Article

Evaluation of Algae-Based Fertilizers Produced from Revolving Algal Biofilms on Kentucky Bluegrass

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Abstract: The revolving algal biofilm (RAB) system is a wastewater treatment method that is effective at removing nutrients from wastewater, and as a result produces algae that could be used as a sustainable fertilizer source. A two-year greenhouse study was conducted to investigate if algae-based fertilizers produced from the RAB wastewater treatment system could be used as an effective and sustainable turfgrass fertilizer. Fertilizer treatments included pure algae (PA), algae + cellulosic filler (Blended), Milorganite, urea, and a nontreated control. Overall, in terms of percent green cover (PGC), Blended and PA performed similar to Milorganite and outperformed urea and the nontreated control. At the conclusion of the study, Blended was the only treatment to have an increased PGC relative to urea, which was a 29% increase. On average throughout the duration of the study, Blended and PA resulted in similar dark green color index (DGCI) relative to Milorganite and urea, and outperformed the nontreated control. Blended, PA, and Milorganite resulted in 50% and 111% greater total root length compared to urea and the nontreated control, respectively. Furthermore, Blended and Milorganite resulted in 107% and 136% greater root surface area and root volume, respectively, compared to urea and the nontreated control. Pure algae resulted in 58% greater root surface area relative to urea and the nontreated control. Blended and Milorganite increased the longest root by 22% compared to urea. Additionally, Blended and Milorganite resulted in 114% and 110% greater root and shoot biomass relative to the nontreated control, respectively. Blended and PA had a similar longest shoot length to Milorganite and urea. Overall, Blended and PA performed similar or better compared to Milorganite and urea in terms of turfgrass shoot growth, cover, color, and rooting. Thus, algae-based fertilizers (PA and Blended) produced from the RAB system should be considered an effective, sustainable turfgrass fertilizer.

Keywords: algae; rooting; slow-release fertilizer; sustainable fertilizer; turfgrass; wastewater treatment

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1. Introduction

Turfgrass requires frequent applications of fertilizers and irrigation to achieve high-quality and uniform turfgrass. However, there are significant negative impacts of the production and usage of conventional synthetic fertilizers. The manufacturing of synthetic N–P–K fertilizers exceeds fossil fuel energy requirements and carbon (C) dioxide emissions of 71 GJ Mg^{−1} and 1142 kg C Mg^{−1}, respectively [1]. Additionally, mismanaged fertilization and irrigation practices can lead to impaired ground and surface water quality, as well as gaseous losses to atmosphere [2–5].

Recently, sustainable turfgrass management has garnered interest, and many entities have adopted nutrient restrictions, bans, or organic management practices [6]. Additionally, Khachatryan et al. [7] found that homeowners prefer eco-friendly fertilizers that reduce negative environmental impacts, compared to synthetic fertilizers. Furthermore, incorporating enhanced efficiency fertilizers (EEF) is another management strategy to reduce nutrient losses to the environment [4]. The definition of an EEF is a fertilizer that increases

nutrient-use efficiency and reduces the potential nutrient losses to the environment [8]. Additionally, changes to organic and/or sustainable fertilizer sources requires less energy consumption and reduces global warming, ozone-depletion, and acidifying emissions compared to conventional fertilizers [9]. Organic fertilizers and EEF reduce turfgrass nitrogen gaseous emissions and leaching compared to conventional fertilizers [10–12]. The utilization of sustainable, organic fertilizers and/or enhanced efficiency fertilizers are a part of the future of eco-friendly turfgrass management practices.

The revolving algal biofilm (RAB) system, which is a series of vertically oriented conveyor belts that grow algae on their surface, has been shown to cost effectively and sustainably remove and recover nutrients from municipal wastewater [13,14]. The RAB system's unique algae cultivation technology allows for rapid growth of algae. The RAB system is strategically placed at publicly owned treatment works (POTWs) that are facing stricter nutrient limits. The RAB system allows the community to keep existing infrastructure in place and simply retrofit their plant to meet the new permits. The algae that are grown on the RAB system is harvested, removing the nutrients from the system. These algae can then be processed into a slow-release fertilizer for turfgrass applications. The fertilizer is produced without the need for any chemicals or artificial additives. The fertilizer simply uses what was considered a waste (municipal wastewater) and converts it into a sustainable and renewable product.

