comparison to the use of waste stream, with no burdens allocated, as raw material. Indeed the latter obtains credits by the avoided waste treatment in the final biopolymer based product (Dacosta et al., 2015). Three studies (Hyde 1998; Hermann et al. 2007; Pietrini et al. 2007) include also the EoL phase after the useful life of the product, being then classified as *cradle-to-grave*. With a more limited scope, Gurieff and Lant (2007), Yu and Chen (2008), Rostkowski (2012), Koller et al. (2013) and Dacosta et al. (2015), Leong et al. (2017) perform a *gate-to-gate* analysis as the feedstock is either a residual stream (e. g. the industrial wastewater considered by Gurieff and Lant 2007 and Dacosta et al. 2015) or the industrial surplus material of the milk processing, whey, used by Koller et al. 2013). In the case of Leong et al. (2017) the *gate-to-gate* boundary is associated with the particular interest of this reference focused on the extraction and recovery stage of the life cycle, so on evaluating the environmental benefit that is possible to obtain using ATPE as primary recovery step. Finally, Khoo and Tan (2010b) and Hermann et al. (2010) perform a gate-to-cradle analysis, being their systems limited to the end-of-life stage. Although they considered the same system boundaries, the comparison between them is a bit difficult since different FU are chosen and because of the exclusion of the collection and transportation phases from Hermann et al. (2010) assessment). However both report the same result according to which composting is the better option compared to incineration. Hermann et al. (2010) also consider anaerobic digestion, the best solution as it combines energy recovery with digestate production.

Inventory phase: origin of primary and secondary data

All the LCA studies reviewed are not based on on-site data, except Kim and Dale (2008) where site specific process information on the corn wet milling as well as PHB fermentation and extra processes was obtained from Telles, a joint venture commercializing PHB biopolymers. Other two studies (Patel et al. 2006 and Hermann et al. 2007) used some data provided by A&F industry, but only to compare them with the data estimated with the Generic Approach[7]. Akiyama et al. (2003) based his/her analysis on computer simulation of PHB production using bioreactor volumes between 300 and 700 m^3. Koller et al. (2013) used information from pilot scale performance (0.3 m^3) and Dacosta et al. (2015) scaled up the PHB production process from

[7] The Generic Approach is a methodology which allows *ex ante* estimation of the environmental impacts and of the basic economic features of new biotechnological processes for which process data are not publicly available (Patel et al., 2006). A specific Generic Approach has been developed and applied to assess the bio-based products because of the very limited availability of process data. It consists in several steps in which the amount of inputs and outputs of bioprocesses were calculated using mass balances derived from process flow diagrams (B. Hermann & Patel, 2007).

lab and pilot scale data and complemented with process modelling in ASPEN Plus software. Due to intellectual property and competitiveness reasons, industry actors find the request to openly share such knowledge a difficult one (EC, 2019) and therefore most of the data used are taken from previous literature and computer simulation. A dense correlation among published papers has been identified (the main connections are shown in figure 15), so it is clear that a great part of primary and secondary data used in the environmental assessment come from preceding LCA studies. Particularly, it is possible to observe that there are two main earlier studies (Gerngross 1999 and Akiyama et al. 2003) which are taken as reference for the data collection of a great part of the following LCA works (orange arrows). These two papers provide information on technology trends in the PHA fermentation and post fermentation processes and the relative parameters used in PHA process calculations. The age of these studies should be noted as they are unlikely to represent the technology currently available, including reductions in energy requirements, like for Khoo et al. (2010a) which has used data from Gerngross (1999) (Yates & Barlow, 2013).

Also Kurdikar et al. (2001) is a key reference for the inventory phase of four studies to obtain information on corn stover, such as the processing (Kim and Dale, 2005) and the corn stover extraction and compounding (Zhong et al., 2009).

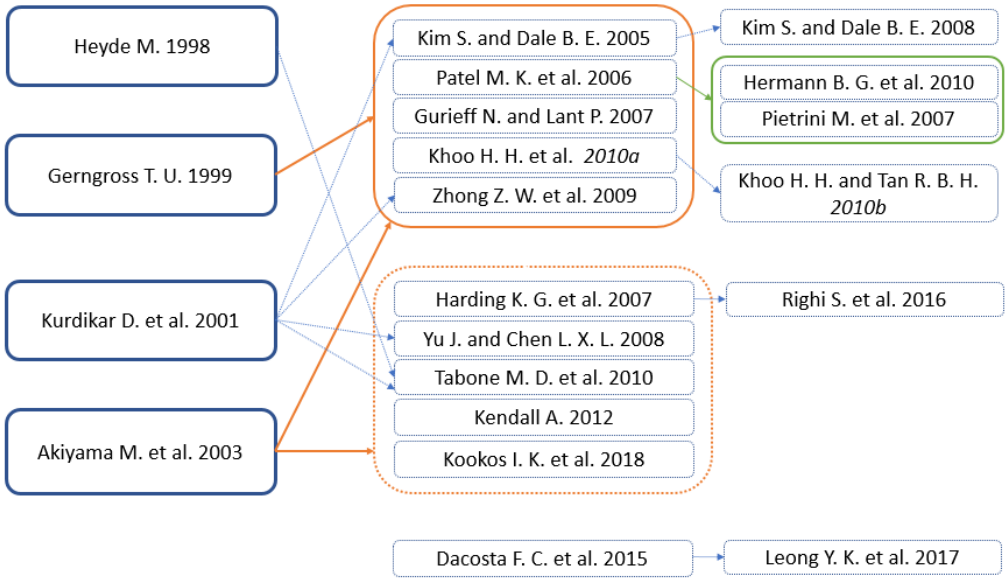


Figure 15 Main connections among the LCA studies based on data used for the inventory

Impact assessment phase: impact categories evaluated and LCIA method selected to do so

The technique of modelling environmental impacts can follow a midpoint or an endpoint approach. The midpoint approach is considered to be links in cause-effect chain of an impact category, prior to the endpoints. All the studies reviewed use the midpoint method to do LCIA, with a unique exception, Zhong et al. (2009). In this latter study the endpoint approach is used so endpoint indicators are chosen further down the cause-effect chain of the environmental mechanism closer to or at the very endpoint of the chains (Hauschild, 2018). The numerous different midpoint indicators (climate change, acidification, ecotoxicity, …)

therefore all contribute to a relatively small set of endpoint indicators, as in the case of Zhong et al. (2009) where they are classified into three damage categories: ecosystem quality (acidification/nitrification, ecotoxicity), human health (carcinogenic effects, climate change, ozone layer depletion, radiation, respiratory-inorganic and organic) and supply of resources (fuels, minerals).

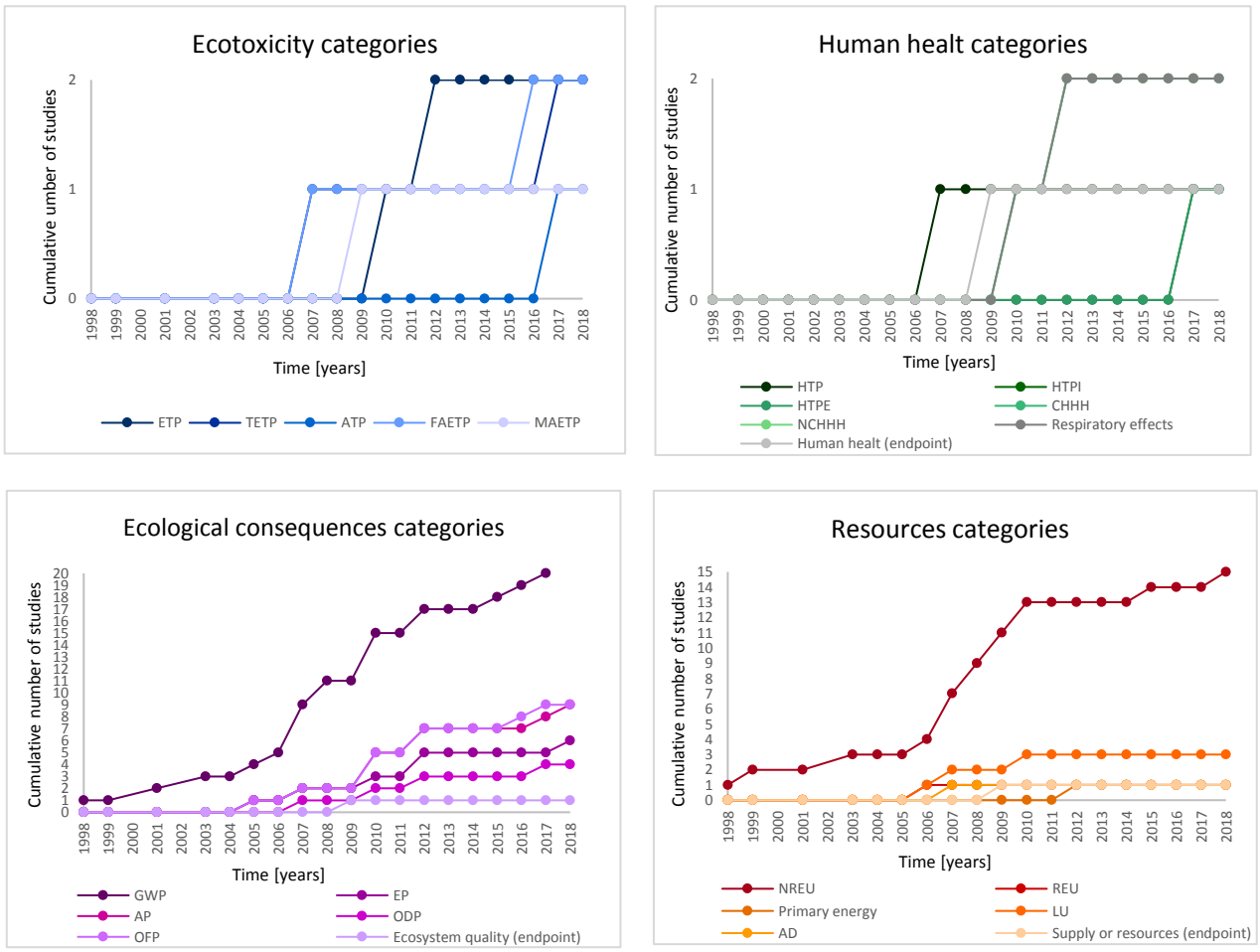


Figure 16 Cumulative number of impact categories assessed over time. The impact categories are grouped on the basis of "macro-categories", according to Nordic Guidelines on Life Cycle Assessment.

A large part of the references reviewed (7/22), especially those published in the earlier period (1998 to 2003), are focused only on two impact categories, GWP and NREU. This choice is often due to a general lack of data about the PHB production process, which is in an early stage of development, without large-scale facilities and publicly available measured results (Pietrini et al., 2007). Three of the papers analysed estimate only one impact category: Kurdikar et al. (2001) and Gurieff and Lant (2007) evaluate just the GWP, while Gerngross (1999) solely the NREU. The energy requirement is evaluated also as primary energy by Kendall (2012), while Patel et al. (2006) calculate the renewable energy use. Other impacts categories assessed are the land use, the eutrophication, the acidification, the abiotic depletion, the photochemical ozone formation and the ozone depletion, the ecotoxicity (terrestrial, fresh and marine aquatic), the human toxicity with also the carcinogenic and noncarcinogenic human health hazards as well as the respiratory effects. Harding et al.

(2007), Tabone et al. (2010), Rostowski et al. (2012) and Leong et al. (2017) provide a more complete environmental assessment compared to the other studies, since a larger number of impact categories are estimated. It is interesting to represent the trend over time of the cumulative number of the categories assessed (figure 16) to have an understanding of how the LCIA phase has developed from the earlier to the latest studies. A major and constant attention is given firstly to GWP which is estimated in all the references, except Gerngross (1999), and, secondly, to NREU, regularly calculated over the years too. Then, the later studies complement the environmental assessment with the calculation of other environmental indicators. In particular, land use (LU), photochemical oxidant creation potential (POCP), acidification potential (AP) and the eutrophication potential (EP) can be critical categories for PHA production from agricultural feedstock. Downstream processes for PHA recovery are the steps with higher energy requirements.

2.2.3 LESSONS LEARNT FROM THE LITERATURE

The main results achieved in the studies reviewed are summarized in table 5.

