Cofermenting Algal Biomass with Municipal Primary Solids to Enhance Carboxylate Production

Taylor Romenesko[1], Erik R. Coats[2†*]

ABSTRACT: As water resource recovery facilities (WRRFs) implement biological nutrient removal (BNR) processes to remove excess wastewater nutrients, carboxylic acid demands increase; resource recovery processes (e.g., struvite, polyhydroxyalkanoate production) also demand carboxylates. In this regard, interest in algae to achieve tertiary treatment creates a new intraWRRF fermentation substrate. Indeed, fermentation potential tests indicated that algal augmentation could prove beneficial; carboxylate concentrations increased 31 % over primary solids. However, unexpectedly, and disproving a key research hypothesis, algal augmentation in a fed-batch fermenter decreased the production of carboxylic acids (26–34% at SRTs of 5–7 d); preliminary analyses suggest heterotrophic algae consumed carboxylates. Disproving a second research hypothesis, algal biomass did not significantly diversify carboxylate speciation. Finally, and unexpectedly, algal fermentation realized significant ammonia removal (39–96 % at SRTs of 5–7 d). Although decreased carboxylate yield is not desired, reduced ammonia load could potentially decrease WRRF energy demands and decrease carboxylic acid demands to achieve denitrification. *Water Environ. Res.*, **90**, 1997 (2018).

KEYWORDS: carboxylates, carboxylic acids, fermentation, heterotrophic algae, municipal primary solids.

doi:10.2175/106143017X15131012188105

Introduction

Municipal water resource recovery facilities (WRRFs) are increasingly being tasked with removing excess quantities of nitrogen (N) and phosphorus (P) present in wastewater in order to protect the environmental integrity of surface and ground waters. Excess quantities of ammonia-nitrogen and phosphorus in WRRF effluent can accelerate surface water body eutrophication; moreover, nitrate/nitrite (NO_x) is a drinking water regulated contaminant, and can also be utilized by certain algal species, thus contributing to eutrophication. In response to intensified nitrogen and phosphorus permit standards, WRRFs often implement biological nutrient removal (BNR) processes, including the enhanced biological phosphorus removal (EBPR) process, targeting removal of NH_3, NO_x, and phosphorus. However, implementing BNR/EBPR processes demands readily biodegradable carbon (specifically carboxylates for EBPR) - for both NO_x reduction and excess phosphorus accumulation (Grady Jr. et al., 2011; Yuan and Oleszkiewicz, 2010). Commensurate with this carbon demand, and recognizing that many municipal wastewaters are carbon limited, WRRFs are adopting fermentation processes to generate carboxylic acids (acetic acid and volatile fatty acids (VFAs; 3C–6C carboxylates)) to improve BNR/EBPR process performance (Burow et al., 2008).

Beyond the need to drive BNR/EBPR, increased quantities of carboxylic acids can enhance resource recovery opportunities in a WRRF. With the scarcity of virgin materials becoming more apparent every year (Hinsinger et al., 2011), there is an increased drive to produce nutrients in new forms (i.e., recycling or resource recovery of nitrogen and phosphorus). In this regard, wastewater nutrients can be recovered as struvite, a naturally occurring crystal; however, the recovery of struvite can be limited by the availability of carboxylic acids, which help drive the release of phosphorus from EBPR biomass (Cullen et al., 2013). Additionally, the mixed microbial culture used to perform EBPR (i.e., phosphorus- and glycogen-accumulating organisms) can potentially be used to store carboxylates in the form of polyhydroxyalkanoates, a biodegradable plastic and valuable commodity that can be produced onsite at WRRFs (Coats et al., 2007; Probst, 2016). Here, again, polyhydroxyalkanoates production vies for the limited amount of carboxylic acids contained within the influent wastewater.

Considering the expanding demand for carboxylates associated with diversifying the WRRF footprint, and the cost to import substrate, some means to maximize onsite production

[1] Carollo Engineers, Inc., Boise, ID, U.S.A. (at the time of the research, was a graduate student in the Department of Civil and Environmental Engineering, University of Idaho, Moscow, ID).

[2] Department of Civil and Environmental Engineering, University of Idaho, Moscow, ID, U.S.A. 83844-1022.

[*] Department of Civil and Environmental Engineering, University of Idaho, Moscow, ID, U.S.A. 83844-1022; e-mail: ecoats@uidaho.edu.

[†] WEF member

are needed. Organic matter fermentation is the primary method currently employed for producing carboxylic acids in WRRFs, and historically organic matter fermentation has focused on primary solids as a substrate (Bouzas et al., 2007). Fermentation of return activated sludge (RAS) has been suggested as an alternative substrate (Tooker et al., 2017), although recent research raised concerns about this practice (Coats et al., 2018 in press). However, growing interest in the use of algae to achieve tertiary treatment of secondary effluent creates a potentially new fermentation substrate (Li et al., 2013). Indeed, the algae might serve multiple purposes: capturing nutrients contained within secondary effluent (Ji et al., 2013), fixing carbon dioxide from the atmosphere (or more ideally, from combined heat and power (CHP) exhaust) to reduce the overall WRRF carbon footprint (Cai et al., 2013; Razzak et al., 2013), and providing another internal fermentation substrate. The growth of algae would provide carbon from an external source (i.e., CO_2 from the atmosphere or anaerobic digester biogas (Cai et al., 2013)), with the algal biomass blended with primary solids and fermented to increase carboxylate production.

Whilst algae is being recognized as a potentially value-producing substrate within a WRRF, investigations into alternative uses for algal biomass focus almost exclusively on refinement of the conversion of lipid-rich algal biomass to biogas, with some research into the potential of the biomass to be used as a fertilizer or feedstock (Lowrey et al., 2015; Madeira et al., 2017; Markou and Nerantzis, 2013). Indeed, there is a gap in knowledge as to the effects a fermented blend of algal biomass and primary solids could have on enhanced carboxylic acid production within a WRRF. Moreover, consideration must be given to carboxylate gains versus potentially adverse effects on WRRF operations. Specifically, there are legitimate concerns about algal fermentation resulting in increased nitrogen and phosphorus loads to the WRRF - that is, internally recycling nutrients that were captured in the algae but released via fermentation.

Recognizing the potentially valuable gains from fermenting intraWRRF produced algal biomass - improved EBPR operations and enhanced resource recovery - research was undertaken to investigate how supplementing algal biomass to a primary solids fermenter might affect production and speciation of carboxylates, soluble nutrient concentrations, and reactor pH. Investigations were driven by the hypothesis that augmenting a primary solids fermenter with algal biomass will both increase carboxylic acid production and diversify carboxylate speciation. Batch fermentation potential tests were first conducted to establish the potential effect of reactor retention time on carboxylic acid production. Subsequently, fed-batch studies were conducted to compare and contrast primary solids versus blended primary solids/algal biomass fermentation, with a focus on carboxylate production and nitrogen/phosphorus production. Bench-scale sequencing batch reactors (SBRs) were operated at primary solids-algal loads designed to emulate conditions that could be realized at full-scale WRRFs. All investigations were performed using real wastewater and algal biomass produced on WRRF effluent.

Methodology

Substrate. Gravity thickened primary solids were collected regularly from the Pullman, WA, WRRF. Pullman operates a Modified Ludzak-Ettinger-based secondary treatment system, with primary clarifiers. The substrate was tested in quadruplicate for volatile and total solids and stored at 4 °C until use. Total solids content in the primary solids ranged from 2.5 to 4.5% (w/w), with volatile solids content ranging from 83 to 89% (w/w). Algal biomass was provided by Clearas Water Recovery in Missoula, MT; according to Clearas, the algal biomass principally consisted of *Scenedesmus* and *Chlorella*. The algal biomass was grown on secondary effluent from the city of Missoula WRRF. Algal biomass was aliquoted into individual bottles for daily feeding to the fed-batch SBR fermenter and frozen at -20 °C. A series of fermentation potential tests were performed to analyze the effect on carboxylate production of freezing the algal biomass (discussed herein).

