Application of Chlorella vulgaris for nutrient removal from synthetic wastewater and MBR-treated bio-park secondary effluent: growth kinetics, effects of carbon and phosphate concentrations

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Abstract

Application of *Chlorella vulgaris* for polishing secondary effluent of a wastewater treatment (containing C, N and P) was investigated. As a first step, batch experiments were conducted in Bold's Basal Media (BBM) to quantify the effects of orthophosphates (0.1–107 mg/L), organic carbon (0–500 mg/L as acetate) and N/P ratio on the growth of *Chlorella vulgaris.* The results revealed that the orthophosphate concentration was found to control the removal rates of nitrates and phosphates; however, both were effectively removed (> 90%) when the initial orthophosphate concentration was 4–12 mg/L. The maximum nitrate and orthophosphate removals were observed at an N:P ratio of ~ 11. However, the specific growth rate (μ) was significantly increased (from 0.226 to 0.336 g/g/day) when the initial orthophosphate concentration was 0.1–4.3 mg/L. On the other hand, the presence of acetate had significantly improved the specific growth and specific nitrate removal rates of *Chlorella vulgaris*. The specific growth rate increased from 0.34 g/g/day in a purely autotrophic culture to 0.70 g/g/day in the presence of acetate. Subsequently, the *Chlorella vulgaris* (grown in BBM) was acclimated and grown in the membrane bioreactor (MBR)–treated real-time secondary effluent. Under the optimised conditions, 92% nitrate and 98% phosphate removals (with a growth rate of 0.192 g/g/day) were observed in the bio-park MBR effluent. Overall, the results indicate that coupling *Chlorella vulgaris* as a polishing treatment in existing wastewater treatment units could be beneficial for highest level of water reuse and energy recovery goals.

Introduction

Around 77% of the domestic sewage in cities and 95% in towns are left untreated in India. About 60% of the domestic wastewater was directly discharged into the nearby water bodies as per studies conducted by the Central Pollution Control Board (CPCB) of India (Kamble et al., 2019). Moreover, population growth and improvement in living standards have led to a rise in wastewater generation. In India, sewage treatment plants (STPs) use conventional treatment

processes predominantly (e.g. activated sludge process, wastewater stabilisation ponds, up-flow anaerobic sludge blanket reactors) (Sahasranaman & Ganguly, 2018). Therefore, the STPs could not meet the standards for effluent discharge or recycling (Moore, 1989). The release of partially treated or untreated nutrient-rich effluent in water bodies causes adverse environmental impacts and health problems. The biological processes generally used for nutrient removal in modern domestic WWTPs are called biological nutrient removal (BNR), with a combination of nitrification, denitrification and biological phosphorus removal. BNR systems require adding external carbon sources to remove nitrates by denitrification completely. Moreover, a separate anaerobic tank is necessary to enrich phosphate-accumulating bacteria to remove phosphates. Physicochemical methods involving the use of chemicals result in high operational costs and the formation of byproducts like chemical sludge, thereby limiting their application (Gao et al., 2016). After the conventional biological treatment process, the secondary effluent is usually transparent and rich in nutrients, making it a good growth medium for algae (Gao et al., 2016). Therefore, secondary effluent becomes a source of excessive nutrients resulting in problems like eutrophication (Beltrán-Rocha et al., 2017). Microalgal cultivation is a potential alternative to conventional biological and physicochemical methods to remove nutrients and residual biodegradable organic matter from the secondary effluent. Algae can utilise dissolved inorganic carbon and nutrients from the wastewater in the presence of visible light through photosynthesis. Photosynthesis releases oxygen into the effluent, thereby oxygenating it in the process. Moreover, algae do not require additional organic carbon for nutrient removal, unlike other BNR processes (Aslan & Kapdan, 2006).

Microalgae are photoautotrophs in which some of the species can survive in mixotrophic conditions. Microalgae need copious micro- and macronutrients for growth, making them ideal for removing nutrients in tertiary treatment (McGriff & McKinney, 1972). Mixotrophic microalgae can switch their metabolism between autotrophic and heterotrophic conditions depending on the availability of organic carbon and nutrients. This helps algae to thrive in harsh and extreme environments. Microalgae systems effectively remove pollutants by various mechanisms like metabolism, biosorption, bioaccumulation and stripping. Compared to conventional aerobic treatment systems, mechanical aeration may not be necessary for microalgal-dominated consortia (Kim et al., 2014). Microalgae also help in carbon dioxide sequestration and result in better nutrient removal. Unlike the traditional activated sludge and chemical sludge, microalgal biomass is considered a valuable raw material (Villaseñor Camacho et al., 2018). The various products from microalgal biomass include energy sources like biogas (Arias et al., 2018), biodiesel (Beuckels et al., 2015; Xin et al., 2010), biohydrogen, animal feed and value-added bioproducts like vitamins, fatty acids, antioxidants, proteins, lipids and nutrients (Chew et al., 2017; Wang et al., 2010a; Woertz et al., 2009; Yen et al., 2013). Thus, algae-based treatments are considered environmentally friendly and cost-efficient, which can be a sustainable treatment option for secondary effluent compared to conventional methods. So far, the potential of several microalga strains has been studied for tertiary treatment of domestic secondary effluent, including *Chlorella* sp. (Arias et al., 2018; Sheng et al., 2017), *Scenedesmus* sp. (Mohamed et al., 2018), *Oscillatoria* sp. (Hashimoto & Furukawa, 1989; Villaseñor Camacho et al., 2018) and *Euglena* sp. (Gao et al., 2016). Some widely used

strains for nutrient removal are *Chlorella* sp., *Scenedesmus* sp. and *Selenastrum gracile* (Lee et al., 2016).

Being a commonly used species for nutrient removal, *Chlorella vulgaris* was chosen as the model organism for the present study, grown in synthetic media and real-time membrane bioreactor (MBR) effluent. Membrane bioreactors are effective in the removal of COD, NH_3-N and NO_2-N. However, incomplete denitrification results in effluent rich in NO_3-N. Moreover, MBR is not effective in the removal of PO_4-P either. The discharge standards state that PO_4-P must be under 1 mg/L (~ 4 mg/L as PO_4^-), and total nitrogen should be less than 5 mg/L (~ 22 mg/L as NO_3^-). Usually, aerobic MBR effluents have very high orthophosphates and nitrates in the range of 10–30 mg/L and 100–150 mg/L, respectively (Chamberlin et al., 2018; Li et al., 2011; Mutamim et al., 2013; Wang et al., 2010b). The objective of this study is to (a) evaluate the effects of initial concentrations of PO_4-P and organic carbon on the removal of NO_3-N and PO_4-P by *Chlorella vulgaris*, (b) apply *Chlorella vulgaris* for the removal of NO_3-N and PO_4-P from MBR effluent and (c) estimate the growth and substrate utilisation kinetics of *Chlorella vulgaris* in both BBM and MBR effluent.

