Optimizing High-Rate Activated Sludge: 
Organic Substrate for Biological Nitrogen Removal and Energy Recovery

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ABSTRACT

Although the A-stage high-rate activated sludge (HRAS) process destroys some of the chemical energy present in municipal wastewater, this process has been gaining attention as a viable technology for achieving energy neutrality at water resource recovery facilities. In addition to carbon capture for energy recovery, A-stages are also being utilized upstream of shortcut biological nitrogen removal (BNR) processes as these BNR processes often require a controlled influent carbon to nitrogen ratio that is lower than required for conventional BNR processes. While there is extensive knowledge on conventional activated sludge processes, including process controllers and activated sludge models, there has been little detailed research on the carbon removal mechanisms of A-stage processes operated at solids retention times (SRT) less than about one day.

The overall objective of this study was to elucidate the chemical oxygen demand (COD) removal mechanisms of short SRT activated sludge processes with a specific focus on the removal of the different COD fractions under varying operating conditions including dissolved oxygen, hydraulic retention time, temperature, and SRT. Once understood, automatic process control logic was developed with the purpose of producing the influent characteristics required for emerging shortcut BNR processes and capturing the remaining COD with the intent of redirecting it to an energy recovery process.

To investigate the removal and assimilation of readily biodegradable substrate ($S_S$), this study evaluated a respirometric method to estimate the $S_S$ and active heterotrophic biomass ($X_H$) fractions of the raw wastewater influent and effluent of an A-stage pilot process. The influent $S_S$ values were comparable to the $S_S$ values determined using a physical-chemical method, but the effluent values did not correlate well. This led to the measurement of the heterotrophic aerobic yield coefficient and decay rate of the pilot process. The yield coefficient was estimated to be $0.79 \pm 0.02 \text{ gCOD/gCOD}$, which was higher than the accepted value of 0.67 g/g. It was speculated
that the batch respirometry tests resulted in the aerobic storage of $S_S$ and this likely contributed to the error associated with the determination of $S_S$ and $X_H$. Therefore, physical-chemical fractionation methods were used to determine the removal of the individual COD fractions. It was concluded that the SRT was the primary control parameter and below a 0.5 day SRT the dominate COD removal mechanisms were assimilation and oxidation of readily degradable substrate and sedimentation of particulate matter. At SRTs between 0.5-1 days, COD removal became a function of hydrolysis, as adsorption of particulate and colloidal matter was maximized but not complete because of limited adsorption sites. Once adequate adsorption sites were available, effluent quality became dependent on the efficiency of bioflocculation and solids separation. While the SRT of the pilot process could not be directly controlled because of severe biofouling issues when using in situ sensors, a MLSS-based SRT controller was successfully implemented instead. The controller was able to reduce total COD removal variation in the A-stage by 90%. This controller aslo provided the capability to provide a consistent carbon to nitrogen ratio to the downstream B-stage pilot process.

To ascertain the settling, dewaterability, and digestibility of the sludge produced by the pilot A-stage process, several standardized and recently developed methods were conducted. The results from these tests indicated that the A-stage had similar dewaterability and digestibility characteristics to primary sludge with average achievable cake solids of 34.3±0.4% and average volatile solids reduction (VSR) of 82±4%. The A-stage sludge also had an average specific methane yield of 0.45±0.06 m$^3$CH$_4$/kgVS. These results were attributed to low extracellular polymeric substance (EPS) content. However, further research is needed to better quantify EPS and determine the effect of HRAS operating parameters on EPS production. Overall the A/B pilot study was able to capture 47% of the influent COD as waste sludge while only oxidizing 45% of the influent COD. Of the COD captured, the A-stage contributed over 70% as dry solids. Coupled with high sludge production, VSR, and methane yield the A/B process was able to generate 10-20% more biogas and 10-20% less dry solids after anaerobic digestion than a comparable single-sludge BNR process.
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Introduction

The activated sludge (AS) process, developed by Ardern and Lockett (1914) over a century ago, is currently the most widely used method of removing pollutants, like organic carbon, nitrogen, and phosphorus, from used water. Nitrification and biological nutrient removal (BNR), from a regulatory and water quality standpoint, has been one of the main drivers behind the optimization of the AS process. However, as global concerns focus more on water scarcity, food production, and energy use, the new paradigms are resource recovery, energy neutrality, and reducing carbon footprint all while meeting stringent nutrient removal requirements. This includes recovering energy by anaerobically converting organic carbon to methane and its subsequent combustion to co-generate heat and electricity, recovering the macronutrients nitrogen and phosphorus as a fertilizer product, producing treated water for reuse, and reducing carbon footprint by reducing energy and chemical use.

While AS is a cost-effective means of pollutant removal, it requires the input of energy in the form of aeration to remove stored chemical energy in the form of soluble, colloidal, and particulate organic carbon compounds. The energy present in these organic compounds is approximately 14 MJ/kg COD (chemical oxygen demand) and is lost as metabolic heat when aerobically oxidized (Jetten et al., 1997). In addition, the removal of nitrogen and phosphorus through biological means requires the input of energy and, more importantly, organic carbon. The use of influent carbon for BNR decreases the energy recovery potential, especially as nutrient limits continue to become more stringent. As the cost of supplemental carbon and energy increases, the desire to use the influent organic carbon for BNR and energy production has also increases.

In order to obtain energy neutrality a plant must not only increase biogas production but also decrease energy use by managing the influent carbon (McCarty et al., 2011). Therefore, the design and operation of a treatment plant must balance organic carbon utilization and the demographics of a plant typically dictate which side of the balance a plant leans. If permit limits are stringent and energy relatively cheap, the focus is typically on directing the influent carbon to the BNR process. If permit limits are not very stringent but energy cost high, the desire may be to generate as much energy as possible albeit without adversely affecting BNR. Holistically, the
objectives of any treatment plant should be efficient utilization of influent organic carbon for
meeting total nitrogen (TN) and total phosphorus (TP) limits and redirect the remainder carbon
in its most reduced form to energy recovery processes. Carbon savings have been realized using
mainstream simultaneous nitrification denitrification (SND) and nitrite shunt type AS processes
(Bertanza, 1997; Collivignarelli and Bertanza, 1999; Marcelino et al., 2011; O'Neill and Horan,
1995). However, fully autotrophic nitrogen removal processes could potentially achieve the
ultimate carbon and energy savings.

One particular AS process that is receiving a lot of attention is mainstream deammonification,
where autotrophic anaerobic ammonia oxidizing bacteria (AMX, anAOB, or anammox) convert
nitrite and ammonia directly to nitrogen gas without consuming organic carbon. The full
potential of a completely autotrophic mainstream deammonification process compared to
conventional BNR employing complete nitrification and heterotrophic denitrification is a 100%
reduction in the organic carbon required for denitrification and a 67% reduction in aeration
energy required for nitrification. The challenge for this type of process and nitrite shunt
processes is to out-select the nitrite oxidizing bacteria (NOB) that compete with anammox for
nitrite and convert it to nitrate. Preliminary studies suggest that NOB out-selection in the
mainstream process may not be possible at very low C/N ratios (<6) and that heterotrophic
denitrification may contribute to initial NOB out-selection by substrate (nitrite) competition
(Regmi et al., 2013) and continued repression by partial denitrification and production of nitrous
acid (HNO₂), nitric oxide (NO), or nitrous oxide (N₂O), which are known inhibitors of NOB
(Udert et al., 2008). Therefore, a controlled influent C/N ratio between 6 to 10 (COD/TAN, total
ammonia nitrogen) may be required for successful NOB out-selection.

Furthermore, in a combined nitritation/anammox system, some organic carbon may enable
higher TN removal efficiencies if the ordinary heterotrophic organisms (OHOs) preferentially
utilize the nitrate that is produced by anammox and NOB (Udert et al., 2008). It has been
demonstrated in Escherichia coli that nitrate is preferentially used over nitrite as an electron
acceptor, presumably because nitrate has a more positive redox potential (White, 2007).
Although organic carbon may be required, too high of an organic load to a partial nitritation or
nitrite shunt process can adversely affect the nitrification rates because of excessive
heterotrophic growth and competition for oxygen (Æsøy et al., 1998; Figueroa and Silverstein,
1992). OHOs will also out-compete anammox since their growth rates are more than 30 times that of anammox (Strous et al., 1999). Therefore, controlling the influent C/N is a critical aspect of mainstream deammonification and nitrite shunt processes.

While there are numerous processes that can remove carbon (e.g., primary clarifiers), the adsorption-style high-rate activated sludge (A-stage HRAS) process is one of the only carbon removal processes that lends itself to being tightly controlled through instrumentation, control, and automation (ICA) and has not yet been studied in great detail. The A-stage was developed by Böhnke and Diering (1980) as a cost-effective biological buffer at plants with high industrial inputs and was not intended to completely remove all of the influent carbon. More recently, redirecting organic carbon to anaerobic digestion for biogas production has driven the use and optimization of the A-stage. A renewed interest in the A-stage has also occurred with the emergence of advanced BNR processes that require tight process control, like nitrite shunt and mainstream deammonification. Since the A-stage was never designed to completely remove organic carbon, the process can potentially be controlled by manipulating operating parameters in order to meet certain carbon removal criteria based on the downstream goals. These operational parameters include dissolved oxygen (DO), aerobic duration, hydraulic residence time (HRT), and solids retention time (SRT). While there is a plethora of data on systems operated at greater than 1 day SRT, typically with the objective of meeting 30 mg/L TSS (total suspended solids) and BOD$_5$ (5-day biochemical oxygen demand) limits, there is very limited knowledge on very high-rate systems and how these operating parameters affect carbon removal mechanisms.

Therefore, the overall objective of this research is to fill this knowledge gap and develop an understanding of carbon removal mechanisms in the A-stage process and how ICA can be employed to exploit and control these removal mechanisms to benefit the downstream BNR process. The hypothesis is that carbon removal in the A-stage occurs primarily because of microbial storage of readily biodegradable COD and enmeshment of colloidal and particulate COD through extracellular polymeric substance (EPS) production and bioflocculation. Subsequent wasting of biomass occurs before significant hydrolysis and mineralization of organic carbon takes place, resulting in energy-rich waste sludge available for redirection to
energy recovery processes. Additionally, it is hypothesized that these COD removal mechanisms can be controlled by using advanced ICA to manipulate DO, SRT, and HRT.
References


Research Objectives and Questions

The overall objectives of this work were to:

- Determine the contribution of bioflocculation, mineralization, and storage on the removal of particulate, colloidal, and soluble COD fractions under varying operating conditions that include DO, SRT, HRT, and temperature in a high-rate activated sludge process operated at less than 1 day SRT.
- Develop the control logic and strategies necessary to operate an A-stage HRAS process to achieve the desired effluent characteristics needed for emerging BNR processes that employ SND, nitrite shunt, and/or anammox and redirect carbon that is not needed for BNR to energy recovery processes.

The specific questions of this work were:

1. **What are the mechanisms of COD removal in high-rate activated sludge systems operated at sludge ages less than one day?**

   The current knowledge on COD remove mechanisms in very low SRT HRAS systems is rather limited. Due to the long SRT requirements for BNR, these mechanisms have largely been overlooked since carbon removal is rapid and almost complete even in low SRT processes (i.e., <2 days). While many have hypothesized that the primary COD removal mechanisms in the A-stage are intracellular storage of rbcCOD and adsorption by enmeshment of cCOD and pCOD, no definitive evidence has been produced that supports these claims. It is the goal of this work to elucidate the physical and biological mechanisms responsible for the removal of COD in HRAS processes operated at sludge ages less than 1 day. Understanding the COD removal mechanisms will support future design and modeling efforts and provide insight into how these mechanisms can be used to achieve different COD removal goals.

2. **What control parameters (i.e., DO, HRT, and SRT) can be effectively used to control COD removal with the goal of providing a desired input to a subsequent BNR process?**

   The successful operation of an AS process is dependent on the manipulation of control variables in order to meet certain removal criteria by selecting for desirable microbial populations.
Considering the fact that OHOs have fast metabolic and growth rates, it is actually quite difficult to limit COD removal in an AS process. The operating parameters not only affect the rate and amount of COD removal but they also affect the removal of different types of COD. Since the COD type being feed to a BNR process is important, controlling the COD fractions could help further optimize BNR processes. For example, rbCOD results in rapid phosphorus release and denitrification rates but sbCOD may be better suited for SND/nitrite shunt systems because of the slow release of rbCOD from hydrolysis of sbCOD.

3. Is it feasible to utilize ICA for the control of an A-stage HRAS process?

The ability to meet stringent permit limits on a consistent and reliable basis is in part due to the advancement of process control and associated instrumentation. While not as critical for an A-stage, since it always precedes another treatment process, some measure of ICA could benefit a plant’s overall nutrient removal or resource recovery goals. Providing a more consistent C/N ratio and load to a BNR process could also potentially provide greater process stability. Since no level of ICA has been documented on the A-stage, it is the goal of this work to develop simple process control strategies using advanced instrumentation and automation.

4. What modeling questions can be addressed through this work?

The current process simulators are not able to accurately model high-rate systems operated less than about 1 day SRT. This is primarily because enmeshment and bioflocculation was assumed to occur instantaneously and therefore was not included in the model matrices. While model development will fall on others, effort will be made to support their work in any way possible. This includes wastewater characterization of the influent and effluents, kinetic and stoichiometric parameter determination, and routine data collection.
Dissertation Outline

The literature that is available on high-rate activated sludge processes operated at low solids retention times is rather limited and typically only available in German or Dutch. Therefore, the aim of Chapter 1 was to summarize the existing literature as it relates to COD and nutrient removal mechanisms in the A-stage process and how high-rate processes can be used to capture COD for energy recovery. Additionally, the purpose of this chapter was to make connections between what is known about COD removal mechanisms in all activated sludge processes and the gaps of knowledge about very high-rate processes. Chapter 2 provides additional literature review of the topics not covered in Chapter 1.

The first step in identifying the COD removal mechanisms of the A-stage pilot study was to properly characterize the raw wastewater influent and effluent of the process. Chapter 3 covers the results from comparing physical-chemical and respirometry-based methods for the estimation of readily biodegradable substrate and the active fraction of heterotrophic organisms. While the respirometric methods were not accurate enough to properly characterize the influent and effluent of the A-stage, the batch tests provided some insight into the COD removal mechanisms, such as aerobic storage. Using the full physical-chemical influent and effluent characterization data and additional batch tests, COD removal mechanisms were further investigated as covered in Chapter 4. The primary COD removal mechanisms were determined to be near complete consumption of readily biodegradable COD, removal by sedimentation of the larger more flocculent particles including inert and slowly biodegradable COD, and production of colloidal material that was presumably unflocculated biomass resulting from the rapid consumption of readily degradable material. Aerobic storage was believed to be occurring, but was not quantified during this work.

Based on the results from Chapter 4 it was determined that the primary control parameter of the A-stage was the solids retention time and this could directly controlled using mixed liquor suspended solids based automatic process control as discussed in Chapter 5. Although not as
critical, dissolved oxygen set point control was also developed using a cascade controller. Part of
the motivation for the development of automatic controllers was that the downstream biological
nutrient removal process required a consistent influent carbon to nitrogen ratio for optimal
performance.

While optimal nitrogen removal was the primary overall objective of the A/B pilot study, a
secondary goal was recovery of excess carbon for energy recovery purposes. Recovery of this
carbon was in the form of excess sludge produced in the A-stage process a practical aspect of
operating a high-rate activated sludge processes, like the A-stage, is the settleability and
dewaterability of the produced sludge. **Chapter 6** summarizes the results from evaluating
settling and dewatering of the A-stage and B-stage sludges using conventional settling tests and a
novel dewatering tests. Although a detailed investigation was not conducted, the excellent
settleability and dewaterability of the A-stage sludge was attributed to the low extracellular
polymeric substance content of the mixed liquor.

As mentioned previously, carbon management and capture of the excess carbon is essential for
treatment facilities that have to balance nutrient removal with resource reduction. The purpose of
**Chapter 7** is to provide a holistic look at how carbon management within the A/B pilot can be
controlled to meet not only stringent nitrogen limits but also potentially obtain energy neutral
operation. While there is still considerable work to be done, the results contained within **Chapter
7** provide an insight into the capabilities of the A-stage to produce highly digestible sludge and
when coupled with shortcut nitrogen removal technologies could provide a viable alternative to
energy and resource intensive single-stage biological nutrient removal processes. To summarize,
**Chapter 8** provides the authors opinions on the significance of this work and future research
needs.
Chapter 1: A mechanistic review of high-rate activated sludge and its application for energy recovery from sewage

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Abstract:

Although high-rate activated sludge processes result in the destruction of the chemical energy present in municipal wastewater, this process has been gaining attention as a viable technology for achieving energy neutrality at water resource recovery facilities. To optimize carbon capture by high-rate processes a better understanding of the chemical oxygen demand (COD) removal mechanisms is needed. This paper addresses this by providing a review of the collective knowledge about activated sludge processes and how it relates to COD removal in high-rate systems. This includes design and operational aspects of high-rate systems operated at sludge ages below 1 day, carbon mass balances to determine the fate of COD once removed or captured, and discussions about specific COD removal mechanisms like adsorption, bioflocculation, and intracellular storage.
Introduction

Based on laboratory experiments conducted at the Lawrence Experiment Station (Massachusetts, USA), Arden and Lockett (1914) developed the activated sludge (AS) process where the motivation for its development was intensification of treatment of municipal sewage. In its early days, conventional AS (CAS) was used to achieve what was then considered a high degree of treatment (i.e., nitrification). It was later recognized that the primary pollutants of concern in sewage were total suspended solids (TSS) and dissolved organic matter (i.e., biochemical oxygen demand; BOD) and that nitrification of ammonia was not always required (Chase, 1944). Therefore, the treatment of sewage using the AS process could be further intensified by increasing the loading rate coupled with a reduction in aeration volume (Orhon, 2014). This AS process, where only TSS and BOD removal is performed (i.e., no nitrification), is referred to as high-rate activated sludge (HRAS) (Chase, 1944; Greeley and Dixon, 1943; Pasveer, 1954; Wuhrmann, 1954).

Building upon the work of Wuhrmann (1954), Böhnke (1977) developed the A-stage of the A/B (adsorption/bio-oxidation) process under various patents between 1977 and 1984 (de Graaff and Roest, 2012). The A/B process was born out of water quality regulations in Germany requiring nitrification, which meant longer solids retention time (SRT) and thus increased volumetric requirements (i.e., hydraulic residence time; HRT). The A-stage, and the technique of integrating this process with a subsequent biological nitrogen removal (BNR) B-stage, is the innovative element of the A/B process (Böhnke et al., 1997a). Unlike the HRAS process that was typically designed and operated to meet discharge standards (e.g., 30 mg/L BOD$_5$ and TSS), the purpose of the A-stage was only to remove a portion (30-70%) of the influent BOD (Böhnke et al., 1997b; de Graaff and Roest, 2012; Salomé, 1997), leaving sufficient organic carbon available for nitrogen removal in the B-stage. A comparison of the typical design and operating parameters for conventional AS (CAS), HRAS, and the A-stage are provided in Table 1.1.
The A-stage was a cost-effective means to reduce the organic load to the nitrification process (i.e., B-stage) in order to decrease the overall volumetric requirements by maintaining high nitrification rates and lower solids inventory when compared to a single-sludge nitrifying AS process preceded by primary sedimentation. Due to its benefits, the A/B process became popular in Europe in the 1980s with more than 50 municipal and industrial installations (Böhneke et al., 1998). However, in the 1990s effluent nitrogen limits were becoming commonplace thus requiring more influent BOD sent to the B-stage for denitrification. Therefore, many of the A/B plants were converted to single-sludge BNR plants (de Graaff and Roest, 2012).

Due to the high and rising cost of energy, the current focus of the remaining A-stages in Europe is to maximize the capture and redirection of organic carbon to anaerobic digestion for biogas production and subsequent combined energy and heat generation (Meerburg et al., 2015; Schaubroeck et al., 2015; Smith et al., 2014). The need for a reduced and controlled influent carbon to nitrogen (C/N) ratio has also generated a renewed interest in the A-stage prior to shortcut nitrogen removal technologies, such as mainstream nitritation-denitritation (nitrite shunt) and partial nitritation-anammox (anaerobic ammonia oxidation), referred to as deammonification (Lotti et al., 2014; Regmi et al., 2014; Wett et al., 2013; Winkler et al., 2012; Xu et al., 2015).

Although dozens of A/B plants have been built, literature on the A-stage is rather limited, usually dated, and not easily accessible to the international community (de Graaff and Roest, 2012). The literature that is easily available is usually descriptive of performance but very little insight is provided on the organic carbon removal mechanisms and transformation processes and how to
effectively design and control the process. This paper aims to summarize this literature and provide an outline for future research needs.

**Design and Applications**

The definition of an A-stage (Figure 1.1), according to Malz and Bili (1992), is a separate-stage HRAS process that is not proceeded by primary sedimentation, has an HRT less than one hour, operated at an SRT less than one day, and is loaded at a rate greater than about 2 kg BOD$_5$/kg MLSS·day (mixed liquor suspended solids). However, the authors acknowledge that this definition has only been loosely followed in practice (e.g., (Xu et al., 2015)) and proposes that the term A-stage should refer to any HRAS process that precedes a separate-stage BNR process. Conventional HRAS should be used when describing a non-nitrifying activated sludge plant that discharges directly after secondary treatment and disinfection.

![Figure 1.1](Image)

**Figure 1.1** – Process flow diagram of the A/B process where the B-stage is a Modified Ludzack-Ettinger (MLE) process. Aerobic (AER); Anoxic (ANX); Mixed Liquor Recycle (MLR); Primary Clarifier (PC); Return Activated Sludge (RAS); Secondary Clarifier (SCL); Waste Activated Sludge (WAS).

Some of the advantages of the A/B process when compared to single-stage BNR plants with primary sedimentation are (Böhnke, 1983; Müller-Rechberger et al., 2001; Winkler and Widmann, 1994):

- Improved treatment efficiency coupled with higher process stability for BOD and COD removal and nitrification

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1.5-2 times higher nitrification rates in the BNR process
30-35% lower specific volume required
50-70% lower specific aeration volume required
Lower specific energy costs as a result of less aeration required
Increased sludge production with higher organic content
Increased degradation of seemingly unbiodegradable compounds

The A-stage has also been used as a cost-effective biological buffer against nitrification inhibition at a plant with highly variable industrial inputs (Böhnke and Diering, 1978) and has been used to successfully treat high-strength industrial streams to include textile dying, pulp and paper mill, and leather tanning (Schulze-Rettmer and Kim, 1988; Schulze-Rettmer et al., 1992; Schulze-Rettmer and Zuckut, 1998).

A-stage designs have never been formally standardized as they were often just integrated into existing infrastructure. A-stages have been retrofitted into existing aerated grit removal tanks and wet-weather settling tanks (Diering, 1992). The Stolberg, Germany plant was configured with two A-stage trains in between existing aerated grit removal tanks and primary settlers (Feyen, 1992). The Utrecht, Netherlands (NL) sewage treatment plant (STP) A-stage is in square, complete-mix tanks while the Dokhaven (Rotterdam, NL) and Nieuweer (Breda, NL) STP A-stages are in rectangular, plug-flow tanks (de Graaff and Roest, 2012). The Garmerwolde STP (NL) A-stage was retrofitted into circular tanks (de Graaff and Roest, 2012).

**Process Control and the Impact of Operational Parameters on Performance**

**Hydraulic Residence Time**

Temperature, HRT, MLSS, and dissolved oxygen (DO) are all important aspects of HRAS (Chase, 1944). The HRT of the A-stage has been defined as 30 minutes (Malz and Bili, 1992), although it is not clear where this value originated from. The authors believe it is just a function of the loading rate, desired removals, and reasonable MLSS concentration for clarifier operation. Using a process model to analyze A-stage data from seven full-scale plants Demoulin (1998)
found that HRT had the greatest impact on COD removals while DO had a very limited impact, likely because heterotrophic organisms have a high affinity for DO and thus were not rate limited even at low DO concentrations. Wahlberg et al. (1994) evaluated the batch flocculation time of 30 full-scale AS plants and found that turbidity removal was 99% complete within 10 minutes of those plants. In a high-rate contact stabilization pilot, Zhao et al. (2000) demonstrated using batch tests on mixed liquor from a high-rate contact stabilization pilot study that MLSS impacted COD, sCOD, and turbidity removal (aerated for 30 minutes; G = 45 s⁻¹). The MLSS concentration had the most impact on sCOD removal increasing from 0 to 30% at 0.5 g/L MLSS to over 6 g/L, respectively. COD and turbidity removal both only increased from 25% to approximately 45%. This suggests that COD removal is dependent on MLSS but it is more important for sCOD removal. The same authors also saw that RAS flow had an impact on COD removal efficiency but not until it was less than 30% of the influent flow. Zhao et al. (2000) also showed that the resulting sludge age will be between 0.2-0.5 days. At these short sludge ages, the bacteria rapidly adapt to influent variations and environmental conditions and therefore prove to be an excellent buffer against shock loads (Böhnke et al., 1997b; Schulze-Rettmer and Zuckert, 1998).

At short SRTs, complex organisms, like flagellates and ciliates, which consume bacteria without actually reducing BOD (i.e., lower observed yield), are not retained in the A-stage because of their low growth rates (Böhnke et al., 1997b). Böhnke et al. (1997b) proposed that the A-stage bacterial community consists primarily of the same bacteria already present in the collection system, however, this has never been validated.

Solids Retention Time and Biomass Inventory

At an HRT of approximately 30 minutes and loading rate between 2-10 kg BOD₅/kg MLSS/day, the resulting sludge age will be between 0.2-0.5 days. At these sludge ages, the bacteria are rapidly adapted to influent variations and environmental conditions and therefore prove to be an excellent buffer against shock loads (Böhnke et al., 1997b).

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using molecular methods in a HRAS process. Using batch tests Henze (1989) showed that the influent biomass has a significant impact on the respiration rates of the AS process where the influent biomass can account for almost 50% of the active biomass in the process (Henze, 1986). Haider et al. (2003) determined that only a portion of the influent soluble biodegradable COD ($S_S$) is removed in the A-stage because the fastest growing organisms are selected for and they are not capable of degrading all the $S_S$. It may be that the organisms completely lacked the ability (i.e., genes) to express exoenzymes capable of hydrolyzing the remaining $S_S$ or the bacteria were never substrate limited and therefore those pathways were not active. Regardless, Haider et al. (2003) suggests the key to A-stage control is SRT based on microbial selection. Lee et al. (2015) investigated the influence of influent wastewater on the temporal dynamics of bacterial communities in full-scale activate sludge bioreactors and found significant differences between the bacterial communities but the impact of the influent communities on the AS communities was weak. This was attributed to the fact the AS processes were operated at long SRTs (6-20 days) and there was no correlation of SRT on community structure, possibly because the SRT was too long to see an impact. Lee et al. (2015) did note that the generalists bacteria that entered with the influent were still seen in the AS, suggesting they thrive under various environmental conditions or at least persist in the AS process.

**Dissolved Oxygen**

One of the least documented A-stage control methods is bulk DO control that is common in most AS processes (Åmand et al., 2013). Böhnke et al. (1997a) even suggests that a few of the early A-stages did not actually add additional air, meaning the only DO provided was already present in the raw wastewater influent (RWI) or after aerated grit removal. Böhnke (1983) clearly suggests that A-stages can be operated near zero DO in a facultative mode; however, actual DO concentrations were never reported and it seems unlikely that an A-stage would not be aerated. Based on a recent review of A/B plants in the Netherlands, de Graaff and Roest (2012) reported wide ranges of DO from 0.1-2 mg O$_2$/L but no indication was given if DO was controlled or the A-stages were simply aerated at a constant design airflow. Although not really a form of DO control but rather aerobic volume control, several A-stages include unaerated zones (e.g., Stolberg STP, Germany) or use intermittent aeration (e.g., Strass STP, Austria) for COD removal control.
What has been reported in the literature is the specific oxygen requirements as kg of O₂ consumed per kg of COD removed. Böhnke (1994) gave a range of 0.23-0.33 kg O₂/kg COD (assuming COD/BOD = 1.5) and Demoulin (1998) reported that the Salzburg STP (Austria) averaged 0.17 kg O₂/kg COD (design value of 0.4 kg O₂/kg COD). Jetten et al. (1997) also reported a value of 0.2 kg O₂/kg COD for A-stage processes. However, these values do not account for the actual amount of aeration required (i.e., airflow) that may result from diffuser fouling and reduced alpha factors typical of short SRT processes (Rosso et al., 2005). Böhnke et al. (1998) estimated a 20% overall energy savings of the A/B process compared to a similarly designed single-stage plant meeting the same treatment objectives. Böhnke et al. (1998) also reported that the A-stage accounted for 18-25% of the total specific aeration requirements in the full-scale Rheinhausen and Baesweiler STPs (Germany).

**Organic Carbon Removal Mechanisms**

Although it is not clear how these values were obtained, Böhnke et al. (1998) estimated that COD removal across an A-stage achieving 55% overall removal was 30% by settling, 15% by biological means (assimilation and oxidation), and 10% by adsorption onto settleable particles. These mechanisms have not been extensively studied in detail specifically for the A-stage, but many inferences can be made from the existing literature on the AS process.

**Sedimentation**

Böhnke et al. (1997b) states that the A-stage should not be preceded by primary sedimentation because influent active biomass is removed that would otherwise contribute to COD removal. Henze (1986) determined that influent VSS could consist of a wide range of active biomass (6-78%) and a portion of the active biomass was associated with the settleable material (Henze, 1989). Using enzymatic assays, Nybroe et al. (1992) found that settling and chemical precipitation of wastewater seemed to remove a larger fraction of the inert material leaving behind more of the active biomass. Influent biomass has been reported to range from 7-25% of the influent COD (Orhon and Çokgör, 1997), although a fraction of the biomass may originate from excess solids recycled within a plant (Kappeler and Gujer, 1992). The settleable, biodegradable material that would be removed by primary sedimentation is likely not degraded
in the A-stage because the retention time is too short for significant hydrolysis and subsequent oxidation to take place. The settleable material, both inert and biodegradable, may also act as a ballasting agent when enmeshed with lighter bioflocs leading to faster settling sludge as reported in the literature (Böhnke, 1994). This is akin to the dense-sludge high-rate clarification processes except the recycled solids are chemically conditioned by adhesion of bacterial biopolymers instead of by the addition of metal salts (Tchobanoglous et al., 2003).

**Bioflocculation**

Bioflocculation is the bacterial conversion of biodegradable substrate into extracellular polymeric substances (EPS) that form the structural matrix of aggregated bacteria referred to as flocs. EPS also enmeshes particulate and colloidal material and retains the necessary exoenzymes needed for the subsequent hydrolysis to transportable substrates (Frølund et al., 1995; Morgenroth et al., 2002). Although the A-stage is referred to as the adsorption process, Böhnke et al. (1998) only cited 10% COD removal by adsorption. This is likely because the SRT of the A-stage is too short for enough EPS production that would result in complete bioflocculation of the majority of colloidal and particulate material. Bisogni and Lawrence (1971) showed that at low SRTs, below about 0.5-1 days, in a complete-mix AS reactor fed synthetic wastewater, that 10-35% dispersed growth occurred suggesting a lack of adequate EPS for complete aggregation. The 30-minute HRT of an A-stage is not limiting in terms of bioflocculation time. It has been demonstrated by many researchers that when adequate EPS with available adsorption sites is available (i.e., SRT > 1.5 days), adsorption is rapid and complete within 15-20 minutes (Jimenez et al., 2007; La Motta et al., 2004; Wahlberg et al., 1994; Zhao et al., 2000). However, the HRT does limit the maximum reasonable SRT and MLSS concentration because of solids loading on the clarifiers.

Many researchers have found that the EPS in various microbial aggregates increases as SRT increase, which implies that bacteria produce more EPS under endogenous conditions (Sheng et al., 2010). Logan and Hunt (1988) proposed that the only common condition inducing aggregation is microbial starvation, since in batch cultures, bioflocculation was frequently observed at the end of logarithmic growth. Ehlers and Turner (2011) found a negative correlation between EPS production and F/M (food-to-microorganisms) ratio and a positive correlation
between EPS and aggregation. Since the A-stage is highly loaded, substrate is rarely limiting and thus EPS production may not be fully induced. Jimenez et al. (2007) compared SRT to EPS content in a AS pilot process (HRT=30 mins; DO=1.0 mg O$_2$/L) and found that EPS production increased linearly from 50 mg EPS/g VSS (as COD) to 105 mg EPS/g VSS with SRT values ranging from 0.3 to 1.0 days but remained constant after reaching 2 days SRT at about 125 mg EPS/g VSS. In a contact stabilization process operated under similar conditions (SRT=1.1-2 days; HRT=36 mins), Zhou et al. (2006) measured the EPS content of 16 mg EPS/g VSS (as COD).

