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The Efficiency of Moving Bed Bioreactor Carrier (MBBRC) Hybrid with Algae and Bacterial Strains for Ammonia Removal in the Rosetta Branch of the River Nile

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ABSTRACT

In the current study, laboratory experiments were conducted using a moving bed bioreactor carrier (MBBRC) to investigate the ability of two blue-green algae genera (*Anabaena* sp. and *Scenedesmus* sp.) and two bacteria genera (*Lactobacillus* sp. and *Azotobacter* sp.) and remove ammonia from contaminated water. The results of this study revealed that using single algal species achieved a maximum removal percentage of 30% for ammonia concentrations ranging from 5 to 7.5mg/ l, while the utilization of two mixed algal species resulted in ammonia removal percentages ranging from 6% to 12% at retention times from 15 to 60 minutes. On the other hand, using each of the two algal species separately at retention times of 2 and 5 hours resulted in removal percentages ranging from 85% to 100%. Additionally, the study found that the application of bacterial genera (*Lactobacillus* sp. and *Azotobacter* sp.) on raw water samples with an average ammonia concentration of 7.8 mg/l, and the utilization of bacterial content within the range of 100-200 ml per 1-liter volume of raw water samples demonstrated a remarkable maximum removal efficiency of 100%, with varied retention times up to 2 hours.

INTRODUCTION

The Nile River is the primary source of freshwater used for drinking water, agricultural activities, industrial purposes, navigation, recreation and fish production in Egypt. Hence, it has a dominating influence on different aspects (economic, cultural, public health, social and political) **(Ibrahim *et al.*, 2018)**. The Nile receives enormous amounts of environmental pollutants, including fertilizers, fishing activities, pesticides, and significant amounts of industrial waste in addition to municipal and domestic materials draining directly or indirectly into it. Due to climatic change impacts and their limitations, freshwater resources worldwide required more attention, especially in Egypt, to overcome the harmful effects of such pollution on all vital activities **(Hasaballah *et al.*, 2021; Elemam & Eldeeb, 2023)**. The Nile pollutants are derived from sources such as industrial wastewater, oil pollution, municipal wastewater and agricultural drainage.

The pollution status of the water of the Nile River is an important indicator of water quality **(Abou-Elela, 2017; Hasaballah *et al.*, 2019a)**.

The Rosetta branch of the Nile River serves as a source of drinking water, fishing and irrigation, with a daily flow averaging 21,500,000m^3/ day **(Negm, 2017)**. However, it is affected by the discharge of domestic, industrial and agricultural wastes that hasn't been treated, posing serious environmental and health risks. Reports indicate that over 900m^3 of organic, domestic and industrial wastes from Greater Cairo are monthly discharged into the Rosetta branch without any treatment (**Eissa *et al.*, 2021)**. Recent studies revealed that the El-Rahawy drain is a significant source of contamination which is heavily polluted with both organic and inorganic pollutants. The aquatic life is harmed as a result of the pollution caused by these contaminants **(Ezzat *et al.*, 2012; Elmahdy *et al.*, 2020)**. Furthermore, it faces significant challenges due to escalating ammonia concentrations, stemming from agricultural drains along the river, industrial effluents from industrial activities and fish farming cages. One particular issue arises during low-demand periods when the flow in the Rosetta branch diminishes. Annually, the Egyptian authorities close water flows in a series of channels to facilitate maintenance, resulting in reduced water levels which has impacted Kafr El Sheikh and El Beheira, located in northern Egypt, as the drainage of industrial wastes during this period has caused a high pollution load of ammonia and other pollutants **(Omran & Negm, 2019)**.

The quick development of human activities has greatly increased the input of ammonia into water bodies **(Dong & Xu, 2020)**. Effluents from secondary domestic and agricultural wastewater treatment plants contain high concentrations of inorganic nitrogen that may lead to eutrophication of the water bodies that they discharge. This input induces eutrophication and causes deterioration in natural water quality. Therefore, the removal of nitrogen from water sources is a fundamental way to prevent eutrophication and water bloom **(Mishra, 2022)**.

