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Aerobic bacterial pretreatment to overcome algal growth inhibition on high-strength anaerobic digestates

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ABSTRACT

Coupling anaerobic digestion and algae cultivation has attracted attention as a sustainable means of treating high-strength wastewaters. In such a scenario, nutrients from the liquid anaerobic digestate are used by algae to produce biomass. However, use of full-strength digestate results in poor algal growth and nutrient removal. Most researchers have overcome this challenge by diluting digestate 10–30 fold prior to algae growth but such dilution rates demand large amounts of fresh water, posing challenges for scale-up. The objectives of this study were to 1) assess whether ammonium, turbidity, and heavy metals in digestate were the primary sources of inhibition for a highly-nutrient tolerant strain of *Chlorella sorokiniana*, and, 2) develop a biological pretreatment strategy to overcome algal growth inhibition on full strength digestate. Ammonia toxicity, turbidity, and heavy metals have been commonly hypothesized as the source of algal growth inhibition, but our results showed that these factors were not critical inhibitors of *C. sorokiniana*. Dose response studies showed that *C. sorokiniana* could grow robustly on 3,500 mg/L ammonium. Regardless, full strength digestates of wastewater sludge and food waste were very inhibitory to *C. sorokiniana*. We utilized a pretreatment approach using activated sludge which led to robust algal growth on full-strength digestate. High growth rates of 250–500 mg/L/d were achievable on pretreated digestates despite very high ammonium levels of ~2,000 mg/L. Pretreating digestate also led to significantly faster algal nutrient uptake compared to untreated digestate ($p < 0.001$).

1. Introduction

With increasingly stringent nutrient discharge standards, municipal wastewater treatment plants (WWTPs) and industrial wastewater generators are seeking innovative nutrient removal technologies. Utilization of algae in wastewater treatment has gained attention for its ability to remove and recover nutrients in their fixed form, mostly as amino acids (Cai et al., 2013). Use of algae also reduces greenhouse gas emissions through CO_2 sequestration and the resulting algal biomass has a variety of beneficial uses (Spolaore et al., 2006).

There are increasing numbers of municipal and industrial treatment systems that employ anaerobic digestion to convert organic matter and bacteria biomass (e.g. excess sludge from aeration tanks) into biogas and digestate. The liquid digestate (LD) fraction is rich in nutrients which can lead to environmental nutrient pollution if not adequately treated. Municipal WWTPs typically reintroduce the LD back into the headworks of the treatment plant (personal communication, William Kent, Manager of Environmental Services, Columbus Water Works), creating a parasitic load on the system. The elevated nutrient concentration not only puts a burden on downstream tertiary treatment but also potentially impacts the efficiency of downstream secondary treatment and anaerobic digestion (Chen et al., 2008).

A variety of algal species are known to quickly assimilate inorganic nutrients (Franchino et al., 2016), and algae have been studied for nutrient recovery from a variety of anaerobic digestates (Ruiz-Martinez et al., 2012; Wang et al., 2010). In fact, digestates are rich in the nitrogen and phosphorus nutrients that typically limit algal growth in nature (Stanley et al., 1990). However, most researchers have found that full strength digestates severely inhibit algal growth (Cho et al., 2013; Franchino et al., 2016), a finding that was common across a wide range of digestate types. In these cases, dilution rates of 10–30 fold were typically used to alleviate inhibition of LD in lab-scale experiments (Cho et al., 2013; Franchino

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et al., 2016). However, diluting LD with freshwater is a non-starter in water-scarce regions and may even be suboptimal given the simultaneous dilution of nutrients needed for algal growth. We hypothesize that removal or transformation of inhibitory compounds in LD will lead to rapid algal growth rates without the need for dilution water. Knowledge regarding specific inhibitors in LD is limited. Most studies cite ammonia (Cho et al., 2013), turbidity (Wang et al., 2010), and heavy metals (Wong et al., 1994) as the primary sources of algal inhibition on LD. A few others have mentioned unknown organic constituents and COD as potential inhibitors of algae (Franchino et al., 2016; Tigini et al., 2016).

The objectives of this research were to 1) assess whether ammonium, turbidity, and heavy metals in digestate were the primary sources of inhibition for a highly-nutrient tolerant strain of *Chlorella sorokiniana* and 2) test the effectiveness of aerobic activated sludge pretreatment of digestate as a means of reducing inhibitor concentrations in full-strength anaerobic digestates.

