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**DOI**
10.1016/j.biteb.2024.101782

**Publication date**
2024

**Document Version**
Final published version

**Published in**
Bioresource Technology Reports

Bioresource Technology Reports 25 (2024) 101782

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

# Full nitrogen and phosphorus removal in the PASDEBPR system

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ARTICLE INFO

*Keywords:*
Microalgae-bacteria consortium
Enhanced biological phosphorus removal
Denitrification
Nitrous oxide

ABSTRACT

Photo-activated sludge (PAS) system aims to utilize microalgae to deliver oxygen for bacterial respiration, eliminating the need for external aeration. However, research on the treatment potential of PAS systems in the removal of nutrients is limited. In this context, a research study was devised to evaluate the possibility of developing a microalgae-bacteria consortium to achieve the simultaneous removal of organic carbon, nitrogen, and phosphorus. A successful PAS system capable of removing phosphorus was established at the end of the first phase, with an effluent phosphorus (P) concentration of 1.6 mg P $L^{-1}$. In the subsequent stage, during the introduction of the nitrification-denitrification process, the system lost stability and deteriorated. Interestingly the system recovered via the sparging of nitrogen gas reaching effluent concentrations of 1.22 mg P $L^{-1}$ and 0.88 mg N $L^{-1}$. Thus, the system was capable of removing phosphorus and nitrogen via biological means without the need for external aeration. It is hypothesized that the inhibition caused was due to the production of a gaseous compound during the nitrification/denitrification process.

## 1. Introduction

Treating wastewater is one of the most visible and sought-after activities in our society in the 20th century with growing awareness about the health and nuisance risks associated with wastewater in the built environment (Henze et al., 2008). One of the major concerns is the accumulation of nutrients (mainly, nitrogen and phosphorus) through wastewater being discharged into the natural environment. Discharging wastewater containing nutrients into water bodies leads to algal blooms causing an imbalance of physicochemical properties resulting in bad taste and foul odor of water and the production of toxins by cyanobacteria (Kehoe et al., 2015; Graham et al., 2016). Hence, with the growing human population and chronic water shortages being witnessed around the globe, the preservation of water bodies in our natural environment is of high significance. The treatment of wastewater has evolved with growing scientific knowledge and research looking at the removal of nutrients.

The conventional activated sludge (CAS) process is the most common biological wastewater treatment method utilized to remove organic carbon, nitrogen, and phosphorus (Gernaey et al., 2004). Phosphorus is commonly removed via the enrichment of polyphosphate-accumulating organisms (PAOs) in wastewater treatment plants (WWTP). PAOs, have a dual metabolism that allows them to over compete ordinary heterotrophic organisms (OHOs). In the absence of electron acceptors, PAOs are capable of intracellular storing volatile fatty acids (VFAs) as polyhydroxyalkanoates (PHAs) by generating adenosine triphosphate (ATP) via the hydrolysis of Poly-phosphate (poly-P). On the contrary, when electron acceptors (such as nitrate, nitrite, or oxygen) are available, PAOs oxidize the stored PHA to replenish their pools of poly-P. These versatile organisms are capable of storing more phosphorus than the one released to the liquid phase, resulting in a net phosphorus uptake of the system. This process is commonly known as the enhanced biological phosphorus removal (EBPR) process (Smolders et al., 1994).

Likewise, nitrogen is biologically removed via 2 groups of organisms. The first group of organisms oxidize ammonia into nitrite and later on nitrate. This group is known as ammonia oxidizers and is mainly autotrophic. The produced nitrate is then reduced to nitrogen gas (considered a complete reduction) by nitrate reducers, a heterotrophic group of organisms (Rada-Ariza et al., 2017).

But, one of the major downsides of the CAS processes is the high operational cost requirements associated with mechanical aeration

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https://doi.org/10.1016/j.biteb.2024.101782
Received 5 February 2024; Accepted 5 February 2024
Available online 16 February 2024

systems (Tandukar et al., 2007; Young and Koopman, 1991). Furthermore, its energy dependency makes this type of system inapplicable to developing countries or rural zones where the energy grids are unstable (Strande et al., 2014). There is an urgent need to conceptualize technologies that are cost-effective and efficient in providing the desired level of nutrient removal.

Owing to the high cost associated with mechanical aeration, there has been a growing interest in microalgae-based technologies used for wastewater treatment. These technologies harness the photosynthetic oxygen produced by microalgae for treating wastewater in the presence of natural light sources (Craggs et al., 2014). Furthermore, microalgae have shown the potential to achieve advanced nutrient removal from secondary treated municipal wastewater (Wang et al., 2017). However, in the case of pure microalgae-based wastewater treatment facilities, the land area requirement is very high which makes them unattractive as compared to activated sludge systems (Craggs et al., 2012). The concept of developing microalgae-bacteria consortia has gained prominence owing to their mutual interaction yielding benefits of nutrient removal in wastewater treatment processes as well as its increased settleability. The photosynthetic oxygen produced by microalgae acts as a key driver of bacterial treatment processes thereby providing effective treatment of wastewater (Muñoz and Guieysse, 2006; de Godos et al., 2009). Moreover, several laboratory-scale studies have been successfully conducted to show the potential of microalgae-bacteria consortia in achieving nitrogen removal (Manser and otros, 2016; Rada-Ariza et al., 2017) and phosphorus (Carvalho et al., 2018; Mohamed and otros, 2021), but not together. Moreover, the studies conducted by Carvalho et al. (2018) depended mostly on external aeration to remove phosphorus biologically. While their last experimental stage where aeration was removed, was operated only for 1 SRT. Thus, limiting the possibility for microbial adaptation and selection. Even further the high VSS/TSS value of 0.8 at the end of this stage, is an indication of low polyp content in the biomass normally associated with changes in the metabolism of PAOs (Welles et al., 2015).

The understanding of microalgae-bacteria consortia to perform nutrient removal can be conceptualized from the equation of algal photosynthesis (Oswald, 1998). The C: N:P ratio provides insight into the nutrient requirements for growing microalgae as well as the potential nutrient assimilation process by the overall microalgae-bacteria consortium. This study aims to develop a microalgae-bacteria consortium that removes carbon, nitrogen, and phosphorus without the addition of external aeration in a photo-activated sludge denitrifying enhanced biological phosphorus removal (PASDEBPR) system.

