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The Utilization of Post-chlorinated Municipal Domestic Wastewater for Biomass and Lipid Production by *Chlorella* spp. Under Batch Conditions

Taurai Mutanda · Subburammu Karthikeyan · Faizal Bux

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Abstract South Africa has a rich microalgal biodiversity which has the potential to be used for renewable bio-fuel production in the region. Bioprospecting for oleaginous microalgae in KwaZulu Natal Province, South Africa, resulted in the establishment of a microalgal culture collection system for alternative energy research in the country. A potential hyper-lipid-producing *Chlorella* spp. strain was isolated, purified, and cultured in supplemented post-chlorinated wastewater for biomass and lipid production at the laboratory scale under batch mode. The microalgal strain was cultivated in different strengths of BG-11 media supplemented with wastewater from a local municipal domestic wastewater treatment plant. The *Chlorella* spp. was grown using ambient dissolved carbon dioxide in shake flasks under photosynthetically active radiation (±120 $\mu molm^{-2}s^{-1}$). Microalgal biomass and lipid productivity were monitored at 24-h intervals in the batch mode. The microalgal biomass was analyzed by direct light microscopy and indirectly by spectrophotometry at 600 nm, and the lipids were extracted and quantified. The growth rate of the *Chlorella* spp. was enhanced in post-chlorinated wastewater supplemented with 5 mM $NaNO_3$ with maximal biomass productivity. A dramatic increase in lipid yield was achieved with the post-chlorinated wastewater supplemented with 25 mM $NaNO_3$. Low dosages of free chlorine were found to enhance microalgal growth. These findings serve as a basis for further scale-up trials using municipal wastewater as a medium for microalgal biomass and lipid production.

T. Mutanda (✉) · S. Karthikeyan · F. Bux
Institute for Water and Wastewater Technology, Durban University of Technology, Steve Biko Campus, P.O. Box 1334, Durban 4001, South Africa
e-mail: taurai7@yahoo.com

F. Bux
e-mail: faizalb@dut.ac.za

S. Karthikeyan
Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

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Introduction

The dwindling crude petroleum reserve has caused an energy crisis concern that requires an impending solution [1, 7]. Current research to develop sustainable, cost-effective, and renewable bio-based fuels for the future has long been underway. However, to date, no feasible full-scale commercial process has been reported [4, 5]. One attractive alternative of producing biodiesel and other useful metabolites is the use of microalgae. A simple transesterification process of the microalgal lipids to biodiesel is economically feasible [9, 24, 35]. Biodiesel generated from microalgae is advantageous because of several reasons such as widespread availability of microalgae and higher neutral lipid yields [16, 21–23, 25, 27]. The feasibility of microalgal biomass production is advantageous as they do not require a large area of land for cultivation, they possess high growth rates, and they accumulate satisfactory amounts of lipid for biodiesel production [7, 14, 15, 35]. Microalgal lipids are mostly neutral lipids due to their lower degree of unsaturation and their accumulation in the microalgal cell at the early or late stage of growth depending on the microalgal strain [7]. This makes microalgal lipids a potential biodiesel fuel substitute as compared to oils derived from oil crops which can lead to food security and other socio-political concerns [4, 26]. The main drawback of using microalgae is that they form a relatively dilute suspension which makes biomass recovery and dewatering an expensive exercise [7, 10].

Various strategies are used to increase microalgal biomass yields. Media supplemented with varying concentrations of urea as a nitrogen source were investigated and this resulted in an increase in biomass and lipid content of *Chlorella* spp. [13]. In a separate study, some co-workers [22] supplemented $NaNO_3$ to the soil extract medium for the growth of the green alga *Neochloris oleobundans* and found that it enhanced biomass and lipid accumulation. The effect of chlorinated compounds on microalgal growth has also been reported [28]. These researchers established that *Chlorella* VT-1 showed some tolerance to all the chlorophenols tested at a concentration of 10 mg/L except for 2,4,5-trichlorophenol, which was toxic at all concentrations investigated. The use of post-chlorinated wastewater for microalgal growth has not been fully investigated although some reports indicate that chloride ions act as microalgal micronutrients [8]. Previous reports focused on the use of chlorinated organic compounds in wastewater as a pollution monitoring strategy. A study was carried out to investigate the effects of chlorine-containing organic compounds formed from the chlorination of domestic wastewater on phytoplankton growth [31]. In an effort to prevent nutrient depletion and enhance biomass yield, Costa et al. [6] supplemented nutrients (carbon as sodium bicarbonate, nitrogen as urea, phosphate, sulfate, ferric iron, magnesium, and potassium) into the raceway pond. The feasibility of using municipal domestic wastewater as feed for microalgal cultivation for biomass production has been reported and shows great potential as a cheap and cost-effective medium [18, 34].

