Hydrodynamic regime considerations for the cultivation of periphytic biofilms in two tertiary wastewater treatment systems

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Abbreviations: ATS – algal turf scrubber; TP – total phosphorus; TSS – total suspended solids

Abstract

As the demand for biofuel products continues to rise along with the need for tertiary wastewater treatment processes to facilitate the removal of phosphorus, increasing importance is placed on the design of cultivation systems for the production of algal biomass. In addition to traditional open ponds and photobioreactors, attached algal growth systems can provide high rates of biomass production while simultaneously removing nutrients from wastewater effluent. The potential economic viability of these attached growth systems is dependent on the optimization of bed flow characteristics, inflow water quality parameters, and hydrodynamic regimes in order to maximize biomass productivity. This study monitored the productivity and nutrient removal rates of a pilot-scale attached growth system (AGS1) used to remove phosphorus from wastewater effluent at a municipal wastewater treatment plant in Fayetteville, Arkansas. These results were compared with the results from a similar system in the same watershed that was monitored in a previous study (AGS2). The performance of AGS2 was documented in a companion article in *Ecological Engineering*. In spite of the similarities between the systems' locations and influent characteristics, the productivities of the two systems were very different. The rates of biomass production in AGS1 and AGS2 were 4.4 ± 4.8 and 26.7 ± 16.0 g dry weight $\text{m}^{-2} \text{d}^{-1}$, respectively. Potential reasons for the dramatic differences in performance between the two systems are explored in this article.

1. Introduction

The ecological services provided by algal growth systems are combined with the potential economic value of algal biomass for a number of industrial applications. For example, the potential for high lipid contents in algal cells, in addition to productivities that are superior to other bioenergy crops, makes algae a promising feedstock for biofuel production (Chisti, 2007; Dismukes et al., 2008; Bajpai and Tyagi, 2006; Hu et al., 2008). In their 2008 study, Dismukes et al. noted that biomass cultivation from aquatic phototrophs would require only a fraction of the amount of land that would be required for the cultivation of corn in order to displace gasoline consumption in the United States. In addition, algal growth systems have also been shown to effectively treat wastewater while generating large amounts of biomass (Adey et al., 2013; Adey et al., 2011; Adey et al., 1993; Craggs, 2001). The United States Department of Energy's Aquatic Species Program's study of the potential biofuel feedstock applications of algae concluded that systems which coupled wastewater treatment with algal biomass production were the most compelling in terms of economic value (Mulbry et al., 2008).

There are several types of artificial growth systems that have been investigated for the industrial-scale production of algae. These include photobioreactors, open ponds, and attached growth systems (Eriksen, 2008; Ugwu et al., 2007; Lee, 2001). While enclosed bioreactors and open pond or raceway systems constitute a more traditional approach to algae cultivation, they are generally used for the monoculture of microalgae. Attached growth systems allow for the cultivation of macroalgae in a mixed assemblage (Adey et al., 2013; Sandefur et al., 2011). Macroalgae, with its relatively high sugar content, is potentially desirable for the production of bioethanol and butanol (Jones and Mayfield, 2012; Cai et al., 2010). In addition, attached

growth systems, which involve the cultivation of a polyculture, do not require the expensive environmental controls found in enclosed systems (Sandefur et al., 2011).

Attached growth systems are designed to simulate a stream environment for the production of periphytic biofilms (Johnson and Wen, 2010; Hoffman, 1998). These oxygenic phototrophic films are composed of microbial communities that are attached to a growth surface. Microorganisms present in the periphytic assemblages include cyanobacteria, benthic diatoms, and green algae (Roeselers et al., 2008). These organisms use carbon dioxide to assimilate nutrients in biomass production. The photosynthetic activity in biofilm growth systems shifts the equilibrium of carbon dioxide in the water, resulting in higher pH levels that drive phosphate precipitation from the water column (Craggs et al., 1996a, 1996b). The removal of phosphorus by precipitation, in addition to removal via biomass assimilation, can lead to large reductions in the phosphorus concentrations of the irrigation water (Roeselers et al., 2008; Craggs et al., 1996a, 1996b).