## 2. Material and methods

### 2.1. Steady-state model design

For setting up the enhanced biological phosphorus removal photo-activated sludge (EBPR-PAS) system, a steady-state model was developed based on the biological phosphorus removal stoichiometry model developed by Wentzel et al. (1990). The optimal conditions that were identified included the composition of synthetic wastewater, light intensity, and operation conditions. The stochiometric model was developed in Microsoft Excel and is provided in Appendix A of the supplementary information. As seen in Mohamed and y otros (2021), the purpose of this model was to determine the composition of synthetic wastewater to achieve a balanced microalgae-bacteria consortium. However, in this study, the composition of synthetic wastewater was modified to contain 150 mg COD $L^{-1}$, 25 mg $NH_4$-N $L^{-1}$, 15 mg $PO_4$-P $L^{-1}$, and 550 mg $HCO_3^-$ $L^{-1}$. In addition to this, the aerobic SRT applied here was 4 days. The resulting solids concentration was estimated to be 3.78 g $L^{-1}$ VSS and 5.78 g $L^{-1}$ TSS, respectively. While the required inorganic carbon concentration was estimated as 467 mg $HCO_3^-$ $L^{-1}$ by the model, the actual concentration in the synthetic feed was 550 mg $HCO_3^-$ $L^{-1}$. This modification was made considering the theoretically estimated 84 mg $HCO_3^-$ $L^{-1}$ that would be retrieved by PAOs as $CO_2$ during the aerobic phase (Smolders et al., 1994).

### 2.2. Reactor configuration and operational setup

For setting up the PASDEBPR system, a cylindrical double-jacketed glass reactor was utilized. The reactor had a diameter of 12.5 cm and an effective volume of 2.5 L. Activated sludge from the wastewater treatment plant (WWTP) located at Harnaschpolder, Den Hoorn, the Netherlands was used for inoculation in this study. 1.24 L of activated sludge was mixed with 100 mL of five algal species. The five species of microalgae were *Scendesmus quadricauda*, *Anabaena vaariabilis*, *Chlorella* sp.,*Chlorococcus* sp., and *Spirulina* sp.

The EBPR-PAS system was operated under alternating dark and light conditions to represent anaerobic and aerobic conditions, respectively. The purpose of illuminating the reactor setup during aerobic conditions was to provide favorable conditions for microalgal growth. The reactor was operated as an SBR comprising four cycles in a day. Each cycle was divided into a 2 h of anaerobic (dark) stage, a 3 h aerobic (light/illuminated) stage, a 0.5 h settling stage, and a 0.5 h decanting (effluent withdrawal) stage. With the help of the ADI controller and BioXpert software (Applikon Delft, the Netherlands), the entire reactor operation including data collection and storage was automated. The contents of the reactor were constantly mixed during anaerobic-aerobic stages using a stirrer operating at 500 rpm. The temperature within the reactor was maintained at 20 ± 1 °C throughout the reactor operation using a LAUDA system (Lauda-Königshofen, Germany). To ensure that the growth of PAOs is favored over the growth of glycogen-accumulating organisms (GAOs), the pH was maintained at 7.5 ± 0.1 (Lopez-Vazquez and otros, 2009). The pH control was done by auto-dosing either 0.4 M HCl or 0.4 M NaOH using peristaltic pumps. The illumination was provided using two light-emitting-diode (LED) lamps (Series E27 rated 75 W, Philips, the Netherlands) each located on opposite sides of the reactor. The SRT was controlled at 8 days by removing 75 mL of MLSS. Half of the working volume from the reactor was removed after the settling time (HRT 12 h). During the start-up, oxygen was supplied during the aerobic stage at a maximum saturation limit of 20 %. Once the bacterial community was able to be sustained via the photosynthetic oxygen supplied, the use of an air compressor was discontinued.

### 2.3. Synthetic media

The influent wastewater supplied in this study was made up of a carbon source, a mineral medium, and deionized water. Each of these individual components was provided in separate containers. The containers containing the carbon source and mineral medium were autoclaved for one hour at 115 °C before being used as influent feed. The final composition of influent wastewater was 400 mg COD $L^{-1}$, 15 mg $NH_4$-N $L^{-1}$, 15 mg$PO_4$-P $L^{-1,}$ and remaining nutrients and trace elements solution as described by Smolders et al. (1994).

### 2.4. Experimental phases

The reactor was first set up to represent an EBPR-PAS system followed by introducing nitrogen removal resulting in the eventual development of the PASDEBPR system. The reactor was operated in three experimental phases for a total duration of 103 days. In the first phase (P1), synthetic wastewater was provided to the EBPR-PAS system to build the microalgae-bacterial consortia that could perform phosphorous removal. While the system showed signs of biological phosphorus removal, it was aided by an external air supply instead of photosynthetic oxygenation by microalgal species. After correcting for the limitation to microalgal growth, the external aeration was gradually reduced and the EBPR-PAS system was allowed to stabilize. With the effluent parameters monitored and found to remain unchanged for 24 days (3*SRT), the EBPR-PAS system was assumed to be in a pseudo-

