



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Evaluation of the effect of reaction time on nutrients removal from secondary effluent of wastewater: Field demonstrations using algal-bacterial photobioreactors

Tahseen Sayara^{a,*}, Saed Khayat^a, Jebreel Saleh^a, Peter Van Der Steen^b^a Department of Environment and Sustainable Agriculture, Faculty of Agricultural Sciences and Technology, Palestine Technical University-Kadoorie, PO BOX 7, Tulkarm, Palestine^b IHE Delft Institute for Water Education, Department of Water Supply, Sanitary and Environmental Engineering, PO BOX 3015, Delft, the Netherlands

ARTICLE INFO

Article history:

Received 19 August 2020

Revised 13 October 2020

Accepted 19 October 2020

Available online 28 October 2020

Keywords:

Photobioreactor

Wastewater

Nutrients removal

Algal-bacterial

Reaction time

ABSTRACT

Real field demonstrations to assess the removal efficiency of nutrients and organic matter from domestic wastewater were carried out using algal-bacterial photobioreactors. The reactors which consisted of three basins of 200 L were fed with secondary effluent of domestic wastewater and operated under natural day light/dark cycles. The results demonstrated that reaction time (RT) has a substantial role on the whole process performance. Whereas inoculation with nitrifiers affected the process only in some aspects. The enhancement in the dissolved oxygen production rate ($1.15 \text{ mg O}_2 \cdot \text{L}^{-1} \cdot \text{h}^{-1}$) was in alignment with growing higher algal biomass concentrations due to the increase in RT. COD removal rates were significantly increased ($p < 0.05$) with increasing the RT, and removal rates of 27%, 46% and 50% were obtained under RTs of 2, 3 and 4hrs. respectively. Meanwhile, 30%, 84% and 95% of the phosphorus was removed under the same studied RTs. No significant effect was recorded due to the addition of nitrifying bacteria on the removal of both COD and phosphorus. Ammonium ($\text{NH}_4\text{-N}$) removal rates were also increased with increasing RT and by the addition of nitrifiers, such that removal rates of 13%, 21% and 31% were obtained in basins inoculated with nitrifiers, but 11%, 14% and 19.5% were obtained in non-inoculated basins under RT of 2, 3, and 4 hrs. respectively. These results provide some new insights into algal-bacterial symbiosis systems under real field conditions which could be helpful for further process development.

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1. Introduction

Conventional wastewater treatment technologies like activated sludge are being widely used all over the world. However, this aerobic conventional technology is characterized by its extensive energy consumption mainly due to mechanical aeration that accounts for about 45–75% of the total used energy (Manser et al., 2016; Nordlander et al., 2017). Moreover, this technology is sometimes associated with other drawbacks like producing a bulky sludge that is characterized by its poor settleability, which

in turn prolong reaction time for particles sedimentation. Also, the different emissions resulting from the process, particularly CO_2 would have a role in expanding the global warming issue (Mamais et al., 2015; Van Den Hende et al., 2014). On the opposite side, anaerobic technologies are characterized by their inferior capacity in nutrients removal, and even in some cases nutrient effluent concentrations were found to be higher than influent ones (Khan et al., 2011).

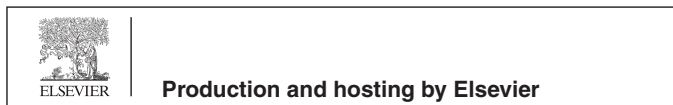
Advanced wastewater treatment for nutrients removal are usually applied in conventional plants, where nitrification and denitrification process are employed for nitrogen removal. Nevertheless, and as suggested by simplified nitrification reactions, a large amount of oxygen is needed for this process ($4.2 \text{ g O}_2/\text{g NH}_4\text{-N}$), and typically at least 2 mg/L of oxygen is required for nitrification. On the other hand, if denitrification process is placed after activated sludge and nitrification in series, an external carbon source is normally needed to be re-introduced into the water.

Some of the aforementioned drawbacks of conventional approaches in wastewater treatment and nutrient removal could

* Corresponding author.

E-mail address: t.sayara@ptuk.edu.ps (T. Sayara).

Peer review under responsibility of King Saud University.



be overcome by the application of algal-bacterial symbiotic association, which is recognized as sustainable and cost-effective strategy in wastewater treatment (Shahid et al., 2020). Indeed, this approach is receiving more attention as an alternative technology for wastewater treatment, whereas, high rate algal ponds are the most common configurations used in such type of treatments (Lu et al., 2020; Manser et al., 2016; Shahid et al., 2020; Van Den Hende et al., 2014). The process fundamentally depends on the intrinsic symbiotic interactions between algae and bacteria within the treatment mixture. In this context, nitrogen and organic carbon in wastewater are oxidized by bacteria using oxygen that is produced by algal photosynthesis activity, meanwhile, the produced CO₂ from bacterial activity is consumed by algal cultures (Gonzalez-Fernandez et al., 2011; Manser et al., 2016). Accordingly, this cycle process is assumed to contribute effectively in the simultaneous removal of nutrients and organic carbon from wastewater with minimum costs, as well as mitigate greenhouse gas emissions (Lu et al., 2020; Shahid et al., 2020). However, it should be noted that the efficiency of this approach is highly dependent on the interactions between various biotic and abiotic factors (Lu et al., 2020; Meng et al., 2018). For instance, Valigore et al. (2012) studied the stability of algal-bacterial system under different food to microorganism ratio and reaction time (RT) and demonstrated that the system works better under longer RT (4 days). Furthermore, the removal rate of pollutants was enhanced by increasing the light intensity (Meng et al., 2018).