Experimental Setup. *Fermentation Potential Tests*. Fermentation potential tests were performed in 500 mL screw-top Wheaton glass bottles covered with aluminum foil to prevent light penetration (and minimize phototrophic algae growth). The beakers were capped, and an air lock was applied to maintain 2.54 to 5.08 cm (1–2 inches) of water pressure to prevent oxygen entrainment into the headspace of the fermenters. Each fermentation potential test was conducted with an organic load of 10 g volatile solids and filled to 500 mL with tap water; the substrate served as the inocula. Tests were conducted in triplicate, and three substrates were compared: a fresh (never frozen) algae and primary solids blend (44% algae, 56% primary solids; on a volatile solids weight basis), a frozen algae and primary solids blend (same loading), and a control reactor of only primary solids. Beakers were placed on a New Brunswick Scientific Co. model G-25 shaker table (Edison, NJ, U.S.A.), operated to achieve mixing of the beaker contents; the investigation was conducted at room temperature (21.5–23.5 °C). Samples were collected at the same time each day for carboxylic acid and pH analysis. In total, the fermentation potential tests were conducted for a 10 d period. Results from the fermentation potential tests were analyzed statistically using an equal sample size, equal variance t-test; for each statistical data set, the null hypothesis was that the two data sets' averages were equal.

Bench-Scale Fermenters. Two bench-top fermenters were operated for a period of 230 d, at room temperature (23.5 ± 1.1 °C). Both fermenters were inoculated from an existing bench-scale fermenter that was principally used to support historical and ongoing EBPR research (e.g., Coats et al., 2011b, 2011c, 2015, 2017; Winkler et al., 2011); the original fermenter had been inoculated with anaerobic digester biomass obtained from the Pullman, WA WRRF. Control fermenter MF1, which received only primary solids, was operated at an organic loading rate (OLR) of 2.25 g volatile solids/L•day and at a 15 L working volume. The 6 L working volume algal-primary solids fermenter, identified as fermenter MFA, was operated at an OLR of 2.50 g volatile solids/L•day, receiving a 10%:90% algae

and primary solids blend (volatile solids weight basis). The proportion of OLR associated with the substrate added to each fermenter was based on theoretical estimates on production of algae and primary solids at a full-scale WRRF; the goal was to load each fermenter in a manner that aligned well with a real WRRF operation. Moreover, the OLR was generally comparable to that applied in the fermentation potential tests (averaged OLR of 2 g volatile solids/L•day). The mass of primary solids added to the fermenters was adjusted based on the substrate total solids and volatile solids content, to maintain the target OLR. The fermenters were operated as fed-batch SBRs on a 24-h cycle. Each batch of substrate was prepared daily by measuring the appropriate mass of primary solids, based on the design OLR, with tap water added to achieve the target feed volume. Solids residence time/hydraulic retention time (SRT/HRT) was maintained by daily wastage of a fixed volume of reactor contents; each fermenter was fed immediately after daily waste was removed. Fermentation investigations were conducted at three SRTs: 5 (for 74 d), 6 (for 56 d), and 7 (for 56 d) d. Fermenter MFA was mixed with an axial flow impeller, whereas fermenter MF1 was mixed using a 9.53 cm (3.75 inch) diameter helical impeller; both impellers were driven by an Oriental Motor (San Jose, CA, U.S.A.) USM315-401W 15 W AC speed control motor connected to a 3GN35SA reduction gearbox operated at a speed sufficient to provide uniform mixing of the reactor contents. Fermenter influent and effluent was regularly monitored for NH_3-N, PO_4-P, carboxylates, pH, and total solids/volatile solids content. Carboxylate and nutrient results were comparatively analyzed statistically using the Welch's t-test (equal or unequal sample size with unequal variance); for each statistical data set, the null hypothesis was that the two data sets' averages were equal.

Analytical Techniques. Samples were monitored for soluble reactive phosphorus, ammonia (NH_3), carboxylates, total solids, and volatile solids as described in Coats et al. (2015). Measurement of pH was accomplished with a Thermo-Fisher Scientific Accumet AP85 Waterproof pH/Conductivity Meter. Dissolved oxygen measurements were collected using a Hach HQ30d Meter with a LDO101 dissolved oxygen probe. Three-day composite samples of the influent and effluent of both fermenters were collected and shipped to the Dairy One Forage Laboratory (Ithaca, New York, U.S.A.) for quantification of acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), starch, crude fat, total phosphorus, and crude protein. The methods and equipment used by the Dairy One laboratory are available at http://dairyone.com. The results of the Dairy One analysis were used to estimate the total carbohydrate content (NDF + starch).

Results and Discussion

The principal purpose of this study was to establish the potential value-add of algal biomass fermentation on carboxylate production within a municipal WRRF; the concept has received minimal research attention, but exhibits potentially significant benefits to WRRF operations. It was hypothesized that a relatively significant increase in carboxylate production would be realized as a result of increased fermentable carbon associated with algal biomass being recycled to the fermenter; additionally, with algal biomass potentially being more lipid rich compared with primary solids, it was also hypothesized that more VFAs (vs. just acetic acid) would be produced. Fermentation potential tests were first undertaken to assess carboxylic acid production potential and guide fed-batch reactor design; subsequently, extended duration fed-batch fermentation studies were performed.

Establishing Algal Biomass Fermentation Potential. Although it has been demonstrated that algal biomass can enhance carboxylate production via fermentation when integrated with organic substrates (Smith et al., 2016), the specific potential in a municipal WRRF environment commingled with municipal primary solids remains undefined; moreover, carboxylate production potential from algae grown on WRRF effluent has not been considered. Investigations were conducted to examine the fermentation potential of algal biomass produced on real WRRF secondary effluent; the aim of the fermentation potential tests was to examine production potential relative to primary solids alone as well to contrast fresh versus frozen algae, while also generating data to inform bench-scale design and operational criteria. Regarding the fresh versus frozen algae comparison, previous research has suggested that freezing can increase soluble COD and thus the availability of potentially readily biodegradable substrate for conversion to carboxylates (Smith et al., 2016); Ward et al. (2014) similarly noted that freezing might increase algal biomass biodegradability (albeit related to anaerobic digestion). Although it is not anticipated that frozen algae might be used in a full-scale WRRF application, the source of algal biomass used in this study was not in near proximity, and thus sufficient quantities of algal biomass were obtained to complete the bench-scale investigations. Considering the duration of the bench-scale studies and the potential for biomass degradation when stored at 4 °C, the decision was made to freeze the algal biomass, thereby preserving its viability over the length of the study (Tsubu, 1973). The primary purpose of the fresh-frozen comparison was to elucidate potential differences in carboxylate synthesis. Two metrics were used to evaluate fermentation potential: carboxylate yield (calculated and expressed as mg VFA (as COD) per mg volatile solids; for ease of presentation, VFAs included acetic acid) and carboxylate speciation.

Conventionally fermentation potential tests are performed in sealed vessels (e.g., crimp topped) and under pressurized conditions (Gungor et al., 2009; Lie and Welander, 1997). However, such conditions are not representative of conditions realized in full-scale fermentation reactors, which are typically under minimum pressure, and thus can generate misleading results. Specifically, by overpressurizing the fermentation reactor, resultant reactor partial pressures can lead to hydrogen gas accumulation in bulk solution and cessation of acetogenic reactions, commensurate with an accumulation of VFAs. Consequently, data obtained from pressurized fermentation potential tests can be misleading as to the speciation of carboxylic acids generated in the fermentation of organic

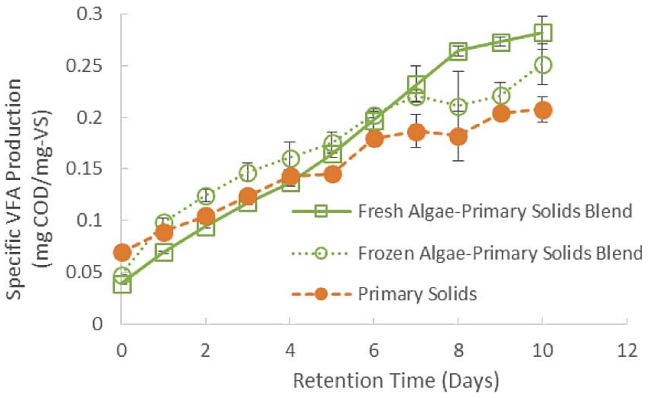

Figure 1—Specific VFA production results for fermentation potential tests.

matter. Indeed, preliminary research employing pressurized vessels (data not shown) revealed an excess accumulation of VFAs, over acetic acid, that would not typically be realized in reactors operated at near-atmospheric pressure conditions. To best replicate real-world fermentation conditions, a nominal pressure was applied to the fermentation potential vessels.

