doi: 10.2166/9781780409566

Chapter 8

Upscaled and validated technologies for the production of bio-based materials from wastewater

Çağrı Akyol[1,2], E. Gozde Ozbayram[3], Anna Laura Eusebi[1], Alessia Foglia[1], Giulia Cipolletta[1], Nicola Frison[4], Vincenzo Conca[4], Cinzia Da Ros[4], Coos Wessels[5], Ramon Ganigué[6,7], Ilje Pikaar[8], Adrian Oehmen[9], Gilda Carvalho[10] and Francesco Fatone[1]

[1]Department of Science and Engineering of Materials, Environment and Urban Planning-SIMAU, Marche Polytechnic University, Via Brecce Bianche 12, 60100 Ancona, Italy
[2]Department of Green Chemistry and Technology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[3]Department of Marine and Freshwater Resources Management, Faculty of Aquatic Sciences, Istanbul University, Fatih, 34134 Istanbul, Turkey
[4]Department of Biotechnology, University of Verona, 37129 Verona, Italy
[5]CirTec BV, Nijverheidsweg 26, 1442 LD Purmerend, The Netherlands
[6]Center for Microbial Ecology and Technology (CMET), Campus Coupure – Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[7]Centre for Advanced Process Technology for Urban REsource recovery, Frieda Saeysstraat 1, 9052 Gent, Belgium
[8]The School of Civil Engineering, The University of Queensland, St Lucia, QLD 4072, Australia
[9]The School of Chemical Engineering, The University of Queensland, St Lucia, QLD 4072, Australia
[10]Advanced Water Management Centre (AWMC), The University of Queensland, St Lucia, QLD 4072, Australia

8.1 INTRODUCTION

The most commonly implemented and established method for the valorization of organics present in wastewater streams is anaerobic digestion (AD) as a means to recover chemical energy present in organic matter as electricity and/or heat via biogas (see Chapter 5). However, the current value of biogas is relatively low. As such, in recent years there has been an increasing interest in the development of technologies that can recover organics embedded in the wastewater matrix in the form of higher value products. Initially, R&D efforts were predominantly restricted to smaller scale testing with only few concepts tested at large and/or full scale. In order for these innovative concepts to become mature technologies that can be implemented by the water industry, long-term demonstration testing under real-life conditions at a practically releveant scale is essential. Within this context, significant efforts are currently underway through public–private collaborations and large scale EU funded projects. A plethora of organic compounds can be recovered from drinking water, municipal wastewater and various industrial wastewater streams. These include, but are not limited to, polysaccharides, cellulose, volatile fatty acids (VFA, also referred to as short-chain carboxylic acids), polyhydroxyalkanoates (PHA), amino acids, proteins, aliginate-like compounds, and so on. In this chapter, key achievements and progress made with respect to the recovery of organics from municipal wastewater are presented. In particular,

The chapter is from the book *Resource Recovery from Water: Principles and Application*, Ilje Pikaar, Jeremy Guest, Ramon Ganigué, Paul Jensen, Korneel Rabaey, Thomas Seviour, John Trimmer, Olaf van der Kolk, Céline Vaneeckhaute, Willy Verstraete (Eds.)

this chapter focusses on two specific products, namely, (i) cellulose and (ii) polyhydroxyalkanoates (PHA). The fundamental principles, applications and design of approaches enabling the recovery of these compounds are discussed, including engineering considerations and implications for practice in a wastewater context. In addition to the technological aspects, the market potential of each of these compounds in terms of product value and requirements (e.g. quality, volumes and market demand) are discussed as well. Lastly, the research needs are highlighted, focused on challenges and opportunities for these concepts to become mature technologies that can be implemented by the water industry and replicated in different areas of the world.

8.2 LEARNING OBJECTIVES

At the completion of this chapter you should be able to:

- Understand the rationale, conceptual design and benefits of implementing approaches that enable the recovery and valorization of organics from municipal and industrial wastewater streams.
- Explain the fundamental principles and key design criteria of approaches for recovery of cellulose and PHA from wastewater streams.
- Explain key specific challenges of the development and implementation of each technology.
- Use waste characteristics and site-based constraints to select appropriate forms of organic recovery technology for a given situation.
- Set a design basis for organic recovery technologies, conduct preliminary reactor sizing and estimate their potential performance.

8.3 CONCEPTUAL OVERVIEW OF CELLULOSE RECOVERY FROM MUNICIPAL WASTEWATER

Cellulose is a polymer of glucose constituted by a linear chain of glucose, also defined as the β-1,4-polyacetal of cellobiose (4-O β-D-glucopyranosyl-D-glucose) with $(C_6H_{10}O_5)_n$ as its chemical formula (Huang, 2013). It is a renewable, biodegradable, and non-toxic material that constitutes many environmental-friendly and biocompatible products (Nechyporchuk *et al.*, 2016). Cellulose fibers are the major components (78.1% of virgin pulps and 67.4% of recycled pulps) of toilet paper (as well as hygiene tissues) together with smaller amounts of hemicellulosic fibers and lignin from wood pulps (Chen *et al.*, 2018). The average consumption of toilet paper per capita in Europe is estimated to be around 10–15 kg per year, which is three times more than the global average consumption (4.4 kg/capita·year) (Crutchik *et al.*, 2018). The toilet paper useage in the USA is even higher with an average use of about ≈23 kg/capita·year (Table 8.1). Moreover, although the annual consumption of toilet

Table 8.1 Per capita consumption of toilet paper in the United States, China, Japan, Western Europe, Latin America, and Africa (Li *et al.*, 2020).

Country/region	Estimated per capita consumption of toilet paper (kg) (2017 data)
United States	≈23
China	≈3
Japan	≈11
Western Europe	≈10–13
Latin America	≈4
Africa	≈0.4

paper per capita is considerably lower in China (i.e. 2.9 kg/capita/year), the total amount of toilet paper entering WWTPs is also significant because of its vast number of consumers (Li *et al.*, 2020).

Despite the large consumption of toilet paper, cellulose fibers originating from toilet paper usage are often overlooked as a contributor to total COD and total suspended solids (TSS). These fibers represent a significant amount of the total COD and TSS load in municipal wastewater. In fact, the influent cellulose content typically accounts for approximately 30 and 25–40% of the TSS and influent COD, respectively (Ahmed *et al.*, 2019). Due to the characteristics of cellulose fibers and the prevalent conditions in sewer networks, these cellulose fibers typically enter WWTPs in suspended solids form. Depending on the design of the wastewater treatment plant and process configuration, the cellulose is either aerobically oxidized to CO_2, used as a carbon source for denitrification, incorporated in heterotrophic biomass or partly converted to biogas during anaerobic digestion. While there is no consensus in the literature regarding the fate of cellulose during AD, it is generally accepted that long solids retention times (SRT) are needed to convert most of the cellulose into biogas (i.e. above 40 days may greatly improve the degradation to over 80%). Such long SRTs are typically not applied nor economically viable. Instead of burdening the biological processes during the secondary treatment in WWTPs, toilet paper fibers could be physically recovered as a potential resource during primary treatment (Li *et al.*, 2020). The latter opens up opportunities for recovery of these fibres through solid–liquid separation approaches. Within this context, the use of microsieves for cellulose recovery has gained significant interest and has shown great practical potential. Microsieves such as rotating belt filters (RBF) enable efficient separation of the cellulose fibers (as well as overall TSS) at high surface loading rates and are regarded as an interesting alternative to traditional primary sedimentation as a pre-treatment step prior to activated sludge processes (Ruiken *et al.*, 2013).

8.3.1 Fundamental principles

The working principle of RBFs is based on physical separation through size exclusion. As a pre-treatment step in municipal WWTP applications, commonly used mesh size ranges from 200 to 500 μm. It should be noted that this is not a strict design parameter and smaller or larger sizes can be used as well (Behera *et al.*, 2018; Palmieri *et al.*, 2019; Rusten *et al.*, 2017). In addition to the microsieve's mesh size, it is important to understand that the size exclusion principle imposed in RBFs also relies on the effect of cake filtration, which allows the removal of solid particles up to three times smaller than the nominal pore size of the filter mesh. The speed of the rotating belt can be adjusted to the applied hydraulic surface loading and solids loading as a means to manipulate the cake thickness, thereby changing the effective 'operational pore size' and thus the solids removal efficiency. Prior to entering the RBF, municipal wastewater goes through a grit removal step, in which the easily settable solids (larger solids) that may cause damage to the RBF as well as equipment and processess further downstream at the WWTP are removed (e.g. sand and coarse grit material). The wastewater in the RBF flows through the filter cloth and its particles create a 'pre-coat' on top of the filter cloth, thereby reducing the effective pore size of the microsieve. The sievings filtered out of the wastewater are typically pumped into a screw press where they are dewatered to a total solids (TS) content of typically 40–50%. After dewatering, the sievings are subsequently dried to achieve a dry solids content over 90% prior to being transported to a hammer mill for further processing and volarization. The quality of the produced sievings can be determined based on various parameters such as ash content, solids content, cellulose, and so on. In general, the sludge collected from an RBF treating municipal wastewater contains a fraction of cellulose fibers as high as ≈80–90% of the recovered organic fraction (Behera *et al.*, 2018; Ruiken *et al.*, 2013). A simplified schematic representation of the working principle of an RBF unit is provided in Figure 8.1.

8.3.2 Applications and design

RBFs are considered a high-rate primary treatment process for the treatment of municipal wastewater with typical surface loading rates of 100–200 $m^3/m^2/h$, offering a small-footprint alternative to primary

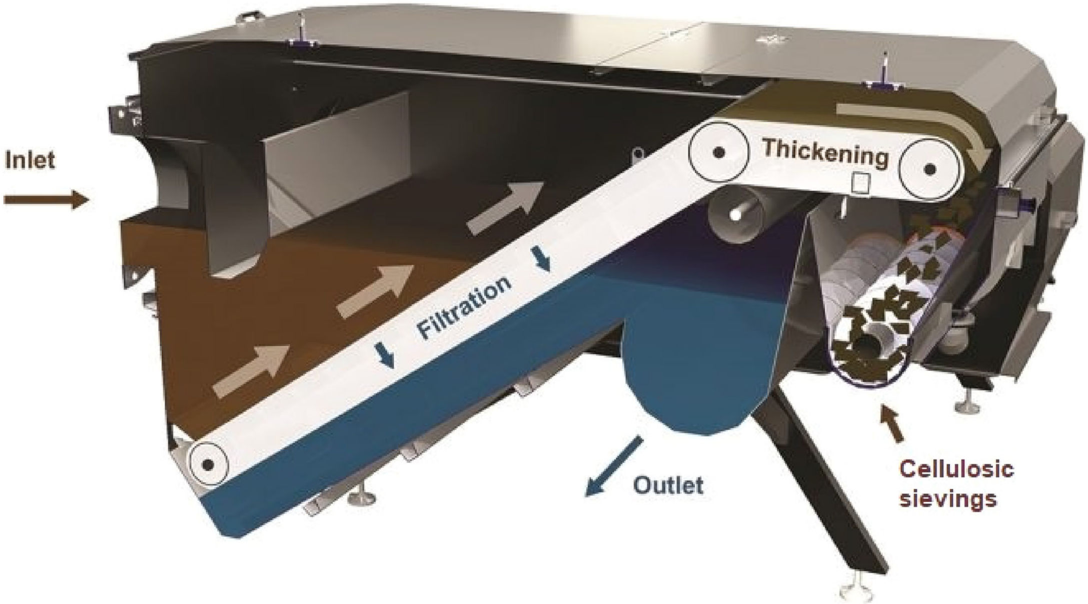


Figure 8.1 Simplified schematic representation of the working principle of microsieving, adapted from Salsnes (Norway) filter product sheet.

settling (with surface loading rates in the order of 1.5–2.5 $m^3/m^2/h$) (Franchi and Santoro, 2015). Interestingly, RBFs were initially developed in Norway as a mechanical low-footprint alternative to primary settling to achieve high TSS and BOD removal due to the lack of space rather then being developed with the aim of becoming a cellulose recovery approach. Importantly, in those days only COD and TSS removal was required without nitrogen or phosphorous discharge limits being enforced (Rusten and Odegaard, 2006). In addition, due to the low temperatures in Norway and its typical steep sewers with low hydraulic retention times, hydrolysis of solids (and toilet paper) was limited, enabling high TSS and COD removal efficiencies.

