Nonoxidative removal of organics in the activated sludge process

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\textbf{ABSTRACT}

The activated sludge process is commonly used to treat wastewater by aerobic oxidation of organic pollutants into carbon dioxide and water. However, several nonoxidative mechanisms can also contribute to removal of organics. Sorption onto activated sludge can remove a large fraction of the colloidal and particulate wastewater organics. Intracellular storage of, e.g., polyhydroxyalkanoates (PHA), triacylglycerides (TAG), or wax esters can convert wastewater organics into precursors for high-value products. Recently, several environmental, economic, and technological drivers have stimulated research on nonoxidative removal of organics for wastewater treatment. In this paper, we review these nonoxidative removal mechanisms as well as the existing and emerging process configurations that make use of them for wastewater treatment. Better utilization of nonoxidative processes in activated sludge could reduce the wasteful aerobic oxidation of organic compounds and lead to more resource-efficient wastewater treatment plants.

\textbf{KEYWORDS}

Adsorption; colloids; contact-stabilization; DLVO theory; high-rate activated sludge; polyhydroxyalkanoate; triacylglyceride

1. Introduction

The activated sludge process is common in wastewater treatment plants. It was developed over 100 years ago (Arden and Lockett, 1914) and is primarily used for removal of biodegradable organic compounds, which could otherwise cause oxygen depletion of receiving waters if discharged in the treatment plant effluent. Certain process configurations also allow simultaneous biological removal of nitrogen and phosphorous from the wastewater (Tchobanoglous et al., 2004). A typical process configuration for an activated sludge plant is depicted in Figure 1. The activated sludge comes in contact with the presettled wastewater in an aerated tank.
Live microorganisms present in the sludge oxidize the organics into carbon dioxide and water. The oxidation is coupled to microbial growth by which the microorganisms assimilate a fraction of the organics. The activated sludge is separated from the treated water in a settling tank. Alternatively, a membrane bioreactor (MBR) can be used to separate the treated effluent using membrane filtration (Yamamoto et al., 1989). A portion of the sludge corresponding to the net amount of microbial growth in the system is wasted while the rest is returned to the inlet of the aerated tank. The wasted sludge is often converted into biogas in an anaerobic digester.

The separation and recirculation of sludge from the treated water makes it possible to decouple hydraulic and solids retention times (HRT and SRT). Traditionally, long SRT has been considered beneficial as it allows retention of slow-growing microorganisms such as nitrifiers and results in lower sludge production because of endogenous decay. Long SRT can also improve the removal of emerging contaminants such as pharmaceuticals and endocrine disruptors, increase the oxygen transfer efficiency, and reduce the number of particles in the effluent (Leu et al., 2012). During operation at long SRT, most of the organic compounds entering the process with the wastewater will be oxidized to carbon dioxide and water. This is a drawback since the energy content of the organic carbon is lost rather than recovered, and a lot of electrical energy is needed to aerate the activated sludge tanks. The energy content of wastewater organics has been estimated to around 14.7–28.7 kJ per g chemical oxygen demand (COD) for different types of wastewater (Heidrich et al., 2011; Shizas and Bagley, 2004). This means that a municipal wastewater with a COD concentration of 400 mg/L could contain an energy value of 1.6–3.2 kWh/m³. In a conventional treatment plant very little of this energy is recovered, instead about 0.6 kWh/m³ is consumed to treat the wastewater (McCarty et al., 2011).

Recently, there has been a rising interest in new activated sludge processes based on nonoxidative mechanisms for removal and valorization of the organic content in wastewater. This means that the organic compounds are not mineralized (i.e., they are not oxidized to carbon dioxide and water). Instead, they are removed from the wastewater in reduced form. There are both environmental and economic drivers for the utilization of nonoxidative removal mechanisms for organics. Today we know that climate change is a reality (IPCC 2013) and wastewater treatment...
plants, like other parts of society, aim to reduce greenhouse gas emissions (Gustavsson and Tumlin, 2013). Reduction of energy consumption by lower aeration requirements and generation of biogas or other chemical products that could replace fossil fuel-based alternatives have clear environmental benefits. Treatment plants could also reduce costs by minimizing energy consumption and potentially even generate profits by producing high-value products. Rising price of petrol makes biodiesel generated from, e.g., wastewater sludge more competitive (Kargbo, 2010). By having a high-rate activated sludge configuration with short SRT and HRT, sorption of organic carbon onto the activated sludge flocs enable nonoxidative removal. Examples of established processes utilizing sorption are the contact-stabilization and adsorption–biooxidation (AB) processes. High-rate activated sludge configurations that minimize aeration requirements, maximize organics removal through sorption, and allow valorization of the produced sludge through methane production have also been investigated in several recent studies (e.g., Ge et al., 2013; Faust et al., 2014b) and have been identified as a possible way toward more resource-efficient wastewater treatment systems (Verstraete et al., 2009; Batstone and Virdis, 2014). Microbial conversion of soluble organic compounds into intracellular polymeric inclusions such as polyhydroxyalkanoates (PHA) (Chua and Yu, 1999) and triacylglycerides (TAG) (Revellame et al., 2013) is another type of nonoxidative removal mechanism. Production of PHAs has received a lot of attention because they could potentially replace conventional plastics and represent a high-value product that could be generated from wastewater (Chua and Yu, 1999; Salehizadeh and van Loosdrecht, 2004). Inclusions of PHAs, TAGs, and wax esters could serve as precursors for biodiesel production (Zhang et al., 2009; Revellame et al., 2012).

There are also technological drivers for using nonoxidative mechanisms in activated sludge. MBRs have recently developed into a mature technology with full-scale installations all over the world (Hai et al., 2014). Previously, applications of high-rate, sorption-based activated sludge processes were hampered by poor settling properties of the produced sludge. However, with membrane separation, high effluent quality can be produced irrespective of the settleability of the sludge. The development of anammox-based processes for nitrogen removal is another technological driver because it eliminates the need of organic carbon oxidation for nitrogen removal through denitrification. Anammox processes are currently used in full scale for sludge liquor treatment (Lackner et al., 2014) and are being developed for main-stream applications (De Clippeleir et al., 2013; Lotti et al., 2014).

The aim of this paper is to review nonoxidative removal mechanisms and the process configurations that utilize these to treat wastewater. The paper starts with a description of organic compounds in wastewater and activated sludge flocs. This is followed by an in-depth review of nonoxidative removal mechanisms and the activated sludge process configurations that are based on these mechanisms. The paper ends with an outlook highlighting some of the research needs in the field. Special emphasis is placed on sorption of organic compounds as this is an
important process in many existing and emerging activated sludge systems and, as far as we know, a review in this area is missing.

2. Characteristics of wastewater organics and activated sludge

2.1 Composition of organic compounds in wastewater

The composition of organic compounds in wastewater is complex. Usually the organic content is quantified using bulk parameters such as COD, biochemical oxygen demand (BOD), total organic carbon (TOC), suspended solids, and turbidity. More detailed characterizations of wastewater organics have been made based on biodegradability, size, and chemical composition of the constituents.

In mathematical modeling of activated sludge systems, the organic compounds are classified based on biodegradability (readily biodegradable, slowly biodegradable, or inert) and physical state (soluble or particulate) (Henze et al., 2000). The biodegradability can be measured using, e.g., oxygen uptake rate tests (Vanrolleghem et al., 1999). The physical state relates to the tendency of compounds to be sorbed to activated sludge flocs. Soluble compounds are assumed to remain in the liquid phase unless they are transformed by microorganisms in the sludge whereas particulate compounds are assumed to be sorbed to the activated sludge (Henze et al., 2000).

Some researchers have done more detailed investigations of size fractions and chemical composition of wastewater organics. Different methods have been used for size fractionation. Early studies often used sedimentation and centrifugation (e.g., Rickert and Hunter, 1971) and the results from several studies are tabulated by Levine et al. (1991) and Sophonsiri and Morgenroth (2004). More recent studies have typically used micro- and ultrafiltration membranes with defined pore sizes (e.g., Karahan et al., 2008). The results from 10 such studies are compiled in Figure 2. In municipal wastewater (Figure 2A), the size fraction <0.001 μm appears to account for around 15–40% of the total COD whereas around 40–60% is associated with the size fraction >1 μm. Industrial wastewaters have highly variable size fractionation patterns (Figure 2B). For example, in textile wastewater about 70% of the COD is <0.001 μm, whereas in swine manure nearly 80% of the COD is associated with the fraction >10 μm.

