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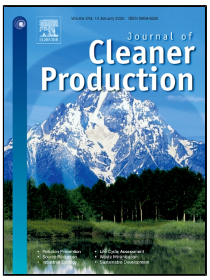

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Arsalan Sepehri, Mohammad-Hossein Sarrafzadeh, Maryam Avateffazeli

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Interaction between *Chlorella vulgaris* and nitrifying-enriched activated sludge in the treatment of wastewater with low C/N ratio

Arsalan Sepehri[1, 2], Mohammad-Hossein Sarrafzadeh[1*], Maryam Avateffazeli[3]

[1] UNESCO Chair on Water Reuse, School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
[2] Department of Chemical Engineering, University of Toledo, OH, USA
[3] Department of Mechanical Engineering, University of Toledo, OH, USA

[*]Corresponding author:

Mohammad-Hossein Sarrafzadeh, Ph.D.
Associate Professor
School of Chemical Engineering, College of Engineering
University of Tehran
P.O. Box: 11155-4563, Tehran, Iran
Tel: +98-21-61112185, Fax: +98-21-66958874
E-mail: sarrafzdh@ut.ac.ir

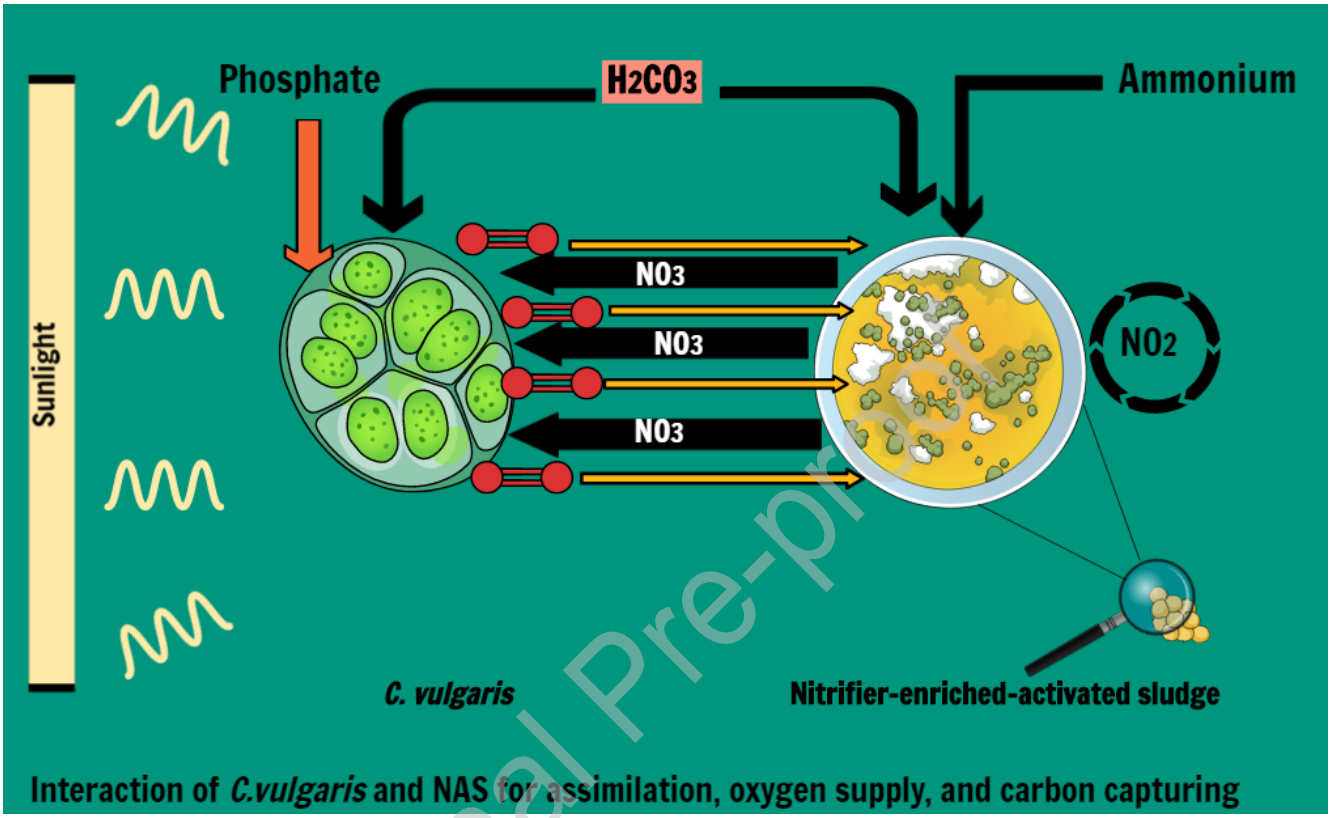
Abstract

Wastewater treatment based on algae-bacteria consortia is expected to find use among other biological processes. In this report, the effects of the inoculation ratios of a *Chlorella vulgaris* and nitrifier-enriched-activated-sludge (NAS) consortium on nutrient removal, carbon capture, and metabolite generation were investigated under autotrophic conditions. The maximum NH_4^+-N removal (100%) was observed within 7 days in a photo-bioreactor containing 10% *C. vulgaris*: 90% NAS (w/w); this consortium was referred to as B10. The maximum PO_4^{3-}-P removal (87.5%) was observed in a pure culture of *C. vulgaris* after 14 days. Due to the fast nitrification achieved using B10, this system showed the highest nitrate accumulation value of 19.73 mg NO_3^--N/L at the end of the experiment. The DO concentration in B10 reached 4 mg/L due to the

photosynthetic activity of *C. vulgaris*, providing appropriate conditions for the NAS. Among all the tested consortium ratios, B90 (90%:10% C. *vulgaris*/NAS, w/w) showed the highest carbon capture (156 mg), while B10 captured only 43.2 mg of carbon. Furthermore, metabolite analysis showed a positive correlation between the proportion of *C. vulgaris* and the generation of metabolites in the cultures. Thus, a trade-off was observed between nutrient removal and carbon capture in *C. vulgaris* and NAS co-cultures. Overall, our study shows that the aeration system in conventional nitrification processes could be replaced by a cleaner process based on microalgae, resulting in enhanced nutrient removal, increased carbon capture, reduced metabolite generation, and a decrease in excess sludge production.

Keywords: Carbon capture, Metabolite, Microalgae, Nitrification, Nutrient removal, Photosynthesis, Wastewater

Graphical Abstract



Highlights

- Using a *C. vulgaris*-NAS system, no external oxygen or CO_2 are needed.
- Complete ammonium nitrogen removal was achieved using 10% *C. vulgaris*:90% NAS (w:w)
- Maximum carbon capture as dissolved carbon was obtained at 90% *C. Vulgaris*:10% NAS
- EPS and SMP concentrations low, ensuring long-period autotrophic growth and prevention of excess sludge production
- Cooperation of *C. vulgaris* and NAS can enhance carbon-capture without compromising nutrient removal

1. Introduction

Symbiotic algae-bacteria systems represent a unique strategy for nutrient recycling (Ji et al., 2018) and the removal of contaminants in municipal and industrial wastewater treatment (WWT) (Liu et al., 2017). Through oxygenic photosynthesis (Zhang et al., 2018), algae produce O_2, which is utilized as an electron acceptor by aerobic bacteria, thus stimulating the biodegradation of the substrates (Liu et al., 2018; Mehrabadi et al., 2015). Additionally, algae have a networking capability for the elimination of many competing microbial species such as pathogens (Muñoz et al., 2003), and also enhancement of the dissolved oxygen concentration (Hanifzadeh et al., 2018), pH, and temperature (Rezvani et al., 2019). Due to assimilation through biological reactions and the symbiotic mechanisms, the accumulated biomass at the end of the WWT process is enriched in potential sustainable energy sources, including biodiesel, fertilizers, and biogas (Cardeña et al., 2017; Quijano et al., 2017). Various unicellular microalgae, including *Chlamydomonas*, *Chlorella vulgaris*, and *Phormidium*, have been used for algae-bacteria culture (Lin and Lin, 2011; Rawat et al., 2011). Algae not only govern the consumption of CO_2 (Gurbuz et al., 2004), but also provide a safe and cost-effective alternative to mechanical aeration (Rawat et al., 2011; Slade and Bauen, 2013). Su et al. (2012a) investigated the influence of the algae and sludge inoculation ratios in the heterotrophic mode. They concluded that nitrogen and phosphorus removal efficiencies of more than 90% could be achieved using a 5:1 (algae/sludge, w/w) culture. Ji et al. (2018) also measured the nutrient removal efficiency of *C. vulgaris* and *Bacillus licheniformis* in the heterotrophic mode and demonstrated that the highest nutrient removal was obtained using a 1:3 (w/w) ratio of these species. More recent evidence (Liu et al., 2017) has demonstrated that an algae-bacteria granular consortium (*C. vulgaris and Scenedesmus*) in six photo-sequencing batch reactor can remove more than 90% of ammonium and 31% of phosphate on average. These authors also reported the

generation of 112 mg extracellular polymeric substances (EPS) per gram of volatile suspended solids (VSS) on average.

However, most research on algae-bacteria cultures has been conducted in the presence of high organic loadings, and the role of autotrophic bacteria has been neglected. In the conventional activated sludge process, a high organic loading rate causes excess sludge production. Management of the excess sludge is a particularly critical challenge in the sewage treatment field (Foladori et al., 2010). At a high influent chemical oxygen demand (COD)/N ratio, the growth rate of the algae will be reduced (Perez-Garcia et al., 2011). Additionally, at high influent COD/N ratios in a WWT system, the amount of hydrophilic organic materials will be greater than at low COD/N ratios. In the disinfection stage, the presence of dissolved organic matter in the effluent, including EPS and soluble microbial products (SMP), leads to the production of genotoxic or mutagenic/carcinogenic by-products (Xia et al., 2016). Furthermore, at high COD/N ratios, the concentration of nitrifying bacteria cannot be enriched, and the nitrification process efficacy will be reduced due to the domination of heterotrophs (Anzola-Rojas et al., 2015; Sepehri and Sarrafzadeh, 2018). A recent study by He et al. (2018) showed that the presence of a small amount of EPS and SMP could enhance the granulation process. We previously demonstrated that nitrifier-enriched activated sludge (NAS) produced significantly lower concentrations of organic metabolites (EPS and SMP) than conventional activated sludge (CAS) (Sepehri and Sarrafzadeh, 2018), which can be attributed to the low growth rate of autotrophic bacteria. In autotrophic mode, both nitrifiers and algae rely on carbon dioxide for their growth requirements. One neglected area in the field of algae-bacteria culture is the interaction of *C. vulgaris* and NAS in organic-carbon-depleted media. The nitrification process is aerobic (Seuntjens et al., 2018); *C. vulgaris* might be able produce sufficient oxygen for the

ammonium-oxidizing-bacteria (AOB) and nitrite-oxidizing-bacteria (NOB). Ammonium can be removed through nitrogen dissimilation (nitrification) and assimilation (uptake by cell growth) (Li and Irvin, 2007). Interaction between *C. vulgaris* and NAS might intensify the simultaneous assimilation and dissimilation of ammonium. However, as mentioned above, the collaboration of *C. vulgaris* and NAS cultures under autotrophic conditions has not been dealt with in-depth.

