Aerobic granular sludge: Impact on nutrient reduction based on seasonal BioWin modeling

Ashley Geesman

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Aerobic granular sludge: Impact on nutrient reduction based on seasonal BioWin modeling

by

Ashley Geesman

A creative component submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Civil Engineering

Program of Study Committee:
Timothy Gage Ellis, Major Professor
Kaoru Ikuma
Elizabeth Swanner Smith

Iowa State University
Ames, Iowa
2019

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# Table of Contents

*Table of Contents* .................................................................................................................. 0

*Introduction* ......................................................................................................................... 1

*Cultivation* ............................................................................................................................. 3

  - Specifics .................................................................................................................................. 3
  - Importance of EPS ................................................................................................................. 4
  - Proposed Formation Mechanisms ....................................................................................... 4

*Nutrient Reduction* .................................................................................................................. 5

  - Function of Microenvironments .......................................................................................... 5
  - Nitrogen ................................................................................................................................. 6
  - Phosphorous .......................................................................................................................... 8

*Real World Implementation* ..................................................................................................... 9

*BioWin Modeling* ..................................................................................................................... 11

*Simulation Results and Discussion* .......................................................................................... 16

*References* ................................................................................................................................ 23

*Appendix* .................................................................................................................................. 26

  - Appendix A. Model Setup ..................................................................................................... 26
  - Appendix B. Simulation Data ................................................................................................ 27
INTRODUCTION

Traditional wastewater treatment techniques attempt to exploit natural processes to treat water more rapidly. The purpose for treating wastewater is to limit the impact on the natural environment, most commonly in Iowa, lakes and rivers. Treatment efficiency is quantified using certain parameters that refer to the strength of the waste (BOD, COD, TOC), turbidity caused by solids (TSS), and nutrient build-up (N, P). These pollutants can cause oxygen depletion, limited light penetration, increased algae growth, and eutrophication, which negatively impact the environment. While regulations have been in place for BOD, TSS, and ammonia reduction, recent policy changes in Iowa, are pushing wastewater treatment plants (WWTPs) to address a common shortcoming of discharging total nitrogen (TN) and total phosphorus (TP).

Figure 1. Morphology of activation sludge (a) versus aerobic granular sludge (b). Scale bar: 1 mm. Cross-section view of an AGS granule (c) Source: Nancharaiah et al., 2019; Wang et al., 2005

Aerobic granular sludge (AGS) or granular activated sludge (GrAS), shown in Figure 1, is categorized as a "self-immobilized microbial consortium" (Xia et al., 2018). First reported in 1991, this technology has improved significantly to focus on current biological nutrient reduction (BNR) limitations. The most commonly researched and developed aerobic granular sludge has been used for aerobic degradation of organics and nitrogen removal (Liu et al., 2004). Another well-researched aerobic granular sludge was developed in aerobic conditions but consists of aerobic and anoxic zones. The granules are most efficiently developed using a sequencing batch reactor (SBR) (Liu et al., 2005). AGS has shown the capability to treat not only traditional pollutants, but also toxic pollutants at high loading rates (Nancharaiah & Kiran Kumar Reddy, 2017). Current research includes treatment efficiency, cultivation conditions, granulation factors, and identifying microbial communities present within the granule (Gao, Liu, Liang, & Wu, 2010; Nancharaiah & Kiran Kumar Reddy, 2017).

To better understand AGS, a comparison to activated sludge is often used because traditionally activated sludge has been the most widely adopted technique implemented for wastewater treatment. Activated sludge is defined as a flocculated microbial community that floats freely. Similarly, AGS is also defined by these constraints but includes granulation and therefore the microbial populations vary. Two physically separated tanks are used for treatment with AS, an aerated basin, responsible for biological removal of organic carbon and nitrification, and a settling tank, where the AS separates from
the treated water by settling the flocculated biomass. Disadvantages of implementing AS include low biomass concentrations in the aeration basin and a large footprint requirement for two tanks (aeration and settling). Additionally, enhanced biological nutrient reduction (BNR) is used to remove TP and TN. Supplementary tanks are required to efficiently cultivate the necessary microbial communities further increasing the footprint. Furthermore, the poor settling ability of activated sludge is termed ‘sludge bulking’ and can deteriorate the quality of the final effluent by losing excess sludge with the effluent (Nancharaiah & Kiran Kumar Reddy, 2017).

Aerobic granular sludge treatment provides a combination of a decreased mandatory footprint shown in Figure 2, shorter settling time, and on-site cultivation. These factors overcome the common limitations of a conventional activated sludge treatment in terms of a more sustainable and efficient treatment option.

Figure 2. A comparison of conventional activated sludge treatment using BNR (top) versus aerobic granular sludge treatment (bottom) in a single tank reactor design. (Kerstens et al., 2017)

There are defined critical characteristics of aerobic granular sludge to ensure successful wastewater treatment. Firstly, the rapid settling rate depends on the high density and large size of the granules. Next, the formation and preservation of aerobic, anoxic, and anaerobic redox layers are mandatory for effective organic matter degradation, nitrification, denitrification, and phosphorus removal, all in one tank. Additionally, the use of dissolved oxygen to regulate the metabolic reactions provides a tuning fork for treatment efficiency. Lastly, the cooperation between autotrophic and
heterotrophic microorganisms is pertinent in developing a functioning granule (Nancharaiah & Kiran Kumar Reddy, 2017).

