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ELLIPSE: EFFICIENT AND NOVEL WASTE STREAMS CO-PROCESSING TO OBTAIN BIO-BASED SOLUTIONS FOR PERSONAL CARE AND AGRICULTURAL SECTORS

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Abstract

Sustainability is a pillar to develop and maintain global strategies such as the EU Bioeconomy Strategy, the EU Green Deal and the SDGs. The transition from a linear to a circular economy including resource recovery, reuse and recycling is essential. However, meeting the requirements for converting bio-based waste streams into renewable raw materials, like bioplastics, with strict purity and high performance to ensure both proper processing and meet product request, demand inter-disciplinary cooperation among high skilled experts from EU. ELLIPSE project will address the valorisation of two heterogeneous waste streams: slaughterhouse waste and paper & pulp sludge, to produce cost-efficient polyhydroxyalkanoates for agricultural and personal care applications, by the coprocessing with other organic waste such as sludge from the dairy industry and glycerol from the biodiesel industry, as well as recovering nutrients to produce bio-based fertilizers. This will be achieved by applying the cascade biorefinery approach using acidogenic fermentation where, by one hand a VFA enrich stream will be generated from the selected feedstocks and coupled to PHB fermentation production system, and by other hand, the solid fraction produced after the acidogenic fermentation will be used to recover nutrients such as N and P. The integration of these waste streams as biorefinery feedstocks will allow reducing the volumes of landfilled waste, opening new avenues for platform chemicals and bioplastics production while creating additional revenue for the related industries generating them, with added advantages of water recycling, decreased soil degradation, groundwater pollution and methane emissions. ELLIPSE approach will be based on lab-scale optimization of organic waste mixtures and operation conditions, then these conditions will be up-scaling to treat at least 100 tonnes of slaughterhouse waste and 20 tons of wastewater sludge derived from pulp and paper industry.

Key words: bioeconomy, waste management, bioplastic, bio-based fertilizers

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1. Introduction

Heterogeneous organic wastes are challenging waste streams due to the presence of impurities that can compromise valorisation and are therefore usually sent to landfill. By one hand, slaughterhouses are one of the parts of the meat production chain where most waste is produced. In these places large volumes of solid waste and wastewater rich in organic contaminants and nutrients (particularly phosphorus) are produced. However, several problems are associated with the end of life of slaughterhouse waste such as waste segregation, high moisture content, presence of infectious pathogens (bovine spongiform encephalopathy) or heavy metal contaminations (Bhunia et al., 2022).

Among them digestive tract content (belly grass) is a major waste produced at cattle slaughterhouses and is comprised of partially digested cattle feed, mainly containing grass and grain. It has been reported that approximately 92 kg of wet belly grass are produced per animal (Smyth et al., 2011). Such a high volume of production makes it a waste that is difficult to manage and whose disposal, most of the time entails economical losses.

By the other hand, a global review of manufacturing sectors divulged that 17% of the global waste comes from paper industries (Camberato et al., 2006). The waste produced from pulp and paper industry is categorized in rejects such as pulping, bleaching, washing, primary sludge, and secondary sludge. Pulp and paper sludge is produced from the treatment of wastewater. The industrial sludge composition has been reported as highly variable with carbohydrate contents varying between 20% and 75% but also it contains heavy metals and toxic chemicals, which leads to toxicity of water bodies and impurities as filler, stickies, dye or ink which makes a waste difficult to manage (Gupta and Shukla, 2020).

At the same time, the environmental damage caused by petroleum-based plastic and the diminution of fossil reserves is translated into an increase in general public awareness of environmental issues and a necessity of a transition to a circular economy, where resources be renewable. This growing awareness in the population has increased industry's interest in the production of plastics from materials that can be eliminated from our biosphere in an "environmentally friendly" way (Leal Filho et al., 2021).

Bioplastics offers many benefits over traditional plastics including reduced greenhouse gas emissions, biodegradation and usage of sustainable materials. In addition, they can be produced from residues which allows to achieve two objectives simultaneously: produce a more sustainable polymer and reduce the huge quantities of waste generated at urban an industrial level. Despite the many advantages of bioplastics, they are not yet as well integrated into the market as fossil-based plastics, mainly because of their price, but also because their properties may not be adequate in certain cases (high fragility, low thermal stability and low nucleation density) (Atiwesh et al., 2021).

Among the most widely produced bioplastic worldwide is polyhydroxybutyrate (PHB), the most common bioplastic from polyhydroxyalkanoates (PHAs) family, due to its biodegradability in all environments. In addition, PHB can be obtained from different heterogeneous wastes as belly grass, sludges from different industries or glycerol (Brojanigo et al., 2021; Thirumala et al., 2010; Wendy et al., 2022). In this process, a first step is carried out where the organic waste is subjected to a pretreatment to avoid presence of impurities and an anaerobic digestion to produce volatile fatty acids (VFAs) and a digestate. In a second step, the VFAs streams is converted into PHB by bacteria present in mixed microbial cultures or pure cultures (Sirohi et al., 2020).

2. Why ELLIPSE's solutions?

The production of PHB a biobased polymer with versatile biodegradability properties in most environments is addressed in the framework of ELLIPSE project. PHB represent a promising material for a wide range of applications: PHB is biodegradable in soil which makes it ideal for agricultural applications (mulching films or fertilizer coatings) where products are disposed of in the soil and recycling system cannot be implemented (Chen et al., 2021). PHB is also a polymer that can be used in the manufacture of certain types of packaging and can be easily recycled by chemical recycling or enzymatic recycling, producing the monomer 3-hydroxybutyric acid that can be reincorporated into the fermentation as fermentation substrate (Parodi et al., 2021).

At the same time, in the process of PHB/PHBV production from sludge VFAs, a large amount of nitrogen (N) and phosphorous (P) remains in the digestate. Non-renewable mineral fertilizers, formed by N, P, K are the base of the current agricultural system, and the European Union agriculture is highly dependent of imports for fertilizing purposes. According to Fertilizers Europe (https://www.fertilizerseurope.com/wp-content/uploads/2022/09/Industry-Facts-and-Figures-2022.pdf) the total fertilizer consumption of European Union was about 17 million tons. Among them 32 % of nitrogen had to be imported as well as 65 % of P_2O_5 (Fertilizer Europe, 2022). Some of them such as P or Mg have been qualified as Critical Raw Materials by the EU COM(2017)490 and these are crucial for EU growth, competitiveness and especially for a sustainable food industry. In a context of migration to production models based on the postulates of the Circular Economy, it is essential to close the cycle by making use of all by-products generated during the industrial processes (Könjnger et al., 2021). For this reason, a good strategy that leads to a complete valorisation of the waste produced at industrial level is essential to reduce dependence on fossil fuels and foreign imports.

ELLIPSE project emerges as a solution for the coprocessing of heterogeneous waste streams generated in significant amounts in Europe (slaughterhouse waste, paper and pulp sludge, dairy sludge and glycerol) to produce cost-efficient PHAs for agricultural and personal care applications, as well as recovering nutrients to produce bio-based fertilizers (BBFs). The integration of these waste streams as biorefinery feedstocks will allow reducing the volumes of landfilled waste, opening new avenues for platform chemical and bioplastics production while creating additional revenue for the related industries, with advantages of water recycling, decreased soil degradation, groundwater pollution and methane emissions.

2. Case studies

2.1. Current state of the art

2.1.1. Acidogenic fermentation (AF) to produce VFAs

Acidogenic fermentation is considered as an efficient method for VFA production using a variety of organic waste as a substrate (sludge, food waste or organic fraction of municipal waste among others). Various methods to promote VFAs yield in waste acidification step have been developed, e.g., using pre-treatments, through in-situ processing control such as the regulation of pH, temperature and additives (Chen et al., 2021; Karki et al., 2021). pH control is widely used strategies to inhibit methanogenesis and enhance VFA production through anaerobic digestion of sludge or other organic matters, and it is found that pH was the main factor affecting the various indicators, especially alkaline pH regulation facilitated solubilisation, hydrolysis, and acidogenesis while simultaneously inhibiting methanogenesis. In recent years, zero-valent iron, Fe(0), has also been reported to enhance the biological processes by stimulating microbial activity and creating favourable reducing conditions (Luo et al., 2014). The presence of Fe(0) can, for example, serve as a buffering agent to react with and compensate for the acidity, and then relieve the low pH inhibition on microbial activities for the generation of VFA (Cao et al., 2019). On the other hand, anaerobic membrane bioreactors (AnMBR) have proven to be effective in treating waste streams from different sources, even food and pulp and paper industry, improving yield and quality of the VFAs produced.

