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Nitrogen removal from wastewater by an immobilized consortium of microalgae–bacteria in hybrid hydrogels

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ABSTRACT

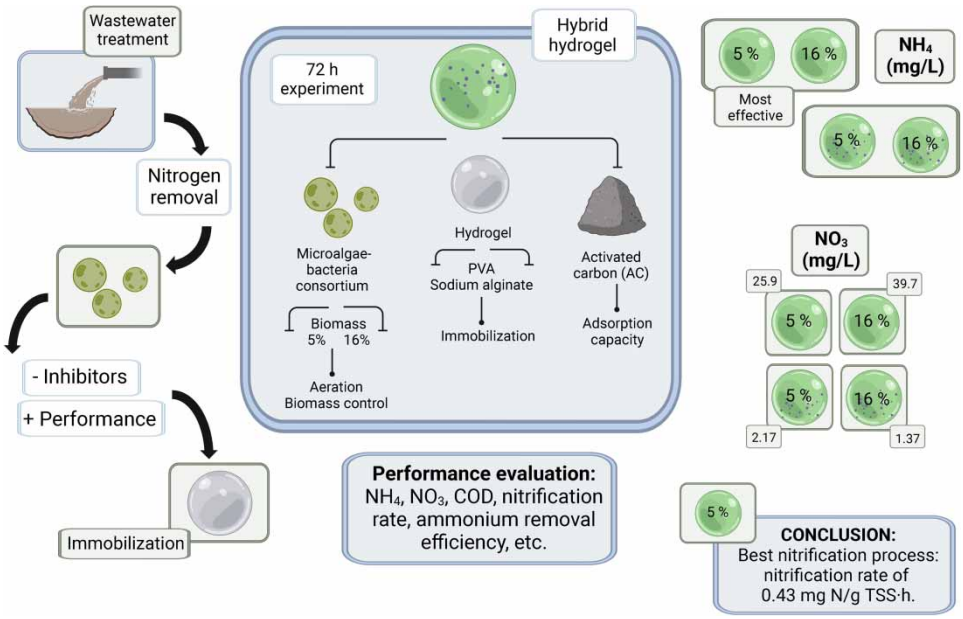
The high content of nitrogen in wastewater brings some operational, technical, and economical issues in conventional technologies. The aim of this study was to evaluate the nitrogen removal by hybrid hydrogels containing consortium microalgae–nitrifying bacteria in the presence of activated carbon (AC) used as an adsorbent of inhibitory substances. Hybrid hydrogels were synthesized from polyvinyl alcohol (PVA), sodium alginate (SA), biomass (microalgae–nitrifying bacteria), and AC. The hybrid hydrogels were evaluated based on the change in ammonium (NH_4), nitrate (NO_3), and chemical demand of oxygen (COD) concentrations, nitrification rate, and other parameters during 72 h. Results indicated that NH_4 removal was more effective for hydrogels without AC than with AC, without significant differences regarding consortium biomass concentration (5 or 16%), presenting final concentrations of 3.13 and 3.75 mg NH_4/L for hydrogels with 5 and 16% of the biomass, respectively. Regarding NO_3 production, hydrogels without AC reached concentrations of 25.9 and 39.77 mg NO_3/L for 5 and 16% of the biomass, respectively, while treatments with AC ended with 2.17 and 1.37 mg NO_3/L. This confirms that hydrogels can carry out the nitrification process and do not need AC to remove potential inhibitors. The best performance was observed for the hydrogel with 5% of biomass without AC with a nitrification rate of 0.43 mg N/g TSS·h.

Key words: hybrid hydrogel, microalgae, nitrification, nitrifying bacteria, wastewater treatment

HIGHLIGHTS

- Hydrogel made of PVA and SA presented notable persistence and integrity in wastewater.
- Hybrid hydrogels containing microalgae–bacteria removed up to 95% of ammonium.
- The nitrogen removal rate from wastewater was up by 0.4 mg N/g TSS·h via hybrid hydrogel.
- Activated carbon affected the nitrification process of immobilized microalgae–bacteria.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Inorganic ions such as SO_4^{2-}, NH_4^+, NO_3^-, NO_2^-, and PO_4^{3-} are considered relevant pollutants in water once their concentration in water exceeds the permissible established limits (Singh *et al.* 2020). These ions are present in many water bodies as a result of discharges of municipal and industrial wastewater (treated and untreated), such as those from tanneries, mining, refining, and manufacture of fertilizer and paper (Akizuki *et al.* 2020; Kwon *et al.* 2020; Singh *et al.* 2020). High loads of these ions, especially those based on phosphorus and nitrogen, cause serious eutrophication and a decrease in dissolved oxygen concentration of water bodies, threatening aquatic organisms, balance of ecosystems, and human health (Kwon *et al.* 2020; Nguyen *et al.* 2020).

