Image caption: Sunflower (left) and pea shoot (right) microgreens growing on Biostrate mats. After harvest, the Biostrate mats with remaining roots were evaluated as a potential carbon source for the treatment of spent hydroponic nutrient solution. Photo courtesy of Kristen Gibson.

Utilization of Biodegradable Hydroponic Growth Media as a Carbon Source for Greenhouse Wastewater Denitrification

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Abstract: Denitrification of spent hydroponic nutrient solution discharged from greenhouses poses a potential environmental hazard. Aerobic denitrification requires a source of organic carbon, which can be costly for treating large volumes of wastewater. This study proposes the use of spent biodegradable hydroponic grow mats as a source of organic carbon. We compared various anaerobic conditions for the digestion of spent organic growth substrate as a source of organic carbon for the potential treatment of hydroponic wastewater. Grow mats in tap water demonstrated the greatest concentration of organic carbon produced during anaerobic digestion. Anaerobic digestion of grow mats resulted in peak levels of organic carbon after 7 days and decreased thereafter. Leaf waste in addition to Biostrate (type of grow mat) and tap water is capable of producing 5 liters of 5454 mg/L (ppm) total organic carbon. Based on the above results, approximately 12.5 L of nutrient solution (2.5:1 C:N ratio) containing 218 mg/L N can potentially be treated. Our findings provide are the first steps in a sustainable and cost-effective treatment for hydroponic wastewater through utilization of another waste stream (i.e. spent, organic grow mats).

Key Points:

- Nutrient concentrations in hydroponic wastewater can be much greater than typical agricultural wastewater.
- Organic grow mats used in hydroponic cultivation can be a source of organic carbon for treatment of hydroponic wastewater.
- Under anaerobic conditions, digestion of grow mats resulted in peak levels of organic carbon after 7 days and decreased thereafter.

Introduction

For the cultivation of horticultural and food crops in hydroponic systems, nitrogen and phosphorus must be added in excess of what is needed (Sonneveld, 1981). Hydroponic nutrient water cannot be used indefinitely due to risk of plant pathogen accumulation and nutrient depletion. As a result, spent hydroponic wastewater rich in nitrogen and phosphorus is often released directly into the environment. The nutrient levels in hydroponic wastewater can potentially be much higher even than that of typical agricultural wastewater, landfill leachate, and municipal sewage (Yamamoto-Ikemoto, 2000). Combined with other wastewater streams and non-point sources of pollution, the water quality of proximal waterbodies can become compromised.

Researchers have explored various treatments to reduce nitrogen and phosphorus levels in hydroponic and greenhouse wastewater to concentrations that are safe for the environment. These technologies include high performance ultrafiltration membranes (Liu et al., 1999; Seo et al., 2010), mineral treatments (Yi et al., 2003, 2005; Seo et al., 2008; Dunets et al., 2014), bioreactors (Yamamoto et al., 2000; Park et al., 2008, 2009; Seo et al., 2008), absorbers (Dunets et al., 2014), and constructed wetlands (Prystay et al., 2001; Seo et al., 2008, 2010; Gruyer et al., 2013). A major limiting factor in the removal efficiency of biological methods (e.g., bioreactors or constructed wetlands) is an abundant, inexpensive carbon source for denitrifying bacteria to use when reducing NO_3-to N_2. Although rich in nitrogen, hydroponic wastewater typically has very little organic carbon available.

