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Polyhydroxyalkanoates (PHAs) from Household Food Waste: Research Over the Last Decade

Klempetsani Stavroula*[1], Malamis Simos[2], Haralambous Katherine-Joanne[1]

[1]*School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou St., 15780 Athens, Greece*

[2]*Department of Water Resources and Environmental Engineering, School of Civil Engineering, National Technical University of Athens, 5 Iroon Polytechniou St., 15780 Athens, Greece*

Abstract

Polyhydroxyalkanoates (PHAs) are the ideal candidates for the substitution of the conventional plastics, as they present similar properties with them, but are also environmentally friendly. Regardless of their benefits, these biopolymers still face challenges today, due to their high market price, which is related to the raw material selected for their production. The utilization of low cost and readily available substrates - like organic wastes - for PHA production is a promising solution, which can decrease the total biopolymer production cost. However, PHA products from waste-derived materials need to have a consistent quality. This currently remains a challenge. The last years, PHA production from organic wastes, like food waste, has acquired growing attention. This work reviews the literature of the last decade on the use of household food waste as feedstock for PHA production. Household food waste has been divided in three categories: composite food waste, spent oils and spent coffee grounds. Both pure and mixed microbial cultures have been employed. The review focuses on the feedstock's and the culture's pre-treatment methods, the biopolymer's production and the purification of the final product. It also refers to the PHA content obtained in each scientific work. Household food waste has proven to be a good substrate, especially when combined with pure microbial cultures, like *Cupriavidus necator* that resulted in PHA accumulation from around 37% to 90%. This scientific work also provides informations concerning PHA applications, industrial production and market prices.

Abbreviations

®, registered trademark symbol; ATCC, American Type Culture Collection; FFW, fermented food waste; FWC, composite food waste; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; HA, hydroxyalkanoates; HDPE, high density polyethylene; HPLC, high performance liquid chromatography; HRT, hydraulic retention time; LB, Luria-Bertani; LDPE, low-density polyethylene; MM, mineral salt medium; MMC, mixed microbial cultures; MSB, minimal salt basal medium; OLR, organic loading rate; PHAs, polyhydroxyalkanoates; PHB, poly(3-hydroxybutyrate); P(HB-HV), poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PHO, poly(3-hydroxy-octanoate); PLA, poly (lactic-acid); PP, polypropylene; SBRs, sequencing batch reactors; SCG, spent coffee grounds; SEC, size-exclusion chromatography; SRT, sludge retention time; TM, unregistered trademark symbol; TSA, tryptone soya agar; TSB, tryptone soya broth; UASB, upflow anaerobic sludge blanket; UCO, used cooking oil; UFW, unfermented food waste; VFAs, volatile fatty acids; WCO, waste cooking oil

Keywords: Household food waste, Biopolymers, Polyhydroxyalkanoates (PHAs), Microbial Cultures, Commercial PHAs, Market prices

Corresponding author: **Klempetsani Stavroula**

School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou St., 15780 Athens, Greece.

E-mail: klepetsani@windowslive.com

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Introduction

PHAs are unique polyesters synthesized naturally by many species of bacteria within their cellular structure under growth-limiting conditions imposed by the scarcity of a nutrient, electron donor or acceptor[1]. These biopolymers are suitable for the replacement of synthetic plastics in many sectors, as they present similar properties with them. Nowadays, they have penetrated mostly in packaging, agriculture and in the medical sector. Besides their good characteristics, PHAs are also environmentally friendly. Unlike conventional plastics that are fossil fuel-based, PHAs come from renewable carbon sources and they decompose naturally in the environment after their disposal. Suitable environments for PHA degradation are those with high microbial activity, like soil and sewage sludge.

PHAs industrial production today is mainly based on the use of sugar-based compounds and the employment of pure microbial cultures. Both the raw material and the microorganisms raise the biopolymers production cost, making them less competitive in the market due to their high price. The utilization of low-cost organic

wastes as feedstock for PHA production is a promising solution for the reduction of the biopolymers production cost, even though organic wastes - due to their variable composition - may not allow the production of a uniform product. Agricultural wastes, agro-industrial wastes, food wastes etc.[2] have been used a lot in the recent literature for PHA production achieving high PHA accumulation, especially when combined with pure microbial cultures. Mixed microbial cultures have also been employed in many cases. As opposed to pure microbial cultures, they do not need aseptic conditions for their use, though they result in lower PHA accumulation than the pure cultures.

Food waste is a rich organic material capable of providing needful compounds to the microorganisms for their maintenance, growth and the accumulation of PHAs. Its high moisture content and high biodegradability[3], turn it into a troublesome waste material, as it is responsible for the creation of odors, water-polluting leachate and greenhouse gas emissions. In the European Union approximately 88 million metric tons of food waste are generated annually[4], so food waste management is of great importance today. Its valorization for PHA production contributes in the reduction of the food waste amounts that end up in landfills. Though, it should be mentioned that in the literature, the estimation of the PHA content is based on the biomass weight unit, i.e. grams PHAs per grams of biomass and not on the food waste weight unit, which would be a helpful information for the correlation of the food waste amount used for the production of PHAs and the amount of the produced biopolymer.

This review focuses on recent literature advances of PHA production from household food waste with the employment of both pure and mixed microbial cultures. Household food waste comprises of inhomogeneous food wastes (composite food waste) and homogeneous waste streams, like cooked oils and spent coffee grounds. Food waste in general includes also the side streams of the food processing industries, like whey and molasses[5], but this review focuses only on the first two mentioned streams arising from a household. The overall procedure comprises of the substrate's and the culture's pre-treatment, the biopolymer's production and the downstream processes, such as the extraction and the purification of the final product.