2. Material and methods

2.1. Anaerobic digestate and activated sludge collection

Municipal anaerobic digestate (MAD) was collected from a mesophilic anaerobic digestor at the South Columbus Water Resources Facility (Columbus GA, USA) which is used to treat excess wastewater sludge and waste cooking oil. Activated sludge (AS) was collected from an aerated activated sludge tank used for secondary wastewater treatment at the same facility. Both MAD and AS were immediately transported back to the lab and stored in a cold room (4 °C) until use. Food waste anaerobic digestate (FWAD) was collected from a commercial-scale high-solids anaerobic digester at UC Davis (Davis, CA, USA), and was shipped to the lab overnight on ice and was stored in a freezer (−80 °C) until use. LD was prepared by a combination of centrifugation and filtration to remove solid components from the digestate as follows. The upper liquid portion of the anaerobic digestate (both MAD and FWAD) was centrifuged at 4,696 x g for 30 min. The supernatant was then passed through a series of filters of the following sizes using a vacuum filtration apparatus: Whatman No.4 filter paper (20–25 μm), No.1 (11 μm), No.2 (8 μm), No.5 (2.5 μm), Advantec GA-55 glass fiber (1.6 μm), GC-50 glass fiber (1.2 μm), Advantec mixed cellulose ester membrane (0.8 μm), and Whatman GF-F glass fiber (0.7 μm). The resulting liquid was termed “clarified” digestate. Sterile filtered digestate was later prepared by passing clarified digestate through Advantec mixed cellulose ester membranes (0.45 μm and 0.2 μm), and a 0.2 μm sterile filtration apparatus (VWR PES filter). Filtration was used to control turbidity and to isolate treatment effects of algae without the assistance of wastewater bacteria.

2.2. Algae culture experimental plan

The first experiments tested *Chlorella sorokiniana* (UTEX 2805) on different dilutions of MAD and FWAD to determine the extent of inhibition. This strain of *C. sorokiniana* was originally isolated from a wastewater treatment plant (de-Bashan et al., 2008) and has successfully been used in treatment of winery wastewater (Higgins et al., 2017). Given the frequently-cited hypothesis that ammonia is the most important inhibitor in digestate, we next cultured *C. sorokiniana* on different concentrations of ammonium chloride in chemical N8-NH_4 medium (Higgins and VanderGheynst, 2014). The pH of this medium was adjusted to 7.5. Next, *C. sorokiniana* was cultivated in AS-pretreated LD (treatment 1) and non-treated LD (treatment 2) in bubble column bioreactors (Wang et al., 2019) to study algal growth and inhibition. This experiment was carried out for both types of anaerobic digestate (MAD and FWAD). Specific culture methods for AS-pretreatment and algae cultivation are described in subsequent sections. Control cultures were cultivated in defined chemical N8 medium (Tanadul et al., 2014). Because AS-pretreatment resulted in decreases in the ammonium concentration, a third treatment group was tested with addition of ammonium chloride to restore the ammonium level to that of the untreated LD. The purpose of this third treatment was to confirm if ammonium removal during AS pretreatment had a meaningful impact on algal growth inhibition. All experimental treatments and controls were tested in biological triplicate. All LD was sterile filtered and supplemented with micronutrients and magnesium (same final concentration as in the control chemical medium) to ensure trace metals were not limiting growth.

2.3. Activated sludge pretreatment of anaerobic digestate

Clarified digestate (passed through a 0.7 μm filter) was treated with activated sludge by adding 2% (v/v) activated sludge slurry (0.67% solids content) to digestate. pH of the digestate was adjusted to 7.5 with 3M HCl and aerated with 1 vvm air for 4–5 days. The AS-treated anaerobic digestate was then sterile filtered through a VWR 0.2 μm sterile PES vacuum filtration unit for use in the algae cultivation test.

2.4. Algae cultivation method

The algae cultivation method has been described previously (Higgins and VanderGheynst, 2014; Higgins et al. 2018a, 2018b; Wang et al., 2019). Briefly, *C. sorokiniana* was initially cultured on a modified Bold 3N agar plate for 5–7 days to isolate single colonies. Colonies were selected and used to grow 1 L stock cultures in N8 medium under a fluorescent light bank and aeration (0.5 vvm, 2% CO_2) until the optical density (550 nm) reached 0.2–0.3. Stock cultures were then settled for 24–48 h at room temperature. After removal of 90% of the supernatant, the concentrated algae slurry was evenly transferred into each bioreactor to inoculate the experiment. Algae were grown in bubble column bioreactors over 5 days with light (170 μmol photons/m^2/s on a 14 h:10 h light-dark cycle) at 25 °C. Bioreactors were aerated at 0.5 vvm and air was supplemented with 2% CO_2. pH was controlled at 7.5 for all cultures using either 3M HCl or 3M NaOH. Daily samples (2 ml) were taken from each bioreactor for optical density (OD) measurement at 550 nm and 680 nm. The samples were then centrifuged, and the supernatant filtered through 0.2 μm syringe filters and stored at −80 °C until further analysis.