Many hydroponic systems use inorganic substrates such as rockwool, perlite, expanded clay, gravel, or simply suspend plant roots directly in nutrient water. However, some systems, particularly those that grow microgreens, will use fibrous mats made of biodegradable materials such as hemp (Dannehl et al., 2015), felt (Van Quy et al., 2016), or wood (Weber et al., 2016). While inorganic substrates can be washed and reused, organic substrates cannot be reused and present a significant disposal challenge (personal communication), which undermines sustainability goals of these systems. In the present study, we used spent organic growth substrate as a source of organic carbon for hydroponic wastewater treatment and to enhance reusability of the growth substrate waste stream. Therefore, the objectives of this study were to: 1) Characterize hydroponic wastewater from a large commercial hydroponics facility in Northwest Arkansas as well as two small research systems at University of Arkansas to determine volume and nitrogen concentrations to be treated, 2) Determine concentrations of bioavailable carbon that can be obtained from soaking or digesting several types of organic growth mats, and 3) Compare nitrogen removal efficiency in both the commercial and the research system as well as a control "synthetic" wastewater mix using carbon from digested mats. Based on the above objectives, we hypothesized that extraction of bioavailable carbon from spent growth mats for treatment of hydroponic wastewater will reduce nitrogen concentrations to levels equivalent to currently utilized technologies. We also hypothesized that bioavailable carbon and time to extract the carbon will vary based on the material type.

Methods

Bucket digestors for organic carbon generation

To obtain organic carbon from anaerobic digestion, three 20-L polyethylene Nalgene buckets were used to hold the treatments. Spent grow mats and wastewater were collected at a commercial scale nutrient film technique (NFT) hydroponic lettuce system in Winslow, Arkansas. Spent Biostrate™ mats used to grow microgreens were prepared by first removing all of the remaining leaves and stems from the harvested crop and setting them aside. The collected mats were cut into 1 in^2 pieces. Each of three independent digestions lasted 4 weeks. All experiments were conducted at ambient temperature of 20 to 22°C. Water samples were tested for Nitrate-N, Nitrite-N, Ammonium-N, and Total Organic Carbon (TOC) (EPA 412.1). All water samples were analyzed in the Arkansas Water Resources Center. The pH of each treatment was recorded on a weekly basis.

First digestion (aerobic versus anaerobic)

Two treatments were set up as a proof of concept. One treatment included 750 g of Biostrate mat pieces and 250 g of microgreen leaf waste in 8 L of autoclaved tap water. The second treatment included 750 g of Biostrate™ mat pieces and 250 g of microgreen leaf waste in 8 L of wastewater drawn from the waste valve of the hydroponic farm. Wastewater was poured directly into a 20-L bucket on-site and closed for transport back to the lab. One 50 mL sample from each bucket was collected every 7 days and stored at -20°C until processing. An identical set of buckets were set up but included an air bubbler to inject oxygen to compare an aerobic versus anaerobic process. The oxygen levels were maintained at 83-87% dissolved oxygen (DO) and measured using a DO meter. At the end of the 4-week period, all of the samples were processed and analyzed for Total Nitrogen (TN) and Total Organic Carbon (TOC). The TOC samples were not acidified prior to storage at 4°C. The samples were not filtered prior to submission; however, 1:10 and 1:100 dilutions were prepared for TOC analysis.

Second digestion (anaerobic)

The second digestion was set up to determine if a different type of growing mat made from hemp could be an alternate source of organic carbon. Four treatment conditions were set up in 20-L Nalgene buckets under anaerobic con-

ditions: 1) 750 g of Biostrate mats plus 250 g of microgreen leaf waste in 8 L of hydroponic wastewater; 2) 8 L of just hydroponic wastewater; 3) 750 g of hemp mats plus 250 g of microgreen leaf waste in autoclaved distilled water; and 4) 750 g of Biostrate mats plus 250 g of microgreen leaf waste in distilled water. The samples were collected and processed for TN and TOC on the same day each week. The TOC samples were acidified with 40 uL of HCl in 40 mL of sample prior to processing. Samples were collected in duplicate.

