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Algae-Based Biofilm Productivity and Treatment of Dairy Wastewater: Effects of Temperature and Organic Carbon Concentration

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ALGAE-BASED BIOFILM PRODUCTIVITY AND TREATMENT OF DAIRY WASTEWATER: EFFECTS OF TEMPERATURE AND ORGANIC CARBON CONCENTRATION

by

Zachary T. Fica

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ABSTRACT

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by

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Utah State University, 2017

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Biofilm-based microalgal growth was determined as functions of organic chemical loading and water temperature utilizing dairy wastewater from a full-scale dairy farm. The dairy industry is a significant source of wastewater worldwide that could provide an inexpensive and nutrient rich feedstock for the cultivation of algae biomass for use in downstream processing of animal feed and aquaculture.

Algal biomass was cultivated using a Rotating Algal Biofilm Reactor (RABR) system. The RABR is a biofilm-based technology that has been designed and used to remediate municipal wastewater. The RABR was applied to treat dairy wastewater, through nutrient uptake, and simultaneously provide biomass for the production of renewable bioproducts.

Algal biomass was grown at temperatures ranging from 7-27 °C, and organic carbon concentrations ranging from 300-1200 mg/L of Total Organic Carbon (TOC). Analysis of Variance (ANOVA) calculations indicated that both the temperature of the wastewater and the level of organic carbon contributed significantly to the rate of biomass growth in the system. However, the interaction of temperature and organic carbon content was not significantly related to the biofilm-based growth rate. Equations were developed that can be used to evaluate algal biomass productivity and nutrient removal rates in future work.

(60 pages)

PUBLIC ABSTRACT

Algae-Based Biofilm Productivity and Treatment of Dairy Wastewater: Effects of Temperature and Organic Carbon Concentration

Zachary T. Fica

Production of dairy and associated products is a source of millions of gallons of wastewater every year. Water used in cleaning feeding stalls as well as the liquid component of the animal waste are two of the major volumetric components of this wastewater. This water is nutrient rich, often limiting the viability as a land applied fertilizer. However, these same nutrients could be used as an inexpensive feedstock for the cultivation of algae, which can then be used to produce downstream products including animal feed and aquaculture.

As part of this study, algal biomass was cultivated on dairy wastewater from the Utah State University Caine Dairy. A Rotating Algal Biofilm Reactor (RABR) system was used to grow the biomass. The RABR is a biofilm technology designed and developed at Utah State University and has been applied to the treatment of municipal wastewater. In this study, the RABR was adapted for use in a dairy wastewater stream.

The RABR was operated at temperatures ranging from 7-27 °C, and organic carbon levels in the wastewater ranged from 300-1200 mg/L of Total Organic Carbon (TOC). Areal algal biofilm growth rates were calculated, and statistical analysis showed that both increasing temperature and levels of organic carbon contributed to an increase in biomass growth and an increase in nutrient removal.

Equations were then developed using a linearization method and corresponding constants and equations were generated that can be used to evaluate algal biomass productivity and nutrient removal rates in future experiments and designs for dairy wastewater.

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CHAPTER 1

INTRODUCTION – LITERATURE REVIEW AND EXPERIMENTAL DESIGN

Dairy Production and Waste Management – USU Caine Dairy

Dairy production is a significant source of wastewater worldwide. A lactating dairy cow can produce over 50 L of wastewater per day [1]. At a dairy farm the size and scale of the Caine Dairy at Utah State University, where this research was conducted, over 10 m^3 of wastewater can be produced daily.

The Utah State University Caine Dairy Teaching and Research Center is among the nation's leading dairy production research centers (https://uaes.usu.edu/farms/caine-dairy). Research at the Caine Dairy focuses on animal nutrition and reproduction, waste-handling, animal health, and irrigated pasture for intensive rotational grazing. The center also houses three-hundred head of cattle used for dairy production. The Caine Dairy employs a traditional flush system to clean the feed stations for the cattle, which is a common practice in agriculture [1]. Cattle are fed while standing on large concrete slabs set at a very slight incline. Two thousand gallons (7.57 m^3) of water are used to flush the stalls twice daily. The flushed waste is directed through a coarse filter grate, and the large solids are removed, dried, and hauled away. Then, the liquid phase is pumped into a one acre settling lagoon, which empties into a one acre evaporation pond, where the wastewater is held until it is pumped back to the feed stations and recycled to flush the feeding stalls (Figure 1). Recycling used wastewater for this kind of flush system conserves water resources and reduces costs for dairy operators; however, recycling water creates a closed system for the liquid waste, where solid waste is the only stream

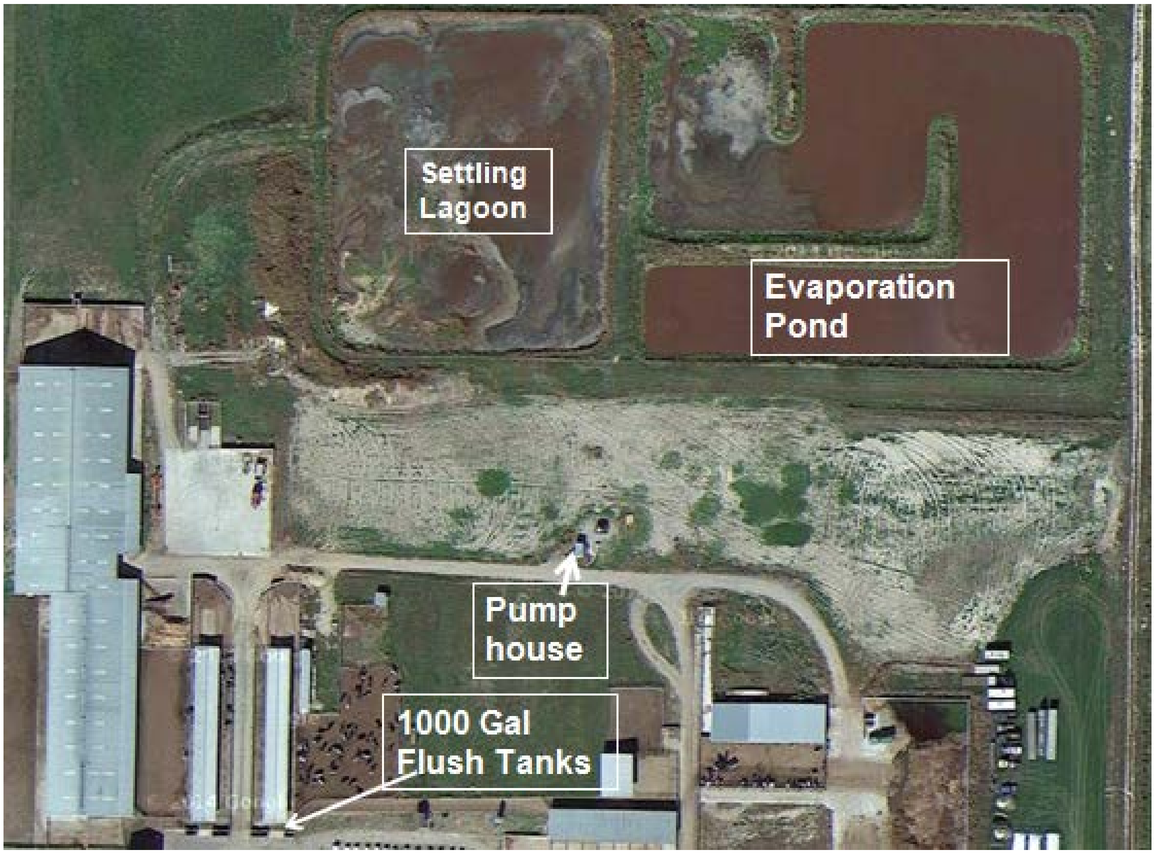

Fig. 1 Caine Dairy areal photo. Waste management system is highlighted within the figure.

leaving the system. The result is a buildup of water soluble nutrients, such as phosphorus and nitrogen, and turbid wastewater.

One strategy that can be employed with agricultural wastes is land application. Incorporation of dairy waste into soils used for crop production has been shown to not only have a positive effect on crop growth, but also reduce the amount of leached phosphorus waste when compared to using traditional fertilizers [2]. Land application provides the advantage of offsetting costs of fertilizers, as well as being a non-specific treatment method for dairy wastes that often vary in composition [3].

However, the levels of nitrogen, phosphorus, and other compounds in many dairy wastewaters, such as the wastewater at the Caine Dairy, require more land for land application than is available to the dairy. The assimilation capacity of the soil for land application is such that only a certain amount of nitrogen and phosphorus, on a mass basis, can be applied per year. The total amount of waste that can be land applied is calculated using the land limiting constituent (LLC) approach [4]. This approach provides a land requirement for a waste to be land applied, given the waste properties and soil assimilative capacity, which includes nutrients that are already in the soils [5,6]. If the land requirement exceeds the land available, the waste must undergo pretreatment solutions if they are to be land applied, which can be costly. This research explores algal biofilm treatment as a possible low cost pretreatment solution to lower the land requirement for land application. The Caine Dairy wastewater contains nutrient levels that cause the land requirement for application to exceed the land available to the dairy for land application. The composition of the Caine Dairy wastewater can be found in the methods section.

If, instead of land application, the wastewater was treated and then discharged, the wastewater would be required to meet the State of Utah standards for waste treatment. According to the State of Utah administrative code R317-3-11, there are various requirements in order to consider a wastewater as having been treated [7]. Some of these requirements include: less than 25 mg/L biological oxygen demand (BOD), less than 35 mg/L total suspended solids (TSS), and turbidity lower than 5 Nephelometric Turbidity Units (NTU). As part of this stud, the wastewater at the Caine Dairy was found to exceed all three of these standards.

Considering the information presented, the management of this particular wastewater would require either the removal of nitrogen and phosphorus in order to qualify for land application, or the removal of organics and suspended solids in order to be discharged.

Algae-Based Wastewater Treatment

Benefits of Algal Systems

Previous studies have been conducted using dairy wastewater as a nutrient source in algae systems [8], and it has been suggested that an algae-based alternative could provide a more cost effective treatment process for dairy and other livestock wastewater sources than traditional lagoons [9]. Algae-based treatment strategies have been successful in removing nutrients, specifically nitrogen, from waste streams [10]. One advantage that algae provide is that algal cultures can grow at different rates based on waste loading rates, and then algal biomass can be removed from the waste system thereby removing nitrogen and other nutrients in the form of biomass [11]. Algae cultivation therefore could provide a possible solution for the Caine Dairy wastewater management problem by reducing the amount of nitrogen and phosphorus in the wastewater in order to qualify the water for land application.