Effect of Retention Time on Carboxylate Production Potential. Firstly, considering specific carboxylate yield, as shown in Figure 1, up to a retention time of approximately 6 d, all substrates (fresh algae/primary solids blend, frozen algae/primary solids blend, and primary solids) exhibited similar fermentation potential (0.18–0.20 mg VFA_{COD}/mg volatile solids). However, between a retention time of 6 and 10 d, the carboxylate production potential exhibited by the algal-augmented fermentation reactors exceeded that of primary solids alone. Overall, for the 10 d retention time, algal biomass produced more carboxylic acids than observed for the municipal primary solids; the fermentation potential for fresh algae, frozen algae, and primary solids was 0.28 ± 0.02 (n = 3), 0.25 ± 0.02 (n = 3), and 0.21 ± 0.01 (n = 3) mg VFA_{COD}/mg volatile solids, respectively. When comparing specific carboxylate production results for the duration of the assessment (10 d), there was no observed statistical difference in fermentation potential between fresh and frozen algae ($t = 2.06$; $t_{crit} = 2.77$). Conversely, a statistical difference was realized between both the fresh algae and the primary solids ($t = 6.43$), and the frozen algae and primary solids ($t = 3.31$). Ultimately, increased carboxylic acid concentrations in the algae-augmented reactors suggested using this substrate could be beneficial in a WRRF.

Effect of Retention Time on Carboxylic Acid Speciation. Beyond specific yield, should algal fermentation be integrated into a WRRF, carboxylate speciation is equally, if not more, important. For example, research strongly supports that VFAs (specifically propionic acid) can enhance EBPR (Carvalheira et al., 2014; Shen and Zhou, 2016). Moreover, should polyhydroxyalkanoates production become a focus, a diverse array of carboxylic acids will result in a better-quality polymer (Anderson and Dawes, 1990; Dias et al., 2006).

Overall, carboxylate speciation was generally typical for the fermentation of organic matter such as primary solids, in that acetic acid dominated in concentration, followed by VFAs (Figure 2). However, the algal-based batch fermenters (Figure 2A,B) exhibited a more diverse distribution of carboxylic acids, particularly at retention times exceeding 5 d. Of even greater interest, the algal fermenters (fresh and frozen) both produced markedly higher concentrations of valeric (C5) and caproic (C6) acids when compared with the primary solids reactor (Figure 3). In regard to valeric acid, the fresh algae reactor yielded an increase of 39%, when normalized to total carboxylic acids (mg valeric $acid_{COD}$/mg carboxylic $acid_{COD}$), over primary solids, whereas the frozen algae yielded an increase of 28% at a retention time of 10 d. Caproic acid was present at an even greater relative concentration (mg caproic $acid_{COD}$/mg carboxylic $acid_{COD}$) in the fresh algae reactor, achieving 977% and 164% greater than observed in the primary solids reactor and frozen algae reactor, respectively. Smith et al. (2016) similarly reported that cofermentation of algae and organic waste (dairy manure) produced more valeric and caproic acids than manure alone. The divergence in speciation - primary solids only vs. algal biomass augmented - occurred most dramatically after a 6 d retention time (Figures 2 and 3).

The excess of VFAs present in the algal-based fermenters can potentially be explained from a substrate and related thermodynamics perspective. Considering substrate, algal biomass (vs. municipal primary solids) is typically more enriched in lipids; research has shown upwards of 22% greater lipid content (on dry weight basis) (Razzak et al., 2013). Indeed, the dominant algal species present in the biomass used - *Scenedesmus* and *Chlorella* - typically exhibit high lipid content (Zhang et al., 2016). Anaerobic digestion theory dictates that lipids contained within the biomass will be hydrolyzed to long chain fatty acids (LCFAs), and subsequently be anaerobically oxidized (acidogenesis) to VFAs (principally), acetate, and hydrogen; this metabolism is typically observed to commence beginning around a 6 d SRT (Grady Jr. et al., 2011; Gujer and Zehnder, 1983), associated with enrichment of lipid degrading microorganisms. VFAs are subsequently oxidized to acetic acid via beta oxidation. Whereas beta oxidation of VFAs is thermodynamically favored over anaerobic oxidation (Grady Jr. et al., 2011), elevated bulk solution hydrogen partial pressure can inhibit acetogenic reactions (Grady Jr. et al., 2011), resulting in VFA accumulation; such was suggested by Bouzas et al. (2007) in fermentation potential studies. In this study, the contents of the batch reactors were not significantly perturbed (other than continuous gentle shaking) or otherwise augmented/replaced; thus, some hydrogen produced via lipid degradation could have accumulated. Consequently, the higher lipid content in the algal biomass may explain the increased concentration of caproic and valeric acids (i.e., undigested VFAs) observed in the algal-based fermentation potential tests.

As a final point of interest, the primary solids fermenter maintained a relatively constant proportion of acetic acid (relative to total carboxylates) over the course of the test (Figure 2D). In contrast, the frozen algae reactor experienced a slowly