Materials and methods

Microalgae

Chlorella vulgaris was obtained from Phytochemical Lab, Chennai, Tamil Nadu. The culture was then repeatedly sub-cultured in Bold's Basal Media (BBM) to maintain a stock culture in the exponential growth phase. The composition of BBM is as follows: NO_3^- 2.94 mM (given as $NaNO_3$), $CaCl_2.2H_2O$ 0.17 mM, $MgSO_4.7H_2O$ 0.3 mM, K_2HPO_4 0.43 mM, KH_2PO_4 1.29 mM, NaCl 0.43 mM, alkaline EDTA solution: EDTA 17.10 mM, KOH 55.30 mM, acidified iron solution: $FeSO_4.7H_2O$ 0.179 mM, H_2SO_4, boron solution 0.185 mM, trace metal solution: $ZnSO_4.7H_2O$ 8.82 mM, $MnCl_2.4H_2O$ 1.44 mM, MoO_3 0.71 mM, $CuSO_4.5H_2O$ 1.57 mM, $Co(NO_3)_2.6H_2O$ 0.49 mM. *Chlorella vulgaris* was also sub-cultured in the real-time MBR secondary effluent for acclimatisation of algae.

Secondary effluent

The real-time MBR-treated secondary effluent was collected from a sewage treatment plant located at TICEL Bio Park, Chennai, India. The wastewater sample for conducting batch studies was collected from the outlet of MBR, and the collected wastewater was stored at 4 °C. The wastewater had a pH of 7.15 with an organic content of 26 mg/L as COD. The effluent had a nitrate content of 105 mg/L and phosphate of 18 mg/L. Chloride and sulphates were at 112 mg/L and 1.6 mg/L, respectively.

Experimental scheme

Borosilicate conical flasks of 250 mL capacity were used for conducting the batch experiments. Mixing was provided at 100 rpm with an orbital shaker (Remi, India) to eliminate mass transfer limitations and keep the algal culture in suspension. The wooden chamber was provided with fluorescent lamps offering a light intensity of 3000–4000 lx, with the duration of illumination controlled by a timer. Intermittent illumination was provided with 12-h light/dark cycles. Initial alkalinity of ~ 4 g/L as $CaCO_3$ (~ 3.4 g/L as $NaHCO_3$) was added in all the experiments

conducted in BBM in the current study. Sterile conditions were maintained throughout the study period.

Phosphate gradient experiment in BBM

BBM without phosphorus was prepared as base media to which varying phosphate concentrations (0.1–107 mg/L) were added using $NaHPO_4$ as the orthophosphate source. The nitrate source was provided as $NaNO_3$. The batch experiments were conducted at a pH of 6.8–7. When the culture was in the logarithmic growth phase (3–6 days after inoculation), 1 mL of the stock culture was centrifuged at 3000 rpm for 15 min. After centrifugation, the biomass pellet was washed and resuspended in 2 mL physiological saline solution (0.85% NaCl) 3–4 times to remove any nutrients adsorbed on the walls of the algal cell surface. One hundred microlitre of the resuspended biomass pellets was then used to inoculate the batch reactors. The samples were collected for analysis of biomass, nitrates and orthophosphate concentrations.

Organic carbon gradient experiment in BBM

The organic carbon concentration in BBM was varied as 0, 10, 25, 50, 100, 150, 200 and 500 mg C/L, with sodium acetate as the organic carbon source. Subsequently, the media were sterilised by autoclaving at 121 °C for 15 min. Similar to the phosphate gradient experiment, the reactors were mixed at 100 rpm throughout the study period. A light–dark cycle of 12 h each with a light intensity of 3000–4000 lx was maintained throughout the experiment. An operating volume of 100 mL was kept to ensure uniform mixing under sterile conditions. Samples were taken for the analysis of nitrates, orthophosphates and biomass.

Growth studies in secondary effluent

The secondary effluent was filter-sterilised through a 0.45-µm sterile nylon filter to remove suspended solids, bacteria, fungi and other microorganisms. The acclimatised strain of *Chlorella vulgaris* was inoculated in the filter-sterilised secondary effluent without any nutritional supplement. BBM was run as control and run simultaneously for comparison. The batch reactors were mixed at 100 rpm, and a light intensity of 3000–4000 lx was provided with a 12-h/12-h light–dark cycle.

Analytical techniques and data analysis

Analytical procedures

Algal biomass densities: 330 µL of the well-mixed sample was collected every day for measuring algal biomass. The light attenuation was measured at a wavelength of 800 nm using a UV–visible spectrophotometer (SpectraMax® M3 Microplate Reader (Molecular Devices LLC)). The light attenuation at 800 nm was then related to the algal biomass concentration (in mg/L estimated gravimetrically) according to Eq. (1).

$$X=4{\cdot}14\times OD800 \quad (R2=0.96) \quad (1)$$

where X_A is the biomass concentration (mg/L), and OD_{800} is the light attenuation at 800 nm.

On the other hand, the nitrate and orthophosphate concentrations in the samples were analysed by APHA 4500 NO_3^- and APHA 4500-P, stannous chloride method, respectively. All the

analyses were done in SpectraMax® M3 Spectrophotometer (Molecular Devices LLC). Whilst APHA 4500-NO_3^- cannot be used for samples with high organic content, a double derivative method was used to determine nitrates in such a case (Crumpton et al., 1992). These nutrient concentrations were calculated according to Eqs. (2)–(4).

Nitrate concentration when organic carbon is less:

$$NO{-}3(mg/L)=19{\cdot}86\times(OD220-2\times OD275)(R2=0.99) \quad (2)$$

Nitrate concentration when organic carbon concentration is high:

$$NO{-}3(mg/L)=2067.60\times P200{-}350(R2=0.99) \quad (3)$$

where $P_{200–350}$ is the peak of the double derivative curve for the spectra from 200 to 350 nm. The peak was obtained at 221 nm in this case.

Orthophosphate concentration:

$$PO3{-}4(mg/L)=3.99\times OD690(R2=0.99) \quad (4)$$

Calculation of bio-kinetic parameters

For the calculations of specific growth rates (μ), the growth profiles were fitted to a Boltzmann curve (Fig. S1) using OriginPro software (version 2019b), and the slope of the curve was used to find μ. The substrate utilisation rates were then calculated for the logarithmic growth phase obtained for each reactor. The yield coefficient was calculated from the total biomass produced and the corresponding consumption of substrate. The removal of nitrates and phosphates was calculated from their initial and final concentrations. These parameters were calculated according to Eqs. (5)–(9). The half-saturation constant (K_s) and maximum specific growth rate (μ_{max}) were calculated from a Lineweaver–Burk plot of Monod's model (Eq. 10).

$$\mu=1/X*dX/dt \quad (5)$$

where X is the average biomass and dX is the biomass produced at the time interval dt.

$$q=1/X*dS/dt \quad (6)$$

where dS is the substrate consumed at the time interval dt.

$$Removal(\%)=C0-CC0*100 \quad (7)$$

where C_0 is the initial concentration and C is the final concentration.