**Mineralization/Oxidation**

Böhnke (1994) estimated that 20% of the COD removal in the A-stage is attributed to biological processes (i.e., growth). The fraction of oxidized COD is of course dependent on the HRT and SRT of the process. By performing a COD mass balance on a lab-scale SBR (sequencing batch reactor) HRAS process (12 hour HRT, 2-4 day SRT) treating abattoir wastewater Ge et al. (2013) found that the fraction of mineralized COD increased from approximately 25% at 2 days SRT to nearly 50% at 4 days. (Haider, 2002) estimated only 12% COD oxidation in a pilot-scale A-stage operated at 0.1 day SRT and Müller-Rechberger et al. (2001) estimated 14% COD oxidation in the A-stage of the Main Treatment Plant of Vienna (1 day SRT; after primary sedimentation).

Haider (2002) proposed that two different types of heterotrophic populations exists in an A/B process, ones which were able to degrade most of the soluble substrate but grow at slower rates and ones that only utilized the most easily degraded substrates like short chain fatty acids (SCFA) and grow at much faster rates. This was based on batch tests where A-stage ML with raw influent was aerated for periods longer than the HRT of the A-stage and no additional soluble substrate removal was observed beyond what was typically seen in the continuous flow reactor. The supernatant from this batch test was then transferred to B-stage ML and soluble substrate was rapidly consumed until reaching the estimated inert soluble COD concentration. Haider (2002) also confirmed that physical adsorption of soluble substrate was not occurring by spiking cyanide as a biotoxicant to stop all biological activity and measuring soluble COD (as flocculated and filtered COD according to (Mamais et al., 1993)) uptake, which did not occur.
Based on the work by Voncken (1985), Böhnke (1994) showed that difficult to degrade or longer carbon chain compounds were either removed or converted into shorter carbon chain compounds in facultative anaerobic A-stage. However, the produced compounds consisted of fermentation products (i.e., organic acids), which would easily be removed under aerobic conditions. Therefore, the statement that the A-stage results in more degradation of the sparingly degradable substances (Böhnke, 1983) may only be valid under facultative conditions and not fully aerobic conditions of most modern A-stages.

**Intracellular Storage**

Internal storage of substrate is a well-known phenomenon occurs in AS systems, most notably under feast/famine conditions (van Loosdrecht et al. 1997). Internal storage only occurs when electron acceptors (i.e., nitrate, nitrite, oxygen) are available because active transport and storage requires the expenditure of energy through the respiratory pathways. The known exceptions to this are phosphorus accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs), which derive their energy under anaerobic conditions from poly-P and glycogen, respectively. The two primary factors that result in storage are higher uptake rates than consumption rates when substrate is non-limiting or under substrate limitation such as nutrients or electron acceptors (Majone et al., 1999). In the A-stage process, depending on influent characteristics, substrate is generally non-limiting and storage has often been cited as a mechanism of COD removal. Haider (2002) only observed 3-12% storage of the influent $S_S$ in batch tests with A-stage ML (0.5 day SRT) and raw wastewater or acetate. Only 2% was measured in the pilot process, however, only PHB (polyhydroxybutyrate) was measured and no other storage compounds.

Carucci et al. (2001) found that aerobic storage (as PHB) only accounted for 18-22% of the $S_S$ removed in an AS plant operated around 16 days SRT. As soon as the $S_S$ was depleted degradation of the stored PHB occurred resulting in little accumulation of PHB in the waste sludge (Carucci et al., 2001). Aerobic storage kinetics have been evaluated in continuously fed pure cultures at short SRTs (van Aalst-van Leeuwen et al., 1997) but not in mixed culture processes <2 days SRT (Beccari et al., 2002; Beun et al., 2002; Beun et al., 2000; Carta et al., 2001). When the dilution rate or SRT of a process approaches the maximum specific growth rate
of an organism, PHB accumulation decreases (van Aalst-van Leeuwen et al., 1997). What is clear from the literature is that fast growing organisms, like those in the A-stage, store less substrate than slower growing organisms, therefore, storage may not play that important of a role in the A-stage process.

**Nutrient Removal Mechanisms**

A critical design parameter for achieving the desired nitrogen removal in the B-stage is the BOD and COD removal efficiency of the A-stage (Böhnke et al., 1997a). Maximizing yield in the A-stage increases nitrogen and phosphorus removal by assimilation (Böhnke et al., 1997a; Jetten et al., 1997), however, these nutrients will be recycled to the head of the plant if the solids are anaerobically digested and the centrate from dewatering is not treated (e.g., sidestream deammonification) or the nutrients are not recovered (e.g., struvite precipitation).

**Nitrogen**

The two primary mechanism of nitrogen removal in the A-stage are assimilation of ammonia (i.e., conversion to organic nitrogen; ON) and settling of solids containing ON. Given the short HRT and SRT of the process, it is unlikely that significant decay and ammonification is occurring, however this has never been investigated. The Main Treatment of Plant of Vienna, Austria (MTPV) averages 40% total nitrogen (TN) removal (85% TN removal in A/B process, effluent ammonia < 5 mg/L) via sludge withdrawal evenly split between primary solids (settled ON) and the combined waste activated sludges (WAS; assimilation of ammonia) from the A/B process (Wandl et al., 2009). Total Kjeldahl nitrogen (TKN) removal ranging from 12-44% has been reported for full-scale A-stages in the Netherlands (de Graaff and Roest, 2012; Jetten et al., 1997), however, all of these plants have effluent recycle that returns nitrate to the A-stage for denitrification.

**Phosphorus**

Several A-stages were built with anaerobic zones for the cited purpose of enhancing biological phosphorus removal (EBPR) (Bio-P) (Diering, 1992; Feyen, 1992). However, Bio-P has been shown to have a temperature dependent critical SRT where release and uptake of P ceases at an
SRT around 1.5 days at 23°C increasing linearly to 3.5 days at 5°C (Erdal et al., 2006; Ge et al., 2015; Mamais and Jenkins, 1992; McClintock et al., 1993). The Krefeld STP (Germany) was upgraded to three A-stage trains with an anaerobic RAS tank that was intended to enhance Bio-P removal but iron salts were added for P removal (Diering, 1992). A report by de Graaff and Roest (2012) gave iron addition rates between 0.24-0.79 (mol Fe$^{3+}$/mol influent P) corresponding to TP removals ranging between 48-68% in full-scale A-stages.

Like nitrogen, phosphorus is also removed in the A-stage by assimilation. Böhnke (1994) estimated a single-stage C and N removal plant (with primary sedimentation) only assimilates 40% of the influent TP compared to the A/B process that averaged 53% TP removal. In this comparison, the A-stage accounted for 38% of the TP removed. Böhnke et al. (1997b) proposed that the A-stage also achieves some TP removal by adsorption, however, this was never quantified.

**Methods in Improve Nitrogen Removal in the A/B Process**

**A-stage Bypass**

A/B plants have bypassed raw wastewater influent (RWI) around the A-stage as a strategy to increase nitrogen removal in the B-stage (Figure 1.2). Similarly, single-sludge plants have bypassed primary sedimentation and sent RWI directly to anaerobic or anoxic zones. However, there are negative impacts of bypassing settleable solids directly to the BNR process. Note that unlike the A-stage bypassing primary sedimentation does not increase the soluble organic load (i.e., soluble BOD) since soluble compounds are not removed by settling. van Loosdrecht et al. (1997a) compared the performance of a full-scale modified UCT (University of Cape Town) process without and with 100% bypass. The nitrification efficiency decreased from 84% to 73%, presumably because the SRT rapidly decreased from 45 to 17 days, although nitrogen removal increased due to ammonia assimilation by heterotrophs. In addition, bypassing the settleable material drastically altered the energy balance of the plant because the particulate matter increased aeration demands and was oxidized to CO$_2$ instead of being directed to anaerobic digestion for methane and energy production. Similarly, Gori et al. (2011) came to the same conclusion when modeling the impact of influent sCOD/COD ratio on a full-scale BNR (anaerobic/aerobic; A/O with anaerobic digestion) facility’s carbon and energy footprint. Also,
secondary sludge is less digestible than primary sludge further reducing methane production and increasing the amount of solids for ultimate disposal (Gossett and Belser, 1982; Parkin and Owen, 1986; van Loosdrecht et al., 1997a). At another full-scale modified UCT plant, Puig et al. (2010) bypassed 27% of the RWI around the primary clarifiers and did not see an improvement in effluent quality or nutrient removal efficiency. Winkler and Widmann (1994) demonstrated that nitrogen removal in an optimized A/B pilot operated with RWI bypass could be matched to that of a single-stage BNR process. In this case, RWI was bypassed directly to the A-stage clarifier in order to remove the settleable material.

![Diagram](image)

Figure 1.2 – A/B process including RWI bypass.

During pilot-scale testing of an A/B process for the upgrade of the Main Treatment Plant of Vienna (MTPV), Austria, Müller-Rechberger et al. (2001) found that overall nitrogen removal was enhanced with a bypass of 10–40% of settled influent because of increased denitrification, however, nitrification rates were reduced by 30% (Wandl et al., 2002). Wandl et al. (2002) proposed that the reduction in rates was due to either a reduction of autotrophic biomass because of higher SVIs (sludge volume index; increased from 90 to >150 mL/g) and lower SRT or inhibitory effects on autotrophs. In a two-stage moving bed biofilm reactor (MBBR), Åsøy et al. (1998) reported that a COD to total ammonia nitrogen ratio (COD/TAN) greater than 3.5 resulted in decreased nitrification rates (20–30% reduction) when compared to the maximum nitrification rates and the reduction was attributed to particulate and colloidal COD. Similarly, in a rotating biological contactor (RBC) pilot, Figueroa and Silverstein (1992) measured reduced
nitrification rates when the organic load was increased except both particulate and soluble BOD had an equal impact. Figueroa and Silverstein (1992) postulated that competitive inhibition by heterotrophs (i.e., for DO) was responsible for the reduction in rates.

**B-stage Effluent Recycle**

Returning B-stage effluent (Figure 1.3) to the head of the plant is an efficient method to improve N removal because the ammonia is oxidized in the B-stage (i.e., high nitrification rates) and sent to the A-stage where rbCOD is available for rapid denitrification (Emde et al., 1992). Another possible benefit of effluent recycling is that maintaining a constant flow to the plant by adjusting the recycle flow based on the influent flow results in a constant hydraulic load on the clarifiers minimizing the potential of solids loss during wet-weather events (Armbruster et al., 2001). The main disadvantage is that effluent pumping can be quite costly (WEF, 2009). Additionally, if the plant is trying to reach very low nitrate concentrations the recycle flow must increase thus decreasing the A-stage performance by diluting the influent.

![Figure 1.3 – A/B process including effluent recycle.](image)

All of the A/B plants in the Netherlands use effluent recycling and these recycle flows vary from 0.3-1.6 times the influent flow (de Graaff and Roest, 2012). Denitrification in the A-stages of these plants accounted for 1-6% of the influent COD removed (de Graaff and Roest, 2012).
During pilot testing for the upgrade of the MTPV, 15-20% N removal by denitrification in the A-stage was achieved and 25-30% in the B-stage with an average overall TN removal efficiency > 80% (Wandl et al., 2009).

**Mixed Liquor Transfer**

Mixed liquor (ML) transfer between the A- and B-stages (Figure 1.4), patented as the Hybrid process, was developed as a result of increasing the nitrogen removal efficiency of a A/B package plant (Matsché and Moser, 1993). Prior to implementation of ML transfer, both waste sludges were aerobically stabilized before disposal. This stabilization tank was incorporated into the A-stage effectively making it a contact stabilization process except for the fact that B-stage waste solids were also sent to the stabilization tank. This resulted in nitrification and denitrification in the A-stage that was otherwise devoid of autotrophic organism because of the short sludge age. Further modification of this process included directly pumping ML from each process to the other to provide organic carbon for denitrification in the C-limited B-stage. It is possible that the enhanced denitrification observed in the B-stage (Matsché and Moser, 1993) was due to the transferred A-stage heterotrophic organism utilizing internally stored carbon for denitrification (van Loosdrecht et al., 1997b). Unlike effluent recycling, the flow from the A-stage to the B-stage was only 15% as not to impact the hydraulics (Matsché and Moser, 1993). For the MTPV, the Hybrid process does not impose a considerable hydraulic load on the plant as the ML transfer flows are operated in the range of 3-5% of the influent load (Winkler et al., 2004). The diluting effect of recycling B-stage ML to the A-stage did result in a reduction of COD removal efficiency of the A-stage, which necessitated altered operation to obtain a higher loading rate (i.e., shorter HRT) (Matsché and Moser, 1993).
Figure 1.4 – A/B process including MLSS recycles.

The Hybrid process has been applied at A/B plants that have primary sedimentation (e.g., Saalfelden WWTP, Austria) and average COD removal of 80% in the A-stage (Winkler et al., 2004). The MTPV was upgraded from a conventional HRAS process (SRT=1.5 days) with primaries to an A/B process by adding a B-stage. To improve nitrogen removal, the plant was designed with the option to operate in Hybrid mode (Müller-Rechberger et al., 2001). This includes sending all of the B-stage waste sludge to the A-stage not only to enhance nitrogen removal and COD removal but also to blend the sludges before thickening and incineration (Wandl et al., 2006).

Shortcut Nitrogen Removal in the B-stage

With the development of reliable on-line and in situ sensors that measure DO and nitrogen (i.e., nitrate, nitrite, ammonium) more cost-effective methods to improve nitrogen removal in the B-stage were developed. A prime example of this is ammonia-based aeration control (ABAC) where the DO setpoint or airflow rate is determined based of an effluent ammonium setpoint (Åmand et al., 2013). This provided the ability for a B-stage to operate in a simultaneous nitrification/denitrification (SND) mode because the DO was low enough in the aerobic zones to allow denitrification to occur (Feyen, 1992). SND has generally been used in plants that are extended aeration or oxidation ditch style configurations because adequate HRT is available for to account for the decreased nitrification rates at lower DO concentrations (Bertanza, 1997; Collivignarelli and Bertanza, 1999; O’Neill and Horan, 1995). Another technique that has been used that is similar to SND, except that nitrification and denitrification occurs at different times.
but not necessarily spatially, is aerobic/anoxic HRT or volume control. Using on-line ammonium and nitrate values, Sorensen et al. (1994) achieved a reduction in effluent TN from 7.8 mg/L to 5.1 mg/L in an A/B plant (Marselisborg WWTP Aarhus, Denmark) where the B-stage was a Biodenitro™ process.

The MTPV uses optimal aerobic volume control (OAV-control) based on continuous in-line oxygen uptake measurements to provide the minimum aerobic volume to achieve an effluent ammonium setpoint while maximizing the anoxic volume for denitrification (Svardal et al., 2003; Wandl et al., 2009).

Since the discovery of anammox bacteria (Mulder et al., 1995; van de Graaf et al., 1995) and its incorporation into sidestream treatment of anaerobically digested sludge liquor streams, now considered a mature technology (Lackner et al., 2014), the focus has been on the inclusion of anammox bacteria into the mainstream process (e.g., B-stage). However, the challenge is the out-selection or repression of nitrite oxidizing bacteria (NOB) that compete with anammox bacteria for nitrite (Xu et al., 2015). One primary strategy is the selective retention of anammox bacteria and decoupling of SRT to promote washout of NOB (Regmi et al., 2014). This has resulted in a variety of configurations for both the A-stage and B-stage that will not be discussed in detail in this paper. Briefly, the Strass WWTP (Austria) is bioaugmenting ammonia oxidizing bacteria (AOB) and anammox bacteria from their sidestream DEMON® process to the mainstream (Wett et al., 2013). Lotti et al. (2014) investigated the feasibility of retaining anammox bacteria in aerobic granules in the B-stage of the Dokhaven WWTP (Rotterdam, Netherlands). Similarly, Ma et al. (2011) operated a bench-scale two-stage process where the second stage was a granular UASB (up flow anaerobic sludge blanket) process with anammox. De Clippeleir et al. (2013) evaluated the performance of a bench-scale rotating biological contactor (RBC) process operated under oxygen-limited autotrophic nitrification-denitrification (OLAND) conditions and treating diluted raw influent (simulate treated sewage). Following a pilot-scale A/B where nitrite shunt was occurring in the B-stage, Regmi et al. (2014) operated a fully anoxic anammox MBBR. In all cases, the goal was to develop a process that uses very little to no organic carbon for nitrogen removal so that the influent carbon could still be captured for energy recovery via biogas production.
Settling and Solids Handling

**Settling**

Full- and pilot-scale experience has shown that A-stage ML settles well with reported SVIs typically below 60 mL/g (Böhnke, 1994). This is contrary to the misconception in the United States that the A-stage process does not settle well and the authors believe this is rooted in the experience of longer SRT and completely mixed (CM) HRAS processes that do settle poorly unless adequately aerated (Palm et al., 1980). For example, several authors measured SVI at SRTs ranging from 0.5-12 days in CMAS pilots and observed high SVIs around 2-4 days but lower SVIs at longer (>4 days) and shorter (<2 days) SRTs (Bisogni and Lawrence, 1971; Chao and Keinath, 1979; Stewart, 1964). Similarly, when compared to loading rate, SVIs increased as loading rate increased but declined when loading rates increased to 2 kg BOD₅/kg MLSS·day. Tomlinson (1976) also observed that when the contact zone loading rate of a contact stabilization process rate was greater than 2 kg BOD₅/kg MLSS·day, SVIs decreased.

Although it is not clear why A-stage processes settle well it seems likely that the short SRTs and high loadings either results in out-competition of filamentous bulking organisms or the SRT is too short to retain them in the process. One of the only cited instances of a poorly settling A-stage was during the pilot- and full-scale testing at the MTPV when operated in bypass mode (Wandl et al., 2002; Wandl et al., 2009). Bypass mode resulted in SVIs >200 mL/g in the B-stage and >150 mL/g in the A-stage attributed to decreased loading below 2 kg BOD₅/kg MLSS·day. When the operation of the MTPV was returned to Hybrid mode settling of both stages improved (Wandl et al., 2002; Wandl et al., 2009).

**Solids Handling**

The A-stage produces 75-90% and the B-stage 10-25% of the total sludge produced in an A/B process (Böhnke, 1983; 1994; Schulze-Rettmer and Zuckut, 1998). A typical split for single-stage plants is 60% primary solids and 40% secondary WAS. The specific amount of sludge production in the Stolberg STP (Germany) was estimated as 60 g/PE/day in the A-stage and 15 g/PE/day in the B-stage (Feyen, 1992). When compared to single-stage BNR plants with primary sedimentation, the A/B process produces 10-15% more sludge overall as dry solids.
The sludge produced by an A-stage has better digestion characteristics compared to normal secondary sludge (i.e. single-stage WAS), which results in a lower overall sludge production after digestion when compared to a single-stage BNR process with primary sedimentation (van Loosdrecht et al., 1997a). Gossett and Belser (1982) found that decreasing the SRT of the AS process (from 30 to 5 days) increased sludge production and methane production but overall very little change in the quantity of solids for disposal because of the increased degradability during anaerobic digestion. The specific sludge production in the A-stage per kg COD removed (i.e., observed yield) is higher than single-stage BNR processes, which results in higher specific gas production (Winkler et al., 2008).

(Böhnke, 1994) states that A-stage sludge can easily be thickened to 6-8% solids while the B-stage only to 3.5-4% although no explanation as to why the A-stage thickens well is provided. One theory is that A-stage solids contain very little EPS (Jimenez et al., 2007; Zhou et al., 2006) and filamentous bacteria (based on low SVIs). Houghton et al. (2001) proposed that the effect of EPS on the dewatering ability of sludge depended on the content of EPS in sludge. Using sludge samples from eight wastewater treatment plants, it was determined that the dewatering ability of activated sludge initially increased with the EPS content, but then decreased once the EPS content exceeded a certain threshold (35 mgEPS/ gSS) . Li and Yang (2007) also found that loosely bound EPS impacted settling and dewaterability. The fact that EPS are highly hydrated and are able to bind a large volume of water was previously confirmed by Forster (1983).

**Energy Recovery**

Several attributes of the A/B process poise it for energy self-sufficiency and even positive energy operation. These include high observed yields in the A-stage of highly digestible sludge and energy-efficient removal of COD and nitrogen if advanced process control, like ammonia-based aeration control, is used in the B-stage. A prime example is the Strass WWTP (Austria) that achieves energy self-sufficiency (Schaubroeck et al., 2015).

When compared to a single-stage BNR process, the A/B process requires 20-35% less aeration volume (Böhnke, 1994; Winkler and Widmann, 1994). COD removal in the A-stage has been reported to only require 0.20 kg O₂/kg COD removed or 0.039-0.169 kWh aeration/kg COD
removed accounting for the aeration system and oxygen transfer efficiency (Jetten et al., 1997; de Graaff and Roest, 2012). The MTPV reported a specific energy consumption of 21.9 kWh/PE (Partaj et al., 2008) and recovers heat and generates electricity by incinerating solids (Wandl et al., 2009).

Several researchers have demonstrated that as SRT decreases (30 to 0.4 days) the rate of degradation, degradability, and methane production from anaerobically digested sludge increases (Ge et al., 2013; Gossett and Belser, 1982; Mottet et al., 2010). Gossett and Belser (1982) predicted that at very low SRTs the ultimate VSR (volatile solids reduction) would be between 65-70% with 250-300 L methane (at 35°C)/kg COD fed. In a HRAS pilot treating abattoir wastewater, Ge et al. (2013) achieved 60% and 80% VSR at 4 and 2 days SRT respectively and approximately 250 L methane (at 35°C)/kg COD fed at 2 day SRT. By redirecting carbon to anaerobic digestion the A-stage at the Dokhaven WWTP (Rotterdam, Netherlands) is able to produce 0.5 kg methane/kg COD removed (Jetten et al., 1997).

Coupled with lower energy requirements for carbon capture and less aerobic carbon oxidation, the lower the SRT of the A-stage the greater the energy production potential per unit carbon removed (Gossett and Belser, 1982). However, there is an optimal SRT where carbon capture (i.e., sludge production as WAS) is maximized as demonstrated in Figure 1.5. This plot is based on the work by Jimenez et al. (2015) who measured the COD removal efficiency of a complete-mix HRAS pilot fed unsettled municipal wastewater at varying SRTs. The difference between COD removal efficiency and the fraction of COD captured as WAS is the fraction of influent COD that is oxidized.

This data demonstrates that COD capture is maximized between an SRT of 0.25-0.4 days. Below about a 0.2 day SRT less COD is oxidized but only a small fraction is removed from the wastewater. This figure also suggests that beyond an SRT of 1 day an increasing fraction of COD is hydrolyzed and oxidized, including a fraction of primary solids. Therefore, if a HRAS process is operated at longer SRTs it should be preceded by primary sedimentation in order optimize carbon capture for energy recovery. Similar studies, although typically with limited SRT ranges, report comparable COD removal efficiencies (de Graaff and Roest, 2012; Ge et al., 2013; Haider, 2002; Müller-Rechberger et al., 2001). However, similar trends of COD captured
as WAS are not discernable, likely because influent wastewater characteristics play a significant role in the fractions of COD oxidized versus captured.

![Figure 1.5 – COD mass balance of a complete-mix HRAS pilot study without primary settling adopted from Jimenez et al. (2015). The HRT of the process was 30 minutes and DO was maintained at 1.0 mg O$_2$/L. The horizontal line (▬) represents assumed 30% COD removal by primary sedimentation; ■ effluent COD; ▣ COD oxidized; □ COD captured as WAS.]

### Modeling

The IWA (International Water Association) activated sludge models (No. 1, 2, 2d, and 3) do not include EPS production and bioflocculation because bioflocculation is assumed to be instantaneous and complete at SRTs greater than 3 days (Gujer et al., 1999; Henze et al., 1986; Henze et al., 1995; Henze et al., 1999). However, A-stages are operated well below 3 days SRT and bioflocculation is limiting, therefore, these models do not accurately predict effluent quality, particularly soluble and colloidal COD. Also, these models assume single substrate kinetics that are independent of SRT and based on observations this assumption may not be applicable to the A/B process (Haider et al., 2003). Initial attempts to model the A/B process using ASM1 (Henze et al., 1986) included fractionation of the influent COD into soluble readily degradable (S$_S$),
particulate slowly degradable ($X_S$), and soluble unbiodegradable ($S_I$) fractions (Freund et al., 1996). Demoulin (1998) also attempted to model the A/B process but with limited success because the difficulty with fractionation of the COD leaving the A-stage. Winkler et al. (2001) made an attempt to model the A/B process (MTPV) by combining ASM1 and ASM3 into an “ASMVienna” model but had very limited success due to the complexity of COD removal when using bypass or Hybrid mode. These early models did not properly predict COD removal particularly through the intermediate clarifiers.

Haider (2002) proposed using a dual substrate model by splitting $S_S$ into $S_{SA}$ ($S_S$ in A-stage) and $S_{SB}$ ($S_S$ in B-stage) where $S_{SB}$ is considered $S_I$ in the A-stage. This was based on the hypothesis that the A-stage retains the fastest growing organisms that are only capable of degrading a portion of the influent $S_S$ and the B-stage retains slower growing organisms that can consume the remaining substrate (Haider et al., 2003). A higher maximum specific growth rate was also used for the A-stage than the B-stage but the same half-saturation could be used for both processes provided it was sufficiently low to allow complete degradation of the respective $S_S$ fractions (Haider et al., 2003). Haider (2002) accounted for bioflocculation by including a function that converted a portion of the influent particulate matter into settleable material that was removed by the clarifier model.

Using ASM1 as the initial framework, Nogaj et al. (2015) developed and compared two different model structures where both incorporated dual substrates like Haider (2002). The influent $S_S$ was further fractionated into $S_{BF}$ (soluble biodegradable; fast) and $S_{BS}$ (slow) where $S_{BF}$ was measured as the influent SCFA (i.e., volatile fatty acids; VFAs) and $S_{BS}$ was the difference between $S_S$ and $S_{BF}$. One model was analogous to diauxic growth in terms of substrate consumption (i.e., $S_{BF}$ prior to $S_{BS}$) and the second with simultaneous consumption but at different rates. EPS production and subsequent bioflocculation of particulate and colloidal COD and intracellular storage of $S_{BF}$ were incorporated into both models. Nogaj et al. (2015) found that the dual substrate model with simultaneous consumption of $S_S$ better predicted the results from two A-stage pilot studies.
Research Needs

While there is certainly a wealth of knowledge on activated sludge processes there is only a very limited amount detailed research specific to the A-stage process. The following are specific research areas that would further the understanding and operation of A-stages:

- Quantify intracellular storage and the impact of wastewater characteristics and operational parameters on storage compound types and quantity.
- Determine the impact of intracellular storage on biogas production from anaerobically digested sludge.
- Develop process control strategies using on-line instrumentation that targets a COD removal goal or optimal COD capture as WAS.
- Better quantify EPS production and EPS components as a function of influent characteristics and A-stage operation.
- Determine the impact of influent bacteria on COD removal and the bacterial communities in the A-stage.
- Develop mechanistic models that can accurately simulate COD removal across the A-stage including the intermediate clarifier.
References


de Graaff, M.; Roest, K. (2012) Inventarisatie van AB-systemen - optimale procescondities in de A-trap, STOWA.


Wandl, G.; Schaar, H.; Papp, M.; Svardal, K. (2009) The first two years of full scale operation of the two-stage Main Wastewater Treatment Plant of Vienna. *Water Practice & Technology* 4(1).


Chapter 2: Literature review of topics not covered in Chapter 1

Treatment facilities utilize three general types of carbon removal unit processes either separately or in some combination depending on the treatment objectives. These include removal by physical means such as gravitational settling, the use of chemicals to either degrade compounds or enhance physical removal (coagulation/flocculation), and removal by biological means. A few physical and chemical processes will be discussed briefly as they relate to this work, while biological mechanisms will be reviewed in detail.

Physical Removal Mechanisms

The removal of suspended materials from wastewater by gravity separation is one of the most widely used unit operations in wastewater treatment because of its simplicity and low energy demand. Efficiently designed and operated primary sedimentation tanks should remove from 50 to 70% of the TSS, 25 to 40% of the BOD, and 20 to 35% of the COD (Tchobanoglous et al., 2003). One of the limitations of primary sedimentation processes is that soluble and colloidal constituents are not removed, which constitutes a large fraction of organic carbon that could otherwise be used for biogas production.

Chemical Removal Mechanisms

Chemical coagulation and flocculation is used as a means of improving the performance of primary sedimentation processes, commonly referred to as chemically enhanced primary treatment (CEPT), with potential removals of 80 to 90% TSS including some colloidal particles, 50 to 80% BOD, and 45 to 80% COD (Tchobanoglous et al., 2003). Chemical phosphorus removal by precipitation using metal salts may also be performed when a plant does not do biological phosphorus removal or has stringent discharge limits. The most common inorganic coagulants used for CEPT are ferric chloride, alum, and lime. Additionally, flocculating agents, like organic polyelectrolytes (polymers), are used to enhance flocculation of particulate and colloidal material. Some of the disadvantages of CEPT are the production of additional solids that contain inert compounds (metal salts) and decreased dewaterability of the primary sludge.
Additionally, when CEPT is used for phosphorus removal, chemical addition to the raw influent is not the most efficient in terms of dose required (Tchobanoglous et al., 2003). El-Gohary et al. (1991) introduced alum, ferric chloride, and lime in domestic wastewater treatment and obtained turbidity removal of 83 to 98%, and a corresponding COD removal in the range of 77 to 86%. Gambrill et al. (1992) reported that single-stage lime treatment of raw domestic wastewater resulted in high removal efficiencies of turbidity, COD, total phosphorous, and suspended solids of 92%, 89%, 96%, and 91 %, respectively. Polymer addition to secondary settling processes to enhance solids capture is also common since the majority of organic matter discharged from activated sludge systems is particulate. In addition to being oxygen demanding, effluent solids contain significant concentrations of the nutrients nitrogen and phosphorus (Wahlberg et al., 1994).

**Biological Removal Mechanisms**

Biological removal of constituents from used water is the most cost-effective and widely used method of constituent removal. This can occur by retaining and keeping biological material or biomass in suspension (i.e., activated sludge) or by providing a surface for the biomass to adhere to referred to as attached growth. The following sections speak directly to suspended growth type systems; however, the mechanisms are generally the same for both.

*Oxidation and Growth*

All organisms require carbon, energy sources, and other macro and micronutrients that include nitrogen and phosphorus. Bacteria replicate by binary fission and during batch growth experiments undergo several growth phases that include lag, exponential, stationary, and death. Biomass concentration and substrate concentration both play a role in substrate utilization (Lawrence and McCarty, 1970). Microbial metabolism and growth have been study extensively and typically following Monod kinetics.

*Bioflocculation and Enmeshment (Biosorption)*

The premise of activated sludge is the retention of bacteria in suspension at a concentration necessary to remove the influent constituents of concern within the provided HRT of the process.
The bacteria aggregate to form biological flocs and this act of forming flocs or aggregates is termed bioflocculation. Good bioflocculation enhances the settling characteristics and allows for proper physical separation such that effluent standards can be met. Physical conditioning of activated sludge to improve suspended solids capture without the addition of chemicals was first studied by Fischer and Hillman (1940). Parker et al. (1971) showed that diffused and mechanical aeration results in shearing of the flocs and reflocculation is required to reduce the amount of dispersed solids leaving a solids separation process. Consequently, the minimization of fine flocs in the activated sludge process is essential for better effluent quality and for improved dewatering properties of sludge (Neyens et al., 2004).

Activated sludge flocs are considered to be a collection of several particles, (e.g., primary particles and microcolonies), held together by different kinds of interparticle forces such as bridging by divalent cations and extracellular polymeric substances (EPS) and hydrophobic interactions (Higgins and Novak, 1997; Sobeck and Higgins, 2002; Urbain et al., 1993). Forster (1983) confirmed that one of the most significant surface polymers for activated sludge is a polysaccharide and has shown that surface proteins are also important. Due to the prevalence of EPS in AS flocs, Li and Ganczarczyk (1990) suggested that EPS should be treated as the third major floc component besides microorganisms and water. Once formed, these flocs enmesh particulate and colloidal compounds present in the liquid medium resulting in the rapid removal of unbiodegradable and biodegradable particulate material without significant mineralization depending on the retention time of the bacteria.

