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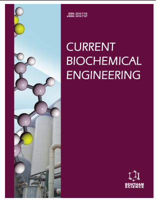
RESEARCH ARTICLE



# Utilization of Semi-continuous Algae Culture for the Treatment of Recycled Dairy Lagoon Wash Water

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**Abstract:** ***Background*:** Utilization of animal wastes in algal culture has proven to be challenging. The utilization of "free" nutrients has drawn researchers and industry to develop business models that call for the use of these free nutrients, which comes at a cost. Some of these costs include reduced productivity, increased contamination, lower value target markets, and lower treatment capabilities (for wastewater treatment applications). This paper evaluates the impact of dairy lagoon effluent on productivity and wastewater treatment ability.

***Methods*:** Screened dairy lagoon wash water was fed to four three square meter outdoor open paddle-wheel algal cultivation reactors. The units were operated semi-continuously for one and a half years. Seasonal productivity and nutrient uptake rates, for nitrogen (N) and phosphorous (P) were measured against wastewater dilution requirements. Seasonal algal species dominance was also recorded. Wastewater was added at two levels, and the lower level was supplemented with synthetic fertilizer.

***Results*:** Seasonal N uptake rates ranged from 0.5 to 1.2 grams of N uptake per square meter per day, while P uptake ranged from 0.17 to 0.3 grams of P per square meter per day depending on season and Hydraulic Residence Time (HRT). N removal efficiency ranged at 40 to 70% for semicontinuous operation, depending on HRT, season, and dilution of influent wastewater, which was made up from 1.5% to 13% of the daily water exchange.

***Conclusion*:** Algal reactors tended to be N limited due to the inability to add enough dairy wastewater to mitigate the high turbidity and dark color. Treatments with lower levels of added dairy wastewater tended to show higher nutrient removal. Algal culture from dairy wash water could benefit from a pretreatment step to reduce turbidity and color, thereby promoting algal growth and productivity.

**Keywords:** Microalgae, dairy wastewater, nutrient removal, algal productivity, high rate ponds, wastewater recovery.

## 1. INTRODUCTION

As population pressure crowd's agriculture, Concentrated Animal Feed Operations (CAFOs) have increased dramatically in number and size. Rather than animals grazing in pastures and rangeland, feed is brought to the animals confined in small areas. Dairies, cattle feed lots, swine and poultry farms generate large amounts of waste. Dairy cows in California create twice as much fecal waste as the human population (dry weight basis). For many operations, this manure waste is spread on local farmland, at rates that exceed agronomic nutrient uptake rates. In 2-5% of all counties in the U.S., including the agricultural regions of California, Virginia, and much of the southeast, the amount of nutrients present in the manure produced by CAFOs is greater than the entire assimilative nutrient capacity of all the cropland and pastureland available in those counties [1]. Roughly 20% of the nitrogen (N) and 35% of the phosphorus (P) are recovered from manure in CAFOs using land application as crop fertilizer. Substantial amounts of nutrients in the manure remain unused in the fields, and find their way into ground

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and surface waters [2]. Nutrient contamination, primarily N and P, generated by CAFOs is leached from soils and can percolate into ground waters and/or gets washed into freshwater reservoirs and the ocean through urban storm water systems. N enhances algal growth, resulting in uncontrolled algae blooms in freshwater, and red tide blooms are frequently observed off the coast of southern California and are reported globally [3]. Algal toxins and the hypoxia produced by decay of such blooms are associated with the mortality of marine fauna [4]. The primary source of agricultural non-point source contamination in California's groundwater is the improper disposal of manure wastes by CAFOs [5]. Moreover, many groundwater basins in the arid southwest have been historically under-used due to high nitrate concentrations. Excess P levels in waters are also associated with cyanobacterial blooms [6]. The need to control manure-derived nutrient pollution is straining the CAFO industry.

The lack of affordable large tracts of land for manure application to soils has led to continued groundwater contamination. There is a need for an alternative highly productive crop, compared to corn or other forage crops, to remove manure N and P, thus preventing nutrient pollution in the smaller land areas. Microalgae are photosynthetic organisms with high productivity that remove eutrophying nutrients from water sources. Microalgae are more efficient for N and P reclamation than higher plants, due in part to higher rates of biomass production but also because algae lack the large stores of structural carbon (*i.e.*, cellulose) characteristic of land plants [7]. Thus, the C/N ratio of higher plants ranges from 18 to 120 (by atoms) while microalgae range from 5 to 20 [8] indicating that water reclamation and nutrient recovery can be accomplished more rapidly and in a smaller area using algae rather than terrestrial plants. Algae have been used for over 50 years in municipal wastewater treatment [9] and more recently for bioremediation of manure effluents [10, 11].

Many studies have shown that algal treatment of livestock wastewater has promising nutrient removal efficiencies. Numerous authors have cited exceptional removal efficiencies for both N and P, and some reported removal efficiencies of Chemical Oxygen Demand (COD). However, the majority of the removal efficiencies have come from either batch culture systems or very small lab scale (*i.e.*, 1 liter or less) algae growth reactors. In addition, in many of these studies, the digester effluent or CAFO wastewater was filtered with a much smaller pore filter than is economically practical for commercial application, before the studies were conducted. It was discovered that filtration, centrifugation, and autoclaving are the most common pretreatment methods for many of these studies [12]. This would potentially remove some of the contaminants that might inhibit algal production. Franchino *et al.* [13] found greater than 90% removal efficiency for total N and P in highly diluted piggery digestate (5% and 10% digestate to 95% and 90% tap water). These experiments were conducted indoors in 250 mL flasks. The experiment was operated for 11 days and the removal efficiency was calculated after that period of growth. In their previous studies, it was found that after 11 days, nutrients were almost completely removed in anaerobic digester effluent from dairy and cheese whey. Dilutions of at least 10:1 (water: digester effluent) were required for successful algal growth [14]. The digestate for this study was centrifuged as a pretreatment. Many other authors reported very good removal efficiencies of total N (greater than 80%), total P (greater than 70%) and total organic carbon (measured as COD, greater than 50%) in small lab scale reactors or with pretreated animal wastewater [15-18].