Third digestion (anaerobic)

Three treatment conditions were set up in 20-L Nalgene buckets: 1) 8 L of autoclave tap water to serve as a control; 2) 8 L of autoclaved tap water, 750 g of spent BioStrate™ mats, and 250 g of previously removed leaf and stem waste; and 3) 8 L of autoclaved tap water and 750 g of spent BioStrate™ mats only. The mixtures were then left to anaerobically digest (i.e., no air bubbler was included) for 30 days. Triplicate samples (50 mL) were collected from each bucket on a weekly basis and duplicate samples were processed for TN and TOC on the same day each week. The TOC samples were acidified as described previously.

Impact of pH on anaerobic digestion of Biostrate™ mats

In order to determine the optimal pH for anaerobic digestion of Biostrate™ grow mats, 5 phosphate buffers at different pH levels were prepared (Table 1).

Following buffer preparation, 20 g of Biostrate™ grow mats were cut into four equal sections (~5 g each) and then each section was cut into small pieces. The small pieces from each section were placed in individual 1 L glass bottles. Each bottle was filled with the appropriate buffer (pH 5.4, 5.8, 6.2, 6.6). An additional control treatment at pH 7.4 was also prepared. Bottles were capped and sealed with parafilm to maintain anaerobic conditions and held under ambient conditions (20-22°C). Samples (5 mL) from each bottle were collected weekly in duplicate over 28 days as well as a final sample collected on day 56. The 5 mL samples were added to 45 mL of Millipore water in a 50 mL conical tube for a final 1:10 dilution. The TOC samples were acidified. Actual TOC values were recorded by multiplying the reported TOC values by 10 due to the dilution factor.

Analysis of grow mat composition

BioStrate™ mats and hemp mats were analyzed at the Fayetteville Agricultural Diagnostic Laboratory to characterize the material. Parameters measured included percent neutral detergent fiber (hemicellulose, cellulose, and lignin) and acid detergent fiber (cellulose and lignin) in the mats (AOAC 1990a,b) as well as percent nitrogen, percent carbon, and pH. A saturation analysis was also performed on the grow mats to obtain electrical conductivity, nitrate, phosphorus, potassium, calcium, magnesium, sulfur, sodium, iron, manganese, zinc, copper, and boron.

Anaerobic denitrification (bucket, non-pumped system)

While generating organic carbon for the aerobic denitrification experiment, an unexpected result occurred. Denitrification of residual nitrogen in greenhouse-derived wastewater occurred in a low oxygen environment using undigested Biostrate™ mats (see Results). Thus, we decided it was necessary to determine if anaerobic digestion of the grow mats to yield organic carbon was necessary prior to denitrification, or if using the mats directly was feasible. Three 20-L Nalgene buckets were set up for anaerobic denitrification (Figure 1). The following conditions were prepared: 1) synthetic wastewater-only control; 2) spent grow mats with synthetic wastewater; and 3) organic carbon liquid generated by anaerobic digestion of the spent grow mats and synthetic wastewater. Duplicate sampling was conducted weekly.

Table 1: Phosphate buffer formulations.[1]

pH	Na_2HPO_4-$7H_2O$ (mw: 268.07 g/mol)	Molarity	NaH_2PO_4-H_2O (mw: 137.99 g/mol)	Molarity
5.4	0 g	0 M	1.557 g	0.0110 M
5.8	0.131 g	0.0005 M	1.313 g	0.0095 M
6.2	0.603 g	0.0023 M	1.069 g	0.0077 M
6.6	1.076 g	0.0040 M	0.826 g	0.0060 M
7.4 (Control)	2.021 g	0.0075 M	0.339 g	0.0025 M

[1]Recipe calculator: https://www.aatbio.com/resources/buffer-preparations-and-recipes/phosphate-buffer-ph-5-8-to-7-4

Figure 1: Example of the pump-included hydroponic wastewater system.

Data Analysis

The changes in total organic carbon (TOC), total nitrogen (TN), Nitrate-N and Nitrite-N (N+N), and ammonia-N and ammonium-N(A+A) were plotted against the experiment time for each treatment. Furthermore, the pH of the treatments was plotted against time. Basic description statistics such as mean and standard deviation were also determined.