A second advantage of using algae based systems for wastewater treatment is that algae biomass that is removed from the system can be used as a feedstock for downstream bioproducts. Various downstream processing procedures have been developed to produce bioproducts including bioplastics, biofuel feedstock, high value pharmaceutical compounds, acetone, and nutrient rich animal feed [12-16]. These bioproducts can help offset costs associated with wastewater treatment. The overall costs and energy requirements associated with these downstream products have been extensively studied and modeled [17]. The use of downstream protein products as an animal feed is of special interest in this research, as the algal biomass produced in this project was grown on animal waste.

Limitations of Algal Systems

Algal systems also have certain limitations, including the presence of organic carbon, reduced efficiency at low temperatures, and inadequate light. In some algal growth systems, organic carbon has been shown to be antagonistic to phototrophic growth often due to nutrient competition with heterotrophic bacteria [18,19]. This is not true of all algal systems with high organic carbon; some systems with algal cultures grown in the presence of heterotrophic bacterial cultures have exhibited a symbiotic effect [20]. It is hypothesized that this symbiotic effect is due to heterotrophic bacteria supplying carbon dioxide to phototrophic algae species, which in turn provide oxygen for heterotrophic respiration. In summary, high levels of organic carbon have an impact on algal growth that is unclear based on the refereed literature. The effect of organic carbon on algal growth appears to vary from system to system.

Another limitation of algal wastewater management systems is temperature range. The effect of temperature on growth rates and nutrient uptake rates of many different algal species is well documented in primary literature [21,22]. The consensus and common trend across all algal species is that growth rates are limited at low temperatures and increase with increasing temperature. The Caine Dairy is in a region that undergoes seasonal temperature changes ranging from below freezing in winter to an average above 30°C in the summer [23]. If an algal system is to be used at the Caine Dairy, the nutrient removal rate would not be constant because of the seasonal variation in temperature.

The third limitation of algae-based wastewater treatment is light penetration in turbid water. Many waste streams, such as the wastewater at the Caine Dairy (Figure 2) are turbid. This turbidity can be due to suspended solids or other soluble colored compounds in the water, but turbidity level has been shown to directly correlate to light penetrance in water with no dependence on the cause of turbidity [24]. This limited light penetrance due to turbidity has been shown to limit algal growth [25].

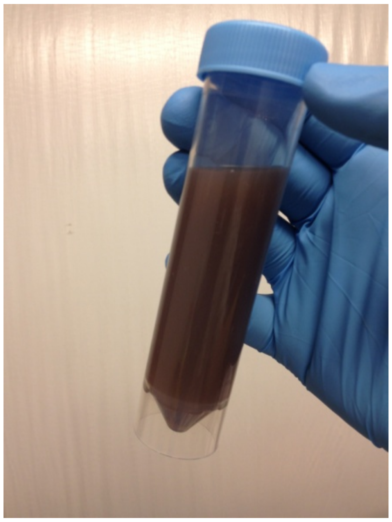

Fig. 2 Caine Dairy wastewater influent stream sample (40 mL). Showing turbidity of 890 NTU.

These three limitations (organic loading, temperature, and light penetration) were the limitations that were considered in this project.

Biofilm-based Algal Solutions

In order to overcome light limitation of algal growth due to turbidity in Caine Dairy wastewater, a biofilm based system could be considered. The system selected for testing with this particular wastewater was the Rotating Algal Biofilm Reactor (RABR) system designed at Utah State University. The RABR is a biofilm-based reactor system using a partially submerged rotating cylinder with growth substratum attached to the outside of the cylinder and with a novel harvesting mechanism [26]. Biofilm growth is possible even in turbid wastewater systems, because the RABR rotates the biofilm in and out of the water, thereby exposing it to both light and a nutrient source.

The RABR provides the possibility for wastewater nutrient sources to be utilized for algae-based systems that could not support suspended algal growth due to turbidity, color, or water depth limitations. Applications of biofilm engineering compared with suspended growth systems offer additional benefits by eliminating the need for polymers, sedimentation, and centrifugation when harvesting [27], and therefore reduce costs associated with harvesting when compared to traditional suspended growth systems [28]. Other biofilm systems, such as the Algal Turf Scrubber and the Rotating Algal Bioreactor, have been investigated as possible algal production strategies; however, these biofilm systems are often limited by turbidity [29].

Arrhenius Linearization in Biological Systems

The Arrhenius equation is a model often used to describe the temperature dependence of a rate constant of a chemical reaction. Its variables, usually named pre-exponential factor and activation energy, can be estimated using several methods [30]. The form of the equation used in this study was

$$K = A * e^{\frac{-E_a}{RT}} \qquad \text{(A)}$$

where K is the biomass productivity (g m^{-2} day^{-1}), E_a is the activation energy of the reaction (J mol^{-1}), A is the pre-exponential factor (g m^{-2} day^{-1}), and R is the universal gas constant (8.314 J K^{-1} mol^{-1}). The application of this equation can be found in chapter 3.

In biological systems, it has been shown that there is a similar relationship between temperature and the rate constant of a biological reaction within certain temperature ranges [31]. This relationship can be modeled, and rate constants and other important variables can be estimated using analytical methods. The adapted equation has been termed the Van't Hoff-Arrhenius equation [32] and the form used in this study was

$$\frac{K_2}{K_1} = \theta^{T_2 - T_1} \qquad \text{(B)}$$

where theta (Θ) represents the temperature correction coefficient. The application of this equation is presented in chapter 3.

CHAPTER 2

RABR TREATMENT OF CAINE DAIRY WASTEWATER

Introduction

Wastewater from the Caine Dairy was collected from the evaporation pond at the same location used for flushing stalls and was analyzed. The composition of the wastewater is shown in Table 1. Biomass produced from the study was also analyzed for elemental composition and the results can also be found in Table 1. Turbidity of the water was also measured at 890 NTU using a Hach 2100Q turbidimeter.

An algal based wastewater remediation system could provide the means for removing nitrogen and phosphorus from the wastewater, while providing biomass for downstream product production. There are possible limitations of an algae based system including light, organic levels, and temperature and three limitations were considered in this study. Application of the RABR system has been proposed as a solution to overcome turbidity of the wastewater and has been studied and demonstrated by Christiansen and Sims [26].

Table 1 Composition of influent Caine Dairy wastewater and cultivated biomass from the RABR system. (Analysis by Chemtech-Ford Laboratories – Sandy, UT).

Chemical Composition	Water[1] ($mg\ L^{-1}$)	Biomass[2] ($mg\ kg^{-1}$ dry wt.)
Total Organic Carbon	1200	648500
Total Nitrogen	155[3]	140400
Total Phosphorus	12	19100
Aluminum	7.67	776
Boron	1.73	132
Barium	0.63	75.7
Cobalt	0.03	1.96
Chromium	0.42	45.3
Copper	3.05	85.2
Iron	6.80	624
Manganese	0.67	107
Molybdenum	0.09	27
Sodium	460	23455
Nickel	0.25	24.1
Lead	0.05	6.41
Silica	106	5572
Strontium	1.18	125
Zinc	1.15	116

[1]Dairy wastewater influent stream
[2]Produced algae-based biofilm
[3]Total Kjeldahl Nitrogen (Organic nitrogen, ammonia, and ammonium)

Because nitrogen and phosphorus ratios are important to understand and monitor in an algal system, a comparison of molar ratios for carbon, nitrogen, and phosphorus in algal biomass, heterotrophic biomass, and Caine Dairy wastewater was performed and is presented in Table 2. Algal biomass nutrient content was taken from the analysis performed in Table 1, and heterotrophic biomass was assumed to be homogeneous ($C_5H_7O_2NP_{0.1}$) where half of the carbon required for growth is expelled from the system as CO_2. As can be seen from the table, the C:N:P ratios of both algal and heterotrophic biomass are similar. This could be cause for concern, as bacterial species in the wastewater could outcompete the algae for nutrients, especially considering the high levels of organic carbon available for heterotrophic metabolism and the high growth rates for bacteria compared with algae. In addition, the elemental molar ratios of the wastewater indicated that nitrogen would be the limiting nutrient for both algal and bacterial growth in the system. This nitrogen limitation was estimated by comparison of the nitrogen:phosphorus ratio of the Caine Dairy wastewater compared to the Redfield ratio of 16:1 (N:P) [33].

Table 2 Calculation of molar ratios for algal biomass, heterotrophic biomass, and Caine Dairy wastewater. C:N:P mass ratios were taken from analysis in Table 1. Molecular mass: C = 12g/mol, N = 14 g/mol, P = 31 g/mol.

Material	Mass Ratio as C:N:P	Molar Ratio as C:N:P
Algal Biomass	34:7:1[1]	88:16:1
Heterotrophic	19:5:1[2]	100:10:1
Caine Dairy	100:13:1	258:28:1

1 – Analyzed and measured from RABR biomass.
2 – Chemical composition assumed to be $C_5H_7O_2NP_{0.1}$. Taken from *Bioprocess Engineering: Basic Concepts* Kargi (1992).

In order to test the effect of organic carbon on algal cultivation on the wastewater, total organic carbon (TOC) was calculated and considered a variable for growth. Water temperature was also investigated as a variable for algal growth. *The hypothesis tested through this research is that abundant organic carbon in the system used by* *heterotrophic bacteria allows the bacterial cultures to outcompete the algal species for nitrogen and phosphorus*.

Materials and Methods

Algal Culture Selection and Analysis

Algae biomass inoculum for the laboratory RABRs was collected from the pilot scale RABR systems operated at the Logan City municipal wastewater treatment facility, a 460 acre (1.86 km^2) open lagoons system [34]. Visual microscopy of the pilot scale RABR based biofilm was keyed using the "PhycoKey" tool at the University of New Hampshire (http://cfb.unh.edu/phycokey/phycokey.htm). Microscopy indicated that the collected algae biomass was a poly-culture that contained a variety of algae species, with *Pseudanabaena*, *Oscillatoria*, and *Chroococcus* as the predominant species.