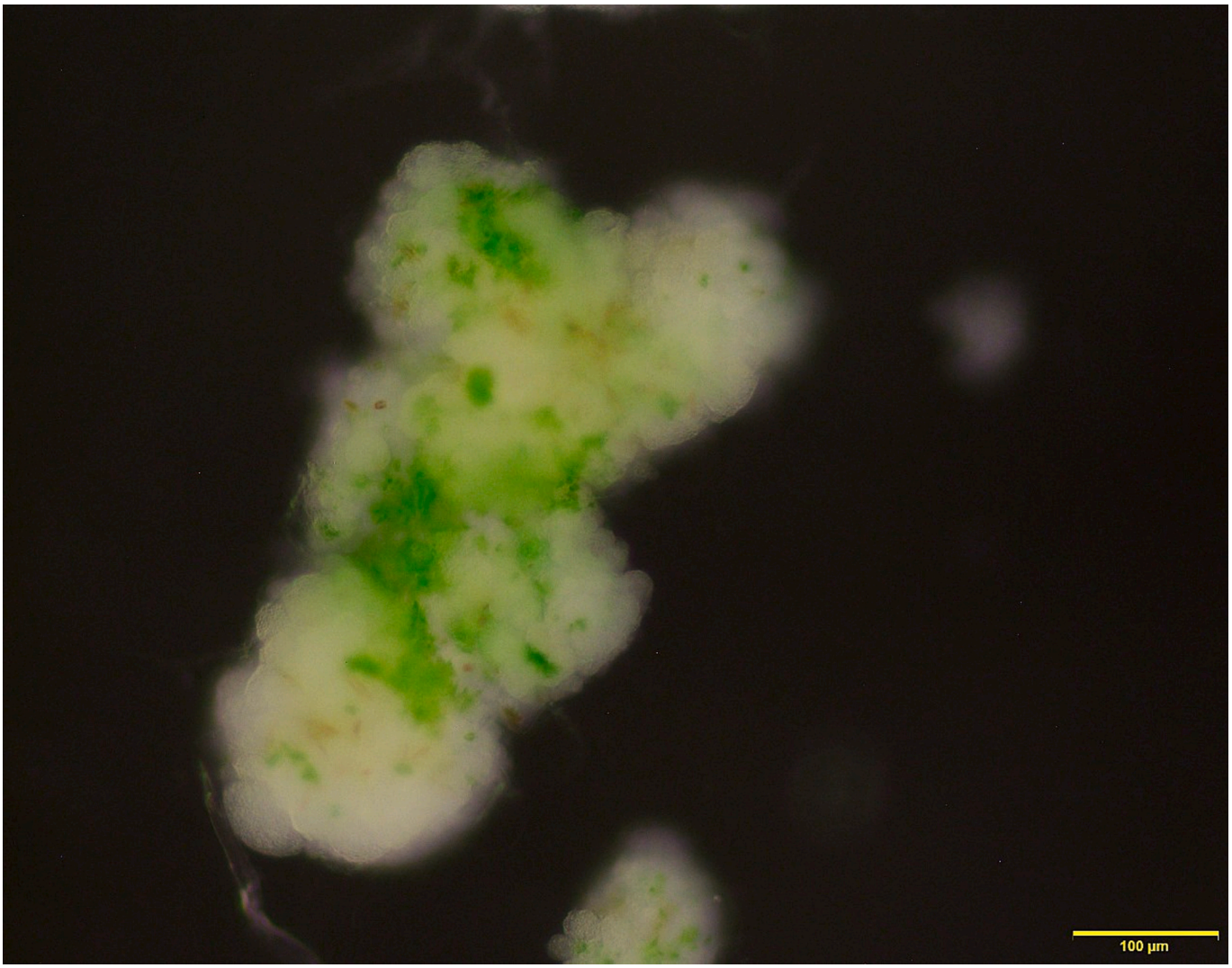


**Fig. 1.** Dark phase microscopic image of biomass from reactor showing the coupling of microalgal species with bacteria.

steady state. In the following phase (P2), to introduce nitrogen removal in the EBPR-PAS system, 120 mL of fresh activated sludge was added to the reactor as an inoculum for the cultivation of nitrifiers. Interestingly, the PASDEBPR system was found to efficiently remove organic carbon, phosphorus, and nitrogen but with the help of nitrogen gas being sparged for 5 min during the aerobic cycle of system operation. It was hypothesized that the system was unable to achieve steady-state conditions in this phase because of some limiting factor. Therefore, in the final phase (P3), the aim was to determine the limiting factor that hampered the performance of the PASDEBPR system. In this phase, the system operation was undertaken with sparging of nitrogen gas during the aerobic cycle and additional monitoring of nitrogen-based compounds was undertaken.

### 2.5. Analysis

The carbon source was measured in terms of acetate and propionate using the Varian 430-GC Gas Chromatography instrument (Varian BV, the Netherlands). This instrument was equipped with a split injector (200 °C), and a WCOT fused silica column (105 °C). And, the internal standard and carrier gas used were butyric acid and helium gas, respectively. Ammonium measurement was performed by the spectrophotometric methods described in NEN 6472 (1983). Nitrate and nitrate measurements were done using the spectrophotometric methods as described in (APHA, 1992b) and (APHA, 1992a), respectively. Phosphate measurement was performed by the ascorbic acid method using a spectrophotometer described in APHA (1992c). Total suspended solids and volatile suspended solids were measured using the gravimetric method (USEPA, 1983). Chlorophyll-*a* was measured by the ethanol extraction spectrophotometric method as described in NEN 6520 (1982). And, the off-gas emissions were measured using the salting-out method using gas chromatography instrument (Varian BV, the Netherlands) as described by (Gal'chenko et al., 2004).

### 2.6. Parameters of interest

At the start of this research, the parameters of interest monitored were volatile fatty acids (VFAs), $PO_4$-P, $NH_4$-N, and dissolved oxygen to establish the PASDEBPR system. These parameters were useful in providing insights into transitioning from the EBPR-PAS system to the PASDEBPR system. Additionally, in the final phase, the additional parameters related to nitrogen removal were also monitored namely, $NO_2$-N, $NO_3$-N, NO, and $N_2O$.

The kinetic profiles of VFAs and $PO_4$-P were observed to assess the phosphorus removal activity with respect to the organic carbon uptake. The VFA uptake rate (in mg COD $L^{-1}$ $h^{-1}$) was determined using the profile representing VFA consumption during the anaerobic stage (dark conditions). The slope of the VFA consumption profile was adjusted