Applications, industrial production and market prices

The composition of the PHA, which is directly related to the biopolymer's structural and mechanical properties, depends on the carbon substrate, the metabolic pathway utilized and the specificity of the PHA synthase[6], the key enzyme that polymerizes the monomeric hydroxyalkanoates[7]. PHAs are categorized in small, medium and long-chain-length PHAs according to the number of the carbon atoms in the monomeric block. They are also divided in homopolymers, that consist of one type of PHA throughout their structure, copolymers that are made up of two different types of monomers and heteropolymers that contain 3-hydroxy fatty acids of many different chain lengths[8]. PHB, which is a typical short-chain-length PHA, is brittle and stiff, while the medium-chain-length PHO is more flexible. So, each one of them can be applicable in different areas in accordance with its characteristics. Different monomers can be combined to form a copolymer with the desirable properties[9]. For example, the combination of HB and HV monomers forms the copolymer P(HB-HV), which is more flexible than the PHB[10]. In 2014, Bugnicourt et al. referring to the PHA general characteristics described them as biocompatible, nontoxic, soluble in chloroform, resistant to ultraviolet radiation and insoluble to water[10]. Figure 1 demonstrates the economic fields in which PHAs are commercialized today.

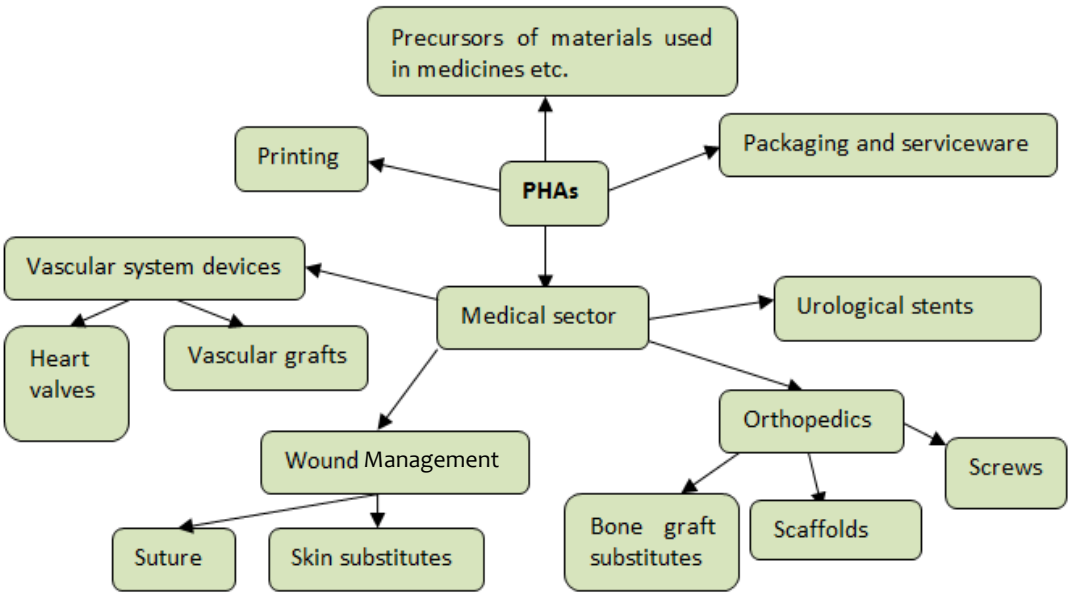

Fig. 1: PHAs applications [11,12].

PHAs are suitable for a large number of applications and it is estimated that the PHA market will be worth 290 million USD by the end of 2025[13]. The industrial production of PHAs is generally comprised of several steps including fermentation, the separation of the biomass from the broth, the biomass drying, the extraction of the PHA, its drying and its packaging[14]. The most common industrially produced PHAs are limited to the combination of PHB and another PHA as the co-monomer[15]. Table 1 shows a list of industrially produced PHAs that are available in the market today and provides a short description of their applications.

PHA trade name	Producer, Region	Year active	Applications
AirCarbon ®	Newlight Technologies, USA	Since 2013	Use in many market segments including furniture, electronics and apparel.
AONILEX ® (X131A)	Kaneka Co., Japan	Since 2011	Improves the strength and tear resistance of packaging films, especially agricultural mulch films and composting bags.
Biocycle ®	PHB Industrial S.A., Brazil	Since 2000	Use in fibers, coating paper and cosmetic packaging.
Biomer	Biomer, Germany	Since 1994/1995	Use in cardiovascular applications, in blood-contact applications or as tissue engineering scaffolds[16].
Biopol	Yield10 Bioscience (formerly Metabolix, Inc.), USA	Since 1990 (First release by the Imperial Chemical Industries- ICI)	Use in fabric and film production, food industry (cups, plates) and in disposable items (razors, rubbish bags).
Ecomann ®	Shenzhen Ecomann Biotechnology Co., China	Since 2008	Use in packaging, paper coatings, cosmetics, personal care and footwear.
Enmat ™	Tianan Biologic, China	Since 2007	Use in plastic fibers, films, sheets, bars, rods, powder, mulching films for agricultural purposes and in packaging materials of rubber.
Hydal	Nafigate Corporation, Czech Republic	Since 2012	Utilization of waste cooking oil to produce PHB. The company has created a coconut peeling milk with PHB instead of microbeads and a sunscreen with PHB with natural UV protection[17].
Minerv-PHA ™	Bio-On, Italy	Since 2007	Use in cosmetics, automotive, electronics, food pack, fibers, toys and in the pharmaceutical sector.
Mirel ™	Yield10 Bioscience (formerly Metabolix, Inc.), USA	Since 2006	Commodity applications, shampoo and cosmetic bottles, cups and food containers.
Nodax ™	Danimer Scientific, USA	Since 2007	Use in packaging, laminates and coatings, nonwoven fibers.
TephaFlex®	Tepha Inc., USA	Since 2007	Use in sutures, films and textile products.
Versamer ™	PolyFerm Canada, Canada	Since 2013	Use as adhesives, plastic additives, inks, toners, paints, coatings and in the medical sector.

