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PHA's (Polyhydroxyalkanoates): General information on structure and raw materials for their production

A running document for "Kleinschalige Bioraffinage

WP9: PHA", Task 5

Authors: Maarten Kootstra, Hellen Elissen, Sander Huurman

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Abbreviations

3HV = 3-hydroxyvalerate

BOD = biological oxygen demand

CHP = combined heat and power

COD = chemical oxygen demand

CFSTR = continuous flow stirred tank reactor

DOC = dissolved organic carbon

EPS = extracellular polymeric substances

GAO = glycogen-accumulating organisms

HRT = hydraulic retention time

LAB = lactic acid bacteria

MC = moisture content

Mcl-PHA= medium chain length PHA

OFMSW= organic fraction of municipal solid waste

OLR = organic loading rate

PAO = phosphorus accumulating organisms

p.e.= person equivalent

PHA = polyhydroxyalkanoate

PHB = polyhydroxybutyrate

PH2MV = poly-beta-hydroxy-2-methylvalerate

PHV = polyhydroxyvalerate

PS = primary sludge

SBR = sequencing batch reactor

SCOD = soluble chemical oxygen demand

SRT = sludge retention time

TCOD = total chemical oxygen demand

UASB = upflow anaerobic sludge blanket

VFA = volatile fatty acids

VS = volatile solids

VSS = volatile suspended solids

WAS = waste activated sludge

WWTP = wastewater treatment plant

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1 Introduction

This report provides background information on structure and diversity of different polyhydroxyalkanoates (PHA) and on feedstocks for their microbial production. The information that is contained in this report was compiled as a running document for the project “TKI-AgriFood Kleinschalige Bioraffinage” Work Package 9: “Fatty acid and PHA production based on residues” (In Dutch: “Vetzuur- en PHA-productie op basis van residuen”) (TKI-AF-12040), and should be seen as such: a compilation of information regarded as interesting for the project partners. The authors kindly refer interested readers to the original publications.

Industrial partners involved in this project are: Rodenburg RB Biobased Institute B.V. (Jeroen van Soest; from 01-02-2015: Aaik Rodenburg), Nuplex Resins BV (Richard Brinkhuis), Maan Research & Development (Agata Zielinska), Feyecon Development & Implementation BV (Daniela Trambitas), KNN Advies BV (Cor Kamminga, Onno de Vegt), Opure (Arnaud Duine, Harry Brouwer), Waterschap Brabantse Delta (Leon Korving, Etteke Wypkema), Waterschap De Dommel (Jarno de Jonge, Alexandra Deeke), Attero (Adrie Veeken), and BIONND (Henk Doddema).

2 General information on structure, production and cost price of PHA

2.1 Structure of PHAs

The general structure for polyhydroxyalkanoates (PHAs) is shown in Figure 1.

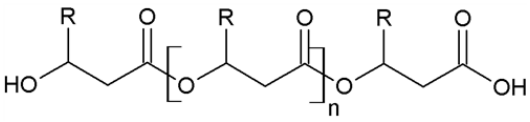
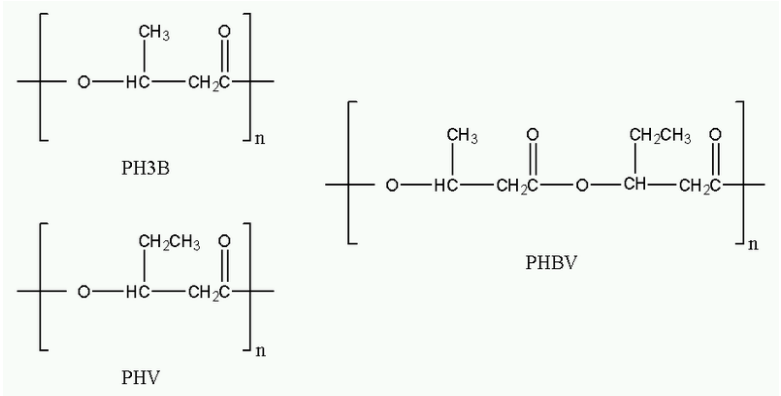


Figure 1 General structure of polyhydroxyalkanoates (PHAs)

Between brackets one monomer (hydroxyalkanoate) is shown, 'R' denotes residual group. The residual group can consist of different carbon chain lengths. The type of PHA depends on both the structure and length of the main chain, but also on the side chains (Figure 2). Examples of different monomers with different residual chain lengths can be found in Figure 3.



PH3B: Polyhydroxybutyrate
PHV: Polyhydroxyvalerate
PHBV: Copolymer of the above

Figure 2 Different types of polyhydroxyalkanoates (PHAs) (From: Wikipedia, 2016)

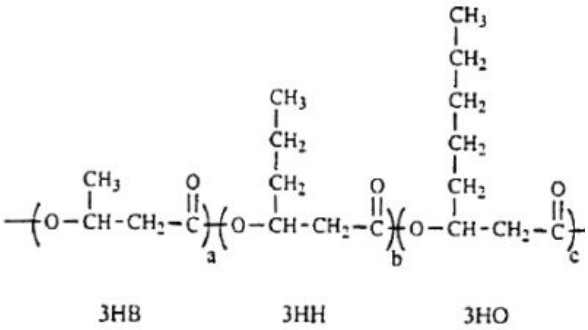


Figure 3 Examples of different monomers with different residual chain lengths (Kato et al, 1996; via Cyberlipid, 2016)

The chain-length of the VFA is of great influence on the composition and properties of PHA (Lee et al, 2014). In mixed culture PHA production, the presence of acetic and butyric acids results in the production of 3-hydroxybutyrate (3HB) while propionic and valeric acids lead to the production of 3-hydroxyvalerate (3HV). Poly(3-hydroxybutyrate) (P(3HB)) is brittle and stiff, and has limited applications. The incorporation of 3HV into P(3HB) results in P(3HB-co-3HV) which is more flexible and tougher. It is less permeable to oxygen compared to PE and PP, making it suitable for food packaging.

2.2 PHA accumulation in mixed and monocultures

A limited number of pathways for PHA accumulation exist, assuming that other growth parameters are constant:

Mixed culture is the culture of many different PHA accumulating microorganisms in the same reactor. Serafim et al (2008) give an overview of the mixed culture processes used for PHA production by reporting the important achievements regarding the use of complex substrates and providing future prospective of this technology. These authors describe a three-step PHA production process consisting of: 1) Feedstock production, where waste-based substrates are converted through anaerobic digestion to substrates for PHA production: organic and fatty acids. 2) Selection of PHA producing microbial cultures under changing feeding conditions. 3) The PHA production.

Monoculture is the culture of a single PHA accumulating microorganism. Tamis (2015) notes that treatment of waste streams with pure cultures is often not economic due to a.o. the requirement for sterile conditions. This is hard to combine with the open (nonsterile) bioconversion processes that are required for efficient valorisation of waste streams.

Furthermore, several modes of feeding can be applied, such as:

1. Mixed culture with constant feed

Part of a stock culture is used to produce PHAs in batch, which results in a constant mixture of PHAs (even though some variability cannot be prevented). When applying continuous fermentation, a mixed culture will contain a limited amount of microorganisms due to natural selection. This will result in changes in the mixture of accumulated PHAs, until steady state conditions are reached. After reaching steady state the composition of the PHA mixture will remain relatively constant.