Raceway pond technology is a cost-effective method for growing microalgae for biomass and lipid production and biomass productivities of 0.5 g/L can be achieved in some commercial raceway ponds [11, 36]. The operation of this outdoor culture open system is easy and requires minimal maintenance [2, 21]. The system has few operating costs, minimal power consumption, and little overheads as compared to photobioreactors [29]. The advantage of this method is that readily available wastewater can be used as

media for cultivation with the added benefit of bioremediation. In addition, if the system is located near a power plant, cheaply available flue gas can be used to speed up the photosynthetic rates in the pond or pure carbon dioxide can be bubbled into the pond [15].

In order to avoid contamination from debris and rainfall, the raceway pond can be covered similar to the agricultural greenhouse concept. This allows maximum sunlight intensity. The depth of water in the pond should not exceed a maximum level of 30 cm to allow sufficient light penetration. Harvesting and dewatering of the microalgae can be performed by a microfiltration system, settling after adding flocculants, or centrifugation which is however power intensive and therefore expensive at a large scale [7, 10]. After harvesting, drying of biomass can be achieved by cheaply using natural sunlight on drying beds. In order to access the physiological status of the microalgal cells, the following parameters must be closely monitored in the raceway pond: cell density, conductivity, pH, light intensity, temperature, salinity, evaporation rates, dissolved carbon dioxide, dissolved oxygen, TDS, ORP, and nitrate and phosphate levels [27].

South Africa is endowed with attractive climate for growing microalgae and has a huge untapped microalgal biodiversity which can be used as a feedstock for biomass and lipid production. The lack of precursor oil feedstocks limits the large-scale development and exploitation of biodiesel use globally [21, 22]. The aim of the current research was to evaluate the use of post-chlorinated municipal domestic wastewater for the growth of a hyper-lipid-producing *Chlorella* spp. strain for biomass and lipid production. This research work is a preliminary study for the feasibility of using supplemented post-chlorinated wastewater for growing microalgae at a commercial scale using raceway ponds for biodiesel production in South Africa.

Materials and Methods

Materials and Reagents

All reagents and solvents were purchased from Sigma-Aldrich (South Africa) and Merck (South Africa) and were of analytical grade unless otherwise stated.

Microalgal Strain and Growth Conditions

Bioprospecting for microalgae was undertaken in the Kwa-Zulu Natal Province (South Africa) freshwater systems. Sampling was conducted over a period to determine the dominant microalgal populations. A temporal and spatial collection strategy was employed and the highest lipid producer was screened and selected for further research [27]. The microalgal cultures were grown and maintained on BG-11 medium [32] which contained (g/L): $NaNO_3$, 1.5; $K_2HPO_4{\cdot}3H_2O$, 0.04; $MgSO_4{\cdot}7H_2O$, 0.075; $CaCl_2{\cdot}2H_2O$, 0.036; $C_6H_8O_7$, 0.006; ammonium ferric citrate, 0.006; EDTA (disodium magnesium), 0.001; Na_2CO_3, 0.02, and a trace metal mix (1 ml). The trace metal mix contained the following (g/L): H_3BO_3, 2.86; $MnCl_2{\cdot}4H_2O$, 1.81; $ZnSO_4{\cdot}7H_2O$, 0.22; $Na_2MoO_4{\cdot}2H_2O$, 0.390; $CuSO_4{\cdot}5H_2O$, 0.079, and $Co(NO_3)_2{\cdot}6H_2O$, 0.049. An aliquot of tetracycline (0.5 μl/ml) was added to the growth medium to prevent any bacterial contamination in the microalgal samples. The pH was adjusted to 7.4 and trace metals were added separately after autoclaving. The BG-11 medium was used for the entire screening exercise. The culture flasks were incubated in a growth chamber with gentle shaking of 50 rpm under an 8:12 h light-to-dark photoperiod regime using plant grow lamps at 25±1 °C under ambient CO_2

concentration. In all experiments contacted, light intensity was kept constant at ±120 μmolm^{-2}s^{-1} using the 18 W Syvania Gro-Lux lamps (Germany).

Screening and Purification of *Chlorella* spp.

