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PHARIO

STEPPING STONE TO A SUSTAINABLE
VALUE CHAIN FOR PHA BIOPLASTIC
USING MUNICIPAL ACTIVATED SLUDGE

New Horizons
in Biological Treatment

RAPPORT

2017
15

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PHARIO:

STEPPING STONE TO A SUSTAINABLE VALUE CHAIN FOR
PHA BIOPLASTIC USING MUNICIPAL ACTIVATED SLUDGE

RAPPORT 2017 **15**

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COLOFON

TEN GELEIDE

De Nederlandse waterschappen werken hard aan het terugwinnen van grondstoffen uit rioolwaterzuiveringen. Dat gebeurt in het kader van een Green Deal Grondstoffen die de waterschappen en het Rijk in november 2014 sloten. De ambities op dit gebied zijn in januari 2017 nog eens vastgelegd in het nationale Grondstoffenakkoord dat mede werd ondertekend door de Unie van Waterschappen.

Waar dat harde werken toe kan leiden, laat het PHARIO-project (PHA uit RIOolwater) goed zien. In dit project is in de praktijk aangetoond dat actief slib uit rioolwaterzuiveringen kan worden ingezet als grondstof voor de productie van PHA, een hoogwaardige kwaliteit biologisch afbreekbaar én biobased plastic.

PHA is een bijzonder bioplastic, omdat het relatief snel afbreekt in een waterig milieu. Deze eigenschap maakt het zeer interessant voor het bestrijden van microplastics in oppervlaktewater, de zogenoemde plastic soup. Daarmee dragen de waterschappen zelf ook bij aan een goede oppervlaktewaterkwaliteit, waar zij medeverantwoordelijk voor zijn.

De milieu-impact van PHA-plastic uit actief slib is fors lager dan plastics van petrochemische oorsprong en zelfs lager dan vergelijkbare bioplastics die nu op de markt aanwezig zijn. PHA bioplastic gemaakt uit actief slib concurreert bovendien niet met de voedselketen. Het huidige PHA bioplastic, afkomstig van landbouwproducten, doet dat wel.

De resultaten van dit project geven meer dan voldoende reden om te onderzoeken hoe we dit concept verder kunnen opschalen. Die opschaling moet vermoedelijk in stappen plaatsvinden, waarbij de productie eerst op demonstratie schaal wordt beproefd, waarna de stap naar een commerciële schaalgrootte gezet kan worden.

De benodigde investeringen voor deze opschaling zijn fors. Het is daarom belangrijk in overleg met waterschapsbestuurders de rol van het waterschap bij het terugwinnen en verwaarden van grondstoffen uit afvalwater verder uit te werken. Door slimme samenwerkingen en gebruik van bestaande faciliteiten kunnen de kosten van deze stappen hopelijk verlaagd worden, zodat we met elkaar deze veelbelovende ontwikkeling met beheersbare risico's verder kunnen verkennen.

Joost Buntsma,
Directeur STOWA

SUMMARY

Production of biopolymers of the polyhydroxyalkanoate (PHA) family by mixed microbial cultures (MMC) in association with wastewater treatment (WWT) has been identified as a valorization route of residual organic material. Since 2011, AnoxKaldnes has been benchmarking technologies for MMC PHA production from WWT at pilot scale at three different sites and with both industrial as well as municipal organic residuals as feedstock − Eslöv (Sweden), Brussels (Belgium) and Leeuwarden (the Netherlands). This experience has lead to methods to up-scale unit processes for MMC PHA production with various strategies of technology integration to existing infrastructures for WWT.

These milestones of technical success have lead in 2015 and 2016 to a PHA production and biobased value chain demonstration project, PHARIO, together with the Dutch water authorities under STOWA and lead by water authorities Brabantse Delta, de Dommel, Fryslan and Scheldestromen. Other project partners were AnoxKaldnes (Veolia Water Technologies AB), sludge incinerator SNB and KNN. PHARIO was centred on processing surplus biomass from the Bath full-scale municipal wastewater treatment plant (WWTP) in the Netherlands to produce PHA polymers.

The biological municipal WWT at the Bath site is a biological nitrogen removal process with anoxic pre-denitrification and chemical phosphorus removal. The bioprocess facilitates feast and famine conditions, which have been shown to favor the selection of a biomass with PHA accumulating potential. A PHARIO pre-investigation using the pilot scale facility in Brussels and using the full-scale secondary activated sludge from Bath WWTP have similarly been shown to produce a biomass with a PHA content of up to 0.47 gPHA/gVSS. Generally, a PHA accumulation potential above 0.40 gPHA/gVSS has been identified as a threshold for achieving a promising business case of integrating PHA production in the material flows of municipal and/or industrial wastewater treatment plants.

FIGURE 1   BUSINESS CARD HOLDER MADE WITHIN THE PHARIO PROJECT USING A BIOPLASTIC FORMULATION CONTAINING 74% PHARIO PHA
(ACKNOWLEDGEMENT: MADE POSSIBLE BY COURTESY OF PEZY)

For the PHARIO project, the full-scale surplus activated sludge was fed into a pilot facility at Bath to consistently produce PHA rich biomass with greater than 0.40 gPHA/gVSS. In order to produce PHA, the biomass was fed with VFA rich liquors derived from a local candy industry or primary sludge. As a benchmark mixtures of pure acetic and propionic acids were also fed. The PHA in the biomass was recovered in a pilot refinery process located at AnoxKaldnes in Lund Sweden. A routine of a weekly kilogram scale batch wise production was established over a 10-month period and the recovered polymers were evaluated for their material properties and market potential.

The results of the project show that the harvested activated sludge can consistently produce a high quality PHA polymer that has interesting and meaningful application potentials. Within the PHARIO project a large set of material property data (thermal, mechanical) were generated, thus making it possible for PHA end-users to evaluate the potential of the material.

The project showed that PHA can be produced at a competitive cost prize, compared to current market prices. Further cost reductions are possible. Additionally, the produced polymer has a 70% lower environmental impact compared to currently available PHA bioplastic.

DE STOWA IN BRIEF

The Foundation for Applied Water Research (in short, STOWA) is a research platform for Dutch water controllers. STOWA participants are all ground and surface water managers in rural and urban areas, managers of domestic wastewater treatment installations and dam inspectors.

The water controllers avail themselves of STOWA's facilities for the realisation of all kinds of applied technological, scientific, administrative legal and social scientific research activities that may be of communal importance. Research programmes are developed based on requirement reports generated by the institute's participants. Research suggestions proposed by third parties such as knowledge institutes and consultants, are more than welcome. After having received such suggestions STOWA then consults its participants in order to verify the need for such proposed research.

STOWA does not conduct any research itself, instead it commissions specialised bodies to do the required research. All the studies are supervised by supervisory boards composed of staff from the various participating organisations and, where necessary, experts are brought in.

The money required for research, development, information and other services is raised by the various participating parties. At the moment, this amounts to an annual budget of some 6,5 million euro.

For telephone contact number is: +31 (0)33 - 460 32 00.
The postal address is: STOWA, P.O. Box 2180, 3800 CD Amersfoort.
E-mail: stowa@stowa.nl.
Website: www.stowa.nl.

PHARIO:
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INHOUD

1

INTRODUCTION

Production of biopolymers of the polyhydroxyalkanoate (PHA) family by mixed microbial cultures (MMC) in association with wastewater treatment (WWT) has since long been identified as a valorisation route of residual organic material. Since 2011, AnoxKaldnes has been benchmarking technologies for MMC PHA production from WWT at pilot scale at three different sites and with both industrial as well as municipal organic residuals as feedstock – Eslöv (Sweden), Brussels (Belgium) and Leeuwarden (the Netherlands). This experience has lead to methods to up-scale unit processes for MMC PHA production with various strategies of technology integration to existing infrastructures for WWT.

All these previous projects showed the potential for production of PHA using activated sludge harvested from municipal wastewater treatment plants. However more information was needed about the quality of the PHA that can be produced in this way. This has led to a PHA production and biobased value chain demonstration project, PHARIO. Project partners in the project were water authorities Brabantse Delta, de Dommel, Fryslan and Scheldestromen and the other Dutch water authorities under STOWA. Other project partners were AnoxKaldnes (Veolia Water Technologies AB), sewage sludge incinerator SNB and KNN Advies. This project was made possible through a financial contribution from the Dutch government, represented by RVO under the subsidy program "Subsidieregeling energie en innovatie, Biobased Economy: Innovatieprojecten".

PHARIO was centred on processing surplus biomass from the Bath full-scale municipal wastewater treatment plant (WWTP) in the Netherlands to produce PHA polymers.

Main objectives of the PHARIO project were to:
- Prove that a high quality PHA product can consistently be produced using biomass harvested from a full-scale municipal wastewater treatment plant.
- To provide enough PHA material to be able to evaluate product quality and identify possible applications.

In this way the PHARIO project is a stepping-stone towards further up scaling of the technology to a demonstration phase where the technology can be demonstrated on a continuous basis producing enough material to test market application.

1.1 GUIDE TO THE READER
Within the scope of the PHARIO project several work packages were defined covering different aspects of the scope of the PHARIO project.

Work package 1 (WP1) was the core of the project and involved a **10 month pilot operation** at the waste water treatment plant of Bath. During this period activated sludge was continu-

ously harvested from the full scale waste water treatment plant and then fed with different sources of volatile fatty acids (VFA). As a benchmark synthetic VFAs were fed to evaluate the performance of the biomass without other influences. Furthermore waste water was obtained from a local candy factory and this waste water was fermented on site at Bath and the fed to the biomass. Also primary sludge was obtained from the sewage treatment plant of Tilburg of water authority De Dommel. The primary sludge was fermented at Bath and then fed to the secondary sludge of Bath.

In this way typically two batches of PHA rich biomass were produced per week. These batches were dried and then transported to Anoxkaldnes in Lund, Sweden. Here part of the batches were extracted in a pilot extraction unit to produce pure PHA.

The quality of the extracted PHA was characterized and part of the extracted PHA was used to test potential applications of the PHA and to assess mechanical properties of the PHA. The results of the work in workpackage 1 are discussed in chapters 4 and 5.

In **work package 2** (WP2) the **scalability of the process to other wastewater treatment plants** was tested. Based on previous knowledge the wastewater treatment plant of Bath was selected because it had shown high PHA accumulation potential in lab scale tests. Within WP2 a large number of different wastewater treatment plants were sampled and tested at lab scale for their accumulation potential to understand how often high accumulation potentials are encountered at waste water treatment plants and to learn more about the factors that favour high accumulation potentials. The results of this work are reported in chapter 3.

In **work package 3** (WP3) a basic engineering design and **cost estimate** was made for a **first full scale reference plant** (FFSR) with a capacity of 30-100 ton PHA/year. This plant would be a next step in scale after the PHARIO project and has as objective to demonstrate the value chain for the produced PHA and test all unit operations at such a scale that the testing would allow further up scaling to a commercial plant. The FFSR-facility would not yet be a commercial operation but demonstrates the potential of the technology. The results of this work package are discussed in chapter 7.

Work package 4 (WP4) studied the **business case for a first commercial reference** (FCR) plant with an anticipated capacity of 5000 ton PHA/year. A basic engineering design of all process elements was made within this framework and based on that design operational and capital costs were estimated. Furthermore a Life Cycle Analysis study was performed to evaluate the environmental benefit of the PHARIO concept. The results of this work package are discussed in chapter 6.

1.2 ACKNOWLEDGEMENTS

This report summarizes the results of the PHARIO project and is based on a set of deliverables with further and more detailed information on the different results of the PHARIO project.

PHARIO was a large team and a multifaceted coordinated effort from the AnoxKaldnes Cella™ team (Veolia Water Technologies AB) including Simon Bengtsson, Markus Hjort, Simon Anterrieu, Lamija Karabegovic, Peter Johansson, Per Magnusson, Tomas Alexandersson, Anton Karlsson, and Fernando Morgan-Sagastume. There were many who contributed and made a difference. The project was managed by a project management group with participation of

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THE PHARIO CONCEPT

During the past decade, the technical feasibility of the production of biodegradable thermo-plastic polyesters, poly-hydroxyalkanoates (PHAs), by open mixed microbial cultures (MMCs) has been repeatedly demonstrated using waste and residual carbon sources as substrates. These MMC systems generally include three biological process elements (PEs): PE1 - acidogenic fermentation, PE2 - enrichment and production of biomass with PHA-storing capacity, and PE3 - PHA accumulation using PE2 surplus biomass and feedstocks with easily degradable organics. Polymers may be recovered from a PHA-rich mixed culture biomass in a fourth process element (PE4) by means of solvent extraction.

FIGURE 2   MUNICIPAL WASTEWATER TREATMENT PLANT BATH. THE PLANT TREATS WASTEWATER FROM 500.000 PEOPLE EQUIVALENTS AND HAS THE POTENTIAL TO SUPPLY BIOMASS TO PRODUCE 2000-2500 TON PHA/YEAR.

The PHARIO project is based on the understanding that full-scale municipal wastewater treatment plants can serve as process units that produce functional biomass (activated sludge) with PHA storing capacities without modifications to the sewage treatment plant (they already are PE2s). In this way the need of a separate process element is avoided, as well as the need to dedicate raw material to the production of the functional biomass (Figure 3).

FIGURE 3   SIMPLIFIED DIAGRAM COMPARING THE PHARIO APPROACH TO THE PRODUCTION OF PHA RICH BIOMASS USING PURE CULTURES.
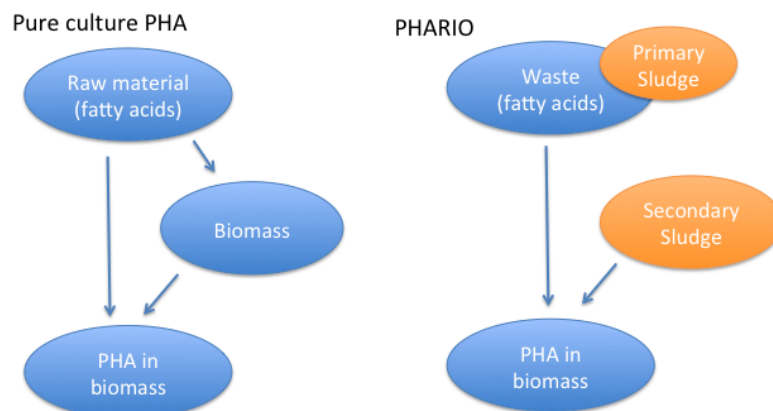
A value chain based on this concept is shown in Figure 4. Secondary sludge is harvested from a municipal sewage treatment plant and then used as functional biomass to produce PHA. Organic residuals from the region around the sewage treatment plant can be collected, fermented (PE1) and fed to the sludge (PE3) to produce a PHA rich biomass, with a typical PHA content of 40-50% of the total volatile solids. The water authorities themselves can supply part of the required organic waste in the form of the primary sludge they produce. Normally the accumulation potential of the secondary sludge is much larger than the amount of fatty acids that can be produced from the primary sludge. Therefore it is advantageous to also resource regional organic waste streams as feed to make optimal use of the PHA accumulation potential of the secondary sludge.

FIGURE 4   PROCESS STEPS TO PRODUCE PHA USING BIOMASS HARVESTED FROM A MUNICIPAL WASTEWATER TREATMENT PLANT.
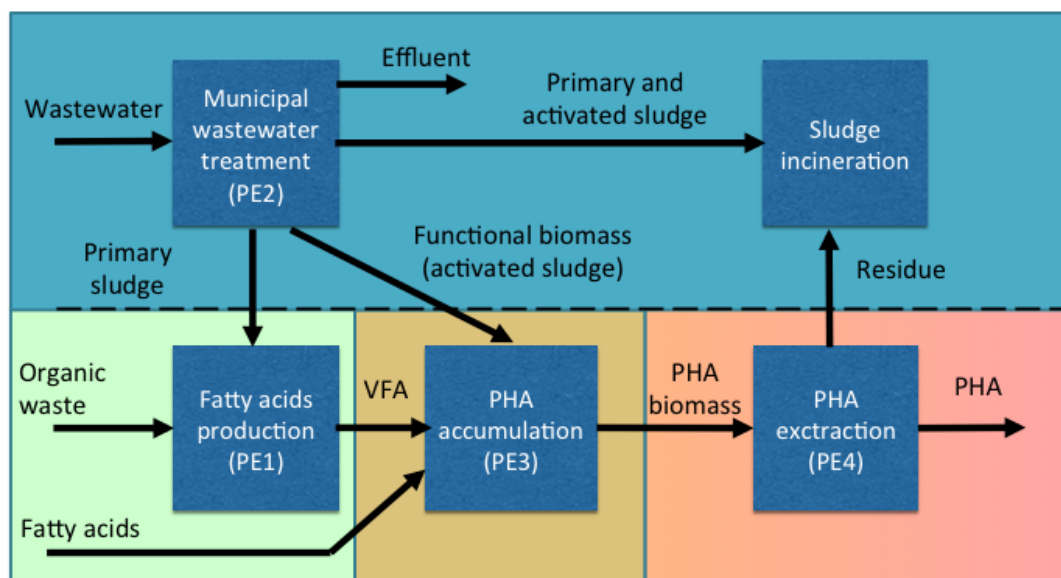
The PHA rich biomass is acidified to conserve the PHA and then dewatered in centrifuges or belt filter presses. The dewatered PHA rich biomass is then dried in a thermal dryer to a dry matter content of at least 90%. Following the drying the PHA is then extracted in an extraction facility where solvents like butanol are used to extract the PHA from the biomass. The extraction takes place at elevated temperature to dissolve the PHA in the solvent. Through cooling of the solvent the PHA can be recovered from the solvent and the solvent reused (see Werker, 2015). An important advantage of this type of extraction is that it safeguards the

quality of the PHA and provides opportunities to control the quality and blend different PHA batches to a compound.

The residual matter after extraction is then incinerated in a similar way as the original sludge would have been. There is future potential for the extraction of lipids from this residue, but this was not investigated within the framework of the PHARIO project.

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PHA ACCUMULATION POTENTIAL OF WWTPS

3.1 INTRODUCTION

Production of polyhydroxyalkanoate (PHA) biopolymers integrated with municipal wastewater treatment may be accomplished with four process elements (PE1-4). In PE2, a surplus biomass with the potential to accumulate PHA is produced while wastewater is concurrently treated. The possibility to produce such a biomass with synthetic streams and process waters having relatively high concentrations of readily biodegradable chemical oxygen demand (RBCOD) has been already studied for a long time. More recently, was found that the relatively low concentrations of RBCOD generally found in municipal wastewater are sufficient to produce biomass with a high PHA accumulation potential (PAP) given that a selective pressure for storage of RBCOD is established in the biological treatment process. It has been estimated that the PAP should be at least 40 % g-PHA/g-VSS for economical down-stream recovery of the polymer.

Enrichment of a biomass with high PAP in municipal wastewater treatment has been observed at laboratory, pilot and, in one case, at full-scale treatment, namely at the wastewater treatment plant (WWTP) Bath. However, until now it has been unclear how common enrichment of PAP is at existing wastewater treatment plants and what factors with respect to process configuration and operation could influence the PAP level. Therefore, this study aimed to clarify the wider scope for sourcing municipal activated sludge for PHA production by investigating fifteen wastewater treatment plants across the Netherlands with respect to PAP and the related process operating conditions. Activated sludge biomass grab samples were obtained from all the plants and assessed for PAP under standardized conditions in Sweden. Information about the biological treatment processes was gathered from the ten associated water boards and site visits were made to most of the treatment plants as part of the survey.

3.2 SELECTION OF PLANTS

Fifteen wastewater treatment plants were selected with preference for large plants treating over 100 000 PE with variety of process configurations spread over a range of geographic locations within the Netherlands. Efforts were made to both include plants with factors that are known to be favorable for enrichment of PAP and some without. Consideration was given to factors such as instances with and without primary treatment, high and low fractions of industrial discharge, and high and low influent organic concentrations.

Information was gathered from the ten water authorities responsible for each of the respective treatment plants. The type of information that was requested included process configurations, process flow diagrams, water quality data (influent and effluent concentrations), operating

conditions and additional information such as separate streams discharged to the plant. Concentrations of total suspended solids (TSS) and solids retention times (SRTs) were obtained and nominal values for volumetric and specific organic loading rates (OLR and SOLR, respectively) were estimated. The sludge management procedures were determined to identify for any potential influence of return dewatering streams or similar. Special attention was devoted to identifying zones in the existing bioprocess that would tend to stimulate a feast-response in the biomass.

In addition to the desktop evaluations based on the information received from the water boards, dedicated site-visits were made to thirteen of the fifteen treatment plants. These visits gave the benefit of direct and visual impressions of the process operations, a chance for identification of undocumented quirks of operations, and more direct interaction with the responsible engineers and operators.

Most of the WWTPs included in the survey were of large size of 100 000 – 1 000 000 P.E. (TOD-150) although a few smaller plants were also included such as WWTPs Kootstertille and Workum. The WWTPs were operated at between 65 and 115 % of their respective design load. The influent total chemical oxygen demand concentrations were in the range 288 to 977 mg/L and the biochemical oxygen demand (BOD) concentrations were in the range 113 to 324 mg/L. The specific organic loading rates were between 0.08 and 1.7 g- COD/g-TSS/d and the SRTs between 0.3 and 29 days. The specific consumption of precipitation chemicals for P removal with respect to incoming P (mol-Me/mol-Pin) was from zero to 1.05 mol-Me/mol-Pin. The types of process configurations at the respective WWTPs are summarized in Table 1 as well as some key performance data. In all the selection embraced a wide range of process types, influent water qualities, and operating conditions. Detailed process information and descriptions are available in the full WP2 report.

TABLE 1   MAIN CHARACTERISTICS OF THE SAMPLED WASTEWATER TREATMENT PLANTS.

WWTP	Water authority	Actual capacity (P.E.-150 g TOD)	Process	Primary settling	SRT (days)	SOLR (g-COD/g-TSS/d)
Almere	Zuiderzeeland	200 000	Selector-Carrousel	No	16	0.18
Amsterdam W	Waternet	999 371	Modified UCT	Yes	12	0.15
Bath	Brabantse Delta	415 346	Predenitrification-nitrification	Yes	20	0.15
Beverwijk	HH Hollands Noorderkwartier	207 651	Predenitrification-nitrification	Yes	15	0.10
Dokhaven	Hollandse Delta	442 806	AB-system with predenitrification	No	0.3 *	6.2
Dordrecht	Hollandse Delta	203 264	A^{2}O with carrousel for polishing	No	18	0.13
Ede	Vallei en Veluwe	311 694	Modified Biodenipho™	Yes	21	0.11
Eindhoven	Dommel	610 286	UCT	Yes	18	0.13
Heerenveen	Fryslan	94 310	Selector-Carrousel	No	26	0.08
Kootstertille	Fryslan	42 857	A^{2}O	No	29	0.09
Land van Cuijk	Aa en Maas	158 957	A^{2}O	Yes	17	0.18
Nijmegen	Rivierenland	333 078	A^{2}O/Predenitrification-nitrification	Yes	16	n.a.
Sint Oedenrode	Dommel	91 224	Selector-Carrousel	No	15	0.13
Workum	Fryslan	14 663	Carrousel	No	25	0.08
Zaandam O	HH Hollands Noorderkwartier	109 059	UCT with carrousel	Yes	n.a.	n.a.

* A-stage

3.3 PHA ACCUMULATION POTENTIAL TESTS

3.3.1 METHOD

Activated sludge grab samples were collected from the aeration tanks at the 15 different WWTPs. The samples were transported to Lund and stored refrigerated (4°C) pending the PAP evaluation. Assessments for PHA accumulation potential were started within three days after the sampling.

The activated sludge samples (1.6-1.7 L) were incubated in a PLC-controlled fed-batch feed-on-demand reactor system. The temperature of the mantled 2-L reactors was controlled at 22±1°C. Mixing was provided with magnetic stirrer and the reactors were aerated through a glass membrane diffuser. DO as well as pH were monitored continuously. A substrate of acetic acid (100 g/L) supplemented with NH_4Cl and KH_2PO_4 was dosed in pulses based on a well-established respiration feed-on-demand control (Valentino et al., 2015a; Werker et al., 2011a) during the incubation of the biomass. The nutrient composition COD:N:P was 100:1:0.05 (mass basis) except with the biomass from WWTP Kootstertille that was without N (COD:N:P = 100:0:0.05).

Repeated feed pulses generated 90 - 165 mg-COD/L peak concentrations to maintain the biomass under feast conditions. The trends in DO signal were used to indicate depletion of substrate which triggered the feed pump to add the next pulse of substrate. The pH of the substrate was regulated to 5.0±0.5 by addition of NaOH. Allylthiourea (10 mg/L) was added to the activated sludge samples (except the biomass samples from WWTPs Workum, Kootstertille and Heerenveen) in order to inhibit nitrification for the laboratory based evaluation (note that allylthiourea is not added in any pilot scale work).

Acclimation of the biomass to the substrate was conducted according to a patent pending method (Werker et al., 2016) before each PAP assessment test to establish the same biomass history prior to the same applied accumulation conditions. The substrate for this acclimation was the same as for the accumulation. The acclimation consisted of a feast period with an initial reactor substrate concentration of 50 mg COD/L followed by a period of famine that was 3 times the duration of the feast. This acclimation procedure was repeated three times and lasted, in total, 2 to 4 h. The DO signal was used to monitor and control these feast and the famine acclimation periods. The PHA accumulation assessments were started immediately after the acclimation. Acclimation was not conducted before the accumulations with the biomass samples from WWTPs Workum, Kootstertille and Heerenveen.

The PHA accumulation assessments were conducted over at least 46 hours and grab samples were taken at selected times to monitor trends in biomass PHA content and water quality (COD, VFA and ammonium).

3.3.2 CALCULATIONS

Trends in biomass PHA content over time (PAPt in g-PHA/g-VSS) were fitted by least-squares regression analysis to the empirical function:

$$PAP_t = A_0 + A(1-e^{-kt})$$

with A_0, A and k as constants to facilitate quantitative comparison of the progress in biomass PHA accumulation as a function of time. A time constant was defined as $\tau = 1/k$ [h].

Trends in VSS and substrate consumption (St in g-COD) over time were fitted to linear and quadratic empirical functions by least squares regression, respectively. Yields and specific production rates of PHA were calculated as initial values as well as average values. Initial values were calculated over 0.2т (typically around 2 h of accumulation) and average values were calculated over 3т. The period 3т represents a time at which the biomass was nearly saturated and had nominally reached 95 % of its maximum PHA content. The initial (i) and average (a) yields of PHA on substrate $(Yi_{P/S}$ and $Ya_{P/S}$ in g-COD/g-COD) were calculated according to:

$$Y_{PS} = \frac{k(P_t - P_0)}{S_t}$$

with P_t and P_0 being the mass of PHA produced at time t and time 0, respectively, and t being 0.2т and 3т for $Yi_{P/S}$ and $Ya_{P/S}$, respectively. The conversion factor for polyhydroxybutyrate mass to COD, k_p, is equal to 1.67 g-COD/g-PHB.

The active biomass (X in g/L) was calculated by subtracting the concentration of PHA from the measured VSS. The initial (maximum) specific PHA production rate (qi_{PHA} in mg-PHA/g- X/h) and the average specific PHA production rate (qa_{PHA}) were calculated by estimating increase in the PHA concentration over time and dividing by the active biomass concentration for time periods of 0.2т and 3т for qi_{PHA} and qa_{PHA}, respectively.

3.4 RESULTS

3.4.1 GENERAL BEHAVIOUR OF THE ACCUMULATION EXPERIMENTS

The PHA content in the biomass samples gradually increased over the course of the accumulation tests until saturation levels of PHA content were reached. The majority of the PHA storage was achieved in the first 24 h. The 46 h accumulation time was applied to be conservatively long enough to reach PHA saturation for all biomass samples. With two biomass samples, from WWTPs Nijmegen and Eindhoven the PHA content reached a saturation level, that then became consumed by the biomass. The reason for this consumption was most likely related to a shift in physiological state in the biomass from PHA storage to active biomass growth. The external substrate was consumed alongside the consumption of the internally stored PHA and thus, this observed onset of PHA consumption was not related to an interruption in supplied external substrate consumption in these cases.

The PAP observed for WWTP Bath was 39% g-PHA/g-VSS. Testing of the WWTP Bath biomass was an experimental control given the wealth of experience for the same biomass during pilot testing. This particular PAP result confirmed that the standardized laboratory-scale PAP assessments were representative of outcomes at pilot scale made under realistic field conditions. The 39 % PAP level observed with the WWTP Bath biomass matched very closely the 39-42 % g-PHA/g-VSS (Hjort et al., 2016) PHA content obtained with the Cella™ pilot on site and during the same month of production.

3.4.2 ACCUMULATION RESULTS

Table 2 summarizes the results from the PAP-assessments for the different WWTPs. Figure 5 shows the full curves for the seven best performing WWTPs.

The PAP-assessments in the present study give a "snapshot" of the surplus activated sludge from the different locations and at the time of sampling. The PAP of a biomass may vary over

time as a function of variations in environmental and operational conditions at the WWTP. It may also be influenced by conditions of accumulation as well as the feedstock VFA composition. For example, the experience with biomass from WWTP Bath is that it exhibits a consistently high PAP although the PAP has varied from between 40 to as high as 55% g-PHA/g-VSS over the course of the pilot operation.

It is estimated that for an economical viable PHA recovery the biomass should have a PAP of at least 40 % g-PHA/g-VSS. The outcomes show clearly that high PAP in biomass at existing WWTPs is not a feature that is unique for one specific plant but is relatively wide spread. Among the fifteen plants tested in this study, four plants (27 %) had a PAP level of at least 38 % g-PHA/g-VSS. The PAP level for the biomass from WWTP Beverwijk was well above the threshold criterion and is therefore already today a very interesting resource for PHA production. It is noteworthy that these positive outcomes from the survey exist without any specific attention being applied at the respective WWTPs for maximizing PAP. A well-tuned municipal WWTP could possibly achieve up to 60 % PAP given that specific attention is placed on the existing bioprocess to ensure an optimal selection pressure for PHA accumulation.

Besides the biomass from WWTPs Beverwijk and Bath, the biomasses from WWTPs Dordrecht, Workum, Dokhaven and Heerenveen should also be considered as already potential sources of biomasses for PHA production since they exhibited PAP levels that are approximately at the 40 % threshold for an economic polymer recovery. The other biomasses exhibited PAP levels that are lower, and these facilities would therefore require the inclusion of potentially rather simple adjuncts for bioprocess optimization in order to stimulate for PAP as will be discussed later.

FIGURE 5   PAP DURING THE ACCUMULATION ASSESMENTS FOR THE SEVEN BEST PERFORMING WWTPS. CURVES FOR THE OTHER WWTPS ARE AVAILABLE IN THE DETAILED WP2 REPORT.
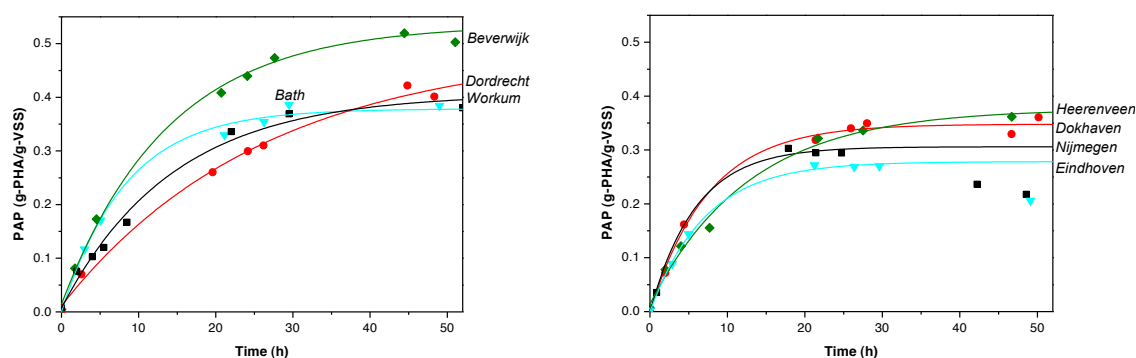
The initial (maximum) specific PHA storage rate was between 12 and 47 mg-PHA/g-X/h and the average specific storage rate was between 4.6 and 21 mg-PHA/g-X/h for all the assessments. Overall, there was no correlation between PAP and storage rates. Therefore, the rate of PHA accumulation by a biomass is not necessarily tied to the amount of polymer the biomass can accumulate.

The yields of PHA on substrate in the PAP assessment were generally initially high (0.37-0.70 g-COD/g-COD) to then decrease when the biomass approached saturation of PHA. The theoretical maximum yield is about 0.7 g-COD/g-COD in complete absence of growth (Beun et al., 2002). Since the PHA storage rate is generally lower when the biomass approaches PHA saturation levels, the PHA yields also tend to decrease over time. Average PHA yields, measured until near saturation, were in the range 0.19 to 0.39 g-COD/g-COD.

It should be noted that PHA yields and production rates may vary with the type of substrate. A biomass can have a higher yield and production rate when using a real fermented substrate than with a synthetic and single model substrate such as applied in these assessments (Bengtsson et al., 2017; Morgan-Sagastume et al., 2015). Therefore the values of yields and rates with the WP2 bioassays are reflective of differences between locations but not necessarily absolute values for the respective biomass sources.

TABLE 2   RESULTS FROM THE PAP ASSESSMENTS REGARDING THE MAXIMUM PHA CONTENT (PAP), INITIAL PHA YIELD (YI$_{P/S}$), AVERAGE PHA YIELD (YA$_{P/S}$), INITIAL SPECIFIC PHA PRODUCTION RATE (QI$_{PHA}$) AND AVERAGE SPECIFIC PHA PRODUCTION RATE (QA$_{PHA}$).

| Biomass | PAP | Yi$_{P/S}$ | Ya$_{P/S}$ | qi$_{PHA}$ | qa$_{PHA}$ |
	g-PHA/g-VSS	g-COD/g-COD	g-COD/g-COD	mg-PHA/g-X/h	mg-PHA/g-X/h
Beverwijk	0.52	0.70	0.32	35	21
Dordrecht	0.42	0.46	0.23	17	12
Bath	0.39	0.52	0.30	41	21
Workum	0.38	0.57	0.29	25	15
Heerenveen	0.36	0.53	0.22	26	8.8
Dokhaven	0.36	0.41	0.30	42	19
Nijmegen	0.30	0.61	0.27	47	18
Eindhoven	0.27	0.37	0.30	36	10
Ede	0.26	0.47	0.23	20	7.5
Kootstertille	0.24	0.56	0.39	27	10
Land van Cuijk	0.24	0.49	0.22	17	6.3
Amsterdam West	0.24	0.59	0.27	23	9.2
Sint-Oedenrode	0.21	0.46	0.23	16	5.6
Almere	0.17	0.40	0.19	12	4.6
Zaandam Oost	0.15	0.47	0.34	33	11

3.4.3   FACTORS INFLUENCING PHA ACCUMULATION POTENTIAL
3.4.3.1   PROCESS CONFIGURATIONS
The WWTPs were categorized with respect to process configuration according to the following:
- Predenitrification-nitrification (WWTPs Bath and Beverwijk)
- A2O (Anaerobic-anoxic-aerobic) process (WWTPs Dordrecht, Kootstertille, Land van Cuijk and Nijmegen)
- UCT (University of Cape Town) or Modified UCT process (WWTPs Amsterdam-West, Eindhoven and Zaandam-Oost)
- Carrousel with or without selector (WWTPs Almere, Heerenveen, Sint-Oedenrode and Workum)
- Other (AB-system, WWTP Dokhaven and Modified Biodenipho™, WWTP Ede)

When comparing process configurations with PAP outcomes (Figure 6), it was found that PAP levels were generally higher for biomasses from processes with only predenitrification and nitrification than for biomasses from processes with anaerobic and anoxic tanks in series. The latter category includes A^{2}O and UCT/MUCT processes.