2. Mixed culture with variable feed

When feeding a mixed culture with variable feed, the mixture of accumulated PHAs will vary accordingly. The extent to which this occurs depends on the composition of the mixed culture and the feed.

3. Monoculture with constant feed

A specific microorganism will accumulate a specific PHA or PHA mixture when feed is constant, assuming other growth conditions remain constant as well. The type of PHA or composition of the PHA mixture will depend on feed and microorganism.

4. Monoculture with variable feed

When feed is variable, the accumulated type of PHA or composition of the PHA mixture will vary.

Generally speaking, using monocultures to produce PHA will result in higher PHA concentrations but fermentation costs are also higher. When mixed cultures are fed with suitable VFA types or when process conditions are optimised, higher PHA content (40-77 %) can be achieved from fermented food waste, waste activated sludge, sugar cane molasses and paper mill effluent (Lee et al, 2014).

2.3 Cost price

To date, the price of PHAs is in general much higher than that of starch polymers and other bio-based polyesters due to high raw material costs, high processing costs (particularly purification of the fermentation broth), and small production volumes (Wolf et al, 2005). The PHA market price in 2014 was €4-5 per kg of biopolymer, for which applications vary from high-value medical products to low-value consumables for agri- and horticultural purposes (Hart, de et al, 2014). Currently, raw material costs account for as much as 40 % to 50 % of the total production costs for PHA. Use of cheap carbon sources, recombinant *E.coli* or genetically engineered plants should lead to cost reduction (Wolf et al, 2005). However, production of PHA generates a large amount of biomass waste: about 5 kg of raw material is required to obtain 1 kg product. As such, both low conversion rates as well as waste management are issues.

3 Fatty acids as substrate for PHA accumulating microorganisms

3.1 Short chain fatty acids: PHAs by 'de novo' pathways

Generally speaking, waste streams may be acidified to convert persistent (and highly variable) carbon sources into short chain fatty acids (Table 1), which can then be used by PHA accumulating microorganisms. PHA production takes places by the 'de novo' pathway. Formation of PHB and PHV may take place starting from different substrates.

Table 1 Structure and name of short-chain fatty acids

	Structure	**Name**
C1	HCOOH	Formic acid
C2	CH_3-COOH	Acetic acid
C3	CH_3-CH_2-COOH	Propionic acid
C4	$CH_3(CH_2)_2COOH$	Butyric acid
C5	$CH_3(CH_2)_3COOH$	Valeric (pentanoic) acid

The report "Bioplastic uit slib" (Hart, de et al, 2014) describes varying PHA composition during accumulation in mixed cultures (secondary sludge) that is achieved by feeding different short chain fatty acids. The fatty acids mentioned are mainly acetate and propionate and the PHAs are PHB and PHV (and to some extent PH-2-methylbutyrate and PH-2-methylvalerate). However, the mentioned PHA accumulation takes place under aerobic conditions, while the mentioned literature regards anaerobic PHA accumulation (Salehizadeh and van Loosdrecht, 2004). It should be further investigated in literature to what extent the composition of PHA accumulated under aerobic conditions can be influenced by the short chain fatty acid composition of the feed. Literature on aerobic PHA accumulation focuses almost entirely on acetate. There is literature on the influence of ethanol, glucose and glutamic acid during aerobic PHA accumulation, but it focuses mainly on removal of these compounds and not on composition of the produced PHAs (Majone et al, 2001; Beccari et al, 2002).

Dias et al (2006) explain how PHA can be produced under aerobic conditions from acetate via acetyl-CoA, propionate via propionyl-CoA, butyrate and valerate via hydroxylacyl-CoA, and longer fatty acids via the β-oxidation route.

VFA molecules are assimilated by the cells through active transport (1 mol ATP/mol VFA) following their activation to acyl-CoA molecules. Simpler VFA (acetate and propionate) are activated directly to acetyl-CoA and propionyl-CoA; meanwhile the remaining VFA go through the β-oxidation pathway to be converted to acetyl-CoA and propionyl-CoA by consumption of one more ATP molecules (Pardelha et al, 2014).

Marang et al (2013) mention that butyrate is an interesting substrate for PHA production. The stoichiometric yield of PHA on butyrate (0.94 mol/mol on carbon basis) is 40 % higher than the yield on acetate. Also, butyrate is produced in large amounts during acidogenic fermentation of organic waste streams, like fermented sugar cane molasses, olive oil mill effluents, paper mill wastewater, waste activated sludge, or food waste. Their results show that for optimised waste-based PHA production the pre-fermentation process should be directed towards butyrate production. Cerrone et al (2014) describe a two-step biological process for the conversion of ensiled grass biomass to mcl-PHAs through the use of anaerobic and aerobic microbial processes. The main FA produced was butyric acid and the VFA mixture was concentrated and converted into PHA (mainly 3-hydroxydecanoic acid).

Chakraborty et al (2009) tested growth of *Ralstonia eutropha* and production of PHAs on acetic, butyric, lactic and propionic acids. They concluded that butyric and propionic acids were the most efficient carbon sources to maximise PHA production when added at a concentration of 5 g/L.

Hassan et al (1996) investigated a two-stage process for the production of PHA from palm oil mill effluent, combining anaerobic treatment to obtain mostly acetic and propionic acid, followed by conversion into PHA by the phototrophic *Rhodobacter sphaeroides.* During the first step formic acid was formed when the pH was maintained below 4. The results clearly indicated that the presence of formic acid substantially decreased PHA yield and content in the cells.

Production of PHA by enriched cultures of phosphorus-accumulating (PAO) or glycogen-accumulating organisms (GAO) with acetate or propionate as the sole carbon source has been described often (Jiang and Chen, 2009). To produce PHA with a high 3-hydroxyvalerate (3HV) content they investigated enriched cultures of GAO using different ratios of propionate and acetate (P/A) as carbon sources in this study. With increasing P/A ratio, total PHA decreased and more 3HV was incorporated into PHA. Zhang et al (2008) found that phosphorus removal by PAO increased with P/A ratio. Phosphorus removal also had a linear positive relation with the concentration of (PHV + PH2MV).

Jiang et al (2011) modeled SBR and fed-batch experiments with a microbial community dominated by *Plasticicumulans acidivorans* and found that propionate induced mainly PHV production whereas only PHB was produced on acetate. Accumulation experiments with acetate-propionate mixtures yielded PHB/PHV mixtures in ratios directly related to the acetate and propionate uptake rate. The model they developed can be used as a useful tool to predict the PHA composition as a function of the substrate composition for acetate/propionate mixtures.

Physical and mechanical properties of PHA can be improved by incorporating 3-hydroxyvalerate (3HV) into the polymer (Jiang and Chen, 2009). Cai et al (2001) found that production of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)) from glucose and propionic (or valeric) acid by *Alcaligenes eutrophus* yielded the same results.

3.2 VFA production

As mentioned earlier volatile fatty acids (VFAs) can be used as substrate for PHA production by PHA accumulating micro-organisms. In general, the production of storage compounds with microbial cultures from diluted waste streams improves recovery efficiency since the product is concentrated inside the biomass and can be readily separated from the water using standard sludge settling (or other separation) methods (Tamis, 2015). At present VFAs are mostly produced through chemical routes[1], but there is increasing attention to produce them through biological routes (Lee et al, 2014). Waste materials are especially interesting for this purpose, such as wastewater sludge, food waste, organic fractions of municipal solid waste, and industrial wastewaters. Koller et al (2009) produced an overview of several organic waste streams of different composition that can be converted into PHA through different intermediate compounds and via lactic acid. Medium chain length (mcl) PHA are mentioned to be formed through fatty acids coming from triglycerides that in turn originate from waste lipids.