Flow rate and nutrient concentrations are generally considered to be the major factors driving biomass production (Blersch et al., 2013; Adey and Loveland, 2007). Growth has been shown to increase with increased velocity in artificial stream systems, and investigations of periphyton growth has suggested that there is a critical flow velocity at which the growth and chlorophyll-a content is maximized (Labiod et al., 2007; Barr et al., 2008; Carpenter et al., 1991; Cronk and Mitsch, 1994). Potential explanations for this phenomenon include altered light regimes and the breakdown of diffusion boundary layers resulting from higher levels of turbulence (Grobelaar, 2009; Dodds and Bigs, 2002; Blersch et al., 2013). The altered light regimes resulting from turbulence in the water column have been shown to occur at Reynolds numbers in excess of 3000. This intermittent lighting has been shown to increase photosynthetic

efficiency in algal cells, and can be driven by turbulence (Grobelaar, 2009). This is coupled with increased nutrient and metabolite exchange between algal cells and the surrounding environment under more turbulent flow regimes, which can also stimulate productivity (Grobelaar, 2009; Dodds and Biggs, 2002).

In addition to increasing photosynthetic efficiencies and community metabolism, turbulent flow also has a role in the colonization of biofilms (Labiod et al., 2007). In their investigation of the relationship between flow regime and periphyton dynamics, Labiod et al. (2007) noted that during the initial stage of periphyton accumulation, diatom species tend to dominate. However, after approximately three weeks of growth, the authors observed a selection of benthic algae, followed by chlorophytes and cyanophytes in the film. Finally, after a one-month development period, a periphytic mat composed largely of long filamentous algae species was observed (Labiod et al., 2007). This climax community is the goal of attached growth systems, with the filamentous species acting as a support structure for other organisms and forming the base of a larger community. However, it should be noted that the climax community will vary by season in outdoor systems (Adey, 1982).

While turbulence is important for production and community development in attached growth systems, site constraints can limit the slope and resulting velocity in retrofitted algal flow-ways. This study presents performance data for an attached growth system that used municipal wastewater treatment plant effluent as an irrigation source, and compares the system's performance to that of another attached growth system that was operated in the same watershed. Factors that could have contributed to differences in performance between the two systems, principally hydrodynamic regime and influent nutrient concentrations, are explored.

2. Materials and Methods

The first algal growth system (AGS1) consisted of a 30 m long by 3 m wide flow-way, and was located at a municipal wastewater treatment plant in Fayetteville, Arkansas. The plant's treatment processes included screening, clarification, biological water treatment, filtration, and disinfection with UV light. The algal turf received effluent after the UV disinfection step and before the effluent was discharged into the Illinois River (see Figure 1). The effluent was redirected to the initial sedimentation step after passage over the growth system. The flow-way was lined with outdoor carpet on which the algal community was cultivated. Effluent was discharged onto the flow-way at a rate of 12.5 L s^{-1} from a broad-crested weir located at the top of the system.

Operation began in June of 2010 and continued through July of 2011. During a one-month startup phase, the flow-way was seeded with filamentous green algae from the genus *Cladophora*. The algae used to seed the system were taken from streams within the local watershed. The filaments were attached to stones and placed in the flow-way at 3 m intervals. By July 1, 2010 the algal community was established and data collection began.

