Sustainable Microalgae Growth in Jamaican Industrial Wastewater for Biofuel Production

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Sustainable Microalgae Growth in Jamaican Industrial Wastewater for Biofuel Production

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Abstract

Purpose

The study determined the feasibility of combining phytoremediation of industrial wastewater and biofuel production from microalgae cultivation.

Methods

Seed cultures of algal species *Botryococcus braunii* and *Chlorella sp.,* cultivated in nitrogen-phosphorus-potassium (NPK) fertilizer medium, were used to inoculate wastewater samples collected from an abattoir and a creamery. The resulting change in nutrient concentration in the wastewater samples was determined using the American Public Health Association (APHA) standard methods. Algal growth was method measured by gravimetric analysis. Bio-oil extraction was conducted using APHA standard methods, while biodiesel was analysed using gas chromatography – mass spectrometry.

Results

When the microalgae species *Botryococcus braunii* and *Chlorella sp.* were cultured in dairy and chicken-processing plant wastewaters, their growth rates increased as Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and nutrients (nitrates and phosphates) depleted, in accordance with the Droop model. The oil content of the *B. braunii* species was 26.25% (w/w) and 33.60% (w/w) in dry biomass weight from the abattoir and creamery respectively; while the oil content of *Chlorella sp.* species was 47.27% (w/w) and 26.42% (w/w) in dry biomass weight from the abattoir and creamery

respectively. The analysed lipid extracts indicated the presence of fatty acid methyl esters (FAMEs), hexadecanoic acid methyl ester and octadecadienoic acid methyl ester, which were found to be suitable for use in biodiesel production.

Conclusion

It is technically feasible to treat industrial wastewaters with microalgal strains, *Botryococcus braunii* and *Chlorella sp.*, which in turn accumulates oil that can be extracted and converted to bio-diesel.

Keywords: microalgae, biofuel, phytoremediation, wastewater, photobioreactor

Statement of Novelty

This paper provides a detail study into the technical feasiblity of treating two sources of industrial wastewater in Jamaica – wastewater from a chicken farm, and wastewater from a creamery, using two different microalgal species in a photobioreactor. The aim was to determine whether this treatment method could be used to achieve national standards for wastewater discharge, and to determine the design parameters for microalgae cultivation in wastewater medium using a continuous photobioreactor at industrial scale.

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1. Introduction

Microalgae has gained attention as a feedstock source for biofuels because, unlike its first and second generation biofuel source counterparts, it does not impact food security and global food markets; nor result in water scarcity and deforestation[1–4]. The drawbacks of crop-based fuel limit their potential to replace fossil fuels[5], an urgent problem that needs to be addressed as the world struggles to deal with global warming and fuel crisis.

Microalgae, dubbed as miniature biochemical factories[6], hold the potential of being the solution to biofuel production problems because of its outstanding properties. That is, it possesses almost twice the photosynthetic efficiency of higher plants[1]; grows rapidly and has many species that are exceedingly rich in oil[2,7]; is able to double its biomass within about 3.5 hours during the exponential phase[8]; and produces an energy content of around 35,800 $kJ.kg^{-1}$ (approximately 80% of the average energy contained in petroleum)[1]. In addition, the biodiesel produced from microalgae is biodegradable; while providing improved lubricating properties, is less toxic than fossil fuels; and has an ignition point that is considerably higher than diesel, making it easy and safe to manipulate[7].

In order to cultivate microalgae, sunlight, water, carbon dioxide and nutrients are needed. While sunlight, water and carbon dioxide can be found in abundance in the natural environment, nutrients, principally nitrates (N) and phosphates (P) are finite and require fossil fuel for their production[1]. Bearing this in mind, microalgae-based biofuel production using the nutrient rich source, wastewater, may be the solution. That is, wastewater is usually loaded with nitrates and phosphates, which should be removed before final disposal

into the environment. Since microalgae need these nutrients for growth, the wastewater can effectively function as a culture medium, while the microalgae perform phytoremediation[9].

The benefits of combining wastewater phytoremediation and biofuel production are many[9]. In fact, according to Pittman et al. (2011), based on current technologies, algae cultivation for biofuels without the use of wastewater is unlikely to be economically viable or provide a positive energy return[10]. When wastewater is treated with microalgae, the energy cost related to aeration, which usually represent 45-75% of energy cost, is significantly reduced because microalgae produce oxygen free of cost[9]. Also, because wastewater treatment using microalgae does not involve the use of chemicals, the sludge production is significantly less; and the cost of maintaining conditions suitable for growth is lowered[9,11]. Finally, microalgae reduce greenhouse gas emissions by fixing approximately 183 tons of carbon dioxide to produce 100 tons of biomass. As such, it is emerging as a biological CO_2 mitigation strategy, in light of major concerns in global warming and the establishment of the Kyoto protocol to curb problem of greenhouse gases[9].

Wastewaters from domestic sources, animal feeding operations and industries such as dairies, distilleries and wineries are great candidates for algae-based treatment due to their composition and the existing need for treatment[12]. Effluents from dairy industries contain very high levels of COD, BOD, total dissolved solids (TDS) and total suspended solids (TSS). This high organic load is due to the typical presence of fat, grease, flesh, manure and blood[13]. The total amount of waste produced per animal slaughtered is approximately 35% (w/w) and this represents a major source of pollution if not treated

effectively[14]. Creameries handle large volumes of milk, or milk by-products such as cheese or yogurt, with the major source of waste material being the water used in processing[15]. The effluent contains organic matter such as fats, protein and lactose, in addition to inorganic matter such as nitrogen and phosphorous. Due to the availability of samples, both sources shall be explored.

The cultivation of micro-algal biomass can be carried out in different types of systems, namely, open ponds, channels or fully contained photobioreactors (PBRs)[16]. PBRs can produce yields of five times that of open systems[17], and have many advantages compared to other systems[18], although these types of bioreactors are generally not considered for use with wastewater when the goal is phytoremediation due to low performance[19]. However; because this research is not just focused on phytoremediation, but will also incorporate the production of microalgae biomass from which bio-oil will be extracted, the use of laboratory scale PBR for culturing microalgae in wastewater will be explored.

The present research aims to investigate the possible use of two type of microalgae in the treatment of industrial wastewater to maximize microalgae growth, and to facilitate nutrient reduction to meet national wastewater discharge standards. To this end, a two-level experimentation was designed to achieve the following: 1). identify the kinetic parameters used to design an efficient microalge growth process; 2). satisfy pollutant removal requirements, while growing microlage to be use in the extraction of oil for the production of biofules.

Despite the numerous advantages of combining phytoremediation and biofuel production described above, Chisti (2013) pointed out that there are limitations in the supply of sufficient domestic wastewater. However, this argument may be challenged by the following points. Firstly, in addition to domestic wastewater, industrial and agro-industrial wastewaters, which though difficult to treat[9], are also loaded with nutrients, and as such can be used as nutrient sources for algal growth. The results of this research will provide a basis for validating this point, as industrial wastewaters will be used to culture the microalgae species. Secondly, even after nutrients have been depleted from culture media, algal growth is still maintained according to the Droop model[20]. The Droop model has been proven to be appropriate for representing the effects of illumination and macronutrients, such as nitrogen or phosphorus, on microalgae growth rate[20–22]. One of the objectives of this research is to prove the Droop model using two microalgae species: *Chlorella vulgaris* and *Botryococcus braunii*, when cultured in wastewaters obtained from an abattoir and a creamery in Jamaica. Wastewaters from these industrial sources contain high organic load, which are major sources of pollution for receiving water bodies if not treated effectively[14,23].

Before treated wastewater is released into the environment, it has to be characterized to ensure that it meets environmental standards. In Jamaica, standards for the wastewater effluent parameters, such as Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Nitrates (N), and Phosphates (P) are prescribed by the National Resources Conservation Authority, NRCA (2013)[24]. This research will involve the quantification of these parameters for samples collected at the discharge of the

photobioreactor to determine if they meet the discharge standards. This finding will reinforce the hypothesis that while growing microalgae the pollutants in a wastewater stream will also be reduced and meet these discharge standards.

2. Results and Discussion

The research focuses on the cultivation of two microalgal species in two different wastewater sources, to harvest bio-oil while simultaneously providing phytoremediation for the wastewater sources. The two-algal species used in the investigation include *Botryococcus braunii*, and *Chlorella vulgaris*, while the wastewater sources were taken from a chicken processing plant and a creamery (Dairy Industries).

Some of the key parameters that were used in the characterization of wastewater quality include nitrates, phosphates, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and pH. As a result, a reduction in these parameters over the growth period of the microalgae was used to provide an indication of the efficiency with which the microalgal species can be utilized in wastewater treatment. Of the nutrients present in the wastewater, nitrates and phosphorus are the two main components which can support micro-algal growth. Therefore, both components were monitored daily to assess their absorbance in relation to the growth rate of the microalgae. The proceeding discussion will show that microalgae cultivation in wastewater offers viable biofuel generation along with treatment and significant nutrient removal. Consequently, this feature makes microalgae

growth feasible for industrial wastewater treatment. Table 1 illustrates initial concentration in the wastewater used for the investigation.

2.1. Nutrient Observation, Uptake and Analysis in Batch Experiment

In the analysis of the wastewater, the nitrates and phosphates concentrations experienced an overall decrease with time, which is in line with the results from previous literature. Figures 1 to 4 show trend of nutrients reduction in the batch cultivation.