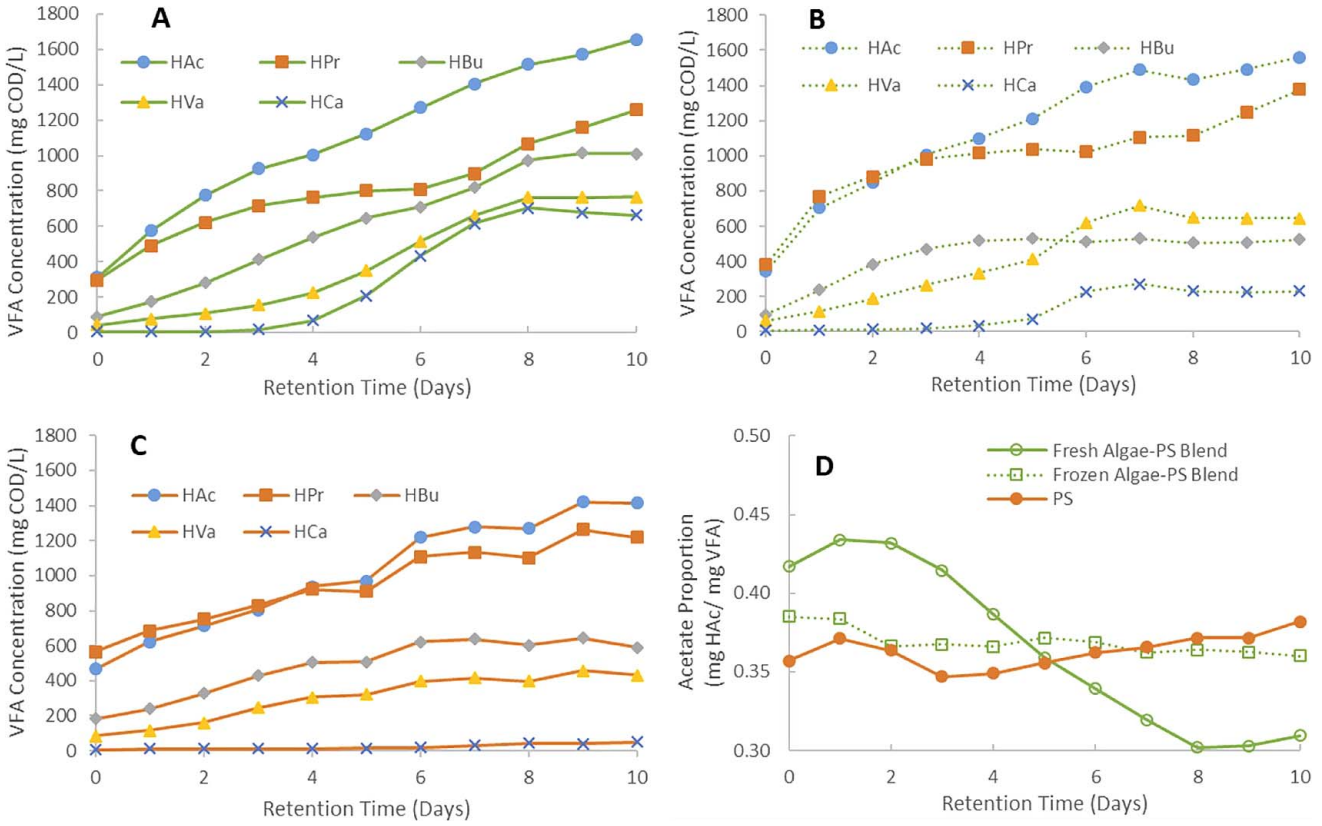

Figure 2—Speciation of VFAs in fermentation potential tests for (A) fresh algae-primary solids blend, (B) frozen algae-primary solids blend, and (C) primary solids (PS). (D) presents acetate fraction of total VFAs in each fermenter potential vessel. (HAc: acetic acid; HPr: propionic acid; HBu: butyric acid; HVa: valeric acid; HCa: caproic acid.)

decreasing acetic acid fraction, whereas the fresh algae reactor exhibited more variability, in that the acetic acid fraction decreased markedly beginning at a retention time of approximately 3 d. Ultimately, the acetic acid fraction in the fresh algae fermenter decreased from over 40% of total VFAs to 30% at a retention time of 8 d. The drastic decrease in the acetic acid ratio in the fresh algae fermenter is best illustrated in the speciation (Figure 2A). Whereas acetate synthesis continued after 3 d, propionic and butyric acid were generated at generally comparable rates (Figure 2A); moreover, after 4 d significant valeric and caproic acid synthesis commenced. One potential explanation for the variability in acetic acid proportion - and the observation that frozen algae did not exhibit a similar pattern - is the effect of freezing on the algae. Freezing causes both intra- and extracellular ice crystals to potentially lyse the cells (Taylor and Fletcher, 1999); also, freezing increases soluble COD (Smith et al., 2016), which could increase substrate bioavailability at earlier retention times. Indeed, as shown in Figure 2B versus 2A, propionic, butyric, and valeric acid production occurred at earlier retention times in the frozen versus fresh algal fermenters, and the resulting acetic acid fraction was markedly lower, respectively. The earlier VFA production appeared to facilitate more rapid onset of acetogenesis, as evidenced by acetic acid concentrations peaking at approximately 7 d in the frozen algal fermenter versus 10 d in the fresh algae fermenter. Conversely, delayed bioavailability of algal substrate (e.g., lipids) ultimately led to late-stage VFA synthesis and decreasing acetic acid ratio.

Fed-Batch Fermentation and the Effects of Algal Biomass Augmentation. Fermentation potential tests are an effective method to quickly evaluate substrate versus operational effects, but ultimately fermentation at a full-scale WRRF will occur in a fed-batch or continuous flow configuration, and not as a batch operation. Thus, using results from the fermentation potential investigations, bench-scale fed-batch algae-primary solids fermenters were designed, operated, and tested as a next step in the research. As indicated by the fermentation potential test results, specific carboxylate yield increased with retention time for all substrates studied, and carboxylate speciation was also enhanced by the augmentation with algal biomass. Indeed, SRT, which represents the bulk average retention time of the solids in a fed-batch reactor, can affect carboxylic acid production and speciation associated with (1) preventing the onset of methanogenesis (i.e., reduced carboxylates concentration, generally observed at SRTs $\geq$ 6 d, depending on temperature (Grady Jr. et al., 2011)); (2) increasing biomass (specifically fermenting bacteria) concentration, which can enhance disintegration, hydrolysis, and fermentation (yielding more carboxylates); (3) inhibiting anaerobic oxidation reactions

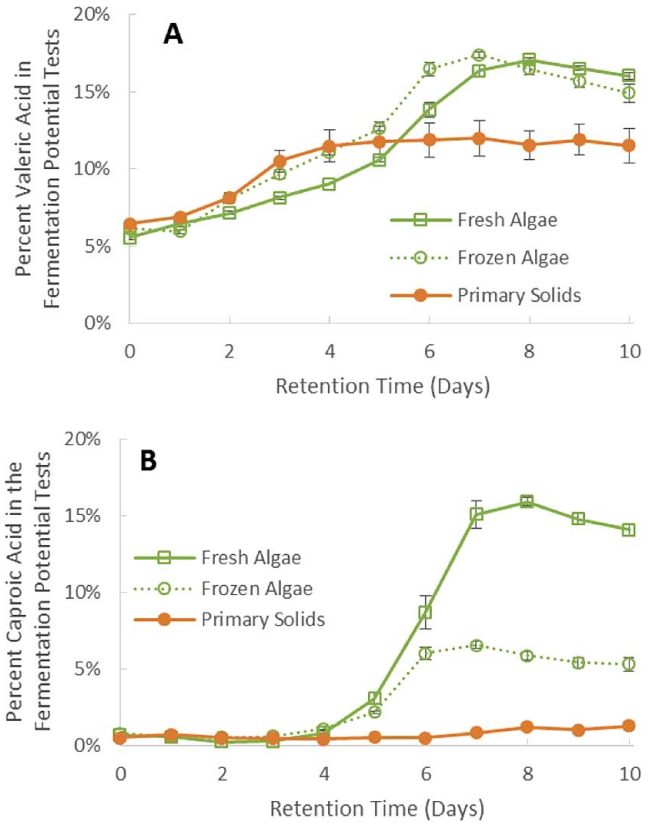

Figure 3—(A) Percent valeric acid and (B) percent caproic acid in the fermentation potential tests; values are relative to total carboxylate concentrations, on a mg COD basis, at each retention time.

associated with elevated hydrogen partial pressures (less acetic acid (HAc); more VFAs)); or (4) a combination of all the above. Considering the fermentation potential results and observed effect of retention time, three SRTs were evaluated in this study (5, 6, and 7 d), applied to two bench-top fed-batch fermenters (labeled fermenter MF1, which received only primary solids, and fermenter MFA, which received primary solids augmented with algal biomass).

Effect of SRT on Carboxylic Acid Production. Maximizing total carboxylic acid production in a WRRF is potentially significant because of the downstream implications discussed. In this regard, theoretically, and empirically based on the fermentation potential data, the additional carbon supplied by the algal biomass should increase total carboxylate production versus the lesser organically-loaded primary solids fermenter. However, and quite unexpectedly, the addition of algal biomass did not increase carboxylate production (concentration or yield basis; Table 1 and Figure 4) relative to primary solids alone. Figure 4 shows both the specific carboxylate yield and the carboxylate concentration of both fermenter MF1 and MFA over the duration of the experiment. Specifically, fermenter MFA exhibited reduced carboxylic acid concentrations of 30%, 34%, and 26% when compared with MF1 at 5, 6, and 7 d, respectively. However, SRT did affect fermentation productivity in both reactors.