The extracellular material that is external to the bacterial cell wall is called glycocalyx (plural glycocolyces), which includes the extracellular matrix (ECM). The ECM includes extracellular polysaccharides or more generally extracellular polymeric substances (EPS). The term EPS is used as a more general and comprehensive term for different classes of organic macromolecules such as polysaccharides, amino sugars, proteins, nucleic acids, uronic acids, lipids, and other polymeric compounds which have been found to occur in the intracellular space of microbial aggregates, more specifically at or outside the cell surface (Bitton, 2011; Flemming and Wingender, 2001a). One of the main components of EPS, polysaccharides, are polymerized from nucleotide derivatives of the sugars that are made in the cytoplasm and then transported outside of the cell (White, 2007). Synthesis of exoenzymes occurs as part of protein synthesis system
(PSS). In natural environments the predominate component of EPS is polysaccharide (White, 2007). However, it has been well documented that activated sludge has a much higher protein content attributed to the abundant amount of substrates in sewage and the presence of large quantities of exoenzymes (Flemming and Wingender, 2001a; Frølund et al., 1995).

EPS can either be capsular (closely associated), slime (loosely associated), or not associated at all and free in solution. Capsular EPS is covalently bonded (hydrophobic anchor) to the phospholipids (or lipid portions) imbedded in the cell wall. The slime layer EPS is thought to only be associated with the cells through hydrophobic forces and cation bridging (White, 2007). Li and Yang (2007) demonstrated that slime layer EPS did not vary significantly between solids retention times (SRTs) of 5, 10, and 20 days or feed type (glucose and acetate). However, glucose fed reactors did show more loosely bound EPS. Because of the analytical difficulty of extracting EPS, it is not well understood whether free EPS is excreted or simply detached from the slime or capsular layers.

There are several theories why bacteria produce and excrete EPS with the overarching purpose being to maintain an environment in which microbial life is possible. These theories include:

- Protection from predation and phagocytosis
- Hydraulic selection (resulting in biofilm, granule, or floc formation)
- Live in close association in order to exploit synergistic or symbiotic relationships
- Increase substrate availability and uptake
- Remain in favorable niches
- Protection from desiccation
- Protection from toxins such as metals and antibiotics
- Protection from oxidative stresses

Recent studies have also found that some bacteria use quorum sensing after adherence to a surface and population enlargement to initiate matrix or EPS formation (Madigan and Brock, 2009). The fact that EPS are highly hydrated and are able to bind a large volume of water was confirmed by Forster (1983). EPS therefore play an important ecological role in protecting biofilm organisms from desiccation (Flemming and Wingender, 2001b). Logan and Hunt (1988)
proposed the only common condition inducing aggregation is microbial starvation, since in batch cultures, bioflocculation was frequently observed at the end of logarithmic growth. Ehlers and Turner (2011) also found a negative correlation between EPS production and F/M (food-to-microorganisms) ratio and a positive correlation between EPS and aggregation. It is also common in lab cultures that EPS is nonexistent because the conditions are rarely substrate limiting. However, a review by Sheng et al. (2010) concluded that bacteria would produce more EPS under unfavorable conditions like substrate type (e.g., inhibitory compounds).

It used to be thought that EPS production only occurred during the endogenous phase of growth (Parker et al., 1971), however, advances in analytical techniques prove otherwise. Ni et al. (2009) demonstrated that EPS formation occurred during external substrate consumption and EPS concentrations did not change significantly after the substrate was depleted. Long-term experiments (>20 hours) did show a slow decline in EPS concentrations, presumably due to EPS degradation because of microbial starvation. This could possibly explain pin floc and effluent quality degradation in systems operated with excessively long SRTs. Studies by Van Dierdonck et al. (2012) observed bioflocculation in reactors with different loading rates. A nominal reactor loading rate (RLR) of 0.70 g COD/L·d led to a well-flocculated activated sludge, whereas a RLR of 0.175 g COD/L·d yielded fragmented sludge, and a RLR of 1.75 g COD/L·d led to increased erosion presumably because of more collisions between the sludge flocs. The fragmentation of the sludge under low RLR is possibly the result of the change in the EPS.

Hydrolysis

Since only small and simple molecules (e.g., acetate and glucose) can be transported across bacterial cell membranes, complex substrate must undergo stepwise depolymerization and hydrolysis in order to be available for metabolism (Chróst, 1991). Extracellular enzymes are produced as part of the PSS and released by the organisms, many of which are hydrolases (add or remove water without oxidation or reduction), but they can be specific cellulases (cellulose), amylases (starch), lipases (lipids), and proteases (protein), or they can be general or nonspecific enzymes capable of degrading a variety of compounds. The production of extracellular enzymes cost the organism in terms of energy and materials (Vaccari et al., 2006).
Work by Frølund et al. (1995) suggests that exoenzymes most likely accumulate in the ECM and should be considered as an integral part of the matrix and not strictly connected to bacteria’s outer membranes. The finding of enzymes in the EPS matrix also suggests that EPS has an additional role in activated sludge, not only to combine the activated sludge floc components, but also to act as a sink for immobilized exoenzymes. This immobilization leads to the biodegradation potential of the activated sludge being much higher than that accounted for by the number of bacteria present in the sludge. Considerable advantages may arise if the enzymes are adsorbed in the EPS matrix, for example, enzymes trapped in the EPS of activated sludge may be less susceptible to degradation and are in close proximity to any macromolecular substrates adsorbed in the floc (Frølund et al., 1995). It has been known for some time now that hydrolysis is typically the rate-limiting step in substrate metabolism pathways. Using both pure and mixed cultures under different electron acceptor conditions Goel et al. (1999) showed little change in the specific hydrolysis rate when the extracellular enzymes were EPS bound and stable with low turnover rates. However, hydrolytic enzyme production was affected by electron acceptor conditions but was less pronounced in the mixed culture system.

*Intracellular Storage and Accumulation*

The phenomenon of bacterial storage can be defined as either biosorption or bioaccumulation, which is simply accumulating soluble substrate inside of a bacterial cell before being utilized for growth or energy. It has been postulated that bacteria accumulate substrate in temporary intracellular pools simply because the rate of transport can at times exceed utilization. However, this has not been studied because of analytical limitations. What has been studied in depth is the ability of bacteria to store energy in the form of insoluble polymers like polyhydroxybutyrate (PHB) and other polyhydroxyalkonates (PHAs), glycogen (polymer of glucose), and elemental sulfur. In the absence of an external energy source, organisms break down the polymers for cell maintenance and make new cell material (Madigan and Brock, 2009).

PHB is a lipid formed from hydroxybutyric acid units (Madigan and Brock, 2009). Abundant carbohydrates results in the production of glycogen stores but carbohydrates are typically low in raw influent (Carucci et al., 2001). Some suggest that substrate storage only occurs when an organism is exposed to feast/famine conditions and not just feast conditions (Insel et al., 2012).
When an activated sludge process imposes a kinetic pressure on the biomass (i.e., the concentration gradient of the carbon sources), a storage response is usually established without the necessity of other external limitations (e.g., lack of nutrients or electron acceptors) (Majone et al., 1999). Under such conditions, storage of intracellular polymers is usually the main mechanism for the removal of readily biodegradable carbon sources in activated sludge systems operating under dynamic conditions (Oehmen et al., 2005).

Insel et al. (2012) hypothesized that during short-term transient loading, substrate uptake down-regulates storage and growth on storage products. This is counter to substrate uptake during feast conditions up-regulating storage and that storage product utilization does not occur until after external substrate depletion. Ni and Yu (2007) demonstrated in a dynamic system that promoted feast/famine conditions, rapid transport and storage of substrate while simultaneously maintaining high growth rates (30-40% external substrate converted to storage polymer). Once the external substrate was consumed, storage compounds were then used for energy and growth but at a reduced rate. Jørgensen et al. (1992) showed that the optical density (biomass) stopped increasing after the external substrate was consumed but not immediately, suggesting growth on stored substrate until exhausted. However, the substrate type may yield different results.

Carucci et al. (2001) demonstrated in a system fed primary effluent that PHB degradation after readily biodegradable COD (rbCOD) exhaustion did not readily occur, potentially because the presence of slowly biodegradable COD (sbCOD). However, in acetate spiked samples, PHB degradation began almost immediately. One explanation could be that hydrolysis of sbCOD resulted in slow generation of rbCOD and until the sbCOD was exhausted, PHB degradation did not proceed. Beccari et al. (2002) demonstrated in aerobic batch tests with AS and synthetic feed that acetate resulted in approximately 45% storage and ethanol 25% storage. When more complex glutamic acid and filtered and non-filtered primary effluent was used, storage products were not even detected, suggesting storage is limited by the rate of substrate hydrolysis. Carucci et al. (2001) also demonstrated that some acetate removal was by storage as PHB and that storage of other compounds was less prevalent. Majone et al. (1999) determined that the storage capacity of pure cultures fed acetate to vary between 0.35-0.75 g COD stored/g COD removed and glucose between 0.32 to 0.71 g/g. Goel et al. (1998) demonstrated maximum storage...
capacities of 0.45, 0.68, and 0.36 g/g of total acetate, glucose, and starch COD removed respectively.

Storage only occurs when electron acceptors (i.e., nitrate, nitrite, oxygen) are available because active transport and storage requires the expenditure of energy through the respiratory pathways. The known exceptions to this are phosphorus accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs). Under anaerobic conditions, PAOs obtain energy from cleaving phosphates from stored polyphosphates (poly-P) in order to uptake volatile fatty acids (VFAs, particularly acetate and propionate) and store them as PHAs. Under aerobic conditions, PAOs use the stored PHAs as a substrate source and uptake phosphorus and store as poly-P. GAOs obtain energy from the anaerobic hydrolysis of stored glycogen and uptake acetate and store as PHAs. Under aerobic conditions, the stored PHAs are used for synthesis and some portion is converted to glycogen.

**High-Rate Activated Sludge (HRAS)**

Prior to the development of the activated sludge process, the predominant treatment processes were gravitational settling, fixed-film processes (e.g., rock trickling filters), media filtration, land irrigation, and direct aeration of the sewage. Because of the immense footprint or energy required to treat sewage using these processes, especially if nitrification was required, the AS process was developed by Ardern and Lockett (1914) as a means to reduce the footprint by retaining bacteria in the aeration basins. What is termed high-rate activated sludge was developed shortly after the AS process for the same reason, to reduce the footprint even further (Chase, 1944). Simply put, the AS process was pushed until it could almost no longer meet the effluent requirements (i.e., 30/30 TSS/BOD₅ limits) and this became HRAS (Figure 2.1). The typical SRT of a HRAS process is between 1-4 days SRT, depending on temperature (Tchobanoglous et al., 2003). By taking advantage of the high-rate of bacterial consumption, HRAS became a relatively affordable method of removing particulate and soluble carbon constituents from used water. HRAS plants are still in operation to this day, mainly at medium to large treatment plants that do not require nitrification or nutrient removal. However, the majority of these facilities have been converted to nitrification or BNR processes or are now proceeded by a nitrification or BNR process.
The COD removal mechanisms were not well understood until much later after the development of the HRAS process. Pearson (1968) coined the term mean cell residence time (MCRT) and Jenkins and Garrison (1968) recommended using MCRT as a kinetic basis for the design, control, and operation of treatment plants. If a specific microorganism or consortium of microorganisms is required for the degradation of specific organic compounds, the SRT will significantly affect the effluent organic quality (Saunders and Dick, 1981). Lawrence and McCarty (1970) demonstrated the relationship of biological SRT to characterize the metabolic activity level of activated sludge and compared SRT to other process factors like F/M. The COD removal efficiency does not depend significantly on the COD loading rate or the F/M ratio. Only a comparison with the SRT shows that with a SRT longer than 1 day COD removal efficiencies of more than 75% and with a SRT longer than 2 days COD removal efficiencies of more than 80% can be expected (Kroiss et al., 1992).

The mixed liquor also has a “history” based on the selective pressures to include substrate gradients (i.e., DO, organic carbon, TN), hydraulic forces (e.g., suspended growth, attached growth, and granulation), and retention time (i.e., SRT). Long SRT systems (20-45 days) typically have old sludge and very little carbon source gradients and low concentrations throughout the AS process (e.g., extended aeration process), therefore, storage is likely not occurring. The EPS/VSS fraction is also high to provide protection from higher life forms and to capture more particulate and colloidal substrate. At very long SRTs (>45 days) the active
biomass fraction continues to decrease and very little EPS is present resulting in poor bioflocculation and pin flocs. On the other hand, young sludge (<3 days) in a plug flow system may rapidly store soluble substrate and have high growth and EPS production rates. However, EPS/VSS is generally low because the SRT is too short to allow significant accumulation of EPS. In extremely short SRT systems (<1 day), only the fastest growers are maintained, EPS/VSS is low, and storage is assumed to be maximized. Another interesting point is that at short sludge ages and low temperatures, the stored COD becomes an appreciable fraction of the mixed liquor volatile suspended solids (MLVSS) that is discharged with the daily sludge wastage (Ekama and Marais, 1979). Furthermore, because of the continuous high level of stored COD, the fluctuation of the oxygen consumption rate tends to be highly attenuated and the oxygen uptake rate (OUR) remains at a low level. In effect, at low sludge ages and temperatures, the plant operates principally as a bioflocculation process (Ekama and Marais, 1979).

In some cases, HRAS processes experienced filamentous bulking because of over loading and low DO in the first portion of the aeration basins that were plug-flow. Additionally, in the US in the 1960s, increased industrial inputs resulted in toxicity events and led designers to construct complete-mix AS plants (CMAS), which also experienced settling issues (Tchobanoglous et al., 2003). Because of bulking, anaerobic selectors (Figure 2.2) were used since they provided better selection pressure than compartmentalization of the aeration basin (Tracy et al., 1986). Anaerobic selectors utilize both kinetic and metabolic selection processes to promote good settling floc-forming bacteria (Jenkins et al., 2004). The anaerobic selectors were generally effective even if biological P removal by PAOs was not occurring because of the presence of GAOs (Jenkins et al., 2004).

![Figure 2.2](image-url) – Process flow diagram of a HRAS process with primary sedimentation and anaerobic selectors. Anaerobic (ANR).
Step Feed (Aeration) Activated Sludge

Step feed or the step aeration process was first referenced by Gould (1939) as a way to distribute the load in an HRAS processes solely based on reducing shock loads of oxygen demand in beginning of aeration basins (Figure 2.3). The influent is distributed, or step fed, along the length of a plug-flow AS reactor. Step feed allows more efficient use of air because of this load distribution and provides flexibility in accommodating loads (Torpey, 1948). This configuration also results in improved SVI (sludge volume index) caused by low DO filaments and reduction of aeration tank volume. This configuration also allowed storage of solids under aeration by diverting the influent away from the front of the aeration basin and thus not diluting the RAS, which is beneficial during wet weather events.

![Figure 2.3 – Process flow diagram of a step feed AS process with primary sedimentation.](image)

Contact Stabilization Activated Sludge (CSAS)

The contact stabilization activated sludge (CSAS) process was developed independently by Ullrich and Smith (1951) to increase capacity and by Eckenfelder and Grieh (1955) to reduce shock loads from industrial inputs. Termed the biosorption process by Ullrich and Smith (1951), unsettled raw wastewater is brought into contact with aerobically stabilized mixed liquor, mixed briefly, and sent to a clarifier (Figure 2.4). The underflow from the clarifier is returned to a mixed liquor aeration basin operated under high MLSS concentrations and long retention times.
Bunch and Griffin Jr. (1987) were the first to demonstrate rapid removal of colloids (defined as 0.03 μm<colloidal COD<1.5 μm) in a contact stabilization process and showed that colloidal removal was complete within 5 minutes and occurred more rapidly than soluble substrate removal. Bunch and Griffin Jr. (1987) suggested cCOD removal is a physical zero-order process and that sCOD (<0.03 μm) removal was first-order and probably biological based. In a CSAS process, La Motta et al. (2003a) determined a pCOD removal efficiency of 72% at a contact time of 5 minutes and 86% removal at 30 minutes.

Since the primary removal mechanism of CSAS is bioflocculation of pCOD and cCOD in the CT, the process is not very effective for wastewaters with high sCOD concentrations. Therefore, in some cases the CSAS process was preceded by a trickling filter for sCOD removal (Figure 2.5). The effluent leaving a trickling filter typically has high pCOD and cCOD fractions. In a trickling filter/solids contact (TF/SC) process, La Motta et al. (2004) determined that a minimum HRT of 20 min in the solids contact tank (CT) is needed for stable operation. La Motta et al. (2003b) also determined that bioflocculation was optimal at DO concentrations greater than 0.5 mg/L with adequate mixing. Experiments demonstrated that mechanical flocculation did not improve bioflocculation when the DO was less than 0.5 mg/L suggesting the impact of the DO concentration was microbial based and not inadequate mixing.
More recent than the CSAS process, Zhao et al. (2000) proposed the bioflocculation-adsorption, sedimentation, and stabilization (BSS) process system to be used as an advanced primary treatment process. The BSS is identical to the CSAS process except the contact tank is not aerated and therefore soluble substrate removal does not occur in the contact tank. Under the optimum operating conditions, the removal efficiencies obtained in the BSS system were COD (70-80%); sCOD (30%); TSS (80-95%); TAN (6%); TKN (total Kjeldahl nitrogen, 18%); and turbidity (30-50%). This process has been proposed as a means to concentrate the influent particulate and colloidal substrate while conserving the readily biodegradable substrate for use in a downstream BNR process.

**Anaerobic and Aerobic Membrane Bioreactors (MBRs)**

A current area of research is the use of synthetic membranes for direct filtration in an AS process. While aerobic MBRs have been successfully used in BNR and CAS processes (Figure 2.6), use in HRAS and anaerobic processes has still not been implemented full-scale. The desire to use membrane filtration in these processes is that by using filtration one could concentrate more COD is the WAS stream and redirect it anaerobic digestion for biogas production and
generate a solids free filtrate for either direct reuse or discharge. However, the primary issue associated with HRAS and anaerobic MBRs is the rate of membrane fouling and the energy required to address it currently exceeds the energy recovery potential.

![Process flow diagram of a MBR process with micro screening. Micro Screen (MS); Membrane Tank (MT).](image)

**Figure 2.6** – Process flow diagram of a MBR process with micro screening. Micro Screen (MS); Membrane Tank (MT).

Al-Malack and Anderson (1997) evaluated the use of crossflow microfiltration using woven polyester fabric and found it was not feasible when using settled municipal wastewater. In theory, crossflow filtration should allow higher fluxes because the crossflow velocity continually scour the membrane, however, when using primary effluent the scour was not sufficient. Ng and Hermanowicz (2005) compared an aerobic MBR to a CMAS process at different SRTs (0.25, 0.5, 2, and 5 days) and 3 and 6 hours HRT and found the MBR could reliably remove 97.3 to 98.4% COD where the CMAS system only removed 77.5 to 93.8% COD. The lower removals of the CMAS process was attributed to poor bioflocculation and solids in the effluent when operated at 0.25 and 0.5 days SRT. This study did not look at membrane fouling. In an anaerobic MBR treating synthetic wastewater, Hu and Stuckey (2006) demonstrated a sCOD removal of 90% at a 3 hour HRT at 35°C. Fouling was the result of fine particles (0.04-0.15 μm) resulting from the lack of bioflocculation. Work by van Voorthuizen et al. (2008) also demonstrated that the absence of bioflocculation under anaerobic conditions resulted in the accumulation of mainly colloidal matter resulting in excessive membrane fouling.
Akanyeti et al. (2010) operated a bench-scale aerobic MBR at various SRTs (0.25, 0.5, and 1 day) and obtained a 21% recovery of the influent COD as methane-COD at 1 day SRT (similar to CAS with primary sedimentation). Using this data, Akanyeti et al. (2010) estimated a 35% recovery at 0.25 day SRT. However, membrane fouling was more significant at lower SRTs presumably due to decreased bioflocculation. Hernández Leal et al. (2010) used a lab-scale aerobic MBR treating grey water to concentrate the influent COD and estimated mineralization to only be 3, 5, and 11% at SRTs of 0.2, 0.4, and 1 day. Again, as with the other studies, rapid membrane fouling occurred, but the flux rates could be recovered after physical cleaning of the membranes.

**Biological Nutrient Removal**

Nitrogen removal through the heterotrophic denitrification pathway requires 4-6 kg COD/kg N removed. The rate of denitrification is also dependent on the type and availability of the COD. Therefore, efficient use of the influent COD is critical and knowledge of the influent COD fractions is of primary importance because it determines oxygen demand, sludge wastage, denitrification, and the capacity of biological phosphorus removal. The relative magnitudes of the fractions can differ greatly between wastewaters (Ekama et al., 1986). Although organic carbon may be required, too high of an organic load to a BNR process can adversely affect the nitrification rates because of heterotrophic competition for space and dissolved oxygen (Æsøy et al., 1998; Figueroa and Silverstein, 1992). In a single-stage BNR process, Gori et al. (2011) demonstrated that as the fraction of sCOD/COD increased, the energy demand for oxidation of sCOD increased and energy production potential decreased because less pCOD was available for removal by primary sedimentation. Additionally, if more carbon is redirected to anaerobic digestion more particulate nitrogen is also redirected there and therefore the energy and carbon requirements in the BNR process are reduced (Siegrist et al., 2008). Another example of efficient carbon use is the treatment of sidestream liquor by partial nitritation/anammox resulting in 10-25% of TN load removed without additional carbon.
Multi-stage versus Single-sludge C and N Removal

The premise behind multi-stage systems is that the separation of carbon removal from nitrogen removal allows each process to be optimized independently. A high reliability of treatment can be achieved either by low loaded systems or multi-stage treatment (Böhnke et al., 1983). Two-sludge systems were implemented in order to decrease the infrastructure required for adequate nitrification since autotrophic doubling rates are half that of OHOs. It was also common for a carbon removal or nitrification plant to receive total nitrogen limits and instead of expanding the existing infrastructure, new tanks were constructed for the BNR or post-denitrification process. One of the major disadvantages of the multi-staged approach is that the influent carbon is removed prior to the BNR process and therefore is not available for heterotrophic denitrification, requiring the addition of supplemental carbon to meet stringent nitrogen limits. At the time, this was not a major concern since these types of upgrades occurred when supplemental carbon and energy was fairly inexpensive, however, this is no longer the case.

Multi-stage plants still in operation today stand to benefit the most from emerging treatment technologies, like complete autotrophic nitrogen removal, because supplemental carbon would not be required and it would allow more carbon redirection to energy recovery processes. Since the cost of methanol has increased over the past few decades and since multi-stage processes do not utilize the energy rich influent carbon efficiently, current BNR processes are typically single-sludge systems that use pre- and post-anoxic zones for denitrification. The inclusion of denitrification in two-stage plants is in principle possible but the separation of the removal of carbonaceous compounds in the first stage and nitrification in the second stage reduces the availability of carbonaceous compounds for the nitrate reduction in the second stage (Matsché and Moser, 1993).

Nitrite Shunt and Mainstream Deammonification

One of the limitations of the A/B process is its ability to meet stringent nitrogen limits because of the carbon-limited conditions in the B-stage. Previously, this has been partially overcome by operating the B-stage with ammonia-based aeration control in order to optimize the denitrification capacity and promote SND conditions. Further nitrogen reductions without additional carbon could be obtained by implementing mainstream deammonification or nitrite
shunt. Since OHOs would out-compete AMX for substrate and space, a mainstream anammox process may actually need to be carbon-limited (Jetten et al., 1997). Also, mainstream deammonification and nitrite shunt could prove to be cost-effective processes that could easily be implemented utilizing existing infrastructure (McCarty et al., 2011).

While the potential benefits of nitrite shunt are not as great as fully autotrophic nitrogen removal, nitrite shunt still results in carbon savings and may even be required for successful implementation of mainstream anammox. Preliminary work by Al-Omari et al. (2012), Wett et al. (2013), and Regmi et al. (2013) suggests that a reduced and controlled influent C/N (6-10) is required for NOB out-selection and that anammox may need to be coupled with nitrite shunt. Therefore, the ability to control COD removal is a critical aspect of mainstream deammonification and nitrite shunt and the A-stage of the A/B process is a potential candidate for this application. Others have suggested pre-removal of organic carbon either by physicochemical pretreatment or after anaerobic treatment (Kartal et al., 2010). However, the idea of converting all the current plants to mainstream anaerobic treatment would be costly (McCarty et al., 2011).

Current pilot investigations of mainstream deammonification and nitrite shunt include an A/B process at the Dokhaven WWTP in Rotterdam, Netherlands, where the B-stage is a granular sludge process (Winkler et al., 2012), a two-stage process with bioaugmentation of AOB and anammox from a sidestream process at the Blue Plains WWTP, DC Water (District of Columbia Water and Sewer Authority) (Al-Omari et al., 2012), and an A/B nitrite shunt process with tertiary anammox MBBR polishing at the Chesapeake–Elizabeth Treatment Plant, HRSD (Hampton Roads Sanitation District) in the United States (Regmi et al., 2013). A full-scale demonstration and investigation, in which the mainstream process is augmented by transferring anammox bacteria from the sidestream process, is occurring at the Strass WWTP (A/B process), Austria (Wett et al., 2013).

**Instrumentation, Control, and Automation**

Advancements of instrumentation, control, and automation (ICA) over the past few decades have provided the opportunity to closely monitor and dynamically control almost all aspects of a
treatment facility from flow to ammonia-based aeration control. Some of the most recent advancements have been with *in situ* online analyzers that are capable of measuring multiple parameters at once. One of the benefits of using *in situ* measurements is they help eliminate errors caused by sampling, sample pretreatment, and transport and storage (van den Broeke et al., 2006).

**Sensors**

One class of multivariable sensors uses UV/Vis spectroscopy to measure the absorbance over a certain wavelength range. For example, the Carbo::lyser by s::can (Messtechnik GmbH, Vienna, Austria) measures the absorbance of visible and ultraviolet light from 220 to 720 nm with an optical path length of 5 mm. Physically, 256 wavelengths are measured between 220 and 720 nm (resolution approximately 1.9 nm) and are converted to a resolution of 2 nm for calculating the concentrations. Gruber et al. (2006) evaluated these UV/Vis spectral sensors directly in sewer mains and in offline sampling stations and found good correlation. However, because of the extreme variability of dry and wet weather, the local calibration was not always accurate. Therefore, different calibration protocols were used depending on flow conditions with good success. Rieger et al. (2005) evaluated a Carbo::lyser in primary effluent and found the 95% prediction interval based on an ideal correlation to be ±50.7 mg COD/L at the mean lab value of 207 mg COD/L. Rieger et al. (2008) did a long-term (1.5 years) evaluation of a UV spectral sensor (Spectro::lyser, s::can) and found it was reliable enough for system monitoring and potentially process control. However, this UV sensor was only evaluated for nitrate and nitrite and not organics. One takeaway was that the sensor worked well *in situ* and only required minimal maintenance. UV/Vis spectroscopy is currently being used to monitor the influents to treatment plants to estimate the influent COD, TSS, and nitrate loads (van den Broeke et al., 2006). However, UV/Vis sensors have not been used in the control and automation of carbon removal processes like the A-stage.

**Aeration Control**

Since aeration energy is commonly responsible for around half of a treatment plant’s power usage any measure to reduce aeration intensity can provide significant savings (WEF, 2009). The most common form of aeration control is to automatically control the airflow to a process by
comparing the basin DO to a user specified setpoint. The airflow is adjusted so that the user DO setpoint is maintained at all times. An additional layer of control can be added using an *in situ* ammonium sensor located in the last portion of an aeration basin. Under this configuration, the DO setpoint is changed based on the difference between the ammonium sensor values and an ammonia setpoint. A review of aeration control strategies by Åmand et al. (2013) concluded with proper application, implementation, and maintenance ICA can provide the opportunity to optimize not only air use but treatment performance in terms of nitrogen removal based on operational flexibility. While DO control is common place in most activated sludge systems, DO control has not been applied to the A-stage process. The primary reason is that biofouling of the sensors is so rapid that the DO signal is rarely accurate. Another reason is the A-stage is so highly loaded that the DO is always near zero even when aerating at maximum capacity (Böhnke et al., 1997b). One form of aeration control that has been implemented in the A-stage is to control the aeration duration by cycling the air on and off or by providing an anaerobic zone. These forms of aeration control have been used to decrease the COD removal efficiency of the A-stage so that more carbon is available for denitrification in the B-stage (Feyen, 1992).

**SRT Control**

Automating SRT by controlling the WAS flow rate is also common practice. This method uses online MLSS and TSS sensors to monitor solids in and leaving the activated sludge process to determine the current SRT and adjust the WAS flow rate to target a user SRT setpoint. SRT control not only provides better process stability but it also helps stabilize downstream processes like sludge thickening (Tchobanoglous et al., 2003). SRT control becomes more and more important the shorter SRT. Therefore, the A-stage should utilize SRT control.

**Modeling**

The activated sludge model 1 (ASM1) primarily aims at low loaded activated sludge plants with nitrification and denitrification (Henze et al., 1986). ASM2 and ASM2d incorporate biological phosphorus removal (Gujer et al., 1995; Henze et al., 1999). ASM3 includes modifications to ASM1, incorporates storage of organic substrate as an independent process, and replaces the death-decay process for OHOs with an endogenous respiration process (Gujer et al., 1999). In
ASM3 (Figure 2.7), the storage of readily biodegradable substrate \( (S_S) \) requires energy from respiration and it is assumed that all the readily biodegradable substrate is first taken up by the heterotrophic organisms and converted to stored material \( (X_{STO}) \) that is subsequently assimilated to biomass \( (X_H) \) (Ni and Yu, 2007). In all of these models, no processes are included that describe carbon substrate uptake due to adsorption to the floc or incorporation into the sludge floc. The ASMs assume that biofloculation of particulate and colloidal substrate \( (X_S) \) occurs instantaneously and therefore is ignored in the model structure.

Therefore, ASM3 cannot be used for highly loaded systems (<1 day SRT) where flocculation or adsorption of particulate and colloidal biodegradable organics and storage become limiting (WERF, 2003). Additionally, in systems with short HRTs, biotransformation may not be complete as assumed in the models (Nogaj et al., 2013). ASM-type models also do not accurately model the hydrolysis of soluble substrate. Karahan et al. (2006) demonstrated that feeding SBRs with starch resulted in a rapid apparent uptake of the starch (adsorption) but transport for primary growth and storage was rate limited by hydrolysis, which the ASM model did not capture. This also suggests the methods for wastewater characterization may need to be evaluated since soluble substrate is generally assumed to be readily transported and biodegraded. Models of parallel growth and storage, growth and biosorption, and growth and EPS production have been proposed (Beccari et al., 2002; Insel et al., 2012; Jimenez, 2002; Ni and Yu, 2008; Ni et al., 2009; Sin et al., 2005). Other workarounds have also been proposed, for example, Haider et al. (2003) demonstrated in an A/B pilot system that the readily biodegradable fraction \( (S_S) \) of the wastewater should be split into two fractions: \( S_{SA} \) and \( S_{SB} \). The latter is not degradable in the A-stage and therefore increases the soluble inert fraction \( (S_I) \) leaving the A-stage. When entering

**Figure 2.7** – Flow of COD in ASM3 (Gujer et al., 1999).
the B-stage the $S_{SB}$ fraction is set free for degradation. The model was modified by keeping the maximum growth rate of OHOs the same in both stages but adjusted the substrate half-saturation coefficient ($K_S$) so that each $S_S$ fraction would be completely degraded.

Part of the collaborative efforts for advancing mainstream deammonification and nitrite shunt processes includes the development of model structures that mathematically describe both the carbon removal processes of the A-stage and NOB out-selection mechanisms in short-cut nitrogen removal processes. A preliminary model that describes EPS production and bioflocculation has been proposed by Nogaj et al. (2013). As shown in Figure 2.8, readily biodegradable substrate ($S_S$) is simultaneously consumed for growth ($X_H$) and EPS production ($X_{EPS}$). When excess $S_S$ is available, it is converted to storage products ($X_{STO}$) for later consumption when $S_S$ is depleted. Particulate and colloidal biodegradable substrate ($X_{SP}$, $X_{SC}$), both influent and cell debris ($X_S$), must first be bioflocculated and then hydrolyzed before becoming available for transport and consumption. In this model, the rate of EPS production is considered proportional to the rate of substrate utilization. Considerable work still remains including, model calibration, sensitivity analysis, and model verification.