The principal difference is that in the predenitrification process, nitrate becomes available to the biomass together with the influent RBCOD and thus, an anoxic feast response may be more readily established. In the A^{2}O and UCT/MUCT processes, RBCOD from the waste-water is first exposed to the biomass under anaerobic conditions. Although storage of RBCOD occurs also under anaerobic conditions, anaerobic storage metabolism is distinctly different compared to those under aerobic and anoxic conditions.

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RBCOD supplied to a biomass under anaerobic conditions promotes enrichment for phosphate-accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) in the biomass. These organisms also store PHA but with a metabolism that also includes storage of glycogen and, in case of PAOs, polyphosphate. PAOs and GAOs are generally considered to utilize specific low-molecular-mass organic compounds as substrates such as VFAs (Oehmen et al., 2007) and glucose (Kristiansen et al., 2013)how similar their ecophysiology is to 'Candidatus Accumulibacter phosphatis' is unclear, although they may occupy different ecological niches in EBPR communities. The genomes of four Tetrasphaera isolates (T. australiensis, T. japonica, T. elongata and T. jenkinsii. In contrast, anoxic storage may occur with a broader range of organic compounds that contribute to the RBCOD fraction of municipal wastewater influent. Therefore, it can be hypothesized that predenitrification on the incoming wastewater under anoxic conditions can be made to promote for a stronger feast stimulation when compared to anaerobic conditions. Notwithstanding, enrichment for high PAP with A^2O and UCT/MUCT processes may still be possible but with some further optimization or minor process modifications (see also paragraph 3.5.2).

FIGURE 6   PERFORMANCE IN PAP ASSESSMENTS FOR BIOMASS FROM DIFFERENT TYPES OF PROCESS CONFIGURATIONS.
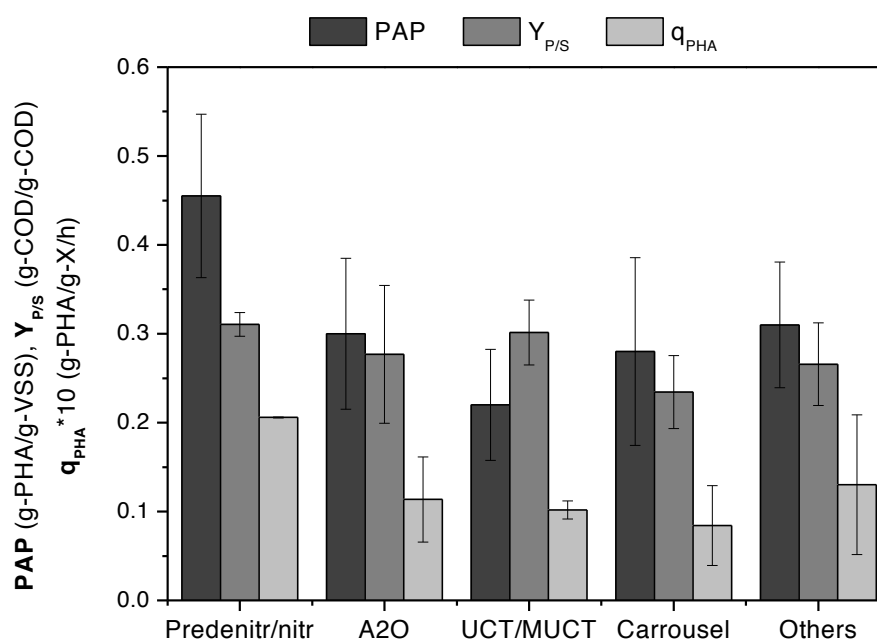
3.4.3.2 INFLUENT WASTEWATER CONCENTRATIONS

Since it is known that exposure of relatively high concentrations of RBCOD to the biomass stimulates storage, it could be expected that higher influent COD and BOD concentrations generally lead to higher PAP. However, and surprisingly, there was no such correlation found in the present investigation (Figure 7). Actually, the highest PAP levels that were observed (WWTPs Beverwijk and Dordrecht) were from treatment plants with influent organic concentrations in the lower range (around 425 mg-COD/L and 180 mg-BOD/L). And the high influent COD concentration at WWTP Almere (977 mg/L) due to the almost storm-water-free effluent, did not at least on its own, promote a high PAP level.

FIGURE 7   PERFORMANCE IN PAP ASSESSMENTS FOR BIOMASS FROM DIFFERENT TYPES OF PROCESS CONFIGURATIONS.
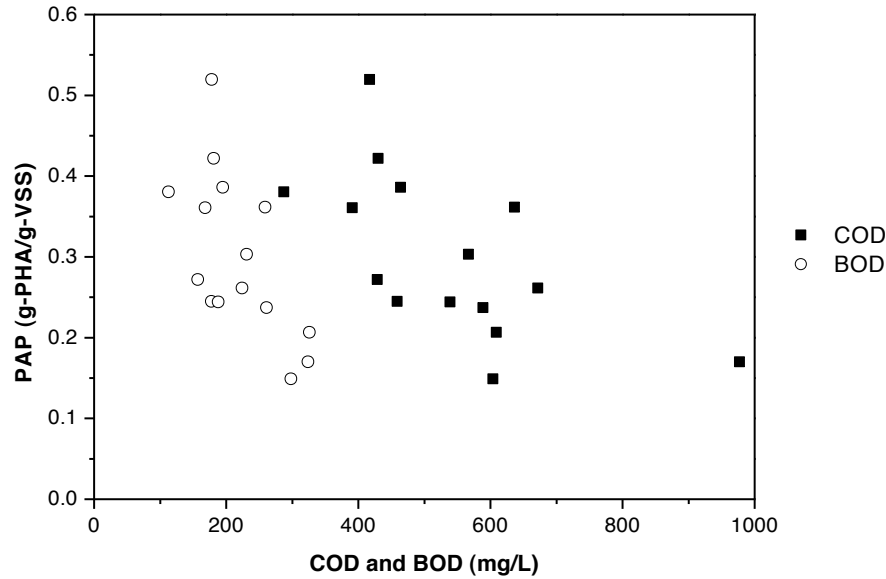
These observations strengthen the impression that rather than the concentration of organic matter in the wastewater itself, it is the RBCOD level that the biomass is periodically exposed to that is crucial for a feast stimulation and, feast stimulation is a critical factor controlling the level of enrichment with respect to PAP.

3.4.3.3   OPERATING AND ENVIRONMENTAL CONDITIONS

Operating conditions such as SRT (solids retention time or sludge age) and organic loading rates were evaluated for a potential correlation with PAP. Here it as also found that no such correlations existed within the present study, neither for SRT nor for volumetric or specific organic loading rates (Figure 8). Biomass from treatment plants operated under intermediate loading rates (0.3-0.5 g-COD/L/day) exhibited PAP levels varying over a wide range from 15 to 52 % g-PHA/g-VSS. The biomass from the A-stage of WWTP Dokhaven, that is operated under extremely low SRT (0.3 days) and high organic loading rates (9.3 g-COD/L/day and 6.2 g-COD/g-TSS/day) compared to the other plants, exhibited a much less extreme PAP level of 36 % g-PHA/g-VSS. Again, while low SRT and high organic loading rates can be understood to be helpful towards PAP and PHA accumulation kinetics, these factors are not essential criteria towards producing a surplus activated sludge from municipal wastewater treatment with high PAP.

FIGURE 8   LEFT: PAP LEVELS VERSUS SOLIDS RETENTION TIME (SLUDGE AGE) IN THE TREATMENT PLANTS. RIGHT: PAP LEVELS VERSUS VOLUMETRIC AND
                  SPECIFIC ORGANIC LOADING RATES IN THE TREATMENT PLANTS.

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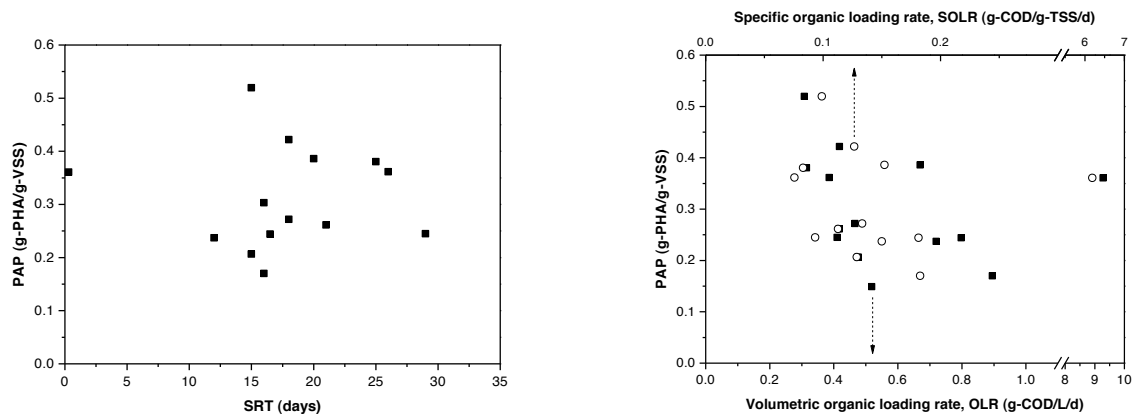
Thus, based on these observations, and somewhat unexpectedly as an exciting outcome from PHARIO, we find that enrichment for PAP should be feasible within a wide range of loading rates and process operating conditions. The broad range of loading rates and sludge age that are typically applied for municipal wastewater treatment are generally sufficient to drive selection pressures for a biomass with significant PAP.

Nevertheless, a low SRT for the biomass may have advantages in the context of PHA production due to a higher fraction of active cells in the biomass which ultimately may lead to a higher PAP and/or more rapid accumulation kinetics. Short SRTs also mean greater biomass yield and lower oxygen demands. Thus, a shorter SRT can lead to a higher PAP and increase the amount of surplus biomass that is available as a resource for PHA production. With the pace of development for mainstream Anammox nitrogen treatment, a high rate heterotrophic biomass can be produced for carbon removal (Morgan-Sagastume et al., 2015), while the Anammox processes manages the effluent nitrogen water quality (Laureni et al., 2016)including over 5 months at 15 degrees C. The two systems consisted of a moving bed biofilm reactor (MBBR).

Five of the plants were operated under temperatures that were considered to have been elevated compared to the norm. In some of these cases, treated effluent is used as cooling water by nearby facilities and returned to the biological process (WWTPs Beverwijk, Dordrecht and Nijmegen). In case of WWTP Almere, the temperature is elevated due to the high extent of sewer separation between wastewater and storm water flows. The relatively high temperature at WWTP Workum is due to an influence of the relatively warm dairy effluent discharged directly to the plant. From the survey, there was nothing to suggest a difference for treatment plants operated at relatively higher temperatures (36±13 % g-PHA/g-VSS) and the other plants (27±8 % g-PHA/g-VSS). Thus, high temperature is not a prerequisite for enrichment of PAP.

3.4.3.4   PRIMARY TREATMENT

It is generally considered that primary treatment of influent wastewater is a benefit to the goals of producing a biomass for PHA production value chains. In the first instance, primary treatment creates for an opportunity to recover suspended solids for bioenergy or VFA production and cellulose as a renewable resource. For PAP enrichment, primary treatment is advantageous since otherwise, influent solids that are not degraded in the process (fibers, inert organic solids, etc.), and that end up associated with the biomass solids, detract from the harvested "functional" biomass *quality*. Quality is reduced since the active fraction of the biomass may become "diluted out" by associated inert suspended solids. Furthermore, without primary treatment, more colloidal COD that is degraded more slowly can reduce the extent to which the biomass will be disposed to sufficiently stringent famine conditions. A stringent famine condition is the compliment to feast that creates for a selective advantage for PHA-storing microorganisms in the biomass.

Eight of the plants in the present survey were with primary clarifiers. These plants had average PAP levels (31±11 % g-PHA/g-VSS) and this average was similar to those without primary clarifiers (29±9 % g-PHA/g-VSS). Thus, even though primary settling is generally considered advantageous for high PAP, it was not a determining factor for the surveyed facilities and a significant PAP may still be possible for an activated sludge without the benefit of a primary clarifier.

3.4.4   SEPARATE COD-RICH STREAMS

The eight treatment plants that receive contributions in form of separate streams with relatively high concentrations of COD are listed below (Table 3). Although it is not possible to determine

the influence of these separate streams on the enrichment for PAP, it is clear from this and previous work that significant contributions from such streams are not necessary for high PAP as evidenced by the relatively high PAP levels observed for WWTPs Bath, Beverwijk and Dordrecht.

This principle is perhaps most convincingly demonstrated by observations from the pilot plant project carried out at WWTP Leeuwarden before the PHARIO project. Treatment of the Leeuwarden wastewater, which is dominated by domestic household wastewater, in the Cella™ pilot plant resulted in a PAP level of 49 % g-PHA/g-VSS. This high PAP level may be compared to 15 % for the surplus activated sludge produced from treating the same wastewater in the full-scale wastewater treatment plant (Bengtsson et al., 2017).

TABLE 3   SUMMARY OF CONTRIBUTIONS FROM SEPARATE COD-RICH STREAMS TO THE TREATMENT PLANTS.

WWTP	Type of contribution	Fraction of TCOD loading (%)	PAP (g-PHA/g-VSS)
Bath	Condensate from SNB	3	0.39
Beverwijk 1-6	Condensate/SHARON	13	0.52
Dokhaven	Centrate/Anammox	n.a.	0.36
Dordrecht	Condensate from HVC	6	0.42
Eindhoven	Centrate from Mierlo	n.a.	0.27
Heerenveen	Filtrate from SOI	22	0.36
Nijmegen	Dewatering supernatant	n.a.	0.30
Workum	Dairy industry	50	0.38
Others PHARIO	None	0	0.22 ± 0.04
Leeuwarden Full-scale	None	0	0.15[1]
Leeuwarden Cella™ pilot	None	0	0.49[1]

[1] (Bengtsson et al., 2017)

Although such separate RBCOD rich streams may not be necessary, they can still serve to benefit the PAP enrichment in the biomass. It appears likely that separate streams facilitated the enrichment in two cases. For WWTP Heerenveen and Workum, the process configurations alone were not expected to provide for a strong selective pressure but PAP levels were still relatively high. These plants both receive contributions from the separate streams that contribute to relatively high fractions of the COD load to the plants, namely 22 and 50 %. If the process configuration does not by design provide for PAP enrichment, a separate COD-rich stream may facilitate an improvement in the selective pressure by generating a feast zone. Hence, although a separate COD-stream is not necessary for enrichment of PAP, if such a stream is used purposefully and strategically to stimulate a periodic feast response for at least some of the biomass for some of the time, then the process will enrich a biomass with significant PAP.

3.5 APPROACHES TO IMPROVE PHA ACCUMULATION POTENTIAL

3.5.1 ESTABLISHING DISTINCT FEAST CONDITIONS FOR THE BIOMASS

From the comparison of PAP outcomes and pilot plant operating conditions, it became clear that in order to achieve enrichment of high PAP, there is no need for very unusual or special conditions with respect to SRT, loading rates or temperature. Furthermore, enrichment is feasible with different types and concentrations of wastewater. Separate COD-rich streams may improve the enrichment for PAP but this is by no means a necessary factor. Wastewater dominated by domestic household contributions have sufficient levels of readily biodegradable COD to promote PAP enrichment given that the process configuration is somehow made to be favorable for enrichment.

Based on the observations made from all the surveyed treatment plants, it furthermore was considered that the most crucial factor for PAP enrichment regardless of the process configuration is how the influent RBCOD in the wastewater is brought in contact with the biomass. It seems as if the contact zone for RBCOD and biomass is such that at least some of the biomass and some of the time periodically experiences a relatively high concentration of RBCOD, then storage is induced which ultimately leads to a selection pressure for a higher PAP in the surplus biomass.

Process configurations that lead to feast-stimulating conditions can be exemplified by WWTP Beverwijk, the plant that exhibited the highest PAP levels from in this survey. At this plant, the return activated sludge (RAS) is directed to the initial part of the biological treatment (Figure 9). The main part of the RAS is not brought in contact with the influent wastewater but directed to a large tank for predenitrification (and intermittently nitrification for treatment lines 1-2). A minor part of the RAS is directed to a small contact zone in which the influent wastewater also enters. By only bringing a minor part of the RAS in contact with the influent RBCOD rather than all the RAS, a higher RBCOD concentration can be periodically established for at least some of the biomass than otherwise would be the case. In this way, fractions of the biomass will repeatedly be exposed to relatively high concentrations of RBCOD. Over time, all the biomass contained in the process will eventually experience this relatively high RBCOD concentration, which will lead to a general benefit from a storage response and ultimately enrichment for PAP.

FIGURE 9   SCHEMATIC DRAWING AS TOP VIEW OF TREATMENT LINES 1-2 AT WWTP BEVERWIJK.
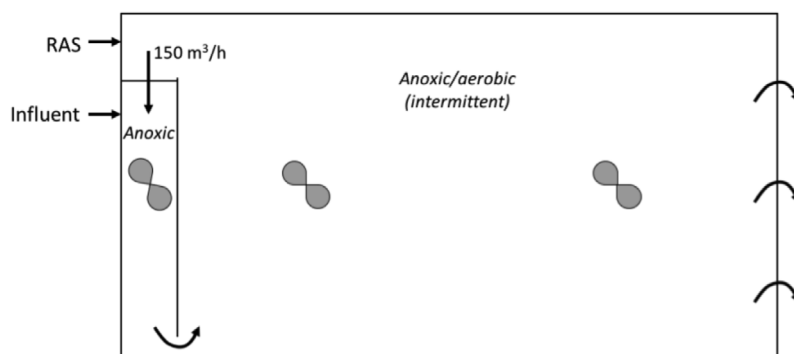
Although all treatment plants may not have configurations that are purposeful in order to create feast-stimulating zones, process modifications that are relatively minor and simple can achieve the same effect and thereby provide significant improvements in enrichment performance without changing configuration or water quality management strategy. Examples of how a feast-stimulating zone can be established are:
•   A small tank up-front of the main treatment volume where influent wastewater and RAS, or a fraction of the RAS, are brought in contact.
•   Modification of the confluence point between return biomass and influent such that the contact zone is well defined and small enough to provide an elevated RBCOD concentration exposed to the biomass.
•   A side-stream sequencing feast reactor (SFR) in which return biomass is mixed with influent wastewater batch-wise at given intervals.

The principle behind the side-stream SFR relies on intermittently feeding return biomass to the reactor, followed by feeding wastewater to the reactor and thereafter allowing the

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biomass to take up the RBCOD for storage (Figure 10). The relatively high concentration of RBCOD will stimulate the biomass to feast conditions. Each sequence can be terminated after depletion of RBCOD. At this point, the reactor contents can be pumped back to the main treatment volume, in which famine conditions are inherently maintained and thereafter, a new cycle can be initiated. The influent to the SFR may be the main wastewater or a separate stream rich in RBCOD. The latter alternative may help to further improve PAP enrichment if such a stream is available.

The side-stream SFR approach may in many cases represent a flexible and cost effective means of upgrade for improved PAP. The advantages include relatively small modifications and large operational flexibility. Since the reactor is controlled by an operational sequence, the feeding of biomass and wastewater can be individually controlled in order to establish an optimized feast response in the biomass. A cost effective and tunable adjunct to the process permits to optimize the selection pressure without influencing the main hydraulic flows in treatment.

FIGURE 10   A SIDE-STREAM SEQUENCING FEAST REACTOR AS AN EXAMPLE OF A MINOR PROCESS MODIFICATION TO IMPROVE SELECTIVE PRESSURE FOR PAP ENRICHMENT.
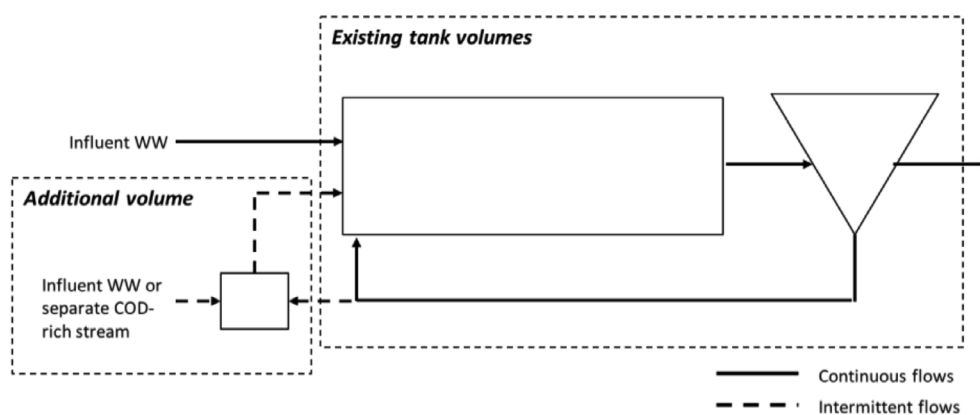
3.5.2 INTEGRATION OF EBPR AND PHA PRODUCTION

The treatment plants in this survey rely to a varying degree on enhanced biological phosphorus removal to meet their effluent P demands. Four of the plants have no design elements that favor EBPR such as a selector that could be expected to become anaerobic, namely WWTPs Bath, Beverwijk, Dokhaven and Workum. As described above, the plants with A^2O or UCT/MUCT process configurations were generally with lower observed PAP levels.

Feast stimulation for PAP enrichment with a municipal wastewater appears to be most favorable under aerobic or anoxic conditions. Anaerobic feast conditions will favor polyphosphate-accumulating organisms (PAOs) and PAOs are a subpopulation of the PHA-storing organisms that are clearly not required for achieving high PAP. The anaerobic uptake of RBCOD that occurs by PAOs in the EBPR process have been observed to rely mainly on some specific organic compounds such as VFAs (Oehmen et al., 2007) and glucose (Kristiansen et al., 2013). Anoxic storage, on the other hand, can occur with a broader range of organic compounds that make up the RBCOD. It may be suggested that within the UCT/MUCT process, stronger selective pressure for PHA storage could be established if it would be possible to utilize the "break through" residual RBCOD after the initial RBCOD uptake by PAOs for a second, anoxic, feast stimulation. Such feast stimulation would require a well-defined contact zone in the initial part of the anoxic volume of the UCT/MUCT process. Improvement of the PAP in surplus biomass from EBPR processes require further optimization. However, given that storage is

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already an integral component of the EBPR mechanism, necessary process modifications are most likely to be minor.

Integration of PHA production with EBPR require specific consideration and strategies for P management. When subjected to aerobic PHA accumulation (in PE3), PAOs with stored poly-P will release ortho-P back into the water phase (Anterrieu et al., 2014; Guisasola et al., 2004; Pijuan et al., 2005; Rodgers and Wu, 2010). If this P release is strategically placed then, P can be harvested for recovery by precipitation as an integral part of the PHA accumulation process.

3.6 POTENTIAL FOR PHA PRODUCTION

Estimations were made to assess the quantity of PHA that may be produced from the WWTPs included in the survey given regionally available VFA feedstocks. The calculations were made based on the assumption that the enrichment for PAP could be conservatively improved such that the harvested excess biomass from the treatment plants was able to accumulate at least 40% g-PHA/g-VSS. Conservative assumptions of yields for biomass and PHA as well as COD removal were made based on previous experience (Table 4).

It was estimated that most of the plants could potentially supply biomass for the production of between 1 000 and 6 000 ton PHA per year (Table 5), provided enough VFA feedstocks can be sourced from the region. The total potential considering all the 15 plants is in the order of 25 000 ton-PHA/y. Thus, each of the large plants (around 100 000 P.E. and above) could be providing raw material in form of surplus biomass for production of commercially significant quantities of PHA.

TABLE 4   ASSUMPTIONS MADE BASED ON EXPERIENCE FOR CALCULATING POTENTIAL PHA PRODUCTION.

Parameter	Unit	Value
Sludge yield. Biomass harvested per COD removed	g-VSS/g-COD	0.25
PHA content in the biomass after PE3	g-PHA/g-VSS	0.40
COD removal efficiency in PE2	g-COD/g-COD	0.93
PHA yield on VFA in PE3	g-PHA/g-COD	0.187

TABLE 5   ESTIMATED POTENTIAL PHA PRODUCTION FROM THE WWTP INCLUDED IN THE STUDY BASED ON THE ASSUMPTIONS IN TABLE 4.

	PE	ton-PHA/y
Almere	200 000	1 700
Amsterdam-West	999 371	5 800
Bath	415 346	2 700
Beverwijk	207 651	1 200
Dokhaven	442 806	2 500
Dordrecht	203 264	1 100
Ede	311 694	1 600
Eindhoven	610 286	3 600
Heerenveen	94 310	450
Kootstertille	42 857	250
Land van Cuijk	158 957	1 000
Nijmegen	333 078	2 300
Sint-Oedenrode	91 224	600
Workum	14 663	75
Zaandam-Oost	109 059	700
Total	4 234 566	25 575

3.7 SUMMARY & CONCLUSION

It was found that four of the investigated plants (27 %) already exhibited PAP levels close to the threshold level of 40%. The high PAP level in biomass from WWTP Bath (39% g-PHA/g- VSS) was confirmed which proved the relevance of the standardized test procedures that were applied for larger scale PHA accumulations. Two other WWTPs, namely WWTPs Dordrecht and Workum, exhibited similar PAP levels and the biomass from WWTP Beverwijk exhibited the most significant PAP of 52%. Therefore, even without specific optimization of the biopro-cess, a relevant high PAP for PHA production may be possible at several locations.

Plant configurations based on pure predenitrification and nitrification were generally associated with higher PAP than plants with anaerobic and anoxic tanks in series. This confirmed, as expected, that feast stimulation of the biomass with wastewater RBCOD under anoxic (or aerobic) conditions more naturally leads to enrichment of high PAP whereas other configurations may need some optimization for periodic feast to at least some of the biomass some of the time.

No apparent correlations were found between operating conditions such as sludge age and loading rates and PAP or between influent concentrations and PAP. Thus, enrichment for high PAP was found, to our surprise, to be generally feasible and for a broad range of operating conditions, bioprocess configurations, and with different types of wastewaters. Based on the outcomes and the observations made on the sites, we consider that the initial contact zone for biomass and wastewater RBCOD is a critical factor for driving PAP enrichment. Several alternative and relatively simple strategies are available for process modifications to improve feast stimulation of the biomass. Optimization for PAP is likely to be readily possible with case to case attention to detail, and only minor process modification.

With 4 of the 15 plants investigated having PAP levels near or above 40%, the potential for water board biomass for PHA value chain development is already significant. Already these four plants show a production potential of 7.500 ton PHA/year which is more than suffi-cient for a commercial production scale for which a minimum scale of 5.000 ton PHA/year is suggested to be required.

One can estimate that PHA production from just the treatment plants surveyed for the present investigation could be brought to at least conservative improvements in PAP levels to reach 40% g-PHA/g-VSS. In this way, a potential annual PHA production capacity of 1 000 – 6 000 ton/year for most of the surveyed plants would be possible and this translates to a production of 25 000 ton PHA per year for a total treatment capacity of 4,2 million people equivalents. Thus, each of the water boards involved in this survey could be stakeholders within a biobased biopolymer supply hub providing surplus biomass as a raw material to produce commercially relevant amounts of PHA. Given that methods for improved PAP are expected to be possible with only minor process modifications, this potential could be readily expanded and spread nationally.

4

TESTING THE PHARIO PROCESS

4.1 PILOT PLANT OPERATION

Production of PHA in PHARIO involved 4 process elements, or PE1 to PE4 (Figure 3 to Figure 5). PE1 was a pilot scale (1200 L) well-stirred and thermostated batch anaerobic fermentation vessel called BioVAP that was operated on site at WWTP Bath. BioVAP had been previously employed in a collaborative project with AnoxKaldnes, KNN and Wassenaar (https://www.nom.nl/wassenaar-maakt-bioplastic-van-plant-enresten-2/, Karlsson et al. 2014). PE2 was a full-scale WWTP process for active biomass production (WWTP Bath). PE3 was a pilot-scale (500 L) fed-batch process for PHA-rich biomass production using surplus PE2 activated sludge, and fermentation filtrate from PE1 as feedstocks (Morgan-Sagastume et al. 2015, Bengtsson et al. 2017). PE3 was also operated on site at WWTP Bath. PE4 was a pilot scale (10-L) batch green-solvent PHA recovery process located in Sweden (Magnusson et al. 2016).

In the normal routine of production operations, two accumulation batches were performed each week from June 2015 to March 2016. For each batch, a grab sample of freshly thickened (50 to 70 gTS/ kg) surplus WWTP Bath activated sludge was obtained for the biomass supply. The grab sample was assessed for solids content and about 1 kilogram of thickened activated sludge (as dry volatile solids) was delivered to the accumulation process along with dilution water. The accumulation pilot process comprised a working aeration volume of about 400 L coupled to a clarifier volume of 120 L (Morgan-Sagastume et al. 2015). The aerated volume was mechanically stirred and air was supplied via a blower with coarse bubble aeration. Active pumping to, and return from, the clarifier maintained a short solids residence time in the clarifier. Depletion of dissolved oxygen for the biomass while in the clarifier maintained oxygen limiting kinetics which are known to prolong the metabolic activity associated with accumulation processes (Pratt et al. 2012). Therefore, time for the fractions of biomass in the clarifier at any one time was simply an accumulation dead time.

There is no specific requirement that the activated sludge biomass source should be thickened. Thickened biomass was used as the source for PHARIO to simplify the material flow control and to decouple the pilot operations from the full-scale wastewater treatment plant operations. Furthermore, in the process scale up, an accumulation process may not be directly adjacent to the wastewater treatment plant.

4.1.1 VFA FEEDSTOCKS

PHA was accumulated in the biomass by semi-continuous supply of a VFA rich feedstock. Organic sources for VFA feedstocks used in the project were either a fermented carbohydrate rich process effluent delivered from a local candy factory, fermented primary sludge centrate from primary municipal sludge delivered from Waterschap De Dommel (Tilburg facility), and defined mixtures of acetic and propionic acids.

The process water from the candy factory was delivered batch wise once per week to WWTP

Bath in IBC containers. The BioVAP pilot fermenter was used to maximize the fermentation product content in a well stirred and temperature controlled volume of 1200 L. The process water was trimmed with dilution water to about 18 gCOD/L and was incubated at 37°C for up to 7 days with a pH controlled nominally between 5.5 and 6.0. A 7-day incubation was not required for the fermentation, but this was time given conservatively and for simplicity with respect to all other aspects of the hands-on piloting production activities. The pH was maintained with a PID controller and pulse wise additions of concentrated NaOH (45 % w/w). The fermentation process biomass (nominally about 1.2 gTSS/L) was maintained within the process at an SRT of between 7 and 10 days. Suspended solids from the candy factory process water fermentation effluent were removed by means of pre-settling with added flocculent polymer (Flopam FO 4800 SH, SNF Floerger), followed by a Hydrotech drum filter model 801 (HDF 801, with 10 μm screen). Suspended solids levels in and out of the HDF were on average 0.6 and 0.2 g/L, respectively. The fermentation product water for the accumulation process contained on average 16 gCOD/L as soluble RBCOD (readily biodegradable COD). Decrease in COD across the fermentation process was primarily due to water entrainment from the pilot HDF backwash operations. The feedstock nutrient balance of COD:N:P was 100:0.5:0.1 (by weight).

Primary sludge was also delivered fresh to WWTP Bath from the De Dommel Tilburg facility in IBC containers. Solids content was variable, but on average it was 45 g/L. The batches of delivered primary sludge were fermented with continuous mechanical stirring for 6 days at 37°C, with pH monitoring but with no pH control. The sludge matrix was self-buffering and generally the pH was inherently maintained between 4.8 and 5.5. No solids were retained in the BioVAP process between batches because unlike the process water from the candy factory, such sludge is already richly inoculated for anaerobic fermentation microbial activity. The fermenter batch was first discharged to a holding tank and then under automation control pumped to a centrifuge decanter (Morgan-Sagastume et al. 2015) with rotating bowl (3000 rpm) and a relative velocity between bowl and screw of 10 rpm. Cationic polymer (Flopam FO4800SH, SNF/ FLOERGER) was added in-line for coagulation/flocculation to improve the solids/liquid separation. The polymer addition was set and controlled by PLC to 50 g-polymer/kg-TSS. The solids reject cake was collected and disposed to the full-scale anaerobic digestion facility on site. Centrate was collected in a holding tank and iron chloride was added from concentrated solution (44% w/w) to precipitate excess soluble phosphorus based on a molar dosing ratio of 1.42 mol Fe:mol PO4. The centrate was further processed through the pilot Hydrotech drum filter (HDF 801, with 18 μm screen) to produce feedstock batches for the pilot-scale accumulation. This fermented primary sludge feedstock was about 7 gCOD/L with a COD:N:P balance of 100:5:0.1 (by weight).

So-called "synthetic" feedstocks were generated by diluting and blending proportions of concentrated acetic and propionic acids in IBC containers to about 10 gCOD/L. A targeted COD:N:P (by weight) of 100:1:0.05 nutrient balance was obtained by chemical additions of NH_4Cl and KH_2PO_4. pH was adjusted to 5 by addition of NaOH (33 or 45% w/w).

4.1.2 PHA ACCUMULATION

Prior to each accumulation the process biomass was subject to an acclimation phase. This acclimation comprised a sequence of three feast and famine cycles. The feast in each of the cycles was generated by stimulating the biomass to near maximal respiration with a pulse input of substrate with sufficient volume to a targeted maximum COD concentration of 200 mgCOD/L. The feast respiration response duration was measured based on changes in

dissolved oxygen concentration. A period of famine was provided to last 4 times as long as the time of feast. The acclimation phase was designed as a controlled assessment of the biomass respiration response from batch to batch over the 10 months of operations, and as a means to provide all the biomass batches with the same perturbation history before the onset of the accumulation response. Previous work suggests that feast-famine acclimation before an accumulation response generally results in an improved PHA accumulation potential (Werker et al. 2016).

At the end of the last acclimation famine period, the accumulation process was started automatically. Accumulations were at 25°C and were sustained using semi-continuous feedstock supply based on feed-on-demand biomass respiration control methods (Werker et al. 2013a). The control method objectives were designed to sustain a prolonged period of near maximal and continuous feast respiration with pulse wise inputs of substrate. There is no requirement that the temperature should be 25°C and equally successful results have been demonstrated for mixed culture accumulations over a wide temperature range from 15 to 30°C (De Grazia et al. in progress). The accumulation or "PHA production process" was typically maintained from 16 to 20 hours in duration.

The accumulations were performed with constant process volume, meaning the influent volume inputs displaced an equal volume that was produced as effluent from the process clarifier. Therefore, some loss of added substrate was to be expected due to hydraulic short-circuiting. Accumulation process operating strategies exist to minimize "substrate leakage" but these were not implemented as part of the present investigation. In fact, this and several other bioprocess optimisation and performance efficiency improvements were not included nor undertaken during the present PHARIO investigation because the main goal was to produce polymer as routinely, and as often, as possible. All efforts were focused on producing materials and evaluating the material quality with respect to the routine of production that was applied.