Table 1: Industrially produced PHAs, available in the market today.

PHAs drawback is their high market price. Table 2 demonstrates a comparison of the market prices of PHAs, PLA and petroleum-based polymers, as it was reported by the Organization for Economic Co-operation and Development (OECD) in 2014. OECD also provides the information that PE (polyethylene) cost is €1.58/kg and PET (polyethylene terephthalate) cost is € 1.73/kg. As it is seen, PHAs are approximately four times more expensive than the petroleum-based polymers. So, the development of cost-effective technologies for the generation of PHAs in order to make them more competitive in the market is of high priority.

Product	Company	Location	Capacity (metric tons)	Price (EUR per kg)
PHAs	Metabolix	United States	300/50,000	4.3 - 4.6
P(HB-HV)	Tianan	China	2,000	4.1 - 4.3
PLA	NatureWorks	United States	140,000	1.5 - 2.0
PLA	Hisun	China	5,000	2.1
Mater-Bi (biopolymer)	Novamont	EU	75,000	3.4 - 5.1
HDPE/LDPE/PP	Braskem	Brazil	200,000	1.3-1.7

Table 2: Prices of biopolymers [18].

PHA production from household food waste the last decade

Household food waste categories

The utilization of food waste has been reported in the literature for the production of high added value products, such as lactic acid and PHAs. Prior to its use for PHA production, food waste must undergo a suitable pre-treatment process in order to be available to the microorganisms. The most frequent pre-treatment process is fermentation. The last decade, scientific papers referring to PHA production from household food waste have focused on composite food waste, spent coffee grounds and spent oils like waste frying oil, used cooking oil and spent palm oil. As it is derived from tables 3 and 4, composite food waste was the most employed waste for PHA production (47%), while spent oils and spent coffee grounds were used at a percentage of 41% and 11%, respectively. The methods employed for the food waste pre-treatment depend on its characteristics (Fig. 2).

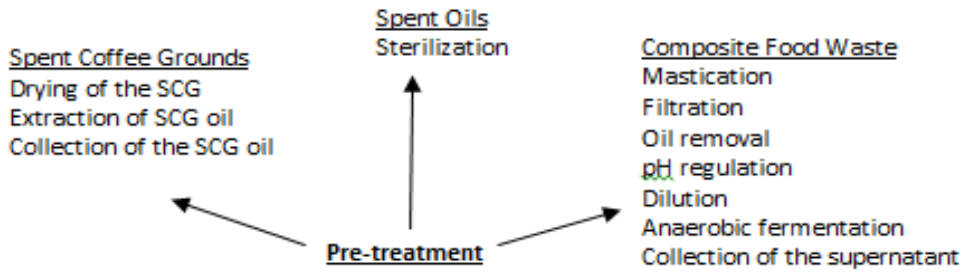

Fig. 2: Pre-treatment methods employed for each category of household food waste

Spent coffee grounds: Spent coffee grounds are an important waste product of the coffee industry[19]. It is estimated that 6 million metric tons of spent coffee grounds are generated worldwide every year [20]. They contain large amounts of organic compounds like fatty acids, amino acids, polyphenols, minerals and polysaccharides[21]. In recent studies, it is seen that the use of spent coffee grounds for PHA production has resulted in very high PHA content, approximately from 70% to 90%. Their useful part for PHA production is their oil. So, it first needs to be extracted in order to be utilized in the procedure. In one study, the spent coffee grounds were dried and then their oil was extracted through supercritical extraction in a semi-continuous high pressure extraction pilot unit[22] and in another, it was extracted with the use of n-hexane in an extractor apparatus[23].

Spent oils: Spent oils are considered to be very good candidates for PHA production. The utilization of fatty acids derived from plants is economically more feasible than the utilization of oily purified acids [25]. The advantage of utilizing plant oils is their high carbon content as well as their high conversion rate to PHA[25–27]. Due to the fact that oils have a high organic carbon content, low flow rate streams can be applied reducing the dilution of the fermentation broth[28]. Usually, spent oils require no pre-treatment[28–32] or they may just require to be sterilized before the PHA production step[33] or to be sonicated for the obtainment of a homogenized mixture [35].

Composite food waste: Composite food waste needs to be fermented for the production of a liquid full of easily consumable volatile fatty acids. In the literature, the composite food waste was directly fermented or it was pre-treated prior to fermentation. Eshtaya et al. (2013) diluted the composite food waste in water before its fermentation, while in other scientific works, it went through mastication, filtration, oil removal and pH adjustment[34] or dilution in sewage sludge to the required OLR[35]. The fermentation took place at ambient temperature[35,36], mesophilic temperature[37,38] or the reactor was subjected in different temperature and pH regimes for the investigation of their effect on the fermentation process[39]. In some cases, the acidogenic fermentation was conducted with the use of anaerobic consortia[35,38]. The reactor operated in a fed-

batch mode[35,37] or in a batch mode[38]. In almost all cases anaerobic conditions prevailed in the reactors[34,35,38], but microaerobic conditions have also been reported[37]. The pH value was usually left uncontrolled throughout the procedure, but it is also reported that it was adjusted to 6 prior to fermentation[34]. Some fermentations were accompanied with agitation[39] and others took place with no agitation[36]. After fermentation, the produced liquid was either filtered[36] or the organic acids were recovered by the freezing thawing method[39]. Finally, the hydrolysate obtained from the acidogenic fermentation was used as influent substrate for the culture's enrichment step and the production of PHA. Figure 2 illustrates schematically the pre-treatment methods that were employed for each category of household food waste before its use in PHA production.