2.5. Heavy metal analysis

Inductively coupled plasma – optical emission spectrometry (ICP-OES) (Spectro Ciros ICP, SPECTRO Analytical Instruments, Kleve, Germany) was used to analyze the metal concentrations in the anaerobic digestate. The digestate was first filtered through Whatman No. 42 filters, and then 5 ml of filtered samples were digested with 1:1 (v/v) nitric acid in a microwave digestion system as described previously (Chaump et al., 2018). Digested samples were analyzed via ICP-OES for metals (Cu, Mn, Al, Ca, Zn and Fe).

2.6. Chemical oxygen demand (COD) and total nitrogen tests

A HACH DR900 was used to measure the soluble COD concentration in the sterile-filtered digestates (5x diluted in DI water). A HACH total nitrogen assay was also used to measure the nitrogen content of harvested algae cells following a previously published procedure (Higgins et al., 2015). Nitrogen content was multiplied by growth rate to determine the rate of nitrogen assimilation into algal cells.

2.7. Optical density and spectrum absorbance

A SpectraMax M2 Plate reader was used for OD and spectrum absorbance measurements. OD was measured in triplicates for each sample at 550 nm and 680 nm. Spectrum absorbance was conducted on membrane-filtered LD (0.2 μm) from 200 nm to 1,000 nm in 10 nm increments in order to assess interference of LD absorbance with chlorophyll absorbance. Pigment and lipid extracts from *C. sorokiniana* were analyzed as a point of reference when assessing absorption interference by digestate. *C. sorokiniana* extracts were obtained using a previously-published modified Folch method (Folch et al., 1956; Wang et al., 2019).

2.8. Ion chromatography for nutrients analysis

A Prominence Liquid Chromatography (LC) system coupled with a conductivity detector (Shimadzu, Japan) was used to analyze ion concentrations (sodium, potassium, ammonium, calcium, magnesium, chloride, nitrite, nitrate, phosphate, and sulfate) in digestate samples based on a previously published method (Chaump et al., 2018). Briefly, A Dionex IonPac CS12 column (4 × 250mm, Thermo science) and a Dionex IonPac AS22 column (4 × 250mm) with suppression (Dionex CERS 500 4 mm and Dionex AERS 500 4 mm, respectively) were used for ion separation. Acidic eluent (20 mM methanesulfonic acid) was used on the CS12 column, and basic eluent (4.5 mM sodium carbonate and 1.4 mM sodium bicarbonate solution) was used on the AS22 column.

2.9. Data analysis and statistics

Experiments were all conducted in biological triplicate except where noted. Statistical analyses (ANOVA and Turkey's HSD test) were carried out in R with the 'car' package and 'agricolae' package. Standard deviations were calculated in Microsoft Excel.

3. Results

3.1. Algal inhibition in anaerobic digestate

The growth of *C. sorokiniana* was severely inhibited in both municipal sludge and food waste anaerobic digestates (Fig. 1). Diluting digestates with deionized water helped alleviate some inhibition, with a 16x dilution of MAD yielding ~1.5 g/L dry algal mass. However, the defined algal growth medium (N8) yielded the highest overall growth at ~2.1 g/L dry mass after the 5-day cultivation period. In full strength MAD, *C. sorokiniana* did not have a detectable biomass increase until the last day of cultivation. Algal growth in diluted MAD had a relatively faster growth rate than the chemical medium in the first 48–72 h, but they reached an early growth "ceiling," suggesting potential nutrient limitation at high dilutions despite supplementation with micronutrients. The most diluted MAD (16x) had the highest algal growth rate in this experiment. Similar trends were observed when cultivating *C. sorokiniana* in FWAD except algae cells experienced complete inhibition in both full strength and 2x diluted FWAD. The growth "ceiling" was also higher in dilutions of FWAD compared to MAD.