The growth of microalgae may be impeded by many contaminants in the bioreactors or in High Rate Algae Ponds (HRAP). A review by Gupta *et al.* [12] found that one of the major bottlenecks for algae used to treat CAFO wastewater is the presence of total solids and high turbidity, as they affect the growth of microalgae by hindering light penetration. In addition, this review found that the presence of other organisms, such as bacteria, also hinders microalgae growth through nutrient competition. Pond performance can be negatively affected by the establishment of zooplankton grazers that can consume much of the algal bio-mass within a few days [19]. However, for nutrient removal efficiency calculations, nutrient uptake by bacteria will suffice as long as the bacteria can be separated from the wastewater (similarly, algae must also be separated). Many of the mentioned studies are performed indoors under constant light and temperature conditions. This is not economically possible for commercial application for this type of technology. Seasonal effects have not been characterized, but researchers have studied the impact of temperature and light effects and found that microalgae growth can be inhibited by less than optimum light and temperature [20-22].

For this study, four three square meter outdoor paddle-wheel mixed ponds were constructed and operated semi continuously for two seasons. The ponds were operated at a thirty cm depth and exchanged three times per week, a semi-continuous flow mode, and they were located adjacent to the 250 head Cal Poly dairy (San Luis Obispo, CA) (CPSLO). The main objectives of this study were to quantify dilution rate requirements for successful algae production in ponds fed Dairy Lagoon Wash Water Effluent (DLE) and to evaluate seasonal nutrient removal rates, of N and P, for algal ponds fed DLE as the primary nutrient source. Ponds were operated as duplicates. In addition to seasonal temperature and solar radiation, Hydraulic Residence Times (HRTs) were changed as well as DLE dilution rates, to determine the influence of those variables.

## 2. MATERIALS AND METHODS

### 2.1 Dairy Lagoon Effluent (DLE) Characterization

Cal Poly DLE effluent is created at the 250 head Cal Poly dairy and a free stall flush lane manure collection system is utilized. This is a system similar to industry, where flush water is captured and pumped through a screen separator. The liquid then flows into a lagoon and is stored, while the screened solids are composted or used as bedding. The lagoon storage is utilized as recycled flush water when flushing the concrete feed lanes. New, fresh water is added daily to the lagoon from the milking parlor, and DLE is also applied to multiple crops throughout the year as needed to meet nutrient requirements. Typically, the lagoon builds up storage until summer, when most of the DLE is used for irrigation.

The DLE was collected at the inlet of the Cal Poly dairy lagoons. DLE was typically characterized weekly, but its composition varied seasonally. DLE was collected as a grab sample and carried to the lab for characterization. The sample was immediately filtered with a TSS 1.5-micron glass fiber filter (Whatman filters, Hach Company), typically used to measure total suspended solids (TSSs) in wastewater. Wastewater characterization occurred on both the filtered and non-filtered samples, to see what portion of the nutrients might be available to the algae. However, non-filtered samples were measured less frequently. Typically, Total N, Total P, Alkalinity, COD, Nitrate, Nitrite, and Total ammonia N were measured. Samples were measured using Hach Test N Tube vials for all of the tests except for alkalinity, which used an acid titration cartridge. A DR 5000 Hach spectrophotometer (Hach) was used to read the TNT vials containing samples, and a DRB 200 heating block (Hach) was used as required per test. A summary of the DLE characterization data can be seen in (Table **1**).

## 2.2. System Design and Operation

Data was collected from Fall 2014 through Fall 2016 in the outdoor pilot scale reactors. Hydraulic and organic loading rate were recorded to determine the impact of those operating parameters on algal growth. In addition, the data was grouped by season (Table **2**).

Four identical three-square meter HRAPs were installed adjacent to the DLE lagoons at the 250-head CPSLO dairy (Fig. **1**). The HRAPs were paddle wheel-mixed, open, raceway ponds operated at a 30-cm depth (1 foot), containing a volume of 1,000 liters per reactor. The units measured 3.25 m (10.7 feet) in length and 1 m (3.3 feet) in width, and 0.37 m (1.2 feet) in height. The reactors were made of a wooden frame with flexible plastic liner. A variable low speed motor was used to drive 6-blade plastic paddlewheels. The paddlewheels for all four ponds were connected with a single shaft so that all paddlewheels were turning at the same rate. Uniform water velocity was maintained to ensure good mixing. The ponds were operated as individual units to conduct experiments for multiple conditions to determine nutrient removal rates in DLE at various DLE dilution rates and pond HRTs, seasonally. Physical parameters of the units, such as, the relationship between paddle wheel RPM and channel water velocity, as well as cross sectional flow patterns that are critical for developing bio-flocculating algal communities were analyzed (data not shown).