The combination of advanced technologies in previous studies, such as the use of Fe(0) and AnMBR, has allowed to improve the production of VFAs and to validate the industrial viability of these technologies. These trials have provided experimental evidence that the process is scalable and cost-effective, offering a practical solution for the circular bioeconomy (Mahmood et al., 2022). The advantages include a stable and high yield of VFAs, improved product purification due to continuous separation, and more effective control of organic loading by regulating the waste stream. For the ELLIPSE project, this technology could be critical in the valorisation of heterogeneous waste, increasing the efficiency of nutrient recovery and maximising the production of specific VFAs. Unlike other anaerobic systems, AnMBRs can process waste with high concentrations of solids, such as complex industrial waste. This makes it possible to work with more challenging waste streams while maintaining high efficiency in the production of VFAs. These innovations in AnMBRs represent a significant improvement over traditional anaerobic fermentation methods, allowing for a more efficient and pure production of VFAs. However, membrane fouling can emerge as a serious problem in the AnMBR especially when the system is operated at acidic or alkaline conditions, those suitable to produce VFAs (Parchami et al., 2020). A variation in operating pH directly affects the cell morphology along with a major alteration in adhesion and flocculation phenomena.

Considering the selected feedstocks to be treated in ELLIPSE project (slaughterhouse waste and pulp and paper sludge), there are not many reports on VFAs production in spite of their potential carbohydrate content. But reports on VFA yields during biogas generation confirm the feasibility of VFAs production from this waste. Optimizing a process for VFAs production from primary sludge alone or in combination with secondary sludge under favourable acidogenic conditions, will open new avenues for platform chemicals and bioplastics production from paper and pulp mills waste. In this context, ELLIPSE plan to valorise 100 tonnes of slaughterhouse waste, 10 tonnes of sludge derived from pulp and paper industry, 10 tonnes of glycerine, and 20 tonnes of waste reject from deinking process. Nowadays, these waste streams are derived to landfill, so ELLIPSE will provide solutions to empower the circular economy and valorisation of this wastes.

2.1.2. Production of PHAs (PHBV) from VFAs

Bacterial fermentation processes using VFAs as carbon source have emerged as a sustainable approach for PHA production due to their abundance in waste streams from organic sources, such as agricultural residues, food processing, and wastewater treatment (Montiel-Jarillo et al., 2021). Thus, harnessing the bacterial metabolism to convert VFAs, such as acetic acid, propionic acid, butyric acid, valeric acid and caproic acid into PHA, offers a greener alternative to traditional petroleum-derived plastics (Yin et al., 2016). Further, by using waste-derived streams, the cost of carbon is lower than using refined materials and the competition for raw materials used in food and feed applications, such as plant-derived sugars and oils, is avoided (Vu et al., 2021).

The process starts by screening and selecting strains known to efficiently use VFAs and convert them into PHA. Strains from genera such as *Cupriavidus, Bacillus* and *Pseudomonas* have been extensively studied and optimised for this purpose (Pradhan et al., 2018; Sabarinathan et al., 2018).

Optimizing fermentation conditions is critical for maximizing PHA production efficiency and reducing production costs, in addition to tailoring of the polymer composition, which results in the production of a polymer with specific mechanical properties. Parameters such as VFA concentration and composition, pH, temperature, aeration, agitation, and nutrient supplementation are carefully controlled to maintain optimal microbial growth and PHA accumulation (Mengmeng et al., 2009). The main challenge of the ELLIPSE project is the production of a PHB with the appropriate characteristics for agricultural and packaging applications. PHB usually has quite poor mechanical properties for use in applications such as blow molding (weak elongation break and melt strength and high brittleness), which are widely used in the plastic industry (Di Lorenzo and Righetti, 2013). By optimizing fermentation parameters, the type of PHB obtained can be modulated, thus varying its mechanical properties.

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is a type of PHA formed by chains of hydroxybutyrate and hydroxyvalerate. The presence of valerate (a larger carbon chain) in PHBV disrupts the crystalline structure that is characteristic of PHB (Grousseau et al., 2014; Policastro et al., 2021). This disruption increases the amorphous regions in the polymer, leading to enhanced flexibility (Avella et al., 2000). Odd-chain fatty acids (valeric acid and propionic acid) are precursors of the 3-HV fraction during PHBV accumulation (Policastro et al., 2021). Therefore, in the ELLIPSE project, by modulating and increasing the proportion of valeric and propionic acids during fermentation, we will obtain PHBV with a higher hydroxyvalerate content, making it suitable for applications where PHB is not feasible due to its poor mechanical properties (such as packaging or mulching films).

After fermentation, the bacterial biomass with PHBV granules is harvested from the culture medium followed by PHBV recovery and purification. Several recovery approaches include two main strategies: PHA recovery with solvents (aqueous solvents, halogenated solvents, alkanes, alcohols, esters, carbonates and ketones) and PHA recovery by cellular lysis (with mechanical treatments, oxidants, acid and alkaline compounds, surfactants and enzymes or combinations thereof) (Koller et al., 2013). One of the main challenges for the green production of PHA is the development of solvent-free recovery processes. ELLIPSE uses aqueous-based methods for the digestion with environmentally friendly re-agents of non-PHA cellular material, more concretely, specific enzymatic methods for extraction are attractive alternatives for purification of PHA.

2.1.3. Transformation of PHBV into valuable products

More than 150 different PHAs building blocks constitute the PHAs family. However, the commercial portfolio is mainly restricted to five types of PHAs, including PHBV. The current commercially available PHAs are mainly produced from sugars and/or vegetable oils. Besides, the PHBV building block contains only between 1-2% of valerate content, which is limited for further processability.

Several technical difficulties arise when processing current PHA compounds into different end applications. PHB has a narrow processing window, during processing (particularly at temperatures of 160 degrees and higher, and with high shear and/or long residence time), thermal degradation and chain scissions occur. This results in an undesirably large decrease in molecular weight (Mw) and viscosity. PHB has also low nucleation density making a crystalline polymer at room temperature, with around 60-70 % of crystallinity and fast crystallization rate between 80 and 100 ºC, but slow below 60ºC or above 130 ºC, so that the material remains amorphous and sticky (dos Santos et al., 2017).

Apart from that secondary crystallization can occurs, which results in brittle and stiff material and slow processing cycles. The material properties then change over the next few days, so you do not immediately have the final properties. To overcome these technical challenges, it is possible to combine the different commercial PHA building blocks and transform the polymer into suitable material that can be processable into injection moulding, extrusion blow moulding, coating and film blowing technologies to end applications.

Within ELLIPSE, the customized produced PHBV with different valerate contents (5-25%) and different Mw (150-500 KDa) is investigated, formulated and transformed into end-applications, including bottles for cosmetic personal care, coating & encapsulates fertilizers and mulch films for agricultural sector.

2.1.4. Nutrients recovery and coated fertilizers

The unsustainability of the fossil-based fertilizer production, the depletion of the conventional P-based fertilizer reserves, combined with the increasing of the geopolitical scenario, highlight the urgent need for new sources of bio-based alternatives for fertilizer production.

The intensive agriculture and livestock concentration across Europe could offer a valid source of nutrients and an alternative to classic synthetic fertilizers. Typically, the related residues and livestock effluent also represent the feedstock to feed more than 1600 anaerobic digestion (AD) plant around Europe. In the AD process, the organic matter is degraded while N and P are released in the mixed liquor which leads to high concentration of nutrients in the effluent. The latter is called anaerobic digestate which includes the stabilized organic matter, N and P compounds, potentially exploitable to produce bio-based fertilizers (Lorick et al., 2020).

