Development of an Integrated Biological Wastewater Treatment System for the Full Utilization of High Nitrogen Livestock Waste

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Abstract:

Livestock wastes such as Liquid Swine Manure (LSM), when discharged improperly, contribute to ground and surface water contamination. These wastes are also full of valuable nutrients that can be converted to bioproducts such as fertilizers, fuels, and feed. For this reason, increasing attention has been focused on utilizing and treating this waste so that it can be discharged without detrimental environmental effects. In Chapter 1 the significance of this study and the purpose of each major component in the system are explained. Then Chapter 2 examines the obstacles apparent from literature to successful biological waste treatment as it regards each component. Finally in Chapter 3 a series of methods including thermal vacuum stripping pretreatment, mesophilic anaerobic digestion, microalgae treatment, and hydroponic cultivation are evaluated for full utilization of wastewater through nutrient removal and recovery, and a balance of mass and nutrients throughout the system is proposed. Overall, the system was capable of reducing the key nutrient parameters (COD, TN, ammonia, TP) to a large degree (>98%) while producing valuable side products. This approach has the potential to sustainably treat agricultural wastewater while offsetting treatment costs with the production of valuable bioproducts.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

Background and Significance of Research

The improper disposal of swine manure contributes to environmental issues such as excessive nutrient loading of water-bodies which leads to eutrophication, increasing oxygen demand, and the destabilization of the homeostatic balance of the affected environment [1]. As of 2020, there are 667 million pigs on earth creating hundreds of millions of gallons of wastewater each day [2]. The conventional approaches to dealing with this waste are storage ponds and anaerobic lagoons as well as liquid-solid separation with the solid fraction going to aerobic compost and the liquid fraction being directly applied as fertilizer to fields [3]. These approaches release odorous, greenhouse gasses, and many of the nutrients applied to fields are washed away by rain and irrigation before they can be fully utilized, with the nutrients contributing to ground and surface water contamination. Due to the environmental issues, as well as increasing regulation regarding manure application, new methods are required to treat and utilize swine manure. The biological methods proposed require conditioning of the feedstock through pretreatment to improve bioavailability of nutrients in the waste. After pretreatment, the waste can be more easily digested anaerobically. This is followed by microalgae and hydroponic biomass cultivation using wastewater nutrients. Together, these systems treat the waste through full utilization of valuable compounds.

Thermal Vacuum Pretreatment

While anaerobic digestion of livestock manure is an established method, its effectiveness suffers from a variety of inhibiting factors, including lower than optimal carbon:nitrogen (C:N) ratios, ammonia nitrogen and hydrogen sulfide inhibitions, and antibiotic and heavy metal contamination. Thermal vacuum pretreatment with the addition of high carbon containing agricultural byproducts such as corn stover has been proposed to address many of these issues. By applying high temperature, pH, and vacuum, free ammonia and hydrogen sulfide can be stripped off and recovered from the vapors in acid and caustic washing vessels. In addition to removing those inhibitions, the heat and high pH can hydrolyse lignocellulosic material into more available carbon to improve the C:N ratio for methane production, while high temperatures are able to degrade some residual antibiotics [4]. This approach has been shown to be able to remove and capture 98% of ammonia and increase methane production in anaerobic digestion by 50%. [5] While the method requires chemical inputs for pH adjustment; caustic for the pretreatment and acid to neutralize the pH before digestion, the chemical requirements may be able to be reduced by increasing temperature. After this step, the waste is ready for digestion.

Anaerobic Digestion

The anaerobic digestion (AD) process relies on four main biological processes to convert volatile solids to methane and carbon dioxide. These processes are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These processes occur simultaneously in single stage anaerobic digestion, but each step is limited by the previous steps, e.g. acidogenesis relies on hydrolysis to have broken down carbohydrates, lipids, and proteins into sugars, long chain fatty acids, and amino acids [6]. For this reason, pretreatment including hydrolysis can accelerate the other downstage anaerobic digestion processes.

While the AD process reduces chemical and biological oxygen demand, the resulting digestate byproduct is a concentrated nutrient solution that is currently most often applied directly to fields as fertilizer. The high nutrient content (primarily nitrogen, phosphorus, and carbon) has attracted significant interest and study into using the digestate as a medium for microalgae cultivation. The obstacles to profitable algae growth in digestate include: inhibitors such as ammonia, dark color, high turbidity, and unfavorable nutrient ratios in the digestate. A number of approaches have been used to mitigate these issues.

Microalgae Treatment

Microalgae are considered to be one of the most promising technologies for the advanced treatment and nutrient recovery of wastewater. [7] Microalgae are capable of rapid growth and are able to assimilate nutrients such as chemical oxygen demand (COD), phosphorus, nitrogen, and some heavy metals. Additionally, they are able to add value by converting nutrients into biomass that can be used as feedstock for biorefineries, or as feed or fertilizer. Profitable cultivation of microalgae relies on inexpensive nutrient sources; anaerobically digested manure can fill that role if the challenges associated with its use can be mitigated.

Digested swine manure is high in ammonia; it has a dark color and high turbidity, and it has a less than optimal nutrient ratio for microalgal growth. Ammonia has inhibitory effects on microalgal growth, and the dark color of the digestate limits photosynthetic activity by lowering light intensity on the cells. A number of approaches have been applied to addressing these issues including biochar filtration, dilution, flocculation, and nutrient supplementation [8]. Following nutrient removal by algae, there are still nutrients present that can be utilized by hydroponic plants.

Hydroponic Cultivation

Hydroponic cultivation of produce has several advantages over traditional land agriculture. Hydroponic systems are capable of faster growth, higher productivity, lower water usage, and they lack the risks of nutrient runoff and water contamination that traditional agriculture faces. Essentially, more plants can be grown faster and safer using hydroponics when compared with land agriculture [9]. Hydroponic systems are also capable of reducing nitrogen, phosphorus, and COD (as much as 77, 86, and 87% respectively) when cultivated in wastewater and serve as an additional biofilter [10]. The main obstacles to productive cultivation in digestate are high salinity, imbalanced nutrient ratios, and potential for pathogen contamination of produce.

Objectives

The overall goal of this study is to develop an integrated biological wastewater treatment system for full utilization of liquid swine manure. Each subsystem is evaluated for nutrient removal as well as required inputs and outputs. A mass and nutrient balance is constructed for the system as a whole, and opportunities for improvement are proposed.

Chapter 2: Literature Review

Anaerobic Digestion

Thermophilic vs Mesophilic Anaerobic Digestion

Thermophilic AD is defined as AD occurring at temperatures greater than 45 degrees Celsius (and typically 50 to 60 C) while mesophilic AD occurs at temperatures below 45 (and typically 35 to 38 C). In general, thermophilic digestion is considered to have superior performance compared to mesophilic AD. The performance parameters that are improved under thermophilic conditions include higher organic matter degradation, and higher methane yield, as well as a greater reduction in pathogen load and lower odor emissions [11]. Despite these advantages, mesophilic AD is still largely preferred for livestock wastes due to higher operational stability and robustness. While thermophilic AD allows for a lower hydraulic retention time (and a correspondingly smaller required vessel volume), it is also prone to instability and failure, especially as long chain fatty acids build up and inhibit methanogenesis [12]. In contrast, mesophilic AD is able to maintain stability with a wider range of organic loading rates and with more varied influent. The reasons for this instability at thermophilic temperatures is not well known, but one hypothesis points to a lower biological diversity which is less able to adapt to changes in the substrate. This makes mesophilic AD more useful for processing livestock manures which are often not always well balanced feedstocks and require long term stability and reliability [13].

The microbial composition of thermophilic and mesophilic digesters has been studied extensively. One study using food waste-recycling water operated one thermophilic and one mesophilic digester for a year and genomic DNA was extracted for analysis. The study found that the bacteria were more diverse in mesophilic AD. Additionally, it found the dominant species in the mesophilic digester contribute to hydrolysis and fermentation, while the dominant species in thermophilic AD are believed to contribute to hydrolysis and acid production. Furthermore, the dominant methanogens in each digester changed as the pH changed over time [14].

Another study investigated similar parameters in a solid-state food waste digester. Again, it was found that the mesophilic conditions allowed for a more diverse bacterial community, and additionally found that temperature was the most significant factor affecting the composition of the biological community. In both cases, Firmicutes dominated the bacterial community, composing 60% of the bacteria in the mesophilic digester and 82% in the thermophilic. On the other hand, *Methanothermobacter* composed the greatest share of the archaea in thermophilic AD, but *Methanoculleus* was the most prominent in mesophilic AD [15].

The performance of solid-state anaerobic digesters under each condition was also studied. Thermophilic anaerobic digestion reduced cellulose and volatile solids significantly faster after twelve days, but by thirty-four days the difference was negligible. Thermophilic AD also resulted in a five times larger buildup of volatile fatty acids which caused a significant pH drop and a corresponding decrease in biogas output. Mesophilic gas production peaked early at day 8 and then slowly declined. Thermophilic gas production took longer to peak (day 12) with a higher gas output but fell quickly afterwards to lower than mesophilic conditions. A significant shift in biological composition also occurred over time. Again, temperature was found to be the largest influencer on microbial composition, and it was correlated to VFA accumulation and pH drop [16].

While the temperature has been shown to influence microbial composition as well as VFA accumulation and biogas output, it also affects the optimal organic loading rate (OLR), or the rate at which organic material is fed to the digester. One study investigated the effect of ORL on mesophilic and thermophilic anaerobic digestion. The optimal loading rate was found to be higher for thermophilic AD at 2.5 grams volatile solids per liter per day and a lower 1.5 grams for mesophilic. Furthermore, applying loading rates higher than the optimal resulted in rapid and severe inhibition of methane production, while lower than optimal loading resulted in lower, but steady biogas yield. The decline of biogas production under high loading rates was attributed to rapid hydrolysis and acidification which led to the accumulation of VFAs [17].

Efforts have been taken in the research community to address the inhibition of biogas production and process instability. One approach employed is the addition of activated carbon to anaerobic digesters. Under mesophilic conditions and low loading rate, the addition of activated carbon showed no positive effect, and the digester eventually collapsed as the organic loading rate increased and the soluble COD in the digester increased from less than 1000 mg/L to more than 8000 mg/L. Under higher loading rates, the addition of activated carbon showed limited positive effects. On the other hand, under thermophilic conditions, the addition resulted in higher methane yield (150% higher) and lower soluble COD levels [18].