The process of biological removal of organic matter from sedimented wastewater is carried out by microorganisms, which are capable of decomposing organic matter through two different biological processes: biological oxidation and biosynthesis **(Paul *et al.*, 2023)**.

Blue green algae offer a low-cost and effective approach to remove the excess nutrients and other contaminants due to their high capacity for inorganic nutrient uptake, while producing potentially valuable biomass. However, one of the major drawbacks of using algae in wastewater purification is the harvesting of biomass **(Hasaballah *et al.*, 2019b; Liu & Hong, 2021)**.

Earlier studies have consistently recorded efficient and rapid removal of nitrogen from wastewater by immobilized algae**.** These algal systems often use Carrageenan, chitosan, and alginate aid polymer. **(Hoffmann, 2002; Moreira *et al.*, 2006; Zhang *et al.*, 2008;Vasilieva *et al.*, 2021)**. Algae have many advantages in the removal of nitrogen; these include: (1) low cost due to sufficient solar energy, (2) simultaneous CO_2fixation,

(3) non-requirement for extra-organic carbon (as compared to biological nitrification–denitrification), (4) oxygenated effluents discharge into water bodies, (5) lack of sludge handling problems, and (6) high economic potential for harvested algal biomass (for feedstock, fertilizers, biogas, biofuels, among others) **(Ahmed *et al.*, 2022)**.

On the other hand, bacteria can improve microalgae growth and metabolism, making them a viable option for various biotechnology applications. Studies have shown beneficial effects since the 1930s, particularly in wastewater treatment and biofuels. These beneficial relationships between microalgae and bacteria involve harmonious and cooperative interactions, allowing the former to synthesize organic matter from simple chemical substances **(Osorio-Reyes *et al.*, 2023)**.

Azotobacter chroococcum and Bacillus megatherium were considered as algae growth-promoting bacteria (MGPB); these bacteria are capable of fixing atmospheric nitrogen and solubilizing phosphorus; it was demonstrated that the observed growth promotion might improve the capabilities of algae to remove nutrients from natural wastewater **(Hernandez *et al.*, 2009; Ali *et al.*, 2012).**

Microalgae used ammonium to create proteins, nucleic acids and pigments for photosynthetic processing. However, nitrifying bacteria primarily use NH_4 as a source of electrons during the nitrification process, which results in the production of nitrite (NO_2) and nitrate (NO_3). In high NH_4-loaded wastewater treatment systems based on microalgae cultivation, a balance between microalgae and ammonium oxidizing bacteria frequently exists **(González-Camejo *et al.*, 2022)**.

Smaller beads/capsules have the advantage of a higher surface-to-volume ratio, allowing good transport of essential nutrients and are less fragile **(Chai *et al.*, 2021)**. Diffusion limitations within larger beads may limit cellular metabolism as the lack of essential substances such as oxygen supply to the interior of the beads that may cause cell death as a result of consumption from the surrounding cells, better dispersion, better mechanical strength, easier implantation and potential access to new implantation sites. Therefore, good control of bead size and shape is crucial and should be carefully controlled **(Mehrotra *et al.*, 2021)**.

The present study involved using a bioreactor column (Moving bed bioreactor carrier, BBRC) that was constructed to investigate its efficiency for the removal of ammonia from contaminated surface water from January to October 2022. A series of microalgae-bacteria inoculation potential ratios were studied, and the pollutants removal performances were monitored. Samples containing ammonia were tested for the efficiency of biological treatment for ammonia removal by means of: (1) isolating certain types of algae (*Anabaena* sp., and *Scenedesmus* sp.) and cultivating them in media; (2) isolating bacteria (*Lactobacillus* sp. and *Azotobacter* sp.); (3) studying the effect of bacteria in the removal of ammonia; (4) examining the effect of algae in the removal of ammonia, and (5) addressing the effect of mixing algae and bacteria in the removal of ammonia.

The findings of this study would offer a new insight into the relationship between microalgae and bacteria while confirming the feasibility of microalgae-bacteria bioreactor columns for highly efficient wastewater treatment.