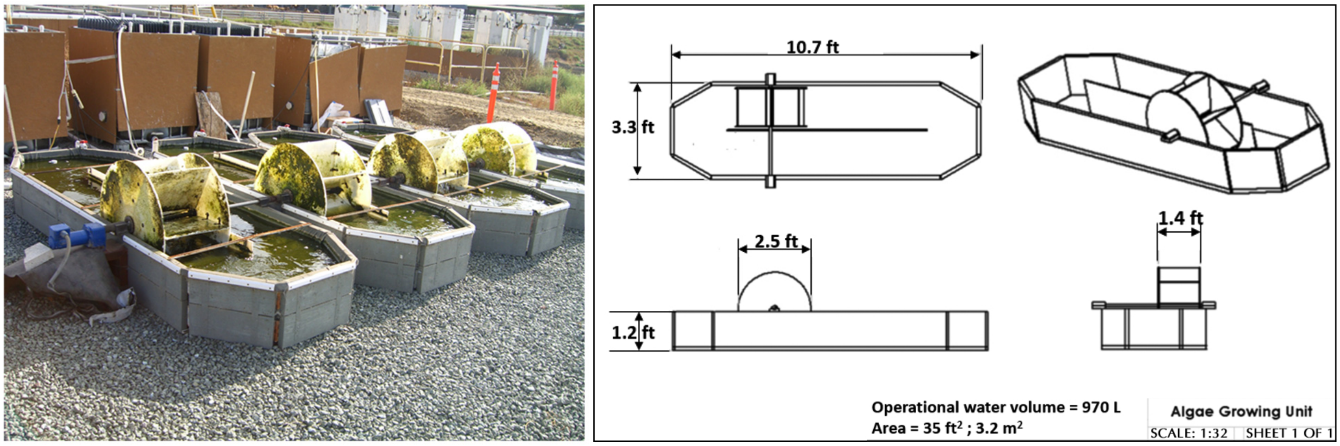

**Fig. (1).** Four outdoor paddlewheel mixed high rate algal ponds used for this study. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

**Table 1. Average physico-chemical characterization of dairy lagoon effluent used in the study from July 2015 through November 2016. Testing methods can be found on the Hach website.**

| Water Quality Parameter | Non-filtered (mg/L) | Filtered (mg/L) | Hach Test Method |
|---|---|---|---|
| Total Nitrogen | 410 | 290 | TNT 827, 2714100 |
| Total Inorganic Nitrogen | 270 | 220 | 2604945 |
| Total Phosphorous | 200 | 90 | TNT 844,845 |
| Alkalinity | 3,100 | 3,200 | 1.6 N cartridge (1438901) |
| COD | 5,600 | 3,200 | TNT 825 |
| Nitrate | 13 | 11 | TNT 835,836 |
| Nitrite | - | 0.9 | TNT 839,840 |
| Total Ammonia Nitrogen | 220 | 190 | TNT 831,2604545 |

The HRAPs were operated in a semi-continuous (semi-batch) mode with exchanges occurring daily. Water was removed daily, volume removed was dependent on the experimental residence time. The algae ponds were then fed a combination of screened dairy flush water and fresh water to meet the required experimental HRT and daily nutrient input. No pretreatment was conducted on the dairy lagoon flush water (DLE); it was utilized directly from the commercial dairy screen separator. Nutrients added to the HRAPs came from either the DLE (Table **1**), or from water soluble miracle grow fertilizer (N-P-K: 24-8-16). Nutrient additions were based on a target total available N, which ranged from 1.5 to 2.5 grams of N per square meter per day fed to the ponds. Ponds were operated in duplicates and either received 80-100% of the nutrients from DLE or 40-50% of the nutrients from DLE. The remainder was delivered from the miracle grow fertilizer which was added as supplemental nutrients to determine if the units fed DLE were inhibited due to the dark color of the units. Data were collected from the outdoor pilot scale HRAPs under steady-state conditions during winter, spring, summer, and fall (Table **2**).

Water quality data that were collected and sorted by season includes Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), total N concentration, total inorganic N concentration, total P, alkalinity, Secchi disk visibility, oxygen concentration, pH, and temperature. The data were analyzed to determine nutrient uptake rates for each season at multiple HRTs, ranging from 2.5 to 10 days. Both influent and effluent samples were collected at least once per week for the following tests: TSS and VSS, total ammonia N, total N, total P, and alkalinity. The data were analyzed to determine nutrient uptake rates for each HRT and season. Water quality testing was conducted using a Hach DR 5000 and DRB 200 as needed. Hach Test N Tube vials were used for each test; test numbers are summarized in (Table **1**).

### 2.3 Determination of Nutrient Uptake Rate

Nutrient uptake was evaluated in the outdoor ponds weekly. Outdoor nutrient uptake evaluation was conducted by taking a water quality grab sample immediately after a water exchange, referenced as time zero, waiting twenty-four hours and then taking a second grab sample before the next

**Table (2). Seasonal hydraulic retention times, percent nitrogen delivered from DLE, seasonal solar radiation, and seasonal water temperature. T1 (treatment 1) was supplied 80-100% of nutrients from DLE while, T2 (treatment 2) was supplied 40% of the nutrients from DLE and the remainder from miracle grow fertilizer.**

| Season | HRT (Days) | % N from DLE | | Average Daily Solar Radiation (W/m2) | | | Water Temperature (°C) |
|---|---|---|---|---|---|---|---|
| | | T1 | T2 | Season Avg. | Daily Max | Daily Min | |
| Winter (Nov. 15th - Feb. 15th) | 4 | 100% | 40% | 125 | 200 | 25 | 7-14°C |
| | 6 | 100%, 80 % | 40% | | | | |
| | 7 | 80% | 40% | | | | |
| | 10.5 | 80% | 40% | | | | |
| Spring (Feb. 15th - May 15th) | 3 | 100% | 40% | 240 | 345 | 35 | 11-18°C |
| | 3.5 | 80% | 40% | | | | |
| | 4 | 100% | 40% | | | | |
| | 6 | 100%, 80% | 40% | | | | |
| Summer (May 15th - Aug 15th) | 2.5 | 100% | 40% | 310 | 360 | 125 | 18-25°C |
| | 3.5 | 80% | 40% | | | | |
| | 4 | 100% | 40% | | | | |
| | 6 | 100% | 40% | | | | |
| Fall (Aug 15th – Nov. 15th) | 2.5 | 80% | 40% | 215 | 300 | 40 | 12 - 23°C |
| | 4 | 80% | 40% | | | | |
| | 6 | 100% | 40% | | | | |

HRT - Hydraulic Residence Time
DLE - Dairy Lagoon Effluent

daily exchange, referenced as time one. The samples were filtered and total N, total P, TSS and VSS were measured on the day of collection of each sample. The delta (change) in N, P, TSS, and VSS represented one day in time, and was calculated by subtracting the time zero reading from the time day one reading. Nutrients were measured using the Hach test equipment as stated previously. TSS and VSS were conducted following Standard Methods 2540 D and E [23]. Data was recorded and logged into a spreadsheet for further analysis. The delta represented the mg of N or P uptake per day. The data obtained for delta TSS and delta VSS were not as consistent as the deltas represented by the breakdown of COD, algal growth or decay, bacterial growth or decay. Therefore, the TSS and VSS data were not used for the nutrient uptake calculations. The mg/L of nutrient uptake per day was then converted to an aerial uptake rate by using a conversion factor of 300 L of reactor per square meter converting to g/L. The depth of 30 cm converts to 300 L per square meter of HRAP surface area.