All nutrients decreased rapidly due to fast assimilation by microalgae in the first culture day followed by slight increases because of release of cellular nutrients. This suggested that a retention time of three days was enough to achieve maximum nutrient reductions when the microalgal strain under the current settings was used.

The concentration of phosphates, in particular, fluctuated continuously throughout batch experimentation. This was expected, because according to Casadevall (1985), a number of species are able to absorb large quantities of phosphate in excess of normal growth requirements, decreasing the phosphate concentration in the growth medium[25]. Later, the phosphates were released into the medium causing increase in concentration once again. These experiments have proven that the species involved are capable of displaying this phenomenon. This was especially evident in the case where wild type *Chlorella* was cultured in chicken processing plant wastewater. Here, *Chlorella sp.* displayed tremendous ability to absorb and release phosphates, as indicated by its sharp increases and decreases in phosphate concentration. These fluctuations, however, had no noticable impact on the

growth rates of the species in each culture. Also, the removal of phosphate was found to be a much slower process than that observed for nitrates.

Among all the nutrient reduction parameters, the reduction of BOD was the greatest. This may be attributed to the fact that carbon is a macronutrient necessary for algae growth. BOD experienced an average reduction of 96.76% over time. The highest reductions were seen in the chicken processing plant wastewater as both species resulted in a 98.30% reduction in the BOD. These cultures also experienced the lowest level of BOD (18 mg/L) after treatment. The dairy industries samples inoculated with wild type *Chlorella* and *B. braunii* saw reductions of 95.09% and 95.35% respectively.

On average, there was a 95.62% reduction in the COD levels over time. The greatest reduction of 97.62% was seen in the dairy industries sample inoculated with *B. braunii*; while the wild type inoculated sample experienced a reduction of 95.27%. The COD reductions in the *B. braunii* and wild type *Chlorella sp.* inoculated chicken processor wastewater were 94.95% and 95.00% respectively. In addition, the lowest concentration after treatment was found in the chicken processing wastewater sample (132 mg/L), even though it did not have the greatest reduction rate.

With regards to the wastewater effluent standards as stipulated by NRCA (2013), this was not achieved in all cases even though the reduction rates were very high. The effluent standard for COD is 100 mg/L. However, none of the cultures were compliant with the permissible trade effluent discharge standards even though reduction rates were up to 97.62%. The BOD effluent standard is 20 mg/L, which was achieved in the chicken

processing cultures (with both species achieving 18 mg/L BOD). The other cultures were also in close range, with BOD levels just over 50 mg/L. The phosphate standard is 4 mg/L, however, none of the cultures could deplete phosphate to this amount or lower. Residual phosphates for the cultures after 11 days ranged from 13 – 43 mg/L. Finally, the nitrate effluent standard of 10 mg/L was met by the wild type *Chlorella sp*.– chicken processing plant and *B. braunii* – Dairy Industries cultures with residual concentrations of 7 mg/L and 8 mg/L respectively. The results for other cultures were within similar range. In general, it was observed that the wild type was more efficient at reducing the wastewater parameters to values that either met the NEPA standards or brought them within range of the permissible standard values.

2.2 Batch Cultivation

The microalgae species were cultivated in the batch experimental setup as described in the methodology. In all cases, the growth of the microalgae exhibited the expected lag phase follow by the growth/exponential phase as predicted in the literature.

2.2.1 Algae Growth and Biomass Productivity in Batch Experiment

2.2.1.1 Wild Type Chlorella sp.

The growth patterns of the wild type *Chlorella sp*. in both the Chicken processing plant and Dairy Industries wastewaters are illustrated in figures 2 and 4. In both wastewaters, the culture was in a lag phase during which algae cells were slowly adapting to the new environmental conditions. The lag phase for the species in the Dairy Industries wastewater continued for approximately three days and in this period, inoculated culture

tended to settle at the bottom of the conical flask, which may have been due to the environmental shock[26]. Further, the stationary phase of the *Chlorella sp.* in the Chicken processing plant sample was relatively short between one to two days when compared with growth in the Dairy Industries Sample. This shorter lag phase in the Chicken-processing plant is significant, as it indicates a higher compatibility of the processing plant wastewater to support microalgae growth, possibly since its nitrogen, nitrate and phosphate concentrations were like the seed culture medium the microalgae were grown in before. The difference in lag phase would not be affected by any characteristic of the microalgae itself, as the same species was used to inoculate both wastewater samples, and was taken from the same seed culture, which was observed to have uniform density. As a result, difference in lag phase, would be due to the growth media, in this case, the wastewater sources.

Around the third day after inoculation, the concentrations of microalgal biomass started to increase rapidly. The colour of the wastewater from chicken processing plant and the dairy industry turned from brown and grey respectively to green as more microalgal cells started growing and were suspended in the culture. In the Dairy Industries sample, rapid growth was observed during the third to the eighth day after the microalgae were inoculated. This growth was characterized by an exponential growth rate of 435.16 mg/ L/day on a dry weight basis. However, in the Chicken processing plant sample, the observed exponential phase was slower than those cultivated in the Dairy Industries sample, with a rate of 128 mg/L/ day on a dry weight basis. This decreased growth rate is likely because, there may be substances in Dairy Industries wastewater that were growth

promoters for the wild type species, but were not present in the chicken processing wastewater. Alternatively, there may have been some amount of growth inhibition in the latter that contributed to the growth retardation.

Furthermore, as illustrated in Table 1, initial concentrations for the dairy industry were higher with respect to COD and BOD parameters, while the sample taken from the chicken processing plant had the higher concentrations of both nitrates and phosphates. Therefore, theoretically, it was expected that growth would be proportional to the concentration of the limiting substrate (nitrates). According to Travieso et al. (2006), the growth rate should be influenced by the substrate concentration for almost constant values of light intensity and temperature[27]. These parameters were kept fairly constant; however, this theory did not directly apply to the microalga species used, and growth rates seemed to not be influenced by substrate concentration. That is, even though more nutrients were available in the chicken processing wastewater, this was not reflected in the growth rate. Consequently, the biomass productivity for the wild type in dairy industries wastewater was significantly higher than in the chicken processing plant, reported as 18,907.56 g/g and 5099.01 g/g respectively. A greater number of wild type cells were produced per gram of nitrate for the Dairy Industries wastewater than for the chicken processing plant. In addition, the maximum specific growth rate (μ_{max}) of the *Chlorella species* in the wastewaters was determined to be 1.54 in the Dairy Industries sample and 1.44 in the sample obtained from the chicken processing plant, with overall specific growth rates of 0.2030 day^{-1} and 0.1429 day^{-1} respectively. Maximum and overall specific growth rates were proportional, as the Dairy Industries culture showed both higher final biomass and a

steeper log phase. Additionally, the higher value obtained for Dairy Industries supports its much higher productivity realized. There was therefore greater compatibility of wild type growth in the Dairy Industries wastewater. Tables 2, 3 and 4 illustrate the biomass productivity during the experimental runs for all species of microalgae.

The growth rates of all cultures decreased into the stationary phase, which was graphically represented as a plateau. This was followed by the death phase starting on the twelfth day, during which the lyses of algal cells by bacteria and rupture of dead bacterial cells would release nutrients out to fuel the remaining viable cells. Throughout the observation period, the highest algal density was observed in the Dairy Industries sample on the eleventh day with the biomass concentration value of 2,520 mg/L for wild type *C. vulgaris*. However, as indicated in the literature, the biomass concentration should increase substantially when the nutrients, such as nitrogen, have been depleted[28]. This was therefore proven to be in accordance with expected results, as the cultures underwent nutrient starvation for ten days, after which the biomass yield was found to be 331.15% and 148.98% higher in the chicken processing plant sample and the Dairy Industries sample respectively. It is suspected that this increase in lipid content in the microalgae was a survival mechanism. That is, fat is an energy storage molecule, the accumulation of which is often triggered by a threat to starvation in higher animals. Here, cells convert other macronutrients (proteins and carbohydrates) to fats, which are not used up immediately but can be efficiently applied to maintain essential body functions within the short-term. It is expected that the microalgal system operates in the same manner, resulting in the phenomenon where lipid content and therefore biomass significantly increase upon

depletion of nitrates[22]. The microalgae yielded higher for the sample from the chicken processing plant than the dairy industry sample.

2.2.1.2 Botryococcus braunii

Figures 1 and 3 show the growth patterns of *Botryococcus braunii* in the Chicken processing plant and Dairy Industries wastewaters respectively. The lag phase lasted for four days in the Dairy Industries sample and two days in the chicken processing plant sample. This suggests that the shorter lag phase was due to the fact that the nutrients available in the pure culture were indeed similar to the nutrients present in the chicken processing plant samples. The exponential phase of the growth curve for the Dairy Industries and Chicken processing plant lasted for 4 days and 3 days respectively, before the stationary phase. Also, the growth rate was faster in Dairy Industries potentially for the same reasons mentioned above for the wild type *Chlorella species*. On a dry weight basis, growth was characterized by a 145.33 mg/L/day increase in the chicken processing plant sample and a 240.95 mg/L/day increase in the Dairy Industries sample. The maximum specific growth rate of *B. braunii* in the wastewaters was calculated to be 1.28 in the Dairy Industries sample and 1.30 in the sample obtained from the chicken processing plant. Refer to Table 3. Overall growth rates were 0.1932 day^{-1} and 0.1638 day^{-1} respectively. In this case, the specific growth rate was not proportional to the maximum growth rate. There was some amount of growth fluctuation for the chicken processing culture, which could have been the reason for this result.