Rada-Ariza et al. (2016) found that this symbiotic process under field conditions in a high-rate algae pond and treating wastewater with 54 mg NH₄⁺-N L⁻¹ could remove on average only 0.7 mgNH₄⁺ https://elsevier.proofcentral.com/en-us/landing-page.html?token=e732ce42003cb651757810f3c8df2a⁻¹h⁻¹ with a hydraulic retention time of 2.9–3.9 days due to the low temperature of 16 °C that reduced the nitrification rates and algae growth. At laboratory scale and 28 °C however, Rada-Ariza et al. (2019) found near complete nitrification by a micro-algal bacterial consortium. It was also found that the solids-retention time of the consortium was a crucial operational parameter. If the solids-retention time was too high, this resulted in too high algae concentration resulting in poor light penetration and low oxygen concentrations, hindering ammonium removal via nitrification. Further process optimization was shown at laboratory scale by Wang et al. (2015), who showed that short-cut nitrogen removal through oxidation to nitrite and denitrification is possible in algal-bacterial consortia.

However, and to the best of our knowledge, these promising laboratory results have not been confirmed under real open-field conditions. Consequently, in this present investigation work, a photo-bioreactor was designed, constructed and operated in the field in order to study the performance of this system under such conditions. Meanwhile, the work aimed to assess the effects of different reaction times and nitrifiers inoculation on nutrients (N, P) and organic carbon (COD) removal rates, biomass and oxygen production and the combined effect of the different physico-chemical factors. The reactor was fed with real secondary effluent of domestic wastewater and was operated under natural day light/dark cycles.

2. Materials and methods

2.1. Photobioreactor

For algal-bacterial symbiosis treatment of wastewater, a photobioreactor was designed and constructed in the field as part of the research wastewater treatment plant of Palestine Technical University, Tulkarm, Palestine. The reactor was operated under sequencing batch reactor (SBR) mode and exposed to natural

light/dark day cycles and environmental conditions. It consisted of 3 identical basins (Fig. 1), which were designed and constructed using straight high quality acrylic fiberglass plastic bathtub (115*50*35 cm) with a working volume of about 200L. Each basin was equipped with electrical water level sensors to control filling and drainage of water to the desired levels, and was also supplied with water pumps for filling and drainage of wastewater. The basins top layer is 30 cm deep and is equipped with a propeller mixer (60 rpm), to provide continuous agitation to keep the biomass in suspension and ensure the frequent exposure of the algae to sunlight, whereas, the bottom layer is a 5 cm layer of gravel to allow the growth of a biofilm with denitrifying bacteria. The reactor operation for all phases and steps was automatically programmed using PLC system, and mechanical aeration was totally replaced by algae photosynthesis.

2.2. Wastewater and inoculation

Domestic wastewater from Tulkarm municipal sewer network was used during the treatment experiments. As shown in Fig. 1, the raw domestic wastewater passes through a grit removal unit, then to a sedimentation tank, after that it goes to stabilization pond units. Secondary effluent from the anaerobic pond was used as the influent for the photobioreactor (Fig. 1).

Inoculating with water from an outdoor pond containing a wide spectrum of algae and other species is recognized as the easiest way to start a microalgae treatment process. In this way the best suited species will grow faster and dominate the treatment units. Therefore, the basins were inoculated with a mixed culture obtained from the top surface of the stabilization pond fed with domestic wastewater. In this regard about 5 L of wastewater containing a mixture of various algae strains (that naturally dominate the ponds surfaces) were inoculated directly to each basin. This approach is considered more robust and resistant to variation in the surrounding environment and requires less supervision and operation than if a particular alga species is chosen to be cultivated for any purpose. A drawback is that the faster growing microalgae are most often unicellular green algae which are difficult to be harvested (Mara and Pearson 1998; Tang et al., 1997). Additionally, in one basin, about 3 L of inoculant containing nitrifying bacteria (denoted as (+Ntf.)) were introduced in order to assess their role in the process performance and enhancement. These nitrifiers consisted of both *Nitrosomonas* and *Nitrosospira* genera as ammonia oxidizing bacteria (AOB) and *Nitrobacter* and *Nitrospira* genera as nitrite oxidizing bacteria (NOB), were obtained from the activated sludge of Nablus-West wastewater treatment plant, Nablus, Palestine.

2.3. Photobioreactor operation

For nitrogen removal under the applied SBR operational conditions, it is assumed that ammonium will be oxidized to nitrite through nitrification process. Therefore, a mixture of ammonium and nitrite will be present in the top layer and will be transported by turbulence and diffusion into the gravel layer, and the dual action of algal and bacterial cultures will work simultaneously to remove the different forms of nitrogen.

After inoculation, the three basins were operating in parallel and fed with secondary effluent of wastewater during the course of the experiment. Before initiating the daily operation cycles which start at 8:00 am, about 60 L are drained from each basin, after that, they are filled (Fill phase) with the same quantity (60 L) of secondary effluent of wastewater (top 10 cm of the basin active volume) into the 140 L of mixed liquor that remains from the previous cycle. The influent during this phase brings food to the microbes in the reactor, creating an environment for biochemical reactions to take place. The second phase "React phase" which

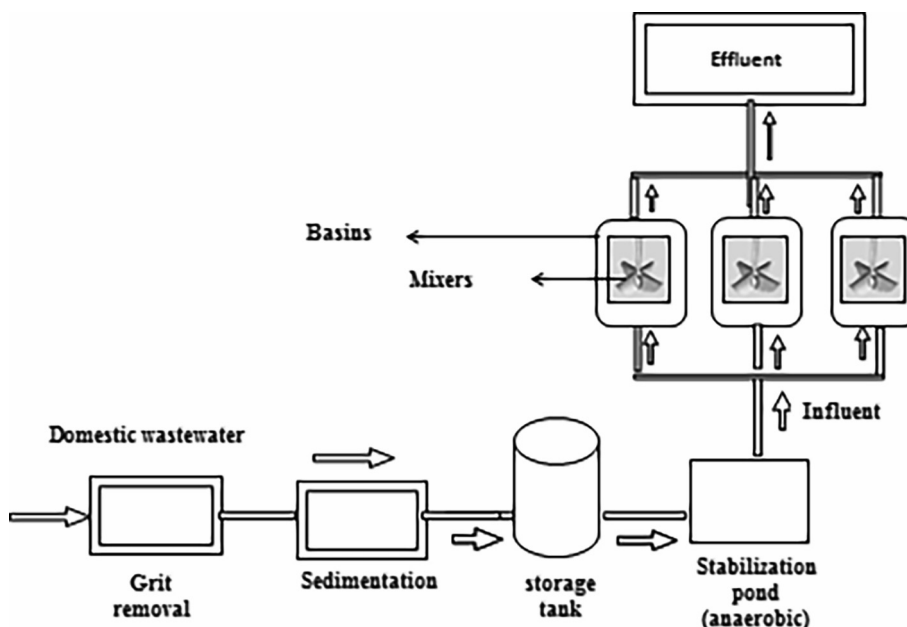


Fig. 1. Schematic diagram of the photo-bioreactor.