The selection of RBF technology over conventional primary clarification depends on several factors such as carbon and energy footprints, plant capacity, primary effluent quality, influent SS concentration, and selective particle size fractionation (Behera *et al.*, 2018). An important advantage of microsieves is their small footprint; for a given hydraulic load, they occupy less than 5% of the footprint of conventional clarifiers (Palmieri *et al.*, 2019). TSS removal/cellulose recovery and cellulose purity depend on multiple operating conditions such as loading rate, sieving rate, water flow rate, fine mesh size and influent wastewater constituents. RBFs are sometimes equipped with a cleaning system made up of an air knife and compressed hot water to remove the residual sludge cake from the belt (Da Ros *et al.*, 2020). Important occupational health and safety (OH&S) concerns to consider are the potential formation of dust and fire hazards during the drying step. Therefore, adequate ventilation and the avoidance of any contact between the heaters and the cellulose are critical design criteria. While important, these OH&S issues can be appropriately managed and should not be considered a limiting factor.

It is important to highlight that the performance of RBFs in terms of removal efficiencies can be highly site-specific and depends on the wastewater characteristics. For example, higher solids concentration may lead to the formation of a thicker 'filtering' layer on the surface of the belt, thereby reducing the effective pore size and thus enhancing the TSS removal efficiency. The presence of fibrous materials may lead to the formation of a thick and porous filtration layer that, in turn, also enhances TSS and organics removal. One can appreciate that the site-specific nature means that pilot scale testing is highly recommended at the target location prior to full-scale implementation in order to accurately determine the expected process performance. Obviously, the latter is not possible in greenfield applications and a performance assessment based on expected wastewater composition is necessary.

In addition to the benefits of recovering cellulose, by decreasing the TSS and organic loading to the subsequent activated sludge step for biological nutrient removal, the power consumption needed for aeration in the WWTP can be significantly decreased (Franchi and Santoro, 2015). Another advantage of cellulose removal prior to aerobic treatment is the increase in aerobic sludge age, thereby improving the nitrification process by 10–15% (Rusten *et al.*, 2016). The latter may be particular beneficial for colder climates. Lastly, the organic carbon recovered by an RBF can be fed to an anaerobic digester, contributing to a significant increase in biogas production (Behera *et al.*, 2018). Obviously, the latter is only possible in WWTPs that are equipped with an anerobic digester. It should also be noted that the use of cellulose for bioenergy production is positioned at the bottom of the so-called 'biomass value pyramid' (Gavrilescu, 2014). The biomass value pyramid is a concept specific to the bio-economy, in which different biomass applications and sectors (such as food, chemicals, biofuels) are ranked in terms of value added (Bout *et al.*, 2019). Therefore, biogas production from recovered cellulose should only be pursued if none of the aforementioned valorization alternatives is technically possible and/or economically feasible. For instance, recovered cellulose can be refined and used as a raw material in paper products (Kehrein *et al.*, 2020), as fibrous reinforcement material in bricks (Kim *et al.*, 2017), as an adhesion binder for asphalts (Makron, Finland) or in various biocomposites together with (bio) plastics such as PHA (Akyol *et al.*, 2020). Alternatively, cellulose can be utilized to produce valuable chemicals or biofuels, such as short-chain carboxylic acids (SCCAs, also referred to as volatile fatty acids (VFA)), polylactic acid, and bioethanol (Crutchik *et al.*, 2018; van der Hoek *et al.*, 2015).

8.3.3 Successful case studies of implementation

The cellulose recovered from toilet paper in municipal wastewater has found its way to the market through various technology providers of microsieves including Salsnes, Eco Mat RBF, Hydrotech Beltfilters and CirTec. For instance, CirTec has developed a dedicated process called Cellvation® that separates the cellulose from the incoming municipal wastewater, followed by a cleaning step that allows the recovery of the cellulose fibers as a clean product commercialized under the name Recell®, which can be used in various applications such as in asphalt, concrete, insulation material, and other building materials (Palmieri *et al.*, 2019). Within the EU Horizon 2020 SMART-Plant project, Recell® was successfully used to produce a variety of biocomposites such as façade, compression-resistant plates, and insulation flakes.

A simplified schematic representation of the overall Cellvation® process is presented in Figure 8.2. Raw wastewater enters the treatment plant and flows through a coarse screen and grit that removes large particles. After that, the Cellvation® process starts when the wastewater is pumped through a grit chamber to remove the easily settleable solids. The remaining wastewater goes through a cellulose washer that separates the cellulose fibres from larger particles and hairs. The influent wastewater is then fed to an RBF which significantly improves the water quality and wastewater discharge options. The filtrate is discharged and the solids are removed from the filter by means of a cleaning system that uses air pressure at the end of the filtration area. The cellulosic fibres coming from the RBF are pre-dewatered in a dewatering unit coupled to the filter and then are further dewatered by a CellPress. The dry solids leaving the CellPress are hygienized, dried and polished to either fluff or pellets.

The Cellvation® was installed at the WWTP of Geestmerambacht of the Water Authority Hollands Noorderkwartier located in the Netherlands (see Figure 8.3) with a capacity of up to 90 m^3/h. The medim-size plant treats a dry weather flow of 4160 m^3/h and maximum flow of 16 300 m^3/h and is

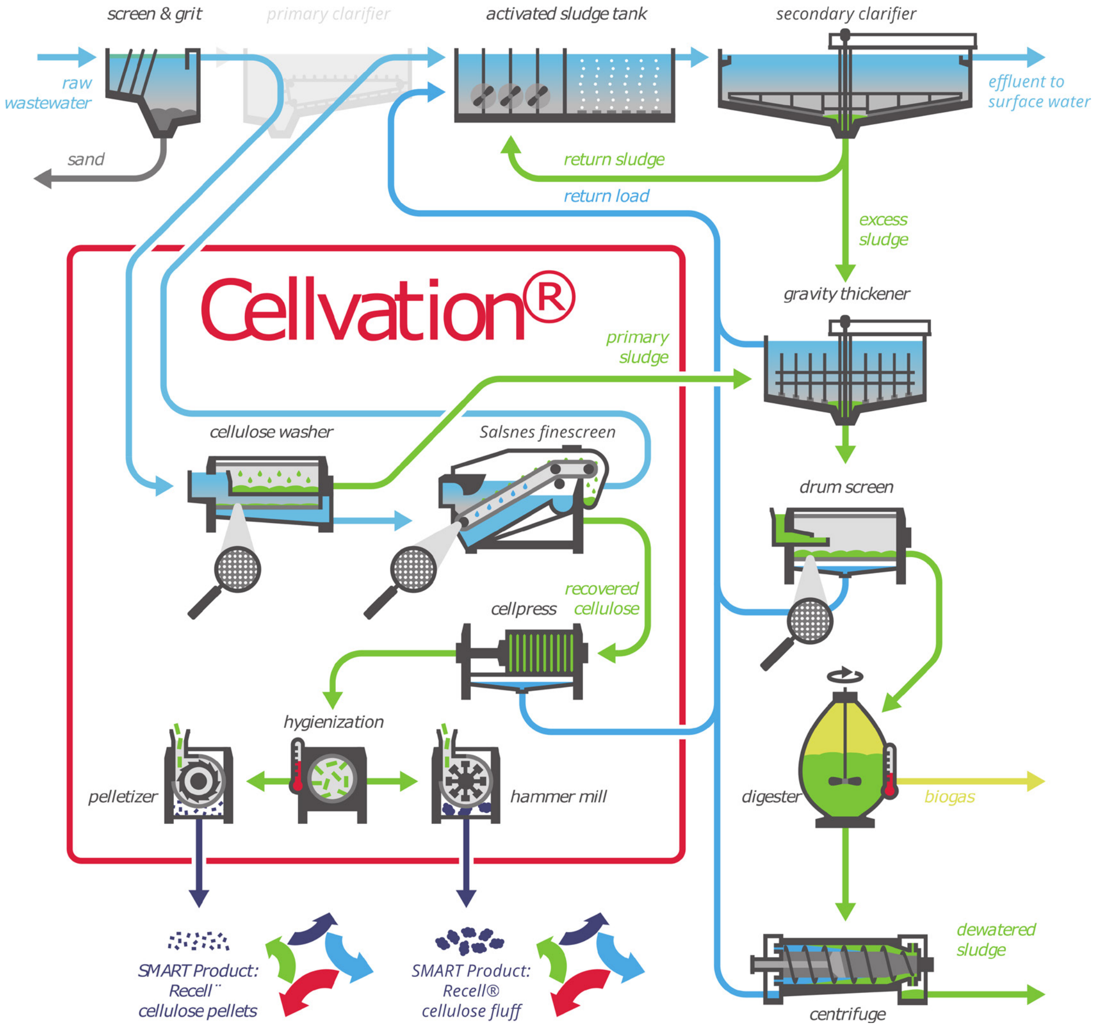


Figure 8.2 Schematic diagram of the Cellvation® process. The Cellvation® process is demonstrated in the WWTP Geestmerambacht within the H2020 SMART-Plant project.

a good example of cellulose recovery which harvests up to 400 kg cellulose per day. A Cellvation® installation, among others consisting of a fine screen, is installed in the feed, and treats the screened material. The screenings generally consist of fibres (80% of which are cellulose fibers), and remaining portions of protein, fats and ash. The amount of sludge produced on site is reduced by 35% when using the Cellvation® installation. The primary cellulose harvesting has a further impact on the overall treatment train, such as reduction in aeration energy of up to 20%, reduction in sludge volume up to 10%, reduction in TSS in treated influent up to 55%, reduction in COD in treated influent up to 30% as well as reduction of CO_2 footprint of up to 15% (http://www.smart-plant.eu/).

In what concerns process economics, the installation of a Cellvation® process at a small-to-medium sized new plant (50 000 PE) is estimated to have an associated additional capital expenditure (CAPEX) of €18/PE, albeit it decreases the operating expenditure in terms of aeration energy by

Figure 8.3 An RBF system is demonstrated at the sewage treatment plant of Geestmerambacht (The Netherlands).

about 20% and also reduces excess sludge production, resulting in a cost saving of about €0.5/PE/year. Besides the potential cost savings, the treatment capacity of an existing plant can be increased due to the reduction in organics loading to the activated sludge tank. In addition to the energy savings and increased treatment capacity, the reduction in the amount of sludge results not only in a lower polyelectrolyte requirement during sludge dewatering, but it also substantially reduces the amount of dewatered sludge to be disposed. The latter is estimated to result in substantial savings in the order of €2.7/PE/year (highly site-specific and depending on location and ultimate disposal route). That, in combination with an estimated revenue for high-quality recovered and cleaned cellulose (which has an EPA rating of class A) valued at €265/ton, would lead to an estimated payback time of approximately six years (SMART-Plant 2020a). It should be emphasized that the payback time highly depends on the local situation, wastewater characteristics, WWTP size and potential revenues of the recovered cellulose. As such, the value provided for the WWTP Geestmerambacht should only be seen as an indicative pay back time. Nevertheless, it provides some indication of the economic competitiveness of the use of microsieves for cellulose recovery. Moreover, many WWTPS are currently reaching their capacity in terms of solids loading due to expansion of the sewer catchment. Especially in these cases and where space is an issue, the retrofitting of existing plants with microsieves may provide an interesting alternative due to their low footprint.

In the WWTP of Falconara Marittima (Ancona, Italy), a pilot-scale RBF recovered cellulosic sludge from municipal wastewater at an influent flow rate of 15–78 m^3/h (Palmieri *et al.*, 2019). Different mesh sizes from 90 to 350 μm were tested in the pilot plant, and the recovered cellulosic material reached concentrations up to $\approx 27\ g/m^3$ (average $15.9 \pm 10.1\ g/m^3$) with maximum solids removal of 74% at 90 μm. After the final washing, the cellulosic material composition was, on average: 9 ± 1% of lipids; 6 ± 1% of ashes; 9 ± 1% of hemicellulose; 38 ± 6% of pure cellulose; 40 ± 3% of lignin and humic compounds; 0% of protein and other compounds. The recovered cellulose was then valorized as reinforcing components in building materials. The flexural strength in mortars was improved with the addition of 20% of recovered cellulose fibers. Based on a feasibility study, it was estimated that a WWTP with a capacity of 150 000 PE could potentially produce 305–1069 sacks per day of pre-mixed mortar with 5–20% of recovered cellulosic fibers by volume, respectively. This amount corresponds to an average production of a small-medium cementitious products company.