Lee et al (2014) reviewed various wastes for VFA production, factors influencing VFA production, and various applications of the resulting VFAs. The type of VFA produced can be manipulated by adjusting process parameters. Wastes in general should be rich in organic matter with COD concentrations greater than 4 g/L. Also the ammonium content of the waste should be lower than 5 g/L to prevent inhibition of VFA production. Availability (e.g. due to seasonal harvests) and amounts are important parameters as well.

Before fermented waste that is rich in VFA can be used for PHA production, ammonium and phosphorus concentrations should be low, since these compounds promote microbial growth and decrease the conversion of VFA to PHA. N and P limitations lead to higher PHA contents and yields. They can be

[1] The Dutch production of refinery fatty acids (from 'soapstock') was 123 ktonne/year in 2008 (Elbersen et al, 2010). Fatty acids can be used in animal feed (24 k tonne) and for technical applications (34 ktonne). Technical applications include energy, soap production, lubricants, cosmetics etc. The surplus of fatty acids is exported. The authors estimated that in 2004, 2005 and 2007 around 100 ktonne of fatty acids per year were imported and applied for stationary energy production. Soapstocks are emulsions of fat in water. To extract the fat from soapstocks the fatty acids are hydrolysed with sulphuric acid. As a result, free fatty acids (acidoils or refinery fatty acids) will float and can be separated from the water phase. Fatty acids with methyl esters (so called fatty matter), collected after the recovery of methanol during biodiesel production, are banned from feed applications. Lipophilic additives applied in biodiesel production could have concentrated in these fatty acids (Ovocom, 2012).

removed by struvite precipitation. Sludge particles can lead to failure of PHA production and should therefore be filtered out (Lee et al, 2014).

Waste materials (e.g. primary and secondary sludge from WWTPs, see 4.3) can be used as a source for VFA production under anaerobic fermentative conditions. This can be achieved in reactors with either attached or suspended growth (Lee et al, 2014). The VFAs are produced in a two-step process: the first step is the hydrolysis phase and the second step is the acidification phase. During hydrolysis, complex organic matter is converted into soluble amino acids, sugars, and higher (long-chain) fatty acids. In the acidification phase these components are converted to VFAs and alcohols (acidogenesis or acidogenic/dark fermentation) which are then converted into acetate, hydrogen and CO_2 (acetogenesis). Both processes involve a complex group of obligate and facultative anaerobic bacteria such as *Bacteriocides*, *Clostridia*, *Bifidobacteria*, *Streptococci* and *Enterobacteriaceae* (Lee et al, 2014). Zhang et al (2015) describe several bacterial species that produce different VFAs. *Fusibacter*-related organisms for example produce acetate and butyrate from carbohydrates, while *Clostridium aminobutyricum* and other *Clostridium*-like organisms use amino acids. *Dethiosulfatibacter aminovorans* produced acetate and propionate from various organic compounds. Zhang et al (2015) suggest to investigate, for example by tag-pyrosequencing, types and functions of microbes which could improve VFA production. When conditions are favourable, methanogenic bacteria will produce methane (Nieuwenhuijzen, van et al, 2011). For PHA accumulation purposes the methane production should however be prevented as this means loss of VFAs. Pretreatment of wastes –examples of which can be found in Table 2– can improve the rate limiting hydrolysis step (Lee et al, 2014), as e.g. cell walls and extracellular polymeric substances (EPS) pose chemical and physical barriers. Also, lignocellulosic material, fats, and proteins lead to lower biodegradation rates. Mixing in other types of waste (e.g. starch-rich industrial wastewater) can enhance VFA production due to better synergistic hydrolysis waste.

Table 2 Overview of methods for improvement of hydrolysis steps in waste streams

Chemical	
	Acid
	Alkali
	Ozone
	H_2O_2
Biological	
	Hydrolytic enzymes
	Cellulomonas uda, *C. biazotea*
	Aspergillus awamori
	Mature compost
	WWTP activated sludge
Physical	
	Microwave
	Thermal (60-180°C)
	Ultrasound
Combinations	
	e.g. combined alkaline and ultrasonic pretreatment

Zhang et al (2015) also describe the different pretreatment methods for VFA production (during anaerobic digestion of sludge). Several process parameters are known to influence the concentration, yield and composition of VFAs from waste: pH, T, retention time, organic loading rate, and additives (Lee et al, 2014).

pH is important for acidogens, since these cannot survive at pH values below 3 or above 12. Optimal pH values for VFA production are usually in the range between 5.25 to 11, but this is dependent on the feedstock used. Research suggests that alkaline conditions are beneficial for VFA production from sludge, and neutral and acidic conditions for food waste and wastewater respectively. pH can also influence the type of VFA (particularly acetic, propionic and butyric acid) produced. For example: propionic acid formation from dairy wastewater is favoured at pH 4-4.5, while acetic and butyric acid are favoured at pH 6-6.5. The same was found for gelatine-rich wastewater. However, it was different for whey and

other substrates. The optimal pH for the production of a specific VFA is thus dependent on the type of feedstock used (Lee et al, 2014).

Different temperature ranges have been applied for the production of VFAs: psychrophilic (4-20 °C), mesophilic (20-50 °C), thermophilic (50-60 °C) and extreme/hyper-thermophilic (60-80 °C). The concentration, production rate, and yield of VFAs increased when increasing temperature within the lowest two ranges, due to increased hydrolysis. The effect of still higher temperatures is not clear, as different organisms were probably present in the investigations. Also, higher production temperatures invoke higher costs, which may not be outweighed by the profits of the improved VFA yield. It seems that temperature has a relatively small effect on VFA production (Lee et al, 2014).

The retention times of the feedstock (hydraulic retention time = HRT) and the microbial population (solids retention time = SRT) are important process parameters. They are the same during VFA production from sludge since feedstock and microbes are present in the same phase. HRT has an influence on both VFA production and composition, but depends on the feedstock and has an optimum above which VFA production stagnates. A longer HRT also requires a larger reactor volume and as such should be carefully evaluated, since this constitutes the main cost factor of the process. In addition, HRT influences the propionic/butyric acid ratio. According to Lee et al (2014) research showed that shorter SRTs lead to higher VFA production, since growth of methanogens is prevented. With primary sludge it was found that acidogenic conditions prevail at an SRT < 8 days, while methanogenic conditions prevail at an SRT > 10 days. As with other process parameters, the influence of SRT on the VFA composition varies between studies and are often contradictory.

The organic loading rate (OLR) can be expressed as COD, VS, VSS, or DOC fed daily to the reactor. There seems to be a linear increase of VFA concentration with OLR until an optimum is reached. The frequency of feeding also has an influence, with lower frequencies leading to higher VFA concentrations. It seems that higher OLR leads to a decreased proportion of acetic acid in the VFA mixture. However, mainly pH and HRT influence the VFA composition, and these factors should also be taken into account.