The available technology developed and exploited at industrial level, for nutrient recovery from anaerobic digestate, comprise on chemical/physical steps aiming firstly at the solid/liquid separation of the organic and the mineral and soluble forms (Selvaraj et al., 2022). This step is accomplished mainly by screw-press, centrifuge and belt filter, which lead to the production of a solid fraction characterized by a total solid concentration between 20-25% and high amount of organic N and P (Munasinghe-Arachchige et al., 2020). The liquid fraction is much more pure on suspended solids but is rich in ammoniacal nitrogen and orthophosphate.

Among the other technologies, the ELLIPSE project will explore and develop a nutrient recovery technique based on membrane separation. Specifically, the system comprises on a preliminary solid/liquid separation aiming the removal of all the fibers and particles and an ultrafiltration unit (UF) with a consequent obtaining of a concentrate fraction rich in suspended solids, microorganisms and organic nitrogen. The free ammonia gases permeate the wall of the shell side of the membrane contactor and dissolved from the counter current flow of sulphuric acid recirculated in the lumen side. The final product will be a solution of ammonium sulphate with a final nitrogen up to 7%.

Another promising option are photosynthesis-based technologies that incorporate the use of microalgae to recover nutrients while simultaneously sequestering carbon dioxide (CO_2) and producing beneficial biomass. Cultivating microalgae on wastewater has gained popularity in recent years as a novel method to recover nutrients before they are released into the environment (Acien Fernandez et al., 2018). Algae has strong potential for use in large-scale systems for upcycling N and P into biomass due to their rapid growth rates (Arumugam et al., 2018). A sustainable farming system promoting the circular N and P-bioeconomy concept could involve growing these aquatic species on either diluted manure or bio-digester effluents and harvesting them for use as a mineral fertilizer substitute (Köninger et al., 2021; Rajagopal et al., 2021). Currently, the scale-up of autotrophic cultures is a time-consuming process occupying a significant area of the industrial production plants. Several studies had proposed a two-stage cultivation strategy involving heterotrophic growth in the first stage for high-density inoculum preparation and to achieve a decrease in the photobioreactors (PBRs) volume required to start (Wang et al., 2017). Even so, this type of cultivation in PBRs still represents an infrastructure and land space greater than the heterotrophic cultivation which can take place in any type of closed fermenter. The technology consists of three main stages, (i) microalgae cultivation in two stages, which consume the organic matter and nutrients contained in the digestate; (ii) harvesting or separation phase to recover the solid fraction of microalgae as high value by product and the liquid fraction as reusable water; (iii) a drying stage by spray dryer to recover the dry microalgae (Barros et al., 2019).

The European Union produces an estimated 180 million tons of digestate annually, containin high concentration of nitrogen (2-5 kg/m^3) and phosphorous (0.5-1.5 kg/m^3) compounds. By recovering these nutrientes, the project can reduce reliance on synthetic fertilizers, thereby decreasing environmental pollution and promoting soil health. Utilizing these waste streams as biorefinery feedstocks for the recovery of N and P, the ELLIPSE project not only addresses waste management challengues but also contributes to a circular economy. This approach reduces the volumens of landfilled waste, open new avenues for a platform chemical and bioplastic production, and creates additional revenue for related industries.

The escalating demands of a growing global population coupled with diminishing arable land per capita necessitate continual advancements in fertilizer application practices. However, conventional nutrient application strategies have faltered in optimizing the use efficiency of key elements like N and P. To address this challenge, the adoption of innovative fertilizer products capable of enabling a controlled release of salts and micronutrients, the controlled release fertilizers (CRFs) has emerged as a promising tailored nutrient management solution to curtail environmental risks while preserving crop productivity.

This granulated fertilizers still represent niche and high-valorizable products with applications in horticulture, lawn maintenance (including sport fields), landscaping (including green area in municipalities) and agriculture (Shaviv et al., 2000). These fertilizer products are based on different technologies which create a barrier around the granules, protecting the salts and nutrients from immediate washing and controlling at some extent the release of nutrients into the soil up to 24 months. This strategy of "doing more with less" has several advantages: (a) avoiding an excessive use of fertilizer salts; (b) Reduced nutrient losses (N, P, K and micronutrients) to the environment ('run-offs') and the associated impacts (e.g. accelerated eutrophication in aquatic environments from P) (c) Maintained /increased crop yield rates at a lower nutrient application rate (d) Improved quality of plants that need a continuous supply of nutrients at a low rate (e) Cost saving and reduction of labour associated to a reduce number of applications on field. On the other hand, these fertilizer products are obviously more technological challenging (spray coating from organic solvents, fluidized beds), they are produced at higher cost, and even small defects hinder functionality (high quality needed).

In this context, the ELLIPSE project will scale-up well-known technologies for the preparation of biodegradable in soil granulated products in which a biodegradable matrix will protect and act as a barrier to control the release of nutrient in soil and soil. ELLIPSE partners has been improving a technology that combine biopolymeric materials and mineral fertilizer in a one-step extrusion process to obtain a granule in which the salts are finely dispersed in the matrix. The PHBV biodegradable biopolymeric matrix will protect the minerals from run-off and will enable a gradual intake of nutrient to the plant. After the nutrients have been released and in-taken by the plant, the remaining empty biopolymeric matrix will biodegrade in the substrate or soil without bioaccumulation and without the generation of microplastics, satisfying the requirements by the EU fertilizer products regulation. As the same time, the ELLIPSE project will foresee the upscale of a technology that enable the coating of pristine mineral granules by spraying solutions of biodegradable materials in rolling drums.

2.2. Challenges and innovations

ELLIPSE addresses different challenges that involve different topics such as organic waste valorization into VFA, PHBV production and purification or biofertilizers, among others. The main challenges and innovations associated are depicted in Table 1.

2.3. Relevance and significance.

The European Green Deal is the European Union Strategy to transform the economy and society to achieve net-zero GHG emissions by 2050. It emphasizes the transition from linear to a circular economy through the Circular Economy Action Plan. ELLIPSE is fully aligned with this approach, where waste streams are bioconverted into high-value products closing the loop of these organic wastes and providing sustainable materials such as biobased and biodegradable bioplastics and new sustainable fertilizers.

Besides, the Green Deal also aims to decarbonize the industry and foster the adoption of clean and sustainable technologies. ELLIPSE promotes the innovation in clean technologies providing alternatives to waste streams that are directly landfilled or incinerated.

Considering the fertilizers, the new European regulation on controlled released fertilizing products address different aspects as the soil health and microplastic pollution, as currently the main controlled released fertilizing products are based on non-biodegradable products, the new fertilizer produced in ELLIPSE based on biodegradable polymers suitable in soil conditions will be a big breakthrough in this field promoting the sustainability avoiding the microplastic pollution.

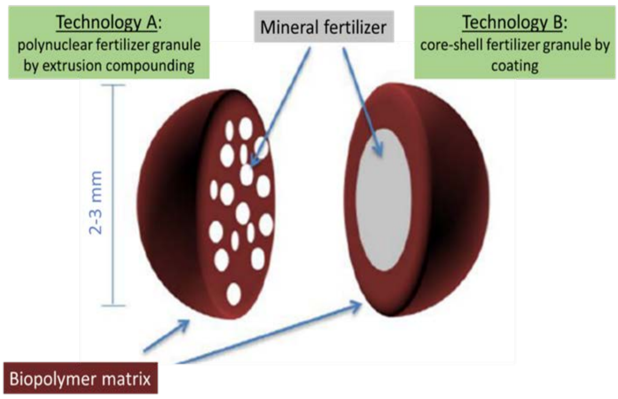

Fig. 1. Technology for bio-coated fertilizers

Table 1. Main challengues of ELLIPSE project and innovations related

Challenge	***Innovation***
Maximize VFA production to improve PHAs production efficiency.	A combined strategy of the following approaches will be implemented: • Use of AnMBRs • Addition of zero-valent iron nanoparticles to enhance the microbial metabolic activity • Optimization of the ratios between the main waste streams and other OW (glycerol or dairy sludge).
PHBV purification through more efficient and sustainable processes	Organic solvent-free methodology to purify PHBV including: • Use of aqueous-based methods for the digestion with environmentally friendly reagents of non-PHA cellular material. • Fine-tuning the processing conditions to tailor the final average molecular weight of the PHA to the envisaged specification. • Use of specific enzymatic methods for extraction and purification of PHA, adapting a previously developed method to the specificities of the organisms to be used.
Maximize the production of VFA of interest (propionic and valeric) to produce PHBV copolymers with tailored content in valerate.	• Optimization of the global operational, including waste and inoculum pretreatment needs, co-digestion ratios and operational conditions to produce PHBV grades with valerate content between 5-25 %. • Use of pre-selected microbial strains known to use VFAs for PHA accumulation and selection of those strains that more efficiently metabolise the specific VFA mixes produced and yield PHA with the required monomeric compositions.