The biomass was then cultivated on cotton rope in shaker flasks on a shaker plate rotating at 120 rpm using dairy wastewater as the nutrient source. The algal biomass was allowed to grow in the shaker flasks for seven days before application to the RABR system to allow the culture to adapt to the nutrient source. The carbon:nitrogen:phosphorus molar ratio of the adapted biofilm calculated in Table 2 was 88:16:1, which is comparable to other algae-based systems [33].

Reactor Design and Waste Preparation

Rotating Algal Biofilm Reactors of 1-Liter volume were constructed and operated according to Christenson and Sims [26], and the biofilm reactors were wrapped with premeasured lengths of 3/16 in. dia. (0.476 cm dia.) solid braid cotton rope. In order to test the effect of organic carbon concentration on biomass productivity, the reactors were filled with different dilutions of wastewater and balanced to match the total nitrogen and phosphorus concentrations in the undiluted dairy wastewater influent stream using sodium nitrate (Thermo Fisher, Pittsburgh, PA) and potassium phosphate (Thermo Fisher, Pittsburgh, PA). The final organic carbon content of the wastewater dilutions was set to 1200, 600, and 300 mg L^{-1} of total organic carbon. The N:P ratio was measured (Hach Method 10127 and 10072) and balanced weekly to the same 155mg:12mg ratio of the Caine Dairy wastewater, and water loss due to evaporation was replaced with double distilled water. This experiment was conducted utilizing a semi-batch system, with a hydraulic retention time (HRT) of seven days. The RABR setup with algal growth is presented in Figure 3.

A water bath (VWR) with ¼ in. dia. (0.635 cm dia.) stainless steel tubing was used to maintain the water temperature of each reactor at 7° C, 17° C, or 27° C (±0.5° C). This range of temperatures was chosen as a representative range of seasonal water temperatures in Northern Utah [23]. Constant light was provided from eight 40W fluorescent lamps (GE) that provided a total of 200 µEinstein photons m^{-2} s^{-1} of continuous photosynthetically active radiation to the upper surface of the RABR systems. Two grams of centrifuged wet weight of adapted inoculum were added to the cotton rope growth substratum upon initiating rotation of the reactors.

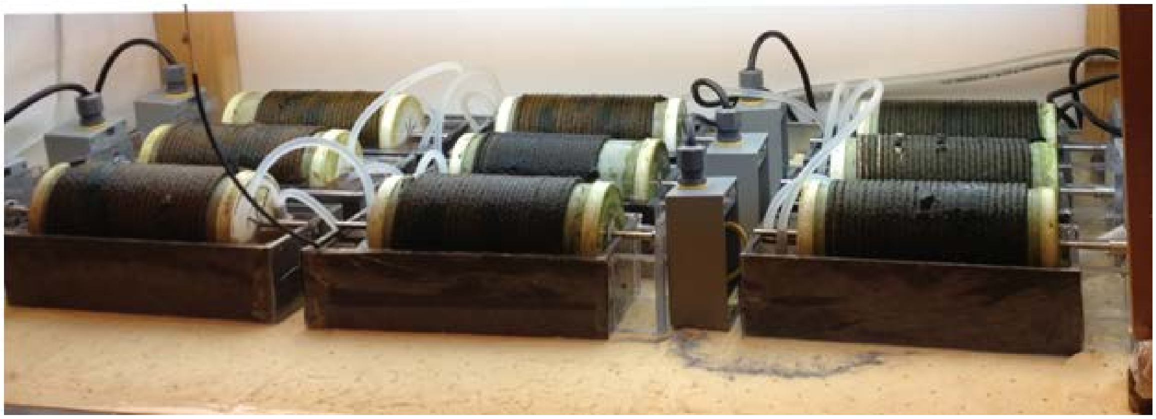

Fig. 3 RABR experimental setup. RABRs with This photograph depicts the algal biofilm on the RABRs 9 days after initial inoculation. Areal footprint of each individual reactor was measured to be 0.0338 m^2 and total rope surface area was calculated to be 0.0305 m^2.

Biomass Harvesting and Analysis

Biomass was harvested from the rope substrata weekly by mechanical scraping and lyophilized for biomass determination, ash free dry weight (AFDW) measurements, and chemical composition. AFDW calculation was determined using lyophilized biomass at 550° C. Biomass productivity was calculated using the AFDW of the biofilm divided by the areal footprint of the reactor (0.0338 m^2). The substrate surface area on which the biomass grew was calculated as 0.043 m^2, a factor of 1.27 larger than the areal surface area. Therefore, using substrate surface area to calculate biomass productivity would yield productivity values 1.27 times higher. Productivity was defined as the overall rate of biomass growth at steady state. Growth rates were calculated, and an Arrhenius plot of the data was used to obtain the temperature correction coefficient. ANOVA calculations were based on using biomass productivity as the dependent variable. Total theoretical productivity for the reactor was also calculated using measured growth rates. The Arrhenius equation was used to model the effect of temperature on the biofilm growth rate.

Number of Reactors and Statistical Analysis

Triplicate RABR trials for each combination of organic carbon concentration and temperature were conducted for statistical analysis. Each temperature was evaluated by testing three levels of organic carbon in triplicate for a total of nine reactors at each temperature and each organic carbon level. The total number of RABRs was 27, three for each combination of organic loading and temperature. In this study, biofilm productivity was the quantitative outcome, and temperature and organic loading were the explanatory variables. Analysis of Variance (ANOVA) was used to analyze the data because ANOVA not only provides a means to see how both of the independent variables, temperature and concentration of organic carbon, impact the dependent variable, biomass productivity, but also how the interaction of the two independent variables impacts the dependent variable [35].

Results and Discussion

After harvesting the produced biofilm, the productivity of the system was calculated. Temperature was made to be a limiting factor for the growth of algae by providing nutrients at a constant initial concentration for all RABRs under constant light

[36,37]. Controlling temperature as the limiting factor allows for the evaluation of the effect of temperature on biofilm productivity, as shown in Figure 4.

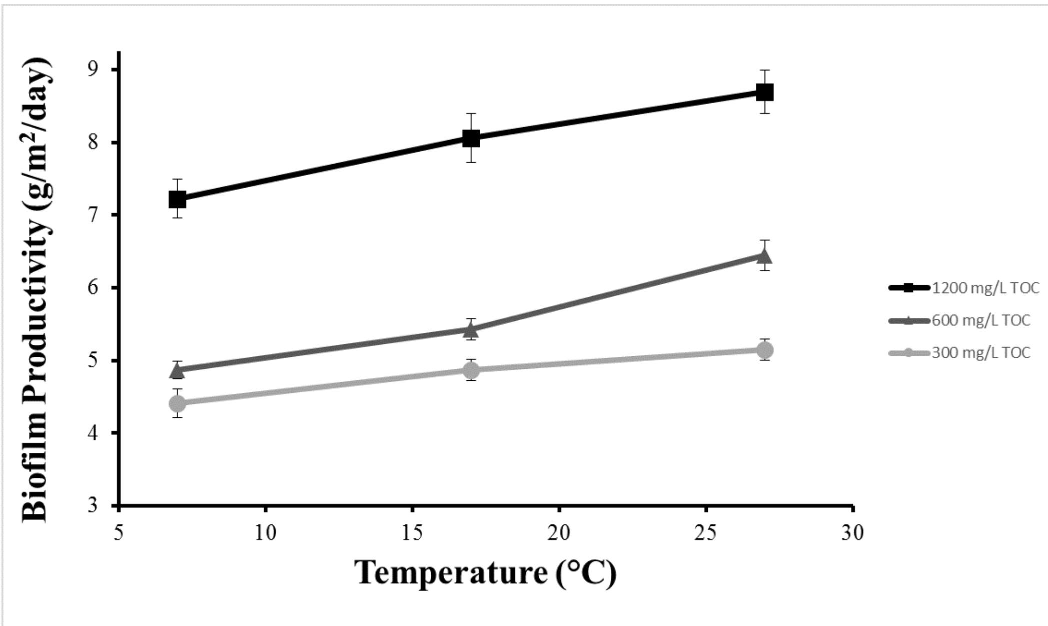

Fig. 4 Areal biofilm productivity (AFDW) as a function of temperature at three levels of organic loading. Error bars represent ± 95% confidence interval. n=3 for each data point. n=27 for entire system.

Results of ANOVA are shown in Table 3. With p-values of less than 0.005, both increasing temperature and increasing organic carbon concentration were correlated with an increase in biofilm productivity. However the interaction of temperature and organic loading did not contribute to a statistically significant increase in productivity (p-value 0.8871). This indicates that both increasing temperature and organic carbon increased biomass growth through different mechanisms. Error bars show 95% confidence intervals from the mean in all figures. ANOVA was performed using triplicate data points in each trial.

Table 3 Summary of the Analysis of Variance (ANOVA) results for the effect of temperature and organic carbon concentration on productivity of RABR based algae biofilm.

Source	**Sum of Squared Deviations**	**Degrees of Freedom**	**Mean Square**	**F-Statistic**	**P-Value**
Temperature	14.12	2	7.06	8.87	**0.0021**
TOC	30.2	2	15.1	18.98	**<0.0001**
Interaction	0.88	4	0.22	0.28	**0.8871**
Error	14.32	18	0.8		
Total	59.52	26			

In addition to correlating biomass productivity with temperature, ANOVA results also indicated that increasing the concentration of organic carbon had a positive correlation with biofilm productivity. This effect of organic carbon concentration of biofilm productivity is presented in Figure 5, by plotting the same biomass growth rates vs. organic carbon concentration instead of vs. temperature as in Figure 4. Application of an algae-based biofilm system for nutrient uptake in dairy wastewater requires that the biofilm be capable of growth in the presence of high levels of organic carbon. The positive correlation between productivity and organic carbon concentration indicates that a biofilm system could be used to remove nutrients from a waste stream with elevated levels of organic carbon. This positive correlation could be due to a symbiotic effect of natural bacteria providing carbon dioxide for phototrophic growth. In a dairy waste stream of similar elemental composition to that of the Caine Dairy, which produces 4000 gal day^{-1}, the theoretical yield for AFDW biomass is 9.5 kg day^{-1} of algae-based biofilm.

