Influence of pathogenic bacterial activity on growth of *Scenedesmus* sp. and removal of nutrients from public market wastewater

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ABSTRACT

The present study aims to investigate the influence of *Staphylococcus aureus, Escherichia coli* and *Enterococcus faecalis* in public market wastewater on the removal of nutrients in terms of ammonium (NH_4^-) and orthophosphate (PO_4^3) using *Scenedesmus* sp. The removal rates of NH_4^- and orthophosphate PO_4^{3-} and batch kinetic coefficient of *Scenedesmus* sp. were investigated. The phycoremediation process was carried out at ambient temperature for 6 days. The results revealed that the pathogenic bacteria exhibited survival potential in the presence of microalgae but they were reduced by 3–4 log at the end of the treatment process. The specific removal rates of NH_4^- and PO_4^{3-} have a strong relationship with initial concentration in the public market wastewater ($R^2 = 0.86$ and 0.80, respectively). The kinetic coefficient of NH_4^- removal by *Scenedesmus* sp. was determined as $k = 4.28$ mg NH_4^- 1 $\log_{10}$ cell mL^{-1} d^{-1} and $k_m = 52.01$ mg L^{-1} ($R^2 = 0.94$) while the coefficient of PO_4^{3-} removal was noted as $k = 1.09$ mg NH_4^- 1 $\log_{10}$ cell mL^{-1} d^{-1} and $k_m = 85.56$ mg L^{-1} ($R^2 = 0.92$). It can be concluded that *Scenedesmus* sp. has high competition from indigenous bacteria in the public market wastewater to remove nutrients, with a higher coefficient of removal of NH_4^- than PO_4^3.

Key words | batch kinetic coefficient, growth rate, pathogens, public market wastewater, removal rate

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INTRODUCTION

Public market wastewater is defined as the wastewater generated from fish cleaning, washing floors and raw materials as well as melting ice packs. The public market wastewater is also contaminated with blood residues from fish or meat (Verheijen *et al.* 1996). Public market facilities are provided by the local government for a variety of daily necessities, especially raw goods at reasonable prices.

Public markets represent one of the main significant challenges for environmental issues due to the relatively high constituents of organic matter which may reach the range of 71 and 122 mg/L for biological oxygen demand (BOD) and 381–560 mg/L for chemical oxygen demand (COD) (Zulkifli *et al.* 2012). Both COD and BOD result in the decrease of dissolved oxygen (DO) levels in the public market wastewater receiving water bodies because the oxygen available in the water is being consumed by the bacteria. Therefore, the direct discharge of these wastes without prior treatment into the environment negatively affects the biological diversity in the environment and natural water bodies due to the decrease of DO necessary for survival of organisms (ReVelle & ReVelle 1998).

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Discharge of public market wastewater into aquatic bodies poses a serious eutrophication threat, leading to slow degradation of the water resources. Jais *et al.* (2017) indicated that the high levels of nutrients in the public market wastewater, which included the presence of phosphates and nitrates, lead to pollution and hypertrophication because the nutrients improve the microalgae growth which causes the water bloom. The adverse effects associated with eutrophication include the reduction of biodiversity and replacement of dominant species, increased water toxicity, turbidity and lifespan of the lakes. Therefore, the nutrient pollutants in the public market wastewater must be reduced before being discharged to prevent undesirable effects (Ruiz-Martinez *et al.* 2012).

Phycoremediation of wastewater is a green technology and eco-friendly process with no secondary pollution or chemical additives. The process relies on the algae that have the potential to assemble nutrients from the wastewater (Prajapati *et al.* 2013). However, the efficiency of phycoremediation is dependent on the nature of the relationship between microalgae species introduced into the wastewater and the indigenous organisms such as bacteria (Rhee 1972). The public market wastewater has high loads of bacterial species resulting from the washing process of meat, chicken, fish and vegetables. The high content of nutrients in the public market can also support the bacterial growth. Therefore, the presence of bacteria in these wastes may negatively or positively affect the phycoremediation process. Meanwhile, phycoremediation may cause significant changes in the concentrations of those bacteria.

Ma *et al.* (2014) stated that the bacteria and algae in the co-existing system have a mutually beneficial relationship where bacteria can help degrade unruly compounds to ammonium, nitrogen, phosphate and carbon dioxide, which can easily be used by algae. In contrast, microalgae cells produce the oxygen required by the aerobic bacteria to mineralize organic pollutants (McGriff & McKinney 1972; Oswald 1988). Nonetheless, both bacteria and microalgae cells have a negative effect against each other. Several bacterial species such as *Bacillus* sp., *Pseudomonas* sp. and *Aeromonas* sp. have high algicidal agent production (Sakata *et al.* 2011). Algicidal agents are biochemical substrates produced by some bacterial species as a result of the competitive interactions between bacteria and toxic algae. In contrast, Syed *et al.* (2015) indicated that *Chlorella vulgaris* has antimicrobial activity against *Klebsiella* sp., *Scenedesmus* sp., *Microcystis* sp., *Oscillatoria geminate* and *C. vulgaris* while *Nostoc* sp. exhibited antimicrobial activity against *Staphylococcus aureus*, *B. subtilis*, *Sarcina lutea*, *Klebsiella pneumoniae* and *B. megaterium* (Olfat *et al.* 2014). So far, many factors include nutrient availability, mixing conditions, light and temperature play an important role in the interactions between algae and bacteria in the environment (Granéli *et al.* 2008).

The present study aims to determine the bacterial load available in the public market wastewater as well as investigate the influence of bacterial activity on the efficiency of phycoremediation of public market wastewater.

MATERIALS AND METHODS

Sampling

Grab sampling was used for the collection of public market wastewater in which one sample was collected at a specific time. The samples were collected in 5 L polyethylene container bottles at 9 am, which represents the peak time for high production of public market wastewater. These containers were used to avoid the negative effects on analytical parameters as recommended by APHA (2005). The samples were obtained from the discharge point where the effluent is thoroughly mixed and close to the discharging public market outlet. The collected samples were immediately transferred into the laboratories and analysed for all parameters according to APHA (2005).

Chemical and microbiological characteristics of public market wastewater

The characteristics of wastewater including pH, DO, ammonium (NH_4^-) and orthophosphate (PO_4^{3-}) were conducted as described by APHA (2005). The pH was determined by using the 4500–H-B method with an Oakton pH 700 Benchtop Meter (Oakton, USA). NH_4^- was measured according to the 4500-NH_4^--B method using the DR 5000 Spectrometer (UV-VIS Hach, USA) while PO_4^{3-} was determined based on method 1060. *Escherichia coli*, *Enterococcus faecalis* and *S. aureus* were enumerated by the direct plating technique on appropriate selective media (APHA 1999; HPC 2004). The viable count was calculated per plate and followed by 100 mL of the original sample in terms of $\log_{10}$ CFU of the bacteria per 100 mL of public market wastewater sample.

Microalgae strain

Scenedesmus sp. (Accession No. JQ315576.1) is an indigenous strain isolated from freshwater (Table 1). In order to identify the strain based on 18S rRNA sequencing, a pure culture of microalgae on Bold Basal medium (BBM) was sent to Axil Scientific Pte Ltd (The Gemini, Singapore). The microalgae inoculum was prepared by sub-culturing in a BBM broth and incubated at room temperature ($25 \pm 2\ ^\circ C$, 12 h L:12 h D period) for 7 days as described by Bischoff & Bold (1963). The culture medium was centrifuged (6,000 rpm) to separate the microalgae cells; and the media residue was removed by washing using sterilized deionized water. The microalgae cells were then suspended in 10 mL of sterilized normal saline water. The concentrations of the cells were counted using a haemocytometer. Three inoculum concentrations were prepared with concentrations of 7, 8 and 9 $\log_{10}$ cells mL^{-1}.

Experimental set up of batch reactor system

Factorial complete randomized design (3×3×2) in triplicate was used to study the influence of pathogenic bacterial activity on phycoremediation of public market wastewater. Three experimental batch reactors were inoculated with *Scenedesmus* sp. (7, 8 and 9 $\log_{10}$ cells mL^{-1}) and supplied with an air pump. Two of the experimental batch reactors were used as control; one batch reactor without *Scenedesmus* sp. was used as the control (CB) to investigate the response of pathogenic bacteria during the phycoremediation of public market wastewater while the other batch reactor contains autoclaved public market wastewater (to inactivate the pathogenic bacteria) inoculated with *Scenedesmus* sp. (7 $\log_{10}$ cell mL^{-1}) which was used as control (CA) to study the efficiency of the phycoremediation process in the absence of indigenous pathogenic bacteria.