Another study performed a similar experiment by the addition of granular activated carbon to digesters under different temperatures. This addition was found to contribute to a shorter lag time and faster biogas output. The added carbon also resulted in faster volatile fatty acid consumption. At higher rates, the effect is reversed when the granular activated carbon concentration reaches 8g/L which results in a large decrease in biogas production. These effects on lag time and VFA consumption are attributed to activated carbons properties as an adsorber which can relieve VFA inhibition by adsorbing acids [19].

In summary, thermophilic anaerobic digestion typically has superior performance in regard to biogas output and volatile solid consumption, but it suffers from long term instability due to the higher proportion of acid producing microbes that can lead to volatile fatty acid accumulation and corresponding digester failure. This effect is amplified by the lower microbial diversity of thermophilic AD which makes it difficult for the community to adapt to changes in the substrate. Activated carbon has been shown to alleviate some of the inhibitory effects of VFAs and increase biogas yield. While mesophilic AD is more popular in industry now, further improvements to thermophilic AD stability will likely make higher temperature AD more suitable for many applications.

Carbon:Nitrogen Ratio

The C:N ratio in an anaerobic digestion feedstock is a crucial parameter affecting biogas production. Excessively high C:N ratios (high carbon) can cause low pH and lead to volatile fatty acid accumulation in the digester. Excessively low C:N ratios (high N) lead to ammonia accumulation and reduced biogas production. Thus, a balance must be struck to maximize digester effectiveness. Numerous studies have found optimal C:N ratios between 20:1 and 30:1 [20]. This is much higher than the typical C:N ratios of livestock wastes which can be as low as 5:1 in LSM and approximately 8:1 in cattle and poultry manure, although the ratios vary [21] [22] [23]. Additionally, C:N ratios tend to decrease as the substrate is digested and carbon is consumed until it reaches a plateau at which point digestion stalls [24]. Inexpensive agricultural byproducts are the most common addition to manure due to their availability and low cost. These include rice husk, corn stover, and barley straw, all of which are rich in carbon and can increase the C:N ratio, but also contain lignocellulosic material that must be hydrolysed to be made bioavailable [25].

VFA inhibition

The accumulation of Volatile Fatty Acids (VFAs) is considered the main factor that contributes to biogas decline and system failure [26]. VFAs are released as an intermediate in the acidogenesis and acetogenesis stages of anaerobic digestion. Due to the rate limiting nature of the methanogenesis stage, these VFAs can accumulate and cause a drop in pH and system instability [27]. Of the VFAs, propionate has been identified as the primary inhibitor [28]. Solutions to reestablish and maintain the balance between acetogenesis and methanogenesis have been explored and they include the supplementation of trace elements and co-digestion with photosynthetic bacteria. One study using food waste stabilized a digester for a year under the organic loading rates 2.19–6.64 g VS (volatile solids)/L day without VFA accumulation by introducing the elements cobalt, iron, molybdenum and nickel. Their investigation found that iron was essential in maintaining methanogenesis. Cobalt was also found to compound that effect in the presence of iron [29]. Other feedstocks may be deficient in different trace elements, but supplementation can improve process stability. A study found that VFA inhibition could be alleviated by the introduction of photosynthetic bacteria with and without light. Both conditions stimulated methane production with the lighted condition being superior. The bioaugmentation using photosynthetic bacteria had the added effect of reducing soluble COD by more than eighty percent with the presence of light [30]. Both of these methods have the potential to recover a digester from VFA accumulation but trace element supplementation is a more widely studied and established method. These studies were both under mesophilic AD but other studies have found similar effects of trace element supplementation in thermophilic AD using calcium and magnesium in addition to the previously mentioned elements [31] [32].

Organic loading rate

The organic loading rate (OLR) of a system is the rate at which organic material is added per liter of system volume. This is a key factor in digester stability and VFA accumulation, methane production, and COD removal. Lower ORL tends to favor COD removal and more process stability while higher ORL tends to favor biogas production at the cost of increased instability due to the potential accumulation of VFAs [33]. One study found the optimal ORL in a mesophilic swine manure digester to be 1.89 g volatile solid (VS)/(L.d) [34]. A study using poultry manure at ORLs of 1.6 and 2.5 g (VS)/(L.d) and under thermophilic and mesophilic conditions found that mesophilic conditions had higher biogas production at the higher loading rate than thermophilic AD at the same rate. Under the lower rate, performance was similar between the two [35]. Feedstocks with much lower ammonia (<100 ppm), such as cattle slaughterhouse wastewater, were able to achieve stability at much higher loading rates between 2 and 10 g (VS)/(L.d) [36]. Unlike the C:N ratio, which has been consistently found to be optimal at similar ratios for different digesters, the optimal loading rate is influenced by a number of factors including influent composition and whether the priority is COD removal or biogas output. A waste treatment process is likely to favor a lower ORL to facilitate greater COD conversion efficiency, improved stabilization and more complete treatment over a higher absolute gas production rate.

Ammonia Inhibition

Ammonia is produced as a result of biological degradation of nitrogen containing material including proteins and urea. It exists as an ammonium ion (NH4+) and as free ammonia (FA) (NH_3) which are together referred to as Total Ammonia Nitrogen (TAN). While small amounts of each can benefit microbial growth, both forms can cause inhibitions in biological systems, with free ammonia being identified as a more potent inhibitor. [37] A study investigated the effects of ammonia concentrations on a swine manure digester at mesophilic to thermophilic temperatures. An inhibition was found at FA concentrations greater than 1.1 g/L with bacterial growth slowing and found the limiting step under inhibition to be acetate-utilizing methanogenesis [38]. Another study examined the effects under varying concentrations of TAN ranging from 0.4 to 5.77 g/L. While the TAN was added progressively with time allotted for the bacteria to acclimate, the higher concentrations (4.92 and 5.77 g/L) caused large reductions in methane output (39 and 64% respectively). It was also found that acclimating bacteria to higher TAN concentrations improved the tolerance to TAN and pH variations [39]. The concentration of TAN, at which inhibition occurs in a digester is influenced by the bacterial community composition, the ratio of free ammonia to ammonium, and the degree of acclimation.

Salinity inhibition

Another factor affecting the microbial processes for AD is salt concentration and electrical conductivity (EC). Research has identified cations such as Na, K, Ca, Mg, and Fe as inhibitors in high concentrations [40]. Similar to ammonia, in low concentrations (<200 mg/L) cations such as Na can enhance digestion, but at high concentrations can significantly inhibit it by dehydrating cells and causing cell death through osmosis [41]. Dilution is the most obvious method to address this problem, although the dilution rate must be minimized to reduce freshwater consumption.

Microalgae Cultivation

Microalgae Cultivation in Wastewater

Microalgae has been studied for its ability to remove nutrients from wastewater as well as its potential for biofuel, feed, and cosmetic applications. Thus far, the application of microalgae wastewater treatment has been limited by the cost of nutrients as well as harvesting, but cultivation in wastewater has the potential to use low-cost waste nutrients while treating water [42]. In addition to nitrogen, phosphorus and COD, microalgae are capable of immobilizing metal contaminants such as lead, zinc, and mercury [43]. For these reasons, algae-based wastewater treatment plants have begun to emerge to address the environmental issues associated with nutrient discharge [44]. Despite this, obstacles to full adoption remain, including ammonia inhibition, high turbidity, and improper nutrient ratios.

Ammonia Nitrogen Inhibition

While ammonium is the most readily available form of nitrogen for microalgae to assimilate, high ammonium concentrations can inhibit microalgae growth [45]. Total ammonium nitrogen represents the sum of free ammonia and ionic ammonium, but free ammonia has been found to be the primary inhibitor. A study of *Chlorella Vulgaris* found a strong (R^2 = 0.9694) negative correlation between free ammonia and specific algae growth rate. While growth under free ammonia concentrations below 36.8 mg/L showed no obvious inhibition whereas a strong inhibition was observed at and above 184 mg/L [46]. Low concentrations of free ammonia are beneficial to microalgae growth, but higher concentrations will inhibit biomass production.

Color and Turbidity

Anaerobic digestate contains suspended solids and humic substances that cause high turbidity and dark brown color respectively. These factors are key in affecting light transmittance and limiting the quantity of light that can reach the algae cells for photosynthesis. Marcilhac et al. [47] used experimentation to construct a model of the relationship between digestate color, optical density, and nitrogen removal. It was found that lower optical density; and correspondingly higher light transmittance, resulted in improved algae growth and nitrogen removal. Additionally, a theoretical approach found that the average growth rate of microalgae is determined by the maximum and minimum light intensities in the bioreactor and determined formulas to optimize optical depth and incident light for maximum microalgae growth [48].

Nutrient Ratios

Another key parameter in microalgae growth are the ratios of nutrients contained in the medium. These ratios of nutrients affect biomass growth and accumulation as well as nutrient removal efficiency. One such important ratio is the ratio between total organic carbon (TOC) and total nitrogen (TN). Gao et al. found that increasing the TOC:TN of simulated wastewater promoted algae growth and nutrient removal. The positive effects on growth rate did not continue past a ratio of 24 TOC:TN, but at a ratio of 24 the microalgae were able to remove 99.58% of nitrogen. These conditions also resulted in a high lipid productivity of 35.4mg/L*D [49]. By increasing the C:N ratio, the nutrient removal rate and lipid productivity can be significantly improved.

Another important nutrient factor is the ratio of nitrogen to phosphorus (N:P). The optimal value for this ratio varies by species and depends on the source of nitrogen. For example, the optimal N:P ratio for *Thalassiosira sp.* was found to be 30:1 when using ammonium chloride as a nitrogen source, but 20:1 when provided nitrogen in the form of urea. In contrast, *Chaetoceros gracilis* growth is optimal in an N:P ratio of 40:1 when using ammonium chloride but 60:1 when using urea [50]. Not only is the optimal N:P nutrient ratio dependent on species, but it is also dependent on the form of nitrogen available and ecological conditions. Optimizing these factors is necessary for productive cultivation and nutrient removal. A study of *Chlorella vulgaris* cultivated in wastewater found that the N:P ratio had no obvious relationship with the total nitrogen removal. However, the total phosphorus removal depended heavily on the N:P ratio with higher N:P ratios resulting in lower phosphorus removal. The cellular N and P compositions were also found to be proportional to the N:P ratio in the wastewater [51]. Analysis of internal cellular composition can inform species selection for nutrient remediation [52].