Additives such as surfactants and enzymes can be used to improve VFA production. They are added during acidogenic fermentation. Surfactants enhance the production of EPS (mainly carbohydrate and protein). Examples are SDS and SDBS. At higher doses however, surfactant can be toxic. Combining surfactants with enzymes, e.g. α-amylase and neuter protease, has more effect than the individual substances. Enzymes are costly and recycling them is difficult, therefore immobilisation may be a good alternative. A third option is the use of specific (2-bromoethanesulfonate, 2-chloroethanesulfonate, mevastatin, lovastatin) or non-specific (chloroform, fluoracetate and methyl fluoride) inhibitors of methanogenesis. The drawback of the latter option is their possible toxicity to other bacteria (Lee et al, 2014).

Zhang et al (2015) describe mixing as a pretreatment method to increase the contact surface area between substrates and microbes. According to the authors, some studies mention agitation speeds ranging from 350-650 rpm. The optimal mixing speed for VFA production from excess sludge is not yet known.

Conversion of proteins, sugars, and fats results in different VFAs during fermentation. Miron et al (2000) investigated the effect of SRT on hydrolysis, acidification, and methanogenesis of domestic sewage. Carbohydrates in general can be easily and rapidly hydrolysed to simple sugars and subsequently fermented to VFA. Proteins are hydrolysed to amino acids and degraded to VFA by either anaerobic oxidation linked to hydrogen production, or by fermentation according to the Stickland reaction. The first route depends on the presence of hydrogen scavengers while the second route is independent of the methanogenic activity in the reactor. Triglycerides (lipids) can be hydrolysed to (medium and long chain) fatty acids (mcFA & lcFA). Accumulation of hydrogen inhibits β-oxidation since it is thermodynamically unfavourable under standard conditions, which means that β-oxidation only occurs in the presence of hydrogen scavengers. It is not clear whether the presence of hydrogen also affects hydrolysis of lipids, although recent research shows that the presence of methanogenic activity enhances hydrolysis of lipids. In addition, the lipid concentration influences lipid hydrolysis. LCFA are known to inhibit both their own degradation as well as the methane production from acetate. Miron et al (2000) found that the hydrolysis of lipids and carbohydrates increased with increasing SRT, whereas protein hydrolysis only occurred

under methanogenic conditions. Hydrolysis was found to be the rate limiting step for the conversion of carbohydrates. Under acidogenic conditions, acidification was the rate limiting step for conversion of lipids, while both hydrolysis and acidification were limiting for the conversion of proteins.

Alibardi and Cossu (2015) evaluated the effects of carbohydrate, protein, and lipid content of organic waste on hydrogen yields, volatile fatty acid production, and the fate of organic carbon from a dark fermentation process. Dark fermentation is the fermentative conversion of organic substrate to hydrogen, with as its main challenge the relatively low energy conversion efficiency from the organic source. Typical H_2 yields range from 1 to 2 mol of H_2 per mol of glucose, which results in 80-90 % of the initial COD remaining in the wastewater in the form of various volatile organic acids (VFAs) and solvents, such as acetic acid, propionic acid, butyric acid, and ethanol. Biogas and hydrogen production were linearly dependent on substrate carbohydrate concentration, but not on protein and lipid concentrations. The end products of dark fermentation were also dependent on the composition of the substrate: the main products were acetic and butyric acid and their ratio was dependent on carbohydrate and protein concentrations.

4 Waste streams to be used for PHA production

4.1 General

Wolf et al (2005) describe four different cheap resources with complex growth and production media for PHA production.

- Carbohydrates: Molasses, starch and whey hydrolysates (maltose), lactose from whey, cellulose hydrolysates (e.g. paper industry waste)
- Alcohols: Wastes from biodiesel production: methanol plus glycerol, methanol
- Fats and oils: lipids from plant and animal wastes
- Organic acids: lactic acid from the dairy industry

Some of these streams can be used directly for PHA formation, while others first need to be converted into fatty acids (Nikodinovic-Runic et al, 2013). Khosravi-Darani et al (2013) give an overview of cheap waste streams that were used in the past for PHA production: they include whey, wheat bran and rice bran, corn steep liquor as well as starch, molasses, waste water from olive mills and starch, waste glycerol, waste of vegetable, sweet sorghum, enzyme hydrolysate of potato starch, sesame oil cake, groundnut oil cake, cassava powder, jackfruit seed powder and corn flour, waste of potato starch, canola oil and waste oil. Nikodinovic-Runic et al (2013) made a comprehensive review of the use of wastes for PHA production.

4.2 Agrofood waste streams

Lee et al (2014) mention solid waste streams for the production of VFA such as waste activated sludge (WAS), primary sludge (PS), food waste, kitchen waste, organic fraction of municipal solid waste (OFMSW) and cattail. They mention liquid wastes such as palm oil mill effluent, olive oil mill effluent, wood mill effluent, paper mill effluent, cheese whey, dairy wastewater, gelatine-rich proteinaceous wastewater and pharmaceutical wastewater. Suitable mixtures of wastes are for example PS + WAS, PS + starch-rich wastewater, food waste + sludge, sugar industry wastewater + pressed beet pulp.

Nikodinovic-Runic et al (2013) supply an overview of potential pathways for processing food-based waste and residues, for PHA production. These include lignocellulosic materials and molasses from food processing an sugar industry, bran and starch residue from cereals, whey, waste lipids, and mixed food waste.

The United Nations Food and Agriculture Organization (FAO) has estimated that globally one-third of food produced for human consumption ends up as waste, which amounts to about 1.3 million ktonnes per year (www.fao.org).

Figure 4 shows an overview of waste and by-product streams from the different braches in the F&B (Food and Beverages) sector in the Netherlands compiled from several data sources. The main by-product streams originate from potato/starch/flour, fats/oils, and sugar production. Most of these streams are suitable for and currently used in animal feed applications, which means that they represent a certain value. In other words, they may be more expensive than e.g. sewage sludge when used as feedstock for PHA production.

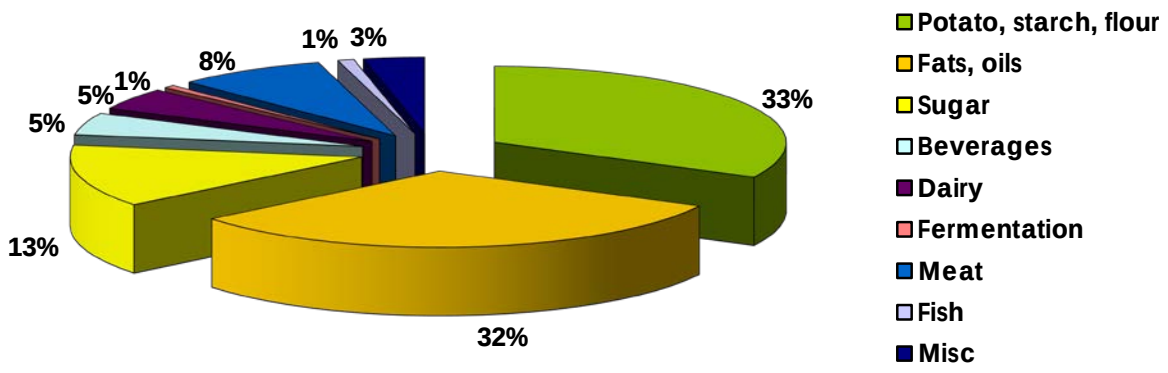


Figure 4 Overview of waste streams from food and beverage industries in the Netherlands (based on de Boer and van Doorn, 1997; Boersma and Hemmes, 2001; Elbersen et al, 2002; Vis, 2002; Bouwmeester et al, 2006; Bondt and Meeusen, 2008; Boekhoff et al, 2008; OPNV, 2009). Data are indicative only as they are averages of several reports and originate from different years. The total estimated amount produced is ~12,000 ktonnes per year.