The experimental batch reactors were incubated for 6 days in an open area where direct sunlight is available as needed for microalgae growth. Four litres of raw public market wastewater samples were transferred into each reactor and inoculated in a separate mode with three concentrations of *Scenedesmus* sp. (7, 8 and 9 $\log_{10}$ cells 100 mL^{-1}). The values of the pathogenic bacteria (*E. coli*, *E. faecalis* and *S. aureus*), pH, DO, ammonium (NH_4^-) and

Table 1 | Accession number of *Scenedesmus* sp

Description	Max score	Total score	Query cover	E value	Ident	Accession
Scenedesmus sp. KMMCC 1534 18S ribosomal RNA gene, partial sequence	3,528	3,528	70%	0.0	98%	JQ315576.1
Scenedesmus sp. KMMCC 1533 18S ribosomal RNA gene, partial sequence	2,165	3,023	56%	0.0	99%	JQ315577.1
Scenedesmus sp. KMMCC 178 18S ribosomal RNA gene, partial sequence	2,159	3,017	56%	0.0	99%	JQ315566.1
Scenedesmus sp. KMMCC 406 18S ribosomal RNA gene, partial sequence	2,154	3,017	56%	0.0	99%	JQ315581.1
Scenedesmus sp. KMMCC 1211 18S ribosomal RNA gene, partial sequence	2,146	2,993	56%	0.0	99%	JQ315570.1
Scenedesmus sp. KMMCC 406 18S ribosomal RNA gene, partial sequence	2,154	3,017	56%	0.0	99%	JQ315581.1
Scenedesmus sp. KMMCC 872 18S ribosomal RNA gene, partial sequence	2,154	3,017	56%	0.0	99%	JQ315579.1
Scenedesmus sp. KMMCC 1258 18S ribosomal RNA gene, partial sequence	2,154	3,017	56%	0.0	99%	JQ315574.1
Scenedesmus sp. KMMCC 1211 18S ribosomal RNA gene, partial sequence	2,146	2,993	56%	0.0	99%	JQ315570.1
Scenedesmus sp. UKM 9 18S ribosomal RNA gene, partial sequence	2,141	3,065	57%	0.0	99%	KU170547.1
Scenedesmus sp. KMMCC 1297 18S ribosomal RNA gene, partial sequence	2,135	2,993	56%	0.0	99%	JQ315599.1
Scenedesmus armatus isolate B 18S ribosomal RNA gene, partial sequence	2,121	3,121	58%	0.0	99%	KR082490.1
Scenedesmus sp. Lake Las Vegas 18S ribosomal RNA gene, partial sequence	2,111	3,049	57%	0.0	99%	JX910112.1
Scenedesmus abundans 18S ribosomal RNA gene, partial sequence	2,065	2,868	53%	0.0	99%	KT868823.1

orthophosphate (PO_4^{3-}) in the raw public market wastewater were used as initial concentrations. The bacterial and microalgae density as well as the values of pH, DO, NH_4^- and orthophosphate (PO_4^{3-}) during the phycoremediation process were determined as described above under 'Chemical and microbiological characteristics of public market wastewater'.

The removal efficiencies by *Scenedesmus* sp. during the phycoremediation period were calculated according to the method used by Larsdotter *et al.* (2007).

Determination of removal rate of NH_4^- and PO_4^{3-} and batch kinetic coefficient of *Scenedesmus* sp.

The coefficient for removal rate of NH_4^- and PO_4^{3-} from public market wastewater by *Scenedesmus* sp. was investigated in a batch reactor inoculated with $8\log_{10}$ cell mL^{-1} of *Scenedesmus* sp. The public market wastewater used was autoclaved to inactivate the indigenous bacteria and other organisms to avoid the competition process with *Scenedesmus* sp. The coefficient for removal rate of NH_4^- and PO_4^{3-} was calculated as described by Aslan & Kapdan (2006). In brief, the concentration of NH_4^- and PO_4^{3-} in raw public market wastewater was used as initial substrate (S_0). The removal rate for the initial substrate (R_i) was calculated as given in Equation (1):

$$R_i = \frac{(S_0 - S_t)}{(t_0 - t_t)} \tag{1}$$

where S_i is the removal rate of substrate (NH_4^- and PO_4^{3-}), S_0 is the initial substrate concentration, S_t is the final substrate concentration at the tested time (t_t).

The specific removal rate (R_{xi}) for NH_4^- and PO_4^{3-} separately were calculated according to Equation (2):

$$R_{xi} = \frac{R_i}{\log_{10} N_0} \tag{2}$$

where $\log_{10}N_0$ is Scenedesmus sp. concentrations inoculated into the public market wastewater at the beginning of the phycoremediation process.

The kinetic coefficients which included the half saturation constant (k_s) and reaction rate constant (k) were calculated according to the Michaelis–Menten kinetic relationship (Equation (3)):

$$R = \frac{R_{\max} S}{K_s + S} \tag{3}$$

where $R_{\max}$ represents the maximum removal rate of the substrate from public market wastewater.

Both initial substrate concentrations and removal rates were considered in the batch reactor system according to Equation (4):

$$R_{so} = \frac{R_{m0} S_0}{K_s + S_0} \tag{4}$$

where $R_{m0} = k.x_0$ represent the maximum initial rate of the substrate removal from public market wastewater. This equation is expressed as the following:

$$R_{so} = \frac{k X_0 S_0}{K_s + S_0} \tag{5}$$

where k represents the reaction rate constant (time −1), X_0 represents the initial concentration of *Scenedesmus* sp. In order to calculate the specific rate of NH_4^- and PO_4^{3-} from public market wastewater, the terms of Equation (5) were divided by the initial concentration of *Scenedesmus* sp., as presented in Equation (6):

$$R_{xi} = \frac{R_{S0}}{X_0} = \frac{k S_0}{K_s + S_0} \tag{6}$$

Equation (7) was linearized in double reciprocal form as in Equation (8) and a plot of $1/R_{xi}$ versus $1/S_0$ yields a linear line with a slope of k_m/k and y-axis intercept of $1/k$:

$$\frac{1}{R_{xi}} = \frac{1}{k} + \frac{k_s}{k}\frac{1}{S_0} \tag{7}$$

The apparent specific growth rate (μ) was calculated according to Equation (8):

$$\mu = \frac{Ln\left(\dfrac{log_{10}N_t}{log_{10}N_0}\right)}{t_t - t_0} \tag{8}$$

Statistical analysis

The data obtained with three determinations from the batch reactor systems of raw public market wastewater were subjected to linear regression analysis to find the coefficient of reduction (R^2). The coefficients for removal of nutrients and reduction of pathogenic bacteria were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Characteristics of public market wastewater

The characteristics of the public market wastewater collected from Ringgit public market centre at Parit Raja are illustrated in Table 2. It can be noted that the public market wastewater has high concentrations of nutrients in terms of NH_4^- (220.66 ± 10.03 mg L^{-1}) and PO_4^{3-} (67.31 ± 7.33 mg L^{-1}). These findings are consistent with many reports in the literature (Zulkifli *et al.* 2012; Jais *et al.* 2015) which are due to blood residues that resulted from the washing of fish, chicken or meat. The high concentrations of nutrients are associated with high density of pathogenic bacteria that ranged from 8.841 ± 0.451 $\log_{10}$ CFU 100 mL^{-1} for *E. faecalis* to 10.32 ± 0.245 $\log_{10}$ CFU 100 mL^{-1} for *S. aureus* while *E. coli* was available in concentrations of 9.563 ± 0.327 $\log_{10}$ CFU 100 mL^{-1}. These bacteria resulted from the wastewater generated from the washing of the public market floor and vegetables as well as chicken and fish products. Moreover, high levels of nutrients induced their growth. *E. faecalis* and *E. coli* are indicator bacteria that have high potential to survive in the environment (Al-Gheethi *et al.* 2013). *S. aureus* is used as an infectious agent indicator for microbiological hygiene of foods (Jin *et al.* 2013).