Treatment of wastewater with high content of nitrogen, mainly ammonium, brings some operational and economical issues to conventional wastewater treatment plants (WWTPs), such as that based on activated sludge. These issues include high energy consumption due to aeration required for nitrification, adaptation of facilities with additional basins and recirculation flows, management of the excess biomass, and the low nitrification rates for un-controlled and un-monitored treatment systems (Kwon *et al.* 2020; Nguyen *et al.* 2020). Among cost-effective alternatives for water treatment with high ammonium loads is the shortcut nitrification process. This technology consists of two steps: partial nitrification and anammox process. First, ammonium is partially oxidized to nitrite under aerobic conditions by the ammonium oxidizing bacteria (AOB). Then, the anammox bacteria (AMB) oxidize the remaining ammonium to gaseous nitrogen involving nitrite as an electron acceptor. Although AMB requires lower energy levels in terms of successful maintenance of the two-stage systems (conventional WWTP), a vital point is the achievement of an effective nitrite oxidizing bacteria (NOB) suppression. This step is necessary because of the competition between NOB and AMB for available nitrite, as well as the limitation of nitrate production, which increases the operating costs for nitrogen elimination (Shourjeh *et al.* 2020). Little research has been done so far regarding the suppression of NOB in the main nitrification/denitrification systems (Zajac *et al.* 2022), and optimizing the nitrification process of other microorganisms (Otondo *et al.* 2018). Therefore, research addressed to develop efficient technologies that contribute to remove nitrogen from wastewater is needed.

Immobilization of microorganisms has gained importance in wastewater treatment, especially to upgrade conventional WWTPs because this enables to overcome technical and economical limitations, e.g. cost of aeration, management of biosolids, and besides it is considered a biotechnological strategy to optimize nitrification and denitrification in wastewater treatment (Bouabidi *et al.* 2019; Nguyen *et al.* 2020). Among several approaches to immobilize microorganisms, hydrogels of organic and inorganic material are promising because they can embed microorganisms, are permeable, and have affinity

to microorganisms (Subhan *et al.* 2021), while other carriers, e.g. those for biofilm growth, can take a long time to develop the biofilm and are sensitive to changes in the organic loading. Hydrogels have been used to immobilize microorganisms such as microalgae (Cao *et al.* 2020; Kube *et al.* 2021), bacteria (Dolejš *et al.* 2019), and even activated sludge (Cruz *et al.* 2020), which maintained their metabolic functions under controlled environment and model solutions; thus evaluations of these hydrogels in real environments are needed. Hydrogels containing selected microorganisms and exposed to real conditions of bioprocess could improve the removal of conventional contaminants in wastewater such as carbon, nitrogen, and phosphorus (Kumar *et al.* 2022). This is because microorganisms immobilized in hydrogels can present higher tolerance for external stressors, higher residence times, as well as an effective spatial–temporal control of biomass (Mehrotra *et al.* 2021), compared to the suspended microorganisms.

However, a limitation in the use of hydrogels for wastewater treatment is the short duration before disintegration, which is a result of the interaction between the components used for their synthesis and complex water media (Tsai *et al.* 2019). Therefore, hydrogels may present drawbacks such as poor durability alongside low mechanical strength (Pham & Tho Bach 2014). For hydrogels to acquire long operating time and high effectiveness, the polymer matrix must be strong enough to immobilize biomass, powders, and others within the structure (Tuyen *et al.* 2018), inducing the synthesis of multicomponent hydrogels, namely hybrid hydrogels. Hence, it is important to combine polymeric materials, e.g. synthetic and natural, to develop the best matrix corresponding to its purpose (Kumar *et al.* 2022).

Few experiments have evaluated the performance of hydrogels with microorganisms that perform a specific process, e.g. nitrification or nitrogen removal, in complex matrices as wastewater. Majority of experiments have been addressed only embedding microalgae, and hydrogel provides a controlled environment to promote microalgae growth, and protection from undesirable biological species, as well as from a series of pollutants that come from different harmful effluents (Cao *et al.* 2020; Kube *et al.* 2021). Kube *et al.* (2021) immobilized *Chlorella vulgaris, Scenedesmus abundans, Selenastrum capricornutum,* and *Coelastrum microporum* in hydrogel beads based on sodium alginate (SA) for N and P uptake and removal. Results indicated that the increased concentration of microorganisms in reactors and the hydraulic retention time (HRT) lower than 12 h, will allow the design of compact systems that simultaneously remove N and P (Kube *et al.* 2021). In addition to pollutants removal, when hydrogels with microalgae are in membrane bioreactors, there is a delay in membrane fouling due to limited organic matter in the mixed liquor (Cao *et al.* 2020). However, nitrogen removal via microalgae can be enhanced up to two times when bacteria interact with microalgae in photo-bioreactors (Rada-Ariza *et al.* 2017).