Results and Discussion

Anaerobic digestion of grow mats

First Digestion

In the tap water (TW) treatment, TN increased slightly relative to the increase in TOC, from 0.79 mg/L to 121.34 mg/L after 4 weeks. The increase in TOC was dramatic, from 0.49 mg/L to 545.44 mg/L in 4 weeks. In the hydroponic wastewater treatment, a significant concentration of nitrogen was present at the start of the experiment (214.76 mg/L) due to residual from the nutrients added for lettuce production. The TN decreased from 214.67 mg/L to 81.7 mg/L by the end of the 4 weeks. The level of TOC increased from 19.48 mg/L to only 78.36 mg/L. No replicates were taken for this experiment, so it is unknown if the differences between treatments were statistically significant. The aerobic digestion resulted in no change in TN or TOC and was deemed an ineffective strategy for generating TOC from Biostrate (data not shown).

Second Digestion

For all treatments, the TOC levels peaked at week 1 and declined thereafter (Figure 2). The total nitrogen increased slightly for each treatment.

The starting nitrogen concentration for distilled water and wastewater were similar, indicating that the day we collected the wastewater, it was more "spent" than on the collection day from the first digestion (i.e., more nitrogen had been used up by the microgreens). There appeared to be no difference between hemp and Biostrate as an organic carbon source; however, the anaerobic digestion also yielded no usable organic carbon by the end of the 4-week period.

Third Digestion

In the TW control treatment, TOC remained consistent, ranging from 0.91 to 1.71 (Figure 3). The TOC in both TW+Biostrate™ and TW+Biostrate+LW treatments increased from 69.65 and 114.76 mg/L with in the first week, respectively. After Week 1, the TOC concentrations decreased to 15 mg/L for the TW+Biostrate treatment and 50.89 mg/L in the TW+Biostrate+LW treatment.

When comparing TN across treatments, the TW control treatment remained consistent and ranged from 0.82 to 1.06 mg/L (Figure 4). TN increased in the TW+Biostrate treatment from 41.05 to 49.76 mg/L from Week 0 to Week 3, and then decreased to 41.25. In the TW+Biostrate+LW treatment, TN increased throughout the experiment, starting at 61.48 mg/L and ending at 85.00 mg/L.

As for N+N, the TW control and TW+Biostrate+LW treatment remained consistent throughout the experiment, ranging from 0.64 to 0.67 and 0.008 to 0.05 mg/L respectively (Figure 5). The N+N for the TW+Biostrate treatment remained consistent from Week 0 until Week 3 where the N+N concentration increased from 0.023 to 2.88 mg/L on Week 4. Like other previous parameters, A+A for the TW control treatment remained consistent, ranging from 0.01 to 0.046 mg/L across the experiment (Figure 6). The A+A in

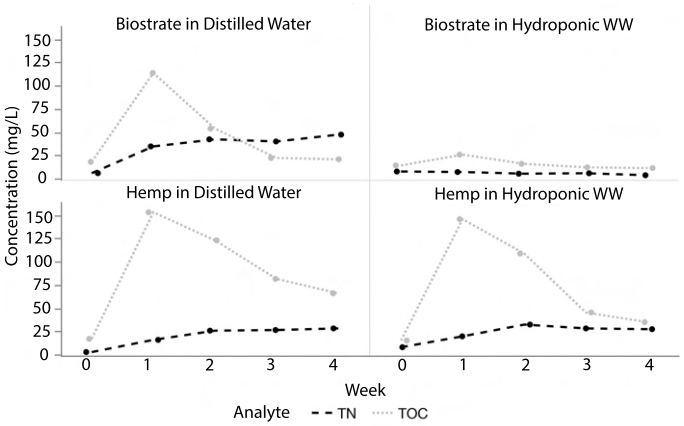

Figure 2: Impact of anaerobic digestion conditions on total nitrogen (TN) and total organic carbon (TOC) over time. WW=wastewater