The sampling regime involved the monitoring of productivity as well as total phosphorus (TP) removal across the system. The biomass was harvested by scraping the flow-way every 4 to 7 days, depending on the season. The interval between harvests depended on the rate of reestablishment of the biofilm, with the goal being to collect the biomass immediately after the growth peak. It was important to harvest before the end of the accrual phase and the beginning of autogenic sloughing in the loss phase which would decrease the amount of recoverable biomass (Labiod et al., 2007). During each harvest, samples were taken from three 1 m^2 sections

roughly at 7.5 m, 15 m, and 22.5 m down the flow-way from the system inflow. The biomass from each sampling section was scraped off of the carpet and onto 1 mm screen for draining. The remainder of the flow-way was also harvested in this manner, and the total weight of the wet biomass was measured. The algae samples from the 1 m² sections were dried at 105°C for 24 hours to determine the dry weight of the algae (Greenberg, 1992). Dry weights from the three samples were used to determine an average productivity value on a dry weight basis for each growth period.

The phosphorus concentrations at the system inflow and outlet were monitored from July 2010 through January 2011. The effluent used to irrigate the growth system was sampled daily at 1400 hours, and analyzed for TP, total suspended solids (TSS), and ammonia. The laboratory methods for the characterization of the effluent included the colorimetric method for TP, the Nesslerization method for ammonia, and a filtration method for TSS (USEPA, 1971; APHA, 2005a; APHA, 2005b). Grab samples of the effluent entering and exiting the system were collected twice a week and were analyzed for TP (Greenberg, 1992). In addition, multi-parameter water quality monitoring devices (YSI, Inc.) were deployed at the system inflow and outflow to monitor pH, dissolved oxygen, specific conductivity, and water temperature. These instruments recorded values for each parameter at ten minute intervals during three one-week long deployments in the summer and fall. These methods were similar to those used to monitor AGS2, and are described by Sandefur et al. (2011).

The second attached algal growth system (AGS2) was operated in Springdale, Arkansas from March through November of 2009. The system was located approximately 23 km to the north of AGS1. This system was 0.3 m wide, 90 m in length, and maintained a 2% slope, and received irrigation water in a surging mode. The irrigation source for AGS2 was the Spring

Creek, with the inlet to the system located immediately downstream of a municipal wastewater treatment plant effluent discharge point. The system was seeded with *Cladophora* in the same manner as AGS1. Water was discharged onto the flow-way in a surging mode, with an equivalent steady flow rate of approximately 1.3 L s^{-1} (Sandefur et al., 2011). The concentration of TP and nitrogen species in the irrigation water, in addition to the ambient temperature and cultivated algae species, were similar at AGS1 and AGS2, though they were not operated at the same time.

In order to assess the flow regime, the Reynolds number was calculated for each system using the following equation:

$$N_R = \frac{vR}{\nu} \quad (1)$$

where v is the flow velocity, R is the hydraulic radius of the channel, and ν is the kinematic viscosity (Haan et al., 1994). Due to site constraints, the flow-way was relatively flat, with only a 0.5% slope. This, combined with a method of water discharge onto the turf that lacked wave generation, resulted in a Reynolds number of approximately 2200. The steeper flow-way slope, combined with the wave-generating method of water discharge, generated more turbulence in AGS2 compared to AGS1, with AGS2 flow characterized by a Reynolds number of approximately 3500. Given the distinct differences in flow regime and system length, production and water quality data reported by Sandefur et al. (2011) for AGS2 was contrasted with the AGS1 performance on the basis of spatial extent and hydrodynamic regime.

3. Results

The system productivity for AGS1 averaged 4.4 ± 4.8 g dry weight $\text{m}^{-2} \text{d}^{-1}$ over the one year period, much lower than the average productivity of 26.7 g dry weight $\text{m}^{-2} \text{d}^{-1}$ observed at AGS2 (Table 1). The results of a two-sample t-test showed the measured productivities at each system to be significantly different ($p < 0.01$). The total mass of wet solids recovered from each harvest event averaged 9.9 kg. While formal counts were not performed on the AGS1 turf due to funding limitations, qualitative taxonomic observations noted that the system was mostly dominated by diatoms, with a few filamentous green algae species, such as those in the genus *Cladophora*. This is similar to the taxonomic data reported by Sandefur et al. (2011) for AGS2. AGS2 was consistently dominated by diatoms, with *Bacillariophyta* and *Chlorophyta* accounting for approximately 60% and 37% of the community, respectively (Sandefur et al., 2011).