Carbohydrates, proteins, and lipids are major constituents of the organic compounds in municipal wastewater. Other compounds that have been measured in some studies include humic acids, surfactants, amino acids, tannic acids, volatile fatty acids (VFAs), DNA, RNA, lignin, and fibers. The results from 11 studies on different municipal and industrial wastewaters are shown in Table 1. In municipal wastewater, all chemical groups appear to be present whereas industrial wastewaters may be dominated by specific compounds. For example, wastewater from a starch factory is dominated by carbohydrates while slaughterhouse wastewater has high content of proteins and lipids (Gorini et al., 2011). A few studies have investigated the chemical composition of
Sophonsiri and Morgenroth (2004) found that carbohydrates were more associated with larger particles (>63 μm) than smaller particles (1.2–63 μm). Data from Raunkjaer et al. (1994) also indicate that particles >0.45 μm had slightly higher carbohydrate content than substances <0.45 μm. Huang et al. (2010) observed that for the size fraction smaller than 0.1 μm, 62% of the TOC could be associated with proteins or carbohydrates. However, for the fraction below 0.001 μm, the protein and carbohydrate part of the TOC was only 15%.

Both the particle size distribution and chemical composition of wastewater can vary significantly between different sampling occasions and due to geographical differences.

**Table 1. Chemical composition of different wastewaters.**

| Type of wastewater | COD conc. (mg/L) | Carbohydrates (%) | Proteins (%) | Lipids (%) | Others* (%) | Unidentified (%) | References |
|--------------------|------------------|-------------------|--------------|------------|-------------|------------------|------------|
| Municipal          | 462 ± 174        | 16 ± 5            | 24 ± 3       | 15 ± 5     | NM          | 47 ± 9           | (Raunkjaer et al., 1994) |
| Municipal          | 249              | 36                | 16           | 1          | 35          | 12               | (Huang et al., 2010) |
| Municipal          | 520 ± 230        | 15 ± 2            | 18 ± 6       | 38 ± 7     | 3           | 26 ± 10          | (Gorini et al., 2011) |
| Municipal          | 967 ± 49         | 17                | 20           | NM         | 8           | 54               | (Dignac et al., 2000) |
| Municipal          | 309              | 6                 | 12           | 82         | NM          | 0               | (Sophonsiri and Morgenroth, 2004) |
| Municipal          | 259 ± 13         | 6 ± 1             | 11 ± 4       | 19 ± 3     | 13          | 52 ± 6           | (Tanaka et al., 1991) |
| Slaughterhouse     | 4600             | 12                | 29           | 28         | NM          | 31               | (Gorini et al., 2011) |
| Cheese dairy       | 1060             | 5                 | 17           | 26         | 4           | 48               | (Gorini et al., 2011) |
| Starch factory     | 3720             | 78                | 4            | 0          | 2           | 16               | (Gorini et al., 2011) |
| Dairy              | 7249             | 11                | 6            | 31         | NM          | 52               | (Sophonsiri and Morgenroth, 2004) |
| Swine manure       | 67,444           | 8                 | 9            | 0          | NM          | 83               | (Sophonsiri and Morgenroth, 2004) |

NM: Not measured.

*Include some or all of the following substances: humic acids, surfactants, amino acids, tannic acids, volatile fatty acids, DNA and RNA, lignin and fibers.
conditions. The sewer network and wastewater flow rate affect transformation of organic compounds in the sewers. For example, in flat landscapes where the sewers are full most of the time because of wastewater pumping, particulate organic compounds may be hydrolyzed into organic acids under anaerobic conditions. In hilly landscapes, the wastewater may be aerated because of rapid gravity flow through the sewers and soluble compounds could be oxidized (Odegaard, 1998). Gravity sewers are typically designed for a flow velocity of 1 m/s. The oxygen concentration in Danish gravity sewers was determined to be 1–4 mg/L, which allows aerobic microbial activity to take place. Pressure mains, which are full-flowing sewers where the wastewater is pumped intermittently, typically have lower mean flow velocities and longer residence time than gravity sewers, and anaerobic conditions occur (Nielsen et al., 1992).

Narkis et al. (1980) found a higher VFA content in wastewater from a sewer system with long residence time (6–12 hr) compared to one with short (10–60 min). The concentration of total VFAs was 89–104 mg/L in the wastewater with long residence time in the sewer, and 22–48 mg/L in the wastewater with short residence time. Acetic acid made up 72–95% of the VFA content, propionic acid 5–25%, butyric acid 0–9%, and isovaleric acid 0–5%. There was no clear difference in VFA composition between the wastewaters with long and short residence time. In an experiment exposing municipal wastewater to anaerobic or aerobic conditions for a period of 20 hr, no significant changes in the protein pool were observed under anaerobic conditions whereas under aerobic conditions the soluble protein content decreased and the particulate content increased because of microbial growth (Nielsen et al., 1992).

The terms soluble or dissolved, colloidal, and suspended or particulate compounds are often used to refer to the different fractions of organics in wastewater. There is no clear definition of what each term mean. For example, many researchers refer to organic compounds passing through a 0.45-μm pore size filter as being soluble or dissolved whereas compounds retained by the filter are called particulate (e.g., Jorand et al., 1995; Modin et al., 2015). Another method for defining soluble compounds was developed by Mamais et al. (1993). They added ZnSO₄ and NaOH to flocculate colloids and particles in the wastewater and then filtered the sample through a 0.45-μm pore size filter. They showed that the COD concentrations in the filtrate of different wastewaters correlated with the concentrations of readily biodegradable COD measured using respirometry (Mamais et al., 1993). In this review, we define soluble organics as compounds that can pass through microbial cell membranes. This requires a size of less than about 0.001 μm or a molecular weight of less than about 600–5000 Da (Hu et al., 2002). COD concentrations measured using the method developed by Mamais et al. (1993) was shown to correspond closely to COD concentration < 1000 Da defined as soluble by Hu et al. (2002). The distinction between colloidal and suspended compounds also varies between different studies. Elimelech et al. (1995) states that 1 μm is a generally accepted cutoff.
2.2 Composition of activated sludge flocs

Activated sludge flocs are amorphous structures consisting of microorganisms embedded in a matrix of extracellular polymeric substances (EPS) (Li and Ganczaczyk, 1990; Ni and Yu, 2012). These macromolecules bind bacteria together with rather weak forces at the outer part of the sludge flocs whereas the interior is made up of a more rigid backbone of fibers, filamentous bacteria and bacterial colonies. The EPS is produced by the microorganisms or sorbed from the wastewater and can contain proteins, carbohydrates, humic acids, uronic acids, and DNA (Frolund et al., 1996). EPS carry a net negative charge at neutral pH due to acidic functional groups (Sheng and Yu, 2006). Divalent cations such as Ca$^{2+}$ and Mg$^{2+}$ as well as metal ions bind to the EPS together forming bridges onto which cells and particles can adhere (Bruus et al., 1992). The size of activated sludge flocs can range from less than 10 $\mu$m up to several hundred $\mu$m (Andreadakis, 1993; Barbusinski and Koscielniak, 1995; Wilén and Balmér, 1999). Andreadakis (1993) estimated floc densities to 1.015–1.034 g/cm$^3$ and specific surface areas to 100–200 m$^2$/g dry sludge. Microbial cells may only make up 10–15% of the organic content of activated sludge, and the rest can be EPS (Frolund et al., 1996). The composition of the EPS affects the surface properties of the activated sludge flocs, e.g., surface charge, hydrophobicity, and floculating activity (Wilén et al., 2003).

3. Nonoxidative mechanisms for organic carbon removal

Activated sludge can contribute to nonoxidative removal of organic compounds from wastewater in several ways. The major mechanisms are assimilation, storage, and sorption.