The aim of our work is to broaden the current knowledge of the use of *C. vulgaris*-NAS cultures for municipal wastewater treatment and carbon capture under autotrophic conditions. Additionally, the influence of *C. vulgaris* and the nitrifier on metabolite generation (EPS and SMP) was investigated at different inoculum ratios.

2. Materials and methods

2.1. Microbial strains and inoculum procedures

2.1.1. *C. vulgaris* preparation and growth medium

To study the behavior of *C. vulgaris*, an initial inoculum of *C. Vulgaris* was obtained through ACECR, Research Institute of Applied Science, in the local area. The *C. vulgaris* was pre-cultivated photo-autotrophically in sterile N-6 media at a fixed temperature of 27 °C using a water-jacketed glass tube (Borowitzka et al., 1988). The medium was continuously illuminated at a light intensity of 2000 lux by fluorescent lamps. For adaptation, the *C. vulgaris* was fed with synthetic wastewater enriched with N and P according to the methods in our previous study (Sepehri and Sarrafzadeh, 2018). For the growth of *C. vulgaris*, the following components were used: $MgSO_4 \cdot 7H_2O$ (50 mg/L), $CaCl_2 \cdot 6H_2O$ (10 mg/L), Fe-EDTA (10 mg/L), and 1 mL/L of a trace element solution containing: $Al_2(SO_4)_3$, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, and $CuSO4 \cdot 5H_2O$. Plexiglas photo-bioreactors with an operational volume of 300 mL containing different

proportions of *C. vulgaris* and NAS were used. The experiments were conducted for 14 days to allow sufficient growth of the bacteria and nutrient removal.

2.1.2. Nitrifier-enriched Activated Sludge (NAS) preparation

To prepare the nitrifier-enriched activated sludge, aerobic activated sludge was obtained from the local municipal WWT plant. Fig. 1 presents a flowchart of the nitrifier enrichment process for the activated sludge. Experiments involving bioprocesses such as sludge management are commonly conducted in batch mode (de Azevedo et al., 2018; Zhao et al., 2019). Two bioreactors with working volumes of 20 L each were prepared for the enrichment of the activated sludge: one was used for CAS, while the other was used for the development of nitrifier-enriched activated sludge, i.e., NAS. The same activated sludge population was used to inoculate the two systems initially. The bioreactors were opaque to ensure that the measured energy corresponded solely to chemical oxidation. Aeration was provided using an airlift bioreactor for uniform aeration. The systems were also equipped with a heater rod to maintain the temperature at approximately 30 °C (Gerardi, 2006). To accelerate the growth of nitrifying bacteria in the NAS, an influent with a C/N ratio of zero was applied by using a feed containing only mineral chemical compounds. To validate the nitrifier enrichment, the performance of the NAS was investigated in terms of COD removal. The incapability of the NAS to achieve COD removal and the low amount of EPS and SMP generated after 75 days confirmed the enrichment of nitrifying bacteria. More information regarding the enrichment process can be found in our previous work (Sepehri and Sarrafzadeh, 2019, 2018).

2.2. Experimental system, cultures, and operation

The total suspended solids (TSS) of the initial cultures of *C. vulgaris* and the nitrifying bacteria were both equal to 0.3 g/L. The main reason for choosing this concentration was the low growth rate of the nitrifiers. At higher biomass concentrations, it is difficult to distinguish the growth of microalgae and the nitrifying community. The photo-bioreactors were loaded with *C. vulgaris* and nitrifying bacteria inocula to obtain the *C. vulgaris*-nitrifying bacteria systems with the following weight ratios: B100 (100% *C. vulgaris*), B90 (90% *C. vulgaris*), B70 (70% *C. vulgaris*), B50: (50% *C. vulgaris*), B10 (10% *C. vulgaris*), and B0 (100% nitrifying bacteria). External aeration was not used in these six photo-bioreactors. Synthetic wastewater was used as the feed for all the reactors. The initial concentrations of NH_4^+-N, PO_4^{3-}-P, and $CaCO_3$ (as a carbon and alkalinity source) in the reactors were chosen according based on the composition of domestic wastewater (Boelee et al., 2014). To avoid biomass sedimentation, light limitation, anaerobic zone formation, and nutritional gradients, magnetic stirrers were used as mixers. Mixing enhances the contact between cells and nutrients and improves removal efficiency. While excessive mixing can cause cell damage and shear stress, insufficient mixing can adversely affect cell growth. Based on these considerations, a mixing velocity of 300 rpm was chosen. This velocity was expected to improve the nutrient removal efficiency and the algae growth rate, as suggested by Su et al. (2012b). Fig. 2 shows the overall biochemical reactions as a result of the interactions between NAS and *C. Vulgaris*.

Inorganic carbon was measured using the method proposed by Nguyen and Rittmann (2016). The inorganic carbon was determined using a calibration curve obtained using a 50/50 mass ratio of $NaHCO_3$ and Na_2CO_3. To calculate the concentrations of different carbon species, Henry's law was applied. The well-known equations presented below demonstrate the calculation of different carbon compounds:

$$[H_2CO_3] = C_i \frac{[H^+]^2}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad \text{Eq. (1)}$$

$$[HCO_3^-] = C_i \frac{K_{a1}[H^+]}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad \text{Eq. (2)}$$

$$[CO_3^{2-}] = C_i \frac{K_{a1}K_{a2}}{[H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2}} \quad \text{Eq. (3)}$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \, , K_{a1} = 10^{-6.3} \quad \text{Eq. (4)}$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \, , K_{a2} = 10^{-10.3} \quad \text{Eq. (5)}$$

$$\text{Inorganic carbon} = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] \quad \text{Eq. (6)}$$

2.3. Analytical procedures

The dissolved oxygen (DO) and pH were measured near the center of the photo-bioreactors using a DO probe (WTW 340i, Germany) and a pH probe (240, ISTEK, Korea). Chemical analyses of the nutrients phosphate, ammonium, nitrate, and nitrite were conducted every 48 hours. Additional media was added to each photo-bioreactor to compensate for the removed nutrients. The samples were prepared for analysis by centrifugation at 10000 rev/min for 10 min at 4 °C. The concentrations of MLSS, NH_4^+-N, NO_3^--N, and PO_4^{3-}-P were measured in accordance with standard methods for the examination of water and wastewater (Aljerf, 2018; Rice et al., 2012). An Agilent 8453 UV-Vis spectrophotometer was utilized; NH_4^+ was monitored at 640 nm, NO_3^- at 275 and 220 nm, and NO_2^- at 543 nm.

The protein (EPSp, SMPp) and carbohydrate (EPSc, SMPc) components of the metabolites were measured using to the well-developed method reported by Azami et al. (2012) and Le-Clech et al. (2006). The decrease in the volume due to sample withdrawal was compensated by adding fresh media to the photo-bioreactors. The SMP values in the supernatant

were measured after centrifuging the algae-bacteria culture samples at 5000 g for five minutes then filtering the remaining supernatant using a 0.45 μm filter. Deionized water was added to the samples, which were then mixed for 10 min. A series of heating and centrifuging processes were applied to remove impurities. The heating and centrifuging processes were carried out at 80 °C and 7000 g for ten minutes. Both forms of EPS content were measured through the supernatant filtration process.

3. Results and discussion

3.1. Ammonium removal performance at different *C. vulgaris*/NAS ratios

Fig. 3 and Table 1 list the specific NH_4^+-N removal rates and average daily NH_4^+-N removal for the different *C. vulgaris*/NAS ratios. The initial ammonium concentration in all the photo-bioreactors was 40 mg/L. As illustrated in Fig. 3, as the proportion of *C. vulgaris* was increased from B10 to B90, the ammonium removal decreased. The specific and average daily ammonium removal rates over the whole experiment were as follows: B0: 40.21 mg NH_4^+-N/L/gMLSS, 1.50 mg NH_4^+-N/L; B10: 133.33 mg NH_4^+-N/L/gMLSS, 6.67 mg NH_4^+-N/L; B50: 124.33 mg NH_4^+-N/L/gMLSS, 4.46 mg NH_4^+-N/L; B70: 117.50 mg NH_4^+-N/L/gMLSS, 3.87 mg NH_4^+-N/L; B90: 109.21 mg NH_4^+-N/L/gMLSS, 3.22 mg NH_4^+-N/L; B100: 99.83 mg NH_4^+-N/L/gMLSS, 2.03 mg NH_4^+-N/L. Similarly, as the NAS proportion was decreased, the rate of nitrification decreased. Fig. 4 shows the average ammonium removal rate over two weeks. B0 showed the lowest ammonium removal due to the lack of oxygen to support the activity of the nitrifier. This was expected, as nitrification is an aerobic process (Neethling et al., 2007). In B10, the photosynthetic activity of *C. vulgaris* provided sufficient oxygen for the nitrifiers, and the *C.*

vulgaris-NAS co-culture efficiently removed NH_4^+-N. The interaction between *C. vulgaris* and the nitrifiers in B10 led to the highest and fastest NH_4^+-N removal (100% within 8 days). Therefore, B10 represented the optimum *C. vulgaris*-NAS proportion for NH_4^+-N removal. Another significant point is that based on the cell growth data (Section 3.7), most of the conversion of ammonium to nitrate was due to the nitrifier activity, not that of *C. vulgaris*, as the MLSS variations in B10 were lower than at other ratios. The cell growth data demonstrates the greater role of the nitrifier in nitrification than role of algae in the assimilation of ammonia. Due to the nitrification, the alkalinity of the bioreactors dropped. In this situation, microalgae tend to consume nitrate more than ammonium nitrogen. The nitrogen assimilation process by microalgae involves the conversion of nitrate to nitrite and the subsequent conversion of nitrite to ammonium (Perez-Garcia et al., 2011). Thus, in the *C. vulgaris*-NAS community, the activity of the microalgae proceeds in the opposite direction to nitrification. Thus, exceeding the optimum proportion of *C. vulgaris* did not support NAS through additional oxygen supply, and also reduced its activity due to the reduction in the NAS population. Based on the NH_4^+-N removal results of these bioreactors, the conditions for the AOB were very favorable, allowing the conversion of ammonium into other forms of nitrogen. The ammonium removal in B100 correlates satisfactorily with the report of Tam and Wong (1996). They concluded that the nitrogen requirements of *C. vulgaris* were supplied through their reserve N, lipids, and carbohydrates to maintain their growth.

Comparison between B10 and B100 suggests that the NAS consumed the majority of the ammonium, and that *C. vulgaris* played a supportive role in ammonium removal. The ammonium removal results are scarcely distinguishable from those of Su et al. (2012a), who investigated the influence of *C. vulgaris* and sludge inoculation ratios. They also demonstrated

that the combination of *C. vulgaris* and bacteria can improve the rate of ammonium removal compared with that of *C. vulgaris* alone or bacteria alone in the absence of external aeration. It should be noted that due to the C/N ratio of zero in the influent, there was no opportunity for heterotrophic activity.