Table 2 | Characteristics of raw wet market wastewater collected from Ringgit public market centre at Parit Raja, Johor in the period between August 2015 to April 2016 ($n = 7$)

			Environmental quality Act 1974 (*Regulation 2009*)	
Parameters	**Unit**	**Parameters concentrations**	**Standards A**	**Standards B**
pH		6.16 ± 0.121	6.0–9.0	5.5–9.0
NH_4^-	mg L^{-1}	220.66 ± 10.03	10	20
PO_4^{3-}	mg L^{-1}	67.31 ± 7.33	5	10
DO	mg L^{-1}	2.44 ± 0.168	NA	NA
E. coli	Log_{10} CFU 100 mL^{-1}	9.563 ± 0.327	NA	NA
E. faecalis	Log_{10} CFU 100 mL^{-1}	8.841 ± 0.451	NA	NA
S. aureus	Log_{10} CFU 100 mL^{-1}	10.32 ± 0.245	NA	NA

Ammonia (NH_3), Orthophosphate (PO_4^{3}–), Dissolved oxygen (DO); Standard A: Discharge upstream of water supply sources; Standards B: Discharge downstream of water supply sources. NA = Not available.

Response of pathogenic bacteria during the phycoremediation process and nutrient removal

The phycoremediation process was conducted for the removal of NH_4^- and PO_4^{3-} by using three inoculum sizes of *Scenedesmus* sp. and the investigation of pathogenic bacteria influence on the process efficiency. The concentrations of *S. aureus* in public market wastewater during the phycoremediation process is depicted in Figure 1. *S. aureus* was reduced significantly ($p < 0.05$) as determined by the analysis of variance (ANOVA) analysis by more than 4 log after 6 days of the phycoremediation process. The reduction level in aerated reactors was more than that in the non-aerated reactors with the presence of 7 and 8 $\log_{10}$ cell mL^{-1} of *Scenedesmus* sp. However, at high concentrations of *Scenedesmus* sp. (9 $\log_{10}$ cell mL^{-1}) the reduction in the non-aerated reactor was more than that in the aerated reactor. *S. aureus* is a facultative anaerobic which could grow in the presence or absence of oxygen. Nevertheless, the presence of high levels of oxygen may inhibit their competitive ability with *Scenedesmus* sp. in terms of nutrient uptake. In the control reactor without microalgae inoculation and in aerated conditions, *S. aureus* reduced rapidly by 4 log at the end of the incubation period of 6 days.

Similar trends were noted for *E. coli* with the differences in the reduction level (Figure 2). The maximum reduction of *E. coli* was 3.5 log in the non-aerated reactor with 9 $\log_{10}$ cell mL^{-1} of *Scenedesmus* sp., thus revealing the ability of *E. coli* to survive more than *S. aureus*. *E. coli* has a high ability to survive in a stressful environment and high competition levels with the indigenous microorganisms in the wastewater as a secondary medium (Partridge *et al.*

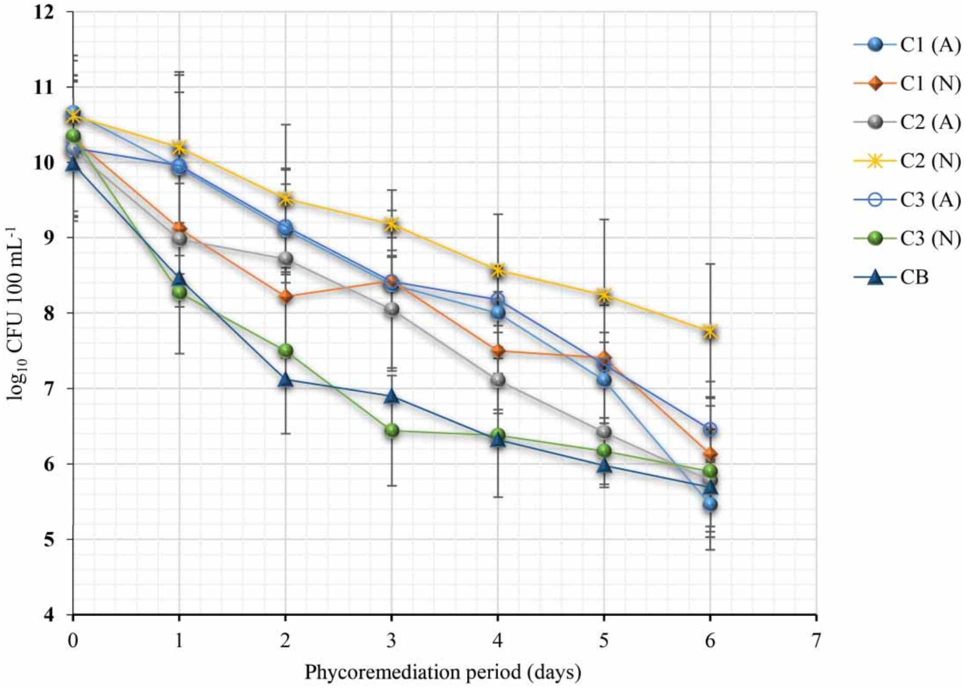

Figure 1 | *Staphylococcus aureus* concentrations during the aerated (A) and non-aerated (N) phycoremediation process of public market wastewater for 6 days and with different concentrations of *Scenedesmus* sp. (C1, 7 $\log_{10}$ cell 100 mL^{-1}; C2, 8 $\log_{10}$ cell 100 mL^{-1}; C3 9 $\log_{10}$ cell 100 mL^{-1}); control (CB).

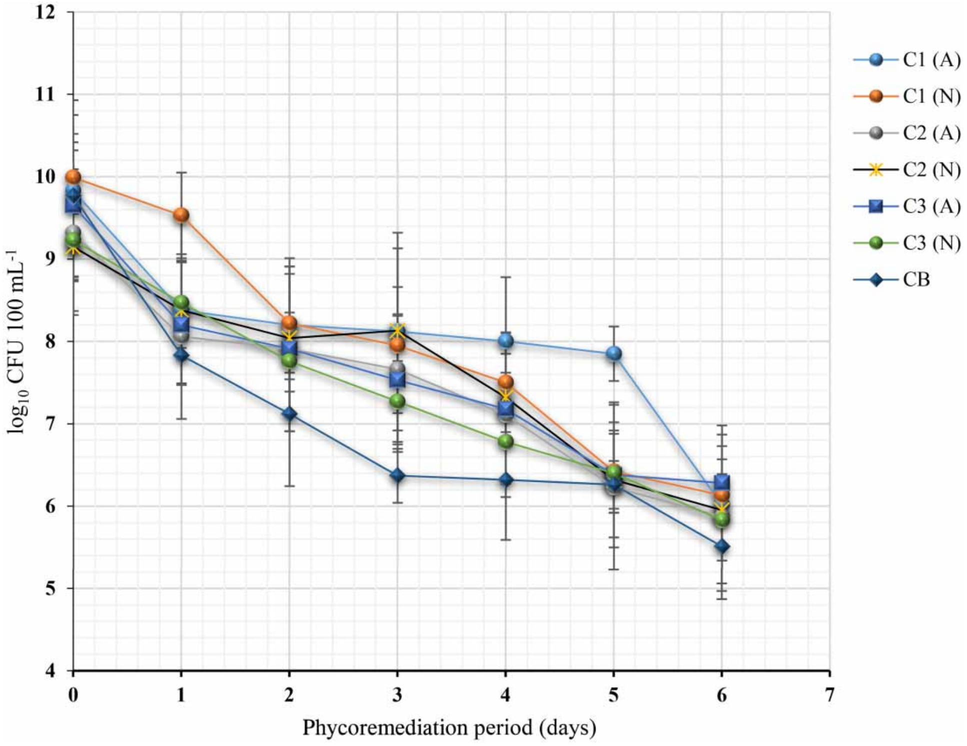

Figure 2 | *E. coli* concentrations during the aerated (A) and non-aerated (N) phycoremediation process of public market wastewater for 6 days and with different concentrations of *Scenedesmus* sp. (C1, 7 $\log_{10}$ cell 100 Ml^{-1}; C2, 8 $\log_{10}$ cell 100 mL^{-1}; C3 9 $\log_{10}$ cell 100 mL^{-1}), control (CB).