3.1 Assimilation

Assimilation is a mechanism that removes part of the soluble, biodegradable fraction of the wastewater organics. Growth-associated assimilation of organic carbon is coupled to the energy-generating oxidation of an electron donor and reduction of an electron acceptor. Typically, organic compounds present in wastewater serve as both carbon source and electron donor for the microorganisms in the activated sludge. This means that while a fraction of the organic carbon is assimilated, a fraction is always also oxidized to carbon dioxide and water. The ratio between the microbial growth rate and the oxidation rate of the electron donor is called yield. Thermodynamic analysis of the energy required to produce biomass from available carbon and nitrogen sources and the energy that can be liberated based on available electron donor and electron acceptor can be used to predict biomass yield (Heijnen et al., 1992; von Stockar et al., 2006). Aerobic heterotrophs growing on organic compounds present in municipal wastewater typically have a yield coefficient of about 0.42 g volatile suspended solids (VSS) per g COD (Rittmann and McCarty, 2001).
3.2 Storage

Many microorganisms can store soluble, biodegradable organic compounds as insoluble polymers which form intracellular inclusions. By converting soluble organic compounds into insoluble polymers, microorganisms avoid the buildup of high osmotic pressure across the cell membrane and can store large amounts of carbon for later use (Majone et al., 1999). Examples of such storage polymers are glycogen (Preiss and Romeo, 1989), PHA (Madison and Huisman, 1999), TAG (Alvarez et al., 1996), wax esters (Fixter and Fewson, 1974), and cyanophycin (Obst and Steinbüchel, 2006) (Figure S1, Supplemental Material). Storage of glycogen, PHA, TAG, and wax esters has been shown to occur in activated sludge systems. For example, both PHA and glycogen play important roles in the process for enhanced biological phosphorous removal (EBPR) (Oehmen et al., 2007). Dynamic process configurations, such as the sequencing batch reactor (SBR) in which the activated sludge is exposed to periods of feast and famine, induce storage behavior. Microorganisms capable of rapidly storing large amount of organics have a competitive advantage during the famine phase (van Loosdrecht et al., 1997; Ni et al., 2015). VFAs such as acetate will result in storage of PHAs whereas carbohydrates such as glucose will result in storage of glycogen (Carta et al., 2001). Storage of TAG and wax esters appears to be accomplished mainly by members of the actinomycetes group and by Acinetobacter sp. (Alvarez and Steinbüchel, 2002; Waltermann and Steinbüchel, 2005). Sludge can be enriched in TAG by feeding with, e.g., glucose or xylose at high carbon-to-nitrogen ratio (Mondala et al., 2013; Revellame et al., 2013).

3.3 Sorption

Different terms such as biosorption, bioflocculation, enmeshment, entanglement, coagulation, and agglomeration have been used to describe the physical-chemical adhesion between activated sludge flocs and organic compounds. In this paper, we will use the term sorption. Sorption is a mechanism that theoretically could affect all of the organic compounds in wastewater, both biodegradable and inert fractions. The commonly used mathematical models developed by the International Water Association (IWA) for activated sludge processes assume that slowly biodegradable organic matters such as particles and colloids are instantaneously sorbed to the activated sludge flocs (Henze et al., 2000). However, research has shown that not all particulate organics are sorbed (Alexander et al., 1980) and the process could be described using both instantaneous sorption and first-order kinetics (Jorand et al., 1995; Modin et al., 2015). Several studies have shown that sorption primarily affects the colloidal and particulate fraction of the wastewater organics (i.e., >0.001 μm) (e.g., Bunch and Griffin Jr., 1987; Jimenez et al., 2005).

For sorption to take place, organic compounds and activated sludge flocs must first collide and then adhere to each other. The collision rate depends on Brownian motion, shear rate, and differential settling (Gregory, 1989; Elimelech et al., 1995).
In activated sludge tanks, the shear rate is the most important factor (Section S1 and Figure S2, Supplemental Material). Increased shear rate leads to higher collision rate and Jimenez et al. (2005) did indeed observe increasing sorption rate coefficients with increasing shear rate up to 30 s\(^{-1}\). At higher shear rates the sorption rate did not increase, possibly because of particle erosion from the activated sludge floc surfaces (Jimenez et al., 2005). Increased erosion at higher shear rates leads to smaller size of the activated sludge flocs (Biggs and Lant, 2000). This could also mean higher sorption capacity because of larger exposed surface area. There is equilibrium between adhesion and erosion of primary particles to aggregates such as activated sludge flocs when exposed to shear forces (Mikkelsen and Keiding, 1999).

The collision between an organic compound and an activated sludge floc must result in adhesion for sorption to occur. Because of the importance of secondary sedimentation for the effluent quality at wastewater treatment plants, many studies have investigated mechanisms affecting the aggregation and stability of activated sludge flocs. All interparticle forces governing interactions in colloidal system are also assumed to be of importance in activated sludge (Wilén et al., 2000). These include van der Waals and electrostatic interactions, hydration, hydrophobic and steric interactions, as well as polymer bridging (Gregory, 1989).

The well-known Derjaguin–Landau–Verwey–Overbeek (DLVO) theory takes van der Waals and electrostatic interactions into account. The van der Waals interactions are attractive and can be explained by attraction between electric dipoles in molecules on the two surfaces. The dipoles may be permanent or instantaneously induced because of a moment of uneven charge distribution in one of the molecules (Nir, 1976). The electrostatic interactions are generally repulsive and depend on the particles’ surface charge. Bacteria and particles in water usually have a negatively charged surface that attracts a layer of oppositely charged ions (Hunter and Liss, 1982; Neu and Marshall, 1990). This is called the electrical double layer. The thickness of the electrical double layer is reduced at higher ionic strengths, which means that van der Waals interactions may dominate and adhesion can take place even though both surfaces have negative potentials (Hermansson, 1999). The DLVO theory has been shown to explain microbial adhesion to a certain extent (van Loosdrecht et al., 1990; Hermansson, 1999). For example, Zita and Hermansson (1994) observed that ionic strength positively affected the stability of activated sludge flocs up to a concentration of about 0.1 M after which the stability decreased. Similarly, Esparza-Soto and Westerhoff (2003) showed that ionic strength was correlated with the capacity of activated sludge to sorb humic and fulvic acids. This suggests that electrostatic double-layer interactions are important for the structure and sorption capacity of activated sludge flocs.

Hydration means that layers of water molecules orient themselves around a charged particle surface. As two particles approach each other, the pressure in the overlapping water layers increases and results in repulsion (Grasso et al., 2002). Hydration forces have been shown to affect the stability of microbial products in water (Wang et al., 2013).
Hydrophobic interactions are attractive and occur when particles offer little opportunity for hydrogen-bonding with water. Humic acids, with higher degree of hydrophobicity, were shown to be more sorbed to activated sludge than fulvic acid, with lesser degree of hydrophobicity (Esparza-Soto and Westerhoff, 2003). The hydrophobicity of the bacteria themselves also affects adsorption of humic acids (Wang et al., 2012) as well as the flocculating ability of activated sludge (Wilén et al., 2003). Hydrophobic cell surfaces increased bacterial sorption to activated sludge flocs when tested in laboratory systems (Zita and Hermansson, 1997b; Olofsson et al., 1998). In situ measurements also showed that the cell surface characteristics of nonattached bacteria in wastewater effluent were hydrophilic (Zita and Hermansson, 1997a).

Steric interactions refer to the spatial effect of polymers attached to the surface of particles. This is highly relevant in activated sludge systems as EPS is a major component of activated sludge. Steric interactions can either be repulsive or attractive (Rijnaarts et al., 1995). Polymer bridging means that polymers, such as EPS, attach to several different particles causing aggregation. Divalent and trivalent cations are especially important to enhance bridging in activated sludge flocs by binding to negatively charged groups on the EPS matrix. Additions of Ca$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, and Al$^{3+}$ have been shown to enhance floc structure (Sobeck and Higgins, 2002; Li et al., 2012), while addition of too high concentrations of monovalent ions, e.g., Na$^+$, can deteriorate floc structure by replacing divergent cations in the EPS (Higgins and Novak, 1997). It has been hypothesized that the anionic polysaccharide alginate is especially important for floc structure (Bruus et al., 1992). Alginate has high affinity to Ca$^{2+}$, which promotes cross-linking of the molecules (Lattner et al., 2003). Addition of di- and trivalent cations to activated sludge can also enhance adhesion by reducing electrostatic repulsions (Zita and Hermansson, 1994). Multivalent cations reduce the electrical double layer to a greater extent than monovalent cations (Gregory, 1989).