Microalgae and bacteria consortium is a very attractive alternative for nitrogen removal from wastewater in photo-reactors (González-Camejo *et al.* 2021). During photosynthesis, microalgae produce oxygen by using light as a source of energy, CO_2 as a source of carbon, and a partial consumption of nitrogen and phosphorous for their cell functions. Oxygen generated by the microalgae is used by the nitrifying bacteria, which provide the CO_2 in return to microalgae (Akao *et al.* 2021). This results in the oxidation of ammonium, an increase of nitrate content in wastewater (Otondo *et al.* 2018; González-Camejo *et al.* 2021), and in a higher survival rate than individual cultures (Phong Vo *et al.* 2020). Hence, the implementation of microalgae–bacteria systems for wastewater treatment results in lower cost, better effluent quality, and higher nitrogen and phosphorus removal, when compared to activated sludge (Morillas-España *et al.* 2021). However, microalgae and bacteria systems have operating conditions that must be properly controlled for efficient application in real wastewater treatment, such as biomass/nutrient ratio (Kwon *et al.* 2020), HRT (Jiang *et al.* 2018), and sludge retention time (SRT) (Fallahi *et al.* 2021). Hydrogels containing microalgae–bacteria can contribute to control these operating conditions and enhance the nitrogen removal.

Immobilized co-cultivation of microalgae with bacteria has mainly been investigated as biofilm in conventional carriers for degradation of pollutants in wastewater (Akao *et al.* 2021*)* and not in a hydrogel. Although synergy between microalgae and nitrifying bacteria has been successfully applied for the nitrification process, nitrification rates when co-culture is in biofilm have been low compared to suspended consortium (Mehrotra *et al.* 2021). This has been attributed to the inhibitory effect of heavy metals and recalcitrant organic compounds over bacteria (Yu *et al.* 2020), and due to direct exposure of biofilm (Grandclément *et al.* 2017). Enhancement of the nitrification process can occur in presence of activated carbon (AC), which improves nitrifying activity (Ng & Stenstrom 1987). This is because AC can adsorb organic compounds responsible for inhibiting nitrification, which are characterized by their resistance to biological degradation as well as being adsorbable by AC (Ng & Stenstrom 1987). To decrease the toxic effects of the inhibitors over nitrifying microorganisms and increase the nitrification rates, addition of AC to bioreactors can be considered. The aim of this study was to prepare stable hydrogels made of PVA and SA, and to evaluate for the first time the performance of hydrogels embedding a consortium of

microalgae–bacteria at different biomass concentrations, and AC as the adsorbent of inhibitory substances, in the nitrification process during treatment of real wastewater.

2. MATERIALS AND METHODS

2.1. Hydrogel synthesis

Hydrogels containing biomass (microalgae and nitrifying bacteria) were produced from a solution of 10 wt% polyvinyl alcohol (PVA), 2 wt% SA, and a fraction (5 or 16 wt%) of biomass in deionized water (DI). SA was selected as it provides an ideal environment, it is permeable, non-toxic, and can protect organisms from extreme physical and chemical conditions (Xue *et al.* 2020). On the other hand, PVA has been widely used for the immobilization of microorganisms and its implementation in wastewater treatment for its accessibility, low toxicity, porosity, and ability to diffuse oxygen and substrate (Bouabidi *et al.* 2019).

For hydrogel preparation, PVA and SA were first dissolved in 50 mL of distilled water on a hot plate magnetic stirrer at 40 °C. Resulting solution was autoclaved for 15 min at 121 °C. Subsequently, the solution was poured into a 100-mL beaker, and temperature was maintained at 40 °C. Water was added to reach 10 wt% of PVA and 2 wt% of SA. Biomass was collected from the adapted culture (see details in Section 2.2), and centrifuged at $F = 1{,}792\ g$, and 28 °C for 20 min to obtain 5 or 16 g of wet biomass, equivalent to 1.35 and 4.35 g/L of TSS and to 5 and 16 wt%, which have been plausible concentrations of biomass embedded in hydrogels to treat wastewater (Wang *et al.* 2019). Then, biomass was incorporated to the hydrogel solution. 0.44 g of AC (<45 μm, HYCEL) was also added to hydrogel solution to minimize the effect of inhibitory substances to the nitrification process, obtaining hydrogels with AC. The hydrogel solution containing biomass (with or without AC) was dripped into a crosslinking solution, containing 5.6 wt% of boric acid and 2 wt% of calcium chloride at room temperature. The hydrogel beads with 5 and 16 wt% of biomass and with or without AC were kept in the crosslinking solution for 24 h under constant stirring at 150 rpm. After, the hydrogel beads were transferred to a 7.1 wt% of sodium sulfate buffer solution where they were stirred again (150 rpm) for 48 h. Then, hydrogels were transferred to DI water until experimentation.

The stability of the synthetized hydrogels without biomass was tested before the nitrification experiment according to methodologies using both tap water and DI water (Tuyen *et al.* 2018; Wang *et al.* 2019). Stability of the hydrogels was judged based on the two parameters of mechanical strength (durability exposed to mechanical stirring) and agglomeration of the gel beads. The test consisted of 150 hydrogels beads that were placed in a 250-mL beaker with 100 mL of DI water and 150 beads in 100 mL of tap water. Each beaker was put on a stirring plate where the hydrogels were exposed to 200, 400, 600 and 800 rpm for 15 min. At the end, the hydrogels were counted and determined how many beads remained undamaged and if they were agglomerated.