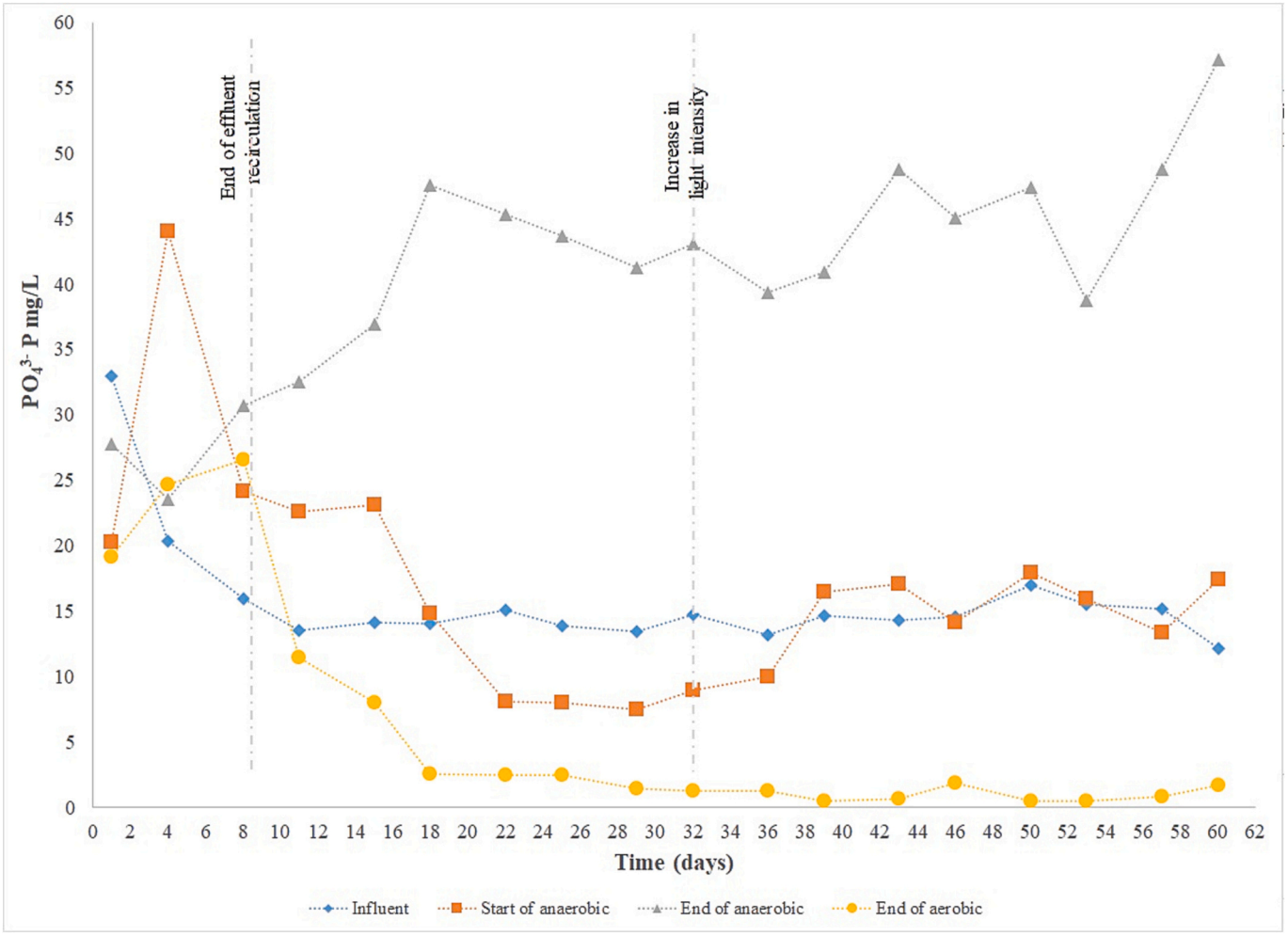


**Fig. 2.** Phosphate profile in EBPR-PAS system during the culture enrichment stage.

using linear regression to the concentrations measured during the experiment. The VFA consumption (in mg COD $L^{-1}$) was calculated as the difference between COD concentration through the anaerobic stage

$$\left(VFA_{consumption} = VFA_{start\ of\ anaerobic\ stage} - VFA_{end\ of\ anaerobic\ stage}\right) \quad (1)$$

Concerning the phosphorus removal, the phosphorus concentrations through the anaerobic and aerobic stage were monitored to calculate the phosphorus release (in mg $PO_4$-P $L^{-1}$ $h^{-1}$; Eq. (2)) and phosphorus uptake (in mg $PO_4$-P $L^{-1}$ $h^{-1}$; Eq. (3)), respectively. In the anaerobic stage, the P release rate (in mg $PO_4$-P $L^{-1}$ $h^{-1}$) was calculated with the help of the phosphorus profile by adjusting the linear regression line to the experimentally determined concentrations.

$$PO_4 - P_{release} = PO_4 - P_{end\ of\ anaerobic\ stage} - PO_4 - P_{start\ of\ anaerobic\ stage} \quad (2)$$

$$PO_4 - P_{uptake} = PO_4 - P_{start\ of\ aerobic\ stage} - PO_4 - P_{end\ of\ aerobic\ stage} \quad (3)$$

With respect to nitrogen removal activity in the PASDEBPR system, the $NH_4$-N uptake rate (in mg $NH_4$-N $L^{-1}$ $h^{-1}$) was calculated using the profile of $NH_4$-N to adjust the linear regression line to experimentally observed concentration of $NH_4$-N through the anaerobic and aerobic stages. The net nutrient removal in the PASDEBPR system was calculated as the difference between P and N concentrations in the influent and at the end of a 6 h cycle (Eqs. (4) and (5))

$$PO_4 - P_{net\ removal} = PO_4 - P_{influent} - PO_4 - P_{effluent} \quad (4)$$

$$NH_4 - N_{net\ removal} = NH_4 - N_{influent} - NH_4 - N_{effluent} \quad (5)$$

## 3. Results and discussion

### 3.1. Phase 1 - culture enrichment and EBPR-PAS system

The development of the EBPR-PAS took about 60 days and it reached a net phosphorus removal of 37.5 mg $PO_4$-P without the need for external aeration. During the initial 18 days, the enrichment of polyphosphate accumulating organisms (PAOs) was slow with poor P-uptake in aerobic stages. Also, the need for external aeration during aerobic stages pointed toward the poor development of algae in the system. This was confirmed through the microscopic examination of biomass on day 12. There was very limited growth of microalgal species mainly, *Scenedesmus quadricauda* and *Chlorella* sp., and a low degree of coupling between microalgae and PAOs. Owing to poor settling characteristics, microalgae was being washed out of the system. Hence, in the initial 8 days of reactor operation, the effluent was recirculated to help retain microalgal species in the system. As a result, the microscopic examination of biomass from the EBPR-PAS reactor system on day 12 indicated an enmeshed growth of microalgae with bacterial biomass (Fig. 1).

Furthermore, an indicator of the photosynthetic oxygenation process by microalgal species in the reactor system was bulk oxygen production. In principle, when the oxygen production by algae surpasses its consumption by bacteria, would be possible to measure an increase in the dissolved oxygen concentration. By increasing the average light intensity to 580 μmol $m^{-2}$ $s^{-1}$ (day 32), there was an immediate rise in oxygen production with DO being recorded in mixed liquor during aerobic stages.