Due to the high levels of particulate solids and COD in the influent DLE, traditional algae productivity analysis was not valid for this study. Typical algae productivity can be measured through volatile suspended solids measurements. It was found that the addition of DLE to the pond impacted both the TSS and VSS. Thus, measuring TSS and VSS would not accurately represent the algae productivity. In addition, measuring the N and P uptake over a fixed amount of time and claiming the difference in nutrients was due solely to algal productivity and was also not correct due to the bacterial presence from the high COD loading of the DLE. The nutrient uptake rate calculations in this study represent a combination of algal assimilation as well as bacterial assimilation and possibly denitrification.

HRAP exchanges were conducted daily for a given HRT and DLE input. Experiments (Table **2**) were conducted with two treatments (T1 and T2), 80-100% of the nutrients delivered from DLE (T1) and 40-50% of the nutrients delivered from DLE (T2). Dilution rates were high, actual DLE daily volume delivered to the HRAPs ranged from 1.6 to 10.2%, depending on season and HRT. Dilution rates were calculated by dividing the liters of DLE added daily by the liters of total liquid added daily (the sum of freshwater and DLE). It was observed that dilution rates of 10% or greater, over time, could not sustain a visible algal culture. For these experiments, minimal nutrient remediation was observed (data not included in this study) when greater than 10% DLE was added daily. Experiments were conducted over a 1.5-year period with various HRT's (Table **2**). Seasonality was separated by examining solar radiation to determine the seasonal dates. Due to the nature of both algal and bacterial productivity decreasing with temperature, longer HRT's were tested during the winter period.

### 2.4. Microscopy

Dominant algae strains were identified over the course of the project. A Leica DM 750 light microscope with attached Accu-Scope 3000 digital camera was acquired and utilized beginning in spring. Samples were collected weekly during warmer seasons and monthly during cooler seasons and examined. The algae were identified using various books [24, 25] and online resources and were identified morphologically to the family level, as best as possible.

## 3. RESULTS AND DISCUSSION

### 3.1. Nutrient Removal

It was found that VSS measurements were not a good indicator of algae growth due to the introduction of organic matter with the addition of DLE. A more representative parameter to determine the productivity was through nutrient assimilation measurements as this would combine algal assimilation as well as bacterial removal of targeted nutrients (N and P). N uptake rates peaked in the summer and when the temperature was the highest, however, HRT was the lowest. This uptake rate represents both algal and bacterial assimilation. A peak N uptake rate of just under 1.4 g N / $m^2$ / day was achieved during these conditions. (Figs. **2** and **3**), respectively, show N and P uptake rates per square meter per day for varying HRTs, seasons, and percent of nutrients supplied by DLE.

Seasonal N uptake rates peaked in summer and fall, while the lowest nutrient uptake was observed in winter and spring. Summer and fall N uptake were almost twice that of winter and spring, 0.9 versus 0.45 grams of N removed per square meter per day. There was a slight variation between

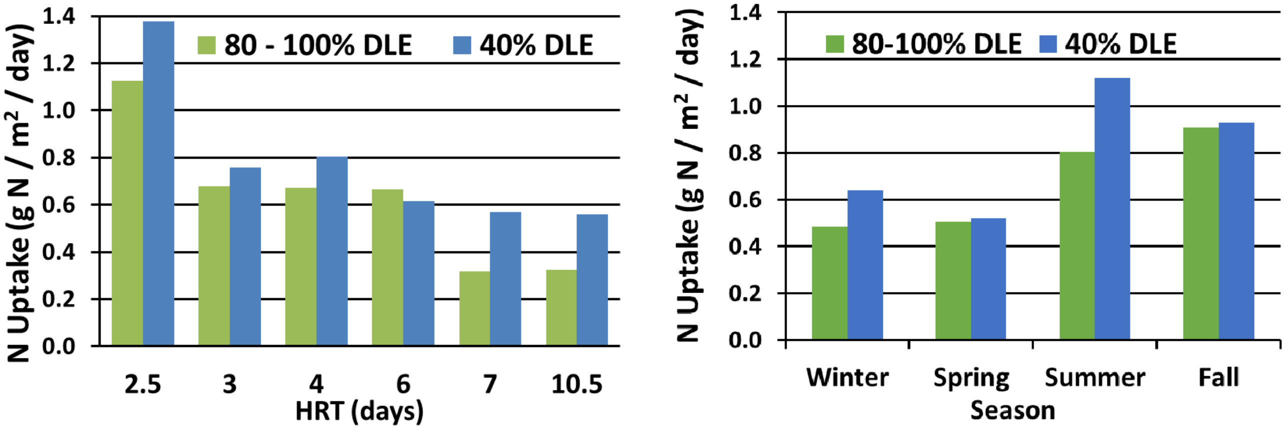

**Fig. (2).** Nitrogen uptake rate (g N / $m^2$ / day) as a function of Hydraulic Residence Time (HRT), left, and as a function of season, right. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