4. Extent of storage in activated sludge

4.1 Polyhydroxyalkanoates

The ability of bacteria to produce PHAs was discovered already in the 1920s. Partially driven by the oil crisis in the 1970s, a commercial production process was developed and introduced in 1982. Most of the research on PHA production and the development of industrial processes has been carried out with isolated or engineered bacterial strains and pure substrates (Madison and Huisman, 1999; Lenz and Marchessault, 2005). However, storage of PHA has also been shown to take place in activated sludge systems. In the 1970s, researchers reported PHA concentrations of 0–2% (dry weight) in activated sludge samples (Deinema, 1972; Wallen and Rohwedder, 1974). Takabatake et al. (2002) collected activated sludge from four different treatment plants and investigated their ability to store PHA when fed with acetate. The PHA content after 24-hr acetate feed was 6–29.5%
(Takabatake et al., 2002). During the past 10–15 years, there has been an increasing interest in producing PHAs from wastewater organics. Activated sludge with high capacity to store PHA can be obtained by enrichment under feast-famine condition (Majone et al., 1996; van Loosdrecht et al., 1997). Serafim et al. (2004) operated an SBR fed with an acetate-containing medium under feast-famine conditions and obtained a culture that contained up to 78.5% PHA, while PHA storage capacity of enriched mixed cultures in SBRs receiving real wastewater (Morgan-Sagastume et al., 2014) or fermented waste activated sludge liquor (Coats et al., 2007; Mengmeng et al., 2009) has been a bit lower, at 52–56.5%. Low supply of oxygen has been shown to cause a larger fraction of the organic substrate to be directed to storage products (Third et al., 2003).

The rate of PHA production depends on the activated sludge and the carbon source. In most studies, acetate has been used as carbon source. Serafim et al. (2004) achieved a maximum rate of 435 mgC/gTSS·hr with enriched PHA-producing sludge whereas Takabatake et al. (2002) measured rates between 2.1 and 27.3 mgC/gTSS·hr for activated sludge samples collected directly from full-scale treatment plants. Production of PHAs and consumption of acetate starts almost immediately when a pulse of acetate is fed to a PHA-producing activated sludge culture. The ratio between PHA production and acetate consumption is typically 0.6 molC/molC for aerobic conditions (Beun et al., 2002), which means that 60% of the consumed acetate is used to produce PHAs whereas 40% is used for growth or cell maintenance. In the initial period of the feeding phase (feast), the ratio between PHA production and acetate consumption can be as high as 96% (Carta et al., 2001).

Different bacteria have different preferences for substrates. Consequently, the feed composition of organic acids affects the composition of the microbial PHA-storing community (Lemos et al., 2008; Albuquerque et al., 2013). The carbon source also determines the polymer type being stored by the bacteria. Acetate is favorably stored by mixed cultures as the homopolymer of poly-3-hydroxybuturate (PHB), while propionate results in polyhydroxyvalerate (PHV) or copolymers of P (HB/HV) (Dionisi et al., 2004; Lemos et al., 2006). Complex substrates, such as fermented waste activated sludge, mainly results in storage of P(HB/HV) (Coats et al., 2007; Morgan-Sagastume et al., 2010). For the production of bioplastics, the composition is important. Homopolymers of PHB is a brittle material, while copolymers, particularly P(HB/HV) have thermoplastic properties comparable to petroleum-based plastics such as polypropylene and polyethylene (Lee, 1996).

### 4.2 Glycogen

Microorganisms can store glycogen at over 50% of the cell dry weight (Preiss and Romeo, 1989). In experiments with activated sludge fed with glucose, glycogen levels were about 18% after the feed phase and the glycogen production rates reached 846 mgC/gTSS·hr. The ratio between glucose consumption and glycogen
formation was 0.88 (Dircks et al., 2001). Ratios as high as 94% have been observed (Carta et al., 2001). Takii (1977) tested the ability of different activated sludges from plants treating various types of wastewater to accumulate glycogen when fed with glucose. The glycogen content accumulated in the sludge ranged from 6.5% to 18.1%. The capacity to accumulate glycogen could be correlated with the fraction carbohydrates in the wastewater normally fed to the activated sludge plant, with sludge treating fruit juice and soft drink wastewater having the highest capacity to accumulate glycogen (Takii, 1977).

4.3 Lipids

TAG and wax esters together with other lipids present in sludge, such as phospholipids and fatty acids, can be used to produce biodiesel by a transesterification reaction with an alcohol resulting in a fatty acid alkyl ester (Dufreche et al., 2007). Certain bacteria have been shown to accumulate high concentrations of lipids. *Rhodococcus opacus* was shown to accumulate 87% (dry weight) of fatty acids when grown on olive oil (Alvarez et al., 1996). Revellame et al. (2012) investigated the lipid content in activated sludge from a plant treating municipal wastewater. The lipid content ranged from about 5.9% to 16.3%. Both TAG and wax esters were present in the samples, as were PHAs and steryl esters. Huynh et al. (2011) analyzed wax esters in sludge from a plant treating food processing wastewater. Wax accounted for 2.75% of the dry solids and wax esters with chain lengths of 48 or 50 carbon atoms dominated. By feeding activated sludge with a nutrient medium having a high carbon-to-nitrogen ratio (C/N >40), the lipid content has been enhanced (Mondala et al., 2012; Mondala et al., 2013; Revellame et al., 2013). For example, Revellame et al. (2013) increased the overall lipid content in sludge from an oxidation ditch treatment plant from 6–8% to 15–18% by feeding with a

### Table 2. Extent of sorption of organic compounds in wastewater by aerobic activated sludge.

| Organic fraction | Contact time | Amount sorbed | References |
|------------------|--------------|---------------|------------|
| 0.03–1.5 μm      | 5 min        | 0–45 mgCOD/gTSS | (Bunch and Griffin Jr., 1987) |
| Total COD in presettled domestic wastewater | 10 min | 0–30 mgCOD/gTSS | (Rensink and Donker, 1991) |
| Filtrate after 3 min centrifugation and filtration | 10 min | up to 50 mgCOD/gTSS | (Pujol and Canler, 1992) |
| 0.1–50 μm        | 15 min       | 40–81 mgCOD/gTSSb | (Torrijos et al., 1994) |
| Supernatant after 1 hr sedimentation | 1 min | 23–100 mgCOD/gTSS | (Tan and Chua, 1997) |
| <0.45 μm         | 15 min       | 0.9–16 mgTOC/gTSSc | (Jorand et al., 1995) |
| Supernatant after 2 hr sedimentation | 60 min | –40–100 mgCOD/TSS | (Guellil et al., 2001) |
| Nonsetttable >0.45 μm | 5 min | 6.5 ± 10.8 mgTOC/gVSS | (Modin et al., 2015) |
| <0.45 μm         | 5 min        | 5.0 ± 4.7 mgTOC/gVSS | (Modin et al., 2015) |

*a* Relative centrifugal force and filter pore size were not specified in the article.

*b* In the article, the amount sorbed was specified as a COD drop from 184 to 35 mg/L. The possible range for sorption per mass TSS was calculated based on data given in the article.

*c* Note that some studies report the organics amount in COD and some in total organic carbon (TOC). Some report the biomass amount as TSS and some as VSS.
synthetic wastewater containing glucose and ammonium at a C/N mass ratio of 70. The TAG content in the lipid extracts increased from 1–2% to 20–24%.

5. Extent of sorption in activated sludge

5.1 Sorption capacity

The mass of organic compounds in wastewater that can be sorbed per unit mass of activated sludge has been quantified in a few studies summarized in Table 2. Although the fractionation of organic compounds and the contact time between wastewater and activated sludge has varied in the different studies, the sorption capacity typically falls in the range of 0–100 mgCOD/gTSS. Several factors could affect sorption including the size of the organic compounds, the characteristics of the activated sludge, and the organic loading.

The size of the organic compounds affects whether they are sorbed by the sludge. Bunch and Griffin showed that the organic size fraction between 0.03 and 1.5 μm was rapidly sorbed whereas the fraction below 0.03 μm or between 1.5 and 10 μm were not sorbed to a significant extent (Bunch and Griffin Jr., 1987; Bunch and Griffin Jr., 1992). Torrijos et al. (1994) measured both removal of organics and oxygen uptake rate and showed that the fraction between 0.1 and 50 μm was mainly sorbed whereas the fraction below 0.1 μm was mainly removed by oxidation (Torrijos et al., 1994). Jimenez et al. (2005) fractionated the organic compounds into particulates that are nonsettleable after 30 min sedimentation but larger than 0.45 μm, high-molecular weight colloids between 0.45 and 0.01 μm, low-molecular weight colloids between 0.01 and 0.001 μm, and soluble below 0.001 μm. Tests with and without the presence of oxygen showed that the soluble organics were mainly removed by oxidation whereas the particulates and colloids in all sizes were mainly removed by sorption. The particulates (>0.45 μm) had the highest removal rate. Interestingly, low-molecular weight colloids had higher removal rate than high-molecular weight ones. The authors suggested that the low-molecular weight colloids could represent microbial EPS in solution, which could attach more rapidly to bacteria than other organic particles in the wastewater (Jimenez et al., 2005). Guellil et al. (2001) measured sorption of nonsettleable organics. Of the total sorption of organics, the fraction >0.22 μm contributed to 58 ± 19% and the fraction <0.22 μm contributed 42 ± 19%.