These samples were also subjected to nutrient deprivation, and after the 10-day starvation period the biomass yield was found to be 303.67% and 244.09% higher in the chicken processing plant sample and the Dairy Industries sample respectively. On a whole, *B. braunii* showed higher biomass productivities. However, the growth rate for the *Chlorella species* was generally higher. This was expected because according to the literature, *B. braunii* usually has higher oil contents than *C. vulgaris*. As it is extremely energy-intensive to produce high-energy hydrogen carbon as the desired product, the cell growth rate is usually low[29]. *C. vulgaris* instead, usually has less oil content and is therefore able to exhibit a faster growth rate. This generates an intrinsic limitation, where oil yield may have to be sacrificed for biomass productivity, and vice versa.

Nutrient depletion rates were correlated to biomass productivity showing a negative value indicating that the growth of biomass is inversely proportional to the nutrient depletion, which was consistent for both species and wastewater type.

When the nutrients are depleted, the biomass productivity increased up to 3 folds and produces more lipids, indicating that nutrient depletion is favorable to algal growth and indeed may be regarded as necessary for maximizing algal growth and bio-oil production when scaling up for commercial operations. This also nullifies the point presented by Chisti (2013), who mentioned that the volume of wastewater produced may be insufficient for supporting microalgal growth. Based on the results of this research, wastewater sustains the growth of microalgae very well.

2.3 Continuous Microalgae Cultivation

A microbial candidate for wastewater treatment has to show a significant capacity to remove pollutants under the intended environmental conditions. In this research, it was determined that the wild type *Chlorella* species was the most efficient, with a maximum specific growth rate of 1.25 day^{-1} when grown in Dairy Industries wastewater. Therefore, the maximum allowable flowrate of wastewater entering the reactor, was determined to be 0.014 m^3/day for a 0.014 m^3 continuous photobioreactor.

2.3.1 Analysis of the Microalgae Growth

During the experimental runs, the wastewater overflow from the reactor was analytically tested for total nitrogen to determine the performance of the process. The only parameter monitored was the limiting substrate, nitrate content. An 80% reduction in total nitrogen was achieved in the continuous reactor, similar to the result obtained from the batch experimentation. The nutrients decreased and the microalgal biomass increased. The residual substrate concentration increased with an increase in the flow rate. The maximum specific growth rate was calculated to be 1.23/day and the saturation constant (Ks) was 109.55 mg/L. The maximum growth rate obtained was similar to the maximum growth rate obtained by the graphical method (1.54/day). However, the value of Ks appeared to be very high, and even above the concentration available as the initial concentration of nitrates for Dairy Industries was 85 mg/L. This result indicated that at concentrations greater than 109.55 mg/L, the growth rate of the species would start to decrease. These results indicate that the microalgae species used in this research have high affinity for nitrates, with growth rate increasing as nitrate concentration decreases. This point was also proven during the

culturing phase, when the species of microalgae were inoculated by a medium containing higher concentration of nitrates, and microalgae growth was inhibited. However, in much lower concentrations of nitrates, the microalgae growth increased rapidly, indicating high affinity for nitrates. The results obtained were contrary to reviewed theory for similar types of wastewater and microalgae.

2.4 Extraction of Lipids from Microalgae

The oil yield from microalgae cultured using industrial wastewaters was also investigated. Based on our experiments, two solvent extraction methods were used to determine lipids extraction yields. The solvent/co-solvent method achieved 31% higher yields on average than the traditional APHA (2017) method[30]. Tables 5 and 6 outline the oil yields for both types of wastewater and microalgae species.

Results showed that, on average, the wild type *Chlorella species* cultured in Dairy Industries and the Chicken processing plant wastewater contained 26.42% oil; and 47.27% oil respectively. The *Botryococcus braunii* species contained 33.60% oil and 26.25% in the Dairy Industries and Chicken processing plant wastewater respectively. The final experimental lipid yields calculated were 0.292g lipid extract/g dried microalgae for *B. braunii* and 0.347 g lipid extract/g dried microalgae for the wild type *Chlorella sp*. The value obtained for *B. braunii* was close to the yield for *Botryococcus braunii,* which was reported at 0.286 g lipid extract/g dried microalgae[31]. The reported yields for *Chlorella sp.* have been recorded at 0.079 g lipid extract/g dried microalgae, much lower than the value obtained in this research.

Nitrogen starvation has been demonstrated to increase the amount of lipids in microalgae, including the genus *Chlorella*[32]. *B. braunii* also showed higher biomass productivities after nitrogen starvation, although the oil yield was lower in comparison to the wild type species. Theory suggests that the oil content of *Botryococcus braunii* can go up to 70%; however, due to the rigidity of the cell walls, the solvent may have been unable to penetrate the cells even after drying and crushing. As such, lower than expected oil yields were seen for *B. braunii*.

Analyses of extracted oil samples were also performed with the Gas Chromatograph/ Mass Spectrum (GC/MS) and procedure described previously. One of the common compounds in all variations of the analyzed lipids was hexadecanoic acid (or palmitic acid), which has good biofuel properties and thus is a desirable biodiesel component. Octadecanoic acid methyl ester (or stearic acid methyl ester), used in biofuel preparation, was also present.

An interesting compound that was also identified was limonene, which is a naturally occurring fragrance and cleaning agent found in citrus fruits. *B. braunii* is therefore a potential source of the compound, which may be another bi-product of the bio-reactions that could increase the economic feasibility of the process, an issue which is continuously being addressed[33].

In order to further investigate the possibility of producing bio-fuel from microalgae cultivated in wastewater, one sample of the extracted bio-oil was transesterified into bio-diesel. The transesterified lipids were also analyzed by GCMS to identify the fatty acid

methyl esters (FAMEs). Figure 10 shows the results of the chromatography. Hexadecanoic acid methyl ester, octadecanoic methyl ester, and heptadecanoic methyl ester were present in the sample. This confirms that useful bio-oil was indeed present in the extracted content from the microalgae.

3. Conclusion

Wastewaters from the dairy and chicken-processing industries were able to adequately support the growth of *B. braunii* and wild type *C. vulgaris* species. Even under nutrient depletion conditions, the microalgae species exhibited high biomass growth. This point must be taken into consideration when designing and optimizing bioreactors for microalgae production. The biomass accumulation and oil yield for the microalgae species in the different wastewasters were quite high and comparable to values reviewed in previous literature. This indicate that combining phytoremediation and biodiesel production using microalgae is very viable for Jamaica and the Caribbean region.

4. Materials and methods

4.1 Microalgae Isolation and Growth

A pure strain, *Botryococcus braunii*, donated by local cultivators and a wild-type *Chlorella sp.* strain isolated from a gully in St. Catherine, Jamaica were used in this study. Both species were cultured in a batch mode on artificial culture media made of NPK Fertilizers under continuous light (120 μmol photons per square meter per second) at ambient temperature, (25±1 °C) and were continuously bubble aerated by a commercial aquarium pump.

4.2 Wastewater Sample Collection

Effluent samples were collected from a local abattoir and creamery. Wastewater samples were collected in five gallon plastic containers, previously washed with non-ionic detergent and rinsed with deionized water prior to usage. During sampling, the containers were conditioned with the sample and filled. The samples were then transported to the laboratory and stored in the refrigerator at approximately 4ºC.

4.3 Determination of wastewater physical and chemical properties

Both samples of wastewater had a high concentration of total suspended solids and thus high turbidity, which reduced light transmission. The samples had to undergo treatment to remove the suspended solids in order to effectively grow microalgae. This was accomplished by rapidly mixing the sample, followed by settling. In order to analyze the nutrient profiles, nitrate nitrogen (NO_{3-} -N), total nitrogen (TN), total phosphorus (TP), and

COD were determined for all samples, following the analytical methods as stated in the water and wastewater standards method[30]. During experimental procedures, samples were analytically tested using similar standard methods.

4.4 Experimental procedure for Microalgae Growth

The procedure was carried out using two experiments set-ups, namely batch and continuous processes.

4.4.1 Batch Experiments

500 ml of each wastewater sample was aseptically inoculated with 100 ml of each algal strain from the fertilizer growth culture. Continuous illumination was provided by fluorescent lamps. Carbon dioxide and aeration provided by an aquatic pump was also part of the experimental operation. Chemical Oxygen Demand (COD), Biological Oxygen Nemand (BOD), nitrates (NO_{3-}) and Phosphates were monitored daily. The collected samples were centrifuged at 2,400 rpm for 5 minutes. The supernatant was analyzed to determine levels of NO_{3-}, Total Nitrogen, Total Phosphorous, and COD according to standard methods[30]. The mass of the settled solids was measured in order to obtain the daily biomass growth using gravimetric analysis. After the supernatant was removed, the tubes were then dried in the oven at 105^{o}C for approximately two hours, and subsequently re-weighed. Nutrient removal rates were also calculated and expressed as percentage. This batch experimentation provided the best microalgae-growth configuration for industrial wastewater treatment.

The main objective of this experimental set-up was to obtain the valuable kinetic information about the two species and their growth performance in both types of wastewaters used in the experiments.

The specific growth rate of the species in each culture was determined using the following formula:

Specific Growth = $(\ln x_2 - \ln x_1)/ \Delta t$. day

Where x_1 and x_2 are the initial and final dried cell weigth.