3.2. Variations in the NO_2^--N removal at different *C. vulgaris*/NAS ratios

Fig. 5 shows the changes in the concentration of NO_2^--N, which follow a uniform pattern. The same pattern was observed for the nitrite concentration in NAS culture in our previous study (Sepehri and Sarrafzadeh, 2019). The concentration of nitrite in the nitrifier community follows a boom and bust (feast and famine) cycle. This variation in the nitrite is a remarkable feature of the nitrification, and results from the link between the AOB and NOB (Schrantz et al., 2013; Yao and Peng, 2017). As the proportion of NAS was increased, the nitrite production peaked. This peak can be rationalized by the higher rate of nitrification at higher NAS proportions. It should be noted that algae can convert nitrogen into nitrite form (Wang et al., 2010). The AOB could be responsible for nitrite production throughout the entire experiment, while the reduction in NO_2^--N from day 8 to day 14 could be due to NOB activity. The nitrite reduction could also be due to the autotrophic conversion of nitrite to nitrate by *C. vulgaris*, which proceeds in the opposite direction to the nitrifier activity. However, nitrification by NAS and the N-assimilation process by *C. vulgaris* are two different nitrite consumption pathways that did not interfere with one another(Perez-Garcia et al., 2011; Sepehri and Sarrafzadeh, 2019). When the *C. vulgaris* proportion was increased, the nitrifier activity was reduced due to its lower weight percentage, enhancing the assimilation and carbon capture activity of *C. vulgaris*. In a different study, Su et al. (2012a) examined the influence of different *C. vulgaris* and bacteria inoculation ratios on

nutrient removal. Their analysis revealed that bacterial activity, rather than *C. vulgaris*, was mainly responsible for nitrite production, as *C. vulgaris* cannot carry out the nitrification process.

3.3. NO_3^--N accumulation at different *C. vulgaris*/NAS ratios

The variations in NO_3^--N are demonstrated in Fig. 6 and Fig. 7. The minimum nitrate production was observed using B0 due to the insufficient DO for the nitritation and nitratation processes. For this reason, the NOB was not activated and could not carry out nitratation. In B100, the capability for nitrate production was also limited, as *C. vulgaris* does not have the intrinsic potential to carry out the nitrification process. The decrease in nitrate in B100 was attributed to NO_3^--N consumption by *C. vulgaris* via assimilation. As shown in Fig. 7, as the proportion of NAS was increased from B90 to B10, the amount of nitrate production was enhanced due to the greater abundance of AOB and NOB. Karya et al. (2013) conducted a relevant study involving a mixture of microalgae and nitrifying bacteria, in which they observed the nitrate increased in the culture due to nitrification. Fig. 6 also clearly demonstrates the higher efficiency of B10 for nitrate removal. As shown in Fig. 7, B10 was found to have the optimum composition for the complete nitrification process. The ammonia and nitrate results show that B10 exhibited a rapid nitrification rate without an external CO_2 gas supply. Efficient nitrate uptake by *C. vulgaris* depends on several factors, including the cell density, alkalinity, and illumination intensity (Hu et al., 2000).

3.4. Variation of PO_4^{3-}-P at different *C. vulgaris*/NAS ratios

Phosphate is an essential component for the sustainable growth of *C. vulgaris*, and a lack of phosphorous compounds in media leads to the suppression of photosynthesis (Rezvani et al.,

2017). Fig. 6 depicts the average daily PO_4^{3-}-P removal in the photo-bioreactors. As shown, B0 did not remove P compounds due to the inability of the nitrifier to remove phosphorus. Increasing the proportion of *C. vulgaris* improved the photo-bioreactor performance in terms of P removal. As Table 1 illustrates, the maximum phosphorus removal was 85.5% for B100. There are two possible explanations for this result: First, *C. vulgaris* had access to all the CO_2 in B100, without competition from other microorganisms. Moreover, the high capacity of microalgae for nutrient removal, especially phosphorous, is well-proven (Rezvani et al., 2018, 2017). Secondly, B100 contained sufficient P and N, and thus the endogenous and declining phases did not occur. This can be attributed to the lack of extreme food shortage, which promoted the activity of *C. vulgaris*. This value correlates well with that of Perez-Garcia et al. (2011), who showed that *C. vulgaris* in autotrophic conditions could remove more than 50% of phosphorus. They made several assumptions that seem to be well-grounded: 1- polyphosphate accumulating organisms represent an alternative means of PO_4^{3-}-P removal; 2- assimilation of phosphorous as $H_2PO_4^-$ and HPO_4^{2-} occurs. To obtain more information regarding the behavior of the *C. vulgaris*-NAS system, the variations in the DO and pH were also investigated. It should be noted that because the pH during the experiment was lower than 9, abiotic phosphorus removal was negligible (Su et al., 2012a).

3.5. DO accumulation at different *C. vulgaris*/NAS ratios

To gain further insight into the interaction between the nitrifier and *C. vulgaris*, the DO profiles in the bioreactors were monitored. At the beginning of the test, the DO concentration was 2.5 mg/L for all the systems. Table 2 shows a clear correlation between the decrease in the DO concentration on the first day and the proportion of NAS. This can be attributed to the

nitrification process. The nitrifier requires approximately 4.6 mg O_2/mg NH_4^+ for nitritation and nitratation (Gerardi, 2006). The DO deprivation in B0 was attributed to the absence of external oxygen and the high oxygen affinity of the nitrifier (Babu et al., 2010). The maximum DO (7.40 mg O_2/L) was observed for B100 due to 1- its having the highest rate of photosynthesis among all the bioreactors, and 2- the absence of DO-consuming species in the photo-bioreactor. From B90 to B10, the trend in the DO concentration is relatively consistent. The DO results demonstrate that the interaction of the nitrifier and *C. vulgaris* can prevent O_2 depletion due to the generation of O_2 via photosynthesis. A significant positive correlation between the NAS proportion and DO consumption is apparent from the data in Table 2. A greater proportion of NAS leads to a greater DO requirement for the nitritation and nitratation processes. Microalgae also requires oxygen for respiration, which can be used for organic compound assimilation (Rezvani et al., 2018). In B10, all the NH_4^+-N was converted to other nitrogen compounds efficiently, and as a result, the DO concentration increased slowly between the 8th and 14th days. The oxygen concentration data are in agreement with those of Karya et al. (2013), who reported a range from 0.3 to 2.4 mg/L. As a result, the generation of dissolved by *C. vulgaris* can mitigate expenses related to external oxygen supply in nitrification (Boelee et al., 2014).

3.6. Effect of different *C. vulgaris*/NAS ratios on the pH

The changes in the pH are presented in Table 2. It should be noted that when the pH dropped, a sufficient amount of NaOH was added to compensate for the deficiency in the alkalinity. Greater pH fluctuation was observed for B10 than for the other NAS ratios because nitrification has a tremendous alkalinity demand (González et al., 2008). B100 exhibited the smallest pH variation, with the pH increasing from 8.5 to 9.5. The photosynthetic activity of *C. vulgaris* led to the higher pH compared with the other ratios (Luo et al., 2017). The increase in

pH can be due to the intensive utilization of the inorganic carbon in the medium for autotrophic algal growth. As the NAS proportion was increased from B90 to B10, the variation in the pH increased because the NAS population required more alkalinity, and the nitrification process lowered the pH of the medium significantly. This observation was consistent with previous studies in which inorganic carbon uptake led to a higher pH (Van Den Hende et al., 2011b, 2011a). Moreover, the continuous illumination provided an energy source for photosynthesis, which in turn led to higher pH values.

3.7. Biomass growth at different *C. vulgaris*/NAS ratios

The variation in the MLSS values corresponds to the cell growth (Su et al., 2012b). The biomass concentrations in the reactors were measured, and the results are detailed in Fig. 8. The photo-bioreactor containing only nitrifying bacteria (B0) did not show any growth, and the MLSS in this reactor was almost constant. Nitrifying bacteria are well-known to have low growth rates (Gerardi, 2006). All of the reactors containing *C. vulgaris* experienced an exponential growth phase with different growth rates. The biomass growth in the total volume is presented in Table 3. The photo-bioreactors containing 70 and 90 wt% *C. vulgaris* had higher growth rates than the other photo-bioreactors. The reactor containing 10 wt% *C. vulgaris* had the lowest growth rate. These results revealed that a greater proportion of *C. vulgaris* led to a higher growth rate. In *C. vulgaris*-NAS systems, the NAS can excrete vitamins or other substances that enhance the growth of algae (de-Bashan et al., 2007). Another significant finding in the growth results was that the heterotrophic growth was limited, since the MLSS values did not exceed 0.65 g/L at any of the *C. vulgaris*/NAS ratios.

3.8. Carbon capture for cell requirements

One of the fundamental parts of the carbon cycle is the conversion of CO_2 into other soluble carbon forms. The chemistry of CO_2 after being dissolved in water is completely dependent on the pH via the equilibrium reactions presented in Section 2.2. These equilibria are well-known for carbon dioxide sequestration. Researchers have demonstrated that for every kilogram of algae, 1.8 kilograms of CO_2 or its equivalent are needed (Slade and Bauen, 2013). To obtain further information regarding the carbon requirements, the dissolved inorganic carbon (DIC) was measured for all the samples; the amount of carbon capture for each is reported in Fig. 9. As discussed in the previous section, there is a positive correlation between CO_2 capture and *C. vulgaris* proportion when nitrifiers are present. This correlation can be rationalized by the fact that nitrifying bacteria are aerobic, and are not able to fix and assimilate CO_2 in the absence of dissolved oxygen.

The tiny amount of CO_2 captured by NAS in B0 corresponds to the small amount of DO present at the beginning of the experiment. For the remainder of the experiment, no significant variation was observed in the CO_2 capture of B0 due to the lack of DO. As the *C. vulgaris* proportion was increased, the demand for dissolved inorganic carbon started to increase due to assimilation. The inorganic carbon demand increased from B10 to B90 due to the nitrification activity of the nitrifier and the higher assimilation by *C. vulgaris*. As is clear from Fig. 9, the CO_2 capture of B100 was lower than that of B90 due to the absence of AOB and NOB. This might have been due to environmental conditions that favored *C. vulgaris*, with no competition for carbon.