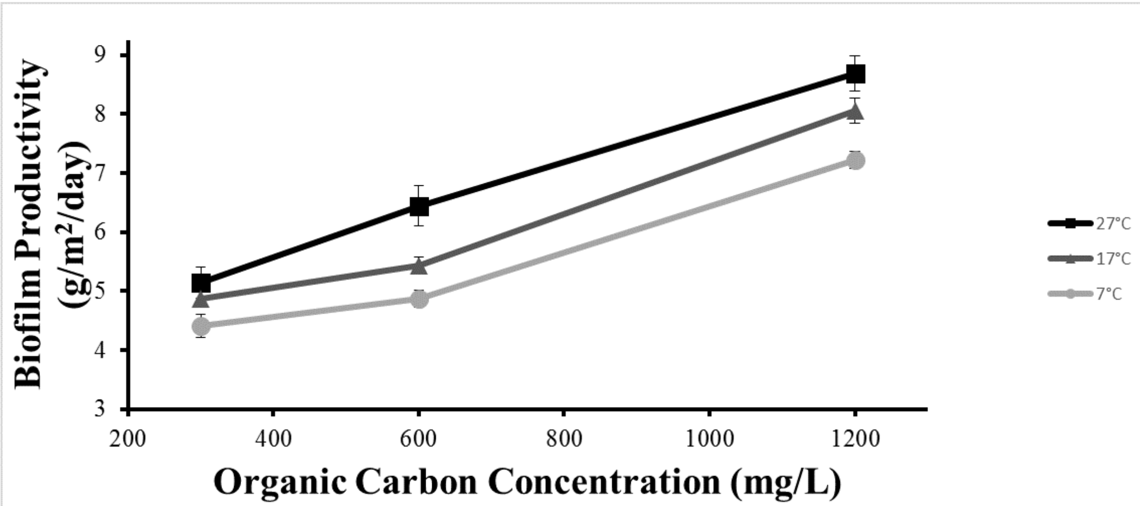

Fig. 5 Areal biofilm productivity (AFDW) as a function of organic loading at three different temperatures. Error bars represent ± 95% confidence interval. n=3 for each data point. n=27 for entire system.

Conclusions

Areal algal biofilm growth rates in dairy wastewater ranging from 7-27°C with organic levels ranging from 300-1200 mg L^{-1} were calculated and statistically analyzed using ANOVA. Results indicated that both an increase in water temperature and an increase in organic carbon level contributed significantly to the rate of biomass growth in the system. However, ANOVA results indicated that the interaction of organic carbon and temperature was not statistically related to the biomass productivity. Productivity was plotted against both temperature and organic carbon. The data generated in this research was used to generate equations related to assessing and predicting biomass productivity on dairy wastewater. *The hypothesis that abundant organic carbon in the system used by heterotrophic bacteria allows the bacterial cultures to outcompete the algal species for nitrogen and phosphorus was demonstrated to be not be true*.

CHAPTER 3

GENERATION OF EQUATIONS

Arrhenius Linearization

Because ANOVA results indicate that temperature was a contributing factor to biomass productivity, the productivity rates at the three specified temperatures can be applied to Equation A (the Arrhenius equation) which is shown again here for convenience.

$$K = A * e^{\frac{-E_a}{RT}} \tag{A}$$

Using the variables and constants described in chapter 1, this equation can be linearized by taking the natural log of both sides to yield:

$$lnK = \frac{-E_a}{RT} + ln\,A \tag{C}$$

Temperature was converted from Celsius to Kelvin, and the slope of the line formed after plotting lnK vs. - $\frac{1}{T}$ provides the value for activation energy of biofilm productivity at a specified organic carbon concentration (E_a). These linearizations can be seen in Figure 6. This activation energy represents the energy input necessary for formation of algal biomass. As will be shown in this chapter, the activation energy values generated using this method can be applied to equation B (the Van't Hoff equation) to generate useful, predictive constants for biomass productivity.

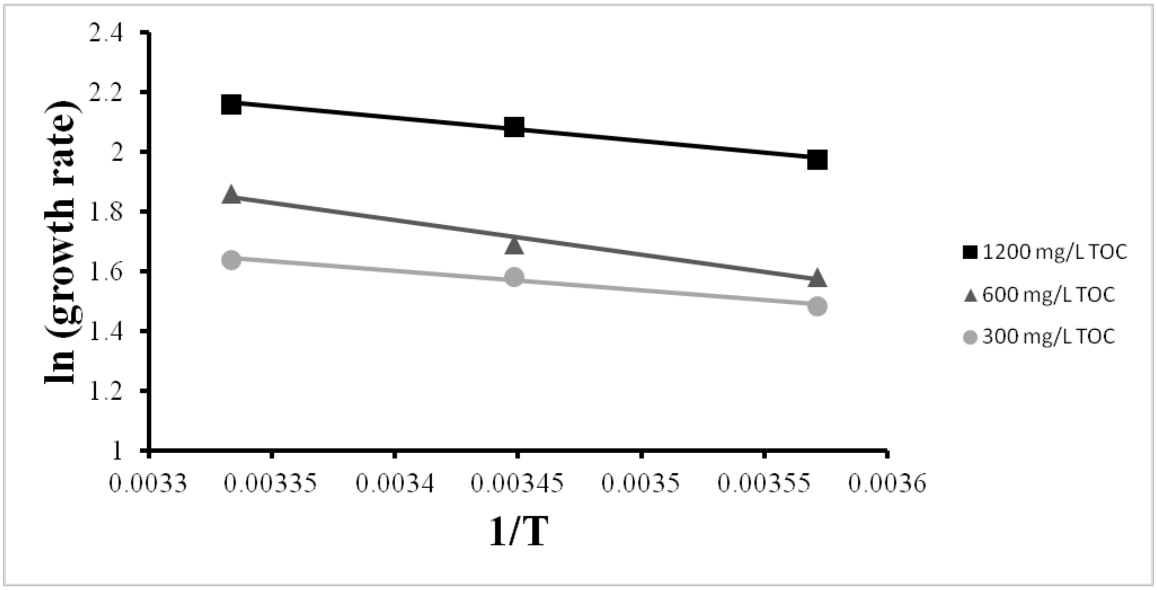

Fig. 6 Arrhenius plot of RABR productivity (g · m^{-2} day^{-1}) as a function of temperature (K). The slope of the best fit line for each concentration represents $\frac{-E_a}{R}$ where $R = 8.314\ J\ K^{-1} mol^{-1}$ and E_a was calculated (Table 4).

Temperature Correction Coefficient and Other Constants

Knowing the activation energy of biomass growth at a specific organic carbon loading level allows for the calculation of biomass productivity given the temperature of the waste water. Biomass productivity (K) values were calculated at the given organic carbon and temperature levels and can be found in Table 4. Applying the activation energy and biomass productivity to equation B (the Van't Hoff equation), shown again here for convenience, allows for calculation of the temperature correction coefficient; theta (Θ) [38].

$$\frac{K_2}{K_1} = \theta^{T_2 - T_1} \quad \text{(B)}$$

This is done by rearranging equation B to:

$$\ln\theta = \frac{\ln\frac{K_1}{K_2}}{T_2 - T_1} \quad \text{(D)}$$

This temperature correction coefficient is calculated using reference productivity (K) and temperature (T) values. The derived temperature correction coefficient values are reported in Table 4, and were found to be consistent with values for other biological systems [39-41].

Table 4 Temperature correction coefficients, activation energies, and constants of biofilm productivity and nutrient uptake at three levels of organic loading (TOC).

Level of TOC (mg/L)	Symbol	1200	600	300
Biomass Productivity (g m^{-2} day^{-1})	K	8.69	6.44	5.152
Activation Energy (J K^{-1} mol^{-1})	E_a	6473	9739	5440
Temperature Correction Coefficient (unitless)	Θ	1.0096	1.0145	1.0081
Nitrogen Uptake Rate (g m^{-2} day^{-1})	K_N	1.22	0.91	0.723
Nitrogen Correction Coefficient (unitless)	Θ_N	1.0098	1.0151	1.0078
Phosphorus Uptake Rate (g m^{-2} day^{-1})	K_P	0.17	0.12	0.098
Phosphorus Correction Coefficient (unitless)	Θ_P	1.0101	1.0149	1.0116

Uptake and Growth Equations

At a known concentration of organic carbon, the temperature correction coefficient (*Θ*), derived from equation D, can be used to predict biofilm productivity at any temperature within the range evaluated as part of this study (7° C-27° C). Replacing the variables in equation B (Van't Hoff) with the calculated constants yields:

$$\boldsymbol{K_{prediction} = 5.152 * \theta^{T_{water}-280}} \tag{E}$$

which predicts algal biofilm productivity (K) when T_{water} is water temperature and $\boldsymbol{\theta}$ is the temperature correction coefficient calculated in the previous section.

Using the molar ratios of the biomass (Table 2) it was also possible to derive a temperature correction coefficient from the biofilm productivity values in order to predict nitrogen and phosphorus uptake by the biofilm. These values were calculated by taking the total biomass productivity (K), and converting the total mass to mass of nitrogen and phosphorus using molar ratios. The calculated rates of nitrogen and phosphorus removal are reported in Table 4 as K_N and K_P respectively. The temperature correction coefficients that were calculated for nitrogen and phosphorus, Θ_N and Θ_P respectively, are also shown in Table 4. Therefore, at a known water temperature and concentration of organic carbon, the Θ_N values found in Table 4 can be applied to:

$$\boldsymbol{K_{N,prediction} = 0.723 * \theta_N{}^{T_{water}-280}} \tag{F}$$

in order to predict the rate of nitrogen uptake (g m^{-2} day^{-1}) from the wastewater. The Θ_P values from Table 4 can be applied to:

$$\boldsymbol{K_{P,prediction} = 0.098 * \theta_P{}^{T_{water}-280}} \tag{G}$$

in order to predict the rate of phosphorus uptake (g m^{-2} day^{-1}) from the wastewater.

These equations allow biomass generation, nitrogen uptake, and phosphorus uptake to be predicted in Caine Dairy wastewater or wastewater with similar qualities. For example, in a similar wastewater with 600 mg L^{-1} of TOC at 283 K the predicted biomass productivity would be:

$$\boldsymbol{K_{prediction} = 5.152 * 1.0145^{283-280}}$$

K = 5.38 m\g m^{-2} day^{-1}

And the associated removal rates of nitrogen and phosphorus would be:

$$\boldsymbol{K_{N,prediction} = 0.723 * 1.0151^{283-280}}$$

$$\boldsymbol{K_{P,prediction} = 0.098 * 1.0149^{283-280}}$$

K_N = 0.756 g m^{-2} day^{-1}

K_P = 0.102 g m^{-2} day^{-1}

These predicted rates of nutrient removal and biomass growth can then be used for application of this system to accomplish various engineering objectives. The biomass productivity rates are given with units of mg m^{-2} day^{-1}, which allows for utilization of the productivity values to predict biomass growth, areal footprint requirements, and time requirements. The equations can also be used to estimate upscale costs and resources, including heating requirements of a remediation system or nutrient requirements for biomass growth.