![Figure 2.8 – Proposed COD flow model (Nogaj et al., 2013).](image)
**OHO Kinetic and Stoichiometric Parameters**

Some of the default BioWin (EnviroSim Associates Ltd. Hamilton, Ontario) kinetic and stoichiometric parameters for OHOs are included in Table 2.1. These parameters are generally accepted for typical activated sludge processes. However, these parameters have not been explicitly determined for short SRT systems like the A-stage. A maximum growth rate of 3.2/d would suggest that the washout SRT of OHOs is 0.31 d (7.5 hrs). However, it is known that the influent active biomass continuously seeds the process meaning the operating SRT can be lower than 7.5 hours as seen in full-scale A-stage processes. The half saturation coefficient is also of importance when modeling short SRT systems. Work by Insel et al. (2012) demonstrated in a bench-scale AS SBR an increase in the $K_S$ from 5 to 25 g COD$_S$/m$^3$ when the SRT was changed from 10 to 2 days. This suggests that $K_S$ will be even higher in shorter SRT systems like the A-stage.

<table>
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<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
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</table>

**Bioflocculation Kinetics**

Because bioflocculation of pCOD and cCOD is rapid and complete in most systems operated at $>3$ days SRT, modeling bioflocculation has not received a lot of attention. Some of the earliest work on flocculation kinetics focused on floc breakup and reflocculation for improving suspended solids removal through secondary clarifiers (Parker et al., 1971; Wahlberg et al., 1994). Building upon this work, La Motta et al. (2003a) and associates developed a first-order rate expression that described the removal of pCOD and cCOD in an aeration tank. Using this model, Jimenez et al. (2005) demonstrated in a HRAS pilot that the removal of cCOD and pCOD is rapid but not instantaneous and cCOD removal was slower than pCOD and comparable to the rate of rbCOD removal. The limitation of the model is that it is dependent on MLSS concentration with the assumption that EPS/MLSS is constant and adsorption sites are available,
which is not the case in short SRT processes. Laspidou and Rittmann (2002) developed a non-steady state model where the EPS production rate is proportional to substrate utilization rate. However, this model only describes consumption of soluble substrate for EPS production and does not address pCOD and cCOD removal associated with EPS production and bioflocculation. Work by Nogaj et al. (2013) is one of the first attempts to couple EPS production with pCOD and cCOD removal (Figure 2.8).
References


Chapter 3: Estimating the influent and effluent readily biodegradable COD and active biomass fractions of the A-stage process using physical-chemical and respirometric methods

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Abstract:

Activated sludge models are used routinely in the design and optimization of activated sludge processes. However, their use is limited to processes operated at solids retention times (SRT) greater than 3 days. A growing interest in short SRT processes, like the A-stage of the A/B process, has led to the development of more complex models that require detailed characterization of not only the raw wastewater influent but also the effluent. This study evaluated a respirometric method to determine the readily degradable substrate (Sₘ) fraction and active heterotrophic biomass (Xₗ) content of the influent and effluent of a high-rate activated sludge process operated below a 0.5 day SRT. While the influent Sₘ was similar to the Sₘ values determined using a physical-chemical method, the effluent values did not correlate well. This led to the measurement of heterotrophic aerobic yield coefficient and decay rate, which did not conform to the typical cited values of 0.67 gCOD/gCOD and 0.62 day⁻¹, respectively. Therefore, this study concluded that aerobic storage of Sₘ likely contributed to the error associated with the respirometric methods for Sₘ and Xₗ determination.
Introduction

Activated sludge models (ASM) are valuable tools for assisting with process selection and design optimization but they require a detailed characterization of the wastewater to be treated. Additionally, ASMs are generally limited to activated sludge processes operated at an solids retention time (SRT) between 3 to 30 days (Henze et al., 2000). This is primarily because certain chemical oxygen demand (COD) removal mechanisms, like bioflocculation and solids separation, are either simplified or intentionally excluded for the sake of reducing model complexity (Hauduc et al., 2013). Typically, the more complex a model the more a wastewater and activated sludge mixed liquor must be characterized. Recently, there has been an increased interest in high-rate activated sludge (HRAS) processes, like the A-stage of the A/B process, which require more complex models and incorporate the COD removal mechanisms that are usually ignored (Nogaj et al., 2015; Smitshuijzen, 2014). As a result, the influent, effluent, and mixed liquor of these HRAS processes, especially when operated below a 1-day SRT, need to be appropriately characterized in order to calibrate the models. Two influent parameters of particular concern are readily biodegradable substrate (SS) and the heterotrophic active biomass fraction (XH).

While there are a myriad of wastewater characterization methods, they generally fall into two categories: physical-chemical methods or respirometry-based methods (WERF, 2003). While respirometry-based methods for the determination of readily biodegradable substrate (SS) are generally considered more accurate than their physical-chemical counterparts, respirometry methods are often more complex and time consuming (Fall et al., 2011; Spanjers and Vanrolleghem, 1995; Wentzel et al., 1999). For example, filter pore size determines the separation between soluble, colloidal, and particulate substrates and filters with nominal pore sizes are less accurate than filters with absolute pore sizes (Gatti et al., 2010). The flocculated and filtered COD (ffCOD) method developed by Mamais et al. (1993), tends to overestimate SS, especially when the wastewater contains industrial inputs with complex soluble compounds that require acclimated bacterial cultures to completely degrade (Germirli et al., 1991). Several studies have also demonstrated that HRAS processes operated below a 1-day SRT do not completely remove SS when quantified using physical-chemical methods (Fall et al., 2011; Gatti et al., 2010; Haider et al., 2003).
Unlike readily biodegradable substrate, the active biomass fraction is more difficult to estimate and can only be done so using respirometry-based methods or microbiology and molecular methods. For example, ATP analysis (Nelson and Lawrence, 1980), DNA quantification (Liebeskind and Dohmann, 1994), FISH analysis (Ismail et al., 2007), acridine orange and DAPI staining, and microautoradiography (Vollertsen et al., 2001) have all been used to estimate the active biomass present in activated sludge samples. Kappeler and Gujer (1992) developed a relatively simple respirometry-based batch test to determine the influent $X_H$ using unseeded raw wastewater samples. This test method was expanded to include the estimation of $S_S$ by Wentzel et al. (1995), soluble and particulate inert COD and slowly biodegradable COD by Wentzel et al. (1999), and autotrophic active biomass by Cronje et al. (2002). While these batch test methods are useful, Lee et al. (2006) found that kinetic parameters and biomass fractions determined using short-term batch tests seemed to represent only the fastest growing organisms although the parent activated sludge system (SRT >10 days) was dominated by slower growing organisms. However, this may not be the case with short SRT (i.e., <1 day) processes, like the A-stage, because it is unlikely that slower growing organisms are retained in the process (Böhnke et al., 1997).

In this study, the influent and effluent of an A-stage pilot process treating municipal wastewater and operated below a 0.5 day SRT, were characterized using physical-chemical and respirometry-based methods. Using the methodology outlined in Wentzel et al. (1995), the initial readily biodegradable substrate ($S_S$) and heterotrophic active biomass fraction ($X_H$) were estimated and compared to the physical-chemical results. While this method has been used to characterize raw wastewater influents (RWI), effluent containing active biomass has never been characterized using this method. The objective of this project was to confirm that the existing methods were accurate enough to be used on the effluent of HRAS process operated at short SRTs.

**Materials and Methods**

**Pilot configuration and operation**

The A-stage pilot consisted of two trains operated in parallel. Since these trains were identical in construction and fed the same influent, no distinction is made between the sample results
throughout this paper. Each train consisted of three vertical, complete-mix bioreactors in series followed by a clarifier and effluent storage tank (Figure 3.1). The total working volume of the bioreactors was 0.51 m$^3$ and each bioreactor had a side water depth of 3.4 m. The clarifier had a working volume of 1.7 m$^3$ with a surface overflow rate of 17 m$^3$/m$^2$·day at the design flow of 17 L/min. At the design flow, the HRT of the bioreactors and clarifier was 30 minutes and 1.7 hours, respectively. The pilot was fed screened (2-3 mm openings) and degritted municipal wastewater after the wastewater temperature was adjusted to a user set point between 15-25°C using submersible heaters (OEM OTS, Minneapolis, MN) or a water chiller (Aqualogic MT-9, San Diego, CA). The influent and return activated sludge (RAS) flows were flow-paced using progressive cavity pumps (Seepex BW5, Bottrop, Germany) with variable frequency drives (VFDs) and magnetic flow meters (Rosemount 8705, Houston, TX). Waste activated sludge (WAS) was removed from the underflow of the clarifier using a digital, speed-controlled peristaltic pump (Masterflex L/S, Vernon Hills, IL). Aeration was provided using compressed air through a single mechanically operated valve (MOV; v-notch ball valve) to fine-pore membrane disc diffusers (17.8 cm diameter) mounted on the bottom of each bioreactor as shown in Figure 3.1. DO control was achieved using a DO cascade controller and optical DO sensor (InsiteIG Model 10, Slidell, LA) installed the last bioreactor. DO was typically maintained at 0.5 mgO$_2$/L. MLSS-based SRT control was achieved using an infrared MLSS sensor (InsiteIG Model 15, Slidell, LA) installed in the middle tank. The SRT of the A-stage pilot process was maintained between 0.1 to 0.3 days. A detailed description of the automatic process controllers is provided in Chapter 5. A detailed description of the B-stage and anaerobic ammonia oxidizing (anammox) moving bed bioreactor (MBBR) is provided in Regmi et al. (2015a) and Regmi et al. (2015b), respectively.
Sample collection and analysis

Performance of the A-stage pilot was assessed by collecting 24-hr flow-weighted composite samples from the influent, effluent, waste active sludge (WAS), and mixed liquor as shown in Figure 3.1. All composite samples were maintained at 4°C during collection. These samples were analyzed for COD, soluble COD (1.5 μm glass microfiber filtered), carbonaceous biochemical oxygen demand (cBOD₅), soluble cBOD₅ (1.5 μm glass microfiber filtered), total, volatile, and mixed liquor suspended solids (TSS, VSS, MLSS), total ammonia nitrogen (TAN), nitrate, and nitrite according to Standard Methods (APHA, 2012). Flocculated and filtered COD (ffCOD) was measured according to Mamais et al. (1993) using 0.45 μm cellulose membrane filters. The cellulose membrane filters were soaked in distilled water for 24 hours and then rinsed prior to use. Particulate COD was calculated as the difference between total COD and sCOD. Volatile fatty acids (VFA) were determined using the gas chromatography method (5560D) outlined in Standard Methods (APHA, 2012).
COD fractionation using physical-chemical methods

Figure 3.2 summarizes the COD fractions and how they were measured or calculated. A simple COD fraction includes measuring total and soluble COD and calculating the particulate fraction as the difference between the two. A full COD fractionation includes the determination of the soluble unbiodegradable or inert fraction ($S_I$), soluble readily biodegradable fraction ($S_S$), particulate slowly biodegradable fraction ($X_S$), and particulate unbiodegradable fraction ($X_I$). The soluble inert fraction of a wastewater is considered equal to the effluent soluble COD of a biological processes fed that wastewater and operated at an SRT $>3$ days (Germirli et al., 1991). For this study, $S_I$ was assumed to be equal to the soluble COD (measured as ffCOD) of the B-stage process. The $S_S$ fraction of the RWI and A-stage effluent was calculated as the difference between the RWI or A-stage effluent ffCOD and $S_I$ (i.e., B-stage effluent ffCOD). The $X_I$ fraction was calculated as the difference between pCOD and particulate cBOD$_5$ (i.e., cBOD$_5$ – scBOD$_5$). While the standard BOD method is considered a respirometric method, it does not use seed inoculum from the process of interest (e.g., A-stage) and therefore does not determine the actual fraction that is biodegradable in the A-stage. However, since the seed inoculum is the same for the influent and effluent samples, the BOD measurement is useful in determining the fraction of biodegradable material that is removed by the A-stage. The final fraction $X_S$ was calculated as the difference between the total COD of the sample and $S_I$, $S_S$, and $X_I$. $S_S$ was further fractionated into VFAs ($S_{SV}$) and soluble complex substrate ($S_{SC}$) by subtracting the measured VFA values from $S_S$. $X_S$ was further fractionated into particulate ($X_{SP}$) and colloidal ($X_{SC}$) slowly biodegradable substrate where the colloidal fraction was the difference between pCOD and ffCOD of the sample.
Figure 3.2 – Schematic representation of full and simple wastewater fractionation and calculation method of each.

**Batch oxygen uptake rate tests**

Batch oxygen uptake rate (OUR) tests were performed in 500 mL Wheaton bottles that were maintained at 20°C using a water bath. Each bottle was sealed with a screw-type septum cap and mixed using a magnetic stir bar and stir plate. Bottle inserts containing potassium hydroxide (30% w/v) were used to scrub respired carbon dioxide from the headspace of the bottles. Oxygen consumption was measured using a pulse-flow respirometer (PF-8000; RSA, Inc. Springdale, AR) as described in Young and Cowan (2004). COD mass balances were performed to confirm validity of the tests and were generally within ±5% error. Allylthiourea (ATU) was added (20 mg/L) to long-term tests and mixed liquor samples containing nitrifying bacteria to prevent nitrification. Short-term batch tests were conducted without ATU and the absence of nitrification was confirmed by measuring nitrate and nitrite at the end of the tests.

**Measurement of $S_S$ and $X_H$ using batch OUR tests**

The batch test used to estimate initial $S_S$ and $X_H$ concentrations is described in detail in Wentzel et al. (1995) including equation derivation. Composite samples (500 mL) of RWI and pilot A-stage effluent were run in duplicate. RWI and A-stage effluent samples were ran without adding

<table>
<thead>
<tr>
<th>Fractionation Method</th>
<th>Calculation Method</th>
<th>Simple Fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble Unbiodegradable ($S_t$)</td>
<td>B-stage effluent ffCOD</td>
<td>COD</td>
</tr>
<tr>
<td>Soluble Biodegradable ($S_b$)</td>
<td>Soluble VFA ($S_{sv}$)</td>
<td>VFA GC method</td>
</tr>
<tr>
<td>Soluble Complex ($S_{sc}$)</td>
<td>ffCOD – $S_{sv}$ – $S_t$</td>
<td>cCOD</td>
</tr>
<tr>
<td>Slowly Biodegradable ($X_s$)</td>
<td>Colloidal ($X_{sc}$)</td>
<td>sCOD – ffCOD</td>
</tr>
<tr>
<td>Particulate ($X_{sp}$)</td>
<td>$X_s$ – $X_{sc}$</td>
<td>pCOD</td>
</tr>
<tr>
<td>Particulate Unbiodegradable ($X_p$)</td>
<td>COD – cBOD$_5$ – scBOD$_5$</td>
<td>COD</td>
</tr>
</tbody>
</table>
seed or dilution water while a second set of A-stage effluent samples were spiked with sodium acetate. Typical acetate spikes were 100 or 200 mg/L as COD. The respirometry tests were typically complete after 8 hours, however, the tests were ran for approximately 24 hours. Using the OUR results from each test, the OUR during exponential growth was linearized by taking the natural logarithm of OUR and plotting against time. The linearized OUR can be expressed as seen in equation 3–1.

$$\ln \text{OUR}_t = \ln \left[ \frac{1-Y_H}{Y_H} * \hat{\mu}_H * X_{H,0}/24 \right] + (\hat{\mu}_H - b_H) t / 24$$

(3-1)

Where \(\text{OUR}_t\) is the OUR (mgO\(_2\)/L·hr) at any time \((t; \text{hours})\), \(Y_H\) is the heterotrophic aerobic yield coefficient, \(\hat{\mu}_H\) (day\(^{-1}\)) is the maximum specific growth rate of heterotrophs, \(X_{H,0}\) (mgCOD/L) is the initial heterotrophic active biomass concentration, and \(b_H\) (day\(^{-1}\)) is the heterotrophic aerobic decay rate. The slope and y-intercept of the lnOUR curve during exponential growth can be expressed as equation 3–2 and 3–3, respectively.

$$\text{slope} = (\hat{\mu}_H - b_H)/24$$

(3-2)

$$y \text{ intercept} = \ln \left[ \frac{1-Y_H}{Y_H} * \hat{\mu}_H * X_{H,0}/24 \right]$$

(3-3)

The decay rate and yield coefficient were assumed to be equal to 0.62 day\(^{-1}\) at 20°C and 0.67 gCOD/gCOD, respectively (Henze et al., 2000). The OUR for each tests was modeled according to equation 3–4.

$$\text{OUR}_t = \frac{1-Y_H}{Y_H} * \hat{\mu}_H X_{H,0}/24 * e^{\left[ \frac{(\hat{\mu}_H-b_H)}{24}\right] t}$$

(3-4)

The modeled OUR was then fit to the measured OUR curves of both duplicate samples using the method of least squares and solver in Excel (Microsoft, Redmond, WA). The sum of the error squared was minimized by changing \(X_{H,0}\) and \(\hat{\mu}_H\). The initial readily biodegradable COD concentration \((S_{S,0})\) was then calculated using equation 3–5.

$$S_{S,0} = \frac{\hat{\mu}_{HSS}*X_{H,0}}{Y_H*\text{slope}+24} * \left( e^{(\text{slope}*t_d)} - 1 \right)$$

(3-5)
Where \( t_d \) was the time when a precipitous drop in OUR occurred coinciding with the depletion of \( S_S \) in the batch tests and \( \hat{\mu}_{H, S_S} \) is the maximum specific growth rate of heterotrophs on \( S_S \). This value was calculated according to equations 3–6 and 3–7.

\[
\hat{\mu}_{H, S_S} = \hat{\mu}_H - \hat{\mu}_{H, X_S}
\]  
(3-6)

\[
\hat{\mu}_{H, X_S} = \frac{OUR_{X_S, t} \times 24}{\frac{1}{Y_H} - \frac{X_{H, 0}}{Y_H} \times e^{(slope \times t_d)}}
\]  
(3-7)

Where \( \hat{\mu}_{H, X_S} \) is the maximum specific growth rate of heterotrophs on \( X_S \) and \( OUR_{X_S, t} \) is the OUR due to consumption of \( X_S \) at the time of the precipitous drop \( (t_d) \) in OUR. The OUR due to consumption of \( X_S \) was also modeled according to equation 3–8 to visually confirm total OURT model fit.

\[
OUR_{X_S, t} = \frac{1 - Y_H}{Y_H} \times \hat{\mu}_{H, X_S} X_{H, 0} / 24 \times e^{(slope \times t)}
\]  
(3-8)

**Measurement of heterotrophic aerobic yield**

To determine \( Y_H \) for readily biodegradable substrate (\( S_S \)), batch OUR tests were performed according to Brands et al. (1994). A-stage and B-stage mixed liquor samples were added to the batch test bottles and ran until an endogenous respiration rate was measured. For B-stage samples, ATU (20 mg/L) was added to prevent nitrification. After reaching endogenous conditions, sodium acetate was spiked in each bottle at 400 mg/L as COD resulting in a significant increase in the OUR. The test was continued until the OUR returned to endogenous conditions. The area between the endogenous rate and the OUR when acetate was being consumed was calculated as the total oxygen used (OU). Yield was then calculated according to equation 3–9.

\[
\frac{COD \ added \ (mg \ O_2) - \ OU \ (mg \ O_2)}{COD \ added \ (mg \ O_2)} = Y_H \left( \frac{mg \ COD}{mg \ COD} \right)
\]  
(3-9)

**Measurement of heterotrophic aerobic decay rate**

The heterotrophic aerobic decay rate was determined according to Ekama et al. (1986). A-stage and B-stage WAS samples were added to OUR batch test bottles and ran for more than 72 hours
under endogenous conditions. Using the OUR results, the endogenous decay rate was calculated as the slope of the natural logarithm of OUR against time. This endogenous decay rate was then converted into the lysis-regrowth decay rate according to equation 3–10. Where \( Y_H \) was assumed to equal 0.67 day\(^{-1} \) and the fraction of inert material \( (f_D) \) was assumed to equal 0.08 (Henze et al., 2000).

\[
b_H = \frac{slope}{1 - Y_H \cdot (1 - f_D)}
\]  

(3-10)

**Statistical analysis**

Statistical analyses including the Pearson product moment correlation, Shapiro-Wilk normality test, Mann-Whitney rank sum test, t-test, linear regressions, cumulative distribution functions, confidence and prediction intervals, mean, standard deviation, and standard error were performed using SigmaPlot 12.5 (Systat Software Inc., Bangalore, India). Confidence intervals were calculated at a \( p \)-value of 0.05. Means are presented with standard deviations unless noted otherwise.

**Results and Discussion**

**RWI and A-stage effluent characterization using physical-chemical methods**

The pilot process was continuously operated for over 600 days. During this time, a full physical-chemical characterization of the RWI and A-stage effluent was performed on a weekly basis. The average results from this sampling campaign are summarized in Table 3.1. The collection system from which the pilot received influent was characterized by long, flat pressure mains resulting in septic sewage with an average influent VFA \( (S_{SV}) \) fraction of 12±4%. \( S_{SV} \) correlated well with the RWI temperature \( (R = 0.52; \ p < 0.001; \ n = 74) \), which indicates that as the RWI temperature increased, fermentation in the collection system increased. The colloidal fraction also correlated with RWI temperature although inversely \( (R = -0.46; \ p < 0.001; \ n = 77) \). The \( X_{SP} \) and \( X_I \) fractions were the main influent fractions averaging 34±11% and 28±7%, respectively. These fractions also varied significantly but did not correlate with seasonal conditions. It is likely that the variability seen was due to operations at the treatment facility that the pilot plant was
located at because the pilot RWI included the treatment facility’s recycles (e.g., solids dewatering centrate).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RWI COD (mgCOD/L)</th>
<th>Fraction of RWI (%)</th>
<th>Effluent COD (mgCOD/L)</th>
<th>Fraction of Eff (%)</th>
<th>Average Removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S_I</td>
<td>21 (± 4)</td>
<td>4 (± 1)</td>
<td>21 (± 4)</td>
<td>7 (± 2)</td>
<td>0 (± 0)</td>
</tr>
<tr>
<td>S_S</td>
<td>121 (± 17)</td>
<td>22 (± 4)</td>
<td>38 (± 16)</td>
<td>12 (± 4)</td>
<td>71 (± 12)</td>
</tr>
<tr>
<td>S_SV</td>
<td>66 (± 20)</td>
<td>12 (± 4)</td>
<td>9 (± 13)</td>
<td>3 (± 4)</td>
<td>88 (± 19)</td>
</tr>
<tr>
<td>X_S</td>
<td>216 (± 50)</td>
<td>40 (± 9)</td>
<td>158 (± 35)</td>
<td>53 (± 8)</td>
<td>30 (± 16)</td>
</tr>
<tr>
<td>X_SP</td>
<td>154 (± 47)</td>
<td>28 (± 7)</td>
<td>77 (± 28)</td>
<td>25 (± 7)</td>
<td>52 (± 17)</td>
</tr>
<tr>
<td>X_SC</td>
<td>62 (± 18)</td>
<td>12 (± 4)</td>
<td>81 (± 18)</td>
<td>28 (± 7)</td>
<td>-27 (± 29)</td>
</tr>
<tr>
<td>X_I</td>
<td>197 (± 106)</td>
<td>34 (± 11)</td>
<td>81 (± 32)</td>
<td>28 (± 7)</td>
<td>58 (± 17)</td>
</tr>
</tbody>
</table>

(± Standard Deviation)

Removal of all fractions, except S_I and X_SC, was seen across the A-stage, which averaged 48±14% total COD removal. Removal of the particulate fractions, X_SP and X_I, averaged 50±17% and 56±16%, respectively. Since the A-stage was not preceded by primary sedimentation, these fractions included settleable solids that would be removed in the A-stage regardless of biological activity. The influent S_S fraction had the highest removal efficiency of 71±12%, which was expected because this fraction is considered readily biodegradable. The X_SC concentration actually increased from 62±18 to 81±18 mgCOD/L resulting in a net increase of 27±29%. As discussed in detail in Chapter 4, this was likely due to conversion of soluble substrate into unflocculated biomass, which is typical of activated sludge processes operated below 0.5 day SRT (Bisogni and Lawrence, 1971). The S_I fraction did not change because the assumption that the B-stage effluent fCOD is the S_I fraction applies to both the influent and effluent of the A-stage.

Similar to the results of Haider et al. (2003), S_S was not completely removed in the A-stage. This was attributed to the inability of heterotrophic organism to consume the S_S because either the organisms never developed the cellular machinery capable of degrading all of the S_S due to non-
limiting substrate conditions or the organisms present in short SRT processes are just not capable of degrading more complex soluble substrate ($S_{SC}$). Regardless of the mechanisms, the result is that the definition of what is typically considered readily degradable substrate does not apply to short SRT processes, like the A-stage, and therefore physical-chemical methods are ineffective at predicting what COD fraction is truly readily degradable in these systems.

**Determination of RWI $S_S$ and $X_H$ using respirometric methods**

Since physical-chemical methods were not capable of correctly estimating the $S_S$ in the RWI, respirometric methods were explored. The method described by Wentzel et al. (1995) was used because sample seeding was not required for the estimation of $S_S$ because bacteria were already present in the samples. The A-stage microbial population has been described as an extension of the microbial population present in the RWI, therefore, batch respirometric tests using only RWI should only estimate the $S_S$ that is degradable in the A-stage (Böhnke et al., 1997). An example RWI batch test (Day 288) with a fitted model to the OUR and InOUR are presented in Figure 3.3. For the RWI tests, the data had to be adjusted for an apparent lag in OUR increase (i.e., non-exponential growth). On average, the first hour to two hours of data had to be removed to provide a better model fit and prediction of the initial $S_S$ and $X_H$ concentrations. This was likely because the organisms present in the anaerobic collection system had to activate their aerobic pathways prior to growth.

**Figure 3.3** – Example OUR profile and model fit to RWI sample (Day 288). (a) OUR versus time and (b) natural logarithm of OUR versus time. (—) total OUR; (——) OUR due to decay and consumption of slowly biodegradable material.
Figure 3.4 compares the results of $S_S$ estimations using the respirometric and physical-chemical methods along with VFA measurements ($S_{SV}$). As expected, both methods predicted higher $S_S$ values than the measured $S_{SV}$ concentrations. $S_{Sphys}$ varied between 89-142 mgCOD/L and $S_{Sresp}$ between 57-145 mgCOD/L. $S_{Sresp}$ correlated with $S_{Sphys}$ ($R = 0.54; p = 0.017; n = 19$) although $S_{Sresp}$ correlated better with $S_{SV}$ ($R = 0.83; p < 0.001; n = 18$). Interestingly, the absolute values of $S_{Sresp}$ and $S_{Sphys}$ were closer when the ambient RWI temperature was greater than about 20°C. However, as seen in Figure 3.5a, $S_{Sphys}$ did not vary significantly based on the RWI temperature ($R^2 = 0.11; p = 0.158; n = 19$). Typically, there is an increase in biologic activity with increased temperature, however, the respirometric results demonstrated an inverse correlation as seen in Figure 3.5b. The small variations of $S_{Sphys}$ may have been associated with transformations in the sewer depending on seasonal temperature variations. That is, complex soluble material ($S_{SC}$) and slowly degradable material ($X_S$) was fermented to VFA (mainly acetate). However, increased fermentation coincides with an increase in methane production from VFA, which represented a loss of $S_S$ (Guisasola et al., 2008). Therefore, only small changes in the total $S_S$ concentrations were detectable using the $S_{Sphys}$ method. The fact that $S_{Sresp}$ correlated well with $S_{SV}$ suggests that the respirometric method was capable of detecting variations in the soluble complex and VFA substrate concentrations based on seasonal variations. This would likely be useful in estimating the soluble substrate removal efficiency of short SRT processes depending on the RWI temperature.
Figure 3.4 – Comparison of physical-chemical and respirometric methods for $S_S$ determination and measured $S_{SV}$ concentrations versus the ambient temperature of the RWI when the samples were collected. *$S_{SV}$ was not measured.

Figure 3.5b contains the calculated initial active biomass concentration and maximum growth rate for each batch test. Although the batch tests were run at 20°C, there was a clear linear correlation of the maximum specific growth rate ($R^2 = 0.40; p < 0.01; n = 19$) with ambient RWI temperature when the samples were collected. This suggests that the organisms regulated anabolic activity based upon the RWI temperature and the organisms did not immediately acclimate during the batch tests. What was not expected was a decrease in the active biomass fraction with an increase in RWI temperatures. If higher temperatures result in faster growth rates the active fraction should also have been higher. However, the batch tests only measured the fraction of aerobic and facultative organisms and not the obligate anaerobes. Since fermentation also increases with increasing temperature, it was likely that the anaerobic
population increased in the collection system while the aerobic or facultative populations decreased.

Figure 3.5 – Comparison of initial readily biodegradable substrate, active biomass, and maximum growth rate (a) using different methods for (●) $S_{\text{phys}}$ ($R^2 = 0.11; p = 0.158; n = 19$); (■) $S_{SV}$ ($R^2 = 0.55; p < 0.01; n = 18$); (▼) $S_{\text{resp}}$ ($R^2 = 0.67; p < 0.01; n = 19$); and (b) respirometry method for (●) maximum growth rate ($R^2 = 0.40; p < 0.01; n = 19$); (△) $X_{\text{NH}}$ ($R^2 = 0.52; p < 0.01; n = 19$).

**Determination of A-stage effluent $S_S$ and $X_H$ using respirometric methods**

The same batch test used to characterize the RWI $S_S$ and $X_H$ was evaluated to see if the test could also be used to measure $S_S$ and $X_H$ in A-stage effluent samples. If successful, this method would also be useful for determining the $S_S$ removal efficiency of the A-stage and the amount of unflocculated biomass present in the effluent. Example OUR profiles (Day 288) of an effluent
sample without and with an acetate spike are provided in Figure 3.6. The effluent samples did not experience the same OUR increase lag seen with RWI samples suggesting that the organisms present in the effluent were already adapted to aerobic conditions. As seen in Figure 3.6a, the effluent sample on day 288 did not register any detectable S_s. This indicated that all the S_s that could be degradable by the A-stage was degraded. Since these tests utilized the existing A-stage organisms, the tests were not able to quantify the substrate that would be readily degraded in the B-stage process. Additionally, to estimate the active fraction of the same effluent sample since S_S was not present, acetate had to be spiked in order to generate the same OUR response as if S_S was present (Figure 3.6b).

Figure 3.6 – Example OUR profile of (a) effluent sample and (b) effluent sample with acetate spike and model fit. (▬) total OUR; (—) OUR due decay and consumption of slowly biodegradable material.

The S_Sresp results of the effluent batch tests, accounting for the acetate spike, was compared to S_Sphys and is shown in Figure 3.7a. Unlike the influent samples, the effluent S_Sresp did not correlate well with S_Sphys (R = 0.31; p = 0.07; n = 35). However, S_Sphys did correlate with S_SV (R = 0.87; p < 0.001; n = 30) as seen in Figure 3.7b. Although, if S_SV was below approximately 10 mgCOD/L, S_SV did not appear to have an impact of the measurement of S_Sphys. Since a known amount of COD as acetate was spiked in the effluent samples that did not contain any measurable S_S, the calculation method could be confirmed by estimating the initial S_S. Almost all estimates of S_S were two to three times greater than the acetate spike and often well in excess of
the $S_{\text{phys}}$ values. This indicated that either the assumptions for yield and decay were incorrect or $S_S$ was removed via aerobic storage.

![Graph](image)

**Figure 3.7** – Comparison of $S_S$ measure using physical-chemical method to (a) respirometric method with (—) ideal correlation line and (b) VFA method.