Similarly, a pilot-scale RBF installed at the Carbonera municipal WWTP (Treviso, Italy) produced cellulosic sludge by the sieving of around 400 m^3 wastewater/day. The microsieving was accomplished through an RBF SF1000 (Salsnes Filter SA, Norway) with a flow capacity up to 15 L/s and a submerged sieve cloth area of 0.24 m^2 using two mesh sizes (350 and 210 μm). The RBF removed approx. 44% of TSS and 35% of COD. The cellulosic sludge had high content of cellulose (41% TS) and lignin (18% TS), while lipids and proteins accounted for 9 and 13% of TS, respectively. The cellulosic sludge was then utilized as a feedstock for the production of SCCA (see Chapter 7 for more detailed information on SCCA production). By adjusting the pH of the fermentor to 9 before the feeding and operating with a hydraulic retention time (HRT) of 6 days under mesophilic conditions, the system achieved bio-based SCCA productivity of 2.57 kg COD/m^3/day (Da Ros *et al.*, 2020). A first indicative economic assessment of that particular pilot trial highlighted that the production of bio-based SCCA from cellulosic primary sludge could be more profitable than its conversion to biogas.

A last case study to be considered is an RBF installed in the WWTP of Aarle-Rixtel, the Netherlands (Ahmed *et al.*, 2019). Without entering into the specific details of the case study, technical specifications, and so on, this specific case study is worth mentioning to illustrate the impact of site-specific conditions and their relation to the overall cellulose recovery potential and product quality that can be achieved. The cellulose content in the RBF sludge was found to be only 35% of the TSS (Ahmed *et al.*, 2019), which is (much) lower than the cellulose content achieved elsewhere (i.e. around 55–75%), significantly reducing product quality and potential for volarization of cellulose as a marketable commodity. This highlights the importance of, and need for, site-specific pilot studies to verify the practical feasiblity of RBF as a cellulose recovery and valorization approach.

8.4 CONCEPTUAL OVERVIEW OF POLYHYDROXYALKANOATES (PHA) PRODUCTION

PHA are microbial intracellular storage polymers that can serve as precursors of bioplastics. The development of technologies to produce PHA as biopolymer precursors has been ongoing for decades, but the high cost of PHA production using pure microbial cultures and refined feedstocks stands as a major obstacle to broader PHA applications. In recent years, this problem has been approached by using waste feedstock materials as carbon source, mixed microbial cultures (MMC) which do not require aseptic conditions, and aqueous two-phase systems for purification and recovery to make PHA production more economical (Pakalapati *et al.*, 2018). Rapidly increasing population, urbanization, and industrialization lead to the production of large quantities of sludge in WWTPs every year, making it a readily available and more economical feedstock for bioplastic production compared to the typical carbon sources used in commercial production (Bengtsson *et al.*, 2008). Hence, PHA production through MMC stands as a perfect example of resource recovery in WWTPs, combining conventional municipal wastewater treatment with innovative advanced technologies. Biological treatment of municipal wastewater and sludge management for recovering wastewater organic carbon as PHAs is a route to transform the 'treat and discharge' approach into a biorefinery approach.

8.4.1 Fundamental principles

MMC present in wastewater sludge hold great potential for PHA production (Reddy *et al.*, 2012). Microbial species in the sludge, such as bacteria, yeasts, and fungi, naturally synthesize biopolymers such as triacylglycerol (TAG), wax esters (WEs) or PHA in the presence of excess carbon source, particularly when either nitrogen or phosphorus (or both) are limiting in the growth media (Kumar *et al.*, 2017, 2018). Bacteria are the major contributers to PHA storage in MMC PHA processes, and many bacterial genera commonly found in activated sludge have been identified as PHA-storing microorganisms. This suggests that there is great potential for practical PHA production using activated sludge-derived MMC. However, the productivity of the process can be substantially improved with an enrichment step prior to accumulation. For example, PHA accumulation from acetate by activated sludge taken from municipal WWTPs was reported to be between 45 and 67 wt% with fed-batch feeding (Cavaillé *et al.*, 2013), while activated sludge subjected to enrichment can accumulate PHA up to 90 wt% from acetate (Johnson *et al.*, 2009). PHA storing oragnisms in MMC need to be fed with a feedstock rich in readily biodegradable organic matter, most preferably dominated by SCCAs, which are readily convertible to PHA, to minimize the production of other products such as glycogen.

A PHA-production process from wastewater is, thus, composed of three steps (Figure 8.4): a first anaerobic step, in which SCCAs are produced from wastewater or sludge (see Chapter 7 for more information on their production from wastewater), a second step where the microbial community is enriched in PHA storing organisms, and a final accumulation stage, using enriched biomass, where PHA storage is maximized. The second and third steps are usually aerobic and are supplied with the SCCAs produced in the first stage. Intracellular PHA needs to be then extracted and purified using physico-chemical methods.

Typically, feast and famine conditions are alternated to achieve a good enrichment of PHA accumulating organisms (Dionisi *et al.*, 2001). A relatively prolonged famine phase is necessary to impose a limitation of internal growth factors (RNA, enzymes, etc.) on the organisms that are not able to store PHA. In the subsequent feast phase, the uptake of carbon by these organisms will be delayed, as they re-establish the level of growth factors necessary for regular metabolic activity. These organisms will gradually be outcompeted by PHA-storers, which can remain active during the famine phase. This selection approach is known as the feast-famine strategy or aerobic dynamic feeding (Dionisi *et al.*, 2001).

Scaling-up PHA production from waste streams still requires further research to become economically feasible, which is currently the main challenge for the implementation of PHA production from waste or wastewater at the industrial scale (Rodriguez-Perez *et al.*, 2018). The selection of an appropriate waste stream is critical to ensure an adequate and constant carbon supply and obtain satisfactory recovery efficiencies. Recently, wastewater, cheese whey and organic wastes such as

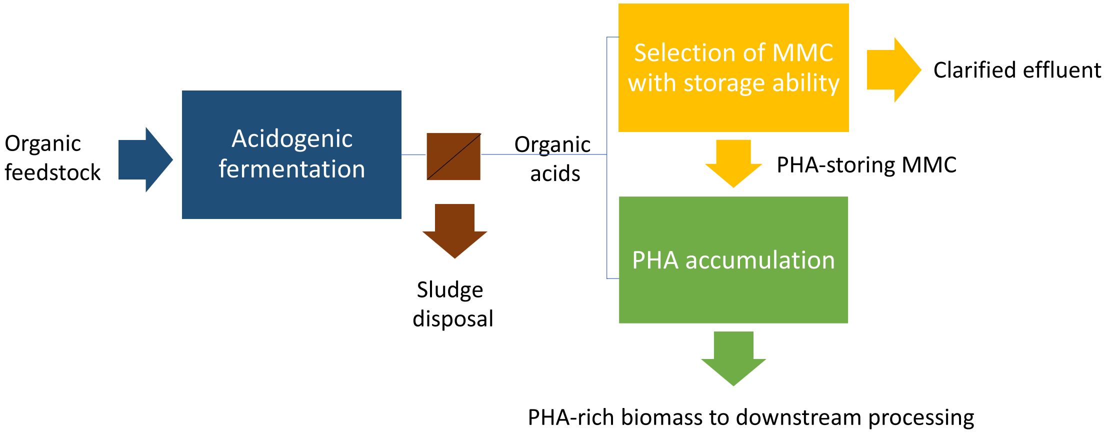


Figure 8.4 Three-step MMC process for PHA recovery: (1) acidogenic fermentation to obtain a SCCA-rich stream, (2) a dedicated biomass production yielding MMCs enriched with PHA-storing potential, and (3) a PHA accumulation step where (1) and (2) outputs are combined in a final biopolymer production bioprocess (modified from Valentino *et al.*, 2017).

fruit and vegetable wastes, have gained great attention as carbon sources. Hence, the process can be optimized in terms of the nutrient balance by combining two or more of these waste streams during the integration of PHA production into WWTPs (Rodriguez-Perez *et al.*, 2018).

8.4.2 Applications and design

PHA production from wastewater can be applied in different configurations, with the two most typical being illustrated in Figure 8.5. An enrichment-based process (Figure 8.5, left) relies on the common three-stage process detailed above. The configuration that relies on accumulation with sludge (Figure 8.5, right) skips step 2 associated with PHA biomass selection, using secondary WWTP sludge directly in a fed-batch accumulation reactor, with fermented wastewater as the SCCA source. While higher PHA contents (>80%) are potentially achievable in the enrichment-based process, the savings in capital costs associated with the elimination of the selection reactor can be advantageous in certain cases. In other situations, PHA selection can be combined with simultaneous side-stream treatment of nitrogen and phosphorus, lowering the nutrient loading rate recycled back to the mainstream WWTP following treatment of anaerobic digester supernatants (see section 8.4.3 for detailed case studies).

In the three-step process configuration, adequate PHA-storers selection is key for a productive system. The feast and famine approach usually used as a microbial selection pressure is normally established in sequencing batch reactors (SBRs), although it can also be imposed through a sequence of two continuous reactors, one with carbon supply and another without (Albuquerque *et al.*, 2010). An effective selection of PHA storing biomass is accomplished when the feast/famine ratio is lower than 0.2 min/min. Generally, the length of the feast phase increases at lower SRTs, as more substrate is supplied per unit of biomass per cycle and the cultures do not increase their SCCA uptake rate (Johnson *et al*. 2010). The SRT is also known to shape the selected microbial communities composition, which can affect the overall performance of the process. SRTs ranging from 1 to 6 days have been typically

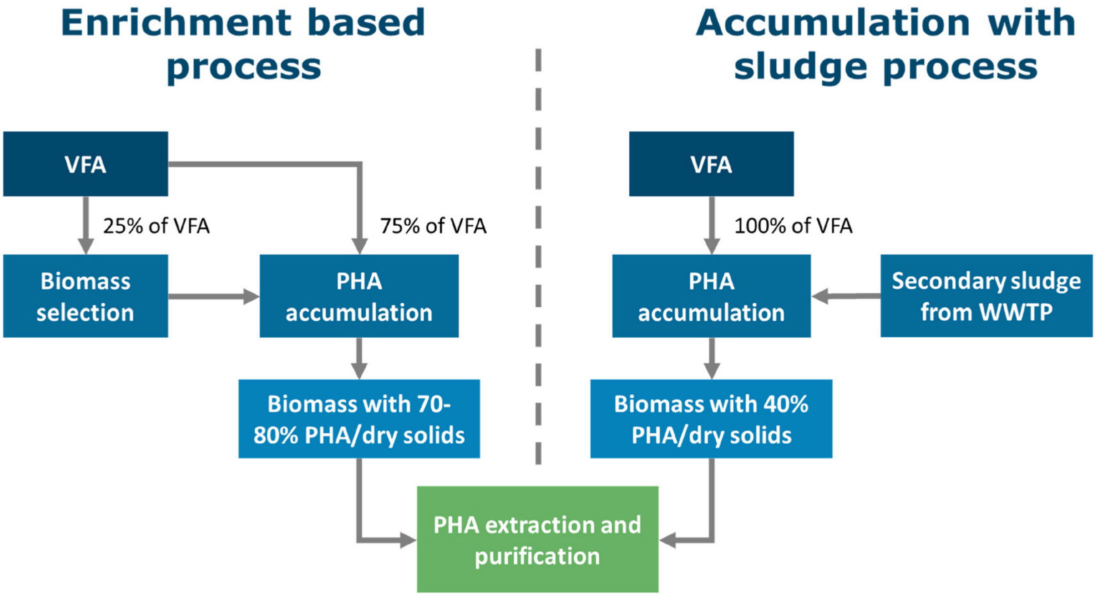


Figure 8.5 Main processes for producing PHA using mixed cultures fed with organic acid from fermented waste-based feedstocks. Left) PHA production based on biomass selection and enrichment; Right) PHA production from VFA (volatile fatty acids, which are SCCA) using secondary sludge as PHA accumulation biomass.

reported for PHA production from wastewater. Besides SRT, the organic loading rate (OLR) is a key parameter influencing the performance of a MMC PHA process. It is normally desired to maximize both the acidogenic and the selection reactor OLR. Higher OLR in the selection step normally leads to higher volumetric productivities due to increased productivity of PHA-storing biomass. However, above a certain OLR threshold, the process can either become inhibited or the feast phase be extended to a point that the selection pressure is inefficient (Campanari *et al.*, 2014; Carvalho *et al.*, 2014; Khatami *et al.*, 2021). Other parameters that can impact the success of the PHA accumulation process are pH, temperature, dissolved oxygen, cycle length and HRT (Kourmentza *et al.*, 2017; Oehmen *et al.*, 2014; Wang *et al.*, 2019). The decoupling of the nitrogen and the carbon sources is another means to drive the enrichment in PHA-storing organisms. If there is no nitrogen available during the feast phase, organisms can only take up and store carbon, which gives PHA-storers an additional competitive advantage when nitrogen is supplied in the famine phase (Oliveira *et al.*, 2017).