is responsible for reduction of wastewater parameters, begins immediately after the fill phase, and the reactor is left with mixing according to the set reaction times (RTs) which were set to be 2, 3 and 4 h for each cycle. Then the mixers are stopped and samples are taken from the basins for analysis, while wastewater is left to settle for 15 min (Settle phase). After that, a decant phase was employed such that the supernatant of the treated wastewater (the top 10 cm) was drained from each basin, and then they were refilled with secondary effluent and starting a new cycle of treatment. During every daylight, several consecutive cycles were performed depending on the set RT (4, 3 and 2 cycles for RT of 2, 3 and 4 h respectively), and in the last cycle and after drainage the top 10 cm, the mixers were operated again, and additional 5 cm were drained in order to discharge part of the algal-bacterial biomass to maintain certain level of total suspended solids. After discharging 15 cm of water from the total depth, the basins are refilled again with secondary effluent and left with mixing all the night.

2.4. Analytical methods

Samples were periodically collected from influent and effluent for further analysis needed for process monitoring and evaluation. Accordingly, total suspended solids (TSS) were measured based on standard gravimetric method (USEPA 8158). Concentrations of Nitrate (NO_3^- -N), Ammonium (NH_4^+ -N), Nitrite (NO_2^- -N), were determined spectrophotometrically using HACH DR 6000. HACH vials LCK 340, LCK 303, and LCK 342 were used respectively according to manufacturer's instructions. Dissolved oxygen (DO) and pH were measured daily using oxygen meter Lutron device (DO – 5510) and HACH pH (HQ 11d) meter respectively. Chemical oxygen demand (COD) was analyzed according to method 410.1 (Titrimetric). Organic and oxidizable inorganic substances in the sample were oxidized by potassium dichromate in 50% sulfuric acid solution at reflux temperature. Silver sulfate was used as a catalyst and mercuric sulfate was added to remove chloride interference. The excess dichromate was titrated with standard ferrous ammonium sulfate, using orthophenanthroline ferrous complex as an indicator. Spectrophotometer analysis was carried out at 320nm for the determination of algal biomass growth rate within the reactor.

Meanwhile, light microscope at 10X and 40X focus was used to study algal species morphology, color, shape of cells and motility.

The experiments were carried out in September 2019, where average daylight and night temperatures were 29 ± 2 °C and 24 ± 2 °C respectively, average light radiation time and dark time were 13 and 11 h respectively and average solar radiation during that period was 0.18 ± 0.03 kW/m². The duration of the experiment was 10 days for each set RT (2, 3 and 4 h), and One-Way ANOVA test was used for statistical analysis of the obtained results.

3. Results

Before initiating the experiments, the reactor was left to work for two weeks, until stable algal – bacterial symbiotic state was observed. In this regard, the reactor was filled and drained (2 cycles of 4 h RT per day) until algal-bacterial cultures were developed. After that, removal efficiencies for nutrients and organic matter under different set RTs were investigated. The reactor worked under natural daily conditions regarding solar radiation and air temperature.

3.1. Algal growth

Biomass evolution within the basins was measured through different analysis. As illustrated in Fig. 2, the average initial algal biomass concentrations in the basins (measured as absorbance at 320 nm) was 0.31 ± 0.03 , 0.34 ± 0.06 and 0.4 ± 0.01 for basins with RT of 2, 3, and 4 h respectively. These concentrations were found to be increased to reach 0.52 ± 0.02 (+67%), 0.68 ± 0.02 (+100%) and 0.74 ± 0.03 (+85%) for basins that were inoculated with nitrifiers (+Ntf) under RT of 2, 3 and 4 h respectively. However, the values were 0.48 ± 0.01 (54%), 0.64 ± 0.01 (88%) and 0.62 ± 0.06 (55%) for non-inoculated basins under the same RT respectively. The same trend was also observed when culture biomass was measured through turbidity and TSS. For instance, the average initial turbidity values were around 25 ± 3 NTU, and increased to reach 91 ± 3 , 89 ± 3.5 and 97 ± 3 in basins with nitrifiers and 80 ± 2 , 79 ± 4 and 82 ± 4 in basins without nitrifiers (Fig. 3). Analysis of algal species demonstrated that *Chlamydomonas*, *Spirogyra*, *Euglena* and *Dinoflagellates* are the dominant species in all basins. These species