**Batch respirometry test to determine $Y_H$ and $b_H$**

To determine if the A-stage heterotrophic yield coefficient ($Y_H$) and decay rate ($b_H$) were significantly different than the assumed values of 0.67 gCOD/gCOD and 0.62 day$^{-1}$ (20°C), batch tests were conducted on A-stage and B-stage sludges. Decay tests were performed on day 361 and 375 and yield tests were performed on day 364 and 378. Figure 3.8a contains example OUR profiles of the decay rate test on A-stage WAS samples ran in triplicate. Figure 3.8b contains two example OUR profiles of the yield test on A-stage mixed liquor samples ran in duplicate.
The decay test results for A-stage sludges (Figure 3.8a) did not conform to typical decay rate curves, which generally follow first order kinetics. Instead, the OUR started increasing after 15 hours from 12 to 25 mgO₂/L·hr. The OUR then began to decline again until the test was stopped. This occurred during all tests with A-stage sludge. The B-stage samples exhibited typical first order decay rates (data not shown). To determine the decay rate for the A-stage samples, the declining tail after the OUR peaked was used and these results are in presented Table 3.2 along with the measured B-stage decay rates. The average A-stage decay rate was 1.87±0.42 day⁻¹, which is more than double the generally accepted lysis-regrowth decay rate of 0.62 day⁻¹ (at 20°C) (Henze et al., 2000). The B-stage average decay rate (0.69±0.11 day⁻¹) was closer to the 0.62 day⁻¹ value.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A-stage</th>
<th>B-stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_H$ (day⁻¹)</td>
<td>1.87 (± 0.42; n = 5)</td>
<td>0.69 (± 0.11; n = 4)</td>
</tr>
<tr>
<td>$Y_H$ (gCOD/gCOD)</td>
<td>0.79 (± 0.02; n = 8)</td>
<td>0.90 (± 0.05; n = 4)</td>
</tr>
</tbody>
</table>

(± 95% Confidence Interval; count)
Interestingly, the calculated decay rate for the A-stage was higher than all the values provided in the literature as summarized by Friedrich and Takács (2013). Several studies found that the decay rate is relatively constant and independent of the process SRT (Marais and Ekama, 1976; Ramdani et al., 2010; van Haandel et al., 1998). However, these studies only measured the decay rate of activated sludge processes operated between 2.5 and 30 day SRT. Wentzel et al. (1995) suggested that in very low SRT processes, like the A-stage, the decay rate may actually be lower due to the lack of predators (i.e., higher lifeforms that graze on the bacteria and consume oxygen). Although, organisms with high maximum growth rates are known to have high death rates (Grady Jr. et al., 2011) and the A-stage only selects for the fastest growing organisms. Therefore, it was postulated that decay rate batch tests were actually measuring the OUR associated with the consumption of stored products or hydrolysis and consumption of adsorbed substrate. Friedrich and Takács (2013) also measured high decay rates in the first two days of batch decay rates tests, which was attributed to the degradation of stored (both internal and external) material. However, what was not clear in the A-stage tests was why the OUR increased. One theory is that the bacteria adapted to the test conditions by increasing hydrolytic activity and thus an increase in OUR was seen due to increased substrate availability. The rate at which OUR increased seemed to follow first order kinetics suggesting it was a function of hydrolysis as opposed to exponential growth. It was also postulated that prior to the OUR peak, utilization of readily hydrolysable substrate was occurring and then the declining curve represented the more difficult to hydrolyze and degrade compounds. What is clear is that the decay rate tests were not a true representation of the decay rate. As demonstrated by Friedrich and Takács (2013), once the hydrolysable substrate is consumed the rate at which OUR declines decreases and this represents the true decay rate of active organisms. Extending the A-stage decay rate tests to capture this decline may not have accurately predicted the true decay rate of the A-stage because the test duration would have been several orders of magnitude greater than the A-stage sludge age. Meaning that the bacterial population in the batch tests could have completely changed as the faster growing organisms decayed while slower growing organisms with lower decay rates established themselves as the dominated population.

The A-stage and B-stage yield coefficients average 0.79±0.02 and 0.90±0.05 gCOD/gCOD, respectively. The yield values for both processes were higher than the accepted value of 0.67 gCOD/gCOD (Henze et al., 2000). High yield values using the calculation method described in
this paper indicates that aerobic storage of acetate may have been occurring during the batch tests. This is because internal storage of carbon requires less energy and thus less oxygen (i.e., OUR) than if the carbon were completely oxidized. Using the same method as this study, Third et al. (2003) reported observed yield coefficients ranging from 0.77 to 0.83 for an activated sludge process operated with an SRT of 11 days where intracellular storage of acetate as polyhydroxybutyrate (PHB) was confirmed. The fact that the B-stage had a higher yield coefficient during the batch tests indicates that the B-stage had a greater storage potential than the A-stage. This was likely due to the B-stage process being operated under feast-famine conditions, which is known to promote intracellular storage (Majone et al., 1999). Additionally, the A-stage retains only the fastest growing bacteria, which have a lower propensity to store because the process SRT is maintained near the bacteria’s maximum growth rate (van Aalst-van Leeuwen et al., 1997). Although, the batch tests indicated the A-stage and B-stage had the ability to aerobically store acetate, these tests do not confirm that storage was actually taking place in the pilot process.

Conclusions

This study compared a physical-chemical method for the determination of readily biodegradable substrate to a respirometric method in order to ascertain if the same methods used for influent wastewater characterization could also be used to characterize the effluent of HRAS processes operated below a 1-day SRT. The respirometric method was also used to quantify the active biomass fraction of the influent and effluent of the HRAS process. The RWI temperature had a significant effect on the estimations of SS using the respirometric method while no effect was seen with the physical-chemical method likely due to COD transformations in the collection system. The respirometry method was not capable of estimating the effluent SS and XH fractions because the respirometric test method inherently provided optimal conditions for aerobic storage to occur. Therefore, the model that was used to estimate the wastewater fractions needs to be modified to account for aerobic storage. Once this is accomplished, the respirometric method could be an accurate tool for characterizing the influent and effluent of high-rate processes and ultimately support efforts in the development of mechanistic models by providing a means to evaluate COD removal mechanisms in high-rate processes.
Acknowledgments

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References


Chapter 4: COD removal mechanisms in a highly-loaded activated sludge process

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Abstract:

To achieve carbon and energy neutrality, carbon management within a water resource recovery facility must be understood and controlled. Energy neutrality has been successfully achieved in two-stage activated sludge processes where the first stage is a highly-loaded activated sludge process referred to as the A-stage. This study explored the mechanisms responsible for chemical oxygen demand (COD) removal in an A-stage pilot process treating municipal wastewater by conducting long-term COD fractionations on the influent and effluent of the process. Solids retention time (SRT) was found to be the primary control parameter and below a 0.5 day SRT the dominate COD removal mechanisms were assimilation and oxidation of readily degradable substrate and sedimentation of particulate matter. At SRTs between 0.5-1 days, COD removal became a function of hydrolysis, as adsorption of particulate and colloidal matter was maximized but limited based on available adsorption sites. Once adequate adsorption sites became available, effluent quality was dependent on bioflocculation efficiency and solids separation.
Introduction

Carbon management and capture is a critical aspect of nutrient removal and resource recovery at water resource recovery facilities (WRRF) that are trying to achieve energy and carbon neutral status. While the activated sludge process still remains the most widely used unit process, it typically requires a substantial amount of energy in the form of aeration (0.3 kWh/m$^3$ of wastewater treated) and results in the chemical destruction of influent organic carbon and thus loss of energy recovery potential and reducing power for denitrification (McCarty et al., 2011). The paradigm shift to energy and carbon neutrality has created a renewed interest in the understanding of carbon removal mechanisms in high-rate activated sludge (HRAS) processes with the goal of reducing carbon oxidation and maximizing the quantity of carbon that can be captured and redirected to energy producing processes, like anaerobic digestion.

One of the most notable activated sludge processes is the two-sludge process known as the adsorption bio-oxidation (A/B) process where the A-stage achieves 50-80% chemical oxygen demand (COD) removal and the B-stage is operated to accomplish biological nitrogen removal (BNR). This configuration, along with advances in automatic process control, development of shortcut BNR technologies, and implementation of co-digestion of sludge and food scraps or fats, oils, and grease, has enable facilities to produce more energy than it consumes. One prime example is the Strass WRRF located in Austria (Schaubroeck et al., 2015; Wett et al., 2013). The A-stage is unique in that it is operated below a 1-day solids retention time (SRT) with a 30-minute design hydraulic retention time (HRT) and is typically not preceded by primary sedimentation. Although the Strass WRRF is energy neutral, COD removal and capture mechanisms are not well documented and understood in HRAS processes, like the A-stage.

Although it is not clear how these values were obtained, Böhnhke et al. (1998) estimated that the COD removal mechanisms of an A-stage achieving 55% COD removal were 30% by sedimentation, 15% by biological means (assimilation and oxidation), and 10% by adsorption onto settleable particles. The settleable material, both inert and biodegradable, likely acts as a ballasting agent when enmeshed with lighter bioflocs leading to fast settling sludge as reported in the literature (Böhnhke, 1994). However, there still exists a significant fraction of slowly and nonsettleable solids present in the effluent of these processes. This indicates that adsorption and
biofloculation is incomplete at short SRTs. Adsorption kinetics of particulate and colloidal material has been shown to be rapid in high-rate processes operated at SRTs >1 day (Jimenez et al., 2007; La Motta et al., 2004; Zhao et al., 2000). Therefore, it is likely that the A-stage is biofloculation or adsorption limited because of low concentrations of extracellular polymeric substances (EPS) or total biomass (i.e., mixed liquor suspended solids; MLSS). EPS not only forms the structural matrix of aggregated bacteria but it is also responsible for the enmeshment of particulate and colloidal material and the retention of exoenzymes required for their subsequent hydrolysis and degradation (Frølund et al., 1995; Morgenroth et al., 2002). EPS components have been measured in high-rate aerobic membrane bioreactor (MBR) processes, however, the solids separation mechanism (i.e., membrane) likely plays a significant role in determining the EPS content of the sludge (Faust et al., 2014a; b; Ng and Hermanowicz, 2005). Therefore, the results of these studies may not be applicable to HRAS processes utilizing gravitational settling tanks for solids separation.

Readily biodegradable compounds (Sₜ) are generally the first substrates to be utilized for growth and energy via oxidation. Oxidation of influent COD in HRAS processes has been estimated to be as low as 12% at a 0.1 day SRT increasing to over 50% oxidation at SRTs longer than 4 days (Ge et al., 2013; Haider, 2002; Jimenez et al., 2015; Müller-Rechberger et al., 2001). However, at short SRTs the Sₜ fraction, when measured using physical-chemical methods, is not completely removed (Fall et al., 2011; Gatti et al., 2010; Haider et al., 2003). This has led to the theory that different microbial populations exist within the different stages of the A/B process (Haider et al., 2003). The microbes in the B-stage are capable of degrading all of the SS coming from the A-stage but these microbes grow at slower rates compared with the microbes in the A-stage. On the other hand, due to the low SRTs, the A-stage retains only the fastest growing organisms that can only utilize the rapidly degradable substrates, like volatile fatty acids (VFAs).

Another mechanism of VFA removal that is not well understood in high-rate processes is the uptake of VFAs under aerobic conditions and storage as intracellular inclusions. The two primary factors that result in intracellular storage is higher uptake than consumption rates when substrate is non-limiting or under substrate limitations such as nutrients or electron acceptors (Majone et al., 1999). Intracellular storage typically occurs in activated sludge processes operated with feast/famine conditions and is most notable in biological phosphorus removal processes. Although storage is commonly cited as a primary mechanism of COD removal in the
A-stage process, Haider (2002) only observed 2% storage of influent COD in an A-stage pilot process. However, Haider (2002) only measured polyhydroxybutyrate (PHB) and did not include other storage compounds like glycogen and other polyhydroxyalkonates (PHA). Carucci et al. (2001) found that aerobic storage as PHB only accounted for 18-22% of the readily biodegradable substrate removed in an activated sludge plant operated with a 16 day SRT, but as soon as the readily biodegradable substrate was depleted, degradation of the stored PHB occurred resulting in little accumulation of PHB in the waste sludge (Carucci et al., 2001). While aerobic storage kinetics have been studied in continuously fed pure cultures at short SRTs (van Aalst-van Leeuwen et al., 1997) there is literature available on aerobic storage in mixed-culture processes at SRTs <2 days (Beccari et al., 2002; Beun et al., 2002; Beun et al., 2000; Carta et al., 2001). What is clear from the literature is that when the dilution rate or SRT of a process approaches the maximum specific growth rate of the organisms, PHB accumulation decreases (van Aalst-van Leeuwen et al., 1997).

In this study, COD removal mechanisms were quantified in an A-stage HRAS pilot process treating municipal wastewater. This study is the first of its kind to complete a long-term detailed COD fractionation on both the influent and effluent of a HRAS process operated below a 0.5 day SRT. Additionally, the impact of HRT, temperature, and dissolved oxygen on the COD removal mechanisms was evaluated.

**Materials and methods**

**Pilot configuration**

The A-stage pilot consisted of three vertical, complete-mix bioreactors in series followed by a clarifier and effluent storage tank (Figure 4.1). The total working volume of the bioreactors was 0.51 m$^3$ and each bioreactor had a side water depth of 3.4 m. The clarifier had a working volume of 1.7 m$^3$ with a surface overflow rate of 17 m$^3$/m$^2$·day at the design flow of 17 L/min. At the design flow, the HRT of the bioreactors and clarifier was 30 minutes and 1.7 hours, respectively. The pilot was fed screened (2-3 mm openings) and degritted municipal wastewater after the wastewater temperature was adjusted to a user set point between 15-25°C using submersible heaters (OEM OTS, Minneapolis, MN) or a water chiller (Aqualogic MT-9, San Diego, CA). The influent and return activated sludge (RAS) flows were flow-paced using progressive cavity
pumps (Seepex BW5, Bottrop, Germany) with variable frequency drives (VFDs) and magnetic flow meters (Rosemount 8705, Houston, TX). Waste activated sludge (WAS) was removed from the underflow of the clarifier using a digital, speed-controlled peristaltic pump (Masterflex L/S, Vernon Hills, IL). Aeration was provided using compressed air through a single mechanically operated valve (MOV; v-notch ball valve) to fine-pore membrane disc diffusers (17.8 cm diameter) mounted on the bottom of each bioreactor as shown in Figure 4.1. Airflow was balanced between the three bioreactors using separate needle valves. An optical DO sensor (InsiteIG Model 10, Slidell, LA) was installed in Tank 3 (the last bioreactor). This is similar to full-scale plants that install DO sensors at the end of aeration tanks or controlled diffuser fields. An infrared MLSS sensor (InsiteIG Model 15, Slidell, LA) was installed in the middle bioreactor (i.e., Tank 2) as that reactor should have represented the average MLSS of the entire A-stage process. SRT control was achieved by maintaining a constant waste flow or using a MLSS-based SRT controller as described in Chapter 5.

![A-stage pilot schematic](image)

Figure 4.1 – A-stage pilot schematic. (—) process water; (—–) solids; (—) air.
Sampling and analysis

Performance of the A-stage pilot was assessed by collecting 24-hr flow-weighted composite samples of the RWI and effluent and analyzing for COD, soluble COD (1.5 μm glass microfiber filtered), carbonaceous biochemical oxygen demand (cBOD$_5$), soluble cBOD$_5$ (1.5 μm glass microfiber filtered), total, volatile, and mixed liquor suspended solids (TSS, VSS, MLSS), total Kjeldahl nitrogen (TKN), total ammonia nitrogen (TAN), total phosphorus, and alkalinity according to Standard Methods (APHA, 2012). Particulate COD (pCOD) was calculated as the difference between total COD and sCOD. Process pH was determined using a handheld pH meter (Beckman Coulter Φ 400 Series, Brea, CA). Volatile fatty acids (VFA) were determined using the gas chromatography method (5560D) outlined in Standard Methods (APHA, 2012). Mixed liquor and WAS samples were also analyzed by collecting 24-hr flow-weighted composite samples and measuring COD, TKN, TP, MLSS, and mixed liquor volatile suspended solids (MLVSS). All composite samples were maintained at 4°C until analyzed.

Raw wastewater influent and A-stage effluent COD characterization

A full COD fractionation was conducted on a weekly basis for the duration of this study (>600 days). This included the determination of the soluble unbiodegradable or inert fraction (S$_I$), soluble readily biodegradable fraction (S$_S$), particulate slowly biodegradable fraction (X$_S$), and particulate unbiodegradable fraction (X$_I$). The soluble inert fraction of a wastewater is considered equal to the effluent soluble COD of a biological processes fed that wastewater and operated at an SRT >3 days (Germirli et al., 1991). For this study, S$_I$ was assumed to be equal to the soluble COD (measured as ffCOD) of the B-stage process. The S$_S$ fraction of the RWI and A-stage effluent was calculated as the difference between the RWI or A-stage effluent ffCOD and S$_I$ (i.e., B-stage effluent ffCOD). The X$_I$ fraction was calculated as the difference between pCOD and particulate cBOD$_5$ (i.e., cBOD$_5$ – scBOD$_5$). While the standard BOD method is considered a respirometric method, it does not use seed inoculum from the process of interest (e.g., A-stage) and therefore does not determine the actual fraction that is biodegradable in the A-stage. However, since the seed inoculum is the same for the influent and effluent samples, the BOD measurement is useful in determining the fraction of biodegradable material that is
removed by the A-stage. The final fraction $X_S$ was calculated as the difference between the total COD of the sample and $S_I$, $S_S$, and $X_I$. $S_S$ was further fractionated into VFAs ($S_{SV}$) and soluble complex substrate ($S_{SC}$) by subtracting the measured VFA values from $S_S$. $X_S$ was further fractionated into particulate ($X_{SP}$) and colloidal ($X_{SC}$) slowly biodegradable substrate where the colloidal fraction was the difference between pCOD and fCOD of the sample.

**Oxygen uptake rate**

The A-stage bioreactors were completely sealed and vented to a common off-gas header (Figure 4.1). The off-gas was continuously monitored for oxygen content using a paramagnetic gas analyzer (Servomex 1440D, Crowborough, UK). Prior to measurement, the off-gas sample passed through moisture and carbon dioxide scrubbers to reduce interference of the gas analyzer. The total airflow to the A-stage bioreactors was measured using a gas mass flowmeter (Alicat M-Series, Tucson, AZ). The airflow, off-gas oxygen content, process DO, and process temperature values were then used to calculate the oxygen transfer efficiency (OTE), actual oxygen transfer rate (AOTR), and finally oxygen uptake rate (OUR). Specific OUR (SOUR) reported as mgO$_2$/gSS·hour was calculated as OUR (mgO$_2$/L·hour) divided by the mixed liquor VSS (gSS/L) concentration.

**Mass balances**

Weekly COD and TP mass balances were calculated for the A-stage pilot based on the influent, effluent, and WAS flows. COD balances were only accepted if the TP balance on the same samples closed within a ±10% error. The difference in the COD mass balance was considered to be the oxidized or mineralized COD fraction unaccounted for in the off-gas. The total COD mass balance was extended by performing a mass balance on the individual COD fractions. However, since the WAS samples were not fully fractionated, WAS pCOD was considered to equal the sum of $X_I$ and $X_{SP}$ and sCOD the sum of $S_S$, $S_I$, and $X_{SC}$. The soluble fractions were assumed to be equal to the effluent soluble fractions.

**Batch test**

To determine COD removal mechanism and rates, batch test were carried out in 4 L containers on magnetic stir plates. DO was monitored using a Hach IntelliCAL™ luminescent DO (LDO)
sensor (Loveland, CO). The reactors were covered during batch tests to prevent oxygen intrusion. Batch tests were conducted at the same temperature as the process. Samples for soluble parameters (i.e., sCOD, ffCOD) were collected at 5 to 10 minute intervals using 60 mL syringes and immediately filtered using the appropriate filter size and type as described in section 2.2. All other samples, including COD, turbidity, TSS, and VSS, were collected using a 100 mL beaker and settled for 30 minutes. The supernatants were then carefully extracted using 60 mL syringes so not to disturb any settled solids.

Statistical methods

Statistical analyses including the Pearson product moment coorelation, Shapiro-Wilk normality test, Mann-Whitney rank sum test, t-test, linear regressions, cumulative distribution functions, confidence and prediction intervals, mean, standard deviation, and standard error were performed using SigmaPlot 12.5 (Systat Software Inc., Bangalore, India). Confidence intervals were calculated at a $p$-value of 0.05. Means are presented with standard deviations unless noted otherwise.

Results and Discussion

General performance of the A-stage pilot

Average RWI and effluent characteristics of the A-stage pilot over a two-year period are shown in Table 1. The RWI fed to the pilot was considered medium strength with total COD averaging 562±88 mg/L and septic in nature because the sewage collection system is comprised of long anaerobic pressure mains (Table 4.1). Due to fermentation in the sewage collection system, the average $S_{SV}$ concentration was high around 66±20 mgCOD/L. The $X_S$ and $X_I$ fractions averaged 216±50 and 197±106 mgCOD/L, respectively. The A-stage was operated in such a way that COD removal was not maximized at each operating condition but rather controlled to produce the effluent necessary for optimal nitrogen removal in the downstream BNR process. Therefore, the A-stage pilot only averaged 48±13% COD, 52±13% cBOD$_5$, and 55±14% TSS removal. Compared to primary sedimentation that typically removes 20-30% COD, 25-40% cBOD$_5$, and 50-70% TSS, the A-stage removal efficiencies were higher except for TSS. It was speculated that conversion of soluble substrate to colloidal material, which is included in the TSS measurement
method, was the reason TSS removal was not higher in the A-stage compared to primary sedimentation. It is also worth noting that TKN (14±7%) and TP (24±11%) were also removed in the A-stage, primarily due to sedimentation and assimilation into new biomass. However, TP removal was also high because the facility at which the pilot was located fed ferric chloride for odor control resulting in some chemical phosphorus removal through the A-stage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RWI</th>
<th>Effluent</th>
<th>Removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>562 (±88)</td>
<td>287 (±69)</td>
<td>48 (±13)</td>
</tr>
<tr>
<td>cCOD (mg/L)</td>
<td>213 (±29)</td>
<td>137 (±32)</td>
<td>36 (±12)</td>
</tr>
<tr>
<td>pCOD (mg/L)</td>
<td>350 (±86)</td>
<td>151 (±50)</td>
<td>55 (±16)</td>
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<tr>
<td>S&lt;sub&gt;i&lt;/sub&gt; (mgCOD/L)</td>
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<td>21 (±4)</td>
<td>0 (±0)</td>
</tr>
<tr>
<td>S&lt;sub&gt;s&lt;/sub&gt; (mgCOD/L)</td>
<td>121 (±17)</td>
<td>38 (±16)</td>
<td>71 (±12)</td>
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<td>S&lt;sub&gt;SV&lt;/sub&gt; (mgCOD/L)</td>
<td>66 (±20)</td>
<td>9 (±13)</td>
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<tr>
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<td>158 (±35)</td>
<td>30 (±16)</td>
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<tr>
<td>X&lt;sub&gt;SP&lt;/sub&gt; (mgCOD/L)</td>
<td>154 (±47)</td>
<td>77 (±28)</td>
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<tr>
<td>X&lt;sub&gt;SC&lt;/sub&gt; (mgCOD/L)</td>
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<td>81 (±18)</td>
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<td>X&lt;sub&gt;I&lt;/sub&gt; (mgCOD/L)</td>
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<td>81 (±32)</td>
<td>58 (±17)</td>
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<tr>
<td>cBOD&lt;sub&gt;S&lt;/sub&gt; (mg/L)</td>
<td>250 (±55)</td>
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<tr>
<td>scBOD&lt;sub&gt;S&lt;/sub&gt; (mg/L)</td>
<td>97 (±15)</td>
<td>45 (±18)</td>
<td>54 (±14)</td>
</tr>
<tr>
<td>TSS (mgSS/L)</td>
<td>207 (±71)</td>
<td>94 (±29)</td>
<td>55 (±14)</td>
</tr>
<tr>
<td>VSS (mgSS/L)</td>
<td>183 (±63)</td>
<td>81 (±25)</td>
<td>54 (±15)</td>
</tr>
<tr>
<td>TKN (mgN/L)</td>
<td>43 (±4)</td>
<td>38 (±4)</td>
<td>14 (±7)</td>
</tr>
<tr>
<td>TAN (mgN/L)</td>
<td>35 (±4)</td>
<td>32 (±4)</td>
<td>11 (±5)</td>
</tr>
<tr>
<td>TP (mgP/L)</td>
<td>5.8 (±0.7)</td>
<td>4.4 (±1.1)</td>
<td>24 (±11)</td>
</tr>
<tr>
<td>Alkalinity (mgCaCO&lt;sub&gt;3&lt;/sub&gt;/L)</td>
<td>178 (±18)</td>
<td>167 (±17)</td>
<td>8 (±5)</td>
</tr>
<tr>
<td>pH</td>
<td>6.6 (±0.1)</td>
<td>7.0 (±0.1)</td>
<td>-5 (±2)</td>
</tr>
</tbody>
</table>

Overall performance of the A-stage pilot, in terms of COD removal efficiency, is depicted in Figure 4.2, which compares the specific removal rate (SRR) to the specific loading rate (SLR) of the process. When these values are equal, the process is removing all of the influent COD. However, since the COD measurement includes the unbiodegradable soluble fraction (S<sub>i</sub>) and nonsettleable solids (i.e., effluent TSS), the SRR will never equal the SLR. This can be seen in Figure 4.2 when the SRR parallels the complete removal line until the SLR increases above approximately 8 kgCOD/kgMLSS·day. This indicated that beyond this SLR, the process became overloaded and the COD removal efficiency became more a function of the influent load rather
than the specific operation conditions, like SRT or DO. Figure 4.2 also indicates that the maximum achievable SRR was approximately 15 kgCOD/kgMLSS·day.

![Graph showing specific removal rate vs specific loading rate](image)

**Figure 4.2** – Comparison of the A-stage specific loading rate to the specific removal rate. Line (▬) represents complete removal of influent COD load.

**Impact of SRT, HRT, and temperature on COD removal efficiency**

The A-stage pilot was operated for over 600 days at various HRTs (20-60 minutes) and RWI temperatures (15-25°C). For the majority of the study, the HRT was maintained constant at 30 minutes. Although HRT correlated with COD removal efficiency ($R = 0.26$, $n = 686$, $p <0.001$), the role of HRT in determining the operational range of SRT was more important. That is, SRT affected COD removal efficiency more significantly ($R = 0.47$, $n = 646$, $p <0.001$) and increasing the HRT just allowed the process to be operated at a longer SRT. As with most full-scale processes, the limiting factor of SRT is the solids loading of the clarifiers and the ability to maintain adequate bulk DO. The impact of SRT on COD removal efficiency at different RWI temperatures is shown in Figure 4.3. As SRT increases, COD removal increases until reaching a maximal removal between 70-80%. Higher COD removal efficiencies near 85-95% would be expected if the SRT was increased beyond a one day SRT, as has been demonstrated by other similar studies on HRAS (Ge et al., 2013; Jimenez et al., 2015).
Temperature did not have a significant impact on COD removal efficiency until the A-stage was operated at 15°C. The lower removal efficiencies associated with the lower temperatures is likely due to reduced growth rates and metabolic activities (Grady Jr. et al., 2011). It is well understood that chemical reactions, enzymatic hydrolysis, and cell division are inherently slower at lower temperatures (Bitton, 2011). Due to the reduced reaction rates at 15°C, the A-stage would need to be operated at a longer SRT in order to achieve similar COD removals as seen at 20-25°C. Depending on the requirements of the treatment facility, seasonal fluctuations of RWI temperature could have an impact on the design capacity and tank redundancy in order to accommodate the ability to operate at various HRTs. Seasonal HRT control could be achieved by maintaining treatment trains in or out of service as needed.

Comparison of the A-stage MLSS concentration to COD removal efficiency (Figure 4.4) yielded a similar curve as seen in Figure 4.3 since MLSS is a function of SRT and HRT. However, COD removal performance did not increase beyond a MLSS concentration around 3000 mgSS/L. This suggests that the A-stage was not limited because of biomass inventory, but rather some other metabolic or degradation pathway. For example, the rate of extracellular hydrolysis has been well established as a rate limiting process (Dold, 1980). However, hydrolysis is typically modeled as a function of the biomass concentration (i.e., MLSS) using first-order kinetics (Morgenroth et al., 2002). EPS also plays a key role in the immobilization of extracellular
enzymes allowing for localized hydrolysis inside of bioflocs as opposed to if the enzymes were released to the bulk solution (Frølund et al., 1995). However, the EPS content of mixed liquor has been shown to be low (<75 mgCOD/gVSS) in shorter SRT (<0.5 day) HRAS processes (Jimenez et al., 2007). Faust et al. (2014b) also measured low protein content of 40 mgEPS/gVSS in a highly loaded membrane bioreactor (MBR) operated at an SRT of 0.125 days. These observations would suggest that the initial rapid increase in COD removal efficiency from approximately 20 to 60% with increasing MLSS was likely just a function of readily biodegradable substrate consumption. After the readily biodegradable substrate was depleted, COD removal efficiency became reliant on EPS production and hydrolysis and thus a slower increase in COD removal efficiency with increasing MLSS and SRT was seen.

To determine the impact of SRT on the removal of specific COD fractions, the influent and effluent of the A-stage pilot was characterized. All the COD fractions, except for $S_I$ and $X_{SC}$, followed similar trends as total COD removal (Figure 4.3). The $S_I$ fraction did not change since it was assumed to be the same as the effluent ffCOD of the B-stage and this fraction passed through the entire A/B pilot process without undergoing any changes. Unlike the other fractions (i.e., $S_S$, $X_{SP}$, $X_I$) that were removed, $X_{SC}$ actually increased as SRT decreased. This is similar to the results by Jimenez et al. (2015), where $X_{SC}$ removal approached zero percent near a 0.2 day
SRT. Figure 4.5 contains the cumulative distribution of the removal efficiencies of $S_S$, $X_{SC}$, $X_{SP}$, $X_I$. The slope of the lines in Figure 4.5 for $X_I$, $X_{SP}$, and $S_S$ indicate that the removal of each fraction was impacted similarly by SRT. $S_S$ had the highest removal efficiencies with 90% of the removal values greater than 50%. $X_I$ and $X_{SP}$ had nearly identical removal efficiencies suggesting that the removal mechanisms of each fraction are the same. This was expected since these fractions are removed by adsorption to the bioflocs and subsequent removal through solids separation (Jimenez et al., 2005).

Figure 4.5 – Cumulative distribution of COD removal efficiencies. (▲) $X_{SC}$; (▼) $X_{SP}$; (■) $S_S$; (▲) $X_I$.

**Impact of DO on COD removal efficiency**

The DO concentration in the last reactor (Tank 3 on Figure 1) was continuously monitored and 24-hr daily averages were compared to COD removal efficiencies. Bulk DO did not correlate with COD ($R = -0.02$, $n = 242$, $p = 0.74$), sCOD ($R = -0.15$, $n = 241$, $p = 0.017$), and pCOD ($R = 0.03$, $n = 241$, $p = 0.64$) removal efficiencies. This was likely due to the fact that heterotrophic organisms (OHO) have such a low DO half saturation coefficient ($K_{DO} <0.05$ mgO$_2$/L) that DO only impacted OHO growth rates when the DO concentration was less than 0.2 mg/L. In a complete-mix activated sludge pilot process, Jimenez et al. (2015) demonstrated that sCOD removal efficiency was only significantly impacted when the bulk DO of the process as
maintained below 0.1 mgO₂/L. DO has also been shown to impact adsorption of colloidal and particulate material and EPS production. Wilén and Balmér (1998) found that short-term (1.5 hours) changes in DO from high (>2 mgO₂/L) to low (<1 mgO₂/L) resulted in an increase in turbidity. However, this was attributed to deflocculation and release of adsorbed material and not associated with total EPS content as the duration of the tests were too short (<8 hours). Wilén and Balmér (1998) also determined that the specific turbidity removal rate was not statistically different when the DO ≥5 mgO₂/L compared to <0.5 mgO₂/L. However, these tests only looked at impact of DO on the adsorption kinetics and not EPS production. In a highly loaded MBR (SRT = 0.5 days), Faust et al. (2014a) measured a difference in EPS production of 122 and 175 mgEPS/gVSS when operated at a DO of 1 and 4 mgO₂/L, respectively. These differences translated into bioflocculation efficiencies of 65% at 1 mgO₂/L and 91% at 4 mgO₂/L, where bioflocculation was calculated as colloidal COD removal. The authors attributed the higher EPS content with higher sCOD removal efficiencies at higher DO (i.e., faster growth rates) with the assumption that EPS production is a function of substrate consumption as suggested by several studies (Jimenez et al., 2015; Laspidou and Rittmann, 2002). Another factor that was not considered is that hydraulic conditions are also associated with increased EPS production and at higher DO concentrations the mixing intensity is higher potentially leading to increased EPS production and generation of bioflocs capable of handling higher shear forces (Sheng et al., 2010).