Previous research has investigated benefits of organic and biofertilizers on turfgrass. Organic fertilizers and biosolids enhanced turfgrass performance over an extended time period compared to quick-release fertilizers [15]. Biosolids improved or provided similar turfgrass establishment, color, and rooting compared to conventional fertilizers [16,17]. Furthermore, biosolids increased turfgrass yield and quality, soil carbon and nutrient concentrations, and microbial biomass and activity [18,19]. An eco-friendly fertilizer derived from slurry composting and biofiltration (SCB) improved turfgrass quality, growth, color, and nutrient uptake [20–22]. Additionally, the SCB fertilizer had minimal soil and pond contamination when applied at a golf course [23]. Fertilizer produced from fermented turfgrass clippings increased turfgrass quality and decreased disease damage [24,25]. Squid-based fertilizers provided higher quality and uniform turfgrass and enhanced microbial activity compared to synthetic fertilizers [26].

Minimal research has been conducted on algae-based fertilizers produced from the RAB wastewater treatment system. In general, algal biomass can be applied to the soil as an organic, slow-release fertilizer [27]. Additionally, algae biomass has also been used as a soil amendment to increase soil nutrients and improve soil biological properties [27]. In a container study, RAB produced algae provided similar crop (marigold, tomato, and sweet corn) performance to synthetic fertilizer and was better than commercial bio-based fertilizers [28]. Research is needed to determine if algae-based fertilizers produced from the RAB system could be used as a turfgrass fertilizer. We hypothesized that the algae-based fertilizers would perform similarly or would outperform industry standards, Milorganite (biofertilizer), and urea (synthetic fertilizer) in terms of turfgrass shoot and root performance. The objective of this greenhouse study was to determine if algae-based fertilizers produced from wastewater treatments could be a sustainable and effective turfgrass fertilizer.

2. Materials and Methods

2.1. Turfgrass Greenhouse Establishment and Maintenance

Rooting tube experiments were conducted in 2019–2020 and 2020–2021 in the Department of Horticulture greenhouse at the Iowa State University (Ames, IA, USA). Rooting tube construction and turfgrass establishment was conducted following methods described by Lindsey et al. [29]. Clear polyethylene tubes (2.5 cm diameter × 60 cm length) were filled with sand that conformed to United States Golf Association (USGA) specifications and packed to the USGA recommended density of 1.6 Mg m⁻³ [30,31]. Sand-filled rooting tubes were placed into polyvinyl chloride tubes at a 45° angle to promote gravitropic growth along the clear polyethylene tube [32]. After rooting tube construction and setup,

‘Rush’ Kentucky bluegrass (*Poa pratensis* L.) was established from seed at a seeding rate of 122.1 kg ha⁻¹. Overhead mist irrigation was applied to the rooting tubes daily throughout the duration of the experiments. Kentucky bluegrass was not clipped during the experiments due to shoot lengths not exceeding 7.6 cm.

In the greenhouse, daily relative humidity ranged from 24.3 to 44.7%, and daily air temperature varied from 22.3 to 23.6 °C. Peak daytime irradiance ranged from 350 to 385 µmol m⁻² s⁻¹ in the greenhouse, and supplemental radiation was applied when daytime irradiance fell below 200 µmol m⁻² s⁻¹ (LightScout Quantum Meter, Spectrum Technologies Inc., Aurora, IL, USA).