MATERIALS AND METHODS

1. Area of study

The Rosetta Branch represents the main freshwater stream of the River Nile (Fig. 1). It extends from the Delta barrage at 30° 11' 04.4" N and 31° 07' 00.4" E for about 256 km on the western boundary of the Nile Delta **(Kaiser *et al.*, 2013)**. It ends with the Edfina barrage at 31° 18' 22.8" N and 30° 31' 07.9" E, with a distance of 30km upstream of the Mediterranean Sea **(Omar *et al.*, 2022; El-Amier *et al.*, 2015)**. The Rosetta Branch has an average width of 180m and provides water for agricultural, industrial, domestic and fishery sectors **(Nada *et al.*, 2021)**.The collection and disposal of drainage water into the Rosetta branch are facilitated by five primary drains (Fig. 1), including El Rahawy, Zaweit El Bahr, El Tahrir, Sabal and Tala, which receive effluents from secondary drains **(Mostafa *et al.*, 2015; Eissa *et al.*, 2021)**. Pollution along the branch mainly originates from these five main drains. The Rosetta branch is subjected to various forms of pollution stemming from diverse sources, including but not limited to the discharge of sewage and domestic effluents from the El-Rahaway drains, which is estimated to produce more than $5 \times 108\ m^3$ of effluent daily. Discharging agricultural wastes from the Soble drain and industrial effluents from El-Malya and Soda Companies in Kafr El-Zayat City significantly impacts the Rosetta Branch aquatic environment **(Sayed *et al.*, 2020).**

2. Collection of water samples and microorganisms

Subsurface water samples were collected from the sampling site of "Ezbet-Sherif" at a distance of 5km before the El-Rahawy drain (Fig. 1). Water samples were collected in 2022 during an interval of 10 months from January to October 2022. The microalga strain of blue-green algae species (*Anabaena* sp. and *Scenedesmus* sp.) was provided by the Reference Laboratory for Drinking Water, Holding Company for Water and Wastewater. The bacterial strains of *Lactobacillus* sp. and *Azotobacter* sp. were isolated and provided by the Egyptian National Research Center (Table 1).

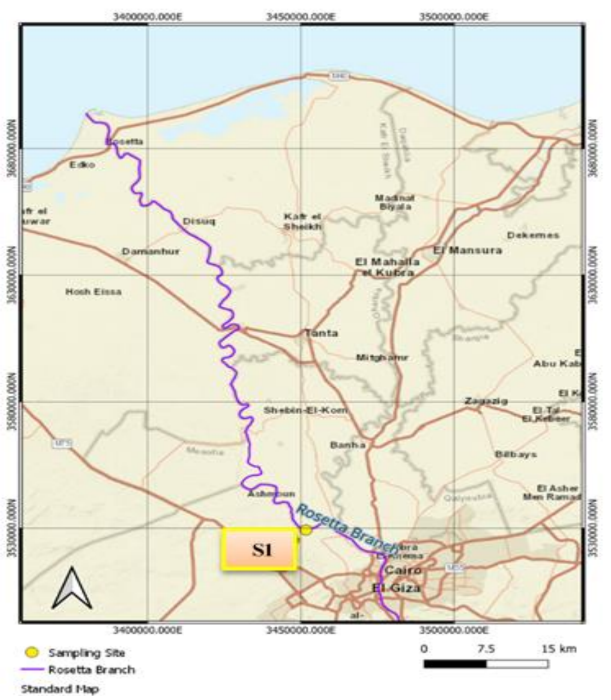

Fig. 1. Geographic map of Rosetta branch and agricultural drains (S1: Sampling site)

Table 1. Different types of microorganisms applied in the bioreactor column

Microorganisms	Code	Species	Source
Algal	A	*Anabaena* sp.	Holding Company for Water and Wastewater
	S	*Scenedesmus* sp.	
	M	Mix (A +S)	
Bacterial Sp	MS	*Lactobacillus* sp.	Egyptian National Research Center
	SS	*Azotobacter* sp.	