$$YS=Totalbiomassproduced/Totalsubstrateconsumed \quad (8)$$

$$\mu=\mu max*S/KS+S \quad (9)$$

$$1/\mu=KS/\mu max*S+1/\mu max \quad (10)$$

Results and discussion

Kinetics of microalgal growth at varying phosphate concentrations

The phosphate gradient experiment in BBM was done at varying orthophosphate concentrations, namely 0.11, 0.55, 1.10, 1.20, 4.30, 6.60, 12, 17, 25, 54 and 107 mg/L. Figures 1, 2, and 3 show the growth profile, nitrate utilisation profile and orthophosphate utilisation profile of *Chlorella vulgaris* at varying orthophosphate concentrations. The results of the phosphate gradient experiment are shown in Table 1. It was observed that the growth profile and nitrate utilisation profile remained almost unchanged at orthophosphate concentrations above 4.30 mg/L. After 16 days, nitrate removals were 42%, 55%, 65%, 74%, 90%, 98%, 97%, 97%, 98%, 97% and 99%, respectively. Orthophosphate removals were observed to be 74%, 89%, 86%, 91%, 87%, 90%, 94%, 77%, 83%, 57% and 29%, respectively. With the increase in orthophosphate concentration up to 12 mg/L, the removal efficiency of orthophosphates increased and then declined for an initial nitrate concentration of 192 ± 15 mg/L. However, nitrate removal showed an increase with increasing orthophosphate concentrations. There was significant nitrate removal even at low orthophosphate concentrations (0.11–1.10 mg/L). Both phosphates and nitrates were effectively removed (> 90%) when the initial orthophosphate concentration was 4–12 mg/L. The total orthophosphates concentration in secondary effluent is usually 4–10 mg/L (Safoniuk, 2004). Therefore, algae are a potential solution for removing nutrients from secondary effluent.

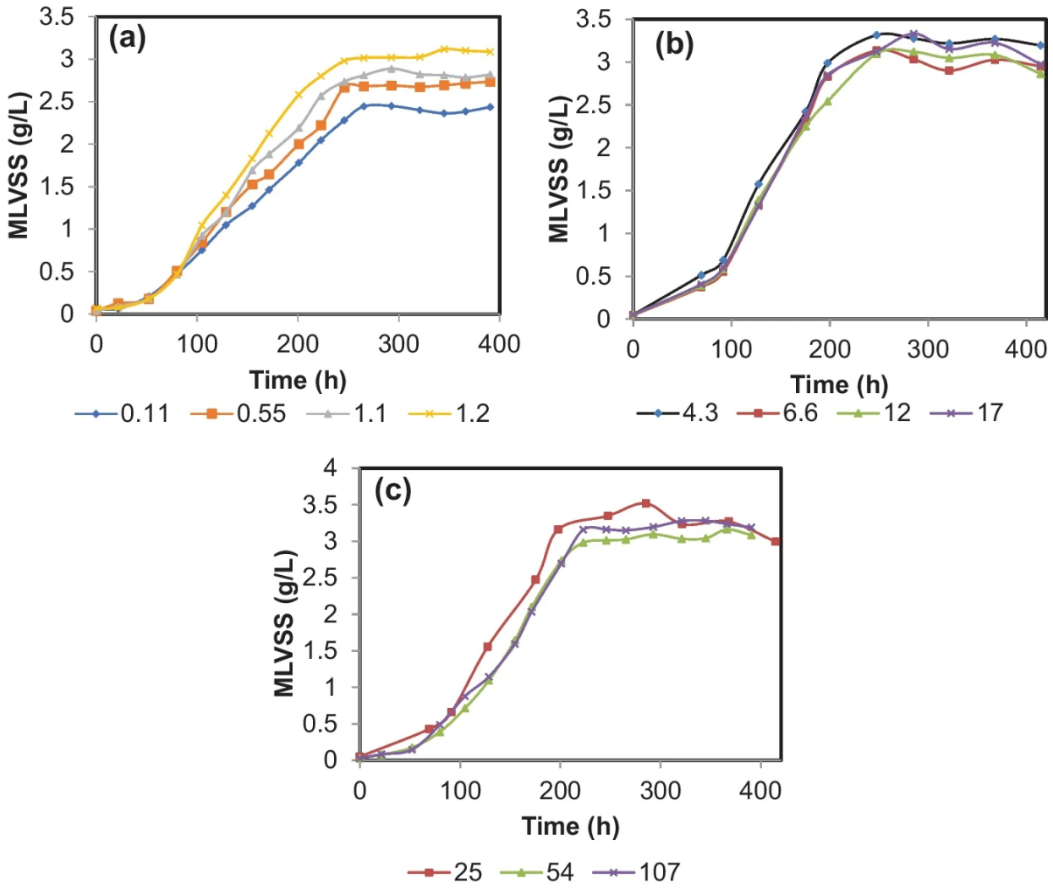

Fig. 1 a–c Algal growth profile at varying phosphates in BBM (0.11, 0.55, 1.10, 1.20, 4.30, 6.60, 12.00 and 17.00, 25.00, 54.00 and 107.00 mg/L)

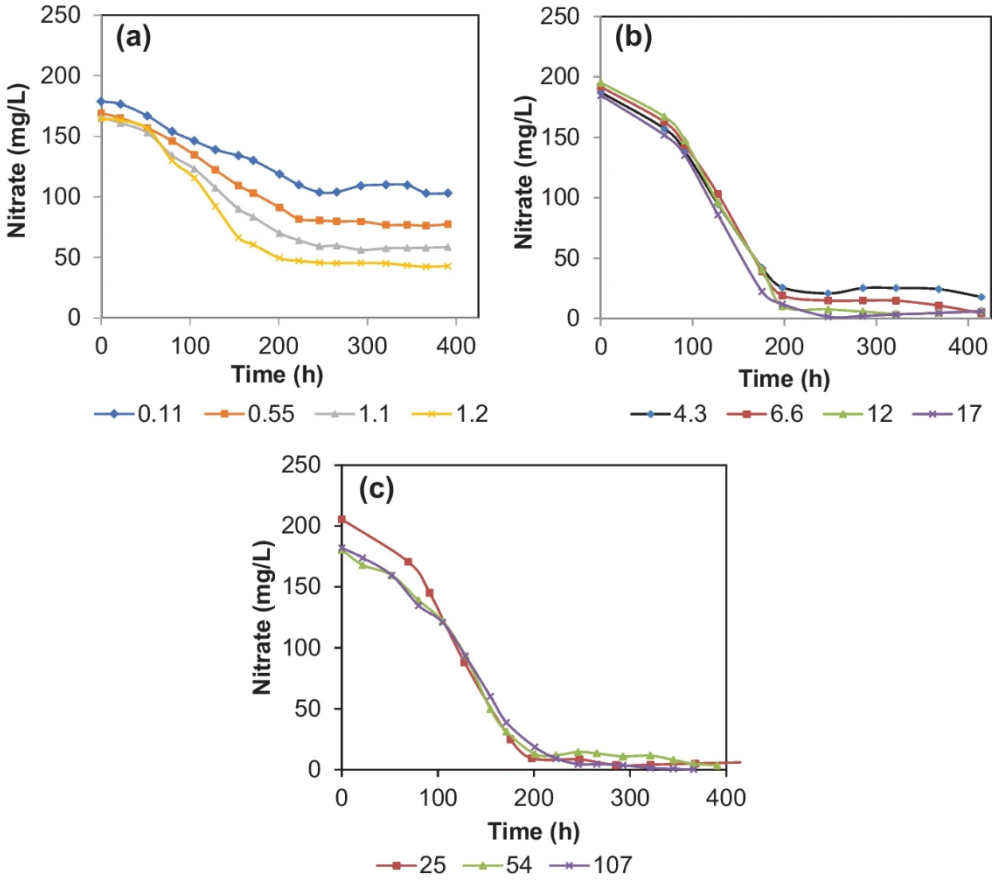

Fig. 2 a–c Nitrate utilisation profile at varying phosphate concentrations in BBM (0.11, 0.55, 1.10, 1.20, 4.30, 6.60, 12.00 and 17.00, 25.00, 54.00 and 107.00 mg/L)