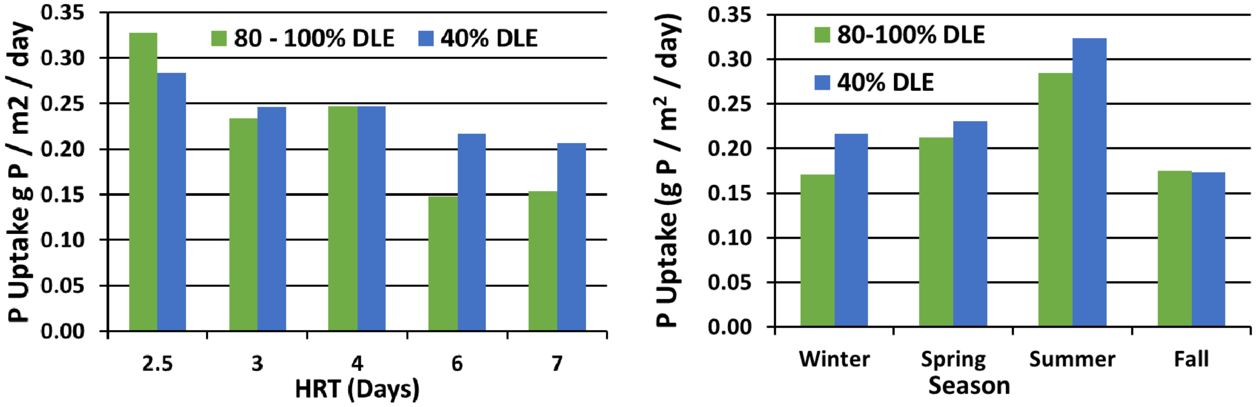

**Fig. (3).** Phosphorus uptake rate (g P / m$^2$ / day) as a function of Hydraulic Residence Time (HRT), left, and as a function of season, right, for reactors fed 80-100% and 40% of nutrients supplied by Dairy Lagoon Effluent (DLE). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

**Table 3. Seasonal HRT and DLE dilution rates for units supplied 80-100% of nutrients from DLE (T1, treatment 1) and units fed 40% of the nutrients from DLE (T2, treatment 2). Also raw DLE (brown) and algae reactor water (green) are shown.**

| Season | HRT (Days) | % N from DLE | | Effective DLE fraction % | |  |
|---|---|---|---|---|---|---|
| | | T1 | T2 | T1 | T2 | |
| Winter (Nov. 15th - Feb. 15th) | 4 | 100% | 40% | 5.0% | 2.5% | |
| | 6 | 100% 80 % | 40% | 7.4% | 3.7% | |
| | 7 | 80% | 40% | 8.7% | 4.3% | |
| | 10.5 | 80% | 40% | 13.0% | 6.5% | |
| Spring (Feb. 15th - May 15th) | 3 | 100% | 40% | 3.6%, 5.4% | 1.8%, 2.7% | |
| | 3.5 | 80% | 40% | 4.3% | 2.2% | |
| | 4 | 100% | 40% | 3.8% | 1.9% | |
| | 6 | 100% 80% | 40% | 5.0% | 2.5% | |
| Summer (May 15th - Aug 15th) | 2.5 | 100% | 40% | 3.6%,6.0% | 3.1% | |
| | 3.5 | 80% | 40% | 4.3% | 2.2% | |
| | 4 | 100% | 40% | 7.2%,9.6% | 5.0% | |
| | 6 | 100% | 40% | 9.7% | 3.6% | |
| Fall (Aug 15th – Nov. 15th) | 2.5 | 80% | 40% | 3.1% | 1.5% | |
| | 4 | 80% | 40% | 5.0% | 2.5% | |
| | 6 | 100% | 40% | 7.4% | 3.7% | |

HRT – Hydraulic Residence Time.
DLE – Dairy Lagoon Effluent.

units fed 80-100% DLE nutrients compared to units fed 40% DLE nutrients and the remaining 60% of nutrients supplied by miracle grow synthetic fertilizer. The 40% DLE combined with miracle grow had slightly higher uptake rates, with larger differences during longer HRTs. Chu *et al.*, 2015 [20], found that in outdoor photobioreactors, shorter HRT's during warmer months had greater productivity, however, they found that longer HRT's had higher removal fractions. Chu *et al.* concluded that longer HRT's is preferable for treating wastewater using microalgae.

P uptake rates peaked in the summer at about 0.30 grams of P per square meter per day, when the HRT was the lowest, 2.5 days, and water temperatures were the warmest. The 40% DLE fed reactors showed higher uptake rates than the counterpart 80-100% DLE fed reactors at the longer HRTs. Seasonal P uptake mirrored N uptake, and both followed water temperature, with higher uptake rates during warmer water temperatures in summer and fall.

In general, the highest seasonal uptake rates occurred, with only 40% of the nutrients coming from the DLE, the remainder coming from miracle grow fertilizer. One exception was for the 2.5-day HRT, where the 80-100% DLE units showed higher P removal than the counterpart 40% DLE and 60% synthetic fertilizer. Seasonal P uptake was greatest in summer, followed by spring, winter, and then fall. Seasonal uptake in the fall was almost two times lower than summer, 0.17 *versus* 0.3 grams of P per square meter per day.

### 3.2 Dilution Required due to High DLE Turbidity

Daily exchange rates were performed based on the desired HRT. DLE addition was based on delivering 1.5 to 2.5 grams of N per square meter per day. The DLE made up between 1.5% and 13% of the daily volume exchanged, see (Table **3**), while the remainder of the exchanged water was clean freshwater, depending on season and HRT. Due to high turbidity and dark color of the DLE, higher algal productivity was observed for higher DLE dilution rates as seen through afternoon dissolved oxygen concentrations (Fig. **4**).