Firstly, considering fermenter MFA, the carboxylic acid concentration did increase, both when SRT was increased from 5 to 6 d (49% increase) and again from 6 to 7 d (8% increase). The increase in carboxylate concentration was statistically significant between 5 and 6 d ($t = -11.0$) and between 6 and 7 d ($t = -3.29$). As suggested by the fermentation potential results and also by others (Banister and Pretorius, 1998; Cokgor et al., 2009; Gungor et al., 2009; Lie and Welander, 1997), longer SRTs will result in increased carboxylate production until the methanogenic population (HAc consumers) reaches a critical mass. Contrasted with fermenter MFA, in fermenter MF1 the carboxylate concentration increased when SRT increased from 5 to 6 d (57% increase), but decreased slightly when the SRT increased to 7 d (3% decrease); these latter results suggest potential onset of methanogenesis in MF1 at an SRT of 7 d. There was a statistical difference in carboxylic acid concentrations within MF1 between an SRT of 5 and 6 d ($t = -17.7$), but no statistical difference was realized between 6 and 7 d ($t = 1.28$). Whereas fermenter MFA realized an increase in carboxylate concentration at a 7 d versus 6 d SRT, the increase was indeed smaller than between 5 and 6 d SRT, which indicates MFA may also have been establishing a methanogenic population. Comparing the bench-scale fermenter results to the fermentation potential test results suggests that the fed-batch fermenters began showing signs of methanogenesis at a shorter SRT; this result is most likely a consequence of operational time, in that a methanogenic population likely will not reach critical mass over the relatively short duration (10 d) of the fermentation potential tests. Finally, the observation that a 6 d SRT is near-optimal for maximizing carboxylic acid production is consistent with other investigations (Bouzas et al., 2007; Coats et al., 2011a).

Some research has indicated there may be a positive correlation between carboxylate production and pH, with production increasing as pH increases (Cokgor et al., 2009; Wu

Table 1—Total carboxylate production and specific carboxylate yield versus SRT [Avg. ± SD (number of samples)].

SRT	MF1		MFA	
Days	mg COD/L	mg COD/mg VS	mg COD/L	mg COD/mg VS
5	1953 ± 195 (53)	0.17 ± 0.02 (53)	1367 ± 150 (11)	0.11 ± 0.01 (11)
6	3073 ± 229 (19)	0.23 ± 0.02 (19)	2036 ± 170 (19)	0.14 ± 0.01 (19)
7	2966 ± 323 (38)	0.19 ± 0.02 (38)	2193 ± 183 (38)	0.12 ± 0.01 (38)

Note: VS, volatile solids.

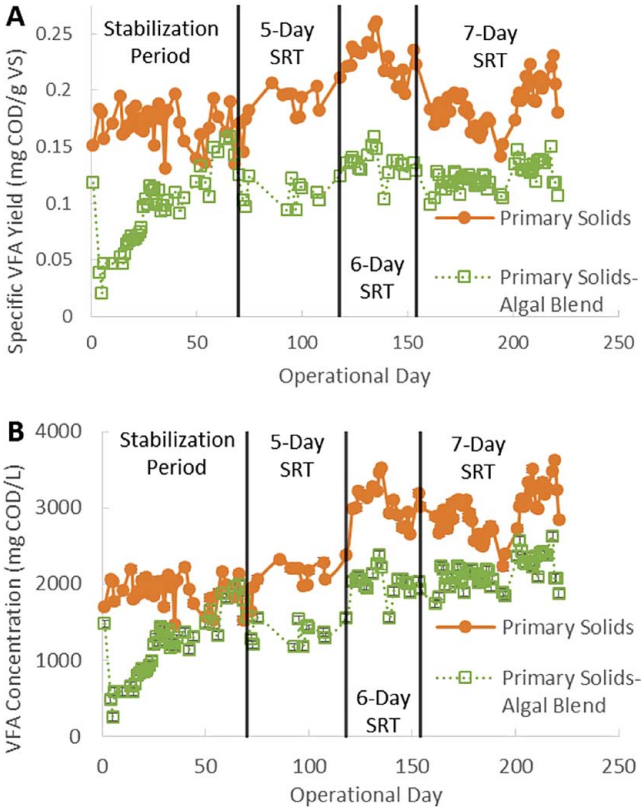

Figure 4—(A) Specific carboxylate production, and (B) total carboxylate production in bench-top reactors.

et al., 2009); however, such investigations involved active pH control through addition of a base. Conversely, for a minimally buffered substrate (Batstone et al., 2002), pH will decrease as more carboxylic acids are produced. Research herein did not control pH; as a consequence, the pH generally converged toward the pK_a for the carboxylates. Moreover, the pH decreased concurrent with increasing SRT (Table 2) and increasing carboxylate concentration - well aligned with Batstone et al. (2002). Comparing fermenters, the reactor MFA pH was statistically higher than observed in fermenter MF1; similar pH results were observed in the fermentation potential tests. The higher pH in fermenter MFA indicates the algal biomass might have provided additional buffering capacity, although the reduced carboxylate production could also explain the higher pH. Ultimately, it appeared the pH was determined by the quantity and species of carboxylic acids, and not vice versa.

Inasmuch as carboxylate concentration in the fermenters is an important metric, specific carboxylate yield sheds light on the efficacy of the cultured biomass to ferment the substrate. Results from this study (0.11–0.23 mg VFA_{COD}/mg volatile solids) align well with reported specific carboxylate yields on a complex organic substrate, which ranged from 0.09 to 0.20 mg VFA_{COD}/mg volatile solids (Coats et al., 2011a; Grady Jr. et al., 2011; Skalsky and Daigger, 1995; Yuan and Oleszkiewicz, 2010). Specific yields of MF1 also aligned well with the values obtained

Table 2—pH for each bench-scale fermenter.

SRT (Days)	MF1	MFA
5	4.83 ± 0.12 (83)	5.27 ± 0.42 (20)
6	4.65 ± 0.07 (44)	4.89 ± 0.07 (44)
7	4.58 ± 0.05 (49)	4.80 ± 0.07 (50)

for the fermentation potential of primary solids. However, the specific yields in fermenter MFA were less than observed in the corresponding fermentation potential analyses; one explanation is the potential establishment of heterotrophic algae in the bench-scale fermenter that did not occur in the fermentation potential tests because of limited time to acclimate - this topic is discussed in greater detail in a following section. Comparing fermenters, similar to the differential carboxylate concentrations observed, but in contrast to the fermentation potential analyses, the primary solids only fermenter (MF1) realized the largest specific yields (Table 1). Regarding SRT, in both fermenters the specific carboxylate yields increased when SRT was increased from 5 to 6 d (MF1, 35% increase, $t = -11.82$; MFA, 27% increase, $t = -5.92$). However, when the SRT was increased to 7 d, although VFA concentrations increased in both fermenters (Table 1), there was an apparent loss in fermentation observed through reduced specific yields (MF1, 17% decrease, $t = 7.76$; MFA, 14% decrease, $t = 3.54$); this decrease in fermentation yield could have been the result of (1) onset of methanogenesis and/or (2) increased volatile solids loading, both of which are affected by longer SRT. Statistical analysis showed there was a significant difference between specific carboxylate yields for both MF1 and MFA at all SRTs, as well as between MF1 and MFA at all SRTs tested (5 d SRT, $t = 14.84$; 6 d SRT, $t = 19.59$; 7 d SRT, $t = 17.69$).