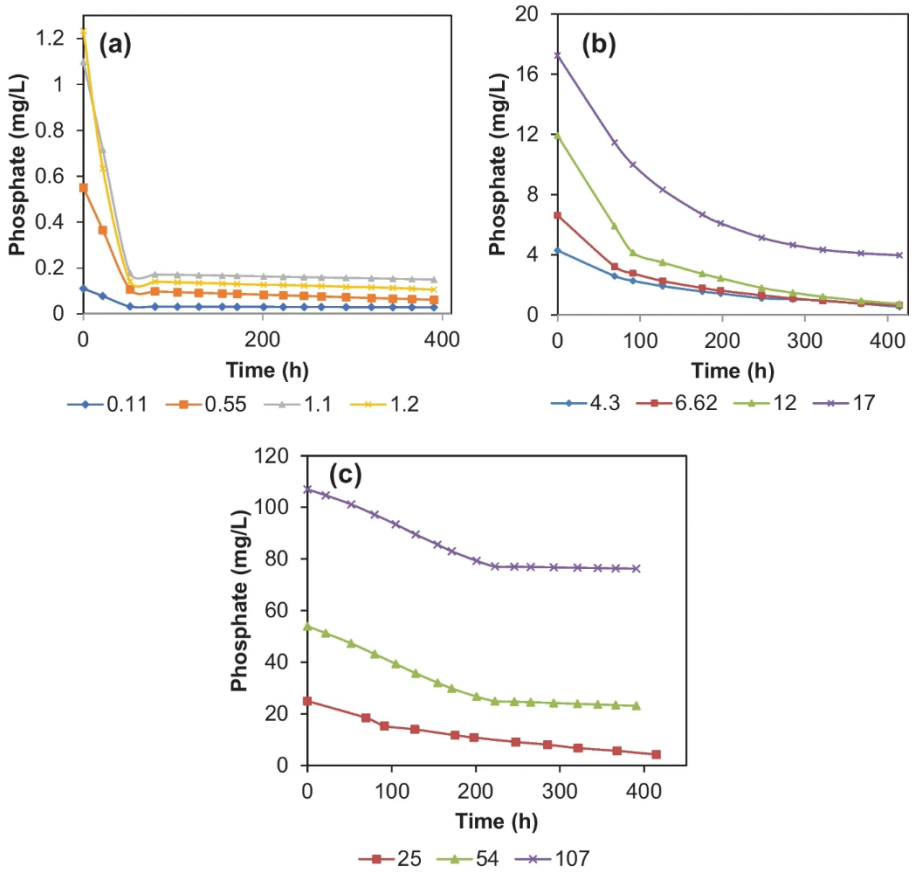

Fig. 3 a–c Phosphate utilisation profile at varying phosphate concentrations in BBM (0.11, 0.55, 1.1, 1.2, 4.3, 6.6, 12, 17, 25, 54 and 107 mg/L)

Table 1 Kinetics of algal growth in varying phosphate concentrations (0.11–107 mg/L), after a time period of 16 days

Parameter	Phosphate concentration (mg/L)										
	0.11	0.55	1.10	1.20	4.30	6.60	12	17	25	54	107
Nitrates (mg/L)	179	170	165	164	187	192	195	185	206	181	182
NO_3-N (mg/L)	40	38	37	37	42	43	44	42	47	41	41
PO_4^{3-}-P (mg/L)	0.04	0.18	0.36	0.40	1.39	2.16	3.88	5.63	8.15	17.60	34.80
N/P	1125	213	104	92	30.2	20	11.3	7.4	5.7	2.3	1.2
Nitrate removal (%)	42	55	65	74	91	98	97	97	98	97	100
Phosphate removal (%)	74	89	86	91	87	90	94	77	82	57	29
μ *10^{-2} (h^{-1})	0. 94	1.09	1.30	1.30	1.40	1.42	1.45	1.48	1.54	1.60	1.50
q_{NO3} (mg/g-h)	0.25	0.35	0.33	0.29	0.56	0.68	0.48	0.67	0.66	0.79	0.77
q_{PO4} (mg/g-h)	$6.9*10^{-6}$	$9.1*10^{-5}$	$4.6*10^{-5}$	$4.9*10^{-5}$	$4.1*10^{-3}$	$6.1*10^{-3}$	$7.6*10^{-3}$	$2.0*10^{-2}$	$2.2*10^{-2}$	$8.4*10^{-2}$	$10.8*10^{-2}$
Y_N (g MLVSS/mg NO_3)	0.031	0.029	0.026	0.025	0.019	0.017	0.016	0.017	0.016	0.018	0.018
Y_P (g MLVSS/mg PO_4)	49.00	8.90	5.00	5.80	0.51	0.29	0.18	0.13	0.10	0.11	0.12
dNO3/dPO4 (mg/mg)	1580.6	306.9	192.3	232.0	26.8	17.6	11.3	7.6	5.9	6.2	6.3

In the present study, the optimum N/P ratio was obtained as 11.3, where a maximum nitrate and orthophosphate removal of 96.76 and 93.85% was obtained. For an N/P ratio between 11.30 and 20.00, the removal of nitrates and orthophosphates exceeded 90%. It is to be noted that along with the N/P ratio of the effluent, the initial NO_3-N and PO_4-P concentrations also impact nutrient removal efficiencies (Aslan & Kapdan, 2006; Xin et al., 2010).

The specific growth rate of algae increased with an increase in the initial orthophosphate concentration or a decrease in the N/P ratio. The specific growth rate (μ) increased from 0.23 to 0.34 g/g/day as the initial orthophosphate concentration was increased from 0.11 to 4.30 mg/L, respectively. The specific growth rate slightly increased from 0.34 to 0.36 g/g/day, with a further rise in initial orthophosphate concentrations from 6.60 to 107 mg/L, respectively.

From the Lineweaver–Burk plot ($1/\mu$ vs $1/s$) (Fig. S2 (a) and (b)), the saturation constant K_s for orthophosphates was obtained as 0.027 mg/L, and the maximum specific growth rate (μ_{max}) of *C. vulgaris* was found to be 0.34 g/g/day, whilst the half-saturation constant for nitrates and μ_{max} by considering the nitrate kinetics was 24.90 mg/L and 0.36 g/g/day respectively.