CO_2 Feedstocks

Highly productive cultivation of microalgae requires supplemented CO_2 beyond the CO_2 diffusion from ambient air. The growth rate of the microalgae is often limited by the quantity and availability of that carbon source. The addition of pure carbon dioxide has been shown to significantly increase microalgal biomass production and lipid content, but it is largely cost and energy prohibitive due to the energy needed for compression and transportation. Lower cost flue gas shows promise as well but comes with its own problems. Transporting flue gas is even more cost prohibitive than pure CO_2 and it contains toxic inhibitors such as NOx, SOx, and heavy metals. The utilization of gaseous CO_2 by microalgae is also limited by the low solubility and diffusivity of carbon dioxide in water.

Another promising source of CO_2 for microalgae cultivation is biogas from anaerobic digestion. Biogas is typically composed of between 20 and 60% CO_2 with the majority of the remainder being methane as well as a smaller fraction of H_2S. Biogas can be bubbled into algae reactors to provide carbon for algal growth and improve nutrient removal while simultaneously upgrading the methane composition and higher heating value of the biogas through selective absorption of CO_2. An airlift photobioreactor cultivating microalgae *Tetradesmus obliquus* in anaerobically digested livestock manures was able to achieve a 70% increase in biomass productivity with biogas bubbled in when compared to air, while increasing the lower heating value of the gas from 22,554 to 33,294 kJ $m-3$ (47.6%). The cultivated microalgae also reduced N, P, and BOD to a level adequate for livestock consumption.

Injecting biogas into microalgae cultivated in anaerobic digestion effluent results in increased biomass production, higher nutrient removal, and yields a more concentrated methane product [53].

While biogas shares the prohibitive cost of transportation with pure CO_2 and flue gas, greater opportunities exist to build microalgae cultivation infrastructure near agricultural waste anaerobic digesters when compared to flue gas. Sources of flue gas tend to be limited in surrounding space due to their location in industrial and power generating areas while agricultural anaerobic digesters are located near sources of livestock waste where land is often less expensive and more available.

Hydroponic Cultivation

Hydroponic Cultivation Advantages

Hydroponic plants are grown without soil or in soilless media with the nutrients provided by a nutrient solution; the plants' roots need to be anchored in a substrate [54]. This method of horticulture eliminates and mitigates many of the risks faced by traditional soil agriculture. For one, the risk of soil-borne contaminants, pests, and diseases is eliminated, and the risk of nutrient runoff into groundwater is prevented provided it is a closed system [55]. Additionally, hydroponic cultivation of lettuce has been found to require only 8% of the freshwater required for similar yields of conventional agriculture, although it required significantly more energy. Hydroponic production was also found to require only 10% of the land required for traditional agriculture and the land does not need to be arable, which offers significant advantages in terms of space and location flexibility [56]. While hydroponic cultivation has currently not replaced conventional agriculture to any large degree, the potential exists to lower costs by supplying inexpensive wastewater nutrients and using renewable energy sources.

Salt Inhibition

One issue limiting the application of hydroponic cultivation is the inhibition caused by high salinity in the nutrient solution [57]. Additionally, there is interest in utilizing seawater entirely or mixed with freshwater for hydroponic cultivation to reduce freshwater usage [58]. High salinity has been found to reduce plant growth and photosynthetic activity in terrestrial and hydroponically-grown plants [59]. One study examined Swiss chard, *Beta vulgaris* growth under varying salt concentrations (0, 100 and 200 mM NaCl) and found that growth was inhibited at 100 and 200 mM NaCl (28.5 and 49.5% reduction in shoot and fresh weight respectively). The study identified two mechanisms of salt tolerance in *Beta macrocarpa* (wild Swiss chard): osmotic and apoplastic water adjustment [60]. Another study examined the effect of NaCl stress on hydroponically grown *Bruguiera parviflora* at salt levels of 0, 100, 200 and 400 mM. It was found that higher salt concentrations resulted in reduced nitrogen reductase and phosphatase production as well as a corresponding decrease in nitrogen and phosphorus uptake [61]. The inhibitory levels of EC and NaCl concentration vary by plant species; even tolerant plants such as Swiss chard are inhibited by excessive concentrations.

Chapter 3: The Development of the Integrated Biological System for Full Utilization of Livestock Waste

Introduction

In this chapter, a series of technologies are demonstrated that are designed towards mitigating the environmental problems faced by animal production facilities in Minnesota by reducing water pollution and producing value-added products and energy from waste. The technology is intended to completely treat and utilize animal manures using a multi-stage approach consisting of the following processes:

(1) Novel manure pretreatment to improve the digestibility of swine manure through C:N ratio adjustment with corn stover and conditional stripping

(2) Mesophilic anaerobic digestion (AD) of pretreated swine manure after ammonia and hydrogen sulfide reduction to improve methane and nitrogen fertilizer production

(3) Flocculation to remove suspended solids, struvite precipitation, and dilution to improve water clarity, and capture organic insoluble particles for conversion to bio-oil

(4) Advanced microalgae cultivation to convert remaining nutrients (Nitrogen, Phosphorus, and COD) in the manure to valuable algal biomass for biofuel production or for high quality animal and fish feed

(5) Hydroponic cultivation of salt tolerant Swiss chard to further remove nutrients and produce biomass in the post-microalgae culture broth

System Design

The system is designed to treat two liters of liquid swine manure (LSM) per day. It includes a thermal vacuum pretreatment followed by three biological treatment systems:

anaerobic digestion, microalgae cultivation, and hydroponic cultivation. In the process diagram (Figure 1) each subsystem has a specific role in nutrient removal. The pretreatment system captures ammonia and hydrolysis carbohydrates for digestion, anaerobic digestion removes COD and organic solids and produces biogas; microalgae and activated sludge bacteria remove large amounts of nitrogen and phosphorus whereas hydroponic plants further condition the water and produce biomass.

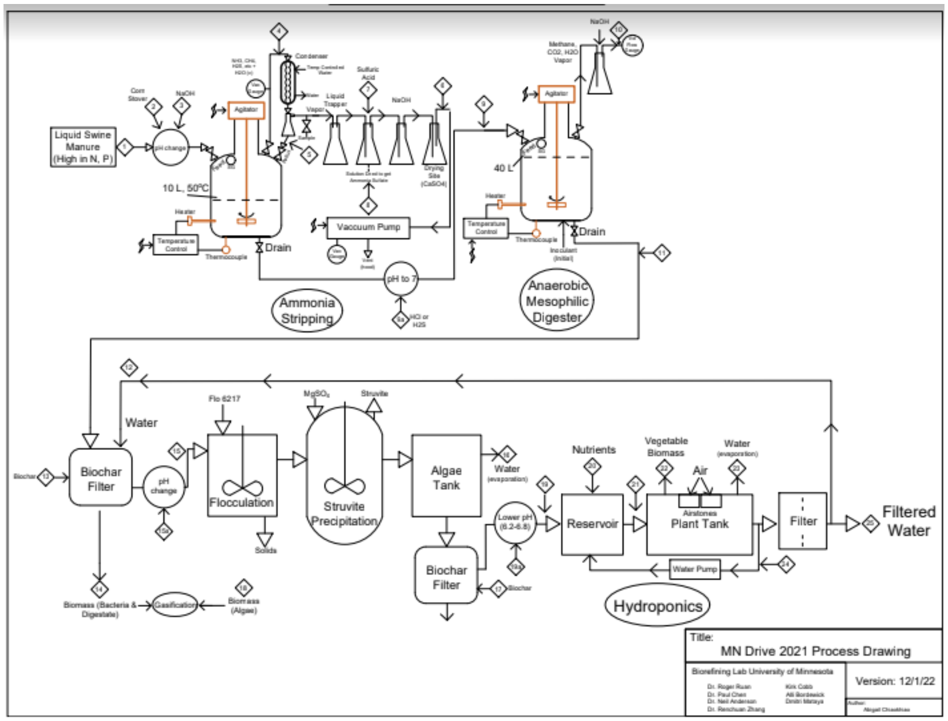


Figure 1: The combined process diagram for the integrated systems. The inputs and outputs of each process are shown. Process diagram was developed with the contribution of[Abigail Chiaokhiao]

Thermal Vacuum Stripping

Previous research done by Dr. Renchuan Zhang in the University of Minnesota’s Bioproducts and Biosystems Engineering Department found that AD is inhibited by ammonia levels higher than 1000 mg/L and microalgae and hydroponic cultivation are inhibited at even lower levels of ammonia. Liquid swine manure (LSM) was found to have an ammonia concentration over 5000 mg/L; a level that would significantly inhibit AD. In order to maximize the biogas production and digestion of the AD process, pretreatment of the LSM is necessary. Thermal-assisted vacuum stripping when operated between a pH range of 9-10 for 60 minutes was found to remove 85-95% of ammonia from a small lab scale system [62]. This pretreatment process will allow the digestion process to occur without ammonia inhibition. The pretreatment similarly reduces hydrogen sulfide concentration, another inhibitor in the digestion process. The heat and caustic also contribute to the hydrolysis step of AD by hydrolyzing lignocellulosic corn stover, reducing carbohydrates to simple sugars.

Methods and Materials

While the research mentioned above by Dr. Renchuan Zhang investigated thermal vacuum stripping in a 1 liter lab scale apparatus, this vacuum stripping pretreatment process occurs in a 26 liter sealed stainless-steel vessel with a heater and thermocouple in a feedback loop, drain port, feed port, sight glass, and condenser/reflux port as shown in the process sub-diagram in Figure 2 and the vessel diagram in Figure 4. The vessel is agitated with the assistance of stainless-steel baffles to improve mixing and prevent distinct liquid layers from forming. The vacuum pump pulls through the condenser which will remove much of the gaseous water and returns it through the reflux port. The remaining gas is pulled through a series of vessels including a sulfuric acid vessel that reacts with the gaseous ammonia to form ammonium sulfate which can be recovered for use as nitrogen fertilizer. A caustic solution is used to capture hydrogen sulfide and carbon dioxide and a final drying vessel ensures that any remaining moisture will be removed before entering the vacuum pump itself. These gas washing and drying vessels will extend the life of the vacuum pump by capturing corrosive gasses and moisture before it enters the pump..