Table 5 Chronological summary of the main results achieved by each review study

SOURCE	MAIN RESULTS ACHIEVED
Hyde, 1998	Due to the lack of data (this study is only based on one estimating study), there are not consistent results according to the aim. However, what emerge from the analysis is that the NREU associated to the cradle-to-gate life cycle of PHB production is lower than HDPE and PS *"under advantageous technological boundary conditions",* while *"under the worst conditions the energy demand of the PHB system reaches another order of magnitude".* Regarding to the end-of-life phase, conventional waste management of PHB-based products deliver an high contribution to GWP (5.1÷6.3 $kgCO_2$/kg PHB) than HDPE (0.51 $kgCO_{2eq}$/kg HDPE) and PS (0.72 $kgCO_2$/kg PS). This contribution is lower than HDPE and PS if the PHA-based products are incinerated or composted.
Gerngross, 1999	The amount of fossil fuels required to produce 1 kg of PHA (2.39 kg) exceeds that (2.26 kg) required to produce an equal amount of PS.
Kurdikar et al., 2001	The production of PHA from genetically modified corn produce higher GHG emissions than PE production, if fossil resources are used for electricity generation. Using biomass power (corn stover), PHA is preferable to PE from a greenhouses gas perspective (in this case the GWP for the PHA production is negative and equal to –2.5 gCO_{2eq}/kg PHA).
Akiyama et al., 2003	Production cost between P(3HB-*co*-5mol% 3HHx) from soybean oil and P(3HB) from corn is comparable (3.53÷4.77 $/kg and 3.88÷4.24 $/kg respectively). The LCI estimated that CO_2 emission are relatively smaller using soybean oil as substrate (-0.04÷0.82 $kgCO_{2eq}$/kg) than corn (0.48÷1.39 $kgCO_{2eq}$/kg), as well as the energy consumption (41.88÷57.04 MJ/kg for soybean oil and 59.17÷68.37 MJ/kg for corn). The LCI values of CO_2 emission and energy consumption are much smaller for the bio-based PHB polymers than for petrochemical ones (HDPE, LDPE, PP, PS, b-PET).
Kim and Dale, 2005	GWP associated with corn grain based PHA is 1.6÷4.1 $kgCO_{2\,eq}$/kg and it overcomes the GWP relative to the PS production (2.9 $kgCO_{2\,eq}$/kg). However, PHA produced in an integrated system, in which corn stover is harvested and used as raw material for PHA along with corn grain, offers global warming credits, ranging from –0.28 to –1.9 $kgCO_{2\,eq}$/kg, and lower OFP, AP and EP values. The main contributing process to GWP and AP is PHA fermentation and recovery; for OFP and EP, it is corn cultivation due to nitrogen related burdens from soil.
Patel et al., 2006	NREU and GHG are lowest for sucrose from sugar cane (with power co-production from bagasse), and highest for dextrose from maize.

	Land use is relatively lower for sucrose from sugar cane than for sugars derived from maize starch. In average PHAs production leads to non-renewable energy savings compared to HDPE.
Harding et al., 2007	PHB production is more environmentally favourable than PP and PE: • PHB production has lower environmental impacts than PP in all LCA categories: GWP of PP production is over 80% more than PHB production; ODP is over 50 times lower in PHB production; TETP and MAETP are respectively almost 10% and 50% higher in PP production; OFP, HTP, FAETP, AD of PP production give LCA scores approximately double those of PHA; AP is 100% higher and EP is 12% higher in PP. • PHB production shows reduced environmental impact in six categories (GWP, AD, FAETP, TETP, HTP, OFP, ODP) compared to PE (both LDPE and HDPE). Particularly, GWP is just less than 50% higher for PE than PHB. AP and MAETP are essentially equal for both PP and PHB. EP is 500% lower for PE than for PHB, mainly due to the NOx emissions partially attributed to the agricultural component of PHB production.
Hermann et al., 2007	Assuming full substitution of HDPE with PHA production from corn stover, the GHG saving potential is 2.9 kg/kg (162 730 tCO_2/year). The value of GHG saving potential for the future (20-30 years of time horizon from 2007) is estimated to be 2.8 kg/kg (159 640 tCO_2/year).
Pietrini et al., 2007	Regarding the monitor housing, their cradle-to-grave LCA shows lower NREU and GWP if they are produced using PHB rather than conventional HIPS. Concerning internal car panels, NREU and GWP score worse for PHB than for conventional PP-GF. The reason is the higher weight of the composites, which leads to higher fuel consumption during the use phase.
Gurieff and Lant, 2007	The use of wastewater for PHA_{MC} production has a higher GWP (34,583,731 kg CO_{2eq}/year) than its use for the biogas option (12,526,015 kg CO_{2eq}/year). However, considering the savings (the CO_2 emissions from displaced resources: HDPE for PHA_{MC} and natural gas for biogas), the net impact of the PHA_{MC} option is lower than that of biogas. The GWP of PHA_{MC} production (20.4 kgCO_{2eq}/kg PHA_{MC}) is lower than that of HDPE (25.2 kg CO_{2eq}/kg HDPE), but it is higher than that for PHA from pure culture production (15.3 kg CO_{2eq}/kg PHA).
Yu and Chen, 2008	The gate-to-gate LCA of PHA coproduction in cellulosic ethanol biorefineries (using the black syrup as substrate) shows a GWP of 0.49 kgCO_{2eq}/kg PHA, and a value of NREU equal to 44 MJ/kg PHA. The results obtained from the comparison with others polymers and biopolymers shows that PHA production from black syrup has: • lower NREU but a slightly higher GWP than PHA from both glucose and vegetable oils; • better environmental performance in terms of GWP and NREU over PLA; • much lower GWP and lower NREU than LDPE and PET; • much lower GWP but higher NREU than LDPE and PP.
Kim and Dale, 2008	The PHB production system consumes 2.5 MJ/kg of non-renewable energy and offers greenhouse gas credits, approximately -2.3 kg $CO_{2\,eq}$/kg, thanks to the use of renewable energy in the corn wet milling and PHB fermentation and recovery processes, and utilization of fermentation residues as fuel. Compared with the values of NREU (69-101 MJ/kg) and GWP (1.9-5.4 MJ/kg) of most petroleum-derived polymers production, PHB has better environmental characteristics.
Zhong et al., 2009	PHA production from transgenic corn has much higher environmental scores in ecosystem damage (0.66 PDF m^2 a), human health damage (17.79 DALY) and resource supply (3.59 MJ surplus energy) compared with PHA from corn and whey, whose values are comparable for all the three categories (respectively 0.16 and 0.17 PDF m^2 a for ecosystem quality indicator, 4.5 and 4.71 DALY for human health damage, 2.29 and 2.28 MJ of supply energy). The respiratory (inorganic), acidification/nitrification, and fossil fuels impact indicators are the major contributors causing the significant differences in the total impacts of the three scenarios in the three damage categories.
Khoo et al., 2010 Part 1	PHA bags production process shows that the GWP is nearly six times the amount of GHG generated from PP bag production system. The highest contribution is linked to the energy requirement for making bio-bags. The AP and the OFP are also much more higher for PHA bags than for PP bags production and for both the indicators the main contributor is the PHA production.
Khoo and Tan 2010 Part 2	The worst end-of-life option for bio-bags is landfill in terms of GWP (about 0.025 kg CO_{2eq}/bag), OFP (approximately 3.2 UES ppm hrs/bag), LU (In m^3/bag) and AP (1.3 m^2 UES/bag). Next highest disposal impacts are from incineration, and the minimal from composting, with both option having much more smaller values in the impact categories evaluated than landfill.

	The landfill of PP bags generates approximately half of the GHG emissions of the landfill of bio-bags, but the emissions of acidic gases contributing to the AP are higher AP (1.7 m^2 UES/bag). The incineration of PP bags generates about double the amount of GHG compared to the incineration of bio-bags, negligible AP and OFP.
Hermann et al. 2010	The best waste treatment option for PHA is anaerobic digestion, resulting in better scores in GWP (about 0.8 kg $CO_{2\,eq}$/kg PHA) and NREU (approximately -18 MJ/kg PHA), followed by incineration with energy recovery (approximately GWP=1.25 kg $CO_{2\,eq}$/kg PHA and NREU=-15 MJ/kg PHA), home composting (about GWP=1.4 kg $CO_{2\,eq}$/kg PHA and NREU=-7 MJ/kg PHA) and finally industrial composting (about GWP=1.6 kg $CO_{2\,eq}$/kg PHA and NREU=-2 MJ/kg PHA).
Tabone et al, 2010	When compared by volume, PHA from corn grain has the highest AP than PET, b-PET, PVC, PLA, HDPE, LDPE, PP and PHA from corn stover. It has moreover higher impacts than PP and PE in all categories (GWP, NREU, EP, ODP, OFP, ETP, CHHH, NCHHH, respiratory effects) other than POC. Its shows instead environmental benefits compared to PET and b-PET in all categories, except AP and ODP.
Kendall, 2012	Producing PHB from a dedicated agricultural feedstock - as corn - is estimated to require approximately **twice** the primary energy (about 110÷130 MJ/kg PHB) and GWP (about 6÷12 kg $CO_{2\,eq}$ /kg PHB) than PHB from material recovery facility residuals (whose indicators values are approximately 2÷3 kg $CO_{2\,eq}$/kg PHB). It has been demonstrated that diverting organic from landfills reduces fugitive emissions of CH_4, but it can also reduce landfill gas generation. So additional electricity from more carbon-intensive sources is required and it increases the energy and GHG intensity attributable to PHB.
Rostkowski et al., 2012	90% contribution of GWP, AP, CHHH, NCHHH, respiratory effects, EP, ODP, ETP, OFP is due to PHB recovery when solvent extraction is used and most of the impacts are primarily attributed to the energy use. By the comparison with PHB produced from corn, it has been found that GWP results lower for PHB production from waste CH_4 (-1.94 kg CO_{2eq}/kg PHB versus -0.1 kg CO_{2eq}/kg PHB for corn) and the total energy requirement too (37.4 MJ/kg PHB for CH_4 and 41.9 MJ/kg PHB for corn).
Koller et al., 2013	The hotspot of the PHA production process is the fermentation step and, both for the fermentation than for the whole PHA process, electricity input is the largest contributor to the entire ecological footprint of the life cycle. The ecological impact of PHA production from industrial surplus whey material (10000 m^2 a/kg PHA) is approximately five times higher than that of the production of conventional polymers (PE, PET, PP, PE). Compare the ecological footprint of the use of whey for PHA or for whey powder production, it has been found that PHA production leads to a decrease of the environmental impact per € earned over the value chain compared to whey powder.
Dacosta et al., 2015	The economic analysis reveals that the total production cost of PHB from wastewater is 1.56 €/kg PHB_{MC}, whose main contributor is the downstream process, accounting for the 73% of the total cost. This cost is lower if compared to the cost of PHB production from crops, but higher than PET market price (1.3 €/kg). The environmental analysis shows that the overall GHG emissions are 1.97 kg CO_{2eq}/kg PHB_{MC} and NREU is 109 MJ/kg PHB_{MC}. The main contributors to GWP and NREU are the downstream process accounting for, respectively, 60% and 72% of the total GWP and NREU. GHG emissions are in line with those associated with sugar-based PHA production (3 to 5 kg CO_{2eq}/kg PHA) and only 4% lower than PET (2.15 kg CO_{2eq}/kg PET). The NREU is around 35% higher than the values associated with sugar-based PHA (81 MJ/kg PHA) and 58% higher respect to PET production (69 MJ/kg PET). The key points for economic and environmental sustainability are the utilities of the downstream processes.
Righi, 2016	The extraction process from microbial slurry and dried biomass using DMC show better environmental performances than using DCE for all the impact categories considered (GWP, OFP, FAETP). GHG emissions due to extract process through DCE (around 58 kg CO_{2eq}/kg PHB) are about six times higher than scenarios representing extraction via DMC. The contribution to FAETP of "DCE" scenario (17 CTUe/kg PHB) is from 2 to 8 times higher than those of "DMC" scenarios. OFP score of "DCW" scenario is from 2 to 3 higher than novel protocol scenarios.
Leong, 2017	PHAs production process with ATPE as primary recovery step outperformed the other (which does not include ATPE) in terms of both economically and environmentally. The production cost of PHAs for the process that include ATPE is 5.77 US$/kg, while for the other it is 6.12 US$/kg. The values of

	the impact categories are not reported in the paper. However it is stated that process without ATPE has worse environmental performance than the other and the major contributing process to the environmental impacts is PHA fermentation.
Kookos, 2018	Sugarcane is the most promising raw material for PHB production in term of GWP (−2.58 $kgCO_{2eq}$/kg PHB) and NREU (−28.4 MJ/kg PHB), compared to soybean oil, corn and HDPE. PHB production from soybean oil shows lower GHG emissions (-2.37÷1.67 $kgCO_{2eq}$/kg PHB) and NREU (55.29 ÷62.67 MJ/kg PHB) than corn (GWP=3.95 kg CO_{2eq}/kg PHB and NREU=75.97 MJ/kg PHB) and HDPE (GWP=1.8 kg CO_{2eq}/kg HDPE and NREU=79.39 MJ/kg HDPE), however its values depend on the allocation (energy, by value or mass) method. In term of AP, PHB production from soybean oil has higher values (24.22÷27.9 $kgSO_{2eq}$/kg PHB) than HDPE (6.39÷22.50 $kgSO_{2eq}$/kg HDPE) and corn sugar (24 $kgSO_{2eq}$/kg PHB). Also the EP (5.09÷11 kgP_{eq}/kg PHB) scores worse for soybean oil than HDPE (0.43÷0.81 kgP_{eq}/kg HDPE) and corn sugar (5.19 kgP_{eq}/kg PHB).