![Figure 3. 16S rRNA gene DGGE profiles of microbial communities for seed sludge flocs (S) and aerobic granules (G) on day 63. (Younmei et al., 2014)](image)

**CULTIVATION**

**SPECIFICS**

To cultivate granules, column reactors are inoculated with activated sludge. Granular formation is achieved by selection-based techniques. Hydrodynamic shear force or up-flow aeration velocities and specific feeding regimes have the largest impact on the end product, AGS. The high shear force of up-flow velocity is considered important in forming a dense and stable granule (Tay et al., 2001). Similarly, research has shown an increase in the production of extracellular polymeric substances (EPS), in cell surface hydrophobicity, and in specific gravity of AGS with high air velocities (Lochmatter & Holliger, 2014). The shear hydrodynamic force also compacts the surface of the aggregate and aids in shaping the outer surface of the granule by detaching loosely attached microorganisms. A high shear force is believed to promote the formation of slow-growing microorganisms, which is a crucial characteristic of granule formation (Wilen et al., 2018). However, lower up-flow velocities have been shown to cultivate granulation in combination with a feeding
regime of feast-famine (Devlin et al., 2017). This gives rise to the assumption that AGS formation results from a multi-parameter cultivation effort and, as seen in Figure 3, has a dramatically different phylogenetic makeup than traditional activated sludge. Based on Illumina MiSeq sequencing of the microbial communities, AGS granules exhibit higher microbial diversity and richness than activated sludge due to cultivation efforts (Wilén, Liébana, Persson, Modin, & Hermansson, 2018).

**IMPORTANT OF EPS**

Extracellular polymeric substances (EPS) are credited with playing a central part in the aggregation of microorganisms. The EPS matrix also provides stability to the structure of the granule (Aday et al., 2008; McSwain et al., 2005; Sarma, Tay, & Chu, 2017; Wang, Liu, & Tay, 2005). Specifically, β-polysaccharides form the stable structure within the granules (Adav et al., 2008). Using ex-situ chemical analysis, in-situ visualization using specific fluorophores, and confocal laser scanning microscopy (CLSM), the makeup of a granule was determined to be microbial cells and EPS made of proteins, polysaccharides, and lipids (Figure 4) (Aday et al., 2008; McSwain et al., 2005; Wang et al., 2005; Liang et al., 2019). The EPS producers comprise one of the most abundant functional groups within AGS, approximately 40% on average, while its cumulative relative read abundance, or how plentiful those species are in comparison to the others, added up only to approximately 13% in the seed sludge (Szabó et al., 2017). This difference is indicative of the importance of EPS in AGS function and formation.

![Figure 4](image)

*Figure 4.* Confocal laser scanning microscope (CLSM) images of granule cross sections showing the distribution of protein (A), α-polysaccharide (B), β-polysaccharide (C), and lipid (D). (Liang et al., 2019)

**PROPOSED FORMATION MECHANISMS**

The method of formation is predominantly unknown, but there are several proposed mechanisms. Initially, the aggregation of the microbial communities is significant for the optimization of the treatment performance. Sludge granulation occurs in a set of four steps: cell-to-cell contact, attractive forces between cells, development of the microbial aggregates with formation of EPS matrix, and granule formation (Wilén et al., 2018). Interactions such as Van der waals forces and cell surface
hydrophobicity between cells control the cell-to-cell contact. The ratio of protein to polysaccharide increased with increasing EPS production and corresponding changes in microbial community composition (Gao et al., 2011). This change in ratio is a phenomenon that leads to increased cell surface hydrophobicity.

Cellular interactions have been shown to play an important role in granule formation. Quorum sensing, microorganism’s ability to regulate gene expression via autoinducer molecules when reaching a critical cell density, has been proposed as one the main types of microbial community interactions. *Pseudomonas, Aeromonas, and Acinetobacter*, known quorum sensing species, were all present in an AGS microbial community investigated by Tan et al. (2014). Moreover, there was a positive correlation between the relative abundance of known quorum sensing molecules (N-acyl-honoserine lactones), known quorum-sensing species, and the abundance of EPS.

![Figure 5](image.png)

Figure 5. Schematic drawing of an aerobic granule with the different conversion processes for organic material, nitrogen, and phosphorous, taking place within different redox zones. Reprinted from “The Mechanisms of Granulation of Activated Sludge in Wastewater Treatment, Its Optimization, and Impact on Effluent Quality” by Wilén, B. M., Liébana, R., Persson, F., Modin, O., & Hermansson, M., 2018, *Applied Microbiology and Biotechnology*, 102(12).

**Nutrient Reduction**

**Function of Microenvironments**

As shown in Figure 5, there are three layers that provide the ability for effective comprehensive wastewater treatment. The outer layer of the granule has the largest interaction with the outside of the granule and the highest concentration of oxygen. Labeled the Aerobic zone in Figure 5, it offers an environment fitting for the microbial communities that perform oxidation of organic matter and nitrification, both dependent on oxygen as the terminal electron acceptor. This outer layer is capable of adequately oxidizing organic matter in the form of chemical oxygen demand (COD).
removal by 94.46 ± 3.59% (He et al., 2016). To the interior of the aerobic zone is the anoxic zone with corresponding low oxygen concentrations. The Anoxic zone is the microenvironment responsible for denitrification and biological phosphorus removal. Both of these metabolic reactions use terminal electron acceptors other than oxygen. Lastly, the core of the granule is termed the Anaerobic zone made of EPS and completely void of microbial cells (Nancharaiah & Kiran Kumar Reddy, 2017). This layer does not have any species that can be used for redox reactions. Due to the structural composition of the granule and the stability of the three microenvironments there is a large diffusion gradient of electron donors and acceptors.

**Figure 6.** Graphical representation on segregated distribution of (a) microorganisms, (b) carbohydrates and proteins of the EPS matrix, and (c) nitrogen removal pathways in an individual aerobic granule. Reprinted from “Aerobic Granular Sludge Technology: Mechanisms of Granulation and Biotechnological Applications” by Nancharaiah, Y. V, & Kiran Kumar Reddy, G., 2018, *Bioresource Technology, 247*, 1133.