Various characteristics of the activated sludge affect its sorption capacity. Tan and Chua (1997) showed that the presence of available sorption sites on the sludge can affect the amount of organics it can sorb. Sludge collected from the beginning of an activated sludge tank, which was expected to be highly loaded with organic compounds, had lower sorption capacity than sludge collected from the end of the tank were most of the organics were expected to have been oxidized (Tan and Chua, 1997). Activated sludge containing more filamentous microorganisms and having higher sludge volume index has been shown to have higher sorption capacity. Sludge containing filamentous microorganisms may have a more open
structure that provides larger surface area for sorption (Pujol and Canler, 1992; Jorand et al., 1995). A high content of EPS can also be positively correlated with sorption capacity (Jorand et al., 1995). Lim et al. (2015) found that sludge exposed to higher dissolved oxygen (DO) concentration resulted in smaller flocs with less negative charge, which was positive for the surface sorption capacity with a synthetic wastewater.

The organic loading or food-to-mass (F/M) ratio has an important effect on sorption. Several workers have noted higher sorption at higher loading (Banerji et al., 1968; Crombie-Quilty and McLoughlin, 1983; Rensink and Donker, 1991; Pujol and Canler, 1992; Guellil et al., 2001). Sorption isotherms relate the amount of sorbed material to the concentration of material present in the liquid under equilibrium conditions. The Freundlich and Langmuir isotherms are two frequently used models, which have also been employed for activated sludge. However, sorption of organics by activated sludge is a complex phenomenon that is difficult to model using the classical adsorption isotherms. As the sorbed organics are constantly being degraded, true equilibrium conditions are difficult to reach. Crombie-Quilty and McLoughlin (1983) investigated sorption of bovine serum albumin protein by activated sludge and found that a modified version of the Freundlich equation, which they call the activated sludge adsorption equation (ASAE) (Eq. (1)), resulted in a better fit than the classical Freundlich and Langmuir isotherms. They also confirmed the general applicability of the ASAE with data from Banerji et al. (1968). However, Guellil et al. (2001), who studied sorption of nonsettleable wastewater organics, could not use the ASAE to fit the data.

\[
m = k \times (c / b)^{1/n}
\]  

where \( m \) is the amount sorbed (g organics/g sludge), \( c \) is concentration of organics remaining in solution (g/L), \( b \) is the sludge concentration (g/L), and \( k \) and \( 1/n \) are constants.

### 5.2 Sorption kinetics

A first order equation (Eq. (2)) has been shown to accurately describe the kinetics of sorption onto activated sludge (Jorand et al., 1995; Guellil et al., 2001; La Motta et al., 2004; Jimenez et al., 2005; Modin et al., 2015). The sorption rate constant, \( k \), can be found by measuring the concentration of organic compounds with time and fitting the data to Eq. (3).

\[
r = -k \times (C - a) \times X
\]  

where \( r \) is the rate of sorption (mg/L-min), \( k \) is the rate constant (L/mg-min), \( C \) is the concentration of sorbate, i.e., organic compounds in the wastewater (mg/L), \( a \) is the concentration of organics that cannot be sorbed, i.e., the concentration
remaining in solution after an infinitely long contact time (mg/L), and \( X \) is the activated sludge concentration (mg/L).

\[
C_t = a + (C_0 - a) \times e^{-k \times t \times X}
\]  

(3)

where \( t \) is time (min), \( C_t \) is the concentration of organic compounds at time \( t \) (mg/L), and \( C_0 \) is the initial concentration of organic compounds (mg/L).

The sorption rate coefficient, \( k \), and the nonsorbable organics concentration, \( a \), determined by different workers are summarized in Table 3. The sorption rate constant appears to be in the order of \( 10^{-4} \) L/mg-min. In general, sorption of larger particles is faster than sorption of smaller particles. The concentration of nonsorbable organics varies but appears to depend on loading. Figure 3 shows a positive correlation between organic loading and nonsorbable organics concentration based on data compiled from several studies. Temperature differences in the range 4–33°C has been shown to have very little effect on sorption kinetics by activated sludge (Tsezos and Wang, 1991; Jorand et al., 1995). Sorption kinetics is usually not considered in activated sludge models. However, La Motta et al. (2007a) included kinetic expression in a mathematical model for the preliminary design of activated sludge systems (La Motta et al., 2007a, 2007b).

### 5.3 Sorption onto other types of sludge

The studies reviewed above focus on sorption onto aerobic activated sludge. However, organic compounds will also sorb onto other types of sludge. Riffat and Dague (1995) studied sorption of organic compounds in milk onto anaerobic granular sludge. The sorption appeared to reach equilibrium after only 2 min of contact time. Temperature, granule size, and organic loading affected sorption capacity. At 28°C, the sorption capacity was 48 and 11 mgCOD/gTSS for granules of sizes 0.84 and 1.81 mm, respectively. At 20°C, the sorption capacity was approximately half. The sorption capacity could be related to organic loading using the Langmuir isotherm model (Riffat and Dague, 1995). Another study showed that primary and

---

**Table 3.** Kinetic coefficients for sorption of organic compounds by activated sludge.

| Organic fraction | \( k \) (L/mg-min) | \( a \)b | References |
|------------------|-------------------|-------|-------------|
| <0.45 \( \mu \)m | 0.01–0.56 \( \times 10^{-4} \)a | 6–27 mgTOC/L | (Jorand et al., 1995) |
| Nonsettleable > 0.22 \( \mu \)m | 1.0–5.5 \( \times 10^{-4} \)a | ~40 mgCOD/L | (Guellil et al., 2001) |
| <0.22 \( \mu \)m | 0.3–4.5 \( \times 10^{-4} \)a | ~40 mgCOD/L | (Guellil et al., 2001) |
| Nonsettleable | 0.88 \( \times 10^{-4} \)a | 29.1 mgCOD/L | (La Motta et al., 2004) |
| Nonsettleable > 0.45 \( \mu \)m | 1.5–1.54 \( \times 10^{-4} \)a | 8.5–10 mgCOD/L | (Jimenez et al., 2005) |
| 0.001–0.45 \( \mu \)m | 0.3–0.5 \( \times 10^{-4} \)a | 9.3–10 mgCOD/L | (Jimenez et al., 2005) |
| Nonsettleable > 0.45 \( \mu \)m | 0.94–1.59 \( \times 10^{-4} \)a | 53–109 mgTSS/L | (Modin et al., 2015) |
| <0.45 \( \mu \)m | 0.14–0.65 \( \times 10^{-4} \)a | 12.5–19.1 mgTOC/L | (Modin et al., 2015) |

aIn these studies, the \( k \) values were given in units min\(^{-1}\). We recalculated to L/mg-min based on information on suspended solids concentration given in the articles.

bNote that depending on the study, the organic content was measured as either COD, TOC, or TSS.
anaerobic sludge both had net release of organics when mixed with raw municipal wastewater (Modin et al., 2015). In trickling filters and rotating biological contactors, sorption of particles was shown to follow first order kinetics. Inorganic and organic particles had similar kinetics showing that the sorption was indeed a physical-chemical phenomenon (Boltz et al., 2006; Boltz and Motta, 2007).

6. Established process configurations dependent on nonoxidative removal mechanisms

There are several configurations of the activated sludge process that function based on the ability of sludge to remove organic compounds using nonoxidative removal mechanisms.