The production of PHAs is a biological procedure consisting of three major phases. The first phase is the substrate's pre-treatment, in this case the household food waste's pre-treatment, for the obtainment of a liquid rich in volatile fatty acids, the precursors of PHAs. In the second phase, the culture is cultivated under specific operating conditions for the production of a large microbial population that is capable of producing PHAs. If the culture is pure, which means that it consists of only one species of bacteria that are already known in the literature as PHA accumulators, homogeneity is achieved. This homogeneous population only needs to multiply before PHA production for the obtainment of a sufficient amount of active biomass capable of producing PHAs. The growth of the bacterial population is achieved through the acclimatization phase (Fig. 3). If the culture is mixed, consisting of many species of microbes, a selection process needs to be applied in the reactor for the enrichment of the culture in PHA-storing microorganisms before its utilization in PHA production. In this case, the second phase is referred to as the culture's selection and enrichment step (Fig. 4). In the third phase, the cells harvested from the second phase are fed with the rich in organic acids liquid obtained from the first phase, usually without the addition of nutrients, for the optimization of the culture's capacity in accumulating PHAs. After the accumulation, PHAs are extracted from the microorganisms and purified through chemical methods.

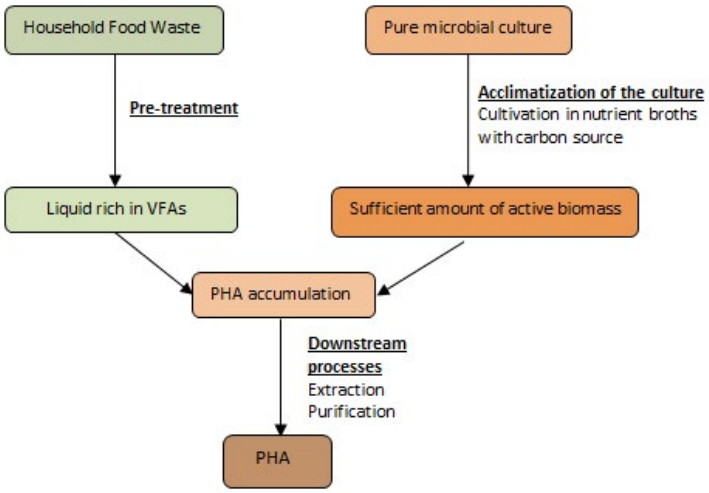

Fig. 3: Flow diagram of PHA production from household food waste and pure microbial cultures

A wide variety of bacteria is capable of accumulating PHAs. *Alcaligenes latus, Cupriavidus necator and Pseudomonas putida* are the most commonly used species in the industrial production of PHAs today[40]. In the recent bibliography referring to PHA production from household food waste, pure microbial cultures were much more employed than the mixed ones for the production of these biopolymers. As it results from tables 3 and 4, *C. necator* of the genus *Cupriavidus* was the most widely employed culture used for PHA production (53%), followed by the activated sludge and aerobic consortia equally used (12% each). The rest scientific works employed *E. Coli, Pseudomonas* and isolates obtained from the environment (23% in total). *E. Coli* is uncapable of producing PHAs when it is in its natural form, but it is the most studied genetically modified strain in the research of PHA biosynthesis[41]. Eshtaya et al. (2013) that employed recombinant *E. Coli* for PHA production with the use of organic acids from fermented restaurant waste reported PHB accumulation equal to 44% (w/w)[39]. Isolates are not considered to be competitive with the commercial strains for the production of PHAs in the literature[42], though Vijay et al. (2019) observed a slightly higher PHA accumulation from a bacterial isolate with the utilization of onion peels as substrate in comparison with the PHA content obtained from a reference strain with the use of the same substrate[43].

Before the acclimatization phase, a pure culture is usually maintained in a synthetic medium, which is mainly composed of peptone, meat or yeast extract, sodium chloride and the solidity provider - agar [23,31,44]. These ingredients offer key compounds to the microorganisms to support their growth like carbohydrates, nutrients and salts[45]. The exact composition of the medium is related to the strain requirements[41]. Besides the culture preservation, a nutrient broth is also used for the culture development in the acclimatization phase[23]. The medium usually contains nutrients and an easily biodegradable carbon source[9,30,36,44]. In most of the cases, the carbon source is the VFAs-rich stream obtained from the first stage[22,30,31]. Many types of media have been used for the acclimatization phase in the recent

literature, such as a mineral medium supplemented with the liquid obtained from the food waste fermentation[22,31], a nutrient rich medium[9], a Luria-Bertani medium[31,39], a tryptone soya broth[33] etc. The incubation of the cultures mostly took place at 30°C[9,31,33,44] and it lasted 16 h[9], 24 h[22,33] or 48 h[30,31].

In the PHA accumulation stage, the hydrolysate from the composite food waste fermentation, the extracted oil from the spent coffee grounds or a waste oil obtained from the first phase was added in the reactor containing the cells from the second phase. In some cases, an extra carbon source was supplemented along with the principle raw material for the achievement of feedstock availability[9]. Both batch and fed-batch cultivations were performed for the accumulation of PHAs. In one study, the fed-batch fermentation for PHA production resulted in much higher PHB content than the batch one[44], while in another one three feeding regimes were investigated – pulse, stepwise and continuous feeding – and the highest PHA accumulation was achieved from the continuous feeding regime[36]. Temperature was mostly maintained at 30°C[30,43,46,48]. In the scientific paper of Eshtaya et al. (2013), the temperature increased from 34 to 38°C to induce PHB production, while in Hafuka et al. (2011) it was maintained at 20°C. The pH value was maintained at 6.8[31], 7 [9,23,39,43,44] and 7.5[36]. Aerobic conditions prevailed throughout the process[30,31,36].

The downstream processes for PHA extraction and purification are not related with the type of the carbon source and the microbial culture used for PHA production, as they take place after the biological production of PHA. The cells from the third phase are harvested-mainly by centrifugation-and then subjected in a pre-treatment method for the softening of the cell structure that is around the PHA granules. Pre-treatment can be chemical, physical or biological[10]. The most common pre-treatment methods are the thermal drying and the lyophilization. Chemical pre-treatment requires the use of sodium chloride (NaCl) or sodium hypochlorite (NaOCl), while physical methods require high temperature and ultrasonication methods[10]. The extraction and the precipitation of PHAs in a lab scale are mostlly achieved with a use of a solvent – mainly chloroform – and the addition of an alcohol, mostly acidified methanol or propanol, respectively. After their extraction from the biomass, the biopolymers are analyzed with the use of GC, HPLC or SEC.