Microalgal samples were obtained from maturation ponds from various wastewater treatment plants in KwaZulu Natal, South Africa. The microalgal cultures were screened and isolated using standard microbiological methods for culturing microalgae as reported [12]. A micromanipulator (Nikon Eclipse 80i coupled to an imaging-Nikon Digital-Sight DS-01, South Africa) was also employed to purify the microalgal cultures. The microalgae were presumptively identified microscopically by their morphological characteristics with keys according to published literature [17]. The Nile red method [20] was used to qualitatively screen for potential hyper-lipid-producing microalgae as described in "Lipid Screening by the Nile Red Technique". Based on preliminary screening, a purified *Chlorella* spp. was observed as the highest biomass and lipid producer and was selected for further research.

Wastewater Supplementation for Microalgal Growth

Wastewater (pre- and post-chlorinated) collected from the Kingsburgh Wastewater Treatment Plant in Durban, South Africa, was evaluated as a potential medium for microalgal growth. Initial pH and free chlorine, nitrate, and phosphate concentrations were measured before using the water as described in "Determination of Free Chlorine, Nitrates, and Phosphates". The response was monitored by determining growth kinetics, biomass production, and lipid content of the microalgal cultures under laboratory conditions. In order to establish the optimal media formulation for microalgal growth, the post-chlorinated wastewater was diluted in various ratios to the artificial media (BG-11) (0–100%). The media were sterilized by autoclaving. *Chlorella* spp. was cultivated in the batch mode under ambient CO_2 levels and temperature using plant grow lamps. The microalgal suspension at 10% inoculum concentration at a cell load of 1.2×10^7 cells/ml was added to 1 L working volume of the growth medium in a 3-L capacity conical flask. Growth was monitored for 8 days and aliquots were retrieved daily. Absorbance was measured spectrophotometrically at 600 nm (Spectroquant® Pharo 300, Merck, South Africa). The samples were analyzed microscopically and any absorbance increase due to contamination by other microflora in the broth was ruled out since it was an axenic culture and no contamination was observed. Biomass and lipid yields were determined at the end of the 8-day growth period following the standard protocols as reported in "Determination of Dry Weight and Cell Density" and "Lipid Extraction", respectively. Growth rate (h^{-1}), division per day, and generation time of the *Chlorella* sp. was calculated as described [34, 37].

Sodium Nitrate Supplementation for Microalgal Growth

The post-chlorinated wastewater was supplemented with sodium nitrate in the range of 0–40 mM. The *Chlorella* sp. (1.2×10^7 cells/ml, 100 ml) was added to 1 L working volume of the supplemented post-chlorinated wastewater in a 3-L conical flask and 5 ml sample aliquots were retrieved daily for biomass and lipid analysis. The *Chlorella* spp. was cultured as previously described under the same conditions in "Wastewater Supplementation for Microalgal Growth" and growth was monitored for 8 days. At the end of the growth period, biomass was harvested and lipids were extracted as described in "Determination of Dry Weight and Cell Density" and "Lipid Extraction", respectively.

Determination of Dry Weight and Cell Density

The biomass was harvested from the culture broth by centrifugation. An aliquot (5 ml) was centrifuged at 4,000 RPM (20 min, 4 °C) and the microalgal biomass was placed in a pre-weighed watch glass. The biomass was dried in an oven at 60 °C for 12 h. The watch glass with the biomass was weighed and the net mass of the microalgal cells was determined by subtracting the final weight from the weight of the watch glass. The microalgal cell density was quantified microscopically by using a counting chamber (haemocytometer) with Neubauer rulings as instructed by the manufacturer.

Lipid Extraction

The dry extraction procedure according to [35] was used to extract the lipid in microalgal cells with minor modifications. The biomass was harvested by centrifugation as described in "Determination of Dry Weight and Cell Density". The dried samples were pulverized in a mortar and extracted using a mixture of chloroform–methanol (2:1, *v*/*v*). A volume of 50 mL of solvent was used for every 1 g of dried sample in each extraction step. The sample was stirred using a magnetic stirrer bar for 6 h and the samples were centrifuged at 3,000 rpm for 10 min. The solid phase was separated carefully using filter paper (Whatman filter paper, no. 1) in which two pieces of filter paper were applied twice to provide complete separation. The solvent phase was evaporated in a rotary evaporator under vacuum at 60 °C. The procedure was repeated three times until the entire lipid phase was extracted.

Determination of Free Chlorine, Nitrates, and Phosphates

Total free chlorine was determined using the pocket colorimeter (Hach) and the *N*, *N*-diethyl-*p*-phenylenediamine-free chlorine reagent as recommended by the manufacturer. Total nitrates and phosphates in the wastewater were determined by the Spectroquant test kits (Merck) as recommended by the manufacturer.