Crawshaw (2004) supplies an extensive overview of by-products from food and beverage industries in the UK that are used for animal feed (Table 3).

Bastein et al (2013) classify waste streams in the Netherlands in three categories. Primary streams are generated during harvesting, storage and transport prior to primary processing. Secondary streams are generated during primary processing within the agro-food industry. Tertiary streams are generated during production or consumption by end users. Predominant crops in the Netherlands are maize for animal feed, sugar beet and potatoes. In addition, the country is a net importer of grain, oilseeds, meat and vegetables. Beef, pork, poultry and milk are produced in large quantities. provide an overview of 34 agro-food waste streams (in total 42.9 Mt wet weight per year), with subdivisions into primary, secondary and tertiary waste streams. These include streams that are (e.g. straw, sugar beet pulp) as well as those that are not suitable for feed applications (e.g. household waste, manure, sewage sludge). A large proportion of organic waste streams is already used as cattle feed or raw materials for biogas or second-generation biodiesel. The authors mention that novel applications and processes that can potentially generate more added value are biorefinery, insect breeding, production of C5 and C6 sugars, solid state fermentation and more efficient biogas production processes. Streams interesting for technical use of paper/packaging/bioplastics are feather meal, horticultural crop residues, maize stalks and cobs, and straw (wheat, barley).

Wang et al (2013) have investigated the production of PHB by *Alcaligenes latus* using sugar beet juice. Media with partial nutrient addition were shown to be optimal for PHB production, with a final PHB concentration 4.01 ± 0.95 g/L, PHB content 38.66 ± 7.28 %, Yp/x (g PHB produced per g dry cell weight) 0.39 ± 0.07 and a maximum PHB productivity of 0.22 ± 0.01 g/L h.

Waste potato starch can be used as a carbon source for PHB production and is cheaper than glucose (Koller, 2009). Most processes for PHA production based on starch require the conversion of starch to easily convertible substrates such as glucose by enzymatic or chemical hydrolysis. Alternatively, VFAs can be produced as fermentation substrates by acidogenesis. In 2011, the total consumption of starch and starch derivatives was 70,000 ktonnes in the EU (Nikodinovic-Runic et al, 2013).

Table 3 Waste streams (by-products) from food and beverage industries in the UK that are used for animal feed (total 1,700 ktonnes of moist products and >1,000 ktonnes of liquid products) (Compiled from: Crawshaw, 2004)

Process	Co-product
Apple processing *20 ktonnes moist products*	apple pomace
Bread and baking *125 ktonnes moist products*	processed bread, biscuit meal, breakfast cereals, cake
Brewing and malting *750 ktonnes moist products and 150 ktonnes liquid products*	barley screenings, malt powder, malt culms, malt residual pellets, brewers' grains, grains pressings, brewers' yeast, ullage (feed quality beer) and other beer, vinegar still bottoms (with liquid yeast), liquid malt extract (LME)
Citrus and tropical fruit processing *42 ktonnes moist products*	citrus pulp, co-product fruit salad, citrus molasses
Distilling *343 ktonnes moist products and 20 ktonnes liquid products*	draff, pot ale syrup (PAS), barley distillers dark grains/ malt distillers dark grains, grain distillers grains- wheat, grain distillers grains-maize, Supergrains, Vitagold, Loch Lomond Supers, Loch Lomond Gold, and evaporated spent wash
Maize fractionation *150 ktonnes moist products*	corn steep liquor (CLS), maize gluten feed, moist maize gluten feed, maize gluten meal (MGM), broken maize/maize screenings, maize fibre, maize germ meal
Milk processing *517 ktonnes liquid products*	whey, whey concentrate, whey permeate, delactosed whey, ice cream, yoghurt, salvage milk
Potato processing *153 ktonnes moist products and 119 ktonnes liquid products*	potato feed (steam peel), potato off-cuts (potato hopper) and canning potatoes, potato off-cut/steam peel skins mix (potato slice), potato mash, potato chips, other fried products (jacket wedges, southern fries, hash browns), prime potato puree (PPP) and potato starch, potato puree feed, co-products of potato crisp production (abraded peel, peel and trim, primary sludge (uncooked)/feed-grade starch, potato crisps), co-products of dehydrated potato production (potato flakes, granules, slices and flour), peeled canning potatoes
Sugar beet processing *190 ktonnes moist products*	molassed sugar beet feed, pressed pulp, sugar beet tails, beet molasses, condensed molasses solids (CMS)
Wheat fractionation *200 ktonnes liquid products*	wheatfeed, Greenwich Gold™, Roux™, Promanna™, Abracarb Plus™, C Starch™, AmyPlus™
Other food industry co-products *25-30 ktonnes moist products and 10 ktonnes liquid products*	liquid chocolate, stockfeed fruit juice, jam

PHB can be produced from sugar beet molasses (Koller et al, 2009) and the production yields of biomass and PHB from this carbon source were comparable to those on pure substrates like glucose. Molasses also contain growth enhancing components such as vitamins and biotin. The global production of molasses amounts to around 50,000 ktonnes per year (Nikodinovic-Runic, 2013). Bagasse is a by-product of sugar production from sugar cane. Vinasse is a by-product of ethanol production from molasses.

In Europe and North America, large amounts of surplus whey from dairy industries are available, containing lactose for the production of a.o. PHAs (Koller et al, 2009). This stream also contains minor components such as minerals and protein residues that are beneficial for microbial growth. Globally, annual production is between 115,000 and 140,000 ktonnes. The yield PHA/C-source = 0.33 g/g. Wolf et al (2005) mention that the 50,000 ktonnes of whey produced annually in Europe could be used to produce 618 ktonnes of P(HB-*co*-15%HV).

Waste streams consisting of fats and oils of vegetable or animal origin are e.g. used frying oils, waste of oil mills, slaughterhouses, fat melting/rendering plants and dairy industries but also fats from sewer systems and grease separators of restaurants, hotels etc. Fernández et al (2005) reported that residual

frying waste and other oily wastes are suitable substrates for PHA production. In the EU, the total cooking oil waste production was 1,000 ktonnes per year (Nikodinovic-Runic et al, 2013). Not all PHA producing bacteria are capable of using fats and oils directly for PHA synthesis. In some cases, it is necessary to break down the triglycerides into fatty acids and glycerol to make the fatty acids available for uptake. This can be done by saponification with a strong alkali or by hydrolysis by means of steam.

During the last two decades, fish silage, resulting from fish waste by adding either strong (sulphuric or formic) or organic (acetic, lactic, propionic) acids, has been successfully used as a low cost ingredient in fish feeds (Vázquez et al, 2011). The waste is ensiled with an external source of carbohydrates. Vázquez et al (2011) studied the efficacy of an alternative option: adding several types of lactic acid bacteria (LAB) as bio-silage inoculants of fish by-products. Batch cultivations without re-neutralisation (pH control) led to the highest production and yields of the main metabolites of LAB fermentation (lactic and acetic acids). The dynamics of these metabolites followed a conversion pattern from lactic to acetic acid with a final joint concentration over 16 g/L and final pH lower than 4.5. Solli et al (2014) examined the effects of an increased load (3-19 volume %) of nitrogen-rich organic material (fish waste silage) on anaerobic digestion and methane production from cow manure. With the highest loadings, biogas processes failed due to overloading and accumulation of ammonia and VFAs, such as acetic and propionic acid, up to total VFA concentrations of 19 g/L.