2.2. Treatment Structure

The experimental design was a randomized complete block with four replications. Fertilizer treatments included pure algae (PA; Gross-Wen Technologies, Slater, IA, USA), algae + cellulosic filler (Blended; Gross-Wen Technologies, Slater, IA, USA), Milorganite (Milorganite, Milwaukee, WI, USA), urea (The Andersons, Inc., Maumee, OH, USA), and a nontreated control (Table 1). PA and Blended were produced from the RAB wastewater treatment system (Gross-Wen Technologies, Slater, IA, USA). Milorganite is a biosolid fertilizer composed of heat-dried microbes that have digested organic matter in wastewater and is produced from the Milwaukee Metropolitan Sewerage District [33,34]. Fertilizer treatments were surface-applied at 24.4 kg N ha⁻¹ every 28 days, starting at the time of establishment (four applications total).

Table 1. Fertilizer treatments and nutrient analysis.

Treatment	Nitrogen %	Phosphorus %	Potassium %	Calcium %	Iron %
Pure algae (PA)	5.6	1.9	0.3	3.8	1.6
Algae + cellulosic filler (Blended)	2.9	1.1	0.2	2.3	1.8
Milorganite	6.0	1.7	0	1.2	2.5
Urea	46.0	0	0	0	0
Nontreated	0	0	0	0	0

2.3. Data Collection

Digital images were collected bi-weekly, starting two weeks after seeding to assess turfgrass cover and color following methods described by Thoms et al. [35]. Digital images were subjected to digital image analysis (DIA; threshold settings: hue 71–176, saturation 10–100, brightness 0–100) to determine percent green cover (PGC) and dark green color index (DGCI) [36,37]. Kentucky bluegrass was harvested from the rooting tubes and cleaned to remove sand 16 weeks after initial treatment (WAIT). After the Kentucky bluegrass was harvested, the roots and shoots were separated at the crown. Post-harvest, the longest root and shoot were measured using a ruler. Total root length, root surface area, and root volume were determined using WinRhizo Reg (Regent Instruments Inc., Quebec City, QC, Canada) following modified methods described by Brosnan et al. [32]. Root and shoot biomass were determined by weighing the roots and shoots after being dried at 80 °C for 3 days [29].

2.4. Statistical Analysis

Post-harvest data were subjected to analysis of variance (ANOVA), and DIA data were subjected to ANOVA with repeating measures using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The experiment was repeated in time. A significant rating date-by-treatment interaction was present for DIA data, and thus data are presented by rating date. Fertilizer treatment means post-harvest data were combined across years due to a non-significant interaction with treatment effect. Fertilizer treatment mean comparisons were separated using Fisher’s protected least significant difference (LSD; $\alpha = 0.05$) at the $p \leq 0.05$ level.

3. Results

Fertilizer treatments had a significant effect on PGC on six of the eight rating dates (Figure 1). There were no treatment differences on two WAIT. On four WAIT, Milorganite had 32% greater PGC relative to the rest of the treatments. On six WAIT, Blended and PA performed equally to Milorganite in terms of PGC. Blended, PA, and Milorganite resulted in 76% greater PGC compared to urea and the nontreated control. There were no treatment differences on eight WAIT. On 10 WAIT, Blended and Milorganite had 85% greater PGC relative to urea and the nontreated control. PA performed similar to fertilizer treatments and had greater PGC compared to the nontreated control. On 12 WAIT, Blended, PA, and Milorganite resulted in 66% greater PGC relative to urea and the nontreated control. Similar results were seen on 14 WAIT, except PA did not result in greater PGC compared to urea. On the last rating date before turfgrass was harvested (16 WAIT), Blended was the only treatment to have greater PGC compared to urea and all fertilizer treatments outperformed the nontreated control in terms of PGC. Blended resulted in a 29% increase in PGC compared to urea at the conclusion of the study. Overall, Blended and PA performed similar to Milorganite and outperformed urea in terms of PGC.