3.2. Ammonium tolerance test on C. sorokiniana

Anoxic conditions and nitrogen-rich organic feed material provide a suitable environment for ammonium production in anaerobic digestors. Most anaerobic digestate contains a large amount of ammonium ranging from 100 to 3,000 mg/L in the liquid fraction (Xia and Murphy, 2016). The syringe-filtered MAD contained approximately 2,000 mg/L of ammonium and the syringe filtered FWAD had approximately 3,000 mg/L of ammonium. Although ammonium is an important nitrogen source for algal growth, excess ammonium combined with high pH can lead to high free ammonia concentrations. Free ammonia is typically harmful for algal growth (Gutierrez et al., 2016) but can be partly controlled through pH. We therefore tested the tolerance of *C. sorokiniana* to high ammonium concentrations while controlling pH at 7.5. An ammonium dose-response test on *C. sorokiniana* revealed it to be highly tolerant to extreme ammonium concentrations (up to 3,500 mg/L) (Fig. 2). Little difference in algal growth was observed on media containing ammonium concentrations ranging from 1,000 mg/L to 3,500 mg/L. Below 1,000 mg/L ammonium, algal growth decreased (Fig. 2A).

3.3. Heavy metals and turbidity

The inhibitory effects of certain metals, such as copper, aluminum, and manganese, have been known for several decades (Wong et al., 1994). These metals could have resulted in algal growth inhibition on digestates. However, the ICP measurement of metals in MAD and FWAD (Table 1) showed that concentrations of copper, aluminum, and manganese in the digestates were lower than those in the defined chemical algal medium (N8). In addition, the high turbidity of raw MAD and raw FWAD should inhibit algal photosynthesis by reducing light penetration. However, filtration through 0.2 μm membranes greatly alleviated the turbidity for both MAD and FWAD (Fig. S1). The spectrum absorption (Fig. S2) also indicated that the filtered MAD did not have strong absorbance at 350-500 and 630–680 nm which are key bands of chlorophyll *a* absorbance. The filtered FWAD had elevated absorbance below

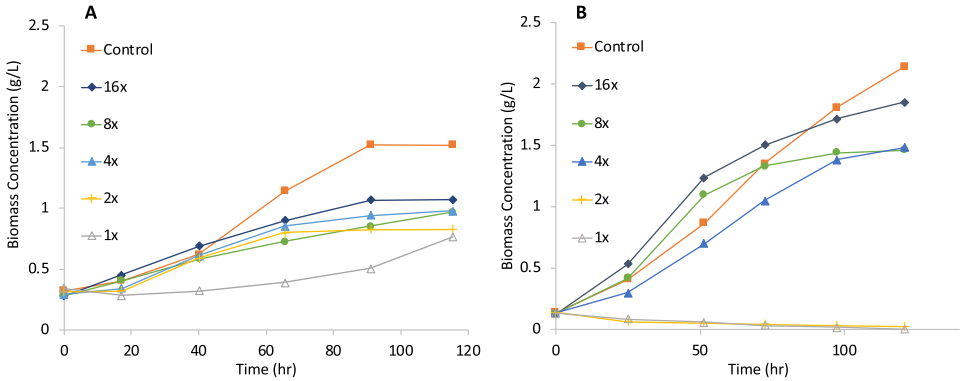

Fig. 1. Growth of *C. sorokiniana* (UTEX 2805) on varying concentrations of LD. A) Response to municipal anaerobic digestate and B) food waste anaerobic digestate. Control cultures were grown on chemical N8 medium. Each data point represents the average of biological replicates (n = 2). Biomass concentration is reported on a dry-weight basis.

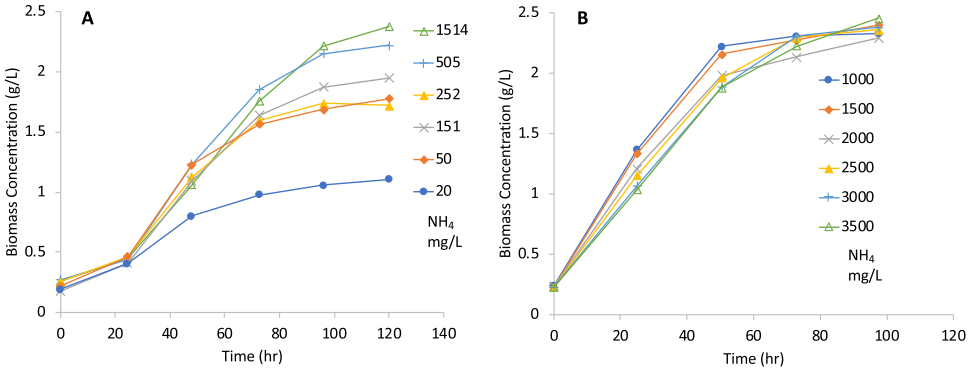

Fig. 2. Growth of *C. sorokiniana* (UTEX 2805) in different ammonium concentrations: A) lower range (20 mg/L to 1500 mg/L) ammonium dose response and B) higher range (1000 mg/L to 3500 mg/L) ammonium dose response. Each data point represents the average of biological replicates (n = 2). Biomass concentration is reported on a dry-weight basis.