PHBV fulfilling the requirements for target applications (rigid packaging, mulching film, paper coating and fertilizers´ encapsulation)	• Formulate the obtained tailored PHBV copolymers in specific new compounds and wet compounds according to the processing technologies to produce the different prototypes and final applications´ requirements.
Maxime nutrient recovery from the digestate through innovative microalgae hybrid cultivation technology	• Optimize the hybrid cultivation system at pilot scale by obtaining in the autotrophic phase a quality inoculum with this type of digestate, capable of improving and completing the recovery of nutrients in the heterotrophic phase, resulting in an algal biomass with fertilising properties. • The combination of these two types of microalgae cultivation modes (PBR+HBR) presents a synergistic effect that will minimize the inherent setbacks of PBRs and HBRs, while harnessing the advantages such as overcoming the inhibition effect with the sufficient sunlight and nutrients supply in the PBR and saving surface area and time.
Zero-waste strategy: biofertilizers algae/ biofertilizers	• Improved recycling/recovering of nutrients from OW by assimilating them into algae biomass with the later collection by spray drying of a product of commercial interest as BBFs. • By supplying recycled nutrients acquired from OW streams to plants and offsetting the use of synthetic fertilisers, algae BBF grown in OW will reduce the environmental impacts. Due to the slow-release, microalgae can be used in organic, low input and conventional farmings with a frequency 3-4 consecutive seasons in the same field and can be combined with compost as a carrier.

3. Results and discussion

3.1. Objectives

Despite all the useful and unique characteristics of PHB, several of which are superior to corresponding polymers, there are still several limitations when PHB is produced at industrial scale capacities.

At the present, the main bottleneck for the large-scale production and incorporation of PHA in the mark is their elevated production cost, ranging from 2.2 to 5.0 € per kilogram. This cost is at least three times higher than that of the predominant fossil-based polymers, which are priced at less than 1.0 € per kilogram (Gholami et al., 2016). Another aspect that can be hinder the possibility of incorporating PHB into the market is their properties are not very suitable for packaging applications. PHB is characterized by a high crystallinity, usually between 55 and 70 % which is an important cause of PHB brittleness and by a melt strength weaker than other non-biodegradable synthetic polymers used in packaging. In order to improve flexibility, the introduction of comonomers such as 3-hydroxyvalerate (3HV), to form the copolymer PHBV, can be carried out. Compared to PHB, PHBV with higher contents (> 2%) is less crystalline and more flexible with improved impact resistance and toughness which makes PHBV more practical for packaging application (Abbasi et al., 2022). To overcome these limitations ELLIPSE project attempts to: (1) reduce the costs of production and achieve economic viability in competition with the current low petroleum manufacturing costs by using free-charge organic wastes as substrate for PHB production and by recovering, (2) enhance physical and chemical characteristics, such as better mechanical flexibility or lower toughness by producing a more versatile polymer as PHBV and (3) provide sustainable performances in the production of PHBV but also considering the end-of-life of the polymer. To achieve this the following objectives are addressed:

1. To apply effective pretreatments for heterogeneous wastes and improve the yield of VFA production.

ELLIPSE project will set an effective pre-treatment system to minimize the presence of the non-biobased impurities on the selected heterogeneous waste according to the waste requirements for subsequent acidogenic fermentation process. The project will also implement a methodology that enhances VFAs accumulation in a liquid stream, especially the accumulation of valeric and propionic acid which facilitates PHBV production by microorganisms in further steps.

2. To obtain PHBV copolymers through efficient and sustainable processes.

The VFAs rich stream from acidogenic fermentation of the processed organic wastes will be directly used as a carbon source for PHAs production, specifically PHBV copolymers, using in-house bacterial strains. Novel green enzymatic method will be employed for PHBV separation and purification to achieve the most efficient and sustainable process of PHBV production.

3. To obtain different PHBV grades and compounds according to the requirements of the final application.

In the ELLIPSE project PHBV copolymers with different contents of valerate will be produced due to the possibility of changing flexible properties and melt strength depending on 3HV content. Copolymers with high valerate content (up to 15%) will be produced for mulch film and rigid packaging applications. Copolymers with lower valerate content (up to 5%) will be produced for coating application and encapsulated fertilizers.

4. To validate new and innovative End-of-life forms to recover monomers than will be reincorporated into the production process.

Both enzymatic and chemical recycling will provide derived 3HB and 3HV monomers that will be additional monomers in the fermentation for PHBV production, besides VFAs from acidogenic fermentation, increasing the yield of bioplastic production and producing the original biopolymer with the same properties.

5. To recover nutrients (nitrogen and phosphorous) to be used as bio-based fertilizers.

To maximize the valorisation of the organic waste, digestate stream will be treated with cutting-edge technologies (microalgae treatment and membrane filtration) to recover nutrients, in particular nitrogen and phosphorus. Recycled nutrients will be used in form of powder or struvite as fertilizer for agriculture and horticulture use, closing the loop.

6. To demonstrate environmental and socio-economic sustainability and apply mature/novel digital technologies in the PHBV production process.

The ELLIPSE project will implement methods and tools for the economic and environmental assessment of the impact from cradle to grave of the compounds/products to guarantee the safety and sustainable production of PHAs for personal care and agricultural applications. This includes the application of life cycle assessment (LCA) and life cycle cost (LCC) analyses and assessment methods of the contribution to a local circular economy. The project will also implement process simulation software and mathematical models to determine and improve the economic and social impacts and reduce the environmental risks associated with the technology process.

3.2. General concept

To achieve the previous objectives ELLIPSE project will apply a 4 phases methodology (Fig. 1) to expand the opportunities for the valorisation of bio-waste in all stages and across all sectors and meet the market requirements for the proposed application in the personal care and agriculture sectors.

3.2.1. Phase 1: Waste stream selection, analysis, pretreatment and VFA production

In ELLIPSE an efficient solution for the excess waste generated in meat production industries and paper and pulp sludges will be implemented. A pre-treatment of the waste streams will be required because slaughterhouse waste normally contains about 5% of different impurities and solids such as plastics that could interfere in the acidogenic fermentation (AF).

Residues from the paper industry, consist of organic matter (e.g. cellulose fibres) but also other impurities such as filler (kaolin, calcium carbonate), ink, binder/stickies and other impurities (e.g. plastics). Before using it as a substrate for VFA production, it is necessary to separate problematic inorganic materials and impurities using a pretreatment step. This separation step will be carried out using existing technologies such as pulper, de-inking processing unit, cavitation unit, among others, and other steps such as sedimentation, sieving will be evaluated if necessary. Then physical and chemical treatments will be implemented to first obtain homogeneous waste stream and adequate particle size and promote the hydrolysis through basic and/or acidic pretreatments. Then, combination of pretreatments and organic wastes will be studied and VFA yield and VFA profile will be evaluated to optimise their production for its use in next phases (Phase 2).

The slaughterhouse bellygrass and paper and pulp sludge will be used as feedstocks to produce VFAs by AF. AF will be optimized through a design of experiments targeting an improvement in the propionic and valeric content. During this phase strategy as mixture of wastes, controlling pH to inhibit methanogenesis and dose iron nanoparticles will be used. ELLIPSE develops AnMBR technology and acidogenic fermentation under pilot-scale conditions to demonstrate the efficiency of membrane bioreactors in the separation of products such as VFAs or biomass and how this technology overcomes traditional limitations by significantly improving organic matter degradation and selectivity of VFA production.