In the recent literature referring to the generation of PHAs from bacterial strains, genetic modified strains or isolates from the environment with the use of household food waste, high percentages of PHA content were reported. 66% of the scientific works demonstrated in table 3 reported more than 50% PHA accumulation, while the highest PHA contents were observed for the employment of *C. necator*. Table 3 presents the overall procedure of PHA production with the employment of pure microbial cultures from scientific works that dealt with PHA production from household food waste the last decade.

Reference No	Authors, Year	Substrate, Substrate's pre-treatment	Substrate's pre-treatment parameters (T, pH)	Culture, Culture's pre-treatment	PHA production	PHA production parameters (T, pH)	Extraction/ Analysis of the samples	PHA content (% g-PHAs/ g-biomass)
[22]	**Cruz et al., 2014**	**Spent coffee grounds (SCG) oil** • Drying of the SCG for 12 h (105°C). • Supercritical extraction of the SCG oil. • Collection, weighing and storage of the oil.	T=105°C	***Cupriavidus necator DSM 428*** • Preparation of the inoculum: Inoculation of LB grown cells in mineral medium with SCG oil and incubation for 48 h. • T=30°C,agitation.	**PHA production** • Cultivation in a bioreactor. • SCG oil as carbon source. • Use of a 10% (v/v) inoculum. • Batch operation, followed by a nitrogen limited fed-batch phase.	T=30°C	**Extraction-Analysis** • Mixing of the broth with n-hexane, centrifugation, washing, lyophilization, extraction, filtration and precipitation of the polymer. •Analysis with GC.	**PHA content 78.4%** (end of the cultivation run).
[23]	**Obruca et al., 2014**	**Spent coffee grounds (SCG)** Drying of SCG for 24 h (80°C) and extraction of the SCG oil with the use of n-hexane in an extractor apparatus. The extraction was stopped when the solvent reflux was clear.	T=80°C	***Cupriavidus necator H16*** • Use of a nutrient broth for culture preservation and inoculum development.	**Cultivations in flasks** Coffee oil compared to other waste oils for PHA production. Cultivation of *C. necator* in a mineral salt medium with an individual waste oil. **Batch cultivation** Coffee oil with mineral salt inoculated with *C. necator.* **Fed batch cultivation** Intermittent addition of coffee oil in the fermenter.	**Cultivations in flasks** T=30°C pH=7 **Cultivations in fermenter** T=30°C pH=7	**Extraction- Analysis** • Centrifugation of the samples and washing of the cells with 5% (v/v) Triton X and distilled water. • Analysis of PHB content with GC.	Cultivations in flasks **PHB maximum: 70.3±0.8%** (for spent coffee grounds). Batch cultivation **90.1±3.5% wt.** (at the end). Fed batch cultivation **89.1±3.1%wt.** (at the end).
[30]	**Cruz et al., 2015**	**Used cooking oil (UCO)**		***C. necator DSM 428*** •Preparation of the inoculum: Inoculation of LB grown cells in mineral medium with SCG oil and incubation for 48 h (T=30°C, agitation).	**PHA production** •Cultivation in a bioreactor. • Use of a 10% (v/v) inoculum. Batch operation with nitrogen excess (growth phase) and nitrogen limitation (PHA production phase). • Aeration, agitation.	T=30°C pH=6.8	**Extraction-Analysis** Collection of the broth, washing with hexane, centrifugation, discharging of the supernatant. Washing of the pellet twice, lyophilization, extraction with chloroform, filtration, precipitation and drying to a constant weight. Analysis by SEC.	**Maximum PHA content 63 ± 10.7%**