3.9. Effect of the *C. vulgaris*/NAS ratio on metabolite generation

One indirect index for the evaluation of *C. vulgaris*-NAS systems in terms of CO_2 fixation is metabolite production. To investigate the generation of metabolites by *C. vulgaris* and NAS, the changes in the concentrations of microbial products such as SMP and EPS were monitored for both proteins (EPSp, SMPp) and carbohydrates (EPSc, SMPc). According to Fig. 10, B0 showed the lowest EPSp production, while B90 showed the highest. As can be seen, a positive correlation can be observed between EPSp and the proportion of *C. vulgaris*. The minimum EPSc production was observed for B10 (4.21 mg/L) and the highest production for B50 (5.49 mg/L). The EPSc results clearly show the superiority of B10 in terms of low metabolite generation. B10 also generated the lowest amounts of SMPc, and the second lowest amount of SMPp after B0. As shown in Fig. 10, B10 exhibited the optimum metabolite generation among the tested photo-bioreactors. These results demonstrate that NAS has a positive influence on the photo-bioreactor in terms of metabolite generation. Sun et al. (2018) demonstrated that interaction between microalgae and sludge had a positive effect in terms of reducing metabolites, and the cooperation between the two can ameliorate the microbial activity. Ramanan et al. (2016) mentioned that the activity of the microalgae is more responsible for the released organic metabolites .

One of the major issues regarding EPS and SMP arises during the disinfection stages in WWT plants. When chlorine reacts with EPS and SMP in effluents, various carcinogenic or genotoxic by-products such as trihalomethanes are formed (Xia et al., 2016). Based on the results of this study, *C. vulgaris*-NAS cultures could be applied to prevent the formation of toxic compounds due to the very low amounts of EPS and SMP produced in the bioreactors.

4- Conclusions and future perspectives

The present study was conducted to determine the autotrophic interaction between *Chlorella vulgaris* and NAS by investigating nutrient removal, carbon capture, and metabolite generation. B10 showed enhanced ammonium removal, requiring only half as much time (7 days) as the other compositions (>14 days). The maximum P removal was achieved using B100. As the proportion of *C. vulgaris* in the culture increased, the amount of carbon captured also increased. The MLSS results demonstrate the low sludge production at the end of the process, ensuring sustainable production due to the prevention of excess sludge generation. Finally, analysis of EPS and SMP demonstrated a positive correlation between the proportion of *C. vulgaris* in the culture and organic metabolite generation.

The selection of chemical components in the influent to the consortium should be considered further. In this study, the influent was based on municipal wastewater, and heavy metals were not considered. Industrial wastewaters that are mainly polluted with heavy metals and their effect on the efficiency of the proposed consortium should be studied.

Although the study investigated the generation of metabolites through the interaction of the two species, the performance of *C. vulgaris*-NAS cultures using advanced technologies such as membrane bioreactors should also be considered. Membrane bioreactors can remove nutrients more efficiently, and could influence the interactions in the *C. vulgaris*-NAS culture.

Reference

Aljerf, L., 2018. ADVANCED HIGHLY POLLUTED RAINWATER TREATMENT PROCESS. J. Urban Environ. Eng. 12, 50–58. https://doi.org/10.4090/juee.2018.v12n1.050058

Anzola-Rojas, M. del P., Gonçalves da Fonseca, S., Canedo da Silva, C., Maia de Oliveira, V., Zaiat, M., 2015. The use of the carbon/nitrogen ratio and specific organic loading rate as tools for improving biohydrogen production in fixed-bed reactors. Biotechnol. Reports 5, 46–54. https://doi.org/10.1016/j.btre.2014.10.010

Azami, H., Sarrafzadeh, M.H., Mehrnia, M.R., 2012. Soluble microbial products (SMPs) release in activated sludge systems: a review. Iranian J. Environ. Health Sci. Eng. 9, 30. https://doi.org/10.1186/1735-2746-9-30

Babu, M.A., Hes, E.M.A., van der Steen, N.P., Hooijmans, C.M., Gijzen, H.J., 2010. Nitrification rates of algal–bacterial biofilms in wastewater stabilization ponds under light and dark conditions. Ecol. Eng. 36, 1741–1746. https://doi.org/10.1016/J.ECOLENG.2010.07.023

Boelee, N.C., Temmink, H., Janssen, M., Buisman, C.J.N., Wijffels, R.H., 2014. Balancing the organic load and light supply in symbiotic microalgal–bacterial biofilm reactors treating synthetic municipal wastewater. Ecol. Eng. 64, 213–221. https://doi.org/10.1016/J.ECOLENG.2013.12.035

Borowitzka, M.A., Borowitzka, L.J., others, 1988. Micro-algal biotechnology. Cambridge University Press.

Cardeña, R., Moreno, G., Bakonyi, P., Buitrón, G., 2017. Enhancement of methane production from various microalgae cultures via novel ozonation pretreatment. Chem. Eng. J. 307, 948–954. https://doi.org/10.1016/J.CEJ.2016.09.016

de-Bashan, L.E., Hernandez, J.P., Bashan, Y., 2007. Microalgae growth-promoting bacteria as "helpers" for microalgae: A novel approach for removing ammonium and phosphorus from

municipal wastewater, in: First International Meeting on Microbial Phosphate Solubilization. Springer Netherlands, Dordrecht, pp. 185–192. https://doi.org/10.1007/978-1-4020-5765-6_28

de Azevedo, A.R.G., Alexandre, J., Xavier, G. de C., Pedroti, L.G., 2018. Recycling paper industry effluent sludge for use in mortars: A sustainability perspective. J. Clean. Prod. 192, 335–346. https://doi.org/10.1016/J.JCLEPRO.2018.05.011

Foladori, P., Andreottola, G., Ziglio, G., 2010. Sludge reduction technologies in wastewater treatment plants.

Gerardi, M.H., 2006. Wastewater bacteria. John Wiley & Sons.

González, C., Marciniak, J., Villaverde, S., García-Encina, P.A., Muñoz, R., 2008. Microalgae-based processes for the biodegradation of pretreated piggery wastewaters. Appl. Microbiol. Biotechnol. 80, 891–898.

Gurbuz, F., Ciftci, H., Akcil, A., Karahan, A.G., 2004. Microbial detoxification of cyanide solutions: a new biotechnological approach using algae. Hydrometallurgy 72, 167–176. https://doi.org/10.1016/j.hydromet.2003.10.004

Hanifzadeh, M., Sarrafzadeh, M.-H., Nabati, Z., Tavakoli, O., Feyzizarnagh, H., 2018. Technical, economic and energy assessment of an alternative strategy for mass production of biomass and lipid from microalgae. J. Environ. Chem. Eng. 6, 866–873. https://doi.org/10.1016/J.JECE.2018.01.008

He, Q., Chen, L., Zhang, S., Chen, R., Wang, H., Zhang, W., Song, J., 2018. Natural sunlight induced rapid formation of water-born algal-bacterial granules in an aerobic bacterial

granular photo-sequencing batch reactor. J. Hazard. Mater. 359, 222–230. https://doi.org/10.1016/J.JHAZMAT.2018.07.051

Hu, Q., Westerhoff, P., Vermaas, W., 2000. Removal of Nitrate from Groundwater by Cyanobacteria: Quantitative Assessment of Factors Influencing Nitrate Uptake. Appl. Environ. Microbiol. 66, 133–139. https://doi.org/10.1128/AEM.66.1.133-139.2000

Ji, X., Jiang, M., Zhang, J., Jiang, X., Zheng, Z., 2018. The interactions of algae-bacteria symbiotic system and its effects on nutrients removal from synthetic wastewater. Bioresour. Technol. 247, 44–50. https://doi.org/10.1016/j.biortech.2017.09.074

Karya, N.G.A.I., van der Steen, N.P., Lens, P.N.L., 2013. Photo-oxygenation to support nitrification in an algal–bacterial consortium treating artificial wastewater. Bioresour. Technol. 134, 244–250. https://doi.org/10.1016/j.biortech.2013.02.005

Le-Clech, P., Chen, V., Fane, T.A., 2006. Fouling in membrane bioreactors used in wastewater treatment. J. Memb. Sci. 284, 17–53.

Li, B., Irvin, S., 2007. The roles of nitrogen dissimilation and assimilation in biological nitrogen removal treating low, mid, and high strength wastewater. J. Environ. Eng. Sci. 6, 483–490. https://doi.org/10.1139/S07-001

Lin, Q., Lin, J., 2011. Effects of nitrogen source and concentration on biomass and oil production of a Scenedesmus rubescens like microalga. Bioresour. Technol. 102, 1615–1621.

Liu, L., Fan, H., Liu, Y., Liu, C., Huang, X., 2017. Development of algae-bacteria granular consortia in photo-sequencing batch reactor. Bioresour. Technol. 232, 64–71. https://doi.org/10.1016/J.BIORTECH.2017.02.025

Liu, L., Zeng, Z., Bee, M., Gibson, V., Wei, L., Huang, X., Liu, C., 2018. Characteristics and performance of aerobic algae-bacteria granular consortia in a photo-sequencing batch reactor. J. Hazard. Mater. 349, 135–142. https://doi.org/10.1016/J.JHAZMAT.2018.01.059

Luo, Y., Le-Clech, P., Henderson, R.K., 2017. Simultaneous microalgae cultivation and wastewater treatment in submerged membrane photobioreactors: A review. Algal Res. 24, 425–437. https://doi.org/10.1016/J.ALGAL.2016.10.026

Mehrabadi, A., Craggs, R., Farid, M.M., 2015. Wastewater treatment high rate algal ponds (WWT HRAP) for low-cost biofuel production. Bioresour. Technol. 184, 202–214. https://doi.org/10.1016/J.BIORTECH.2014.11.004

Muñoz, R., Köllner, C., Guieysse, B., Mattiasson, B., 2003. Salicylate biodegradation by various algal-bacterial consortia under photosynthetic oxygenation. Biotechnol. Lett. 25, 1905–1911.

Neethling, J.B., Willey, B., Harmon, D., Benisch, M., 2007. Phased Nitrification-Denitrification Provides Flexible Nitrogen Removal. Proc. Water Environ. Fed. 2007, 626–639.

Nguyen, B.T., Rittmann, B.E., 2016. Effects of inorganic carbon and pH on growth kinetics of Synechocystis sp. PCC 6803. Algal Res. 19, 363–369. https://doi.org/10.1016/J.ALGAL.2016.03.011

Perez-Garcia, O., Escalante, F.M., de-Bashan, L.E., Bashan, Y., 2011. Heterotrophic cultures of microalgae: metabolism and potential products. Water Res. 45, 11–36.

Quijano, G., Arcila, J.S., Buitrón, G., 2017. Microalgal-bacterial aggregates: Applications and perspectives for wastewater treatment. Biotechnol. Adv. 35, 772–781.

https://doi.org/10.1016/J.BIOTECHADV.2017.07.003

Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., Kim, H.-S., 2016. Algae–bacteria interactions: Evolution, ecology and emerging applications. Biotechnol. Adv. 34, 14–29. https://doi.org/10.1016/J.BIOTECHADV.2015.12.003

Rawat, I., Kumar, R.R., Mutanda, T., Bux, F., 2011. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl. Energy 88, 3411–3424.