Figure 1. Effects of wastewater algae-based fertilizers on Kentucky bluegrass percent green cover (PGC) growing in a greenhouse in Ames, IA, in 2019–2020 and 2020–2021. Fertilizer treatments were applied at $24.4 \text{ kg N ha}^{-1}$ every 28 days (four applications total) starting at the time of establishment, which was 3 December 2019 and 19 November 2020. PGC was determined using digital image analysis. Significant rating date-by-treatment interaction; means are pooled across years. Fertilizer treatment mean comparisons were separated using Fisher’s protected least significant difference (LSD; $\alpha = 0.05$) at the $p \leq 0.05$ level. LSD values are above treatment means for each rating date. NS, nonsignificant at the 0.05 probability level. Means and standard deviation bars are shown ($n = 5$).

Fertilizer treatments had a significant effect on DCGI on seven of the eight rating dates (Table 2). There were no treatment differences on two WAIT. On four WAIT, PA had a 6% increase in DCGI compared to the nontreated control. All other treatments were similar to the nontreated in terms of DCGI. On six WAIT, all fertilizer treatments resulted in a greater DCGI relative to the nontreated control. Blended was the only treatment that had improved DCGI compared to urea, which was 6% greater than urea. On eight WAIT, PA and Blended resulted in 6% greater DCGI relative to the nontreated control. On 10 WAIT, all fertilizer treatments resulted in greater DCGI compared to the nontreated control, and Blended had a 6% increase in DCGI relative to Milorganite. On 12 WAIT, Blended and PA were the only treatments to have a greater DCGI compared to the nontreated control. On 14 WAIT, the only treatments that resulted in an increased DCGI compared to the nontreated control were Blended and urea. On the last rating date (16 WAIT), urea was the only treatment to have greater DCGI relative to the nontreated control. Overall, Blended and PA resulted in similar DCGI compared to Milorganite and urea and resulted in greater DCGI relative to the nontreated control.

Fertilizer treatments had a significant effect on all root and shoot parameters determined post-harvest (Table 3). Blended, PA, and Milorganite increased total root length by 50% and 111% compared to urea and the nontreated control, respectively. Blended and Milorganite had 107% greater root surface area compared to urea and the nontreated control. PA resulted in less root volume compared to Blended and Milorganite; however, PA had 58% greater root surface area relative to urea and the nontreated control. Blended and Milorganite were the only treatments to have an increased root volume compared to urea and the nontreated control, which was an increase of 136%. PA had 83% greater root volume compared to the nontreated control. Blended and Milorganite increased the longest root by 22% and 54% compared to urea and the nontreated control, respectively. PA had a similar longest root length to Blended, Milorganite, and urea. Additionally, PA resulted in 49% greater longest root relative to the nontreated control. Blended and Milorganite were the only treatments that had an increased root and shoot biomass compared to the nontreated control, which was a 114% and 100% increase, respectively. All fertilizer treatments had a greater longest shoot compared to the nontreated control. Blended and PA had similar longest shoot length to urea and Milorganite. Overall, Blended and PA performed similar or better compared to Milorganite and urea in terms of rooting and shoot growth.

Table 2. Effects of wastewater algae-based fertilizers on Kentucky bluegrass dark green color index (DGCI) growing in a greenhouse in Ames, IA, in 2019–2020 and 2020–2021.

Treatment ¹	DGCI (0–1) ²							
	Weeks after Initial Treatment							
	2	4	6	8	10	12	14	16
Pure algae (PA)	0.56 (+/-0.02) ³	0.56 (+/-0.03)	0.55 (+/-0.02)	0.54 (+/-0.04)	0.54 (+/-0.03)	0.53 (+/-0.03)	0.53 (+/-0.02)	0.54 (+/-0.03)
Algae + cellulosic filler (Blended)	0.56 (+/-0.02)	0.54 (+/-0.03)	0.56 (+/-0.02)	0.54 (+/-0.03)	0.55 (+/-0.02)	0.53 (+/-0.02)	0.54 (+/-0.02)	0.55 (+/-0.03)
Milorganite	0.57 (+/-0.02)	0.54 (+/-0.02)	0.54 (+/-0.02)	0.52 (+/-0.03)	0.52 (+/-0.02)	0.51 (+/-0.02)	0.52 (+/-0.02)	0.53 (+/-0.02)
Urea	0.56 (+/-0.02)	0.54 (+/-0.03)	0.53 (+/-0.03)	0.53 (+/-0.04)	0.53 (+/-0.03)	0.52 (+/-0.02)	0.55 (+/-0.02)	0.56 (+/-0.03)
Nontreated	0.56 (+/-0.03)	0.53 (+/-0.03)	0.51 (+/-0.03)	0.51 (+/-0.04)	0.50 (+/-0.04)	0.50 (+/-0.03)	0.51 (+/-0.03)	0.53 (+/-0.03)
LSD _{0.05} ⁴	NS ⁵	0.02	0.02	0.02	0.02	0.02	0.02	0.02