Other researchers have also found the need for dilution due to the high turbidity of the wastewater [13, 14], while Chokshi *et al.*, [26] found that short batch cultures in 1 liter flask did not require dilution. Claims by multiple authors of

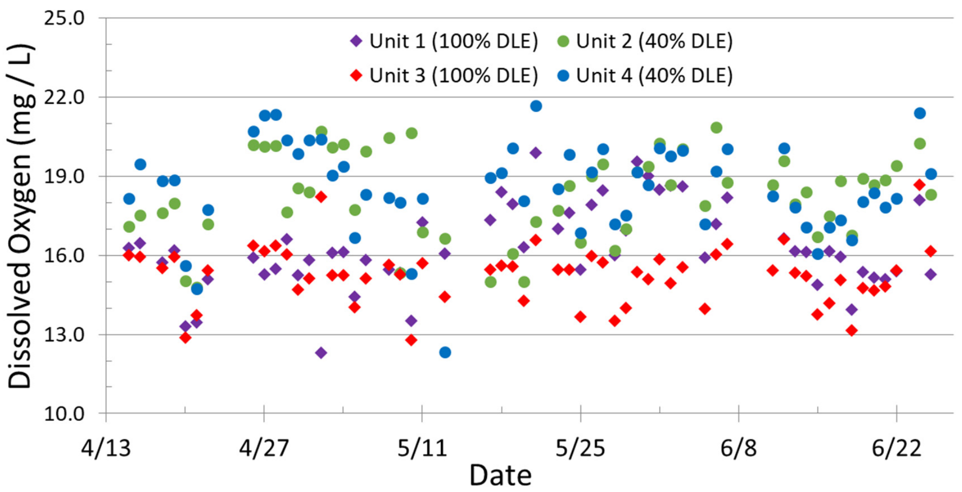

**Fig. (4).** Typical daily afternoon oxygen concentration for each unit during summer 2015. Units 1 and 3 were fed 100% DLE (generally lower oxygen). Units 2 and 4 were fed 40% DLE and synthetic fertilizer (N-P-K, 26-8-16). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

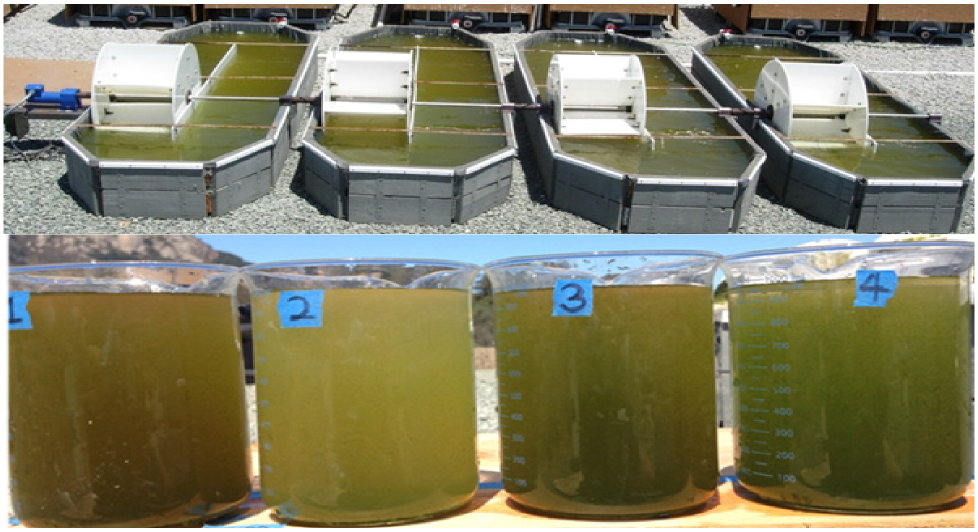

**Fig. (5).** Algal culture for units fed DLE at a rate of 5% and 2,5% of daily exchange. Units 1 and 3 were fed 5% while 2 and 4 were fed 2.5% DLE. Units 1 and 2 were operated at a 4 day HRT while Units 3 and 4 were operated at a 6 day HRT. DLE, dairy Lagoon effluent; HRT, Hydraulic Residence Time. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

"no pretreatment" rather included some type of filtration [15, 17, 18, 20, 26]; while other authors utilized synthetic wastewater [16]. These approaches are too expensive for large scale commercial applications and can only be affordable at lab and research scale. The development of large scale outdoor culture is required and therefore, outdoor continuous culture research is also required.

Daily afternoon oxygen concentrations were generally higher for units fed 40% DLE combined with synthetic fertilizer. In Fig. (**4**), units 1 and 3 nutrients were supplied 100% from DLE, DLE made up 5% of the daily exchanged volume. Units 2 and 4 nutrients were supplied by DLE (40%) and synthetic fertilizer (60%), DLE made up 2.5% of the exchange volume. Units 1 and 3 were operated with a 4-day HRT while units 3 and 4 had a 6-day HRT. Both treatments showed supersaturated oxygen concentration, suggesting algal photosynthesis and autotrophic metabolism, but units 2 and 4 averaged 18.3 mg of dissolved oxygen per liter, while units 1 and 3 averaged 15.7 mg of dissolved oxygen per liter, over the period from April 15th through June 25th, 2015. Higher oxygen concentration suggests higher photosynthesis, possibly due to the lower level of LE addition and thus higher level of light penetration into the water column.

(Fig. **5**) shows how the color changed for the four units, depending on HRT and DLE dilution. Typically, the higher additions of DLE lead to higher turbidity and a darker brown color, leading to lower available light in the unit and less algal productivity [27]. Units 1 and 3 were exchanged with 5% of the exchange from DLE, while units 2 and 4 were exchanged with 2.5% from DLE. Studies were conducted with the DLE fraction ranging from 1.5 to 13% of the daily exchange. The higher the DLE fraction, the darker the reactor watercolor became, leading to a shift from algal based assimilation to a combination of algal and bacterial nutrient