Table 1
ICP-OES metal concentrations in raw digestate.

	Aluminum	Copper	Manganese	Zinc	Calcium	Iron
MAD[a] (mg/L)	ND[d]	0.23	0.00	0.18	15.01	0.25
FWAD[b] (mg/L)	ND[d]	0.10	ND[d]	0.10	5.09	0.41
N8 medium[c] (mg/L)	0.29	0.47	3.60	0.73	3.55	1.52

[a] Raw municipal liquid anaerobic digestate.
[b] Raw food waste liquid anaerobic digestate.
[c] N8 medium is the defined chemical algal growth medium.
[d] ND = not detected.

400 nm, but it only partially blocked the useful spectrum for photosynthesis.

3.4. Pretreating anaerobic digestate with activated sludge (AS)

3.4.1. Anaerobic digestate nutrient composition

The change in anaerobic digestate nutrient composition before and after AS pretreatment is shown in Table S1. There was approximately 1,300 mg/L COD in MAD before and after AS treatment. However, a significant decrease ($p = 0.01$) followed by an increase in soluble COD was observed during the AS treatment process (Fig. S3). This suggests removal of organics followed by degradation of recalcitrant material and release of soluble metabolites by AS bacteria. Ammonium concentration in MAD decreased from approximately 2,000 mg/L to 1,100 mg/L. Increases in soluble phosphate and sulfate were observed during the AS treatment process for MAD, indicating solubilization under the aerobic conditions. Nitrate and nitrite were not detected during the process. Similar changes were observed in FWAD during the AS treatment process: COD and ammonium decreased whereas chloride increased due to pH adjustment. All other ions were relatively constant. The ammonium concentration was originally 3,200 mg/L, and it decreased to roughly 2,000 mg/L after AS treatment.

3.4.2. Suppression of nitrification in full-strength municipal anaerobic digestate

Nitrification carried out by aerobic bacteria during wastewater treatment is well-established (Ge et al., 2015). As activated sludge is known to harbor nitrifying organisms, it was surprising that AS pretreatment did not lead to any detectable increase in the nitrate concentration. This led us to hypothesize that nitrifying organisms were also inhibited in the full strength digestates. We carried out a test on full strength and 10x-diluted MAD (Fig. S4). Nitrification was suppressed in full strength MAD with both nitrate and nitrite concentrations remaining undetectable during the AS pretreatment process. However, significant nitrification was observed during AS-treatment of 10x-diluted MAD ($p < 0.001$). The nitrate concentration increased linearly over time. Moreover, the nitrite concentration began increasing 48 h after the inoculation of activated sludge.

3.5. Algae cultivation in AS-pretreated anaerobic digestate

3.5.1. Algal growth

AS pretreatment greatly alleviated the inhibitory effects of full-strength MAD on algae (Fig. 3A). Culturing *C. sorokiniana* on AS-pretreated MAD resulted in 3.5 times faster growth (532 mg/L/day over a 5 day average) than the culture in untreated MAD (150 mg/L/day) and 1.4 times faster than the control culture. The addition of ammonium to AS pretreated MAD (to compensate for ammonium lost during the pretreatment process) only had a negligible impact on algal growth (516 mg/L/day) compared to the AS-pretreated MAD. We also experimented with simultaneous "co-treatment" of digestate using AS and algae. The result was continued inhibition of algal growth (Fig. S5).

AS pretreatment of FWAD resulted in partial alleviation of algal growth inhibition (Fig. 3B). Pretreatment of FWAD with AS resulted in a decline in ammonium content from 3100 mg/L to 2000 mg/L. Out of concern that such a high ammonium level could have a negative interactive effect with other inhibitors, we diluted untreated FWAD by a factor of 1.4 to reduce the ammonium concentration to the same level as AS-pretreated FWAD (2,000 mg/L ammonium). We also added a third group of reactors in which we diluted pretreated digestate by 1.4 fold and then supplemented it with ammonium chloride to restore the ammonium level to 2,000 mg/L. This treatment was included to control for the dilution benefit afforded to the untreated digestate. Although the highest average growth rate (318.5 mg/L/day) was still observed in the control N8 medium, there was strong cell growth in the diluted AS pretreated FWAD and moderate growth in the full-strength AS-pretreated FWAD. The untreated FWAD completely inhibited algal growth even with the 1.4-fold dilution, consistent with the previous dose-response experiment.