Lipid Screening by the Nile Red Technique

The Nile red technique was followed as per protocol [20] with minor modifications. The Nile red solution (40 μl) was prepared in acetone (250 mg/L) and 0.2 μl of this preparation was added to 10 ml of algal suspension. The mixture was agitated thoroughly on a vortex mixer and placed in the dark for 10 min. Qualitative determination was achieved by using an epi-fluorescent microscope (Zeiss, South Africa) with a narrow band excitation filter of 490-nm narrow band excitation filter and a narrow band emission filter of 585 nm at 5-nm slit. The relative fluorescence intensity of Nile red was obtained after eliminating both the autofluorescence of algal cells and the fluorescence intensity of Nile red in the medium [20]. For the screening of lipid-producing microalgal cells, those that stained yellow indicated lipid globules in the cells and were accepted as lipid producers.

Validation of Experiments

To validate the observed enhanced growth rates, biomass and lipid production in post-chlorinated (0.2 mg/L [free chlorine]) wastewater was serially diluted with deionised water from 0.2 to 0.04 mg/L free chlorine dosages. The *Chlorella* spp. was grown as previously described in "Microalgal Strain and Growth Conditions". Growth rates, biomass, and lipid

yields were determined as previously described in "Determination of Dry Weight and Cell Density" and "Lipid Extraction", respectively. The toxicity concentration of free chlorine was determined by adding sodium hypochlorite solution to the post-chlorinated wastewater in the range of 0.2–1 mg/L NaOCl. *Chlorella* sp. was cultivated as previously described in "Microalgal Strain and Growth Conditions" and growth rates, biomass, and lipid production were measured.

Statistical Analysis

In order to arrive at a valid conclusion, the biomass and lipid yield data obtained using neat post-chlorinated wastewater and post-chlorinated wastewater supplemented with $NaNO_3$ were subjected to a one-way ANOVA to establish if there was any significant difference. The statistical analysis was performed using the SPSS software and all descriptive statistics were analyzed using Microsoft Excel software package.

Results and Discussion

Bioprospecting for Microalgae

Bioprospecting for a variety of microalgae resulted in the collection of microalgal strains which were identified, purified, and stored for later use. Seventy-eight microalgal samples were collected in aquatic environments in Kwa-Zulu Natal Province, South Africa, and out of these 26 were positively identified and characterized for lipid production. It is important to determine parameters such as pH, nitrates, phosphates, and temperature of the water sampled to maintain microalgal strains under same laboratory conditions. In addition, the spatial and temporal microalgal bioprospecting strategy is crucial in order to establish any microalgal succession that may occur in the aquatic environment. The microalgal strain collected from Kingsburgh Wastewater Treatment Plant maturation ponds produced the highest lipid content (18% *w*/*w*) and was identified as *Chlorella* sp. On the basis of lipid content, this microalgal strain was selected for further work.

Feasibility of Wastewater as a Medium for Microalgal Growth

The initial pH, free chlorine, NO_3, and PO_4 concentrations in the post-chlorinated wastewater were found to be 6.79, 0.2, 1.5, and 1.4 mg/L, respectively. The ratio of nitrates to phosphates in the post-chlorinated wastewater was 1:0.93. The Nile red method clearly showed that the *Chlorella* spp. is a lipid-producing strain (Fig. 1). The growth kinetics, biomass, and lipid yields of *Chlorella* spp. were determined and the results are shown in Table 1 and Fig. 2. Experiments with pre-chlorinated wastewater proved ineffective to support microalgal growth. The lack of microalgal proliferation in pre-chlorinated wastewater could be attributed to the presence of protozoans and rotifers which are known and documented as microalgal predators [6, 26].

Current findings support previous research conducted and it was reported that chlorine is a micronutrient element and therefore enhances the growth of *Chlorella* spp. [8]. In addition, sodium ions are also reported to increase microalgal growth as reported [3]. The effect of free chlorine on *Chlorella* spp. cells has not been elucidated. Hence, further research is necessary to explain the interaction of free chlorine influenced by abiotic factors such as temperature, pH, salinity, and dissolved carbon dioxide concentrations on microalgal cells [4].