2006). Moreover, *E. coli* reduced more significantly in the absence of *Scenedesmus* sp. (to below 5.5 $\log_{10}$ CFU mL^{-1}).

The response of *E. faecalis* during the phycoremediation process is presented in Figure 3. It can be noted that the reduction level of this bacteria in the aerated reactors were more than that in the non-aerated ones. There are no significant differences in the reduction level of *E. faecalis* with different concentrations of *Scenedesmus* sp. Moreover, the log reduction was less than *E. coli* and *S. aureus*. This may be due to the ability of *E. faecalis* to survive better in the environment compared to *E. coli* and *S. aureus*. Al-Gheethi *et al.* (2013) indicated that this bacterium survives longer than FC in the environment.

The findings recorded in this study revealed that the presence of low concentrations of *Scenedesmus* sp. may prolong the survival period of pathogenic bacteria. However, they still have less competition potential in comparison to the algae. Cole (1982) reported that the bacterial cells have more activity in the presence of algae. This is because the algae provide the bacterial cells with vitamin B_{12} necessary for growth (Croft *et al.* 2005). Further, the phycoremediation process in the present work contributed significantly in the reduction of these pathogens due to the removal of nutrients by algae that enhanced the reduction of bacteria.

Scenedesmus sp. growth curve in the public market wastewater is depicted in Figure 4. *Scenedesmus* sp. grow better in the presence of bacteria, the maximum growth (10.24 $\log_{10}$ cell mL^{-1}) was recorded after 5 days with 8 $\log_{10}$ cell mL^{-1} of inoculum in the aerated reactor and with 9 $\log_{10}$ cell mL^{-1} in the aerated and non-aerated reactor (10.24 $\log_{10}$ cell mL^{-1}). The growth trends of *Scenedesmus* sp. with 7 $\log_{10}$ cell mL^{-1} of inoculum in the presence and absence of pathogenic bacteria found that no influencing effects of pathogenic bacteria on *Scenedesmus* sp. was recorded. Despite that, Ma *et al.* (2014) revealed that the algae growth in the raw wastewater was much higher than the autoclave samples due to the positive impact of bacteria on algae growth profile. The results in this study indicated the absence of the role bacteria plays on the growth of algae. The explanation for these findings might be related to available high nutrients in the public market wastewater which are enough for microalgae growth.

Based on the data obtained in the current work, it can be indicated that pathogenic bacteria may exhibit some survival in the presence of microalgae. However, they have no

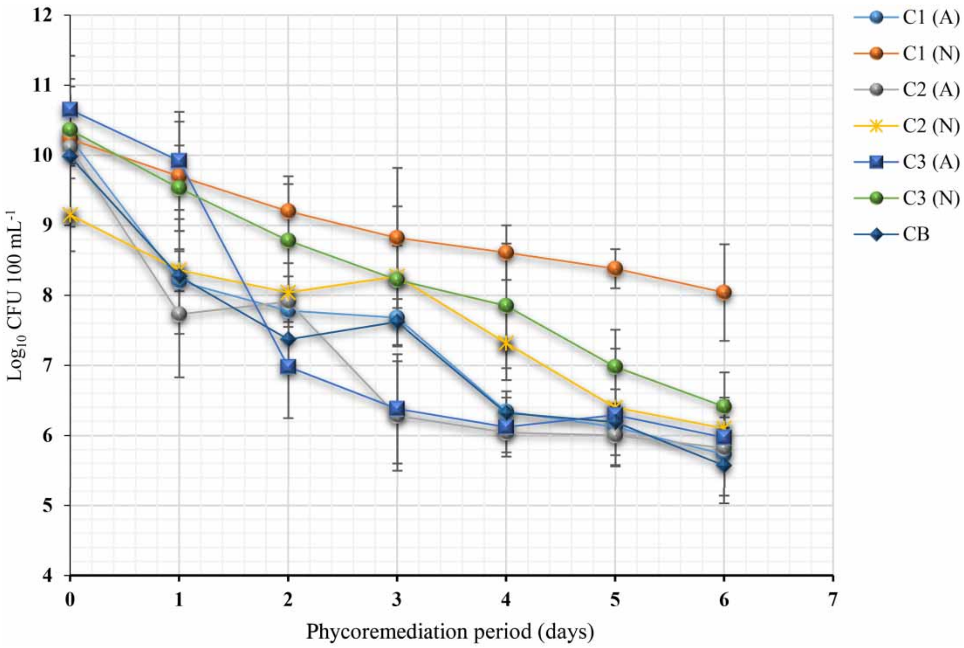

Figure 3 | *E. faecalis* concentrations during the aerated (A) and non-aerated (N) phycoremediation process of public market wastewater for 6 days and with different concentrations of *Scenedesmus* sp. (C1, 7 $\log_{10}$ cell 100 mL^{-1}; C2, 8 $\log_{10}$ cell 100 mL^{-1}; C3 9 $\log_{10}$ cell 100 mL^{-1}).

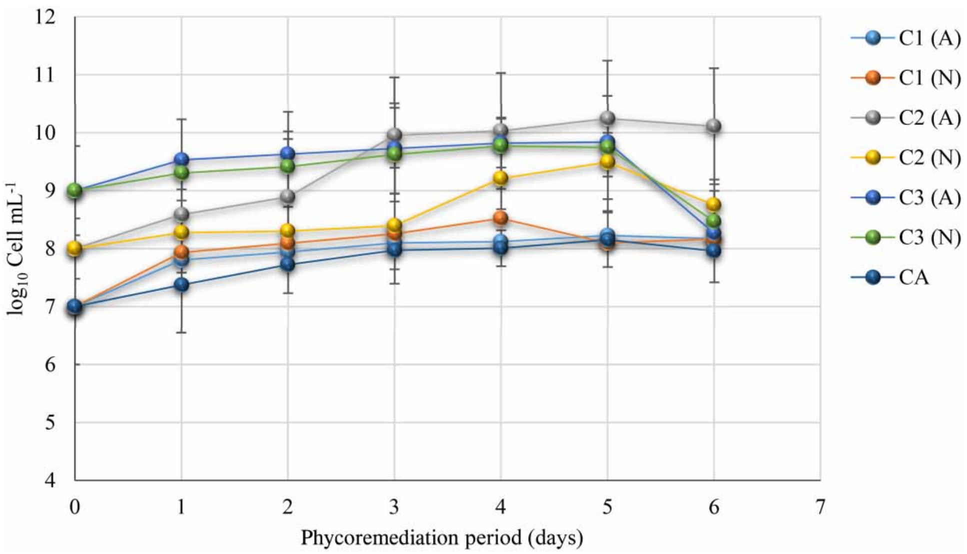

Figure 4 | *Scenedesmus* sp. concentrations during the aerated (A) and non-aerated (N) phycoremediation process of public market wastewater for 6 days and with different concentrations of *Scenedesmus* sp. (C1, 7 $\log_{10}$ cell mL^{-1}; C2, 8 $\log_{10}$ cell mL^{-1}; C3 9 $\log_{10}$ cell mL^{-1}).

effect on *Scenedesmus* sp. growth in the public market wastewater. Nonetheless, the control experiments which were performed without microalgae inoculum achieved a high reduction of pathogenic bacteria. These findings are explained as a result of the sunlight effect where the absence of microalgae growth in the wastewater samples enhanced the penetration of sunlight and then inactivated the pathogenic bacterial cells. In contrast, in the presence of the microalgae growth layer, the penetration of sunlight may have been prevented and thus reduce the inactivation process. The inactivation of pathogenic bacteria by sunlight treatment has been mentioned in the literature (Al-Gheethi *et al.* 2015). Sunlight is one of the single most important disinfection factors of wastewater treated by the stabilization pond (Maynard *et al.* 1999). The efficiency of sunlight in the inactivation of pathogens is due to the solar UV-B, which is absorbed by the microorganism's DNA and has the ability to cause direct damage of the DNA structure by pyrimidine dimer formation (Jagger 1985). The second reason for the inactivation by sunlight is the absorption of shorter wavelength UV-A by cell constituents, including DNA (called endogenous photosensitisers). In the process, the activated constituents are reacted with the DO in the wastewater and generate high reactive photo-oxidised substances which lead to damage of the microorganism's DNA. The third reason may be related to the absorption of visible wavelengths in sunlight by extra-cellular constituents of the pond medium (exogenous photosensitisers notably humic material) (Al-Gheethi *et al.* 2015).