CHAPTER 4

ENGINEERING SIGNIFICANCE

Engineering Application of Equations

The equations developed and described in Chapter 3 can be used to design algal systems at the Caine Dairy, or other dairies with similar wastewater characteristics. For the purposes of understanding how the equations generated as part of this study can be applied to upscale engineering, some examples of applying these equations are given here.

The first example is a dairy that produces a total of 10g of nitrogen waste per day. This dairy would like to remove all 10g of nitrogen waste in the form of algal biomass. The water temperature has been measured at 17℃ (290K) and the organic carbon level has been measured to be 1200 mg L^{-1}. In this example, the nitrogen removal equation will be used:

$$\boldsymbol{K_{N,prediction} = 0.723 * \theta_N{}^{T_{water}-280}} \qquad \text{(F)}$$

From Table 4, at 1200 mg L^{-1} the temperature correction coefficient for nitrogen removal is $\Theta_N = 1.0098$. Inserting these known variables into the equation yields:

$$\boldsymbol{K_{N,\,prediction} = 0.723 * 1.0098^{290-280}}$$

Solving for K_N provides a nitrogen removal rate of 0.797 g m^{-2} day^{-1}. Therefore, the areal footprint requirement for removal of 10g of nitrogen per day is:

10g day^{-1} / 0.797 g m^{-2} day^{-1} = **12.6 m^2 areal footprint requirement.**

A second example is a dairy that is not concerned about nitrogen removal, but wants to produce as much algal biomass as possible for downstream bioproducts. This dairy has 10 m^2 of land on which to grow biomass. The water temperature at this dairy has been measured at 17°C (290K) and the organic carbon level has been measured at 600 mg L^{-1}. In this example, the biomass growth equation will be used:

$$\boldsymbol{K_{prediction} = 5.152 * \theta^{T_{water}-280}} \quad \text{(E)}$$

From Table 4, at 600 mg L^{-1} the temperature correction coefficient for biomass growth is Θ = 1.0145. Inserting these known variables into the equation yields:

$$\boldsymbol{K_{prediction} = 5.152 * 1.0145^{290-280}}$$

Solving for K provides a biomass growth rate of 5.95 g m^{-2}. Therefore, the biomass that can be produced per day is:

5.95 g m^{-2} * 10 m^2 = **59.5 g biomass day^{-1}.**

A final example is a dairy that needs to remove 3g of phosphorus waste per day. This dairy is in a cold climate, but they are willing to heat wastewater in order to keep the temperature high enough for the RABR to remove the 3g of phosphorus per day as long as it is cost effective. The organic carbon concentration in the water has been measured to be 300 mg L^{-1} and the dairy has 25 m^2 of areal space on which to grow biomass. In this example, the phosphorus removal equation will be used:

$$\boldsymbol{K_{P,prediction} = 0.098 * {\theta_P}^{T_{water}-280}} \quad \text{(G)}$$

From Table 4, at 300 mg L^{-1} the temperature correction coefficient for phosphorus removal is Θ_P = 1.0116. In this case, the rate of phosphorus removal per m^2 is known (3g/25m^2) so inserting known variables into the equation yields:

$$\mathbf{0.12\ g/m^{\wedge}2 = 0.098 * 1.0116^{T_{water}-280}}$$

Solving for T_{water} provides a water temperature of 294.6 K or **24.6 °C**. This is relatively warm water, and the dairy will most likely have to expend a significant amount of energy to heat the water to this temperature.

Design of a RABR for the Caine Dairy

Using the rate of waste production at a dairy farm the size of the Caine Dairy as well as the ratio of nitrogen and phosphorus separated along with solid waste [1], in combination with the amount of water use and wastewater characteristics at the Caine Dairy, the areal requirement for a RABR system can be calculated. Some assumptions need to be made, including: wastewater remains at a constant 1200 mg L^{-1} of organic carbon, water temperature is a constant 27°C, light is not a limiting factor for algal growth, and the input of phosphorus waste into the wastewater is constant.

The Caine Dairy has a rate of soluble phosphorus waste production of approximately 39 g day^{-1} [1]. The organic carbon concentration in the water has been measured to be 1200 mg L^{-1}, and the water temperature is assumed to be 27°C. We are interested in phosphorus removal, so the equation we will use is:

$$\boldsymbol{K_{P,prediction}}\left(\frac{\boldsymbol{g}}{\boldsymbol{day*m^2}}\right) = \mathbf{0.098} * \boldsymbol{\theta_P}^{\boldsymbol{T_{water}}-\mathbf{280}} \tag{G}$$

From Table 4 the temperature correction coefficient for phosphorus removal in this case is Θ_P = 1.0101. Inserting all of our known variables into the equation yields:

$$\frac{\mathbf{39\,\frac{g}{day}}}{\mathbf{Areal\ Land}(\boldsymbol{m^2})} = \mathbf{0.098 * 1.0101^{300-280}}$$

Solving for areal land provides a land requirement of **325.5 m^2** or **0.081 acres.** This amount of areal land would also produce 2.03 kg of AFDW biomass per day, and represents 0.868 m^2 per animal unit (AU – 1,000 lb. dairy cow).

The RABR can also be utilized as a pre-treatment or post-treatment step in a process that can be used to recover excess nutrients from a wastewater source. The RABR could be used in combination with other waste management strategies, such as land application. The pre-treatment of the wastewater by the RABR can remove nitrogen and phosphorus, thereby reducing the amount of land necessary for land application.

CHAPTER 5

UPSCALE APPLICATION

Caine Dairy Upscale

The Caine Dairy at Utah State University has already been used as a site for potential upscale of wastewater treatment using the RABR. Because of the wastewater management challenges described in Chapter 1, a pilot scale RABR system was constructed and operated using the effluent wastewater from the evaporation pond, as can be seen in Figure 7.

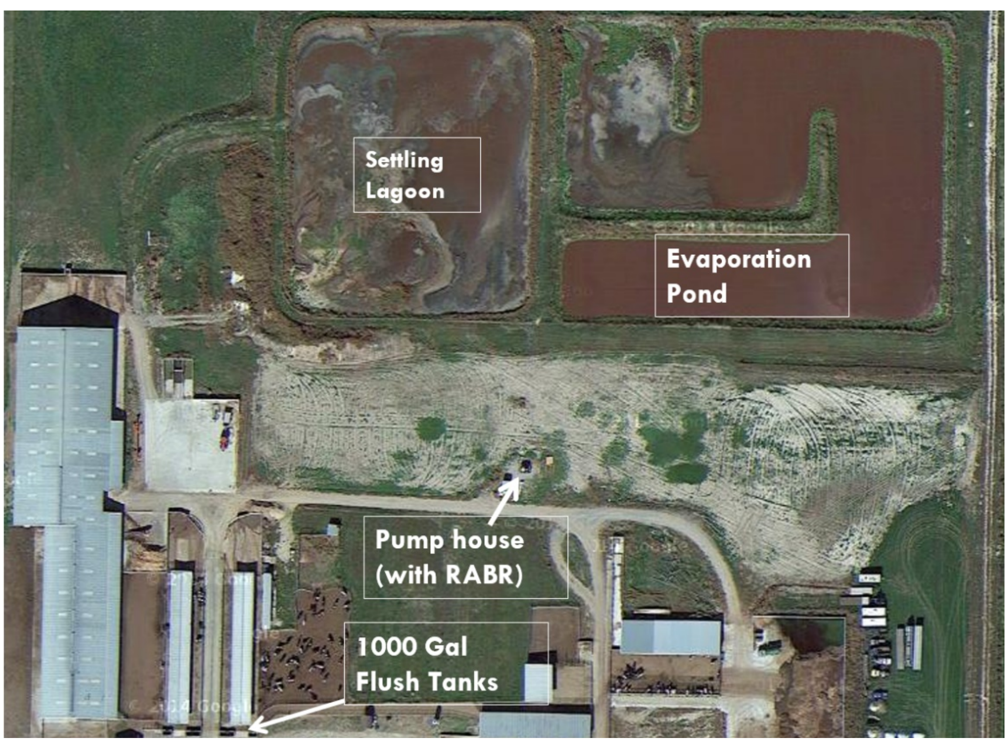

Fig. 7 Caine Dairy overhead photo. Waste management system is highlighted within the figure, including RABR setup at pumphouse.

The RABR is operated in the wastewater at the pump house that is used for filling the flush tanks. Future work at the Caine Dairy should include the adaptation of the equations generated in this study to a continuous flow, large scale system. It should also include optimization of environmental variables at the Caine Dairy, including temperature, light, flow rates, and biomass harvesting rates.

The setup and design at the Caine Dairy should accommodate further evaluation and testing of the RABR system in dairy wastewater. The layout of the RABR station at the Caine Dairy is shown in Figure 8.

Fig. 8 Layout of RABR station at Caine Dairy. Left – Disc style RABR (200 gal, continuous flow) with 8 polystyrene discs of 2-foot diameter Back – Pilot Scale 6-foot diameter RABR (batch mode) Right – Reservoir for continuous flow disc reactor.

As can be seen, the research station at the Caine Dairy includes a structure approximately 15-feet x 40-feet, with a domed roof covering. Currently there is an operating RABR with 6-feet diameter, and a disc-style reactor operating in a 250-gallon tank. The RABR is currently operated in batch mode, and the disc reactor is fed by a second storage tank and is operated in continuous flow setup with a variable rate of water flow through the system.

Future work at the Caine Dairy research station could include comparing disc RABR productivity and removal rates to those of the cylindrical RABR, as well as adapting the systems to be continuous flow. Power requirements, heating requirements, and capability of operation during winter months could also be investigated as the system is further optimized. The equations generated in this experiment could also be validated against a large scale system using the Caine Dairy RABR.