The results achieved can be sometimes different and controversial as they are referred to processes like PHA production that are part of complex life cycles, may use a wide variety of raw materials, consider different system boundaries and allocation methodologies, and are strongly context-dependent because of considerable electricity consumption, so resulting more sustainable in countries that use a great percentage of renewable energy (Narodoslawsky et al., 2015; Koller et al., 2013). Nevertheless, the main teachings that can be extrapolated from the results achieved follow a common thread: the necessity to optimize the system and reduce the production cost, in order to reach the economic competitiveness over the petrochemical counterparts, allowing the commercial diffusion of PHAs based products on the market. To do so, some critical aspects must be challenged. These weak points to overcome emerging from the review are:

- the high energy demand, developing a system integration with biorefineries that leads to reduce the energy consumption from non-renewable energy resources and the GHG emissions, for example producing energy from corn stover residues (Kurdikar et al., 2001; Kim and Dale; 2005);
- the search for extraction and purification steps with lower cost, higher performance, and lower environmental impact since conventional methods are highly energy intensive and requires the use of harmful solvents (Righi, 2016; Leong, 2017);
- the research of a carbon source that leads to low environmental impacts and that is inexpensive. So a recent tendency to evaluate the use of alternative raw materials as substrate emerges, avoiding the use of a dedicated agricultural feedstock. Indeed, the crop cultivation phase is the more environmental sensitive area, especially impacting in acidification, eutrophication and photochemical ozone creation categories (Kim and Dale, 2005; Kim and Dale, 2008). A promising alternative to first generation source is by-products or waste stream.

  Yu and Chen (2008) found that the coproduction of PHA bioplastics in cellulosic ethanol biorefinery (using the black syrup, a by-product as substrate) offers GHG savings over petrochemical polymers as PS, LDPE, PET and PP (0.49 kg CO_{2eq} for PHA over 2-3 kg CO_{2eq} for petrochemical polymers). Kendall (2012) estimates that producing PHB from the cellulosic fraction of organic residuals from material recovery facilities can reduce twice the energy consume and the GHG emission of corn. PHB

production using methane collected from landfills and anaerobic digesters leads to reduce the CO_2 emission and energy investment compared to dedicated corn (Rostokowki et al., 2012). Gurieff and Lant (2007) and Dacosta et al. (2015) evaluates the PHA production from wastewater in mixed—culture conditions, founding that GHG emission are lower than those of conventional polymers (HDPE and PET) and in line with those of PHA corn-based production;

- the reduction of the cost associated to the pure culture production, where sterile conditions and infrastructure for an axenic bioprocess are required, evaluating PHA production through mixed cultures (Gurieff and Lant 2007 and Dacosta et al. 2015). Dacosta et al. (2015) found a reduction of the cost for kg PHA compared to the pure culture production using corn as feedstock. However, PHA_{MC} option had a higher level of non-renewable energy use than the pure culture production process. The high energy costs were caused by the much lower cell and therefore lower PHA density in the accumulation reaction. This lower density requires more energy to be used per kg PHA in the pre-fermentation, accumulation and the downstream process steps. Therefore, the environmental footprint of PHA_{MC} production could be nearly removed, again, using renewable electrical energy (i.e. wind, solar, hydro).

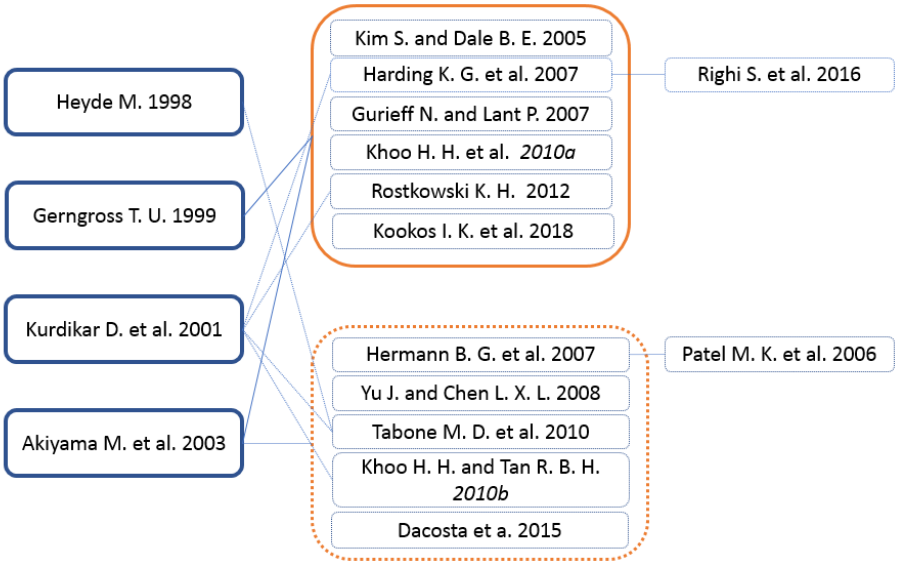


Figure 17 Main connection among the LCA studies based on data used for comparison in results interpretation

Being the investigation field relatively recent, focused on a process whose aspects are not always deeply consolidated, there is a tendency not only to consider the same data taken from previous works (as seen before), but also to compare the results with the previous literature. The main correlations among the authors in terms of comparison and interpretation of the results are shown in figure 17, from which emerges that the earlier study of Gerngross (1999) has been taken as reference to many following papers (grouped in

the up orange rectangular) as was the work done by Akiyama et al. (2003). This second paper is also the key reference for a second group of articles (down orange rectangular. Actually, other links are present also between the more recent studies, but they are not graphed in the figure for clarity of the picture.

2.4 OPEN OR UNRESOLVED QUESTIONS

There are some critical aspects that the investigation line has to face. The main challenge that emerges from this review is the optimization of the process to maximize the yield and minimize the energy consume. For reducing the ecological pressure of the energy use, renewable energy sources should be used (Kho et al., 2010; Koller et al., 2013). The main bottlenecks for the energy saving that require technological efficiency improvement are the downstream process and the fermentation (Kim and Dale, 2005; Gurieff and Lant, 2007).

Another key point is the search for a not expensive source of organic matter, since the raw material accounts for around 40-48% of the total production cost of PHA (Rodriguez-Perez et al., 2018). In this sense, the research studies for the different kinds of wastes show an increment from 2014 to 2016 (figure 18). The proposal of new kinds of wastes and the promotion of useful new knowledge will be necessary for a successful development of a competitive commercial process.

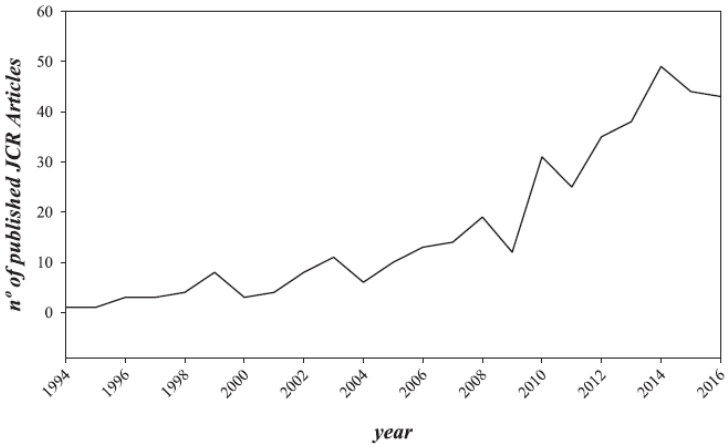


Figure 18 Number of research studies on PHA production from waste materials in the last 24 years (Rodriguez-Perez et al., 2018)

The physicochemical characteristics of the waste materials can make it necessary to include a previous treatment to adapt them to the requirements of the PHA production process. This treatment is previous to PHA production, so it is called the pre-treatment phase. This phase might be used to increase the carbon sources available, dilute the concentration of organic matter, regulate the pH, control the temperature, sterilize waste material and/or remove suspended solids. The necessity of a pre-treatment step is an important challenge for the scaling-up of PHA production due to the additional costs. Therefore, the

optimization of the pre-treatment processes is necessary to avoid unnecessary costs (Rodriguez-Perez et al., 2018).

Another interesting aspect for the scaling up of the PHA production process is the use of mixed-cultures due to the reduction cost compared to aseptic condition. A promising research line is addressed on the integration of the PHA production processes into wastewater treatment plants and the use of wastewater activated sludge as culture (Rodriguez-Perez et al., 2018).

Although the conventional LCA shows some pitfalls in evaluating emerging products and commercial data are often not available, this methodology is the most common used. Therefore the future developments in the PHAs production process have to be evaluated implementing ex-ante LCA, which has the potential to influence technological development and sustainable innovation more than the science of LCA has ever done in the past (Cucurachi et al., 2018).

To consider the key advantage of PHAs, their biodegradability is crucial when including the EoL phase within the system boundaries. So, it is important to provide a more comprehensive estimate of the life-cycle environmental impacts of biopolymers as this phase can indeed greatly influence the GWP results, as explored by Hottle et al. (2013) in a review based on fifteen studies (figure 19).

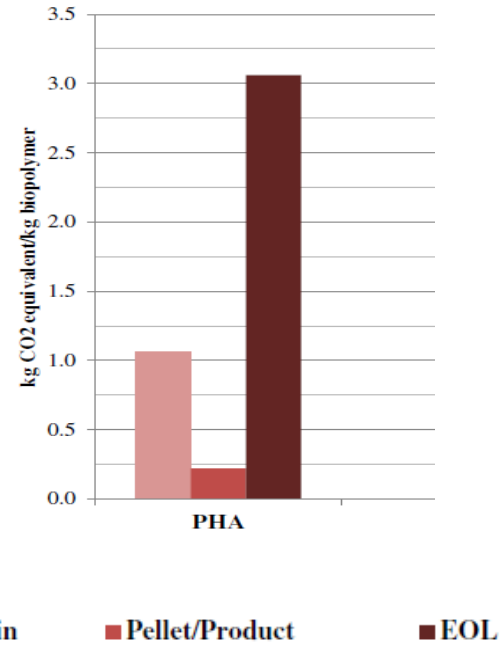


Figure 19 Average global warming potential of biopolymers based on the extent of the system boundaries of the fifteen different LCAs studies (based on the review of Hottle et al., 2013)

At the end-of-life phase, PHAs biodegradability provides a great advantage over conventional plastic, especially considering a long-term horizon. As mentioned in the previous chapter, every year about 10 Mt/y of plastics flow into the oceans. This generates the great environmental problem of the marine litter, a serious threat for the marine biodiversity with over 500 species which are known to be affected by

entanglement, ingestion, and ghost fishing (UNEP, 2018). It would be interesting to evaluate also the long-terms consequences of the PHA based products in the natural environment, particularly in the sea, according to their properties and quantities. However, impacts from marine litter are at present not included in life cycle impact assessment, so the environmental benefits that biodegradable PHAs can offer over plastics in this sense are still not considered. Several initiatives have been recently launched and promising results are expected from their work:

MariLCA (Marine Impacts in LCA), launched in May 2019, will allow to integrate potential environmental impacts of marine litter, especially plastic, in Life Cycle Assessment (LCA) results. It has been structure in three main project phases from 2019 – 2025: the first phase (January 2019 - December 2019) aimed to provide a first framework paper developing and illustrating the different impact pathways associated with marine litter to be developed and identify the gaps and building blocks; in the second phase (2019 - 2022) different research projects (Master's and PhDs) are coordinated and launched in order to fill identified gaps and act as a central scientific reference on the topic to avoid overlaps; finally, the third phase (2023 - 2025) will focus on the consensus building aiming at delivering a harmonized and consensus-based impact pathway framework and methods addressing plastic litter impacts in LCA (MariLCA, 2019).