It was noted that the reduction of *S. aureus*, *E. coli* and *E. fecalis* in C2 (N) was less than that of C1 (N) and C3 (N); these findings could explain that the relationship between *Scenedesmus* sp. and pathogenic bacteria is dependent also on their concentrations in the wastewater and the conditions available in the wastewater medium such as the presence or absence of oxygen.

Furthermore, the results revealed that the wastewater needs to undergo a further disinfection process for the reduction of pathogenic bacteria. However, the storage of the wastewater generated from the phycoremediation process for 2 weeks may be sufficient for the reduction of pathogenic bacteria to less than detection limits. Al-Gheethi *et al.* (2017) indicated that the fecal indicator bacteria in the treated effluents has reduced to below the detection limits after 2 weeks of the storage period at room temperature. The storage system is used to regulate between the wastewater production and demand of treated effluents for irrigation or disposal (Barbagallo *et al.* 2003).

The removal of microalgae biomass generated in the phycoremediation of wastewater is conducted by several methods including flocculation processes (Atiku *et al.* 2016). Hauwa *et al.* (2017) investigated the harvesting of microalgae biomass generated during the phycoremediation of greywater by flocculation using *Moringa oleifera* seed flours and found that the harvesting efficiency was 86.80%.

The concentrations of DO in the public market wastewater increased during the phycoremediation process with slight differences in the values associated with the inoculum size of *Scenedesmus* sp. (Figure 5). DO concentrations in the aerated reactors were slightly more than that in the non-aerated reactors, which are logical results. The DO increased from 2.44 ± 0.168 mg L^{-1} to the maximum values between 7.5 and 8.2 mg L^{-1} after 6 days of the treatment process in the aerated system and ranged from 7 to 7.5 mg L^{-1}. In the control samples (CB) without algae inoculum, the DO increased to 6.83 mg L^{-1} and dropped to 6.3 mg L^{-1} at the end of the treatment system. In contrast, in the control samples (CA) without bacteria, the DO increased slowly and reached the maximum values of 7.78 mg L^{-1} after 4 days, and then dropped to 7 mg L^{-1} on the 6th day which may be due to the die-off of the microalgae cells. Suh & Lee (2003) mentioned that the high DO levels may negatively affect the treatment efficiency by damaging the microalgae cells through photo-oxidative generation.

The pH of the public market wastewater increased from pH 6.12 to the maximum values between pH 8.9 and 9.9 in the aerated reactors and between pH 7.8 and 8.5 in the non-aerated reactors (Figure 6). In the control sample with microalgae (CA), the pH increased to 7.5 at the 6th day of the phycoremediation process. It was mentioned that the pH of public market wastewater during the phycoremediation process depends on the aeration and microalgae initial inoculum (Pahazri *et al.* 2016). The presence of CO_2, H_2CO_3, and HCO_{3-} which occur rapidly in heavy inoculum play an important role to increase pH values (Yaakob *et al.* 2014). In the control samples of bacteria (CB), the pH decreased significantly ($p < 0.05$) from 6.12 to 4.14 at the end of the treatment period. The reduction in the pH values with CB samples may lead to the biodegradation of organic matter by bacterial cells and production of acids (Al-Gheethi & Norli 2014).

The maximum removal of NH_4^- from public market wastewater was recorded in the non-aerated batch reactor with 8 $\log_{10}$ cell mL^{-1} of *Scenedesmus* sp. (Figure 7). The removal percentage was 93.29% on the 5th day of phycoremediation process and was 66.07% in the aerated system. The results have no recorded significant differences in the removal percentage between the aerated and non-aerated reactors inoculated with 7 and 9 $\log_{10}$ cell mL^{-1} of *Scenedesmus* sp.; the removal was 72.88 and 77.48% respectively. These findings indicated that the optimal inoculum was 8 $\log_{10}$ cell mL^{-1} of *Scenedesmus* sp. while high concentrations of inoculum may affect

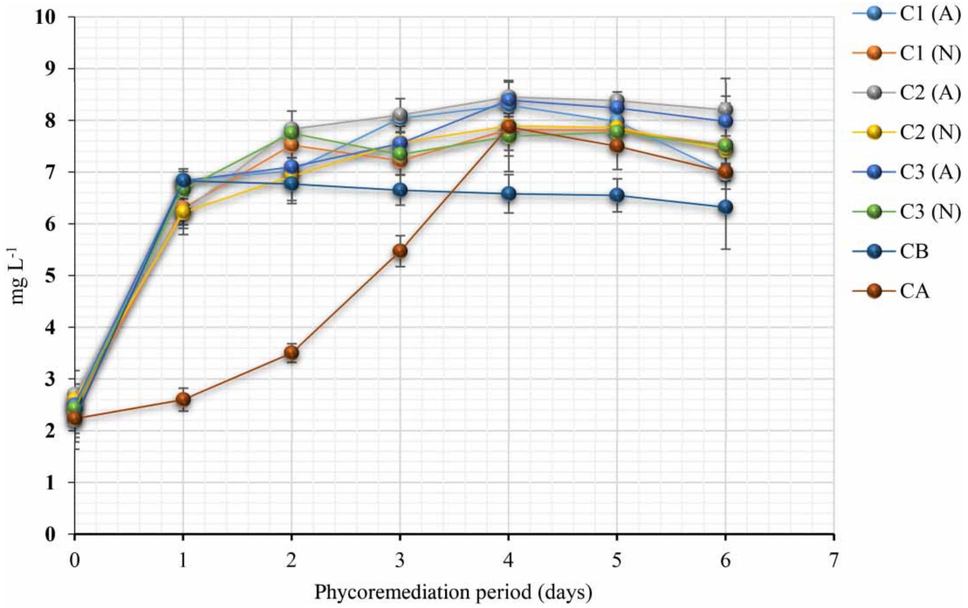

Figure 5 | Dissolved oxygen (DO) concentrations during the aerated (A) and non-aerated (N) phycoremediation process of public market wastewater for 6 days and with different concentrations of *Scenedesmus* sp. (C1, 7 $\log_{10}$ cell 100 mL^{-1}; C2, 8 $\log_{10}$ cell 100 mL^{-1}; C3 9 $\log_{10}$ cell 100 mL^{-1}); control of bacteria (CB); control of algae (CA).

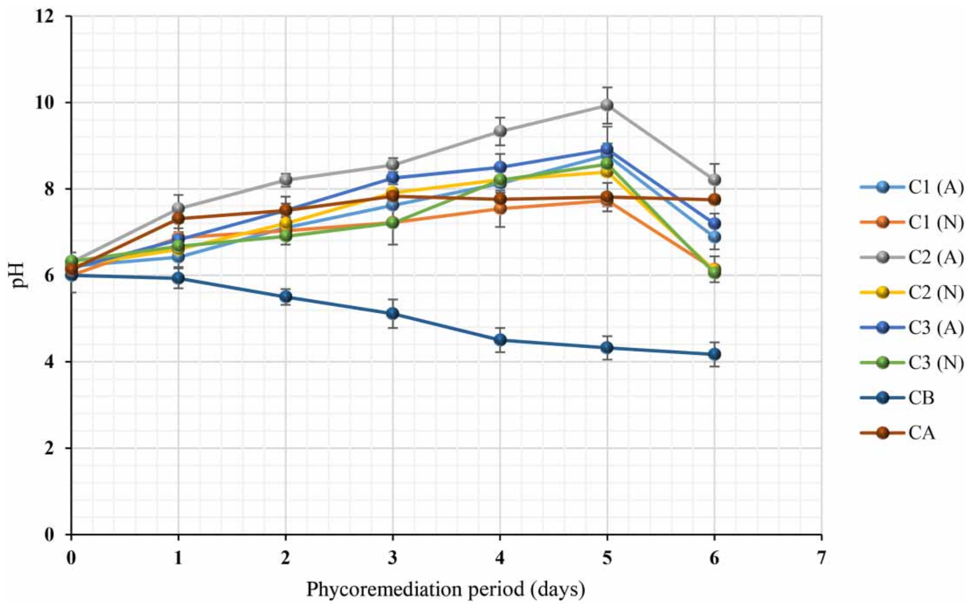

Figure 6 | pH values during the aerated (A) and non-aerated (N) phycoremediation process of public market wastewater for 6 days and with different concentrations of *Scenedesmus* sp. (C1, 7 $\log_{10}$ cell 100 mL^{-1}; C2, 8 $\log_{10}$ cell 100 mL^{-1}; C3 9 $\log_{10}$ cell 100 mL^{-1}), control of bacteria (CB); control of algae (CA).