Bioproduct Production

Another future recommendation is to generate further predictive equations and models to correlate biofilm productivity with the generation of bioproduct generation. As described in Chapter 3, the equations generated as part of this study are useful for the prediction of biomass generation and nutrient removal from dairy wastewater. The addition of further equations and models could allow for the prediction of bioproduct production and downstream value from wastewater composition. This would prove useful as part of a Techno-Economic Assessment, where wastewater quality could be directly linked to a downstream product, through the RABR. Some specific examples of downstream bioproduct opportunities and options include biofuels, protein feeds, nutraceuticals, and bioplastics.

Upscale Challenges

Some steps have already been taken to upscale production of algae from dairy wastewater and to generate bioproducts. The Caine Dairy Algae Treatment Facility has already been developed as part of a goal to upscale algae production and wastewater treatment. There are some challenges to consider when further upscaling the RABR and the production of bioproducts.

One challenge to consider is the proximity of the RABR to the facility where the algae will be processed. Too often at the Caine Dairy the harvested biomass must be transported and stored for an extended period, which may affect the quality of bioproducts downstream.

Another challenge in the processing of algal biomass is the water content of the sample. Some bioproduct generation procedures have been shown to be effective with wet algae [42]. However, many procedures require dry algae samples, and the cost associated with drying biomass samples must be considered in the future.

There will be other issues to consider, however most of the problems with upscale will be specific to the downstream product that is being produced. The author recommends that any future work be carefully considered and planned in order to consider any and all possible barriers that may be create problems, especially when moving onto upscale plans.

CHAPTER 6

CONCLUSIONS

The background and problems described in Chapter 1 were addressed, and a possible solution and design was explored as part of this study. Because of the high nutrient levels of the wastewater at the Caine Dairy that restricted the land application alternative, an algae-based treatment solution was explored. A laboratory scale experimental design was described in Chapter 2 and included the methods used to quantify nutrient removal from the wastewater as well as methods used in analysis of algal biomass harvested. The biomass productivity rates were used to generate useful constants and equations in Chapter 3.

These equations are a meaningful contribution of this study to the general scientific community. The predictive rates of nutrient removal and biomass growth can be applied to future experimental designs and plans. From the perspective of wastewater treatment, the equations can be used to predict nutrient removal from a dairy waste stream. From a downstream perspective, the equations can be used to predict the amount of algal biomass and other nutrients needed to generate downstream products.

In Chapter 4, future upscale applications of the RABR system at the Caine Dairy were discussed, as well as the upscale possibilities of using algal biomass as a downstream feedstock. The results of this study were summarized and published as a peer reviewed article in the Journal of Biological Engineering referenced as: **Fica, Zachary T., and Ronald C. Sims. "Algae-based biofilm productivity utilizing dairy**

wastewater: effects of temperature and organic carbon concentration." Journal of Biological Engineering 10.1 (2016): 18. The published article has been appended to this Thesis in Appendix A.

For future research and upscale, the following projects and ideas are suggested. First, the equations generated in this study could be further developed and adapted to include a larger variety of dairy wastewater, in addition to the Caine Dairy wastewater. Including inputs other than temperature and organic carbon levels in the equations would also be useful for future applications. The equations could be further adapted to include the effects of the following variables: light levels, RABR rotation speed, nitrogen levels, phosphorus levels, toxin levels, and growth substratum.

Another future recommendation is the optimization of the upscale process to minimize energy input and cost associated with treatment. Algal biofilm systems show promise in the treatment of dairy wastewater streams, especially in streams that have high concentrations of nitrogen and phosphorus. The limitations of algal biofilms will be in the cost associated with construction and management of algal reactors and offsetting costs of downstream algal bioproducts. Optimizing the operation of an algae based reactor on a pilot or industrial scale would help to make wastewater treatment through algal biofilms a realistic technology. Algal technologies have a future in wastewater treatment and the production of renewable bioproducts.

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APPENDICES

Appendix A – Published Article

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Journal of Biological Engineering

RESEARCH

Open Access

Algae-based biofilm productivity utilizing dairy wastewater: effects of temperature and organic carbon concentration

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Abstract

Background: Biofilm-based microalgal growth was determined as functions of organic chemical loading and water temperature utilizing dairy wastewater from a full-scale dairy farm. The dairy industry is a significant source of wastewater worldwide that could provide an inexpensive and nutrient rich feedstock for the cultivation of algae biomass for use in downstream processing of animal feed and aquaculture applications. Algal biomass was cultivated using a Rotating Algal Biofilm Reactor (RABR) system. The RABR is a biofilm-based technology that has been designed and used to remediate municipal wastewater and was applied to treat dairy wastewater through nutrient uptake, and simultaneously provide biomass for the production of renewable bioproducts.

Results: Aerial algal biofilm growth rates in dairy wastewater at 7 and 27 °C temperatures were shown to be 4.55 ± 0.17 g/m^2-day and 7.57 ± 1.12 g/m^2-day ash free dry weight (AFDW), respectively. Analysis of Variance (ANOVA) calculations indicated that both an increase in temperature of the wastewater and an increase in the level of organic carbon, from 300 to 1200 mg L^{-1}, contributed significantly to an increase in the rate of biomass growth in the system. However, ANOVA results indicated that the interaction of temperature and organic carbon content was not significantly related to the biofilm-based growth rate.

Conclusion: A microalgae-based biofilm reactor was successfully used to treat turbid dairy wastewater. Temperature and organic carbon concentration had a statistically significant effect on algae-based biofilm productivity and treatment of dairy wastewater. The relationships between temperature, TOC, and productivity developed in this study may be used in the design and assessment of wastewater remediation systems and biomass production systems utilizing algae-based biofilm reactors for treating dairy wastes.

Keywords: Biofilm, Dairy wastewater, Arrhenius, Temperature correction coefficient, Organic carbon, Nutrient uptake

Background

The Utah State University owned Caine Dairy Teaching and Research Center is among the nation's leading dairy production research centers. Research at the Caine Dairy focuses on animal nutrition and reproduction, waste-handling, animal health, and irrigated pasture for intensive rotational grazing. The center also houses three-hundred head of cattle used for dairy production. The Caine Dairy is equipped with a traditional flush system to clean the feed stations for the cattle, and 2000 gallons of water are used to flush twice daily. The flushed waste is directed through a coarse filter grate and the large solids are removed and dried. The liquid phase is pumped into a one acre settling lagoon, which empties into a one acre evaporation pond, where the wastewater is held until it is pumped to the feed stations to be used for flushing stalls. Recycling used wastewater to flush stalls is a common practice in agriculture [1]. However, recycling water creates a closed system for the liquid waste, where solid waste is the only stream leaving the system. The result is a buildup of water soluble nutrients, such as phosphorus and nitrogen, and turbid wastewater.

One strategy that can be employed with agricultural wastes is land application [2]. However many dairy wastewaters, such as the wastewater at the Caine Dairy, contain high

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concentrations of nitrogen and phosphorus that are not appropriate for land application, and must undergo costly pretreatment solutions if they are to be land applied [3]. These same compounds can serve as a nutrient source for the production of algal biomass that can be used as a feedstock for downstream processing into bioproducts [4, 5].

The Rotating Algal Biofilm Reactor (RABR) is a biofilm-based reactor system using a partially submerged rotating cylinder with growth substratum attached to the outside of the cylinder and with a novel harvesting mechanism [6]. Biofilm growth is possible even in turbid wastewater systems, because the RABR rotates the biofilm in and out of the water, thereby exposing it to both light and a nutrient source. The RABR provides the possibility for wastewater nutrient sources to be utilized for algae-based systems that could not support suspended algal growth due to turbidity, color, or water depth limitations. Applications of biofilm engineering compared with suspended growth systems offer additional benefits by eliminating the need for polymers, sedimentation, and centrifugation when harvesting [7], and therefore reduce the costs associated with harvesting when compared to traditional suspended growth systems [8]. The harvested biofilms can be used to generate bioproducts through downstream processing including bioplastics, biofuel feedstock, high value pharmaceutical compounds, and nutrient rich animal feed [4, 9–12]. Other biofilm systems, such as the Algal Turf Scrubber, have been investigated as possible algal production strategies; however these biofilm systems are limited by turbidity [5].

Previous studies have been conducted using dairy wastewater as a nutrient source in algae systems [13, 14], and it has been suggested that an algae-based alternative could provide a more cost effective treatment process for dairy and other livestock wastewater sources [15, 16]. In some other algal growth systems, organic carbon has been shown to be antagonistic to phototrophic growth often due to nutrient competition with heterotrophic bacteria [17, 18]. However there is a lack of information in the literature regarding the effect of temperature and organic carbon content on algae growth in dairy wastewater systems. There is also a lack of published information concerning the potential for biofilms to be used to remove nutrients from dairy wastewater and create a renewable source of bioproducts. The objective of this study was to determine the effect of temperature and organic carbon concentration on biofilm biomass productivity and on associated nutrient uptake into algae-based biomass cultivated on dairy wastewater.

Methods

Wastewater and culture

Wastewater was collected from the Utah State University Caine Dairy Farm evaporation pond. Characteristics of the water and the produced algae-based biofilm are summarized in Table 1. Turbidity of the water was measured at 890 NTU (Hach 2100Q turbidimeter). Algae biomass inoculum for the laboratory RABRs was collected from the pilot scale RABR systems currently operating at the Logan City wastewater treatment facility, a 460 acre (1.86 km^2) open lagoons system [19]. Visual microscopy of the pilot scale RABR based biofilm indicated that the collected algae biomass contained a variety of algae species, with *Pseudanabaena*, *Oscillatoria*, and *Chroococcus* as the predominant species. The biomass was then cultivated on a cotton rope substratum in shaker flasks using dairy wastewater as the nutrient source before application to the RABR system to allow the culture to adapt to the nutrient source. The carbon:nitrogen:phosphorus molar ratio of the adapted biofilm was measured to be 85:16:1, which is comparable to other algae-based systems [20].