2.2. Microalgae–bacteria consortium adaptation

The enriched microalgae–nitrifying bacteria consortium was obtained from microalgae–bacteria cultures that treat wastewater in the laboratory (Akizuki *et al.* 2020), and it was adapted to treat raw wastewater. Microalgae consisted *C. vulgaris* while nitrifying bacteria were collected from the full-scale oxidation ditch WWTP in Guanajuato, Mexico. For adaptation, 250 mL of seed culture was added to four Erlenmeyer flasks of 1 L. The consortium was adapted to treat wastewater (350 mL) from the WWTP of the Tecnológico de Monterrey Campus Puebla, whose characteristics are shown in Table 1. Flasks operated as sequential batch reactors (SBRs), settling biomass of flasks, and replacing 350 mL of supernatant by

Table 1 | Characteristics of raw wastewater used in the experiments from the Tecnológico de Monterrey WWTP

Parameter	Adaptation period	Experiments
pH	7.6	7.5
EC (μS/cm)	1,431	1,492
NH_4 (mg/L)	20.85 ± 1.5	77.3 ± 1.2
NO_3 (mg/L)	0.9 ± 1.1	<0.1
COD (mg/L)	642 ±	597 ± 12
BOD (mg/L)	331.5 ± 6	331 ± 7

wastewater every third day. SRT was complete during adaptation period (14 days). The flasks containing the consortium were placed in an incubator (incubator AlgaeTron AG130) at an average temperature of 27 °C with a constant stirring at 150 rpm, White LED light was irradiated at an intensity of 160 μmol/m^2/s, with a cycle of 12 h of light and 12 h of darkness. Flasks were characterized every 2 or 3 days as the function of pH, electrical conductivity (EC), ammonium (NH_4), and nitrate (NO_3).

2.3. Experimental setup

Hydrogels with 5 and 16 wt% of biomass, with or without AC were evaluated per triplicate in 125-mL Erlenmeyer flasks during 72 h. The flasks contained between 13 and 15 g of hybrid hydrogels and 85 ± 1 mL of wastewater. The wastewater used was the same as that in the adaptation experiment. Flasks were kept in an incubator (AlgaeTron AG130) at an average temperature of 27 °C with constant stirring at 150 rpm for 72 h, white LED light was irradiated at an intensity of 160 μmol/m^2/s, with a cycle of 12 h of light and 12 h of darkness. Parameters such as pH and EC were monitored every 24 h of each flask with hydrogels. The measurements of ammonium and nitrate were carried out per triplicate in all flasks every 24 h, for a total period of 72 h. Within the period specified, the ammonium removal efficiency (ARE) was determined based on the data obtained for each biomass concentration (5 and 16 wt%), using Equation (1) (Akizuki *et al.* 2020):

$$\text{ARE} = \left[1 - \left(\frac{\text{NH}_{4\text{Final}}}{\text{NH}_{4\text{Initial}}}\right)\right] \times 100 \tag{1}$$

The nitrification rate for each type of hybrid hydrogel was evaluated based on the change of nitrate concentration every 24 h. The following Equations (2)–(4 were used to determine the nitrification rate of all treatments. For this purpose, it was considered the equivalent fraction of the atomic weight of nitrogen in the nitrate molecule, where N_1 is the nitrate concentration (mg/L), C_1 is the nitrate production concentration (mg/L), N_2 is the net nitrogen removal (mg N), v is the experimental volume (L), and t stands for time (h).

$$N_1 = 0.226 \cdot C_1 \tag{2}$$

$$\text{N}_2 = \text{N}_1 \cdot v \tag{3}$$

$$\text{Nitrification rate} \left(\frac{\text{mg } N}{g\ \text{TSS} \cdot\ \text{h}^{-1}}\right) = \frac{N_2}{\text{g TSS} \cdot t} \tag{4}$$

2.4. Analytical methods

The parameters analyzed in the experiment were pH, electrical EC, temperature, ammonium, nitrate, chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS), and total volatile solids (TVS). The pH, temperature, and EC values of all flasks were measured with a portable meter using the corresponding probes (HACH HQd). COD, ammonium, and nitrate content in the samples were analyzed according to the commercial methods (HACH). TSS, VSS, TS, TVS concentrations in the consortium were determined according to Standard Methods (APHA 2005), respectively. Light intensity was directly measured using a solar radiation meter (PCE-SPM 1).

2.5. Statistical analysis

A three-way ANOVA analysis was carried out with Minitab using a *p*-value of 0.05, through a factorial design ($2 \times 2 \times 3$), where the response variables were ammonium and nitrate. Results of response variables are shown as the average value ± standard deviation. Parameters were measured every 24 h in a period of 72 h to quantify ammonium removal and nitrate production. Biomass (5 and 16 wt%), AC (without and with), and time (24, 48 and 72 h) were the factors analyzed with their respective levels and the effect on each of the response variables through principal effect plots.

3. RESULTS

3.1. Stability of hydrogels

For the establishment of a suitable immobilization method, stability of hydrogels was tested in DI and tap water under a constant agitation with different stirring speeds (200–800 rpm) (VELP, AREC Heating Magnetic Stirrer), as function of persistence and agglomeration (Table 2).