Rezvani, F., Sarrafzadeh, M.-H., Ebrahimi, S., Oh, H.-M., 2019. Nitrate removal from drinking water with a focus on biological methods: a review. Environ. Sci. Pollut. Res. 26, 1124–1141. https://doi.org/10.1007/s11356-017-9185-0

Rezvani, F., Sarrafzadeh, M.-H., Seo, S.-H., Oh, H.-M., 2018. Optimal strategies for bioremediation of nitrate-contaminated groundwater and microalgae biomass production. Environ. Sci. Pollut. Res. 25, 27471–27482. https://doi.org/10.1007/s11356-018-2777-5

Rezvani, F., Sarrafzadeh, M.-H., Seo, S.-H., Oh, H.-M., 2017. Phosphorus optimization for simultaneous nitrate-contaminated groundwater treatment and algae biomass production using Ettlia sp. Bioresour. Technol. 244, 785–792. https://doi.org/10.1016/J.BIORTECH.2017.08.053

Rice, E.W., Bridgewater, L., Association, A.P.H., 2012. Standard methods for the examination of water and wastewater. American Public Health Association Washington, DC.

Schrantz, K.A., Pressman, J.G., Wahman, D.G., 2013. Simulated distribution nitrification: Nitrification Index evaluation and viable AOB. J. Am. Water Works Assoc. 105, E242–

E254. https://doi.org/10.5942/jawwa.2013.105.0046

Sepehri, A., Sarrafzadeh, M.-H., 2019. Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated sludge process: metabolite reduction and CO2 mitigation intensification process. Appl. Water Sci. 9, 131. https://doi.org/10.1007/s13201-019-1017-6

Sepehri, A., Sarrafzadeh, M.-H., 2018. Effect of nitrifiers community on fouling mitigation and nitrification efficiency in a membrane bioreactor. Chem. Eng. Process. - Process Intensif. 128, 10–18. https://doi.org/10.1016/j.cep.2018.04.006

Seuntjens, D., Han, M., Kerckhof, F.-M., Boon, N., Al-Omari, A., Takacs, I., Meerburg, F., De Mulder, C., Wett, B., Bott, C., Murthy, S., Carvajal Arroyo, J.M., De Clippeleir, H., Vlaeminck, S.E., 2018. Pinpointing wastewater and process parameters controlling the AOB to NOB activity ratio in sewage treatment plants. Water Res. 138, 37–46. https://doi.org/10.1016/j.watres.2017.11.044

Slade, R., Bauen, A., 2013. Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. Biomass and Bioenergy 53, 29–38.

Su, Y., Mennerich, A., Urban, B., 2012a. Synergistic cooperation between wastewater-born algae and activated sludge for wastewater treatment: influence of algae and sludge inoculation ratios. Bioresour. Technol. 105, 67–73.

Su, Y., Mennerich, A., Urban, B., 2012b. Coupled nutrient removal and biomass production with mixed algal culture: Impact of biotic and abiotic factors. Bioresour. Technol. 118, 469–476.

Sun, L., Tian, Y., Zhang, Jun, Li, L., Zhang, Jian, Li, J., 2018. A novel membrane bioreactor

inoculated with symbiotic sludge bacteria and algae: Performance and microbial community analysis. Bioresour. Technol. 251, 311–319. https://doi.org/10.1016/J.BIORTECH.2017.12.048

Tam, N.F.Y., Wong, Y.S., 1996. Effect of ammonia concentrations on growth of Chlorella vulgaris and nitrogen removal from media. Bioresour. Technol. 57, 45–50. https://doi.org/10.1016/0960-8524(96)00045-4

Van Den Hende, S., Vervaeren, H., Desmet, S., Boon, N., 2011a. Bioflocculation of microalgae and bacteria combined with flue gas to improve sewage treatment. N. Biotechnol. 29, 23–31. https://doi.org/10.1016/j.nbt.2011.04.009

Van Den Hende, S., Vervaeren, H., Saveyn, H., Maes, G., Boon, N., 2011b. Microalgal bacterial floc properties are improved by a balanced inorganic/organic carbon ratio. Biotechnol. Bioeng. 108, 549–558. https://doi.org/10.1002/bit.22985

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., Ruan, R., 2010. Cultivation of Green Algae Chlorella sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. Appl. Biochem. Biotechnol. 162, 1174–1186. https://doi.org/10.1007/s12010-009-8866-7

Xia, C., Ma, D., Gao, B., Hu, X., Yue, Q., Meng, Y., Kang, S., Zhang, B., Qi, Y., 2016. Characteristics and trihalomethane formation reactivity of dissolved organic matter in effluents from membrane bioreactors with and without filamentous bulking. Bioresour. Technol. 211, 183–189. https://doi.org/10.1016/J.BIORTECH.2016.03.026

Yao, Q., Peng, D.-C., 2017. Nitrite oxidizing bacteria (NOB) dominating in nitrifying community in full-scale biological nutrient removal wastewater treatment plants. AMB

Express 7, 25. https://doi.org/10.1186/s13568-017-0328-y

Zhang, B., Lens, P.N.L., Shi, W., Zhang, R., Zhang, Z., Guo, Y., Bao, X., Cui, F., 2018. Enhancement of aerobic granulation and nutrient removal by an algal–bacterial consortium in a lab-scale photobioreactor. Chem. Eng. J. 334, 2373–2382. https://doi.org/10.1016/J.CEJ.2017.11.151

Zhao, F., Zhang, C., Nan, W., Lu, W., 2019. An integrated bioprocess for fermentative production of protopanaxadiol by recycling ethanol waste during down-stream extraction process. J. Clean. Prod. 234, 1–8. https://doi.org/10.1016/J.JCLEPRO.2019.06.190

Table. 1.Nutrient removal performance in proportion of *C. vulgaris* during the experiment

Reactor	Proportion of *C. vulgaris*	Average daily NH_4^+-N removal (mg/L)	Total nitrogen removal efficiency (%)	Total phosphorous removal efficiency (%)
B0	0	1.50	2.85	1.25
B10	10	6.67	37.81	45.75
B50	50	4.46	31.63	59.50
B70	70	3.87	29.55	64.00
B90	90	3.22	24.57	60.75
B100	100	2.03	7.13	85.50

Table. 2. DO accumulation and pH variation due to photosynthesis by *C. vulgaris* and chemical oxidation requirement for nitrifier are shown.

Bioreactor # / Time(day)	B0		B10		B50		B70		B90		B100	
	DO (mgO_2)	pH	DO (mgO_2)	pH	DO (mgO_2)	pH	DO (mgO_2)	pH	DO (mgO_2)	pH	DO (mgO_2)	pH
0	2.50	8.73	2.70	8.82	2.12	8.25	2.78	8.50	2.90	8.50	2.65	8.50
1	0.22	8.42	0.77	7.60	0.70	7.53	0.98	7.96	1.30	8.27	2.70	8.90
2	0.12	8.00	0.73	6.65	0.68	6.90	0.87	7.11	1.36	7.22	2.80	8.90
4	0.10	7.72	0.68	4.04	0.65	4.65	0.91	6.03	1.35	6.83	2.90	9.10
6	0.04	8.00	0.98	8.10	2.50	8.22	3.10	8.12	1.63	8.11	4.00	9.20
8	0.00	7.90	1.37	7.10	3.20	7.70	3.87	7.64	3.40	7.78	5.56	9.30
10	0.07	7.98	2.34	6.97	3.40	7.10	3.67	7.21	3.89	7.43	6.90	9.40
12	0.00	7.77	3.49	6.55	3.80	6.64	4.01	6.75	4.67	6.84	7.30	9.50
14	0.01	7.65	3.53	6.30	3.78	6.40	4.25	6.60	5.03	6.76	7.40	9.70

Table. 3. *C. vulgaris* / NAS growth in total volume

Reactor *C. vulgaris* **(w%)**	**Biomass growth in total volume (mg)**
0	0
10	2400
50	6600
70	8100
90	8700
100	6000

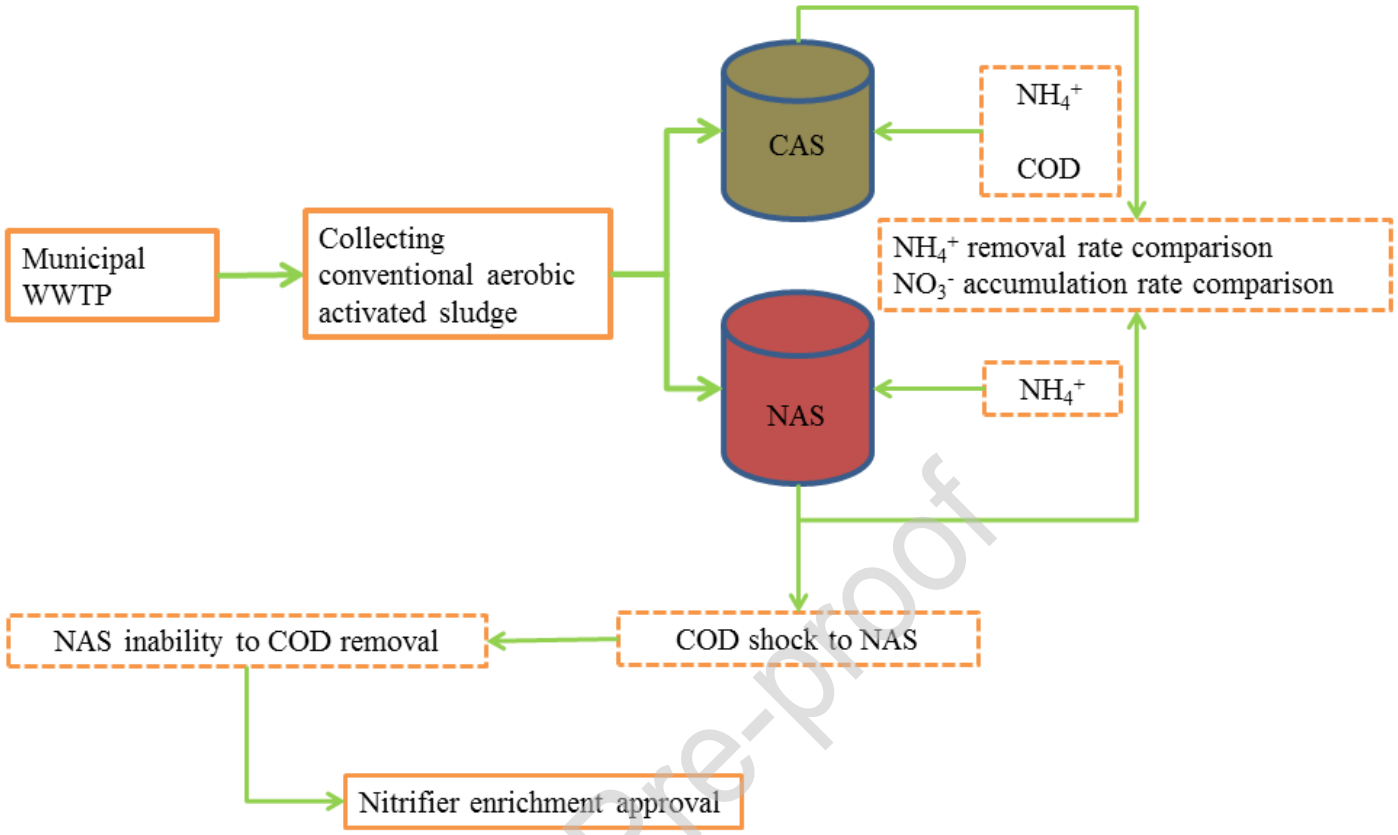


Fig. 1. Flowchart for enrichment of nitrifier in activated sludge and the proof of enrichment. Activated sludge is supplied through local municipal WWTP. Organic carbon-depleted strategy is applied to distinguish the microbial behavior in NAS and CAS communities. Ammonium, nitrate, alkalinity, metabolites generation are compared initially to observe the divergence in two bioreactors. Finally, NAS is exposed to organic loading shock to monitor microbial behavior to new adopted conditions.