4.3 Waste water sludge

The total wastewater treatment capacity in the Netherlands is estimated at 24 million population equivalents (p.e.), with 10 million p.e. represented by wastewater treatment plants (WWTPs) equipped with digesters (Hart, de et al, 2014). One population equivalent is defined as an amount of waste with a Biological Oxygen Demand (BOD) of 54 g O_2 per 24 hours. BOD is a unit commonly used in wastewater treatment, as is COD (Chemical Oxygen Demand), mostly for mass balance reasons and process control. (Note: for people not accustomed to BOD and COD: it should be noted that expressing concentrations as BOD and COD does not carry information about the concentration of specific components. As an example, a COD of 60 mg/L means that 1 L contains an amount of material that would demand 60 mg of O_2 to oxidise).

Lee et al (2014) mentioned that compared with industrial wastewater, domestic wastewater (influent) has low organic content, with typical COD values in the range of 175–600 mg/L, thus it is not attractive for VFA production. Sludge produced during treatment of this wastewater contain higher COD values.

In a case study, de Hart et al (2014) mention a PHA production potential from Dutch wastewater sludge of 2.9 ktonne/year using the rich culture route, and 5.2 ktonne/year if produced in mixed culture. Their estimates for an indicative cost-effective price (break-even) of PHA produced from wastewater sludge are shown in Table 4.

Table 4 Estimates of indicative cost-effective PHA price (€/kg) for PHA produced from sewage sludge in the Netherlands (adapted from: Hart, de et al, 2014).

		Existing sludge facilities	New sludge facilities
Excluding purification	Rich culture	9.1 (4.6-13.7)	6.0 (3.0-9.0)
	Mixed culture	5.3 (2.7-8.0)	2.5 (1.3-3.8)
Including purification	Rich culture	10.9 (5.5-16.4)	7.8 (3.9-11.7)
	Mixed culture	8.5 (4.3-12.8)	5.7 (2.9-8.6)

Between brackets: bandwidth of - and + 50 %

Van Nieuwenhuijzen et al (2011) reported that primary sludge (PS) is collected during primary settling of suspended solids which originate from municipal waste water. Secondary sludge, also known as activated sludge (WAS), is produced in the aerobic zone of a municipal WWTP. The bacteria that make up this sludge mainly remove leftover organic residues after the primary settling step. Secondary sludge consists of flocs composed of a diversity of microorganisms and other organic/inorganic matter .

Zhang et al (2015) review methods that improve the production of volatile fatty acids (VFA) from excess sludge during anaerobic digestion.

Research by Ucisik and Henze (2008) compared the VFA production of PS and WAS from different WWTPs and a sludge mix from one WWTP. In semi-continuous reactors PS showed the greatest VFA potential. They found a soluble COD (SCOD) production of 168-270 mg/g volatile suspended solids (VSS) and VFA yield (VFA/SCOD) that varied from 77 % to 100 %. Fermentation of the sludge mix and WAS showed a SCOD production of 114 and 62 mg/g VSS respectively with a VFA yield of 77 % and 82 % respectively. The solids retention time (SRT) in this research was 5 days in order to prevent methane production. The difference in SCOD production between the different sludge types is explained by inaccessibility of organic matter in WAS, which mainly consists out of living organisms. SCOD in sludge is often low in comparison to total COD, which slows down VFA production, since hydrolysis is the rate limiting step (Lee et al, 2014).

The composition of the produced mixture of VFAs in semi continuous reactors from PS showed little variation between the different WWTPs: 25 % to 30 % acetate, 40 % to 50 % propionate, 15 % to 20 % butyrate, and +/- 10 % other. When comparing PS, WAS and mixed sludge from a WWTP it was observed that both WAS and mixed sludge produced a VFA mixture relatively lower in acetate than PS. The percentages propionate and butyrate were mainly higher in the mixed sludge and the percentage of (mainly C5 and C6 VFAs) increased in the fermentation of WAS and mixed sludge compared to PS .

The fermentation of PS at different pH results in different VFA yields. It was concluded that optimal fermentation pH was PS pH found in the collected research material, which ranged from pH 6.72 to pH 6.84. Both higher (7.5) and lower (5.5) controlled pH had a negative effect on the VFA yield. The lowest VFA-COD/SCOD yields of 58 % and 56 % were observed at controlled pH 6 and 7.5 respectively. The highest yield of 90 % VFA-COD/SCOD was observed at a non-controlled initial pH of 6.72 and during fermentation pH dropped to 5.84. The hydrolysis process seems to be enhanced at higher pH levels. A controlled pH of 7.5 resulted in a 26 % higher SCOD concentration compared to the uncontrolled pH of 6.82 for the same PS composition. VFA composition differed with fermentation pH as the relative concentration of acetate was approximately 37 mass % at pH 6.5, while this was 50 % at pH 7.0, and 70 % at pH 5.5. All results were generated in experiments performed at 20 ^{0}C. Changing fermentation temperature affected VFA production composition. Sludge containing ~15 g/L VSS produced 610 mg/L VFA at 10 ^{0}C while a fermentation temperature of 24 ^{0}C produced 3 g/L VFA in experiments lasting around 120 hours. Relative acetate content dropped from over 80 % at 10 ^{0}C to around 50 % at 20 ^{0}C and practically stabilised between 20 ^{0}C and 24 ^{0}C (Cokgor et al, 2008).

Khardenavis et al (2005) studied P(3HB) production from sludge by using different carbon and nitrogen sources. Maximum accumulation of polymer was observed with carbon source acetic acid and diammonium hydrogen phosphate (DAHP) as nitrogen source. Further studies were carried out to optimise the carbon/nitrogen ratio using acetic acid and DAHP. A maximum of 65.8 % (w/w) P(3HB) production was obtained at a C/N ratio of 50 after 96 hours of incubation.

To improve VFA yield from WAS, Chen et al (2007) investigated its hydrolysis and acidification at different pH settings. pH values ranged from 4 to 11 including a control in which pH was not adjusted or controlled. The research showed that under alkaline conditions (pH 10 and 11) fermentation of WAS yielded a much higher SCOD/TCOD (total COD) ratio and soluble protein and carbohydrate concentrations compared to the control. At room temperature the extent of sludge hydrolysis was in the following order: alkaline (pH 9-11) >acidic (pH 4-5) >(neutral and blank test). It was found that methane production had its optimal rate at pH 6-7. VFA production was highest at pH 10 and the final concentration of VFA was 2770 mg/L VFA-COD after 12 days of fermentation. High pH values enhance the hydrolysis of WAS and decrease or prevent methanogenic conversion of VFAs to methane. Acetic, propionic and iso-valeric acid were the three main products.