**NITROGEN**

Nitrogen removal requires both nitrification of ammonium and denitrification of either nitrate or nitrite. These two microbial metabolisms are done in two different environments; ammonium nitrification is typically an aerobic process while denitrification is an anaerobic process. The presence of both of oxic and anoxic environments over short spatial or temporal scales is imperative for effective
nitrogen removal, as the product of nitrification, nitrate (NO$_3^-$) is a reactant in denitrification. As seen in Figure 6, the granule maintains three microenvironments attributable to microbial diffusion and respiration in the outer region (Nancharaiah & Kiran Kumar Reddy, 2017). Incorporation of periods with high oxygen and low oxygen flux is vital to accomplishing complete nitrogen removal. There are two ways nitrification and denitrification can occur: simultaneous nitrification denitrification (SND) or alternating nitrification denitrification (AND). SND represents a system where both nitrification and denitrification are executed in the same reactor, while AND was developed to introduce an anoxic phase to encourage more complete TN removal (Nancharaiah & Kiran Kumar Reddy, 2017). Alternating aeration is additionally credited for promoting functional redundancy within the microbial community. Common nitrifying microorganisms found in AGS include autotrophic ammonium-oxidizing bacteria (AOB) such as genus *Nitrosomonas* (Szabó et al., 2017; Wang et al., 2018). Heterotrophic nitrite oxidizing bacteria (NOB) were also present, most commonly *Nitrospira* and *Nitrosbacter* (Table 1; Figure 7d). However, their relative abundance varied depending on the granulation conditions. According to Szabó et al. (2017), the denitrifying microorganisms in the anoxic zone were the most abundant with the largest diversity. With a group relative abundance of over 50% the higher abundances were from *Denitromonas*, *Meganema*, *Thauera*, *Devosia*, and *Stenotrophomonas*, which are interestingly all EPS producing microorganisms (Szabó et al., 2017). Total inorganic nitrogen removal efficiencies from AGS totaled 93.88 ± 6.78 % (He et al., 2006).

Table 1

*Key Functional Groups Classification at Genus Level*

<table>
<thead>
<tr>
<th>Key functional groups</th>
<th>Relative abundances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1</td>
</tr>
<tr>
<td>GAOs</td>
<td></td>
</tr>
<tr>
<td><em>Defluviicoccus</em></td>
<td>1.698</td>
</tr>
<tr>
<td><em>Candidatus_competibacter</em></td>
<td>13.524</td>
</tr>
<tr>
<td>AOBs</td>
<td></td>
</tr>
<tr>
<td><em>Nitrosomonas</em></td>
<td>0.010</td>
</tr>
<tr>
<td><em>Norank_f_Nitrosomonadaceae</em></td>
<td>0.276</td>
</tr>
<tr>
<td>NOBs</td>
<td></td>
</tr>
<tr>
<td><em>Nitrospira</em></td>
<td>1.251</td>
</tr>
<tr>
<td>DNBs</td>
<td></td>
</tr>
<tr>
<td><em>Arcobacter</em></td>
<td>0.000</td>
</tr>
<tr>
<td><em>Flavobacterium</em></td>
<td>1.204</td>
</tr>
<tr>
<td><em>unclassified_f_Rhodobacteraceae</em></td>
<td>3.577</td>
</tr>
<tr>
<td><em>norank_f_Rhodospirillaceae</em></td>
<td>0.430</td>
</tr>
<tr>
<td>Species</td>
<td>Oxic</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Rhodobacter</td>
<td>0.089</td>
</tr>
<tr>
<td>norank_f_Rhodocyclaceae</td>
<td>0.003</td>
</tr>
<tr>
<td>Azoarcus</td>
<td>0.031</td>
</tr>
<tr>
<td>Rhodococcus</td>
<td>0.027</td>
</tr>
<tr>
<td>Azospira</td>
<td>1.548</td>
</tr>
<tr>
<td>Zoogloeoa</td>
<td>0.072</td>
</tr>
<tr>
<td>Thauera</td>
<td>0.020</td>
</tr>
<tr>
<td>PAOs</td>
<td></td>
</tr>
<tr>
<td>Candidatus_Accumulibacter</td>
<td>0.119</td>
</tr>
<tr>
<td>DNPAOs</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0.020</td>
</tr>
<tr>
<td>Dechloromonas</td>
<td>0.072</td>
</tr>
</tbody>
</table>


**Phosphorous**

Lastly, phosphorus removal is represented by phosphate accumulating organisms (PAOs) on the intersection between the oxic/anoxic zone because they require both environments for the metabolism of phosphorus (Figure 6a). Selection for PAOs is important during granulation because they are slow growing and are responsible for the bulk of the phosphorus removal. These bacteria accumulate poly-phosphate intracellularly by using the energy from oxidizing poly hydroxyl alkanoates (PHAs). The cycle continues as PAOs use their stored poly-P and glycogen as energy (Henrie, Meunier, Henry, & Mahillon, 2016). The major competition is from glycogen accumulating organisms since they reduce the concentration of glycogen. This competition can be limited by cycling of aeration and a strict feeding-fasting regime. An effluent total phosphorus reduction of 97.71± 3.63% was seen by He et al., 2006.
Figure 6. Composition and diversity of functional groups. Note. Abundant (A) and rare (B) EPS producing taxa; hydrolyzing taxa (C); nitrifying bacteria (D); abundant (E); and rare (F) denitrifying taxa in the reactors (R1-R3) of different carbon-nitrogen ratios and the Seed Sludge. (Szabó et al., 2017)