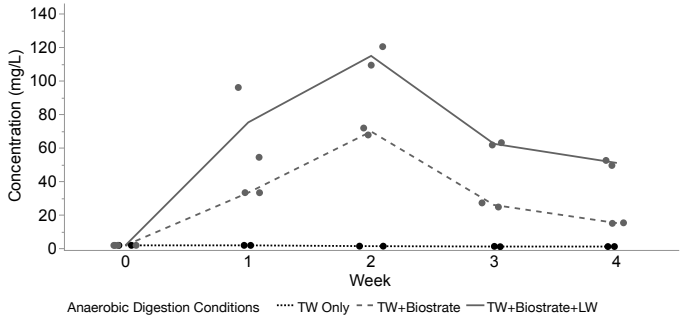

Figure 3: Concentration of total organic carbon (TOC) by anaerobic digestion of condition over time.

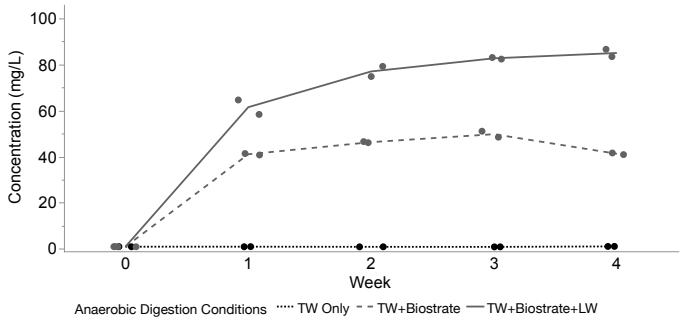

Figure 4: Concentration of total nitrogen (TN) by anaerobic digestion condition over time.

the TW+Biostrate treatment increased from 23.10 mg/L on Week 0 to 30.93 mg/L on Week 3, then decreased to 25.71 mg/L on Week 4. The TW+Biostrate+LW treatment's A+A increased from Week 0 until Week 1. From Week 1 to Week 2 A+A then decreased slightly, then increased from Week 3 to Week 4. Table 2 summarizes the results and C:N ratios of these experiments, wherein only the first was successful at generating sufficient organic carbon for denitrification.

The pH of the treatments followed a similar trend from Week 0 to Week 2 where the pH decreased and then increased (Figure 7). After Week 2, the pH of the TW control treatment continued to decrease and the pH of the TWB and TWB+L treatments increased until Week 3. The pH of the TW+Biostrate treatment then decreased in Week 4 where the TW+Biostrate+LW treatment increased.

Impact of pH on total organic carbon during anaerobic digestion of grow mats.

To assess the impact of pH level on TOC over time during anaerobic digestion of Biostrate™ mats, five pH levels were selected, and TOC was assessed. Overall, the mean level of TOC was 3.66 mg/L (Table 3) with the peak level at 7.99 mg/L for sample pH 5.4 on day 56, whereas 0.95 mg/L was the lowest TOC recovered in the pH 7.4 sample on day 54 (data not shown). The general trend of TOC values over time are presented in Figure 8. For all samples, the mean peak recovery occurred on day 28 then TOC levels declined by day 56 (Figure 9). The pH from day 0 to day 7 increased then dipped down at week 14 for all samples (Figure 10).

Grow Mat Analysis

The BioStrate™ mats had a greater proportion of neutral detergent fiber of 94.82% compared to the hemp mats which were 86.12% (Table 4) (Van Soest et al., 1991; AOAC, 1990). The percent nitrogen was highest in the hemp mats at 0.31% and least in the BioStrate™ mats at 0.09% (AOAC, 1990). The pH of a water extract of the hemp mats was 5.9 and the pH of BioStrate™ mats using the same technique was 6.3 (Cataldo et al., 1975). Table 5 shows the extraction analysis results for Biostrate™ and hemp which indicate low nutrient content overall for these grow mats.