Table 2 | Stability analysis of hydrogels at different rpm values in 15 min

Water media	Speed (rpm)	Quantity of hydrogels		Agglomeration status
		Initial	Final	
Tap water	200	150	150	Complete structure
	400	150	150	Complete structure
	600	150	132	Partially dissolved
	800	132	0	Completely dissolved
DI water	200	150	150	Complete structure
	400	150	124	Complete structure with mild agglomeration
	600	124	103	Partially dissolved with agglomeration
	800	103	0	Completely dissolved

In tap water at 200 and 400 rpm, all the hydrogel beads maintained their original shape without agglomeration. At 600 rpm, the damage of the beads started to appear, and approximately 12% of beads were disintegrated and dissolved, while at 800 rpm, hydrogels were completely dissolved. Whereas, in DI water, approximately 17% of the beads were already disintegrated at 400 rpm and even mild agglomeration was also observed, which was worse at higher speed, reaching complete dissolution at 800 rpm.

The results showed that hydrogels in tap water had higher mechanical durability and resistance for agglomeration than those in DI water. This could be a result of the chemical nature of ions present in tap water, which can stabilize the structure of the hydrogels, while DI water, due to lack of these ions, only caused rapid disintegration and provoke agglomeration (Alcântara *et al.* 2009). Although complete dissolution was observed for both cases at 800 rpm, this speed is an extremely severe condition to test mechanical durability within a short period of time. The best performance of hydrogels was observed at 200 rpm, hence the nitrification experiment was carried out below this speed, at 150 rpm. It can be concluded that the prepared hydrogels had the mechanical durability and resistance for agglomeration suitable for nitrification experiments.

3.2. Consortium adaptation

The concentration of ammonium and nitrate in the influent and the effluent during the adaptation period (13 days) of the microalgae–bacteria consortium is shown in Figure 1. Results indicate a high efficiency of the consortium to perform the nitrification process by decreasing ammonium concentration present in the raw wastewater from an average of 20.84 mg/L in influent to 1.96 mg/L in effluent, which corresponds to approximately 90% of ammonium removal, under pH between 6.7

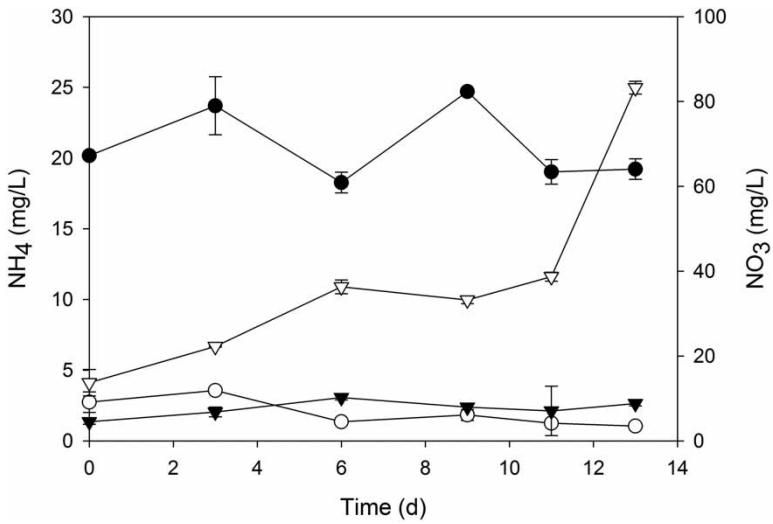


Figure 1 | Temporal variation of ammonium (NH_4) and nitrate (NO_3) during the culture adaptation period. Symbols: NH_4 influent (●), NH_4 effluent (○), NO_3 influent (▼), and NO_3 effluent (▽).

and 7.6 (Supplementary material, Figure S1). The nitrate concentration throughout the adaptation process had an increase from 17 up to 81.9 mg/L, showing a cumulative effect of nitrate in the remaining liquor after each replacement of wastewater. This also confirms that the nitrification process was effective as a result of microalgae–bacteria interaction. In addition, the consortium removed biochemical oxygen demand (BOD) while removing ammonium. It was found that during the adaptation time, the BOD value decreased in average from 331 ($\pm$6.4) to 19.5 ($\pm$0.7) mg/L, equivalent to a removal efficiency of approximately 94%. This also demonstrates the coexistence of heterotrophic bacteria in the consortium which contributed to consume the organic matter present in the wastewater.

3.3. Nitrification in hydrogels of microalgae–bacteria

3.3.1. Ammonium removal

Figure 2 shows variation of ammonium concentration for all experiments during 72 h. The initial ammonium concentration was 77 $\pm$ 0.5 mg/L for all experiments. During the first 24 h, this concentration decreased significantly in all treatments' conditions. For hydrogels without AC, values at 24 h were 28.47 and 23.80 mg/L for 5 and 16% of biomass, respectively. At 48 h, the concentrations for both treatments without AC decreased to 21.93 mg/L for 5% and 16.19 mg/L for 16%. Finally, the values obtained at 72 h were the lowest for all treatments with an average concentration of 3.13 mg/L for hydrogels with 5 wt% of biomass, and 3.75 mg/L for hydrogels with 16% of biomass.