REAL WORLD IMPLEMENTATION

The Nereda® process, patented by Royal HaskoningDHV (RHDHV), is the most well-known commercial application of AGS (Khan, Ahmad, & Giesen, 2015). In the operational form of an SBR, the selection for granular sludge has been optimized using many of the aforementioned techniques. Currently, there are 42 municipal treatment plants, six industrial treatment plants, and three pilot/demonstration facilities using the Nereda process (Khan et al., 2015). As a patented technology, there are limitations to AGS’s implementation without a Nereda system, but AGS has successfully been adopted by at least 5 wastewater treatment plants. The granulation process has been, and continues to be, extensively researched because it is the most difficult, yet essential, step. Nereda commercially packages patented specialized equipment with selection factors to ensure for optimal granulation, simplifying this step.
Anaerobic applications of granular sludge technology include two other commercially available patented processes Paques ANAMMOX® and DEMON®. The Paques ANAMMOX® and DEMON heavily utilize anammox (anaerobic ammonium oxidation) bacteria. Paques ANAMMOX® (Figure 8) granule has layered microenvironments similar to AGS and is cultivated using a membrane (Lackner et al., 2014). DEMON is an anaerobic side stream process illustrated in Figure 9. Separated using a hydrocyclone, the anammox granule with ammonium oxidizing bacteria (AOB) perform nitrite shunt or nitritation and deammonification (Innerebner, Insam, Franke-Whittle, & Wett, 2007). Nitrite shunt of nitritation is the metabolic process of oxidizing ammonia to nitrite. The end product of the ammonia oxidation to nitrite followed by deammonification is nitrogen gas (Brickles, 2017). Unlike AGS, both of these systems require additional treatment for phosphorus removal.

AGS is an excellent solution to an up and coming problem of nutrient reduction for retrofitting current plants with limited footprints. With removal efficiencies above 90%, AGS could be the wastewater treatment of the future. However, there is still substantial research that needs to be done. The microbial mechanisms specifically regarding cell-cell interactions are still mainly unknown. The reliability of efficient cultivation is a main concern and is continuing to be studied. Additionally, excess sludge removal and AGS functionality in low strength wastewater is a concern. Overall, AGS is a promising new wastewater treatment technique for the future.

**BioWin Modeling**

In order to effectively illustrate the nutrient reduction potential of aerobic granular sludge, the modeling software BioWin (version 5.3, EnviroSim Associates Ltd.) was used in conjunction with the data from the City of Ames Water Pollution Control Facility (WPCF). To account for seasonal changes in temperature and its effect on the varying input parameters, all four seasons were modeled with data averaged from 5 years.
BioWin, a biological wastewater modeling software program, was designed as a simulation tool that incorporates several different models. The International Water Association (IWA) task group developed the mathematical activated sludge models (ASM1, ASM2, ASM2d, ASM3) to simulate biological wastewater treatment processes (Elawwad, 2017).

These models are well-known and have been used for over 30 years (WEF). There has been extensive research into optimizing ASM models specifically for BNR (Elawwad, 2017). BioWin is a general model based on these highly researched IWA models. Biowin was initially developed by Barker and Dold and currently being marketed by EnviroSim, Hamilton, Canada. As a general simulation program, BioWin offers the ability to model a complex system with a multitude of parameters. BioWin is “evaluated against an extensive data set” (BioWin Manual) and has been enhanced using empirical records.

Figure 10. Four stage granular sludge sequencing tank phases. BioWin manual.
To simulate AGS implementation at the Ames WPCF, BioWin’s Granular Sludge Sequencing Tank (GSST) configuration was selected with two reactors in parallel and an upstream buffer tank (Figure 11). The hydraulic retention time (HRT) was set to 6 hours for both the buffer tank and the SBRs (Table 2). The buffer tank is used to hold influent wastewater until a reactor is in the feeding stage. Dual GSST tanks offer more flexibility in feeding times since the cycles can be staggered. There are four reactor phases that occur in rotation (Figures 10, Table 2): mixing, settling, feeding, and decanting.

Table 2

*GSST Reactor Phase Timeline*

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>GSST 1 Phase</th>
<th>GSST 2 Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Mix</td>
<td>Mix</td>
</tr>
<tr>
<td>0.25</td>
<td>Mix</td>
<td>Mix</td>
</tr>
<tr>
<td>0.5</td>
<td>Mix</td>
<td>Settle</td>
</tr>
<tr>
<td>0.75</td>
<td>Mix</td>
<td>Settle</td>
</tr>
<tr>
<td>1</td>
<td>Mix</td>
<td>Settle/Waste</td>
</tr>
<tr>
<td>1.25</td>
<td>Mix</td>
<td>Settle/Feed</td>
</tr>
<tr>
<td>1.5</td>
<td>Mix</td>
<td>Settle/Feed</td>
</tr>
<tr>
<td>1.75</td>
<td>Mix</td>
<td>Settle/Feed</td>
</tr>
</tbody>
</table>
Since the model was initially calibrated using the large empirical dataset, the next objective was to adjust the model to represent the influent wastewater at WPCF. The first step to optimize BioWin to mimic WPCF wastewater was completed using the “influent specifier.” BioWin uses wastewater fractions that are calculated using the influent specifier, an excel spreadsheet that balances influent wastewater characteristics from the input data. Figures 11, A-1, and A-2 illustrate the influent specifier and the correlated wastewater fractions estimation. The influent data and wastewater fractions were input into BioWin for each season to most appropriately represent WPCF wastewater during that time of year (Appendix A-1, A-2; Table 3).