Effect of SRT on Carboxylate Speciation. As evidenced by the fermentation potential results, and specifically during algal augmentation, there is potential to leverage the SRT to increase the diversity of carboxylate species in a fermenter; as discussed, enhanced carboxylic acid speciation can benefit both EBPR performance and polyhydroxyalkanoates production. Firstly, considering primary solids fermentation, process stability was realized relatively quickly in MF1 (Figure 5A). Moreover, carboxylate speciation of MF1 did not vary with the SRT, which mimics the results of the fermentation potential assessment (Figure 2C vs. Figure 5A). However, fermenter MF1 did show interesting speciation behavior. Specifically, the dominant carboxylic acid alternated between acetic and propionic acid (Figure 5A); this is in contrast with the literature, which commonly shows acetic acid as the predominant species for this substrate (Banister and Pretorius, 1998; Bouzas et al., 2007; Gungor et al., 2009; Lie and Welander, 1997; Smith et al., 2016), although such a result was suggested by the fermentation potential data (Figure 2C) and other sources (Skalsky and Daigger, 1995; Wu et al., 2009). As discussed, the type of organic matter (i.e., polysaccharides, proteins, lipids) will influence the carboxylate speciation in fermenters. In this regard, the organic matter was not characterized for each batch of primary solids;

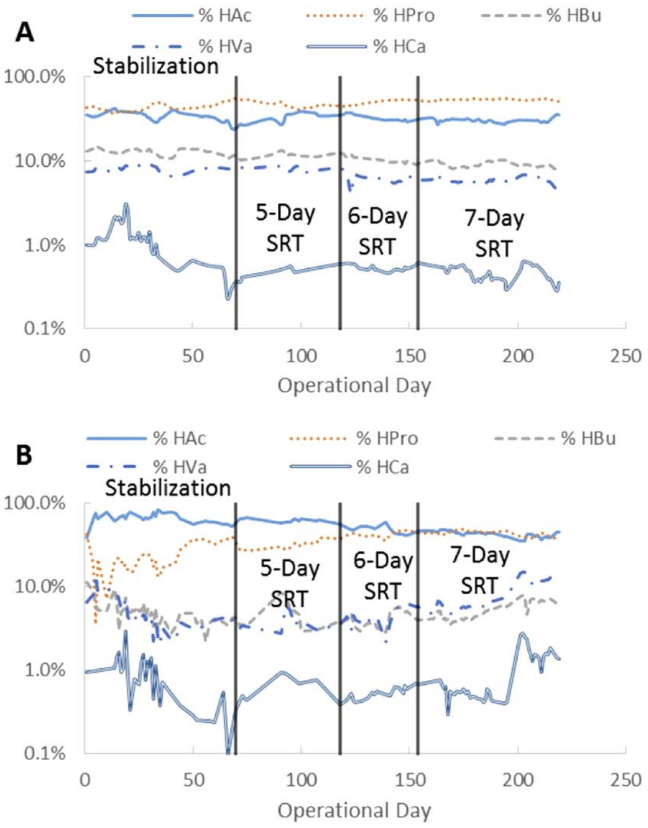

Figure 5—Speciation of carboxylates within fermenter (A) MF1 and (B) MFA; percentages are all based on mg of carboxylate species as COD relative to total mg of carboxylates as COD. (HAc: acetic acid; HPr: propionic acid; HBu: butyric acid; HVa: valeric acid; HCa: caproic acid.)

thus, the acetic to propionic acids behavior could be a result of variations in organic matter characteristics.

Although there was an unexpected decrease in carboxylate production for the algal-augmented system (relative to reactor MF1, and relative to the fermentation potential analyses), which reduces the intrinsic value of algal biomass fermentation, enhanced carboxylic acid speciation could generate some added value of algal fermentation. The bacterial culture in fermenter MFA required a longer period of time to achieve stable operations (fermenter MFA was initially started as a primary solids-only fermenter), as indicated by the convergence of acetic and propionic acid proportions as the research progressed (Figure 5B); this phenomenon was also observed during start-up of a primary solids fermenter by Wu et al. (2009). It was initially considered that higher acetic acid proportions in fermenter MFA (relative to observed fermentation potential results) were potentially because of mixing. As the two fermenters had different mixing systems, it was possible that MFA realized a lower mixing intensity, which would decrease local turbulence (i.e., smaller velocity gradient, G) and potentially reduce the release of gases from solution (including hydrogen); as noted, a larger partial pressure of hydrogen in bulk solution will inhibit acetogenesis of VFAs to acetic acid (Grady Jr. et al., 2011). However, investigations into the mixing - by physically switching fermenter contents between the two reactors - showed no statistical difference in acetic acid proportions between systems, which indicates the mixing intensity in each fermenter was similar. Ultimately, carboxylate speciation in fermenter MFA followed the same trends as observed in the algae-primary solids blend fermentation potential tests - more closely mimicking frozen algae-primary solids blend, as would be expected. There was an increase in valeric and caproic acids at an SRT above 6 d, which supports the hypothesis that lipids in the algae are being hydrolyzed to LCFAs and anaerobically oxidized to acetic acid, causing a buildup of VFAs potentially a result of elevated hydrogen partial pressures in solution (Bouzas et al., 2007). Nevertheless, although the MFA carboxylate speciation was somewhat more diverse than observed in fermenter MF1, when compared with the corresponding primary solids data, the results are likely not a significant value-add to a WRRF.

Assessing Fermenter Nutrient Concentrations - and Potentially Explaining Carboxylate Consumption. Fermentation of organic matter involves a complex array of microbially-mediated processes, including biomass disintegration and subsequent hydrolysis. The breakdown of complex organic matter to simple monomers not only yields carboxylate precursors, but also nitrogen (as ammonia) and phosphorus (as soluble reactive phosphorus). Thus, fermentation of organic matter results in an increase in bulk solution nutrient concentrations in the fermenter effluent (Banister et al., 1998; Bouzas et al., 2007); fermenting algal biomass that was cultured specifically to achieve tertiary nitrogen and phosphorus removal would be expected to increase effluent soluble nitrogen and phosphorus. Any produced nitrogen and phosphorus would need to be reprocessed through the WRRF, therefore potentially increasing energy and carbon demands.

Although phosphorus concentrations did indeed increase in concentration for both fermenters and at all SRTs, an unexpected observation was the reduction in ammonia concentrations in both fermenters (Table 3). Relative to the influent ammonia concentrations, for fermenter MF1, ammonia decreased on average 15% and 61% for 5 and 7 d SRTs; no ammonia reduction was observed at the 6 d SRT. Fermenter MFA realized an even greater ammonia reduction, with 39%, 74%, and 96% for 5, 6, and 7 d SRTs. In full-scale WRRFs, ammonia is commonly regulated in the effluent (EPA, 2010). Thus, any additional generation of nitrogen from fermenting organic biomass only increases treatment requirements. Conversely, reducing the amount of ammonia could have a significant effect on the oxygen requirements of the WRRF, thereby reducing the operating costs associated with treatment; aeration represents approximately 50% of WRRF energy usage (Goldstein and Smith, 2002).

Certainly the observed reduction in ammonia in both fermenters was an unexpected, and unpredicted, outcome. In seeking alternative explanations for the observed decrease in ammonia, the potential for NH_3 off-gassing can be ruled out, as

Table 3—Influent and effluent nutrient concentrations in bench-scale fermenters for 5, 6, and 7 d SRTs [Avg. ± SD (number of samples)].

SRT Days	MF1		MFA	
	PO_4-P (mg/L)	NH_4-N (mg/L)	PO_4-P (mg/L)	NH_4-N (mg/L)
Influent	24.29 ± 0.55 (6)	27.48 ± 2.91 (6)	38.83 ± 5.45 (6)	32.81 ± 4.58 (6)
5	33.78 ± 6.22 (9)	23.37 ± 5.43 (9)	43.91 ± 12.03 (9)	20.10 ± 15.02 (9)
6	45.43 ± 4.81 (12)	27.35 ± 4.02 (12)	47.31 ± 3.97 (12)	8.69 ± 5.30 (12)
7	42.26 ± 4.93 (11)	10.78 ± 3.62 (11)	49.49 ± 6.41 (11)	1.22 ± 1.72 (11)