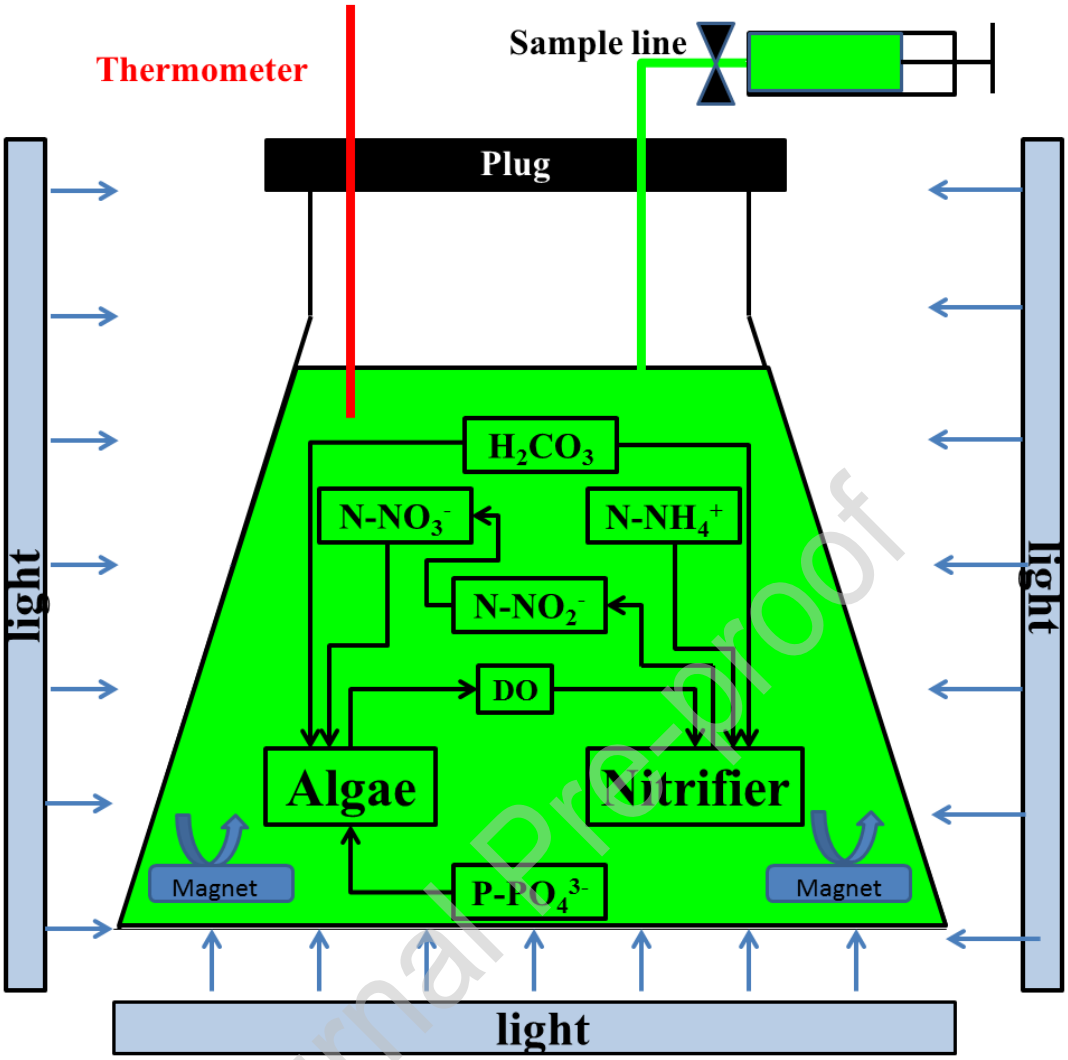


Fig. 2. Schematic of biochemical reactions in *C. vulgaris*/NAS interaction in photobioreactor. The bioreactor consists of a thermometer to measure temperature . The light intensity is supplied with fluorescent lamps under 2000 lux light intensity. The unit operates in batch modes and the bioreactor is clogged with plug.

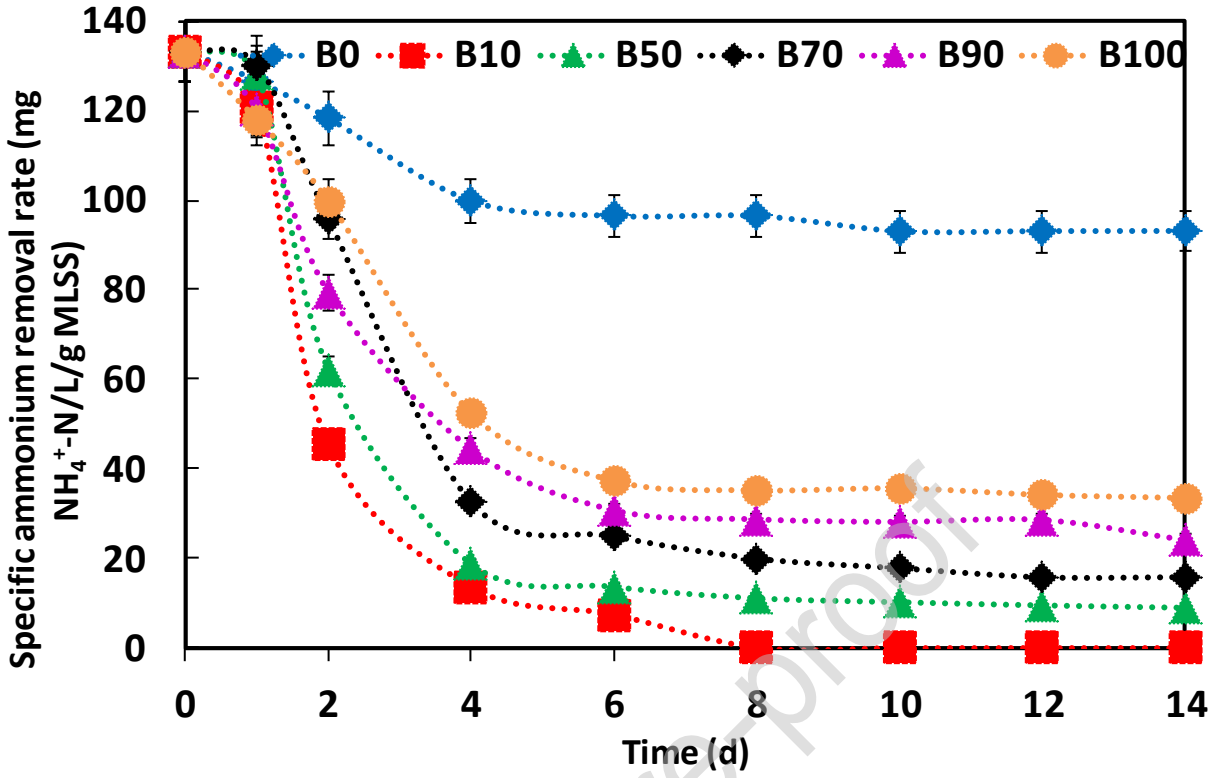


Fig. 3. Variation of specific ammonium removal rate in *C. vulgaris*/NAS culture. All ratios are normalized by corresponding MLSS. B10 could reach complete ammonium nitrogen removal within 8days. The initial MLSS and ammonium nitrogen for all ratios were identical.

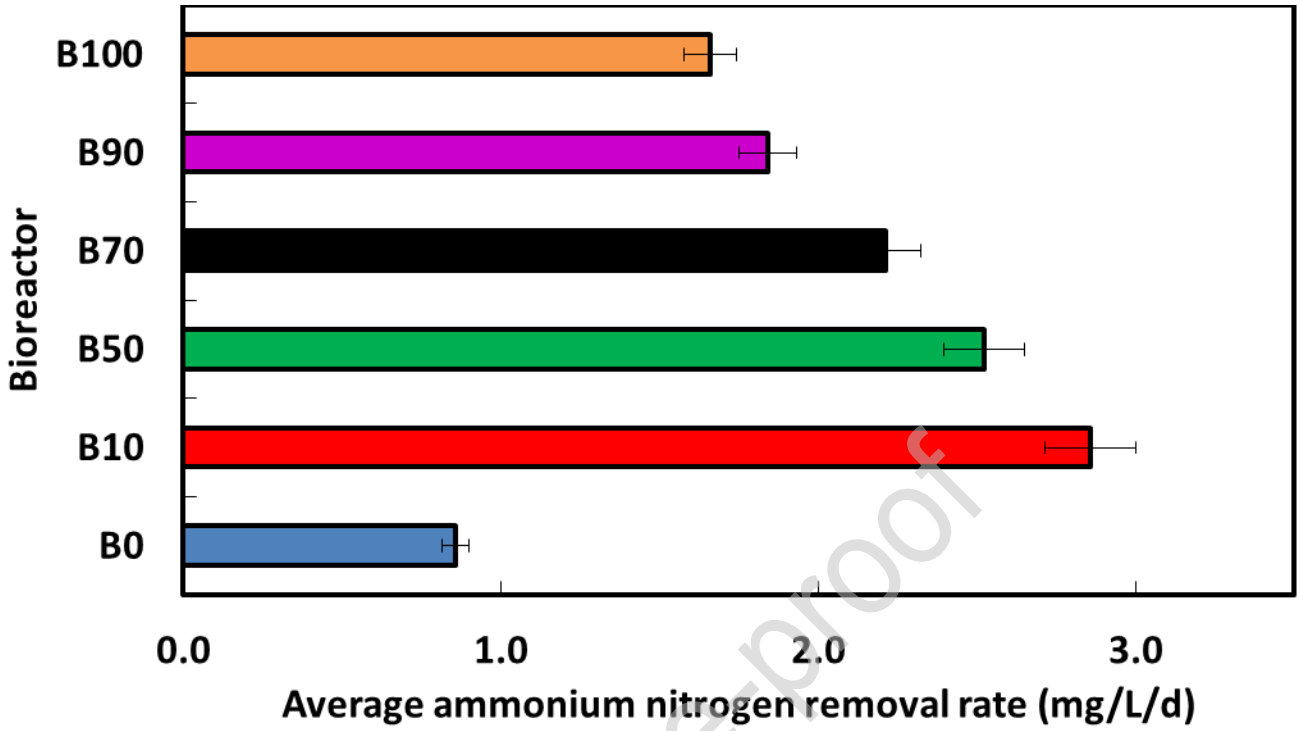


Fig. 4. Comparison among different *C. vulgaris* proportions in terms of ammonium nitrogen removal during 14 days. The initial ammonium nitrogen available to *C. vulgaris* and NAS are similar to 40 mg/L representing the average ammonium nitrogen concentration in municipal wastewater. The average values are calculated based on the day in which the ammonium was totally converted to other nitrogen forms.