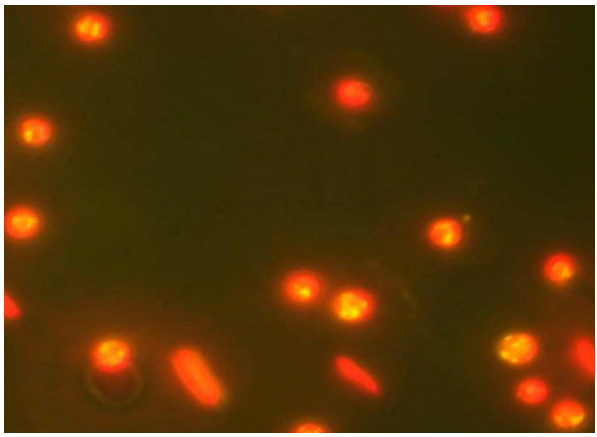

Fig. 1 *Chlorella* spp. cultured in post-chlorinated wastewater for 8 days using ambient CO_2 and fluorescent light. Nile-red-stained *Chlorella* spp. viewed at −1,000 using the fluorescence microscope at 490-nm excitation and 585-nm emission filters

The growth kinetics of the *Chlorella* spp. using modified media from the preliminary investigations indicate that there is no significant difference between the growth rates of microalgae grown in pure BG-11 medium and that grown in unaltered post-chlorinated wastewater (ANOVA, $p<0.05$) (Fig. 2). This demonstrates that wastewater can be used as a growth substrate with some additional nutrient supplementation to support microalgal growth. The growth rate in 100% BG-11 medium was 0.009 h^{-1} as compared to 0.006 h^{-1} in post-chlorinated wastewater. The generation time of the *Chlorella* spp. in 100% BG-11 medium was 76.92 h and 111.11 h in post-chlorinated wastewater (Table 1). The longer generation time in post-chlorinated wastewater can be attributed to the prolonged lag phase which can be explained by the adaptive ability of the *Chlorella* spp. in most conditions.

Biomass and lipid production was quantified and the statistical analysis indicated that there was no significant difference (ANOVA, $p<0.05$) between the two media types, i.e., post-chlorinated wastewater and pure BG-11 medium. The biomass and lipid yields were 69.9 and 7.6 mg/L, respectively, for the unaltered post-chlorinated wastewater. However, the biomass and lipid production were much higher in the BG-11 medium, i.e., 116.3 and 12 mg/L, respectively (Fig. 3). The experimental trials indicated that post-chlorinated wastewater is a potentially effective medium for microalgal growth and therefore can be used for biomass and lipid production by *Chlorella* spp.

The relatively low biomass and lipid yields obtained in the post-chlorinated wastewater are explained presumably by the absence of growth factors in the post-chlorinated wastewater medium. However, for full-scale application, it is recommended to add trace metals and vitamins, which are required for the optimal growth of microalgae, to the

Table 1 Growth kinetics of *Chlorella* spp. in BG-11 medium supplemented with post-chlorinated municipal domestic wastewater. The reported values are means of duplicate experiments with standard deviation values

Medium (%)	Growth rate (h^{-1})	Division/day	Generation time (h)
100	0.009±0.002	0.013±0.003	76.92±5.23
80	0.008±0.001	0.011±0.002	90.9±8.21
60	0.012±0.004	0.018±0.005	55.55±7.46
40	0.015±0.007	0.022±0.008	45.45±6.24
20	0.009±0.003	0.012±0.003	83.33±6.99
0	0.006±0.001	0.009±0.002	111.11±8.47

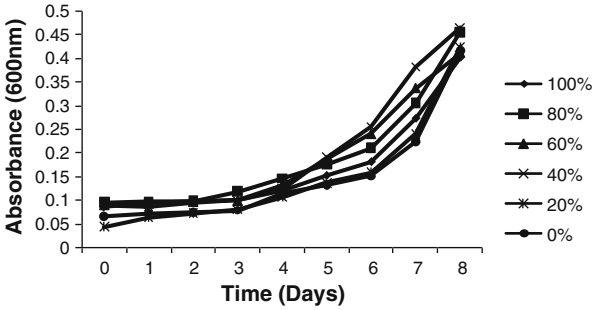

Fig. 2 Microalgal growth in BG-11 medium supplemented with post-chlorinated municipal domestic wastewater. The BG-11 strength was diluted with wastewater from 100% pure BG-11 medium to 0% neat post-chlorinated wastewater. The *data points* are means of duplicate experiments

wastewater. The addition of these trace metals and vitamins to post-chlorinated wastewater will still provide a cheaper support medium for microalgae than BG-11 medium in a large-scale raceway pond. The microalgal cells were observed to aggregate and also to settle at the bottom of the growth flasks and this is mainly caused by insufficient mixing and stirring. This will not be a problem at using the large-scale raceway pond since a paddle wheel will be installed to provide adequate mixing and maintain a constant flow rate in the pond. To establish the necessity of nutrient supplementation for microalgal growth, varying concentrations of sodium nitrate were added to the wastewater, and microalgal growth, biomass, and lipid production were monitored as previously reported in a similar study [21, 22].