3. Cellular growth kinetics and the culture medium

The concentration of the algal species was determined based on the chlorophyll content found in the samples. To prepare the algal species, different species were inoculated into a 5-liter volume of prepared BG-11media, (Fig. 2) which was then incubated for 15 days in PR-R precision incubators (17.8 cu. ft., THOMAS, USA) at a temperature of 26°C **(Pawar *et al.*, 2021)**. Subsequently, the chlorophyll content in 1ml of the incubated media was quantitatively detected using a spectrophotometric method employing a DR6000 spectrophotometer **(Mao *et al.*, 2021)**. In addition to the investigation of algal species, this study encompassed the analysis of raw water samples containing varying volumes of bacterial species. To achieve this, two isolates from *lactobacillus* sp. and *Azotobacter* sp. were prepared and cultivated by inoculating cells into growth media. Burk's medium was selected as the cultivation medium to support the mass production of microbial cells for the purpose of inoculants generation **(Plunkett *et al.*, 2020)**.

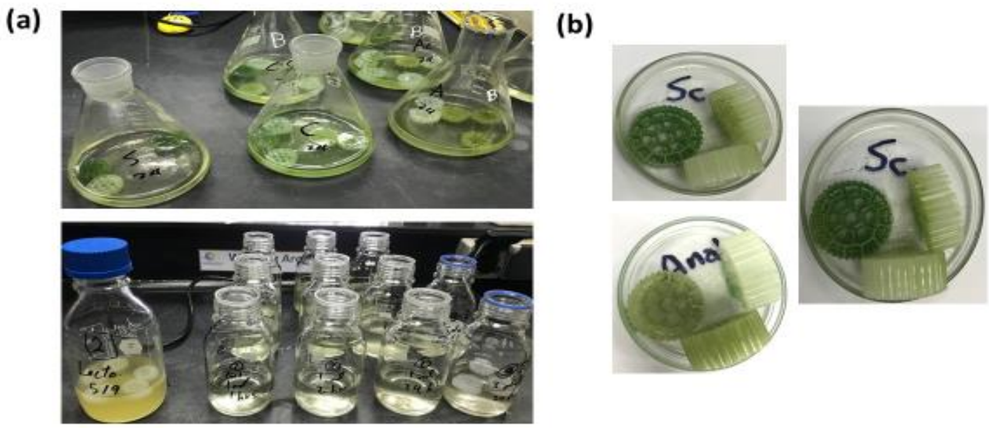

Fig. 2. Moving bed bioreactor carrier's preparation process (a: Batch Technique, b: Algae Carrier)

4. Moving Bed Bioreactor Carrier (MBBRC)

This study depends on applying the moving bed bioreactor (MBBR) process for ammonia removal. MBBRC was prepared and cultivated according to **Yuan *et al.* (2015)** for the following step of the treatment process (Fig. 2).

5. Experimental setup and operational conditions for moving bed biofilm reactor (MBBR) investigation

Fig. (3) shows the MBBR system and the bioreactor columns used in this study. The device was placed in conditions adjusted with variables, where algae were in conditions similar to their optimum environment, with natural air and variable atmospheric temperature (19– 30°C). It consisted of 3 transparent acrylic columns with an outer diameter of 125mm; an inner diameter of 123 mm, and a height of 990mm. The columns were connected with faucets and inoculated with algae discs (or algae with bacterial sp.) and cultivated for 7 days with an influent ammonia concentration of 12 mg/l. The three columns were not connected, and the total effective volume was 11.76 liter for each one. The hydraulic retention time was calculated based on the total volume of the bioreactor. The liquid superficial velocity in the reactor was 5.67 l/min. Data concerning day lengths and ambient temperature were obtained from the Egyptian Meteorological Authority, Atmospheric Science Department **(NWP 2023)**.