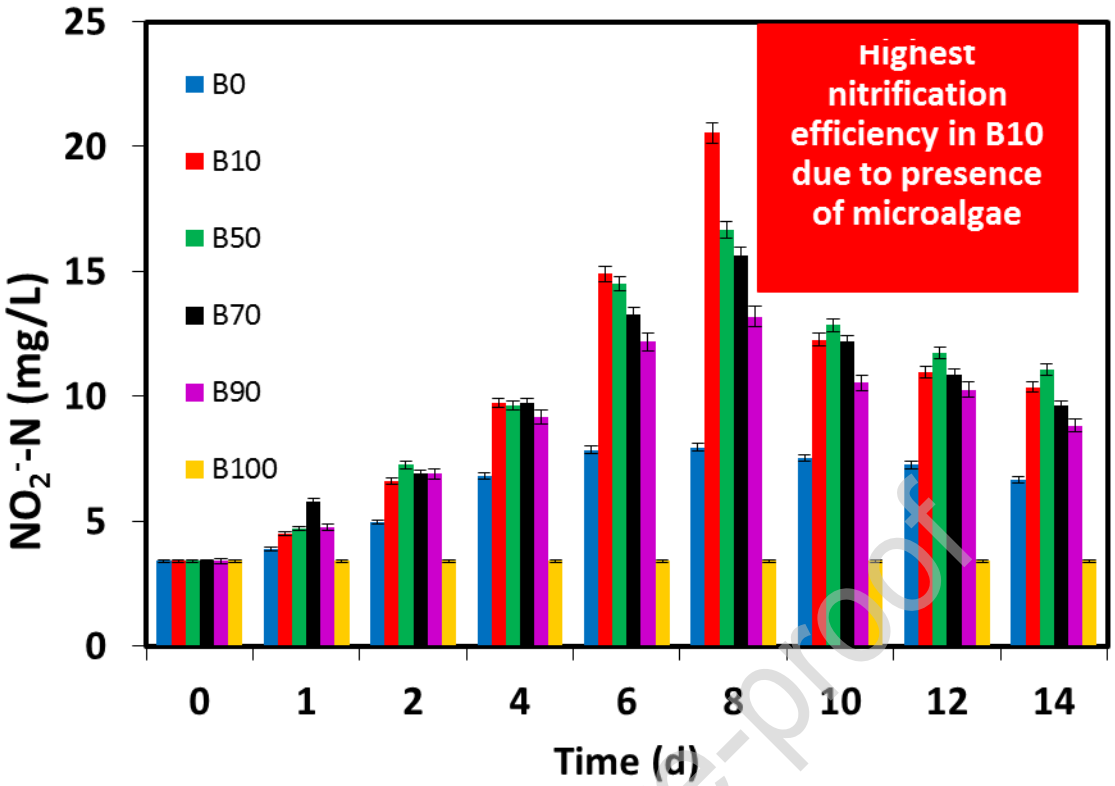


Fig. 5. Effect of *C. vulgaris* proportions on nitrite production. Overall outlook exhibits the presence of boom and bust cycle NAS-included ratios. The maximum nitrite productions among all ratios belong to 8th day. B10 significantly deviates from other ratios in performing nitritation.

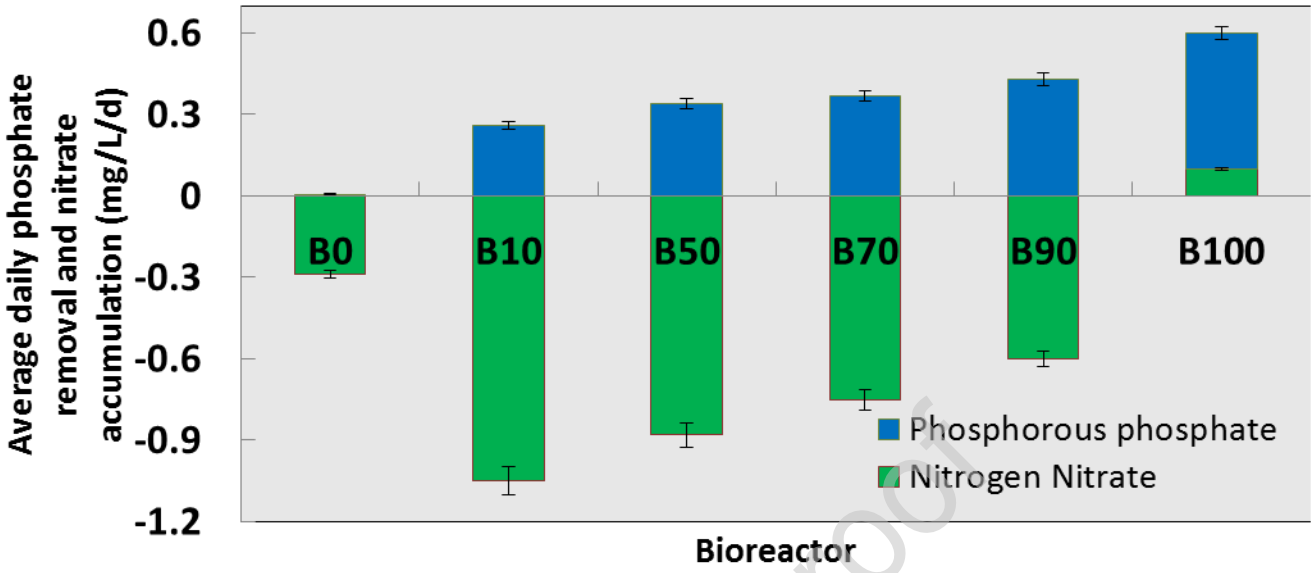


Fig. 6. Effect of *C. vulgaris*/NAS ratio on the average daily phosphate removal rate and nitrate accumulation rate. The inverse correlation between daily accumulation of nitrate and phosphate in microalgae-NAS ratios demonstrates the interaction is able to manage the treatment without compromising of phosphate removal and nitrification. The longer column means the highest efficiency in simultaneous phosphate removal and nitrate production. The columns' order in terms of the length is: B10 > B50 > B70 > B90.

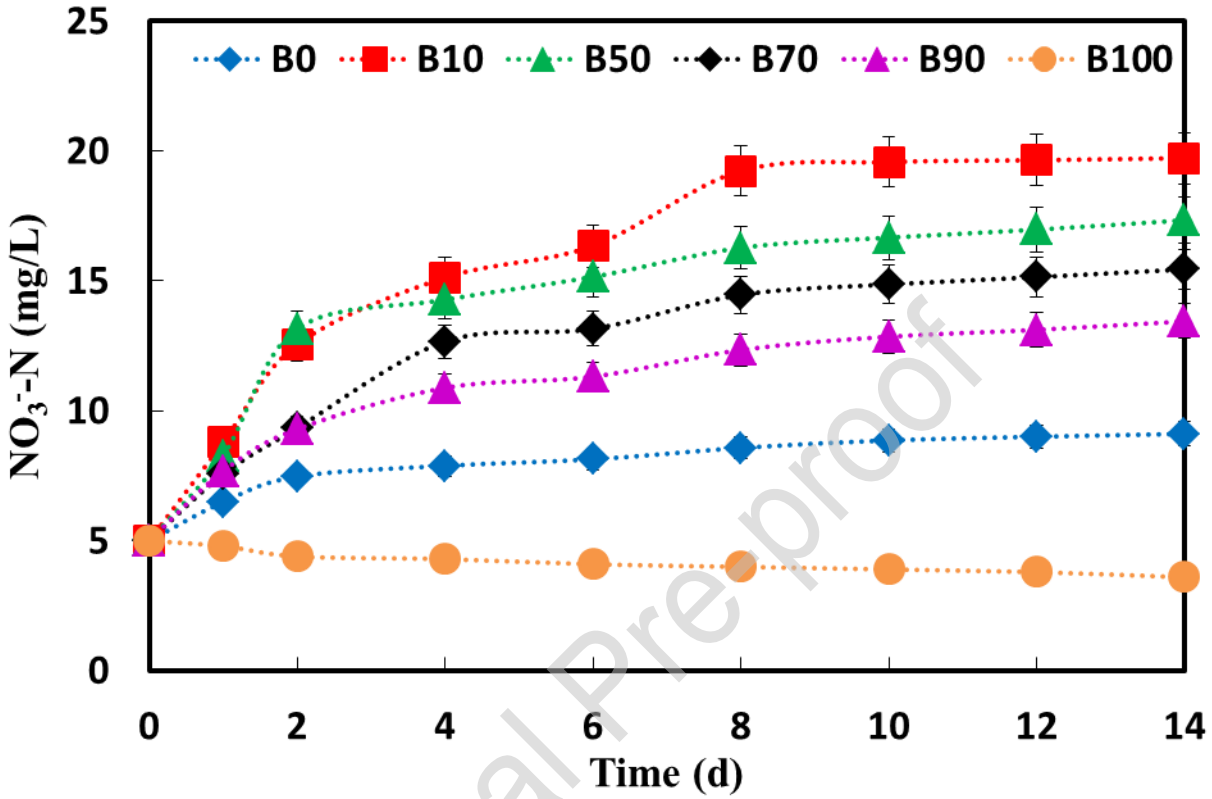


Fig.7. Variation of NO_3^--N in *C. vulgaris*/NAS aggregates. The initial nitrate concentration was adjusted to 5 mg/L of NO_3^--N. The deviation in nitrate production can indicate the efficiency of nitrification efficiency in the presence *C. vulgaris*. B10 could reach to 19.73 mg/L of NO_3^--N being highest among other ratios. The presence of algae could not have inhibitory effect on nitrification.