Treatments with AC had a similar downward trend for ammonium concentration, reaching around 20 mg/L, regardless content. At 48 h, ammonium concentration did not change significantly, while at 72 h, the final concentration of ammonium in assays with AC and both content of biomass reached a value of around 14 mg/L. According to the statistical results (Supplementary material, Figure S2), with a *p*-value greater than 0.05 in ammonium removal, all factors alone, as well as their respective interactions, showed not to have an effect on the response variable. Therefore, it can be concluded with statistical evidence that H_0 is not rejected. This means that biomass, content, presence of AC, and time had no influence on the ammonium removal.

3.3.2. Nitrate production

Figure 3 shows the temporal change of the nitrate concentration in the experiments using hybrid hydrogel with and without AC. The initial nitrate concentration in the wastewater for all treatments using hybrid hydrogels was lower than a detectable level by the used method (<0.1 mg/L). In the first 24 h of the experiment, hybrid hydrogel treatments with 5 and 16% of biomass without AC obtained an average nitrate value of 16.20 and 16.07 mg/L, respectively. At 48 h, the concentration had a

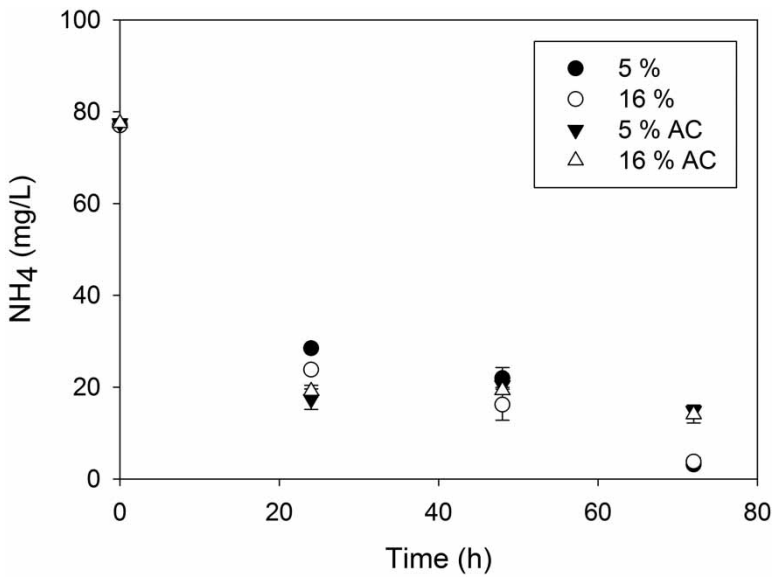


Figure 2 | NH_4 concentration as time course in experiments with hydrogels containing different biomass concentrations (5 and 16%) with and without AC.

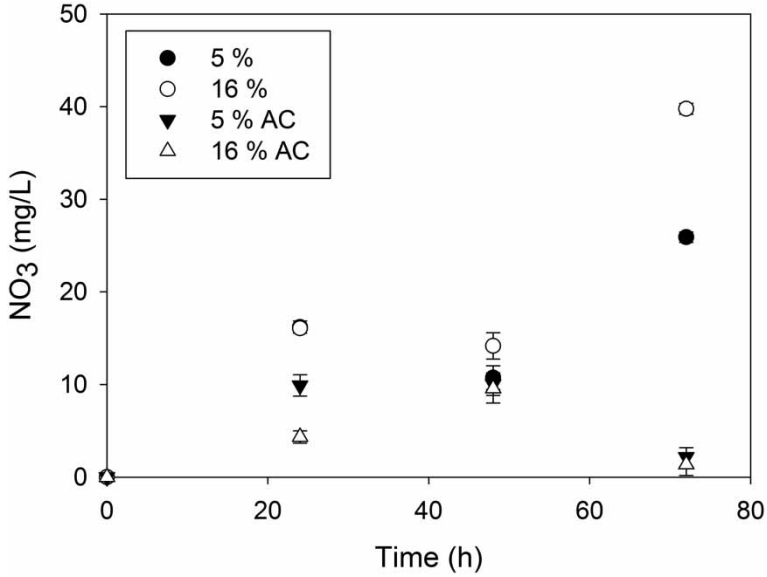


Figure 3 | Time course change of NO_3 in experiments with hydrogels with different biomass concentrations (5 and 16%) with or without AC.

decrease of 33.95 and 11.80%, resulting in 10.7 and 14.2 mg/L, respectively. Finally, at 72 h, the concentration of nitrate was 25.9 mg/L for hybrid hydrogels with 5% of biomass and 39.77 mg/L for hydrogels with 16% of biomass.