With exceptions of a few abnormal events that were detected by the process automatic control, the accumulation process was terminated based on a set time of accumulation. Frequently the automated termination occurred in the middle of the night. After accumulation termination, the aeration was automatically turned off and the biomass was collected in the main process volume and allowed to settle by gravity. Due to the scheduling and logistics of production, as well as the permitted timing for access to the site, process termination usually occurred in the middle of the night. With return of operating personnel to the site in the morning, and now under manual control, about 100 to 150 L of mixed liquor containing gravity thickened solids of PHA-rich biomass were pumped over to a 200 L holding tank and the mixed liquor pH was adjusted to 2 by titration with concentrated H_2SO_4 (Werker et al. 2013b). Following acidification, the solids were further thickened by floatation within the 200 L volume and these thickened solids were then dewatered by means of a filter bag centrifuge after adding sludge dewatering chemicals. The mixed liquor with added dewatering chemicals (Flopam EM 840 TBD) with a bag centrifuge (at 980×g with filter bags defined with 7 L/dm²/min at 200 Pa) were dewatered on average to about 19% dry solids. Under optimized dewatering conditions it is anticipated to reach approximately 25% dry solids content. The sludge cake was transferred manually to drying trays and then set to dry at 70°C.

4.1.3 PHA EXTRACTION

The dried materials were packaged and sent by courier to AnoxKaldnes in Sweden where

the biomass, PHA-in biomass and recovered and purified PHA qualities were assessed quantitatively (Werker et al. 2013b, Werker et al. 2015). Over the course of the process operations including fermentation, accumulation and downstream processing, selected samples were obtained for basic water quality and solids analyses. From these analyses the process mass balances were evaluated and potential influences for the process operations on the recovered polymer product quality were examined.

4.1.4 PRODUCTION BATCHES AND CODING

The WP1 efforts for PHA production thereby comprised 59 activated sludge batches Axx (A01 to A59), accumulated with 3 different types of feedstocks Sxx, Cxx and Pxx over ten months of pilot operations. For Sxx, the xx denotes the propionic weight fraction (as COD) in the mixture of acetic and propionic acids. Thus, S05 denotes 5 percent propionic acid and 95 percent acetic acid as COD in the feedstock. Cxx refers to the fermentation batches with the process water from the candy factory (C01 to C26) where C01 to C06 were made during the initial commissioning and benchmarking period (Hjort et al. 2015). Pxx refers similarly to the primary sludge fermentation centrate batches P01 to P12 that were produced in the final and third phase of operations (Hjort et al. 2015). Exception events happened for the following Axx batches:

- A01 was a first "dry run".
- A07 was terminated due to loss of alkalinity causing undue pH decrease
- A08 was terminated due to pumping errors
- A09 was abnormally terminated early
- A10 required corrections to the PLC program on the that were made on the fly
- A25 was stopped early due to feed running out (higher biomass loading)
- A53 was terminated due to feed running out during accumulation

These batches were not included in the evaluation of product quality and variability. Fifty-two batches of PHA-rich biomass were successfully completed out of the 59 accumulation runs. The quality of the polymer in the biomass (PHA-in biomass quality) and the recovered polymer quality in relation to the feedstock and the operations form the basis of the assessment were performed and are reported.

Out of the 52 successful accumulation trials:

- 20 were with defined mixtures of acetic and propionic acids, or so-called synthetic ("S") feedstock.
- 27 were with the supply of 26 batches of fermented candy factory process water filtrate feedstock, or "C" feedstock.
- 5 were with the supply of 12 batches of fermented primary sludge centrate, or "P" feedstock.

4.2 PHA CHEMISTRY AND CHARACTERIZATION

4.2.1 INTRODUCTION TO PHA POLYMER CHEMISTRY

The purpose of this section is to introduce to non-polymer science engineering professionals the kinds of PHA polymers produced in PHARIO and the parameters evaluated for quality. Polyhydroxyalkanoates or PHAs are a family of linear polyesters produced by many species of bacteria found in nature (Sudesh et al. 2000, Janarthanan et al. 2016). For the bacteria, they represent an efficient means to store energy and carbon intracellularly. More than 150 different monomers can be combined within the PHA family, wherein the respectively

different combinations result in extremely different materials with properties as thermo-plastic or elastomeric materials and with melting points ranging from 40 to 180°C (Laycock et al. 2012). The polymers are biodegradable (Arcos-Hernandez et al. 2012) and they can be used in formulations to make bioplastics for which such things as processing and mechanical properties, as well as features of biodegradability, can be significantly modulated. The mechanical properties and compatibility of PHA can be influenced through surface modification and/or blending and combining PHA with other polymers, enzymes, and/or inorganic materials. Thus, from this interesting class of biopolymer, comes a very a wide and largely unexplored range of possible applications. Most people refer to these biopolymers simply as PHAs, but PHA is a polymer family, wherein the kinds of PHAs produced in PHARIO are specific and well defined, and these specific polymers will be described in this chapter.

In PHARIO polymers accumulated by the biomass comprised two building blocks (Figure 11) poly-(3 hydroxybutyrate) (or PHB) and poly-(3 hydroxyvalerate) or (or PHV). The mixture of these monomer building blocks (3 hydroxybutyrate and 3 hydroxyvalerate, or 3HB and 3HV) on a polymer chain is the co-polymer poly-(3 hydroxybutyrate-co-3 hydroxyvalerate) or PHBV. The intermediary pool of metabolites that provide the precursors to 3HB and 3HV and the microbial storage or "accumulation" of PHB, PHV, and/or PHBV, are related to the type of substrates that are fed to the biomass.

FIGURE 11   THE POLYMERS ACCUMULATED BY THE BIOMASS COMPRISED TWO BUILDING BLOCKS POLY-(3 HYDROXYBUTYRATE) OR PHB AND POLY-(3 HYDROXYVALERATE) OR PHV. THE MIX OF THESE BUILDING BLOCKS ON A POLYMER CHAIN IS POLY-(3 HYDROXYBUTYRATE-CO-3 HYDROXYVALERATE) OR PHBV. N AND M REPRESENT THE NUMBER OF RESPECTIVE REPEATING UNITS.
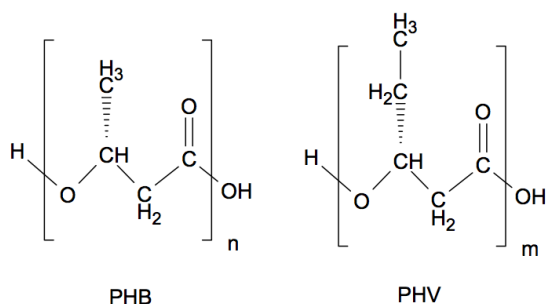
In PHARIO polymer accumulation was achieved by making a substrate with given organic composition continuously available to the biomass for 16 to 24 hours. In this case a random distribution of the monomer types in each polymer chain is to be expected. Thus, in PHARIO, the individual polymer chains are not expected to be with a systematic pattern of monomer type - it will be a random pattern of 3HB and 3HV monomer units. Polymerization of each polymer chain of PHA in the bacteria involves a sequence of chain initiation, chain elongation and chain termination. If the probability of a "chain-termination-event" is kept as low as possible, then the bacteria will tend to produce long polymer chains (high molecular weight). If there is a mixed pool of available monomer precursors during chain elongation, and there is no preference to the precursor selection, then once a chain has been initiated the polymer will become a random co-polymer of PHBV (Figure 10). In PHARIO we produced random co-polymers of PHBV and the accumulation conditions were designed to reduce the probability of chain termination during the accumulation process. As a consequence we found that we could produce polymers with weight average molecular mass as high as 1500 kDa.

FIGURE 12   PHARIO PRODUCED PHBVS COMPRISED CO-POLYMERS THAT WERE NOT REGULAR PATTERNS BECAUSE THE POLYMERISATION IS EXPECTED TO BE RANDOMIZED. THE UPPER SET OF THREE POLYMER CHAINS ILLUSTRATES A RANDOM CO-POLYMER BLEND OF PHBV WHERE EACH POLYMER CHAIN IN THE BLEND CONTAINS A MOLAR AVERAGE OF 33% 3HV MONOMERS. SINCE THE POLYMER CHAINS ARE OF DIFFERENT LENGTH, THERE IS A MOLECULAR WEIGHT DISTRIBUTION FOR THE BLEND. THE LOWER SET OF THREE POLYMER CHAINS ILLUSTRATES A SIMILAR CO-POLYMER BLEND WITH THE EXCEPTION THAT EACH CHAIN DOES NOT HAVE EXACTLY THE SAME AMOUNT OF 3HV UNITS. THEREFORE, IN THIS LOWER SET, THERE IS A DISTRIBUTION OF BOTH 3HV CONTENT AND MOLECULAR WEIGHT BETWEEN THE CHAINS. THE LOWER SET IS ILLUSTRATIVE AS A CO-POLYMER BLEND TO WHAT WAS PRODUCE BY THE ACTIVATED SLUDGE IN PHARIO.

PHBV co-polymer blend with 33 mol% 3-hydroxyvalerate (3HV) content (molecular weight distribution only)
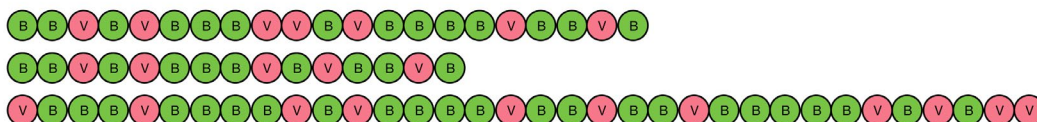
PHBV co-polymer blend with mean 33 mol% 3HV content (molecular weight and 3HV distributions)
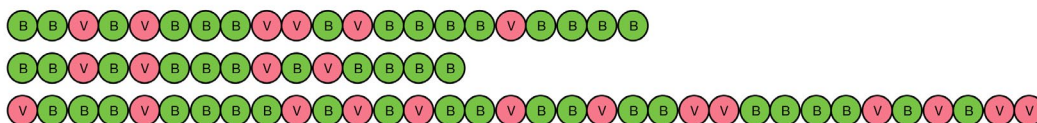
Polymers with a distribution of molecular weights will be generated, and generally one strives to obtain a relatively high molecular weight and a relatively consistent distribution. The weight average or number average molecular mass (M_w or M_n) are typically used to describe this distribution alongside the polydispersity index (PDI). The <u>number average molecular mass</u> (M_n) is just the total weight of all the polymer molecules in a sample, divided by the total number of polymer molecules in a sample. The <u>weight average molecular mass</u> (M_w) compensates for the fact that the longer chain length polymers in the mix contain more of the total mass of the polymer sample than the smaller chain length polymers.

The PDI or breadth of the distribution is 1 if all polymer chains are of the same length. A PDI of 3 already suggests a wide distribution of chain lengths. In general, it is anticipated that PDI will be greater than 1 and approach towards 2 as the chain termination probability approaches zero for the case of step growth linear polymerization, as is the case for PHA polymerization in biomass.

Not all species of bacteria in the biomass will process the supplied substrate in exactly the same way, and/or the nature of the biomass metabolic activity has been observed to shift during PHA accumulation processes (Montano Herrera 2016). Therefore, not all polymer chains will have exactly the same co-polymer composition. The outcome will be a co-polymer blend of PHBV with distributions of both molecular weight and monomer compositions (Figure 12). Generally, the average monomer composition is reported. In the data presented for PHARIO, the PHBV composition for each batch is represented by the average 3HV content of the PHBV. Comparisons of molecular weight between batches are considered with data of M_w in the report. The nature of the distributions of co-polymer and chain length will be discussed as part of the consideration of the product quality.

4.2.2   THERMAL CHARACTERIZATION OF PHA

Whether or not the polymers are homo-polymers or co-polymers, be they alternating, gradient, block or random co-polymers or co-polymer blends, they can be of interest to material scientists for a range of potential meaningful applications. In all cases, most higher value applications definitely require that, from batch to batch, the polymers, notwithstanding particular type, behave in a consistent way.

Rather than considering the consistency of a polymer in terms of the polymer content, it can be more interesting or relevant to evaluate the polymer thermal characteristics (Figure 13). It is these thermal characteristics that are critical to the processing, blending and performance of the materials in applications. In PHARIO WP1 we evaluated glass transition temperatures (T_g), melting temperatures T_m, de-composition temperatures (T_d) and crystallization temperatures (T_c) by means of differential scanning calorimetry (DSC), in combination with thermogravimetric analysis (TGA), and melt rheology. Since the thermal characteristics are so central to the exploitation of a polymer for engineering applications, these characteristics became a principal focus for the evaluations of quality for the batches of polymer produced for PHARIO. Our consideration of quality has been with respect to the consistency of behaviour of the polymer thermal characteristics for the types of polymer produced. The type of polymer was identified by the average 3HV weight content.

FIGURE 13   TEMPERATURE ZONES AND MATERIAL CHARACTERISTICS CHANGE AS A FUNCTION OF TEMPERATURE AS COMMONLY EXPERIENCED FOR THERMOPLASTIC POLYMERS. CHARACTERISTIC TEMPERATURE POINTS ARE THE GLASS TRANSITION TEMPERATURE (T_G), CRYSTALLIZATION TEMPERATURE (T_C), MELT TEMPERATURE (T_M), AND DECOMPOSITION TEMPERATURE (T_D).
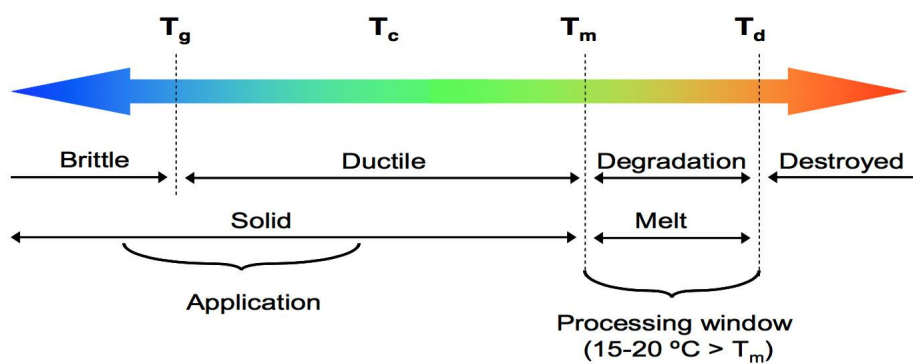
In the solid state, polymers may comprise both crystalline (highly ordered) and non-crystalline (amorphous) phases. Blends of polymers may comprise both miscible and immiscible mixtures. Polymer glass transition, melt, and crystallization behaviour is central in consideration of the material processing and application. Polymers will crystallize depending on the type of polymer, the thermal history, and time. The crystalline nature of the polymer microstructure generally gives the material its mechanical strength. The amorphous nature of the polymer microstructure generally gives the material its ductility. The integrity of the interfaces between crystalline regions (and immiscible phases) can determine the material brittleness. Since the PHARIO PHBVs were co-polymer blends, we paid attention to questions of quality with respect to predictability of crystallinity and the miscibility of the blend contents as a function of the average 3HV content.

Thermal gravimetric analysis (TGA) was used to evaluate the extractable polymer in the PHA-rich biomass (Figure 14). A dried and ground representative biomass grab sample (≈5 mg) was first heated and held at 105°C to remove remaining moisture and then the temperature was raised at a constant ramp of 10°C/min to 550°C with a nitrogen atmosphere. Thereupon the atmosphere was changed to air and the sample was held at 550°C for 30 minutes to determine the biomass inorganic (ash) content. Weight loss during heating occurred due to volatilization of sample biomass components and the shown peak of weight loss rate between 250 and 300°C is indicative of the sample PHA content. The temperature at the peak quantified the thermal decomposition temperature (T_d) of the polymer in the biomass. This method of polymer content determination was calibrated to other methods of quantification including, sample digestion and hydrolysis followed with GC-FID/MS analyses, standardized gravimetric

mass balances of extractable polymer content, in-house methods of polymer-volatile solids analyses, and FTIR.

Quality of the polymer "in-the-biomass" was assessed by extracting the polymer following in-house methods of extraction that were specifically developed to cause negligible changes to the polymer in the process. From all the 52-completed accumulation (Axx) batches, a control and a reference polymer recovery was made at the test tube scale (10 mL) with 10 g/L polymer loading, in acetone, at 125°C and for one hour. This control was intended to check for any influence of the pilot scale materials management methods and/or dewatering chemicals on the polymer quality. The reference sample was take after the pilot scale dewatering.

The melt characteristics of the control and reference polymer samples were made by differential scanning calorimetry (DSC). DSC measures a heat flow signal coming from the crystalline fraction of the polymer which gives an endothermic peak during melting (Figure 14). The glass transition temperature(s) of the polymer by DSC are derived from a heat flow signal coming from the amorphous fraction of the polymer which gives an endothermic shift in the base line. DSC measurement procedures were designed in such a way to first erase any thermal history and get all the samples in the same starting reference condition.

FIGURE 14   IN A DSC MEASUREMENT, A SAMPLE IS TYPICALLY SUBJECTED TO A SERIES OF HEATING AND COOLING (QUENCHING) RAMPS, HEATING UP TO ABOVE THE MELTING TEMPERATURE, AND COOLING TO BELOW THE GLASS TRANSITION TEMPERATURES. DEPENDING ON THE THERMAL HISTORY, AND AGING OF THE SAMPLE BETWEEN SUCH HEATING AND COOLING SAMPLES, AND FROM THE HEAT FLOW MEASURED FROM THE SAMPLE DURING EACH CYCLE, CHARACTERISTIC TEMPERATURES AND PROPERTIES OF THE POLYMER SAMPLE CAN BE QUANTIFIED (STUART 2002).
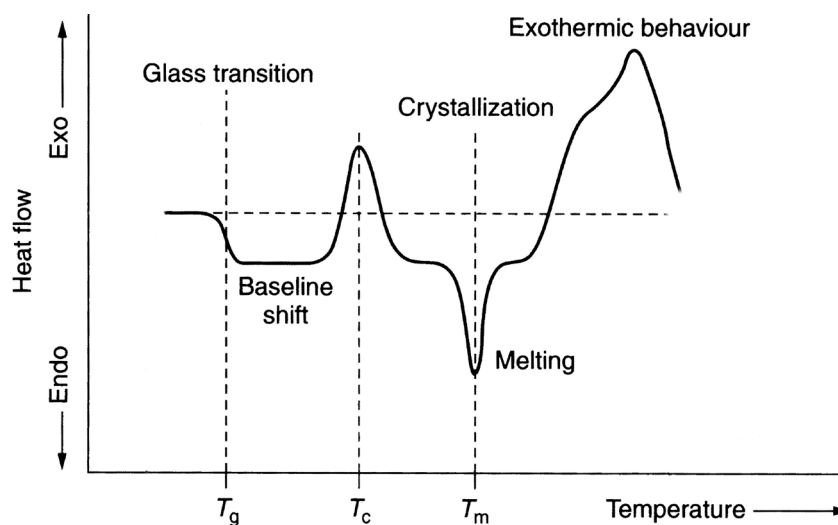
Polymer molecular weights (M_w and M_n) were estimated by **size exclusion chromatography** or SEC (Gustafsson 2016). SEC is also referred to as gel permeation chromatography or GPC. A Malvern Viscotek GPC Max was used with a Viscotek VE3580 RI detector. Polymer was dissolved in chloroform to a concentration of 3 mg/mL. The temperature was 35 °C in both the columns and the detector. A PHA dissolved in chloroform is injected onto the GPC column. The distribution of the polymer molecular weight is quantified by the elution concentration (refractive index detection) as a function of solvent elution volume (Figure 17). The elution volume (time) was calibrated to polystyrene polymer standards of known molecular mass and very narrow PDI. Polymer chains of higher molecular mass elute from the column in a shorter length of time.

Melt rheology of the above-mentioned control and reference polymer samples, was used to assess weight average molecular mass, and thermal stability of the polymers in the melt at 180°C (Malengreaux 2008). A mass of the recovered polymer (nominally 0.5 g) was pressed and melted between the two parallel discs at 180°C in the rheometer (DHR-2 from TA Instruments). One disc is the actuator and the other is the sensor for the instrument. The samples were subjected, over thirty minutes, to an oscillating agitation at 10 Hz with a constant maximum strain (2%). From the transmitted shear force through the sample, the dynamic viscosity and the phase angle (delta) between the stimulus and transmitted response were measured and recorded. Both the initial dynamic viscosity and angle delta were correlated to the polymer mean weight average molecular mass (M_w).

The advantage of the evaluation of the polymer M_w from melt rheometry at 180°C is that it enables a comparison of the polymer molecular weight thermal stability in the melt. Consistency of thermal stability in the melt from batch to batch is an element of quality that is desirable for PHA-refiners, PHA-compounders, and bioplastic-converters.

The decrease of melt viscosity in time was correlated to the "**scission rate**" or rate of decrease of polymer molecular mass. The trend of increase in delta was also correlated to a decrease of molecular mass in the melt. Decrease of weight average molecular mass was interpreted to be due to random scission of the polymer chains. Random scission refers to reactions that will clip individual polymer chains randomly along the chain of coupled monomers. Note that delta can range from 0 to 90 degrees. A delta of zero is expected for a purely elastic material. A delta of 90° is expected for a Newtonian fluid. Interpretation of the trends over the 30 minute measurement become less well-defined in time due to the potential, for example, of material loss from between the parallel plates when the molecular weight becomes very low. Therefore, estimation of scission rates for the polymer in the melt were made based on the initial measurement period (up to about 5 minutes).

The random scission rate was seen to be approximately constant with all other factors maintained constant (temperature and environment). This means that in the extraction process, for example, if the scission rate is known, then given the initial molecular weight, the molecular weight at the end of the extraction process can be estimated with some certainty.

4.3 PHA YIELDS AND SUBSTRATE CONSUMPTION

In the pilot accumulation pilot plant a dried "PHA-rich-biomass" or PRB was routinely produced. Biomass is hygroscopic and normally in storage at room temperature the dried materials will contain about 2 to 4 percent bound water. Neglecting this moisture content the PRB from 52 accumulation batches comprised three fractions: non-PHA biomass (≈53 wt%), inorganic material (10±2 wt%) and PHA (37±5 wt%).

The non-PHA biomass is sometimes approximated as "active biomass" (X_a) in the research literature because this fraction is expected to be dominated by the population of species of bacteria that have accumulated the intracellular biopolymer. X_a is, therefore, anticipated to be principally comprised of components in proportions typical of bacteria including proteins (≈50 wt%), genetic material (DNA, RNA, ≈23 wt%), lipids (≈7 wt%) and polysaccharides (≈7 wt%). This bacterial X_a contains nitrogen (12 wt%) and phosphorus (1-2 wt%). X_a may also comprise extracellular polymeric materials (EPS) being the external matrix of flocculated biomass. It may also contain inert organic materials including municipal wastewater influent cellulose

fibre that has become associated with the floc structures as well as suspended solids introduced with the accumulation feedstock.

Naturally it is desirable to minimize these non-active fractions to the X_a as they will dilute the active biomass that accumulates the PHA. In this way primary treatment including cellulose recovery and reuse is synergistic to the goals of PHA production (Bengtsson et al. 2016). The inorganic content is due to the active biomass, the floc matrix and any entrapped inert materials like grit associated with the suspended solids (Bengtsson et al. 2016). Bath WWTP does include primary clarification, and the suspended solids in the fermented feedstocks for PHARIO were reduced by sedimentation and filtration (including also centrifugation for the primary sludge fermentation).

We consider the PHA accumulation potential (PAP) with respect to the biomass organic content as this most closely reflects what the "active biomass" is able to routinely achieve. This organic content is measured as volatile suspended solids or VSS. Therefore, on average WWTP Bath activated sludge produced PHA with raw product content of 41% gPHA/gVSS. Thus, we found that a kilogram of Bath activated sludge VSS, as the process is operated today, can be expected to reliably accumulate about 700 grams of polymer. There are ways to further improve the PHA accumulation potential, but this was not within the scope of the pilot operation.

The amount of polymer accumulated was with some variation but generally the product PHA content was consistent and not significantly influenced by the type of feedstock used (Figure 15). Variation in the case of the Pxx accumulation batches was due to the need for some measure of accumulation process optimization when feeding a nutrient rich feedstock. We found that a higher specific loading of the Pxx substrate to the biomass (red symbols) gave PAP outcomes that were similar or better to the expectations based on Cxx and Sxx batches. It was further interpreted from the data for observed variation of the PHA contents for the Sxx accumulations where feedstock quality was constant, that managing biomass physiological state before the accumulation process was likely a factor to optimize in the future. By physiological state, we are referring to the state of starvation before the accumulation process.

FIGURE 15   THE PHA ACCUMULATION POTENTIAL (PAP) WAS REPRODUCED WITH THREE DISTINCT TYPES OF FEEDSTOCKS AND ASSOCIATED MATRICES WITH AND WITHOUT NUTRIENTS IN LIMITATION. ALL ACCUMULATIONS WERE RUN AT A NOMINAL TEMPERATURE OF 25°C. CHANGES IN TEMPERATURE ARE NOT EXPECTED TO INFLUENCE THE PAP. TEMPERATURE HAS BEEN FOUND TO INFLUENCE YIELDS AND KINETICS. IMPROVED PERFORMANCE FOR THE PXX ACCUMULATIONS WERE ACHIEVED BY INCREASING THE SPECIFIC LOADING OF SUBSTRATE TO BIOMASS.
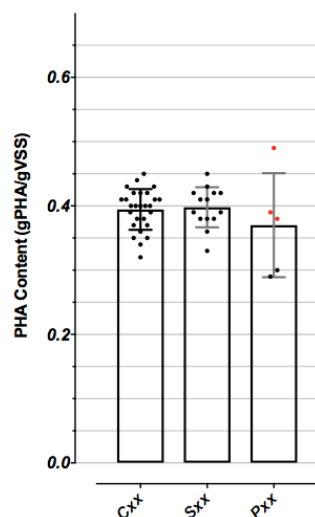
FIGURE 16   LEFT (A) AVERAGE ACCUMULATION YIELD OF PHA PRODUCED PER MASS OF FEEDSTOCK SUBSTRATE CONSUMED. OPTIMIZATION OF THE METABOLIC

RESPONSE OF THE BIOMASS MAY FURTHER OPTIMIZE THIES YIELD. RIGHT (B): EFFICIENCY OF THE USE OF THE COD IN THE FEED. OPTIMIZED

PROCEDURES MAY FURTHER INCREASE THIS EFFICIENCY, BUT THIS WAS NOT WITHIN THE SCOPE OF THE PHARIO PROJECT.
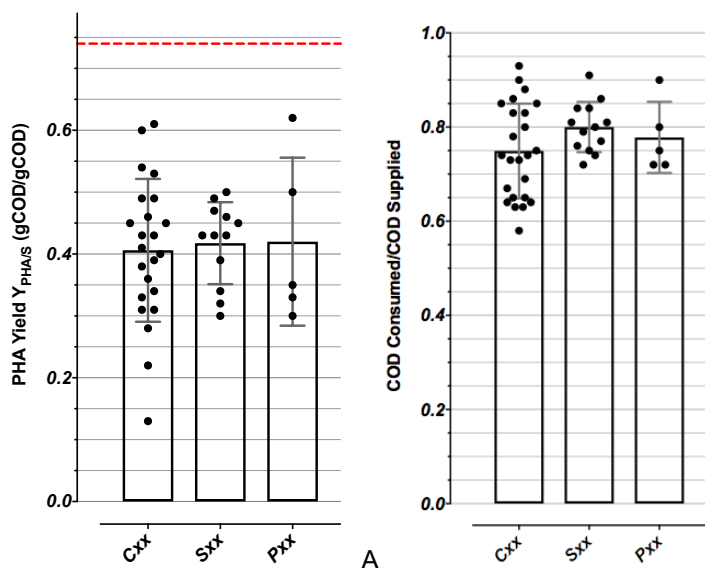
The average accumulation yield of PHA produced per mass of feedstock substrate consumed is shown in Figure 16 A. The efficiency of the biomass to accumulate PHA is best compared on the basis of grams of PHA produced (as COD) with respect to grams of substrate consumed (as COD). A COD basis removes the influence of the fact that different kinds of PHA contain different amounts of chemical energy. For example, PHB is 1.67 gCOD/g and PHV is 1.92 gCOD/g. A maximum theoretical efficiency of PHA storage is predicted to be about 0.74 gCOD/gCOD (Beun et al. 2002). This level of efficiency is generally only possible when the bacteria have little to no stored PHA. As the biomass becomes "fat" with the intracellular granules, increasing maintenance energy requirements means that more of the feedstock carbon is spilled to carbon dioxide rather than being converted and stored as polymer.

Due to the trend of diminished polymer yield with accumulation time, an accumulation process should not be run longer than it needs to be run. The initial yields of PHA on substrate were generally high with reference to a recent review of the mixed culture PHA literature (Valentino et al. 2016). The literature values are with a wide range that depend on substrates but also on the basis of how the yields were calculated (initial versus after an arbitrary length of an accumulation process). Unfortunately, there has been no common standard in reporting such yields in the research literature referenced to, for example, the normalized accumulation time scale, and meaningful comparisons are therefore sometimes difficult to obtain. Reports of "high" and "low" yields in the literature can therefore be misleading if the context of the reported values is not clear. Without any context in the literature, yield values between 0.4 and 0.6 gCOD/gCOD may be considered to be typical for mixed culture systems.

Alongside the potential for improvement and optimization of the biomass polymer yield on substrate, the overall pilot operations efficiency of substrate supply was evaluated. The amount of substrate (as COD) consumed due to biological activity was estimated with respect to the amount of PHA produced for each Axx batch. The pilot system was operated with flow through, under hydraulic balance, meaning that every litre of feed added at the front end resulted directly in a litre of effluent discharged at the back end of the process. Bacterial biomass was retained in the pilot process by means of gravity separation. The estimated

leakage (about 20%), of input feedstock COD short-circuited to the effluent, was not a surprise (Figure 16 B). Methods of bioprocess operation and control to minimize feedstock leakage are known and these may be readily implemented. Such process control efficiency factors were beyond the scope of the pilot operations for PHARIO but are recommended for demonstration scale developments. Bacterial activity will also result in some production of soluble microbial products. This bacterial activity by-product COD that is not feedstock will nevertheless be interpreted as non-consumed COD from the COD mass balance as it was estimated from these data.

The yields in the present work are the average yields over accumulation times from 16 to 24 hours. Kinetics of PHA content during accumulation for mixed cultures generally follow an exponential asymptote (see paragraph 3.3.2). The time constant   gives the time frame for accumulation to the maximum content. For PHARIO, the WWTP Bath Activated sludge at 25°C reached a maximum content after about 12 hours if 3^* is taken to be the metric. Therefore, the yields are lower than they need to be, in part, because the accumulation batches were run to a selected time and conservatively long. For industrial practice, the accumulations can be terminated based on methods of on-line monitoring of respiration and/or PHA content (Werker et al. 2013a).

4.4 STABILITY OF THE PRODUCED PHA

4.4.1 STABILITY DURING STORAGE

The PHA-rich biomass is a product and since everyone talks about how the polymers are biodegradable, one might naturally ask the question of the "shelf-life" of this product. In the commercial scale operations, it may be practical to store batches of the dried PHA-rich biomass for some time. PHA rich biomass representing PHA types of grades that can be mixed and master batched together with other batches and this mixing enables the opportunity to compound these materials as an integral part of the PHA recovery process. Samples of PHA-rich biomass that have been stored for 3 years at AnoxKaldnes (approximately 98% dry, at room temperature, and in the dark) were re-evaluated as part of PHARIO (Figure 17). This PHA-rich biomass was produced using the same piloting facility but with wastewater from Procordia Food AB in Eslöv Sweden in 2013. After three years, the PHA content of the dried biomass had not changed.

FIGURE 17   THE PHA CONTENT WITHIN DRIED PHA-RICH BIOMASS WAS STABLE WHEN STORED FOR OVER THREE YEARS AT ANOXKALDNES (ROOM TEMPERATURE, IN THE DARK).
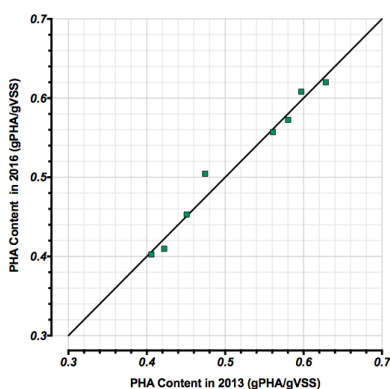
Although the amount of PHA in the dried (70°C) biomass was stable over three years, some degree of molecular weight loss will occur. Molecular weight loss is due to thermally driven and often catalysed reactions that result in random scissions of the polymer chains. The molecular weight stability can be expressed in terms of a "molecular weight half-life", being the time taken for the weight average molecular mass (M_w) to be reduced by half. This half-life was found to be dependent on the type of PHBV in the biomass. While the half-life of a PHB was essentially "infinite" the half-life of a PHBV with 35 wt% HV was approximately 5 years. So the PHBV amount with higher 3HV content is still stable over a long time but there is a measurable rate of molecular weight loss. The difference as a function of 3HV co-polymer content is due to associated differences in crystallinity. A 40 wt.% 3HV PHBV is less crystalline (more amorphous) than a PHB homopolymer. The amorphous regions of a polymer are more "open" for chemical reactions.

Wet, acid stabilized and dewatered PHA-rich biomass (Werker et al. 2013b) is not as stable in storage as is dried biomass. An evaluation was made of the **stability of the polymer before drying**. These data were acquired with the Bath activated sludge, in anticipation of PHARIO, but using the same pilot facility while it was still at the Brussels North WWTP. The PHA-rich biomass was obtained following acidification and dewatering in the same bag centrifuge to between 15 and 20 % DS. The dewatered solids were divided into parallel streams wherein one mass was stored at room temperature (20°C) and the other was stored under refrigeration at 4°C. At 20°C the polymer content decayed exponentially with an exponential rate constant of about 15 days (Figure 18 A). The polymer content was more adequately preserved at 4°C and after a prolonged period of 30 days at lower temperature the polymer content reduced by only 10 percent. Molecular weight loss was estimated and the scission rate at 4 and 20 °C was derived to be 0.01 and 0.13 day^{-1} (Figure 18 B). These scission rates are equivalent to saying half-lives of 100 and 7.7 days for 4 and 20 °C storage of the wet PHA-rich biomass, respectively.