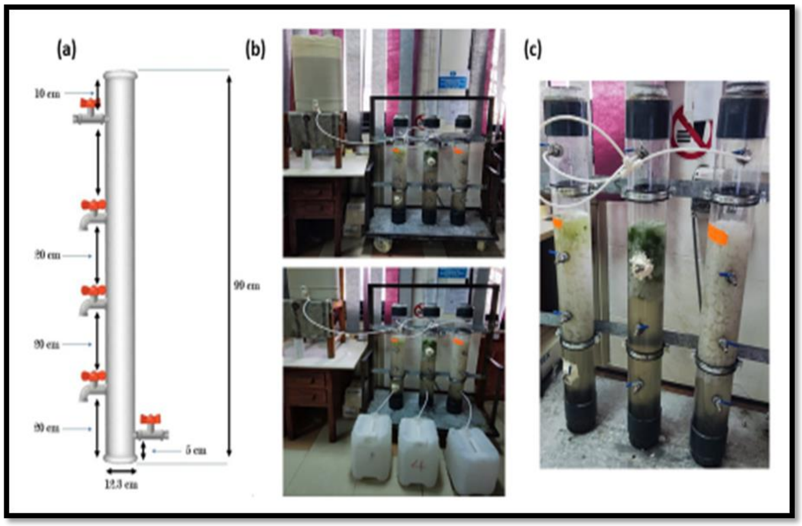

Fig. 3. MBBR experimental system for ammonia removal (a: Schematic Diagram for column dimensions; b: Column technique for biological treatment MBBR, and c: Applied acrylic column (Acrylic is a transparent plastic material with outstanding strength, stiffness and optical clarity)

The effluent sample was collected by fixing a sampling bottle at the outlet of the columns. Ammonia then was measured using a spectrometer (DR-6000, HACH) according to **Zhang *et al.* (2021)**. To ensure consistent testing conditions, the volume of raw water samples was calculated to achieve a final concentration of 100mg chlorophyll per 1 liter of the tested sample, and then effluent water was subjected to testing for residual ammonia concentration at various time intervals, ranging from 15 minutes to 24 hours, following the preparation of the samples. The removal efficiency was determined by comparing the calculated percentage of ammonia removal to the ammonia content present in the raw water (i.e., the matrix of the tested samples).

Water samples underwent testing subsequent to their inoculation with various algal and bacterial species for different retention intervals, with different doses contingent upon the chlorophyll content, as samples were tested for the reduction in ammonia content compared to raw water samples considering the following factors: (1) Different algal types prepared and tested on constant retention time intervals; (2) Different retention times in addition to (3) Content of algal and bacterial species (cooperation).

RESULTS AND DISCUSSION

1. Effect of using algal spp.

Water samples were tested to investigate the efficiency of 2 algal types, *Anabaena* sp (A) and *Scenedesmus* sp. (S) content is correspondent to 100 mg chlorophyll /l for raw water ammonia concentration ranging from 6.492 to 7.5 mg/l. An obvious reduction was observed in ammonia concentration, resulting in average reductions of 20.6 to 31.5 % and 14.7 to 30.8% for A and S sp., respectively. On the other hand, using mixed species of A and S sp. in removing ammonia from water samples increased with the average reduction percent of ammonia from 20 % to 37 % as shown in Fig. (4). **Hasaballah *et al.* (2019c)**, **Mustafa *et al.* (2021)**, **Meril *et al.* (2022)** and **Bhandari *et al.* (2023)** used these algal species in their studies and unveiled the potential of *Anabaena* sp. *Scenedesmus* sp. to reduce ammonia and other nutrients from different types of wastewater.

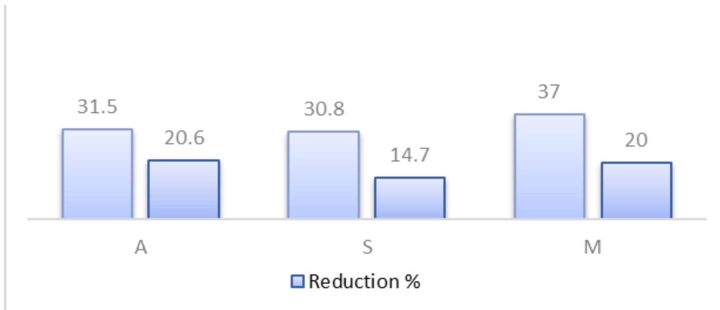

Fig. 4. Ammonia reduction percentage with different algal species

2. Effect of retention time interval with algal spp. content

The doses of algal species were increased; the tested samples were inoculated with 100 and 500mg/ l, and the samples were tested for the reduction in ammonia concentration after different incubation intervals (1=15 min, 2=30 min, 3= 60 min), as shown in Table (2). A slight reduction was detected in ammonia concentration in reference to raw water average ammonia concentration (6.8mg/ l).