Sodium Nitrate Supplementation Trials

The *Chlorella* spp. strain was grown in post-chlorinated wastewater supplemented with sodium nitrate to establish if this compound enhances growth, biomass production, and lipid yields. The highest growth rates were observed in the culture medium supplemented with 5 mM sodium nitrate (Fig. 4) and this is in agreement with the findings of [22]. This concentration of $NaNO_3$ was found to be optimal for microalgal growth. However, with prolonged incubation, there was increased growth in the culture medium supplemented with 25, 30, and 40 mM $NaNO_3$. This indicates that the initial high concentrations of $NaNO_3$ (25–40 mM) might be retarding microalgal growth from day 0–3 possibly due to the increased total dissolved solids and salinity in the medium.

The growth rates observed in the supplemented post-chlorinated wastewater (5–40 mM) are higher than the growth rate of the *Chlorella* cultured in unaltered post-chlorinated wastewater (0 mM) (Fig. 4). The high growth rates observed with $NaNO_3$ supplementation

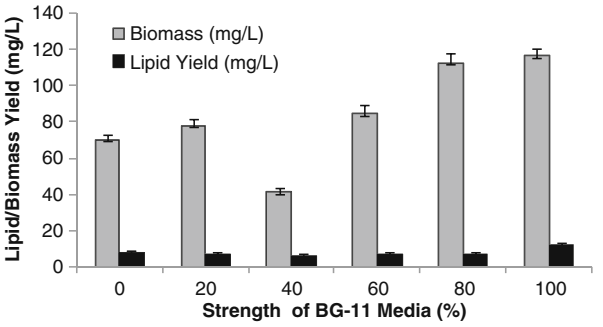

Fig. 3 Biomass and lipid production in supplemented wastewater. The microalgae were grown in varying strengths of BG-11 diluted with post-chlorinated wastewater. The values reported are means of duplicate experiments

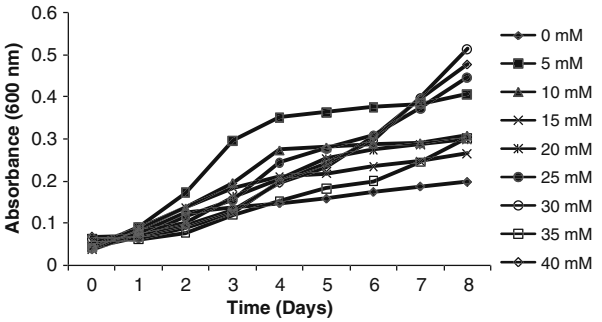

Fig. 4 *Chlorella* spp. growth in post-chlorinated wastewater supplemented with sodium nitrate. The values reported are means of duplicate experiments

can be attributed to the presence of sodium ions which are essential cofactors in the microalgal metabolic pathway. Nitrates are also an essential source of nitrogen which is an important component of proteins and enzymes and hence increases microalgal biomass [22]. This indicates that it will be necessary to supplement the culture medium at a large-scale raceway pond with 5 mM $NaNO_3$ for initial optimal microalgal growth.

Biomass and lipid production was evaluated in the microalgal cultures supplemented with sodium nitrate. The highest biomass production (161.4 mg/L) was obtained at 5 mM sodium nitrate and the lowest biomass production (80.9 mg/L) was achieved in the post-chlorinated wastewater with no added sodium nitrate (Fig. 5). The highest lipid yield (45.8 mg/L) was obtained at 25 mM sodium nitrate and the lowest lipid yield (12.9 mg/L) was achieved in the post-chlorinated wastewater with no added sodium nitrate. At 5 mM sodium nitrate supplementation, the lipid yield was found to be 15.9 mg/L.

To achieve high-quality neutral lipids from microalgae, it is vital to induce the microalgae to produce large amounts of neutral lipids. Documented research evidence indicates that microalgal induction for high lipid production can be done through careful nitrogen deprivation in the nutrient medium [30]. This calls for a fundamental tradeoff between biomass accumulation and lipid production, and some microalgal strains attained 60% lipid content after nitrogen starvation [30].