As outlined in Figure 8.4, the anaerobic fermentation of wastewater or sludge is of key importance for generating the SCCAs necessary for PHA production. The SCCA spectrum also governs the PHA monomeric composition, which in turn influences the properties of the PHA bioplastic product. For instance, the fraction of polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) are linearly dependent on the level of PHB (containing an even number of carbon atoms) and PHV (odd carbon atoms) precursors produced through anaerobic fermentation (Albuquerque *et al.*, 2007). For example, both acetic and butyric acids correspond to PHB precursors and propionic and valeric acids correspond to PHV precursors. Improved mechanical properties of the recovered polymer were noted in co-polymers of PHB and PHV, as opposed to PHB alone (Albuquerque *et al.*, 2011). These properties included decreased stiffness and brittleness, increased flexibility, increased tensile strength and toughness and a lower melting temperature that improved its processability. Thus, tuning the anaerobic fermentation step towards production of a SCCA profile leading to a desirable PHB/PHV fraction can have positive impacts on the PHA produced, where desired polymeric traits depend on the intended final application.

A very important step for use of these polymers is the final extraction and purification process. Several methods exist for extraction of PHA from the PHA-rich biomass produced. They are categorized mainly in physical separation processes, cell lysis-based processes and solvent-based processes (Pagliano *et al.*, 2021). The choice of method is highly dependent on the type of PHA-rich biomass produced. From waste-based feedstocks and mixed cultures, simple physical separation processes are usually not suitable as most of the impurities are still present, although physical separation is often applied in conjunction with cell lysis and solvent-based processes. Cell lysis-based techniques have the advantage of lower investment costs and simplicity of operation but often lead to the production of wastewater that requires additional treatment. Solvent-based methods are robust methods that can handle variations of the PHA-rich biomass but have higher investment costs and are more complex in operation. Therefore, the choice of method will depend on two main factors: type and variation of PHA-rich biomass (e.g. PHA content, type of contaminants, microorganisms present) and the quality requirements for the final PHA polymer (e.g. purity, molecular weight) (Fernández-Dacosta *et al.*, 2015; Samorì *et al.*, 2015). Cell lysis or solvent-based techniques are currently the most commonly applied in downstream processing for PHA extraction from mixed cultures (Pagliano *et al.*, 2021). Some commonly applied examples include chlorinated compounds (such as sodium hypochlorite (NaClO), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), and so on), alkali or acid treatment, and surfactants (e.g. sodium dodecyl sulphate (SDS)). Typically this involves a series of steps based on mixing in a tank with the PHA-rich biomass, followed by separation through centrifugation, filtration or hydrocyclonation and a final drying step (Fernández-Dacosta *et al.*, 2015; Samorì *et al.*, 2015). A similar post-treatment stage to remove contaminants may also be necessary. Some key process factors requiring optimization and control include the temperature, as heating and cooling steps can also be required during downstream processing, retention time in the process tanks combining the biomass and key active ingredients, as well as process conditions specific to the separation and drying stages.

Table 8.2 Market possibilities and features of recovered PHA.

Features	Industrial use	Quality indicators	Price range	Reference
• Biocompatible • Biodegradable • Non-toxic • Appropriate mechanical, thermal, physical properties • Easy surface modification • Noncarcinogenic	• Material industry (packaging, paper coating etc.) • Fuel industry (biofuels additives) • Industrial microbiology	• Purity • Toxicity • Chemical constituents • Blending	2.2–5.0 €/kg	Anjum *et al.* (2016), Możejko-Ciesielska and Kiewisz (2016), Valentino *et al.* (2017)

The multiple stages that are often required to achieve high PHA recovery and purity represent a significant cost to the overall process.

The utilization of wastewater for the synthesis of PHAs can lead to production of a value-added compound; however, the final product quality and application will be dependent on the source of SCCA and the potential presence of contaminants. For instance it is unlikely that PHA obtained from wastewater could be used in medical applications where high purity products with a non-toxic nature are of utmost consideration (Raza *et al.*, 2018). Possible market alternatives and features for recovered PHA are summarized in Table 8.2.

8.4.3 Case studies

Two case studies will be discussed here, the first will focus on an enrichment-based process integrated with side-stream wastewater treatment, while the second deals with a system that uses sludge as source of microorganisms for accumulation.

First, the side-stream Short-Cut Enhanced Phosphorus and PHA Recovery (SCEPPHAR) process offers recovery of both PHA and struvite, efficient nitrogen removal via nitrite from the anaerobic supernatant and reduction of sludge volume, offsetting disposal costs (Figure 8.6). It consists of: (i) (alkaline) fermentation of sewage sludge (pH 6–10) and/or cellulosic sludge to enhance the production of selected SCCA (e.g. propionic acid first) and release nitrogen and phosphorus in soluble forms (ammonia and phosphate); (ii) solid and liquid separation of the fermentation products and recovery of struvite from the sewage sludge fermentation liquid (SFL) by the addition of $Mg(OH)_2$ to favor the precipitation in a crystallizer reactor; (iii) sequential nitritation of the sludge reject water; (iv) selection of PHA storing biomass in a SBR by the alternation of aerobic-feast and anoxic-famine conditions for denitritation driven by internally stored PHA as carbon source; (v) PHA production in a fed-batch reactor to accumulate the intracellular PHA content in the selected biomass using SFL.

In SCEPPHAR, the anaerobic supernatant of an anaerobic digester is fed into the nitritation SBR and then, after nitritation, its effluent (nitrified supernatant) is discharged into a storage tank. Nitrite is later used as electron acceptor by the PHA storing biomass in the selection SBR. PHA-accumulating biomass selection is essential to obtain a microbial community capable of synthesizing and hyper-accumulating PHAs and at the same time, removing nitrogen via nitrite. To achieve this result, biomass is subjected to feast and famine conditions under aerobic and anoxic conditions, respectively. During the feast phase, PHA are synthetized and accumulated by the biomass as internal carbon storage, while under famine conditions nitrite is used as electron acceptor for internal carbon (PHA) degradation under anoxic conditions, thus promoting the microbial growth of denitrifiers and PHA storing organisms without aeration input in the famine period. Aerobic conditions in such processes are known to be energy-intensive; it is estimated that approximately 39 MJ are needed to produce 1 kg of PHA via traditional aerobic accumulation processes (Frison *et al.*, 2015), thus presenting an advantage for the SCEPPHAR process. The accumulation SBR allows maximum PHA storage to be reached (PHA yields ranging between 0.58 and 0.61 g COD_{PHA}/g COD_{VFA}) (Conca *et al.*, 2020), and

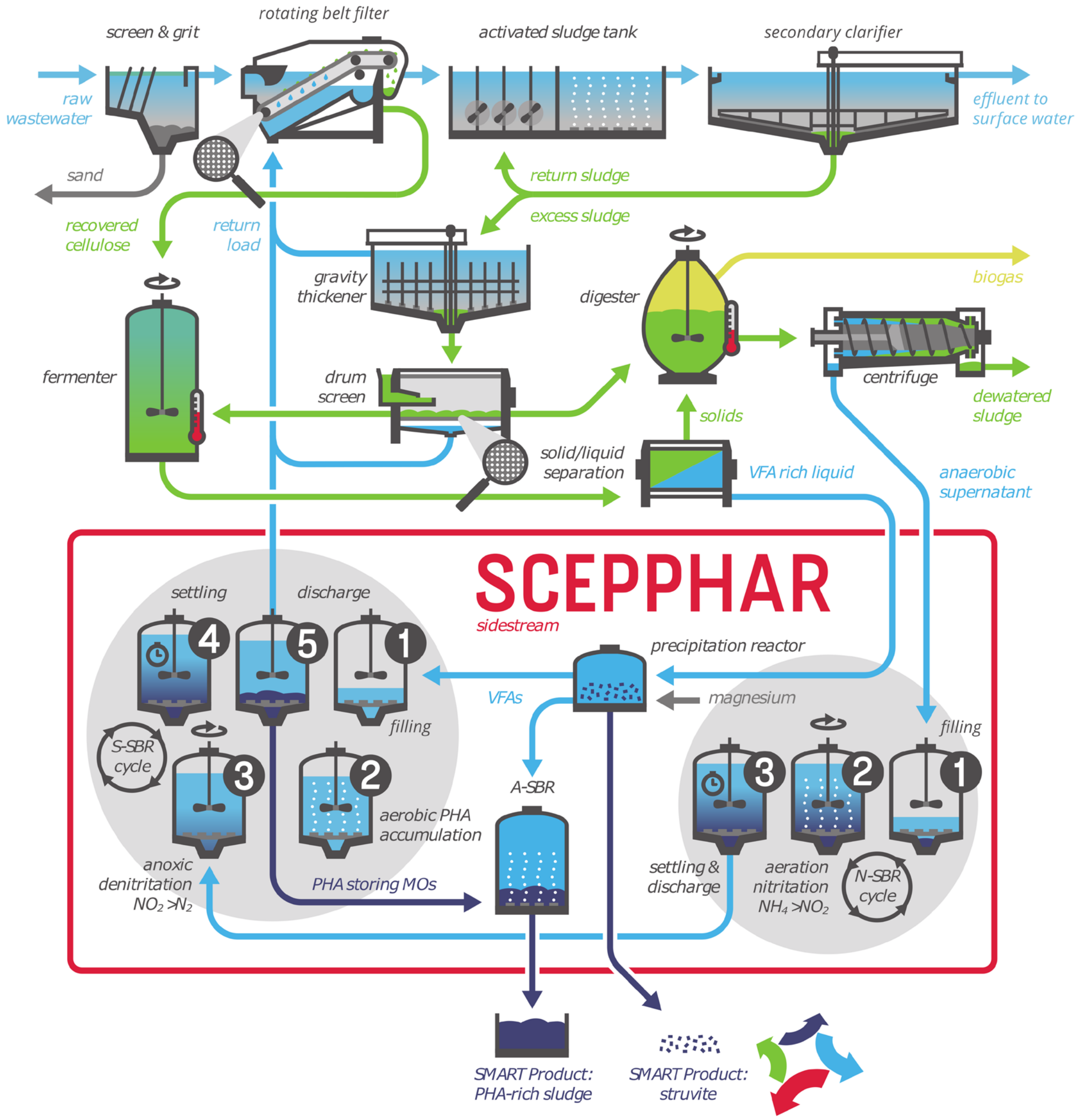


Figure 8.6 Schematic diagram of the side-stream SCEPPHAR process. The side-stream SCEPPHAR process was demonstrated in the WWTP of Carbonera (Italy) within the H2020 SMART-Plant project.

high rates under feast conditions, during which aeration is controlled based on the DO (e.g. 2 mgO_2/L) and pH (e.g. >7.5) profiles. The carbon source is intermittently supplied to the aerobic accumulation SBR for a total batch length of between 4 and 6 hours; at the end of the PHA accumulation step, biomass activity is interrupted through the dosage of a quencher (e.g. sulfuric acid) to cease biological activity and prevent PHA degradation. PHA is obtained as PHA rich sludge, where up to 50% of PHA (PHB + PHV) is accumulated in the bacterial cells. From an operational point of view, side-stream SCEPPHAR provides several advantages, such as: (i) a short start-up period; (ii) high stability of the process under transient conditions; (iii) self-management of the bioprocesses; (iv) simplified operations through on-line monitoring; (v) real time process control based on indirect parameters; and (vi) possibility to switch between PHA production and sole nitrogen removal from reject water via-nitrite.