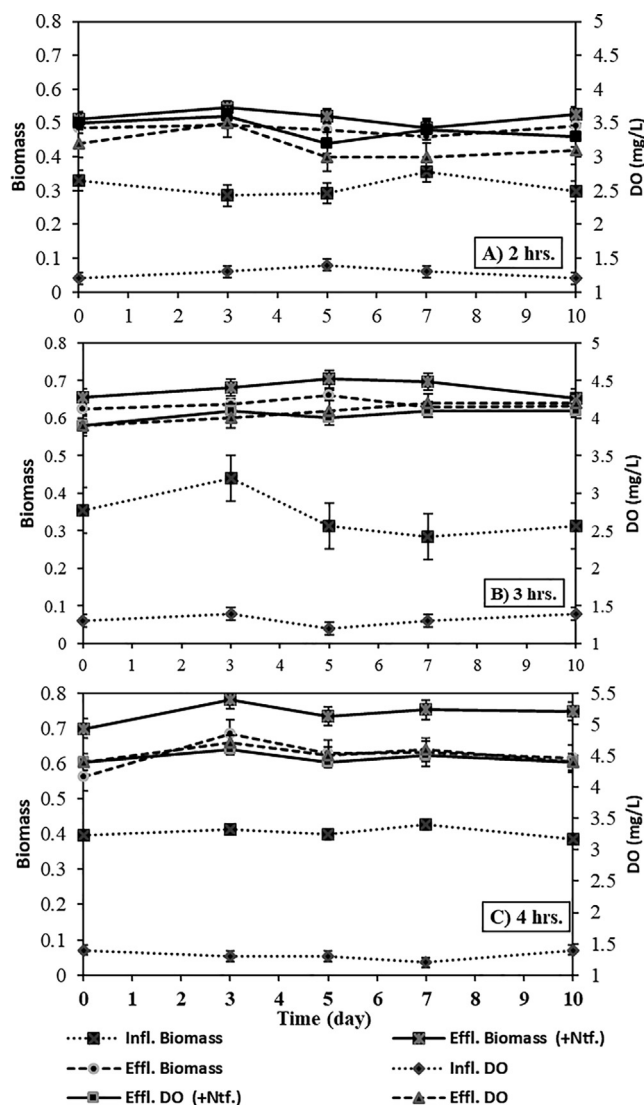


Fig. 2. Influent and effluent biomass (absorbance at 320 nm) and dissolved oxygen (DO) concentrations (mg/L) under: A) 2 hrs. RT, (B) 3 hrs. RT and (C) 4 hrs. RT. (+Ntf) represents treatments with nitrifiers inoculation.

are unicellular that can use a mix of different sources of energy and carbon, instead of having a single trophic mode. These figures show that an almost stable state with a stable number of cultures inhabited the basins throughout the experiment, and no external impact of the surrounding environment has been observed to affect the adaptation of the algae to practical conditions.

3.2. Dynamics of DO and pH in the reactor

Due to the photosynthetic activity of algal cultures, the concentration of DO began to increase in all basins and under all studied RTs (Figs. 2 and 4). This would indicate the prevalence of such activity over heterotrophic carbon-oxidation and nitrification (Delgadillo-Mirqueza et al., 2016). Initial concentrations of DO (mg/L) at the start of the reaction time were around 1.3 ± 0.1 , and by the end of each experiment, the average values of DO were 3.4 ± 0.16 , 4.0 ± 0.06 and 4.5 ± 0.09 respectively for RT of 2, 3, and 4 h in treatments inoculated with nitrifiers (+Ntf). In treatments with no nitrifiers inoculation, the DO concentrations (mg/L) were 3.16 ± 0.2 , 4.0 ± 0.13 and 4.5 ± 0.1 respectively. A significant increase in DO concentration ($p < 0.05$) was observed between

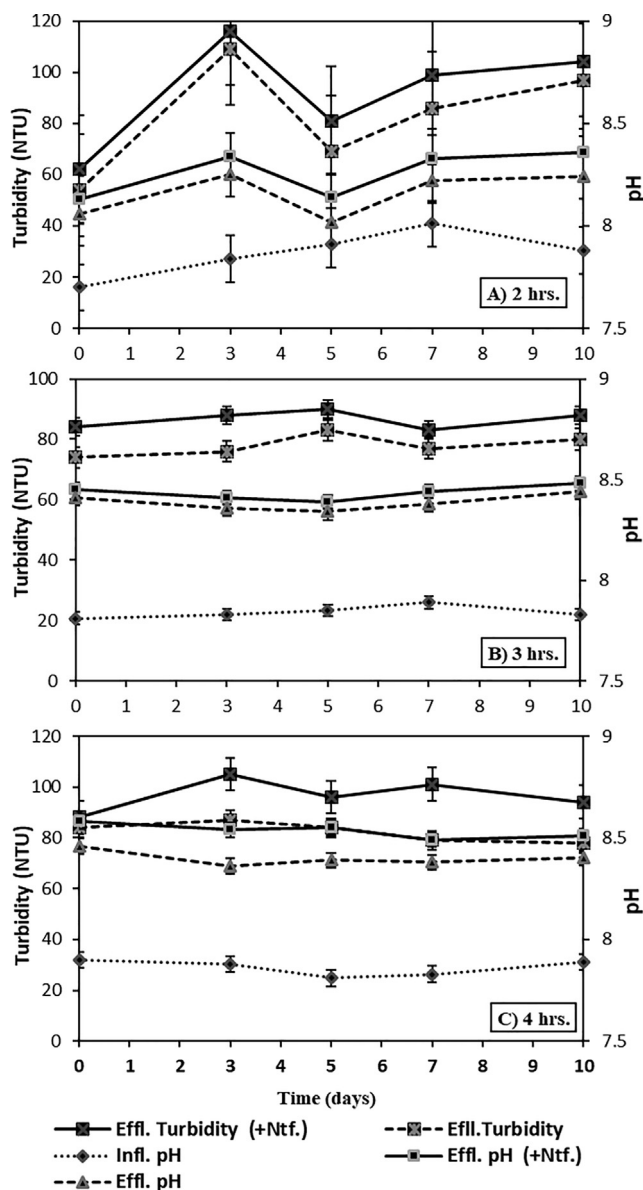


Fig. 3. Influent and effluent turbidity (NTU) and pH values under: (A) 2 hrs. RT, (B) 3 hrs. RT and (C) 4 hrs. RT. (+Ntf) represents treatments with nitrifiers inoculation.

initial and final concentrations in all experiment and under all conditions, but no significant differences ($p > 0.05$) were observed between the nitrifiers inoculated and non-inoculated treatments. The evolution of DO concentration within the basins during one cycle treatment is shown in Fig. 4. The increase in the DO production rate was estimated to be about $1.15 \text{ mg} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ which was in alignment with the observed increase in the biomass concentration and especially the algal biomass (Fig. 2) that is responsible for producing oxygen during its photosynthetic activities (Meng et al., 2018). Actually, the increase in DO is fit with the minimum required oxygen concentration for class B reclaimed water that can be used for irrigation according to Palestinian standards for treated wastewater reuse which is about 4 mg/L. In parallel with the increase in biomass and DO concentrations, pH values were found to be increased also (Fig. 3). Initial values of pH were around 7.8 ± 0.04 and increased to reach about 8.4 ± 0.04 . An additional slight increase of almost 0.1 pH units was observed in basins inoculated with nitrifiers and at all studied RTs, noting that no pH adjustment was applied in the experiment at all.