The yield coefficient for nitrates and phosphates showed a decrease with increasing orthophosphate concentration from 0.11 to 107 mg/L. Variation of q_{NO3}, q_{PO4} and μ with various concentrations of nitrate, phosphate and biomass is shown in Fig. 4.

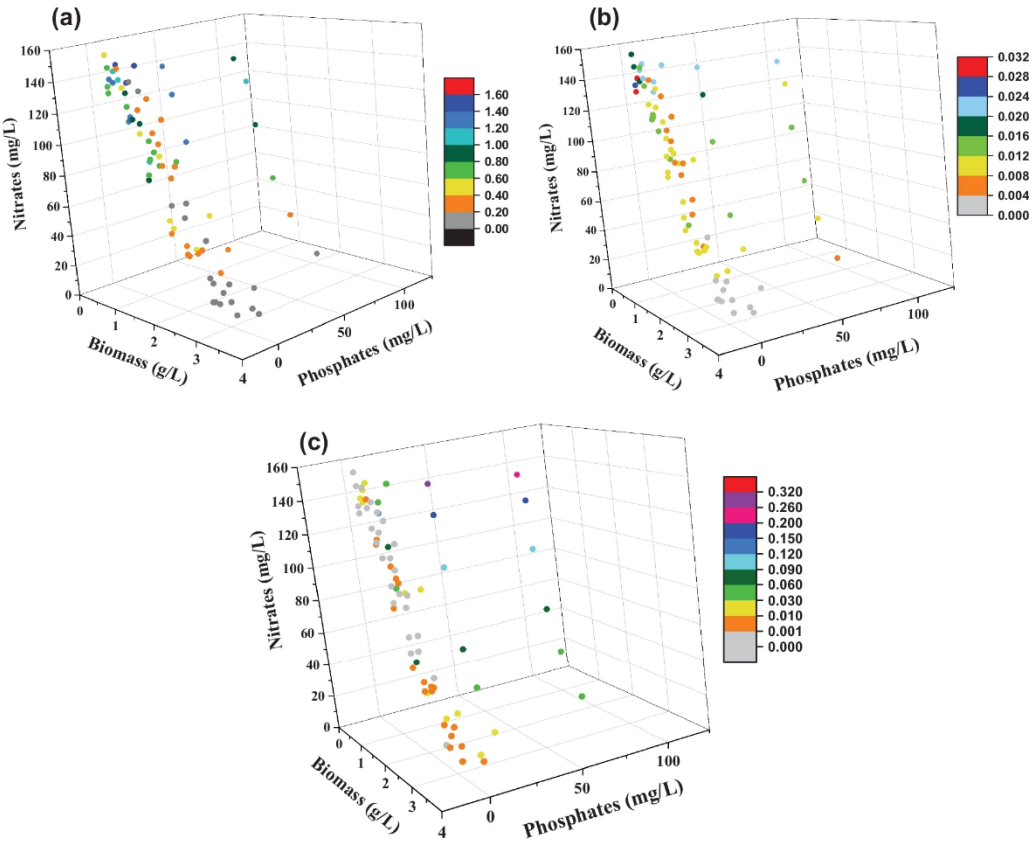

Fig. 4 a Variation of specific nitrate utilisation rate (q_{NO3}), **b** specific phosphate utilisation rate (q_{PO4}) and **c** specific growth rate (μ) with nitrates, phosphate and biomass concentration during log phase

Variation of specific nitrate utilisation rate (q_{NO3})

The specific nitrate utilisation rate (q_{NO3}) increased with increasing orthophosphate (P_0) and nitrate concentrations (N_0). At the stationary phase, where the biomass was greater than 3 g/L, nitrate utilisation was the least. However, around 0.5–1 g/L biomass concentration (initial log phase), q_{NO3} is high and increases with an increase in nitrate concentration. At high initial orthophosphate concentration (P_0 around 100 mg/L), q_{NO3} increased with increasing initial nitrate concentration (N_0). At P_0 of around 50 mg/L, q_{NO3} remained the same (1.85 mg/g.h) even with an increase in N_0 beyond 80 mg/L. However, the highest q_{NO3} was observed at P_0 about 15 mg/L and N_0 around 125–140 mg/L. At low P_0 (< 4 mg/L), q_{NO3} showed an increase with an increase in N_0.

Variation of specific phosphate utilisation rate (q_{PO4})

At high P_0 (> 50 mg/L), q_{PO4} increased with an increase in N_0. At an initial P_0 around 10 to 25 mg/L, q_{PO4} showed an increase with an increase in N_0, and a further decrease, with the corresponding point of maxima at N_0 of 120 mg/L. However, the highest q_{PO4} was obtained at P_0 of 50 mg/L and N_0 of about 140 mg/L. At $P_0 < 10$ mg/L, the q_{PO4} remained the same (around 0.049 mg/g.h) even with an increase in N_0. This implies that at low P_0, q_{PO4} remained unaffected even with an increase in N_0.

Variation of specific growth rate (μ)

At $P_0 < 25$ mg/L, μ increased with increasing N_0 and slightly decreased with a further increase in N_0. With an increase in P_0, beyond 50 mg/L, μ showed a notable increase, with a significant increase at increasing values of N_0. The highest value of μ was obtained at a P_0 of about 25 mg/L and N_0 of 130 mg/L. However, at a high value of P_0, around 100 mg/L, μ showed a continuous increase with an increase in N_0. At low P_0 (< 12 mg/L), μ was low.

Kinetics of microalgal growth at varying organic carbon concentrations

In the organic carbon gradient experiment conducted, the organic carbon was varied from 0 to 500 mg/L (0, 10, 25, 50, 100, 150, 200 and 500 mg/L) using sodium acetate as the organic carbon source. $NaHCO_3$ was provided as the inorganic carbon source. The experiment showed a nitrate removal of 96.1%, 97%, 98%, 96%, 95%, 98%, 98% and 97%, respectively, at the end of 10 days. However, the orthophosphate removal percentages showed no trend and varied between 6 and 15%. The specific growth rate was observed to increase proportionally from 0.48 to 0.70 g/g/day as the organic carbon was increased from 10 to 500 mg/L, respectively (Table 2). It was evident that μ improved with the addition of organic carbon to BBM. It was also observed that with the increase in organic carbon concentrations, the exponential growth phases shortened due to the faster depletion of nitrates at higher organic carbon concentrations. However, as observed from the growth profiles (Fig. 5), the stationary phases were not distinguishable as the growth was not logistic. In the absence of organic carbon, algal growth halted as soon as nitrates got depleted. However, it was not the case in the presence of organic carbon. Algae grew significantly but with a reduced growth rate and orthophosphate utilisation rate after the depletion of nitrates. This might be due to the ability of algae to switch nutrition between mixotrophic and heterotrophic growths in the absence of nitrates.