The side-stream SCEPPHAR process was demonstrated in the Carbonera WWTP (as part of the SMART-Plant project), in a pilot installation that treated 4–5 m^3 of sludge liquor per day. The process achieved up to 85% N removal and enabled the recovery of phosphorus as struvite, in addition to producing PHA-rich sludge. The carbon source for optimized biopolymer production was produced on-site by fermentation of cellulosic sludge captured using an RBF (sieving of around 400 m^3/day of municipal wastewater, and an acidogenic fermentation unit with a working volume of 2.6 m^3 for the production of SCCA). SRT and HRT were maintained as 6–7 and around 2 days, respectively. Average PHA yields of 0.58–0.61 $gCOD_{PHA}/gCOD_{SCCA}$ were obtained, while the maximum PHA production was up to 1.2 kgPHA/PE/y when treating solely municipal wastewater (Conca *et al.*, 2020). In addition, this process decreased the energy costs for side-stream treatment by up to 20%. The recovered struvite can be used as P-based fertilizer while the PHA, which achieved a content in the biomass up to 50% on a dry weight basis, was studied for use in flame retardant formulations for building materials. On-site extraction of PHA was not deemed sustainable in the short term in terms of CAPEX & OPEX (around 1.5 €/kg), as the payoff time was too long. In a scenario where the side-stream SCEPPHAR is installed in an existing plant with a size of 50 000 PE and PHA is not extracted but bio-composites are produced directly from the PHA-rich sludge and sold for 1000€/ton, the additional CAPEX would be €6/PE (€ 300 000) and OPEX savings would range from €0 to €3.7/PE/y, depending on the cost of sludge disposal. The income from PHA sales would represent a maximum of €2/PE/y, while the savings of sludge treatment could be as high as €3.85/PE/y. All together this would result in a short payoff time, that is less than two years. In case PHA-rich biomass is not accepted by the market, PHA-rich sludge could be sent to an already existing anaerobic digester to generate electricity, further sold in the market for €0.10/kWh, where the income from the sale of electricity would be €0.12/PE/y, whilst the OPEX savings could be as high as €1.2/PE/y. In this scenario, the payoff time would still be lower than five years.

Implementing a side-stream SCEPPHAR system for wastewater treatment can also have a positive impact on the net energy balance of the entire plant, although this depends on the valorization route for the PHA-rich sludge. For instance, PHA valorization for biogas production on-site would reduce net energy demand by −15%, mainly by generating additional electricity from the biogas. The drying of dewatered PHA sludge and its direct use bio-composite production would reduce the net energy demand by −18%. The chemical extraction of PHA and production of a purified PHA powder is not beneficial for energy balance, increasing the net energy demand by +6%. For this route, the requirement of chemicals for PHA extraction, and especially the energy required for treatment of the liquid waste (basically dissolved excess sludge), would be substantial and off-set all credits for PHA use downstream. Additional electricity required for the process (+15–18%) is mostly compensated by savings in coagulation chemicals, which are not required with the bio-P removal of SCEPPHAR. However, on the downside, the SCEPPHAR process can increase net GHG emissions, mainly due to the predicted higher N_2O emissions (+33%) in the heterotrophic and autotrophic SBR compared to a conventional activated sludge process (SMART-Plant, 2020b).

As a side-note, similar enrichment-based approaches have been tested at larger scale. A pilot project at Brussels-North WWTP by Aquiris (a subsidiary of Veolia Water) demonstrated the technical feasibility of producing biomass with elevated PHA-accumulation potential from municipal wastewater treatment and fermented waste sludge as feedstock for PHA accumulation (Morgan-Sagastume *et al.*, 2016). The main features of the Brussels-North system were: Sludge fermentation at 42°C, pH = 5.5–6.5; $Y_{SCCA/VS} = 270 \pm 30$ g COD(SCCA)/g VS; 12 cycles per day; Feast/Famine 15/85%; OLR = 3 kg COD/m^3 d; SRT = 1–2 days; Final PHA content = 34% (gPHA/gVSS); Overall PHA accumulation rate = 0.07 g COD(PHA)/g $COD_{treated}$. (Morgan-Sagastume *et al.*, 2014). Following the same process concept, another pilot-scale prototype comprising biomass production from wastewater treatment and accumulation of PHA in the surplus biomass was installed at the demonstration site at Leeuwarden WWTP in Friesland (Bengtsson *et al.*, 2017). The treatment of wastewater and biomass production with PHA accumulation was conducted in one or two SBRs. Pre-denitrification and nitrification was carried out in the first SBR throughout the entire operational period under anoxic feast and aerobic famine conditions, then the first SBR was combined with the second SBR operated under anoxic conditions to achieve further N removal by post-denitrification. In the end, 83% COD and 80% N removal, as well as PHA accumulation up to 49% PHA of VSS with acetic acid or fermented organic residues as substrates, were achieved.

A second potential configuration for PHA production from sewage sludge was initially investigated by Dutch water utilites and other partners like Anox Kaldnes (Veola Water Technologies AB) within the PHARIO project. PHARIO was centred on processing surplus biomass from Dutch full-scale municipal WWTPs in the Netherlands to produce PHA biopolymers. The process steps involved in the PHARIO project consisted of the harvesting of secondary sludge as functional biomass (inoculum) able to produce PHA from SCCA-rich streams, obtained from either fermented organic waste, primary sludge or other sources (Figure 8.7). In the PHARIO concept PHA polymers are recovered via solvent extraction (using acetone) (Werker *et al.*, 2015).

The results of the PHARIO project revealed a PHA rich biomass with on average 0.41 g PHA/gVSS, with reproducible thermal properties and high thermal stability. As the main output of the project, Veolia Water Technologies developed basic engineering designs for a full-scale commercial plant with a production capacity of 2000 ton PHA per year. The results indicated that building the first full scale plant would require around 13 M€ of initial investment for the facilities and another 4 M€ to cover the operational costs for a period of three years. Based on the economic evaluation, PHA could be produced at a price of 4.5€/kg, thus generating a net profit of 1.1€/kg PHA, or 5.5 M€/year. The cost analysis concluded that the produced PHA had a competitive price compared to commercial products

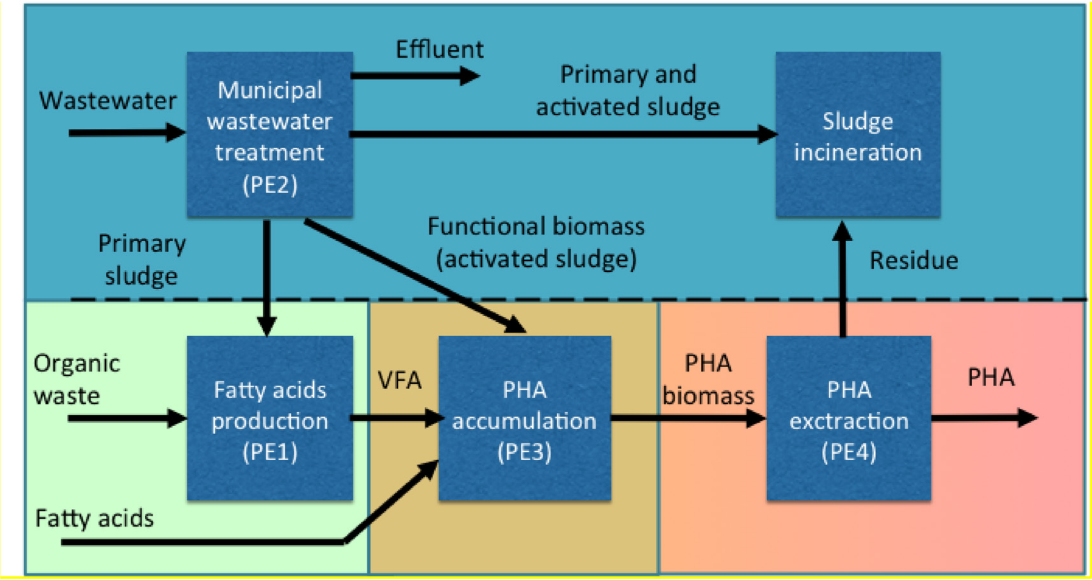


Figure 8.7 Process steps involved in the PHARIO project for PHA production from secondary sludge and organic waste as carbon source (STOWA, 2017).

and could be used as a green alternative in the manufacturing industry (Mannina *et al.*, 2020; Werker *et al.*, 2018). The construction of a demo-scale installation for the production of PHBV has just started in September 2020 in the Netherlands, and is expected to be operational by the end of 2021.

8.5 CHALLENGES, OPPORTUNITIES, AND RESEARCH NEEDS

There are a wide variety of possible alternatives for organic resource recovery from municipal wastewater, and the technical options are growing even in terms of demonstration in the operational environment. However, technical, social and legislation barriers remain and should be addressed by further research and innovative solutions to make and replicate business cases for resource recovery.

From a technical point of view, organic resource recovery technologies should be able to provide materials with high technical quality and stability that can be used in new or conventional industrial or consumer products. The novel technologies should be able to integrate existing infrastructure and allow for a smooth transition from WWTPs to resource recovery facilities. Water utility operators should have the technical skills to integrate and successfully run the resource recovery systems. From the economic and market point of view, cost should be calculated in a holistic manner, considering not only technical costs, but also social aspects, organizational strength, and institutional support. This approach can easily help to better understand the specific market potential of each recovered material.

Resource security should prioritize targeted recovery, and future research should support decisions about policy integration and regulatory change that must be addressed at international, national, and local scales. The major challenge to the overall resource recovery platform is public acceptance, since most of the recovered compounds are still labeled as wastes. The contamination risks associated with pathogens, chemicals, metabolites, and so on. concern society. Thus, new approaches should be developed to change general public opinion to support more sustainable management systems within circular economy frameworks. The communication problem can be overcome by a well-planned strategy focused on the most applicable technologies and geographical features, as well as cultural habits and sensitivities. Another important issue concerns improving markets for the recovered materials. This can be achieved by closing the gap between actual and potential recovery rates, which will directly affect the market price since improved recovery rates can lower the price.

In general, the bottlenecks of organic resource recovery in a WWTP are related to economics and value chain development, environment and health, and society and policy issues.

8.5.1 Cellulose

The implementation of a cellulose recovery process has the potential to reduce the net environmental impact of a conventional WWTP significantly, although to date there are no quantitative indications of the potential GHG emission reduction. Overall, the net energy demand of the system can decrease by 4–23% depending on the efficiency of cellulose removal in the process, while the net GHG emissions can be reduced by 2–19%. The further implementation of this technology (either by the retroffiting existing facilities or in new designs) should provide more real data to support the anticipated benefits of cellulose recovery, strengthening the business case for its implementation.

Beyond the benefits linked to energy and GHG emissions reduction, cellulose recovery can yield a product. The properties of the recovered cellulosic materials vary depending on the characteristics of the raw wastewater as well as to the design and operating parameters of the processes along the treatment line. The selected end-use of a certain cellulosic material dictates the product requirements, in turn determining the process design and operational conditions to be applied (Keijsers *et al.*, 2013). Public acceptance is not often easy for toilet-paper production from recovered cellulose, but valorization of cellulosic sludge via bio-drying and use as as feedstock for SCCA production or biofuel can generate additional credits in avoided energy demand and related GHG emissions.

8.5.2 PHA

While the first commercial full-scale process to produce PHA is currently being constructed with the PHARIO configuration, upscaling of the 3-step configuration is also taking place. In this case, the most critical upstream process factor that requires optimization is the overall process productivity, which depends on the PHA yield and on the PHA content in the biomass. A recent pilot study integrated multiple strategies that had been developed separately for different points of the 3-step process, attaining global productivities above 8 gPHA/L.d (Matos *et al.*, 2021). Based on this unprecedented PHA productivity from real feedstocks, the 3-stage configuration could become more competitive and be taken to the commercial scale in the near future.

In addition to productivity, the long-term stability of the process is another point where further research is needed for implementation of MMC PHA production from wastewater. The functional redundancy of multiple microbial populations within the MMC is one of the positive aspects of this technology with respect to pure cultures (Carvalho *et al.*, 2018). However, future research should validate the resiliency of enriched PHA-storing communities under the variable conditions of flow rate and influent composition that is typical of WWTPs. Moreover, stability relates not only to the productivity but also to the polymer characteristics (Werker *et al.*, 2018). Methods for achieving a stable PHA biopolymer product in terms of quantity and composition would aid downstream processing, and can be challenging with mixed cultures subject to dynamics in wastewater composition.

The economic viability of retrofitting existing WWTPs to produce PHA has been recently evaluated in a study comparing both large (>3 M PE) and small (50 k PE) sized plants. The final production cost of crude PHA (i.e. excluding downstream extraction) was found to be viable in both scenarios, in the range of 1.26–2.26 US$/kg PHA-crude for the large and small WWTP, respectively (Crutchik *et al.*, 2020). Despite the progress attained in the efficiency of the production step, current production costs are not yet competitive compared to conventional plastics, with the cost of recovery and purification currently being the critical bottleneck (Kehrein *et al.*, 2020). Indeed, the cost of downstream processing has been recognized as a major challenge for PHA recovery, and avoiding the use of harmful chlorinated solvents would also simplify the process for wastewater treatment plant operators, reducing the risk associated with hazardous materials. An alkali/surfactant based process has been shown to be more cost effective (1.40 €/kg PHB recovered) with reduced global warming potential (2.4 kg CO_2-eq/kg PHB) as compared to a solvent-based process (1.95 €/kg PHB, 4.30 kg CO_2-eq/kg PHB) (Fernández-Dacosta *et al.*, 2015), where the trade-off is typically reduced purity as compared to solvents (Pagliano *et al.*, 2021). Focussing on final applications necessitating low purity would be beneficial for PHA produced from wastewater, and would facilitate its integration into wastewater treatment plants. Simplification of downstream process flowsheets containing numerous stages also requires further study. The elimination of unit processes during downstream processing would further reduce the costs, increasing its economic viability.