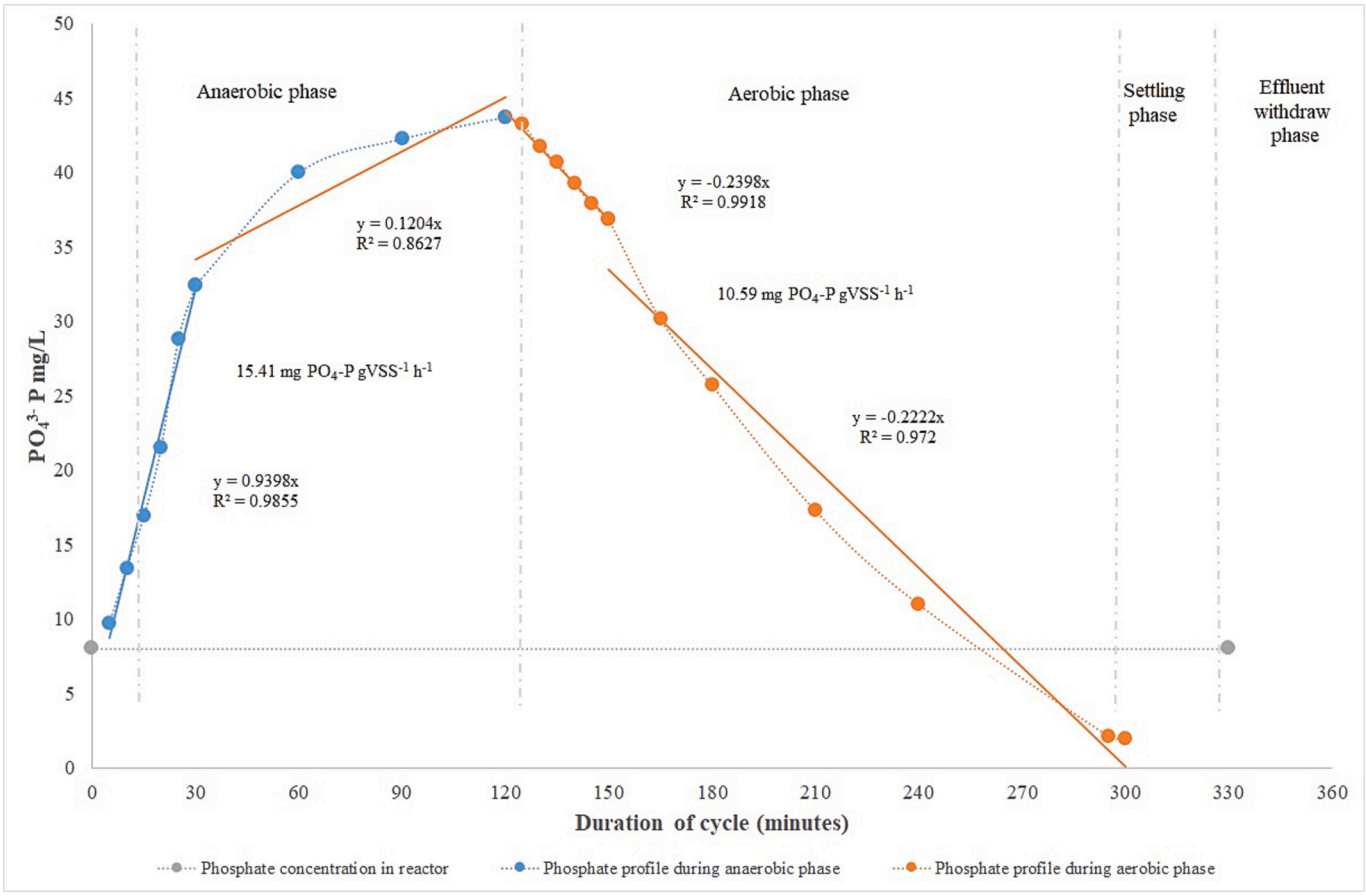


**Fig. 3.** Phosphate profile during cycle test in EBPR-PAS system.

The external aeration during aerobic stages was discontinued from day 37 and reactor operation was monitored daily through monitoring of pH and dissolved oxygen as well as phosphate concentration. With the parameters showing no significant changes over 24 days (3*SRT), the EBPR-PAS system was assumed to achieve pseudo-steady-state conditions. With the modification in reactor operation to promote microalgal development and avoid external air supply, the microalgal-bacterial consortia were able to successfully perform biological removal of phosphorus by day 60 (Fig. 2). On day 8, the P-release and total P-uptake values were 6.5 mg P $L^{-1}$ and 4.1 mg P $L^{-1}$ showing poor activity by PAOs. However, by day 60, the values of P-release and total P-uptake improved to 39.7 mg P $L^{-1}$ and 55.5 mg P $L^{-1}$, respectively. Moreover, the EBPR-PAS system had an effluent P concentration of 1.6 mg P $L^{-1}$ with a net P-removal of 10.5 mg P $L^{-1}$. The P-release/VFA consumed ratio increased from 0.13 P-mmol/C-mmol on day 8 to 0.65 P-mmol/C-mmol on day 60.

On day 57, a cycle test was carried out to further understand the details of biological activity in the EBPR-PAS system. During the anaerobic phase, the net P-release and VFA consumed were found to be at 34 mg P $L^{-1}$ (Fig. 3) and 54 mg COD $L^{-1}$, respectively. These values resulted in a P-release/VFA consumed to be 0.63 P-mmol/C-mmol observed during daily monitoring of the EBPR-PAS system. Similarly, the average TSS concentration was 2060 mg SS $L^{-1}$ with an average VSS concentration of 1265 mg SS $L^{-1}$ and resulting VSS/TSS ratio of 0.61. The ammonium concentration was also important to assess the nitrogen uptake by microalgae in the EBPR-PAS system. While ATU in the influent prevented the growth of nitrifiers, the ammonium uptake rate during the light stage was 1.15 mg $NH_4$-N $gVSS^{-1}$. $h^{-1}$ (4.38 mg $NH_4$-N $h^{-1}$) which results in oxygen production of 74 mg $O_2$ $L^{-1}$ based on the stoichiometric equation shown below. Thus, the oxygen produced by algae was well above the theoretical value of 66 mg $O_2$/L required to oxidize the 200 mg COD/L fed into the system (Henze et al., 2008.). Showing in this way that the oxygen requirements of the system could be supplied by algae.

$$106CO_2 + 236H_2O + 16NH_4^+ + HPO_4^{2-} \xrightarrow{light} C_{106}H_{181}O_{45}N_{16}P + 118O_2 + 171H_2O + 14H^+$$

The net P removal observed in our study correlates with good EBPR activity as conventional EBPR systems have shown the P-release/VFA consumed ratio to be within 0.4 to 0.8 P-mmol/C-mmol and VSS/TSS ratio of 0.59(Smolders et al., 1994; Welles et al., 2015; 2017). The net P removed of 37.5 mg P $L^{-1}$ observed during this study was well above the previous values reported of 10 mg P $L^{-1}$ and 19 mg P $L^{-1}$ reported by Trebuch et al. (2023) and Carvalho et al. (2021). However, is considerably lower than the 64 mg P $L^{-1}$ observed by Carvalho et al., (2018). While all these studies have a similar organic load, the studies of Carvalho et al. (2018) utilized a significantly higher illumination (2760 µmol $m^{-2}$ $s^{-1}$ as compared with the 580 µmol $m^{-2}$ $s^{-1}$ utilized in this