Since the bulk DO concentration did not correlate with COD removal efficiency, the total OUR and SOUR of the A-stage was compared to SRT (Figure 4.6) and COD removal efficiencies. OUR correlated well with COD \( (R = 0.36, n = 232, p < 0.001) \), sCOD \( (R = 0.40, n = 232, p < 0.001) \), and pCOD \( (R = 0.35, n = 230, p < 0.001) \) removal. As shown in Figure 4.6a, OUR increased as SRT increased until the maximum transfer rate of the aeration equipment was met. For the pilot, this was between 150-200 mgO₂/L·hr. Most full-scale aeration systems can only reasonably achieve a maximum OUR of approximately 150 mgO₂/L·hr. This limitation imposed by oxygen transfer efficiency possibly explains why the A-stage COD removal efficiency peaked around 70-80% even with an increase in SRT and MLSS concentration (Figure 3 and 4). The oxygen transfer limitation can only be alleviated by increasing the HRT of the process, which in full-scale WRRF would be in the form of the aeration tank volume.
When comparing SOUR to SRT (Figure 4.6b), SOUR decreases with increasing SRT suggesting lower specific activity rates at longer SRTs. This was likely associated with the rapid consumption of readily biodegradable substrate. Once the readily biodegradable substrate was exhausted, the SOUR became a function of slowly biodegradable substrate resulting in lower SOUR values. However, SOUR may have also been lower at longer SRTs because of the oxygen transfer limitation. That is, when OUR reached the maximum limit but the MLSS concentration kept increasing with increasing SRT, the value of SOUR decreased.

**Role of heterotrophic growth and aerobic storage on S_{S}\text{removal}**

To evaluate the removal mechanisms of readily biodegradable substrate (S_{S}), the RWI and A-stage effluent were analyzed for soluble VFAs (S_{SV}) weekly. The removal efficiency of S_{SV} in the A-stage pilot at various SRTs is presented in Figure 4.7. The RWI S_{SV} was primarily comprised of acetic acid (79 ± 7%; n = 86) and propionic acid (15 ± 4%; n = 86) with only minor contributions (< 3%) of butyric, isobutyric, valeric, isovaleric, and caproic acids detected. Total VFAs in the influent fluctuated from 30-40 mgCOD/L in the colder months (<20°C) to >50 mgCOD/L in the warmer months (25°C) because of increased fermentation rates in the anaerobic sewer collection system. A-stage effluent S_{SV} consisted primarily of acetic acid (86 ± 22%; n = 85), although isovaleric acid contributed a significant portion when it was present in measurable quantities (28 ± 23%; n = 37). S_{SV} was rapidly removed and nearly complete (>95%) at SRTs as low as 0.15 days. This was expected since VFA is easily transported across cell membranes and rapidly consumed or stored by bacteria.
The overall removal efficiency of $S_S$ was not similar to the trend of $S_{SV}$ depicted Figure 4.7. Rather, as seen in Figure 4.8, at SRTs $> 0.2$ days, A-stage effluent $S_S$ was still between 20-40 mgCOD/L. This suggests that only 30-60% of the influent $S_{SC}$ was removed by the A-stage process. However, this residual $S_{SC}$ fraction was rapidly consumed in the downstream B-stage process (SRT $> 3$ days). These results are consistent with the work by Haider et al. (2003) who also demonstrated that not all of the influent $S_S$ was degraded in A-stage processes operated at SRTs between 0.4-1.0 days. Saunders and Dick (1981) also reported similar results for complete-mix process operated at a 0.8 day SRT. An alternative explanation is that the residual $S_{SC}$ is really the byproduct of bacterial growth, substrate consumption, or cell lysis. These byproducts are often defined as soluble microbial products (SMP) or soluble (unbound) EPS (Laspidou and Rittmann, 2002). Although unlikely at shorts SRTs, the $S_{SC}$ could also represent a fraction of partially hydrolyzed slowly biodegradable substrate. What is clear, is that the degradability of influent organic matter increases with SRT (Saunders and Dick, 1981).
To determine if the residual $S_S$ was the result of process loading rates or metabolic limitations, batch tests were carried out on the A-stage mixed liquor as described in Section 2.8. Mixed liquor samples were subjected to continuous aeration for one hour while measuring fDOC (data not shown). These tests did not show a significant decrease in fDOC by the end of the test suggesting that substrate affinity was not the limiting factor. That is, the half saturation coefficient ($K_S$) for the bacteria present in the A-stage was not limiting the degradation potential of $S_S$ but rather degradation was not occurring because either the bacteria were not capable or were not expressing the necessary exoenzymes capable of hydrolyzing the more complex soluble substrate. It has been postulated that different populations of bacteria exists in activated sludge processes depending on SRT and influent characteristics. Although this has not been studied extensively in HRAS processes, Pala-Ozkok et al. (2012) found that the fast growing phylum *Proteobacteria* dominated in a 2 day SRT process as opposed to the slower growing phylum *Actinobacteria*, which was dominate in a 10 day SRT process. Faust et al. (2015) also found that *Proteobacteria* dominated in a high-rate MBR operated between 0.125-1.0 day SRT. Several other studies measured species diversity and found that species diversity increases with SRT (Başaran et al., 2014; Duan et al., 2009; Pala-Ozkok et al., 2013). However, none of these studies explored the degradability of soluble complex substrate ($S_{SC}$) at different SRTs. It is possible that since the A-stage is so highly loaded (Figure 4.2), the bacteria never produced the exoenzymes
necessary for hydrolysis of $S_{SC}$ because the process was rarely substrate limited or the retention time of the process was too short to accumulate enough enzymatic activity.

Since the majority of $S_{SV}$ consisted of acetic acid it was very likely that aerobic storage as polyhydroxybutyrate (PHB) was occurring as demonstrated by several studies (Beccari et al., 2002; Beun et al., 2000; Carucci et al., 2001; Dircks et al., 2001; Majone et al., 1999). To rule out anaerobic storage of $S_{SV}$ by polyphosphate (PAO) and glycogen accumulating organisms (GAO), batch tests were performed where a sample of A-stage mixed liquor was spiked with acetate under anaerobic conditions and ffCOD was monitored (data not shown). The results did not indicate any anaerobic uptake activity. The results of these test were as expected since the SRT of the A-stage (0.1-0.3 days) was typically an order of magnitude lower than the PAO critical SRT of approximately 1 day at 20°C (Erdal et al., 2006; Mamais and Jenkins, 1992). Although the A-stage was not operated under feast/famine conditions that typically promote aerobic storage, the DO was often limiting in the first reactor because of the high ($x$ mgO$_2$/L-hr) OUR. Third et al. (2003) found that under oxygen limited (<0.01 mgO$_2$/L) conditions, storage yield was higher (0.63-0.68 g PHB/g acetate) than when the DO was >0.9 mgO$_2$/L (0.48-0.49 g/g). However, the specific PHB production rates and growth rates decreased. This means a longer HRT would be required for the same overall substrate removal. Therefore, low DO conditions in the A-stage may actually limit storage of acetate given the fact that the HRT is typically only 30 minutes. PHB production rates have also been shown to decrease with increasing temperature because of higher growth rates at higher temperatures (Krishna and Van Loosdrecht, 1999). The SRT of the A-stage may also play a role in storage since only the fastest growing organisms are retained in the process resulting in high growth rates and low PHB production rates (Beun et al., 2002; van Loosdrecht et al., 1997). Therefore, further work is needed to elucidate the role of aerobic storage in $S_{SV}$ removal in the A-stage.

**Role of EPS production and adsorption on removal of $X_S$ and $X_I$**

To determine the fate of $X_S$ and $X_I$, a complete COD fractionation mass balance was performed on the A-stage and these results are presented in Figure 4.9. The effluent contained only minor fractions of $S_{SV}$ (3.0±0.7%; mean ± standard error) and $S_{SC}$ (9.2±0.6%). The sum of $X_I$ and $X_{SP}$ decreased slightly from 61.3±1.3% in the RWI to 54.7±1.4% in the effluent while $X_{SC}$ increased
from 11.8±0.8% to 26.1±1.4%. The WAS COD was predominately $X_1$ and $X_{SP}$ (96.7±0.3%). The increase of colloidal fraction is likely the result of dispersed growth that has been shown to occur in low SRT processes (Bisogni and Lawrence, 1971). Additionally, the mass balance indicated an increase in the $X_S$ fractions at the expense of $S_S$ oxidation. This suggests that soluble substrate was being converted to new un-bioflocculated biomass that was not removed from the process by settling. This also indicates that the A-stage was bioflocculation or adsorption limited. Faust et al. (2015) measured the bacterial population dynamics of the influent, supernatant, and sludge of a high-rate MBR and found that the influent populations were very similar to the supernatant populations at a short (0.125 days) SRT indicating lack of bioflocculation of the influent biomass. Less similarity was observed as the SRT was increased up to 1 day.

![Figure 4.9](image)

Figure 4.9 – COD fractionation mass balance (a) normalized to RWI COD and (b) fraction of process influent COD. Oxi/Prod: oxidized/produced; (n = 20).

To investigate the adsorption potential of the A-stage mixed liquor a series of batch adsorption tests were conducted as outlined by Tan and Chua (1997). However, the results were inclusive and were often negative indicating floc breakup or desorption of substrate during the test. This again suggests that the A-stage process was bioflocculation limited. Adsorption of particulate and colloidal substrate has been shown to follow first order rate kinetics as a function of MLSS concentration (Morgenroth et al., 2002). However, this assumes that the biomass has an adequate quantity of adsorption sites and bioflocculation would proceed until completion. While this may
be the case in longer SRT processes, this is likely not the case in the A-stage because of the shorter SRT. The rate of adsorption may still be rapid until all available adsorption sites are occupied after which the rate of adsorption would likely be governed by the rate of hydrolysis and oxidation of adsorbed substrate.

To observe COD removal rates, batch test where A-stage RAS was blended with settled RWI were conducted and the results from one of these tests in provided in Figure 4.10. Settled RWI was used so that the settleable solids would not be included in the bioflocculation of particulate and colloidal substrate. The initial values represent the predicted COD values of the blended samples. The first 10 minutes of the test were anaerobic to observe just bioflocculation and the last 20 minutes to observe COD oxidation. As expected, bioflocculation was rapid and appeared to have reached a maximum as indicated by no significant concentration change ($p = 0.805$) of all the COD fractions and turbidity between the 5 and 10 minutes samples. After the air was supplied to batch tests, colloidal and soluble COD (measured as ffCOD) decreased at specific rate of 30 and 92 gCOD/kgVSS·hr, respectively. Alternatively, pCOD increased at a specific rate of 137 gCOD/kgVSS·hr. Interestingly, after the first 5 minutes the total COD did not change significantly ($p <0.001$) by the end of the test.

![Figure 4.10](image-url) – Anaerobic/aerobic batch test results considering (a) all COD fractions and turbidity and (b) pCOD, cCOD, and sCOD values with linear regressions during aerobic period only.
The batch adsorption potential test demonstrated that bioflocculation of pCOD occurs rapidly suggesting first-order kinetics can still be used to describe adsorption in A-stage HRAS processes. However, cCOD adsorption did not occur to the same extent, which is similar to the conclusions of Jimenez et al. (2005) who found that adsorption of colloidal material is slower than particulate material. These observations may be associated with particle size and the increased settleability of larger particles rather than the actual rate of adsorption. That is, the larger the initial particle the less bioflocculation that would need to occur before the particle would settle out of solution. Colloidal particles would therefore inherently require additional time to reach a particle size that would settle fast enough to be removed from the process. This could also explain why pCOD increased during the test as cCOD decreased.

**Summary of COD removal mechanisms**

Proposed mechanisms that limit COD removal in HRAS processes are presented in Figure 4.11, which includes the COD removal efficiencies of this study, Jimenez et al. (2015), and Ge et al. (2013). The initial fraction of COD that is removed regardless of SRT represents settleable solids (i.e., primary solids) in the influent. From there, the COD removal efficiency is limited by specific removals rates like oxidation of $S_S$ and adsorption of $X_S$ and $X_I$. After reaching an SRT around 0.3-0.5 days, COD removal becomes dependent on the ability of organisms to degrade complex soluble material and available adsorption sites, which are limited by the hydrolysis rate. After reaching an SRT of approximately 1 day, removal of the remaining colloidal and particulate material likely becomes a function of floc strength and bioflocculation efficiency. As the SRT increases and carbon availability decreases, the bioflocs become more efficient at capturing and retaining any remaining colloidal and particulate material as a means of survival. However, excess shear forces of a poorly designed solids separation process could impact the efficiency of suspended solids removal. The remaining soluble fraction is then defined as the inert fraction of the effluent, although some fraction may still be degradable in longer SRT process.
Conclusions

Managing influent carbon for energy production and biological nutrient removal requires an understanding of the COD removal mechanisms of activated sludge and how to properly control them. This study found that SRT was the primary control parameter in processes operated below a 1-day SRT. Although HRT and temperature did play a role in COD removal, SRT was more dominate, as it is just a function of both HRT and temperature. This study also supports the theory that different microbial populations exist in activated sludge processes operated at different SRTs and that in low SRT (<0.5 day) processes, soluble complex substrate is not completely removed.

Overall, at SRTs below 0.5 days COD uptake and oxidation, dictated COD removal efficiency and as SRT increased, the process became hydrolysis limited. At this point all available adsorption sites were occupied and thus a large fraction of particulate and colloidal substrate remained suspended in the effluent after solids separation. Once the rate of hydrolysis was no longer limiting, effluent quality then became dependent on bioflocculation efficiency and floc strength. Of course, as the SRT is increase the fraction of influent COD that is oxidized also increases.

Figure 4.11 – Graphical representation of limiting factors on COD removal efficiency of HRAS processes as a function of SRT.
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References


Chapter 5: Control of COD removal in an A-stage pilot study using conventional instrumentation and automatic process control

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Keywords: A/B Process, A-stage, High-Rate Activated Sludge, On-line Sensors, Process Control

Abstract:

This study evaluated the use of \textit{in situ} on-line dissolved oxygen (DO) and mixed liquor suspended solids (MLSS) sensors in the A-stage of an A/B (adsorption/bio-oxidation process) pilot study. The objective was to reduce the variability of chemical oxygen demand (COD) removal efficiency of the A-stage so that the overall stability of the A/B process in terms of nitrogen removal could be improved. An optical fluorescent DO sensor and an infrared MLSS sensor were used in an A-stage process with automatic process controllers to achieve this objective. The use of cascade DO control in the A-stage did not impact COD removal at the conditions tested in this study, likely because the DO concentration (>0.5 mg/L) was maintained above the half saturation coefficient (0.2 mg/L) of heterotrophic organisms. MLSS set point control, where MLSS was used as a surrogate for SRT, was able to reduce COD removal variation in the A-stage by 90%.}

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Introduction

The A-stage of the A/B process (adsorption/bio-oxidation) was developed in the late 1970s as a cost-effective means of decreasing the volumetric requirements of nitrifying activated sludge processes (i.e., B-stage) by reducing the influent chemical oxygen demand (COD) load (Böhnke, 1977). The A-stage is a separate-stage high-rate activated (HRAS) sludge process typically designed with a hydraulic residence time (HRT) of 30 minutes and operated at a solids retention time (SRT) between 0.2 to 1 days, depending on the influent COD load, temperature, and desired COD removal efficiency. A-stage processes are not preceded by primary clarifiers and are generally designed to remove 50-70% of the influent COD as opposed to more conventional HRAS processes (i.e., SRT = 1-4 days; HRT = 1-3 hours) that are operated to meet secondary discharge standards (i.e., 30 mg/L total suspended solids (TSS) and biochemical oxygen demand (BOD₅)) (Böhnke et al., 1998; Feyen, 1992; Orhon, 2014; Wandl et al., 2006). The main disadvantage of the A/B process is that the COD removed in the A-stage is no longer available as an internal organic carbon source for denitrification in the B-stage (de Graaff and Roest, 2012).

To cope with this limitation, biological nitrogen removal (BNR) in the B-stage has been optimized using in situ nutrient sensors and automatic process control (Sorensen et al., 1994). One example is ammonia-based aeration control (ABAC), which is a form DO control that ensures the entire treatment capacity of the B-stage is utilized for BNR (Åmand et al., 2013). However, even with these control strategies, it is difficult to obtain effluent total nitrogen (TN) concentrations below 5 mgN/L in A/B processes because denitrification requires approximately 4.5 gCOD/gN removed (Tchobanoglous et al., 2003).

Considerable research has been undertaken in an effort to decrease the amount of COD required for nitrogen removal through the development of shortcut BNR technologies like nitrite shunt (nitritation/denitrification) and deammonification (partial nitritation/anaerobic ammonia oxidation) (De Clippeleir et al., 2013; Lotti et al., 2014; Regmi et al., 2015; Wett et al., 2013; Winkler et al., 2012). One common requirement of nitrite shunt and deammonification processes, other than the out-selection of nitrite oxidizing bacteria (NOB), is that COD removal is required upstream of the shortcut BNR process. This is because of heterotrophic bacterial competition with anaerobic ammonia oxidizing (anammox) bacteria for nitrite or with ammonia oxidizing bacteria (AOB) for dissolved oxygen (DO) (Xu et al., 2015). These shortcut BNR processes may also be
operated at SRTs near the critical or washout SRT of AOB and NOB with high total ammonia nitrogen (TAN) loading rates (>0.2 kgTAN/m³·day). This subjects the shortcut BNR processes to an increased risk of failure in the event of process upsets or load variations (Regmi et al., 2014; Smitshuijzen, 2014). While the development and use of more advanced automatic process controllers in the B-stage, like Ammonia versus NOx-N (AvN) aerobic volume control (Regmi et al., 2014), has improved process stability, nitrogen removal efficiency in the B-stage has still been hampered by the variability of influent COD/total Kjeldahl nitrogen (TKN) from the A-stage or other upstream COD removal processes (Regmi et al., 2015; Xu et al., 2015). Therefore, application of automatic process control using *in situ* on-line sensors in the A-stage with the goal of controlling COD removal and the influent COD/TKN ratio for the B-stage is critical. Additionally, automatic process control could be used to balance COD removal with COD capture in the waste sludge from the A-stage for energy recovery by biogas production (Jetten et al., 1997).

Other than DO set point control, most full-scale A/B plants put little effort into dynamically controlling the A-stage to generate a consistent influent COD/TKN for the B-stage (de Graaff and Roest, 2012). The use of DO and SRT controllers in activated sludge processes operated at SRTs >1 day has been well documented (Olsson, 2012). However, their use in an A-stage process operated with the goal of controlling the C/N input to a shortcut BNR process has not been studied. Therefore, the objective of this study was to evaluate well-established forms of automatic process control using readily available *in situ* on-line sensors so that full-scale adoption would be straightforward and familiar to plant operators. Cascade DO control and MLSS set point control were evaluated in terms of COD removal performance and process variability in the A-stage of an A/B pilot study treating municipal sewage. Prior to the evaluation of these control strategies ten different *in situ* on-line sensors that measured DO, TSS, turbidity, ammonium, oxidation reduction potential, and COD were evaluated for their accuracy, precision, and long-term reliability (Miller et al., 2014).
Materials and methods

A-stage pilot configuration and operation

The A-stage pilot consisted of three vertical, complete-mix bioreactors in series followed by a clarifier and temporary effluent storage tank (Figure 4.1). The total working volume of the bioreactors was 0.51 m³ and each bioreactor had a side water depth of 3.4 m. The clarifier had a working volume of 1.7 m³ with a surface overflow rate of 17 m³/m²·day at the design flow of 17 L/min. The HRT of the bioreactors and clarifier was 30 minutes and 1.7 hours, respectively. The pilot was fed screened (2-3 mm openings) and degritted municipal wastewater after the wastewater temperature was adjusted to a user set point between 15-25°C using submersible heaters (OEM OTS, Minneapolis, MN) or a water chiller (Aqualogic MT-9, San Diego, CA). The influent and return activated sludge (RAS) flows were flow-paced using progressive cavity pumps (Seepex BW5, Bottrop, Germany) with variable frequency drives (VFDs) and magnetic flow meters (Rosemount 8705, Houston, TX). Waste activated sludge (WAS) was removed from the underflow of the clarifier using a digital, speed-controlled peristaltic pump (Masterflex L/S, Vernon Hills, IL). The total SRT, considering only the mass of solids in the bioreactors, was maintained between 0.1-0.3 days. Aeration was provided using compressed air through a single mechanically operated valve (MOV; v-notch ball valve) to fine-pore membrane disc diffusers (17.8 cm diameter) mounted on the bottom of each bioreactor as shown in Figure 4.1. Airflow to each of the three bioreactors was balanced using separate needle valves in order to mimic a single diffuser field. A DO sensor (InsitieG Model 10, Slidell, LA) was installed in tank 3 and a MLSS sensor (InsiteIG Model 15, Slidell, LA) in tank 2. The DO sensor was mounted in the last bioreactor (i.e., tank 3) to match full-scale plants that install DO sensors at the end of aeration tanks or controlled diffuser fields. The MLSS sensor was installed in the middle bioreactor (i.e., tank 2) as that reactor should represent the average MLSS of the entire A-stage process.
**Figure 5.1** – A-stage pilot schematic. (▬) process water; (▬▬) solids; (▬) air; (--) sensor signals; (---) controller output.

**Instrumentation and maintenance frequency**

To evaluate the accuracy of the *in situ* DO and MLSS sensors used in this study the sensor values were compared to either reference DO values measured using a handheld DO sensor or by taking grab mixed liquor samples and measuring TSS. The technical specifications of the *in situ* on-line sensors and the handheld DO sensor used for this reference measurements are listed in Table 5.1. The *in situ* sensors were mounted vertically in the bioreactors at one-third the side water depth from the water surface and equipped with an airblast cleaning mechanism according to the manufacturers’ specifications. Due to persistent biofouling in the bioreactors, the required airblast frequency was every ten minutes. The sensors were also manually cleaned weekly and on an as needed basis after comparison to daily reference DO and lab MLSS values. Initial sensor calibrations were performed according to the manufacturers’ instructions and on an as needed basis. Both *in situ* and reference DO sensors were calibrated using the zero-point (i.e.,
offset adjustment at 0% DO saturation) and reference-point (i.e., slope adjustment at 100% DO saturation) methods. The MLSS sensor was calibrated using the reference-point (i.e., offset adjustment at a known MLSS concentration) method. Since the effluent from the A-stage pilot continuously fed the B-stage pilot, routine calibration of the sensors was required. Therefore, long-term sensor drift was not evaluated in this study.

Table 5.1 – Technical specifications of the in situ and reference sensors used in this study.

<table>
<thead>
<tr>
<th>Sensor Technology</th>
<th>Manufacturer Model</th>
<th>Measured Parameter</th>
<th>Range</th>
<th>Accuracy</th>
<th>Response Time (T_{0.90})</th>
<th>Averaging Time</th>
<th>Auto Cleaning Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminescent Dissolved Oxygen (LDO)</td>
<td>Hach IntellICAL™</td>
<td>DO</td>
<td>0.1-20.0 mgO$_2$/L</td>
<td>± 0.1 mg/L below 8 mg/L</td>
<td>10 secs</td>
<td>0 secs</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>LDO101</td>
<td></td>
<td></td>
<td>± 0.2 mg/L above 8 mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescent Dissolved Oxygen (FDO)</td>
<td>InsiteIG Model 10</td>
<td>DO</td>
<td>0-25.0 mgO$_2}$/L</td>
<td>± 1% of sensor reading or ± 0.02 mg/L, whichever is greater</td>
<td>60 secs</td>
<td>12 secs</td>
<td>Airblast</td>
</tr>
<tr>
<td>Infrared Total Suspended Solids (TSS)</td>
<td>InsiteIG Model 15</td>
<td>MLSS</td>
<td>0.25-30 gSS/L</td>
<td>± 5% of sensor reading or ± 100 mg/L, whichever is greater</td>
<td>60 secs</td>
<td>300 secs</td>
<td>Airblast</td>
</tr>
</tbody>
</table>

*Handheld LDO sensor was used for reference measurements against the in situ DO sensor
N/A: Not available

Process automation and control

A block diagram of the process automation used in this study for DO cascade control and MLSS set point control is provided in Figure 5.2. Process automation and control was achieved in the pilot using a programmable logic controller (PLC) with integrated proportional-integral-derivative (PID) controls (Allen-Bradley SLC 500, Milwaukee, WI). The DO sensor installed in tank 3 and a gas mass flow meter (Alicat M-Series, Tucson, AZ; standard temperature pressure = 25°C, 1 atm) on the compressed air line provided the feedback signals for the cascade DO controller (Figure 5.2a). The first PID loop would compare the DO feedback signal to the user DO set point and output an airflow set point to the second PID loop accordingly. The second PID loop would then act upon the MOV in order to match the process airflow to the airflow set point. The airflow controller was bounded (20-90 standard liters per minute; SLPM) to ensure that the process was always well mixed and to limit the oxygen transfer rates (OTR) to 150 mg/L·hr, which is a typical limit of full-scale aeration systems.
The MLSS sensor located in tank 2 provided the feedback signal for the WAS flow-based MLSS controller (Figure 5.2b). The MLSS PID loop compared the MLSS feedback signal to the user MLSS set point and output a WAS pump speed. To increase the MLSS concentration in the A-stage process, the pump speed was decreased and vice versa to decrease the MLSS concentration of the A-stage.

![Proces control block diagram](image)

**Figure 5.2** – Process control block diagram for (a) cascade DO set point (SP) control and (b) WAS flow-based MLSS set point control (b). Standard: Std; Set point: SP.

**Analytical methods and data analysis**

Performance of the A-stage pilot was assessed by collecting 24-hr flow-weighted composite samples of influent and effluent and analyzing for COD, soluble COD (1.5 μm glass microfiber filtered), TSS, volatile suspended solids (VSS), TKN, and TAN, according to Standard Methods (APHA, 2012). Filamentous bacteria were identified in mixed liquor samples by staining and microscopic observation according to Jenkins et al. (2004). Diurnal profiles of A-stage influent, effluent, and mixed liquor were obtained by either collecting 24 or 12 discrete samples over a 24-hr period using an auto sampler. Reference DO concentrations were determined using a handheld DO sensor (Table 5.1). Outlier determination and calculation of the width of the 95% prediction interval (PI) at the mean lab value was performed according to Rieger et al. (2005). Sensor values are considered outliers when they have the highest absolute residual value between the linear regression function and the measured sensor value and their removal from the dataset.
statistically improves the standard deviation of the residuals. Outliers are presented but were not used included in the statistical analyses of sensor data. All other statistical analyses including Pearson product moment correlation, Shapiro-Wilk normality test, t-test, linear regression, 95% confidence and prediction intervals, mean, and standard deviation of the sample were performed using SigmaPlot 12.5 (Systat Software Inc., Bangalore, India).

Results and Discussion

Initial A-stage performance

Initial diurnal COD profiles of the raw influent and A-stage effluent were performed before the implementation of any process control to evaluate the variability of the A-stage pilot. These diurnal profiles are shown in Figure 5.3. Four separate profiles (1-4) of the influent and effluent were collected during a two-week span with two profiles collected on weekdays (profiles 2 and 4) and two on weekend days (1 and 3). The same two days were profiled each week. For each sample point, 12 samples were collected over a 24-hr period. The influent COD patterns followed a typical diurnal trend (Tchobanoglous et al., 2003). However, the influent pilot samples also included full-scale plant recycles from solids dewatering and therefore were impacted by plant activities. A diurnal trend was also seen in the A-stage effluent (Figure 5.3b). The effluent trends were attenuated due to COD removal in the A-stage and effluent equalization in the clarifier. For the four profiles the influent COD concentrations ranged from 386-542 mg/L (mean (μ) = 460; standard deviation (σ) = 43) and the effluent COD ranged from 251-387 mg/L (μ = 332; σ = 41).
Figure 5.3 – Diurnal sampling results of the A-stage pilot: (a) influent COD; (b) effluent COD; (c) effluent COD/TKN.

The variability in the influent COD concentrations (386-542 mg/L) and COD removal efficiencies (5-44%) resulted in variation of the effluent COD/TKN ratio (Figure 5.3c) ranging
from 6.0-9.1 mgCOD/mgTKN ($\mu = 7.6; \sigma_x = 0.8$). Although the influent TKN followed a similar diurnal pattern to COD (data not shown), the variability of effluent COD/TKN was impacted more by fluctuations of COD rather than TKN. This variability in effluent COD/TKN directly affected the stability and nitrogen removal performance of the proceeding bio-oxidation (B-stage) process when the B-stage was operated aggressively in terms of ammonia loading and SRT (Regmi et al., 2015; Regmi et al., 2014).

The variability of the A-stage effluent COD/TKN ratio was further investigated after selecting a reliable DO sensor. A total of three optical DO sensors (InsiteIG Model 10; Hach LDO sc; WTW FDO® 700 IQ) were evaluated as discussed in Miller et al. (2014). From these, the InsiteIG Model 10 FDO sensor was selected because of its ability to withstand damage from frequent airblast cleaning that occurred every 10 minutes to avoid biofouling. The FDO sensor’s durability was attributed to the fact that the airblast was integrated into the sensor body and the sensing material was embedded in hard epoxy resin. Compared to the FDO sensor, the sensing material of the LDO sensors was delicate and was prone to damage from the required frequent airblast cleaning. Once damaged the LDO sensor response time increased to a point where the sensors no longer calibrated.

To evaluate the impact of diurnal influent COD patterns on DO and MLSS in the A-stage process a 24-hr profile of DO and MLSS in tank 3 was performed using an FDO sensor and collecting discrete mixed liquor samples once per hour and analyzing for TSS (Figure 5.4). The airflow and waste flowrate were maintained constant during this test. Over the 24-hr period, the DO concentration varied between 0.5 and 1.3 mg/L ($\mu = 0.9; \sigma_x = 0.2$) and MLSS varied between 810 and 1270 mgSS/L ($\mu = 1040; \sigma_x = 142$).
Figure 5.4 – Profile of DO and MLSS concentrations in the A-stage pilot. DO was logged from the in situ DO sensor and MLSS concentrations were from discrete samples (n = 24) over 24 hours. A fluctuation in DO around 9:00 was due to daily maintenance procedures that required temporarily stopping the influent feed.

Since the half saturation coefficient of ordinary heterotrophic organisms (OHO) for DO (K_{DO}) is 0.2 mg/L, the variability of DO concentrations on COD removal performance was minor because DO \gg K_{DO} (Grady Jr. et al., 2011; Henze et al., 2000). However, variable MLSS affected A-stage performance because the specific loading rate (SLR; kgCOD/kgMLSS·day) inversely correlates with COD removal efficiency (Böhnke, 1977). Since the A-stage process was operated at a short HRT (30 mins) and short SRT (0.09 days), the COD loading changed before the process MLSS had time to stabilize. The ability of the A-stage bacterial community to quickly adapt to changing COD loads may be advantageous to the overall process performance (Böhnke et al., 1997). As mentioned earlier, this variability in the effluent COD/TKN lead to variability of nitrogen removal in the B-stage.

Based on the variability in the effluent COD/TKN ratio of the A-stage pilot process, several in situ sensors coupled with automatic process controllers aimed at reducing fluctuations in COD removal performance were considered. This included single-loop and cascade DO set point control, SRT set point control using effluent and WAS TSS sensors, oxygen uptake rate (OUR) based WAS flow control, and MLSS-based WAS flow control. Of all the in situ sensors that
were evaluated only the InsiteIG FDO and MLSS sensors (Table 5.1) were reliable enough to be used with automatic process controllers (Miller et al., 2014). The FDO sensor was used to implement cascade DO control and the MLSS sensor for MLSS-based WAS flow control in the A-stage pilot.