In the presence of AC, the hydrogel with 5% of biomass at 24 h had greater production of nitrates (10 mg/L), which was maintained up to 48 h. However, at 72 h concentration had a decrease of 78% resulting in 2.17 mg/L. Hydrogel with AC and 16% of biomass presented 4.3 mg/L of nitrate at 24 h, then increased to 9.6 mg/L at 48 h the concentration. However, similar to the hydrogel with 5% of biomass, the final concentration of nitrate had a decrease of 86%, ending with 1.37 mg/L. This suggests that the decrease in nitrate concentration at the end of the experiment may have resulted from the presence of AC in treatments (Najmi *et al.* 2020). Unlike treatments without AC, nitrate accumulation was evident. AC has presented high adsorption capacity of nitrate, more than 65% when 100 mg/L was fed, in short periods (300 min), which was attributed to positive charge and micro-pores on its surface (Azhdarpoor *et al.* 2019).

According to the statistical results in nitrate production (Supplementary material, Figure S3), the AC factor was shown to have an effect in the response variable with a *p*-value of 0. Therefore, it can be concluded with statistical evidence that H_0 is rejected. The rest of the factors alone, as well as their respective interactions, resulted in *p*-values greater than 0.05, therefore it can be concluded that biomass and time had no effect in the nitrate production.

4. DISCUSSION

The ammonium removal in all treatments indicated that regardless of the incorporated biomass amount, the hydrogels without AC is 14.3% more effective than those with AC for time and conditions applied (Figure 4(a)). However, at 24 h, hydrogels containing AC with 5 and 16% of biomass presented the greatest decrease in ammonium concentration, reaching 77.5 and 75.3% of ARE, respectively. At the end of experiments, same hydrogels just increased ARE by of 3.2 and 6.4%, respectively. In contrast, ARE in hydrogels without AC increased over time reaching up to 95.9 and 94.9% for that containing 5 and 16% of biomass. Interestingly, in the first 48 h, ARE presented better performance for 16% of biomass content but at the end, and even in the treatment with AC, ARE was similar for both biomass contents.

Nitrate concentration at the end of the experiments in all treatments confirm that the nitrification process was carried out, but at different levels for treatments with and without AC. The hydrogels with 16% biomass without AC induced the highest production with 39.77 mg/L. Conversely, the results of treatments with AC, showed lower nitrate concentration. This is in line with the low ARE, 80.7 and 81.7% for 5 and 16% of biomass content, respectively (Figure 4(a)). This demonstrates an adverse effect of AC during the nitrification process by hydrogels containing microalgae–bacteria, regardless biomass content. The main potential reason is because the available space within the hydrogel matrix may have been saturated with the presence of AC, resulting in the inability of microorganisms to develop the nitrification process (Wu *et al.* 2014).

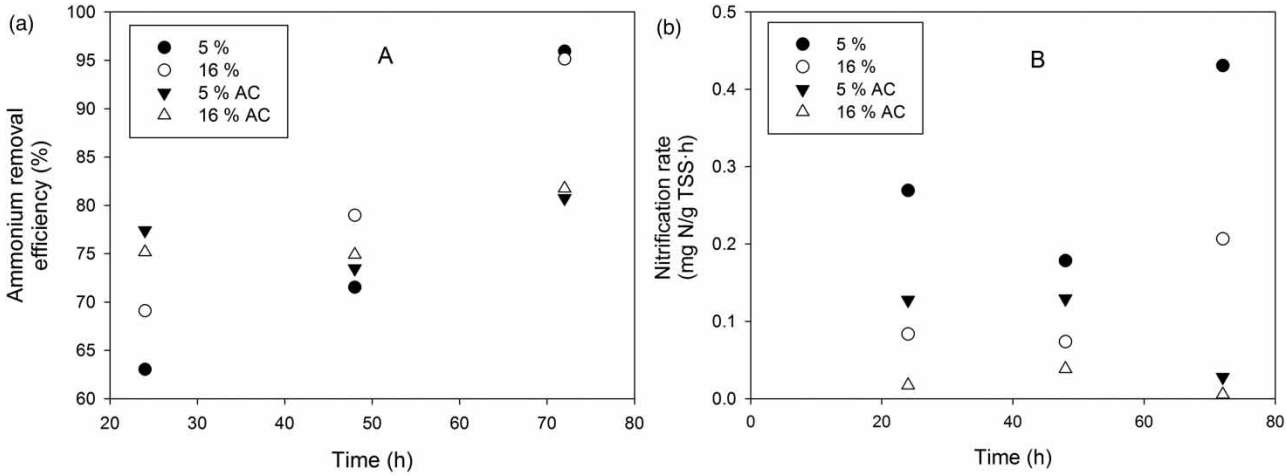


Figure 4 | Temporal change of hydrogels with different biomass concentrations (5 and 16%), with and without AC of (a) ARE and (b) nitrification rate (mg N/g TSS.h).

Considering the nitrification rate per mass of microorganisms values in experiments without AC, hydrogels with 5% of biomass showed better performance than those with 16%. Figure 4(b) shows the highest nitrification rate of 0.43 mg N/g TSS·h for hydrogels containing 5% of biomass without AC addition, while for hydrogels with 16%, it was 0.20 mg N/g TSS·h at the experimental endpoint. It can be concluded that hydrogels without AC and with 5% biomass were more efficient among all treatments. This means that embedded consortium in hydrogel was able to produce oxygen using light as a source of energy and CO_2 as a source of carbon, while oxygen generated by the microalgae was used by the nitrifying bacteria, which provide the CO_2 in return to microalgae, and oxidize the ammonium to nitrate. Besides, high content of biomass did not result in higher efficiency. This can be attributed to a self-shading of microorganisms and, therefore, reduction of the penetration of light necessary for photosynthetic and microbial activity (Foladori *et al.* 2020). Therefore, it could be considered that the microorganisms of the upper layers of the hydrogel were able to perform metabolic activities, limiting the performance of the microorganisms in the core of the hydrogels.