From the data obtained (Fig. 5), the main trends observed are the increase in biomass accumulation at low nitrate levels and a tendency of lipid accumulation in the microalgal cells at a higher $NaNO_3$ concentration. This is in contrast to the observed phenomenon of nutrient starvation whereby microalgal cells tend to accumulate lipids under nutrient-limiting conditions [22]. The observed trend (Fig. 5) could be explained presumably by the presence of free chlorine in the growth medium which enhances lipid production in the

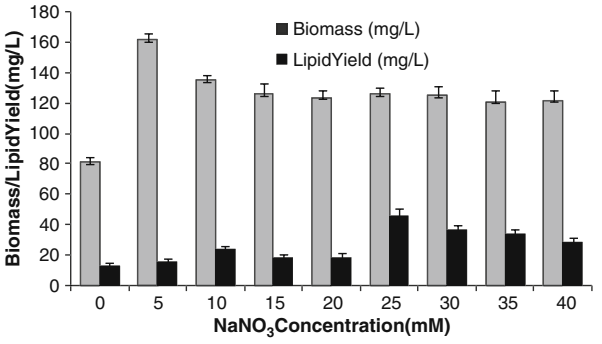

Fig. 5 Lipid and biomass yield of *Chlorella* spp. in post-chlorinated wastewater supplemented with different concentrations of $NaNO_3$ (0–40 mM). The values reported are means of duplicate experiments

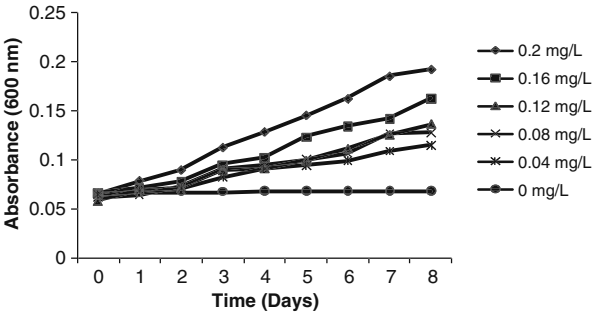

Fig. 6 Effect of chlorine dosage on the growth of *Chlorella* spp. under batch conditions. The initial chlorine dosage (0.2 mg/L) in post-chlorinated wastewater was serially diluted to 0.04 mg/L and chlorella growth was monitored spectrophotometrically at 600 nm. The reported values are means of a duplicate experiment

Chlorella spp. under elevated sodium nitrate concentrations. In the current study, the main limitation to improved biomass and lipid accumulation could be attributed to relatively low ambient carbon dioxide concentration (since no external carbon dioxide source was provided) and lower ambient temperatures. Our findings support previous research where it was shown that biomass production increased at 30°C and elevated CO_2 concentration (6%) [4]. However, at a large scale, this will not be a problem since CO_2 will be bubbled when needed in order to control pH and subsequently improve biomass yields.

Effect of Initial Chlorine Dosage on *Chlorella* spp. Growth

The effects of initial free chlorine concentration on the growth, biomass, and lipid production of *Chlorella* spp. were evaluated. There was no growth of *Chlorella* spp. in pure deionised water (0 mg/L chlorine) and the highest growth rate was achieved at 0.2 mg/L free chlorine dosage (Fig. 6).

Growth rates increased with increase in bioavailability of free chlorine concentration in the post-chlorinated wastewater. The highest biomass yield was 69.8 mg/L at 0.2 mg/L chlorine dosage (Fig. 7). This finding corroborates the finding of [19] who observed that 0.2 mg/L of free chlorine keeps the population of algae constant but does not reduce their numbers appreciably. There was a gradual increase in biomass production with increase in chlorine dosage. Highest lipid yield (13.9 mg/L) was achieved at 0.2 mg/L chlorine dosage and there was negligible lipid production in pure water with no added chlorine (Fig. 7). There was a gradual increase in lipid yield with an increase in chlorine dosage in the substrate. These findings clearly validate the phenomenon that chlorine dosage has a positive effect on microalgal growth [33].