the pH in both fermenters was consistently below pH 5 (Table 2). Similarly, struvite production can be eliminated, as a minimum pH of 7 is required (Doyle and Parsons, 2002); also, no struvite granules were observed, and the phosphorus concentration increased. Ultimately, the significant reduction in ammonia in fermenter MF1 at an SRT of 7 d could not be explained. However, a plausible explanation for the observed reduction in ammonia (without reduction in phosphorus) observed in the algal-fed fermenter is the presence of heterotrophic algae. These algae - including *Scenedesmus*, which were part of the biomass used in this study - are capable of growing and consuming ammonia in the absence of sunlight and/or an inorganic carbon source (CO_2). Not only would the presence of heterotrophic algae potentially explain ammonia consumption, the same could explain reduced carboxylates in fermenter MFA, relative to fermenter MF1. Specifically, *Scenedesmus* and other heterotrophic algae can use carboxylates (most notably acetate and butyrate) as a carbon/energy source (Lowrey et al., 2015; Mohan and Devi, 2012; Ren et al., 2014). As discussed and noted (Table 2), fermenter MFA unexpectedly realized markedly lower carboxylate production compared with MF1, despite being operated at a larger organic loading rate. Carbon utilized for algal growth could account for the lower than expected production of carboxylic acids in MFA, relative to MF1, and the associated ammonia reductions. A theoretical stoichiometric assessment was performed to evaluate the potential of algae to reduce the ammonia and carbon (carboxylic acids) in the fermenters. Assuming a molar ratio of 106:16:1 of carbon, nitrogen, and phosphorus, respectively (Pate et al., 2011), and further assuming that the difference in carboxylate production between MF1 and MFA was associated with heterotrophic algae growth, the resulting ammonia demand would be approximately 39 mgN/L, 74 mgN/L, and 60 mgN/L at SRTs of 5, 6, and 7 d, respectively. As this theoretically-based estimate exceeds the actual observed reduction in ammonia, it is thus feasible that heterotrophic algal growth was responsible for both ammonia and carboxylate consumption in MFA. Ren et al. (2018) similarly observed the potential for a *Scenedesmus* culture to concurrently consume carboxylates and nitrogen. As additional support for this theory, solids analysis performed on the bench-scale fermenters showed an increase in crude protein (31% in MFA vs. 12% in MF1), crude fats (20%, vs. no change), and lignin (93% vs. no change), all of which indicates growth of a high fat/high nitrogen biomass consistent with algae.

Conclusions

WRRFs are increasingly in need of carboxylic acids to support EBNR processes; additional carboxylate production could also enhance resource recovery opportunities. Moreover, tertiary algal treatment has been suggested as a means to capture secondary effluent nitrogen and phosphorus, thereby producing better overall effluent quality. Recognizing the potential synergy, research was conducted to evaluate the potential for algal biomass cofermentation with municipal primary solids to increase carboxylates production. Investigations were conducted using real wastewater primary solids and algal biomass grown on WRRF effluent, and experiments were designed to reflect potential actual conditions that could be realized at a full-scale WRRF. The research results, summarized below, were both insightful and, surprisingly, disproved the central hypothesis driving the investigations.

- Whereas fermentation potential evaluations showed no statistical difference in carboxylate yield on fresh versus frozen algae, results did suggest fresh algae could yield a greater quantity of longer chain VFAs (5C and 6C). Additional investigations would need to be undertaken to thoroughly understand the differences fresh and frozen algae can have on carboxylate speciation.
- Although fermentation potential testing is a useful and expedient method to inform design and operation of fed-batch or continuous flow fermenters, an important observation was that the fermentation potential results appear to overpredict the actual fermentation productivity that can be realized in fed-batch fermentation.
- Quite unexpectedly, and disproving a key research hypothesis, algal augmentation did not increase the production of carboxylic acids. Preliminary analyses suggest growth of heterotrophic algae in the fermenter decreased the concentration of carboxylates compared with primary solids only fermentation.
- Additionally, and disproving the second research hypothesis, augmenting a primary solids fermenter with algal biomass will not significantly increase the speciation diversity of produced carboxylates.
- Beyond the unexpected negative effects on carboxylate production, results also revealed that algal fermentation realized significant ammonia removal. The reduced ammonia load to the WRRF could potentially decrease WRRF energy demands through reduced aeration. Additionally,

reduced ammonia load would decrease the amount of carboxylic acids required to achieve denitrification.

Submitted for publication March 17, 2018; accepted for publication May 22, 2018.

References

Anderson, A. J.; Dawes, E. A. (1990) Occurrence, Metabolism, Metabolic Role, and Industrial Uses of Bacterial Polyhydroxyalkanoates. *Microbiol. Rev.*, **54**, 450–472.

Banister, S. S.; Pretorius, W. A. (1998) Optimisation of Primary Sludge Acidogenic Fermentation for Biological Nutrient Removal. *Water SA*, **24**, 35–41.

Banister, S. S.; Pitman, A. R.; Pretorius, W. A. (1998) The Solubilisation of N and P During Primary Sludge Acid Fermentation and Precipitation of the Resultant P. *Water SA*, **24**, 337–342.

Batstone, D. J.; Keller, J.; Angelidaki, I.; Kalyuzhnyi, S. V.; Pavlostathis, S. G.; Rozzi, A.; Sanders, W. T. M.; Siegrist, H.; Vavilin, V. A. (2002) The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Sci. Technol.*, **45**, 65–73.

Bouzas, A.; Ribes, J.; Ferrer, J.; Seco, A. (2007) Fermentation and Elutriation of Primary Sludge: Effect of SRT on Process Performance. *Water Res.*, **41**, 747–756.

Burow, L.; Mabbett, A.; McEwan, A.; Bond, P.; Blackall, L. (2008) Bioenergetic Models for Acetate and Phosphate Transport in Bacteria Important in Enhanced Biological Phosphorus Removal. *Environ. Microbiol.*, **10**, 87–98.

Cai, T.; Park, S. Y.; Li, Y. B. (2013) Nutrient Recovery from Wastewater Streams by Microalgae: Status and Prospects. *Renewable Sustainable Energy Rev.*, **19**, 360–369.

Carvalheira, M.; Oehmen, A.; Carvalho, G.; Reis, M. A. M. (2014) The Effect of Substrate Competition on the Metabolism of Polyphosphate Accumulating Organisms (PAOs). *Water Res.*, **64**, 149–159.

Coats, E. R.; Loge, F. J.; Wolcott, M. P.; Englund, K.; McDonald, A. G. (2007) Synthesis of Polyhydroxyalkanoates in Municipal Wastewater Treatment. *Water Environ. Res.*, **79**, 2396–2403.

Coats, E. R.; Gregg, M.; Crawford, R. L. (2011a) Effect of Organic Loading and Retention Time on Dairy Manure Fermentation. *Bioresour. Technol.*, **102**, 2572–2577.

Coats, E. R.; Mockos, A.; Loge, F. J. (2011b) Post-Anoxic Denitrification Driven by PHA and Glycogen within Enhanced Biological Phosphorus Removal. *Bioresour. Technol.*, **102**, 1019–1027.

Coats, E. R.; Watkins, D. L.; Brinkman, C. K.; Loge, F. J. (2011c) Effect of Anaerobic HRT on Biological Phosphorus Removal and the Enrichment of Phosphorus Accumulating Organisms. *Water Environ. Res.*, **83**, 461–469.

Coats, E. R.; Dobroth, Z. T.; Brinkman, C. K. (2015) EBPR Using Crude Glycerol: Assessing Process Resiliency and Exploring Metabolic Anomalies. *Water Environ. Res.*, **87**, 68–79.

Coats, E. R.; Brinkman, C. K.; Lee, S. (2017) Characterizing and Contrasting the Microbial Ecology of Laboratory and Full-Scale EBPR Systems Cultured on Synthetic and Real Wastewaters. *Water Res.*, **108**, 124–136.

Coats, E. R.; Eyre, K.; Bryant, C.; Woodland, T.; Brinkman, C. K. (2018) Assessing the Effects of RAS Fermentation on EBPR Performance and Associated Microbial Ecology. *Water Environ. Res.*, in press.

Cokgor, E. U.; Oktay, S.; Tas, D. O.; Zengin, G. E.; Orhon, D. (2009) Influence of pH and Temperature on Soluble Substrate Generation with Primary Sludge Fermentation. *Bioresour. Technol.*, **100**, 380–386.

Cullen, N.; Baur, R.; Schauer, P. (2013) Three Years of Operation of North America's First Nutrient Recovery Facility. *Water Sci. Technol.*, **68**, 763–768.

Dias, J. M. L.; Lemos, P. C.; Serafim, L. S.; Oliveira, C.; Eiroa, M.; Albuquerque, M. G. E.; Ramos, A. M.; Oliveira, R.; Reis, M. A. (2006) Recent Advances in Polyhydroxyalkanoate Production by Mixed Aerobic Cultures: From the Substrate to the Final Product. *Macromol. Biosci.*, **6**, 885–906.