AnMBRs allow for greater retention of microbial biomass due to the physical separation offered by the membranes, which maximises the efficiency of substrate conversion to VFAs. Once optimized operational conditions at lab scale in the 2L reactors for the acidogenic fermentation, an AnMBR will be used to validate the parameters selection and to improve the yield and quality of the VFA produced: (i) check that the operating pH selected does not affect the durability and performance of the AnMBR membranes by previous test at an existing 30L-scale AnMBR plant before scaling the process; (ii) assess degree of retention of Fe(0) nanoparticles in the reactor, favouring the economy of the process; (iii) check the possible effect of undissolved solids in the AnMBR operation, and if required, include a phase separation stage before the fermenter to remove part of the solids entering the system.

A relevant example of AF at pilot and industrial scale is the use of poultry waste in an AnMBR (Yin et al., 2022), which has proven to be very effective for the production of VFAs that achieved a high yield of 0.90 g-VFA/g-VS under weakly alkaline pH conditions and an organic loading rate of 2 g-VS/(L-d), which is in line with the parameters tested at ELLIPSE. In addition, the system was able to maintain stable productivity for more than 50 days under high solids concentration and high flow rate conditions. The best conditions will be upscaled in two pilot plants: in BEST (Austria) the approach is to simulate a direct implementation into pulp and paper production process to produce lower chain VFAs aimed for coating materials for paper/cardboard. In Green Generation (Ireland) the approach is on slaughterhouse waste targeting longer-chain VFAs intended for PHBV for agricultural applications (Placido and Zhang, 2018).

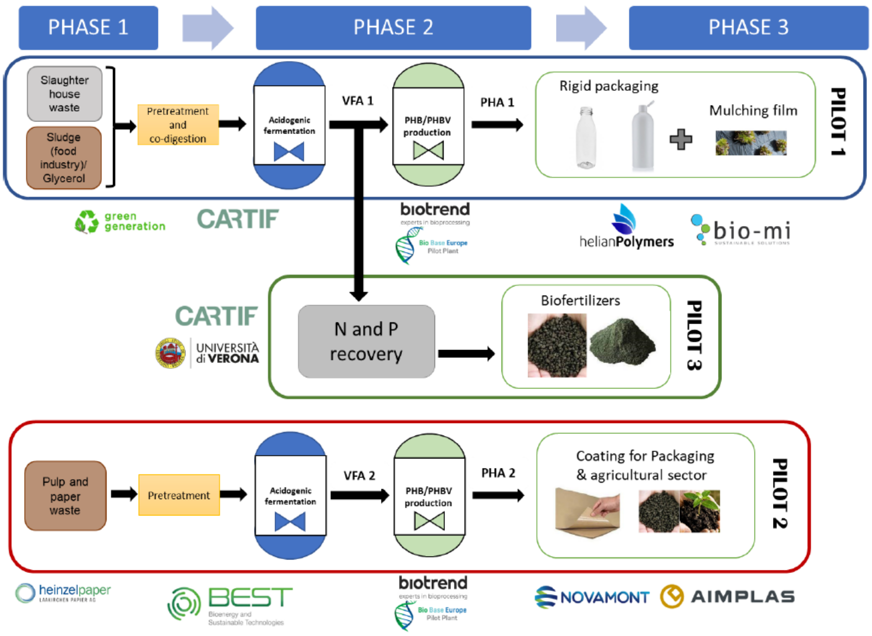

Fig. 2. ELLIPSE methodology

3.2.2. Phase 2: Intermediary products

In this phase, two approaches will be tackled: VFAs produced in the phase 1 will be used to produce PHAs by biotechnological processes. During this phase strains able to growth on carboxylic acid in different concentrations coming from Phase 1 will be selected. The bacterial producers selected will be grown in fed-batch cultures with the VFA mixes obtained from the AF. The cultivation strategy will be optimized to attain high polymer productivities by changing parameters such as carbon source feeding regime to avoid substrate inhibition, the optimum dissolved oxygen profile and the best trigger of polymer accumulation (nitrogen vs phosphate depletion). Different strategies such as pH stat and cell recycling systems, in case a diluted substrate stream will be tested. The most optimal fermentation process on the VFA mixture selected substrates will be scaled in 1500 L bioreactors in BBEPP facilities (Belgium).

The digestate will be treated by two different technologies (microalgae bioreactor and different types of membranes bioreactors) to recover the nutrients, mainly N and P. These nutrients are the base for future formulations of biobased fertilizers (BBFs.)

By one hand, using microalgae bioreactors, the digestate generated in phase 1 will be used as feedstock for microalgae cultivation. Once the algae biomass is harvested it will be separated from the solution by centrifugation and spray-dried to estimate the quantitative potential of nutrient recovery per unit volume of feed. Besides, supernatant will be removed, and remaining biomass heated until completely dried. Algae control will be evaluated with spectrophotometry and optical microscopy. The best operating conditions to maximize the quality and quantity of the recovered nutrients will be selected.

By other hand, nutrients recovery from fermentation digestate will be performed through sequential unit operations including solid/liquid separation of the digestate through a screw-press, ultrafiltration of the permeate, reverse osmosis and ammonia recovery as ammonium sulphate by membrane contactor. The process will be implemented by treating of 100-200 L of digestate per day. At the same time a membrane contactor for ammonia recovery from digestate will be used where NH_3 from the origin solution, passing through the gas-filled porous membranes and recovered by sulfuric acid to obtain ammonium sulphate.

3.2.3. Phase 3: Applications

There are two big sectors in which the focus is set to validate both PHA and nutrients: personal care and agriculture. By one hand, personal care will use the PHBV with less content of valerate for rigid packaging and the PHBV more flexible for paper coated flexible packaging. Agriculture sector will include the use of the PHBV for mulching film production, together with the combination of the recovered nutrients and PHA into a control-release BBF.

3.2.4. Phase 4: End-of-life alternatives

Finally, end of life alternatives of the validated products will be studied to confirm their recyclability and biodegradability respectively: Mechanical recycling route for the PHBV based rigid packaging and the mulch films; chemical recycling for rigid packaging, to check the feasibility of recovering monomers that could be further introduced in the PHB/PHBV production process and enzymatic recycling for PHBV coating in paper. Mulch films and slow-release fertilizers will also be tested for biodegradability in soil according to ISO 23517:2021.

In addition, a full life cycle assessment (at technical, economic and social level) will be carried out to evaluate the whole ELLIPSE value chain. These three phases are complementary in three different pilots, where the starting organic waste and applications are different:

3.3. Pilot phases

3.3.1. Pilot 1

In pilot 1 a co-digestion of feedstocks will be carried out with the aim of ensuring the most optimal conditions to produce VFAs. Slaughterhouse waste will be the main residue used coupled to either sludge from food industry or glycerol. In this pilot 1, the co-digestion of the residues will be optimized to assure the highest odd-chain VFA concentration (valerate and propionate). This is because in the next step when bacterial strains are accumulating PHBV, the presence of odd-chains VFAs trigger the accumulation of a higher fraction of 3-HV in the PHBV polymer making it more flexible and with lower melt strength, which makes it more suitable for application in the personal care and agricultural sectors.

Rigid packaging for the personal care sector and mulching film for agriculture are produced. In this way, valerate high-content polymer will be used to produce compounds to produce bottles by EBM and flexible films (mulch films) obtained by blown film extrusion.

3.3.2. Pilot 2

In pilot 2, different residues produced during the paper production are addressed. This pre-sorted wastepaper industry waste will be used in the production of VFA. For this pilot the requirements of a VFA stream rich in valerate is not so high, as the final applications achieved do not require the same processing conditions than in pilot 1.

In this context, A higher total concentration of VFAs will be sought rather than a higher partial concentration of propionate and valerate. As the valerate fraction is lower in this pilot the valerate-low content PHBV with lower melting point will be used to produce wet compounds suitable for coating application for paper-based flexible packaging for liquid personal care products using conventional printing technologies.

3.3.3. Pilot 3

In pilot 3 a strategy to recover N and P will be implemented with the aim of producing bio-fertilizers. Two different technologies will be validated.

• The technical feasibility of nutrient recovery via hybrid microalgae cultivation process (photoautotrophic and heterotrophic) while treating digestate from acidogenic fermentation will be demonstrated.