In addition, the results of the phosphorus analysis showed lower removal rates across AGS1 when compared to AGS2. For the months from May through September, TP removal averaged 0.023 ± 0.05 mg L^{-1} (0.28 g $\text{m}^{-2} \text{d}^{-1}$) in AGS1, compared to an average removal of 0.076 ± 0.06 mg L^{-1} (0.34 g $\text{m}^{-2} \text{d}^{-1}$) in AGS2. Over the same time period, the average inflow concentrations of TP for AGS1 and AGS2 were 0.27 ± 0.15 mg L^{-1} and 0.17 ± 0.10 mg L^{-1} , respectively. The inflow concentrations of TSS, TP, and ammonia at each system are shown in Table 2. The ammonia levels were higher in the wastewater effluent used to irrigate AGS1 than in the influent at AGS2, while the concentrations of TP were slightly higher at AGS2. The TSS concentrations at AGS2 were also elevated, which was likely the result of the system being irrigated by a natural stream receiving wastewater effluent discharges.

The typical diurnal trends in pH at the system inflow and outflow showed diel ranges of less than one SU. Dissolved oxygen concentrations showed similar trends to pH levels, with peak dissolved oxygen concentrations occurring at midday. The diurnal trends in pH and dissolved oxygen for AGS1 are compared to AGS2 in Figure 2. The maximum pH recorded at the inflow and outflow of AGS1 were 8.3 and 9.5, respectively. This was lower than the maximum observed values for AGS2, which were 9.9 and 10.8 in the water entering and exiting the system. The temperature and specific conductivity of the effluent entering the growth systems were similar. The average water temperatures at AGS1 and AGS2 were $24.7 \pm 3.2^\circ\text{C}$ and $19.2 \pm 3.9^\circ\text{C}$, respectively. The specific conductivity at AGS1 and AGS2 were $403 \pm 237 \mu\text{S cm}^{-1}$ and $492 \pm 107 \mu\text{S cm}^{-1}$, respectively.

4. Discussion

The average rate of biomass production was approximately six times higher in AGS2 than in AGS1. The relatively low rates of production observed in AGS1 can likely be attributed in part to the flow regime and mode of effluent discharge into the system (Carpenter et al., 1991; Cronk and Mitsch, 1994). The slope of the AGS2 flow-way was four times greater than that of AGS1, corresponding to a Reynolds number of 3500 in AGS2, compared to a Reynolds number of 2200 in AGS1. The positive relationship between macroalgae productivity and flow velocity has been well established in the literature (Barr et al., 2008; Carpenter et al., 1991; Cronk and Mitsch, 1994; Blersch et al., 2014). In their laboratory-scale study of the effect of hydrodynamic regime, Carpenter et al. (1991) reported significant increases in algal turf net primary productivity with increasing flow. While more controlled comparative studies would be needed to determine the cause of the decreased productivity in AGS1, it is possible that this phenomenon could have contributed to the system's relative underperformance.

In addition to flow velocity, other studies have also demonstrated the importance of the mode of discharge onto the flow-way (Carpenter et al., 1991; Blersch et al., 2013; Stewart and Carpenter, 2003). AGS2 received water in a surging mode that generated waves down the flow-way. This feature was absent in AGS1, where effluent was discharged from a broad-crested weir which resulted in a relatively uniform flow velocity in one direction across the algal turf. Because unidirectional flow will tend to compact the filaments in the direction parallel to the bulk flow, shading within the turf can result in decreased photosynthetic activity (Stewart and Carpenter, 2003). The periodic change in flow direction at the base of the turf resulting from the wave motion generated by a surge device has been shown to mitigate the effects of self-shading by exposing both sides of the filaments to sunlight (Carpenter et al., 1991). This decrease in productivity with turbulence has been documented in other algal turf scrubber (ATS) systems. Carpenter et al. (1991) reported a 21% increase in algal turf productivity with the addition of oscillatory flow, and Adey and Loveland (2007) reported a 50% drop in the production of oxygen when circulation was maintained and wave-generating mechanisms were not operational. In their study of the effects of varying nitrogen loading rates, wave frequencies, and volumetric flow rates, Blersch et al. (2013) suggested that, while nitrogen availability was the main limiting factor in productivity, there was an optimum volumetric flow rate and surge frequency at which the algal growth was maximized. It is possible that the absence of a wave-generating discharge mechanism could have also contributed to the underperformance of AGS1.