[31]	**Martino et al., 2014**	**Used cooking Oil (UCO)** Characterization (lipid composition, elemental analysis, water and ash content, density). Use of UCO as the sole carbon source for PHA production.		***C. necator DSM 428*** Storage in LB medium with glycerol (-80°C). Reactivation in solid LB through incubation for 2d (30°C). Inoculation of a single colony into LB - incubation for 24h (30°C, agitation). Transfer of the culture into mineral medium with UCO - incubation for 48h (30°C, agitation).	**PHA production** • Batch cultivation of the culture with UCO. • 10% (v/v) inoculum. • Aeration, agitation and addition of antifoam.	T=30°C pH=6.8	**Extraction-Analysis** Washing, lyophilization, extraction, filtration of the solution and precipitation of the polymer. PHA recovery in Na_2HPO_4 buffer solution, centrifugation, determination by SEC.	**PHB content** **37% (w/w)** (at the end of the experiment).
[32]	**Mozejko et al., 2011**	**Waste rapeseed oil**		***Pseudomonas sp. G101 and G106*** • Isolation of the strains from mixed microbial cultures. • Growing of the strains in Luria-Bertani broth for 24h (T=30°C, agitation).	**PHA production** Cultivation of the cells in a nitrogen-limited mineral salt medium for 48h (in a fermenter). Addition of waste rapeseed oil twice (t=0, t=24h). Aeration.	T=30°C pH=7	**Extraction-Analysis** Shaking of the freeze-dried cells in chloroform. Filtration. Dissolving of PHAs in chloroform. Precipitation with methanol and evaporation. Determination by GC.	**Maximum PHA content** • *Pseudomonas sp.G101:* 21% • *Pseudomonas sp.G106:* 19.3%
[33]	**Verlinden et al.,2011**	**Pure vegetable oil, heated vegetable oil and waste frying oil** Separate sterilization before fermentation. Batch fermentations with each oil at a time. After addition of the oil but before inoculation, the sterile medium was sonicated for 10 min to achieve a homogenized mixture.		***Cupriavidus necator H16*** • Sub-culture of *C. necator* on Tryptone Soya Agar (TSA). • Inoculation of a single colony to Tryptone Soya Broth (TSB). • Incubation for 24 h (30°C).	**PHA production** • Batch fermentations in flasks filled with medium including TSB inoculum. • Addition of pure vegetable oil, heated vegetable oil or waste frying oil to the medium. • Incubation.	T=30°C	**Extraction-Analysis** Extraction of the PHAs from lyophilized biomass. Concentration of the polymer's hot solution. Precipitation of the solution in n-hexane. Measurement with SEC.	**Maximum PHB concentration** Pure vegetable oil:**0.62 g/L** Heated vegetable oil:**0.9 g/L** Waste frying oil:**1.2 g/L**
[36]	**Hafuka et al., 2011**	**Composite food waste** • Mixing of fresh food waste slurry with the inoculum in a 2 L reactor. • Fermentation. • Filtration.	Ambient T	***Cupriavidus necator*** • Cultivation of *C. necator* in ATCC medium 3 [47]. • Harvesting of the cells by centrifugation. • Re-suspension of the cell pellet in a quantity of the same medium and dilution in tap water.	**PHA production** Inoculation of the seed culture in 3 air-bubbling reactors. Addition of the filtered fermented liquid in each reactor. 3 feeding regimes: pulse, stepwise (once a day with 7 pulses) and continuous feeding (with the use of peristaltic pump). Aeration, agitation.	T=20°C pH=7.5	**Analysis** Analysis with GC.	**Maximum PHB content** **87%** (continuous feeding regime).
[39]	**Eshtaya et al., 2013**	**Restaurant waste** Dilution in water. Anaerobic fermentation (batch-natural microflora). Agitation. Recovering of VFAs by the freezing-thawing method. Centrifugation. Evaporation 3 folds. Maximum VFAs concentration obtained for: T=30°C, pH=7.	**6 regimes:** • T=30°C pH=7 • T=30°C Uncontrolled pH • T=37°C pH=7 • T=37°C Uncontrolled pH • Room T (21-26°C) pH=7 • Room T Uncontrolled pH	***E. Coli pnDTM2*** • Use of recombinant *E. coli* and of modified LB medium.	• **Batch culture in flask** Mixed pure acids. Fermentation acids. Use of recombinant *E. Coli*. • **Batch culture in the bioreactor:** Use of the fermentation acids and recombinant *E. Coli*. • **Fed batch culture in the bioreactor:** Use of the concentrated fermentation acids and recombinant *E. Coli*.	**Batch culture in flask** pH=7 **Batch culture in the bioreactor** T=34-38°C pH=7 **Fed batch culture** T=34-38°C pH=7	**Extraction-Analysis** • Determination of organic acids and of the PHB concentration by HPLC. • Analysis of the cell dry weight based on a previously determined optical density to CDW relationship.	**Batch culture in flasks** • **45%** for the fermentation acids. • **42%** for pure acids. **Batch culture in the bioreactor: 36.4%** **Fed-batch culture in the bioreactor: 44%**
[43]	**Vijay et al., 2019**	**Kitchen waste** • Use of orange peel and of onion peel, respectively. • Analysis of the total organic carbon content of each substrate.		**Isolates** • Isolation of bacterial strains from polluted environments. • Selection of isolates having the ability to produce PHA. • Cultivation of the isolates in nutrient broth with either onion or orange peel and yeast extract.	**PHA production** • Transfer of actively growing bacterial strains to flasks. Incubation. • The tested parameters were the carbon source, the bacterial strains, the time of incubation and the C:N ratio.	T=37°C pH=7	**Extraction-Analysis** PHA extraction with the chloroform extraction method. After extraction, dissolving of the polymer granules in boiling chloroform, air drying to yield dry powder of PHA and weighing to obtain the amount of the extracted PHA.	**Maximum PHA content** For onion peels, *Bacillus subtilis JCM 1465*, 3:1 C:N ratio, 48 h of incubation: 89%. For orange peels, *Bacillus siamensis PD-A10*, C:N ratio 1:1, 24 h of incubation: 82%.