The plot of these data representing the growth of the species versus the nutrient removal rate will be used to determine whether or not the growth rate is proportional to removal rate for each sample. A graph of removal rate versus growth rate will be plotted for each sample to get a more vivid picture of the relationship between growth rate and removal rate.

In this method, the point at which the substrate curve and the biomass curve cross represents the maximum specific growth rate (μ_{max}). The limiting substrate for the microalgal species used is nitrate.

The amount of cells produced by microorganisms is usually proportional to the amount of limiting substrates consumed, which reflects the biomass productivity of the organism (Lee, 2006). The biomass productivity can be defined by the following formula:

$Y_{x/s} = \Delta X/\Delta S$

Where $Y_{x/s}$ represents the biomass productivity coefficient and the conversion efficiency of the substrate to biomass; ΔX is the change in biomass concentration; and Δs is the change in the concentration of the limiting substrate.

If the values obtained for the effluent components are equal to or less than these limits, or just above by a maximum of 10%, then these species of microalgae can be considered for wastewater treatment.

4.4.2 Continuous Photobioreactor experiments

The maximum specific growth of the algal species was determined and used when conducting the design for the continuous bioreactor, based on the maximum allowable flow for the photobioreactor.

Wastewater was inoculated in the bioreactor and hold for a period of five days to allow maximum microalgae growth under aeration and carbon dioxide supply. Specimens were alowed to settle for two days. Flocculated biomass was then collected by syphening and centrifuged at 3,400 rpm for 10 mins. The cell suspensions were also centrifuged at 3,400 rpm for 10 mins. All biomass was collected in pre-weighed petri dishes and oven dried at 105 °C for four hours and weighed to four decimal places on an analytical balance.

4.5 Oil Extraction procedure

The basic principle that characterizes organic solvent extraction of microalgal lipids is the basic chemistry concept that 'like dissolves like'[34]. The oil extraction process was carried out using the APHA et al. (2017) standard method 9071B, Soxhlet apparatus using n-hexane as solvent, which is supported by Halim et al. (2012) who proposed organic solvent extraction mechanism at the cellular level[34]. A modification of this method was also conducted, where a co-solvent procedure using isopropanol was used to enhance cell wall disruption. Results from both methods were then compared based on yield efficiency.

Extraction performance will be evaluated by two key indicators, namely, lipid yield and GC–MS analysis of the FAME composition of the lipid extract.

Lipid productivity (%) will be calculated based on the ratio of total oil extracted to dry weight of algae as shown below:

Lipid productivity (%) = g of oil/dry weight of biomass

In addition, microalgae culture will be observed for species diversity.

4.6 Analysis of Extracted Oil

The GC–MS analysis was carried out in a Hewlett Packard 6890 gas chromatograph, equipped with a Hewlett Packard 5973 mass spectrometer. Compounds were profiled on a 60 m long, 0.25 mm ID, and 0.25 μm film thickness HP-5MS capillary column. The carrier gas was Helium at 1.0 ml/min. Temperature conditions were: 60 ℃ for 3 min and 280 ^{o}C/min held for 4 min with 250 ℃ injection temperature, and 250 ℃ detector temperature. The sample injection volume was 1 μl and the injection mode was splitless. The mass

spectrometer acquisition mode was SCAN 30 m/z – 550 m/z and the run time was 35.50 minutes. The following standards were used for quantification: hexadenoic acid methyl ester, and octadecadienoic acid methyl ester.

Statements & Declaration

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Competing Interests Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Author Contributions

1. **Jervian Johnson** - is the first author and corresponding author for the research paper; conducted research; and contributed to writing the research paper (abstract, introduction, discussion, conclusion)
2. **Renee English** - conducted research, and contributed to writing research paper (discussion, methodology, graphs)
3. **Nilza Justiz-Smith** – Supervised research, and revised the research paper

Data Availability

A public repository storing the datasets generated during and/or analysed during this study does not exist currently, but are available from the corresponding author on reasonable request.

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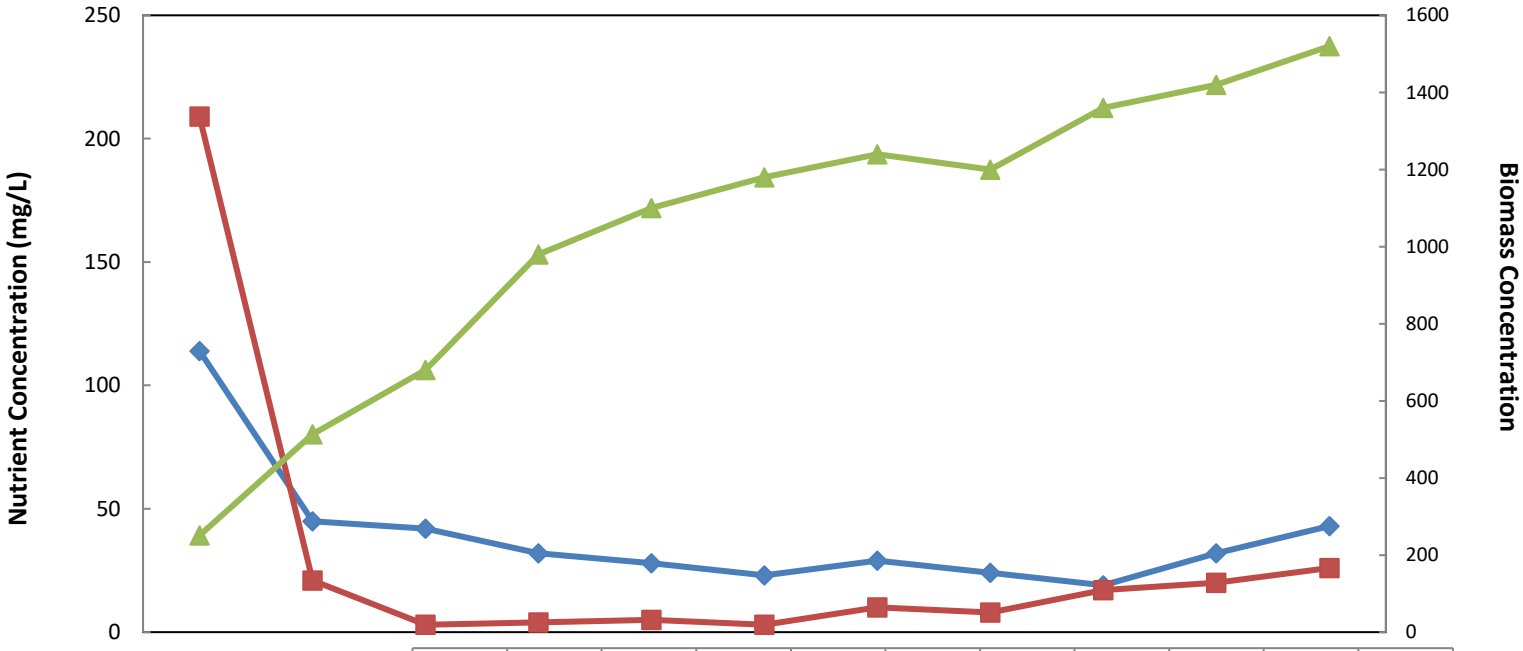
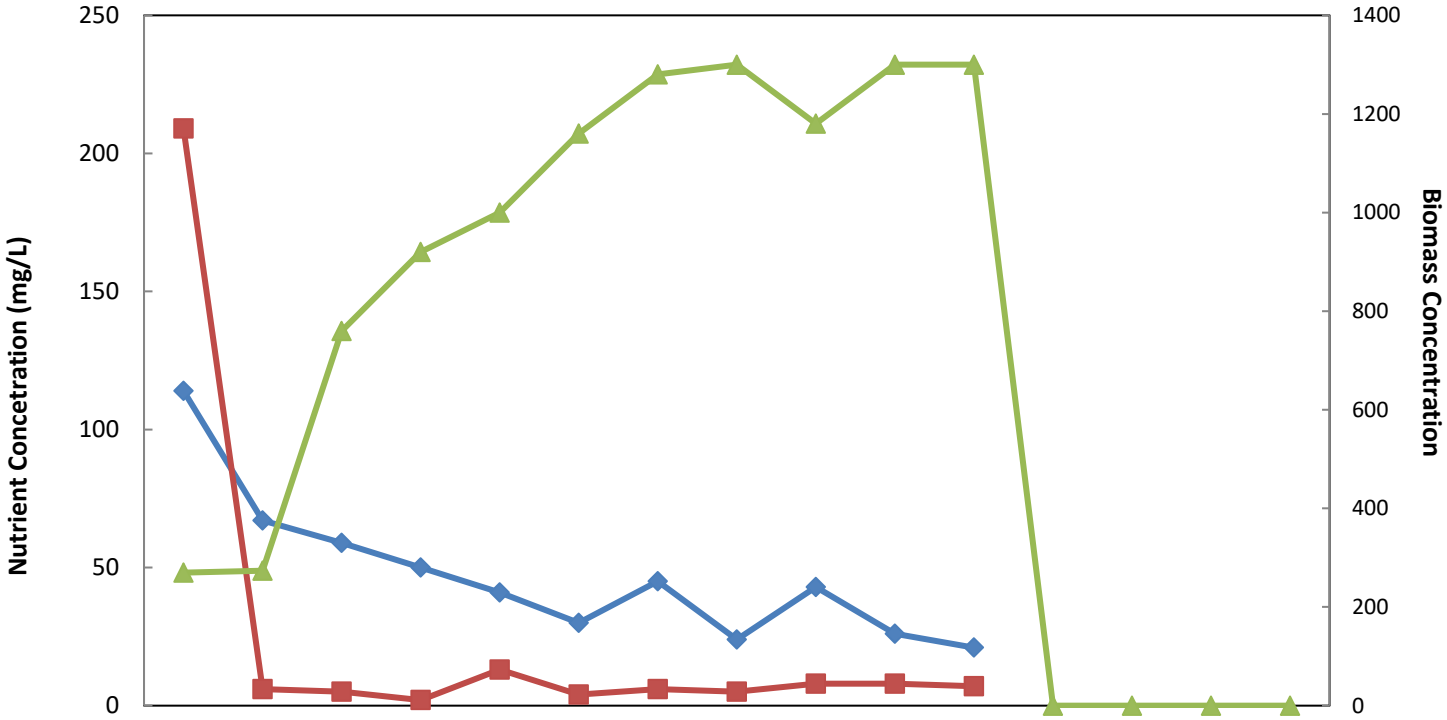
Figures