Table 2. Reduction in ammonia concentration with varied retention time interval (RTI), algal species and content

Content of algal sp.	RTI for the sample	Sample Reduction %		
		S	A	M (Mix of S & A)
100 mg/l	15 min	5.8	3.4	3.7
	30 min	2.2	2.9	6.4
	60 min	4.3	-	-
500 mg/l	15 min	4.3	6.4	9.9
	30 min	3.3	9.0	9.8
	60 min	3.1	11.2	11.9

On increasing the content of algal sp. from 100 to 500mg/ l, in reference to raw water average ammonia content (6.8 mg/l), an obvious increase in reduction percent for sample M containing a mixture of S and A sp. was observed, with an average reduction of 9.9% for the retention time interval of 15min., as displayed in Table (2). While, there was average reduction of 9 % and 9.8% for samples A and mixture (A+S), respectively, for the retention time interval of 30min., and a reduction of 11.2 % and 11.9% was recorded for the retention time interval of 60min. On the other hand, increased retention time intervals of 2 and 5 hours for algal species resulted in an average maximum reduction of 93.22%, 99.6% and 85.03% for A and S species, respectively. It was noticed that, A sp. showed a higher reduction percentage than S sp. for retention time exceeding 1 hour, as shown in Fig. (5).

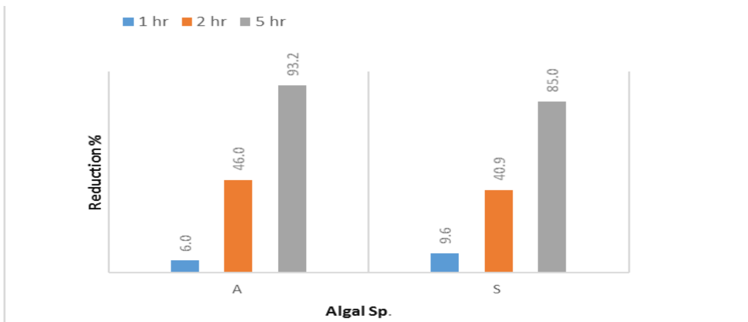

Fig. 5. Ammonia reduction percentage with varied retention time and different algal species

3) Effect of bacterial spp. cooperation

In different experimental trials, upon using different content of different bacterial species (*Lactobacillus* sp. and *Azotobacter* sp.) reaching 100 - 200ml/ l of raw water for varied retention time intervals reaching 2hrs., it was observed that, average maximum reduction percent reached 98% for *Lactobacillus* sp. of content 20ml and 100% for different contents (1,10, 20 ml) of *Azotobacter* sp. at constant retention time intervals of 2 hrs., as shown in Figs. (6, 7). This indicates that, the tendency of bacterial sp. to reduce ammonia is exceeding double that for algae sp., with an average maximum removal of 100% (Fig. 8).

4) Application of the mixture of algal and bacterial sp. in MBBR system

Testing for algal and bacterial sp. and mainly for *Anabaena* sp. and *Azotobacter* sp., respectively, in addition to a mixture for both algal and bacterial sp. for testing the raw water of ammonia content = 12mg/ l for different retention time intervals, as shown in Table (3).

Table 3. Coding for retention time intervals

Code	Retention time interval	Code	Retention time interval	Code	Retention time interval
a	15 min	e	75 min	9	12 hrs.
b	30 min	f	90 min	10	24 hrs.
c	45 min	g	105 min	11	27 hrs.
d	60 min	h	9 hrs.	12	48 hrs.

In the MBBR system, algal (*Anabaena* sp.) and bacterial sp. (*Azotobacter* sp.), as well as a mixture of both algal and bacterial sp. were tested for different retention time intervals ranging from 15 to 105min. Mixed algal sp. (*Anabaena* sp.) and bacterial sp. (*Azotobacter* sp.) for varied retention time interval achieved average maximum reduction efficiency for ammonia of 100 % at most retention time intervals (15 min -105 min), as represented in Fig. (9).