3.5.2. Nutrient removal

With the alleviation of algal growth inhibition by AS pretreatment of anaerobic digestate, there was also a significant increase in nitrogen assimilation into algal biomass. Increased assimilation of nitrogen was observed in both MAD and FWAD (Fig. 4) after pretreatment. Measurements of nitrogen assimilation were used rather than measurements of nitrogen removal in order to understand algae's contribution to removal as opposed to other means,

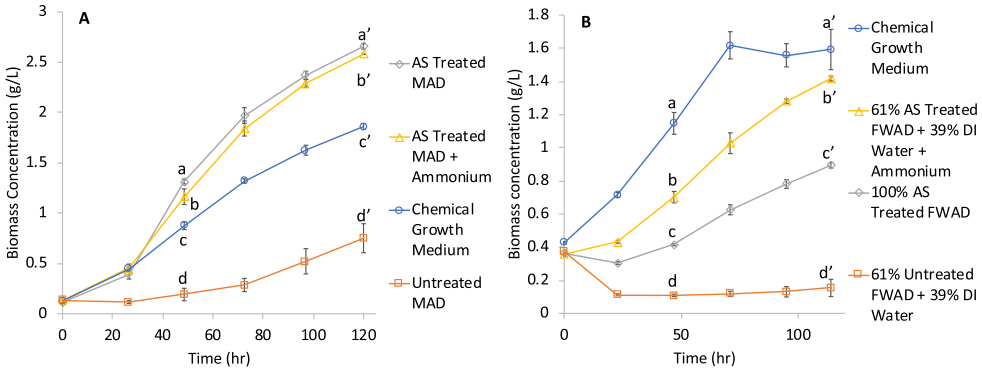

Fig. 3. Growth of *C. sorokiniana* on municipal anaerobic digestate (A) and food waste digestate (B) with and without pretreatment with activated sludge (AS). Note bacteria were removed before algae cultivation so growth is only due to algae. Control cultures were grown on chemical medium (N8). Because activated sludge treatment resulted in removal of some ammonium in the food waste digestate, the untreated digestate was diluted 1.4 fold to equalize ammonium concentrations (~2,000 mg/L) in all digestates. Because of the dilution advantage afforded to the untreated digestate, an additional set of reactors was prepared with 1.4-fold-diluted AS-pretreated digestate. Ammonium was added to this last set of reactors to equalize to other cultures at 2,000 mg/L. Errors bars are SD, n = 3 biological replicates. Letters above data points at 48 h and 120 h of growth indicate statistical significance where data points with the same letter are not significantly different at the 0.05 level. Biomass concentration is reported on a dry-weight basis.

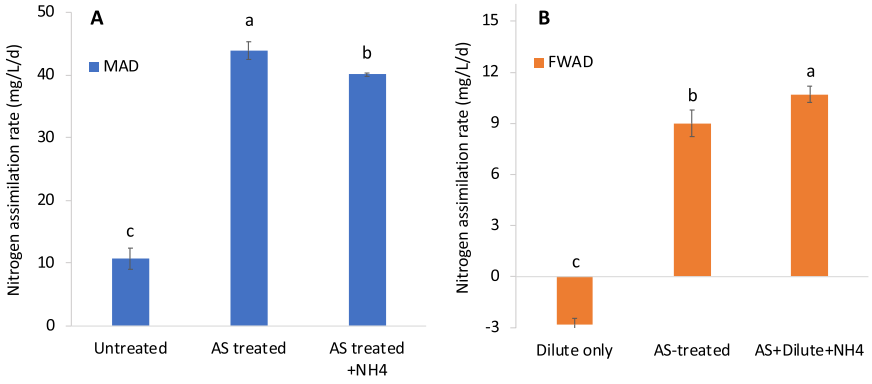

Fig. 4. Nitrogen uptake rates by algae grown on municipal anaerobic digestate (A) and food waste digestate (B). Nitrogen uptake was determined through digestion of algae and analysis of nitrogen content. Negative values indicate net nitrogen release due to net loss of algal biomass over the culture period. Error bars are SD, n = 3 biological replicates. Bars with the same letter are not significantly different at the 0.05 level.

such as volatilization. Over 40 mg/L/day nitrogen removal was observed when culturing algae in AS-pretreated MAD with or without exogenous ammonium addition. This was significantly higher than the algal nitrogen assimilation rate in untreated MAD which was ~10 mg/L/day ($p < 0.001$). Significantly higher nitrogen assimilation ($p < 0.001$) was also observed in AS-pretreated FWAD (~10 mg/L/day) compared to nitrogen assimilation in untreated FWAD (−3 mg/L/day). The untreated FWAD ended with a negative nitrogen assimilation due to net cell death in these cultures.