Plastic Leak Project, launched also in 2019, is a multi-stakeholders initiative interested in taking effectively action on plastic and microplastic leakage. The Project will contribute to the global effort to fight plastic pollution developing a robust metrics which will enable industries and governments to forecast and map plastic and microplastic leakages along their life cycle (within the industry during the use phase and the production process, or even further back along the supply chain). If currently industrial companies miss clear and reliable data on plastic leakage hotspots to tackle this problem with effective actions at systemic level, the Plastic Leak Project will fill this gap by delivering a metrics-driven methodology to assess plastic leakage through LCA and an industry-specific guidance that enables companies to locate and evaluate plastic leakage along their value chains. The project aims at working closely with the scientific community to define reliable plastic leakage inventory data for the LCA. In this way companies will be able to verify that plastic materials, and relative impacts, are not being transferred from one area to another (Quantis, 2019).

In conclusion, the review carried out has enabled to individuate the weak points of the PHA production process, towards which address the future development and researches to put forward the development of a cost-effective system. As has been seen, **the choice of a waste stream as feedstock**, moving then from its treatment to its valorisation following the circular economy principles, is a promising alternative on which recent investigations are currently focusing. A further possibility that, as has been stated, can lead to a more sustainable PHA production system, both in environmental and economic terms, is the use of **mixed culture**, assuming that a technology improvement aimed to minimize the energetic demand is preliminarily achieved.

Further research is needed as the LCA studies that evaluate these new production trends and compare them with the use of *ad hoc* feedstock and pure culture are not so numerous. In fact, only 7 out of the 24 of the papers reviewed (Gurieff and Lant, 2007; Yu and Chen, 2008; Zhong et al., 2009; Kendall, 2012; Rostowski, 2012; Koller et al., 2013; Dacosta et al., 2015) have considered waste streams or by-products as raw materials and only two papers (Gurieff and Lant, 2007; Dacosta et al., 2015) assess the mixed culture production process and compare it with the pure culture alternative. If, from one hand, numerous potential waste streams have been individuated to produce PHA (agro-food wastes such as potato peels, fruit processing water, wastewater from fish canning industry as well as food wastes i.e. spent coffee grounds and used cooked oil) (Rodriguez-Perez, 2018), from the other hand, there is a scarcity of studies that assess these novel production systems through LCA. A major level of awareness on the sustainability of these promising ways to produce PHA is however necessary in order to pursue the technological development and overcome the critical aspect of the scaling-up. Following this aim, this thesis wishes to give its contribution to try to reduce this gap. To do so, in the following chapters LCA methodology is applied to the production of PHA using wastewater from fish canning industry as substrate, assessing and comparing both the use of pure and mixed culture.

3 GOAL AND SCOPE DEFINITION OF THE LCA STUDIES

3.2 GOAL DEFINITION

As pointed out by the bibliographic review, producing PHA from waste stream and using mixed culture are currently the focal points in order to improve the environmental and economic sustainability of the PHA production process. In this line, mussel processing wastewater (MPW) qualifies as a suitable substrate for PHA production, since it is rich in glycogen, an appropriate and viable feedstock for obtaining glucose. Moreover, its valorisation to value-added products is a great advantage since it would avoid the treatment of a wastewater that is produced in large volumes and whose treatment itself is extremely difficult since MPW has a high organic and NaCl content (25 and 18 g/L, respectively) (MusselBioVal Project, 2015). None of the reviewed LCA studies deal with the PHA production using fish canning industry wastewater as raw material, so the assessment of this PHA production pathway turns out to be mandatory in order to determine the sustainability and the hot spots of this process, which can be divided into two main blocks:

1) the fermentation system aimed to the accumulation of the biopolymers into the bacterial cells, which can be carried out following two different pathways: the use of pure culture or mixed culture.

2) the downstream process (DSP) aimed to the extraction and purification of said PHA.

The aim of this LCA study is evaluating the holistic environmental impacts and the hotspots of the PHA production processes using MPW as substrate, comparing the valorisation scenarios using pure and mixed culture with the wastewater treatment scenario. Within the innovative scenarios, a special focus is directed to the fermentation step, for which the two different technologies -the use of pure and mixed culture- have been analysed and compared in order to identify which is the more suitable for a better environmental performance (chapter 4 and 5, respectively). After that, the full production process is assessed, so the DSP is included in the analysis, and its environmental performances are compared with the baseline scenario, i.e. the conventional wastewater treatment (chapter 6).

To reach this objective, LCA methodology (ISO 14010:2006; ISO 14044:2006) has been used due to its validated capacity to identify and quantify the potential impacts of a product or a system like biopolymer production (Koller et al., 2013).

3.3 SCOPE DEFINITION

3.3.1 TYPE OF LCA

An attributional LCA is performed, as the object of the study is to describe the environmentally relevant physical flows to and from a life cycle and its subsystems (Ekvall et al., 2016).

3.3.2 FUNCTIONAL UNIT

The functional unit is defined on a mass basis, 1 kg PHA, for a consistent comparison with data and results of most of the reviewed LCA studies (as presented in chapter 2).

3.3.3 SYSTEM BOUNDARIES

All scenarios start at the MPW generation point. As reported in Table 6, this stress can be considered a high strength water that requires an intensive treatment as described below.

Table 6 Characterization of cooked mussel processing wastewater (MPW)

MPW characterization		
COD_t	16.6	g/L
COD_s	15.3	g/L
NT	790.4	mg/L
N-Protein	330	mg/L
$N\text{-}NH_4$	185.19	mg/L
Total solid	34.8	g/L
Protein	2.2	g/L
Glycogen	5.7	g/L
NaCl	18	g/L
pH	7.5	

3.3.3.1 Wastewater valorisation scenarios

This work presents a gate-to-gate approach, as it has as starting point the gate of the mussel processing industry and ends at the gate of the downstream process (solid green box in figure 20). Therefore, only the production process is considered, while the origin of the substrate as well as manufacturing of PHA based products, the use and end of life phases are not under assessment as they are considered common for the two valorisation routes and therefore left out the boundaries of the system under study (Hospido et al., 2010). It should be noted that only the operational phase has been included in the boundaries, while the emissions related to the construction of the production facilities and the machinery such as the emissions due to administration, maintenance and supervision of their operation are not assessed because of the lack of data at industrial scale.

Within the full production process, this work focuses on the fermentation stage (red box in figure 20), by building up a detailed inventory for both pure and mixed culture and by quantifying and comparing the potential impacts associated to both routes. Downstream processing is also evaluated from an environmental perspective and to do so data taken from the master thesis of Mateo Saavedra del Oso (2020) will be used.

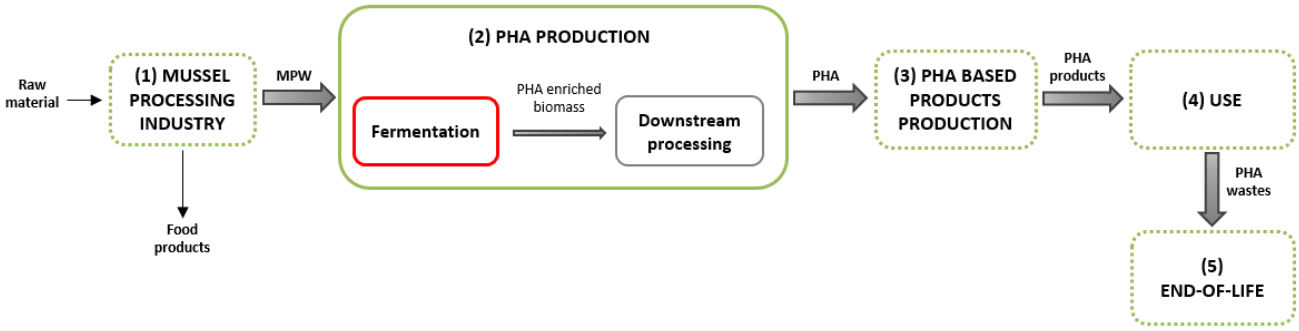


Figure 20 Process flow diagram of the life cycle and system function considered

Considering the LCA of the fermentation subsystem, all the flows associated with this production block are included in the boundaries, from the wastewater derived by the mussel processing industry to the PHA enriched biomass obtainment: the thermal and electric energy production, the use of chemicals and salts, the biogenic CO_2, the waste treatment. Regarding the pure culture production system, there is the co-production of protein that is assumed to be sent to animal feed production, prior purification treatment which is however excluded from the system boundaries.

When the DSP is assessed, the system boundaries extend from the same starting point (i.e. the gate of the industry) to the PHA production.

3.3.3.2 Wastewater treatment scenario

In line with the valorisation scenario, the system boundaries of the baseline one, i.e. wastewater treatment, has as starting point the gate of the mussel processing industry and to the cradle. So, it includes the operational phase of the plant (the treatment of wastewater) and the disposal of the sludge generated by the treatment. The chemical agents as well as the energy requirement are included in the system assessed. For a compatible comparison with the valorisation route, the construction of the WWTP is excluded from the assessment.

Regarding the geographical boundaries and the time horizon, all the defined scenarios are considered to take place in Europe as well as the input material production (whether it is possible), and the long term emissions have been excluded.

3.3.4 DATA COLLECTION

3.3.4.1 Wastewater valorisation scenarios

The inventory of the fermentation subsystems is based on measurements carried out on lab-scale reactors. Primary data for the pure culture were provided by Thelmo Lú Chau, responsible for the production of PHA by *Halomona Boliviensis* within the USABLE Packaging project (https://www.usc.es/biogroup/usable) while

primary data for the mixed culture were provided by Alba Roibás, working within the TREASURE-TECHNOLSAT project (https://www.usc.es/biogroup/treasure). From those data, the scaling-up has been performed assuming an estimated daily production of 85 m^3 of mussel processing wastewater as input of the two different processes.

Detailed inventory data are reported in chapter 4 and 5, but some essential elements are common for the two subsystems and detailed here. The energy consumption has been measured at lab scale and therefore it does not reflect the real consumption of full-scale systems, where the economy of scale means that the treatment of higher flow rate is expected to imply relevant energy savings. For this reason, literature data have been used to estimate the energy requirement of the fermentation process. The biogenic CO_2 emissions derived from fermentation process have been reported from some authors and therefore estimated according to Dacosta et al. (2015) for the mixed culture process and Zhong et al. (2009) for the pure culture production.

Finally, inventory data for the DSP have been provided by M. Saavedra del Oso (2020) and integrated into the system in chapter 6.

Ecoinvent 3.3 is the database used to integrate the background data[3] in the inventory.

3.3.4.2 Wastewater treatment scenario

The data of the conventional treatment process have been gathered and adapted on the basis of the inventory provided by Alba Roibás regarding a MPW treatment plant consisting in a prior homogenisation tank, a DAF (dissolved air flotation) unit and two biological reactors aimed to the removal of the organic matter by heterotrophic bacteria. The DAF system has the following removal efficiency: 80% for the solids, 80% for the protein, 40% for the COD and 31% for the carbohydrates. To adjust the pH at 4, 1L/m^3 of HCl is added. The ratio COD/TN of the effluent of the DAF is equal to 19.94 so two CAS (conventional activated sludge) systems in series have been disposed. After that, a decantation unit allows to improve the solid removal and the biomass recovery. At the end of the treatment the effluent concentrations check the limits established in the environmental authorization of the factory considered as case study for the inventory provided (Alba Roibás), as shown in table 7.

[3] The background data include energy, materials and waste management system that are delivered to the foreground system as aggregated data sets in which individual plants and operations are not identified (EPA, 2006).