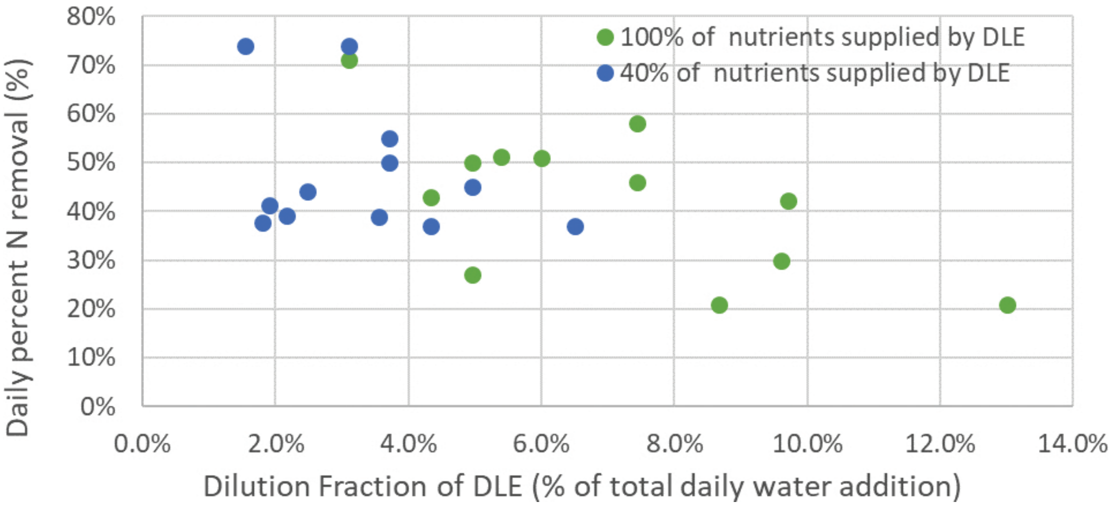

**Fig. (6).** Daily percent nitrogen removal at various Dairy Lagoon Effluent (DLE) dilution fractions observed in semi-continuous culture reactors. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

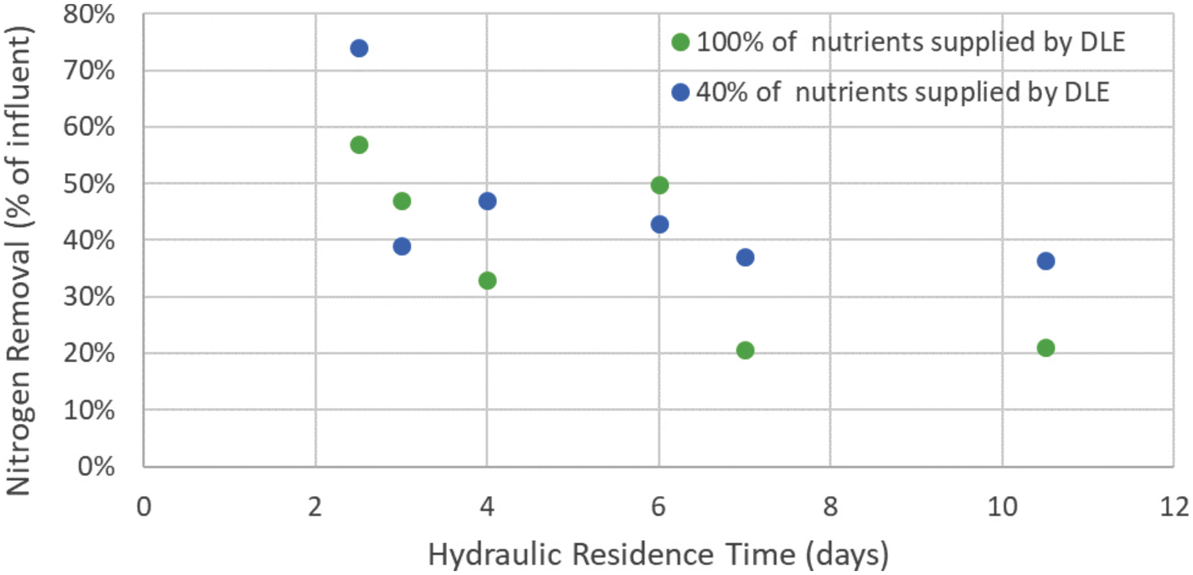

**Fig. (7).** Daily percent nitrogen removal at various hydraulic residence times, observed in semi-continuous culture reactors. DLE, dairy lagoon effluent. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

removal. The DLE was not pretreated in any way prior to being added to the reactors.

Higher dilutions with longer HRTs tended to be the darkest in color. It was observed that in general, DLE fractions higher than 10% did not reduce N very efficiently. Fig. (**6**) shows the daily percent N removal as a function of DLE exchange fraction. As much as 70% N removal was observed in semi-continuous reactors, but more typically ranged from 40-60% as long as the DLE fraction was less than 8% of the total influent. It is suggested that for wastewater with similar characteristics, the daily addition should not be higher than 8% as the daily nutrient removal decreases to about 30%.

Fig. (**7**) shows how the nitrogen removal changed as a function of HRT. Lower (shorter) HRTs showed higher nitrogen removal, but this was partly due to lower HRTs primarily occurring in warmer seasons. Removal efficiency was generally 40 to 50% for HRTs between 3 and six days. From previous studies, it can be noted that lower HRT's lead to higher removal rates (fraction of influent received), however, longer HRT's can lead to lower effluent concentration. For large scale applications, it is important to note if discharge requirements are based on mass or concentration. Large scale applications may include two reactors in series where the first reactor is operated at short HRT's for high mass removal and the second reactor at a longer HRT for low effluent concentration.