8.6 CHAPTER SUMMARY

This chapter summarizes some of the emerging technologies for the recovery and valorization of organics in WWTPs. The main organic products that can be recovered/produced from municipal wastewater in WWTPs are cellulose and PHA. Cellulosic primary sludge, mostly generated from the flushing of toilet papers, can be efficiently utilized to recover cellulose or follow other pathways for the valorization of cellulose and separated organics. Recovered cellulose can be further processed and used as a starting material for SCCA or bioplastic production. Meanwhile, PHA recovery from sewage is another important technology since PHAs have comparable properties to petrochemical plastics. Producing PHA from sewage could provide a cost-competitive route for their production, although further research is needed to strengthen the economic business case of this organics valorization route.

8.7 EXERCISES

Exercise 8.1: A centralized municipal wastewater treatment plant services a city of 750 000 inhabitant equivalents. In order to improve the sustainability of urban wastewater management of the city, the city council has raised the recovery of cellulose from municipal wastewater through micro-sieves as one of the priority resources. Based on a toilet paper usage of 10 kg toilet paper/person/year (i.e. typical consumption in Western European countries):

(a) Calculate the amount of cellulose that can be recovered at the WWTP on a daily and annual basis at a practical relevant cellulose recovery efficiency of a micro-sieve unit of 80%.
(b) Calculate the theoretical amount of methane (in m^3) that can be generated from the amount of cellulose embedded in the wastewater matrix when not being recovered as cellulose but being fed to an anaerobic digestion. Assume that the COD content of cellulose is equal to 1.1 g COD/gram cellulose, that the cellulose can be fully degraded during mesophilic anaerobic digestion, and that 0.5 m^3 methane are produced per kg of COD converted.
(c) Assuming that 1 m^3 of biogas is equivalent to 6.27 kWh, that the conversion of methane to electricity in a CHP unit is 40% and the electricity consumption of a household of four people is 20 kWh/day, how many households can theoretically be powered from the produced electricity from the anaerobic digestion of cellulose.

Exercise 8.2: In various regions in the world existing WWTPs in urban areas are reaching their maximum capacity in terms of solids and COD load due to the increase in population growth within the catchment area of the WWTP during the last decade(s). Some of these WWTPs have serious constraints in terms of available land for extension of the WWTP. In this context, beyond the context of the importance of resource recovery, the implementation of micro-sieves can be of particular interest.

(a) Based on typical surface loading rates for primary settling tanks and micro-sieves of 1.5–2.5 and 100 m^3/m^2/hour, respectively, calculate the footprint of primary settling and micro-sieve for a WWTP with a capacity of 500 000 PE. Assume a wastewater production of 120 L/PE/d.
(b) Based on your answer in (a), what is the percentage in land space saving that can be achieved?
(c) Compare this with the overall size of a typical WWTP (tip: use google earth for an aerial shot of a WWTP near to where your life).

Exercise 8.3: The Canadian city of Halifax is building an activated sludge system at its main wastewater treatment plant that services 170 000 PE. Two resource recovery strategies are considered for the sludge produced from this treatment facility. The first consists of one-stage anaerobic digestion for methane recovery from biogas and the other is polyhydroxyalkanoate (PHA) recovery through a 3-stage process. The 3-stage PHA process consists of: (1) anaerobic sludge fermentation to SCCA; (2) aerobic culture selection through a feast/famine process fed with the effluent of (1); and (3) PHA accumulation from the selected culture in (2) using the effluent of (1). Assume standard (i.e. Table 8.1) wastewater flows and characteristics and that 80 gVSS of sludge are produced per m^3 of wastewater treated by the facility. Considering that the value of PHA is \$3.5/kg and that the value of methane is \$0.4/m^3, where 1 m^3 of biogas (with a methane content of 65%) is produced per kg of VSS, while 0.1 kg of PHA are produced per kg of VSS in the 3-stage process:

(a) Determine the relative value of the sludge stream using either the PHA recovery or biogas recovery strategy.
(b) Discuss the key factors impacting the capital and operational expenditures associated with biogas or PHA production. Which process is likely to incur higher production costs? Why? What would you consider to be the key points impacting your decision on the process to be implemented?

Exercise 8.4: Contrary to popular belief, a large fraction of the COD load entering WWTPs is in the form of toilet paper, depending on the region in the world. For example, the amount in South

American countries is typically much lower as it is often prohibited to flush toilet paper in the toilets as the sewer pipes in the building are not designed for this and as such need to be collected separately in a bin. Let us consider USA, with a consumption of toilet paper of 12.7 kg/person/year:

(a) Calculate the concentration of cellulose based COD assuming a daily water consumption of 200 L/per person per day.
(b) Determine the fraction of $COD_{cellulose}/COD_{total}$ assuming a typical municipal wastewater composition.

Exercise 8.5: In various places in the world, the excess waste activated sludge after dewatering is being transported to landfills for ultimate disposal. Moreover, not all WWTPs have implemented primary settling as a pre-treatment step, as such, in a typical biological nitrogen removal (or biological P removal) configuration all incoming cellulose enters the activated sludge tanks. According to the Foundation for Applied Water Research (STOWA), depending on the WWTP configuration and local conditions (e.g. wastewater composition, local climate, industrial activities in sewer catchment), about 30–70% of the cellulose is being aerobically converted with an accompanying biomass yield of 0.3 kg sludge/kg COD removed. Assume an annual toilet paper usage of 10 kg/PE, 70% aerobic degradation of cellulose, with a COD of 1.1 kg COD/kg cellulose, consider that 0.44 kg O_2 are needed for degradation of 1 kg of COD, a power consumption for aeration of 2.5 kg O_2/kWh for bubble aeration. Based on the above:

(a) Calculate the energy savings that can be achieved for a WWTP with a capacity of 100 000 PE by implementing microsieves as a pre-treatment step.
(b) Calculate the fraction of the incoming toilet paper that ultimately ends up in landfill (in case this is the ultimate disposal route) in case there is no primary treatment.

Exercise 8.6: A pilot-scale WWTP is designed to a fully integrated process to valorize the harvested screenings from municipal wastewater and to produce pure marketable cellulose. In this regard, the plant is equipped with an RBF for enhanced TSS separation and further cellulose recovery. Together with solids, cellulose fibers are separated with RBF and thus recovered. The RBF system works at a flowrate of 30 m^3/d. Calculate the maximum cellulose recovery yield considering that:

(1) The sludge production after 5 hours is 24 kg.
(2) The % of TS is 20%.
(3) The percentage of pure cellulose in the sludge, after a post-treatment of sludge washing (to concentrate the amount of fibers), is 35% of the cellulosic material.
(4) The specific recovery yield of the cellulosic material in terms of g VS after washing to g TS initially recovered in the sludge is 0.88 gVS/gTS.

Exercise 8.7: A given wastewater contains 50 g soluble COD/L, of which 30% are carbohydrates. Assuming that all fermentable fractions of carbohydrates are converted to acetic acid and butyric acid, to be used as substrate for PHA production, what would be their final concentrations in solution, both in g/L and in g COD/L. Assume that all carbohydrates are glucose, and the stochiometries are as given in Chapter 7.

Exercise 8.8: The PHA polymeric properties are strongly influenced by the relative PHB vs PHV fractions. Typically, acetate and butyrate are precursors for PHB production, while propionate and valerate are precursors of PHV production. Assuming that the PHB and PHV content are linearly related to the quantity of their SCCA precursors (on a mass basis) produced during acidogenic fermentation, calculate the relative fraction of PHB and PHV produced for the case of an acidogenic feedstock containing 4.1 g/L acetate, 1.6 g/L propionate, 1.1 g/L butyrate and 0.6 g/L valerate. Assume a constant yield coefficient of 0.7 gPHA/gSCCA for each SCCA, while all acetate and propionate are completely consumed by the PHA producing culture, and 85% of the butyrate and valerate are consumed.

Exercise 8.9: SCCA are an excellent feedstock for PHA bioplastic production. Considering the supply chain for PHA production, approximately how much organic waste (in COD) would be needed to make an industrially-relevant 5000 tonnes PHA per year. Assume an SCCA yield of 0.34 $\text{gCOD}_{\text{SCCA}}/\text{gCOD}_{\text{fed}}$, an overall PHA yield of 0.5 g biomass/$\text{gCOD}_{\text{SCCA}}$, a PHA content of 70% and a total extraction efficiency of 63%.

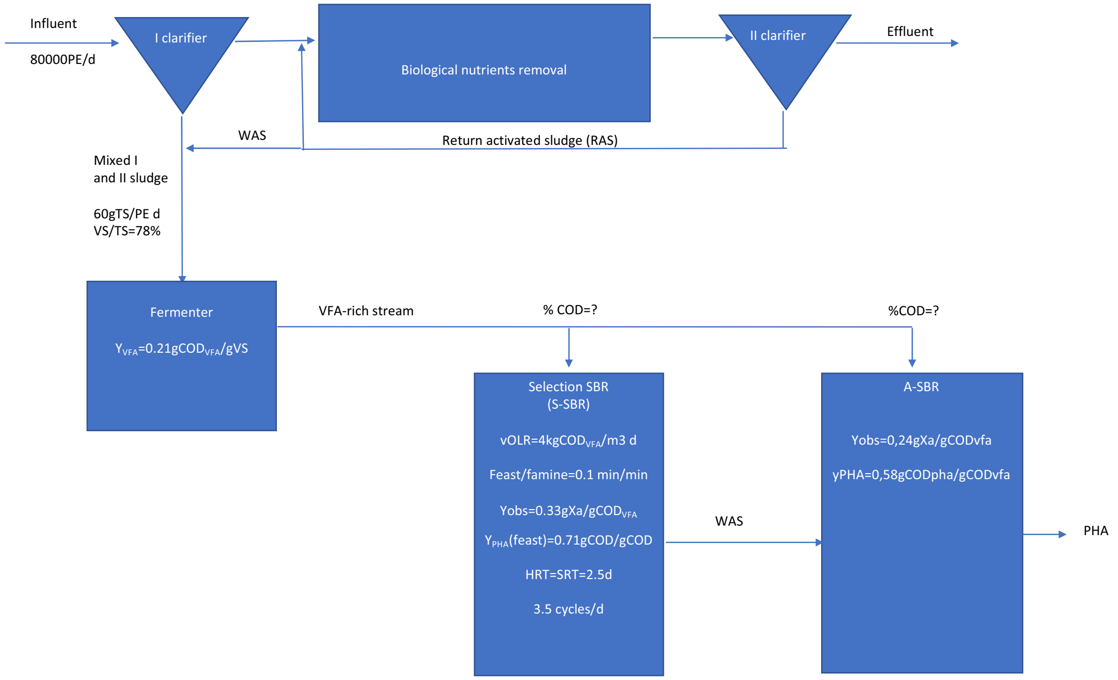
Exercise 8.10: A WWTP with a capacity of 80 000 PE produces around 60 gTS/PE d with a VS/TS ratio of 78%. All the produced sludge is fermented to produce SCCA with an observed fermentation yield of 0.21 $\text{gCOD}_{\text{SCCA}}/\text{gVS}_{\text{fed}}$. The SCCA-rich stream (7500 $\text{mgCOD}_{\text{SCCA}}/\text{L}$) is then used for PHA production with anaerobic feast/famine process. The PHA-production line is composed of two different SBRs reactors:

(a) Selection reactor (S-SBR);
(b) Accumulation reactor (A-SBR).

A vOLR of 4.0 $\text{kgCOD}_{\text{SCCA}}/\text{m}^3$ d is applied to the S-SBR, which is operated at a feast/famine ratio of 0.1 min/min. The Y_{PHA} under feast conditions is 0.71 $\text{gCOD}_{\text{PHA}}/\text{gCOD}_{\text{SCCA}}$, while the Y_{obs} is 0.33 $\text{gXa}/\text{gCOD}_{\text{PHA}}$. The SRT is set at 2.5 days and the SBR works with 3.5 cycles per day. The excess sludge produced from the S-SBR is then fed to the A-SBR for the accumulation test to maximize PHA production.