Table 7 Characterization of the effluent and discharge limits established

	Effluent concentrations (mg/L)			
	COD	TN	NH_4^+-N	TSS
	450	68.8	5.1	150
Discharge limits	700	115	50	250

The energy demand for the agitation of the homogenization tank, as well as the energetic consumption of the pumping system and the compressor providing aeration to the DAF are estimated according to Coulson et al. (2005). Aeration requirement of the CAS has been evaluated according to Metcalf and Eddy (2014).

Hydrochloric acid and sodium hydroxide dosage used to adjust the pH respectively in the DAF and in the CAS systems have been estimated on the basis of experimental data.

The emissions to water have been calculated on the basis of the residual concentration showed in table 7, while emissions to air have been determined as 0.0025 kgN_2O-N/kg N discharged for emissions related to effluent discharge (IPCC, 2006).

3.3.5 CHOICE OF IMPACT CATEGORIES AND METHOD OF IMPACT ASSESSMENT

To carry out the LCIA phase, the software SimaPro 8.3.0.0 was used. SimaPro is a professional tool used to collect, monitor, and analyse the sustainability performance data of both products and services. It is a scientific-based source of information that enables to build the inventory and calculate the impacts in transparent and systematic way (https://simapro.com/about/).

Impact categories have been selected following the literature review performed, which has helped to individuate the more significant environmental issues associated to the system under assessment. So, especially on the basis of the more recent papers reviewed, the impact categories shown in table 8 have been selected. A midpoint approach is used:, being the Hierarchist ReCiPe Midpoint methodology the option selected for all the relevant impact categories, except the global warming potential (GWP) for which the IPCC method (2013) has been used, since its last version (IPCC v. 1.03) is not yet integrated at ReCiPe.

Table 8 Impact categories evaluated

IMPACT CATEGORIES	REASON OF SELECTION	METHOD
Global warming potential (GWP 100a)	Relevant for the estimation of the associated GHG emissions which are the major environmental issue at present.	IPCC 2013
Terrestrial acidification	Relevant for the emissions associated to waste stream treatment and electricity production.	ReCiPe Midpoint (H)
Freshwater eutrophication	Relevant for the phosphates emissions related to the wastewater treatment.	
Human toxicity	Relevant for the use of chemicals in the production process and in the residual wastewater treatment.	

Freshwater ecotoxicity	Relevant for the use of chemicals in the production process and in the residual wastewater treatment.	
Fossil depletion	Relevant for the process energy requirement.	

3.3.6 ASSUMPTIONS AND LIMITATIONS

Main limitations and assumptions are listed below:

- Background data for chemicals used in the process: approximations based on structure or function similarities were applied when a particular compound was not available in the Ecoinvent database:
    - The glucose used in the preparation of the seed media and potassium hydrogen phosphate added both in the preparation of the seed and of the culture media were assimilated respectively to the production of corn and sodium phosphate available at Ecoinvent 3.3.
    - The glucoamylase enzyme required by the pure culture production process was inventoried from Gilpin & Andrae (2017) and some assumption were required as some compounds were not included in the database: polysorbate 80 is approximated to its main component (ethoxylated alcohol); glucose carbon source, corn step liquor and corn oil (used as antifoam) are all approximated to an amount of "sweet corn production" equal to the required quantity of the glucose carbon source. The needed amounts of corn step liquor and corn oil were not taken into account since these two components are both by-products of the corn milling. The approximation related to the corn is conservative, since it is supposed that the corn used for the enzyme production do not derive from *ad hoc* cultivation, but from a second generation crop.
    - The antifoam agent used is pure and culture fermentation and allylthiourea involved in the mixed culture fermentation were approximated to silicone and thiocarbamide, respectively.
- For the MC production system, the background production of monosodium glutamate and the tris(hydroxymethyl)amino methane used in the preparation of the pure culture media are not included within the system boundaries due to the lack of data.
- The wastewater treatment for mixed culture fermentation is assimilated to the treatment of a wastewater with closer characteristics (similar values of chemical oxygen demand and total nitrogen) because a not complete characterisation of said wastewater is detailed predictable.
- The sludge generated from the mixed culture production is assumed to be destined to a compost facility, according to Popa, Ungureanu & Vlăduț (2019), while the sludge generated at WWTPs (both for the conventional scenario than for the wastewater leaving the pure culture fermentation system)

is assumed to be digested. The digestate deriving from the anaerobic digestion is considered to be incinerated while the biogas to be destinated to energy production.

4 LCA OF PURE CULTURE FERMENTATION

4.1 FERMENTATION PROCESS DESCRIPTION

PHB is produced by the halophilic bacterium *Halomonas boliviensis* using MPW as carbon source at laboratory scale as shown in figure 21:

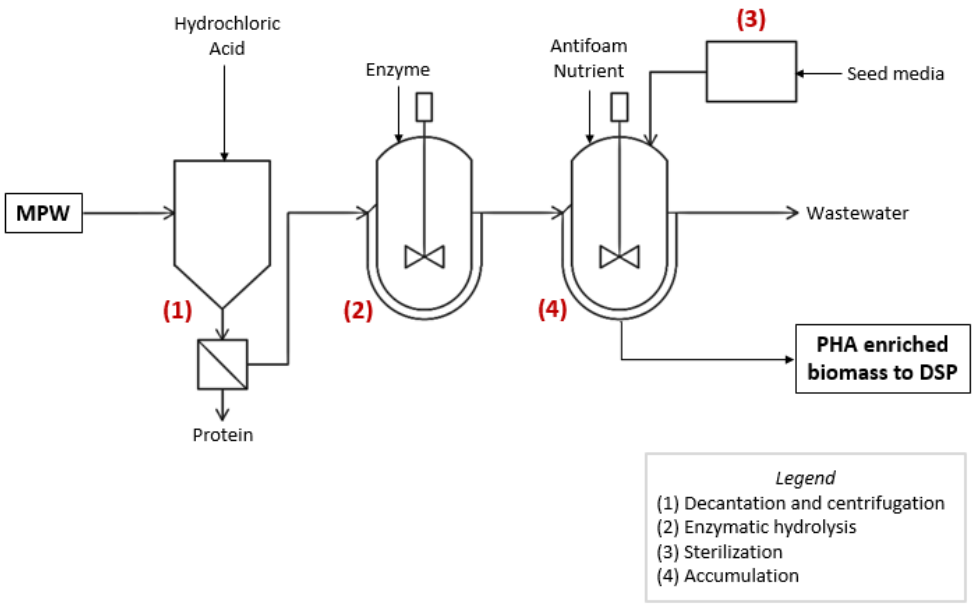


Figure 21 Process flow diagram of the pure culture fermentation

Firstly, a pre-treatment of the substrate consisting in decantation and subsequent centrifugation takes place (1): the MPW is acidified to pH= 4.0-4.5 adding 5 mL of HCl per litre MPW (González et al. 1992). After 3 hours of sedimentation of a precipitate composed mainly of protein, a subsequent centrifugation allows separating the clarified supernatant that will be influent of the second step and the precipitated protein enriched biomass as co-product[4] that, after a proper treatment, can be suitable as animal feed (Chan, Hossain & Brooks, 2007). The removal of the protein is essential to create favourable state or the PHAs accumulation, which occurs under nitrogen limiting condition along with excess carbon source.

[4] A mass allocation has been applied here: 99.91% associated to the MPW and 0.09% to the This allocation regards only this first phase (decantation and centrifugation), while the impacts associated to the other remaining phases are assigned as 100% to the PHA production process.

The three next phases have been modelled taking into account the invention patent of the "procedure for the production of PHA and ectoine by simultaneous saccharification and fermentation from cereal grain hydrolysates" (M. G. Torreiro et al., 2016) and the PhD thesis of M. G. Torreiro (2017).

Phase 2, involving the saccharification of the glycogen by adding glucoamylase enzyme, takes place in a batch reactor for 17 hours at temperature of 50°C. Phase 3 consists of preparation of the seed medium using glucose as carbon source, salts and 5 mol/L of NaOH to adjust the pH at 7.5., according to the amounts provided by Torreiro (2017) (table 9), and of its sterilization in a continuous system at 120 °C.

Table 9 Data about the composition of the inoculum

	Concentration (g/L)
Glucose	10
NaCl	45
$MgSO_4 \cdot H_2O$	1.4
K_2HPO_4	0.55
NH_4Cl	2.3
$FeSO_4 \cdot 7H_2O$	0.005
Monosodium Glutamate	3
Tris(hydroxymethyl)amino methane	15

Finally, the PHA accumulation phase occurs (4): the culture and the seed medium enter in an aerated semi-batch reactor following a ratio wastewater-inoculum 9:1. Here the foam is controlled by the addition of antifoam A (Sigma) and 2.2 g/L of K_2HPO_4 are added to support the microbial growth, which takes place at 30°C, pH=7 for 32 h. At the end of the cycle the solids settle down for 30 minutes to concentrate the product stream. The clarified fraction outflows via the top of the settler and is sent to the wastewater treatment, while the PHA enriched biomass is sent to the DSP. According to the laboratory data, the accumulation yield of PHA into biomass for this process reaches the 48.49%. The outgoing wastewater is characterized by an high organic load (about 7 g/L of total chemical oxygen demand and 251.6 mg/L of total nitrogen) as well as a content of total phosphorous (equal to 413.89 mg/L) whose high value is mainly linked to the addition of nutrient (K_2HPO_4) both in the seed media than in the growth reactor. For this reason, a specific wastewater treatment, consisting in chemical phosphorus removal unit using ferric chloride ($FeCl_3$) and a DAF system, has been considered to reach the discharge limit values considered before (conventional scenario).

4.2 LIFE CYCLE INVENTORY

The process flow diagram of the fermentation system reporting the scaled-up quantified flows for the functional unit (1 kg of PHA) is shown in figure 22.

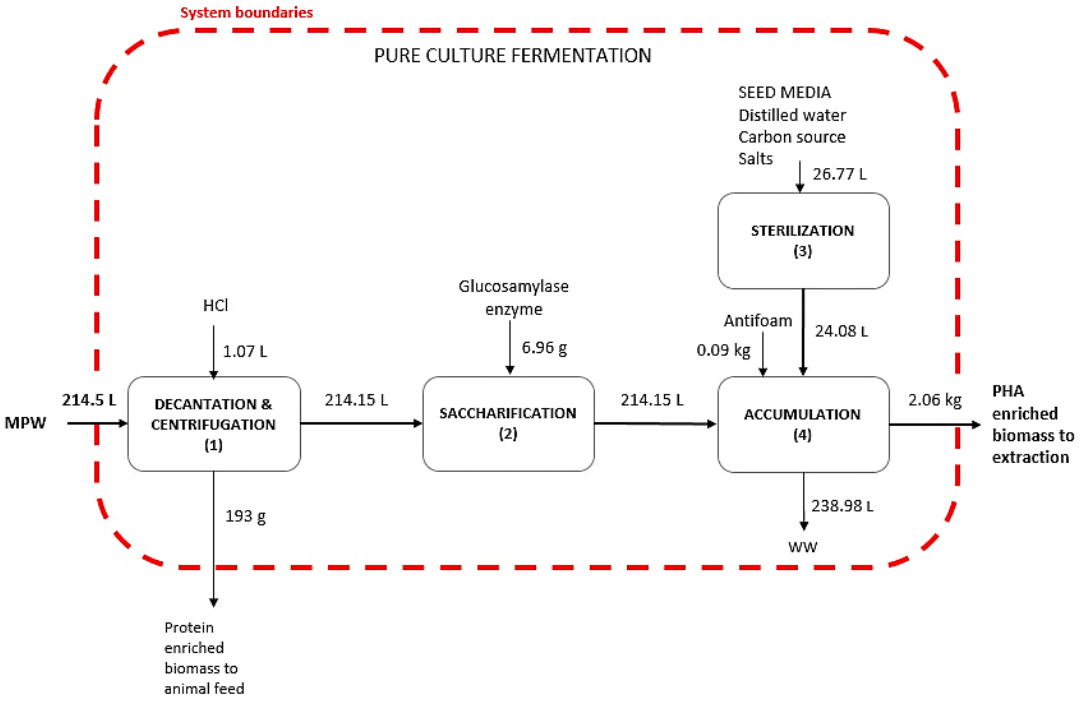


Figure 22 Diagram of quantified flows per F.U. referred to pure culture fermentation system

Antifoam dosage at full scale has been taken from Harding et al. (2007). The amount of glucoamylase enzyme involved in the saccharification process has been adjusted from the lab scale value to full scale treatment according to Rois (2009). The energy demand for the centrifugation has been calculated according to Perry et al. (1984), while the energy for the agitation of the enzymatic hydrolysis reactor has been estimated according to Levett et al. (2016), using a volumetric power of 0.2 kW/m^3. The steam used for heat sterilization, as well as the energy consumption associated to agitation of the accumulation reactor, fermentation temperature, aeration, and water-cooling processes, are estimated as reported by Zhong et al. (2009).