Phosphate removal was likewise faster in the AS-pretreated anaerobic digestates compared to the untreated digestate (Fig. 5). Around 15 mg/L/day phosphate removal was observed in MAD compared to a net negative phosphate removal (release of phosphate into the media) in untreated MAD. Similar observations were found when growing algae in FWAD: algae did not remove a significant amount of phosphate from the untreated FWAD ($p = 0.718$). On the other hand, algae removed all of the phosphate in the 1.4-fold diluted, AS-pretreated FWAD, averaging a phosphate removal rate of 10 mg/L/day. Moreover, positive phosphate removal (5 mg/L/day) was also observed in full strength AS-pretreated FWAD.

4. Discussion

The results obtained through our experiments suggest that the most commonly-cited factors for algal growth inhibition on digestate, namely ammonia (Cho et al., 2013), turbidity (Wang et al., 2010), and heavy metals (Wong et al., 1994), do not provide a complete picture of algal inhibitors present in digestate. High ammonium concentrations are likely to be inhibitory to a range of algae species, however, ammonium does not appear to be a significant problem for nutrient-tolerant species of the genera *Chlorella* and *Scenedesmus* (Ayre et al., 2017), so long as pH is controlled. The *Chlorella* species in this study grew robustly even at ammonium concentrations of 3,500 mg/L. Heavy metals including aluminum and copper, which are known to inhibit algae (Wong et al., 1994), had lower concentrations in the two digestates than in chemical growth medium. Hence, these metals cannot explain algal inhibition on digestate. Finally, the use of filtration can largely alleviate the problem of digestate turbidity, another inhibitor of algal growth. Filtration is already widely used in wastewater treatment processes for separation of solids and liquids. For example, the wastewater treatment plant that supplied the municipal anaerobic digestate in this study employs a belt-press filter to separate digestate solids and liquid.

Nevertheless, we observed strong growth inhibition in *C. sorokiniana* in both digestate types. This inhibition could be partially or, in the case of municipal digestate, fully alleviated through pretreatment with an aerobic bacterial consortium. This finding suggests that organic constituents are likely inhibitors of

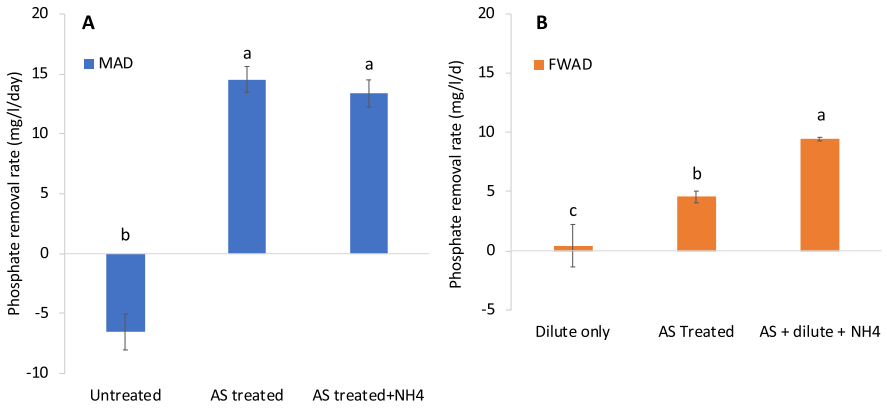

Fig. 5. Phosphate removal rates by algae grown on municipal anaerobic digestate (A) and food waste digestate (B). Phosphate removal was determined through the difference of phosphate concentration between t = 0 h and t = 120 h in the spent medium as measured by anion chromatography. Negative values indicate net phosphate release. Error bars are SD, n = 3 biological replicates. Bars with the same letter are not significantly different at the 0.05 level.

algal growth in the digestates studied. Indeed, Franchino et al. (2016) have suggested that unknown organic constituents may contribute to inhibition. Tigini et al. (2016) have also cited COD in digestate as an inhibitor of algae.