The 26 liter vessel is operated with a maximum working volume of 18 liters in order to mitigate issues caused by potential foaming. The residence time for the manure in the vacuum stripper is less than six hours (compared to the ~20 day residence time of the anaerobic digester). This means that the pretreatment process will not be the volume limiting process in the total system.

Thermal Vacuum Stripping Results and Discussion

The vacuum stripping process in the 26 liter vessel removed and captured 79% of the ammonia in six hours from liquid swine manure when operating at full operational volume at a pH 0f 10, a vacuum pressure of 660 mmHg and temperature of 60 C (figure 3). This is a significant reduction with the downstream effect of reducing ammonia influent into the AD which has been found to increase COD removal and biogas output. This reduction is slower and less complete than the small lab scale experiments, but still represents a major reduction in ammonia. This reduction in efficiency may be the result of an increased depth of the vessel which was identified as a critical factor due to the increased bubble retention time [63]. The efficiency of the system could likely be improved by redesigning the vessel to be wider with less depth.

This process has significant chemical requirements due to the high buffering capacity of the LSM influent. It required 17 grams of NaOH to raise a liter of LSM to a pH of 10, and a subsequent 0.56 moles of HCl to lower the pH to 7 to be suitable for algae cultivation. This chemical requirement is likely prohibitively intensive and TVS efficacy dropped dramatically at lower pHs at the same temperature, so future work on high temperature processes may be able to achieve similar ammonia removal with less chemicals. Since the process is not volume limiting, a smaller pH change and higher temperature may be used which would increase the required residence time of the LSM in the pretreatment but reduce the buildup of salts due to pH changes. It is anticipated that salt build up will inhibit hydroponic plant growth, so less significant pH changes may be beneficial. Further optimization of this process may yield similar or greater ammonia removal with lower chemical requirements, although likely at the cost of greater energy.

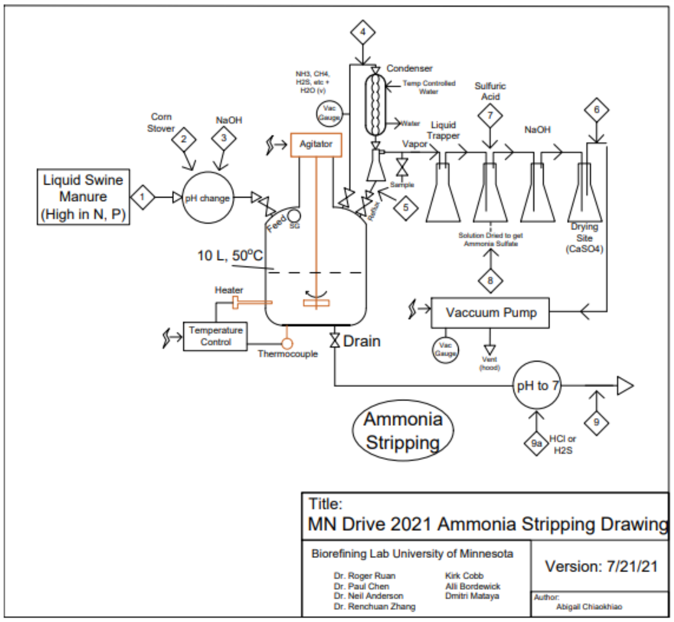


Figure 2: Ammonia stripping process subdiagram showing the vessel, condenser, gas washing vessels, and vacuum pump.

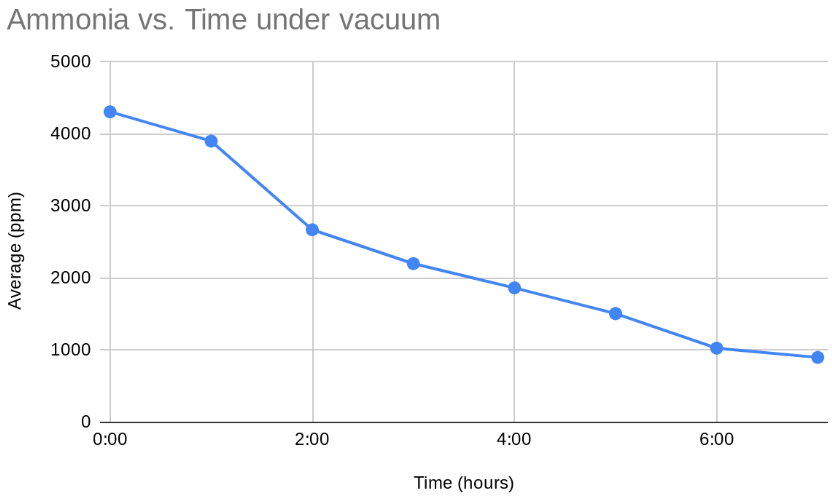


Figure 3: Graph of ammonia concentration over time in hours under vacuum (660 mmHg).

Ammonia is removed over time from the LSM.

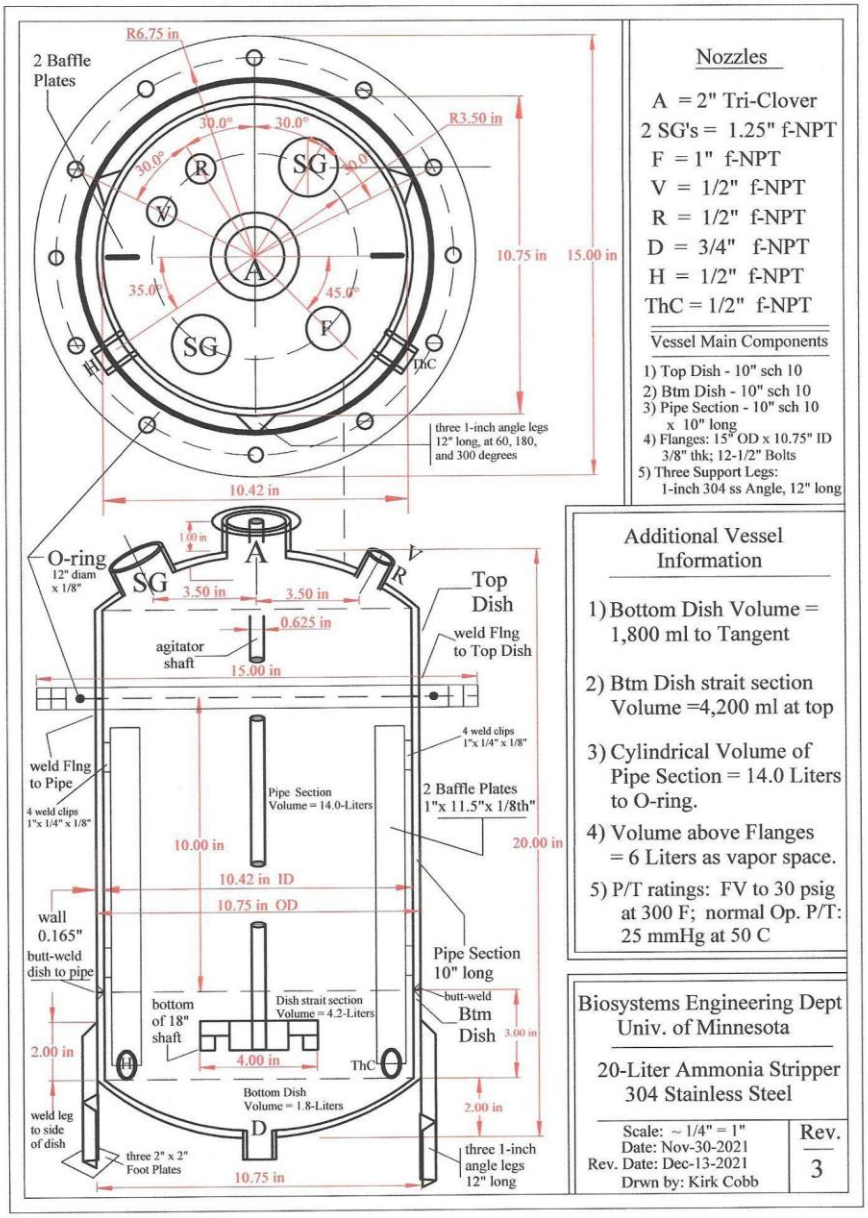


Figure 4: Schematic diagram of the ammonia stripping vessel. 1:4 scale Developed in consultation with Kirk Cobb

Anaerobic Digestion

Anaerobic Digestion has been found to be effective at stabilizing wet organic solids and high strength wastewater and producing biogas. The process works effectively across a large range of moisture contents of the feedstock, which makes it useful for many types of animal wastes. The resulting liquids and solids are unsuitable for direct discharge due to a high nutrient content, but can be separated by phase with the liquid portion being used to cultivate microalgae and hydroponically grown plants while the solid portion can be converted to bio-oil, syngas, and biochar. Pretreatment of the feedstock for AD will mitigate the ammonia and hydrogen sulfide inhibitions on biogas production.

Methods and Materials

The anaerobic digester is a polyethylene vessel that has a heater and thermocouple in feedback to maintain a temperature optimal for mesophilic bacteria (35C) as well as an agitator with baffles for mixing, drain port, feed port, sight glass (SG), and biogas output port (Figure 6). Previous work has focused on thermophilic AD due to its superior process efficiency, though mesophilic conditions were chosen due to the greater long term system stability. The pretreatment process begins hydrolysis and makes the higher thermophilic temperatures unnecessary. The 50 liter vessel operates with a working volume of 40 liters and a residence time of 20 days. It was fed with 2 L/day LSM with an average volatile solids content of 4% and an ash content of 3.5%. The digester seed was a proprietary bacterial community from Riverbend Biolabs and the LSM was obtained from the swine production facility on the UMN St Paul campus. Biogas production was measured one hour before and one hour after feeding with a U tube manometer.