In addition to the systems' flow regimes, the differences in production could have been driven in part by nutrient concentrations in the water used to irrigate the turf. The higher TP concentrations measured at the AGS2 inflow could have contributed to the higher biomass productivity; however, the differences in TP do not appear to be large enough to entirely account

for the dramatic differences in productivity observed between AGS1 and AGS2. It is also important to note that the average removal rate of phosphorus across the AGS1 system was lower than the removal rates reported at similar growth systems. Craggs et al. (1996b) reported an annual average of $0.73 \pm 0.2 \text{ g P m}^{-2} \text{ d}^{-1}$ for an attached growth system used for tertiary wastewater treatment in Patterson, California. In addition, the nutrient loading rates in the systems observed in this study were much higher than those observed by Adey and Loveland (2007), and could account for the more pronounced differences in observed productivity.

In addition to the differences in biomass production, varying nutrient removal rates were also observed between the two systems, with AGS1 underperforming compared to AGS2. The relatively low phosphorus removal rates observed in this study can likely be attributed to a lack of phosphorus precipitation across the system. At high pH levels, a precipitate in the form of a calcium phosphate compound is formed. In traditional wastewater treatment systems, the pH is adjusted chemically to drive phosphorus precipitation with the addition of lime (Metcalf and Eddy, Inc., 2003). In the algal growth systems, the pH is mediated biologically by the algae. As the algal turf undergoes photosynthesis, bicarbonate ions are removed from the water increasing the carbonate concentration and resulting in higher pH levels. At high pH levels the phosphate in the water will react with calcium and other multivalent metal ions to form a sparingly soluble precipitate that is removed with the biomass (see Equation 2) (Larsdotter et al., 2007; Metcalf and Eddy, Inc., 2003).

The algae-driven phosphorus precipitation process was described by Larsdotter et al. (2007) in their lab-scale study of phosphorus removal mechanisms in the production of

microalgae. This phenomena has also been observed in algal turf scrubber systems, and phosphorus precipitation with calcium has been reported at pH levels from 8.9 to 9.5 and greater in attached growth systems (Craggs et al., 1996b). The maximum pH recorded at the outflow of AGS1 was 9.5, and even at times of peak photosynthetic activity, the pH level rarely exceeded 9.0. Higher levels of phosphorus removal were reported at AGS2, with pH values measured in excess of 10.0 (Sandefur et al., 2011).

The lower pH levels and the absence of phosphorus precipitation in AGS1 can likely be attributed to the length of the flow-way in addition to the low productivity of the system. At 30 m long, the flow-way was relatively short. Sandefur et al. (2011) and Craggs et al. (1996b) monitored systems that were 90 m and 152 m long, respectively, and both systems yielded phosphorus removal rates that were higher than those observed in this study. The shorter length would have corresponded to less biomass for the effluent to come in contact with along an individual flow path, which could have resulted in fewer opportunities for carbonic acid removal.

While it is likely that the hydrodynamic regime and spatial extent played a role in the underperformance of AGS1, more data is needed in order to address the cause of the productivity decline with a higher degree of confidence. Qualitative assessment of the algal turf in AGS1 indicated that the algae species were similar to that of AGS2; however, the lack of quantitative taxonomic data for this system weakens the comparison. It is possible that an undesirable species composition in AGS1 contributed to the system's underperformance. Differences in community composition, in addition to the differences in the influent nutrient concentrations, could also have contributed to the differences in performance between the two systems.