**Cascade DO control**

Cascade DO control was evaluated in the A-stage to determine the impact of DO on COD removal efficiency and the effluent COD/TKN variability. The *in situ* DO sensor was checked daily during normal workdays against a handheld LDO sensor (Table 5.1) over a period of 300 days. The results from this period are presented in Figure 5.5 along with the ideal correlation (i.e., slope = 1) and the linear regression function with 95% confidence (CI) and prediction intervals (PI). Outliers were removed according to the method used in (Rieger et al., 2005). The 95% PI at the mean reference value of 0.81 mg/L was ± 0.67 mg/L meaning that a future single reference value has a 95% probability of falling within 0.14 and 1.48 mg/L. This variability is mainly associated with sensor biofouling as the sensor has an accuracy of 1% of the sensor reading or ± 0.02 mg/L, whichever is greater, in clean water (Table 5.1). While the airblast was effective at controlling biofouling, especially when compared to other optical DO sensors, biofouling was often severe and required manually cleaning of the sensor to maintain sensor accuracy. Since sensor biofouling caused the *in situ* sensor reading to be lower than the reference DO sensor (i.e., slope <1) it is likely that the true calibration function was closer to the ideal correlation line than what was predicted by the linear regression function (slope = 0.87; offset = -0.04; $R^2 = 0.96$). Therefore, errors in DO readings were likely associated with biofouling rather than calibration errors.
A 24-hr profile of DO and the standard airflow rate of the A-stage process while under cascade DO control with a DO set point was 0.5 mg/L as shown in Figure 5.6. The DO concentration was held constant while the airflow rate changed according to the influent COD load. To evaluate the impact of DO concentration on COD removal, the A-stage was operated at different DO set points ranging from 0.2 to 1.5 mg/L. However, no statistically significant correlation of DO concentration to COD removal efficiency was detected (Pearson product-moment correlation $R = 0.16; p = 0.042; n = 162$). As mentioned previously, the lack of correlation between the DO concentration and COD removal efficiency was likely due to $\text{DO} \gg K_{\text{DO}}$. Nogaj et al. (2015) reported that DO only significantly impacted substrate removal when the bulk DO was below 0.5 mg/L in an HRAS process operated at a 1 day SRT. Attempts were made to operate the A-stage pilot at DO concentrations below 0.5 mg/L, however, due to sensor biofouling issues, it proved very difficult to precisely control DO in the range (0-0.5 mg/L) that would likely affect COD removal. Operating the pilot at a DO <0.2 mg/L also resulted in bulking conditions with sludge volume indexes (SVI) >150 mL/g.
Bulking episodes also occurred when the process OUR exceeded the aeration system’s oxygen transfer capabilities for an extended period (e.g., >2 times the SRT). The filamentous bacteria were identified as Type 1863, which are known to proliferate under SLR > 0.6 kgCOD/kgMLSS·day and DO < 0.1 mg/L. Bulking was resolved by decreasing the MLSS concentration (i.e., lowering the SRT). Since DO control did not offer the ability to control COD removal and MLSS correlated with COD removal ($R = 0.59; p < 0.01; n = 308$), MLSS set point control was investigated.

**MLSS set point control**

Previous attempts to implement SRT control were unsuccessful because of the lack of reliable in situ MLSS and TSS sensors. The premise of the automatic SRT controller was to monitor MLSS and effluent TSS concentrations and vary the WAS flow to meet the desired SRT set point. However, using two sensors with process control could result in a very unstable controller since the error of each sensor would add to the total error of the controller. In addition, small changes in MLSS would only have had a minor effect on SRT while effluent TSS accounted for almost half of the suspended solids leaving the A-stage. Even a small error in the measurement of
effluent TSS could have detrimental effects on the SRT controller. When compared to more conventional HRAS processes that typically maintain effluent TSS values <20 mgSS/L, the impact of effluent TSS is not as significant. Most activated sludge plants also try to maintain a constant MLSS concentration by adjusting HRT and SRT in order to maintain a consistent solids loading of the secondary clarifiers. However, this is now discouraged in practice because the main variable of concern in terms of treatment performance (e.g., nitrification) is SRT and not MLSS concentration. In the case of the A-stage pilot, MLSS-based WAS flow control was adopted as MLSS directly correlated to SRT ($R = 0.78; p < 0.01; n = 256$) as seen in Figure 5.7. This means that although the controller was based on a MLSS set point, MLSS was just used as a surrogate for SRT.

![Figure 5.7 – Influence of SRT on the A-stage MLSS concentration.](image)

A comparison of MLSS sensor values to grab MLSS samples for the A-stage are presented in Figure 5.8. The results include data from before and after MLSS set point control was used as the controller did not impact sensor performance. The 95% PI at the mean lab value of 2000 mg/L was ±1320 mg/L. It is unclear if the variability was associated with sensor biofouling, grab sample collection and analysis, or the true error of the sensor. The linear regression function (slope = 1.20; offset = -200; $R^2=0.86$) was off from the ideal correlation suggesting that the
sensor calibration function could have been improved. However, this does not explain the variability of the sensor. Regardless the sensor was deemed accurate enough for use with automatic process control.

![Figure 5.8](image)

**Figure 5.8** – Comparison of *in situ* sensor MLSS readings to lab MLSS grab samples. Ideal correlation represents what should be expected (i.e., sensor MLSS = lab MLSS) when the sensor is calibrated correctly. PI: prediction interval; CI: confidence interval.

A 24-hr snapshot of the MLSS set point controller in action is provided in Figure 5.9. The controller was able to maintain the MLSS set point although the PID tuning could have been improved to reduce WAS pumping variability (Figure 5.9). A full-scale facility would experience operational issues such as variable solids and hydraulic loading on downstream thickening processes if the WAS flow was not dampened or equalized. The WAS flow variability also caused the process SRT to be very dynamic. However, this likely had no effect on the A-stage COD removal efficiency because the SRT operating range was so narrow (0.1-0.3 days). While the controller was successful at stabilizing the process MLSS concentration, the effluent diurnal variation did not show any significant improvement.
Due to the frequent changes in operating conditions (i.e., DO, HRT, SRT) and unavoidable mechanically issues, long-term COD removal and process stability was not comparable during the periods before and after implementation of MLSS set point control. Instead, an effort was made to compare COD removal (Figure 5.10) when the pilot was stably operated under similar conditions (20°C; HRT = 30 min; average SRT = 0.15 days; DO = 0.5 mg/L). The influent COD for the period before MLSS control averaged 585±88 while the influent COD averaged 598±54 after implementation of the controller. Although the means of COD removal (49.2% before; 48.4% after) of each dataset were not statistically different ($p = 0.616$), the variances (110.1% before; 20.4% after) between the two periods were statistically different ($p = 0.002$). This means that while both periods averaged the same COD removal efficiency, the use of MLSS set point control reduced the variability of COD removal by 90%. This reduction of variability translated to increased stability and reliability of the B-stage process.

**Figure 5.9** – Profile of MLSS and WAS flow when MLSS set point control is in use. Horizontal line represents MLSS set point of 4000 mg/L.
Further improvement of the MLSS controller could entail the use of either \textit{in situ} on-line sensors or \textit{ex situ} on-line analyzers that measure organics such as COD, BOD, ultraviolet light absorbance at 254 nm (UV254), and total organic carbon (TOC), to provide an organics removal feedback signal. This would be an effluent organics-based cascade SRT controller. Additionally, coupling an effluent organics measurement with a measure of effluent nitrogen (e.g., TAN analyzer) would provide effluent C/N values that could be used for finer control of COD removal in the A-stage. Although the technology is available to monitor parameters of interest such as COD, MLSS and DO, further research needs to be done to fully understand COD removal mechanisms and how they can be effectively controlled in short SRT processes like the A-stage.

\textbf{Conclusions}

This research documented the successful implementation of automatic process control in an activated sludge process operated at an SRT $<0.5$ days in order to meet an effluent COD/TKN ratio of approximately 6-8 mgCOD/mgTKN for optimal shortcut nitrogen removal in the downstream BNR processes. Although cascade DO control did not impact COD removal at the conditions tested in this study, the controller can be used to mitigate excess aeration and energy
consumption in the A-stage. The MLSS set point control was able to reduce COD removal variation by 90%.

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References


de Graaff, M.; Roest, K. (2012) Inventarisatie van AB-systemen - optimale procescondities in de A-trap, STOWA.


Chapter 6: Quantifying the Settling and Dewatering Characteristics of an A-stage High-Rate Activated Sludge Process Preceding a Shortcut Nitrogen Removal Process

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Keywords: A/B Process, A-stage, Dewatering, High-Rate Activated Sludge, Settling

Abstract:

The solids settling and dewatering characteristics of the A-stage of an A/B process pilot study were characterized using standardized and recently developed methods. The pilot settling and dewatering characteristics indicates that the A-stage has similar characteristics to four conventional full-scale plants operated by HRSD including the Chesapeake-Elizabeth (CE) HRAS plant, a Virginia Initiative (VIP) process plant, the Boat Harbor (BH) pre-anoxic plant, and the Nansemond 5-stage Bardenpho plant. SVI values of 85 ± 26 mL/g over an 18-month operating period were obtained by the pilot A-stage, which was in the range obtained by full-scale plants (Böhnke, 1994). The centrifuge cake solids obtained from undigested pilot A/B WAS dewatered to an average value of 25.7% with a range of 23.2-28.0 % DS.
Introduction

To obtain energy self-sufficiency, water resource recovery facilities (WRRFs) must maximize energy recovery through biogas production by diverting influent organic carbon to anaerobic digesters all while reducing energy consumption. However, many WRRFs must also remove nutrients (nitrogen and phosphorus), which is generally energy and resource intensive and may require external organic carbon addition if internal organic carbon is not available for denitrification. To reduce resource consumption (i.e., organic carbon, energy, and alkalinity) for biological nitrogen removal (BNR), research groups have been developing shortcut nitrogen removal technologies aimed at reducing energy requirements for aeration and optimizing influent organic carbon use for denitrification (Al-Omari et al., 2012; Lotti et al., 2014; Pérez et al., 2015; Regmi et al., 2014; Winkler et al., 2012). The A-stage of an adsorption/bio-oxidation (A/B) process is a carbon removal process that typically precedes these shortcut BNR processes. In the A/B configuration the A-stage, which is a high-rate activated sludge (HRAS) process, is operated at an solids retention time (SRT) of less than 1 day and a 30 minute hydraulic residence time (HRT) resulting in 50-70% chemical oxygen demand (COD) capture and removal with low aeration energy input and minimal COD oxidation (Böhnke, 1994; Jimenez et al., 2015).

While the A/B process has been well established in Europe since the 1980s, there is relatively little scientific literature available on the COD removal mechanisms and waste solids characteristics of the A-stage process. This is due in part to the lack of readily available literature in English on the A-stage process and the fact that most of the performance data in terms of settleability, dewaterability, and digestibility of A-stage solids has largely gone unpublished. The majority of the data that has been published is generally limited to overall performance, like COD and total suspended solids (TSS) removal efficiencies and general observations about sludge settleability (Böhnke, 1994; Böhnke et al., 1997; Malz and Bili, 1992).

The cost of sludge treatment and disposal represents a significant portion of a facilities operational and maintenance costs (WEF, 2008). As with many activated sludge processes, solids separation is also usually the limiting factor in terms of design capacity and process loading rates. Solids separation, which is typically done using large settling tanks or clarifiers, is exacerbated when the activated sludge settles poorly (i.e., sludge volume index (SVI) >150
mL/gSS) as a clarifier’s solids removal efficiency is directly related to the sludge quality, like settleability, flocculation, and thickenability (WEF, 2005). The A-stage has been reported to have low SVIs in the range of 40 to 90 mL/gSS, which indicates good settling characteristics (Böhnke, 1994). However, since A-stage systems are designed to remove approximately 70% of the influent COD, the effluent still contains non-settleable solids that are not characterized by conventional settling tests like SVI.

Böhnke (1994) also mentions that A-stage waste sludge can easily be thickened up to 6-8% total solids (TS) but no indication as to why A-stage thickens similarly to primary solids (5-10% TS) was given (Tchobanoglous et al., 2003). One plausible explanation is that A-stage sludge contains low concentrations of extracellular polymeric substances (EPS), which are known to negatively impact settleability and dewaterability (Forster, 1983; Houghton et al., 2001; Li and Yang, 2007). However, it should be noted that the studies that have investigated the EPS content of HRAS processes operated below 1 day SRT only focused on COD capture and removal efficiencies and not the solids handling characteristics (Faust et al., 2014; Jimenez et al., 2007; Zhao et al., 2000).

The lack of detailed literature and design knowledge on A-stage solids handling means worldwide acceptance of the A/B process has been rather limited. More research is needed to characterize A-stage solids settleability and dewaterability to further validate its use in WRRFs for COD capture and removal. Therefore, the goal of this research was to investigate the settleability and dewaterability of sludge produced by a pilot-scale A-stage process treating municipal wastewater. Sludge settling and dewatering characteristics were evaluated using standardized characterization methods, like SVI and zone settle velocity, in addition to novel settling and dewaterability tests. The A-stage results were compared to full-scale activated sludge processes using the same methodology for analyzing settleability and dewaterability. This work represents the first study to characterize the sludge produced by an A-stage pilot process.
Materials and methods

Pilot configuration and operation

A detailed pilot description and overview of the instrumentation, automation, and control strategies are covered in Chapter 5 for the A-stage and in Regmi et al. (2014) and Regmi et al. (2015) for the B-stage. Briefly, the A-stage pilot consisted of three vertical, complete-mix bioreactors in series followed by an intermediate clarifier and effluent storage tank that served as a sample collection point as shown in Figure 6.1. The total working volume of the A-stage bioreactors was 0.51 m$^3$ and each bioreactor had a side water depth of 3.4 m. The clarifier had a working volume of 1.7 m$^3$ with a surface overflow rate of 17 m$^3$/m$^2$·day at the design flow of 17 L/min. The HRT of the bioreactors and clarifier was 30 minutes and 1.7 hours, respectively. The B-stage pilot consisted of four equal volume bioreactors with a combined working volume of 0.60 m$^3$ followed by a clarifier with a working volume of 0.33 m$^3$ and surface overflow rate of 6 m$^3$/m$^2$·day at the design flow of 1.9 L/min.

The pilot received municipal raw wastewater influent (RWI) after screening (6 mm) and grit removal by the Chesapeake-Elizabeth Wastewater Treatment Plant (CE WWTP). After additional grit and scum removal in the pilot, the influent was conveyed through a 2.4 mm basket screen and then adjusted to the desired operating temperature of 20°C prior to entering the A-stage aeration tanks. A-stage and B-stage return activated sludge (RAS) rates were maintained at 100% of their respective influent flow. A-stage waste activated sludge (WAS) was continuously removed from the underflow of the intermediate clarifier. The A-stage solids retention time (SRT), considering only the mass of solids in the bioreactors, was typically maintained between 0.1-0.3 days. To control the B-stage SRT, which varied between 4-10 days, hydraulic wasting from the last bioreactor was performed. Aeration was provided to both stages using compressed air through mechanically operated valves to fine-pore membrane disc diffusers (17.8 cm diameter) mounted on the bottom of each bioreactor as shown in Figure 6.1.
Figure 6.1 – A/B pilot schematic. AvN: ammonia versus NOX-N (nitrate plus nitrite); IMLR: internal mixed liquor recycle.

**Full-scale WWTPs evaluated in this study**

A brief description of each plant’s main liquid and solids treatment processes is contained in Table 6.1. All of these plants are owned and operated by the Hampton Roads Sanitation District located in Southeastern Virginia, USA.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Abbreviation</th>
<th>Liquid Treatment</th>
<th>Solids Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/B Pilot Study</td>
<td>A/B</td>
<td>A/B process</td>
<td>None</td>
</tr>
<tr>
<td>Atlantic</td>
<td>AT</td>
<td>PST and HRAS with anaerobic selectors</td>
<td>Phased acid/gas AD</td>
</tr>
<tr>
<td>Boat Harbor</td>
<td>BH</td>
<td>CEPT and A/O</td>
<td>Incineration</td>
</tr>
<tr>
<td>Chesapeake-Elizabeth</td>
<td>CE</td>
<td>HRAS (no PST)</td>
<td>Incineration</td>
</tr>
<tr>
<td>James River</td>
<td>JR</td>
<td>PST and MLE IFAS (media in aerobic zones)</td>
<td>Mesophilic AD</td>
</tr>
<tr>
<td>Nansemond</td>
<td>NP</td>
<td>PST and 5-stage Bardenpho</td>
<td>Mesophilic AD</td>
</tr>
<tr>
<td>Virginia Initiative Plant</td>
<td>VIP</td>
<td>PST and VIP process</td>
<td>Incineration</td>
</tr>
</tbody>
</table>

PST: primary settling tanks; AD: anaerobic digestion; CEPT: chemically enhanced primary treatment; A/O: anaerobic/oxic; MLE: modified Ludzack-Ettinger; IFAS: integrated fixed-film activated sludge
Pilot and full-scale plant influent characteristics

The HRSD collection network is over 90% pressurized; therefore, the wastewater can be characterized as septic and moderate strength. The average A-stage influent and effluent characteristics and the removal efficiency of each are included in Table 6.2. A-stage effluent represents the influent of the B-stage pilot.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influent</th>
<th>Effluent</th>
<th>% Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total COD (mg/L)</td>
<td>551 (± 74)</td>
<td>301 (± 64)</td>
<td>45 (± 12)</td>
</tr>
<tr>
<td>Soluble COD; sCOD (mg/L)</td>
<td>217 (± 30)</td>
<td>144 (± 31)</td>
<td>33 (± 12)</td>
</tr>
<tr>
<td>Particulate COD; pCOD (mg/L)</td>
<td>335 (± 67)</td>
<td>157 (± 45)</td>
<td>52 (± 16)</td>
</tr>
<tr>
<td>Total Suspended Solids; TSS (mg/L)</td>
<td>201 (± 41)</td>
<td>98 (± 26)</td>
<td>50 (± 16)</td>
</tr>
<tr>
<td>Volatile Suspended Solids; VSS (mg/L)</td>
<td>178 (± 35)</td>
<td>84 (± 21)</td>
<td>50 (± 17)</td>
</tr>
<tr>
<td>Total Kjeldahl Nitrogen; TKN (mg N/L)</td>
<td>42 (± 5)</td>
<td>38 (± 4)</td>
<td>13 (± 7)</td>
</tr>
<tr>
<td>Total Phosphorus; TP (mg P/L)</td>
<td>5.7 (± 0.8)</td>
<td>4.4 (± 0.8)</td>
<td>23 (± 12)</td>
</tr>
</tbody>
</table>

Sludge samples from various full-scale treatment plants were collected and subjected to the same measurements as the A/B pilot. This provided the ability to directly compare pilot to full-scale results using the same standardized methods without relying on operational data collected by the plants. Table 6.3 contains the average RWI characteristics, design flow, and sludge age of the activated sludge process for the full-scale plants that were evaluated in this study. This data represents operational data collected over one year as part of each facilities sampling plan. The wastewater characteristics shown in Table 6.2 and Table 6.3 for the pilot and full-scale systems were analyzed as described below.
Table 6.3 – Average RWI (n >48) concentrations and activated sludge operational parameters of the full-scale plants evaluated in this study. Mean (± standard deviation).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AT</th>
<th>BH</th>
<th>CE</th>
<th>JR</th>
<th>NP</th>
<th>VIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>507 (+37)</td>
<td>379 (+70)</td>
<td>497 (+35)</td>
<td>547 (+18)</td>
<td>469 (+28)</td>
<td>366 (+50)</td>
</tr>
<tr>
<td>sCOD (mg/L)</td>
<td>256 (+21)</td>
<td>180 (+48)</td>
<td>246 (+16)</td>
<td>281 (+8)</td>
<td>229 (+18)</td>
<td>191 (+34)</td>
</tr>
<tr>
<td>pCOD (mg/L)</td>
<td>251 (+22)</td>
<td>199 (+30)</td>
<td>251 (+27)</td>
<td>266 (+27)</td>
<td>241 (+32)</td>
<td>175 (+26)</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>168 (+13)</td>
<td>136 (+20)</td>
<td>161 (+12)</td>
<td>174 (+17)</td>
<td>161 (+10)</td>
<td>117 (+13)</td>
</tr>
<tr>
<td>TKN (mg N/L)</td>
<td>44 (+2)</td>
<td>31 (+5)</td>
<td>41 (+2)</td>
<td>39 (+4)</td>
<td>41 (+3)</td>
<td>28 (+4)</td>
</tr>
<tr>
<td>Design Flow (m³/sec)</td>
<td>2.37</td>
<td>1.10</td>
<td>1.05</td>
<td>0.88</td>
<td>1.31</td>
<td>1.75</td>
</tr>
<tr>
<td>SRT (days)</td>
<td>2.4 (+0.2)</td>
<td>7.6 (+4.9)</td>
<td>3.2 (+0.9)</td>
<td>5.0 (+0.6)</td>
<td>12.5 (+2.1)</td>
<td>9.9 (+1.7)</td>
</tr>
</tbody>
</table>

**Settling characterization methods**

Settling performance was evaluated by a variety of methods. Standard methods (APHA, 2012) were used to measure the SVI (2710D). This method is a commonly used measurement of settling performance at treatment plants. However, SVI results are affected by several factors, such as the mixed liquor suspended solids (MLSS) concentration (Ekama and Marais, 1986). For this reason, the standard method for zone settling velocity (ZSV; 2710E) was the benchmark measure of settleability for this work (APHA, 2012). A solids flux analysis (SFA) was performed on the CE, AT, and VIP plants and the A-stage pilot. A SFA conducted by performing multiple ZSV tests using the same sample of sludge diluted to different initial MLSS concentrations to yield a hindered settling velocity curve plotted as hindered settling velocity versus initial solids concentration. Using this curve, Vesilind settling parameters, $V_0$ and $k$, were estimated by fitting the Vesilind exponential model (Eq. 6–1) to the experimental data by minimizing the sum of the squared error between the model settling velocities and the measured settling velocities.

$$V = V_0 \cdot e^{-k \cdot X} \quad (6-1)$$

Where $V_0$ is the initial hindered settling velocity (m/hr), $k$ is the settling coefficient (m³/kg), $X$ is the solids concentration (kg/m³), and $V$ (m/hr) is the settling velocity at the solids concentration of interest. From these results, a solids flux curve due to gravity was constructed by multiplying $V$ and $X$ and plotting against $X$.  

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Settling column tests

To determine the effect of MLSS on COD removal the method described by Ramalingam et al. (2012) was modified to include flocculent settling by using higher MLSS concentrations. The settling column consisted of a clear cylinder (87 cm) with an Imhoff cone affixed to the bottom of the column. A drain port was located where the Imhoff cone and column met. The column was filled with effluent (5.8 L) from the process of interest and a 0.3 L sample of mixed liquor with a known TSS concentration was added to the top of the cylinder using an open bottom container. This container allowed the mixed liquor sample to be added to the top of the cylinder without inducing hydraulic forces that would result from pouring the sample into the column. The solids were allowed to settle for a set amount of time then the top of the cylinder was drained using the drain port. The solids that collected in the Imhoff cone were removed by draining the cone and quantified by measuring the TSS. The fraction of solids that settled during the test was subtracted from the total amount of solids added to the column at the start of the test including the effluent TSS.

Three different settling times were used that corresponded to three distinct settling velocities. Large particles were defined as particles with a settling velocity >6 m/hr. Medium size particles were defined as particles that settled slower than 6 m/hr but faster than 1.5 m/hr. Finally, small particles were defined as particles with settling velocities <1.5 m/hr. The fraction of solids that did not settle during the 1.5 m/hr test were considered non-settleable.

Dewaterability characterization methods

To determine the obtainable dewatered cake solids from the pilot and full-scale solids, a standardized dewaterability test described in Higgins et al. (2014) was used. This method provided the ability to compare different sludges using the same dewatering apparatus instead of relying on plant operational data, which varies based sludge dewatering equipment. Sludge samples collected from biological phosphorus removal plants were transported to the laboratory under aeration to prevent phosphorus release. First, the optimal polymer (Zetag 7583) dose was determined by dosing increasing polymer concentrations and measuring capillary suction time (CST) according to Standard Method (2710G). The optimal polymer dose was selected as the dose that resulted in the lowest average CST after ran in triplicate. The sludge sample with the
optimal polymer dose was then partially dewatered by gravity on belt filter press fabric and then transferred to centrifuge cups. These cups were custom built and contained an apparatus that supported belt filter press fabric and allowed water to accumulate in the bottom of the cups. The samples were centrifuged at 3000 x g for 10 minutes. The percent dry solids (DS) content of the dewatered cake was measured according to Standard Method (2540G) (APHA, 2012).

**Analytical methods and data analysis**

Performance of the A/B pilot was assessed by collecting 24-hr flow-weighted composite samples of influent and effluent and analyzing for total COD, soluble COD (sCOD) (1.5 μm glass microfiber filtered), TSS, VSS, TKN, TP according to Standard Methods (APHA, 2012). Particulate COD was calculated as the difference between total and sCOD. Filamentous bacteria were identified in mixed liquor samples by microscopic enumeration according to Jenkins et al. (2004). Oxygen uptake rates (OUR) were determined by measuring the total airflow (1 atm, 20°C) to the process and measuring the oxygen content of the off-gas (Servomex 1440D, Crowborough, UK). A detailed description of how OUR was calculated is covered in Chapter 4.

**Data Analysis**

Statistical analyses including Pearson product moment coorelation, Shapiro-Wilk normality test, t-test, linear regression, mean, standard deviation, standard error of the mean, one-way analysis of variance (ANOVA), and confidence intervals were performed using SigmaPlot 12.5 (Systat Software Inc., Bangalore, India). Confidence intervals were calculated at a p-value of 0.05.

**Results and Discussion**

**Average A-stage pilot operation and performance**

The A-stage of the A/B pilot was operated with a 30-minute HRT and the SRT was maintained between 2 to 6 hours (excluding solids present in the clarifier). TSS and VSS removal averaged 50 ± 16% (mean ± standard deviation) and 50 ± 17%, respectively (Table 1). TSS removal was lower than what is typically achieved by primary sedimentation alone, which is approximately 65% (Tchobanoglous et al., 2003). This was attributed to conversion of soluble substrate to nonsettleable particulate matter as discussed in detail in Chapter 4. Total COD removal
efficiency averaged 45 ± 12%, which was also lower than the reported performance of full-scale A-stage processes that remove 55-75% of the influent COD (de Graaff and Roest, 2012). The lower COD removal performance was intentional since COD removal was controlled in the A-stage in order to optimize nitrogen removal in the B-stage pilot (Regmi et al., 2014). TKN and TP were primarily removed by assimilation and sedimentation in the A-stage and averaged 13 ± 7% and 23 ± 12%, respectively. TP was also removed by chemical precipitation because the CE plant doses ferric chloride prior to preliminary treatment (i.e., upstream of pilot RWI intake) for most of the year in order to control odors.

**Impact of A-stage operation on mixed liquor settling**

A-stage settling was quantified by routinely measuring the SVI of the mixed liquor. Mixed liquor was also observed under a microscope on a weekly basis for the presence of filamentous bacteria. The pilot A-stage mixed liquor exhibited an average SVI value of 85 ± 26 mL/g (n = 393) over the 600 days of continuous operation. This value is within the range of 38 to 93 mL/g reported by Böhnke (1994) for pilot- and full-scale A-stage processes. During the 600 days of operation, there were less than 10 days when the SVI was above 150 mL/g and the maximum SVI observed was 217 mL/g. Unlike complete-mix HRAS processes that are known to settle poorly when operated at low sludge ages or high loadings (Bisogni and Lawrence, 1971; Chao and Keinath, 1979; Stewart, 1964), the A-stage pilot was configured in a plug-flow configuration that promoted well settling sludge. Additionally, operation at dissolved oxygen (DO) concentrations less than 1 mg/L did not result in poor settling, which is counter to the classical bulking theory presented by Palm et al. (1980).

During periods of filamentous bulking filamentous bacteria Type 1863 were observed in the A-stage sludge. *Thiothrix* spp. types I and II were also present but never at an abundance that caused bulking sludge. Type 1863 filamentous bacteria are typically seen in activated sludge processes with high sludge loading rates, short sludge ages, and low DO conditions (Jenkins et al., 2004). These are the operational conditions at which the A-stage pilot was operated. Although a statistical analysis did not find a direct correlation between DO concentration and SVI (n = 414, R = -0.05, p = 0.24), bulking was observed when DO was less than about 0.1 mg/L. SVI did correlate with SRT (n = 381, R = 0.32, p <0.001), OUR (n = 144, R = -0.40,


$p<0.001$, and MLSS (n = 242, R = 0.24, $p < 0.001$). These three parameters are interrelated and directly affect the bulk DO concentration. As SRT increases the MLSS concentration increases resulting in an increase in the OUR. Once the OUR exceeded the oxygen transfer capability of the A-stage aeration system, DO would decrease below 0.1 mg/L and bulking would soon onset.

**Characterization of settleability using ZSV and settling column tests**

To further investigate and compare the settleability of A-stage mixed liquor to other full-scale activated sludge processes, ZSV and settling column tests were performed using mixed liquor samples from the A-stage and the AT, CE, and VIP treatment plants. The CE plant was selected because it is a HRAS process like the A-stage and received the same RWI as the A-stage pilot. The AT plant is also a HRAS process except it has primary sedimentation and anaerobic selectors for improved settling. The VIP plant also has anaerobic zones for biological phosphorus removal but is operated at longer SRTs (9.9 ± 1.7 days) to achieve biological nitrogen removal.

Solids flux analyses were conducted using the ZSV test results to experimentally determine Vesilind settling parameters for the mixed liquor samples from the pilot A-stage, and the AT, CE, and VIP treatment plants. The average Vesilind parameter results from these tests are shown in Figure 6.2. The pilot A-stage had a Vesilind $V_0$ of 15.0 ± 6.3 m/hr (mean ± CI) and $k$ of 0.70 ± 0.06 m$^3$/kg. Comparing the pilot $V_0$ to the other plants, the pilot A-stage was slightly higher than the CE (11.3 ± 3.4 m/hr) and VIP (11.2 ± 6.1 m/hr) plants. However, the A-stage $k$ was closer to that of the AT (0.67 ± 0.31 m$^3$/kg) plant. Apart from the AT plant, $V_0$ values for all of the other mixed liquor samples were within the typical ranges for activated sludge processes ($k = 0.2-1$; $V_0 = 5-15$) (WEF, 2005).
Figure 6.2 – Comparison of measured Vesilind initial hindered settling velocity ($V_0$) and settling parameter ($k$) determined using ZSV tests on mixed liquor samples from the A-stage pilot and AT, CE, and VIP treatment plants. Error bars represent 95% CI.

Using all of the ZSV test results for each process, a Vesilind settling velocity model curve (Eq. 6–1) was fitted to each dataset using the sum of least squares method. The resultant curves are displayed in Figure 6.3a. Using the predicted $V_0$ and $k$ for each model curve and the actual MLSS data, a solids flux due to gravity (SFg) curve was generated for each plant and the pilot A-stage (Figure 6.3b).
As indicated previously, the A-stage maximum settling velocity was similar ($p = 0.591$) to CE and VIP. However, the pilot A-stage settling velocity decreased rapidly with increasing MLSS, as was the case with AT mixed liquor. Interestingly, the maximum SFg for the pilot A-stage, CE, and VIP (Figure 6.3b) occurred at an initial MLSS concentration close to their average operating MLSS concentrations of 1592 ± 615, 2710 ± 265, 4145 ± 450 mg/L, respectively. This suggests that the MLSS concentration at which an activated sludge process is operated may play an important role in determining sludge settleability. Moreover, this is likely associated with the solids loading and surface overflow rates of the secondary clarifiers that results in the selection
of solids that settle at certain velocities as dictated by the operation of the solids separation process.

One of the limitations of the ZSV test is that at low initial MLSS concentrations it is very difficult to discern a discrete solids interface and therefore difficult to determine settling velocities. Additionally, during the pilot A-stage ZSV tests, it appeared as if two types of solids existed and these solids settled at differential rates. To quantify this observation, settling column tests were performed on A-stage, CE, and VIP mixed liquor samples and the results are summarized in Figure 6.4. CE and VIP were used to compare the A-stage to a HRAS (short SRT) process and a BNR (long SRT) process for the same reasons discussed previously.

![Diagram](image)

**Figure 6.4** Results of settling column tests comparing initial sample MLSS concentration to particle fractionation based on settling velocities for the A-stage pilot and CE and VIP treatment plants.

For the full-scale facilities when the settling test were ran at high (>1.5 g/L) initial MLSS concentrations, >80% of the solids settled at a rate >6 m/hr with only minor fractions (1-10%) of medium and small particles. However, the A-stage had a high nonsettleable fraction (36%)
compared to the other processes. This was as expected because the A-stage is bioflocculation limited due to its high-rate operation (i.e., SRT<0.5 days) and is reflected in the effluent TSS, which averaged 98 ± 26 mg/L.