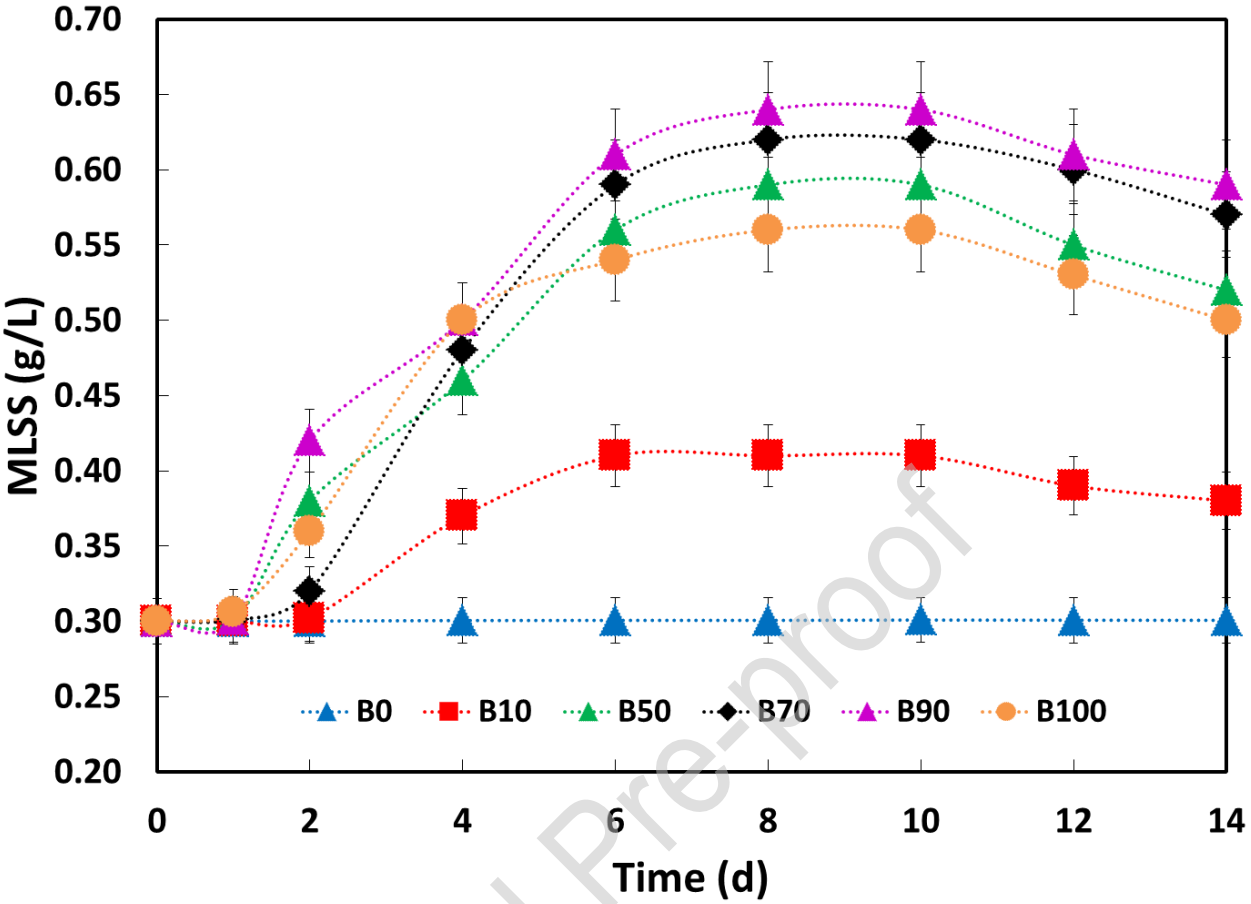


Fig. 8. Growth variation in *C.vulgaris* and nitrifier aggregates. Initial biomass concentration of 0.3 g/L due to microalgae involvement reached to dissimilar ultimate MLSS: B0: 0.30 g/L, B10: 0.38 g/L, B50: 0.52 g/L, B70: 0.57 g/L, B90: 0.59 g/L, B100: 0.50 g/L.

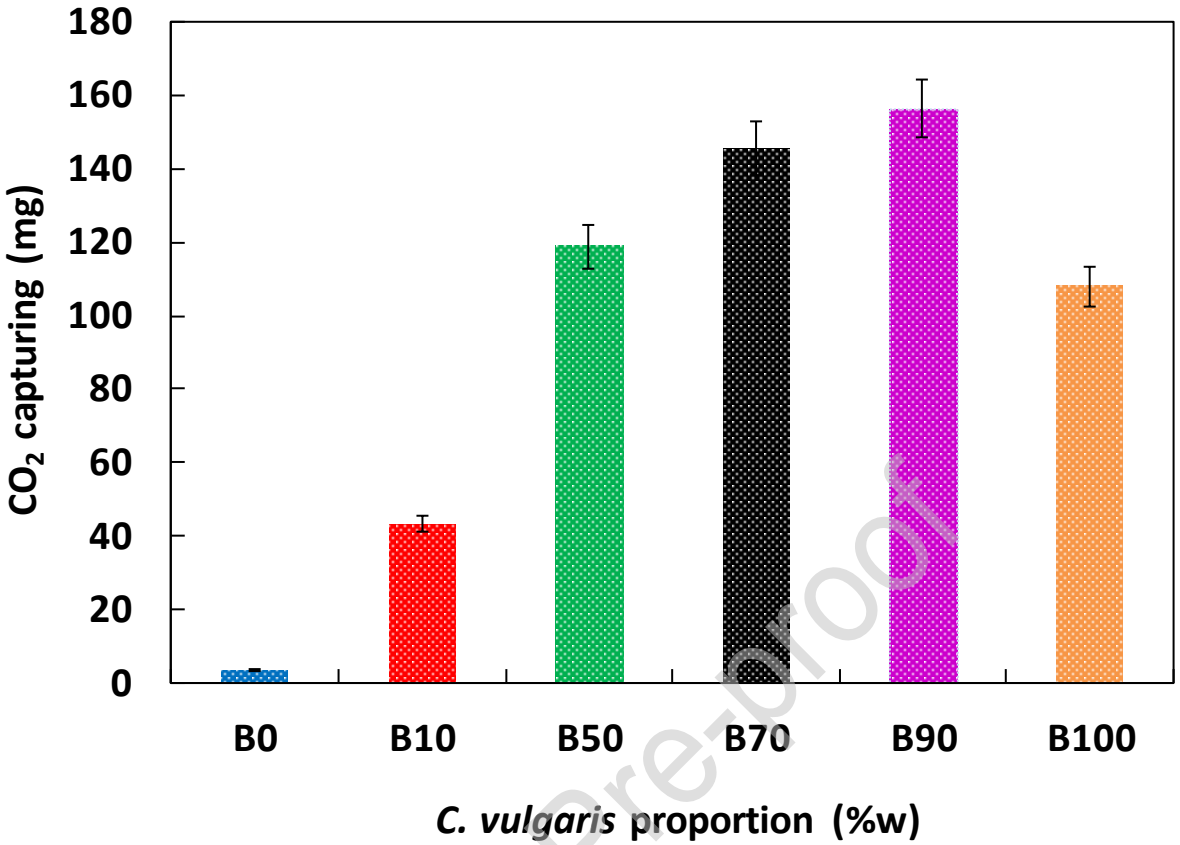


Fig. 9. CO_2 capturing in different proportions of *C. vulgaris*. Positive correlation between captured carbon and algae ratio in algae-included proportion confirms the collaborative interaction of *C.vulgaris* and nitrifier in autotrophic mode. Comparing B100 and other ratios comprised of algae promotes the idea of carbon capturing enhancement through *C.vulgaris* and nitrifier.

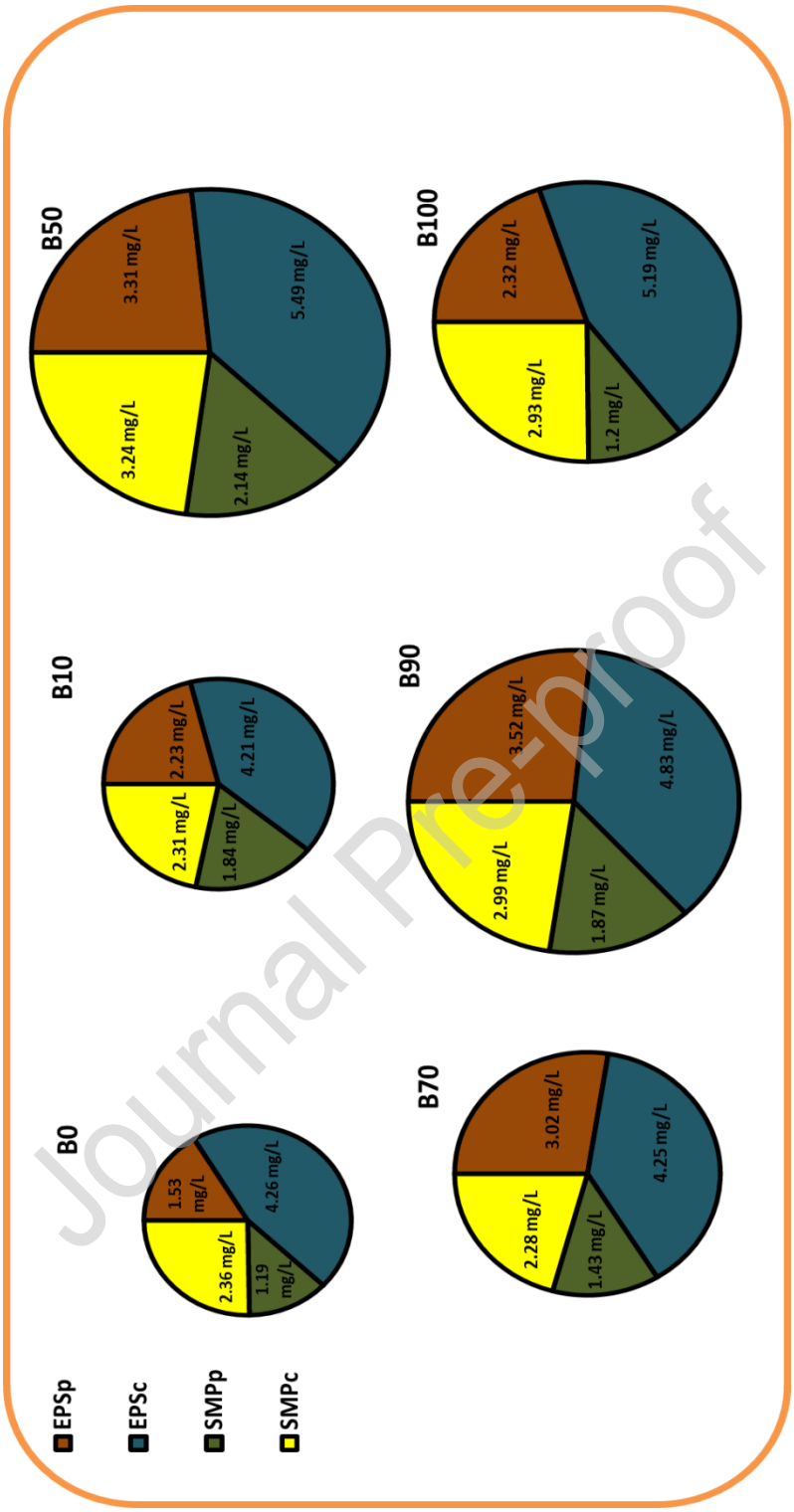


Fig. 10. Distribution of carbohydrate and protein portions of SMP and EPS in consortium. The circle areas exhibit the total metabolite generation. Circular sectors are proportional to EPSp, EPSc , SMPp, SMPc. B50 generated highest metabolites (14.18 mg/L). Among algae-included ratios, B10 produced the lowest metabolites (10.59 mg/L).

Conflicts of interest

There are no conflicts of interest to declare.