5. Conclusions

The performance of the system monitored in this study illustrates the importance of designing attached growth systems with an appropriate spatial extent and hydrodynamic regime. The low rates of biomass production and TP removal measured in AGS1, in addition to the findings of past studies of similar systems, suggest that the flow and length limitations of the system could have had a damping effect on performance. The mode of effluent discharge should also be considered when designing future algal growth systems, as the absence of a surging mechanism in AGS1 could have reduced the system's productivity. In addition to the more commonly addressed issues of community structure and influent water quality, the hydrodynamic regime should also be considered in the design of future attached growth systems.

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Figure Captions

Figure 1. Process diagram of the algal growth system within wastewater treatment plant unit operations (figure not to scale).

Figure 2. Comparison of diurnal trends in pH and dissolved oxygen in algal growth systems at the Fayetteville Wastewater Treatment Plant (Site 1, $N_R \approx 2200$) and the Springdale Wastewater Treatment Plant (Site 2, $N_R \approx 3500$).

Tables

Table 1. Average productivity rates and standard deviations by month for the growth system under study (AGS1) compared with rates reported by Sandefur et al. 2011 (AGS2)

AGS1 ($N_R \approx 2200$)			AGS2 ($N_R \approx 3500$)		
Month	Productivity (g dry weight $m^{-2} d^{-1}$)		Month	Productivity (g dry weight $m^{-2} d^{-1}$)	
	Average	S.D.		Average	S.D.
Jul-10	2.79	0.73	Jul-09	34.0	20.0
Aug-10	8.45	7.28	Aug-09	31.0	18.0
Sep-10	6.78	3.51	Sep-09	26.0	10.0
Oct-10	5.81	3.07	Oct-09	24.0	14.0
Nov-10	1.27	0.98	Nov-09	15.0	5.0
Dec-10	1.42	0.88			
Jan-11	5.20	3.46			
Feb-11	1.89	1.55			
Mar-11	6.17	4.90	Mar-09	13.0	7.0
Apr-11	5.13	2.03	Apr-09	18.0	12.0
May-11	4.90	2.17	May-09	12.0	4.0
Jun-11	3.33	2.92	Jun-09		
Jul-11	2.84	2.62			

Table 2. Comparison of influent concentrations of total suspended solids (TSS), total phosphorus (TP), and ammonia at AGS1 and AGS2. AGS1 was irrigated with wastewater effluent while AGS2 received flow from the Spring Creek. Effluent concentrations at AGS1 were reported as monthly averages. AGS2 data adapted from Matlock et al. (2009).

AGS1				AGS2			
Month	Average Concentration (mg L ⁻¹)			Sampling Date	Concentration (mg L ⁻¹)		
	TSS	TP	NH ₃		TSS	TP	NH ₃
Jul-10	1.75	0.25	0.24	1-Mar-09	2.90	0.16	0.100
Aug-10	1.72	0.23	0.13	18-Mar-09	2.80	0.28	0.090
Sep-10	1.44	0.26	0.13	22-May-09	3.40	0.36	0.060
Oct-10	1.56	0.32	0.13	22-Jun-09	15.6	0.37	0.071
Nov-10	0.93	0.24	0.10	14-Jul-09	2.80	0.34	0.070
Dec-10	2.26	0.19	0.23	31-Jul-09	10.2	0.23	0.065
Jan-11	3.71	0.20	0.15	4-Aug-09	2.10	0.31	0.064
Feb-11	1.84	0.13	0.13	27-Aug-09	2.80	0.23	0.069
Mar-11	2.42	0.11	0.10				
Apr-11	2.14	0.15	0.11				
May-11	2.64	0.16	0.11				
Jun-11	2.05	0.26	0.20				
Jul-11	3.50	0.24	0.16