FIGURE 18: STABILITY OF PHA CONTENT AND THE POLYMER WEIGHT AVERAGE MOLECULAR MASS IN ACIDIFIED BUT WET BIOMASS (15 TO 20% DS) IS TEMPERATURE DEPENDENT.
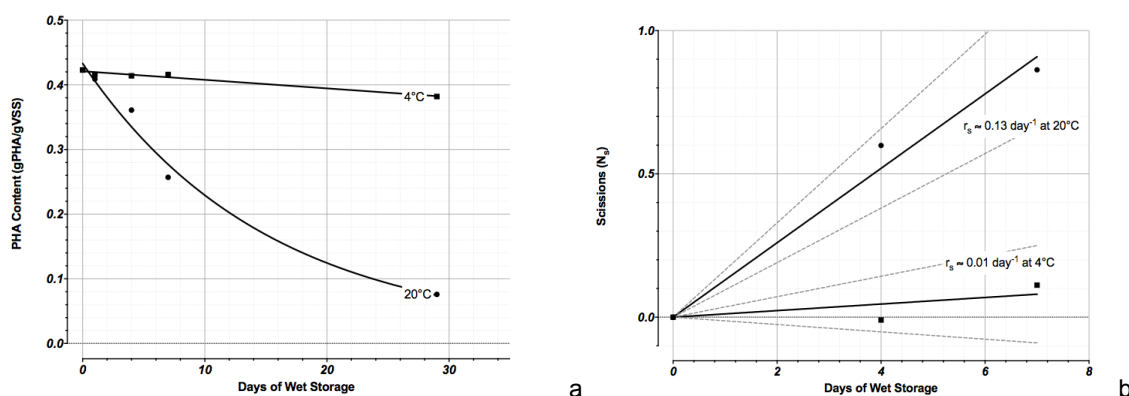
The degradation of the polymer in the PHA-rich biomass while in wet storage was temperature sensitive and the mechanisms of degradation were interpreted to be:
- Enzyme activity within the PHA rich bacteria where the kinetics of activity are temperature dependent and not fully disabled by the condition of acidification before drying.
- An onset of pH tolerant microbial activity, including but not limited to fungal growth.
- Chemical reactivity catalysed by biomass in close association with the polymer.

4.4.2 THERMAL STABILITY

The thermal stability of the produced PHA is important because elevated temperatures are required for drying and extraction of the PHA. It therefore important to understand the consequences of these process steps on the PHA quality. Furthermore, this information is relevant for the further processing of the extracted PHA, for instance during injection moulding.

An important measure for the thermal stability of the produced PHA is the thermal decomposition temperature (T_d). The thermal decomposition temperature of the PHA in the biomass was consistently very high and in the order of 285°C. This high and reproducible T_d (Figure 19) was a consequence of the specifically developed methods applied for after the accumulation process (Werker et al. 2013b).

FIGURE 19   THE THERMAL STABILITY (AS MEASURED BY THE PHA IN BIOMASS THERMAL DECOMPOSITION TEMPERATURE T_D) WAS CONSISTENTLY AND
SIGNIFICANTLY HIGH IN ALL CASES. THE CONTROL VALUES ARE FOR BIOMASS SAMPLES TAKEN DIRECTLY AFTER ACIDIFICATION AND DEWATERED
BY LABORATORY CENTRIFUGE BEFORE DRYING AT 70°C. THE PRODUCT BIOMASS SAMPLES ARE FOR THE MAIN PRODUCTION BATCHES AFTER PILOT
DEWATERING AND DRYING AT 70°C. THE POLYMER IS THE PHA IN ISOLATION AFTER EXTRACTION FROM THE BIOMASS.
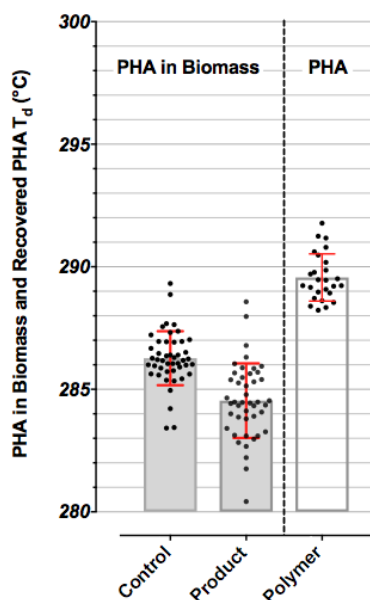
The **chemical reactivity of the PHA polymer at 170°C** was evaluated for the dried and wet biomass. For the PHA-rich biomass dried isothermally at 70°C, subsequent thermal treatment at 170°C for up to 3 hours did not affect the amount of polymer in the biomass but M_w decreased with time (Figure 20). The M_w was initially stable at 170 °C for about 25 minutes but molecular weight loss ensued with a scission rate of 0.01 1/min meaning an estimated M_w half-life of 100 minutes.

FIGURE 20   ISOTHERMAL INCUBATION OF DRIED PHARIO BIOMASS SUGGESTING AN ONSET OF RANDOM SCISSION AFTER AN INITIAL 25 MINUTE INCUBATION PERIOD. PHA CONTENT OF THE BIOMASS WAS CONSTANT BUT MOLECULAR WEIGHT DECREASED.
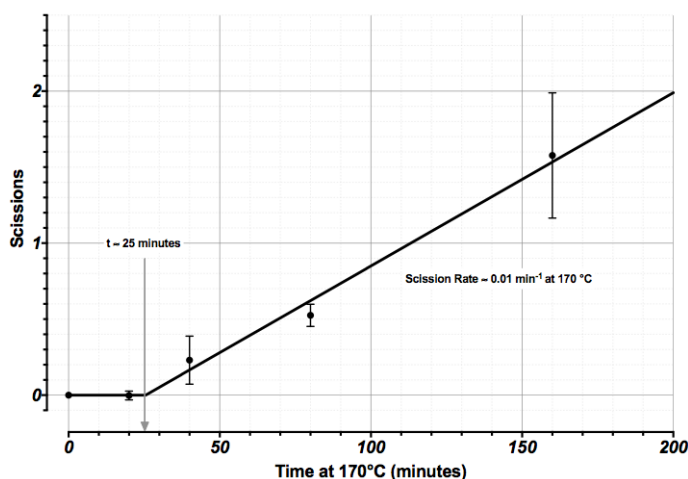
The influence of elevated temperatures on the PHA in the wet biomass was also examined. These tests were motivated by the expectations of scaled up industrial **drying conditions** based on the BioCon™ technology (Magnusson 2016), see also http://technomaps.veoliawater-technologies.com/bioco/en/). The Kruger BioCon™ system is an efficient two stage belt dryer with the wet solids extruded out onto the belt. The belt conveys the biomass into a hot-zone (140 to 180°C) and a lower temperature end zone (60 to 80°C). The above-mentioned heat 170°C test on the already dried materials suggested that the PHARIO PHA with high thermal stability (T_d) in the biomass would be robust to BioCon™ typical hot-zone drying temperatures and times (170°C and up to about 30 minutes). For completeness, a practical evaluation of the BioCon™ drying technology was also performed in collaboration with Kruger AS on the PHARIO freshly acidified and dewatered PHA rich biomass.

For this BioCon™ practical drying evaluation we used Batch A56, which was an S01 accumulation over 20 hours. The accumulation resulted in 39 % gPHA/gTSS, which was typical as compared to the other Sxx accumulation batches. Due to the timing of the process and the access to the Bath site, the biomass settled for 12 hours prior to the routine of acidification to pH 2.0, reference sampling, and centrifugation DSP activities. The final centrifugation was performed on March 16th between 10:00-14:00. The dewatered mass was divided into two and wherein a portion was disposed to isothermal drying over 5 days at 70°C. The remaining material was sent wet to the Kruger office in Århus (Denmark) on March 17 and the package arrived at its destination on March 18th. The materials were extruded in a fashion analogous to the BioCon™ process and 4 different drying conditions were performed on sub-samples of the delivered materials:
- 15 minutes at 120°C followed by 45 minutes at 75°C.
- 15 minutes at 120°C followed by 45 minutes at 80°C.
- 15 minutes at 120°C followed by 45 minutes at 100°C.
- 15 minutes at 170°C followed by 45 minutes at 75°C.

The dried materials from Århus were then sent to AnoxKaldnes in Sweden where the influence of drying condition on biomass composition and polymer qualities was evaluated. All samples, as delivered, to Sweden were dry to 2.5±0.7% moisture content. Replicate samples were available for the isothermal drying. The 70°C isothermal control was the sample taken after acidification and dewatered by laboratory scale centrifugation before drying. The 70°C

isothermal reference is the main batch of pilot PHA-rich biomass dewatered by the pilot scale bag centrifuge before drying. These replicates were very similar and the TGA results indicated that the logistics of transport and different methods of drying did not significantly influence the qualities of thermal stability (T_d) or PHA content (Figure 21).

FIGURE 21   INFLUENCE OF DIFFERENT DRYING METHODS ON THE BIOMASS COMPOSITION AND THERMAL STABILITY. THE 70°C ISOTHERMAL CONTROL WAS THE A56 SAMPLE TAKEN AFTER ACIDIFICATION AND DEWATERED BY LABORATORY SCALE CENTRIFUGATION BEFORE DRYING. THE 70°C ISOTHERMAL REFERENCE WAS THE A56 MAIN BATCH OF PILOT PHA-RICH BIOMASS DEWATERED BY THE PILOT SCALE BAG CENTRIFUGE BEFORE DRYING. THESE TGA RESULTS INDICATED THAT THE LOGISTICS OF TRANSPORT AND DIFFERENT METHODS OF DRYING DID NOT SIGNIFICANTLY INFLUENCE THE QUALITIES OF THERMAL STABILITY (T_d) OR PHA CONTENT.
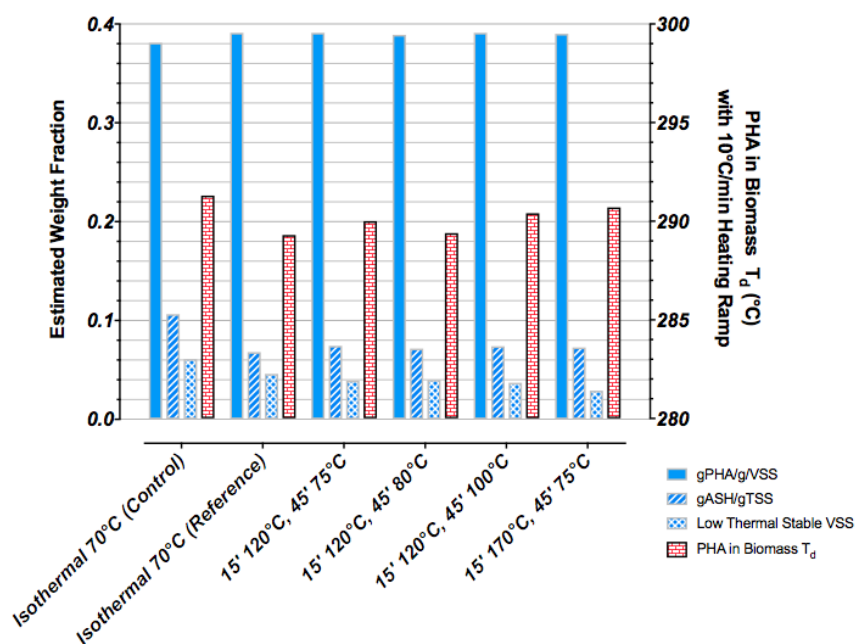
The drying strategy exerted a significant and positive influence on the PHA-in biomass molecular weight. Higher initial drying temperatures resulted in a PHA-rich biomass with higher PHA in biomass molecular weight (Figure 22). Akin to the results of aging of the wet biomass, there is sufficient residual biological activity in the acidified wet mass to degrade the polymer and the kinetics of this activity are temperature dependent. The optimal outcomes of M_w were obtained either with at least a moderate hot zone (120°C) followed by a warmer end zone (100°C), or with a more aggressive hot zone temperature of 170°C.

The drying conditions influenced the product quality in ways that were not initially anticipated and these results showed the need to engineer the drying environment that will limit any remaining biological activity (higher temperatures) without resulting in any thermal degradation of the polymers (time at higher temperatures) during drying. These data suggested that much of the observed variability of M_w in PHARIO was most likely due to vagaries of drying (cake thickness, oven loading, etc.) resulting in prolonged or shortened times in temperature zones that still enabled enzyme activity on the stored polymers before the wet mass came up to 70°. The outcome that the accumulated PHA may exhibit consistent molecular weight in the order of 1500 kDa is significant and very exciting. Higher end applications such as fibre spinning and film blowing require improved polymer melt strength and this property is generally a function of M_w. Therefore, this processing insight to perserve PHAs of significant M_w opens a door to very interesting high value applications.

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The literature suggests that in general PHA depolymerase activity is often catalysed by calcium ions and becomes less favoured under acidic conditions and temperatures equal to or greater than 70°C (Jendrossek 1998). However, acidic conditions do not appear to necessarily eliminate the depolymerase activity altogether (Mao et al. 2013). From previous studies focused on drying temperature trends for the dewatered biomass, we found that the time that the wet solids take to warm up can vary widely from a just couple to as much as several hours depending on such factors including, but not limited to, the wet material position in the oven, and the methods for distributing the sludge cake. Therefore, from PHARIO we have come to interpret a potential for depolymerase activity during the warm up phase of the solids to temperatures above which these enzymes can still be active. From the literature, that threshold temperature is around 70°C. The quicker the solids are warmed the better. Therefore, the initial hot zone of processes like the BioCon™ drier will tend to produce a polymer of improved quality in terms of anticipated higher and more consistent molecular weight for the PHA in the biomass. This outcome considerably widens the opportunity for downstream value from the polymer.

FIGURE 22   EFFECT OF DIFFERENT DRYING REGIMES ON THE MOLECULAR WEIGHT OF THE PRODUCED PHA. HIGHER DRYING TEMPERATURES WILL HEAT UP THE BIOMASS QUICKER THUS INHIBITING DEPOLYMERASE ENZYME ACTIVITY.
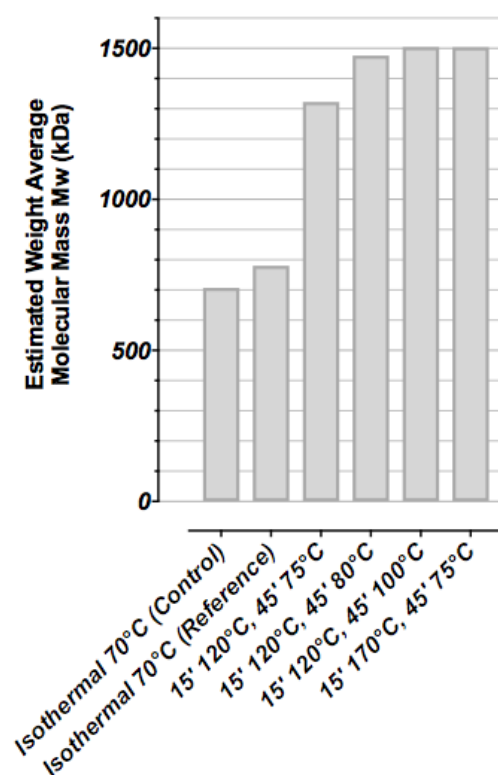
4.5 STEERING THE PHA QUALITY

4.5.1 EFFECT OF THE FEEDSTOCK COMPOSITION

A range of feedstocks were used to produce PHBV co-polymer blends over the 10 months of pilot plant operations. The organic composition of the feedstocks used for respective Axx accumulation batches are shown in Figure 23. Sxx batches were synthetic feedstocks with defined mixtures of acetic acid with 0, 1, 5 and 15 percent COD fractions of propionic acid. Cxx batches were the BioVAP pilot filtrates from fermented process effluent from a local candy factory. Note that shifts in composition for the same Cxx feedstock (for example C25 for

A38 and A39) are due to continued fermentation that took place while the materials were in storage. Pxx batches were the BioVAP pilot centrate from fermented primary sludge delivered by Waterschap De Dommel from Tilburg.

The Pxx feestock fermentation results were largely consistent and reproducible both at pilot and laboratory test tube fermentation scales. The low variability in the case of Pxx fermentate is related, in part, to the fact that hydrolysis is a rate limiting step to the fermentation biological activity. Furthermore, in primary sludge the balance of carbohydrates, lipids and protein in sludge is expected to be relatively consistent.

In contrast, the Cxx feedstock batch fermentation results were variable in nature. This variability was promoted from the onset by the current process effluent management practices at source that stimulated a large and preventable level of yeast fermentation while the effluent is in storage at the candy factory. This alcohol fermentation activity could be readily prevented in the future. The history of the effluent storage and management at the candy factory today inadvertently encourages the maintenance of a significant yeast biomass. In addition to yeast alcohol production, the Cxx fermentation outcomes are also linked to challenges, in general, of fermenting a carbohydrate rich stream.

The BioVAP pilot fermentation process, as it was run for PHARIO, was not a suitable bioprocess method to constrain the possible degrees of freedom of fermentation metabolism. It was the fermentation infrastructure that was made available to the project before the sources of organic residuals were decided upon. The batch dump feed method of BioVAP meant that the carbohydrate conversion bioactivity was unregulated by the substrate supply as it should be. Conditions of pH and SRT could not be as readily controlled as they would be for example in an up flow continuous feed reactor (Zoetemeyer 1982). Notwithstanding, we found over the course of the project that other parameters (in addition to pH, SRT, and substrate limitation) could be used in the future to manipulate and steer primarily the challenges due to alcohol production and secondary fermentation microbial activity. Nutrient regulation and the use of an electronic sink such as iron were both found to be additional effective potential control parameters for these refined and higher sugar content organic residual streams. Therefore, the observed variations in the Cxx feedstock fermentation products were interpreted to be controllable in scaled up fermentation for PHA production, through better source management practices and already well-established methods of bioprocess operations and control.

The combination of a carbohydrate rich stream with a dump feed batch fermentation bioprocess, and the uncontrolled-at-source raw materials management for fermentation products were challenging in PHARIO, but they were a benefit for PHARIO purposes.

Cxx feedstock composition variation facilitated a benefit to the PHARIO objectives because it gave a deeper understanding the reliable nature of polymer quality from an WWTP activated sludge biomass. We found that the activated sludge biomass from the full scale WWTP was a robust and stable raw material for polymer production over 10 months and 4 seasons of operations. The feedstock determined the type of polymer produced, not the biomass.

FIGURE 23   RELATIVE COD COMPOSITION OF THE RANGE OF FEEDSTOCKS THAT WERE USED TO PRODUCE BATCHES (A01 TO A59) OF PHA-RICH BIOMASS OVER THE 10 MONTHS OF PILOT PLANT OPERATIONS. NON-VFA COD IS EXPRESSED AS CARBOHYDRATE. THE FEEDSTOCKS WERE BATCHES OF SYNTHETIC VFA MIXTURES (SXX), FERMENTED CANDY FACTORY PROCESS EFFLUENT (CXX), AND FERMENTED WATERSCHAP DE DOMMEL PRIMARY SLUDGE CENTRATE (PXX).
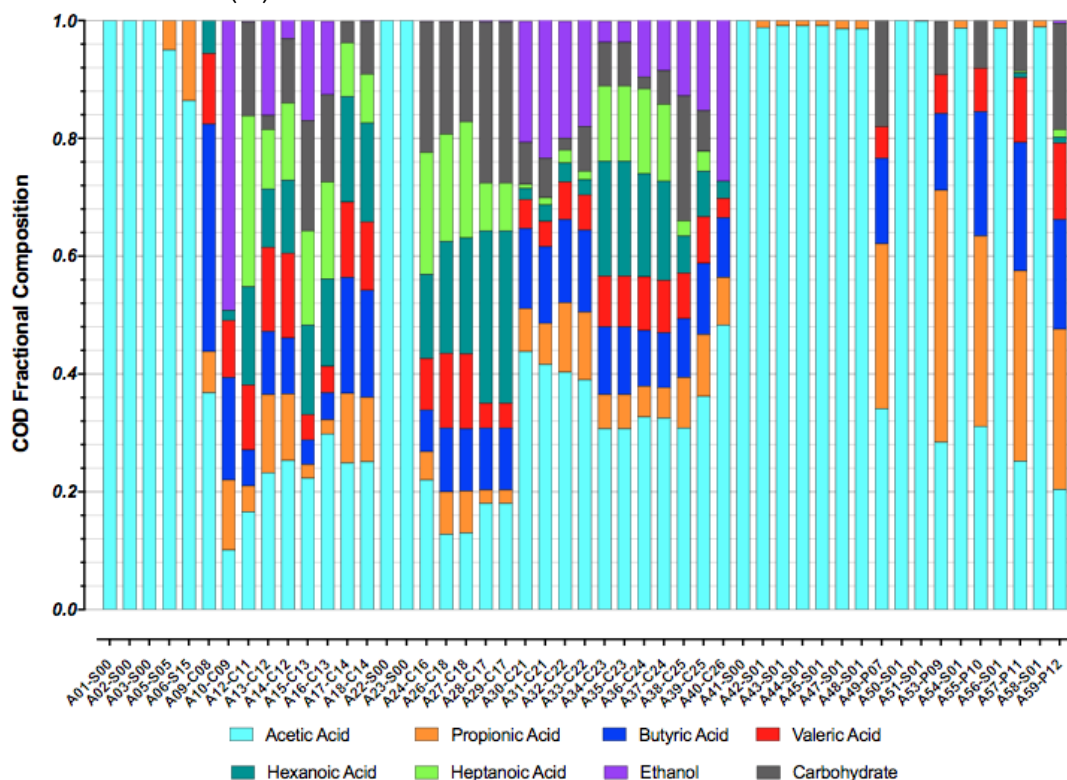
For all Axx batches, co-polymer blends of poly-(3 hydroxybutyrate-co-3 hydroxyvalerate) or PHBV were produced as a function of the feedstock composition. As mentioned previously, the recovered PHAs comprised a polymer chain mixture wherein the mixture is described both by a distribution of molecular weights (chain length) and co-polymer content (3HV wt.% in each respective polymer chain).

Average molecular weights (M_w) were more variable than expected from batch to batch. This observed variability was dominated by the conservative choice to dry the biomass isothermally at 70°C. The PHARIO project showed that a high initial drying temperature of at least 120 °C with well-defined cake morphology under drying is preferable (see paragraph 4.4.2). Future attention to detail for the biomass drying conditions will produce much more consistent and higher values of the recovered polymer molecular weight.

4.5.2   VFA FEED COMPOSITION DETERMINES CO-POLYMER COMPOSITION

Due to the benefit of a broad range of feedstock compositions used in PHARIO, we produced a wide spectrum of co-polymer blends in the range from 0 to 50 wt.% 3HV in PHBV. It was of interest to understand if the feedstock composition was directly related to the average polymer blend composition. The conversions of the individual feedstock components into metabolic precursors for 3HB and 3HV are well described in the research literature. Metabolic models have been proposed to describe the pathways from substrates to polymers. We wished to understand if polymer quality variation in co-polymer content was related to the feedstock composition, or the biomass or both.

If the co-polymer composition was predicted by the feedstock composition, then that would suggest that the biomass is itself a robust raw material for producing the specific co-polymers of PHBV just by controlling the feedstock. The maximal molar contributions for 3HB and 3HV based on conversions of substrates to acetyl-CoA or propionyl-CoA were defined based on the research literature. The fractional contributions of 3HB and 3HV derived from the respective substrates were estimated by minimizing the RMS error for the measured average 3HV polymer content estimated from the polymer melt properties by DSC.

Modelling results (Figure 24), after removal of justified outliers, supported the fact, that for the typical circumstances, biomass response in the accumulated biopolymer average 3HV composition was directly related to the feedstock composition. These are results for a full scale activated sludge sampled over 10 months of WWTP operations, 4 seasons, and with 3 distinctly different feedstock sources. The standard deviation in the error for the predicted 3HV molar content of the PHBV was 1.6%.

FIGURE 24   THE MEASURED PHARIO PHBV MOLAR 3HV CONTENT FROM DSC WAS MODELLED WITH RESPECT TO THE EXPECTED 3HV CONTENT BASED ON THE FEEDSTOCK MOLAR COMPOSITION AND CONSTRAINED BY KNOWN METABOLIC PATHWAYS. FROM A LEAST SQUARES REGRESSION INCLUDING ALL THREE SUBSTRATE DATASETS (CXX, PXX, AND SXX), THE MEASURED VALUES COULD BE READILY PREDICTED BASED ON THE FEEDSTOCK COMPOSITION (PREDICTION ERROR STANDARD DEVIATION OF 1.6%).
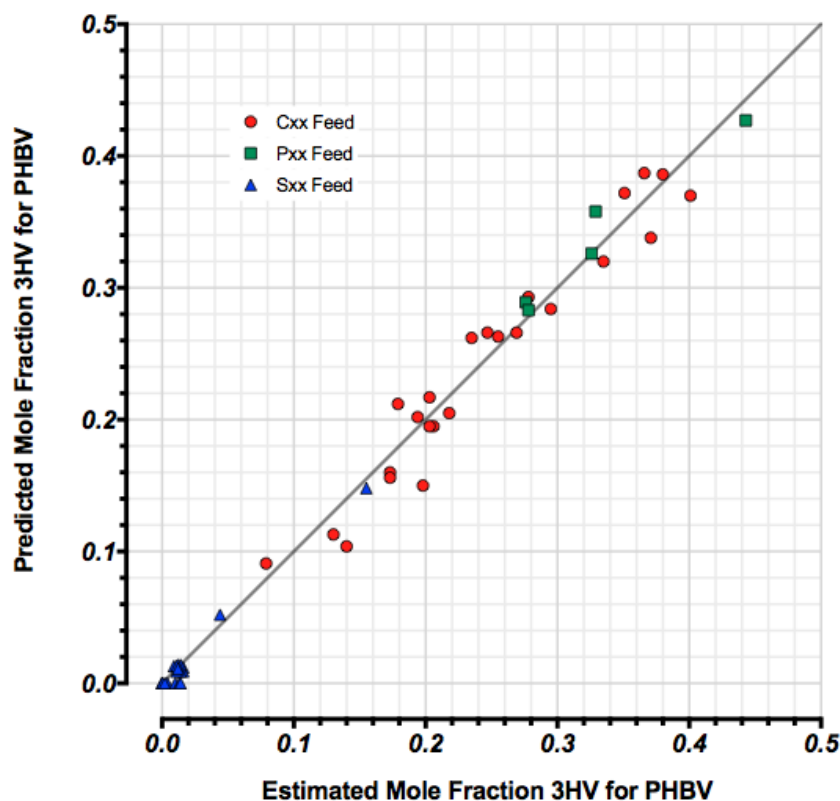
The was no correlation of model errors to biomass PHA content, molecular weight, yields of active biomass or PHA during accumulations. However, higher ethanol feedstock concentrations resulted in poorer predications of the PHBV 3HV content. Model predictions were further improved if the best fit coefficients were determined for Sxx, Cxx or Pxx feeds independently. This improved feedstock-specific model may indicate that other factors, and/or specific presence or absence of feedstock components do exert some different influence on the biomass metabolism and conversion in producing PHBV.

Drift in VFA composition for the same batch of fermented candy factory feedstocks occurred in some cases during storage between replicate accumulations with the same Cxx batch. In general, the type of PHBV co-polymer produced was a deterministic function of the feed-stock quality and the biomass was a stable raw material from which PHA polymers can be produced. This outcome was a very exciting and important result for the proof of concept of mixed culture PHA production methods.

4.5.3 CONSISTENT POLYDISPERSITY

Notwithstanding the observed variability of the recovered polymer M_w, we found that the molecular weight distributions from SEC evaluations were consistent and described by a PDI of 1.71±0.09 (Figure 25). This PDI fits very well from statistical modelling theory for polymerization processes of linear chain elongation and associated probability of chain term events. A consistent PDI is a signature of the product quality (value) as it suggests that polymers of a consistent molecular weight distribution were produced by the mixed culture activated sludge over a wide range of polymer PHBV type (i.e. wt.% range of 3HV) and 10 months of operations.

FIGURE 25   MEASUREMENTS OF PHARIO POLYMER IN BIOMASS MOLECULAR WEIGHTS BY SEC. THE SLOPE IN THE CORRELATION BETWEEN NUMBER AVERAGE (M_N) AND WEIGHT AVERAGE (M_W) MOLECULAR MASS IS THE POLYDISPERSITY INDEX (PDI). THESE DATA SUGGEST A CONSISTENT PDI OF 1.7 WHEREIN THE VARIATION IS INTERPRETED TO BE DOMINATED BY ERRORS ASSOCIATED WITH M_N DETERMINATION.
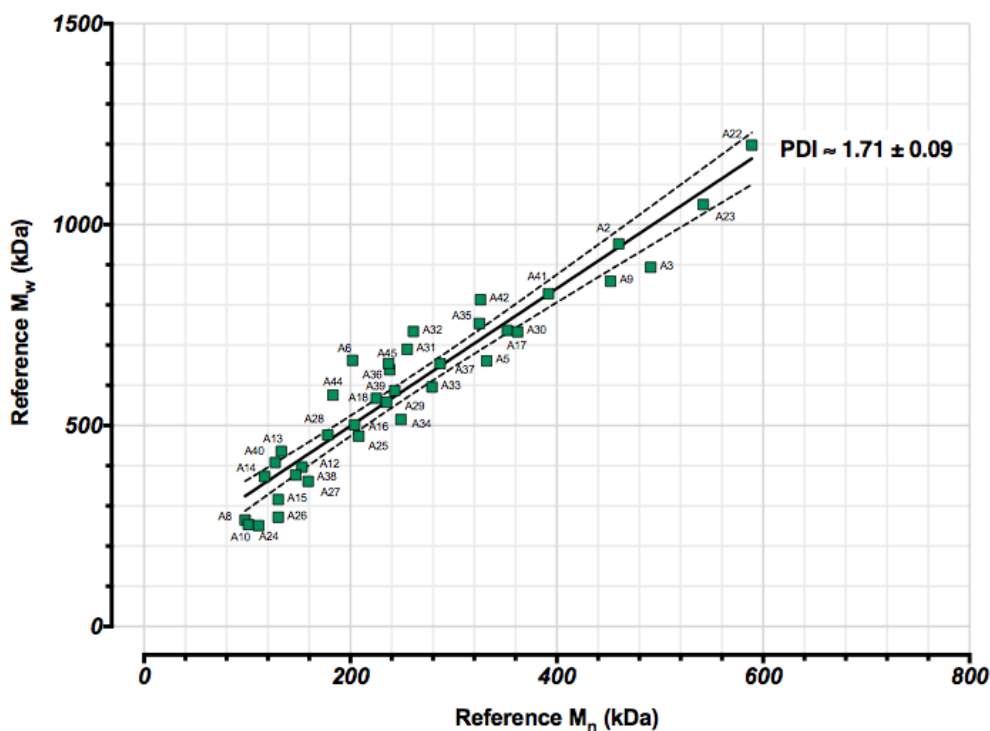
4.5.4 CONSISTENT THERMAL STABILITY

An advantage of the evaluation of the polymer M_w from melt rheometry at 180°C was that it enabled a comparison of the polymer molecular weight thermal stability in the melt. The polymer degrades in molecular weight by random chain scission and the predictability of melt stability is important to the value of the polymers for processing to bioplastics and applications. The scissions (N_s) were defined from the trends in the melt viscosity over 5 minutes. From the plot of the derived rheology initial M_w and M_w after 5 minutes in the melt, the slope minus one was the average scission rate (r_s). To our experience, with all other things being the same, the random scission rate is approximately constant.

Initial melt and 5 minute melt molecular weight data for reference laboratory scale test tube polymer recovery methods are shown with replicate data of the larger 10 L scale pilot recoveries (Figure 26). Both methods are based on PHA-poor solvent recovery at elevated temperatures (Werker et al. 2015). It is expected that polymers will degrade in molecular weight while in the melt. What is most important for reliable processing of these polymers, is that any kinetics of degradation occur in a reproducible way. If the properties are consistent then methods and process protocols can be developed with predictable outcomes. If the outcomes are predictable then quality targets can be readily achieved. If quality targets can be readily achieved, then the materials have value.

These data show, for all polymers recovered in PHARIO by the applied PHA-poor solvent recovery methods, and over a wide range of 3HV contents, that the melt degradation behaviour was very re-producible (constant r_s value). The plot suggests that at 180 °C, these polymers will be reduced to half their original M_w after about 9 minutes in the melt. These were remarkable results based on an overwhelming number of samples, which suggested that methods and protocols applied in PHARIO to produced mixed culture PHA, resulted in polymers with reproducible M_w distribution and consistent performance of stability in the melt. This outcome is synonymous to say that the polymers are of high quality and have value.

FIGURE 26   MELT RHEOLOGY OF PHARIO POLYMERS WITH DERIVED WEIGHT AVERAGE MOLECULAR MASS AND AFTER 5 MINUTES IN THE MELT AT 180°C. THE SLOPE OF THE CORRELATION MINUS ONE SUGGESTS A SCISSION RATE. POLYMER SAMPLES ARE REPRESENTATIVE OF POLYMER M_w IN THE BIOMASS (REFERENCE SAMPLES) AND FOR SELECTED BATCHES AFTER RECOVERY AT PILOT SCALE (EXTRACTED PHA).
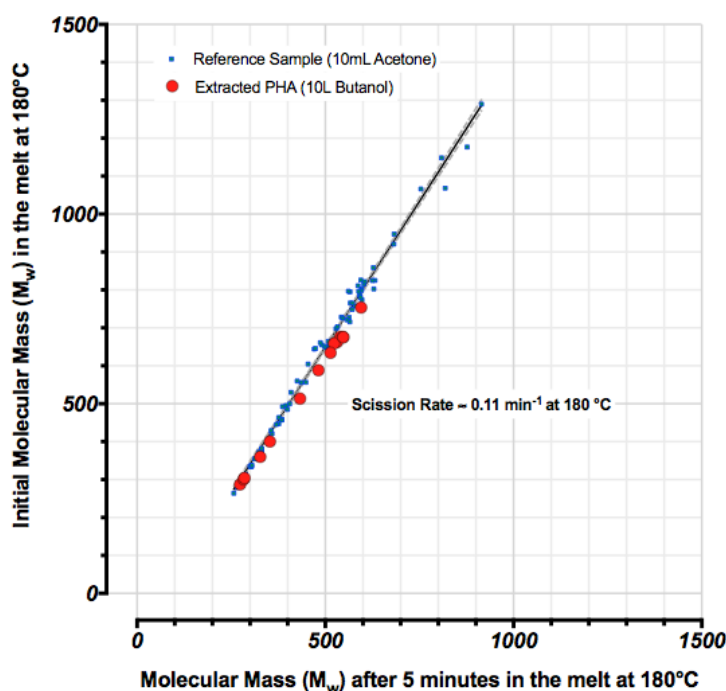
4.5.5 VARYING MOLECULAR WEIGHT BUT CONSISTENT MECHANICAL PROPERTIES

Due largely to the interpreted drying related M_w variation in the recovered polymers, a batch to batch variation of M_w was observed in the range from 200 to about 1200 kDa (Figure 26). The insights gained from the experience in the M_w variation have resulted in an understanding of how M_w outcomes can be readily controlled in the future to a much narrower range. The discovery that the polymers are high (nominally 1500 kDa) M_w opens a wider potential for use of the materials in high end applications that become possible due to the properties of

polymers with high molecular weight for processing (for example, melt strength). In PHARIO, we have come to recognize an unexpected value of the polymers that is made possible by further attention to one element in the chain of methods for the materials management (drying methods).

The range of M_w variation quality that was achieved in PHARIO is nevertheless acceptable within another window of the material value. The polymers after pilot scale recovery were with a M_w range from about 200 to 600 kDa (Figure 26). As will be described later with the discussion on mechanical properties, we confirmed outcomes from earlier studies that the polymer mechanical properties were dependent on the polymer 3HV content but the molecular weight did not exert a measurable effect on mechanical properties in the range from 200 to 600 kDa. The polymers as produced and recovered by the methods and protocols of PHARIO have value in melt processing applications with a wide tolerance in quality of M_w when targeting mechanical properties. Questions of absolute quality (value) cannot be adequately addressed without the context of the material commercial intent. What has been possible to confirm in PHARIO is that the materials can achieve commercial intent because they behave in a predictable manner.