Figure 1-4: Trend of nutrient and biomass concentration



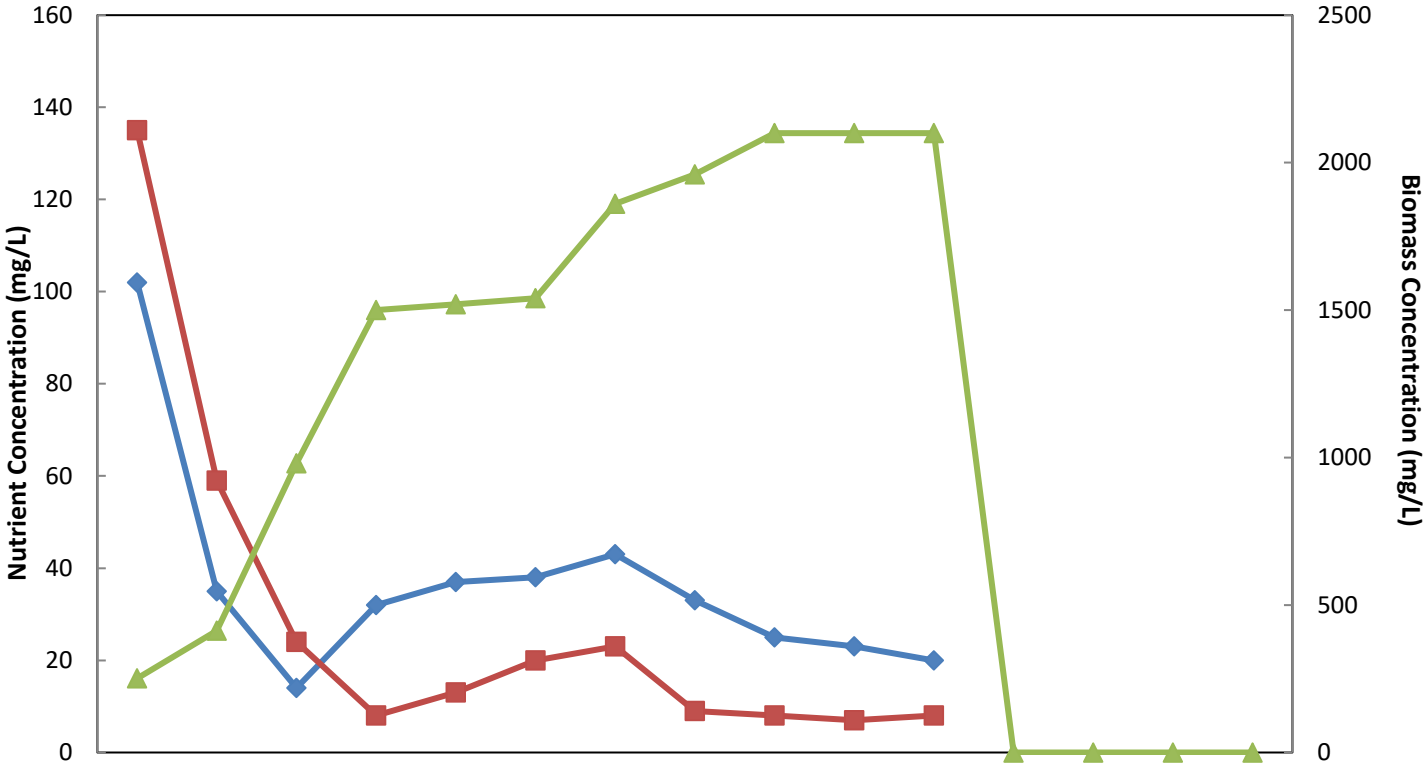
	8-May	9-May	10-May	11-May	12-May	13-May	14-May	15-May	16-May	17-May	18-May
Phosphates (mg/l)	114	45	42	32	28	23	29	24	19	32	43
Nitrates (mg/l)	209	21	3	4	5	3	10	8	17	20	26
Biomass concentration (mg/L)	250.83	513.33	680.00	980.00	1100.00	1180.00	1240.00	1200.00	1360.00	1420.00	1520.00

Figure 1: Trend of nutrient and biomass concentration for Chicken Processing Plant (CPP) - *B. braunii*;



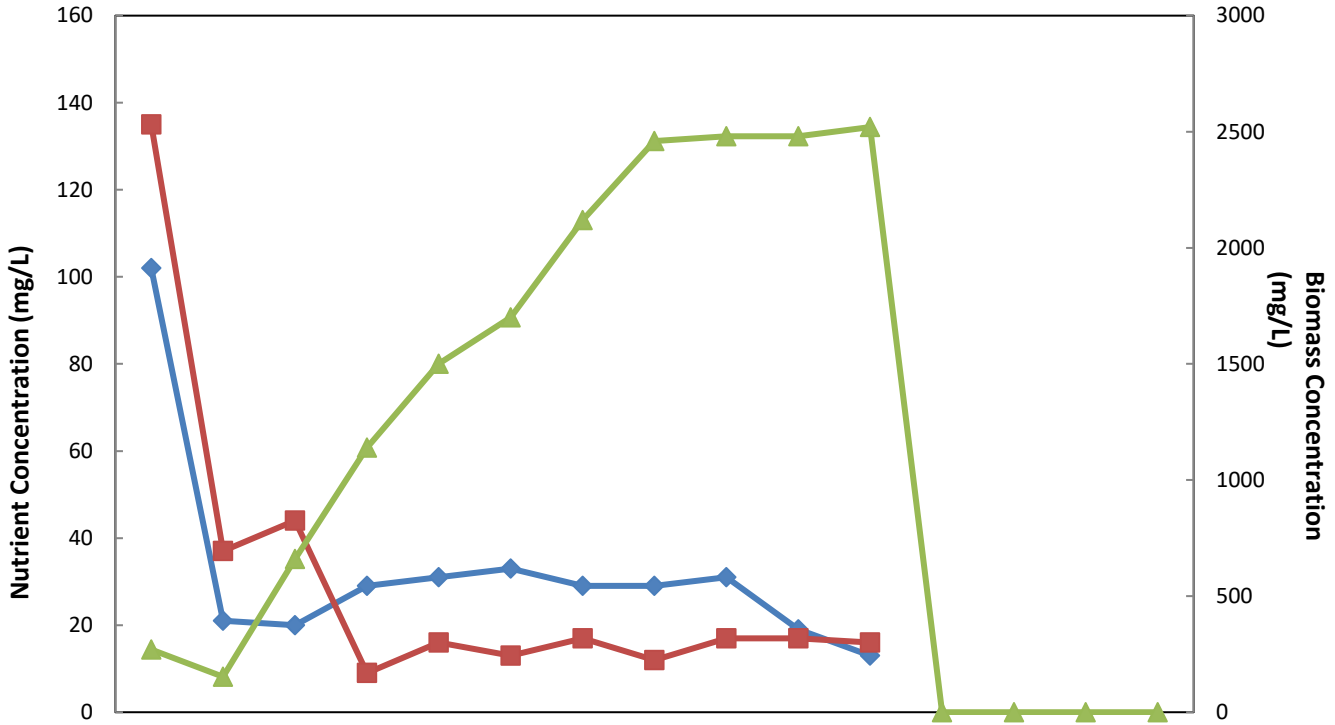
	8-May	9-May	10-May	11-May	12-May	13-May	14-May	15-May	16-May	17-May	18-May
Phosphates (mg/l)	114	67	59	50	41	30	45	24	43	26	21
Nitrates (mg/l)	209	6	5	2	13	4	6	5	8	8	7
Biomass concentration (mg/L)	270.00	273.33	760.00	920.00	1000.00	1160.00	1280.00	1300.00	1180.00	1300.00	1300.00

Figure 2 Trend of nutrient and biomass concentration for Chicken Processing Plant -wild type *Chlorella sp*.