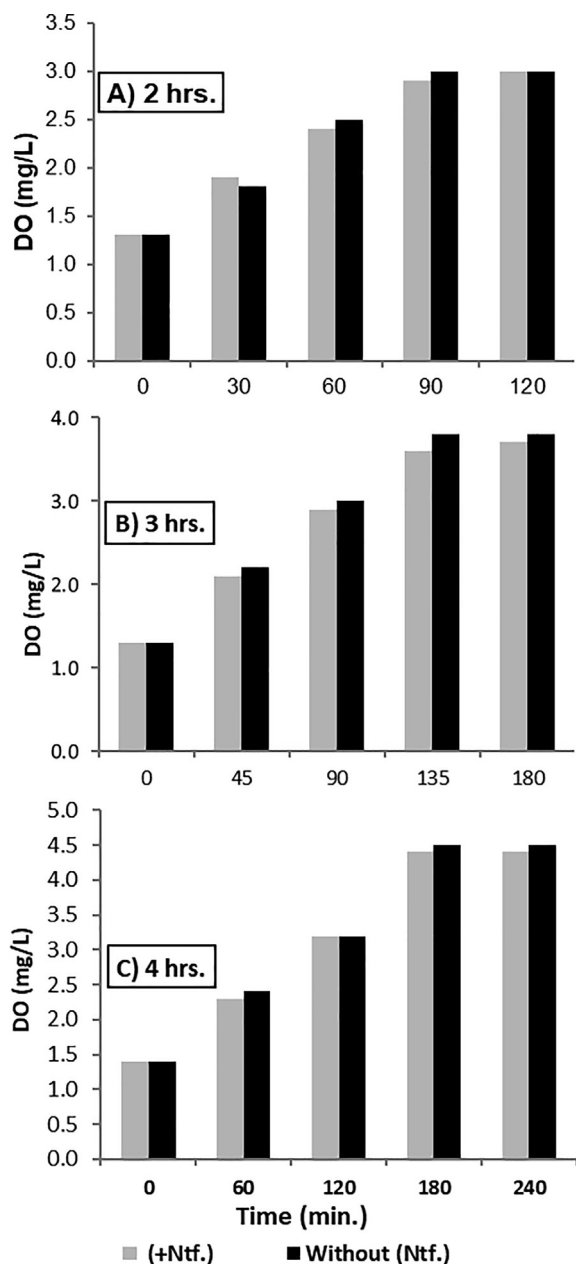


Fig. 4. Dissolved oxygen (DO) evolution in mg/L for one cycle treatment under: (A) 2 hrs. RT, (B) 3 hrs. RT and (C) 4 hrs. RT. (+Ntf) represents treatments with nitrifiers inoculation.

3.3. Nutrients and organic matter removal

A symbiotic interaction between algae and bacteria was observed within the basins. The produced oxygen by algal photosynthesis was used by bacteria to biodegrade organic matter. This was clear in the reduction of COD values during the experimental period and under all studied conditions (Fig. 5). Influent COD ranged between 830 and 860 ± 23 mg/L. Although slight enhancement was observed, no significant differences in COD removal rates were recorded due to the addition of nitrifiers. However, COD removal rates were significantly increased ($p < 0.05$) with increasing the RT. In this context, COD removal rates of 27%, 46% and 50% were obtained under RT of 2, 3 and 4 h respectively, which are considered promising rates especially because influents COD concentrations were elevated (830–860 ± 23 mg/L). It is obvious as appears in Fig. 5 that a relatively stable removal rate of COD was