4.5.6 HOMOGENEOUS BLENDS OF COPOLYMERS: ONE GLASS TRANSITION TEMPERATURE

The recovered polymers were co-polymer blends representing a distribution of 3HV content (Laycock et al. 2012, Arcos-Hernández et al. 2013, Laycock et al. 2014a, Laycock et al. 2014b). The co-polymer blends were characterized by the mean melt temperature which correlated to a mean 3HV weight percent.

The presence of just one detectable glass transition temperature suggested that the polymer blends were miscible. When more than one type of polymer are blended, the T_g of the mixture will depend on the relative amount of both components (Fried 2014). If the mixture is homogeneous in nature, then the blend will exhibit one T_g value. If a blend exhibits more than one T_g value then the mixture is inhomogeneous and the components are phase separated. Inhomogeneous polymer blends may not possess good mechanical properties due to a potential for weakness and crack propagation at the phase boundaries.

Even if the PHAs produced by mixed cultures are a PHBV co-polymer blend, the blend comprises a miscible mixture of polymer chains having a distribution of 3HV content. If the blend contained an immiscible mixture of co-polymers then more than one glass transition temperature would be observed. In all cases, only one single glass transition temperature was observed and this characteristic temperature followed as expected a systematic trend correlated to the mean PHBV 3HV content (Figure 27).

Variation in the data are understood to be related in part to the generally low signal to noise ratio in discerning the T_g. Some of the variation is also explained due to the effect of molecular weight on T_g. The available PHARIO T_g and M_w data suggested that for the same 3HV content, a molecular weight difference of about 700 kDa will decrease the T_g from the theoretical maximum by about 1°C, while a Mw of 350 kDa would result in a 2°C decrease. Molecular weight variation (as previously described) was due to controllable factors of drying conditions and the generically applied feed on demand biomass respiration stimulation methods. Therefore, the polymer quality, as measured by the property of T_g, was a consistent function of the co-polymer type (mean 3HV content).

FIGURE 27   FOR PHARIO, THE RECOVERED POLYMERS EXHIBITED, IN ALL CASES, A SINGLE GLASS TRANSITION TEMPERATURE AND THIS CHARACTERISTIC
TEMPERATURE FOLLOWED AS EXPECTED A SYSTEMATIC TREND CORRELATED TO THE MEAN PHBV CO-POLYMER BLEND 3HV CONTENT. VARIATION IN
THE DATA WERE UNDERSTOOD TO BE RELATED IN PART TO THE GENERALLY LOW SIGNAL TO NOISE RATIO IN DISCERNING THE T_G. SOME OF THE
VARIATION WAS ALSO EXPLAINED DUE TO THE EFFECT OF THE EXPERIENCED MOLECULAR WEIGHT DIFFERENCES ON T_G.
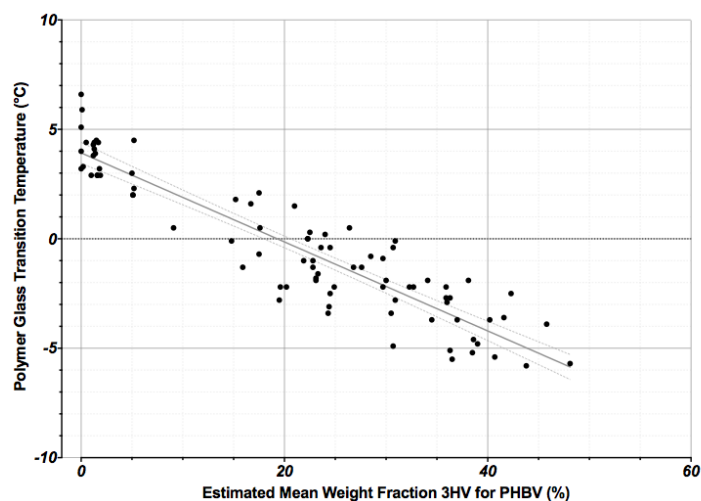
4.5.7   DIFFERENT PHA BATCHES CAN BE BLENDED

Mixed and pure culture systems alike tend to produce co-polymer blends of PHA so that the blend nature of the PHARIO materials is not unique or unusual. Blend characteristics can influence the product mechanical properties. Thus, it may be of interest in the downstream product development to consider to mix polymer batches together towards "master-batching" certain products and product grades.

We undertook to develop specific deeper insight to the PHARIO polymer blends by means of a Masters thesis investigation (Gustafsson 2016). Miscible blends of materials were produced with a single T_g when the percent weight average 3HV content of the blend did not exceed by nominally 9% the 3HV content for the most dominant component. This is inline with the so-called "Fox equation". When immiscible blends were generated the resulting two measurable T_g temperatures suggested distinct phases within the co-polymer blend.

The PHARIO batches of PHBV co-polymer blends could be combined and "compounded" together in following methods that are analogous to the polymer solvent recovery methods used to extract the PHA from the biomass. When miscible blends of materials were produced, a single glass transition temperature resulted and this characteristic T_g for the blend generally followed the trends of expectation based on the Fox Equation. Miscible blends of materials can be produced as long as the percent weight average HV content of the blend does not exceed by about 9% of the 3HV content for the most dominant component. Failure to achieve a miscible blend can be readily identified because two T_g temperatures result for the distinct phases within the co-polymer blend wherein the phases are a selective mixture of parts of the blend components (Figure 28).

Compounding and strategic combination of the polymers to targeted weight average 3HV contents as part of the PHA-recovery process offers significant potential for cost savings as well as a means to preserve other quality parameters of the polymer such as molecular weight. Different biomass batches may be combined and blended for recovery of defined PHBV types. In this way the quality control for master batches of polymer type can be established already

at the PHA recovery step. These findings have far reaching positive practical application for the materials management and product quality control and, as such, the product value.

FIGURE 28   AN EXAMPLE OF THE BLENDING MISCIBILITY RESEARCH AND DEVELOPMENT UNDERTAKEN FOR PHARIO (GUSTAFSSON 2016). PHARIO AXX BATCHES OF COPOLYMER BLENDS WITH 8 (HV$_{08}$) AND 41 (HV$_{41}$) PERCENT 3HV WERE COMPOUNDED ("MASTER BATCHED") WITH METHODS USED FOR THE POLYMER RECOVERY FROM PHA-RICH BIOMASS (WERKER ET AL. 2015). BLENDS WERE MADE IN DIFFERENT PROPORTIONS TO ACHIEVE A NEW MEAN BLEND COMPOSITION. IMMISCIBILITY (2 T$_G$ VALUES SHOWN IN RED) RESULTED WHEN THE MEAN BLEND COMPOSITION WAS GREATER THAN NOMINALLY 9 % OF THE MAJOR BLEND COMPONENT.
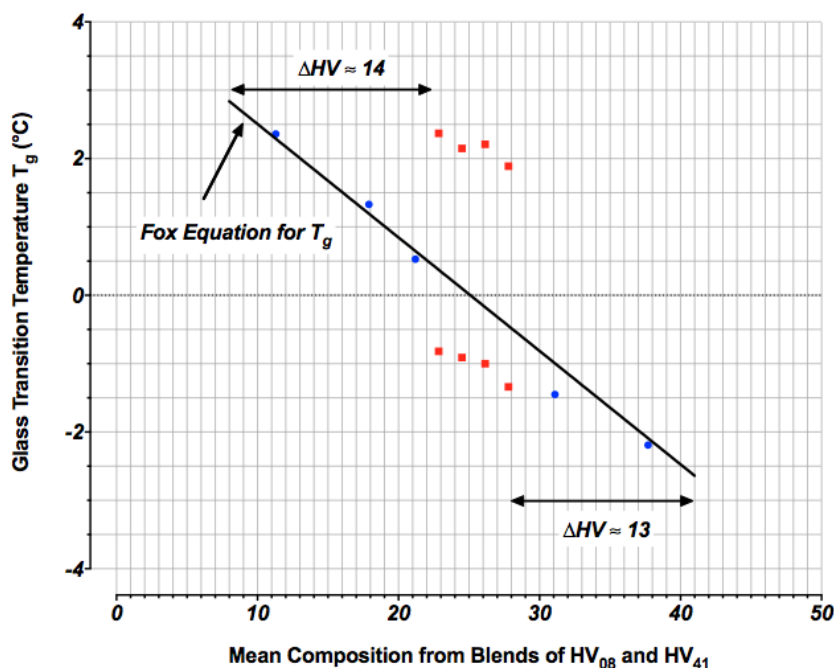
4.5.8   CONSISTENT MELT AND CRYSTALLIZATION BEHAVIOUR

Product value will also be very much linked to the reliability of the polymer melt characteristics. The polymer blend typically melts over a range of temperature wherein the mean melt temperature decreases and the range increases as the co-polymer content goes from lower to higher average 3HV contents up to 50% HV (Figure 29). This outcome suggests that the higher 3HV co-polymer blends contain a wider distribution of co-polymer types. A key factor for downstream utilization of the polymers is that the polymer properties behave systematically such that optimised formulations and processing conditions can be established towards any given value added application for these materials.

For the downstream end users it is important that PHARIO grade (polymer types) behave in a predictable way with respect to:
- melt and crystallization behaviour;
- thermal and chemical stability in the melt.

TYPICAL TRENDS OF PHARIO PHBV MELT FRACTION AS A FUNCTION OF TEMPERATURE DURING HEATING AT 10°C/MIN. DIFFERENT GRADES OF PHARIO PHBV ARE SHOWN WITH GRADE DENOTED BY MEAN 3HV CONTENT NOTED AS HV$_{XX}$. XX REFERS TO THE AVERAGE 3HV CONTENT IN PERCENT WEIGHT.
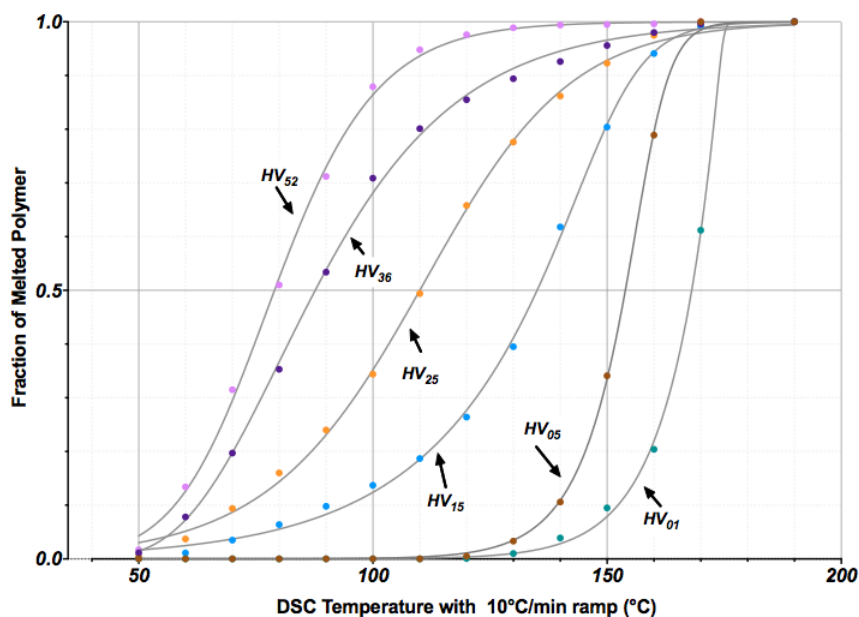
The materials produced and recovered were already shown to exhibit very similar thermal and chemical stability in the melt (Figure 26) and consistent and high thermal stability (Figure 19).

Over a wide range of material type, also the **melt behaviour** of a given polymer type followed a definable trend (Figure 30). The two graphs in Figure 30 show similar information as Figure 29 but now for <u>all</u> PHARIO polymer samples as measured by DSC. The left graph (a) shows the fraction of polymer melted as a function of temperature and average 3HV content. The right graph (b) shows the estimated start and finish melt temperatures, alongside the 5, 50 and 95 % melt temperatures as a function of polymer average 3HV content. Although there is variability in the end melt temperature (b), this variability is not representative of a large fraction of the co-polymer content (a) in the blend. The variability in the end melt temperature can be due to more or less of a component of PHB present in some of the co-polymer blends. Per these data, PHB melts at approximately 180°C. In other work, we have found that a minor amount of PHB in the co-polymer blend can be beneficial as an inherent nucleating agent during the cooling and crystallization of the polymer.

THE LEFT GRAPH (A) SHOWS THE FRACTION OF POLYMER MELTED AS A FUNCTION OF TEMPERATURE AND AVERAGE 3HV CONTENT. THE RIGHT GRAPH (B) SHOWS THE ESTIMATED START AND FINISH MELT TEMPERATURES, ALONGSIDE THE 5, 50 AND 95 % MELT TEMPERATURES AS A FUNCTION OF POLYMER AVERAGE 3HV CONTENT.
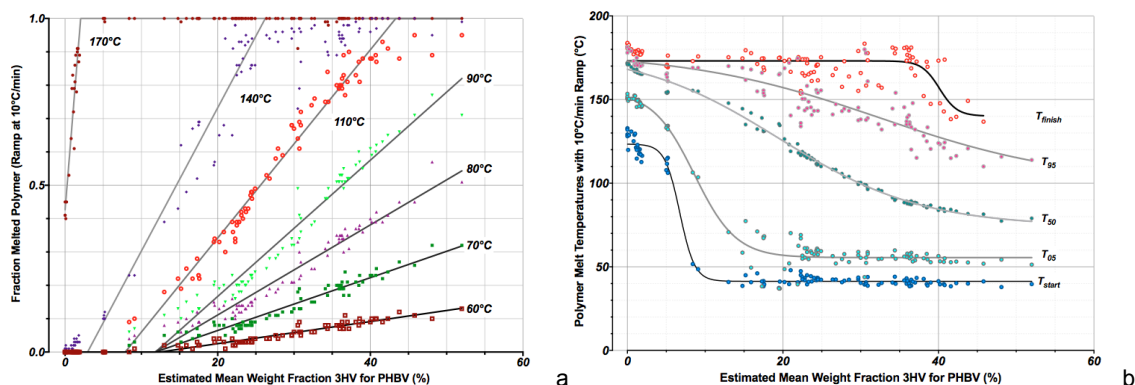
The **cooling rate** of a polymer from the melt will influence the extent to which the polymer forms ordered crystals in the solid phase. It is the crystalline nature of the polymers that gives these materials mechanical strength. If the cooling of the polymer is sufficiently rapid, then no ordered crystalline structure will result and the polymer in the solid phase will be completely amorphous. The amorphous component of a polymer microstructure imparts properties of toughness. Often a polymer microstructure is a mixture of crystalline and amorphous phases that are generated by a combination of the type of polymer, the thermal history of the polymer, time, and the use of additives to influence the rates and distribution of the crystalline phase(s). The interfaces between crystalline and non-crystalline phases are very important to the material properties. For example, a weak interface can be an initiation point for cracks that lead to mechanical failure. In a sense the non-crystalline regions and their entanglements at the interfaces in the microstructure matrix is the glue that keeps the material together. The melt peak ΔH is correlated to the material crystallinity. Higher ΔH means a higher degree of crystallinity.

The degree of crystallinity of the PHARIO co-polymers was systematically related to the mean 3HV content and thermal history (Figure 31). A systematic behaviour is the basis for value (quality) in the downstream compounding and processing of these materials. A consistent behaviour is a starting point towards developing formulations to exploit beneficial properties while working to mitigate for factors that may challenge the material use in processing or in applications.

FIGURE 31   THE MELT PEAK ΔH IS CORRELATED TO THE MATERIAL CRYSTALLINITY. HIGHER NUMBERS MEAN A HIGHER DEGREE OF CRYSTALLINITY. THIS GRAPH SHOWS THAT THE NATIVE PURE PHARIO MATERIALS BEHAVE IN A SYSTEMATIC WAY AS A FUNCTION OF MEAN 3HV CONTENT AND THERMAL HISTORY.
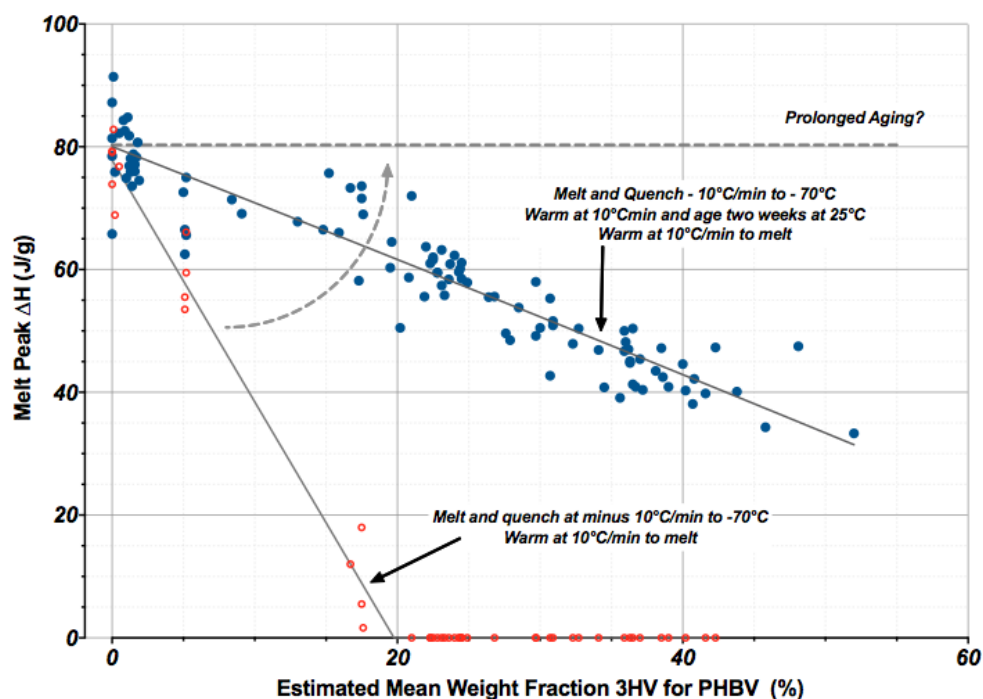
Just as the melt ΔH indicates for the degree of crystallinity, the glass transition C_p is related to the amorphous fraction of the solid. The C_p trends of the raw DSC signal were difficult to estimate precisely because the signal levels were low. A cooling quench rate of -50°C/min will provide very little time for the melt to crystallize upon cooling. Thus, a rapid quench is

intended to maximize the amorphous fraction of the solid. The data suggested (Figure 32) that above a mean 3HV content of 5 percent by weight, the polymer is consistently the same and is anticipated to be 100 percent amorphous (slower crystallization rate). Below a mean 3HV content of about 5% some degree of crystallinity is indicated. This tendency for the lower 3HV materials to more rapidly crystallize is why PHB has application as a nucleating agent in the higher 3HV co-polymer blends. The materials behaved in a consistent way that was dominated in predictability based on the polymer type (average 3HV content).

FIGURE 32   THE PHARIO POLYMERS WERE MELTED AND THEN QUENCHED AT -50 °C/MIN TO - 70°C. JUST AS THE MELT ΔH INDICATES FOR THE DEGREE OF CRYSTALLINITY, THE GLASS TRANSITION C_p IS RELATED TO THE AMORPHOUS FRACTION OF THE SOLID. HIGHER NUMBERS MEANS MORE OF THE QUENCHED POLYMER IS AMORPHOUS. THESE DATA SUGGEST THAT ABOVE A MEAN 3HV CONTENT OF 5 PERCENT BY WEIGHT, THE POLYMER IS CONSISTENTLY THE SAME AND IS ANTICIPATED TO BE 100 PERCENT AMORPHOUS.
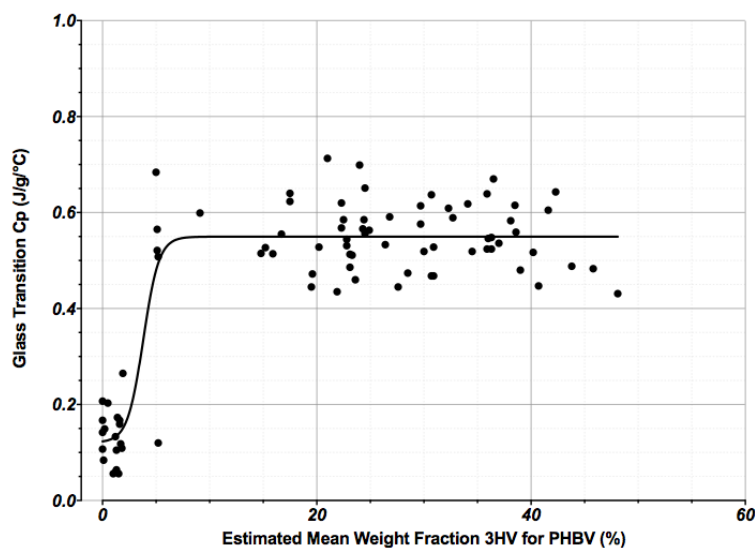
As part of the TKI project Kleinschalige Biomassa the Wageningen Food & Biobased Research group (WFBR) independently verified thermal properties of extracted PHARIO samples (Kappen 2017). For this purpose several samples of non-extracted PHA-rich biomass with different HV contents were supplied to WFBR. PHA was extracted from these samples via Soxhlet extraction. The thermal properties were determined by differential scanning coulometry (DSC) using a Perkin Elmer Series 8000 DSC. Table 6 summarizes the glass transition temperatures and melting points determined for both non-extracted and extracted samples.

From both series it can be seen that higher 3HV contents result in lower glass transition temperatures and melting point (as expected). At very high 3HV contents (30 % and higher) the material shows a melt trajectory (so not only 1 melting point). If the thermal properties of the non-extracted and extracted PHA samples are compared, both Tg and Tm of the extracted samples are significantly higher than the non-extracted samples. A possible explanation is that due to contamination of the non-extracted PHA materials both Tg and Tm are somewhat lower.

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TABLE 6   **THERMAL PROPERTIES TG AND TM OF EXTRACTED PHA MATERIALS AS DETERMINED BY WAGENINGEN FBR (KAPPEN 2017).**

		Non-extracted		Extracted by WFBR	
Sample	%3HV	Tg (°C)	Tm (°C)	Tg (°C)	Tm (°C)
PHA S0	0	-3.9	168.5	-0.2	176.4
PHA S1	1	-6.0	166		
PHA S5	5	-8.3	153.9	-0.1	164.9
PHA C10	9	-7.8	157.4	-2.3	165.2
PHA C20	16	-7.0	159.7		
PHA C30	30	-9.5	73.8 / 109.1[*]		
PHA C40	42	-12.8	72.9 / 104.7[*]	-6.1	70.5 / 112.4[*]

* this is a melt trajectory, not a melting point

Another important thermal property of (semi)-crystalline polymers is crystallisation kinetics. In this research this property has been analysed by determination of the isothermal crystallisation behaviour in DSC equipment (protocol 2 of description of the DSC). Crystallisation behaviour has been studied between 40 and 80°C. Results can be seen in Figure 33. The crystallisation behaviour of sample C40 could not be analysed as the kinetics were too slow for the type of analysis as proposed. Optimal crystallisation temperature of this type of PHA materials is at about 60 – 65°C (= temperature with the lowest crystallisation peak temperature). Moreover crystallisation rate is dependent on PHA co-polymer composition: the higher the 3HV content the lower the speed of crystallisation. Speed of crystallisation of the commercial PHB powder looks like the behaviour of PHA sample S0.

FIGURE 33   **CRYSTALLISATION KINETICS OF VARIOUS PHA SAMPLES AS DETERMINED BY WAGENINGEN FBR (KAPPEN 2017).**
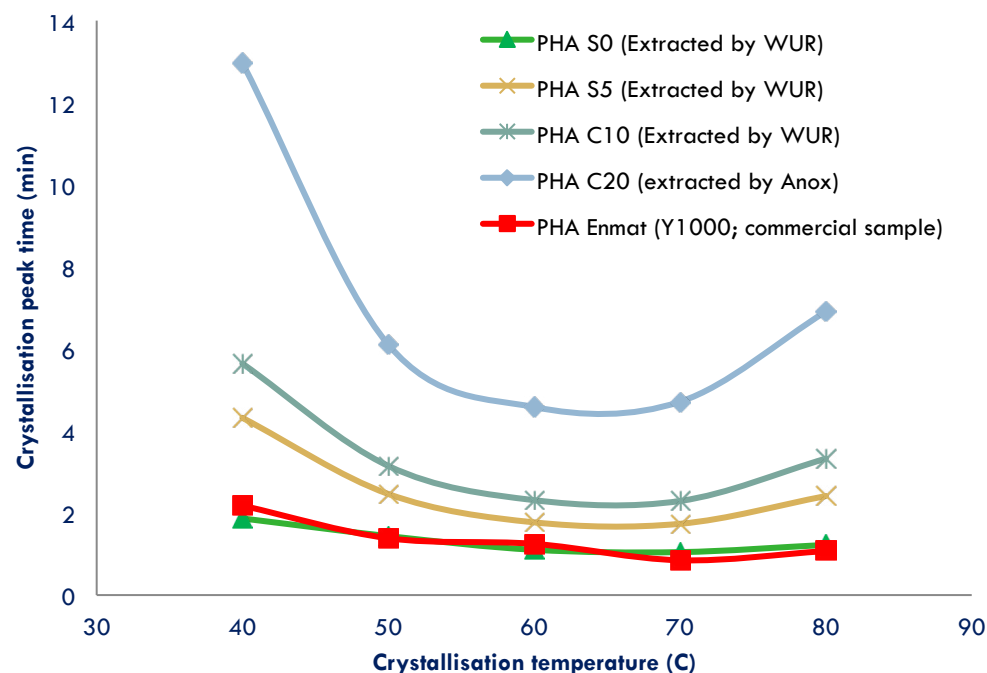
4.6 CONCLUSION

In summary, the body of information for PHARIO was comprised of the successful production of 52 batches of PHAs. These polymers were co-polymer blends of PHBV where the discriminating parameter used to define the different grades of PHBV was the mean 3HV composition. The mean 3HV composition was a direct function of the feedstock organic composition and,

so, the full scale WWTP Bath biomass performance to produce predictable polymer products was robust.

In PHARIO the average molecular weights varied but we learnt that this variability was most likely due, in part to the need for refinements in the accumulation process control, but more significantly due to drying conditions that permitted for some degree of microbial enzyme activity as the wet biomass heated up. Accumulation process control requires a sustained maximal respiration to minimize polymer chain termination probability and control methods can be improved upon for an industrial scale prototype. The drying influence of molecular weight will be eliminated by reducing the time for the warm up period of the wet biomass, and generally by using higher initial drying temperatures.

The molecular weight distribution (PDI), melt stability, and thermal stability were approximately constant and generally independent of polymer grade or molecular weight.

Batches of polymer can be compounded as an integral part of the PHA recovery process and blend miscibility can be ensured based on respective component mean 3HV contents.

Polymer properties of melt and crystallization behaviour that are critical towards developing formulations for compounding and recipes for processing these polymers were consistent and predictable functions of the mean 3HV content.

In conclusion, the qualities of the polymers produced for PHARIO are filled with promise of engineering and predictability. These materials have value.

5

VALORIZATION OF THE PRODUCED PHA

5.1 INTRODUCTION

In this Chapter, we consider the questions of PHA quality through the eyes of stakeholders further down the value chain - the PHA-refiners, the PHA-compounders, and the Bioplastic-converters. The batches of PHARIO PHA rich biomass were refined at pilot scale to produce grades of products. The products were evaluated for consistency in mechanical properties. In addition, five case studies were undertaken within a local European commercial network to examine and report on illustrative routes of how the recovered Water Board polymer could be valorised in the downstream bio economy.

5.2 PILOT SCALE PHA EXTRACTION

5.2.1 PROCEDURE

PHA Rich Biomass from PHARIO (Hjort et al. 2015) were processed for reference and pilot scale (10L) polymer recovery. From all of the successfully 52-completed accumulation (Axx) batches, reference polymer recovery was made for all batches at the test tube scale (10 mL) with 10 g/L polymer loading, in acetone and at 125°C for one hour (Werker et al. 2015). In addition, 23 × 10L PHA extractions were undertaken from selected Axx batches to supply a range of material types for investigations related to the quality and opportunities for commercial developments for these polymers.

The pilot scale recoveries were undertaken as previously disclosed (Werker et al. 2015), and with analogy to the material flow as described for the PHARIO WP3 LCA PHA recovery process mass balance estimations (Magnusson et al. 2016). Recoveries at the 10 L scale represented a range of types of PHBV blends with 3HV contents ranging from 0 to 44 weight percent. Recovered materials were further used in case study evaluations of routes to market in direct interaction with well-established commercial end users and product developers (compounders and processors).

For the pilot solvent extraction methods 2-Butanol was used, but other so-called PHA-poor solvents are possible (Werker et al. 2015). Recovered polymers were rinsed following recovery and pressing to more adequately remove residual solvent and to compensate for the non-industrial process nature of the piloting equipment. Extraction and loadings were performed with the same protocol for comparison of the recovery reproducibility. Thus we did not tune the recovery parameters to be optimal for the different PHBV types, notwithstanding that this is a central and important principle for the developed methods (Werker et al. 2015).

Optimal conditions and challenges to recover PHB are not the same as those for a co-polymer blend of PHBV, nor a homopolymer of PHV. Optimisation of the recovery settings was beyond the scope of PHARIO. The goal was on production and evaluation of the products for value.

A constant recovery solvent loading of nominally 50 g/L polymer was applied in all cases and, therefore, there was some variation in solvent biomass loading due to the minor variation in the batch to batch biomass PHA content. A 52-minute heating program from 50°C to a maximum solvent temperature of 140 °C was used and the average solvent temperature during the extraction heating cycle was nominally 103 °C. We targeted on average about 87% of the extractable polymer based on extraction rate evaluations (see Werker et al. 2015).

Mechanical testing of the pure recovered PHARIO co-polymers was undertaken in order to assess for the consistency of the material behaviour as a function of polymer type. PHARIO PHBVs were recovered at pilot scale from the following accumulation PHA-rich biomass batches A03, A05, A06, A09, A10, A13, A14, A15, A16, A17, A18, A24, A27, A28, A29, A30, A33, A37, A38, A40, A42, A44, and A45. These batches represented a range of polymer types with respect to the mean 3HV content of the PHBV co-polymer blends.

5.2.2 TUNING PHA EXTRACTION TO 3HV CONTENT

Given PHBV polymers in biomass with similar weight average molecular mass, the most dominant factor influencing the extraction kinetics is the polymer mean 3HV content. An "Adam-test" comprises a series of polymer isothermal extractions at selected temperatures given the biomass prepared by granulation to a defined particle size distribution, the same loading to the solvent, and the same extraction time. In PHARIO for these Adam-tests we used 150 mg of PHA-rich biomass with grain size of 0.71 to 2 mm to perform "Adam-test" isothermal reference extractions with 10 mL of 2-butanol over 45 minutes. The polymer rich solvent was separated from the biomass suspended solids and the mass balance was evaluated by TGA.

FIGURE 34   RESULTS OF ADAM-TESTS FOR ASSESSMENT OF PHARIO PHBV RECOVERY FROM PHA-RICH BIOMASS KINETICS AS A FUNCTION OF 3HV CONTENT FROM 0 TO 40 PERCENT. FROM THE SIGMOID TREND ALONG WITH MASS BALANCE CONSIDERATIONS, AN "IDEAL" FIRST ORDER RATE CONSTANT AS A FUNCTION OF TEMPERATURE CAN BE ESTIMATED FOR THAT PARTICULAR BIOMASS WITH DEFINED PHBV TYPE, AND PARTICLE SIZE DISTRIBUTION.
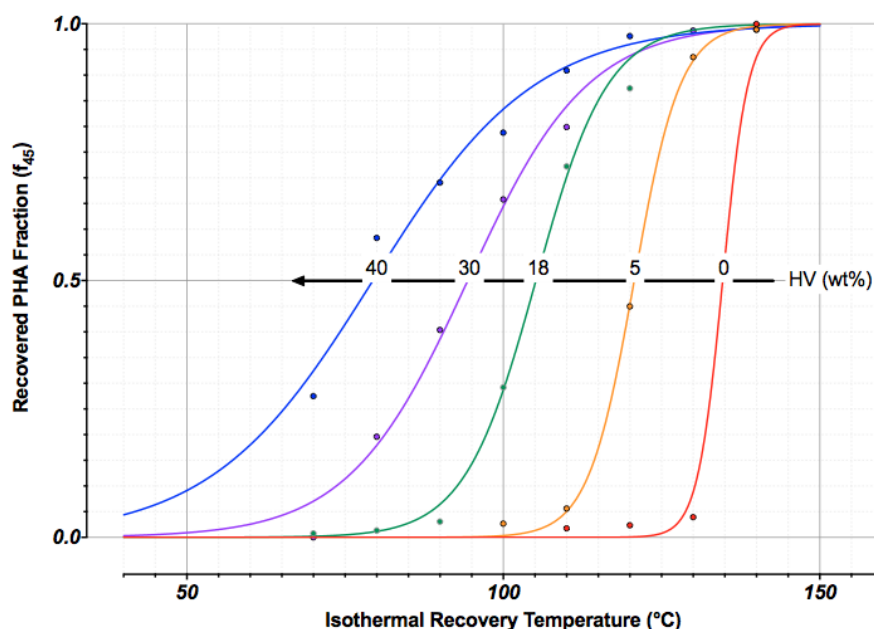
From the sigmoid trend (Figure 33) and with mass balance considerations, an "ideal" first order rate constant as a function of temperature can be estimated for that particular biomass with defined PHBV type, and particle size distribution (Werker et al. 2015). The estimated

Adam test sigmoid trend provided estimates of an inflection temperature and rate that was closely related to 3HV content given otherwise similar M_w distributions of the PHA-in biomass (Figure 34). Higher 3HV PHBV co-polymers are recovered faster and at lower temperatures than pure PHB.

Thus, the Adam-test method can be used to predict the polymer type, or knowledge of the polymer type could be used to predict the required conditions for the polymer recovery from batch-to-batch. Conditions of recovery depend on particle size, the time, temperature and loading to the extraction system. Given the potential for Water Boards to produce a range of interesting PHA types, as we have done for PHARIO, it is important to establish recovery practices that can be systematically tuned to the needs for each PHA-rich biomass batch (Werker et al. 2015, Magnusson et al. 2016).