Table 2 Various parameters obtained from growth experiments at varying organic carbon concentrations (0–500 mg/L) in BBM after a time period of 10 days

Bio-kinetic parameter	Organic carbon concentration (mg/L)							
	0	10	25	50	100	150	200	500
μ *10^{-2} (h^{-1})	1.43	2.10	1.98	1.92	2.00	2.70	2.90	2.90
μ (day^{-1})	0.34	0.49	0.48	0.46	0.54	0.65	0.70	0.70
q_{NO3} (mg/gh)	0.58	1.10	0.90	0.83	1.14	1.25	1.15	0.79
q_{PO4} *10^{-1} (mg/gh)	1.05	0.50	0.38	0.39	0.73	0.50	0.67	1.60
Nitrate removal (%)	96.10	96.52	98.29	96.18	95.42	98.20	97.95	97.30
Phosphate removal (%)	15.01	11.20	13.60	7.20	15.60	6.70	14.51	19.10
Y_N *10^{-2} (g MLVSS/ mg NO_3^-)	1.70	1.75	1.89	1.90	2.00	1.98	1.96	2.00
Y_P (g MLVSS/mg PO_4^{3-})	0.09	0.18	0.16	0.30	0.15	0.20	0.16	0.13
dNO_3/dPO_4 (mg/mg)	5.50	10.30	8.50	15.80	7.60	10.30	8.20	6.50

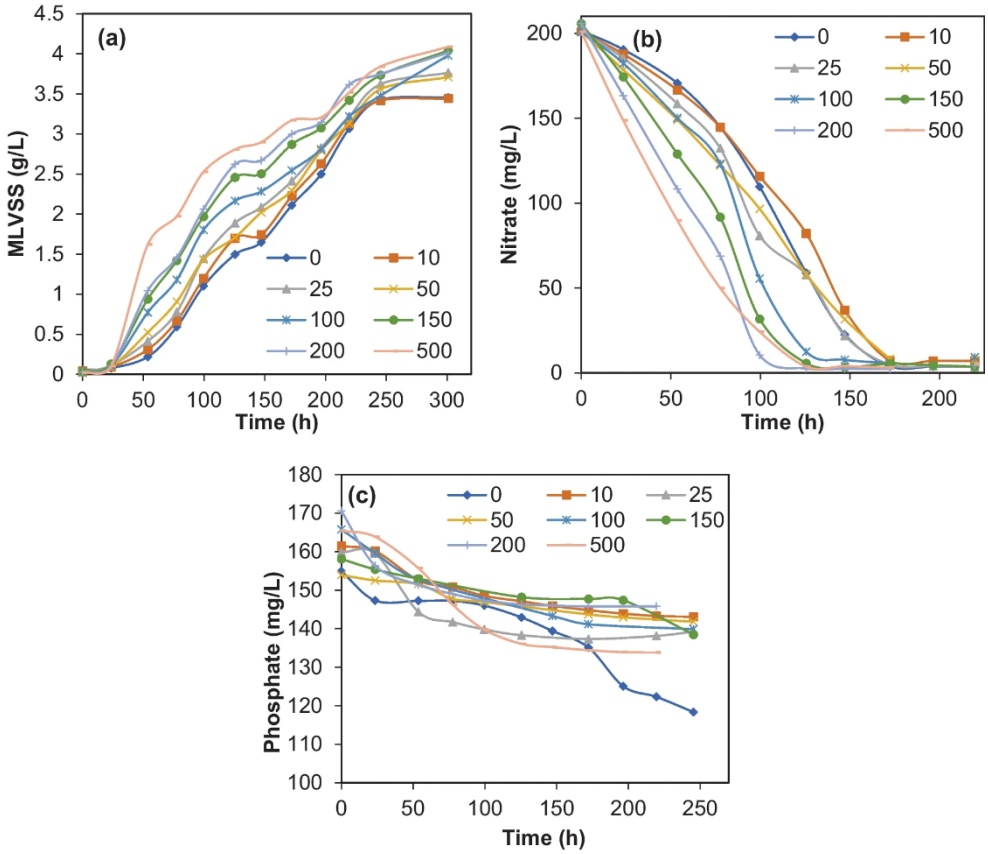

Fig. 5 **a** Algal growth profile, **b** nitrate utilisation profile and **c** phosphate utilisation profile at varying organic carbon concentrations in BBM

The substrate utilisation rates were calculated for the initial mixotrophic phase until the nitrates got depleted. The q_{NO3-} showed values ranging between 0.83 and 1.30 mg/h (Table 2), when the organic carbon concentration was varied between 10 and 500 mg/L organic carbon. The values of q_{NO3-} obtained in the presence of organic carbon were higher than all the values obtained in the phosphate gradient experiment without organic carbon and in BBM. This implies that the presence of organic carbon could bring significant improvement in the specific nitrate utilisation rate. It is also noted that around 90–95% of nitrates was consumed in 5 to 7 days in the presence of organic carbon, whilst the same took at least 8 days in the absence of organic carbon. The growth, nitrate utilisation and phosphate utilisation profiles for *Chlorella vulgaris* at varying organic carbon concentrations are shown in Fig. 5.

The specific orthophosphate utilisation rate q_{PO4} (mg/g/h) showed a variation in a narrow range of 0.038 to 0.067 mg/g.h (Table 2) with the addition of organic carbon between 0 and 200 mg/L. However, a higher q_{PO4} of 0.16 mg/g-h was obtained at 500 mg/L organic carbon. The lower q_{PO4} in the presence of organic carbon signifies that a lesser phosphate is required than that required in an inorganic media for nitrate removal.

The nitrate removal efficiency was consistently above 95%, whilst orthophosphate removal varied from 6.7 to 16%. Yield coefficient for nitrate showed a slight variation from 0.018 to

0.021 g MLVSS/mg NO_3, whereas the yield coefficient for phosphates was around 0.15 to 0.18 g MLVSS/mg PO_4, with an increase in organic carbon concentration from 10 to 500 mg/L.

Algal growth studies in effluent collected from MBR outlet of STP at TICEL Bio Park

The effluent treatment plant in TICEL Bio Park, Chennai, operates with membrane bioreactors (MBR) for secondary treatment of the wastewater effluent. Algal growth and nutrient removal studies were carried out in the MBR effluent after repeated subculturing of algae in the MBR effluent. The algal cultures were inoculated in filtered MBR effluent without adding any external carbon or nutrient source.

Algal growth kinetic studies were conducted in the MBR outlet effluent collected from TICEL Bio Park, Taramani, at the end of 17 days, with a specific growth rate of 0.19 g/g/day (Table 3). Correspondingly, q_{NO3} and q_{PO43} obtained for the MBR outlet were 0.16 mg/g-h and 0.04 mg/g-h, respectively. Compared to the growth in BBM, the algae grew at a relatively slower rate in the MBR effluent. The matrix effects of the effluent could lead to reduced growth of algae than that of a tailor-made growth medium like BBM. Moreover, the nutrient uptake was balanced in MBR effluent such that almost 98% orthophosphates and 92% nitrates were removed during treatment. The orthophosphate utilisation (Fig. 6c) commenced early at the lag phase. However, significant nitrate utilisation (Fig. 6b) commenced only at the exponential phase. Almost 5 mg/L of PO_4 was utilised at the lag phase, similar to the lag phase PO_4 utilisation in BBM experiments. A biphasic growth pattern was observed, similar to studies with organic carbon. The algal growth reached its exponential phase after 98 h and grew exponentially until 400 h when nitrate was almost entirely utilised. After 400 h, there was a significant algal growth but at a slower growth rate. The growth and nutrient utilisation profiles of the secondary effluent and BBM are shown in Fig. 6. Table 4 shows some of the nutrient removal efficiencies of *Chlorella* sp. from domestic wastewater effluent, as obtained from the literature.