• Nutrient recovery will also be performed through physical methods optimizing two different technologies to recover ammonia, as ammonium sulphate: i) pressure drive membrane technologies (ultrafiltration and reverse osmosis) and ii) membrane contactor.

The products will be converted into pellets will be further processed to produce two types of innovative fertilizers with tunable kinetics of nutrient release, applying PHA from pilot 2 in the formulation of polynuclear fertilizer granules by extrusion compounding and core-shell fertilizer granules by coating. These final products will be tested for biodegradability in soil according to ISO 23517:2021.

3.4. Consortium members

The aforementioned issues are being tackled through a collaborative initiative represented by a project consortium, consisting of 1 academic team (University of Verona), 3 technological centers (AIMPLAS, CARTIF and BEST) and 10 industry partners (BBEPP, BIOTREND, NOVAMONT, BIO-MI, ZER0-E ENGINEERING, HELIAN POLYMERS, ENCO, GREEN GENERATION and LARKIRCHEN PAPIER) from 8 different countries. The role and the background are the following (Table 2).

3.5. Preliminary results

During the first period of the project (20 months), the project consortium worked mainly on tasks related to the production of VFAs from different wastes and its optimization. A brief summary of the results is presented below.

3.5.1. Characterization and selection of wastes

The waste streams samples were provided by:

1. Pulp & paper processing industry provided by Heinzelpaper. Residues from pulp and paper industry (rejects from deinking process), OCC (old corrugated containers) process and chemical pulp production. The samples were specifically taken from four different processes (Fig. 3). Two secondary fibres processes: i) De-Inking Process (DIP; used to regenerate raw material from waste paper for the production of white paper), ii) Recycled Fibre (RCF) process (RCF; first step of recycling brown paper, e.g. cardboard), iii) the production of pulp from primary fibres/wood (POELS) and iv) wastewater treatment (ARA).

The characterization provided analytical data for the following parameters of the waste streams: total solids (TS), volatile solids (VS), pH, chemical oxygen demand (COD), soluble chemical oxygen demand (sCOD), total Kjeldahl nitrogen (TKN) and biomethane potential (Table 3) to evaluate the suitability of the samples for the acidification process. Samples from the processes DIP and ARA show a low methane yield. Sample RCF10-04 achieved the third highest methane yield, however most of the sample is inorganic sand which can be very problematic when put inside a reactor. The samples with the most promising results are RCF10-03, RCF10-05, RCF10-06 and POELS-03. These samples are regarded as most interesting for the acidification process and were therefore selected for further analysis and pre-treatment trials.

2. Organic waste provided from the company Green Generation, such as slaughterhouse waste (belly grass or rumen content), wastewater treatment sludge from dairy industry (Glanbia Ballitore WWTP Sludge, Rosderra Edenderry WWTP Sludge) and waste from bioprocess (Glycerine from biodiesel production). The characterisation of glycerine, belly grass and sludge are shown in Table 3.

Fig. 3. List of wastes from Heinzelpaper production process, including ID, description and picture. The abbreviations mark the processes they come from: DIP = deinking process; RCF = recycled fiber; POELS = pulp from primary fibres; ARA = wastewater treatment

Table 2. List of participants in ELLIPSE project

Participant	***State***	***Country***	***Role***
AIMPLAS	Technological center	Spain	Mechanical, chemical and enzymatic recycling of personal care and agricultural products
Bio Base Europe Pilot Plant (BBEPP)	Industry partner	Belgium	Scale up of the PHA fermentation process in 1500 L reactor and scale up of PHA recovery and purification
University of Verona (UV)	Academic team	Italy	Green nutrient recovery through physical methods and ammonia recovery from liquid phase of digestate by membrane contactor
Biotrend (BIOT)	Industry partner	Portugal	Technical scale implementation of the PHA fermentation process from VFA-rich streams.
Novamont S.p.A (NVMT)	Industry partner	Italy	Compounding, coating, prototyping and validation of PHA based bioplastic into innovative fertilizers with tunable kinetics of nutrient release
Fundación CARTIF	Technological center	Spain	Pre-treatments of waste streams. Acidogenic fermentation to produce VFAs as substrate for PHA fermentation. Nutrient recovery from hybrid microalgae cultivation
Zero-Emissions Engineering (Zer0-E)	Industry partner	Netherlands	Full life cycle assessment at the technical, economic, and social levels to evaluate the value chain feasibility and scalability.
Helian Polymers (HP)	Industry partner	Netherlands	Compounding, prototyping and validation of PHA based bioplastic into rigid packaging for personal care products applications
ENCO srl	Industry partner	Italy	Dissemination, exploitation, awareness and market uptake
Bioenergy and Sustainable Technologies (BEST)	Technological center	Austria	Pre-treatment of pulp and paper waste for the acidification process. Acidogenic fermentation in a pilot reactor to produce VFAs
Green Generation Ireland Ltd (GG)	Industry partner	Ireland	VFAs production at pilot scale from co-digested sludge
Laakirchen Papier AG (HEPA)	Industry partner	Austria	Supply of substrates directly from the pulp and paper production process
BIO-MI	Industry partner	Croatia	Compounding, prototyping and validation of PHA based bioplastic into mulch film application

Table 3. Characterization and analysis results of samples from Heinzelpaper production process

ID	*pH [-]*	*TS [%]*	*VS [%]*	*COD [g/kg]*)*	*sCOD [g/L]*	*TKN [g/kg]*	*Biomethane potential [Nm³/t FM**)]*
RCF10-03	7.0	3.2	2.7	20.59 ± 4.54	4.7	0.2	6.7
RCF10-04	6.8	26.0	4.7	82.78 ± 9.21	7.0	0.3	5.6
RCF10-05	6.1	3.3	2.9	36.81 ± 0.79	5.2	0.1	7.1
RCF10-06	6.6	1.4	1.1	13.43 ± 3.09	4.1	0.0	3.3
DIP11-01	6.8	6.2	1.6	23.81 ± 18.02	3.7	0.0	2.8
DIP11-02	6.7	1.3	1.0	11.78 ± 12.73	2.9	0.2	2.2
ARA-A-01	7.2	1.2	0.5	4.07 ± 3.45	0.3	0.5	0.8
ARA-A-02	6.8	4.9	3.9	25.38 ± 2.41	0.7	1.1	2.4
ARA-A-03	7.1	0.5	0.2	1.87 ± 1.21	0.4	0.0	0.4
POELS-02	5.8	1.1	1.1	9.61 ± 0.11	0.2	0.0	2.4
POELS-03	6.3	0.8	0.8	5.93 ± 5.41	0.0	0.0	3.0

**) Samples were inhomogeneous which made sampling difficult. Therefore, the COD values (duplicate analysis) show a high deviation. **) FM = fresh matter*

3.5.2. Impurity removal and pre-treatments

Samples from the pulp and paper industry were inspected to find strategies to remove potential impurities. Common impurities are plastic pieces, polystyrene, stickies, and metal pieces. Those impurities are problematic for microbial processes and should be removed before the acidification. In the RCF process for cardboard recycling, impurity removal is already performed during the process in the production plant. Samples RCF10-03, RCR10-05 and RCF10-06 are almost free from impurities, only small styrofoam pieces are left in RCF10-03. Therefore, it was decided that no additional impurity removal for the RCF-samples is necessary. No impurities were detected in samples from the production of pulp from primary raw material (wood). Thus, no impurity removal is necessary for the sample POELS-03. ARA and DIP samples were not selected for acidification which is why impurity removal of those samples was not considered.

With the chosen samples (RCF10-03, RCF10-05, RCF10-06 and POELS-03, see above), pre-treatment trials were performed to enhance the potential yield of VFAs in a subsequent acidification step. The sCOD was used as an indicator for easily available organic matter; an increase in sCOD was considered a positive effect for acidification. Four methods were investigated: two physical treatments to obtain a homogeneous stream (i) heat (125 ºC), (ii) high-shear mixing (blender), and two chemical treatments to promote hydrolysis and diminish the possibilities of methanogenesis (iii) acid addition (H_2SO_4), pH 5 or 4), (iv) base addition (NaOH, pH 9-11) (Ortner et al., 2015).