FIGURE 35   FROM ALL THE ADAM-TESTS PERFORMED IN PHARIO A CORRELATION WAS MADE BETWEEN THE TEST PARAMETERS OF TEMPERATURE AND RATE SHOWING HOW THE POLYMER TYPE INFLUENCES RECOVERY CONDITIONS. HIGHER 3HV PHBV CO-POLYMERS ARE RECOVERED FASTER (B) AND AT LOWER TEMPERATURES (A) THAN PURE PHB.
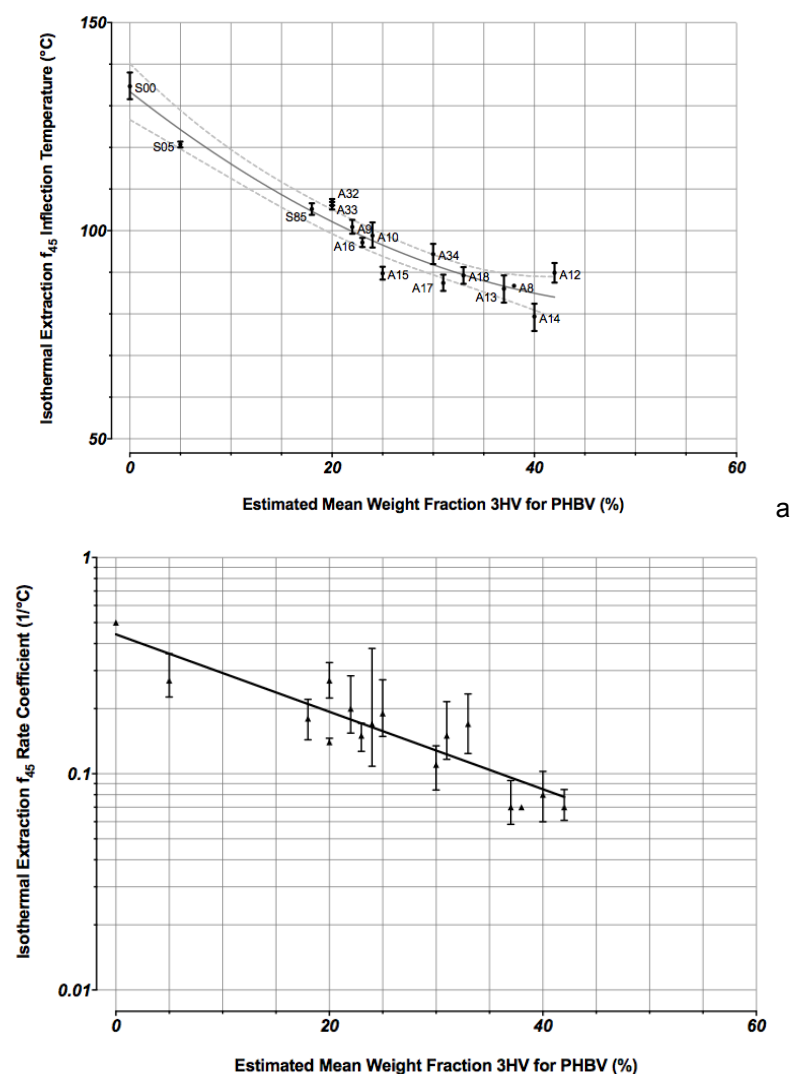
5.2.3   INFLUENCE OF PILOT SCALE EXTRACTION ON MOLECULAR WEIGHT

Reference extractions had been made for all Axx batches to assess the effective molecular weight of the polymer in the biomass before extraction. Molecular weights were also measured

for the pilot scale recovered materials and these were compared to the reference "in-biomass" values (Figure 35). Since all the pilot 10 L extractions were performed similarly, an estimate of the nominal molecular weight loss during the extraction suggested a scission rate for the polymers due to the higher solvent loading of polymer 50 g-PHA/L instead of 10 g-PHA/L) and type of solvent (2-butanol instead of acetone).

The results suggested a consistent influence of the extraction conditions on the recovered polymer molecular weight being a scission rate of 0.276 hr^{-1}. A 1-hour extraction was applied in the pilot recovery, and this scission rate indicates that it would take 3.6 hours for the polymers to be reduced to half the original PHA-in biomass M_w.

Three of the 23 batches of pilot scale PHA recovery were with higher than typical scission rates. In principle, this difference in scission rate can represent a different quality of the PHA rich biomass and such differences can be evaluated a priori based on chemometric models (Werker et al. 2015). Given the overwhelming consistency of scission rate, the differences could also simply have been measurement errors, but a deepened evaluation of these three outliers was not undertaken.

The fact that the scission rate was generally very reproducible during the extraction process is very important to the PHA-refiners. PHA-refiners require delivery of a PHA-rich biomass that behaves in a reliable way as this makes the development of industrial protocols required for the process easier to establish. Process development is easier because the outcomes are predictable and predictable outcomes provide for polymer products that can be refined within a definable quality window.

FIGURE 36   SINCE ALL THE PILOT 10 L EXTRACTIONS WERE PERFORMED SIMILARLY, AN ESTIMATE OF THE NOMINAL MOLECULAR WEIGHT LOSS DURING THE EXTRACTION COULD BE ESTIMATED.
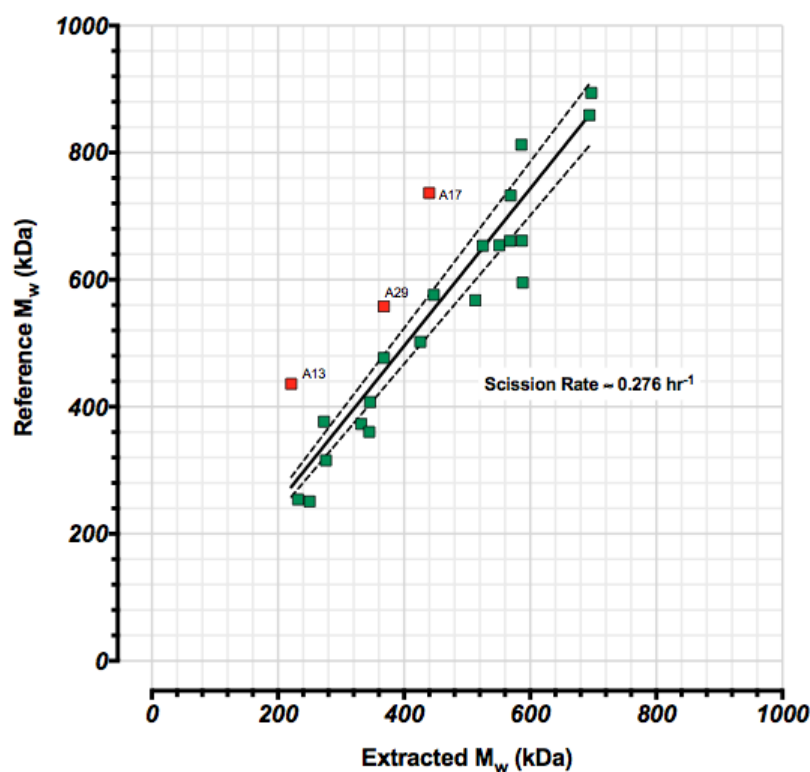
5.3 MECHANICAL PROPERTIES OF THE EXTRACTED PHA

The pilot-scale recovered polymers were melt processed into test elements for evaluating the material mechanical properties. Tensile test "*dog bones*" were made using a hot press. A grab sample of 0.9 g of extracted purified PHBV powder was weighed and loaded into a dog bone mould with a thickness of 2 mm, width of 5 mm and a gauge length of 15 mm. The mould was placed in the hot press at 180 °C for the extracted purified PHBV powder. Pressure was subsequently increased to 30 bars. When pressure had been applied the mould was kept at 180 °C for 5 minutes. After 5 minutes the mould was cooled by water to 70 °C before releasing the pressure and removing the test specimen from the mould. 6 test bars were made for each sample. Tensile testing was carried out according to ASTM D638 standard method using an Instron 430I tensile testing machine. The test specimens were placed in the grips of the testing machine and care was taken to align the long axis of the specimen and the grips along an imaginary line joining the points of attachment of the grips to the machine. The grips were tightened evenly and firmly to the degree necessary to prevent slippage of the specimen during the test. A small pre-load (less than 5 N) was applied to the specimen at a cross-head speed of 0.1 mm/min. A progressively increasing Tensile test force was then applied with a cross head speed of 10 mm/min and the load-extension curve was recorded.

FIGURE 37   THE MECHANICAL PROPERTIES OF ELASTICITY MODULUS AND TENSILE STRENGTH WERE FOUND TO CORRELATE WITH THE POLYMER TYPE (3HV CONTENT). THE REPLICATE TEST SPECIMENS EXHIBITED A DEGREE OF VARIATION AS EXPECTED. ERRORS BETWEEN THE LINE OF BEST FIT AND THE MEASUREMENT VALUES WERE EVALUATED AND SHOWED NO WELL-DEFINED CORRELATION TO M_W DIFFERENCES BETWEEN SAMPLES.
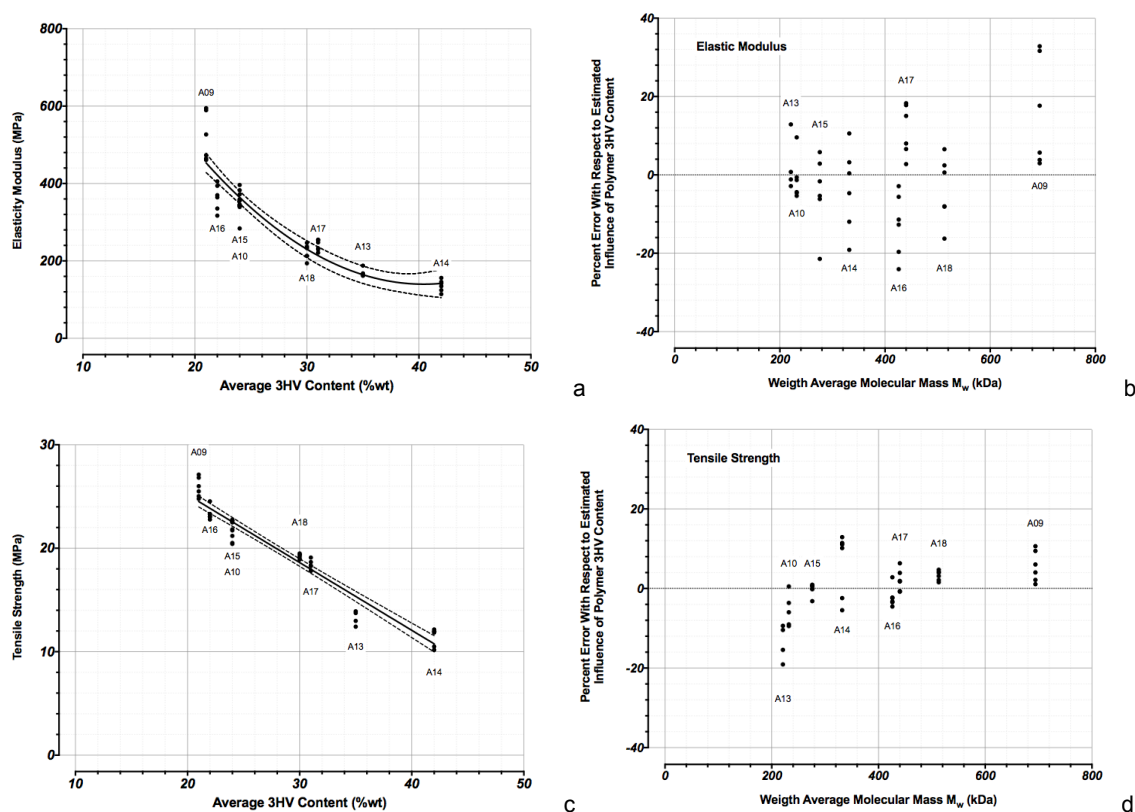
Flexural test bars were also made using a hot press. A grab sample of 0.5 g of extracted purified PHBV powder was weighed and loaded into a test bar mould with a width of 6 mm, a depth of 1 mm and a length of 40 mm. Hot pressing was performed similarly to the tensile test specimens. Flexural testing was carried out according to ASTM D790 standard method with the same Instron 430I tensile testing machine. Test bars were supported at two points over a defined span, and loaded by a nose pressing midway between these supports. The specimen

was deflected until rupture occurred in the outer surface or until a maximum strain of 5.0 % was reached, whichever came first.

Note that it was a challenge to manufacture, on small scale and with limited materials identical test specimens without any mould defects. Therefore, the mechanical test data results and trends were expected to exhibit a degree of variability. Notwithstanding this variability, the purpose of the testing was to evaluate the extent to which the mechanical properties were correlated with the polymer type and/or variation in the source material molecular weight. The absolute values of the mechanical properties were of less interest than the display of systematic trends. With the benefit of the wide range of polymer types produced in PHARIO it was possible to evaluate this influence of polymer type on the material mechanical properties. All specimens were aged for two-weeks at room temperature between production and testing in order to allow for secondary crystallization.

Replicate specimens exhibited a degree of variation as expected in elasticity modulus and tensile strength (Figure 36). The mechanical properties of elasticity modulus and tensile strength were found to correlate with the polymer type (i.e. 3HV content). The weight average molecular weight (M_w) of the polymers that were tested varied between 200 and 600 kDa. Therefore, it was of further interest to see if the test specimen mechanical properties were sensitive or insensitive to M_w values in this range. Errors between the line of best fit, in the correlation to 3HV content, and the measurement values were evaluated for potential correlation to M_w differences between test specimens. Absence of a secondary correlation to M_w suggested that the mechanical properties were insensitive to M_w in this molecular weight range. This outcome is supported by other in-house observations and evaluations, made previously and in collaboration with the University of Queensland, where we found that mechanical properties of these PHBV polymers were insensitive to M_w down to about 150 kDa. The scatter in the mechanical test results for the pure materials is believed to be mostly due to an influence of test specimen sample-to-sample variations and imperfections.

Results of flexural modulus and stress at 3.5% strain suggested that beyond about 32 % wt 3HV, for the tested range, the material stiffness is about the same (Figure 37). Otherwise stiffness increases with decreasing 3HV content. Changes in behaviour of flexural modulus as a function of 3HV content were in some ways analogous to results of elongation to break (Figure 38). PHBVs with higher 3HV content are expected to be more ductile. However, an influence of 3HV content on the elongation to break seems to require a significant amount of 3HV (> 30% wt). Increased variability with increased elongation to break is understood to be due to increased probability of the influence of a test sample defect as the material stretches plastically.

FIGURE 38   RESULTS OF FLEXURAL MODULUS AND STRESS AT 3.5% STRAIN SUGGESTED THAT BEYOND ABOUT 32 %WT HV, FOR THE TESTED RANGE, THE
MATERIAL STIFFNESS IS ABOUT THE SAME. OTHERWISE, STIFFNESS INCREASED WITH DECREASING 3HV CONTENT.
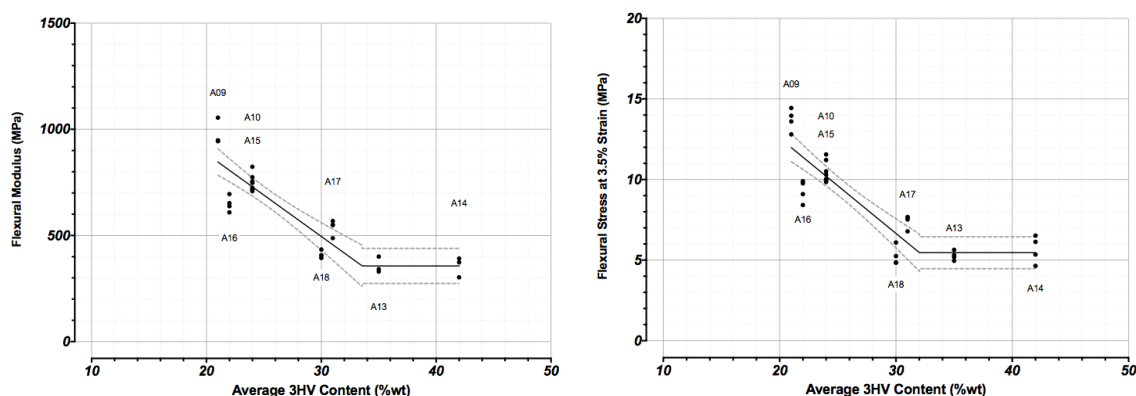
FIGURE 39   PHBVS WITH HIGHER 3HV CONTENT WERE MORE DUCTILE. INFLUENCE OF 3HV CONTENT ON THE ELONGATION TO BREAK SEEMED TO REQUIRE A
SIGNIFICANT AMOUNT OF 3HV (> 30% WT). INCREASED VARIABILITY WITH INCREASED ELONGATION TO BREAK WAS UNDERSTOOD TO BE DUE TO
INCREASED PROBABILITY OF THE INFLUENCE OF A TEST SAMPLE DEFECT AS THE MATERIAL STRETCHES PLASTICALLY.
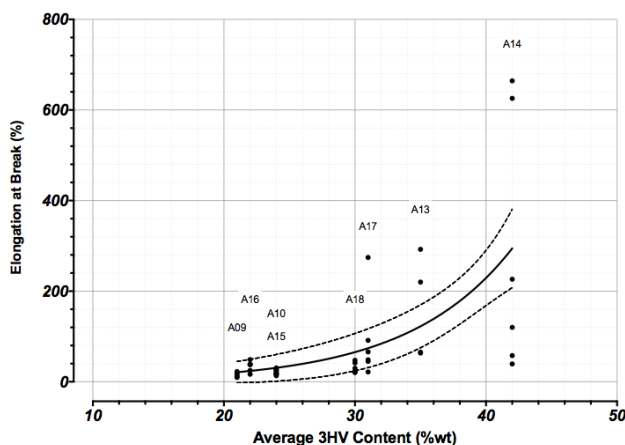
5.4 TESTING APPLICATIONS OF PHARIO PHA

So, the PHA-refiners can apply methods and adjust to changing polymer type in the PHA-rich biomass supplied by the PHA-producers to recovery polymer products. The polymer products are characterized by a mean 3HV content in a co-polymer blend of PHBV. The melt and crystallization behaviour of these polymer products follows systematic trends as do the mechanical properties. The polymers should have value to downstream PHA-compounders and Bioplastic-converters. To assess the question of potential for downstream value as part of WP1, four cases studies were undertaken where real-life links in the value chain for commercial intent in the downstream of PHARIO PHBVs could be made in the future:

1   PHARIO PHBVs were compounded according to long-standing commercial compounding formulations used by Biomer (www.biomer.de). In this exercise we wished to evaluate the potential use of PHARIO materials within a well-established business activity. PHARIO polymers could become an alternate and biobased polymer source to an existing commercial value chain.

2   PHARIO PHBVs were also compounded with commercial poly-lactic acid biopolymers (PLAs) from Corbion (www.corbion.com). PHAs can be used as modifiers in bioplastic formulations and to this end, PHAs can improve the impact resistance of PLA bioplastics. There are many elements to optimising such formulations but, at least as a case study, we wished to demonstrate that PHARIO PHBVs could be used as biobased functional additives in bioplastic formulations.

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3 The first two cases concerned the compounding of PHARIO PHBVs. In the third case study, PEZY (www.pezy.nl) was supplied with PHARIO produced "Biomer-like" and PLA-modified bioplastics formulations as pellets. PEZY is a product developer and in that sense a "Bioplastic-converter" turning bioplastics into products and services. The objective of this case study was to gain direct feedback from PEZY on the practical experience of using PHARIO bioplastics in injection moulding their reference product – a business card holder.

4 PHARIO PHBVs were delivered to the company Bioplastech in Ireland (www.bioplastech.eu). Bioplastech was founded by two entrepreneurs developing capacity for production of medium chain length (mcl) PHAs by pure culture methods. They have established formulations and products from their polymers. The objective of this case study was to obtain independent feedback on the PHARIO PHBV polymer quality and insight to the potential of these PHBVs as ingredients for film applications.

The PHARIO PHBV product grades used for the case studies are summarized in Table 7.

TABLE 7   THE POOL OF MATERIALS USED FOR THE CASE STUDIES ARE SUMMARISED. A RANGE OF PHARIO PHBV GRADES WITH 3HV CONTENTS FROM 0 TO 40 PERCENT WERE USED. [1]DETERMINED BY GC, [2]WEIGHT AVERAGE MOLAR MASS DETERMINED BY SEC, [3]POLYDIS-PERSITY INDEX DETERMINED BY SEC, [4]DECOMPOSITION TEMPERATURE MAXIMUM RATE OF WEIGHT LOSS DETERMINED BY TGA, [5]GLASS TRANSITION TEMPERATURE DETERMINED BY DSC, [6]MELTING RANGE DETERMINED BY DSC, [7]AVERAGE MELTING TEMPERATURE DETERMINED BY DSC. [8]ENTHALPY OF THE MELTING PEAK DETERMINED BY DSC.

Grade	Feed	Composition [1] 3HV by wt.%	Mw [2] (kDa)	T_d [4] (°C)	T_g [5] (°C)	$T_{m\text{-start to end}}$ [6] (°C)	$T_{m\text{-mean}}$ [7] (°C)	ΔH [8] (J/g)
S00	Synthetic	0	980	285	4,7	127-182	167	81
S01	Synthetic	2	810	283	3,2	113-177	162	81
S05	Synthetic	5	660	288	2,0	112-171	151	67
S20	Synthetic	18	660	286	0,5	40-168	117	69
C10	Candy factory process water	9	410	285	0,5	49-176	139	69
C20H	Candy factory process water	18	670	284	-1,5	40-175	116	63
C20L	Candy factory process water	20	380	283	-2,2	41-173	111	50
C30H	Candy factory process water	29	670	285	-1,7	42-163	96	52
C30M	Candy factory process water	25	510	284	-1,6	43-169	103	57
C30L	Candy factory process water	25	320	285	-2,5	42-162	103	59
C40u	Candy factory process water	38	400	285	-4,2	40-167	84	44
C40o	Candy factory process water	42	350	285	-4,7	40-143	8-	39

5.4.1 BIOMER-LIKE THERMOPLASTICS FROM PHARIO PHA

Biomer thermoplasts (www.biomer.de/IndexE.html) are polyesters obtained from renewable resources. The products are waterproof, heat resistant, have appealing surfaces, and are biodegradable. The pellets are processed on standard machines. The melt behaves like liquid crystalline polymers. This property allows moulding of thin walled or complex structures even on small machines. Plastics processors emphasize responsibility to the environment by using Biomer thermoplasts. The company profiles products having high consumer esteem.

Biomer has proprietary technology and methods for compounding PHB (or PHBV with low HV-content) into PHA based bioplastics having properties similar to PP and HDPE. It was therefore of interest to test PHARIO polymers using the commercial Biomer proprietary compounding know-how. These PHARIO Biomer-like bioplastics could then be tested and compared to the commercial Biomer materials that are sold in the market today.

Compounding was carried out following Biomer proprietary compounding methods and supplied ingredients but with PHARIO PHBV instead of the commercially available pure culture PHB/PHBVs. It is our understanding that Biomer commercial bioplastic products are based on commercially available pure culture PHAs. Our aim was to base our comparison from mechanical properties of Biomer bioplastic grades to equivalent materials made with PHARIO PHBV.

Biomer provided three different compounding recipes for PHBV to give PHARIO PHA mechanical properties that were similar to high density polyethylene (PE), polypropylene (PP) and Acrylonitrile Butadiene Styrene (ABS). These PHARIO PHBV compounds were then analysed for tensile, flexural and impact mechanical properties.

Extracted, purified and dried S01 PHBVs with 2 wt.% 3HV content were combined with Biomer ingredients to make three different Biomer formulations mimicking properties of PE, PP and ABS plastics. The mixed materials were each melt blended separately in a Collin Teachline ZK 25T twin screw extruder. The temperature profile used in the four heating zones in the extruder was 180 (inlet), 170 (zone 2), 160 and 155 °C (outlet). The blended melt was extruded in strands that were collected and allowed to cool and crystallize at room temperature for 24h. The crystallized strands were then pelleted using an Alpine Augsburg shredder. The pelleted materials were left at room temperature for two weeks to be allowed to fully crystallize before mechanical testing.

Tensile and flexural test specimens were produced as previously described for the pure materials but now using the PHARIO Biomer-like pellets. Tensile and flexural testing was carried out per ASTM D638 and ASTM D790 standards using an Instron 430I tensile testing machine.

Impact test bars were made using a hot press. Four grams of pelleted sample were introduced into a test bar mould with a length of 6.3, a width of 1.2 and a depth of 0.4 mm. The mould with added material was placed in the hot press 180 °C. Pressure was subsequently increased to 25 bar. When pressure had been applied the mould was kept at 180 °C for 5 minutes. After 5 minutes the mould was water cooled to 70 °C before releasing the pressure and removing the product from the mould. The test bars were then notched in the middle of the bar with a 2-mm notch from the bar side end to the notch end. Notched Izod impact testing was carried out per ASTM D256 standard method using a Zwick impact testing machine.

The compounding formulation differences result in slightly different amounts of final PHA content, and these differences also influence elasticity modulus, and tensile strength. In Figure 39, the notations of PE, PP, and ABS represent the Biomer bioplastics made with PHARIO S01 PHBV. P226 is the "equivalent" PP commercial material obtained from Biomer. The slight difference in PHA content between PP and P226 is a result of slight formulation adjustments and challenges in the small-scale formulation work performed by us at Avans Hogeschool. The PHARIO Biomer-like PP performed as expected compared to the commercial P226 bioplastic.

FIGURE 40   RESULTS OF MECHANICAL TESTING OF THE PHARIO BIOMER-LIKE BIOPLASTICS AND BIOMER COMMERCIAL P226. PE, PP, AND ABS REPRESENT

                                 BIOPLASTICS MADE WITH PHARIO S01 PHBV. P226 IS THE "EQUIVALENT" PP COMMERCIAL MATERIAL OBTAINED FROM BIOMER.
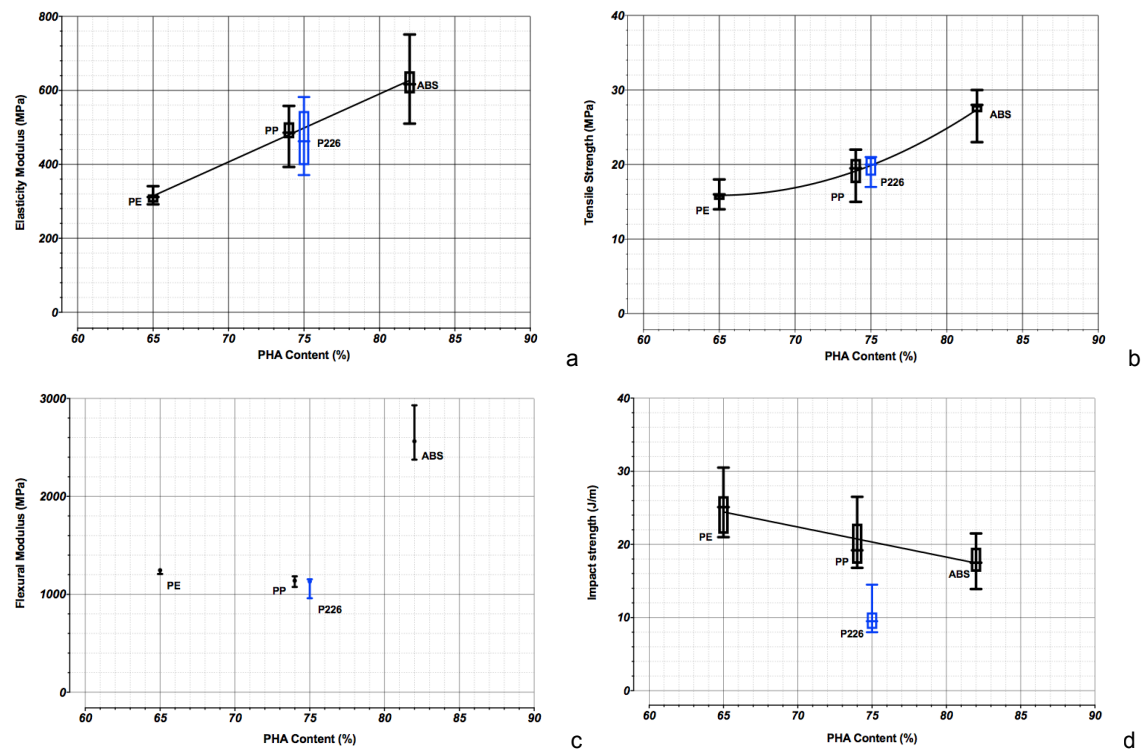
It was surprising to observe that the PHARIO Bioplastics exhibited improved impact strength in comparison to the P226. One possible explanation for the difference is a higher molecular weight of the PHARIO compounded materials (Figure 40). With all other things assumed to be the same, the rheology data suggested that the Biomer P226 was with lower weight average molecular mass when compared to the PHARIO S01 compounded PP like bioplastic. A factor in the preservation of M_w during compounding and moulding is the thermal stability of the polymer. If the PHARIO materials have superior thermal stability then this provides a benefit (value) for the final product and in application.

FIGURE 41   RESULTS OF MELT RHEOLOGY FOR BIOMER AND PHARIO BIOMER-LIKE BIOPLASTICS AT 180°C AND 2% STRAIN. THE INITIAL VISCOSITY IS

                                 RELATED TO THE MATERIAL AVERAGE MOLECULAR WEIGHT AND THE SLOPE IN TIME INDICATES FOR A RATE OF MOLECULAR WEIGHT LOSS. WITH

                                 ALL OTHER THINGS ASSUMED TO BE THE SAME, THE RHEOLOGY DATA SUGGESTED THAT THE BIOMER P226 WAS WITH LOWER WEIGHT AVERAGE

                                 MOLECULAR MASS WHEN COMPARED TO THE PHARIO PP LIKE BIOPLASTIC. VISCOSITY OF ABS, PP, AND PE PHARIO BIOMER LIKE BIOPLASTIC

                                 FORMULATIONS ARE IN ORDER OF RESPECTIVE PHA CONTENT.
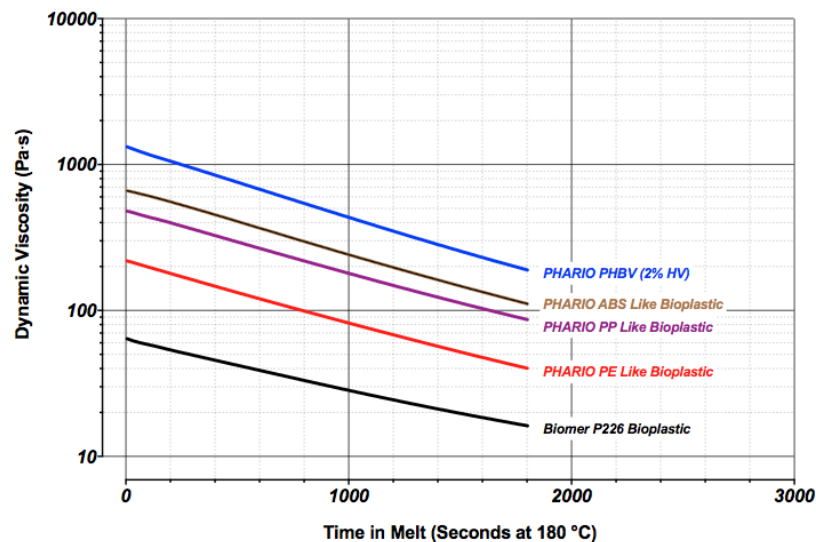
5.4.2 PHARIO PHA TO IMPROVE IMPACT RESISTANCE OF PLA

Corbion is said to be a global market leader in lactic acid, lactic acid derivatives, and lactides. It is a leading company in functional blends containing enzymes, emulsifiers, minerals, and vitamins. Corbion delivers biobased products made from renewable resources. These materials are applied in global markets like bakery, meat, culinary, pharma, home and personal care, and resins. Corbion generates annual sales of around € 770 million and employs 1,893 people in 20 countries. They work across two distinct segments: Biobased Food Ingredients and Biochemicals.

PHBV produced within the PHARIO project was used as property modifier of PLA supplied by Corbion. To do this, Corbion PLA was blended with various grades of PHARIO PHBV and the blend properties were evaluated to confirm the strategic use of PHBVs as a PLA property (toughness) enhancer. The research literature has established that blending of PHA and PLA brings important modification to PLA based bioplastics especially for improving the impact strength of PLA. The high HV content PHBV produced within PHARIO was anticipated to be an effective toughness enhancer of PLA.

Various grades of extracted, purified and dried PHARIO PHBV powder and commercial PHBV powder were dry mixed in a 2 L beaker with PLA pellets (Corbion PLA L130, general purpose injection moulding grade). The mixed materials were then melt blended in a Collin Teachline ZK 25T twin screw extruder. The temperature profile used in the four heating zones in the extruder was 180 (inlet), 170 (zone 2), 160 and 155 °C (outlet). The blended melt was extruded in strands that were collected and allowed to cool and crystallize at room temperature for 24h. The crystallized strands were then pelleted using an Alpine Augsburg shredder. The pelleted materials were then left at room temperature for two weeks to be allowed to fully crystallize before analysis.

Tensile and Flexural test specimens were produced as previously described for the pure materials but now using the PLA-PHARIO PHBV pellets with a melt temperature of 190°C. Tensile and Flexural testing were carried out according to ASTM D638 and ASTM D790 standards using an Instron 430I tensile testing machine.

Impact test bars were made using a hot press with 4.0 g of pelleted sample each. Methods for specimen production were as reported for the Biomer-like bioplastic specimen preparation but with a mould temperature of 190°C. Notched Izod impact testing was carried out per ASTM D256 standard method using a Zwick impact testing machine.

The added PHBVs were found to modulate the PLA elasticity modulus and tensile strength (Figure 42). The observed influence appeared to be more strongly related to the amount of PHBV rather than the type of PHBV. The PHARIO PHBVs did exhibit on average an expected positive influence on the PLA impact resistance (Figure 43). This effect was coupled most strongly to the amount of PHBV rather than the type of PHBV added in the formulation. Variability in the results is, in part, due to the vagaries of the methods of generating the test specimens. It could be observed that the moulded specimens were not completely homogeneous indicating that the specimen preparation by pressing was not optimal. From the literature, it is also reported that methods to compatibilize PLA compounded with PHBV are beneficial to the material mechanical properties.

FIGURE 42   RESULTS OF MECHANICAL TESTING FOR COMPOUNDED CORBION PLA WITH PHARIO PHBV POLYMERS SHOWN AS A FUNCTION OF PHARIO PHBV

CONTENT. THE ADDED PHBVS WERE FOUND TO MODULATE THE PLA ELASTICITY MODULUS AND TENSILE STRENGTH. THIS INFLUENCE FOR THESE

COMPOUNDED MATERIALS APPEARED TO BE MORE STRONGLY RELATED TO THE AMOUNT OF PHBV RATHER THAN THE TYPE OF PHBV.
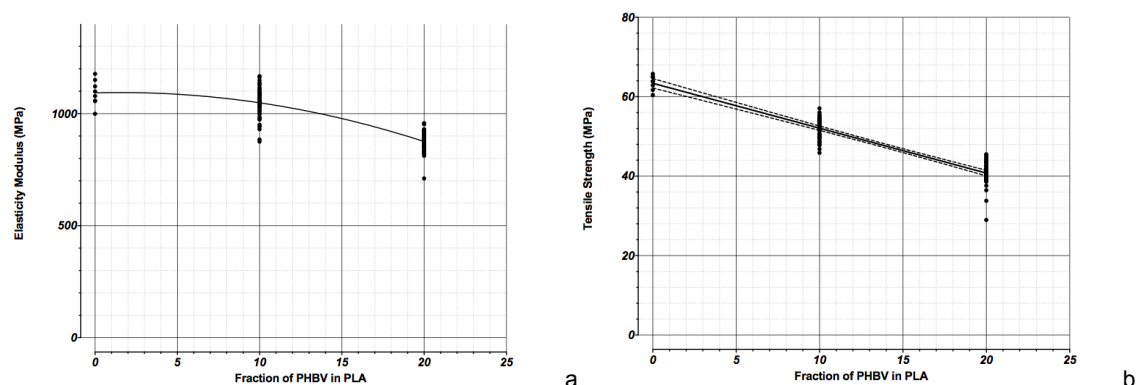
FIGURE 43   RESULTS OF IMPACT TESTING FOR COMPOUNDED CORBION PLA WITH PHARIO PHBV POLYMERS SHOWN AS A FUNCTION OF PHARIO PHBV CONTENT.