	8-May	9-May	10-May	11-May	12-May	13-May	14-May	15-May	16-May	17-May	18-May
Phosphates (mg/l)	102	35	14	32	37	38	43	33	25	23	20
Nitrates (mg/l)	135	59	24	8	13	20	23	9	8	7	8
Biomass concentration (mg/L)	250.83	413.33	980.00	1500.00	1520.00	1540.00	1860.00	1960.00	2100.00	2100.00	2100.00

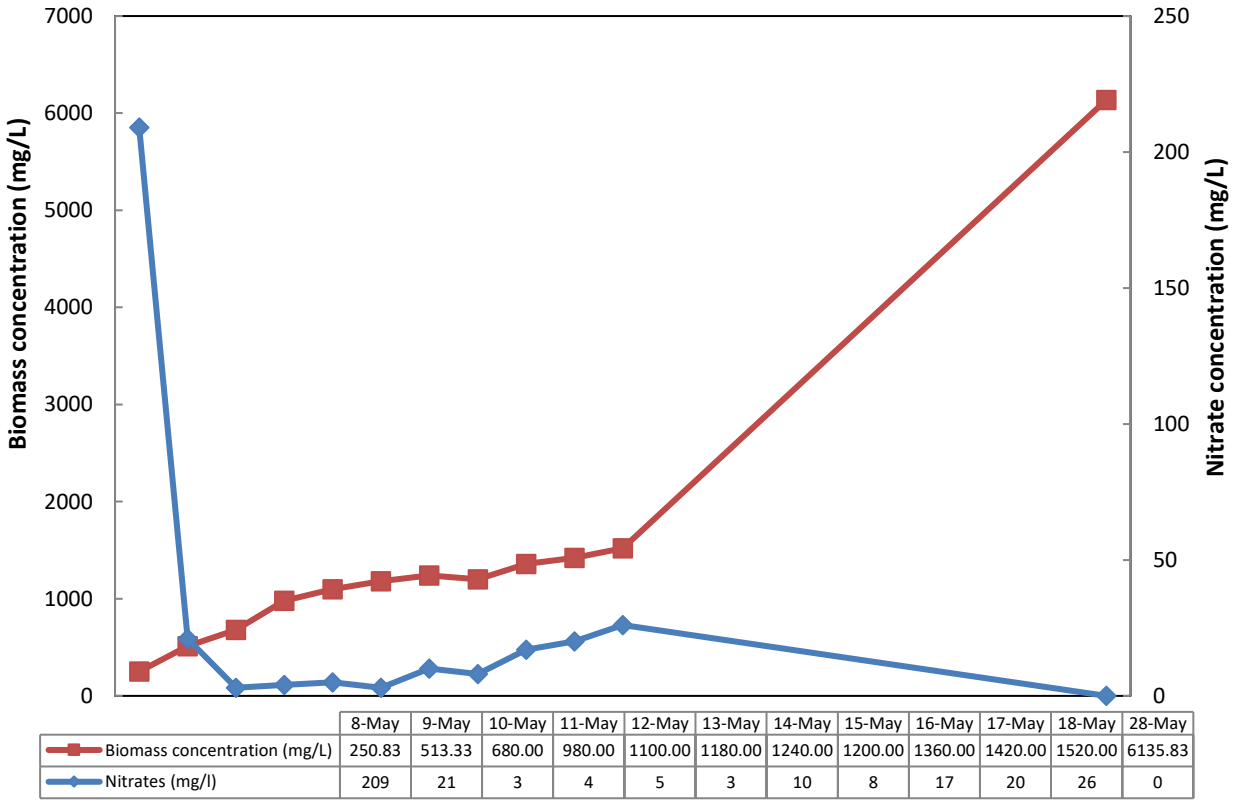
Figure 3 Trend of nutrient and biomass concentration for Dairy Industries (DI) - *B. braunii*



	8-May	9-May	10-May	11-May	12-May	13-May	14-May	15-May	16-May	17-May	18-May
Phosphates (mg/l)	102	21	20	29	31	33	29	29	31	19	13
Nitrates (mg/l)	135	37	44	9	16	13	17	12	17	17	16
Biomass concentration (g/L)	270.00	153.33	660.00	1140.00	1500.00	1700.00	2120.00	2460.00	2480.00	2480.00	2520.00

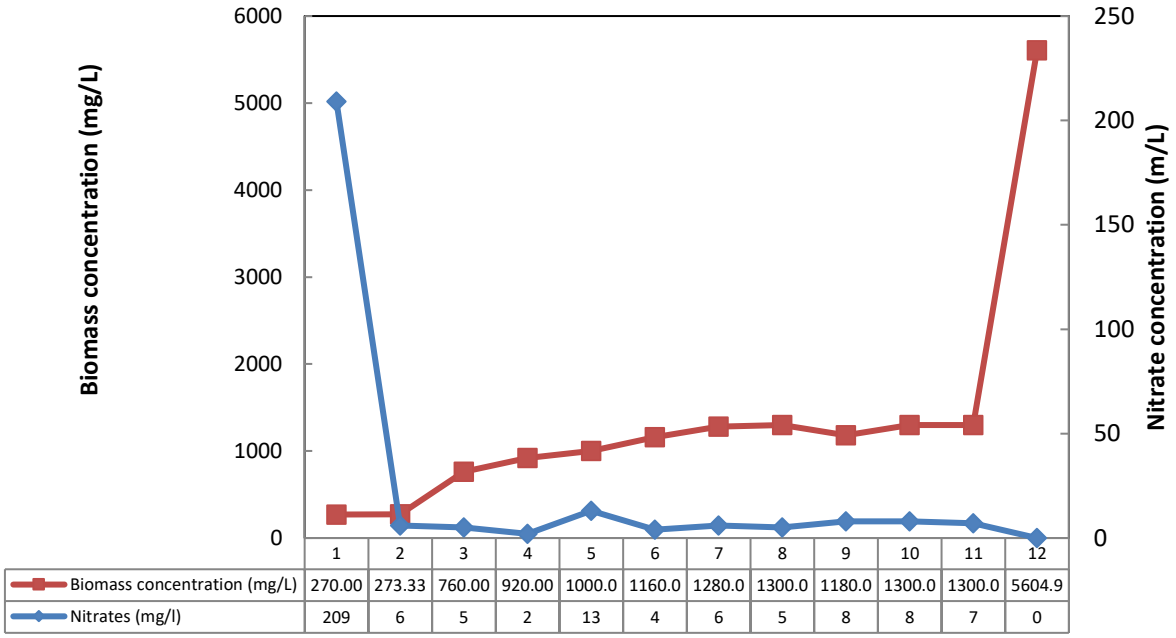
Figure 4 Trend of nutrient and biomass concentration for Dairy Industries -wild type *Chlorella sp*.

Figure 5-8: Effect of limiting substrate depletion on biomass concentration



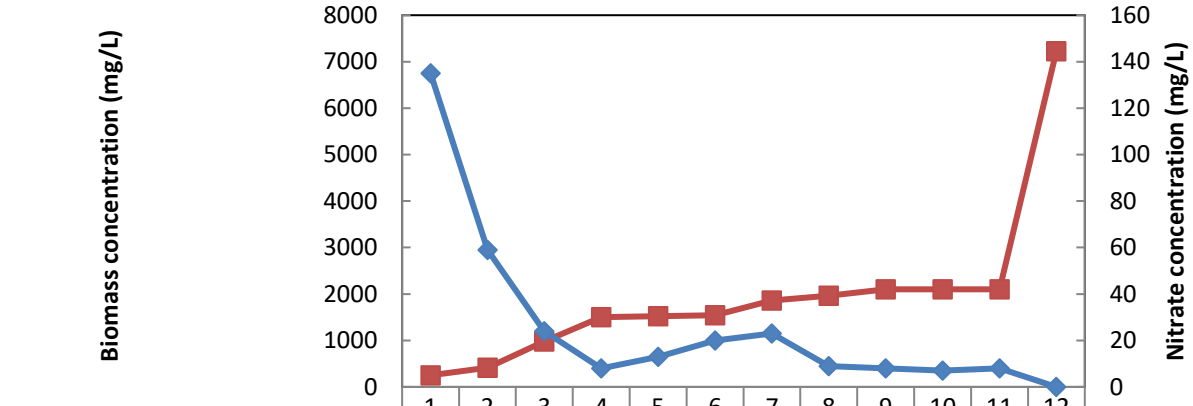
	8-May	9-May	10-May	11-May	12-May	13-May	14-May	15-May	16-May	17-May	18-May	28-May
Biomass concentration (mg/L)	250.83	513.33	680.00	980.00	1100.00	1180.00	1240.00	1200.00	1360.00	1420.00	1520.00	6135.83
Nitrates (mg/l)	209	21	3	4	5	3	10	8	17	20	26	0

Figure 5 Effect of limiting substrate depletion on biomass concentration - Chicken processing plant inoculated with *B. braunii*



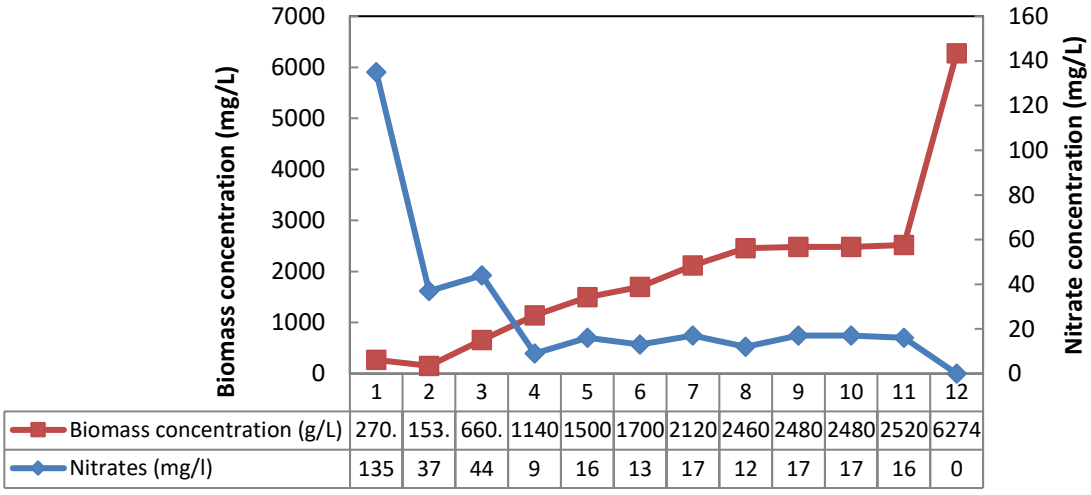
	1	2	3	4	5	6	7	8	9	10	11	12
Biomass concentration (mg/L)	270.00	273.33	760.00	920.00	1000.0	1160.0	1280.0	1300.0	1180.0	1300.0	1300.0	5604.9
Nitrates (mg/l)	209	6	5	2	13	4	6	5	8	8	7	0

Figure 6 Effect of limiting substrate depletion on biomass concentration - Chicken processing plant inoculated with wild type *Chlorella sp*.