Table 3 Various bio-kinetic parameters obtained from growth experiments in bio-park MBR effluent after a time period of 17 days

Bio-kinetic parameter	BBM	Secondary effluent
μ (h^{-1})	0.014	0.008
μ (day^{-1})	0.34	0.19
q_{NO3} (mg/gh)	0.58	0.16
q_{PO43-} (mg/gh)	0.10	0.04
Nitrates (mg/L)	202	105
Phosphates (mg/L)	155	18
Nitrate removal (%)	96	92
Phosphate removal (%)	15	98
Y_N (g MLVSS/mg NO_3)	0.017	0.027
Y_P (g MLVSS/mg PO_4)	0.09	0.16

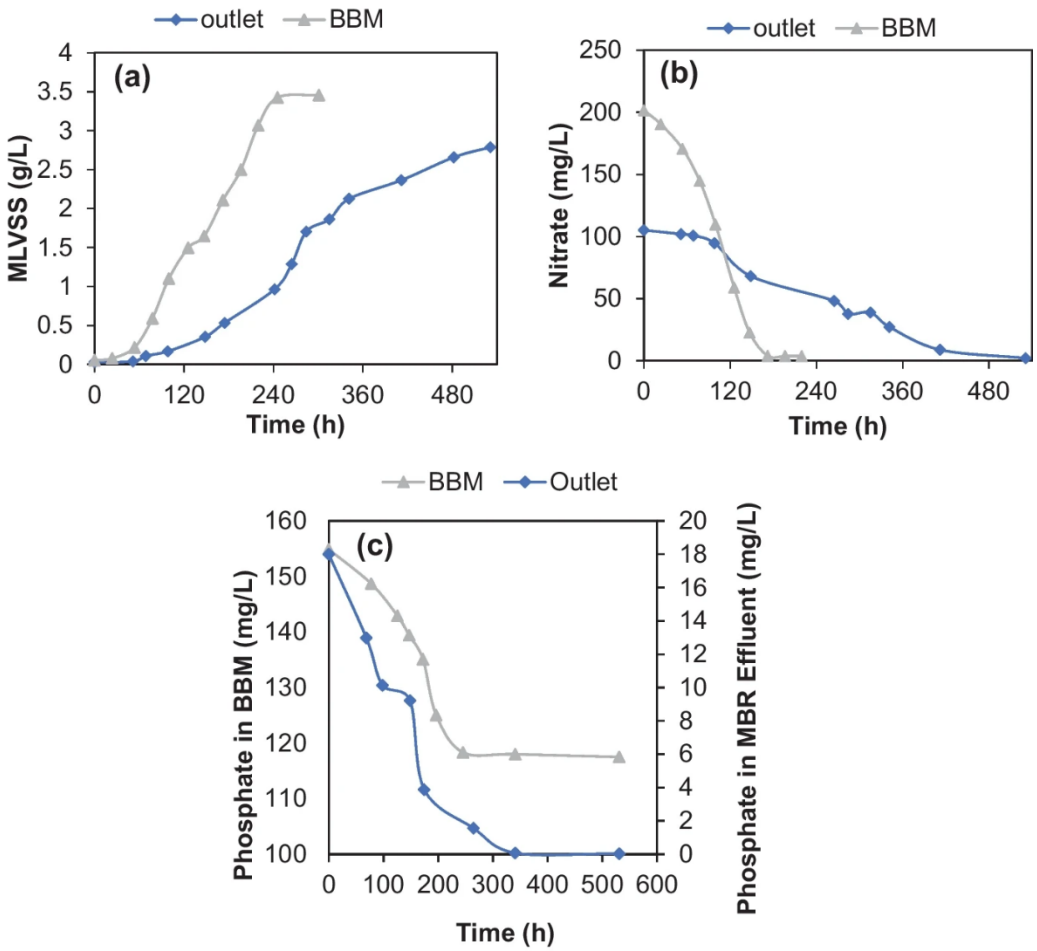

Fig. 6 a Algal growth profile, **b** nitrate utilisation profile and **c** phosphate utilisation profile in BBM, raw WW and MBR effluent collected from TICEL Bio Park, Taramani

Table 4 Comparison of nutrient removal efficiency of *Chlorella vulgaris* from MBR effluent with literature

Species	Source of wastewater	Removal efficiency (%)		Reference
		NO_3-N	PO_4^{3-}-P	
Chlorella sp. IM-01	Outlet of primary treatment	98	89 (TP)	(Kiran et al., 2014)
C. vulgaris	Effluent after secondary treatment	67	31	(AlMomani & Örmeci, 2016)
C. ellipsoidea	Secondary effluent after activated sludge process	99 (TN)	95 (TP)	(Yang et al., 2011)
Native microalgal consortia	Secondary municipal effluent	91	79	(Beltrán-Rocha et al., 2017)
C. vulgaris	Bio-park MBR effluent	92	98	Present study

Conclusions

Chlorella vulgaris species was successfully grown in synthetic wastewater and real-time wastewater sample collected from an ETP. In batch studies conducted in synthetic medium (BBM), the model organism successfully removed nitrates even at a low orthophosphate concentrations. Nitrate removal of above 90% was obtained at a minimum initial orthophosphate concentration of 4 mg/L. The kinetics for nitrates and orthophosphate consumption was established and followed Monod's model. The presence of organic carbon

(specifically acetate) improved the specific growth rate and specific nitrate utilisation rates. Faster depletion of nitrates was also observed. This becomes significant in the case of secondary effluent with organic carbon, mainly in the form of organic acids after the biological treatments. In the growth studies conducted in secondary effluent, the model organism was acclimatised to the secondary effluent from TICEL Bio Park, Chennai. It showed significant nutrient removals (92% nitrates and 98% orthophosphates) and a biomass growth up to 2.7 g/L in batch. This study showed that *Chlorella vulgaris* could treat MBR effluent for nutrient removal under fluctuating nutrient conditions.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

References

AlMomani, F. A., & Örmeci, B. (2016). Performance of *Chlorella vulgaris*, *Neochloris oleoabundans* and mixed indigenous microalgae for treatment of primary effluent secondary effluent and centrate. *Ecological Engineering, 95*, 280–289 S0925857416303640. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.ecoleng.2016.06.038

Arias, D. M., Solé-Bundó, M., Garfí, M., Ferrer, I., García, J., & Uggetti, E. (2018). Integrating microalgae tertiary treatment into activated sludge systems for energy and nutrients recovery from wastewater. *Bioresource Technology, 247*, 513–519. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.biortech.2017.09.123

Aslan, S., & Kapdan, I. K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering, 28*(1), 64–70. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.ecoleng.2006.04.003