Anaerobic Digestion Results and Discussion

The process reduced volatile solids by 57.5% and COD by 49% in twenty days, although a longer residence time may be capable of greater reductions. The volatile solid content of the influent and effluent for a five-week period after achieving steady state are shown in figure 4.

Biogas output averaged 0.465 L/hr with a composition of 64% CH_4 and 36% CO_2. H_2S was not measured. The difference in biogas production before and after feeding for a time period is shown in Figure 5. This equates to a cumulative methane gas production of 139.5 mL/g VS which is lower than expected at this volatile solid consumption rate. The measurement timing may not have captured the peak production times. Despite the measured gas output, the COD and VS reduction rates are fairly high and consistent with the literature which have volatile solids contents around 50% [64].

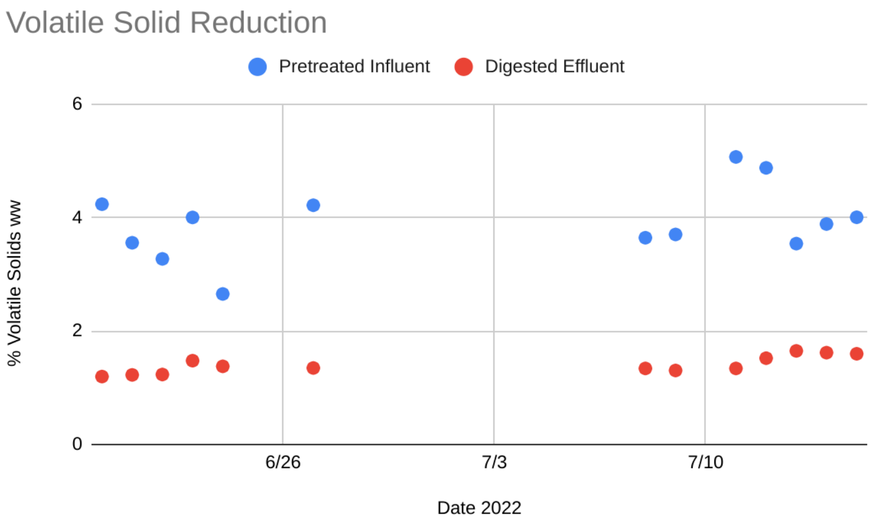


Figure 4: A graph of volatile solids entering and exiting the anaerobic digester after achieving steady state. The difference between VS in and out is the consumed VS.

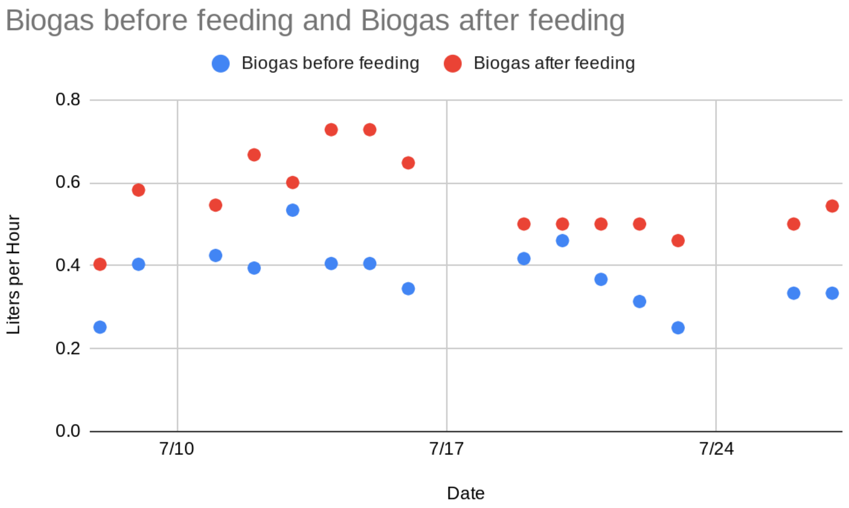


Figure 5: A graph of measured biogas output an hour before and an hour after adding pretreated LSM. The measurements were intended to capture peak and minimum gas production times.

Figure 6: The 40 L AD vessel has an agitator, feeding port, sample port, heater, thermocouple, and gas port as shown above. The digester vessel is approximately 35 cm by 53 cm and the temperature is maintained at 35 C. Height is approximately a meter with the agitator.

Struvite Precipitation

Struvite precipitation has been found to be an effective way to capture phosphorus and nitrogen from AD effluent [65]. In order to precipitate struvite, Mg 2+ is added and reacts with phosphorus and ammonia to form struvite in the reaction:

$$\mathrm{Mg2+ + NH4+ + H2PO4\text{-}1 + 6H2O \rightarrow MgNH4PO4\cdot 6H2O + 2H+}$$ [66]

Struvite acts as a slow release fertilizer with lower risk of nutrient runoff, and it can be transported more economically than LSM [67]. For this reason, struvite precipitation was applied to the integrated wastewater treatment system and evaluated.

Methods and Materials

Prior to precipitation, suspended solids were removed through flocculation. The flocculant used was 10 mL/Liter LSM of Flo-6217 (0.5%, SNF Inc., US) which was mixed in at 350 rpm for one minute before being stirred at 60 rpm for two minutes and being allowed to settle for 4 hours. Following that process, MgSO4 was added at a 1:1 Mg:P molar ratio and stirred at 150 rpm for 10 minutes and allowed to settle. This ratio was chosen from literature which found that a 1;1 molar ratio could remove 60% of PO_4-P which leaves a more optimal N:P ratio for the microalgae and produces struvite crystals which can be recovered for fertilizer. The process also removes ammonia (NH_4+) at a 1;1 molar ratio with phosphorus [68] [69].

Results and Discussion

In the post-flocculation AD effluent, the NH_4+ and PO_4-P are at 1630 and 691 mg/L respectively. After the struvite precipitation using 875 mg (7.3 mmol) $MgSO_4$ per liter LSM, the NH_4+ is reduced by 31% to 1120 mg/L while the PO_4-P is reduced by 47.9% to 331 mg/L. This 360 mg P/Liter reduction represents a 3.79 mmol reduction while 3.49 mmol Mg 2+ remains in the system. A higher concentration of Mg 2+ could increase the ammonia and phosphorus removal but would have higher chemical requirements and could remove too much of the phosphorus and reduce algae growth. Further optimization of the Mg addition ratio for variety influent compositions may be able to maximize struvite precipitation in a way that provides optimal nutrient ratios to the algae.

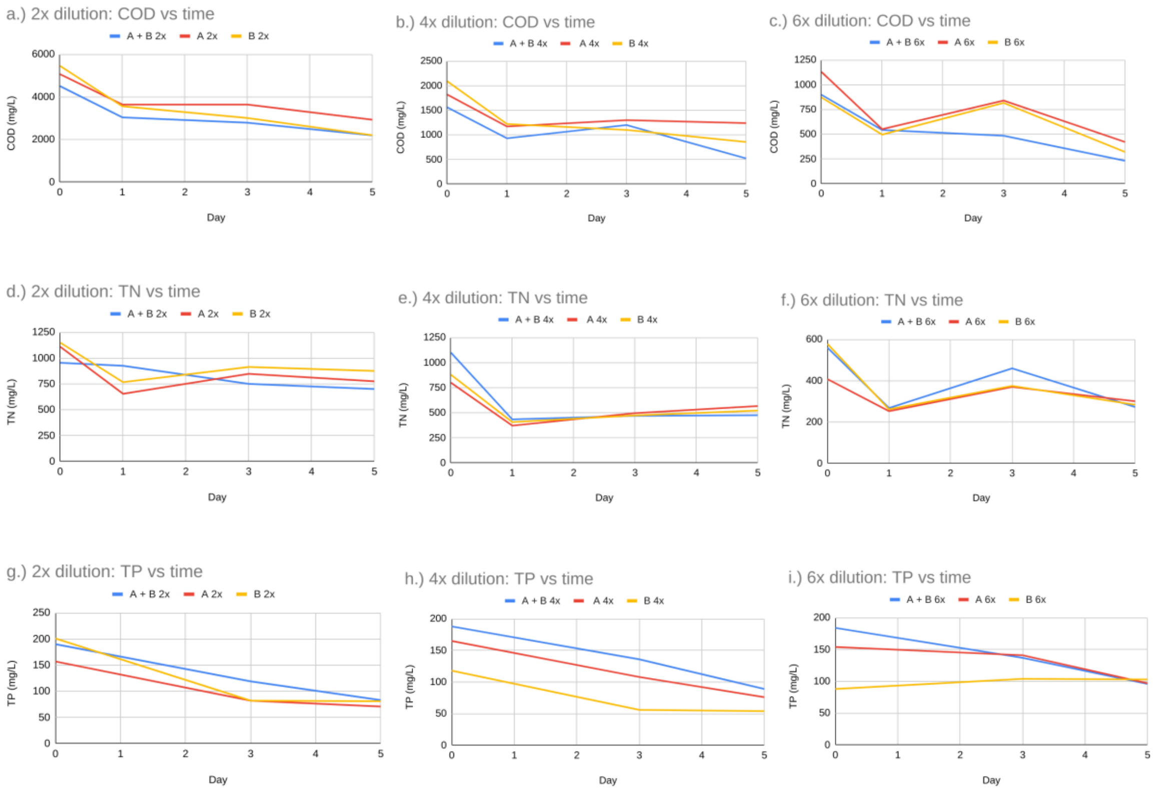
Microalgae and Activated Sludge

Methods and Materials

The algae and bacteria experiments were inoculated with *Chlorella vulgaris* and activated (secondary) sludge from the Metropolitan wastewater treatment plant. A 20ml/L LSM activated sludge was used for each of the bacteria conditions and 100mL/L LSM of algae medium at 3 g biomass/L of were used for inoculation.. The laboratory scale experiments used 250 mL flasks with a 150 mL working volume and were cultivated in a controlled shaker at 33 C and 60 rpm with artificial light. Nutrient concentrations were determined by Hach test kits. Three dilution rates (2x, 4x, and 6x) were used and three test conditions: algae and bacteria (a+b), algae (a), and bacteria (b), were applied. The initial pH was set at 6.7 for each condition.