In past work, we have found that volatile fatty acids (VFAs), particularly propionic and butyric acid that are sometimes present in anaerobic digestate significantly inhibit algae (Wang et al., 2018). Those studies revealed EC50 concentrations of propionate and butyrate of roughly 450 mg/L (Wang et al., 2018) which are within the ranges found in many digestates from commercial-scale operations (Franke-Whittle et al., 2014). However, the digestates used in the present study did not contain detectable VFAs, making this an unlikely explanation for inhibition observed in the present study. Many digestates also contain long chain free fatty acids as a result of lipid hydrolysis (Alves et al., 2009; Sousa et al., 2013) and these are known to be lethal to certain algae including *Chlorella* (Wu et al., 2006). Lipids are present in food waste, and large volumes of waste cooking oil are processed in the anaerobic digester at the municipal wastewater treatment plant. Thus, free fatty acids, even at low concentrations could contribute to algal inhibition. A range of phenolic compounds are also present in digestates (Hecht and Griehl, 2009; Hernandez and Edyvean, 2008) and many algal species have been shown to be severely inhibited by a wide range of phenolics (Nakai et al., 2001; Pillinger et al., 1994; Wang et al., 2016). However, clear links between specific phenolics found in digestates and algal inhibition are the subject of ongoing investigations.

It is possible that organic constituents interact with ammonium to suppress algal growth. However, our results show that removal of inhibitory constituents by aerobic bacteria alleviates inhibition even at very high ammonium concentrations (e.g. 2,000 mg/L). Praveen et al. (2018) also utilized aerobic bacteria to pretreat anaerobic digestate prior to algae growth and found that this approach reduced inhibition. However, they largely attributed this effect to nitrification of ammonium, which they assumed to be the primary inhibitor. During treatment of digestate with activated sludge, there were indeed reductions in ammonium. However, no concomitant increase in nitrite or nitrate was observed suggesting little to no ammonium oxidation during pretreatment. Instead, much of the ammonium loss was likely due to ammonia volatilization. Our results showed that full-strength digestate completely inhibited ammonium oxidizing organisms: ten-fold dilution of the digestate allowed for a resumption of ammonium oxidation and production of nitrate. However, observation of significant nitrite production also indicates continued partial inhibition of nitrite oxidizing bacteria. Indeed, Praveen et al. (2018) used dilution rates of 10-fold for all of their inhibition studies which likely explains their observation of nitrification. It is interesting to note that algae, but not nitrifying bacteria, could process ammonium in full-strength digestate, underscoring the potential niche that hyper-eutrophic algae can play in treatment of high-strength wastewaters.

There are several major problems that make the dilution approach impractical for advancing algae treatment of anaerobic digestate. First, freshwater is a scarce and valuable resource, particularly for agricultural and industrial wastewater generators, who may lack access to large quantities of dilution water. Second, critical nutrients needed for algal growth are diluted at the same rate as the inhibitors, leading to sub-optimal algal growth rates and nutrient removal, as we observed in digestate dosing studies. It was interesting that dilution led to slightly faster initial algal growth rates than the chemical control medium. However, this effect was likely due to the presence of ammonium in the digestate versus nitrate in the control medium. *C. sorokiniana* preferentially consumes ammonium over nitrate (Ogbonna et al., 2000) and our results indicate that growth on ammonium (Fig. 2) was faster than that on nitrate medium (Fig. 1). Moreover, dilution led to a lower plateau in growth, indicative of nutrient limitation despite supplementation with a micronutrient solution. Finally, sub-optimal growth rates necessitate a large reactor volume for algal growth. This leads to greater cost and thus lower likelihood of technology adoption. A better approach is to remove or destroy the inhibitors present in the digestate, thus allowing rapid algal growth on full-strength digestate. Fast growing algae, in turn, remove nutrients more quickly, shrinking the footprint (and cost) of the required treatment facility. That said, our results suggest that aerobic bacterial treatment does not always fully remove inhibitors in the digestate, as was the case with FWAD. In such cases, mild dilution can be helpful in maximizing algal growth and nutrient removal rates. The pretreatment approach discussed here would benefit from additional process optimization. Moreover, given the very high nutrient levels in digestate, a multi-stage algal treatment system is likely required in order to reduce nutrient concentrations to levels acceptable for environmental discharge.

5. Conclusions

1. Severe algal inhibition was observed on high-strength LD, but the main source of inhibition was not due to the commonly-

cited reasons of ammonium toxicity, turbidity, or heavy metal toxicity.
2. Using aerobic bacteria as a pretreatment step effectively alleviated algal inhibition and increased nutrient removal rates. Pretreatment was more effective with municipal sludge digestate than with food waste digestate.
3. Organic compounds in LD are likely to be important algal inhibitors and the pretreatment process led to initial reduction in digestate COD levels.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2019.07.011.

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