Khiewwijit et al (2015a, b) investigated a combined process of bioflocculation (to concentrate sewage organic matter) and anaerobic fermentation (to produce volatile fatty acids (VFA)). The bioflocculation step was performed in a high-loaded aerobic membrane bioreactor (HL-MBR), after which the concentrate was anaerobically fermented. More than 75 % of sewage COD was diverted to the concentrate, but only 15 % sewage COD was recovered as VFA, due to incomplete VSS degradation at

the short treatment time applied. The VFA mixture produced consisted of 50 % acetate, 40 % propionate and 10 % butyrate. In their next paper they discussed the optimisation of VFA production with respect to SRT and alkaline pH (pH 8–10). Application of pH shock to a value of 9 at the start of a sequencing batch cycle, followed by a pH uncontrolled phase for 7 days, gave a 50 % higher yield of 440 mg VFA-COD per g of VSS. The authors explained this by (1) a reduction of methanogenic activity, or (2) a high degree of solids degradation or (3) an enhanced protein hydrolysis and fermentation. They suggest that VFA production can be further optimised by fine-tuning pH level and longer operation, possibly allowing enrichment of alkalophilic and alkali-tolerant fermenting microorganisms.

Zoetemeyer et al (1982) studied the anaerobic formation of fatty acids from glucose in a continuous flow stirred tank reactor (CFSTR) inoculated with activated sludge over the temperature range 20-60 °C. A mesophilic and a thermophilic region could be distinguished, with optimum temperatures of 37 °C (D_{max} = 0.51/h) and 52 °C (D_{max} = 0.71/h) respectively. Maximum sludge loading rates at these temperatures were 77 and 112 kg COD per kg dw per day, respectively. In the liquid phase, mainly acetate, propionate, butyrate, lactate and ethanol were found. They concluded that separate acidification should be designed at mesophilic temperature below optimal temperature and at high, almost maximal, sludge loading rates. Suboptimal temperatures lead to more stable conditions (broader optima and stable product distribution, e.g. prevention of lactate formation) and require less energy. High sludge loadings lead mainly to the formation of butyrate instead of acetate/propionate.

4.4 Garden and vegetable biowaste (at Attero)

In Venlo, Attero operates a two-stage digestion and post-composting unit of bio-waste, of which 90 % is the separately collected organic fraction of household waste (Fruit, Vegetable and Yard waste (FVY) or Groente-, Fruit- en Tuinafval (GFT) in Dutch) (Veeken, 2014). The site processes 90 ktonnes per year.

Volume and composition varies over the year, and on average the material consists of 25 % indoor organic waste and 75 % yard waste. The average composition is: 40 wt % dry matter, and 50 wt % volatile matter (also known as organic matter) in the dry matter. About 50-60 % of the organic matter is biodegradable in four to five weeks, which is the maximum residence time in anaerobic digestion and/or composting. Indoor food waste has a higher biodegradability than tree branches from garden waste.

The Attero process consists of the following steps:

1. Fresh bio-waste is mixed with a bulking agent (course fraction of processed bio-waste) and loaded into nine air-closed 500 m^3 hydrolysis tunnels.
2. The bio-waste is leached with water for seven days. The leachate is collected and recirculated over the tunnels. The tunnels are operated in batch mode: every day, one to two tunnels are filled and the leachate cycle started.
3. Because of the hydrolysis and acidification of the bio-waste (first steps of the anaerobic digestion process), VFA accumulate in the leachate.
4. VFA in the leachate are kept at a constant level by draining leachate water to the UASB at a rate that depends on the VFA production rate.
5. In the UASB VFA are converted into biogas which is burned in the combined heat and power (CHP) plant, producing green electricity and heat. Part of the heat is used in winter to heat up the leachate water.
6. The effluent of the UASB is fully recycled in the process by returning it to the leachate collection tank.
7. After seven days the material is taken from the hydrolysis tunnels and transported to eight post-composting 500 m^3 tunnels where it is composted for 14 days. Water loss in the process occurs through evaporation in the composting process.
8. Compost is sieved and visible impurities are removed to produce high-quality compost for agriculture and horticulture.

Some key features of the process:

- Per tonne of bio-waste in seven days of hydrolysis, 41 m^3 of biogas is produced from the VFAs formed. This is about 55 % of the biogas potential (as average bio-waste has a biogas potential of 75 m^3/tonne).
- The leftover energy potential in bio-waste is needed for the composting process, i.e. the produced heat is used to evaporate water from the bio-waste.
- About 90 % of the biogas is produced in the UASB and 10 % in the tunnels; both are collected and sent to the CHP.
- About 80 % of degradable COD in the leachate water consists of VFAs. The level of VFAs in the leachate is approx. 5 g/L.

The VFA composition of the leachate water is somewhat variable, with acetate as the predominant VFA (50-75 mass % of total VFA), and together with propionate these amount to 80-90 mass % of total VFA. 2 to 10 mass % can be ascribed to butyrate, and 1 to 2 mass% each for iso-butyrate, valerate, and iso-valerate.

4.5 Silage leachate

In a standard ensiling process the used biomass (grass, maize, cereals or other) is dry enough to prevent leachate/effluent but still contains enough moisture to support lactic acid bacterial (LAB) growth. The lactic acid produced by the lactic acid bacteria lowers the pH which prevents biomass spoilage. Depending on the of type, LAB produce lactic acid only or a combination of lactic- and acetic acid as preservative. Homofermentative LAB only produce lactic acid which preserves the silage by lowering pH. Heterofermentative LAB produce both lactic- and acetic acid. Acetic acid decreases sensitivity to deterioration of the silage (Nicolasen, 2009).

An optimal dry matter (DM) content for maize to prevent leachate is 31-32%. Lower DM content causes leachate, as for example ensiling maize at 28% DM generates 14-25 litre leachate per tonne biomass (Werkgroep Snijmais, 2014). Grass silage effluent is high in biochemical oxygen demand (BOD), nitrogen (N), phosphorus (P), and has an average pH of 4.3, with lower and upper limits reported as 3.7 and 5.8, as reported by Gebrehanna et al (2014). These authors also reported organic matter concentrations in the leachate of 16 to 81 g/L COD and 33 to 170 g/L BOD, and pointed towards high concentrations of carbohydrates (e.g. 32 g/L) and proteins (e.g. 19 g/L) in general, and specifically to acetic acid (1.4 g/L) and lactic acid (5.8 g/L).

Silage effluent production typically occurs if the ensiled crop is high in moisture (>75-85 %) over a 30-60 day period, with 90 % occurring within the first 20-26 days. Peak flows typically occur within 10 days after ensiling due to the time required for plant cell walls to be broken down. The pre-ensiled crop moisture content (MC) is influenced by plant factors, weather conditions and mechanical or chemical harvesting treatments. There is high variability in reported effluent production rates for different crops of varying MC, however, typical values are 0-100 L/t for corn silage (70-75 % MC), 180-290 L/t for fresh grass or clover (78-83 % MC), with wilting grasses to <78 % MC preventing leachate formation. Up to 55-75 L/tonne of silage can be lost as leachate in the case of maize, with increasing DM percentages leading to lower losses (Werkgroep Snijmais, 2014).

It is further estimated that with increasing DM percentages of 25, 30, 35 and 40 %, the amount of leachate produced is 2, 3, 5 and 11 kg per tonne ensiled corn respectively (Baarda and Feenstra, 2012).