Table 3

<table>
<thead>
<tr>
<th>Defined Seasons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

14
### Table 4

**Seasonal Data from the AMES WPCF**

<table>
<thead>
<tr>
<th>Influent wastewater characteristic</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Flowrate, MGD</td>
<td>6.7</td>
<td>6.9</td>
<td>7</td>
<td>5.9</td>
</tr>
<tr>
<td>BOD, mg/L</td>
<td>177.4</td>
<td>149</td>
<td>180.1</td>
<td>207.4</td>
</tr>
<tr>
<td>COD, mg/L</td>
<td>446.7</td>
<td>335</td>
<td>373.3</td>
<td>435.7</td>
</tr>
<tr>
<td>Total Nitrogen Kjeldahl, mg/L</td>
<td>38.5</td>
<td>28</td>
<td>33.3</td>
<td>44</td>
</tr>
<tr>
<td>Total Phosphorus, mg/L</td>
<td>4.6</td>
<td>3.6</td>
<td>4.4</td>
<td>5</td>
</tr>
<tr>
<td>Nitrate, mg/L</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>pH, s.u.</td>
<td>7.9</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Alkalinity, mg/L</td>
<td>271</td>
<td>257.1</td>
<td>293</td>
<td>270.3</td>
</tr>
<tr>
<td>Total suspended solids – TSS, mg/L</td>
<td>208.8</td>
<td>201.7</td>
<td>212.2</td>
<td>231.1</td>
</tr>
<tr>
<td>Volatile suspended solids, mg/L</td>
<td>202</td>
<td>202.9</td>
<td>162.8</td>
<td>182.7</td>
</tr>
<tr>
<td>Calcium, mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium, mg/L</td>
<td>15</td>
<td>18.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved Oxygen, mg/L</td>
<td>6</td>
<td>4.4</td>
<td>4.2</td>
<td>6.8</td>
</tr>
</tbody>
</table>
The buffer tank outflow flow rate was adjusted based on the average seasonal influent flow. Without this step the wastewater could overflow from the buffer tank and/or reactor, which would diminish treatment efficiency because the wastewater would be moving through too fast to be treated.

The next step was to determine the most efficient aeration schedule for nutrient reduction. Figures 13, 14, 15, and 16 exhibit the varying schedules that were simulated and their corresponding effluent TN and TP concentrations. A simulation time of 14 days was suggested to produce steady-state results (BioWin AGS Simulation Notes). In comparison to a 40 day simulation, 14 days was shown to be sufficient and produce constant results. Dissolved oxygen concentrations varied from 0.15 mg/L to 2.0 mg/L depending on the season (Appendix B-1). A range of DO concentrations were run with aeration start times of 30 minutes and 45 minutes to determine the highest nutrient reduction potential. An aeration start time of 30 minutes equates to 3 hours of aeration time and similarly, a start time of 45 minutes corresponds to 2 hours and 45 minutes of aeration. Nitrification is temperature sensitive; therefore, a longer aeration time with a higher DO concentration would be expected to be more efficient in the winter and vice versa for summer.

**Simulation Results and Discussion**

According to the latest revision of the Iowa Nutrient Reduction Strategy (Iowa Department of Natural Resources, 2017), section 3 on “Point Source Nutrient Reduction Technology Assessment,” IDNR will likely set permit requirements for nutrient reduction equal to or less than 10 mg/L TN and 1 mg/L TP. The simulation results determined using seasonal data from the City of Ames WPCF and BioWin fell below these hypothetical discharge limits using optimized aeration schedules.

---

**COD Influent data**

<table>
<thead>
<tr>
<th>Name</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow</td>
<td>6.7</td>
</tr>
<tr>
<td>Total COD mg/L</td>
<td>446.7</td>
</tr>
<tr>
<td>Total Kjeldahl Nitrogen mgN/L</td>
<td>38.5</td>
</tr>
<tr>
<td>Total P mgP/L</td>
<td>4.6</td>
</tr>
<tr>
<td>Nitrate N mgN/L</td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>7.9</td>
</tr>
<tr>
<td>Alkalinity mmol/L</td>
<td>5.42</td>
</tr>
<tr>
<td>Inorganic S.S. mgTSS/L</td>
<td>31.4</td>
</tr>
<tr>
<td>Calcium mg/L</td>
<td>220</td>
</tr>
<tr>
<td>Magnesium mg/L</td>
<td>15</td>
</tr>
<tr>
<td>Dissolved oxygen mg/L</td>
<td>0</td>
</tr>
</tbody>
</table>

*Figure 12. Influent specifier—COD influent data*
Figure 13. Effluent ammonia.

Figure 14. Effluent nitrite and nitrate.

Figure 15. Effluent total nitrogen.
Figure 16. Effluent total phosphorous.

As previously mentioned, nitrification is temperature sensitive and the simulation results supported an aeration schedule dependent on season. All of the seasons showed a favorable dissolved oxygen concentration that correlated with temperature. For the spring and fall seasons, the most favorable DO concentration was 1.0 mg/L (Figures 13, 14, 15, 16, 17, and 19). While the spring season showed better nitrification with a longer aeration, the fall season showed more TN reduction with a shorter aeration time (Figures 13, 14, 15, 16, 17, and 19). For spring, the impact of a decrease in aeration time correlated to the decrease in nitrification is shown by an increase in effluent NH₃ and decrease in effluent NOₓ (Figure 16, Appendix B-1). Specifically, an increase in ammonia is seen in the spring simulation runs from 1.2 mg/L for 30 minutes aeration start time to 3.24 mg/L for 45 minutes and a decrease of effluent NOₓ from 5.69 mg/L to 2.81 mg/L (Appendix B-1). Both of these seasons had comparable, mild temperatures and correlating TN and TP concentrations. The window for simulated effluent nitrogen concentrations below the proposed discharge limit of 10 mg/L was limited but manageable.
Figure 17. Spring simulation nutrient removal optimization.

Figure 18. Summer simulation nutrient removal optimization.
The summer season showed the most efficient nutrient removal, this was likely due to higher temperatures and therefore higher biochemical reactions, such as growth and microbial metabolisms. The summer simulations favored a shorter aeration time, and a lower DO concentration (Figures 18). The seasonal data simulated the lowest TN with sufficient nitrification and phosphorus reduction at a DO concentration of 0.25 mg/L (Figure 15 and 18; Appendix B-1). As DO concentration increased from 0.25 mg/L to 2 mg/L effluent ammonia decreased, and effluent NOx increased representing nitrification efficiency (Figures 18).