Doyle, J. D.; Parsons, S. A. (2002) Struvite Formation, Control and Recovery. *Water Res.*, **36**, 3925–3940.

EPA, U. S. (2010) *National Lakes Assessment Report*. U.S. Environmental Protection Agency, Washington, D.C.

Goldstein, R.; Smith, W. (2002) *Water & Sustainability (Volume 4): U.S. Electricity Consumption for Water Supply & Treatment—The Next Half Century*. Technical Report for Electric Power Research Institute, Palo Alto, CA, U.S.A.

Grady Jr., C. P. L.; Daigger, G. T.; Love, N. G.; Filipe, C. D. M. (2011) *Biological Wastewater Treatment*, 3rd ed.; IWA Publishing/CRC Press: Boca Raton, FL.

Gujer, W.; Zehnder, A. J. B. (1983) Conversion Processes in Anaerobic Digestion. *Water Sci. Technol.*, **15**, 127–167.

Gungor, K.; Muftugil, M. B.; Ogejo, J. A.; Knowlton, K. F.; Love, N. G. (2009) Prefermentation of Liquid Dairy Manure to Support Biological Nutrient Removal. *Bioresour. Technol.*, **100**, 2124–2129.

Hinsinger, P.; Betencourt, E.; Bernard, L.; Brauman, A.; Plassard, C.; Shen, J. B.; Tang, X. Y.; Zhang, F. S. (2011) P for Two, Sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species. *Plant Physiol.* **156**, 1078–1086.

Ji, M. K.; Abou-Shanab, R. A. I.; Kim, S. H.; Salama, E.; Lee, S. H.; Kabra, A. N.; Lee, Y. S.; Hong, S.; Jeon, B. H. (2013) Cultivation of Microalgae Species in Tertiary Municipal Wastewater Supplemented with CO_2 for Nutrient Removal and Biomass Production. *Ecol. Eng.*, **58**, 142–148.

Li, Y.; Hua, D.; Zhang, J.; Zhao, Y.; Xu, H.; Liang, X.; Zhang, X. (2013) Volatile Fatty Acids Distribution during Acidogenesis of Algal Residues with pH Control. *World J. Microbiol. Biotechnol.*, **29**, 1067–1073.

Lie, E.; Welander, T. (1997) A Method for Determination of the Readily Fermentable Organic Fraction in Municipal Wastewater. *Water Res.*, **31**, 1269–1274.

Lowrey, J.; Brooks, M. S.; McGinn, P. J. (2015) Heterotrophic and Mixotrophic Cultivation of Microalgae for Biodiesel Production in Agricultural Wastewaters and Associated Challenges - A Critical Review. *J. Appl. Phycol.*, **27**, 1485–1498.

Madeira, M. S.; Cardoso, C.; Lopes, P. A.; Coelho, D. (2017) Microalgae as Feed Ingredients for Livestock Production and Meat Quality: A Review. *Livestock Science*, **205**, 111–121.

Markou, G.; Nerantzis, E. (2013) Microalgae for High-Value Compounds and Biofuels Production: A Review with Focus on Cultivation under Stress Conditions. *Biotechnol. Adv.*, **31**, 1532–1542.

Mohan, S. V.; Devi, M. P. (2012) Fatty Acid Rich Effluent from Acidogenic Biohydrogen Reactor as Substrate for Lipid Accumulation in Heterotrophic Microalgae with Simultaneous Treatment. *Bioresour. Technol.*, **123**, 627–635.

Pate, R.; Klise, G.; Wu, B. (2011) Resource Demand Implications for U.S. Algae Biofuels Production Scale-Up. *Appl. Energy*, **88**, 3377–3388.

Probst, D. (2016) Polyhydroxyalkanoate Production within a Novel WRRF Configuration, Civil and Environmental Engineering. M.Sc. Thesis, University of Idaho, Moscow, ID, U.S.A.

Razzak, S. A.; Hossain, M. M.; Lucky, R. A.; Bassi, A. S.; de Lasa, H. (2013) Integrated CO_2 Capture, Wastewater Treatment and Biofuel Production by Microalgae Culturing - A Review. *Renewable Sustainable Energy Rev.*, **27**, 622–653.

Ren, H.-Y.; Liu, B.-F.; Kong, F.; Zhao, L.; Xing, D.; Ren, N.-Q. (2014) Enhanced Energy Conversion Efficiency from High Strength Synthetic Organic Wastewater by Sequential Dark Fermentative Hydrogen Production and Algal Lipid Accumulation. *Bioresour. Technol.*, **157**, 355–359.

Ren, H.-Y.; Kong, F.; Ma, J.; Zhao, L.; Xie, G.-J.; Xing, D.; Guo, W.-Q.; Liu, B.-F.; Ren, N.-Q. (2018) Continuous Energy Recovery and Nutrients Removal from Molasses Wastewater by Synergistic System of Dark Fermentation and Algal Culture under Various Fermentation Types. *Bioresour. Technol.*, **252**, 110–117.

Shen, N.; Zhou, Y. (2016) Enhanced Biological Phosphorus Removal with Different Carbon Sources. *Appl. Microbiol. Biotechnol.*, **100**, 4735–4745.

Skalsky, D. S.; Daigger, G. T. (1995) Waste-Water Solids Fermentation for Volatile Acid Production and Enhanced Biological Phosphorus Removal. *Water Environ. Res.*, **67**, 230–237.

Smith, S. A.; Hughes, E.; Coats, E. R.; Brinkman, C. K.; McDonald, A. G.; Harper, J. R.; Feris, K.; Newby, D. (2016) Toward Sustainable Dairy Waste Utilization: Enhanced VFA and Biogas Synthesis via Upcycling Algal

Biomass Cultured on Waste Effluent. *J. Chem. Technol. Biotechnol.*, **91**, 113–121.

Taylor, R.; Fletcher, R. L. (1999) Cryopreservation of Eukaryotic Algae - A Review of Methodologies. *J. Appl. Phycol.*, **10**, 481–501.

Tooker, N. B.; Guangu, L.; Bott, C. B.; Dombrowski, P.; Schauer, P.; Menniti, A.; Shaw, A.; Barnard, J. L.; Stinson, B.; Stevens, G.; Dunlap, P.; Takacs, I.; Phillips, H.; Analla, H.; Russell, A.; Ellsworth, A.; McQuarrie, J.; Carson, K.; Onnis-Hayden, A.; Gu, A. Z. (2017) Rethinking and Reforming EBPR strategy - Concepts and Mechanisms of Side-stream EBPR. *Proceedings of the Annual Water Environment Federation Technical Exposition and Conference*; Chicago, IL, U.S.A.; 2547–2564.

Tsubu, S. (1973) Preservation of Marine and Fresh-Water Algae by Means of Freezing and Freeze-Drying. *Cryobiology*, **10**, 445–452.

Ward, A. J.; Lewis, D. M.; Green, F. B. (2014) Anaerobic Digestion of Algae Biomass: A Review. *Algal Res.*, **5**, 204–214.

Winkler, M.; Coats, E. R.; Brinkman, C. K. (2011) Advancing Post-Anoxic Denitrification for Biological Nutrient Removal. *Water Res.*, **45**, 6119–6130.

Wu, H.; Yang, D.; Zhou, Q.; Song, Z. (2009) The Effect of pH on Anaerobic Fermentation of Primary Sludge at Room Temperature. *J. Hazard. Mater.*, **172**, 196–201.

Yuan, Q.; Oleszkiewicz, J. A. (2010) Biomass Fermentation to Augment Biological Phosphorus Removal. *Chemosphere*, **78**, 29–34.

Zhang, T.-Y.; Hu, H.-Y.; Wu, Y.-H.; Zhuang, L.-L.; Xu, X.-Q.; Wang, X.-X.; Dao, G.-H. (2016) Promising Solutions to Solve the Bottlenecks in the Large-Scale Cultivation of Microalgae for Biomass/Bioenergy Production. *Renewable Sustainable Energy Rev.*, **60**, 1602–1614.