In small flasks, algae and bacterial cultures can exist side by side in various portions because the entire flask contents are being illuminated. However, in the field, bacterial density shades the water column leading to increased inhibition of algal growth with respect to depth, ultimately leading to a "feed forward effect" with rapid collapse of algae photosynthesis. Total solids and turbidity found in these types of wastewater can prevent light penetration. Small scale laboratory studies in 1-liter flasks will not accurately determine the wastewater treatment capability of a larger outdoor continuously operating system. In the field, the impact of organic

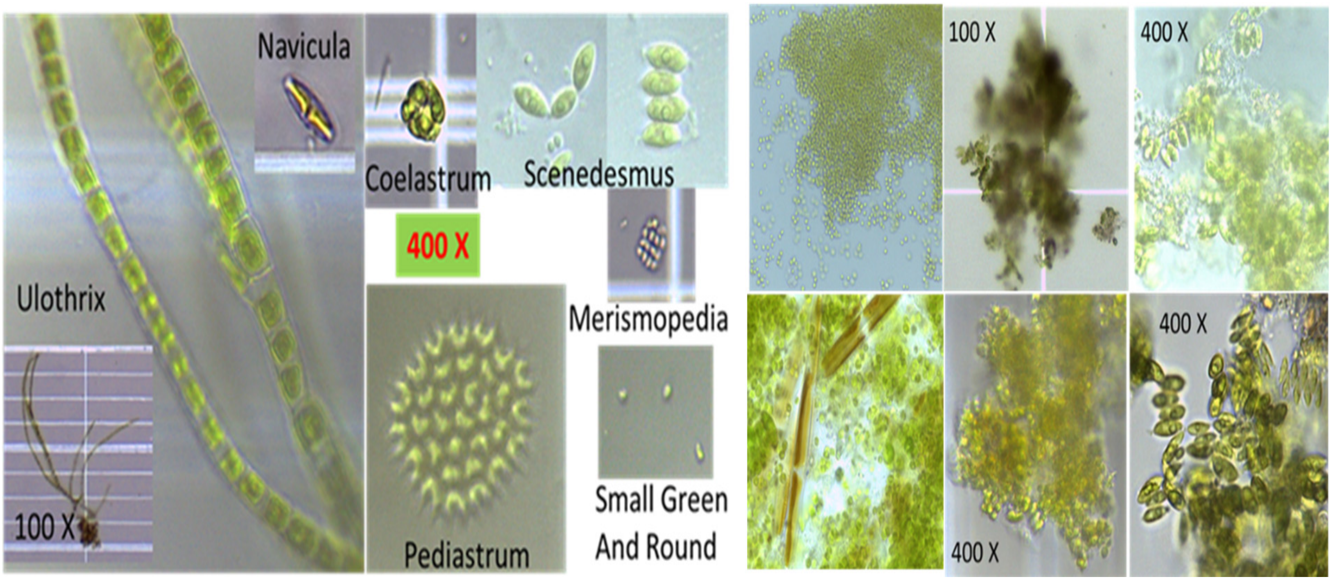

**Fig. (8).** Other algal species present during microcopy examinations of all four bioreactor units (all pictures are at 400x magnification unless otherwise stated). (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

loading is much more catastrophic compared to indoor batch cultures.

### 3.3. Microscopy

Observations and trends were noticed with respect to the type of algae that appeared seasonally. The reactors were operated outdoors in open ponds and no pretreatment was applied to the DLE influent. There was no pre-filtration for color or turbidity control, as many other studies reported prefiltration [17, 20, 26] In addition, the reactors were operated at low available nutrient concentration, less than 20 mg/L of available N, but more typically less than 10 mg/L, so the reactors could have been N limited.

The dominant algal species present shifted seasonally. It appeared that during the spring and fall, *Scenedesmus* strains along with a diatom, such as *Nitchea* or *Navicula,* were dominant. Even the *Scenedesmus* species shifted from 8 cell to 2 cell forms, possibly due to the presence of or lack of predators. Summer tended to be dominated by smaller round, green colonies such as *Chlorella*-like or *Merismopedia*-like organisms. During the winter, species like *Pediastrum* and *Merismopedia* were present, but most significant was the amount of debris compared to other seasons. There were other species present, but none was dominant. Some of the other species present are shown in Fig. (**8**). Further characterization of pond species was conducted by Ibekwe *et al.* (2017) [28].

### 3.4 Limitations of Current Study

Continuous long term outdoor culture was achieved through this study, however, there are some limitations that should be noted. Utilizing raw dairy wash water resulted in many challenges. Most importantly, the added solids and turbidity led to the difficulty in measuring meaningful COD data. It was difficult to quantify the change in COD of the wastewater due to the algae productivity and the bacterial assimilation and transformation of nitrogen. The main mechanism for COD reduction was through dilution, as only 1.6 to 13% of the daily exchange was DLE. Similarly, it was difficult to measure algal productivity using VSS measurements since TSS and VSS changed daily as a result of the DLE additions and also as a result of bacterial growth in the system. The current study results do not distinguish between algae and bacteria, but rather the results include both autotrophic and heterotrophic uptake.

## CONCLUSION

This study demonstrates that algal/bacterial nutrient reduction of diluted dairy wash water can be achieved even when used as a sole nutrient source. During this long term, outdoor, semi-continuous culture study, it was found that HRAPs can remove a significant fraction of the N (20-74%) and P present in the dairy wash water. The best results were obtained with the wastewater making up less than 8% of the daily liquid input, about a 12:1 or higher dilution. Nutrient uptake rates were greatly impacted by the season, with colder seasons limiting the nutrient removal. Seasonal shifts of microalgal species were observed.

To consider HRAPs for commercial scale application for treatment of dairy effluent, future research should examine the following main issues: 1) the high turbidity and dark color present in this wastewater. High dilution is required, resulting in a high volume of freshwater required for treatment. A pretreatment step addressing the turbidity and color may help reduce the supplemental freshwater requirements for dilution but will greatly impact the cost. Previous studies have suggested that a ratio of 1:1 or less, of carbon to available N will promote green water (algal) treatment systems, while greater than 1:1 ratio will promote brown water (bacterial) treatment systems [29]. 2) Seasonal impact on nutrient uptake rate will require great land area during colder months. The development of a pond that can store water during the cooler, lower treatment months, could help as an equalization technique. 3) the development of a reliable algal biomass harvesting strategy is needed to remove the nutrients that are in their new biomass form. This is no longer a technical challenge but rather an economic challenge.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available within the article.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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