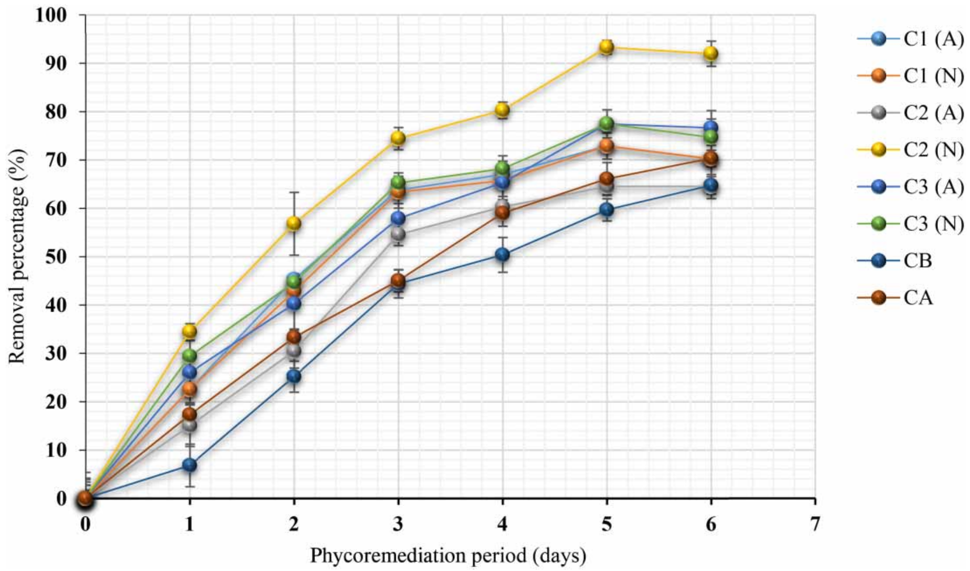

Figure 7 | Removal of ammonium (NH_4^-) during the aerated (A) and non-aerated (N) phycoremediation process of public market wastewater for 6 days and with different concentrations of *Scenedesmus* sp. (C1, 7 $\log_{10}$ cell 100 mL^{-1}; C2, 8 $\log_{10}$ cell 100 mL^{-1}; C3 9 $\log_{10}$ cell 100 mL^{-1}); control of bacteria (CB); control of algae (CA).

negatively because it would lead to a reduced amount of light penetrating through the bioreactor and enhancing the self-shading effects (Zhang *et al.* 2008). It was noted that the maximum removal was recorded at a pH of more than 8.5. Gimeno-García *et al.* (2000) claimed that pH values of more than 9 induced the NH_4^- and PO_4^{3-} through NH_4^- volatilization and PO_4^{3-} precipitation. In the control reactor with microalgae, the removal percentage increased significantly with increasing phycoremediation period. The maximum removal was noted on the 6th day (70.32%). The highest removal percentage in the control bacteria reactor was 64.74% at the end of the treatment process. These results indicated that the bacteria cells have significant contributions in the removal of NH_4^- from public market wastewater. The potential of bacteria to remove NH_4^- from wastewater have been reported before. Farehah *et al.* (2014) revealed that *S. warneri* had removed 97.2% of NH_4^- from wastewater. In comparison to the data presented in Figure 1, it was noted that the least reduction of *S. aureus* was recorded in the non-aerated system with 8 $\log_{10}$ cell mL^{-1} of *Scenedesmus* sp. which may indicate the presence of competition between *S. aureus* and *Scenedesmus* sp. on the uptake of NH_4^-, because these findings have not been observed with *E. faecalis* but were noted with *E. coli* when the *Scenedesmus* sp. inoculum size was 7 $\log_{10}$ cell mL^{-1}.

The highest removal of PO_4^{3-} was observed in the aerated reactor with 8 $\log_{10}$ cell mL^{-1} of *Scenedesmus* sp. (95.64%) after 5 days, which confirms that this inoculum showed the best optimum for the phycoremediation process (Figure 8). There are no significant differences in the removal percentage among the aerated and non-aerated reactors with other inoculum sizes. The microalgae control reactor (CA) exhibited higher removal than the bacterial control reactor (CB); the removal was 52.85 for CA compared to 42.59% for CB, which means that the removal of PO_4^{3-} from public market wastewater takes place by microalgae. The high removal of PO_4^{3-} in the aerated system may be related to the absence of PO_4^{3-} precipitation, which eases the uptake by microalgae cells. These findings are in agreement with Harley & McCready (1981) who had concluded that the oxygen consumption is proportional to phosphate uptake for equivalent times.

Removal rate of NH_4^- and PO_4^{3-} and batch kinetic coefficients of *Scenedesmus* sp.

The specific removal rate of NH_4^- from public market wastewater by *Scenedesmus* sp. is presented in Figure 9(a). The ammonium is the preferred nitrogen source for microalgae. However, in this study the removal rate was calculated and determined for ammonia because it represents the most energetically efficient nitrogen source due to the low energy required for its assimilation by the cells (Delgadillo-Mirquez *et al.* 2016). The optimal removal rate is associated with the increase of NH_4^- concentration ($R^2 = 0.86$). The removal rate increased from 2.81 mg NH_4^- 1 $\log_{10}$ cell mL^{-1} d^{-1} with 7.22 mg L^{-1} to 3.75 mg NH_4^- 1 $\log_{10}$ cell mL^{-1} d^{-1} with 155.34 mg L^{-1}. However, the high concentrations of NH_4^- to 190 mg L^{-1} slightly decreased the specific removal rate to 3.67 mg NH_4^- 1 $\log_{10}$ cell mL^{-1} d^{-1}. The specific removal rate of PO_4^{3-} was less than that recorded for NH_4^-. However, the trends of the specific removal rate were similar to NH_4^- where it increased significantly ($p < 0.05$, R^2, 0.80) from

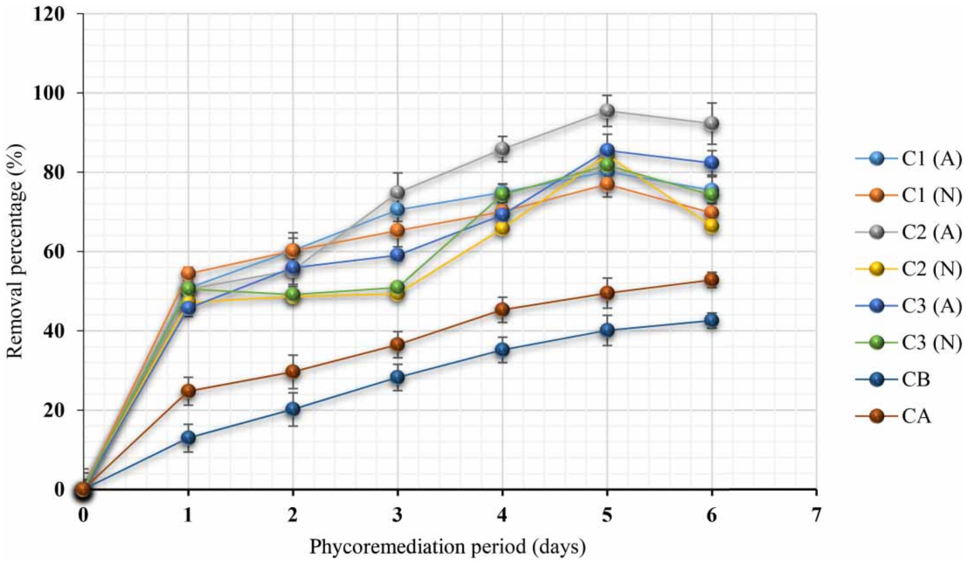

Figure 8 | Removal of orthophosphate (PO_4^{3-}) during the aerated (A) and non-aerated (N) phycoremediation process of public market wastewater for 6 days and with different concentrations of *Scenedesmus* sp. (C1, 7 $\log_{10}$ cell 100 mL^{-1}; C2, 8 $\log_{10}$ cell 100 mL^{-1}; C3 9 $\log_{10}$ cell 100 mL^{-1}); control of bacteria (CB); control of algae (CA).