Table 2. Summary of C:N ratios and effectiveness of anaerobic digestion after 4 weeks

Condition	Carbon (mg/L)	Nitrogen (mg/L)	C:N Ratio	TOC (mg/L)
TW	0.49	0.79	0.62	545
TW + Biostrate	18.15	5.96	3.04	20.09
TW + Biostrate + LW	75.09	61.48	1.22	50.89

TW=tap water; LW = leaf waste; TOC=total organic carbon

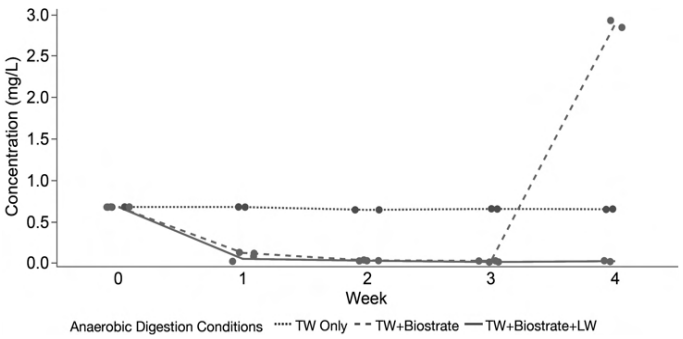

Figure 5: Concentration of nitrate-nitrite by anaerobic digestion condition over time.

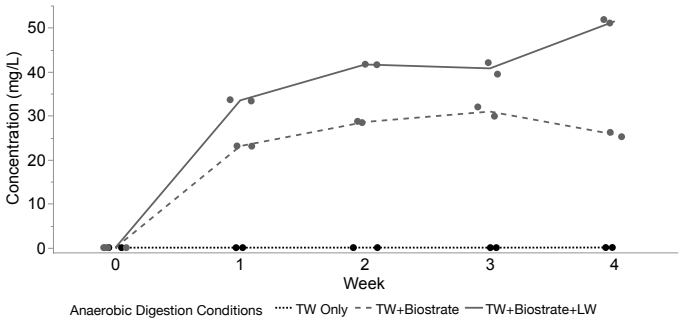

Figure 6: Concentration of ammonia + ammonium (A+A) by anaerobic digestion condition over time.

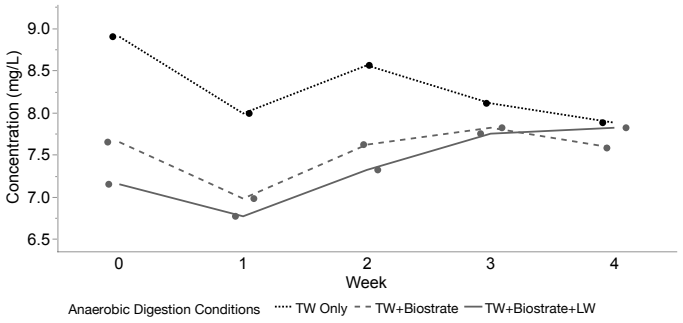

Figure 7: pH by anaerobic digestion condition over time.

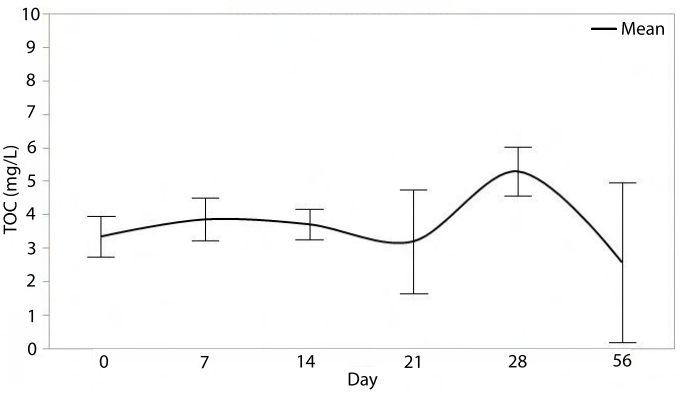

Figure 8: The mean TOC (mg/L) over time. Each error bar is constructed using 1 standard deviation from the mean.