6.1 Sludge-enhanced primary sedimentation

Returning waste activated sludge (WAS) (Figure 4) to the primary settlers is a quite common practice in treatment plants (Tchobanoglous et al., 2004) and was used already in 1928 at the activated sludge plant in Essen-Relinghausen, Germany (Imhoff, 1951). Returning WAS to the primary settlers can improve the thickening properties of the secondary sludge which typically has a lower solids concentration than primary sludge (Tchobanoglous et al., 2004). Sorption onto the activated sludge can also contribute to the removal of organics in the primary settlers. Yetis and Tarlan (2002) investigated the effect of activated sludge addition on suspended solids removal during sedimentation of wastewater. The residual suspended solids concentration decreased from about 160 mg/L without activated sludge addition to about 50 mg/L with addition of 10%
sludge by volume. The best results were obtained using sludge from a reactor operated at an SRT of 8–10 days and a sludge concentration above about 1600 mg/L in the sludge–wastewater mixture (Yetis and Tarlan, 2002). Ross and Crawford (1985) performed tests on two full-scale primary settlers, one was receiving WAS and the other was used as control. The WAS flow rate represented only 1.2% of the total flow to the settler and resulted in about 135 mg/L of sludge in the WAS-wastewater mixture. The effluent from the settler receiving WAS has slightly higher suspended solids concentration and slightly lower BOD and TOC concentration. However, only the difference in suspended solids concentration was statistically significant (Ross and Crawford, 1985).

6.2 Contact-stabilization

In the contact-stabilization process, the wastewater is mixed with activated sludge in a contact tank with low hydraulic retention time. Removal of organics is assumed to take place mainly by sorption onto the activated sludge flocs. The treated water is then separated from the flocs in a sedimentation tank. The concentrated sludge is regenerated in an aerated stabilization tank where the organics sorbed onto the sludge are oxidized (Figure 5) (Rittmann and McCarty, 2001). The process was patented by Coombs (1922), and later also developed by Ullrich and Smith (1951). The latter used hydraulic retention times of 15 min in the contact tank, 65 min in the settler, and 90 min in the stabilization tank and achieved 90–95% reductions of suspended solids and BOD in the influent wastewater (Ullrich and Smith, 1951). As the main removal mechanism of organic matter from the wastewater is sorption onto the activated sludge flocs, it is most suitable for treatment of wastewater with a low fraction of soluble organics and a high fraction of colloidal or particulate organics (Sarioglu et al., 2003). A higher removal of soluble organics can be attained with a higher sludge recirculation ratio. However, then the process becomes similar to a completely mixed reactor (Gujer and Jenkins, 1975).
Variations of the contact-stabilization process have been proposed as an economical enhanced primary treatment technology for developing countries. Huang and Li (2000) compared treatment using only primary sedimentation to treatment using a contact-stabilization type process where the primary sludge was recirculated through an aeration tank and mixed with the raw wastewater in a contact tank with HRT of 25 min. Over time, the primary sludge developed into an activated sludge and this was essential for efficient removal. With 6000 mg/L of sludge in the contact tank, removal efficiencies of suspended solids and COD in the system were 51% and 40%, respectively, which was 11% and 35% higher than primary sedimentation alone. Zhao et al. (2000) investigated the effect of the activated sludge concentration in the contact tank. Removal efficiencies generally improved with higher MLSS concentrations up to 14,000 mg/L. With 4000 mg/L MLSS concentration, they achieved removal efficiencies of 70–80% for COD, 30% for filtered COD, 80–95% for suspended solids, 6% for ammonium, and 30–50% for total nitrogen. Liu et al. (2011) developed a mathematical model based on ASM1 (Henze et al., 1987) with the inclusion of first-order adsorption kinetics of particulates as the main mechanism for COD removal in the contact tank. The model was able to predict effluent COD and ammonium concentrations from a laboratory-scale reactor (Liu et al., 2011).

6.3 Adsorption–biooxidation and other two-stage processes

The adsorption–biooxidation process consists of two activated sludge stages in series (Figure 6), each with its own secondary settler. The first stage (A-stage) receives a high organic load with an F/M ratio ranging from 2 to 10 gBOD/gVSS-day, an SRT of 3–12 hr, and an HRT of 30 min or less. Organic matter is removed primarily by sorption onto the activated sludge flocs. The second stage (B-stage) is low loaded with an F/M ratio of less than 0.1 gBOD/gVSS-day. The B-stage is operated with long SRT of 8–20 days and biological nitrogen removal through nitrification and denitrification can be achieved. The process was developed at the municipal wastewater treatment plant in Krefeld, Germany, which because of industrial discharges received a wastewater with high degree of variability in pH.
and toxicity. The A-stage was able to cope with toxic shocks and made the problematic wastewater of Krefeld biologically treatable. The first full-scale AB plant was installed in 1980. Since then, several full-scale plants have been installed (Versprille et al., 1985; Boehnke et al., 1997a, 1997b; Boehnke et al., 1998). Garcia-Olivares and Becares (1995) developed a mathematical model predicting COD in the final effluent and MLSS concentrations in the reactors. They calibrated the model against data from a pilot reactor by varying the growth rate, specific substrate removal rate, and concentration factor in the settling tank parameters. However, they did not include sorption kinetics in the A-stage as a specific mechanism in the model. Nogaj et al. (2015) developed a modified version of the ASM1 model to describe organics removal in A-stage activated sludge. As previous research has shown that activated sludge operated at very short SRT is only able to utilize the most easily biodegradable fraction of the organics (Haider et al., 2003), they divided the soluble organics into two fractions: rapidly and slowly biodegradable substrate. They also included EPS production, storage of soluble organics, and sorption of colloidal organics as specific mechanisms in the model. The model was calibrated and validated against process data from two high-rate activated sludge pilot plants (Nogaj et al., 2015).

Two-stage activated sludge systems other than the AB process also exist. Imhoff (1951) describes recirculating excess sludge from the second stage to the beginning of the first stage to improve the organics removal capacity of the first stage. As the second stage of a two-stage activated sludge process usually receives quite a low organic load, denitrification can be limited. To improve biological nitrogen removal, Matsché and Moser (1993) developed a hybrid plant in which some of the sludge from the first stage was passed to the second stage to improve denitrification, and some of the sludge from the second stage was recirculated to the first stage to enable some nitrification there. Another concept is the bypass configuration in which a portion of the influent wastewater is bypassed the first stage and fed directly to the second stage to support denitrification (Winkler et al., 2000). The hybrid and bypass configurations were evaluated for the extensions of
Vienna’s municipal wastewater treatment plant. Pilot-reactor tests found that the hybrid configuration resulted in more stable nitrification performance whereas the bypass configuration achieved more thorough denitrification. The sludge volume index increased in both stages in the bypass mode because of reduced loading on the first stage and increased growth of filaments (Müller-Rechberger et al., 2001; Wandl et al., 2002). A modeling study by Constantine et al. (2012) suggested that two-stage activated sludge processes, including the AB process, could be more energy efficient than a conventional single-stage process both in terms of reduced aeration energy requirements and increased biogas production.

6.4 Enhanced biological phosphorous removal

The purpose of the EBPR process is to remove phosphorous from wastewater. Intermittent intracellular storage of organic compounds plays an important role for the biochemistry of the microorganisms involved. However, it should be noted that the organic compounds are not harvested from the wastewater as storage products, but are oxidized in the microbial cells during the aerobic or anoxic phase of the process. In the EBPR process, polyphosphate accumulating organisms (PAOs) are responsible for removing phosphorous from wastewater by storing it inside the cell as polyphosphate (Oehmen et al., 2007). Enrichment of PAOs can be achieved by cycling the activated sludge through anaerobic and aerobic conditions (Figure 7) (van Loosdrecht et al., 1997; Seviour et al., 2003). Under anaerobic conditions, the activated sludge is mixed with wastewater containing VFAs. The PAOs take up the VFAs and store them as PHAs. The energy and reducing power required for PHA storage is obtained from intracellular reserves of polyphosphate and glycogen. Under aerobic conditions, the PAOs oxidize the stored pools of PHAs and instead store polyphosphate and glycogen (Mino et al., 1998). In EBPR systems, the PAOs can face unwanted competition from glycogen accumulating organisms (GAO), which take up VFAs under anaerobic conditions and store glycogen under aerobic conditions without storage of polyphosphate (Mino et al., 1995). The competition between PAOs and GAOs is, for example, affected by the VFA composition in the anaerobic phase where PAOs appear to have more rapid uptake of propionate. The propionate uptake rate was $0.18 \pm 0.02 \text{ mol C/mol bio-}$

![Figure 7. Schematic of an EBPR process.](image)
mass C per hour irrespective of acetate concentration whereas the acetate uptake rate decreased from $0.21 \pm 0.02$ to $0.06 \pm 0.01$ mol C/mol biomass C per hour with increasing concentration of propionate in the VFA mixture (Carvalheira et al., 2014). Denitrifying glycogen accumulating organisms (DGAO) also compete for the available VFAs (Zeng et al., 2003).