In the A-SBR, the SCCA-rich stream is added seven times (once per hour) to achieve an initial COD concentration of around 1 $\text{gCOD}_{\text{SCCA}}/\text{L}$. The volume of each addition is 0.56 m^3. The PHA yield is about 0.58 $\text{gCOD}_{\text{PHA}}/\text{gCOD}_{\text{SCCA}}$ and the growth yield is 0.24 gXa/ $\text{gCOD}_{\text{SCCA}}$.

Conversion factor for PHA: 1.67 gCOD/gPHA



Calculate:

(1) The daily SCCA amount produced from the whole WWTP;
(2) The length of both the SBR cycle and feast/famine of the S-SBR;
(3) The volume of the S-SBR to sustain a production of 10 kgPHA/d, assuming a PHA content in the biomass of 40% (gPHA/gTS);
(4) The biomass concentration of S-SBR;
(5) The flowrate of carbon source to S-SBR and A-SBR, respectively (for the production of 10 kg PHA);
(6) The volume of the A-SBR (considering seven carbon source additions);
(7) The maximum annual PHA production potential of the whole WWTP.

8.8 DISCUSSION QUESTIONS

Question 8.1 (*technology and economy*): As the innovation manager of a large water utility, you are in charge of reorganizing the existing water infrastructure from its current situation to a more circular approach within a timeframe of 25 years. The current wastewater treatment infrastructure comprises three large-scale wastewater treatment plants with capacities in the order of 500 000 PE each. In addition, it comprises more than 20 small scale WWTPs with a capacity of around 10 000 PE. You are asked to give a presentation to the board of directors in which you evaluate the current status and justify your masterplan. Where would you focus on in terms chosen technology, location (i.e. which WWTP to focus on) and which resources discussed in this chapter would you target? What are your key considerations/motivations?

Question 8.2 (*social license to operate*, *risks and market analysis*): A wastewater utility operating a WWTP with a capacity of 1 000 000 PE is ending its service life. Assume a hypothetical situation in which the WWTP will be completely rebuilt from scratch and you are the business development manager of the wastewater utility in charge of selecting the most appropriate resource to recover. You are asked to give a presentation to the board of directors in which you evaluate the economic potential of the recovery of cellulose, SCCA or energy recovery through anaerobic digestion. Based on your assessment, from an economic perspective, recovery of cellulose is the most promising followed by SCCA recovery. Nevertheless, in your presentation to the board of directors you recommended the implementation of an anaerobic digester for the production of biogas. Discuss what the key criteria and considerations the business development manager most likely considered as critical?

Question 8.3 (*market analysis*, *risks and economy-of-scale*): In developed countries, the wastewater infrastructure is aging and would require major upgrades in the coming decades. In less developed countries and emerging economies a significant amount of new infrastructure will need to be realized in the coming decades. Considering the above, there is an ongoing debate regarding how our future urban waster infrastructure will look like, that is more centralized or more modular and decentralized. Evaluate the general characteristics of centralized and decentralized systems and provide advantages and disadvantages for each of these scenarios in the context of cellulose and PHA recovery, taking into account economy of scale, practical feasibility and maintenance, monitoring and quality control and market requirements of the two resources.

Question 8.4 (*product quality*, *chemical industry and economy-of-scale*): Plastics are currently produced from oil and other fossile resources. PHA could be one biodegradable alternative to replace them. Considering that petrochemical plastic production can ensure large scale manufacturing at constant quality, discuss the potential impact of the latter in terms of market potential for PHA. In your answer, include the following aspects: (i) quality and quantity aspects; (ii) logistics (i.e. means of storage, transportation and distribution); and (iii) ultimate end-user.

Question 8.5 (*process performance*, *capital costs*): The implementation of new resource recovery schemes and technologies is (partly) dependent on their capital and operational costs and the return on investment period. What are the key operational factors that affect the CAPEX and OPEX of cellulose recovery and PHA production, respectively, and how do they affect the process economics.

Question 8.6 (*design considerations*, *process stability*, *robustness and operator confidence*): Discuss the potential advantages and/or disadvantages of cellulose recovery and PHA production from a process stability/robustness and wastewater operator confidence and overall willingness of wastewater treatment operators to introduce these recovery approaches.

Question 8.7 (*economic considerations*, *market demands*, *decision-making*): You are the Innovation Manager of a large wastewater utility that needs to prioritize the resources to be recovered from your largest wastewater treatment facilities. The resources discussed in this chapter (i.e. cellulose and PHA) cannot be recovered simultaneously. Which of the two would be your priority as an innovation manager? Justify your choice.

Question 8.8 (*social license to operate*, *reduce not recover*): According to the waste management hierarchy, prevention and reduction of the use of resource are more favorable options compered to resource recovery. Within this context, discuss options to prevent the use of toilet paper and its potential impact on municipal wastewater treatment. Also, discuss the importance of social acceptance of finding alternatives for the use of toilet paper. In your discussion, specifically mention the situation in Japan (and other Asian countries).

Question 8.9 (*process stability*, *robustness and operator confidence*): Only in recent years has the recovery of cellulose gained a lot of interest. However, the use of micro-sieves as a pre-treatment step for the removal of suspended solids from municipal wastewater were developed and implemented in Norway as a means to comply with EU regulation with respect to the removal of suspended solids during primary treatment rather than being developed with the purpose of recovering cellulose. The practical feasibility of microsieves as a viable alternative technology for pre-treatment of municipal wastewater treatment was thus already demonstrated prior to discovering its additional benefits of enabling the recovery of cellulose. Discuss the importance of the latter in the context of market uptake and replication.

Question 8.10 (*public acceptance and market uptake*): One of the biggest challenges of resource recovery is to develop new markets and applications for the recovered products. Changing the opinion and practices of stakeholders is an important component of the resource recovery concept. Producers need to rethink the composition and design of their products with respect to the targeted information as well as to the stakeholders' involvement. What is the importance and influence of stakeholders and/or policymakers on helping/hampering the development of markets for recovered organics materials?

FURTHER READING MATERIALS

Lema, J. M. and Suarez, S. (eds), Innovative Wastewater Treatment & Resource Recovery Technologies: Impacts on Energy, Economy and Environment. IWA Publishing, London, UK. https://doi.org/10.2166/9781780407876

Puyol, D., Batsone, D. J., Hülsen, T., Astals, S., Peces, M. and Krömer, J. O. (2017). Resource recovery from wastewater by biological technologies: Opportunities, challenges, and prospects. *Frontiers in Microbiology*, **7**, 1–23. https://doi.org/10.3389/fmicb.2016.02106

REFERENCES

Ahmed A. S., Bahreini G., Ho D., Sridhar G., Gupta M., Wessels C., Marcelis P., Elbeshbishy E., Rosso D., Santoro D. and Nakhla G. (2019). Fate of cellulose in primary and secondary treatment at municipal water resource recovery facilities. *Water Environment Research*, **91**, 1479–1489. https://doi.org/10.1002/wer.1145

Akyol Ç., Foglia A., Ozbayram E. G., Frison N., Katsou E., Eusebi A. L. and Fatone F. (2020). Validated innovative approaches for energy-efficient resource recovery and re-use from municipal wastewater: From anaerobic treatment systems to a biorefinery concept. *Critical Reviews in Environmental Science and Technology*, **50**, 869–902. https://doi.org/10.1080/10643389.2019.1634456

Albuquerque M. G. E., Eiroa M., Torres C., Nunes B. R. and Reis M. A. M. (2007). Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses, *Journal of Biotechnology*, **130**(4), 411–421.

Albuquerque M. G. E., Concas S., Bengtsson S. and Reis M. A. M. (2010). Mixed culture polyhydroxyalkanoates production from sugar molasses: The use of a 2-stage CSTR system for culture selection. *Bioresource Technology*, **101**, 7112–7122. https://doi.org/10.1016/j.biortech.2010.04.019

Albuquerque M. G. E., Martino V., Pollet E., Avérous L. and Reis M. A. M. (2011). Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: Effect of substrate composition and feeding regime on PHA productivity, composition and properties, *Journal of Biotechnology*, **151**(1), 66–76.

Anjum A., Zuber M., Zia K. M., Noreen A., Anjum M. N. and Tabasum S. (2016). Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: A review of recent advancements. *International Journal of Biological Macromolecules*, **89**, 161–174. https://doi.org/10.1016/j.ijbiomac.2016.04.069

Behera C. R., Santoro D., Gernaey K. V. and Sin G. (2018). Organic carbon recovery modeling for a rotating belt filter and its impact assessment on a plant-wide scale. *Chemical Engineering Journal*, **334**, 1965–1976. https://doi.org/10.1016/j.cej.2017.11.091

Bengtsson S., Werker A., Christensson M. and Welander T. (2008). Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater. *Bioresource Technology*, **99**(3), 509–516. https://doi.org/10.1016/j.biortech.2007.01.020

Bengtsson S., Karlsson A., Alexandersson T., Quadri L., Hjort M., Johansson P., Morgan-Sagastume F., Anterrieu S., Arcos-Hernandez M., Karabegovic L., Magnusson P. and Werker A. (2017). A process for polyhydroxyalkanoate (PHA) production from municipal wastewater treatment with biological carbon and nitrogen removal demonstrated at pilot-scale. *New Biotechnology*, **35**, 42–53. https://doi.org/10.1016/j.nbt.2016.11.005

Bout A. E., Pfau S. F., van der Krabben E. and Dankbaar B. (2019). Residual biomass from Dutch riverine areas-from waste to ecosystem service. *Sustainability*, **11**, 1–14. https://doi.org/10.3390/su11020509

Campanari S., e Silva F. A., Bertin L., Villano M. and Majone M. (2014). Effect of the organic loading rate on the production of polyhydroxyalkanoates in a multi-stage process aimed at the valorization of olive oil mill wastewater. *International Journal of Biological Macromolecules*, **71**, 34–41. https://doi.org/10.1016/j.ijbiomac.2014.06.006

Carvalho G., Oehmen A., Albuquerque M. G. E. and Reis M. A. M. (2014). The relationship between mixed microbial culture composition and PHA production performance from fermented molasses. *New Biotechnology*, **31**, 257–263. https://doi.org/10.1016/j.nbt.2013.08.010

Carvalho G., Pedras I., Karst S. M., Oliveira C. S. S., Duque A. F., Nielsen P. H. and Reis M. A. M. (2018). Functional redundancy ensures performance robustness in 3-stage PHA-producing mixed cultures under variable feed operation. *New Biotechnology*, **40**, 207–217. https://doi.org/10.1016/j.nbt.2017.08.007

Cavaillé L., Grousseau E., Pocquet M., Lepeuple A. S., Uribelarrea J. L., Hernandez-Raquet G. and Paul E. (2013). Polyhydroxybutyrate production by direct use of waste activated sludge in phosphorus-limited fed-batch culture. *Bioresource Technology*, **149**, 301–309. https://doi.org/10.1016/j.biortech.2013.09.044

Chen S. S., Wang L., Yu I. K. M., Tsang D. C. W., Hunt A. J., Jérôme F., Zhang S., Ok Y. S. and Poon C. S. (2018). Valorization of lignocellulosic fibres of paper waste into levulinic acid using solid and aqueous Brønsted acid. *Bioresource Technology*, **247**, 387–394. https://doi.org/10.1016/j.biortech.2017.09.110

Conca V., Ros C., Valentino F., Eusebi A. L., Frison N. and Fatone F. (2020). Long-term validation of polyhydroxyalkanoates production potential from the sidestream of municipal wastewater treatment plant at pilot scale. *Chemical Engineering Journal*, **390**, 1–13. https://doi.org/10.1016/j.cej.2020.124627

Crutchik D., Frison N., Eusebi A. L. and Fatone F. (2018). Biorefinery of cellulosic primary sludge towards targeted short chain fatty acids, phosphorus and methane recovery. *Water Research*, **136**, 112–119. https://doi.org/10.1016/j.watres.2018.02.047

Crutchik D., Franchi O., Caminos L., Jeison D., Belmonte M., Pedrouso A., Val del Rio A., Mosquera-Corral A. and Campos J. L. (2020). Polyhydroxyalkanoates (PHAs) production: A feasible economic option for the treatment of sewage sludge in municipalwastewater treatment plants? *Water (Switzerland)*, **12**, 1–12. https://doi.org/10.3390/W12041118

Da Ros C., Conca V., Laura A., Frison N. and Fatone F. (2020). Sieving of municipal wastewater and recovery of bio-based volatile fatty acids at pilot scale. *Water Research*, **174**, 1–10. https://doi.org/10.1016/j.watres.2020.115633

Dionisi D., Majone M., Tandoi V. and Beccari M. (2001). Sequencing batch reactor: Influence of periodic operation on performance of activated sludges in biological wastewater treatment. *Industrial and Engineering Chemical Research*, **40**, 5110–5119. https://doi.org/10.1021/ie001008k

Fernández-Dacosta C., Posada J. A., Kleerebezem R., Cuellar M. C. and Ramirez A. (2015). Microbial community-based polyhydroxyalkanoates (PHAs) production from wastewater: Techno-economic analysis and ex-ante environmental assessment. *Bioresource Technology*, **185**, 368–377. https://doi.org/10.1016/j.biortech.2015.03.025

Franchi A. and Santoro D. (2015). Current status of the rotating belt filtration (RBF) technology for municipal wastewater treatment. *Water Practice and Technology*, **10**, 319–327. https://doi.org/10.2166/wpt.2015.038

Frison N., Katsou E., Malamis S., Oehmen A. and Fatone F. (2015). Development of a novel process integrating the treatment of sludge reject water and the production of polyhydroxyalkanoates (PHAs). *Environmental Science and Technology*, **49**, 10877–10885. https://doi.org/10.1021/acs.est.5b01776

Gavrilescu M. (2014). Biomass potential for sustainable environment, biorefinery products and energy. In: Visa, I. (ed.). Sustainable Energy in the Built Environment – Steps Towards NZEB. Springer International Publishing, Cham, pp. 169–194.