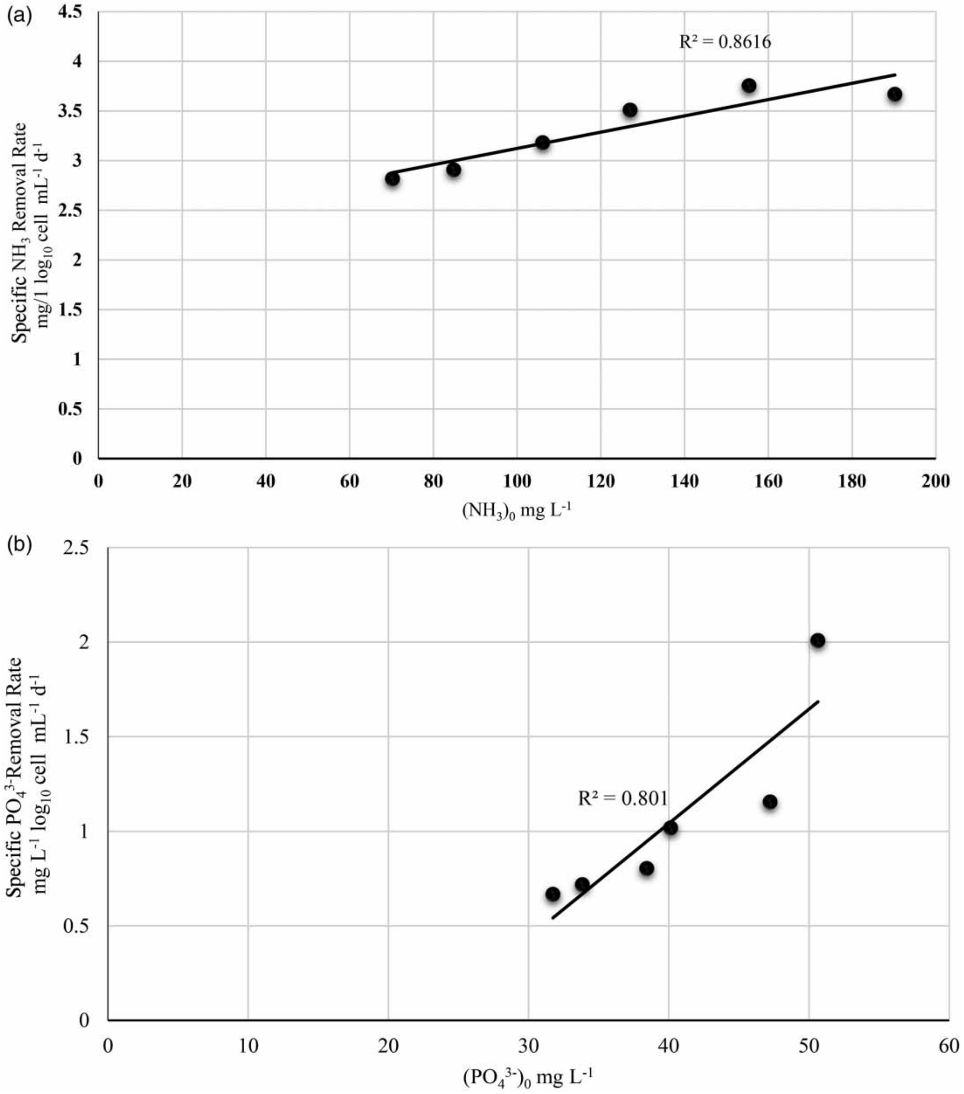

Figure 9 | (a) Effect of initial concentration of NH_3 on the specific removal by *Scenedesmus* sp. with initial concentrations of 8 $\log_{10}$ cell mL^{-1}. (b) Effect of initial concentration of PO_4^{3-} on the specific removal by *Scenedesmus* sp. with initial concentrations of 8 $\log_{10}$ cell mL^{-1}.

0.66 mg PO_4^{3-} 1 $\log_{10}$ cell mL^{-1} d^{-1} at 31.74 mg L^{-1} to 2.01 mg PO_4^{3-} 1 $\log_{10}$ cell mL^{-1} d^{-1} at 50.64 mg L^{-1}. These differences would be related to the affinity of *Scenedesmus* sp. to assimilate NH_4^- and PO_4^{3-} as well as the nature of the batch reactor which was conducted in the non-aerated system. These conditions are in favour of the NH_3 due to the NH_3 volatilization while it leads to the precipitation of PO_4^{3-}, and thus poor distribution of *Scenedesmus* sp. and PO_4^{3-} in the public market wastewater and poor contacts between microalgae cells and PO_4^{3-} substrate. These findings are similar to that reported by Aslan & Kapdan (2006) as well as by Lau *et al.* (1998), in which the specific removal rate of NH_3 from synthetic wastewater samples by *C. vulgaris* was more than that for PO_4^{3-}. Jimenez-Perez *et al.* (2004) revealed that *Scenedesmus* sp. achieved more removal for nitrogen than that for phosphorus. However, Wang *et al.* (2013) revealed that the removal of phosphorus from real municipal wastewater samples by *Chlorella* sp. was more than that of nitrogen.

The slope of specific growth rate of *Scenedesmus* sp. versus the remaining concentrations of NH_4^- and PO_4^{3-} are presented in Figure 10(a) and 10(b), which indicated that the growth rate is correlated with the removal of NH_4^-