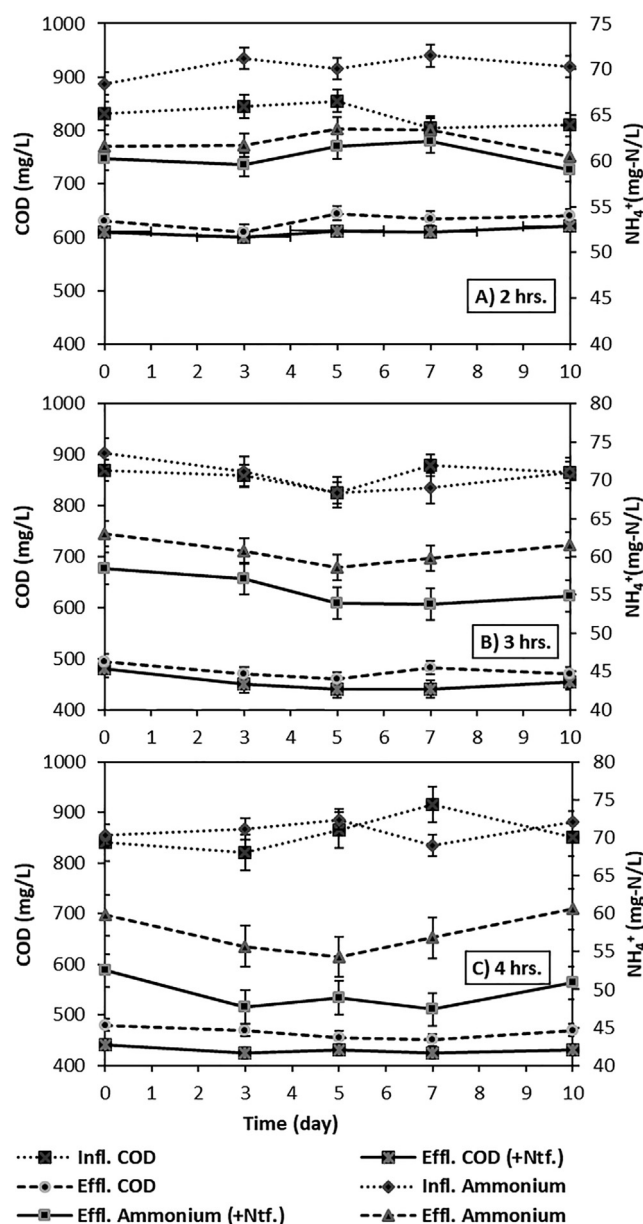


Fig. 5. Influent and effluent COD (mg/L) and ammonium ($\text{NH}_4^+\text{-N}$) concentrations (mg/L) under: (A) 2 hrs. RT, (B) 3 hrs. RT and (C) 4 hrs. RT. (+Ntf) represents treatments with nitrifiers inoculation.

maintained during the experiment and under the different RTs. This observation could be used again to prove that algal-bacterial system achieved the desired symbiosis and could run under almost stable conditions.

Regarding phosphorus removal, a remarkable decrease in phosphate concentration was observed, and the removal rate was found to be proportional with RT (Fig. 6). Influent phosphate concentrations were around 22.3 ± 1.5 mg/L, and a removal rates of 30%, 84% and 95% in nitrifiers-inoculated treatments were obtained under RT of 2, 3 and 4 h respectively. Importantly, despite inoculated treatments achieving higher removal rates, these differences were not significant ($p > 0.05$) compared with non-inoculated treatments that achieved 22%, 79% and 89% removal rates under the same RT. The obtained phosphate removal rates could be attributed to the collectivity of various parameters; the increase in the algal-bacterial cultures that adsorbed part onto the cell surfaces, followed by biomass assimilation for part of the phosphate

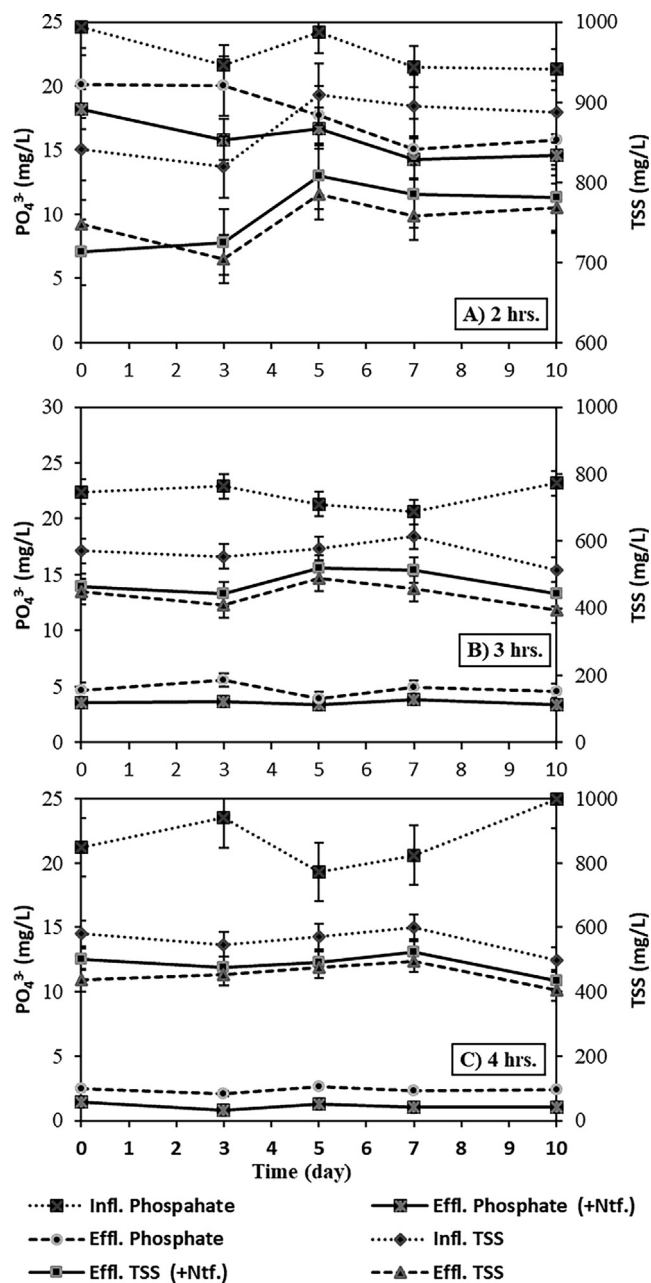


Fig. 6. Influent and effluent phosphate (PO_4^{3-}) and total suspended solids (TSS) concentrations (mg/L) under: (A) 2 hrs. RT, (B) 3 hrs. RT and (C) 4 hrs. RT. (+Ntf) represents treatments with nitrifiers inoculation.

ions. The remaining portion is presumably undergoing chemical precipitation, particularly due to high pH values (Fig. 3) which normally enhance phosphorus precipitation through the formation of hydroxyapatite (Kim et al., 2013; Su et al., 2012).