Huang F. (2013). What is biomass? In: Materials for Biofuels, A. J. Ragauskas (ed.), World Scientific, Singapore, pp. 1–26.

Johnson K., Jiang Y., Kleerebezem R., Muyzer G. and Van Loosdrecht M. C. M. (2009). Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity. *Biomacromolecules*, **10**, 670–676. https://doi.org/10.1021/bm8013796

Johnson K., Kleerebezem R., and Loosdrecht M. C. M. (2010). Influence of the C/N ratio on the performance of polyhydroxybutyrate (PHB) producing sequencing batch reactors at short SRTs. *Water Research*, **44**(7), 2141–2152.

Kehrein P., van Loosdrecht M., Osseweijer P., Garfí M., Dewulf J. and Posada J. (2020). A critical review of resource recovery from municipal wastewater treatment plants – market supply potentials, technologies and bottlenecks. *Environmental Science: Water Research and Technology*, **6**, 877–910. https://doi.org/10.1039/c9ew00905a

Keijsers E. R. P., Yilmaz G. and Van Dam J. E. G. (2013). The cellulose resource matrix. *Carbohydrate Polymersm*, **93**, 9–21. https://doi.org/10.1016/j.carbpol.2012.08.110

Khatami K., Perez-Zabaleta M., Owusu-Agyeman I. and Cetecioglu Z. (2021). Waste to bioplastics: How close are we to sustainable polyhydroxyalkanoates production? *Waste Management*, **119**, 374–388. https://doi.org/10.1016/j.wasman.2020.10.008

Kim M., Lee E.-K., Choi C.-J., Lee M.-H., Kim J.-M., Ji S.-W., Jeon B.-J., Park G.-P. and Song K.-B. (2017). Brick insulation composite and method for manufacturing same. US Pat. 2017/0191264 A1. https://doi.org/10.1016/j.(73)

Kourmentza C., Plácido J., Venetsaneas N., Burniol-Figols A., Varrone C., Gavala H. N. and Reis M. A. M. (2017). Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. *Bioengineering* **4**, 1–43. https://doi.org/10.3390/bioengineering4020055

Kumar M., Morya R., Gnansounou E., Larroche C. and Thakur I. S. (2017). Characterization of carbon dioxide concentrating chemolithotrophic bacterium Serratia sp. ISTD04 for production of biodiesel. *Bioresource Technology*, **243**, 893–897. https://doi.org/10.1016/j.biortech.2017.07.067

Kumar M., Ghosh P., Khosla K. and Thakur I. S. (2018). Recovery of polyhydroxyalkanoates from municipal secondary wastewater sludge. *Bioresource Technology*, **255**, 111–115. https://doi.org/10.1016/j.biortech.2018.01.031

Li S., Wu Z., Zixin Wu. and Liu G. (2020). Enhancing fiber recovery from wastewater may require toilet paper redesign. *Journal of Cleaner Production*, **261**, 121138. https://doi.org/10.1016/j.jclepro.2020.121138

Mannina G., Presti D., Montiel-Jarillo G., Carrera J. and Suárez-Ojeda M. E. (2020). Recovery of polyhydroxyalkanoates (PHAs) from wastewater: A review. *Bioresource Technology*, **297**, 1–12. https://doi.org/10.1016/j.biortech.2019.122478

Matos M., Cruz R. A. P., Cardoso P., Silva F., Freitas E. B., Carvalho G. and Reis M.A.M. (2021). Combined strategies to boost polyhydroxyalkanoate production from fruit waste in a three-stage pilot plant. *ACS Sustainable Chemical Engineering* 9(24), 8270–8279. https://doi.org/10.1021/acssuschemeng.1c02432

Morgan-Sagastume F., Valentino F., Hjort M., Cirne D., Karabegovic L., Gerardin F., Johansson P., Karlsson A., Magnusson P., Alexandersson T., Bengtsson S., Majone M. and Werker A. (2014). Polyhydroxyalkanoate (PHA) production from sludge and municipal wastewater treatment. *Water Science and Technology*, **69**, 177–184. https://doi.org/10.2166/wst.2013.643

Morgan-Sagastume F., Heimersson S., Laera G., Werker A. and Svanström M. (2016). Techno-environmental assessment of integrating polyhydroxyalkanoate (PHA) production with services of municipal wastewater treatment. *Journal of Cleaner Production*, **137**, 1368–1381. https://doi.org/10.1016/j.jclepro.2016.08.008

Możejko-Ciesielska J. and Kiewisz R. (2016). Bacterial polyhydroxyalkanoates: Still fabulous? *Microbiological Research*, **192**, 271–282. https://doi.org/10.1016/j.micres.2016.07.010

Nechyporchuk O., Belgacem M. N. and Bras J. (2016). Production of cellulose nanofibrils: A review of recent advances. *Industrial Crops and Products*, **93**, 2–25. https://doi.org/10.1016/j.indcrop.2016.02.016

Oehmen A., Pinto F. V., Silva V., Albuquerque M. G. E. and Reis M. A. M. (2014). The impact of pH control on the volumetric productivity of mixed culture PHA production from fermented molasses. *Engineering in Life Sciences*, **14**, 143–152. https://doi.org/10.1002/elsc.201200220

Oliveira C. S. S., Silva C. E., Carvalho G. and Reis M. A. (2017). Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: Feast and famine regime and uncoupled carbon and nitrogen availabilities. *New Biotechnology*, **37**, 69–79. https://doi.org/10.1016/j.nbt.2016.10.008

Pagliano G., Galletti P., Samorì C., Zaghini A. and Torri C. (2021). Recovery of polyhydroxyalkanoates from single and mixed microbial cultures: A review. *Frontiers in Bioengineering and Biotechnology*, **9**, 1–28. https://doi.org/10.3389/fbioe.2021.624021

Pakalapati H., Chang C. K., Show P. L., Arumugasamy S. K. and Lan J. C. W. (2018). Development of polyhydroxyalkanoates production from waste feedstocks and applications. *Journal of Bioscience and Bioengineering*, **126**, 282–292. https://doi.org/10.1016/j.jbiosc.2018.03.016

Palmieri S., Cipolletta G., Pastore C., Giosuè C., Akyol Ç., Eusebi A. L., Frison N., Tittarelli F. and Fatone F. (2019). Pilot scale cellulose recovery from sewage sludge and reuse in building and construction material. *Waste Management*, **100**, 208–218. https://doi.org/10.1016/j.wasman.2019.09.015

Raza Z. A., Abid S. and Banat I. M. (2018). Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. *International Biodeterioration and Biodegradation*, **126**, 45–56. https://doi.org/10.1016/j.ibiod.2017.10.001

Reddy K. H. K., Patra M. R., Roy D. S. and Pradhan B. (2012). An adaptive scheduling mechanism for computational desktop grid using GridGain. *Procedia Technology*, **4**, 573–578. https://doi.org/10.1016/j.protcy.2012.05.091

Rodriguez-Perez S., Serrano A., Pantión A. A. and Alonso-Fariñas B. (2018). Challenges of scaling-up PHA production from waste streams. A review. *Journal of Environmental Management*, **205**, 215–230. https://doi.org/10.1016/j.jenvman.2017.09.083

Ruiken C. J., Breuer G., Klaversma E., Santiago T. and van Loosdrecht M. C. M. (2013). Sieving wastewater – cellulose recovery, economic and energy evaluation. *Water Research*, **47**, 43–48. https://doi.org/10.1016/j.watres.2012.08.023

Rusten B. and Odegaard H. (2006). Evaluation and testing of fine mesh sieve technologies for primary treatment of municipal wastewater. *Water Science and Technology*, **54**, 31–38.

Rusten B., Razafimanantsoa V. A., Andriamiarinjaka M. A., Otis C. L., Sahu A. K. and Bilstad T. (2016). Impact of fine mesh sieve primary treatment on nitrogen removal in moving bed biofilm reactors. *Water Science and Technology*, **73**, 337–344. https://doi.org/10.2166/wst.2015.498

Rusten B., Rathnaweera S. S., Rismyhr E., Sahu A. K. and Ntiako J. (2017). Rotating belt sieves for primary treatment, chemically enhanced primary treatment and secondary solids separation. *Water Science and Technology*, **75**, 2598–2606. https://doi.org/10.2166/wst.2017.145

Samorì C., Abbondanzi F., Galletti P., Giorgini L., Mazzocchetti L., Torri C. and Tagliavini E. (2015). Extraction of polyhydroxyalkanoates from mixed microbial cultures: Impact on polymer quality and recovery. *Bioresource Technology*, **189**, 195–202. https://doi.org/10.1016/j.biortech.2015.03.062

SMART-Plant (2020a). D4.5 Socio-economic Assessment Including Life Cycle Costing (LCC) and Cost Benefit Analysis (CBA) Reports. Available at: https://ec.europa.eu/research/participants/documents/downloadPublic?documentIds=080166e5cf44b308&appId=PPGMS (accessed 26 January 2022).

SMART-Plant (2020b). D4.4 Environmental Impact Report, incl. LCA (Life Cycle Assessment). Available at: https://publications.kompetenz-wasser.de/pdf/Remy-2020-1273.pdf (accessed 26 January 2022).

STOWA (2017). PHARIO – Stepping Stone to A Sustainable Value Chain for PHA Bioplastic Using Municipal Activated Sludge. STOWA, Amersfoot, The Netherlands.

Valentino F., Morgan-Sagastume F., Campanari S., Villano M., Werker A. and Majone M. (2017). Carbon recovery from wastewater through bioconversion into biodegradable polymers. *New Biotechnology*, **37**, 9–23. https://doi.org/10.1016/j.nbt.2016.05.007

van der Hoek J. P., Struker A. and de Danschutter J. E. M. (2015). Amsterdam as a sustainable European metropolis: Integration of water, energy and material flows. *Urban Water Journal*, **14**, 61–68. https://doi.org/10.1080/1573062X.2015.1076858

Wang X., Bengtsson S., Oehmen A., Carvalho G., Werker A. and Reis M. A. M. (2019). Application of dissolved oxygen (DO) level control for polyhydroxyalkanoate (PHA) accumulation with concurrent nitrification in surplus municipal activated sludge. *New Biotechnology*, **50**, 37–43. https://doi.org/10.1016/j.nbt.2019.01.003

Werker A., Johansson P. and Magnusson P. (2015). Process for the Extraction of Polyhydroxyalkanoates From Biomass. VEOLIA Water Solutions & Technologies, Saint-Maurice, France, US20150368393A1.

Werker A., Bengtsson S., Korving L., Hjort M., Anterrieu S., Alexandersson T., Johansson P., Karlsson A., Karabegovic L., Magnusson P., Morgan-Sagastume F., Sijstermans L., Tietema M., Visser C., Wypkema E., Van Der Kooij Y., Deeke A. and Uijterlinde C. (2018). Consistent production of high quality PHA using activated sludge harvested from full scale municipal wastewater treatment – PHARIO. *Water Science and Technology*, **78**, 2256–2269. https://doi.org/10.2166/wst.2018.502