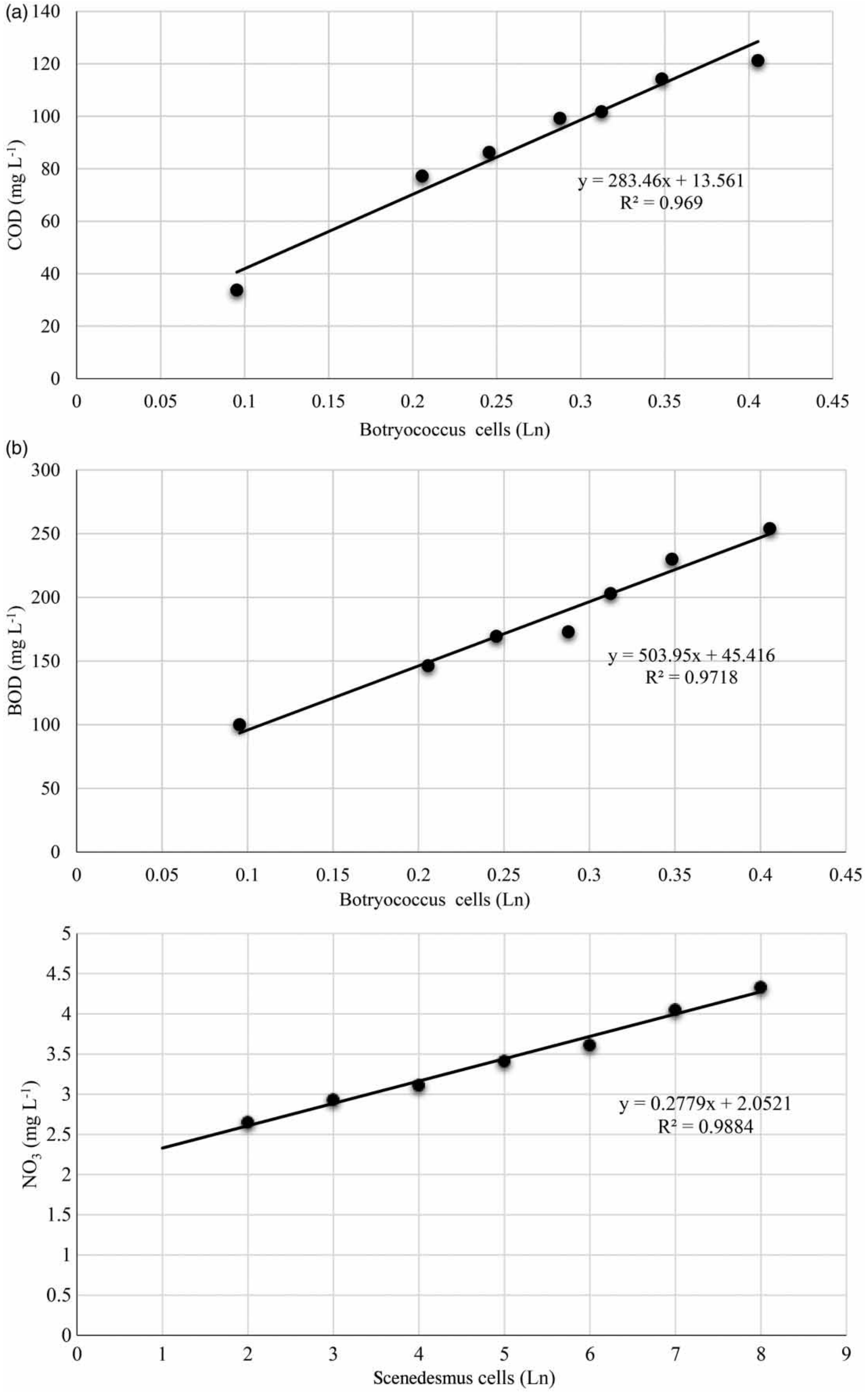

Figure 10 | (a) Determination of coefficient relationship between COD concentrations and *Scenedesmus* sp. specific growth rate. (b) Determination of coefficient relationship between BOD$^-$ concentrations and *Scenedesmus* sp. specific growth rate.

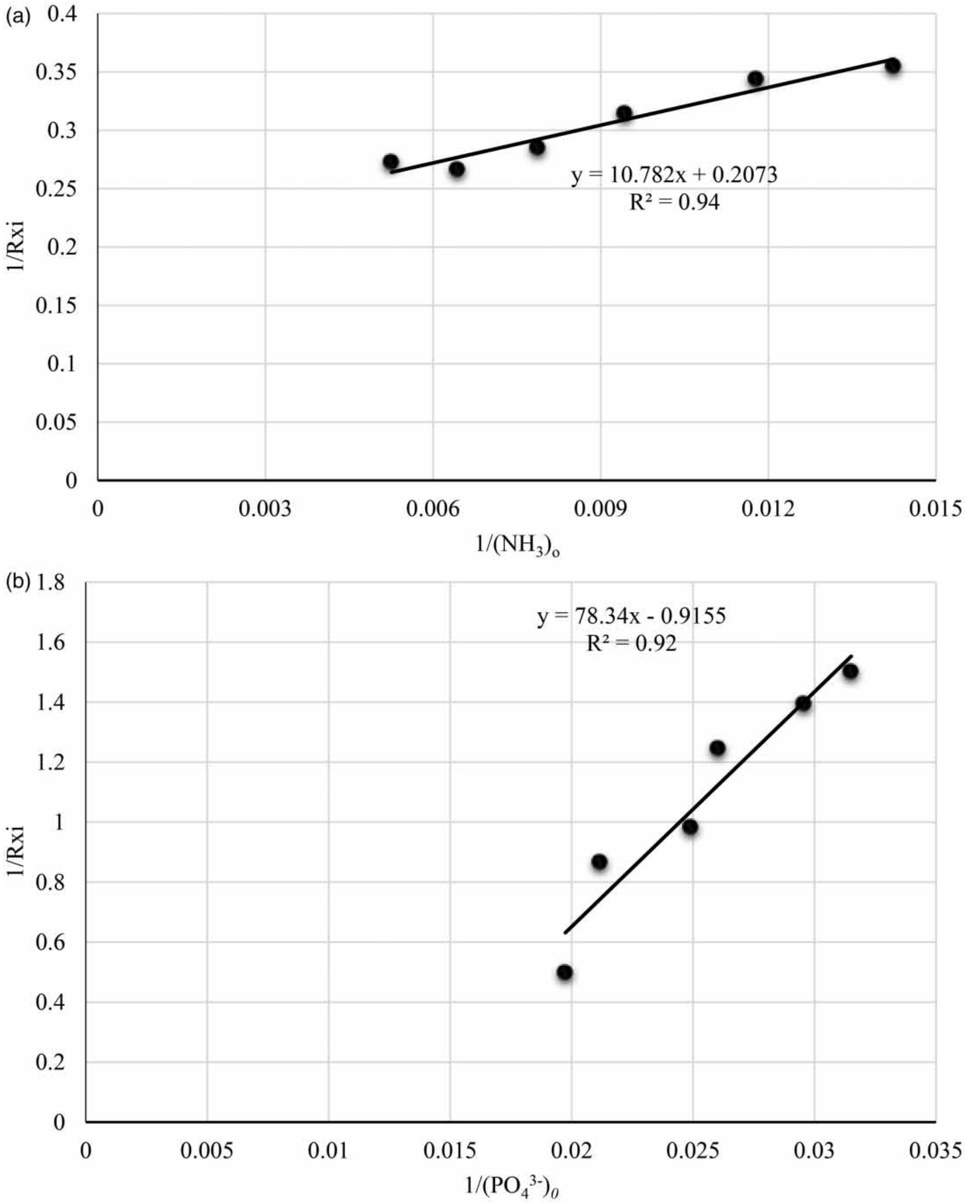

Figure 11 | (a) Effect of NH_3 concentrations on specific removal rate by *Scenedesmus* sp. k = 4.82, km = 52. (b) Effect of PO_4^{3-} concentrations on specific removal rate by *Scenedesmus* sp. k = 1.09, km = 85.56.

($R^2 = 0.64$) and PO_4^{3-} ($R^2 = 0.67$). The values of R^2 in the specific growth rate of *Scenedesmus* sp. was less than that presented with the specific removal rate of both NH_4^- and PO_4^{3-}. This case can be explained according to Roleda *et al.* (2013) who claimed that the microalgae cells have the potential to sustain metabolic activity even without cell division.

The kinetic coefficient of NH_4^- removal by *Scenedesmus* sp. was calculated according to Equation (8) and determined as $k = 4.28$ mg NH_4^- 1 $\log_{10}$ cell mL^{-1} d^{-1} and $k_s = 52.01$ mg L^{-1} ($R^2 = 0.94$) (Figure 11(a)). The coefficient of PO_4^{3-} removal was noted as $k = 1.09$ mg NH_4^- 1 $\log_{10}$ cell mL^{-1} d^{-1} and $k_s = 85.56$ mg L^{-1} ($R^2 = 0.92$) (Figure 11(b)). The low values of k_m in the kinetic coefficient of NH_4^- in comparison to that for PO_4^{3-} indicated that *Scenedesmus* sp. has high efficiency for NH_4^- removal more than PO_4^{3-}.

The biokinetic model for removal of NH_4^- and PO_4^{3-} by microalgae has been used by many authors in the literature. Biokinetic models are used to determine the potential rate and controlling steps necessary for designing a full scale biosorption process. Many kinetic models have been used to investigate the nutrient removal from various wastewaters. Moreover, the Michaelis–Menten model is used to determine the removal of nutrients. The Michaelis–Menten kinetic relationship is used to determine biokinetic coefficients such as k, reaction rate constant, Ks, half saturation constant, and Y, yield coefficient. The best algal substrate utilization was described more accurately and consistently with this logistic model than the Monod's model (Aslan & Kapdan 2006; Stringfellow *et al.* 2006).

CONCLUSIONS

The study aims to investigate the effects of pathogenic bacteria on the phycoremediation of wet market wastewater by *Scenedesmus* sp. The results revealed that the presence of pathogenic bacteria in the public market wastewater may create competition with microalgae in terms of NH_4^- removal due to the potential of bacteria in using NH_4^- as a nitrogen source. However, these concentrations are reduced by 3–4 log. The removal rate of NH_4^- and PO_4^{3-} and batch kinetic coefficient of *Scenedesmus* sp. results revealed that the microalgae have high efficiency for removal of NH_4^- more than PO_4^{3-}.

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