Results and Discussion

All of the test conditions were capable of reducing TP, COD, and TN to varying degrees. The optimal dilution rate was found to be 4x, which experienced an average TP reduction of 54%, an average COD reduction of 52%, and an average TN reduction of 44%. The 6x dilution condition achieved a higher COD reduction of 66% but achieved the same TN reduction and a lower TP reduction (30.5%) (Figure 7). The best condition for inoculation was found to be algae and activated sludge bacteria (A + B). This condition averaged a 52% reduction in TP, a 58% reduction in COD, and a 45% reduction in TN. The next best condition was bacteria alone, which averaged a 42% reduction in TP, a 60% reduction in COD, and a 36% reduction in TN. The best individual condition was algae and bacteria at 4x dilution which achieved a 53% reduction in TP, a 67% reduction in COD, and a 57% reduction in TN. This is the optimal condition for treatment. Two factor ANOVA was performed on the nutrient depletion rates to determine statistical significance. (Table 1) None of the results were significant to $p < 0.05$. The strongest correlation was found between TP removal and dilution rate ($p = 0.11$). The next strongest were between inoculation conditions ($p = 0.17$) and COD removal and dilution rate and COD removal ($p = 0.13$). The TN removal was weaker with p values of 0.21 and 0.25 for dilution and inoculation conditions respectively.



a.) 2x dilution: COD vs time

b.) 4x dilution: COD vs time

c.) 6x dilution: COD vs time

d.) 2x dilution: TN vs time

e.) 4x dilution: TN vs time

f.) 6x dilution: TN vs time

g.) 2x dilution: TP vs time

h.) 4x dilution: TP vs time

i.) 6x dilution: TP vs time

Figure 7 a, b, c, d, e, f, g, h, i: Graphs of nutrient values over time for each of the test conditions. x denotes the dilution factor, A represents algae, B represents bacteria and A + B is both. a through c are COD concentrations over time, d through f are TN concentrations, and g though i are TP concentrations. The best condition was found to be A+B 4x.

COD					
ANOVA	DF	SS	MS	F	P-value
Dilution Rate	2	452.4067	226.2033	2.8197 (2,4)	0.1722
A+B vs A vs B	2	576.5067	288.2533	3.5932 (2,4)	0.1279

Error	4	320.8867	80.2217		
Total	**8**	**1349.8**			
Percent COD depletion					
	A + B	A	B		
2x	51.4	42.2	59.7		
4x	66.6	31.9	58.6		
6x	74.2	62.7	63.3		
TN					
ANOVA	DF	SS	MS	F	P-value
Dilution Rate	2	482.4156	241.2078	2.3344 (2,4)	0.2129
A+B vs A vs B	2	408.1422	204.0711	1.975 (2,4)	0.2532
Error	4	413.3111	103.3278		
Total	**8**	**1303.8689**	**162.9836**		
Percent TN depletion					
	A + B	A	B		
2x	26.6	30.2	23.8		
4x	56.8	29.1	40.1		
6x	51.3	26.2	50.8		
TP					
ANOVA	DF	SS	MS	F	P-value
Dilution Rate	2	166.6467	83.3233	3.8924 (2,4)	0.1152
A+B vs A vs B	2	67.3867	33.6933	1.574 (2,4)	0.3132
Error	4	85.6267	21.4067		
Total	**8**	**319.66**	**39.9575**		
Percent TP depletion					

	A + B	A	B		
2x	56.3	54.8	59.7		
4x	52.6	53.9	54.2		
6x	52	37	50.8		

Table 1: Depletion percentages for each nutrient and each test condition and the results of 2 factor ANOVA. None of the results were statistically significant at $p < 0.05$ but A + B tended to be superior for nutrient depletion, and higher dilution rates tended to improve nutrient removal.

Ozone Application to Algae Effluent

Methods and Materials

The effects of ozone gas on filtered post algae treatment wastewater was investigated in regard to COD, ammonia and phosphorus concentrations. The hypothesis tested was that ozone would be able to degrade recalcitrant COD. The post algae waste had ozone applied directly through an porous airstone in a 4 liter flask containing 2 liters of waste. The ozone came from a Pacific Ozone Technology ozone gas generator at 50% ozone output being fed pure oxygen (99.8%) at a flow rate of 2.8 liters per minute (6 SCFH).

Results and Discussion

The application of ozone gas had obvious effects on wastewater color as well as decreased the COD concentration. As shown in Figure 8, the color of the waste was reduced significantly from nearly opaque before ozone application to nearly clear after 30 minutes. This change in light transmission could have significant positive effects on algae growth if used before that process, although the energy and oxygen requirements are high and the reduced nutrients are not utilized. Additionally, the ozone process affected COD and ammonia. The remaining COD was reduced by 30% after 30 minutes (figure 9) with the ammonia concentration being reduced by 28%. This ammonia and COD is lost and unable to be utilized, so this process is less than ideal for complete utilization, but it can be an option for further reducing recalcitrant COD and reducing pathogen load if needed. There appeared to be no effect on TP. Further study on improving ozonation efficiency by decreasing the ozone required to reduce COD could make this process viable for a biological system, although any nutrients removed are lost.

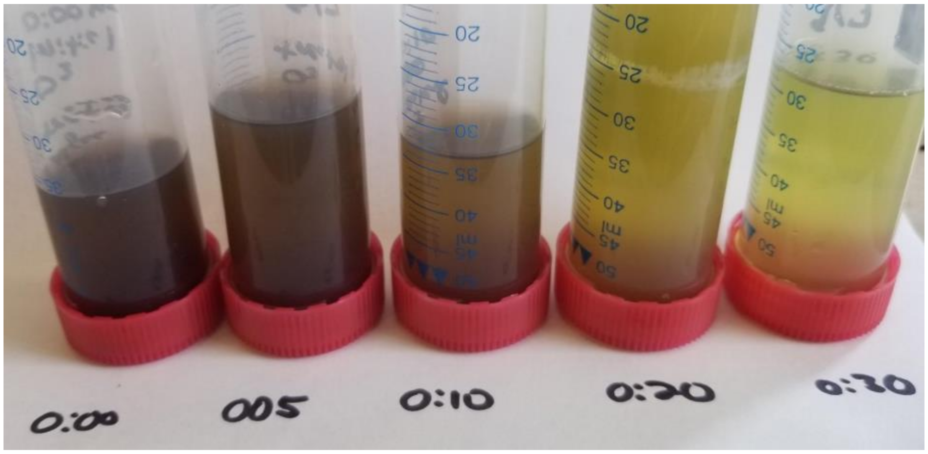


Figure 8: Image of postalgae wastewater samples taken after being treated by ozone for progressive time periods (0, 5, 10, 20, and 30 minutes). The application of ozone resulted in rapid and obvious changes in water clarity.

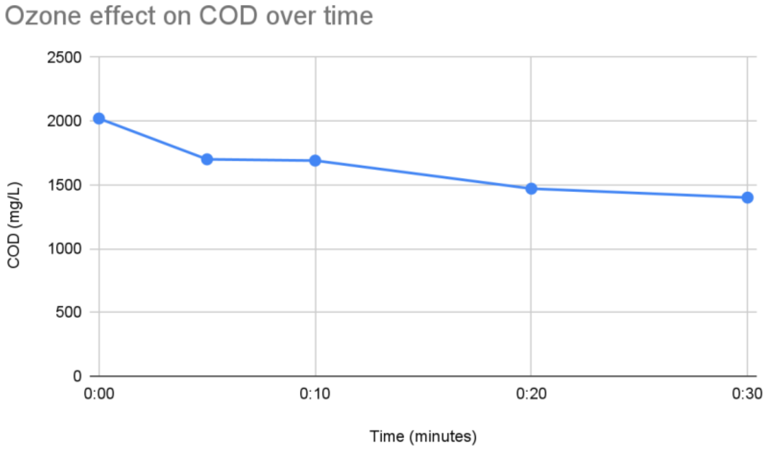


Figure 9: A graph of COD concentration over time under applied ozone. There is an initial decrease in COD followed by a much less rapid decrease over time.

Hydroponics

Declaration of Contributions

The work done in the hydroponics section with the exception of the small-scale hydroponic experiments were done in collaboration with Emily Lefrancois. I designed, modified, and maintained the hydroponic system as well as collaborated on experimental design and analysis of samples and results. Emily Lefrancois planted, measured biomass and analyzed samples and results for the ozone application experiment in the larger hydroponics system. She provided Figures 12 a and b and 13 a and b. The aforementioned sections are all in my own words, but using results obtained collaboratively. The small-scale ozone application section was not done collaboratively.

Figure 10: An image of both hydroponic production systems. The recirculation pump and reservoir are located on the far right. The growing area is approximately 1 x 1.5 m for each system.

Hydroponic Intermittent Ozone Application

Methods and Materials

This experiment tested the effect ozone had on nutrient concentration and biomass production of plants grown in hydroponics amended with algae effluent. Swiss chard was chosen due to its high salt tolerance and overall productivity. The hydroponic cultivation system consists of a 260 liter tank recirculating with a detachable 60 liter reservoir for a total working volume of 320 liters. The solution is aerated by a blower motor connected to airstones and the system is artificially illuminated by two 250 Watt LED lights to support photosynthesis on a long day photoperiod (16 hour day, 8 hour night). Each tank has space for 45 plants. Two of these functionally identical systems are fabricated (Figure 10) for comparison. Alpha tank

(Tank A) functioned as a control while the Bravo tank (Tank B) received the treatment of ozone application. The ozone came from a Pacific Ozone Technology ozone gas generator at 50% ozone output being fed pure oxygen (99.8%) at a flow rate of 2.8 liters per minute (6 SCFH). Ozone was applied for 30 minutes every feeding day (3x per week) through an eductor located at the outlet of the recirculation pump.