THE PHARIO PHBVS DID EXHIBIT AN EXPECTED POSITIVE INFLUENCE ON THE PLA IMPACT RESISTANCE, NOTWITH-STANDING THE VARIABILITY

EXPRESSED BY THE DATA. DUE TO THE VARIABILITY, IT WAS ONLY POSSIBLE TO SUGGEST AN INFLUENCE OF PHBV AMOUNT RATHER THAN THE

TYPE OF PHBV ADDED IN THE FORMULATION. VARIABILITY EXPRESSED IN THE RESULTS ARE INTERPRETED TO BE DUE TO THE FORMATION OF TEST

SPECIMENS WITH INHOMOGENEITIES.
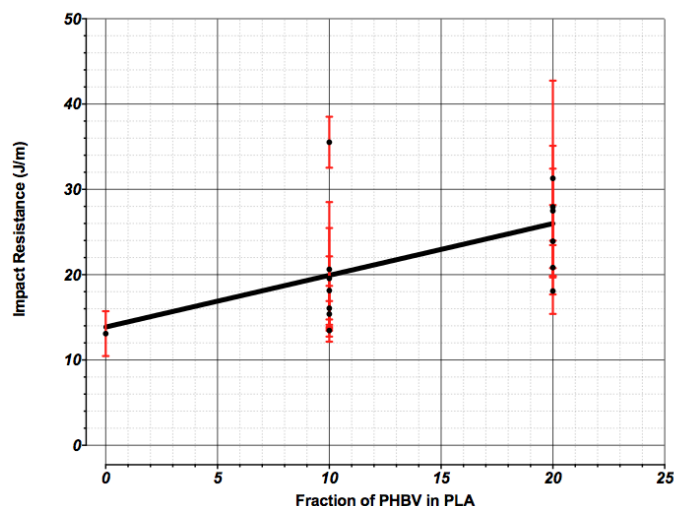
This case study was therefore a first positive indication of a potential application and value that can be further developed and optimised given readily available supply of starting materials and use of more industrial methods for compounding and specimen production. Wageningen Food and Biobased Research repeated and made own independent confirmation of these PHARIO PLA compounding findings (Kappen 2017). This research was performed in the framework of work package 9 of the TKI project "Kleinschalige bioraffinage" (TKI-AF-12040 KBR) project and as a subproject of PHARIO.

For this study seven blends were prepared using a commercial available PLA blend from Corbion (blend B) and two grades of high 3HV PHARIO PHA (see also Table 7).

1   111016-I: 100 % PLA blend B
2   111016-IIB: 90 % PLA blend B + 10 % PHBV C20
3   111016-IIIB: 80 % PLA blend B + 20 % PHBV C20
4   111016-IVB: 90 % PLA blend B + 10 % PHBV C40
5   111016-VB: 80 % PLA blend B + 20 % PHBVC40
6   111016-VI: 90 % PLA blend B + 10 % PHBV Enmat Y1000
7   111016-VII: 80 % PLA blend B + 20 % PHBV Enmat Y1000

Blends were compounded on a Leistritz ZSE 18 HP machine and subsequently injection moulded on a DEMAG NC IV machine (Figure 44). Compounds were injection moulded in both a cold (30 °C) and a hot (110 °C) mould to crystallize the PLA material. After conditioning the materials for at least 2 weeks at 20˚C and 50 %RH the test samples were evaluated on thermo-mechanical properties with help of dynamic mechanical thermal analysis (DMTA), thermal properties with help of DSC and mechanical properties by performing tensile and impact tests.

FIGURE 44   LEFT (A): EXTRUDER AS USED FOR THE PRODUCTION OF (BLEND B-PHA) BASED MATERIALS (LEISTRITZ ZSE 18 MAXX; CO-ROTATING TWIN SCREW EXTRUDER) AND RIGHT (B): INJECTION MOULDING MACHINE AS USED FOR PRODUCTION PHA BASED SAMPLES (DEMAG ERGOTECH 25-80 COMPACT MACHINE).
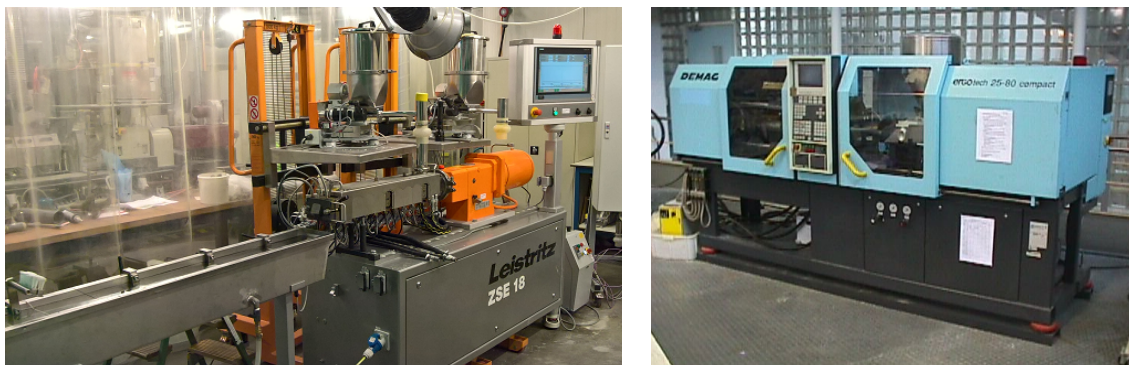
DMTA showed that blends injection moulded at with 30˚C mould temperature were not completely crystallized. When heated further crystallisation occurred. However, when the same materials were moulded at mould temperatures of 103˚C no further crystallization occurred. Maximum crystallisation was confirmed by DSC: during heating no exothermal effects of further crystallization could be detected. Moreover, the effect of crystallized PLA is reflected in the HDT-B values as given in Table 8: crystallized PLA samples showed higher heat deflection temperatures.

Figure 45 shows the effect of the PHA addition to the PLA blend on the impact resistance. These results show a positive effect on the impact resistance of the addition of 10-20% of the C40 PHBV PHARIO material with approximately 40% HV.

TABLE 8   HEAT DEFLECTION TEMPERATURES (HDT-B) FOR DIFFERENT PLA BLENDS COMPOUNDED WITH PHARIO PHA. HDT-B WAS TESTED USING A RAY-RAN HDT ACCORDING TO ISO 75. SAMPLES MADE ACCORDING TO ISO 294 WERE TESTED. THE LOAD APPLIED TO THE SAMPLE WAS 0.45 MPA AND THE HEATING RATE WAS 2°C/MIN (KAPPEN 2017).

Sample	Blend	Mould Temperature (˚C)	Heat Deflection Temperature - B (˚C)
111016-I	Blend B	30	51.2 ± 1.3
		103	105.9 ±1.2
111016-IIIB	80 % Blend B + 20 % C20	30	48.8 ± 0,1
		103	92 ±3
111016-VB	80 % Blend B + 20 % C40	30	48.3 ± 0,6
		103	81.8 ± 2,8

FIGURE 45   MECHANICAL PROPERTIES OF DIFFERENT PLA BLENDS MOULDED AT TWO TEMPERATURES (KAPPEN 2017). AT THE LOWER TEMPERATURE (30 C) CRYSTALLIZATION WAS NOT COMPLETE. LEFT, A: UN-NOTCHED IMPACT RESISTANCE, RIGHT, B: NOTCHED IMPACT RESISTANCE. ALSO STIFFNESS, TENSILE STRENGTH AND ELONGATION AT BREAK WERE DETERMINED (DATA NOT SHOWN).
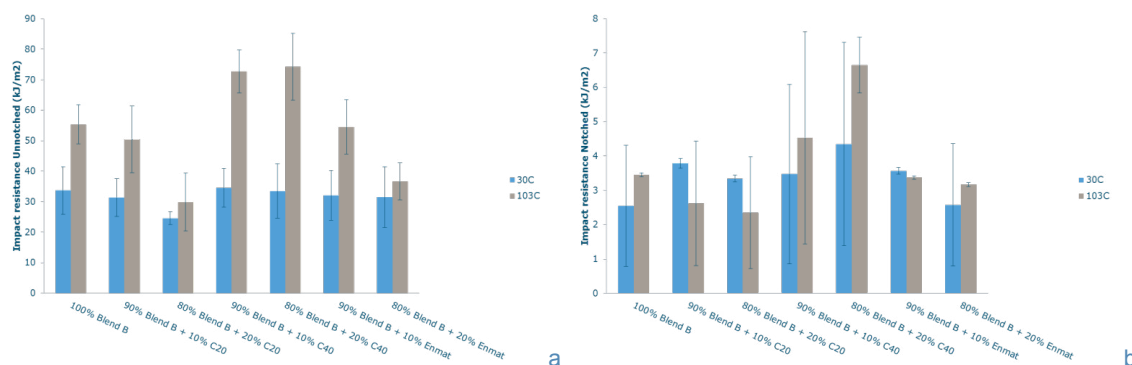
5.4.3   INJECTION MOULDING OF PHARIO PHA

Pezy Group is an international international company that accelerates product innovation within the areas of industrial design, function development, plastics engineering, rapid tooling, industrialization, mould flow, sustainability, testing & verification and liquid silicone rubber. The company has established expertise in product development and production support by collaborating in multidisciplinary teams.

Pezy in Groningen were provided with compounded and pelleted PHARIO PHBV bioplastics for producing business card holders towards getting direct end-user feedback on the bioplastic quality for processing. We were especially interested in the processor comments on the ease, or challenges, to injection mould the PHARIO case study compounded bioplastic materials. Batches of two different types of compounds were used for this case study to produce the business card holders. The two different batches were the PHARIO PHBV based Biomer-like bioplastics (Biomer Case Study – see 5.4.1) and the PLA bioplastics modified with PHARIO polymers (Corbion Case Study – see 5.4.2).

The PLA blends used in the production of the business card holders were a master batch of the compounded materials due to the limited available amounts. The master batch comprised S00 (1.4 wt.-%), C10 (3.0 wt.-%), C20 (6.0 wt.-%), C30 (4.2 wt.-%) and Corbion PLA grade L130 (85.4 wt.-%).

The PHARIO PHBV based Biomer-like bioplastics were also mixed as a master batch due to a limitation of materials. The final blend comprised the Biomer like PE (16.3 wt.-%), PP (71.7 wt.-%) and ABS (12.0 wt.-%) pellets.

Examples of the injection moulded business card holders are shown in Figure 43. The upper picture is the business card holder made with Biomer-like bioplastics. Therefore, we made a commercial bioplastic using biopolymers that were derived from municipal waste management processes. The amount of the PHARIO PHA in the Biomer-like bioplastic was about 74%.

The picture below is the Corbion PLA modified with PHARIO PHBVs. A commercial use of these renewable polymers as strategic ingredients in bioplastic formulations was demonstrated. There was about 15% of PHARIO PHBV in the PLA-PHA bioplastic.

The experience from PEZY's perspective was extremely positive. Thijs Feenstra commented that "…we were positively surprised about the quality and easiness they came out of the

machine. It took about 7 shots to get to good products...." (Personal Communication, August 24, 2016).

FIGURE 46   BUSINESS CARD HOLDERS INJECTED MOULDED BY PEZY GROUP. CARD HOLDERS WERE MADE WITH PHARIO BASED BIOPLASTIC FORMULATIONS WITH BIOMER-LIKE (UPPER), AND PLA-PHBV (LOWER) COMPOUNDS.

5.4.4   PHARIO PHA IN FILM APPLICATIONS

Ireland-based Bioplastech is converting waste, agricultural by-products and petrochemical products into value added biodegradable plastic polyhydroxyalkanoates (PHAs). Started in 2008, Bioplastech is looking at waste food oils, glycerol and biodiesel, though the main driver to begin the 10-employee company was waste plastic (PET). The company is on route to test its lab-proven technology on a larger level. They have several products (coatings and adhesives) based on mcl-PHA formulations and they are looking at developing a broadened suite of products and services. We believe that the PHARIO activities are synergistic to the aims of Bioplastech and the possibility to develop functional materials from both the PHARIO and Bioplastech routes has many win-win attractions. The company has been funded with about €10 million ($14.7 million) from the Irish government, including government development agency Enterprise Ireland, which supports companies and academic research (see Trinity College Dublin looks to bring clean technology innovations to market).

Bioplastech are interested in testing PHBV containing various amounts of 3HV for additives in functional materials. They were willing to make preliminary evaluation of PHARIO PHBV

grades as a property modifier/enhancer for their mcl-PHAs and for other developments that are of interest to the company such as PHA based films. A uniqueness of the PHARIO materials is that the PHARIO higher 3HV grades are not on the market today.

Three different grades (Table 6) of PHARIO PHBVs were sent to Bioplastech for compounding and production of biobased films: S00, C25 and C40 (Charifou and Babu 2016). Based on the properties of the PHBV polymers it was decided to process the S00 on its own and along with other biobased polymers. Similarly other blends were prepared with C25 and C40 using other commercial biobased polymers and Bioplastech proprietary ingredients to evaluate the mechanical properties of the blend films.

Before processing all the materials were dried under vacuum oven overnight at 50 °C. All the composites were prepared using a lab scale melt mixer (Brabender Plastograph) with a capacity of 40 cm^3. Samples were processed at 170 °C with 50 rpm screw speed for 5 min to minimize polymer degradation in processing. The blends were then pressed into thin films (Figure 47) at 170 °C (4 min at 10 Bar to melt the material, then 1 min at 400 Bar). The film formulations were then cut into dumbbell shaped specimens for tensile tests (Zwick Roell) to determine the mechanical properties with a 2.5 kN load cell at 50 mm/min.

TABLE 9   TABLE OF RESULTS SUMMARIZING MECHANICAL TESTING OF PHARIO PHBV BASED FILMS COMPOUNDED BY BIOPLASTECH (CHARIFOU AND BABU 2016).

Film Sample	Young's Modulus (MPa)	UTS (MPa)	Strain at Break (%)	Toughness (MJ/m^3)
S00	2243 ± 78	40.8 ± 1.5	2.8 ± 0.2	0.83 ± 0.12
S00-Blend1	2475 ± 193	47.5 ± 3.1	2.6 ± 0.2	0.71 ± 0.12
S00-Blend 2	1812 ± 100	32.6 ± 1.0	3.7 ± 0.3	0.81 ± 0.07
C25-Blend3	567 ± 88	18.1 ± 0.6	18.1 ± 3.9	1.90 ± 0.49
C40-Blend 4	329 ± 12	12.6 ± 0.4	17.9 ± 7.1	2.01 ± 0.26
C40-Blend 5	832 ± 125	16.1 ± 2.8	7.1 ± 2.4	0.74 ± 0.30
C40-Blend 6	734 ± 96	15.6 ± 1.3	12.2 ± 3.7	1.47 ± 0.40

S00 showed highest modulus and tensile strength (Table 9) with very low strain at break as we also found for the pure materials. Blends prepared with 50% PHARIO (S0) and 50% of commercial biobased polymers show improvement in ultimate tensile strength without affecting the other mechanical properties. Compounding formulations are therefore recommended for S0 type PHAs to optimize for mechanical properties. Processing of C25 and C40 was difficult. Samples were very sticky during the processing and very slow to crystallize. Similarly we undertook to develop specialized methods for C40 together with WUR for the above-mentioned WUR sub-project. Compounding formulations were recommended for C25 and C40 type PHAs to optimize for processing properties. Note the different requirements and purposes for blending - bioplastic properties versus processing characteristics. Blends prepared with 50% PHARIO C25 and C40 showed polymers with improved strain at break. C25 and C40 type bioplastics are expected to have higher strain at break, higher toughness, and will be more elastic but less strong than S0 type polymers.

This initial film feasibility study indicated that PHARIO blends may find application in biobased product lines including films and commodity household items (Charifou and Babu

2016). It makes sense to establish compounding formulations for optimal processing and mechanical properties when there is routine production and a readily available source of the base polymer materials.

FIGURE 47   FLATBED SCANNER IMAGES OF BIOPLASTIC FILM FORMULATIONS PRODUCED BY BIOPLASTECH. TRANSPARENCY OF THE FILMS IS AN IMPORTANT PROPERTY IN THIS APPLICATION. THE REFERENCE PICTURE BEHIND THE FILM THAT IS SHOWN ABOVE WAS PRINTED ON HP GLOSSY INKJET PHOTO PAPER. ALL IMAGES WERE ACQUIRED WITH AN EPSON PERFECTION 4990 PHOTO SCANNER IN REFLECTION MODE USING VUESCAN 9.5 (HAMRICK SOFTWARE) AND SAVED AS TIFF WITH NO FURTHER ADJUSTMENTS FOR WHITE BALANCE OR CONTRAST.
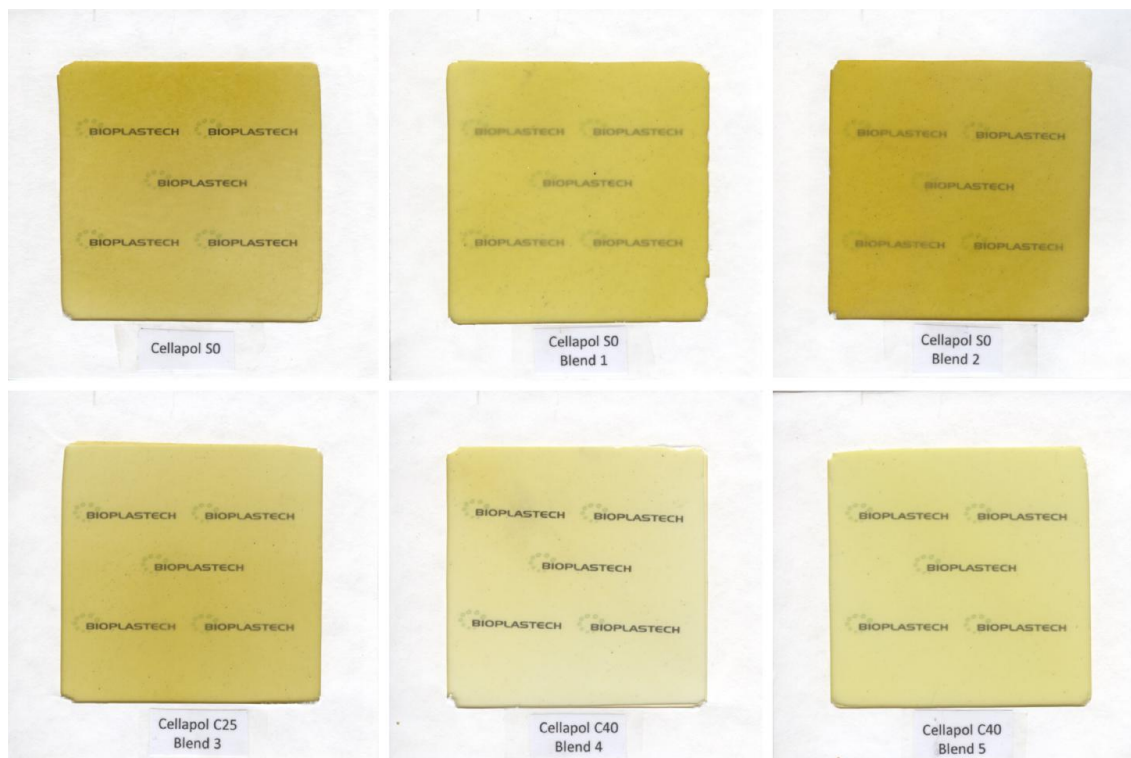
5.4.5 OTHER COMMERCIAL CONTACTS FOR DOWNSTREAM PROCESSING

The **SO.F.TER.** group (www.softergroup.com/en/) is one of the largest independent compounders in the world. The company markets a range of engineering plastics including, thermoplastic elastomers and thermoplastic vulcanizates. SO.F.TER. markets to offer customers innovative, tailor-made solutions with specialization in the automotive, domestic appliance, construction, footwear and sport sectors. Specialties include certified flame retardant compounds, reinforced materials suitable for metal replacement and high temperature resistant and impact resistant compounds for the automotive sector.

Researchers at SO.F.TER. group have developed patented methods for PHA compounding to be used in injection moulding for durable goods applications with the trade name BIODURA™. These grades of bioplastics are being launched on the market with high end designer furniture (http://milan.spacefur-niture.com/milan-news-2016/2016/5/6/kartell-organic-chair). They wish to source biobased supplies of PHA for the future developments of their bioplastic product and market developments. Recently SO.F.TER has announced that it would concentrate its biobased activities in a new company called **Sabio**.

Rodenburg Biopolymers BV (www.biopolymers.nl) activities started with trading of plant derived products in 1945. The company has been a family owned company for three generations. During a period of many years Rodenburg has developed specialized extrusion

production processes for production of biopolymer starch products from residual streams like from the potato industry. The company markets a base for a wide range of products and applications. The product Solanyl has evolved with range of derivatives where focus is reported to be on the development and production of a biodegradable biopolymer product range. Rodenburg products are generally based on reclaimed potato, grain, root or seed flour resources for various applications in the bioplastics, paper and oil drilling industries. For its investments in patented product developments Rodenburg seeks partnerships in value chains and with various companies and universities worldwide.

Rodenburg has used PHA as an ingredient in starch based bioplastic formulations to enhance thermal properties and improve processing of starch blend formulations. An explicitly expressed concern about PHARIO PHAs from mixed culture is the product reliability in supply and consistency of properties (Personal communication, Thijs Rodenburg, March 17, 2016). We believe that the low to medium (1-20 wt.-%) 3HV content PHARIO grades are of interest to develop as modifier ingredients for Rodenburg starch based biopolymers. The results of WP1 suggested outcomes that were a pleasant surprise to the company founders. Notwithstanding, interest of Rodenburg and companies like Rodenburg to invest energy and time to develop specialized formulations based on Water Board supplied PHAs requires at least a demonstration scale supply chain (1 to 100 tPHA/yr). Therefore, the value of the polymers can only be developed once there is a meaningful production scale availability of these materials.

Other potential applications could be the development of dispersion chemicals from biobased resources such as biopolymers of PHA. Oligomeric polymers (low molecular weight) are required and preferentially polymers that are amorphous. Therefore, PHARIO PHBVs with high 3HV content may be applicable starting materials in niche application developments. For this purpose, information and samples were exchanged with a developer in Belgium. The developer represented an independent research company experienced in designing, developing, prototyping and up scaling of dispersions, coatings, inks (Inkjet) and functional fluids. The solubility parameters were reported to be promising but the molecular weight of the supplied materials would need to be reduced further application development. Therefore, future testing would need to include pre-scrambling of the PHARIO polymers to defined oligomeric molecular weights. Such scrambling can be performed systematically based on the observed well-defined scission properties (see Figure 26). Thus, the consistent thermal stability of the PHARIO polymers is of value because it will be possible to process the polymers during recovery to targeted reduced molecular weight.

As a subproject to the PHARIO project a so-called "icon" project was realized to identify interesting applications of PHA bioplastics. The Dutch Value Chain Agreement for Plastics (Ketenakkoord Kunststofkringloop) financed this project. In the framework of this project various meetings were held with stakeholders from the plastics industry to identify bottlenecks and advantages of this material. Industry partners in this project were amongst others: Oerlemans Packaging, Rodenburg Bioplastics, Timmerije, KNN Bioplastics, Pure Hair Concepts, Van Plestik. Interesting idea's for PHA products were:
- PHA based crates for water plants as a means of protection of river banks;
- Biodegradable PHA coins for festivals (prototypes were made);
- PHA-wood composites as an alternative for particle board;
- A biodegradable wellington serving as a plant pot (to symbolize the connection between water and the food chain);
- 3D print filaments from PHA (first filament was made).

The markets for Water Board PHAs as an emerging technology implementation cannot be predicted in advance (Christensen 1997). One thing has been repeatedly has been shown when it comes to disruptive technology developments: expert forecasts will always be wrong and the most interesting market developments are found from doing and strategic learning. Since markets for disruptive technologies cannot be predicted well in advance, it is most likely that initial entry strategies will be wrong. The greatest chances for success and often the most interesting success stories in an emerging technology with new markets is to enable a demonstration activity that can allow for failure and to learn, sometimes by quirks of serendipity, the niches for most interesting markets to build successful value chains. The case studies undertaken for PHARIO WP1 have demonstrated a basis for learning and the breadth of possible routes for success based on a supply of materials that have value in the true context of the material downstream route to products and services. That value is built on predictability of performance, and we find from the pure materials and the bioplastic examples made for PHARIO that the materials behave as a well-defined function of the mean 3HV content. The 3HV content is a function of the feedstock and the content can be master batched further by blending during the recovery process.

6

SUSTAINABILITY OF THE PHARIO PROCESS

6.1 INTRODUCTION

A sustainable PHARIO process combines a low environmental footprint with an economically feasible business case. Both aspects are discussed in this chapter.

6.2 ENVIRONMENTAL IMPACT

6.2.1 INTRODUCTION LCA STUDY

KNN Advies performed a Life Cycle Analysis for the PHARIO process and the main results are summarized in the following paragraphs. A detailed report is available as one of the final deliverables of the PHARIO project.

A life cycle analysis (LCA) is essential to determine the sustainability of a route for such integration. LCA can be used to compare products or processes, and to gain insight in the most important contributions to the environmental impact of a product, process or service. An LCA also provides insight into optimization possibilities in the production chain. Lastly, environmental advantages downstream in the production chain can be shown using LCA; this includes production routes that can be replaced by the use of products obtained from the waste water treatment plant (WWTP), like fossil fuels or other (finite or cultivated) raw materials.

In this study all steps that have to be executed to obtain PHA are taken into account. This means that the whole water purification line from influent to effluent and the sludge processing as well as the end processing was investigated. Three different VFA sources for the PHA production were identified and the effects compared in three scenarios.

6.2.2 METHOD AND LCA FRAMEWORK
6.2.2.1 LIFE CYCLE ANALYSIS

The goal of an LCA is to provide insight in the environmental benefits and damages of products and services, in this case PHA production from sludge from Bath. All interventions, like use of electricity, excipients, heat, transportation, are environmentally rated, usually 'from cradle to grave'. In this LCA all additional operations are included to process used water from Bath to produce PHA.

6.2.2.2 FUNCTION AND FUNCTIONAL UNIT

The main function of WWTP Bath is to treat waste water. Other functions are e.g. production of biogas for internal use. In this LCA biogas production is therefore part of the business as usual (BAU) scenario. It must be noted that addition of PHA production to the existing system will lead to a decrease in biogas production, as secondary sludge will be used for the accumulation of PHA instead of for biogas production. This LCA study will investigate the environ-

mental impact of the difference in operation at the WWTP when PHA production is added to the BAU situation. This difference will give a delta-score per process step. This delta-score can either be an environmental benefit (negative value) or an environmental damage (positive value). The combined process steps, together with the substituted product will yield the net result of a scenario (again, this is not an absolute value, but compared to the BAU situation).

The functional unit is PHA production from the yearly amount of waste-activated sludge produced at WWTP Bath. Bath produces 71.000 ton waste-activated sludge/year from which 2.900 ton PHA pellets can be produced in the end. At SNB the processing of only this amount of sludge will be modeled.

As this LCA has different (technical) starting points, system boundaries, functional unit, etc. than similar studies from literature, they cannot easily be compared. However, we have done our best to outline the framework and assumptions so that comparisons can be more readily made with the present study as a reference.

6.2.2.3 LCA METHOD AND IMPACT CATEGORIES

The LCA is performed using the ReCiPe analysis method (Goedkoop *et al.*, 2009). With this method environmental impacts can be reported at three levels: - Midpoints: problem-oriented environmental impacts, like global warming and acidification. There are 18 midpoints. - Endpoints: damage-oriented environmental impacts: effects on nature, effects on humans, and effects on resources. There are 3 endpoints, wherein all midpoints are incorporated. - Single score: weighted environmental score of the 3 endpoints, expressed in points, or Pt. A more elaborate explanation of the ReCiPe method can be found in Appendix I. In this LCA study the single score will be presented, wherein all impact categories are weighted. The single score is expressed in megapoints (MPt). 1 MPt represents the environmental impact or load (i.e. entire production/consumption activities in the economy) of 1000 average Europeans.

6.2.2.4 AIDS

The used software is Excel for the mass and energy balances, and SimaPro 8 and ReCiPe endpoint Europe H/A, V1.12 for the LCA calculations. The database used is Ecoinvent 3.1 recycled content. The data concerning processing taking place at SNB (except the PHA extraction) are provided by SNB (via L. Sijstermans). The data for the PHA extraction are for the most part provided by AnoxKaldnes (via A. Werker) and for a minor part based on expert-based guesses from KNN. The data concerning the biogas production are provided by Witteveen + Bos (STOWA 2016-22). The data for the other processes taking place at Bath are provided by Waterschap Brabantste Delta (via L. van Dixhoorn).

6.2.3 SUBSTITUTED PRODUCT: PURE CULTURE PHA

6.2.3.1 INTRODUCTION

The produced PHA (from mixed culture) will be put on the market. In LCA analyses it is custom to include avoidance of the use of conventional, fossil products upon introduction of PHA. However, in the case of the bioplastic PHA which has different characteristics from conventional plastics, like biodegradability, it is better to compare it to another bioplastic, in this case PHA via monoculture with crops / crop waste as VFA source. Because PHA using a monoculture is not an entry in the Ecoinvent database, PHA production using a monoculture is also modeled. The following literature was used to compile the monoculture production process: Akiyma *et al.*, 2003; Harding *et al.*, 2007; Kendall, 2012; Kim and Dale, 2005, 2008.

The following process steps are defined for PHA production via a monoculture:

1 Sterilization and production of the nutrient medium
2 Growing of the bacteria culture
3 PHA accumulation
4 Dewatering of the PHA-rich biomass
5 Drying of the PHA-rich biomass
6 PHA extraction, consisting of dissolving PHA, solid-liquid separation, precipitation of PHA
7 PHA extrusion
8 Combustion of biomass residue

6.2.3.2 MAIN ASSUMPTIONS

Energy requirement

The energy consumed during PHA production is an important parameter. To determine the energy consumption the total energy used is determined based on the five literature sources mentioned above. Then this is divided over the different process steps. However, the energy consumed during PHA production using a monoculture varies greatly within the litera-ture. Therefore, an average is determined for the energy consumption. The average energy consumption during PHA production via a monoculture is set at 50 MJ/kg PHA and this energy consumption is for 70% (37 MJ/kg PHA) a heat consumption and for 30% an electricity consumption (13 MJ/kg).

Extraction

Different extraction techniques are used for pure culture PHA production, of which cell digestion (digestion extraction) is the most common. However, PHA produced via a mixed culture is extracted using a solvent (solvent extraction). To allow for a better comparison, the PHA produced in a monoculture is also extracted using butanol. The above calculated energy demand also included cell digestion and the calculation was corrected for this.

Carbon source

In the literature several sources of carbon are used as feed for PHA accumulation. These sources are grain, soya and sucrose. Sucrose can be produced from several sugar-rich crops like sugar beets or sugar cane. During the sugar refining process molasses is produced as a waste product, which makes it sustainable to use as carbon source. Molasses contains around 50% sucrose. For 1 kg of PHA 2 kg of sucrose is required, meaning 4 kg of molasses. In the Netherlands molasses is produced from sugar beets and imported from abroad (Germany, Ukraine, Russia, Basil and India). Each year roughly 200 kton molasses is produced by the Suikerunie, 200 kton is imported from countries that produce molasses from sugar cane (Brazil and India), and 100 kton is imported from countries that produce molasses from sugar beets (Germany, Ukraine and Russia). Based on these figures it is assumed that 40% of the molasses used in this study is produced in the Netherlands, 40% is imported from countries that produce molasses from sugar cane, and 20% is imported from other countries in Europe.

6.2.3.3 RESULTS

The LCA analysis shows that the production of 2907 ton PHA using a pure culture method has an environmental impact of 2,8 MPt/year, or 0,96 Pt/kg PHA. Energy consumption and the feedstock are together responsible for 90% of the impact (energy 50%, feedstock 40%).

FIGURE 48   DIVISION OF THE ENVIRONMENTAL SCORE PHA PRODUCTION USING A MONOCULTURE APPROACH.

Single score result PHA production using a monoculture, divison in aids, total score

MPt/year, based on yearly sludge amount Bath
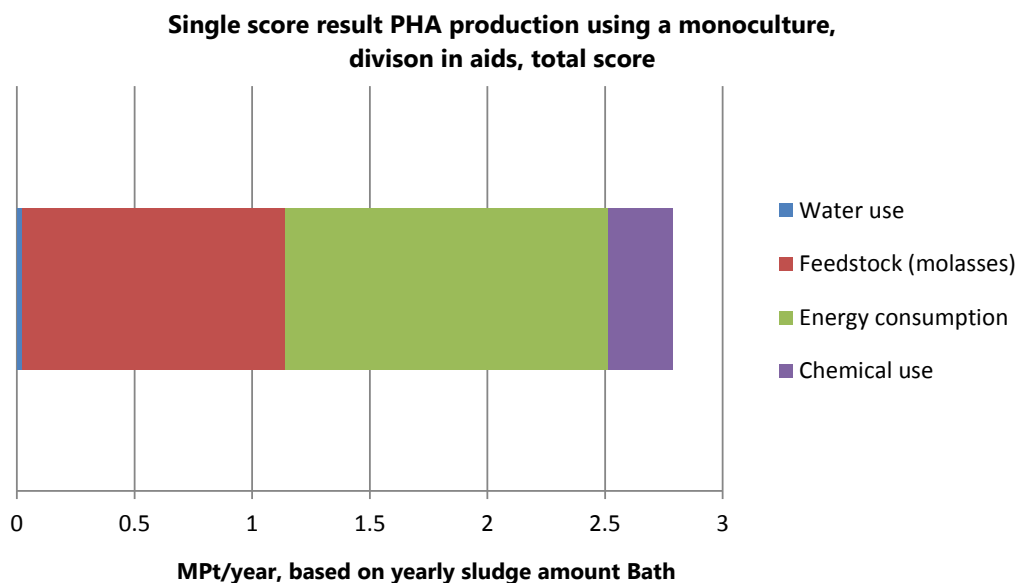
6.2.4 SCENARIO'S

For the PHARIO LCA study a business as usual (BAU) scenario is defined where primary and secondary sludge from the wastewater treatment plant of Bath is digested, dewatered and then transported to SNB for incineration in a dedicated incineration installation for sewage sludge. SNB partially dries the sludge and then incinerates the sludge in fluidized bed incinerators. The heat from the incineration is recovered through steam generation (two different pressures) and electricity is produced in steam turbines. The waste heat is used for the sludge driers and no primary heat source is required for the sludge driers.

In the PHARIO production process the secondary sludge is harvested from the wastewater treatment plant and used as a functional biomass for PHA production. This secondary sludge is the main asset of a municipal wastewater treatment plant in a value chain for PHA production. The potential for VFA production from the primary sludge at Bath is lower than the accumulation potential of the secondary sludge. The functional biomass. Therefore the LCA study assumes the import of external VFA sources. In this LCA study three different sources for the volatile fatty acids (VFA) were considered:

1. The VFA source is bought in (fossil) acetic acid;
2. The VFA source is external organic waste material, which is fermented at Bath to produce VFA;
3. The VFA source is internal & external primary sludge, which is fermented at Bath to produce VFA.