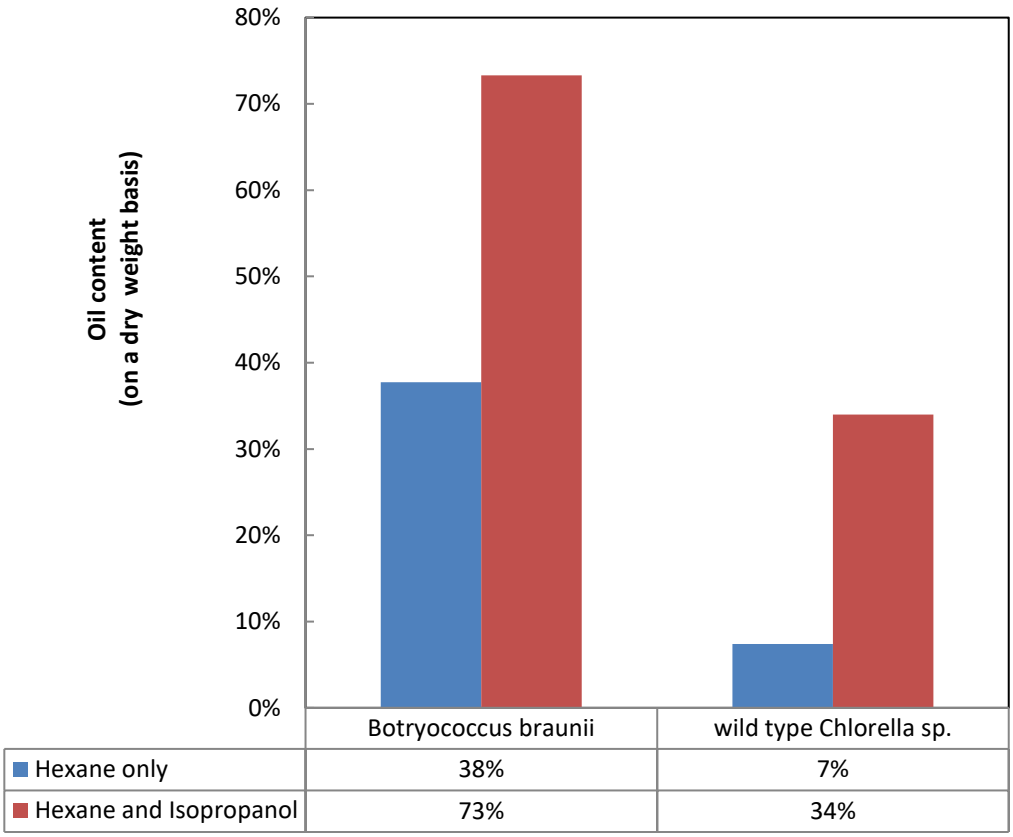
	1	2	3	4	5	6	7	8	9	10	11	12
Biomass concentration (mg/L)	250.	413.	980.	1500	1520	1540	1860	1960	2100	2100	2100	7225
Nitrates (mg/l)	135	59	24	8	13	20	23	9	8	7	8	0

Figure 7 Effect of limiting substrate depletion on biomass concentration – Dairy Industries inoculated with *B. braunii*



	1	2	3	4	5	6	7	8	9	10	11	12
Biomass concentration (g/L)	270.	153.	660.	1140	1500	1700	2120	2460	2480	2480	2520	6274
Nitrates (mg/l)	135	37	44	9	16	13	17	12	17	17	16	0

Figure 8 Effect of limiting substrate depletion on biomass concentration – Dairy Industries inoculated with wild type *Chlorella sp*.



	Botryococcus braunii	wild type Chlorella sp.
Hexane only	38%	7%
Hexane and Isopropanol	73%	34%

Figure 9 Comparison of bio-oil extraction methods used

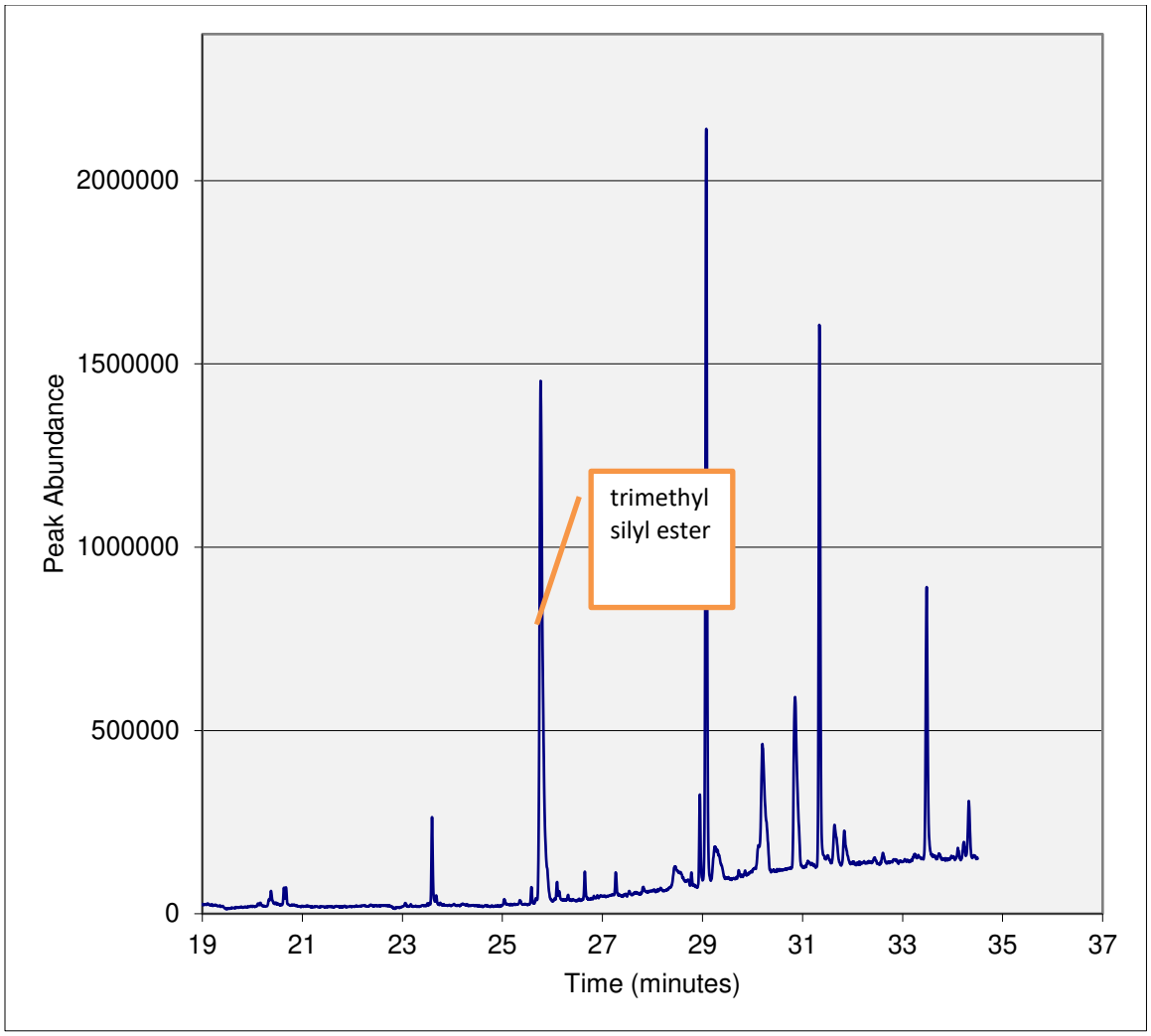


Figure 10 Recreated chromatogram of GCMS analysis of lipid extracts from wild type *Chlorella sp.*

Tables

Table 1 Initial parameters of wastewater

Parameter	Chicken Processing Plant Waste	Dairy Industries Waste
COD (mg/l)	3900	12900
BOD_5 (mg/l)	1060.50	1129.50
pH	7.96	6.15
Total Nitrogen (mg/l)	100	85
Nitrates (mg/l)	270	135
Phosphates (mg/l)	114	102