Three cultivars of Swiss Chard (Bright Yellow, Peppermint, and Rhubarb Chard) were again used due to no significant difference observed in preliminary experiments. Seeds were started in rockwool plugs and germinated under mist in a greenhouse environment (21C day/night) before being transplanted to the hydroponic system at the sign of first true leaves approximately two to three weeks after sowing. Each of the identical tanks were started with an initial half-rate charge of commercial fertilizer (0.32 g/L of Jack's Nutrients 5-12-26 Hydroponic Fertilizer and 0.49 g/L calcium nitrate) and fed with algae effluent three times weekly for a total of 28 L per tank per week. Tap water was used to replace water loss due to evaporation three times per week. Additionally, the volume added through feeding was removed from the tank to maintain water levels and prevent salt accumulation. All of the leaves over 15cm were harvested weekly and dried to obtain fresh and dry biomass measurements. Macronutrient levels (nitrate (NO3-N), phosphorus (PO4-P), potassium (K+), and ammonia) were also acquired on a weekly basis. Algae effluent feeding was paused for two weeks after the plants had achieved steady growth (Figure 12) to obtain nutrient removal rates.

Results and Discussion

In terms of biomass, Alpha tank produced 1588 grams of biomass (135.5 g per week) while Bravo tank produced 1471 grams (120.6 g per week) however this difference was not statistically significant (Table 2). This suggests that intermittent ozone application likely does not affect biomass productivity in hydroponic cultivation. During the course of the experiment, algae effluent feedings were paused for a two-week period to determine nutrient removal rates for both systems (week 5 to week 7; Figure 12). Even after feeding resumed for the last two weeks of the experiment, all macronutrient levels (excepting potassium) remained below the 20 ppm threshold for safe wastewater disposal. In these two weeks, the hydroponic systems were able to remove on average 65% of potassium, 90% of total nitrogen, all of the ammonia, and 86% of phosphorus. The pause in feeding did lead to reduced plant biomass going forward as shown in Figure 11, but also showed the capacity of this system to reduce nutrients to dischargeable levels.

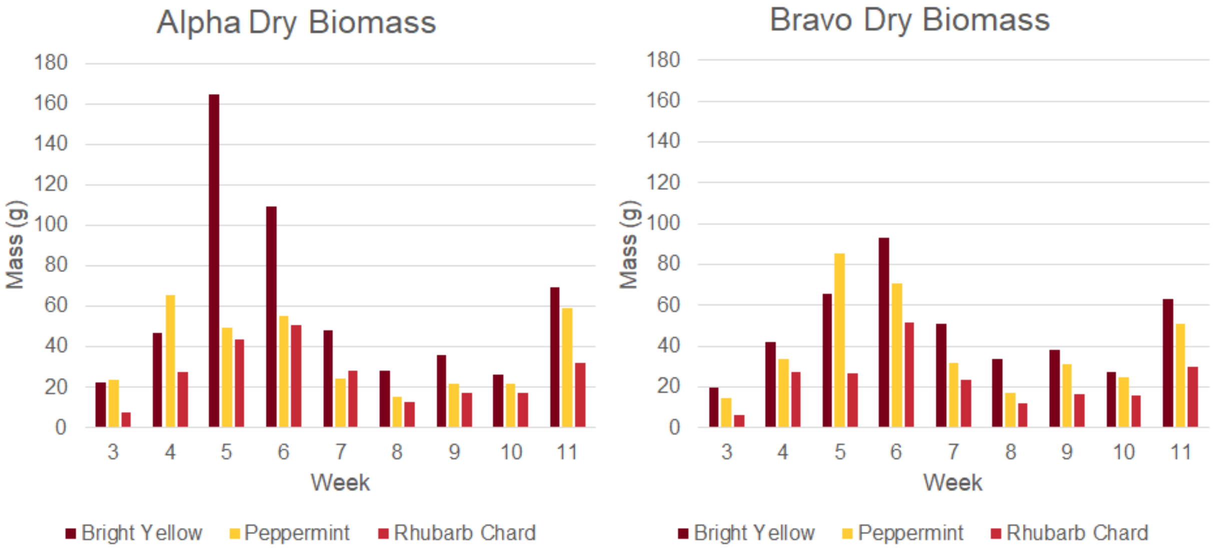


Figure 11 a, b: Dry biomass obtained weekly from each hydroponic tank for each cultivar. Alpha is the control tank and Bravo had ozone applied.

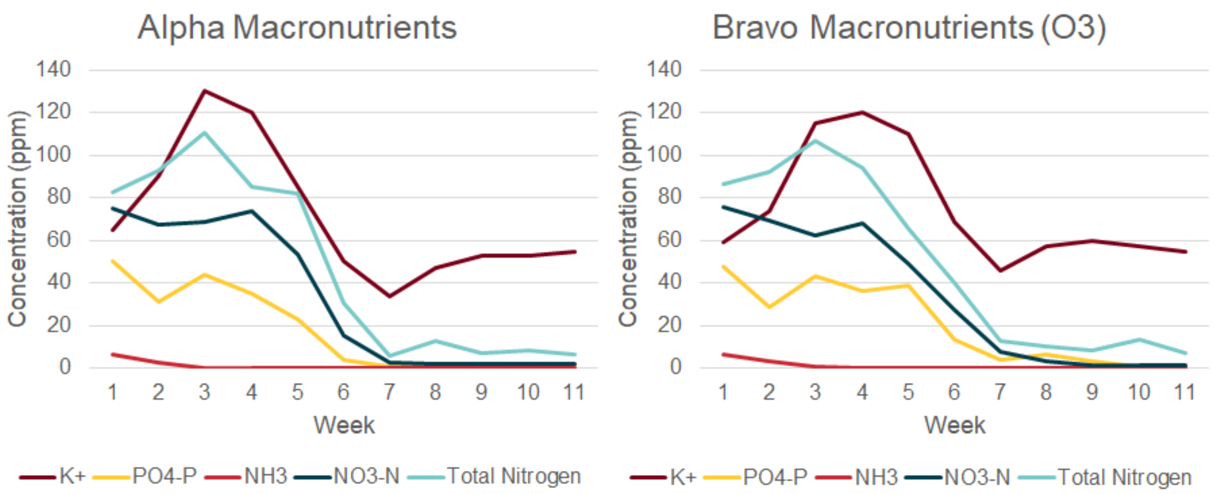


Figure 12 a, b: Nutrient values over time for each hydroponic system. Feeding was stopped for weeks 5-7 to obtain nutrient removal rates.

Groups	N	Mean (g/plant)	Std Dev	Std Error	
Tank A	120	2.54	2.83	0.26	
Tank B	120	2.45	2.58	0.24	
	df	SS	MS	F value	P Value
Between Groups	1	0.415	0.415	0.05	0.812
Within Groups	238	1752	7.36		
Total	239	1753			

Table 2: Data Table for dry biomass harvested per plant each week. There was no significant difference between the treatment and control tank in terms of dry biomass productivity per plant.

Hydroponic Initial Ozone Application

Methods and Materials

Additional study on the effects of one-time initial ozone treatment of algae effluent on hydroponic plant growth was conducted on 3 small 5 liter hydroponic systems. One system was charged with box nutrient solution (0.64 g/L of Jack's Nutrients 5-12-26 Hydroponic Fertilizer and 0.98 g/L calcium nitrate. The second was charged with algae effluent without additional treatment, and the third was charged with algae effluent after being treated with ozone applied directly through a porous airstone in a 4 liter flask containing 2 liters of waste for 10 minutes from pure oxygen (99.8%) at a flow rate of 2.8 liters per minute (6 SCFH). This was done only once initially as opposed to intermittently in the large scale system. The plants were germinated in the same method as previously mentioned, with six plants per system and at the end of three weeks, fresh weights were determined from the plants in each system.

Results and Discussion

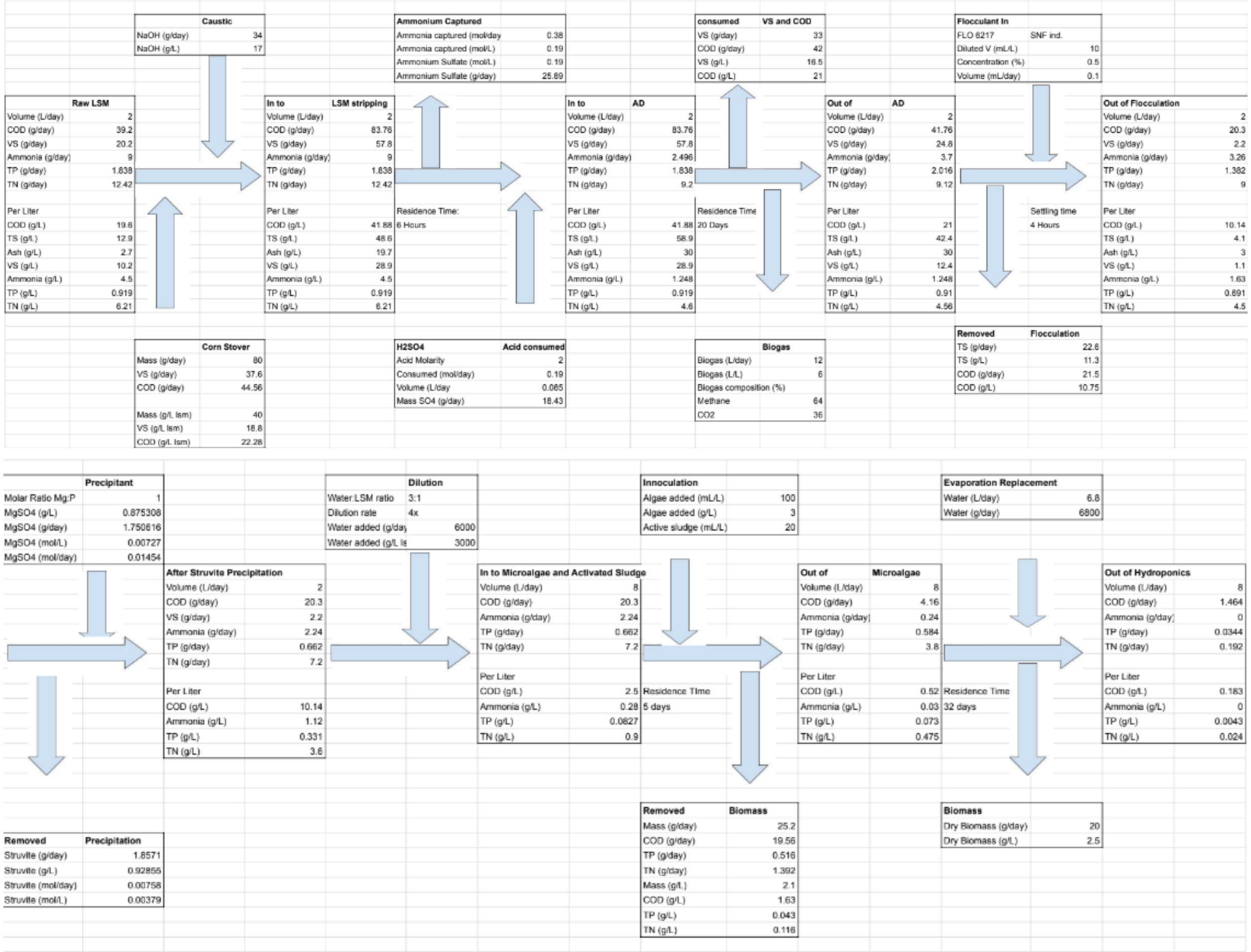
Early on, there were significant differences observed between the systems. As shown in Figure 13, the optimal nutrient tank grew quickly and the biomass filled the available space, while the ozone treated system grew slower, and the untreated algae tank grew the slowest. The final biomass also confirms this observation with the average dry biomass per plant in the optimal tank being 5.5 grams, with the ozone treated tank averaging less than 50% of that biomass at 2.22 grams and the untreated algae effluent system averaging only 1.07 grams. The ozone treated effluent performed significantly better than the untreated, but still was much worse than the optimal conditions. Further steps to optimize nutrient ratios in hydroponics may improve biomass accumulation, but would also require higher chemical inputs into the system.