[44]	**Farah et al.,2011**	**Kitchen waste** Collection. Acidogenic fermentation. Centrifugation and pellet's disposal. Condensation of the organic acids in the supernatant (70°C). Storage of the supernatant (-20°C) until use.	T=37°C pH=7	***Cupriavidus necator CCGUG 52238*** • Growth of *C. necator* on nutrient agar (30°C). • Inoculation of the cells into a growth medium. • Cultivation (30°C). • Aseptic centrifugation and re-suspension of the pellet in distilled water.	**PHA production** **Shake-flask:** The organic acids and mineral salts were inoculated with *C. necator*. Incubation. **Batch fermentation:** Use of organic acids from kitchen waste (agitation, aeration). **Fed-batch fermentation:** Intermittent addition of the concentrated acids from the kitchen waste.	**Shake flask** T=30°C **Batch fermentation** T=30°C pH =7 **Fed batch fermentation** T=30°C pH =7	**Analysis** Analysis of the PHB content in the cell mass with HPLC.	**Shake flask** Kitchen waste organic acids - PHB content: 5g/L-35% 10g/L-42% 15g/L-10% 20g/L-11% **Batch fermentation** Highest PHB content: 52.79% **Fed-batch fermentation** Highest PHB content: **84.54%**
[46]	**Song et al., 2008**	**Waste vegetable oil**		**Isolates** • Isolation of corn-oil-degrading bacteria from a rice field through cultivation in a minimal salt basal medium (MSB) with 1% corn oil. • Growing of the bacteria isolates in LB or MSB supplemented with carbon sources (agitation, T=30°C).	**PHA production** • Culturing of the bacteria in a phosphate and ammonium limited MSB with various carbon sources for 72 h.	T=30°C	**Extraction-Analysis** Drying of the cultures under vacuum using a freeze dryer and dissolving of PHAs with hot chloroform. Filtration of the dried cell mass. Precipitation of the concentrated solution. Purification of PHAs by re-suspension in methanol and drying under vacuum.	**PHA content** **37.34%** with the use of vegetable oil and *Pseudomonas sp. strain DR2.* **23.52%** from waste vegetable oil and *Pseudomonas sp. strain DR2.*
[48]	**Kamilah et al., 2013**	**Waste cooking oil (WCO)** • Sterilization (121°C, 15min)	T=121°C	***C. necator H16*** ***C. necator PHB⁻⁴*** • Growing of *H16* on mineral salt medium (MM) and of *PHB⁻⁴* on MM with canamycin. • Transfer of the two loops from MM plates to nutrient-rich medium. • Incubation (agitation).	**PHA production** • Inoculation of 3% (v/v) of each inoculum in 50 ml MM. • Addition of WCO and nitrogen source. • 72h fermentation (agitation).	T=30°C pH=7	**Extraction-Analysis** • Centrifugation of the cells. • Washing with hexane and distilled water. • Freezing of the cell pellets. • Lyophilization. • Determination by GC.	**Maximum PHA content** • *C. necator H16*: **71%** • *C. necator PHB⁻⁴*: **85%**
[49]	**Urmila Rao et al., 2010**	**Spent palm oil** PHA production medium consisting of metals and trace elements. Addition of spent palm oil to the medium and autoclaving. Addition of sterilized 1,4-butanediol.		***Cupriavidus necator*** • Growth of the culture in a nutrient rich medium (pH=7) and incubation in a rotary shaker for 16 h (30°C). • Harvest of the cells by centrifugation and washing with distilled water.	**PHA production** • PHA accumulation in flasks containing the washed cells and the production medium. • Incubation in a rotary shaker for 144 h (30°C).	T=30°C pH=7	**Extraction** Centrifugation, washing with acetone, drying, washing twice with hexane, extraction, condensation of the solvent, precipitation, recovering by filtration and overnight drying.	**Maximum PHA content** **81 wt%** (at 144 h of cultivation).

Table 3: PHA production from household food waste and pure microbial cultures.

The employment of mixed microbial cultures, such as the bacterial populations from the activated sludge process, has gained growing attention the last years mostly for the scaling up of the process, as it results in lower production cost and provides the advantage of the procedure's integration in the wastewater treatment process. For the selection of a culture with high PHA storage capacity, the mixed microbial population must undergo through a particular pre-treatment method. In all scientific works that dealt with PHA production from household food waste by applying mixed microbial cultures, the cultures employed were subjected in the feast and famine regime for the selection of PHA-storing bacteria [34,37,38]. In this regime, when the reactor operates under periods of substrate excess (feast phase) and relatively long periods of substrate limitation (famine phase), the microorganisms undergo through an internal growth limitation. During the latter, they use external nitrogen and phosphate ions and organic carbon stored internally as PHA for growth and maintenance. Those organisms that do not store PHAs are seriously affected by the long famine period and are eliminated from the reactor, while those that have the capacity to store bigger amounts of PHAs become the prevailing species in the reactor.

The culture's selection and enrichment step was often carried out in sequencing batch reactors (SBRs), compact systems where the full feast and famine cycle were performed in one single reactor and the length of each phase could be varied[49]. During the feast period, the reactor was filled with sludge, carbon substrate and nutrients. The carbon substrate in all cases was the hydrolysate obtained from the food waste fermentation, while the nutrients comprised of ammonium and phosphate ions. If the seed is obtained from the aeration tank of an activated sludge process it is usually aerated for some time, so that the original existing substrate can be exhausted[38]. The reactor operation is divided in cycles. Each cycle lasts for 12 h[38] or 24 h[34] and usually consists of four discrete phases: the influent filling, the aerated phase, the settling of the biomass and the withdrawal of the supernatant. Besides of the cycle length, the sludge retention time, the hydraulic retention time, the organic loading rate and the C:N ratio are also important parameters for the reactor operation.

PHA accumulation requires the hydrolysate from phase I as carbon substrate, which is full of volatile fatty acids and the enriched culture from phase II as microbial population. Batch mode[37] and fed-batch mode[35] were employed. In most cases of MMC PHA production,

aerobic conditions were applied[37,38]. Venkateswar et al. (2012) tested both the aerobic and the anoxic environment, though. The temperature was usually ambient[38] or it was maintained around 30°C[37] and the pH value was adjusted to 7[35,37], 8.8[34] or it fluctuated from 5.5 to 7.5 [38]. Finally, it is reported that, in order to avoid nitrification into the reactor, thiourea was added along with the substrate and the enriched culture[37]. Analytical description of all stages of PHA production from household food waste and mixed microbial cultures is demonstrated in Table 4. The accumulation of PHAs by the mixed microbial populations was lower than that obtained from the pure ones.