¹ Fertilizer treatments were applied at 24.4 kg N ha⁻¹ every 28 days (four applications total) starting at the time of establishment, which was 3 December 2019 and 19 November 2020. ² DGCI (0–1 scale where values close to 1 correspond to darker green color) was determined using digital image analysis. ³ Significant rating date-by-treatment interaction; means are pooled across years. Means (+/- standard deviation) are presented. ⁴ Fertilizer treatment mean comparisons were separated using Fisher's protected least significant difference (LSD; $\alpha = 0.05$) at the $p \leq 0.05$ level. ⁵ NS, nonsignificant at the 0.05 probability level.

Table 3. Effects of wastewater algae-based fertilizers on Kentucky bluegrass root and shoot parameters growing in a greenhouse in Ames, IA, in 2019–2020 and 2020–2021.

Treatment ¹	Total Root Length ² cm	Root Surface Area ² cm ²	Root Volume ² cm ³	Longest Root ³ cm	Root Biomass ⁴ g	Longest Shoot ³ cm	Shoot Biomass ⁴ g
Pure algae (PA)	2403.3 (+/-633.4) ⁵	176.4 (+/-50.0)	1.1 (+/-0.5)	46.8 (+/-8.5)	0.12 (+/-0.06)	4.8 (+/-1.1)	0.12 (+/-0.07)
Algae + cellulosic filler (Blended)	2868.3 (+/-452.1)	231.9 (+/-76.2)	1.6 (+/-0.9)	48.5 (+/-4.8)	0.16 (+/-0.04)	4.7 (+/-1.6)	0.14 (+/-0.07)
Milorganite	2655.9 (+/-265.5)	230.7 (+/-67.4)	1.7 (+/-1.1)	48.3 (+/-4.3)	0.14 (+/-0.06)	4.7 (+/-0.9)	0.14 (+/-0.10)
Urea	1758.5 (+/-366.0)	130.1 (+/-36.4)	0.8 (+/-0.4)	39.6 (+/-11.3)	0.11 (+/-0.03)	6.4 (+/-2.5)	0.10 (+/-0.03)
Nontreated	1252.7 (+/-496.5)	93.6 (+/-62.5)	0.6 (+/-0.6)	31.5 (+/-6.3)	0.07 (+/-0.04)	2.6 (+/-0.9)	0.07 (+/-0.08)
LSD _{0.05} ⁶	473.0	44.9	0.4	8.0	0.05	1.7	0.05

¹ Fertilizer treatments were applied at 24.4 kg N ha⁻¹ every 28 days (four applications total) starting at the time of establishment, which was 3 December 2019 and 19 November 2020. ² WinRhizo Reg (Regent Instruments Inc., Quebec City, QC, USA) was used to determine total root length, root surface area, and root volume. ³ Longest root and shoot were measured using a ruler. ⁴ Root and shoot biomass were determined by weighing the roots and shoots after being dried at 80 °C for three days. ⁵ Means were pooled across years and rating dates due to a non-significant interaction with treatment effect. Means (+/- standard deviation) are presented. ⁶ Fertilizer treatment mean comparisons were separated using Fisher's protected least significant difference (LSD; $\alpha = 0.05$) at the $p \leq 0.05$ level.