Biogenic CO_2 emissions have been roughly estimated according to the reaction's stoichiometry, obtaining a value of 1.6 $kgCO_2/kg_{PHA}$, which is relatively close to that calculated by Zhong et al. (2009): 1.91 $kgCO_2/kg_{PHA}$. Note that although reported in the inventory, those CO_2 emissions do not contribute to the GWP due to their biogenic origin.

The inventory table for producing 1 kg PHA through the pure culture fermentation is reported in Table 10. The treatment of the wastewater leaving the accumulation reactor has been inventoried as follows: the dosage of $FeCl_3$ has been estimated according to Metcalf and Eddy (2014) resulting in 0.5 kg/kg_{PHA}; the amount of HCl added in the DAF unit to adjust the pH has been estimated on the basis of experimental data (Alba Roibas) as 0.29 kg/kg_{PHA}; the total energy consume (0.46 kWh/kg PHA) required for the pumping system and the DAF unit have been evaluated according to Coulson et al. (2005) and Metcalft and Eddy (2014),

respectively. The emissions to water have been estimated on the basis of the total nitrogen and total phosphorous remaining after the treatment (6.8 mg/L and 13 mg/L) resulting in 1.625g TN/kg_{PHA} and 3.107 gTP/kg_{PHA}. The emissions to atmosphere result in 0.0041 gN_2O/kg_{PHA}.

Table 10 Inventory data for the production of 1 kg PHA by pure culture

MATERIAL/FUELS (inputs from technosphere)	
Hydrochloric acid	1.07 kg
Glucoamylase enzyme	6.96 g
Antifoam	0.09 kg
Distilled water	26.77 L
Sodium Chloride	1.2 kg
Magnesium sulphate	37.4 g
Ammonium Chloride	61.5 g
Potassium hydrogen phosphate	14.72 g
Iron sulphate	0.13 g
Sodium Hydroxide	5.35 kg
ELECTRICITY/HEAT (inputs from technosphere)	
Steam	0.45 kg
Electricity	3.084 kWh
EMISSION TO AIR	
Carbon dioxide, biogenic	1.6 kg
WASTE AND EMISSIONS TO TREATMENT (outputs to technosphere)	
Wastewater treatment	238.98 L

4.3 Life Cycle Impact Assessment

In this section the results of the third phase of the LCA, the LCIA, of the pure culture fermentation are presented. They will be further discussed and compared with those obtained for the mixed culture fermentation and also within the framework of the whole production process (i.e. including downstream processing) in Chapter 6.

Table 11 display the characterisation results for the impact categories under evaluation, while Figure 23 presents the relative contribution of the different elements reported in the inventory (table 10) to each impact category.

Table 11 Results of the evaluated impact categories for the pure culture fermentation system relative to 1 kg PHA

Impact category	Unit	Total
IPCC GWP 100a	kg CO_2 eq	6.037
Terrestrial acidification	kg SO_2 eq	0.032
Freshwater eutrophication	kg P eq	0.001
Human toxicity	kg 1,4-DB eq	1.374
Freshwater ecotoxicity	kg 1,4-DB eq	0.004
Fossil depletion	kg oil eq	1.512

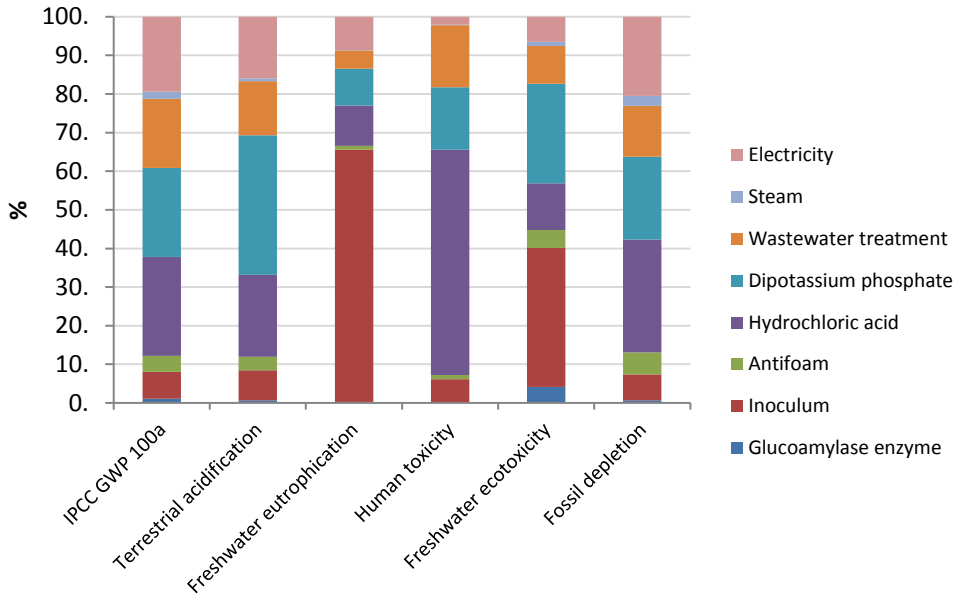


Figure 23 Characterization of pure culture fermentation and contribution of each LCI component

Among the components that most contribute to all the impact categories, there are the dipotassium phosphate and the hydrochloric acid. This last chemical shows a relevant contribution to the human toxicity category, as consequence of the cracking of the hydrogen liquid and hard coal mine operation aimed to obtain the fossil fuel for the generation of the energy involved in the HCl production. Another critical contributor is the inoculum preparation, mainly for freshwater eutrophication category: the distilled water involved in the composition of the seed media is the main responsible of the impact, due to the treatment in surface landfill of residuals derived from the extraction of lignite and hard coal, which are supposedly both raw materials used for the generation of the energy required to sustain the water distillation process. Plus, the inoculum preparation affects the freshwater ecotoxicity category because of the natural gas and the fertilizers, as phosphate and nitrogen, used in the production of the carbon source (corn).

The wastewater treatment burdens on the various impact categories for the following reasons: the treatment of the sludge by anaerobic digestion largely affects the GWP, terrestrial acidification and freshwater

ecotoxicity category for the municipal incineration of the digestate and the heat involved in the operation. The hydrochloric acid added in the DAF unit highly affects the human toxicity, as seen before, and the fossil depletion for the energy requirement associated to its production.

5 LCA OF MIXED CULTURE FERMENTATION

5.1 PROCESS DESCRIPTION

The fermentation process using mixed culture is carried out in a three-step system (figure 24):

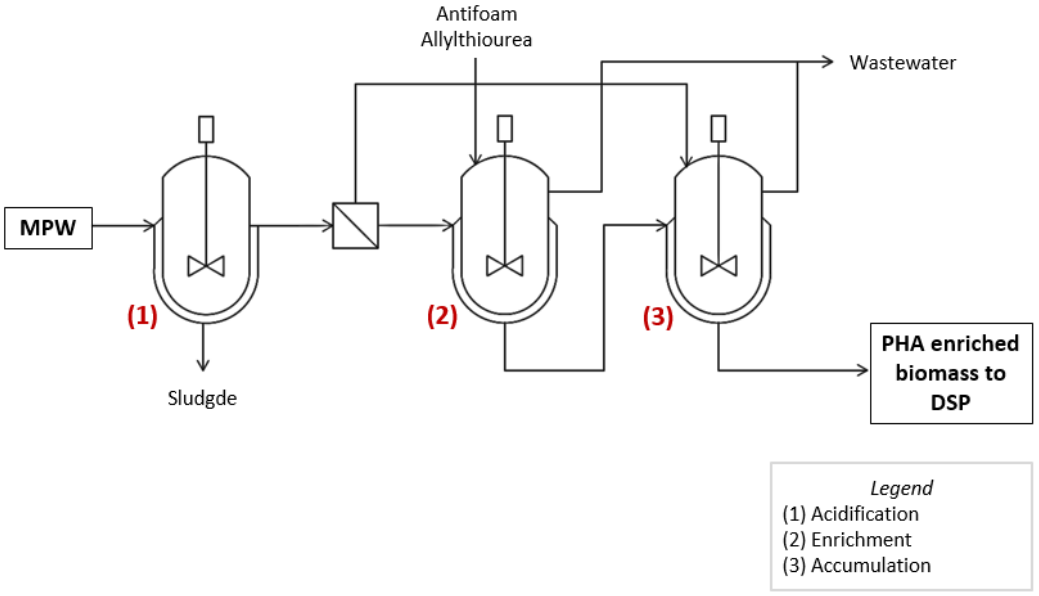


Figure 24 Process flow diagram of the mixed culture fermentation

- the first fermenter (1) is an acidification reactor, where complex organic molecules present in the wastewater are transformed into volatile fatty acids which represent the carbon source for the PHA-accumulating bacteria. This acidification can be considered as a pre-treatment to obtain a substrate with the optimal characteristics for obtaining the PHAs (Valentino et al., 2017). The effluent of this reactor is centrifuged in order to separate the anaerobic biomass and suspended solids from the VFAs-rich stream, which is used as substrate in the enrichment and accumulation phases.

  The separated sludge is assumed to be destined to a compost facility, as trough the composting treatment it is converted into a stabilized product that can be used as organic fertilizer (Gherghel, Teodosiu, & De Gisi, 2019).

- The second fermenter (2) is the selector reactor that is fed with a split fraction of the acidification product, which is rich in substrate and contains the amount of COD needed for the bacterial growth (Fernández-Dacosta et al., 2015). This influent is supplemented with an amount of 1.5 mL/L of 33.0 g/L allylthiourea (ATU) solution to inhibit the nitrification activity, and 0.25 mL/L of antifoam agent (Y-30 Emulsion, Sigma Aldrich) to avoid problems of foam during aerated phases (Argiz, Fra-Vázquez, del Río & Mosquera-Corral, 2020). The aim of this stage is the selection and the enrichment of a bacterial mixed

culture with high PHA accumulation capacity (Valentino et al., 2017). The selector is a sequencing batch reactor (SBR) as the substrate is feed in short periods of time resulting in the so-called feast/famine regime. The cycle length is 0.5 days and the solids retention time 1 day. The operational cycles of the SBR-S comprised the following stages: 1) feeding; 2) aerobic reaction; 3) a settling stage, implemented in order to remove undesired substances (mainly proteins and carbohydrates) that promoted the growth of non-accumulating bacteria; 4) supernatant discharge; 5) reactor refilling with the previous cycle effluent (recirculation), 6) aerobic reaction and 7) effluent withdrawal. Aeration is supplied during all stages, except for settling and supernatant discharge, while the temperature is controlled at 30°C (Argiz et al., 2020).

The supernatant discharge is assumed to be destined to wastewater treatment due to its high nitrogen concentration (0.13 g/L).

- The third step (3) is the fed-batch accumulation reactor, where the content of intracellular PHB on biomass is maximized up to 41.5%wt, according to the laboratory experiments. The accumulator is fed with the biomass rich replacement volume from the selector and the substrate rich remaining fraction from the acidification reactor. At the end of the cycle, the solids settle down during 30 minutes to concentrate the product stream (Fernández-Dacosta et al., 2015). The clarified fraction outflows via the top of the settler and is assumed to be sent to wastewater treatment, while the settled product is the feed to the downstream processes for the PHA extraction.

5.2 LIFE CYCLE INVENTORY

The process flow diagram of the mixed culture fermentation system reporting the scaled-up quantified flows for the functional unit (1 kg of PHA) is shown in figure 25.