Beltrán-Rocha, J. C., Barceló-Quintal, I. D., García-Martínez, M., Osornio-Berthet, L., Saavedra-Villarreal, N., Villarreal-Chiu, J., & Javier López-Chuken, U. (2017). Polishing of municipal secondary effluent using native microalgae consortia. *Water Science and Technology, 75*(7), 1693–1701. https://doi-org.ezproxy.lib.uts.edu.au/10.2166/wst.2017.046

Beuckels, A., Smolders, E., & Muylaert, K. (2015). Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. *Water Research, 77*, 98–106. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.watres.2015.03.018

Chamberlin, J., Harrison, K., & Zhang, W. (2018). Impact of nutrient availability on tertiary wastewater treatment by *Chlorella vulgaris*. *Water Environment Research, 90*(11), 2008–2016. https://doi-org.ezproxy.lib.uts.edu.au/10.2175/106143017X15131012188114

Chew, K. W., Yap, J. Y., Show, P. L., Suan, N. H., Juan, J. C., & Ling, T. C., et al. (2017). Microalgae biorefinery: High value products perspectives. *Bioresource Technology, 229*, 53–62. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.biortech.2017.01.006

Crumpton, W. G., Isenhart, T. M., & Mitchell, P. D. (1992). Nitrate and organic N analyses with second-derivative spectroscopy. *Limnology and Oceanography, 37*(4), 907–913. https://doi-org.ezproxy.lib.uts.edu.au/10.4319/lo.1992.37.4.0907

Gao, F., Li, C., Yang, Z. H., Zeng, G. M., Mu, J., Liu, M., & Cui, W. (2016). Removal of nutrients, organic matter, and metal from domestic secondary effluent through microalgae cultivation in a membrane photobioreactor. *Journal of Chemical Technology and Biotechnology, 91*(10), 2713–2719. https://doi-org.ezproxy.lib.uts.edu.au/10.1002/jctb.4879

Hashimoto, S., & Furukawa, K. (1989). Nutrient removal from secondary effluent by filamentous algae. *Journal of Fermentation and Bioengineering, 67*(1), 62–69. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/0922-338X(89)90088-3

Kamble, S., Singh, A., Kazmi, A., & Starkl, M. (2019). Environmental and economic performance evaluation of municipal wastewater treatment plants in India: A life cycle approach. *Water Science and Technology, 79*(6), 1102–1112. https://doi-org.ezproxy.lib.uts.edu.au/10.2166/wst.2019.110

Kim, B. H., Kang, Z., Ramanan, R., Choi, J. E., Cho, D. H., Oh, H. M., & Kim, H. S. (2014). Nutrient removal and biofuel production in high rate algal pond using real municipal wastewater. *Journal of Microbiology and Biotechnology, 24*(8), 1123–1132. https://doi-org.ezproxy.lib.uts.edu.au/10.4014/jmb.1312.12057

Kiran, B., Pathak, K., Kumar, R., & Deshmukh, D. (2014). Cultivation of *Chlorella* sp. IM-01 in municipal wastewater for simultaneous nutrient removal and energy feedstock production. *Ecological Engineering, 73*, 326–330 S0925857414005059. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.ecoleng.2014.09.094

Lee, J., Lee, J., Shukla, S. K., Park, J., & Lee, T. K. (2016). Effect of algal inoculation on COD and nitrogen removal, and indigenous bacterial dynamics in municipal wastewater. *Journal of Microbiology and Biotechnology, 26*(5), 900–908. https://doi-org.ezproxy.lib.uts.edu.au/10.4014/jmb.1512.12067

Li, Y., Chen, Y. -F., Chen, P., Min, M., Zhou, W., & Martinez, B., et al. (2011). Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresource Technology*, *102*(8), 5138–5144. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.biortech.2011.01.091

McGriff, E. C., & McKinney, R. E. (1972). The removal of nutrients and organics by activated algae. *Water Research, 6*(10), 1155–1164. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/0043-1354(72)90015-2

Mohamed, R. M. S. R., Al-Gheethi, A., Buyong, R., Hashim, N. H., Matias-Peralta, H. M., & Mohd-Kassim, A. H. (2018). Nutrient recovery from domestic effluent using an indigenous strain of *Scenedesmus* sp. *Clean - Soil, Air, Water*, *46*(8). https://doi-org.ezproxy.lib.uts.edu.au/10.1002/clen.201800204

Moore, J. W. (1989). Municipal wastewater management (Vol. 000, pp. 186–216). https://doi-org.ezproxy.lib.uts.edu.au/10.1007/978-1-4612-3496-8_8

Mutamim, N. S. A., Noor, Z. Z., Hassan, M. A. A., Yuniarto, A., & Olsson, G. (2013). Membrane bioreactor: Applications and limitations in treating high strength industrial wastewater. *Chemical Engineering Journal, 225*, 109–119. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.cej.2013.02.131

Safoniuk, M. (2004). Wastewater engineering: Treatment and reuse (Book). *Chemical Engineering, 65*(7), 10–11.

Sahasranaman, M., & Ganguly, A. (2018). *print Wastewater Treatment for Water Security in India*.

Sheng, A. L. K., Bilad, M. R., Osman, N. B., & Arahman, N. (2017). Sequencing batch membrane photobioreactor for real secondary effluent polishing using native microalgae: Process performance and full-scale projection. *Journal of Cleaner Production, 168*, 708–715. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.jclepro.2017.09.083

Villaseñor Camacho, J., Fernández Marchante, C. M., & Rodríguez Romero, L. (2018). Analysis of a photobioreactor scaling up for tertiary wastewater treatment: Denitrification, phosphorus removal, and microalgae production. *Environmental Science and Pollution Research, 25*(29), 29279–29286. https://doi-org.ezproxy.lib.uts.edu.au/10.1007/s11356-018-2890-5

Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J., & Ruan, R. R. (2010a). Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. *Bioresource Technology, 101*(8), 2623–2628. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.biortech.2009.10.062

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., & Liu, Y., et al. (2010b). Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology*, *162*(4), 1174–1186. https://doi-org.ezproxy.lib.uts.edu.au/10.1007/s12010-009-8866-7

Woertz, I., Feffer, A., Lundquist, T., & Nelson, Y. (2009). Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *Journal of Environmental Engineering, 135*(11), 1115–1122. https://doi-org.ezproxy.lib.uts.edu.au/10.1061/(ASCE)EE.1943-7870.0000129

Xin, L., Hong-ying, H., Ke, G., & Ying-xue, S. (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga Scenedesmus sp. *Bioresource Technology, 101*(14), 5494–5500. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.biortech.2010.02.016

Yang, J., Li, X., Hu, H., Zhang, X., Yu, Y., & Chen, Y. (2011). Growth and lipid accumulation properties of a freshwater microalga *Chlorella ellipsoidea* YJ1 in domestic secondary effluents. *Applied Energy, 88*(10), 3295–3299 S0306261910004927. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/j.apenergy.2010.11.029

Yen, H. -W., Hu, I. -C., Chen, C. -Y., Ho, S. -H., & Lee, D. -J. (2013). Microalgae-based biorefinery – From biofuels to natural products. *Bioresource Technology, 135*, 166–174. https://doi-org.ezproxy.lib.uts.edu.au/10.1016/J.BIORTECH.2012.10.099