4. Discussion

4.1. Effect of Fertilizer on Shoot Growth, Cover, and Color

Before the conclusion of the study, the biofertilizers (PA, Blended, and Milorganite) resulted in enhanced turfgrass growth and cover during turfgrass establishment compared to the synthetic fertilizer (urea). This result could be due to the slow-release characteristics of the biofertilizers and is consistent with other studies showing that biofertilizers improved turfgrass growth and cover compared to conventional fertilizers [16,21,22]. Conversely, Aamlid and Hanslin [38] reported that organic fertilizers did not significantly improve turfgrass cover compared to mineral fertilizer treatments. In general, fertilizer treatments improved the turfgrass color (DGCI) compared to the nontreated control; however, the DGCI varied between rating dates. This is similar to other studies that report biofertilizers and conventional fertilizers increased turfgrass color [21,22,39]. Furthermore, Acikgoz et al. [40] reported that biofertilizers did not improve Kentucky bluegrass color compared to a synthetic fertilizer alone. Overall, Blended and PA outperformed or performed similar to the industry standards (Milorganite and urea) in terms of turfgrass growth, cover, and color.

All fertilizer treatments resulted in greater longest shoot length compared to the nontreated control. Blended and PA had similar longest shoot length and shoot biomass relative to Milorganite and urea. These results are similar to other studies showing that biofertilizers and conventional fertilizers perform similarly in terms of shoot yield and growth and outperform the nontreated control [15,16,20,40]. Conversely, Ham et al. [21,22] reported biofertilizers increased clipping dry weight compared to conventional fertilizers; however, the differences between studies could be due to experimental location (i.e., greenhouse vs. golf course). Overall, Blended and PA had similar shoot biomass and longest shoot length relative to Milorganite and urea.

4.2. Effect of Fertilizer on Root Growth

Post-harvest, all fertilizer treatments resulted in greater longest root length compared to the nontreated control. Blended and Milorganite had increased longest root length compared to the synthetic fertilizer. Blended, PA, and Milorganite increased the total root length and root surface area compared to urea. This could be due to the biofertilizers containing phosphorus. Lindsey et al. [29] found that fertilizers containing phosphorous improved turfgrass rooting relative to fertilizers without phosphorus. Furthermore, Blended and Milorganite improved root volume compared to PA and urea. Overall, Blended and Milorganite provided enhanced rooting compared to PA and urea. The increased rooting of Blended and Milorganite compared to PA and urea could have been due to differences in nutritional analysis and/or fertilizer composition (combination of algae + cellulosic filler vs. pure algae) (Table 1). Although there were differences between biofertilizers and the synthetic fertilizer in terms of longest root length, total root length, root surface area, and root volume, there were no differences between fertilizer treatments for root biomass. Only Blended and Milorganite provided increased root biomass compared to the nontreated control. Lee et al. [25] reported biofertilizer increased root dry weight during turfgrass establishment compared to synthetic fertilizers, which is different from the results in this study. The varying rooting response between the studies could be due to differences in fertilizer application (i.e., surface applied vs. incorporated into the root zone). Overall, Blended and Milorganite provided the greatest improvement in turfgrass rooting, and PA offered increased rooting compared to urea.

5. Conclusions

At the conclusion of the study, all fertilizer treatments had greater turfgrass cover (PGC) compared to the non-treated control. Blended and PA outperformed or performed similar compared to Milorganite and urea in terms of turfgrass shoot growth, cover, color, and overall rooting. Thus, algae-based fertilizers (PA and Blended) produced from the RAB wastewater treatment system should be considered a sustainable and effective tur-

turfgrass fertilizer at establishment. Future research is needed to determine if algae-based fertilizers could be considered an EEF, which could be done by determining the N release characteristics and how much N is made plant-available in the soil. Additionally, research should be conducted in a field-based setting to determine nutrient uptake by the turfgrass and changes in soil health.

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