It is possible that captured ammonium sulfate from vacuum stripping could be used to raise nitrogen levels in hydroponics if needed without an external input.

Figure 13: Image of plants on week 2 with the leftmost system in each image containing the control solution, the middle containing ozone treated algae effluent, and the rightmost containing untreated algae effluent. There are obvious differences in productivity on week 2.

Mass and Nutrient Balance

The overall mass and nutrient balance is based on measured data as well as derived calculations therefrom (Table 3). The best treatment conditions were used for the nutrient balance. Ozone application was not included in the final balance due to the limited effectiveness. Inputs required per liter of LSM treated are as follows: 17 grams NaOH, 40 grams of corn stover, 0.19 mol H2SO4, 0.1 mL of flocculant (FLO 6217), 0.87 grams MgSO4, and 6.4 liters of water (3 for dilution, 3.4 to replace evaporation losses in hydroponics). From this the following outputs per liter of LSM can be obtained: 12.4 grams ammonium sulfate, 6 liters of biogas (64% methane), 11.3 grams of organic solids, 0.93 grams of struvite, 12.6 grams of algae biomass, and 10 grams of plant biomass. In addition to those inputs and outputs the nutrient reduction across the whole system in percent is as follows: 96% reduction in COD (using initial COD which does not include added corn stover), 100% reduction in ammonia, 98% reduction in total phosphorus, and a 98.5% reduction in total nitrogen.



	Caustic
NaOH (g/day)	34
NaOH (g/L)	17

Ammonium Captured	
Ammonia captured (mol/day	0.38
Ammonia captured (mol/L)	0.19
Ammonium Sulfate (mol/L)	0.19
Ammonium Sulfate (g/day)	25.89

consumed	VS and COD
VS (g/day)	33
COD (g/day)	42
VS (g/L)	16.5
COD (g/L)	21

Flocculant In	
FLO 6217	SNF ind.
Diluted V (mL/L)	10
Concentration (%)	0.5
Volume (mL/day)	0.1

	Raw LSM
Volume (L/day)	2
COD (g/day)	39.2
VS (g/day)	20.2
Ammonia (g/day)	9
TP (g/day)	1.838
TN (g/day)	12.42
Per Liter	
COD (g/L)	19.6
TS (g/L)	12.9
Ash (g/L)	2.7
VS (g/L)	10.2
Ammonia (g/L)	4.5
TP (g/L)	0.919
TN (g/L)	6.21

In to	LSM stripping
Volume (L/day)	2
COD (g/day)	83.76
VS (g/day)	57.8
Ammonia (g/day)	9
TP (g/day)	1.838
TN (g/day)	12.42
Per Liter	
COD (g/L)	41.88
TS (g/L)	48.6
Ash (g/L)	19.7
VS (g/L)	28.9
Ammonia (g/L)	4.5
TP (g/L)	0.919
TN (g/L)	6.21

Residence Time:
6 Hours

In to	AD
Volume (L/day)	2
COD (g/day)	83.76
VS (g/day)	57.8
Ammonia (g/day)	2.496
TP (g/day)	1.838
TN (g/day)	9.2
Per Liter	
COD (g/L)	41.88
TS (g/L)	58.9
Ash (g/L)	30
VS (g/L)	28.9
Ammonia (g/L)	1.248
TP (g/L)	0.919
TN (g/L)	4.6

Residence Time
20 Days

Out of	AD
Volume (L/day)	2
COD (g/day)	41.76
VS (g/day)	24.8
Ammonia (g/day)	3.7
TP (g/day)	2.016
TN (g/day)	9.12
Per Liter	
COD (g/L)	21
TS (g/L)	42.4
Ash (g/L)	30
VS (g/L)	12.4
Ammonia (g/L)	1.248
TP (g/L)	0.91
TN (g/L)	4.56

Settling time
4 Hours

Out of Flocculation	
Volume (L/day)	2
COD (g/day)	20.3
VS (g/day)	2.2
Ammonia (g/day)	3.26
TP (g/day)	1.382
TN (g/day)	9
Per Liter	
COD (g/L)	10.14
TS (g/L)	4.1
Ash (g/L)	3
VS (g/L)	1.1
Ammonia (g/L)	1.63
TP (g/L)	0.691
TN (g/L)	4.5

	Corn Stover
Mass (g/day)	80
VS (g/day)	37.6
COD (g/day)	44.56
Mass (g/L lsm)	40
VS (g/L lsm)	18.8
COD (g/L lsm)	22.28

H2SO4	Acid consumed
Acid Molarity	2
Consumed (mol/day)	0.19
Volume (L/day	0.065
Mass SO4 (g/day)	18.43

	Biogas
Biogas (L/day)	12
Biogas (L/L)	6
Biogas composition (%)	
Methane	64
CO2	36

Removed	Flocculation
TS (g/day)	22.6
TS (g/L)	11.3
COD (g/day)	21.5
COD (g/L)	10.75

	Precipitant
Molar Ratio Mg:P	1
MgSO4 (g/L)	0.875308
MgSO4 (g/day)	1.750616
MgSO4 (mol/L)	0.00727
MgSO4 (mol/day)	0.01454

	Dilution
Water:LSM ratio	3:1
Dilution rate	4x
Water added (g/day	6000
Water added (g/L ls	3000

Innoculation	
Algae added (mL/L)	100
Algae added (g/L)	3
Active sludge (mL/L)	20

Evaporation Replacement	
Water (L/day)	6.8
Water (g/day)	6800

After Struvite Precipitation	
Volume (L/day)	2
COD (g/day)	20.3
VS (g/day)	2.2
Ammonia (g/day)	2.24
TP (g/day)	0.662
TN (g/day)	7.2
Per Liter	
COD (g/L)	10.14
Ammonia (g/L)	1.12
TP (g/L)	0.331
TN (g/L)	3.6

In to Microalgae and Activated Sludge	
Volume (L/day)	8
COD (g/day)	20.3
Ammonia (g/day)	2.24
TP (g/day)	0.662
TN (g/day)	7.2
Per Liter	
COD (g/L)	2.5
Ammonia (g/L)	0.28
TP (g/L)	0.0827
TN (g/L)	0.9

Residence Time
5 days

Out of	Microalgae
Volume (L/day)	8
COD (g/day)	4.16
Ammonia (g/day)	0.24
TP (g/day)	0.584
TN (g/day)	3.8
Per Liter	
COD (g/L)	0.52
Ammonia (g/L)	0.03
TP (g/L)	0.073
TN (g/L)	0.475

Residence Time
32 days

Out of Hydroponics	
Volume (L/day)	8
COD (g/day)	1.464
Ammonia (g/day)	0
TP (g/day)	0.0344
TN (g/day)	0.192
Per Liter	
COD (g/L)	0.183
Ammonia (g/L)	0
TP (g/L)	0.0043
TN (g/L)	0.024

Removed	Precipitation
Struvite (g/day)	1.8571
Struvite (g/L)	0.92855
Struvite (mol/day)	0.00758
Struvite (mol/L)	0.00379

Removed	Biomass
Mass (g/day)	25.2
COD (g/day)	19.56
TP (g/day)	0.516
TN (g/day)	1.392
Mass (g/L)	2.1
COD (g/L)	1.63
TP (g/L)	0.043
TN (g/L)	0.116

Biomass	
Dry Biomass (g/day)	20
Dry Biomass (g/L)	2.5

Table 3: The calculated mass and nutrient balance of the system including inputs and products. The balance is based on a throughput of 2 L LSM per day.

Conclusions

The system was capable of reducing the key nutrient parameters (COD, TN, ammonia, TP) to a large degree (>98%). Ozone treatment was evaluated and, while it was capable of reducing nutrient concentrations in solution, it was not proven to improve hydroponic growth and nutrient removal, it is likely to be too energy intensive to be a viable part of the system, especially considering the nutrients removed are lost without utilization.. The co cultivation of microalgae and activated sludge bacteria was found to improve nutrient removal. While the nutrient reductions achieved are significant, improvements to the system can be made in terms of both nutrient removal and chemical requirements. All of the nutrient levels exiting hydroponic cultivation were under 20 ppm with the exception of potassium. Methods for further reducing potassium should be evaluated. Additionally, using increased temperature for vacuum stripping pretreatment may be able to achieve similar hydrolysis and ammonia removal at a lower pH (with lower chemical requirements). The magnesium addition for struvite precipitation can be optimized to target a specific nutrient ratio for algae cultivation. Additionally, laboratory adaptive evolution may be an effective method of increasing algae tolerance to ammonia and COD and could allow for less fresh water usage for dilution. The addition of trace nutrients in algae cultivation may improve nutrient reductions as well. Further study can improve and optimize the integrated biological system and increase its economic viability.

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