**Figure 19.** Fall simulation nutrient removal optimization.

**Figure 20.** Winter simulation nutrient removal optimization.
The winter season showed favorable nutrient removal with longer aeration time and higher DO concentrations (Figure 20). The nutrient removal peaked with a DO concentration of 1.75 mg/L for 3 hours but was still slightly above the hypothetical limit of 10 mg/L TN with a nitrogen concentration of 11.18 mg/L (Appendix B-1). Notably, at lower DO levels the effluent NH₃ is significantly lower for an aeration start time of 30 minutes versus 45 minutes (Figure 13).

Effluent total phosphorus concentrations were steadily below 1.0 mg/L for all seasonal simulations (Figure 16). As lower temperatures are expected to decrease the rate of “biochemical transformations” (Gurtekin et al., 2014), a high TP concentration during winter would have been expected but was not seen. This could be due to reduced competition from GAĜs. Phosphorus concentrations were stable for most of the simulations with summer having the highest reduction and spring having the lowest. Interestingly, at low DO concentrations the fall season showed a spike in effluent TP (Figure 16). This could be explained by insufficient oxygen for polyphosphate uptake from PAĜs.

Table 5

**Effluent Nutrient Concentrations and Percent Removal**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Parameter</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>Current Effluent Concentration, mg/L</td>
<td>23.5</td>
<td>18.3</td>
<td>25.5</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td>Simulated Effluent, mg/L</td>
<td>6.05</td>
<td>2.06</td>
<td>7.83</td>
<td>11.18</td>
</tr>
<tr>
<td></td>
<td>Current Percent Removal</td>
<td>39%</td>
<td>35%</td>
<td>23%</td>
<td>37%</td>
</tr>
<tr>
<td></td>
<td>Simulated Percent Removal</td>
<td>74%</td>
<td>89%</td>
<td>69%</td>
<td>69%</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Current Effluent Concentration, mg/L</td>
<td>3.7</td>
<td>2.9</td>
<td>3.6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Simulated Effluent, mg/L</td>
<td>0.54</td>
<td>0.39</td>
<td>0.40</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Current Percent Removal</td>
<td>20%</td>
<td>19%</td>
<td>18%</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>Simulated Percent Removal</td>
<td>85%</td>
<td>87%</td>
<td>89%</td>
<td>88%</td>
</tr>
</tbody>
</table>

The hydraulic retention time (HRT) for the BioWin AGS simulation of 6 hours was similar to the HRT for the Ames WPCF’s intermediate clarifiers, ~5.6 hours. However, in accounting for the additional processes, the Ames WPCF has a much higher HRT. Overall, the BioWin AGS simulation, including the buffer tank has a total HRT of 12 hours. In comparison, the primary clarifiers, solids contact, and final clarifiers, have HRTs of ~4 hours, ~5.6 hours and ~5.6 hours, respectively totally
~15.2 hours and not including the trickling filters. This time savings would allow for larger loading capacity which would be beneficial for urban population growth and potential industrial treatment increases.

To implement AGS at the Ames WPCF it would be most cost effective to retrofit the current plant. The current footprint is adequate for the current capacity with ample room to grow. Using AGS as a treatment option allows flexibility for growth because of the simplicity of adding one additional tank.

The nutrient removal simulated using raw data from the City of Ames WPCF and the AGS BioWin configuration provided sufficient nutrient removal to comply with prospective permit levels. As seen in Table 5, the total nitrogen removal would increase from an average of 33% to 73%. Likewise, the total phosphorus removal would increase from 19% to 87%. Simulated phosphorus effluent concentrations results from all seasons were below the hypothetical discharge limits of 1 mg/L TP from the nutrient reduction strategy (Iowa Department of Natural Resources, 2017). Nitrogen concentrations were below 10 mg/L for spring, summer, and fall using fine-tuned aeration schedules and the winter season was very close to being below the limit at ~ 11 mg/L. Overall, AGS has shown to be a compelling option for nutrient reduction that would be suitable for the City of Ames WPCF.
REFERENCES


Iowa Department of Natural Resources. (2017). Section 3 — Point Source Nutrient Reduction Technology Assessment and Implementation Plan, 1–12.


APPENDIX

APPENDIX A. MODEL SETUP

<table>
<thead>
<tr>
<th>Influent COD fractions</th>
<th>Default</th>
<th>Estimate</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fbs</td>
<td>0.160</td>
<td>0.319</td>
<td>from Step 1</td>
</tr>
<tr>
<td>Fus</td>
<td>0.050</td>
<td>0.075</td>
<td>from Step 1</td>
</tr>
<tr>
<td>Fup</td>
<td>0.130</td>
<td>0.299</td>
<td>affects BOD, VSS</td>
</tr>
<tr>
<td>Fzbh</td>
<td>0.000</td>
<td>0.030</td>
<td>from separate method</td>
</tr>
<tr>
<td>Fss</td>
<td>0.680</td>
<td>0.277</td>
<td>by difference (must be &gt; 0)</td>
</tr>
<tr>
<td>Fxsp</td>
<td>0.750</td>
<td>0.833</td>
<td>affects VSS, scale: 0 to 1</td>
</tr>
</tbody>
</table>