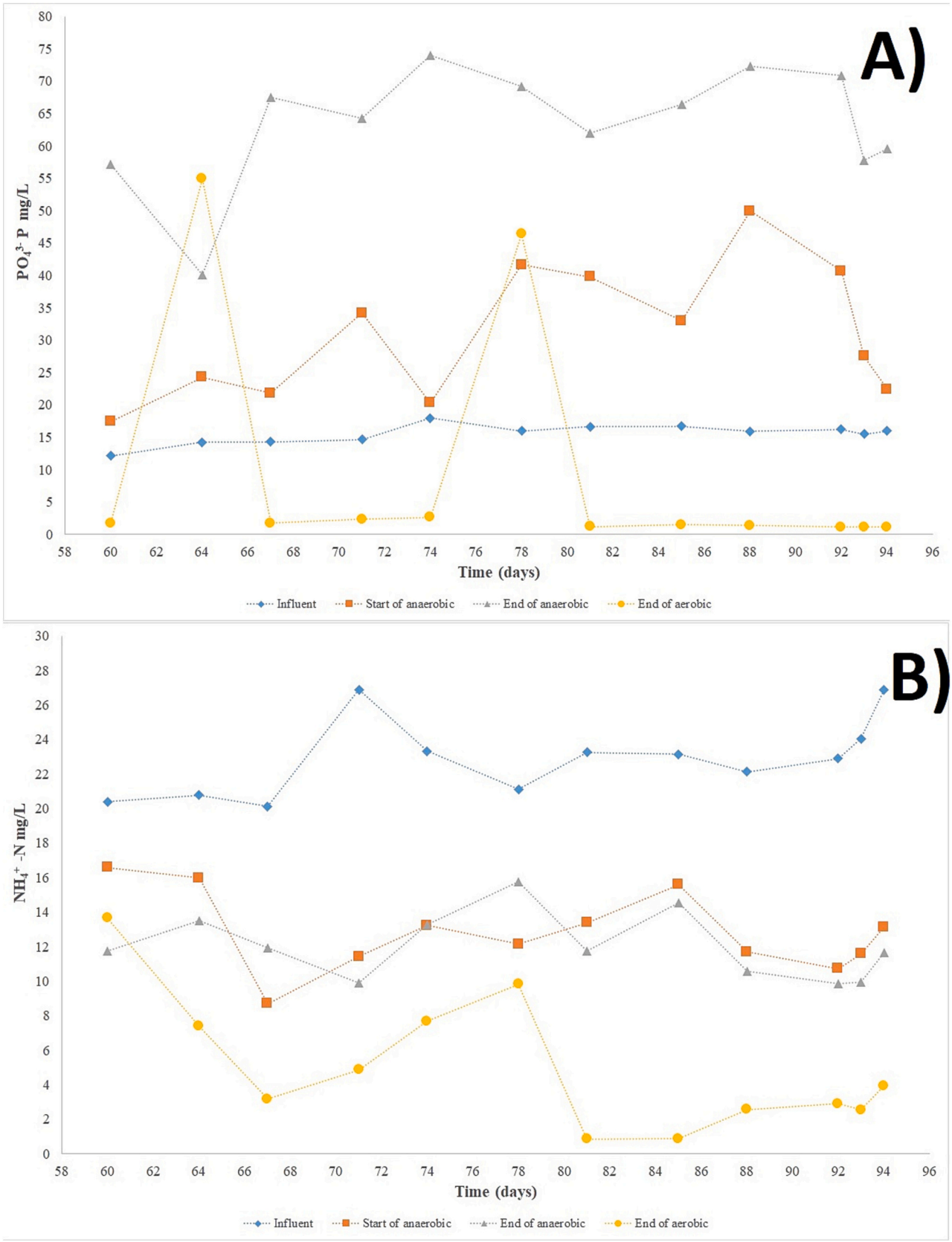


**Fig. 4.** Profile for PASDEBPR system during phase 2 (from day 60 to day 94) for phosphorus (A) and Nitrogen (B).

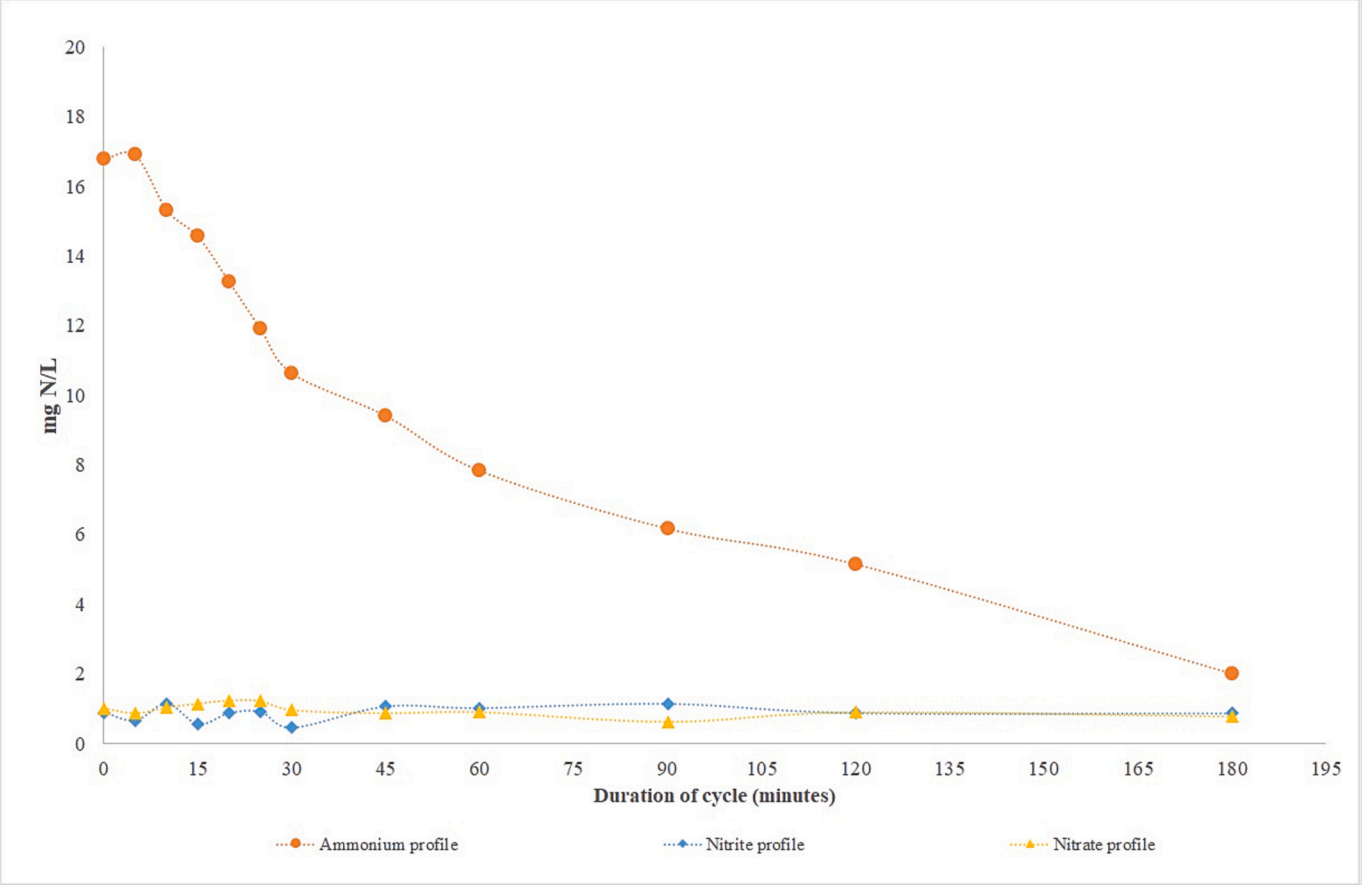


**Fig. 5.** Measurement of ammonia, nitrate and nitrite during the 3 h cycle test of the PASDEBPR.

study). Interestingly, Pope, 1975 postulated that an increase in light intensity should be applied with caution as light per se can be inhibitory for phototrophic organisms. On the contrary, Carvalho et al. (2018) postulated that the excess energy harvested by Algae was directly utilized for the excess of phosphorus removed observed in their system. Without knowing the wavelength of the illumination utilized by Carvalho et al. (2018), it is impossible to correlate both studies as in principle UV light is well known to be used for disinfection process and does not provide energy generation for algae. In our study, light illumination needed to be increased to produce enough oxygen by algae. The limitation of light was also considered in the study by Mohamed and otros (2021) during the initial phase of establishing the EBPR-PAS system. The result of effluent recirculation and an increase in light intensity was an increase in chlorophyll-*a* measurement from 1.07 mg $L^{-1}$ on day 18 to 3.09 mg $L^{-1}$ on day 37. However, as aforementioned this approach requires careful consideration.

### 3.2. *Phase 2 – introducing nitrogen removal to establish the PASDEBPR system*

During the second phase (P2), 120 mL of fresh activated sludge was added to the EBPR-PAS system to allow nitrifiers to grow within the existing microalgal-PAOs consortia. Additionally, the addition of ATU in the influent feed was discontinued. As the nitrifying bacterial community was expected to grow, there would be a rise in demand for photosynthetic oxygen produced by microalgae. Therefore, two additional lamps were added that provided a light intensity of 775 μmol $m^{-2}$ $s^{-1}$. However, the reactor performance deteriorated with bulk liquor dissolved oxygen concentration dropping to 1 %. The effluent concentration of phosphate and ammonium was observed to rise to values of 55 mg P $L^{-1}$ and 7 mg N $L^{-1}$, respectively (Fig. 4). To salvage the biological removal of phosphorus by PAOs, the external air supply was started as a corrective measure on day 64.