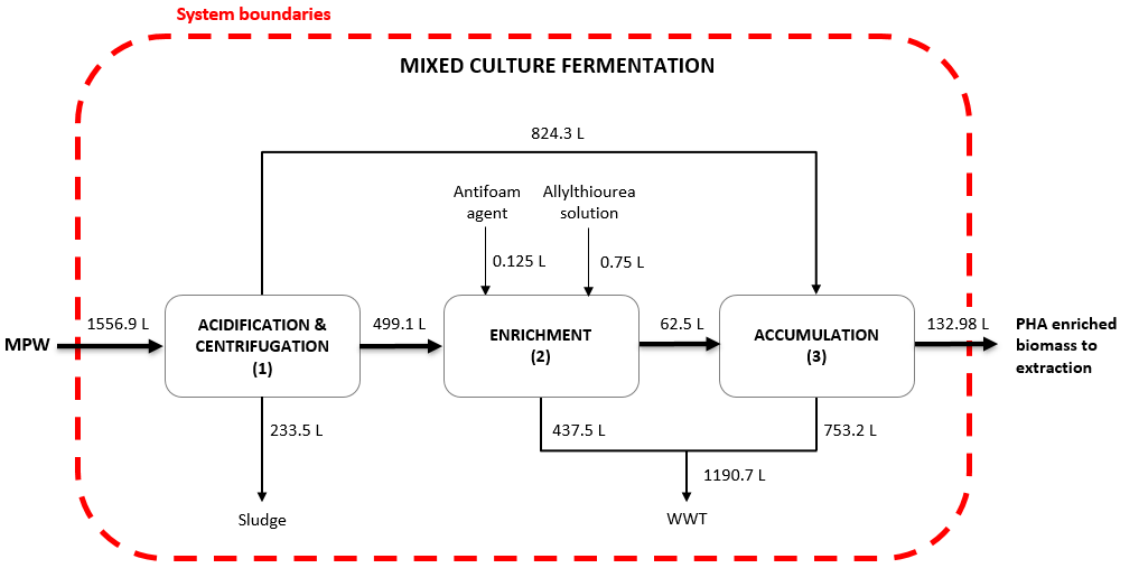


Figure 25 Diagram of quantified flows per F.U. referred to mixed culture fermentation system

The mass balance represented in figure 25 has been modelled on the basis of laboratory data, which have been scaled-up and referred to the F.U. The dosage of antifoam and allylthiourea are taken from Argiz et al. (2020). Data regarding the electricity consume, the steam used for heating the fermenters, as well as the CO_2 biogenic emissions are estimated according to Fernández-Dacosta et al. (2015).

The inventory table for the production of PHA enriched biomass containing 1 kg of PHA using mixed culture production is reported below (table 12).

Table 12 Data inventory of mixed culture production of 1 kg PHA

MATERIAL/FUELS (inputs from technosphere)	
Antifoam agent	0.137 g
Allylthiourea solution	0.82 g
ELECTRICITY/HEAT (inputs from technosphere)	
Steam	3.02 kg
Electricity	1.36 MJ
EMISSION TO AIR	
Carbon dioxide, biogenic	2.04 kg
WASTE AND EMISSIONS TO TREATMENT (outputs to technosphere)	
Wastewater, treatment of wastewater, average	1190.7 L
Treatment of biowaste, composting	12.77 kg

5.3 Life Cycle Impact Assessment

The results of the life cycle impact assessment for the mixed culture fermentation process are presented in this section. The values of the impact categories indicators are shown in table 13, while the relative percentage contribution of each LCI component is represented in figure 26.

Table 13 Results of the evaluated impact categories for the mixed culture fermentation system relative to 1 kg PHA

Impact category	Unit	Total
IPCC GWP 100a	kg CO_2 eq	3.694
Terrestrial acidification	kg SO_2 eq	0.025
Freshwater eutrophication	kg P eq	0.001
Human toxicity	kg 1,4-DB eq	0.118
Freshwater ecotoxicity	kg 1,4-DB eq	0.003
Fossil depletion	kg oil eq	0.389

The characterization of the percentage contribution of each LCI component to the impact categories is represented in figure 26.

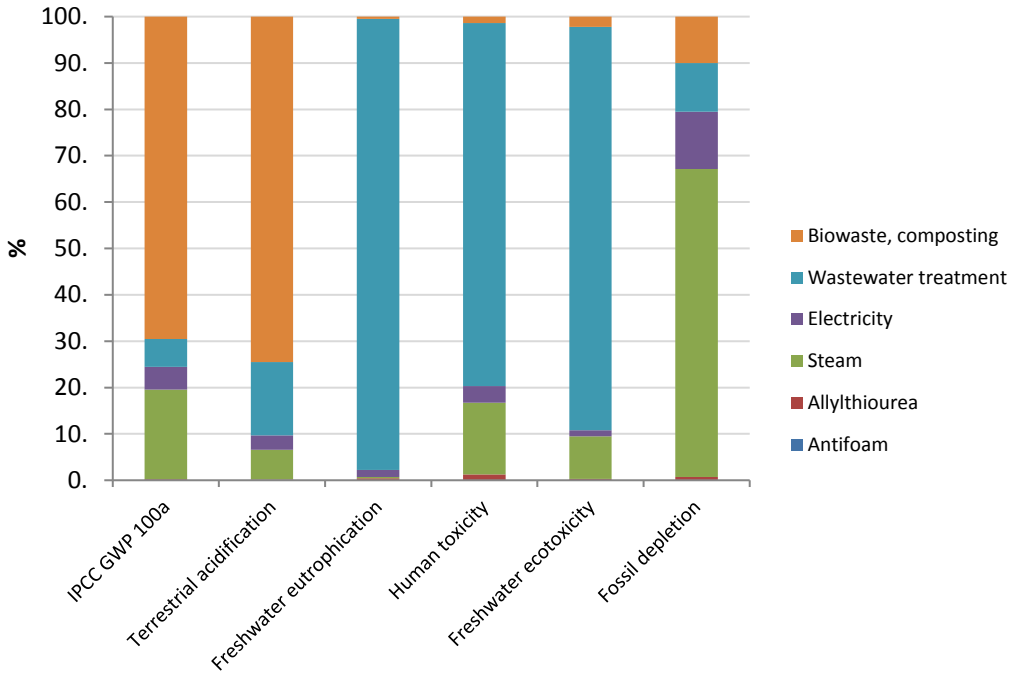


Figure 26 Characterization of mixed culture fermentation and contributions of each LCI component

Regardless the impact category, the management of the waste streams from the process dominate the environmental burdens associated to the mixed culture processes. On the one hand, the composting of the sludge produced at the acidification and consequent centrifugation dominates the GWP and the terrestrial acidification. Both these categories are mostly affected by the emissions related to the production of heat required in the composting treatment plant.

On the other hand, the further treatment of the wastewater leaving the enrichment and accumulation reactors after sedimentation dominates the freshwater eutrophication, human toxicity and freshwater ecotoxicity categories. The main responsible of the environmental burden produced on freshwater eutrophication and the human toxicity is the disposal in surface landfill of the residuals derived from the extraction of lignite and hard coal, which are both used as raw material to supply energy for the WWTP. The WWT highly contributes to the freshwater ecotoxicity too, mainly for the extraction of the natural gas required to sustain the treatment.

In addition to this, the steam production used for heating the fermenter is a significant contributor at the fossil depletion category, mainly as consequence of the high-pressure natural gas production.

6 COMPARATIVE ANALYSIS OF THE FULL PHA PRODUCTION PROCESSES

In order to have a comprehensive perspective of the environmental performance of the full PHA production processes, this chapter presents and discusses the results of the LCA of the entire systems, integrating the environmental analysis of the two different routes for the fermentation step (i.e. Chapter 4 and 5) with the evaluation of the downstream process (Saavedra del Oso 2020).

Among the eight alternative processes for the isolation of PHA evaluated by Saavedra de Oso (2020), the following alternatives have been selected for the present work:

- The LCA of the LV-2 method (appropriate to isolate PHB for low value applications) has been integrated with the analysis of the pure culture fermentation system: LV-2 can be applicable when the feedstock used is a wastewater and with halophilic bacteria, like *Halomona Boliviensis*. This extraction method is based on the hypothesis that an osmotic shock, namely a step of washing with fresh water, combined with a chemical digestion step -SDS- would be enough to isolate the pure PHB.
- The LCA of the HV-2 method (used to obtain P(3HB) suitable for high value applications) has been integrated to the evaluation of the mixed culture fermentation process, since HV-2 results the most sustainable among the methods assessed, besides being appropriate for substrate like food industry by-products and for any kind of strain. HV-2 combines mechanical disruption with chemical digestion by using a mixture of a base and a surfactant. After that, a bleaching step takes place before filtration and spray drying.

6.1 ENVIRONMENTAL ASSESSMENT OF FULL PHA PRODUCTION PROCESSESES

Results from the LCIA stage for the full PHA production processes, using pure and mixed culture, reveals that the mixed culture production system report better results for all the impact categories under assessment (Table 14). This conclusion is in line with the main lessons learnt from the review performed in chapter 2, confirming that the type of culture used is a relevant element to reduce the environmental burdens of the PHA production process.

Table 34 Values of the impact categories assessed for the full PHA production process, using pure or mixed culture (values referred to 1 kg PHA)

Impact category	Unit	Pure culture production process	Mixed culture production process
IPCC GWP 100a	kg CO_2 eq	9.669	4.435
Terrestrial acidification	kg SO_2 eq	0.047	0.028
Freshwater eutrophication	kg P eq	0.006	0.002
Human toxicity	kg 1,4-DB eq	1.516	0.149
Freshwater ecotoxicity	kg 1,4-DB eq	0.005	0.003
Fossil depletion	kg oil eq	2.754	0.664

When comparing the values reported here with the already available in the literature for PHA production from waste streams, it can be stayed that:

- for the pure culture production system, the global warming potential shows an intermediate value between the range of values reported by Kendall (2012) (3.1 ÷ 5.1 $kgCO_{2eq}$/kg PHA) for the cellulosic fraction of the organic residual derived by municipal solid wastes and the value found by Rostkowski et al. (2012) for the waste biogas ($9.42x10^2$ $kgCO_{2eq}$/kg PHA). For terrestrial acidification as well as freshwater eutrophication, the values obtained here are slightly higher, but of the same order of magnitude, that the ones reported by Kendall (2012): 0.016 ÷ 0.028 kg SO_{2eq}/kg PHA and 0.0054÷ 0.005 kg P_{eq}.

- regarding the mixed culture fermentation system, Gurieff and Lant (2007) as well as Dacosta et al. (2015) use the same type of culture and wastewater from food industry (Gurieff and Lant, 2007) and from industrial paper mill and food industry (Dacosta et al., 2015) as substrate, and reported similar values for global warming potential (1.97 $kgCO_{2eq}$/kg PHA found by Dacosta et al. and 20.4 $kgCO_{2eq}$/kg PHA found by Gurieff and Lant). The fossil depletion category results having a lower value than that found from Dacosta et al. (2015) (equal to 2.59 kg oil eq.), since the extraction method considered in this work (HV-2) is less energy intensive than the chemical digestion with sodium hypochlorite and sodium dodecyl sulfate (SDS) used by Dacosta et al (2015).

When looking at the contribution of the fermentation phase in the whole value chain (figure 27), the fermentation process, independently of the type of culture used, reports a higher contribution over the DSP process. The unique exception is the freshwater eutrophication potential of the pure culture production, for which the extraction phase turns out to have a higher impact than the fermentation step because of the phosphate emissions associated to the wastewater treatment.

This result may seem unexpected in relation with the previous literature (Kim and Dale 2005; Gurieff and Lant 2007; Rostkowski et al. 2012; Dacosta et al. 2015; Righi et al. 2016; Leong et al. 2017), which normally points out the DSP as the environmental and economic bottleneck due to the higher energy requirements.