(i) Heat treatment was performed in a 12 L bench autoclave. A volume of 300 mL homogenized sample were transferred to 500 mL bottles and then put in the autoclave. The autoclave was heated up to 125 °C and the temperature was held for 15 minutes. Afterwards it cooled down to room temperature.

(ii)The samples were mixed to break down particles and with that increase the surface available for (bio-) chemical reactions. A blender was used for this and the samples were mixed until no further change in fibre or liquid texture was observed (approximately 30 sec).

(iii)The pH value was adjusted to 5 using H_2SO_4. After the adjustment of the pH, the samples were stored at 4°C for 24 hours, and analysis (sCOD, VFA) was performed afterwards. The samples were stirred during the pH adjustment using either a magnetic mixer or a spoon if the sample was too viscous.

(iv)The pH was adjusted to 9-11 using NaOH. After the pH adjustment, the samples were stored at 4°C for 24 hours, and analysis (sCOD, VFA) was performed afterwards. The samples were stirred during the pH adjustment using either a magnetic stirrer or a spoon if the sample was too viscous and base was added.

At first, the different treatments were performed separately, and original samples were treated using either heat, high-shear mixing, addition of acid or base. In Fig. 4, the sCOD content in the original samples compared to after the treatments is shown.

Each individual pre-treatment method increased the sCOD content, except for high-shear mixing of sample RCF10-03 which had no effect. Heat treatment had the highest impact and increased the sCOD by 15-40 %. The sCOD of the sample POELS-03 was very low in the beginning (0.03 g/L) and was only increased to around 0.1 g/L with each treatment. Considering a subsequent microbial acidification process, those results indicate that the used pre-treatments solubilize solid organic matter and thus make it more available for microbes. The planned conversion of organic matter to fatty acids could be increased if a pre-treatment is performed.

The content and composition of VFA in the samples after the separate pre-treatments is shown in Fig. 5. The VFA content in the original samples was between 1.9 g/L and 2.8 g/L in the RCF-samples and were not considerably increased by the pre-treatments. However, the composition changed in the sample RCF10-05, where lactic acid was formed during heat treatment and acid and base addition. No VFAs were detected in the sample POELS-03. After all treatments were tested separately, a combination of treatments was used. The pre-treatment cascade consisted of high-shear mixing, pH adjustment to pH 4 followed by heat treatment.

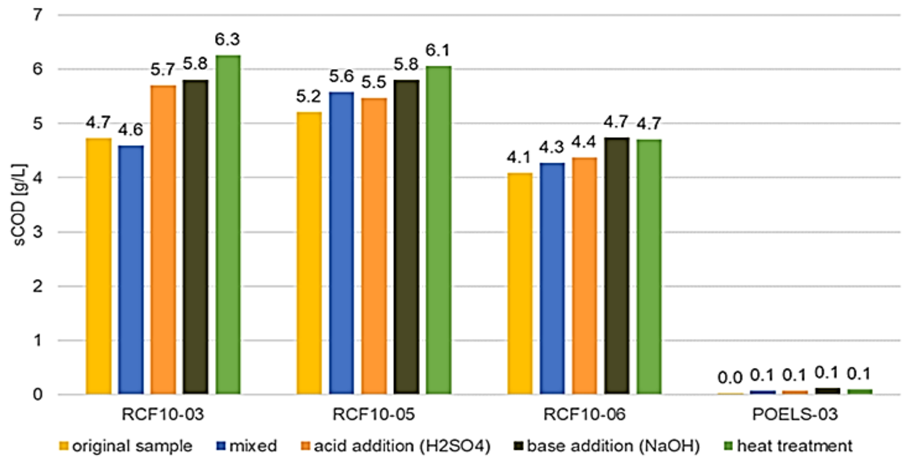

Fig. 4. sCOD content in original samples compared to after separately performed pre-treatments

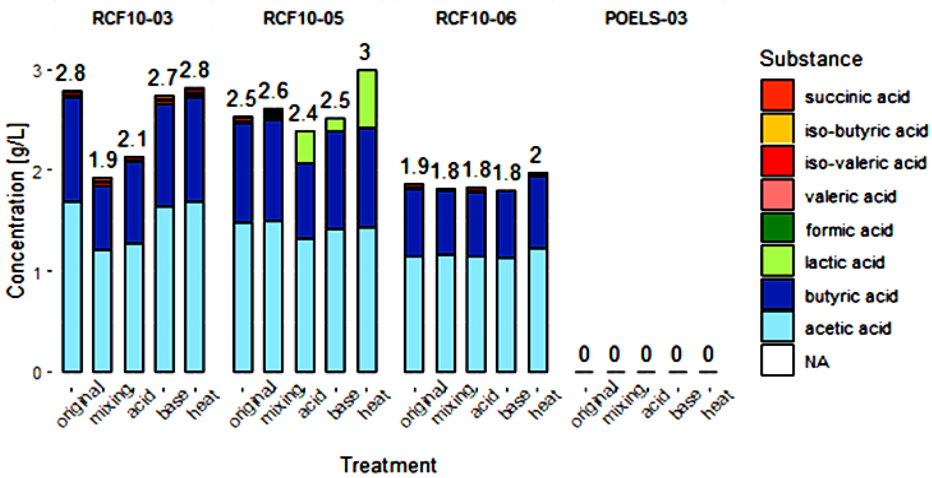

Fig. 5. Volatile fatty acid (VFA) content and composition of original samples compared to after separately performed pre-treatments

After each individual step, the sCOD and VFA were determined (results not shown). Combining the treatments did not increase sCOD or VFA content. In the case of the organic waste samples from Green Generation, physical treatment (blending and chopping) was performed for the homogenisation, for the most solid samples (bellygrass). The goal was to test the samples in the most natural and easy/feasible way possible.

For the chemical treatment, H_2SO_4 and NaOH was applied to the samples adjusting the pH to values 5.5 and 10 respectively. The VFA content was influenced by the modification of the pH, increasing the concentration of acetic and propionic acids mainly at a basic pH of 10 and has reached a yield of 0.5 g VFA/g VS.

3.5.3. Acidogenic fermentation tests

A series of acidogenic fermentation tests were performed to evaluate the feasibility of producing VFAs for the development of marketable bioplastics. The trials were carried out in glass bioreactors with a working volume of 1 and 2 L filled with different volumes between 700 mL and 1100 mL. The total volume depended on the amount of waste (substrate) added to the mixture according to the substrate-to-inoculum S/I ratio, with value of 2 g VS/g VS used as positive control (inoculum from Valladolid's Wastewater Treatment Plant in Spain), and in order to inhibit methanogens, it was chosen to allow O2 to be present in the reactor (Wainaina et al., 2019). A correction of the methane production from the inoculum was done using a blank test containing only inoculum.

For each waste stream and pH condition, replicates were added to ensure that the results obtained were similar under these conditions. With the characterization of the waste streams, the amount of waste and inoculum was calculated based on the VSs. The trials focused on the effects of acidic (pH 5.5 adjustment with H_2SO_4) and alkali pH 10 with NaOH addition) on VFA production efficiency and VFA composition. To maintain a homogeneous mixture, the reactors were placed on an orbital shaker (shaking table) which was located in a temperature-controlled room where the mesophilic reaction temperature was set to 38°C (Fig. 6).

The retention time was 10-13 days, ensuring acidogenic fermentation and avoiding methanogenesis. A minimum of 15 mL samples were daily withdrawn to analyze the evolution of VFA concentration and profile. Samples were centrifuged and stored at 4 ◦C prior to their analysis. Phosphoric acid (150 µL H_3PO_4/15 mL sample) was added to the samples to avoid volatilization of the fatty acids to ensure their preservation. Gas chromatography-flame ionization detection (GC-FID) was the analytical technique used to separate and analyse mixtures consisting of volatile components to quantify VFA in samples. By examining the pH values for the same samples, better results are generally obtained under basic conditions than under acidic conditions. The pH was monitored and adjusted during the tests. The samples of glycerine, sludge and bellygrass with pH 10 has the highest concentration of total VFAs with 1927.64 mg/ L, 1657 mg/ L and 1652.38 mg/ L respectively (Fig. 7). For glycerine the maximum was reached at day 7 and for the bellygrass and sludge was at day 12.