GUIDE
- Change COD fractions (BOLD)
- until match is achieved

Suggestion:
Inhibited cBOD5 = 0.84 x “true” cBOD5

<table>
<thead>
<tr>
<th>Influent values</th>
<th>Measured (From Step 1)</th>
<th>Calculated (Based on fractions above)</th>
<th>Match Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>CODI</td>
<td>447</td>
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</tr>
<tr>
<td>Soluble COD (GFC)</td>
<td>197</td>
<td>197</td>
<td>Excellent</td>
</tr>
<tr>
<td>FF COD</td>
<td>176</td>
<td>176</td>
<td>Excellent</td>
</tr>
<tr>
<td>dCOD</td>
<td>177</td>
<td>177</td>
<td>Excellent</td>
</tr>
<tr>
<td>tCOD (GFC)</td>
<td>115</td>
<td>115</td>
<td>Excellent</td>
</tr>
<tr>
<td>VSS</td>
<td>178</td>
<td>178</td>
<td>Excellent</td>
</tr>
<tr>
<td>TSS</td>
<td>209</td>
<td>209</td>
<td>Excellent</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Important fractions</th>
<th>(can be used as a check)</th>
<th>Fraction</th>
<th>Value</th>
<th>Typical range</th>
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<tr>
<td>COD/cBOD5</td>
<td></td>
<td>2.52</td>
<td>1.9-2.2</td>
<td></td>
</tr>
<tr>
<td>Sol. COD fraction</td>
<td></td>
<td>0.44</td>
<td>0.3-0.5</td>
<td></td>
</tr>
<tr>
<td>VSS/TSS</td>
<td></td>
<td>0.85</td>
<td>0.75-0.85</td>
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</tr>
</tbody>
</table>

FIND Fup & Fxsp
0.01237727025

<table>
<thead>
<tr>
<th>Calculated concentrations (from COD &amp; fractions)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sxs</td>
<td>34</td>
</tr>
<tr>
<td>Xi</td>
<td>133</td>
</tr>
<tr>
<td>Sbs</td>
<td>143</td>
</tr>
<tr>
<td>Xs (g/l)</td>
<td>124</td>
</tr>
<tr>
<td>Zbh</td>
<td>13</td>
</tr>
<tr>
<td>Xsc</td>
<td>21</td>
</tr>
<tr>
<td>Xsp</td>
<td>103</td>
</tr>
</tbody>
</table>

COD/VSS Ratio 1.40

Figure A-1. Influent specifier—Wastewater fraction calculations.

<table>
<thead>
<tr>
<th>Name</th>
<th>Raw defaults</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fbs - Readily biodegradable (including Acetate)</td>
<td>gCOD/g of total COD</td>
<td>0.16</td>
</tr>
<tr>
<td>Fac - Acetate</td>
<td>gCOD/g of readily biodegradable COD</td>
<td>0.15</td>
</tr>
<tr>
<td>Fxsp - Non-colloidal slowly biodegradable</td>
<td>gCOD/g of slowly degradable COD</td>
<td>0.75</td>
</tr>
<tr>
<td>Fus - Unbiodegradable soluble</td>
<td>gCOD/g of total COD</td>
<td>0.05</td>
</tr>
<tr>
<td>Fup - Unbiodegradable particulate</td>
<td>gCOD/g of total COD</td>
<td>0.13</td>
</tr>
<tr>
<td>Fna - Ammonia</td>
<td>gNH3-N/gTKN</td>
<td>0.86</td>
</tr>
<tr>
<td>Fnox - Particulate organic nitrogen</td>
<td>gN/g Organic N</td>
<td>0.5</td>
</tr>
<tr>
<td>Fnus - Soluble unbiodegradable TKN</td>
<td>gN/gTKN</td>
<td>0.02</td>
</tr>
<tr>
<td>FupN - N:COD ratio for unbiodegradable part. COD</td>
<td>gN/gCOD</td>
<td>0.035</td>
</tr>
<tr>
<td>Fpo4 - Phosphate</td>
<td>gPO4-P/gTP</td>
<td>0.5</td>
</tr>
<tr>
<td>FupP - P:COD ratio for influent unbiodegradable part. COD</td>
<td>gP/gCOD</td>
<td>0.011</td>
</tr>
<tr>
<td>FZbh - OHO COD fraction</td>
<td>gCOD/g of total COD</td>
<td>0.02</td>
</tr>
<tr>
<td>FZbh - Non-poly-P heterotrophs</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>FZbm - Anoxic methanolutilizers</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>FZaoa - Ammonia oxidizers</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>FZnob - Nitrite oxidizers</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>FZamo - Anaerobic ammonia oxidizers</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>FZbp - PAOs</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>FZbp - Propionic acid gases</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>FZbmn - Acetoclastic methanogens</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
<tr>
<td>FZbhm - H2-utilizing methanogens</td>
<td>gCOD/g of total COD</td>
<td>1.00E-04</td>
</tr>
</tbody>
</table>

Figure A-2. Influent specifier—wastewater fractions.
### APPENDIX B. SIMULATION DATA

Table A-1. Seasonal BioWin simulations and their effluent concentrations by aeration schedule.