For investigating the drop in EBPR-PAS performance, the limitation of light intensity inside the reactor with mixed liquor was measured. It was found that there existed dark zones within the reactor at a radial distance of 1.8 cm pointing toward the development of anoxic zones during the aerobic stage. Furthermore, the solids concentration in the reactor was visibly high and was confirmed by measuring solids concentration to be 4295 mgTSS $L^{-1}$ and 3179 mgVSS $L^{-1}$. This translated into a VSS/TSS ratio of 0.74 which was higher than the model prediction.

As expected the external addition of oxygen resulted in the performance recovery of the reactor with an effluent concentration of phosphorus and ammonium measured as 2 mg P $L^{-1}$ and 3 mg N $L^{-1}$, respectively. Also, the nitrite and nitrate concentrations were found to be 1.1 mg N $L^{-1}$ and 0.9 mg N $L^{-1}$ in the effluent. Hence, the external aeration was discontinued by day 67 in an attempt to establish the PASDEBPR system. The PASDEBPR system performance was monitored continuously through online measurement of dissolved oxygen concentration alongside phosphorus and nitrogen removal in the effluent. By day 78, the dissolved oxygen concentration dropped precipitously. Instead of supplying oxygen through external aeration, nitrogen gas was sparged for 5 min during the aerobic stage of reactor operation as a corrective measure. Within 3 days, the PASDEBPR performance was recovered with effluent concentrations of phosphorus and nitrogen recorded as 1.22 mg P $L^{-1}$ and 0.88 mg N $L^{-1}$. Consequently, this resulted in the net P and N removal of 13.8 mg $PO_4$-P/L and 24.1 mg $NH_4$-N $L^{-1}$, respectively.

The need for an external gas supply either in the form of oxygen or nitrogen was found to be a necessity for PASDEBPR system performance. Based on the phosphate and ammonium profiles for the system, it was clear that sparging of air aided in faster recovery of reactor performance. On the