Table 3: Comparison of mean TOC (mg/L) values by pH over time

	Mean TOC (mg/L) (±SD) by Day					
Sample	0	7	14	21	28	56
pH 5.4	3.53 (0.85)	3.61 (0.54)	3.50 (0.47)	3.77 (1.35)	4.63 (0.70)	4.00 (3.30)
pH 5.8	3.73 (0.39)	3.70 (0.88)	3.87 (0.53)	2.29 (1.14)	5.37 (0.52)	1.40 (0.14)
pH 6.2	2.92 (0.62)	4.05 (0.62)	3.82 (0.51)	2.96 (1.39)	5.15 (0.49)	4.27 (3.41)
pH 6.6	3.31 (0.37)	3.85 (0.49)	3.68 (0.62)	4.02 (2.49)	5.82 (0.94)	2.05 (1.20)
pH 7.4	3.21 (0.69)	4.06 (0.78)	3.66 (0.19)	2.96 (1.18)	5.47 (0.61)	1.13 (0.33)
Average	3.66					

SD = Standard Deviation

Table 4: The composition analysis of biostrate and hemp

Parameter	Biostrate	Hemp
Acid detergent fiber (%)	91.97	75.91
Neutral detergent fiber (%)	94.82	86.12
Nitrogen (%)	0.09	0.31
Carbon (%)	48.94	42.99
pH	6.3	5.9

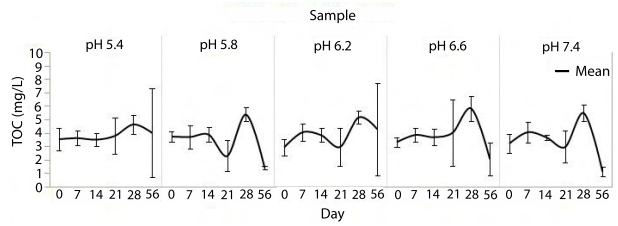

Figure 9: The TOC (mg/L) over time by pH level. Each error bar is constructed using 1 standard deviation from the mean.

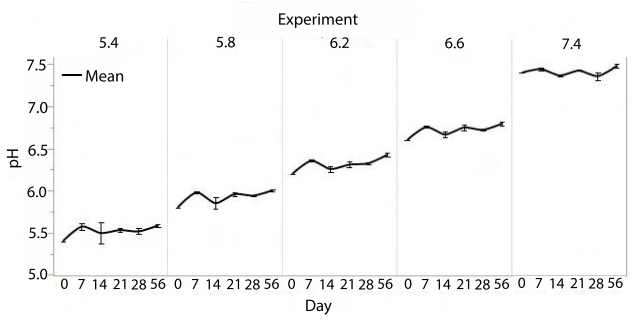

Figure 10: The change in pH over time. Each error bar is constructed using 1 standard deviation from the mean.

Wastewater Treatment System

The wastewater treatment system was only created within this study. Future experiments aim to observe the denitrification potential of the aerated, pump-included system compared to aerated, pump-less system, and an anaerobic denitrification system.

Discussion

The primary purpose of this experiment was to determine the amount of organic carbon that could be extracted from the anaerobic digestion of the BioStrate™ mats and leafy green waste, and if either tap water or hydroponic wastewater was a more appropriate digestion medium. In the first digestion, TOC production occurred in tap water but not in hydroponic wastewater. However, denitrification unexpectedly occurred in the hydroponic wastewater bucket containing Biostrate™, where total nitrogen decreased by 62% over 4 weeks. As previously stated, the organic carbon in subsequent experiments increased initially (Figure 1), then began to steadily decline. This could be due to the increased concentrations of nitrogen and carbon present in the water at the start of the experiment. Increased initial concentrations of nitrogen and carbon could have favored dissimilatory nitrate reduction to ammonium (DNRA) since A+A concentrations increased in the BioStrate™ and BioStrate™ + leafy green waste treatments from Week 0 to Week 4 (see Second Digestion). DNRA is assumed to occur when nitrate is the limiting nutrient compared to organic carbon (Cole and Brown, 1980).