Growth conditions

Rotating Algal Biofilm Reactors of 1-Liter volume were constructed and operated according to Christenson and Sims [6], and the biofilm reactors were wrapped with premeasured lengths of 3/16 in. dia. (0.476 cm dia.) solid braid cotton rope (Fig. 1). In order to test the effect of organic carbon concentration on biomass productivity, the reactors were filled with different dilutions of

Table 1 Composition of influent Caine Dairy wastewater and cultivated biomass from the RABR system. (Analysis by Chemtech-Ford Laboratories – Sandy, UT)

Chemical composition	Water[a] (mg L^{-1})	Biomass[b] (mg kg^{-1} dry wt.)
Total organic carbon	1200	648500
Total nitrogen	155[c]	140400
Total phosphorus	12	19100
Aluminum	7.67	776
Boron	1.73	132
Barium	0.63	75.7
Cobalt	0.03	1.96
Chromium	0.42	45.3
Copper	3.05	85.2
Iron	6.80	624
Manganese	0.67	107
Molybdenum	0.09	27
Sodium	460	23455
Nickel	0.25	24.1
Lead	0.05	6.41
Silica	106	5572
Strontium	1.18	125
Zinc	1.15	116

[a]Dairy wastewater influent stream
[b]Produced algae-based biofilm
[c]Total Kjeldahl Nitrogen (Organic nitrogen, ammonia, and ammonium)

Fig. 1 Laboratory scale RABRs. Each set of three RABRs represents a different organic carbon concentration, and all reactors are held at a constant temperature. This experimental design was replicated at three different temperatures, for a total of 27 different reactors. The rectangular base of each RABR contains 1 L of wastewater and the cylindrical portion of the reactors (76 mm diameter, 200 mm length) rotates at approximately 8 rpm. Biofilm accumulates on the cotton rope surface of the cylinder

wastewater and balanced to match the total nitrogen and phosphorus concentrations in the undiluted dairy wastewater influent stream using sodium nitrate (Thermo Fischer, Pittsburgh, PA) and potassium phosphate (Thermo Fischer, Pittsburgh, PA). The final organic carbon content of the wastewater dilutions was set to 1200, 600, and 300 mg L^{-1} of total organic carbon. The N:P ratio was balanced weekly to the same 155 mg:12 mg ratio to accommodate for the uptake of nutrients by the biofilm. This experiment was conducted utilizing a semi-batch system, with a hydraulic retention time (HRT) of 7 days.

A water bath (VWR) with ¼ in. dia. (0.635 cm dia.) stainless steel tubing was used to maintain the water temperature of the reactors at 7, 17, or 27 ° C (±0.5 ° C). This range of temperatures was chosen as a representative range of seasonal water temperatures in Northern Utah [21]. Constant light was provided from eight 40 W fluorescent lamps that provided a total of 200 μmol photons m^{-2} s^{-1} of continuous photosynthetically active radiation to the upper surface of the RABR systems. Two grams of centrifuged wet weight of adapted inoculum were added to the cotton rope growth substratum upon initiating rotation of the reactors.

Biomass determination and quantification

Biomass was harvested from the rope substrata weekly by mechanical scraping and lyophilized for biomass determination, ash free dry weight (AFDW) measurements, and chemical composition. AFDW calculation was determined using lyophilized biomass at 550 ° C. Biomass productivity was calculated using the AFDW of the biofilm divided by the areal footprint of the reactor (0.0338 m^2). Growth rates were calculated, and an Arrhenius plot of the data was used to obtain the temperature correction coefficient. ANOVA calculations were based on using biomass productivity as the dependent variable. Total theoretical productivity for the reactor was also calculated using measured growth rates. The Arrhenius equation was used to model the effect of temperature on the biofilm growth rate.

Statistical analysis

Triplicate RABR trials for each combination of organic carbon concentration and temperature were conducted for statistical analysis. Each temperature was evaluated by testing three levels of organic carbon in triplicate for a total of nine reactors at each temperature and each organic carbon level. The total number of RABRs was 27, three for each combination of organic loading and temperature. Error bars show 95% confidence intervals from the mean in all figures. ANOVA was performed using triplicate data points in each trial.

Results and discussion

ANOVA

In this study, biofilm productivity was the quantitative outcome, and temperature and organic loading were the explanatory variables. The advantage of performing ANOVA is that it not only provides a means to see how both of the independent variables, temperature and concentration of organic carbon, impact the dependent variable, biomass productivity, but also how the interaction of the two independent variables impacts the dependent variable [22].

Results of ANOVA can be seen in Table 2. With p-values of less than 0.005, both increasing temperature and increasing organic carbon concentration were correlated with an increase in biofilm productivity. However the interaction of temperature and organic loading did not contribute to a statistically significant increase in productivity (p-value 0.8871).

Temperature effects on system productivity

Because ANOVA results indicate that temperature was a contributing factor to biomass productivity, biofilm productivity rates at the three specified temperatures were applied to the Arrhenius equation in order to obtain the activation energy (E_a). The equation used was

Table 2 Summary of the Analysis of Variance (ANOVA) results for the effect of temperature and organic carbon concentration on productivity of RABR based algae biofilm

Source	Sum of squared deviations	Degrees of freedom	Mean square	F-Statistic	*P*-Value
Temperature	14.12	2	7.06	8.87	*0.0021*
TOC Concentration	30.2	2	15.1	18.98	*<0.0001*
Interaction	0.88	4	0.22	0.28	*0.8871*
Error	14.32	18	0.8		
Total	59.52	26			

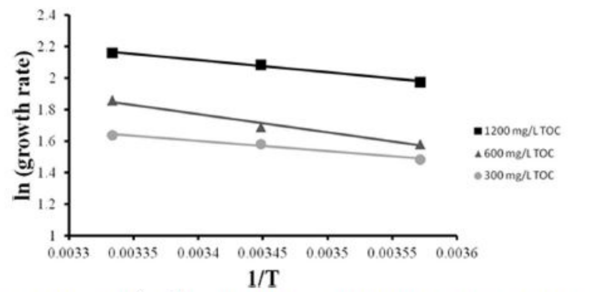

Fig. 2 Arrhenius plot of RABR productivity (g · m^{-2} day^{-1}) as a function of temperature (K). The slope of the best fit line for each concentration represents $\frac{-E_a}{R}$ where $R = 8.314\ J\ K^{-1} mol^{-1}$ and E_a was calculated (Table 3)

$$K = A * e^{\frac{-E_a}{RT}} \quad (1)$$

where K is the biomass productivity, E_a is the activation energy of the reaction, and R is the universal gas constant (8.314 J K^{-1} mol-1). Equation 1 was then linearized by taking the natural log of both sides.

$$lnK = \frac{-E_a}{RT} + lnA \quad (2)$$

The slope of the line formed after plotting lnK vs. - $\frac{1}{T}$ provides the activation energy (E_a) (Fig. 2). Using the Van't Hoff-Arrhenius equation, it was possible to apply the activation energy to derive the equation

$$\frac{K_2}{K_1} = \theta^{T_2 - T_1} \quad (3)$$

in order to find the temperature correction coefficient; theta (Θ) [23]. The activation energies and temperature correction coefficients are reported in Table 3, and were observed to be consistent with values seen in other biological systems [24–26].

Organic carbon concentration effects on system productivity

ANOVA results indicated that increasing the concentration of organic carbon had a positive correlation with biofilm productivity, and the effect of organic carbon concentration on biofilm productivity is presented in Fig. 3. Application of an algae-based biofilm system for nutrient uptake in dairy wastewater requires that the biofilm be capable of growth in the presence of high levels of organic carbon. The positive correlation between productivity and organic carbon concentration indicates that a biofilm system could be used to remove nutrients from a waste stream with elevated levels of organic carbon. This positive correlation could be due to a symbiotic effect of natural bacteria providing carbon dioxide for phototrophic growth. In a dairy waste stream of similar elemental composition to that of the Caine Dairy, which produces 4000 gal day^{-1}, the theoretical yield for AFDW biomass is 9.5 kg day^{-1} of algae-based biofilm.

Effect of interaction of temperature and organic carbon concentration on system productivity

Because ANOVA calculations indicate that temperature and organic loading did not interact to contribute to growth rate, an Arrhenius linearization of the data is

Table 3 Temperature correction coefficients, activation energies, and constants of biofilm productivity and nutrient uptake at three levels of organic loading (TOC)

Level of TOC (mg/L)	Symbol	1200	600	300
Biomass Productivity (g m^{-2} day^{-1})	K	8.69	6.44	5.15
Activation Energy (J K^{-1} mol^{-1})	E_a	6473	9739	5440
Temperature Correction Coefficient (unitless)	Θ	1.0096	1.0145	1.0081
Nitrogen Uptake Rate (mg m^{-2} day^{-1})	K_N	1.22	0.91	0.723
Nitrogen Correction Coefficient (unitless)	$Θ_N$	1.0098	1.0151	1.0078
Phosphorus Uptake Rate (mg m^{-2} day^{-1})	K_P	0.17	0.12	0.1
Phosphorus Correction Coefficient (unitless)	$Θ_P$	1.0101	1.0149	1.0116

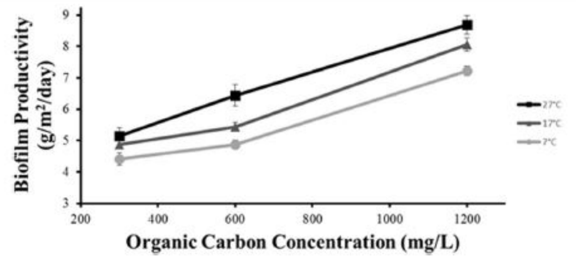

Fig. 3 Areal biofilm productivity as a function of organic carbon concentration at three different temperatures. Error bars represent ± 95% confidence interval. $n = 3$ for each data point. $n = 27$ for entire system

advantageous. Application of biomass productivity (K) to Eq. 2 yields values of E_a. Both K and E_a values are reported in Table 3. These values allow productivity to be compared directly to temperature, creating a predictive system that can be used to estimate biofilm productivity at a given temperature.

At a known concentration of organic carbon, the temperature correction coefficient (Θ), derived from Eq. 3, allows for prediction of biofilm productivity at any temperature within the range evaluated, i.e. 7–27 ° C. At a given water temperature and concentration of organic carbon, the Θ-values from Table 3 can be applied to the equation

$$K_{prediction} = 5.152 * \theta^{T_{water}-280} \quad (4)$$

to predict algal biofilm productivity. A larger Θ value indicates a more significant increase in growth rate as temperature increases.