The fact the DSP where first identified and compared (Saavedra del Oso 2020) and the options here (i.e. LV-2 and HV-2) selected are those reported there as the best alternatives from an environmental perspective might be behind this result. In fact:

- referring to the pure culture system, the chosen extraction method (LV-2) -based on non-cellular PHA mass (NCPM) disruption using cell fragility to hypotonic mediums combined with surfactant treatment- results to be the most sustainable respect to the others PHA isolation processes used to obtain biopolymers for low value applications. Except for the freshwater eutrophication, which is highly affected by phosphate emissions linked to the wastewater treatment, all the others categories related to LV-2 and considered in this study result in better environmental performances compared to the others DSP methods. In particular, the solvent extraction method (LV-4) is found to have the worst environmental performance in all impact categories due to its high solvents consumption as well as intensive energy use; LV-1 and LV-3 (Dacosta et al., 2015) processes, which are based on PHA solvent extraction and NCPM chemical digestion by sodium hypochlorite combined with surfactant treatment respectively, show higher impact related to IPCC GWP 100a (LV-1) and human toxicity (LV-3) if compared to LV-2 (M. Saavedra del Oso, 2020).
- In relation to the mixed culture system, the downstream process chosen (HV-2) -based on NCPM mechanical disruption- is the most sustainable over the alternative extraction methods considered to obtain PHA for high value applications. The methods based on solvent extraction (HV-2 and HV-4)- have a higher impact in all the categories evaluated in this study due to their high solvents consumption as well as intensive energy use. Also the HV-3 alternative, which is based on NCPM chemical digestion by alkali treatment combined with surfactant treatment, shows higher impact than HV-2 in all impact categories, mainly due to the background production of the Lysol involved in the process, and the phosphate emissions related to the wastewater treatment in term of freshwater eutrophication (M. Saavedra del Oso, 2020).

Moreover the fermentation step may have an higher weight on the impacts of the full processes because of the lower PHA accumulation yield achieved until now at experimental level compared to the yield values reported in literature: the pure culture fermentation achieves the 48.49wt% and the mixed culture fermentation reaches the 41.5wt% of yield, while normally other studies, like Dacosta et al. (2015), report a PHA intracellular content of 70wt%.

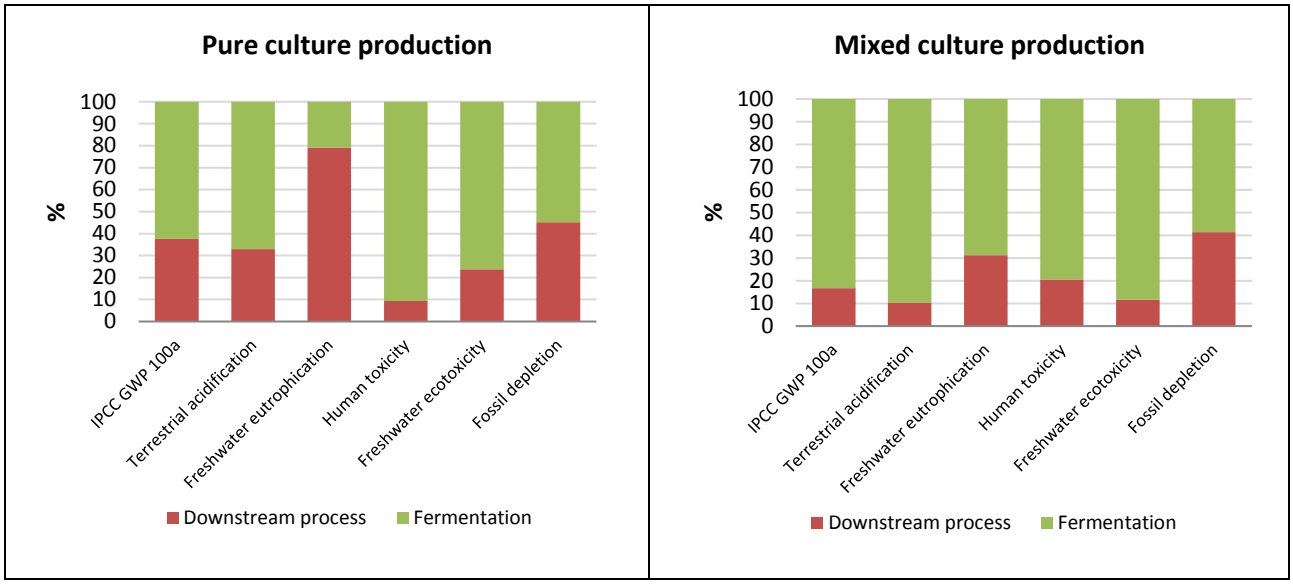


Figure 27 Percentage contribution of the fermentation and DSP steps for the pure and mixed culture PHA production

6.2 COMPARISON WITH THE BASELINE SCENARIO

The valorisation scenarios have the advantage to convert the waste stream into value-added products like biopolymers and to contribute, at the same time, to the treatment of the MPW, which is a hight strength wastewater whose treatment is extremely difficult as mentioned in chapter 3. Indeed, after the fermentation systems considered (chapter 4 and 5), the conventional parameters of the effluents achieve the discharge limits values. Therefore, it should be interesting to check the advantage of the valorisation scenarios over the baseline option respect to the impact categories assed. From figure 28, it is possible to observe that the pure culture scenario has the worse environmental performance in all categories, while for the freshwater ecotoxicity its impact is the same of the conventional treatment. On the other hand, the mixed culture valorisation route proves to be the most sustainable alternative, resulting in better environmental performances in all the categories evaluated.

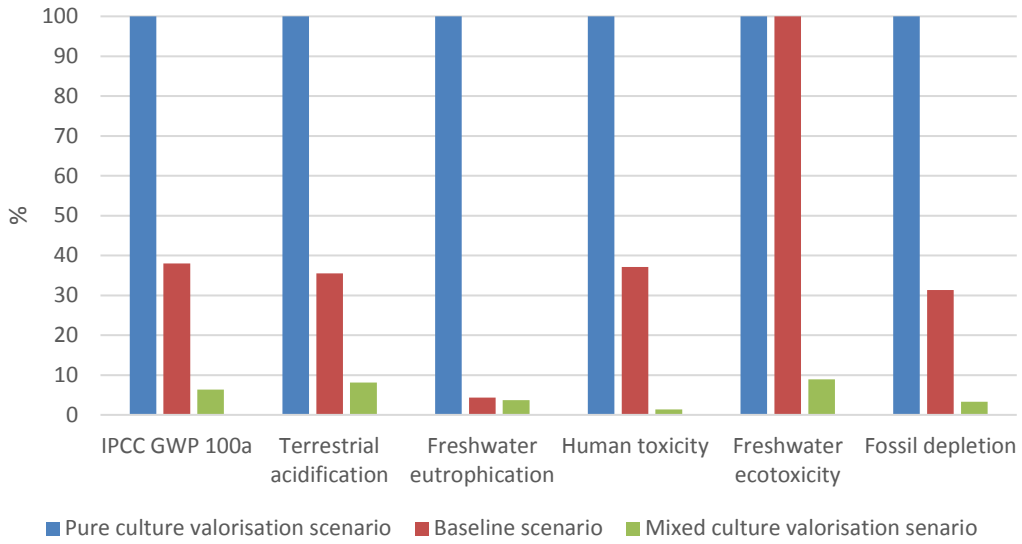


Figure 28 Indexed graph showing the relative impacts of the three scenarios considered (conventional treatment, pure culture PHA production and mixed culture PHA production)

Focusing on the relative contribution of each LCI components of the baseline scenario (figure 29), it can be observed that a high strength water as MPW proves to be highly energy intensive. The main contributor of all the impact categories (except human toxicity and freshwater ecotoxicity) is indeed the energy demand. The major responsible of the energy related impact is the high voltage electricity production from hard coal and lignite (for the GWP and terrestrial acidification categories), the mining operation for the hard coal extraction and the high pressure natural gas production (for the fossil depletion category) and the treatment in surface landfill of the residuals derived from lignite and hard coal mining (for freshwater eutrophication).

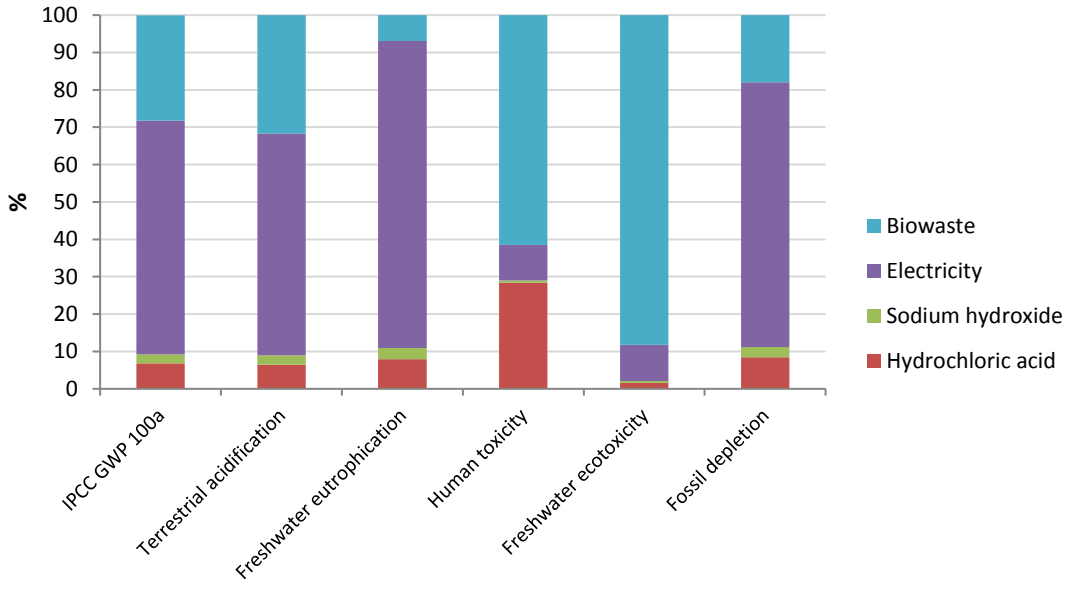


Figure 29 Characterization of the impact categories for the baseline scenario

From this section, it can be concluded that the valorisation of the mussel processing wastewater through the mixed PHA production process represents a promising alternative under a circular economy perspective. The MPW conversion into biopolymers not only avoids the use of dedicated raw materials, but also avoids the conventional WWT which in this case is highly energy intensive. Furthermore, it allows to treat the wastewater to achieve the discharge limits.

7 CONCLUSIONS

A brief overview on the main issues related to the actual plastic production- as the climate-change emissions and natural resource scarcity linked to the dependency on non-renewable fossil resources as well as the critical impacts on biota and environment due to the mismanagement of plastic waste- has highlighted the necessity to shift from an omnipresent linear economy to a bio based and circular economy. In this context, this work has focused on the production of bio-based and biodegradable polymers (PHAs) as promising alternative to the conventional plastics, and on its environmental performance by means of the use of the Life Cycle Assessment (LCA), methodology that has a considerable potential to drive the development of emerging technologies and identify the relative environmental hotspots.

A systematic review of published LCA studies of PHA production has allowed to point out the main challenges of the bioplastic production technologies as well as the focal points towards which address the future development and investigation, in order to make this alternative suitable in economic and environmental terms. From the review performed two main possibilities to achieve a more sustainable PHA production system have emerged: the choice of waste streams as feedstock and the use of mixed culture. The bibliographic review has also helped to take the fundamental decisions to carry out the LCA study.

LCA has been applied to two novel PHA production pathways, i.e. using as substrate the cooking mussel processing wastewater and with pure and mixed culture. The gate-to-gate LCA of the PHA production using mixed culture shows that this option results in better environmental performance compared to the pure culture production. Differently from the main lessons learnt from the review performed, fermentation step shows to have higher environmental impacts than the downstream process. The no yet optimized accumulation yields of the two fermentation processes is probably the reason behind this, which reinforces the need to improve the accumulation yield of the PHAs into the bacterial cells of the two novel processes so as to achieve the values reported in literature. Moreover, the valorisation of the mussel processing wastewater allows to avoid the conventional treatment of a high-strength wastewater, resulting in a better environmental performance if the mixed culture is used.

Finally, even if the fermentation efficiency may be still improved, the valorisation of the mussel processing wastewater aimed to obtain PHA is an attractive alternative, since it allows at the same time to produce value-added products as well as to treat a high-strength wastewater.

ACKNOWLEDGEMENTS

I acknowledge the projects USABLE Packaging and TREASURE-TECNOSALT within which this work has been carried out as well as the Politecnico di Torino for the scholarship.

I thank Professors Almudena Hospido and Silvia Fiore for the constant support and for the opportunity given to carry out this experience that enriched me from both a personal and professional point of view.

My thanks also go to to Alba Roibás, Lucía Argiz Montes, Mateo Saavedra del Oso and Thelmo Lú Chau whose useful support makes me possible to develop this work.

I am grateful to all the colleagues and friends known during this experience which have contributed to making the environment at work and out of work a pleasant place, besides having made me feel welcome right away.

A special thank goes to my family.

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