According to Fig. 5, influent NH_4^+-N was around 71 ± 1.5 mg/L. These concentrations were decreased due to the symbiotic action of the algal- bacterial system, and removal rates of 13%, 21% and 31% were obtained in basins with nitrifiers, but 11%, 14% and 19.5% were obtained in basins without nitrifiers under RT of 2, 3, and 4 h for both conditions respectively. It is obvious from these results that NH_4^+-N removal rates were significantly increased by increasing the RT under both conditions (with/without nitrifiers). However, it was apparent that basins with nitrifiers achieved significant higher removal rates of NH_4^+-N compared with non-inoculated ones. Additionally, under all the studied conditions,

the decrease in ammonium (NH_4^+-N) concentrations was accompanied by an increase in nitrate (NO_3^-) and nitrite (NO_2^-) concentrations (Fig. 7). Influent concentrations of NO_3^- were around 0.6 ± 0.1 mg/L, however, effluent concentrations in nitrifiers inoculated basins were 0.9 ± 0.04 , 1.2 ± 0.05 , 1.6 ± 0.04 and in the effluent of non-inoculated basins were 0.9 ± 0.04 , 1 ± 0.02 , 1.1 ± 0.08 for RT of 2, 3 and 4 h respectively. Almost no difference was observed under RT of 2 h for both conditions, and this trend continued with non- inoculated basins where a slight increase took place to reach 1.1 mg/L. The significant difference was clear in inoculated treatments themselves and particularly among RT 3 and RT 4, also, a similar significant difference was observed between inoculated and non-inoculated treatments. For nitrite (NO_2^-), similar performance as nitrate was recorded under RT 2, nevertheless, a gradual increase was noticed under both treatments with RT of 3 and RT 4,

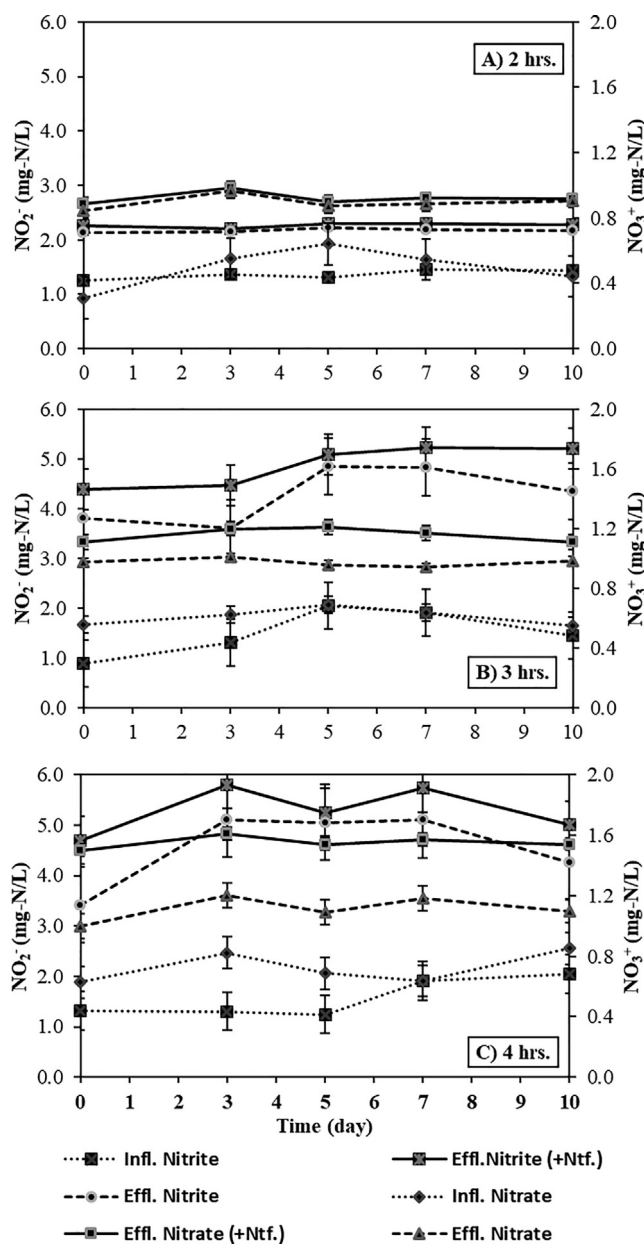


Fig. 7. Influent and effluent nitrate (NO_3^-) and nitrite (NO_2^-) concentrations (mg/L) under: (A) 2 hrs. RT, (B) 3 hrs. RT and (C) 4 hrs. RT. (+Ntf) represents treatments with nitrifiers inoculation.

but significant difference ($p < 0.05$) was clear between inoculated and non-inoculated treatments.

A mass balance for nitrogen removal under nitrifiers inoculated treatment and RT of 4 h shows an ammonium decrease of 22 mg-N/L, while nitrate and nitrite increased by 0.83 and 3.74 mg-N/L respectively, indicating that about 17.4 mg NH_4^+ -N/L was consumed by algal uptake and/or denitrification. However, only 10.6 mg NH_4^+ -N/L were consumed by these two processes under non-inoculated treatments, which implies that these treatments were not able to develop enough nitrifiers that could produce nitrite or nitrate for subsequent denitrification. It should be noted also that the effect of RT was clear on the rate of ammonium consumption which was enhanced by increasing the RT (as mentioned before). In this context, and under nitrifiers inoculated conditions, mass balance indicated that ammonium removal by algal uptake and denitrification was 7.7, 11.1 and 17.4 mg NH_4^+ -N/L under RT of 2, 3 and 4 h respectively.

The total areal removal of ammonium-nitrogen (total daily load in g-N/m² multiplied by the ammonium removal efficiency) was ranging from 4.6 g-N/m²/day removal for RTs of 2 (4 feedings) and 4 h (2 feedings) to 5.3 g-N/m²/day removal for RT of 3 h and 2 feedings. Assuming that one person produces 10 g-N per day, the required area per person for full nitrogen removal is around 2 m². This is still substantially higher than for activated sludge systems, but much less than for waste stabilization ponds.

4. Discussion

Algal growth all over the period of the experiment showed that the biomass increased in all basins, but it was more pronounced with RT of 3 h. Furthermore, it was clear that inoculation with nitrifiers enhanced the algal growth compared with non-inoculated basins. The observed differences in algal biomass concentration between RT 3 and RT of 4 could be attributed to inhibition conditions that could be created by elevated DO concentrations (Fig. 2) and low CO₂ in the case of longer RT (Huang et al., 2015; Zhang et al., 2020) or due to high temperatures (Delgadillo-Mirqueza et al., 2016).