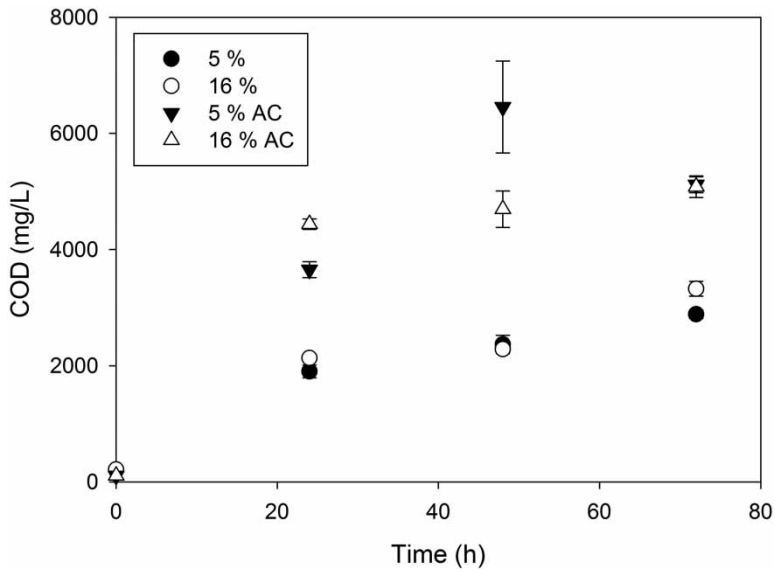


Figure 5 | Treatments of total COD variation with different biomass concentrations (5 and 16%), with and without AC.

Table 3 | Advantages and disadvantages of incorporating AC and different percentages of biomass in hydrogels made of PVA and alginate

Types of hydrogels	5 wt% of biomass	16 wt% of biomass
With AC	*Advantages:* Ammonium removal through adsorption *Disadvantages:* Potential persistence of nitrate in AC. High hydrogel breakdown.	*Advantages:* Ammonium removal through adsorption *Disadvantages:* Potential persistence of nitrate in AC. High hydrogel breakdown
Without AC	*Advantages:* Highest ammonium removal. High nitrate production. Highest nitrification rate (0.43 mg N/g·TSS·h) *Disadvantages:* Long-term disintegration	*Advantages:* High ammonium removal. High nitrate production. Successful nitrification process *Disadvantages:* Self-shading effect. Long-term disintegration

A clear effect of AC over ammonium removal and nitrate production was observed, which can be attributed to the adsorption of these compounds rather than the shortcut nitrification process. Considering that the nitrification process was performed during first 48 h, the low nitrate concentration in AC treatments may have resulted from their adsorption in AC hydrogel (Mehrotra *et al.* 2021). However, change in the microbial community responsible for nitrogen removal caused by the presence of AC has been already reported (Yu *et al.* 2020), and it could be explored as a potential explanation. Hence, more research involving the reusability of hydrogels, together with microbial diversity analysis can be performed.

Figure 5 shows the COD concentration as time course, which resulted in constant increasement for all treatments. The reason for this, regardless of the concentration of biomass in all treatments, could be due to the leaching of the components of hydrogel. Specifically PVA, for its low biodegradability (Sun & Lu 2019), could have caused the increase of COD during the 72 h of the experiment. COD concentrations in AC treatments were always higher than those without AC throughout the experiment. This difference could have resulted from the possible effect that AC can induce over hydrogel structure, releasing higher content of PVA from their treatments. In general, main advantages and disadvantages of hydrogels containing AC and different amounts of biomass are summarized in Table 3.

5. CONCLUSIONS

In this study, microalgae and nitrifying bacteria were immobilized together in hydrogels in different biomass concentrations, with or without AC. The effectiveness of the treatments was analyzed throughout the 72-h experiment, monitoring ammonium removal as well as nitrate production. With the results obtained it can be concluded that the most effective hydrogel was that with 5% of biomass concentration and without AC, which presented a nitrification rate of 0.43 mg N/g TSS·h, and up to 95% of ammonium removal. Presence of AC in hydrogels containing microalgae–bacteria may have interfered the nitrification process of microalgae-nitrifying bacteria, which can be attributed to the sorption of some compounds in the surface of AC. The persistence of hybrid hydrogels can be extended as a result of the components used for synthesis, as well as the type of water media to which it is exposed. However, during application there is the possibility of slow release of some compounds, e.g. PVA or SA. This leachate may affect the quality of the treated effluent in terms of COD concentration. Nonetheless, hybrid hydrogels with immobilized consortium are a feasible option to perform the nitrification process in wastewater.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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