In the Netherlands, it was estimated that in 2013 around 10 million m^3 of run-off from a total of almost 43,000 farms was released (Klein, 2015). This run-off is composed of rainwater with feed and manure residues and silage effluents/leachates. Average N_{total} and P_{total} concentrations in these run-off streams are 96 and 32 mg/L respectively. For comparison, the average emission for a household is 3 p.e. while that of an average farm is 85 p.e. (Broos, 2011). It is not known what part of the run-off is composed of silage leachate/effluent.

Using ensilaged biomass and the leaching method for the production of VFAs to be used by PHA accumulating organisms might not be the most promising option. An alternative approach could be the use of the ensiling process as a long term storage method for biomass, after which the stored biomass is extracted and anaerobically fermented to produce VFAs.

4.6 Paper/pulp waste

Bengtsson et al (2008) examined a process for production of PHA from paper mill wastewater at laboratory scale. The three stage process examined consisted of acidogenic fermentation to convert wastewater organic matter to VFAs, an activated sludge system operating under feast/famine conditions to enrich for PHA producing organisms and accumulation of PHA in batch experiments. After fermentation of the wastewater, 74 % of the soluble COD was present as VFA (acetate, propionate, butyrate and valerate) and the resulting PHA after batch accumulation consisted of 31–47 mol % hydroxybutyrate and 53–69 mol % hydroxyvalerate. The maximum PHA content achieved was 48 % of the sludge dry weight and the three stage process exhibited a potential to produce 0.11 kg of PHA per kg of influent COD treated.

The Dutch paper industry consists of 23 paper factories that produce 65 ktonnes of COD in total, of which 50 % is acidified (Adriaanse, 2014). COD concentrations vary for each factory (500-32,000 mg/L), depending on the type of raw material, extent of water recycling and type of WWTP. In addition, solid waste streams are produced, containing fibres that can possibly be converted into dissolved COD.

According to Luimes (2009), out of the in total 25 paper factories at the time in the Netherlands and Belgium, 9 had anaerobic pretreatment of their process water (in 7 installations). In total more than 14.5 million cubic metres of biogas per year were produced. Their report suggests to investigate the possibilities for pretreatment of the process water with enzymes, controlled pre-acidification, and anaerobic treatment to enhance biogas production.

The European project ProgRESS was focused on the development of organic bio-based PHA derived from paper mill wastewater to be used as barrier coatings and extruded laminates for paper and board (food) packaging (Tiekstra, 2014 & 2015). In this project mixed cultures of non-modified microorganisms were used. There were however some delays with the actual production of PHA from wastewater.

4.7 Waste streams from biodiesel production

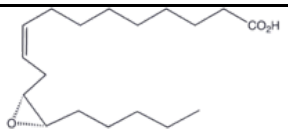
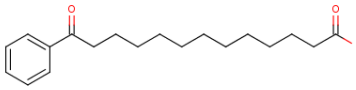
These waste streams are discussed in a separate category from fatty acids of animal and vegetable origin.

1. Glycerol (for de novo synthesis) There are two industrial routes that are currently employed in the production of glycerol, namely chemical synthesis or a microbial fermentation process (Nikodinovic-Runic et al, 2013).
2. Fats and oils can be used as feedstock for biodiesel production. However, different types of fatty acids have an influence on biodiesel properties, such as performance under cold weather conditions. Also, the presence of unsaturated fatty acids leads to higher oxidation levels and insoluble products in the biodiesel (Rajagopal et al, 2016).This means that it is likely that not all of the fatty acids from the fats and oils of a biodiesel producer can be used, hereby creating a source of fatty acids that may be used for PHA production.

4.8 Exotic fatty acids from vegetable or animal origin

Exotic fatty acids are defined as fatty acids that are not usually found in the previously mentioned fats and oils of vegetable or animal origin. They include for example FAs with terminal carbon rings, side chains or rings as side chain. They are found in the seeds of some plants (Eggink et al, 1995; Chempro, 2016). Examples of exotic fatty acids are shown in Table 5.

Table 5 Examples of exotic fatty acids

Group	Name	Structure	Origin	Reference
Epoxy FA	Vernolic acid		seeds of the *Vernonia* plant	Ward et al, 2006; Eggink et al, 1995
Cyclopropene FA	Malvalic acid		cottonseed	AOCS Lipid Library, 2016
FAs with terminal ring	13-Phenyltridecanoic acid		seeds of the calla family	AOCS Lipid Library, 2016

4.9 Modification of fatty acids

Different processes can modify fatty acids. When animal and vegetable fats are heated (e.g. during food frying), the FAs are oxidised (Chempro, 2016; Dostálová et al, 2005). By controlled heating or otherwise modifying an oil or fat, the resulting FAs may be used to produce PHAs with specific characteristics.
In addition, methods for chemical modification of FAs are known such as attaching methyl groups to fatty acids, reactions of FAs with cyclohexane to add a terminal C6 ring to an FA or producing perfluoralkylated FAs (Marinova et al, 2012). It is unknown whether these modifications are preserved in the produced PHAs.

4.10 Other hydrocarbons

Several hydrocarbons can be used as substrates for PHA production. Some microorganisms that are able to use these hydrocarbons are mentioned below for four groups of hydrocarbons (Sabirova, 2010).

1. Saturated hydrocarbons, such as methane (CH_4) and octane (C_8H_{10})
    a. *Methylosinus trichosporium* OB3b can produce PHB from methane
    b. *Pseudomonas oleovorans* ATCC 29347 can produce PHAs from C8-C12 molecules
2. Unsaturated hydrocarbons, e.g. 1-octene and 1-decene
    a. *Pseudomonas oleovorans* and *Pseudomonas cichorii* YN2 can produce PHAs using several alkenes
3. Aromatic hydrocarbons, e.g. toluene and styrene
    a. *Pseudomonas putida* and *Pseudomonas mendocina* can produce mcl-PHA (medium-chain-length PHA) from these building blocks
4. Chlorinated hydrocarbons
    a. *Methylosinus trichosporum* OB3b can convert trichloroethene into PHAs

Sources for hydrocarbons for PHA production can be hydrocarbon containing wastewaters from petrol stations or gas scrubbers or liquid waste streams from petrochemical industries. Lee et al (2014)

mention that effluent from petrochemical industries is deemed unsuitable for VFA production regardless of its high COD value of 11,500 mg/L, due to the presence of toxic and recalcitrant petrochemical pollutants which are harmful to the microorganisms. Methane from digesters could in theory also be used, but it should be investigated whether that is more efficient than PHA production from the VFAs produced during this process. Plastics such as polyethylene (Guzik et al, 2014) and polystyrene can be converted into PHAs by pyrolysis, after which the pyrolysis oil can be fed to a PHA producing bacteria culture (Ward et al, 2006). Biologically persistent plastics can in this way be converted into bioplastics. Nikodinovic-Runic et al (2013) also describe the development of technologies to convert petrochemical plastic waste streams into PHA. The conversion processes have been reported for PS, PET, PE and products present in the pyrolysis products of mixed plastic waste. The technologies are characterised by a two-step chemo-biotechnological route. Firstly, the plastic waste stream is submitted to pyrolysis, the thermal decomposition of a substance in the absence of air to produce pyrolysis oils, solids, and gases. Pyrolysis is one of a number of thermochemical methods proposed for plastic waste management with a high conversion rate of plastic to pyrolysis products. The pyrolysis products are supplied as the carbon substrate for microbial fermentation to produce PHA.

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