The dominant developed local algal populations seemed to be adapted to bacterial communities in the basins, and the whole system exhibited a great compatibility among all components. The observed increase in pH values between feeding and reaction phases indicates intensive photosynthetic activity following the feeding event, and confirm that algal species consumed the produced CO₂ during their photoautotrophic activities and supply the reactor with O₂ (García et al., 2006; Mata et al., 2010; Shayan et al., 2016), which ultimately achieving an efficient algal-bacterial symbiosis leading to nutrients and organic matter removal, along with reducing operational cost and greenhouse gas emissions (Delgadillo-Mirqueza et al., 2016; Shahid et al., 2020). Collectively, the trend of the observed results throughout aforementioned parameters demonstrated the adequacy of the used reactor to enhance and optimize algal activity, and a matching between algal growth and O₂ production with the chemical oxygen demand (COD) of the receiving wastewater was achieved. It was clear that increasing the RT and addition of nitrifiers enhanced in general the performance of the different parameters which would be reflected on nutrient and organic matter removal (Muñoz and Guieysse 2006; Tang et al., 2018).

The obtained moderate results regarding removal of NH_4^+ -N could be attributed to several factors including elevated pH which normally lead to incomplete nitrification (Shayan et al., 2016), or high temperature values as most algal cultures normally prefer temperatures around 25 °C (Delgadillo-Mirqueza et al., 2016). The reduction in NH_4^+ -N concentrations is referred to the

algal-bacterial activities within the reactor which assimilate these nutrients and others to synthesize new biomass, and as energy source also which by the end leads to promote nitrogen removal. Actually, these results are well correlated with algal biomass growth and the trend of other parameters like DO and pH during the entire experiment.

Among the different nitrogen forms present in the basins, particularly ammonium was the most favorable one by algae and bacteria due to its reduced state and energetically favorable assimilation which ultimately lead to reduce its concentrations (Eustance et al., 2013). On the other side, the observed increase in the nitrite concentrations is mainly attributed to the nitrification process in which NH_4^+ is converted to NO_2^- by aerobic ammonium-oxidizing bacteria. Simultaneously, the available ammonia is converting to NO_3^- throughout nitrification and thus increasing its concentration (Mukarunyana et al., 2018). It is apparent that nitrate was not assimilated by algal cultures due the abundance of ammonium which would reduce or even inhibit the enzymes necessary for nitrate reduction. The same behavior was obtained in various studies, which documented a negative effect on nitrate assimilation at both transcriptional and post transcriptional levels in green algal treatments (Fernandez and Galvan, 2008). Actually, when algae absorb NH_4^+ , they immediately incorporate it into amino acids; however, algae can only use NO_3^- after enzymatic reduction to NO_2^- and NH_4^+ , and these enzymatic reactions require cellular energy and thereby affect cell growth. Therefore, algae that use NH_4^+ before NO_3^- may experience inhibition of NO_3^- uptake (Dugdale et al., 2007; Flores et al., 2005). Another explanation could be low CO₂ concentration in the mixture. According to Zhang et al. (2020), stripping of CO₂ resulted in deteriorating algal growth and activity which lead to large amount NO_2^- and NO_3^- . Also, it was argued that competition for CO₂ between algae and autotrophic bacteria might result in reducing nitrifying bacteria efficiency and quantity (Shahid et al., 2020). Throughout the entire experiment and under all conditions, the concentrations of nitrite were almost always in parallel but higher than nitrate concentrations, and in the case of NH_4^+ depletion, algal culture are assumed to utilize NO_2^- before NO_3^- .

The bottom layer of the basins (5 cm of gravel) was supposed to support the growth of denitrifying microbial communities which are responsible for converting NO_3^- to N_2 under anoxic conditions. Nevertheless, and due to the observed concentrations of DO and its evolution in the basins during daylight (Figs. 2 and 4), it would therefore appear imperative that denitrification is likely to be active only in the bottom layer, and consequently, this would be reflected on the rate of nitrate being removed by this process. Furthermore, it is most probably to assume that no nitrification by conversion of nitrite to nitrate took place during the night. The observed removal and transformation of nutrients within the reactor were mostly attributed to the symbioses interaction between algal and bacterial cultures. However, and under real open field conditions, it is noteworthy to highlight that part of the removed nitrogen could be attributed to stripping mainly under elevated pH conditions which promote the volatilization of ammonia under such conditions (Shahid et al., 2020).

Importantly and worth to mention that after finishing the experimental program, the reactor (inoculated with nitrifiers) was left to work without discharging the water, and after 6 days, samples were taken. The analysis showed that a remarkable removal of the nutrients took place during this period, where phosphorous and ammonium achieved a removal rate of 100% and 98.5% respectively, meanwhile nitrate concentration increased by 95%. These observations draw the attention to deeply study the process performance with more retention time, and the evolution of the different parameters under such conditions.

5. Conclusions

The used algal-bacterial photobioreactor demonstrated the efficiency of this approach for nutrient removal from wastewater under real field conditions. The obtained results showed that reaction time (RT) has a key role in the process behaviour, and almost all studied parameters were enhanced by increasing the RT. However, the effect of the inoculation with nitrifiers clearly appeared on the nitrogen removal rates only. Maximum COD removal rates of 50% were achieved under RT of 4 h, while 95% of the phosphorus was removed under the same RT. No significant effect was observed as a result of introducing nitrifiers on the removal of both COD and phosphorus. On the contrary, ammonium ($\text{NH}_4^+\text{-N}$) removal rates were significantly enhanced with the addition of nitrifiers beside their enhancement with increasing RT. Ammonium removal rate of 13%, 21% and 31% were obtained in basins with nitrifiers, but 11%, 14% and 19.5% were obtained in non-inoculated basins under RT of 2, 3, and 4 h respectively.

Acknowledgements

The authors are grateful to the Dutch government for financially supporting this research within the collaboration project: The Palestinian-Dutch Academic Cooperation on Water (PADUCO-2) between Palestine Technical University- Kadoorie (PTUK), Palestine, and the UNESCO-IHE Institute for Water Education, Delft, The Netherlands.

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