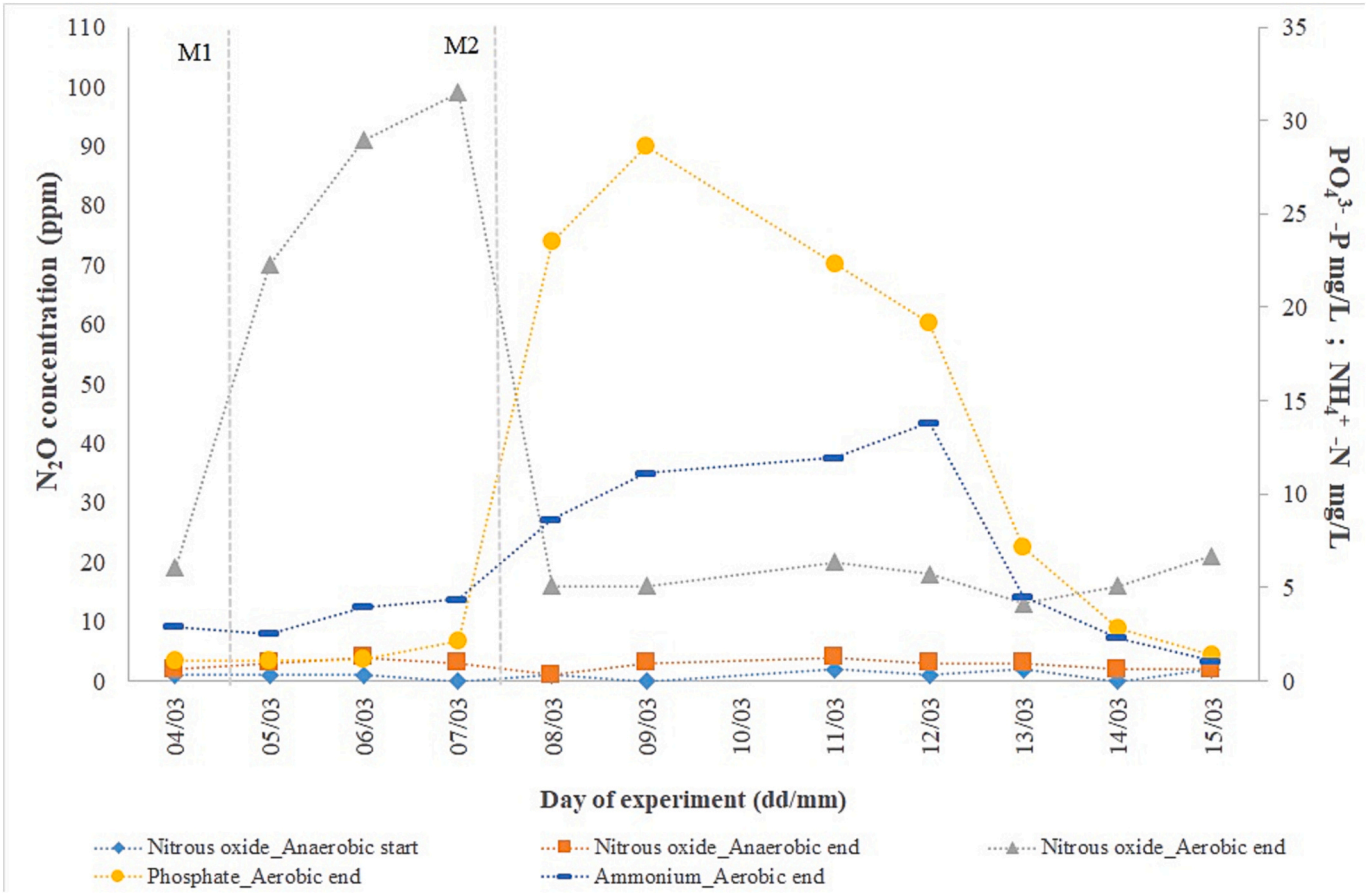


**Fig. 6.** Profile of nitrogen-based compounds during aerobic cycle test of PASDEBPR system on day 82.

other hand, the absence of an external air supply resulted in the slow deterioration of reactor performance. To investigate the impact of denitrification, an aerobic cycle test was conducted on day 82 to measure concentrations of ammonium, nitrite, and nitrate. During the 3-h cycle test, the ammonium concentration showed a gradual decline with time and was recorded to be 2 mg N $L^{-1}$ at the end of the aerobic cycle (Fig. 5).

The low concentrations of nitrate and nitrate in the system indicate that the system was performing a simultaneous nitrification-denitrification process. As there was no leakage of carbon into the aerobic phase, stored PHA by PAOs should have been utilized as a carbon source during the denitrification process. It is not clear, however, if the PAOs in this system utilized nitrate or nitrite as an electron acceptor. Previous studies have indicated the inability of PAOs to fully denitrify (Rubio-Rincón et al., 2019), while others have the contrary (Camejo et al., 2016). Interestingly, the main difference in these studies was the availability of dissolved oxygen from 20 % to microaerophilic, respectively. In our studies, dissolved oxygen concentration was only measurable by the end of the light (aerobic) phase, which would indicate that the system operated at microaerophilic conditions. If or not, the concentration of oxygen limits the denitrification capacities (either via selection or adaptation) of PAOs requires further study.

### *3.3. Phase 3 – identifying the limiting factor in PASDEBPR system PERFORMANCE*

As seen in P2, the PASDEBPR system performance was found to recover in the presence of external nitrogen sparging during aerobic stages. Thus, the limiting factor was assumed to be an unknown compound that was being stripped out due to sparging of nitrogen gas. By measuring the dissolved gases in the mixed liquor, this final phase (P3) focused on identifying this unknown compound. Since the earlier phase introduced the biological removal of nitrogen in the EBPR-PAS system, the focus of investigation in this phase was to investigate individual biological processes that make up the biological nitrogen removal process.

Furthermore, the assumption behind this limiting factor being an unknown compound capable of being stripped off by nitrogen sparging can be understood from biological processes underlying nitrogen removal. The metabolic pathways for ammonium removal resulted in several intermediate products that were measured in this phase. Due to the limitation of analytical methods, the measurement of nitrogen gas was not undertaken in this research study. The nitrite and nitrate concentrations were found to be very low during the aerobic stages of the PASDEBPR system in P2 (Fig. 5). Therefore, the analytical measurement focused on nitrous oxide measurement during the aerobic stages.

The profile of nitrous oxide was compared alongside the profile of phosphate and ammonium by measuring daily for a period of days 92 to 103 (Fig. 6). It is evident that by discontinuing the sparging of nitrogen gas (indicated by M1) the nitrous oxide gas concentration immediately spiked at the end of aerobic stages followed by elevation in phosphate and ammonium concentrations in the effluent. However, the restart of sparging of nitrogen gas (indicated by M2), led to a drop in nitrous oxide being recorded at the end of aerobic stages and subsequently, a drop in phosphate and ammonium concentrations in the effluent. On the other hand, throughout these 12 days, nitric oxide was not measured in the mixed liquor of the PASDEBPR system.

In this phase (P3), the presence of nitrous oxide was found to limit the biological performance of PASDEBPR system. The occurrence of nitrous oxide could be attributed to the presence of dark zones in the center of the reactor during aerobic (light) stages as observed in P2. Such dark zones could potentially act as anaerobic or anoxic zones resulting in the denitrification of nitrite and nitrate in the PASDEBPR system. Elevation in nitrous oxide during dark periods and drop in nitrous oxide

during light periods was observed in a reactor setup comprising microalgae-bacteria consortia (Fagerstone et al., 2011). The production of nitrous oxide observed in this research study was similar to the production of nitrous oxide in anoxic conditions due to partial denitrification of nitrate observed by Fagerstone et al. (2011). Moreover, other studies have observed nitrous oxide production during nitrification and denitrification processes (Alcántara et al., 2015; Guieysse et al., 2013; Hatzenpichler, 2012). In this study, the presence of nitrous oxide in the PASDEBPR system is postulated to inhibit the biological activity of the microalgal-bacterial consortia. While nitrous oxide formation in this system could be caused due to incomplete denitrification, its formation within NDEBPR systems requires further study.

## 4. Conclusion

This study shows that it is possible to biologically remove phosphorus and nitrogen in a photo-activated sludge system. However, its performance is directly affected by the light intensity of the system. In this study, the light intensity needed to be increased to ensure enough oxygen production by algae. Moreover, either during the nitrification or denitrification process a gaseous compound is formed that inhibits the biological removal of phosphorus and nitrogen.

## CRediT authorship contribution statement

**P. Kamath:** Formal analysis, Writing – original draft. **F.J. Rubio-Rincón:** Conceptualization, Supervision, Writing – review & editing. **D. Brdjanovic:** Conceptualization. **C.M. Lopez-Vazquez:** Conceptualization, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2024.101782.

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