7. Emerging process configurations dependent on nonoxidative removal mechanisms

7.1 High-rate activated sludge

While the aims of existing sorption-based processes have been to use smaller reactor volumes such as in contact-stabilization (Sarria et al., 2011) or to treat toxic and highly variable wastewater such as in the AB process (Versprille et al., 1985), high-rate activated sludge processes have recently started to be investigated for the explicit purpose of more resource- and energy efficient wastewater treatment (e.g., Ng and Hermanowicz, 2005). They are also being investigated as a suitable system for decentralized wastewater treatment (Zamalloa et al., 2013). The activated sludge system is operated like the A-stage in an AB process with a short SRT to maximize organics removal by sorption and minimize oxidation. The produced sludge can then be valorized to methane gas through anaerobic digestion while the effluent water may receive further treatment for nutrient removal, be discharged, or reused. Several studies focusing on different types of wastewater and using either sedimentation or membrane filtration for sludge separation have been reported in the literature during the last couple of years.

Ge et al. (2013) investigated a high-rate SBR system for treatment of meat-processing wastewater. The SBR was operated with an SRT of 2–3 days and an HRT of 0.5–1 day. Removal efficiencies of >80% for COD and P, and 55% for nitrogen were achieved. About 70–80% of the COD removal was due to assimilation or sorption rather than oxidation. They found that the anaerobic biodegradability of produced sludge was higher at low SRT with 85% biodegradability at an SRT of 2 days and 63% at 4 days, with an increased methane production at the shorter SRT. The effluent water could be used for irrigation but did not have sufficient quality for environmental discharge. Zamalloa et al. (2013) operated a continuous reactor followed by a settler at an SRT of 1 day and HRT of 1.1 hr. On average 57% COD removal was achieved. The activated sludge stage was followed by a microalgae biofilms reactor for nutrient removal. The system was proposed for decentralized wastewater treatment at the individual building level. Diamantis et al. (2014) operated a continuous reactor followed by a settler at an SRT of 0.3–0.5 day and HRT of 0.9 hr. Better COD removal efficiency (80%) was obtained at a high loading of 10–20 kg/m³·day compared to a low loading of 6.8 kg/m³·day. The effluent from the high-rate system was further treated by ultrafiltration. Jimenez et al. (2015) investigated the effect of SRT, HRT, and DO on the performance of a high-rate activated sludge system treating municipal wastewater. They found
that the removal efficiency of colloidal and particulate COD increased progressively with SRT from 0.1 to 2 days while the soluble COD reached its maximum removal efficiency at SRT over 0.5 day. The soluble COD removal efficiency was positively correlated with DO concentration up to 0.3 mg/L and HRT up to 15 min. The particulate and colloidal COD removal efficiency was positively correlated with DO concentration up to 0.7 mg/L and HRT up to 30 min. They also showed that the EPS content of the sludge was positively correlated with removal efficiency of particulate and colloidal COD up to about 80 mg EPS\textsubscript{COD}/g VSS.

Instead of sedimentation, MBR systems have been used for separation of sludge and treated water in high-rate systems. Ng and Hermanowicz (2005) compared a high-rate conventional activated sludge (CAS) system to a MBR. They varied the SRT in both systems from 5 days to 0.25 day while the HRT was kept at 3–6 hr. The MBR had lower COD concentration in the effluent and COD removal efficiencies of 98%. In the CAS, the COD removal efficiency was 78–94%. At short SRT, lower EPS content in the sludge and more dispersed flocs were observed. Akanyeti et al. (2010) investigated a high-rate MBR operated at SRT of 0.25–1.0 day and HRT of 1.2 hr. They found that the fraction of influent COD that was mineralized, i.e., oxidized to CO\(_2\), was reduced from 54% at 1 day SRT to 27% at 0.25 day SRT. Hernandez Leal et al. (2010) operated a high-rate MBR for grey water treatment and estimated the mineralized COD fraction to 3% at SRT of 0.2 day and 11% at 1 day. Similarly, Faust et al. (2014b) estimated the degree of mineralization to 1% at SRT of 0.125 day, 11% at 1 day, and 32% at 5 days for a MBR fed with wastewater. At higher SRT, they observed better flocculation, higher sludge EPS content, and less membrane fouling. Fouling was correlated with the concentration of small particles, 45–450 nm, in the water. The authors recommended operation at an SRT of 0.5–1 day at which mineralization and permeate COD concentration were low and a large fraction of the COD could be captured as suspended material (Faust et al., 2014b). When comparing operation at DO concentrations of 1 and 4 mg/L, the higher concentration resulted in better flocculation, larger flocs, and better membrane filterability (Faust et al., 2014a).

### 7.2 Production of polyhydroalkanoates

For PHA production from municipal wastewater using naturally occurring bacteria as inoculum, three principal steps are usually proposed (Dionisi et al., 2004; Coats et al., 2007; Serafin et al., 2008), as depicted in Figure 8. First, fermentation of waste activated sludge and/or municipal wastewater is used to transform the organic content into VFA, which can be effectively used by the PHA-accumulating microorganisms. Typically, the VFAs produced by fermentation are mostly composed of acetic acid and some propionic-, butyric-, and (iso)valeric acids (Coats et al., 2007; Mengmeng et al., 2009; Morgan-Sagastume et al., 2014), with yields in the range of 26% TOC\textsubscript{VFA}/VSS (g/g) (Mengmeng et al., 2009) and 27% COD\textsubscript{VFA}/VSS (g/g) (Morgan-Sagastume et al., 2014).
The second step consists of an enrichment of PHA-producing microorganisms to get a microbial community with a high storage potential, using the produced VFA or wastewater amended with VFA as substrate. This is commonly achieved in SBRs at feast/famine conditions but can also be achieved using anaerobic/aerobic conditions. Enrichments using this approach have generally produced biomass with a high storage capacity of PHAs (Coats et al., 2007; Morgan-Sagastume et al., 2010; Coats et al., 2011). Alternatively, enrichment can be performed using the wastewater as the only carbon source, as recently demonstrated (Morgan-Sagastume et al., 2014). Moderate removals of COD (60–90%) in the enrichment reactors (Coats et al., 2007; Coats et al., 2011; Morgan-Sagastume et al., 2014), however, indicates that additional posttreatment for COD removal may be required at the wastewater treatment plant.

The third step consists of the actual PHA production using the enriched biomass and the VFA produced by the fermentation. This step is also performed in an SBR. PHA production using fermented waste activated sludge as carbon source has shown PHA accumulation in the range of 50% PHA/VSS (g/g), indicating the feasibility of using this food source for PHA production (Coats et al., 2007; Morgan-Sagastume et al., 2014). With the integrated approach of fermentation-biomass enrichment-PHA production, the organic content in wastewater has furthermore been estimated to suffice to produce enough PHA precursors for an economically profitable wastewater treatment process (Coats et al., 2011). Besides municipal wastewater treatment, successful PHA production using similar approaches have been demonstrated for a number of other organic waste sources (Dionisi et al., 2005; Bengtsson et al., 2008; Albuquerque et al., 2010a; Chakravarty et al., 2010; Duque et al., 2014).

Additionally, the PHAs need to be recovered and refined. This is commonly performed by extractions using organic solvents to get a highly purified precursor for bioplastic production (Gumel et al., 2013). However, the choice of PHA
extraction has a significant impact on the economic cost and ecological footprint of the entire PHA production process (Koller et al., 2011). Mechanical and enzymatic processes are hence being evaluated as alternatives to solvent-based extractions (Jacquel et al., 2008; Gumel et al., 2013). Alternatively, the end products do not necessarily need high purity PHA as precursors. Natural fiber-reinforced thermoplastic composites would be such an alternative for which the PHA-rich biomass can be used directly in the manufacturing procedure (Coats et al., 2008). Another alternative would be production of biofuels, such as 3-hydroxybutyrate methylester, which also does not require highly purified precursors (Wang et al., 2010).