Table 2 The specific growth rate for the growth cultures

Cultures	X2 (mg/L)	X1 (mg/L)	μ (day^{-1})
Chicken processing plant - *B. braunii*	1520	250.83	0.1638
Chicken processing plant - Wild Type *Chlorella*	1300	270	0.1429
Dairy Industries - *B. braunii*	2100	250.83	0.1932
Dairy Industries - Wild Type *Chlorella*	2520	270	0.2030

Table 3 The maximum specific growth rate of each culture used in the research

Cultures	Maximum Specific growth rate (day^{-1})
Chicken processing plant - *B. braunii*	1.30
Chicken processing plant - Wild Type *Chlorella*	1.44
Dairy Industries - *B. braunii*	1.28
Dairy Industries - Wild Type *Chlorella*	1.54

Table 4 Biomass productivity of the cultures

Cultures	ΔX (g/L)	Δs (g/L)	$Y_{x/s}$ (g/g)
Chicken processing plant - *B. braunii*	1269.17	0.183	6935.36
Chicken processing plant - Wild Type Chlorella	1030.00	0.202	5099.01
Dairy Industries - *B. braunii*	1849.17	0.127	14560.39
Dairy Industries - Wild Type *Chlorella*	2250.00	0.119	18907.56

Table 5 Oil yields for different species inoculated in the waste from the chicken processing plant

	B braunii		**wild type *Chlorella sp*.**	
	1	2	1	2
Dried Biomass (g)	0.5049	0.2884	0.6670	0.1850
Oil yield (g)	0.0900	0.1000	0.2700	0.1000
Oil content (%)	17.83	34.67	40.48	54.05

Table 6 Oil yields for different species inoculated in the waste from Dairy Industries

	B braunii		**wild type *Chlorella sp.***	
	1	2	1	2
Dried Biomass (g)	0.6279	0.7974	0.5479	0.4884
Oil yield (g)	0.1700	0.3200	0.1100	0.1600
Oil content (%)	27.07	40.13	20.08	32.76

Continuous cultivation - Calculations

As stated above, using the Dairy Industries wastewater sample inoculated with the wild type Chlorella sp., a continuous system was designed. This addressed the second research question which was to design and build a continuous photo-bioreactor to allow for the efficient reduction of the parameters being investigated and rapid algal growth. The total volume of the reactor was **11.03 m^3** and its design and set up are illustrated in appendix B.

Table 20 below gives the changes in the limiting residual substrate when the flow rate is manipulated.

Table 7 Results obtained from reactor operated at steady state

Valve stem	Flow rate (L/day)	Dilution (day-1)	Residual Concentration (mg/L)	1/D (day)	1/Sr (L/mg)
Open	3.996	0.3624	45	2.759009	0.022222
2/3 Open	2.952	0.2678	31	3.734756	0.032258
1/3 Open	2.458	0.2229	24	4.485354	0.041667

Several parameters can be determined from a plot of the dilution rate against the concentration of the limiting residual substrate as illustrated on figure 16. The intercept gives the maximum specific growth rate (μ_{max}) and the gradient gives the ration of saturation constant to the maximum specific growth rate ($\frac{K_s}{\mu_{max}}$).

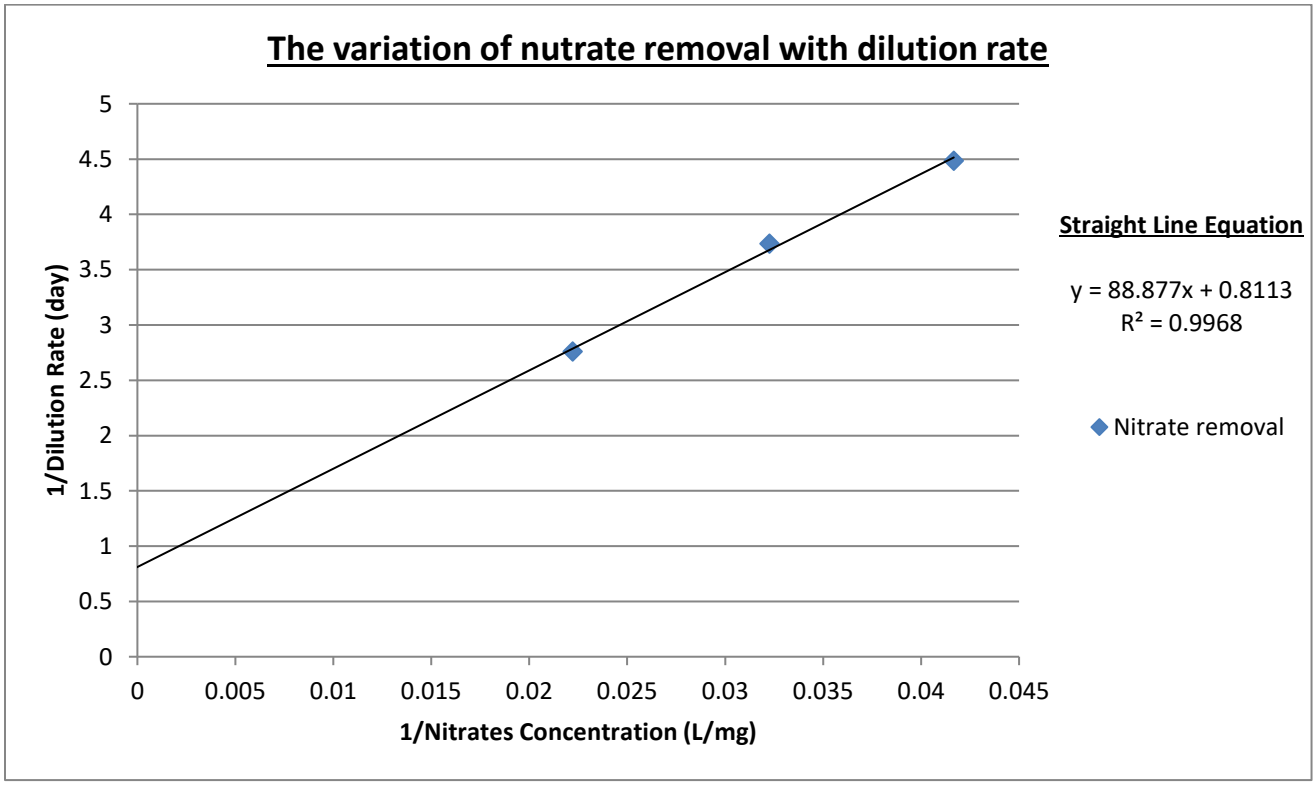


Figure 11 The variation of limiting substrate with dilution rate in a reactor operated at steady state

From 2.4.2 in the reactor design section, the following two equations would be integral for designing the reactor:

$$\rho(s) = \rho_m \frac{s}{s + K_s} \qquad (equation\ 1)$$

$$\mu = \frac{\mu_m}{1 - \frac{Q_o \mu_m}{\rho_m}} \qquad (equation\ 2)$$

Linearizing both equations results in the following:

$$\frac{1}{\rho_{(s)}} = \frac{K_s}{\rho_m}\left(\frac{1}{s}\right) + \frac{1}{\rho_m} \qquad (equation\ 3)$$

$$\frac{1}{\mu} = \frac{1 - Q_o \mu_m / \rho_m}{\mu_m} = \frac{1}{\mu_m} - \frac{Q_o}{\rho_m} \qquad (equation\ 4)$$

From equation 4, the K_s and ρ_m can be determined by plotting $1/\rho_{(s)}$ versus 1/s. The ρ_m could then be plugged into equation 3and the μ_m determined graphically. The problem with this method is that it is difficult to find a suitable determine the absorption rate. As a result, the equation will be simplified to the Monod equation, which is analogous to equation 1, where the absorption rate ($\rho_{(s)}$) will be approximated to the specific growth rate (μ). The design equation is therefore:

$$\mu = \mu_m \frac{s}{s + K_s} \qquad (equation\ 5)$$

Where μ is the specific growth rate; μ_m is the maximum specific growth rate; K_s is the saturation constant; s is the residual nitrate concentration.

Table 8 Dimension of photobioreactor

	Cm
Length	45.0
Width	24.5
Height	31.0

The volume of the reactor used was 11025 cm^3 (the dimensions are shown below); the maximum growth rate for the Dairy Industries wild type Chlorella species, according to the graphical method (the point where growth rate and biomass concentration intersect) was 1.27 day^{-1}. From this, the maximum flow rate can be determined:

$$\mu = D = \frac{F}{V}$$

Where, μ is the specific growth rate of the wild type Chlorella species; D is the dilution rate; F is the flow rate, and V is the volume of the reactor.

$$\therefore F = \mu \times V$$

$$F = 1.27\ day^{-1} \times 11025\ cm^3 = \frac{14001.75\ cm^3}{day} = y14.002\frac{L}{day}$$

14.002 L/day is the maximum allowable flow rate, beyond which washout of the microalgal cells will take place. As seen, in table 18, all the flowrate used were kept below this limiting amount.

From figure 33, the equation for the graph is:

$$y = 88.877x + 0.8113$$

According to equation 5, the following is true:

$$\frac{1}{\mu_{max}} = 0.8113$$

$$\therefore \ \mu_{max} \ = 1.2326 \ day^{-1}$$

$$\frac{K_s}{\mu_{max}} = \frac{K_s}{1.2326 \ day^{-1}} = 88.877 \frac{mg \cdot day}{L}$$

$$\therefore K_s = 109.5498 \ mg/L$$

Based on the results of this graph, the maximum specific growth rate is 1.2326 days, and the residual nitrate concentration constant is 109.5498 mg/L.