The general process schemes for the reference scenario and these three alternatives are shown in Figure 46. In the first two alternatives the primary sludge form the wwtp Bath is still digested in the existing sludge digesters. The secondary sludge is also digested in the BAU-scenario but is diverted from the digester for PHA production in the other scenarios. This leads to a reduction in the biogas production in the digester at Bath. In this last alternative no biogas is produced at Bath.

This LCA study assumed that the available organic waste will contain a certain fraction of non-digestable solids and that therefore a digestate residue will be produced after the VFA production. This residue will have a higher reuse potential than sewage sludge and therefore

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this residue is not mixed with the sewage sludge solids but dewatered separately and sent away following similar routes as the digestate in the case the organic waste would have been used for biogas production.

In the case primary sludge is used for VFA production the normal digestion of the sewage sludge is replaced by a production unit that produces VFA from the primary sludge. This unit will also process additional primary sludge from other sewage treatment plants. These plants would also have brought their sludge to SNB for incineration, but in this scenario they are first diverted to Bath for VFA production. The residue after VFA production will be dewatered together with the primary sludge from Bath and then transported to SNB.

At SNB the PHA rich biomass is dried and the PHA is extracted from the biomass. The drying of the PHA rich biomass is done in a separated installation but steam is used from the incinerator for the drying process. The residue after PHA extraction is mixed with the other sewage sludges processed at SNB and incinerated.

FIGURE 49   **PROCES SCHEMES FOR THE FOUR LCA SCENARIO'S.**
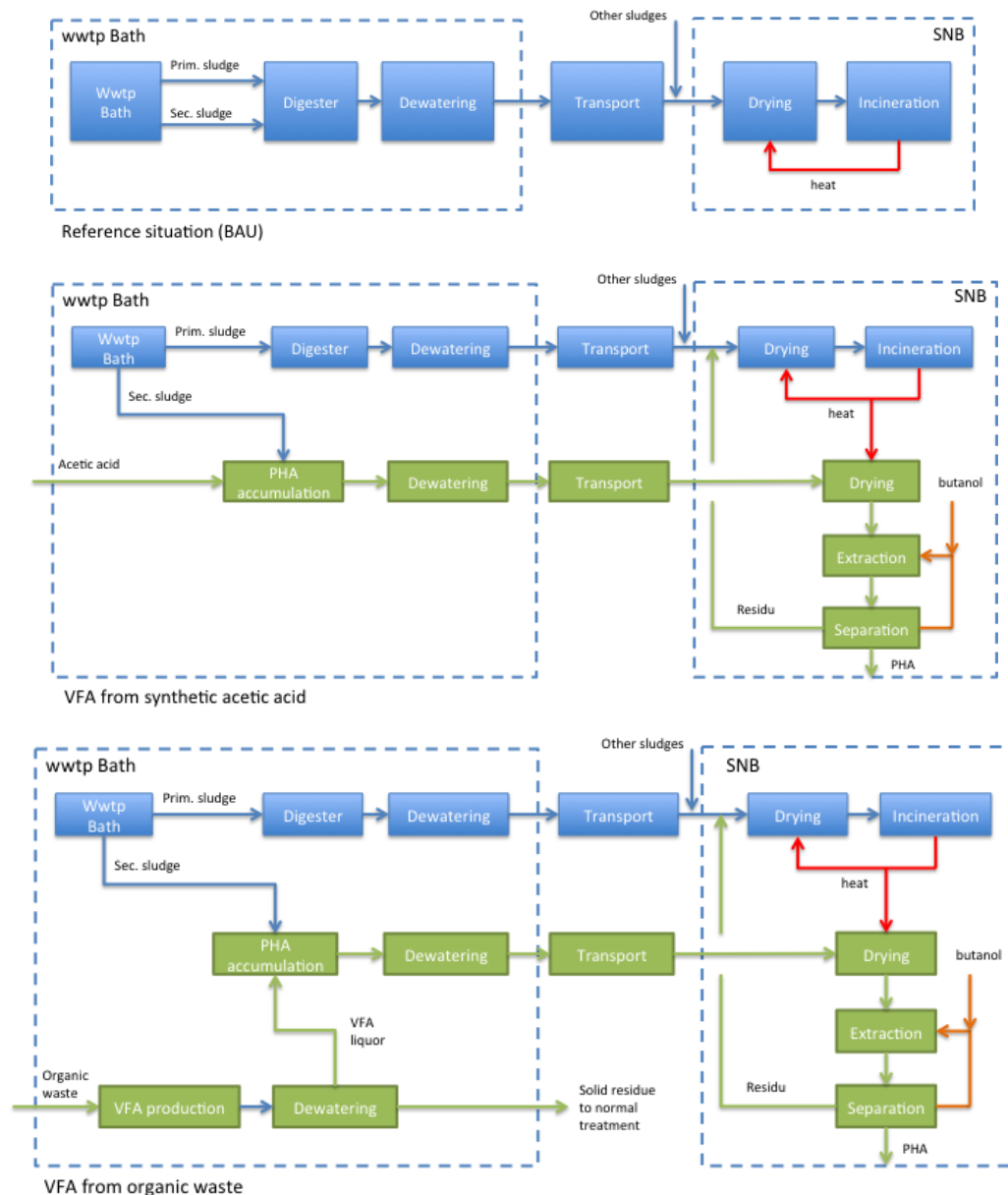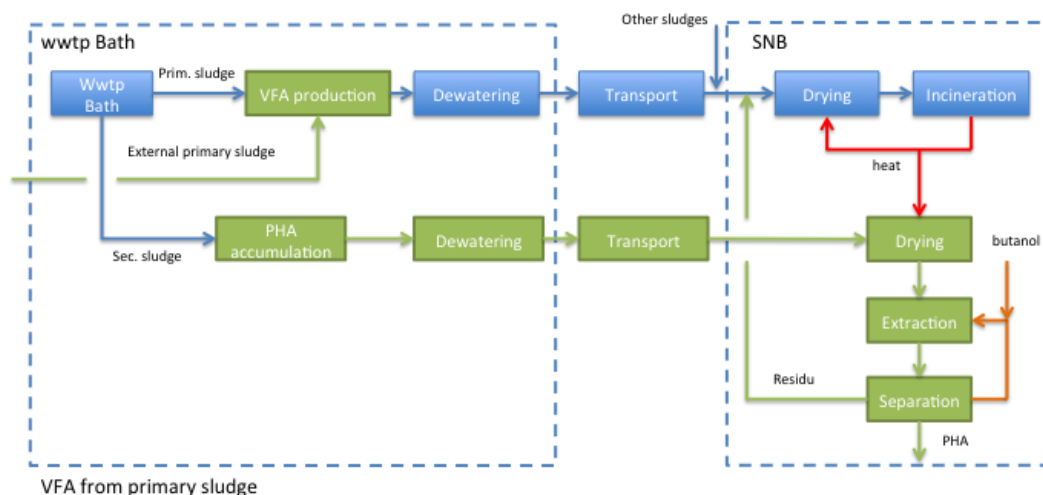
6.2.5 RESULTS

Table 10 and Figure 47 summarize the main results from the PHARIO LCA study. The table and the figure show the average results as well as worst case and best case values. These values were determined by means of a sensitivity analysis on the results.

The environmental effect can be evaluated in two different ways. First of all the new value chain for PHA production can be compared to the situation where the sludge from Bath is treated in the conventional way (business as usual). This comparison assumes there is a environmental benefit through replacement of monoculture PHA by PHARIO PHA. The wwtp Bath can produce ca. 2900 ton PHA per year in the different scenario's. The LCA analysis shows that all scenario's for PHA production lead to a significant increase in the net environmental benefit of from -0,3 MPt/year to +0,3-1,7 MPt/year, depending on the choice of the VFA source. The use of organic waste material as a VFA feedstock gives the highest net environmental benefit.

Producers of PHA plastic may prefer to know the environmental impact of PHARIO PHA compared to PHA produced via monocultures as is currently available on the market. Therefore also the environmental impact is calculated to produce one kilogram of PHARIO PHA. This approach shows that the production of PHARIO PHA has a negative environmental footprint of 0,3 Pt/kg PHA when organic waste or primary sludge is used as a VFA feedstock. This shows that the PHARIO concept has a 70% lower environmental footprint than PHA produced using monocultures. There is still an environmental benefit of 20% if petrochemically produced acetic acid is used as a VFA feedstock to produce PHARIO PHA.

TABLE 10   **MAIN RESULTS LCA STUDY. THE TABLE GIVES THE ENVIRONMENTAL IMPACT COMPARED TO THE CURRENT SLUDGE TREATMENT OF BATH. IN THE DIFFERENT SCENARIO'S 2909 TON PHA IS PRODUCED PER YEAR.**

Scenario	Environmental benefit Bath (MPt/y for 2.900 ton PHA/y)			Environmental footprint to produce 1 kg of PHA (Pt/kg PHA)		
	Average	Best	Worst	Average	Best	Worst
Business as usual (BAU)	-0,3			Not applicable		
PHARIO: Acetic acid as VFA feedstock	0,3	-0,1	0,4	-0,8	-0,9	-0,7
PHARIO: organic waste as VFA feedstock	1,7	1,4	1,8	-0,3	-0,4	-0,2
PHARIO: primary sludge as VFA feedstock	1,5	1,1	1,6	-0,3	-0,5	-0,3
Pure culture PHA production	Not applicable			-1,0		

FIGURE 50   COMPARISON OF THE ENVIRONMENTAL IMPACT FOR THE THREE DIFFERENT SCENARIO'S. THE LEFT FIGURE (A) COMPARES THE ENVIRONMENTAL
IMPACT OF PHA PRODUCTION TO THE EXISTING WAY OF SLUDGE TREATMENT. THE RIGHT FIGURE COMPARES THE ENVIRONMENTAL FOOTPRINT OF
THE PRODUCED PHA TO PHA PRODUCED VIA THE MONOCULTURE ROUTE.
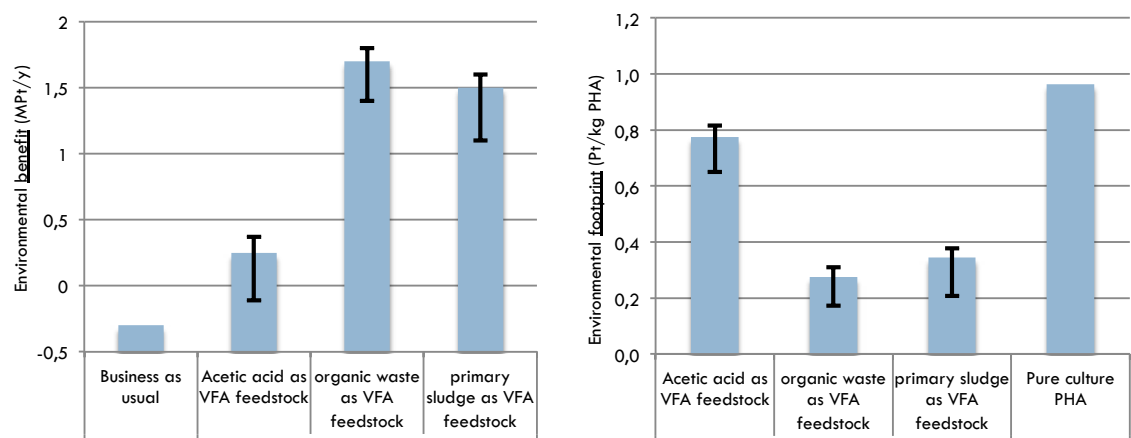
6.2.6   SENSITIVITY ANALYSIS

As an example Figure 48 shows a breakdown of the contributions of different process steps to the overall environmental impact for the scenario that organic waste is used as a VFA feedstock.

The use of the secondary sludge of Bath will lead to a reduction in the biogas production of the wwtp Bath. This reduction has only a small environmental damage of 0.04 MPt. Drying of the PHA- rich biomass and PHA extraction lead to the largest environmental damage (0.76 MPt). The substituted product (PHA production via monoculture using molasses as VFA source) is responsible for the bulk of the overall net environmental benefit.

FIGURE 51   DELTA SINGLE SCORE OF THE SCENARIO WHERE ORGANIC WASTE IS USED AS VFA FEEDSTOCK TO PRODUCE PHARIO PHA.
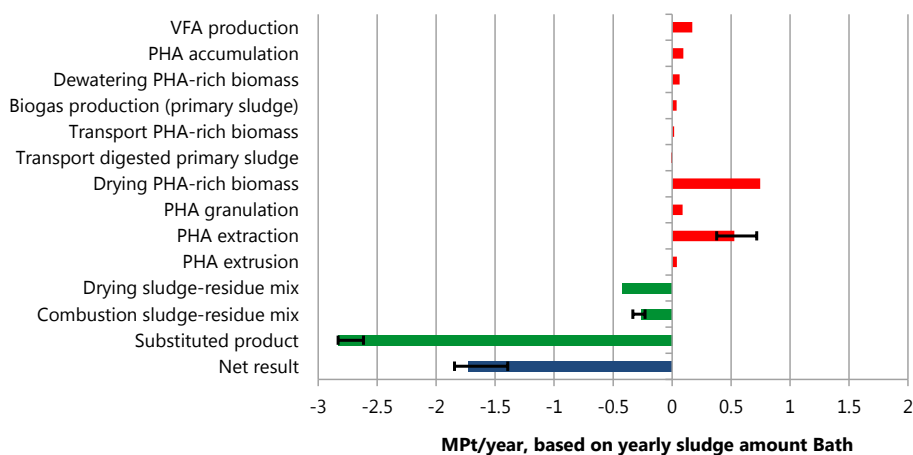
The ranges in the environmental scores are due to uncertainties in some of the process parameters for activities that are not yet practiced at industrial scale. The uncertainty analysis nevertheless gives an important point of reference from which to judge the performance of pilot investigations and to specify as requirements for the process scale up.

The LCA analysis shows that solvent use will have the largest influence followed by PHA loss in the solid-liquid separation step of the PHA extraction. The range in solvent use leads to a net environmental score between -0.09 and -0.37 MPt per year for scenario 2 (organic waste as VFA feedstock). In the same scenario PHA loss in the solid-liquid separation step of

the PHA extraction has a net environmental score between between -1.6 and -1.7 MPt/y for scenario 3.

Solvent use influences the net environmental score by avoiding the use of excipients (butanol itself), while PHA loss influences the net environmental score by influencing the PHA yield, and thereby the amount of substituted product. In the case of butanol use a second argument is important besides the sustainability argument: the economic aspect of buying butanol. The less butanol needs to be bought each year, the cheaper it is. PHA loss also influences both sustainability and economics. The more PHA loss there is, the less PHA pellets that can be obtained, leading to less financial income.

Butanol is not the only solvent that can be used, as other PHA-poor (non-chlorinated) solvents can accomplish an effective PHA recovery (e.g. acetone; Werker *et al.*, 2014). Solvent type and conditions of recovery are dependent on the type of PHAs produced. Different solvents have different environmental impacts, depending on the synthesis method, and allocation within the EcoInvent database (impact is less when co-products are formed). Acetone for example has an environmental impact that is approximately 2x more beneficial than that of butanol. Butanol and acetone are then represented by the worst and best case of solvent use (-0.09 vs. -0.37 Mpt/y). So the uncertainty analysis of solvent use not only displays the influence of the use of butanol itself (and the economics of buying butanol), but also indicates the performance of PHA-poor solvents.

6.3 ECONOMICS OF A FUTURE VALUE CHAIN

6.3.1 MARKET SIZE AND SCALE OF COMMERCIAL PRODUCTION

The market is for biobased polymers is growing and KNN Bioplastics currently experiences an increase in biopolymer quality from suppliers, as well as an increase in demand for (home) compostable packaging. Especially recent changes in legislation in France require many packaging industries and disposable products to switch towards home compostable bioplastic resources. Home compostable bioplastic resources are however scarcely available. In order to achieve a home compostable products many plastic converters are relying solely on PHA and starch-based materials. Within the EU as a whole biodegradable plastic demand is expected to triple from 100 kton in 2015 towards 300 kton in 2020.

Starch-based bioplastics are produced by a more diverse set of organizations, but are only suitable for a limited set of applications. Producers of PHA currently have issues to maintain a continuous and stable quality of PHA. When the PHARIO concept achieves a continuous production of stable quality PHA this material will be desired by industry as an alternative to current sources. If new value chains based on the PHARIO concept are able to produce a stable and sufficient quality PHA at a competitive price (see above) such production capacity would be easily covered by the market demand at that time.

We currently estimate that the actual global PHA production of active producers ranges from 2.000 - 4.000 ton/year. As market demand has started to rise from 2015 and onward we expect these numbers to rise significantly due to scale up activities by various producers. An estimated guess (conservative) would come down to 15.000 - 25.000 ton/y of actual PHA production volumes in 2025.

It is expected that a first commercial reference plant (FCR) would require a capacity of at least 5000 ton PHA per year to be economically successful. If such production facility would come online in 2025, this scale would represent a considerable contribution to the global production capacity of PHA.

The scale of 5000 ton PHA is based on studies by Veolia Water Technologies that showed this scale as the smallest commercially feasible scale based on investment cost evaluations of especially the extraction facility (PE4). Accumulation of PHA could typically be possible at the scale of larger wastewater treatment plants with a capacity of 200.000 to 1.000.000 people equivalents. Several PHA accumulation facilities at two to five wastewater treatment plants would then supplied dewatered or dried PHA rich biomass to a central extraction facility.

For instance the secondary biomass from the wastewater treatment plant of Bath has a capacity of 490.000 people equivalents (based on 150 g total oxygen demand per day per P.E.) and would be able to produce 2700 to 2900 ton of PHA per year. The survey of wastewater treatment plants with similar PHA accumulation potential identified the wastewater treatment plants of Dokhaven and Dordrecht as plant with PHA accumulation potential of respectively 2500 and 1100 ton PHA. These three wastewater treatment plants could therefore together supply more than 5000 ton PHA rich biomass to a central extraction facility. Such an extraction facility could be located next to sewage sludge incinerator Slibverwerking Noord-Brabant in Moerdijk. The PHA rich biomass would then follow more or less a similar route as it currently follows for incineration. Transport distances for the PHA rich biomass would then be comparable to the current situation and not further than 50 kilometres.

6.3.2 AVAILABILITY OF VFA FEEDSTOCK

In the PHARIO concept functional biomass is harvested from a wastewater treatment plant (secondary sludge). This biomass is then fed with a VFA rich feedstock to accumulate PHA in the biomass. Part of the VFA can be obtained through fermentation of primary sludge from the same wastewater treatment plant. In this way typically 30-40% of the accumulation capacity of the harvested functional biomass can be used. Therefore it is of interest to also source other raw materials from the region to produce additional VFA feedstock and to maximize the accumulation potential of the harvested functional biomass. Furthermore VFA may be produced at a lower cost from these materials then from primary sludge.

Within the framework of the PHARIO project (WP3) KNN Advies performed a study to assess the availability of secondary raw materials for VFA production within a 50 km range of the WWTP Bath. A range of 50 km was selected as this was thought to me the maximum economic transport distance for such waste materials.

Suitable VFA feedstocks should be readily fermentable with minimum pre-treatment and be available on a regular and reliable basis. Reliability of supply will be key to produce a consistent PHA quality, but predictability is probably more important. Through careful planning and blending different VFA feedstocks can be produced with different qualities to produced different qualities of PHA products.

The PHARIO PHA accumulation process can accept a range of different fermented feedstocks, varying in concentrations. Some solid material may be acceptable but this will require additional investment in solid separation of the fermented feedstock. Preferably the fermented

VFA feedstock to the PHA accumulation process should meet the quality as specified in the table below.

A first commercial reference plant based on the PHARIO concept is expected to have an annual production capacity of 5.000 ton PHA. Taking into account various efficiencies in the production process this requires the availability of roughly 15.000 ton dry matter of organic materials. Macro numbers for the province of Brabant and Zeeland show the availability of 480.000 ton of organic materials. However a large number of these materials are not suitable or have a low fermentability. Therefore specific industrial organic waste streams were inventoried and this study showed the regional availability of in total 43.000 ton dry matter of organic material. Circa 29.000 ton of this total amount was produced by three regional companies. Therefore there should be enough organic waste in the region to feed a full scale commercial PHA production unit with a capacity of 5.000 ton PHA/year.

TABLE 11 **QUALITY DEMANDS TO THE FERMENTED VFA FEEDSTOCK.**

	Acceptable	Preferred
VFA content (as mg vfaCOD/l)	> 2	5-30
share VFA of total soluble organics (mg vfaCOD/mg soluble COD)	> 0,8	> 0,95
Solids content (mg/l)	< 1	< 0,15
Nutrients (SCOD:TN:TP)	100: <11; <5	100: <1; <0,05

6.3.3 PHA PRODUCTION COST FOR A FIRST COMMERCIAL REFERENCE

6.3.3.1 INTRODUCTION

For the PHARIO project Veolia Water Technologies has made basic engineering designs for a full-scale commercial reference. For the PHA accumulation process (PE3) a production capacity of 2000 ton PHA (in PHA rich biomass) was used for the cost estimate. A central drying and extraction facility (PE4) was designed for a capacity of 5000 ton PHA/year.

As a basis for the cost estimate the following engineering documents were prepared:
- Process flow diagrams;
- Sized equipment lists;
- Plot plans;
- Mass & Energy balances.

In addition KNN Advies made a cost estimate for the production of fatty acids for the production of 2000 ton PHA/year (PE1). This estimate is based on a factorial estimate using an estimate of the main hardware costs as a basis. The accuracy of this estimate is therefore lower than the estimate for PE3 and PE4. The actual costs may also vary significantly depending on the VFA source that is used as a basis. For this study a concentrated waste stream with 20% dry solids (for instance: grey starch) was used as a basis. This waste stream was known to be available in significant quantities in the surroundings of Bath.

It should be noted that the design is based on a conservative estimate and the best available knowledge gained by Veolia in pilot scale testing before the PHARIO project. Already the PHARIO project has gained new insights and the realization of a demonstration facility will further show opportunities for cost reduction.

6.3.2.2   COST ESTIMATES PHA ACCUMULATION AND EXTRACTION (PE3 AND PE4)

The table below shows the capital investment for a 5000 ton/year PHA production facility. The investment costs for the accumulation (PE3) are based on a design with a capacity of 2000 ton PHA/year and were then multiplied by a factor 2,5 to obtain an estimate for 5000 ton PHA/year.

TABLE 12   SUMMARY OF THE INVESTMENT FOR THE REALIZATION OF A 5.000 TON PHA/Y FIRST COMMERCIAL REFERENCE.

FCR Investment Cost (M€) 5000 ton PHA/y	VFA production	Accumulation (PE3)	Extraction (PE4)	Total Investment
Equipment & Installation	1,9	14,6	21,0	37,5
Civil works	0,3	8,2	4,9	13,4
Engineering & Construction	0,6	10,4	10,2	21,2
Contingency	0,1	3,9	4,4	8,1
Total	**2,9**	**36,9**	**40,5**	**80,3**

TABLE 13   SUMMARY OF THE COSTS FOR THE OPERATION OF A 5.000 TON PHA/Y FIRST COMMERCIAL REFERENCE.

FCR Operational costs (M€/ year)		Accumulation (PE3)	Extraction (PE4)	Total Operations
Manpower for operations	0,1	1,0	0,6	1,7
Maintenance	0,1	0,4	0,5	1.1
Chemicals	0,3	1,3	1,3	2,8
Power	0,1	1,2	0,6	1,9
Steam	0,5	0,0	2,5	3,0
Total	**1,0**	**3,9**	**5,5**	**10,4**

The second table summarizes the operational costs for a full-scale commercial plant. The table shows that the operational costs amount to circa 2,1 €/kg PHA produced.

It is expected that capital for the required investment can be attracted at low Interest rates because of the involvement of public partners and by making use of investment funds focussing on green investment. With a 15 year depreciation and a relatively low interest rate of 3% the capital costs would then amount to 6,7 M€/year, or 1,3 €/kg PHA produced. This shows that with the current state of the art the PHARIO concept can produce PHA at a cost price of 3,4 €/kg PHA.

6.3.4   PHARIO BUSINESS CASE

The cost evaluation of a full-scale commercial reference plant shows that the PHARIO concept can produce PHA at a cost price of 3,4 €/kg PHA, based on the current state of the art. It is expected that the PHA can be sold at a price of 4,5 €/kg, thus generating a net profit of 1,1 €/kg PHA, or 5,5 M€/year.

A market price of 4,5 €/kg is in line with current commercial PHA producers. Competitive PHA producers are however also scaling up and therefore PHA price levels are expected to drop towards €4,0/kg in 2018. This price decrease will also continue further downward (~ €3,50/kg in 2025).

Next to PHA producers there is also price competition with other (home) compostable biopolymers, such as PBAT and bioPBS. These biopolymers are currently available on the market at ~€3,5/kg. And are expected to fall below €3,0 from 2025 onward.

Therefore it is advisable to aim for a PHA market price €3,00 - €3,50 / kg, when commencing with commercial PHARIO production around 2025.

The current cost price is based on pilot scale operations and already a number of potential costs reduction factors have been identified. As a first step the current pilot scale operation should be scaled-up to demonstration scale. At this scale the different opportunities for cost reduction should be tested and further developed. This scale will also give a better insight in the market position and value of the produced PHA and help to further defined the business case.

7

A DEMONSTRATION FACILITY FOR PHARIO

7.1 INTRODUCTION

In the PHARIO project pilot plant testing of the process has been performed during a period of 10 months. Activated sludge was harvested from a full-scale plant and sugar water from a local industry was used as a feedstock. This is the first time that such a long period of testing has been performed and this has given a very good insight in the reliability of the process and the quality of the produced materials.

With this project the technology can be considered to be at a Technology Readiness Level of 6, according to European definitions. Given the positive results of the PHARIO project the technology should now be demonstrated at a larger scale, a first full scale reference (FFSR). Such a large scale will make it possible to produce enough PHA material to establish a first small scale value chain and to start pilot production runs at potential customers of the PHA. Furthermore this scale will make it possible to validate that the process still produces the same quality of PHA, but with industrial sizes for the equipment.

Within the framework of the PHARIO project (work package 4) a cost estimate has been made by Veolia Water Technologies for the realization of a demonstration facility that can produce up to 32 ton PHA/year. Veolia selected this capacity as it was considered the minimal scale at which all equipment could be tested in such a way to make a further scale step to 5000 ton PHA/year possible (the first commercial reference, FCR). All equipment selected for the first full-scale reference plant will apply similar principles as would be required for a first commercial reference. This also implies that some of the selected equipment has a larger than required capacity because the equipment is not available at a smaller scale.

7.2 DESIGN APPROACH

Veolia Water Technologies prepared a basic engineering design for a 32 ton/y PHA demonstration facility (FFSR) that would be sited partially at wwtp Bath (VFA production PE1, Accumulation PE3) and sludge incinerator SNB (drying and extraction PE4).

For the design the following deliverables were prepared:
• Process flow diagrams
• Sized equipment lists
• Plot plans

Based on these engineering documents a cost estimate was prepared. Synergies with the existing facilities where evaluated and taken into account in the cost estimate. The most important synergies that were identified were related to the reuse of existing buildings at both sites.

7.3 COST ESTIMATE

Table 12 and Table 13 summarize the results of the cost estimates for the realization and operation of a first full scale reference plant. Approximately 85% of the equipment costs are related to the extraction facility at SNB and therefore the extraction costs are the post significant part of the required investment. In the capital cost estimate synergies with the wwtp Bath and SNB were identified and included in the cost estimates. These synergies reduced the investment costs with approximately 1,1 M€. The siting at two sites however requires transport of the PHA rich biomass between the two sites and this leads to additional transport costs of 0,3 M€/year for a three year operation.

TABLE 14 SUMMARY OF THE COST ESTIMATE FOR THE REALIZATION OF A 35 TON PHA/Y DEMONSTRATION FACILITY.

FFSR Investment Cost	Investment (€)
Manpower for construction	1,173.000
Equipment	5.493.000
Installation Subcontract	560.000
Site costs	689.000
Other costs	100.000
Insurance & allocations for main contractor G&A	2.003.000
Civil Work subcontract	727.000
Process Engineering	300.000
Contingency	1.949.000
Total Capital Investment	**12.992.000**

TABLE 15 SUMMARY OF THE OPERATIONAL COSTS FOR A 35 TON PHA/Y DEMONSTRATION FACILITY.

FFSR Operational costs (€/year)	Accumulation (PE3 @ Bath)	Extraction (PE4 @ SNB)	Total
Manpower for operations	157.000	157.000	314.000
Maintenance	32.000	162.000	194.000
Chemicals	36.000	38.000	74.000
Power	109.000	46.000	155.000
Steam	-	30.000	30.000
Transport	235.000	-	235.000
Other	-	24.000	24.000
Process engineering support	118.000	118.000	235.000
Total Operational costs (per year)	**686.000**	**575.000**	**1.261.000**
For 3 years	2.058.000	1.725.000	3.783.000

7.4 CONCLUSION

A first full-scale reference plant requires an investment of 13 M€ to realize the facility and then needs 4 M€ to cover the operational costs for a period of 3 years. The total investment is therefore 17 M€. This plant would produce 35 ton PHA/year. If this PHA could be sold at a price of 4 €/kg then this would give an additional benefit of at maximum 0,4 M€. This shows that at this scale the production facility is by far not yet economical. It is however a necessary step before a first full scale plant can be realized.

8

SUMMARIZING CONCLUSION

During the past decade, the technical feasibility of the production of biodegradable thermo-plastic polyesters, poly-hydroxyalkanoates (PHAs), by open mixed microbial cultures (MMCs) has been repeatedly demonstrated using waste and residual carbon sources as substrates. These MMC systems generally include three biological process elements (PEs): PE1 - acido-genic fermentation, PE2 - enrichment and production of biomass with PHA-storing capacity, and PE3 - PHA accumulation using PE2 surplus biomass and feedstocks with easily degrad-able organics. Polymers may be recovered from a PHA-rich mixed culture biomass in a fourth process element (PE4) by means of solvent extraction.

The PHARIO project has shown that full-scale municipal wastewater treatment plants can serve as process units that produce functional biomass (activated sludge) with PHA storing capacities without modifications to the wastewater treatment plant (they already are PE2's). Within the scope of the PHARIO project several wastewater treatment plants have been identi-fied that have high PHA storing capacities. Other wastewater treatment plants may be require minor modifications to achieve similar high PHA storage capacities.

Within the framework of the PHARIO project PHA has been accumulated and extracted at pilot scale at the wastewater treatment plant of Bath within a period of 10 months. For this pilot operation secondary sludge was harvested from a full-scale wastewater treatment plant Bath. This sludge was fed with different VFA feedstocks to produce a PHA rich biomass. VFA's were produced from wastewater from a local candy factory and from primary sludge from the wastewater treatment plant of Tilburg. The PHA rich biomass was dried and then extracted in a pilot scale extraction facility in Lund, Sweden.

The pilot operation made it possible to evaluate the full process from waste to resource production. Moreover it made it possible to critically evaluate the product quality and there-fore the value of the produced PHA. The PHARIO project showed that a very high quality product could be made in this way in a consistent manner. Various opportunities for quality control have been identified, thus giving confidence that a consistent quality of PHA can be produced in this way, despite the fact that the starting feedstocks are waste materials. This is a very exiting and promising result. Furthermore the product evaluation showed that the PHARIO concept could consistently produce PHA with a relatively high valeriate (HV) content. This type of PHA is not widely available on the market today, but could be used in some interesting niche applications due to its unique thermal properties. The lower melting point and lower crystallinity of this PHA for instance, make it interesting as an additive to PLA to improve the impact resistance of this material. Currently the low impact resistance is a mayor drawback of this material.

The PHA produced in the pilot was characterized extensively for it's thermal and mechanical properties. This information makes it possible, for the first time, for potential end-users to

evaluate the potential of the material for their value chain. As part of the PHARIO project some first applications were evaluated and compounds with PHARIO PHA were produced. The results of these evaluations showed that the produced PHA was of high quality and comparable or better than currently available on the market.

Within PHARIO a roadmap has been developed for scaling up of this value chain. A first step on this road is a demonstration facility (First Full Scale Reference, FFSR). This FFSR should demonstrate that a value chain can be realized and also validate the technology on a realistic scale. The total investment was estimated at 17 M€ for a demonstration facility with a capacity of 35 ton PHA/y, including three years of operation. This investment may be lower when creative ways can be found to make use of existing pilot facilities for extraction of PHA from the biomass.

A successful demonstration at this scale serves as a step to further improve the business case for the PHARIO concept and to reduce the costs of the value chain. Furthermore it will make it possible to produce enough material for full scale testing of various product applications, thus giving definite evidence of the value of the PHARIO PHA.

In this way a FFSR facility is an important and necessary step towards realization of a First Commercial Reference (FCR). It is estimated that this FCR should have a scale of circa 5000 ton PHA/year to make it commercially interesting. At this scale and in the Dutch context the biomass supply of two to three large municipal wastewater treatment plants like Bath (in total 1.000.000 people equivalents) is required.

The total investment for a FCR is currently estimated at 80 M€. At this scale and based on a conservative estimate based on the current pilot scale knowledge it is projected that PHA can be produced at a cost price of 3,4 €/kg PHA. It is expected that the PHA can be sold at a price of 4,5 €/kg, thus generating a net profit of 1,1 €/kg PHA, or 5,5 M€/year. Through continued development, future market prices may be lower than the current market prices. Therefore also the PHARIO concept should continue to seek further cost reductions during the next demonstration phase. The PHARIO project has delivered important insights in order to be able to realize such cost reductions.

The produced PHA not only has a very good and consistent quality. The LCA study performed within the framework of the PHARIO project also showed that PHARIO PHA can be produced at a 70% lower environmental footprint compared to current PHA plastics due to the use of third generation feedstock.

To conclude, a value chain based on the PHARIO concept has the following advantages:
- It uses the least possible amount of raw material;
- It uses third generation feedstocks;
- The environmental impact of the produced PHA is 70% lower than currently available PHA
- The raw materials can be of low quality and but produce a high quality product;
- The produced PHA shows unique qualities compared to current PHA products;
- A public-private partnership based on this value chain can guarantee a secure supply of the PHA product.

9

ABBREVIATIONS

BAU	Business as usual (LCA study)
COD	Chemical Oxygen Demand, a measure for the organic content of waste water
DSC	Differential scanning calorimetry
DMTA	Dynamic mechanical thermal analysis
FCR	First Commercial Reference
FFSR	First Full Scale Reference
HDT	Heat Deflection Temperature
MMC	Mixed Microbial Culture
LCA	Life Cycle Analysis
PE1	Process element 1: fermentation of organic residues to produce a VFA rich feedstock
PE2	Process element 2: production of a functional biomass that is capable to accumulate PHA
PE3	Process element 3: process that feeds a VFA rich feedstock (from PE1) to a the functional biomass (from PE2) to produce a PHA rich biomass.
PE4	Process element 4: process that extracts the PHA from a PHA rich biomass to produce the PHA product.
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHV	Polyhydroxyvalerate
PHBV	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
PLA	Poly lactic acid
VFA	Volatile Fatty Acids
wwtp	wastewater treatment plant

10

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