### 7.3 Production of biodiesel

Biodiesel consists of fatty acid alkyl esters, for example fatty acid methyl ester (FAME). Direct production of FAME from activated sludge has been investigated in several studies. The lipid component of the sludge undergoes an acid-catalyzed transesterification reaction with methanol resulting in FAME, which can be extracted from the liquid using, e.g., hexane. Yields of about 2.5–6.2% (FAME per dry weight of sludge) have been obtained using waste activated sludge samples (Dufreche et al., 2007; Mondala et al., 2009; Revellame et al., 2010; Revellame et al., 2011). Dufreche et al. (2007) estimated that a yield of about 10% would be needed for sludge biodiesel to be economically competitive with soy-based biodiesel. TAG is an excellent feedstock for biodiesel. In order to increase the TAG content of the sludge, feeding waste activated sludge with a carbohydrate solution has been investigated (Mondala et al., 2012; Mondala et al., 2013; Revellame et al., 2013). Mondala et al. (2012) obtained a biodiesel yield of 10.2% by feeding the sludge with 60 g/L glucose. An activated sludge process solution for generating sludge with enhanced lipid content as proposed by Mondala et al. (2012) and Revellame et al. (2013) is shown in Figure 9. Another strategy was used by Hall et al. (2011) who augmented wastewater with oleaginous microorganisms. However, the results did not show that the added microorganisms could compete with the indigenous microbial population over time. As mentioned in Section 7.2, PHA can also be used to produce biofuels (Muller et al., 2014). Esterification of PHA yields hydroxyalkanoate methyl esters. Wang et al. (2010) showed that 3-hydroxybutyrate methyl ester produced from PHB had similar or better properties than ethanol when used as an additive in gasoline.

### 8. Outlook

The activated sludge process has played a very important role in wastewater treatment plants since its development 100 years ago, and will likely be widely used also in the future. However, because of the increased focus on resource recovery from wastewater, we expect that process configurations designed for a higher degree of nonoxidative removal of organics will become more and more prevalent. The emerging process configurations can be categorized as either sorption-based
or storage-based. The two types of processes have different challenges and opportunities.

### 8.1 Sorption-based processes

Sorption-based processes are essentially high-rate activated sludge systems where the aim is to divert as much as possible of the organic compounds to the sludge and minimize mineralization. The product is a concentrated sludge stream, which can be valorized, e.g., by producing biogas through anaerobic digestion. Sorption works best for particulate and colloidal material (Bunch and Griffin Jr., 1987; Bunch and Griffin Jr., 1992) and is therefore suitable for wastewaters with high particulate content, e.g., municipal, swine, dairy, tannery, and polymer industry wastewater (Figure 2).

High-rate activated sludge, which means operation at short SRT, is assumed to increase nonoxidative organics removal both through assimilation and sorption. Although lowering the SRT does not affect the fraction of the original organic substrate that is assimilated by the microorganisms, which is limited by thermodynamic constraints, it reduces the degree of endogenous respiration in relation to microbial growth and therefore increases the apparent microbial yield in the process. There is also no evidence suggesting a short SRT would allow better sorption of organics onto activated sludge. However, it minimizes hydrolysis and subsequent oxidation of the sorbed organic material, thereby resulting in a larger fraction of the organics being removed by sorption.

Further research is needed about how to model and operate high-rate activated sludge systems. IWA’s activated sludge models are not suited for systems operating at very low SRT (<1 day). Instead modified models accounting for sorption and storage kinetics as well as the lower biodegradability of organic matter at low SRT
should be used (Nogaj et al., 2015; Sin et al., 2005). New models should also consider how changes in SRT affect properties of the sludge. At low SRT, potential benefits in the energy efficiency of the plant may be offset by, e.g., poor effluent quality, foaming problems, or increased membrane fouling (in the case of MBRs). During operation at short SRT, there is a trade-off between removal efficiency and energy efficiency. This was illustrated in a recent article by Jimenez et al. (2015), where a removal efficiency for colloidal and particulate COD of >60% was achieved at SRT > 1.5 days while the specific oxygen requirements for aeration and the mineralization of COD was the lowest at 0.1 day, which was the shortest SRT tested.

Further research is also needed about fundamental aspects of sorption of wastewater organics onto activated sludge. Contrary to the detailed knowledge about microbial uptake of different organic molecules, fundamental knowledge is still lacking about sorption. Thus far, sorption has only been explored for different size fractions of wastewater organics (Guellil et al., 2001; Jimenez et al., 2005). However, the chemical composition and parameters such as surface charge, structure, and hydrophobicity of the wastewater organics are likely also important in determining the kinetics and extent of sorption.

It could potentially also be interesting to investigate enrichment as a strategy to improve the sorption capacity of activated sludge. The sorption process should be highly dependent on the properties of the EPS. For example, interactions between negatively charged carbohydrates and positively charged amino acids of proteins could facilitate sorption (Tielen et al., 2013). Some bacteria can also excrete certain extracellular polymers (the so-called bioflocculants) that facilitate flocculation of wastewater organics (Salehizadeh and Shojaosadati, 2001). Thus, it would be beneficial if high-rate activated sludge processes could be operated to promote the enrichment of sludge that is good at sorption and at producing bioflocculants.

### 8.1 Storage-based processes

Storage-based processes are designed to enrich a microbial community capable of converting the influent organic compounds into specific biological polymers, which could be used as precursors for production of plastics and biofuels. Storage can be enhanced by operating reactors under, e.g., feast-famine regimes or low DO concentration (Beun et al., 2000; Third et al., 2003). Storage-based processes would be suitable for wastewaters with high dissolved content such as olive mill wastewater (Figure 2). If the organic compounds are not dissolved, a hydrolysis step is needed to generate compounds that can be taken up by the microbial cells. Fermentation, which, e.g., could take place in sewer lines (Narkis et al., 1980) or in a dedicated reactor (Coats et al., 2011), results in production of VFAs. These are used by bacteria to store PHAs. On the other hand, carbohydrates result in storage of glycogen (Carta et al., 2001) or lipids (Revellame et al., 2013). Thus, wastewater rich in carbohydrates, such as discharges from juice and soft drinking manufacturers, could be used to store glycogen in the sludge (Takii, 1977).
Research is needed in several areas related to storage-based activated sludge processes. For PHAs, scalable process configurations have already been developed (Albuquerque et al., 2010b; Morgan-Sagastume et al., 2014). However, maintaining a mixed activated sludge population that produces a PHA of desired composition (e.g., the ratio between polyhydrobutyrate and polyhydroxyvalerate) is still challenging. Further understanding of the relationship between feed composition, microbial community selection, and PHA composition is important (Albuquerque et al., 2013).

Developing a process configuration that enhances the production of TAGs is more challenging. While the capacity to store PHA is widespread among bacteria, TAG storage appears limited to a smaller number of bacterial species (Alvarez and Steinbüchel, 2002). Nevertheless, it has been shown that by feeding sludge a nutrient medium containing high concentration of carbohydrate and low concentration of nitrogen, TAG accumulation can be promoted (Revellame et al., 2013). For this process to be economically viable, the carbohydrate feed must be inexpensive and readily available at the treatment plant. Utilizing lignocellulosic waste material has been suggested (Mondala et al., 2013). Ideally, the influent wastewater would have the appropriate composition for TAG accumulation. Industrial wastewater with high carbohydrate and low nutrient content could potentially be suitable. Further research on activated sludge process configurations that promote the accumulation of TAG is needed. Moreover, the feasibility of using activated sludge to produce other storage polymers such as wax esters and cyanophycin is still largely unexplored. In a wastewater treatment context, storing nitrogen as cyanophycin would be an interesting concept as the removed nitrogen would be kept in a useful form instead of being converted to dinitrogen gas. Cyanophycin is mainly produced by cyanobacteria; however, some heterotrophs, most notably Acinetobacter sp., are also capable of its production. Phosphorous or sulfur limitation appear to trigger cyanophycin accumulation (Obst and Steinbüchel, 2006).

8.3 Combination of sorption- and storage-based processes

We do not have to choose between either a sorption- or storage-based process for a given treatment plant. The processes are used for different purposes and for different types of wastewater streams and can therefore be combined in the same plant. Sorption is used for bulk removal of the organics, producing sludge with unspecific chemical composition. Storage results in production of specific chemical products. A process configuration combining the two mechanisms could consist of a high-rate activated sludge process diverting most of the incoming organic compounds to the sludge stream. The sludge could then be fermented into VFAs, which could be used for production of PHAs.

The development of more advanced activated sludge processes based on sorption and storage should be accompanied by life cycle assessment to evaluate the environmental impact of the new process configurations (Heimersson et al.,
2014). If storage processes are used to generate a new product from wastewater, e.g., bioplastics or biodiesel, it is important to investigate the downstream processes required to transform the microbial storage polymer into a product. The potential market for the generated product is also an important aspect to consider.

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