When comparing the results from the low initial MLSS to the high MLSS tests, the fraction of nonsettleable solids increases for all three mixed liquors. This can be explained by the phenomenon of orthokinetic flocculation where large particles settling at higher rates than smaller particles collide with the small particles resulting in the removal of both particles at a higher net settling velocity (Tchobanoglous et al., 2003). This also explains why the fraction of solids that settle at a rate >6 m/hr increases with increasing MLSS. Another trend is that at longer SRTs the fraction of medium and small particles increases while the fraction of nonsettleable and large particles decreases. This is likely attributed to the conversion of denser primary particles to biological flocs that are more porous and settle at slower rates.

**Characterization of dewaterability using optimal polymer dose and centrifugation method**

To characterize the sludge dewaterability of the A-stage pilot and full-scale plants, capillary suction time tests at different polymer doses were performed. The optimal polymer dose for each sludge, which is indicated by the lowest CST, are shown in Table 6.4. These tests were also performed using primary and secondary sludges from the BH, NP, and VIP treatment plants. These plants were selected because they all have primary sedimentation and each plant has different sludge characteristics. Table 6.4 also contains the average fraction of primary to secondary solids produced based on a year’s worth of operational data for each plant and the A/B pilot. Although the pilot A-stage sludge was not sent to an anaerobic digestion system, analysing the dewaterability of anaerobically digested A-stage sludge is important to provide a full scope of solids handling characteristics. In addition, such a test would provide data for direct comparison with full-scale systems that have anaerobic digesters.

CST tests consistently concluded that the A-stage sludge required an optimal polymer dose of 3.9 ± 1.1 g polymer/kg DS at a CST of 10.1 ± 1.1 seconds. CST test results were similar for the full-scale undigested solids streams tested (Table 6.4). All of the optimal polymer dosages were within the typically ranges for primary (1-4 g/kg) and secondary solids (3-10 g/kg) (Tchobanoglous et al., 2003).
Using the optimal polymer doses determined during the CST tests, dewaterability at different blends of primary solids (PS) to secondary solids (SS) for each process was determined as shown in Figure 6.5. As expected the results show that PS dewater better than SS and that a higher blend of PS to SS increases the overall dewaterability of the combined sludges. Although the A-stage is a biological process, A-stage WAS dewatered (34.3 ± 0.4%; mean ± SEM) nearly as well as the PS for the full-scale plants (VIP = 37.4 ± 1.1%; BH = 36.3 ± 0.3%; NP = 35.3 ± 0.01%). This may be attributed to the low EPS content of the A-stage sludge since EPS is known to bind water resulting in increased sludge viscosities (Forster, 1983; Jimenez et al., 2015; Li and Yang, 2007). Houghton et al. (2001) used CST test to demonstrate that EPS benefits dewaterability until around 35 mg EPS/g SS for activated sludge. However, PS dewatered better at lower EPS concentrations and did not benefit from increasing EPS concentrations.

### Table 6.4 – Average (± standard deviation) CST and optimal polymer dose for the A/B pilot and BH, VIP, and NP plants.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Solids Type</th>
<th>CST (secs)</th>
<th>Optimal Polymer Dose (g polymer/kg DS)</th>
<th>PS to SS Fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/B</td>
<td>A-stage</td>
<td>10.1 (± 1.1)</td>
<td>3.9 (± 1.1)</td>
<td>70 (± 15)</td>
</tr>
<tr>
<td></td>
<td>B-stage</td>
<td>10.9 (± 1.7)</td>
<td>4.1 (± 0.4)</td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>Primary</td>
<td>11.0 (± 0.1)</td>
<td>4.5 (± 0.3)</td>
<td>67 (± 9)</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>11.2 (± 0.3)</td>
<td>4.4 (± 1.9)</td>
<td></td>
</tr>
<tr>
<td>BH</td>
<td>Primary</td>
<td>12.0 (± 1.5)</td>
<td>3.8 (± 0.7)</td>
<td>49 (± 5)</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>11.5 (± 1.6)</td>
<td>3.7 (± 1.3)</td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>Primary</td>
<td>11.1 (± 0.5)</td>
<td>3.4 (± NA)</td>
<td>45 (± 15)</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>10.7 (± 0.2)</td>
<td>2.5 (± NA)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.5 – Results from dewatering tests comparing cake solids to fraction of primary to secondary solids blend as dry solids. Error bars represent standard error of the mean.

The overall dewaterability of the A/B pilot plant, as indicated by the line of best fit in Figure 6.5, was less than that of the full-scale plants. However, when considering that the A/B pilot produced a higher fraction of A-stage to B-stage WAS, the dewaterability of the A/B pilot compared well with the full-scale plants in terms of obtainable cake solids. This can be seen in Figure 6.6, where dewatered cake solids for each plant were predicted based on the linear relationship (i.e., slope and intercept) between the PS to SS fractions to cake solids. Actual monthly averages for one year of the fraction of PS to SS for each plant were used to predict dewatered cake solids. For the A/B pilot a full year of data was used.
Figure 6.6 – Predicted cake solids based on monthly averages of fractions of PS to SS for the VIP, NP, and BH treatment plants and a full year of routine measurements of A-stage to B-stage WAS for the A/B pilot.

The predicted dewaterability of blended A/B solids was 26.8 ± 0.3% (mean ± CI), which is lower than the VIP and BH plants that averaged 27.8 ± 0.5% and 29.4 ± 1.0%, respectively. The NP plant was the lowest at 23.5 ± 1.4%. If the fraction of solids produced by the A-stage was closer to the typical split of 75% for full-scale A/B plants (Böhnke, 1994), the predicted cakes solids would have been slightly higher around 28%. The actual lower fraction of 70 ± 15% was likely due to the lower COD and TSS removal efficiency of the A-stage pilot. The BH plant had the highest cake solids because of a higher PS to SS fraction associated with higher solids removal in the chemically enhance primary treatment process and the production of metal precipitates resulting from iron salt addition. The NP had poor dewaterability likely because the plant performs biological phosphorus removal that has been shown to decrease the dewaterability of digested solids (Higgins et al., 2014).
Conclusions

The solids produced by the A-stage process exhibited good settling and dewatering characteristics. Both settling and dewatering performance of undigested A-stage solids indicates that the A-stage activated sludge process is a viable process from a solids handling standpoint. Further research is required to ascertain the dewaterability performance of digested A/B solids. These results are only applicable to undigested solids as they may not be predicative of digested solids since dewaterability changes with digestion.

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References


de Graaff, M.; Roest, K. (2012) Inventarisatie van AB-systemen - optimale procescondities in de A-trap, STOWA.


Chapter 7: Combining high-rate activated sludge and shortcut nitrogen removal for efficient carbon and energy utilization

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\textbf{Keywords:} A-stage, A/B Process, Anammox, BNR, Deammonification, HRAS

\textbf{Abstract:}

Carbon recovery for energy generation while sustainably meeting stringent nitrogen limits has become the focus of many water resource recovery facilities (WRRFs). Obtaining this goal not only requires reducing energy consumption but also managing carbon utilization for biological nutrient removal (BNR). This paper summarizes the findings of an A/B process pilot study that used high-rate activated sludge (HRAS) to recover organic carbon and a two-stage shortcut nitrogen removal process with a post-anoxic anammox reactor to efficiently remove nitrogen under mainstream conditions. The pilot study was able to capture 47\% of the influent COD as waste solids while only oxidizing 45\% of the influent COD. The A/B pilot averaged 79±11\% TIN removal efficiency without supplemental carbon addition to the BNR process. This work represents one of the first attempts to demonstrate the feasibility of mainstream deammonification.
Introduction

In the quest to achieve energy self-sufficiency and sustainable operation, water resource recovery facilities (WRRFs) typically employ some type of energy recovery process, like anaerobic digestion, to produce biogas for onsite heat and energy generation. Since mainstream anaerobic processes are generally limited to tropical climates or concentrated waste streams, facilities treating municipal wastewater must first concentrate then redirect the organic carbon present in the raw wastewater to the energy recovery process in a cost-effective and efficient manner. Typically, it is not possible to obtain energy-neutral operation without concurrently minimizing energy usage and maximizing energy (i.e., organic carbon) recovery. Aerobic biological processes, common to most WRRFs, also mineralize a portion of the organic carbon, which represents a loss of energy recovery potential. Additionally, organic carbon is required for biological nutrient removal (BNR). Therefore, controlling carbon redirection for energy recovery and nutrient management is critical so that facilities can still reliably meet effluent quality criteria, particularly nitrogen and phosphorus limits.

Organic carbon savings have been realized using mainstream simultaneous nitrification denitrification (SND) and nitrite-shunt activated sludge (AS) processes (Bertanza, 1997; Collivignarelli and Bertanza, 1999; Marcelino et al., 2011; O'Neill and Horan, 1995). However, these combined carbon and nitrogen removal systems generally do not take advantage of the carbon savings by recovering the excess organic carbon for energy production. The A/B process (adsorption/bio-oxidation) has demonstrated the ability to recover energy and meet moderate nitrogen discharge limits (e.g., Strass WRRF, Austria). However, with increasingly stringent nitrogen limits, additional measures must be taken in order to maintain the balance between permit compliance and energy recovery. This could include advanced process control coupled with mainstream deammonification, where autotrophic anaerobic ammonia oxidizing bacteria (anammox) convert nitrite and ammonia directly to nitrogen gas without consuming organic carbon (Mulder et al., 1995). The challenge for this type of process and other shortcut nitrogen removal processes is the out-selection of nitrite oxidizing bacteria (NOB) that compete with anammox for nitrite and convert it to nitrate.
Previous work by Regmi et al. (2014) demonstrated that controlling the influent carbon to a BNR process is a critical aspect of mainstream deammonification and nitrite-shunt processes. The A-stage process is one of the only carbon removal processes that lends itself to being controlled through instrumentation, control, and automation (ICA). The high-rate operation of the A-stage (<1 day SRT, ~30 min HRT, <1 mg/L DO) results in concentrating the influent particulate, colloidal, and soluble COD (chemical oxygen demand) to a waste solids stream with minimal energy input in a small footprint by maximizing sludge production (i.e., observed yield), bacterial storage, and bioflocculation. Coupled with nitrite-shunt and anammox it may be possible to approach energy neutral operation while meeting stringent nutrient limits. The Hampton Roads Sanitation District (HRSD, Virginia, USA) piloted an A/B process performing carbon capture and mainstream nitrite-shunt followed by tertiary anammox polishing. This paper is a summary of the many results over several years of work.

**Materials and Methods**

**Pilot Study Configuration**

The HRSD pilot consisted of adsorption-style (A-stage) high-rate activated sludge (HRAS) process followed by a nitrite-shunt BNR (B-stage) process (Figure 7.1). The B-stage effluent then passed through a fully anoxic, anammox moving bed biofilm reactor (MBBR) for nitrogen polishing. The B-stage was operated under ammonia versus NOx-N (AvN) configuration and this process is described in section 2.2. The A-stage consisted of three tanks in series ($V_{tot} = 511$ L) followed by an intermediate clarifier. A single modulating valve controlled airflow to the A-stage. The B-stage consisted of four tanks in series ($V_{tot} = 606$ L) with independent aeration control and mechanical mixing in each tank. The anammox MBBR contained K-3 media (AnoxKaldnes) at a 50% fill ratio and slow speed mechanical mixing. The MBBR tank had variable volume settings (454, 341, and 227 L) so the hydraulic residence time (HRT) could be altered independently from the B-stage HRT. The pilot was fed screened (2.4 mm openings) and degrittred municipal wastewater that was first adjusted to the desired operating temperature (equipment not shown). Table 7.2 contains the average influent characteristics.
Pilot Operation and Process Control

The pilot was continuously operated for over 600 days and the average operating conditions are presented in Table 7.1. Process automation was achieved using various online sensors and a programmable logic controller (PLC). A-stage aeration control was achieved using a cascade DO controller, DO sensor located in the last bioreactor, mass gas flowmeter, and modulating valve. The DO setpoint was typically set at 0.5 mg O₂/L, however, a minimum airflow was also set to ensure adequate mixing which meant the DO was greater than the setpoint during periods of low oxygen demand. The aerobic fraction of the A-stage was altered by operating the first bioreactor under aerobic or anaerobic conditions. The A-stage wastage rate (WAS) was maintained constant because of the lack of reliable instrumentation that would allow accurate automated SRT control. The wastage rate was manually changed as needed to obtain the desired COD removal.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A-stage HRAS</th>
<th>B-stage AVN</th>
<th>Anammox MBBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRT (days)</td>
<td>0.15±0.05</td>
<td>7.0±2.6</td>
<td>-</td>
</tr>
<tr>
<td>HRT (hours)</td>
<td>0.5</td>
<td>2-6</td>
<td>1.1-3.4</td>
</tr>
<tr>
<td>Aerobic Fraction</td>
<td>1 or 0.67</td>
<td>0.5±0.1</td>
<td>Anoxic</td>
</tr>
<tr>
<td>DO (mgO₂/L)</td>
<td>0.91±1.29*</td>
<td>1.6†</td>
<td>Below Detection Limit</td>
</tr>
</tbody>
</table>

*Average DO of all three reactors
†DO setpoint when air is on
In the B-stage, the AvN controller, as described in detail by Regmi et al. (2014), consisted of an aeration duration controller that used on-line \textit{in situ} DO, NH$_4^{+}$,NO$_2^{-}$ and NO$_3^{-}$ sensors. During periods of aeration the DO controller maintained a DO setpoint of 1.6 mg O$_2$/L. The purpose of the AvN controller was to maintain equal parts effluent TAN and NOx-N at all times, which has been shown to give optimal TIN removal for a given influent C/N (Batchelor, 1983) and it provides an influent amenable to the anammox process.

**Biochemical Methane Potential (BMP) Tests**

BMP tests were conducted according to (Angelidaki et al., 2009) in 500 mL Wheaton bottles and cumulative methane production was measured using a RSA PF-8000 respirometer (Springdale, Arkansas, USA) with moisture and CO$_2$ scrubbers. Primary solids, secondary WAS, and digestate was collected from HRSD’s Nansemond, York River, and James River treatment plants. All plants had mesophilic digesters. Each bottle contained 300 mL of inoculum and were degassed for one day prior to starting the test. The bottles were maintained at 37±1°C in a water bath and continuously stirred with magnetic stir bars at 150 rpm. Sludge samples were added to the bottles (30-50 mL depending on VS content) and brought to total volume of 500 mL by adding tap water with a bicarbonate concentration of 10 gHCO$_3^{-}$/L. Triplicates were ran on each sample and blanks were ran using digestate only. The actual methane production for each sample was obtained by subtracting the average methane production of the blanks from the average methane production of the samples. Methane production is expressed at 35°C and 1 atm.

**COD Mass Balances**

COD and total phosphorus (TP) mass balances were conducted on each process to determine the fate of the influent COD. If the difference between the influent TP versus effluent TP for each stage was greater than 10% the COD mass balance was rejected. The difference between COD in and COD out was assumed to be the mineralized or oxidized fraction.

**Laboratory Methods**

Performance of the pilot was monitored by collecting 24-hr flow-weighted composite samples from the influent and effluent of each process and the mixed liquor and WAS of the A-stage. Grab samples were also taken from the B-stage AvN process. These samples were analysed for
total and volatile solids (TS, VS) and suspended solids (TSS, VSS) according to Standard Methods (APHA, 2012). Samples analyzed for NO$_3$-N, NO$_2$-N, and TAN using Hach Test-n- Tube (TNT) kits were first filtered through 0.45 μm cellulose membrane filters. COD and soluble COD were measured using Hach TNT kits where the sCOD sample was first filtered through a 1.5 μm glass microfiber filter. Total Kjeldahl nitrogen (TKN) and TP were determined by flow injection analysis colorimetry after block digestions and acid persulfate digestion, respectively. Settled volume index (SVI) was determined according to Standard Methods (APHA, 2012).

**Statistical Analysis**

Statistical analyses including Pearson product moment coorelation, Shapiro-Wilk normality test, t-test, mean, standard deviation, standard error of the mean, and confidence intervals were performed using SigmaPlot 12.5 (Systat Software Inc., Bangalore, India). A $p$-value of 0.05 or lower indicates that variables being compared are statistically different at the 95% confidence level.

**Results and Discussion**

**Pilot Performance**

During the 600 days of operation, many operational changes occurred that affected performance and for the sake of brevity will not be covered. Performance of the B-stage processes are addressed in greater detail elsewhere (Regmi et al., 2015a; Regmi et al., 2015b; Regmi et al., 2015c; Regmi et al., 2014). The average influent and effluent parameters are presented in Table 7.2. On average, the A-stage removed 45±12% of the total influent COD and the overall TIN removal efficiency of the pilot averaged 79±11%. A-stage and B-stage SVIs were similar to cited values for full-scale A/B plants (Böhnke, 1994).
### Table 7.2 – Average influent and effluent concentrations ± standard deviation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RWI</th>
<th>A-stage HRAS Eff</th>
<th>B-stage AVN Eff</th>
<th>Anammox MBBR Eff</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>552±73</td>
<td>302±63</td>
<td>50±14</td>
<td>41±12</td>
</tr>
<tr>
<td>sCOD/pCOD (g/g)</td>
<td>0.66±0.15</td>
<td>0.97±0.31</td>
<td>2.1±3.8</td>
<td>2.7±5.8</td>
</tr>
<tr>
<td>TKN (mg N/L)</td>
<td>43.0±4.5</td>
<td>37.8±4.2</td>
<td>9.0±3.9</td>
<td>7.7±4.0</td>
</tr>
<tr>
<td>TAN (mg N/L)</td>
<td>34.9±4.0</td>
<td>31.6±3.8</td>
<td>6.48±3.18</td>
<td>4.69±3.11</td>
</tr>
<tr>
<td>NO$_2$-N (mg N/L)</td>
<td>BDL*</td>
<td>BDL</td>
<td>4.44±2.54</td>
<td>3.62±2.57</td>
</tr>
<tr>
<td>NO$_2$-N (mg N/L)</td>
<td>BDL</td>
<td>BDL</td>
<td>1.99±1.08</td>
<td>0.20±0.13</td>
</tr>
<tr>
<td>Sludge Yield (kg VSS/kg COD)</td>
<td>-</td>
<td>0.57±0.18</td>
<td>0.17±0.07</td>
<td>-</td>
</tr>
<tr>
<td>SVI (mL/g)</td>
<td>-</td>
<td>86±26</td>
<td>128±36</td>
<td>-</td>
</tr>
</tbody>
</table>

*BDL = Below Detection Limit

The A-stage also averages 0.22±0.08 kg O$_2$/kg COD removed, which is similar to the value reported for the full-scale A-stage process at the Rotterdam WRIF (Netherlands) of 0.2 kg O$_2$/kg COD removed (Jetten et al., 1997). A typical value for a conventional HRAS process operated at a HRT and SRT of x and y, respectively is 0.6 kg O$_2$/kg COD removed (Jetten et al., 1997).

The TIN removal performance of the nitrite-shunt process was stable during the study, but was mostly dependent on the influent C/N ratio (effluent of A-stage). When the nitrite-shunt process was operated aggressively (i.e., low HRT and short SRT), NOB out-selection was achieved. Maximum activity test were conducted *ex situ* as described in Regmi et al. (2014) and revealed that the ratio of maximum NOB rate to maximum AOB rate was 0.75±0.17 during the study. Although the process was carbon limited, no additional alkalinity or supplemental carbon was added.

**Controlling COD/TAN and its impact on TIN removal efficiency**

The COD removal efficiency, and thus effluent C/N, of the A-stage was controlled by manipulating the SRT. Figure 7.2a is a comparison between the measured total SRT of the A-stage and the effluent COD/TAN (C/N). This trend will continue to approach a C/N ratio near zero as the SRT increases because COD removal is maximized (assuming no nitrification). Given the narrow SRT range at which C/N is controllable means that process control was needed for the A-stage to ensure that an effluent with a consistent C/N ratio was sent to the B-stage. Subsequently, the effluent C/N of the A-stage directly impacts the TIN removal efficiency of the B-stage (Figure 7.2b).
Figure 7.2 – Impact of A-stage total SRT (a) on A-stage effluent COD/TAN and (b) its subsequent impact of on TIN removal efficiency in the B-stage.

Due to the inefficiencies of the B-stage a higher C/N is not necessarily beneficial to TIN removal efficiency. For the B-stage process and the given influent COD fractions (i.e., sCOD/pCOD), this occurred around a C/N of 10 with only marginal increases of TIN removal efficiency beyond that. The reason for this was that the autotrophic fraction of the mixed liquor decreased as the organic load increased because the SRT had to be decreased to maintain a MLSS concentration <4 g/L at the same HRT. Since the B-stage was already operated aggressively in terms of SRT, this resulted in decreased AOB rates because of washout and heterotrophic competition for space and DO. Therefore, any excess organic carbon must be removed prior to the B-stage in order for it to be effectively redirected for energy recovery.

*Nitrogen Removal in the A/B Process*

TIN removal efficiencies for the A and B stage and the anammox MBBR are shown on Figure 7.3. On average, the A-stage process accounted for 10±5% removal of the influent TIN (13±7% TN), which was due to assimilation of ammonia and sedimentation of ON containing matter. Böhnke et al. (1998) reported a full-scale A-stage process averaged 23% TN removal, which was likely higher because of its higher COD removal efficiency and thus larger fraction assimilated. If a plant anaerobically digests its solids, the nitrogen that is removed by the A-stage will end up being returned to the head of plant with the dewatered digestate liquor. Therefore, a sidestream
deammonification process should be used to remove the nitrogen. Alternatively, if anaerobic digestion is not available, the solids could be incinerated without concern of the nitrogen being returned to the head of the plant.

The B-stage removed 56±15% of the influent TIN mainly through BNR processes and some assimilation. Variations between A-stage and B-stage TIN removal were primarily due to the variability of COD removal in the A-stage. When TIN removal of the A-stage increased, B-stage TIN removal decreased. This data implicates that shortcut nitrogen removal processes operated aggressively (i.e., $\text{SRT}_{\text{actual}} \approx \text{SRT}_{\text{critical}}$) using process control may be sensitive to variations in influent composition. Therefore, better control of the carbon removal process, whether it is an A-stage process or other unit process like chemically enhanced primary treatment, is required for stable TIN removal in the B-stage.

The anammox MBBR removed an additional 13±4% of the influent TIN for a combined average TIN removal efficiency of 79±11% for the whole pilot. Influent nitrite was the limiting factor for...
the anammox MBBR throughout the entire study. Final effluent nitrite was typically maintained below 0.2 mgN/L while ammonia and nitrate were generally greater than 1 mg N/L. However, maximum anammox activity rates of 0.6-1.0 g TIN/m²/day, which were about three times higher than the *in situ* rates, suggests that the anammox process could handle much higher nitrite loading rates and therefore obtain higher TIN removal efficiencies. Contrary to anammox stoichiometry, nitrate was actually removed (Table 7.2) and not produced presumably due to endogenous denitrification. Nitrate removal was enhanced by adding acetate to the MBBR. The drawback of using acetate is that it can only be used in limiting amounts or heterotrophic bacteria will outcompete anammox for their substrate, nitrite. The benefit becomes apparent when trying to meet very stringent nitrogen limits (i.e., <3 mg/L TN) as the cost for removing nitrogen to very low levels increases exponentially.

**A-stage Influent and Effluent COD Fractions**

Figure 7.4 contains the average influent and effluent COD and sCOD/pCOD values over the course of the study for the A-stage. These values were averaged (7-day moving average) to reduce variation while still demonstrating the general trend. The average influent COD concentration was 552±73 mg COD/L and the soluble to particulate COD fraction remained constant around 0.66±0.15 g COD/g COD. The influent COD varied slightly with seasonal patterns, however, it does not appear seasonal variations significantly influenced the sCOD/pCOD fraction. The A-stage effluent sCOD/pCOD was affected by the pCOD removal efficiency. That is, as COD removal efficiency increased, a larger fraction of pCOD was removed when compared to sCOD.
The A-stage effluent sCOD/pCOD fraction was also important when considering the downstream B-stage process. Particulate COD was more beneficial to the B-stage because the process did not have designated anoxic or anaerobic zones that utilized the sCOD that is easier to degrade. It was postulated that the pCOD that adsorbed to the flocs persisted through the aerated cycles and was hydrolyzed to readily biodegradable COD during the anoxic periods. Therefore, denitrification occurred throughout the entire B-stage during the anoxic cycles.

**Fate of COD in the A/B Pilot**

The COD mass balance results are presented in Figure 7.5. The A-stage averaged 45±12% COD removal where 12±5% was oxidized and the remainder (32±7%) was captured as WAS. The B-stage oxidized a much larger fraction (56±11%) of its influent COD, which is similar to most BNR processes. However, the COD oxidized in the B-stage process represents a much smaller fraction (32%) of the RWI COD. Since the anammox MBBR was fully anoxic, very little carbon was lost to oxidation (2% of RWI COD). Overall, only 45% of the RWI COD was oxidized, which is less than what has been reported in literature for conventional activated sludge process.

This means that the A/B process was able to capture more COD as WAS (47%), which can redirected to anaerobic digestion for energy recovery as methane.
Normalized sludge production (kg DM/day·m$^3$ influent) was determined for the A-stage and B-stage. On average the A-stage produced 70±15% of the total sludge produced (i.e., WAS) and the B-stage produced 30±15%. The sludge production was higher for the A-stage since this process was operated at a much lower SRT and thus had a higher observed yield (Table 7.2). A single-sludge BNR plant typically produces 40% primary solids and 60% secondary solids with an overall solids production that is about 10% less than the A/B process (cite). However, since the sludge produced by an A-stage has better digestion characteristics when compared to normal secondary sludge, lower overall sludge production after anaerobic digestion is expected when compared to a single-sludge BNR process preceded by primary sedimentation (van Loosdrecht et al., 1997).

**Implications of BMP Tests**

Four separate BMP tests were run on each sludge sample to determine the volatile solids reduction and methane production potential. The average VS removal and CH$_4$ yields results are summarized in Figure 7.6. Volatile solids reduction (VSR) was the highest (82±4%) for the A-stage WAS followed by primary solids (62±12), secondary WAS (45±7), then B-stage WAS (41±10). The COD removal trend was similar except the B-stage WAS degraded slightly more (40±15%) than the secondary WAS (33±11%). As expected, the specific methane yield (SMY)
was highest for the primary solids at 0.52±0.11 m$^3$CH$_4$/kgVS added. The A-stage had the second highest SMY at 0.45±0.06 m$^3$CH$_4$/kgVS. Although the B-stage sludge should be similar to secondary sludge, the SMY for the B-stage (0.29±0.09 m$^3$CH$_4$/kgVS) was higher than the secondary WAS (0.19±0.00 m$^3$CH$_4$/kgVS). This is likely because the SRT of the B-stage was lower than the full-scale facilities.

**Figure 7.6** – Comparison of average (a) volatile solids reduction and (b) COD removal to specific methane yield of A-stage HRAS WAS, B-stage AvN WAS, Primary Solids (PS), and Secondary WAS. Error bars represent standard deviation of the mean. Primary solids and secondary solids were collected from three different full-scale HRSD treatment plants (Nansemond, York River, and James River).
The COD removal results have more variability, especially the PS, likely because they are more susceptible to measurement errors. Another explanation is that it was very difficult to obtain a homogenous sample that was truly representative of all the primary solids. Additionally, there is a difference in the primary solids composition between the full-scale plants because of different industrial inputs.

While these results suggest trends between the samples, they certainly do not represent the actual biogas production that could be expected full-scale. BMP tests provide only a glimpse of degradability and methane yield because they are run at optimal conditions and not the actual design conditions of the digester (i.e., HRT, SRT, mixing, temperature, etc.) (Angelidaki et al., 2009). Although, the A-stage did not have as high SMY as PS, it does digest better, 82±4% VSR compared to 62±12% for PS. When considering that A-stage represents a larger fraction of the total solids produced (70%) compared to single-sludge BNR plant with primary sedimentation (60%) and A/B plants produce on average 10% more total sludge, the A/B process is able to recover more of the influent COD as energy.

Conclusions

Coupled with the low energy requirements for carbon recovery and increased overall sludge production of solids that are easily anaerobic digested with high methane yields the A/B process exploiting short-cut nitrogen removal demonstrated that COD capture for energy production is possible without compromising nitrogen removal performance. While there is certainly room for improvement in terms of process stability and reaching lower final effluent TN values this study represents one of the first attempts to implement anammox in the mainstream process to improve the sustainability of future wastewater treatment facilities.
References


Chapter 8: Engineering Significance

Other than water, organic carbon management is one of the most critical aspects of wastewater treatment as organic carbon removal requires a substantial amount of energy in the form of aeration, is an energy source for nutrient removal, and is the primary constituent of excess sludge. As water resource recovery facilities transition from removal and disposal to recovery and beneficial reuse, organic carbon currently has the greatest recovery potential. This is because of its quantity and value when compared to exogenous carbon sources, like methanol for denitrification and natural gas for heating, which are expensive and petroleum based. While there are certainly other carbon capture technologies, like fine sieve screens and chemically enhanced primary treatment, high-rate activated sludge is currently one of the only proven technologies that is capable of capturing soluble chemical oxygen demand (COD) from wastewater in moderate to cold climates (mainstream anaerobic treatment is emerging). However, the literature and experience available on high-rate processes operated at very low sludge ages, like the A-stage, is limited and often dated.

The objective of this study was to really take a holistic look at the A-stage process and document the findings with a focus on identifying the primary COD removal and capture mechanisms and confirming the solids handling characteristics and digestibility of the sludge produced. As part of this project, automatic process controllers were developed with the purpose of controlling COD removal and capture so that biological nitrogen removal in the downstream B-stage process could be intensified through the use of shortcut nitrogen removal technologies. Although the A-stage is often referred to as the ‘adsorption’ process, the primary COD removal mechanism were determined to be oxidation and assimilation of soluble substrate and sedimentation of particulate matter. While adsorption was certainly occurring, it was relatively minor at the sludge ages investigated. As expected, the primary control parameter that influenced all aspects of treatment, including COD removal and sludge settleability, was the solids retention time (SRT) of the A-stage. The hydraulic retention time and process water temperature were also important as they determined the operating range of SRT. Based on SRT, this study confirmed that there are different optimal SRTs at which carbon capture is maximized or carbon removal is maximized. Additionally, the range at which COD removal is controllable is narrow and occurs at SRTs below the optimal SRT for carbon capture. This means that a facility must balance carbon
capture with nutrient removal as it is unlikely that both energy recovery and nutrient removal can simultaneous be maximized.

This work was able to confirm that A-stage produces significantly more sludge (70-80%) than the B-stage process (20-30%) and the A-stage sludge is highly anaerobically digestible. Overall, the A/B process generates 10-20% more sludge than an equivalent single-sludge process. Due to the quantity and digestibility of the A-stage sludge, the A/B process overall produces less biosolids that need to be disposed. Of course, one of the major benefits of the A/B process is that 10-20% more biogas is recovered because of the efficiency at which COD is removed in the A-stage. What is still lacking, and is one of the major shortcomings of this work, is exactly why the A-stage settles, dewater, and digest so well. The extracellular polymeric substance (EPS) content of the A-stage sludge was measured and found to be low, but a detailed investigation into the types of EPS and the effect of A-stage operation on EPS production was not conducted. Aerobic storage was also not investigated and still remains one of the most unstudied aspects of COD removal in the A-stage. Intracellular storage likely plays a significant role in determining the digestibility of the A-stage sludge. Aerobic storage and EPS production, as it relates to adsorption, are also critical aspects of modeling high-rate processes as these COD removal mechanisms have been excluded or simplified from current activated sludge models.

While the A-stage process is well established, future installations will largely be dependent on the development of shortcut nutrient removal technologies that require little to no organic carbon. The A-stage does have a potential use at facilities with existing high-rate processes or at facilities where the main focus is energy recovery and not nutrient removal. However, it is more likely that the fundamentals of this process will be applied to emerging carbon capture technologies like high-rate aerobic membrane bioreactors and contact stabilization processes. The benefit of membrane filtration is that bioflocculation is no longer a limiting factor. Therefore, this process can capture more carbon with less oxidation by operating at shorter sludge ages. High-rate contact stabilization may also improve bioflocculation by allowing more time for EPS production, but more importantly, the process is poised to maximize intracellular storage and thus improve energy recovery potential. Although activated sludge has been around for over 100 years, there is still a considerable amount of research for the undertaking.