Fig. 6. Anaerobic digestion reactors on the orbital shaker

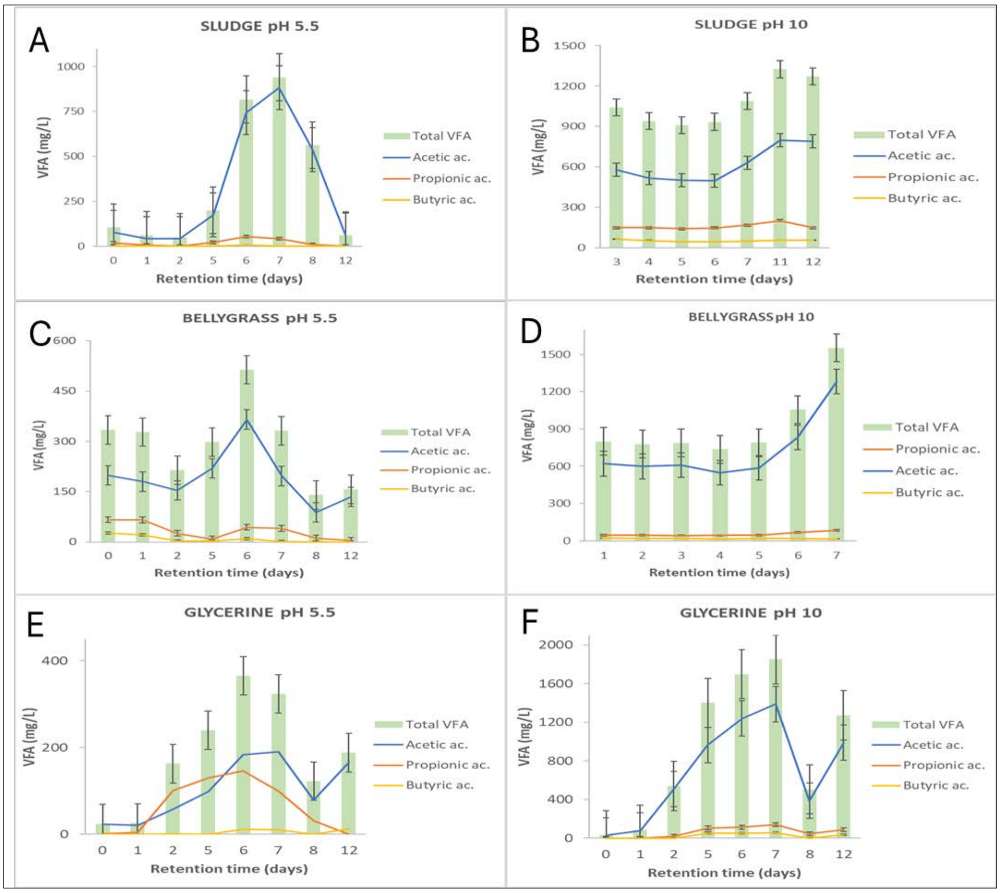

Fig. 7. VFA concentration (mg/L) of bellygrass (A, B), sludge (C, D) and glycerine samples (E, F) for 12 days pretreated at pH 5.5 and pH 10

The soluble COD (sCOD) COD was measured with quick tests using HACH DR 1900 Portable Spectrophotometer and Cuvette Test System with the Dry Thermostat Reactor LT200 (Hach) obtaining values of 2690, 1800 and 4080 (mg/ L) for glycerine, sludge and bellygrass respectively for that residence time. The VFA yield reached a value of 0.33 g VFAs /g VS glycerine, 0.47 g VFAs /g VS sludge and 0.27 g VFAs /g VS bellygrass. Under both pH conditions, VFAs represented a predominance of acetic, propionic and butyric acids. Figure 7 shows the concentration of these acids (mg/ L) over the residence time (days) of two replicates for each organic waste under the basic conditions. Similar concentration around 2 g/L of total VFAs has been founded in other acidogenic fermentation where the substrate was sludge coming from pulp and paper mills. In this case also acetic acid and propionic acid were predominant, but higher concentrations of valeric acid were founded (Li et al., 2022).

4. Conclusions

In general terms, impurity removal is already performed during the production process and the selected samples do not need further removal steps. VFA content depended mainly on the nature of the samples and the pH, as physical pre-treatments in most samples hardly affected the content of VFA in the samples. VFA content is not influenced significantly by the treatments. A combination of more than one method was not beneficial. Mixing the samples do not increase VFA content. As expected, samples in which pH was adjusted to 10 resulted in improved production of VFAs compared to pH 5.5 because the alkaline condition enhances the hydrolysis.

Green Generation’s samples (bellygrass, glycerine and sludge) generated more VFAs than Heinzelpaper samples. This is caused by the higher COD content in the Green Generation’s samples. Most of the VFAs produced are acetic acid though it would be interesting to have higher contents of propionic and valeric acids, which would allow to produce PHBV, a more interesting polymer than homopolymer PHB.

Experimental results in the preliminary phase of the first laboratory scale trials showed that adjusting the pH to 10 during fermentation allows higher production of PHBVs, reaching concentrations of up to 2 g/L in glycerine samples, with high yields also in bellygrass and sludge. These advances are crucial for the production of biopolymers such as PHBV and need to be improved in terms of co-digestion of the different organic wastes and further operational parameters and the addition of Fe(0) nanoparticles will be tested. As initial trials have shown promising results, further tests will be conducted in future stages by adjusting different operational parameters, such as pH, temperature, waste concentration and substrate ratio, in order to maximise efficiency and improve the production of VFAs. The results obtained confirmed the feasibility of obtaining VFA from these different organic and pulp and paper wastes but, in order to obtain PHB from VFA, it is necessary to reach a higher concentration, so it is necessary to further investigate parameters to optimise the acidogenic fermentation and to study possible downstream concentration techniques. Different alternatives also can be applied to increment the concentration of VFAs. For example, in similar experiments, ultrasonic pretreatments have been used to increase the concentration of VFAs from pulp and paper sludges. These type of pretreatments can increase the VFAs production in the acidogenic fermentation by at least double.

These additional tests will be key to optimise the process and ensure its industrial scalability.

In addition, the use of membrane bioreactors will lead to better biomass retention and more efficient product recovery, overcoming the limitations observed in traditional technologies. The combination of these factors positions ELLIPSE as an innovative project in the circular bioeconomy, contributing to sustainability and efficiency in industrial waste management.

At the same time, one of the main objectives in ELLIPSE project is to obtain a PHBV biopolymer with more suitable properties and processability than brittle PHB polymers. To achieve this a strategy focused on the increment of valeric and propionic acids, which are precursors of the 3HV monomers, has been implemented. Thus, valerate and propionic acids concentration in the culture medium strongly affects the 3HV fractions of the PHBV polymer. In experiments with slaughterhouse wastes, as for example animal blood, the production of valeric acid is higher, representing in some cases around 20% of the total VFAs. However, these substrates normally contain higher organic matter, measured both as volatile solids (VS) of 80-100 gVS/kg or as COD of 180-260 gCOD/kg, while the organic matter of the samples used during ELLIPSE experiments was considerably lower. For example, bellygrass samples contains only 15.33 gVS/kg. These findings make it necessary to look for pretreatments that lead to an increase in the organic matter available for the acidogenic fermentation processes, which will be translated into an improvement of fermentation yields and consequently a higher concentration of propionic and valeric acid. Similar results in the content of valeric acid (less than 5% of total VFAs) when glycerol was fermented as the main substrate, but higher concentration of propionic acids was founded, being ever more abundant than acetic acid (45% of propionic acid and 40% of acetic acid of the total VFAs produced). One possibility to improve these results could be the implementation of anaerobic co-digestion which provides an opportunity to overcome the drawbacks of mono-digestion by simultaneously digesting two or more feedstocks. Among the principal benefits of co-digestion, promoting a more diverse microbial community, better nutrient balance or increasing the bioavailability of nutrients could help to achieve a better VFA concentration during acidogenic fermentation.

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