<table>
<thead>
<tr>
<th>Simulation run time, days</th>
<th>Spring</th>
<th>GSST #1</th>
<th>GSST #2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO Concentration, mg/L</td>
<td>time, min</td>
<td>Effluent NH₃, mg/L</td>
<td>Effluent NOₓ, mg/L</td>
<td>Effluent TP, mg/L</td>
</tr>
<tr>
<td>14 0.5 30</td>
<td>9.5 0.21 0.32 9.5 0.21 0.3</td>
<td>19 0.42 19.42 0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 1 30</td>
<td>0.6 2.88 0.28 0.6 2.81 0.26</td>
<td>1.2 5.69 6.89 0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 1.5 30</td>
<td>0.13 6.11 0.28 0.12 6.03 0.26</td>
<td>0.25 12.14 12.39 0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 2 30</td>
<td>0.11 8.52 0.27 0.11 8.44 0.26</td>
<td>0.22 16.96 17.18 0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 0.5 45</td>
<td>11.6 0.14 0.33 11.6 0.15 0.31</td>
<td>23.2 0.29 23.49 0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 1 45</td>
<td>1.62 1.4 0.28 1.62 1.41 0.26</td>
<td>3.24 2.81 6.05 0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 1.5 45</td>
<td>0.18 4.88 0.27 0.18 4.81 0.26</td>
<td>0.36 9.69 10.05 0.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 2 45</td>
<td>0.17 6.9 0.27 0.11 6.89 0.26</td>
<td>0.28 13.79 14.07 0.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Summer</th>
<th>GSST #1</th>
<th>GSST #2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO Concentration, mg/L</td>
<td>time, min</td>
<td>Effluent NH₃, mg/L</td>
<td>Effluent NOₓ, mg/L</td>
</tr>
<tr>
<td>14 0.25 30</td>
<td>0.15 0.87 0.2</td>
<td>0.18 0.86 0.19</td>
<td>0.33 1.73 2.06 0.39</td>
</tr>
<tr>
<td>14 0.5 30</td>
<td>0.07 3.14 0.2</td>
<td>0.07 3.06 0.18</td>
<td>0.14 6.2 6.34 0.38</td>
</tr>
<tr>
<td>14 1 30</td>
<td>0.06 7 0</td>
<td>0.06 6.96 0.18</td>
<td>0.12 13.96 14.08 0.38</td>
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<tr>
<td>14 1.5 30</td>
<td>0.05 8.6 0.19</td>
<td>0.05 8.58 0.17</td>
<td>0.1 17.18 17.28 0.36</td>
</tr>
<tr>
<td>14 0.25 45</td>
<td>0.31 0.99 0.2</td>
<td>0.43 0.97 0.18</td>
<td>0.74 1.96 2.7 0.38</td>
</tr>
<tr>
<td>14 0.5 45</td>
<td>0.1 3.4 0.17</td>
<td>0.1 3.5 0.19</td>
<td>0.2 6.9 7.1 0.36</td>
</tr>
<tr>
<td>14 1 45</td>
<td>0.08 5.97 0.18</td>
<td>0.08 5.92 0.17</td>
<td>0.16 11.89 12.05 0.35</td>
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<tr>
<td>14 1.5 45</td>
<td>0.07 7.4 0.2</td>
<td>0.07 7.3 0.19</td>
<td>0.14 14.7 14.84 0.39</td>
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</table>

<table>
<thead>
<tr>
<th>Fall</th>
<th>GSST #1</th>
<th>GSST #2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO Concentration, mg/L</td>
<td>time, min</td>
<td>Effluent NH₃, mg/L</td>
<td>Effluent NOₓ, mg/L</td>
</tr>
<tr>
<td>14 0.25 30</td>
<td>13.2 0.03 0.35</td>
<td>13.5 0.03 0.32</td>
<td>26.7 0.06 26.76 0.67</td>
</tr>
<tr>
<td>14 0.5 30</td>
<td>3.24 0.77 0.24</td>
<td>3.21 0.72 0.22</td>
<td>6.45 1.49 7.94 0.46</td>
</tr>
<tr>
<td>14 1 30</td>
<td>0.12 4.43 0.21</td>
<td>0.12 4.34 0.2</td>
<td>0.24 8.77 9.01 0.41</td>
</tr>
<tr>
<td>14 1.5 30</td>
<td>0.11 7.8 0.21</td>
<td>0.1 7.7 0.2</td>
<td>0.21 15.5 15.71 0.41</td>
</tr>
<tr>
<td>14 2 30</td>
<td>0.1 9.82 0.21</td>
<td>0.1 9.75 0.2</td>
<td>0.2 19.57 19.77 0.41</td>
</tr>
<tr>
<td>14 0.25 45</td>
<td>14.6 0.02 0.37</td>
<td>14.8 0.02 0.34</td>
<td>29.4 0.04 29.44 0.71</td>
</tr>
<tr>
<td>14 0.5 45</td>
<td>5.54 0.54 0.24</td>
<td>5.64 0.51 0.23</td>
<td>11.18 1.05 12.23 0.47</td>
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<tr>
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<td>0.18 3.77 0.21</td>
<td>0.18 3.7 0.19</td>
<td>0.36 7.47 7.83 0.4</td>
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<tr>
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<td>0.11 6.38 0.21</td>
<td>0.1 6.3 0.19</td>
<td>0.21 12.68 12.89 0.4</td>
</tr>
<tr>
<td>14 2 45</td>
<td>0.1 8.3 0.2</td>
<td>0.1 8.23 0.19</td>
<td>0.2 16.53 16.73 0.39</td>
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</table>

<table>
<thead>
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<th>Winter</th>
<th>GSST #1</th>
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<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DO Concentration, mg/L</td>
<td>time, min</td>
<td>Effluent NH₃, mg/L</td>
<td>Effluent NOₓ, mg/L</td>
</tr>
<tr>
<td>14 1 30</td>
<td>9.59 0.34 0.27</td>
<td>9.48 0.34 0.26</td>
<td>19.07 0.68 19.75 0.53</td>
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<td>3.29 2.5 0.24</td>
<td>6.66 5.1 11.76 0.5</td>
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<td>1.64 3.9 0.24</td>
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<tr>
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<td>1.69 11.1 12.79 0.5</td>
</tr>
<tr>
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<td>11.8 0.21 0.28</td>
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<tr>
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<td>5.8 1.88 0.25</td>
<td>5.69 1.85 0.24</td>
<td>11.49 3.73 15.22 0.49</td>
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<tr>
<td>14 2 45</td>
<td>2.02 4.27 0.25</td>
<td>1.95 4.22 0.24</td>
<td>3.97 8.49 12.46 0.49</td>
</tr>
<tr>
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<td>0.69 6.22 0.25</td>
<td>0.66 6.17 0.24</td>
<td>1.35 12.39 13.74 0.49</td>
</tr>
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