With the increase in ammonium from the anaerobic degradation of organic matter, a different approach may be used to remove nitrogen from the system. Under anoxic conditions, anaerobic ammonium oxidation (anammox) bacteria form nitrogen gas (N_2) from nitrite and ammonium (Jetten et al., 2005). Anammox bacteria have been detected in most aquatic habitats that contain anoxic zone (Dong et al., 2009; Jetten et al., 2005). The chemolithoautotrophic anammox pathway is preferred in habitats that are oxygen-depleted and that are limited in organic matter. As organic matter decomposes anaerobically, ammonification occurs producing ammonium. Furthermore, DNRA can also produce ammonium for the anammox bacteria. Nitrite can be produced by aerobic nitrifying bacteria that inhabit the surface of the water where the oxic and anoxic habitats interact (Hao et al., 2002). Moreover, anammox bacteria were observed to reduce nitrate to nitrite through the oxidation of organic compounds (Guven et al., 2005).

Another possible explanation of the continual loss of TOC in the BioStrate™ treatments is that the anaerobic digestion process continued further than desired. In anaerobic digestion, there are four steps: (1) hydrolysis, (2) fermentation or acidogenesis, (3) acetogenesis, and (4) methanogenesis (Gould, 2015). Step 1 is hydrolysis where large organic polymers including proteins, fats, and carbohydrates, are broken down into smaller polymers including amino acids, fatty acids, and simple sugars. The goal of the anaerobic digestion in this study was to create the simple sugars for the bacteria. While all steps occur simultaneously, step 2 of fermentation or acidogenesis may have outpaced step 1. In step 2, the small polymers previously mentioned

Table 5: The saturation extract analysis biostrate and hemp

Parameter	Biostrate	Hemp
EC (μmhos/cm)	32	96
NO_3-N (mg/L)	0.3	0.1
P (mg/L)	1.0	1.7
K (mg/L)	2.1	3.7
Ca (mg/L)	0.5	4.2
Mg (mg/L)	0.3	1.4
S (mg/L)	0.3	5.7
Na (mg/L)	2.0	7.2
Fe (mg/L)	0.1	0.3
Mn (mg/L)	0.001	0.07
Zn (mg/L)	0.002	0.16
Cu (mg/L)	0.02	0.02
B (mg/L)	0.02	0.02

are broken down further. As fermentative bacteria break down the polymers, an acidic environment and ammonia is created. The increased ammonia content can be observed in Figure 5.

Conclusions

A clear understanding of the carbon and nitrogen cycles are necessary to produce organic carbon for denitrification. Factors including environmental conditions, the concentrations of different nitrogen and carbon molecules, and bacteria present can influence the biochemical reactions within the system. In this study, the first objective was to decompose spent organic substrates to produce organic carbon for denitrification. Future research would be necessary to explain the differences in organic carbon production between the three digestion scenarios. One experiment would be to observe organic carbon production when organic carbon is the limiting nutrient compared to total nitrogen. This may encourage the decomposition of the substrates in the system. Furthermore, a different approach such as using anaerobic ammonium oxidation may be used as another process to produce atmospheric nitrogen for nitrogen removal. As the hydroponic industry continues to grow, organic materials such as the spent substrates and wastewater will be produced. A sustainable and cost-effective method of treatment will become more pertinent to prevent the release of nutrients into soil, with the potential to cause eutrophication within proximal surface waters.

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