Reference No	Reference, Year	Substrate, Substrate's pre-treatment	Substrate's pre-treatment parameters (T, pH)	Culture, Culture's pre-treatment	PHA production	PHA production parameters (T, pH)	Extraction/ Analysis of the samples	PHA content (% g-PHAs/g-biomass)
[34]	**Amulya et al., 2015**	**Food waste (FWC)** •Collection, mastication, filtration and separation of the oil fraction. • pH adjustment to 6. • Acidogenic fermentation in anaerobic bioreactor.	Before fermentation pH=6 After fermentation pH=8±0.05	**Mixed microbial culture** Aerobic consortia from an SBR treating wastewater. SBR: addition of hydrolysate supplemented with ammonium and phosphate ions. 24h and 12h cycle length: fill, feast and famine, settling phase, decant phase.	**PHA production** • Operation of another SBR with the enriched biomass collected from the culture's selection step and the hydrolysate from the acidogenic fermentation. • No addition of ammonium and phosphate ions.	Ambient T pH=8.8	**Extraction-Analysis** Centrifugation, washing with acetone and ethanol, suspension in NaOCl, incubation, centrifugation, washing of the pellet with acetone and ethanol, dissolving in hot chloroform, filtration. Analysis of PHA colorimetrically.	**Maximum PHA content 23.7%** (for the cycle length of 12 h).
[35]	**Venkateswar et al.,2012**	**Food waste (FWC)** **Unfermented:** Mastication, filtration, oil removal, dilution in domestic sewage to the required OLR. **Fermented:** Use of anaerobic consortia (from a lab scale UASB) for fermentation. Fed-batch mode.	**FWC** pH=6.52±1.2 **Unfermented:** (after the dilution in domestic sewage) pH=6.8±0.2 **Fermented:** pH=4.2±0.2	**Mixed microbial culture (aerobic mixed culture)** Washing of the aerobic consortia in saline buffer. Enrichment in a designed synthetic wastewater under aerobic microenvironment (28°C) for 24 h.	**PHA production** SBR1: unfermented food waste/aerobic microenvironment (UFW/A). SBR2: UFW/anoxic (UFW/Ax). SBR3: FFW/ A. SBR4: FFW/Ax. Inoculation of the 4 SBRs with the aerobic consortia (fed-batch mode).	Ambient T (29±2°C) pH=7	**Extraction-Analysis** Same procedure as Amulya et al., 2015[34].	Aerobic microen/ment **Maximum PHA production** FFW: **35.2%** UFW: **32.6%** Anoxic microen/ment **Maximum PHA production** FFW: **39.6%** UFW: **35.6%**
[37]	**Wu et al., 2017**	**Food waste (FWC)** • Collection. • Acidification in microaerobic conditions. • Dilution in water. • Centrifugation. • Storage of the supernatant (4°C).	T=35±1°C	**Mixed microbial culture** Substrate: supernatant and $C_3H_5NaO_2$. Inoculum: aerobic activated sludge. 12 h SBR cycle: feeding, feast/ famine, biomass removal, settling and effluent removal.	**PHA production** Batch system: the enriched sludge, thiourea trace element solution. Substrates: i) acetate/ propionate/ butyrate/NH_4Cl (APBN), ii) APB (no NH_4Cl), iii) acidogenic FWC plus propionate.	T=30°C pH=7±0.1	**Extraction-Analysis** • Analysis of PHA with GC-MS.	**PHA content 27.32%** for APB. **PHA content 2.19%** for APBN. **PHA content 3.76%** for acidogenic FWC plus propionate.
[38]	**Zhang et al., 2014**	**FWC & excess sludge** Batch acidogenic fermentation of the FWC and the excess sludge with the use of an anaerobic sludge from an anaerobic digester as seed culture.	T=35°C	**Mixed microbial culture (activated sludge)** Aeration of the seed culture (4 d). SBR consisting of: the aerated sludge, the fermentative VFAs and minerals. SRT=HRT=10d (T=25°C).	**PHA production** Inoculation of the SBR with the acclimated sludge. Operation with the ratio of feast and famine phase equal to 1:3. Same prevailing conditions with the enrichment stage.	T=25°C pH=5.5-7.5	**Extraction-Analysis** Centrifugation, lyophilization, heating, addition of acidified propanol. Thorough mixing and heat (105°C). Addition of water. Analysis with GC.	**Maximum PHA content 47.65%** (for an optimum organic load: 3600 mgCOD/L).

Table 4: PHA production from household food waste and mixed microbial cultures.

Conclusion and suggestions for further research

Household food waste has shown a good potential as a substrate for PHA production. The review of existing works indicated PHA accumulation from around 19% to 90% (g-PHAs/g-biomass*100%). So, the performance of PHA production from household food waste can be very high, but also presents great fluctuations that may be connected with the different operating parameters employed by each scientific work and the variability of the substrate and should be taken into consideration for the scaling up of the process. Nevertheless, in the scientific works that used spent coffee grounds oil for PHA production, the PHA content was over 70%. Waste cooking oils have also been a promising substrate, as they produced PHAs up to 85%. Pure microbial cultures exhibit higher performance than the mixed ones, but their pre-treatment is more expensive, which is a drawback for the overall production cost in a large scale system. The use of *Cupriavidous necator* for PHA production from household food waste resulted in high PHA accumulation from around 37% to 90%, while the corresponding percentage when mixed microbial cultures were employed was 23-47%.

A key challenge in PHA production is that they have to be cost competitive with the fossil fuel-based polymers. PHA industrial production nowadays is mainly carried out with refined sugar substrates as raw materials and pure cultures or genetically modified strains as microbial populations for the creation of a final material with specific characteristics; this is an expensive choice. The use of low cost organic wastes - like household food waste - for PHA production, is a promising choice for the reduction of their market price, but also a challenge to the production of a uniform product having consistent properties.

Organic wastes are composite raw materials and they provide to the microbial population a variety of different carbon sources like proteins, carbohydrates and lipids making it difficult to foresee or control the composition of the produced biopolymer. Thus, PHAs produced from waste feedstocks are characterized by lack of consistency in properties. Furthermore, the extraction process is currently a bottleneck which increases the overall production cost of PHAs. The current situation results in a limited market uptake for PHAs, in comparison with the market uptake for conventional plastics that are both inexpensive and consistent in properties and therefore still have a primary role in commerce.

Ending, PHAs are of great significance today, as their production aims in decoupling economic growth from resource depletion and environmental degradation. PHA production from food waste offers the extra advantage of utilizing waste for the creation of another product. Subsequently, the food waste amounts that end up in landfills producing large quantities of methane, a powerful greenhouse gas, which is responsible – among others - for global warming and climate change, are reduced. The scientific community and the industrial sector should pay much more attention in PHA production from food waste the following years for the benefit of the environment and the improvement of the quality of life.

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