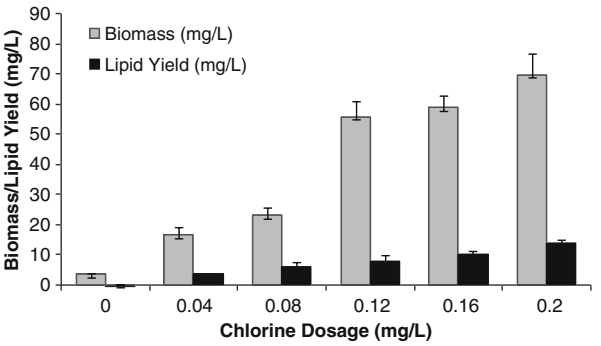

Fig. 7 Effect of free chlorine dosage on biomass and lipid production by *Chlorella* spp. The reported values are means of a duplicate experiment

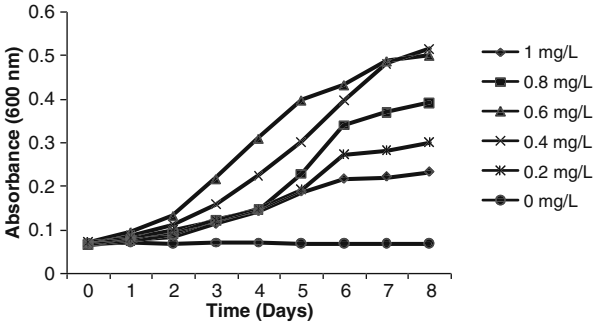

Fig. 8 Effect of free chlorine dosage on microalgal growth in post-chlorinated wastewater. The reported values are means of a duplicate determination

In a separate study, the concentration of hypochlorite in the growth medium was increased from 0.2 to 1 mg/L. The data generated indicate that 0.4 mg/L is the threshold hypochlorite dosage supporting microalgal growth (Fig. 8). This is in agreement with the findings of [19] whereby the chlorine dosage above 0.42 mg/L was algicidal. Above this hypochlorite concentration, the microalgal cells succumbed to the detrimental effects of hypochlorite solution in water and this is in agreement with work done by [26]. There was no microalgal growth observed at 0 mg/L hypochlorite dosage due to possibly the nonavailability of nutrients such as nitrates and phosphates which are commonly found in post-chlorinated wastewater.

Biomass and lipid yields were determined in this aspect of the study. The highest biomass (93.8 mg/L) was achieved in the culture flask with 0.4 mg/L hypochlorite concentration and there was a gradual decrease in biomass with increase in hypochlorite dosage (Fig. 9). At 1 mg/L hypochlorite dosage, a net biomass yield of 30.4 mg/L was achieved. This suggests that increased hypochlorite dosages above the threshold value negatively impact on biomass accumulation in the culture. The highest lipid yield (21.4 mg/L) was achieved at 0.6 mg/L hypochlorite dosage and 9.8 mg/L of lipid yield was achieved at 1 mg/L hypochlorite dosage (Fig. 9).

There was a gradual decrease in lipid yield with increase in hypochlorite dosage in the post-chlorinated wastewater. These findings indicate and validate the phenomenon that the bioavailability of free chlorine in the post-chlorinated wastewater triggers and enhances microalgal growth, biomass, and lipid accumulation at a threshold concentration as indicated in Figs. 8 and 9. The mechanism of the interaction of free chlorine and microalgal cells is not known at this stage. In addition, the species of chlorine residuals in the wastewater were not differentiated and therefore not known. The latter aspects will be the focus of future research.

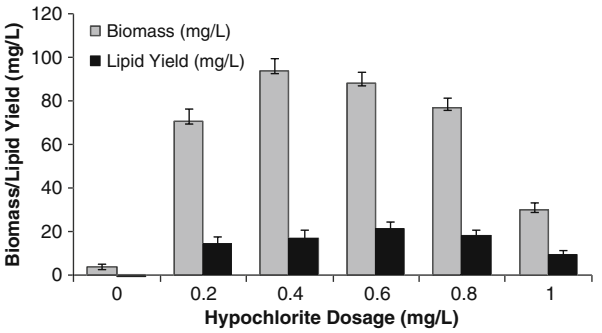

Fig. 9 Effect of hypochlorite dosage on microalgal biomass accumulation and lipid yield in post-chlorinated wastewater. The reported values are means of a duplicate determination

Conclusions

Biomass and lipid yields were comparatively low in these set of experiments. However, it should be noted that the emphasis was not to optimize yields but rather to evaluate post-chlorinated wastewater as a feed source for microalgal propagation. The study proved that it is feasible to use post-chlorinated wastewater for microalgal growth for biomass and lipid production. Furthermore, supplementing the post-chlorinated wastewater with sodium nitrate further enhanced biomass and lipid productivity, which requires further investigation. The lipids extracted from the microalgal biomass could be used for biodiesel production. It was demonstrated that hypochlorite solution enhances microalgal growth at a threshold value of 0.4 mg/L. This study focused on the use of cheaply and readily available post-chlorinated wastewater for biotechnological applications in South Africa. It is important to investigate the mechanisms of action of this halogen on *Chlorella* spp.

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