Using molar ratios of the algae-based biofilm, it was also possible to derive a temperature correction coefficient from the biofilm productivity values in order to predict nitrogen and phosphorus uptake by the system. Rates of nitrogen and phosphorus uptake are reported in Table 3 as K_N and K_P respectively, and the temperature correction coefficients for nitrogen and phosphorus, Θ_N and Θ_P are also reported in Table 3. At a known water temperature and concentration of organic carbon, the Θ_N-values from Table 3 can be applied to the equation

$$K_{N\,prediction} = 0.723 * \theta_N{}^{T_{water}-280} \quad (5)$$

to predict rate of nitrogen uptake (mg m^{-2} day^{-1}), and the Θ_P-values from Table 3 can be applied to the equation

$$K_{P,\,prediction} = 0.098 * \theta_P{}^{T_{water}-280} \quad (6)$$

to predict rate of phosphorus uptake (mg m^{-2} day^{-1}) by the biofilm.

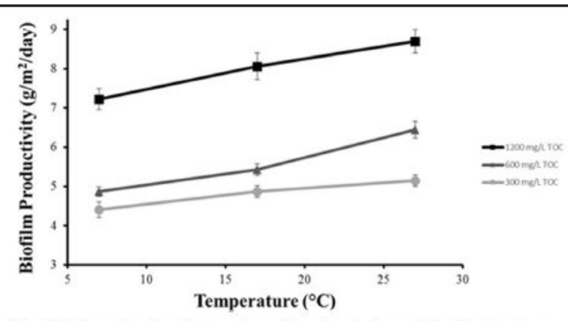

Fig. 4 Areal biofilm productivity (AFDW) as a function of temperature at three levels of organic loading. Error bars represent ± 95% confidence interval. $n = 3$ for each data point. $n = 27$ for entire system

RABR system productivity

After harvesting the produced biofilm, the productivity of the system was calculated. Temperature was made to be a limiting factor for the growth of algae by providing nutrients in excess under constant light [27, 28]. Controlling temperature as the limiting factor allows for the evaluation of the effect of temperature on biofilm productivity, as shown in Fig. 4.

Conclusions

Results of this research are the first in the refereed literature that the authors are aware of that determined growth rates of algae-based biofilm on dairy wastewater at different temperatures for different strengths of the wastewater. Equations 4, 5 and 6 can be used for a waste stream with known organic carbon concentration and water temperature to predict biofilm productivity and nutrient uptake; where Θ is the value taken from Table 3 and T_{water} is water temperature in degrees Kelvin from 280 to 300. The relationships among temperature, productivity, and TOC developed in this study can be applied to the design of dairy wastewater remediation systems and algae-based biomass production systems using biofilm reactors.

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Availability of data and materials
The data that support the findings of the study are available from the corresponding author upon reasonable request.

Authors' contributions
ZF conceived of the experimental design and carried out all setup, analysis, and data collection including RABR harvesting and wastewater preparation and analysis. ZF also prepared drafts of the manuscript, and led the statistical analysis as well as prepared tables and figures. RS prepared and conceived of the study and variables to be considered, assisted in the experimental design and reviewed and drafted the manuscript. All authors have approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

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Appendix B – Presented Poster Examples

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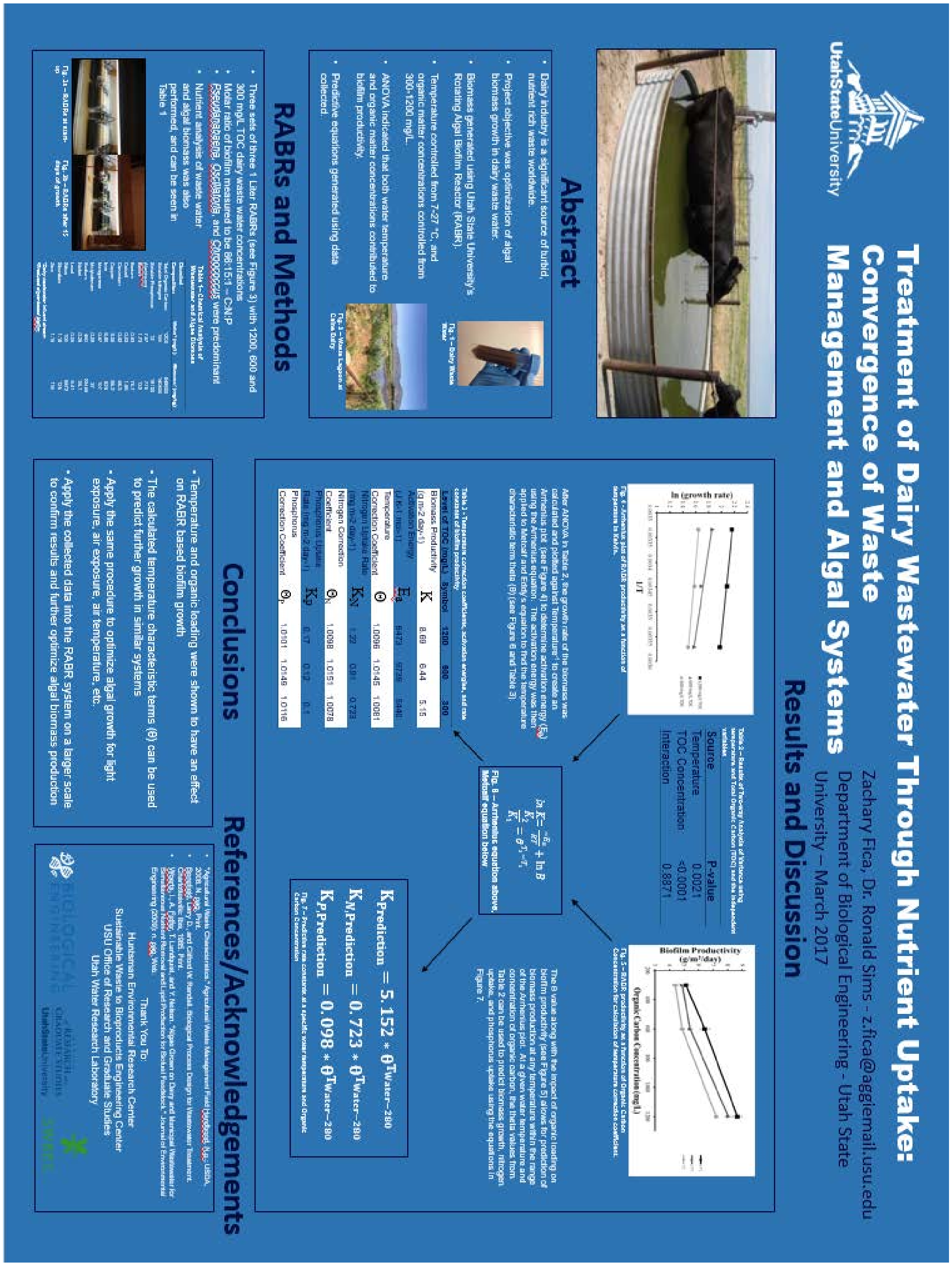

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1

Effects of temperature and organic loading on RABR based algal growth in dairy waste water systems

Caine Dairy Farm

I. Introduction

A significant source worldwide of waste products is the production of dairy. Algal biomass in Utah State University's RABR (Rotating Algal Biofilm Reactor) system is a new biotechnology designed to remediate waste water, such as dairy waste water, while simultaneously providing biomass for the production of renewable bioproducts.

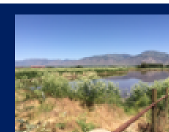
This study focuses on the optimization of the RABR system in dairy waste, by investigating the effects that temperature and total organic carbon have on algal growth. The data and knowledge obtained can be integrated into the RABR system on a larger scale, in order to optimize algal growth in the reactor.

II. Methods

- Three sets of three 1 Liter RABR tanks with 3200, 1600 and 800 mg/L TOC dairy waste water concentrations. (See Figure below)
- Nitrogen and Phosphorus levels balanced to be consistent across all RABRs
- Water bath maintaining the temperature of the waste water at 17°C
- Inoculation with a variety of algal species.
- Weekly testing of soluble Nitrogen, Phosphorus, TOC, and COD levels.
- Biomass harvested and weighed periodically to calculate total growth.
- Retention time used to calculate "efficiency" of each RABR (See Figure to right)

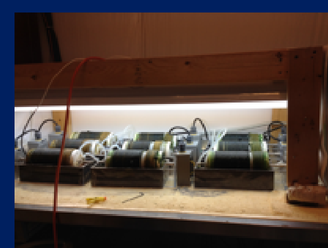
Algae inoculum

Pure dairy waste water



Waste pond at Caine Dairy

RABRs Day 1



RABRs Day 15

Retention Time Data (17°C)

Retention Time = Days necessary to accumulate 60 g/m^2 of biomass.

Waste Type (% Concentration)	First Retention Time	Second Retention Time	Third Retention Time
25%	12 Days ± 1.07	10 Days ± 3.09	9 Days ± 1.53
50%	11 Days ± 1.07	11 Days ± 1.07	11 Days ± 2.08
100%	11 Days ± 1.53	8 Days ± 1.53	8 Days ± 1.53

III. Results

- Lower levels of organic carbon do not necessarily provide a more optimal algal growth environment at 17°C in dairy waste water.
- At 17°C, algal growth accelerates with multiple retention times.

IV. Conclusions

- Initial data indicates RABR based algae grows very efficiently in dairy waste water.
- Additional testing at other temperatures is needed to confirm results.
- Testing at 27°C and 7°C will begin this month

V. Acknowledgements

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USU Caine Dairy farm

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January 2014

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Researcher of the Year Nominee, USU Biological Engineering	Jan. 2016
Department of Biological Engineering Student of the Year	Feb. 2016
1st Place, Engineering Design – Institute of Biological Engineering	Mar. 2017
SWBEC Annual Student Award of Excellence	April 2017

Work Experience
Primrose Home Health and Hospice
Certified Nursing Assistant **2014-15**
Provided patient care and coordinated care plans with other aides, nurses, and doctors

Teaching Assistant **2014-16**
Prepared labs, taught laboratory skills, graded reports, and other teaching duties for undergraduate biomaterials and human anatomy courses

Engineering Ambassador **2015-16**
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Sustainable Waste to Bioproducts Engineering Center

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Developed and carried out experimental designs and maintained satellite research stations

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Institute of Biological Engineering
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Algae-based biofilm productivity utilizing dairy wastewater: effects of temperature and organic carbon concentration.
Journal of Biological Engineering – First Author 2016

Nutrient and suspended solids removal from petrochemical wastewater via microalgal biofilm cultivation

Chemosphere 2017

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