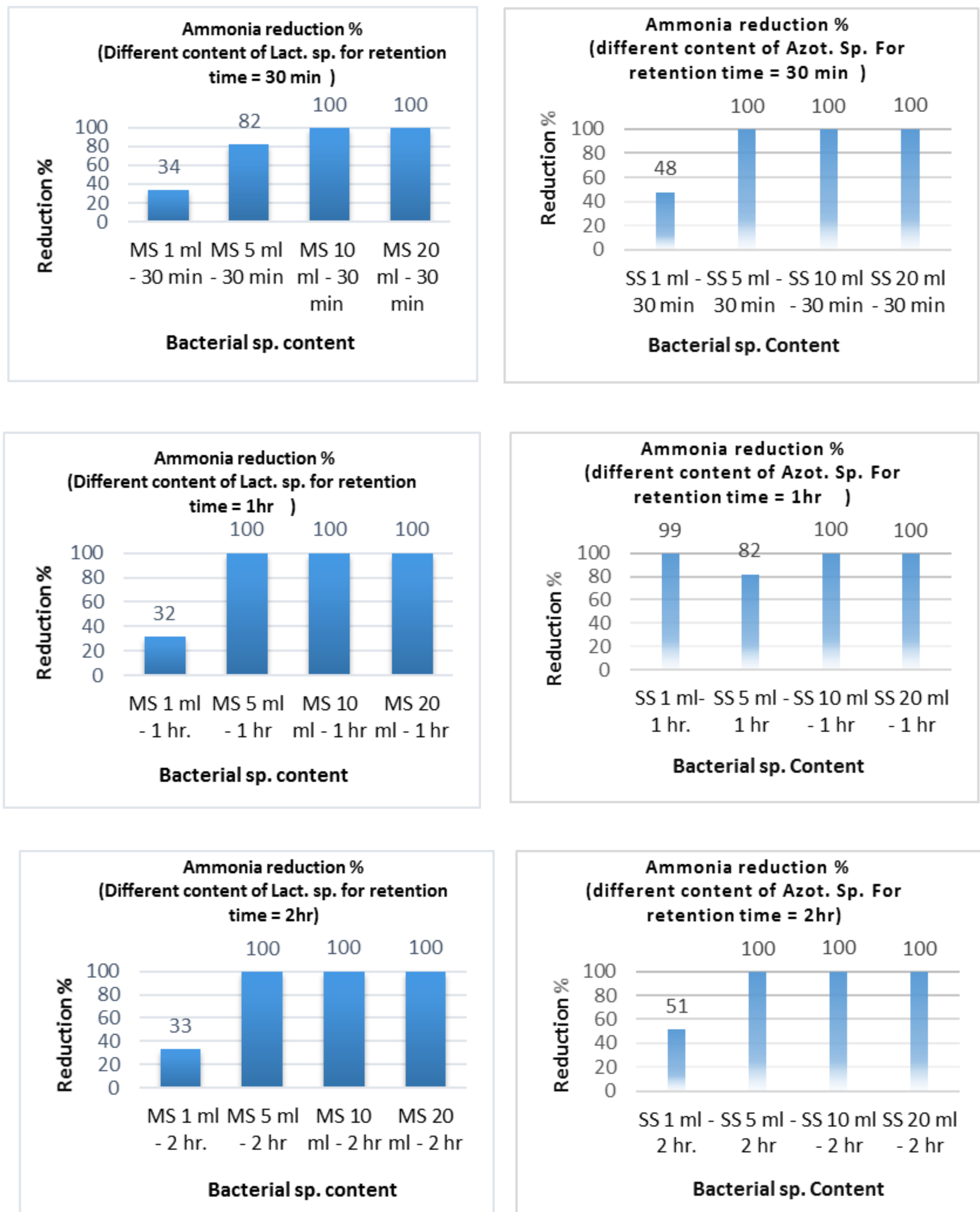

Fig. (6): Ammonia Reduction % for bacteria sp. at different retention time.

Fig. 7. Ammonia reduction % for bacteria sp. at different content

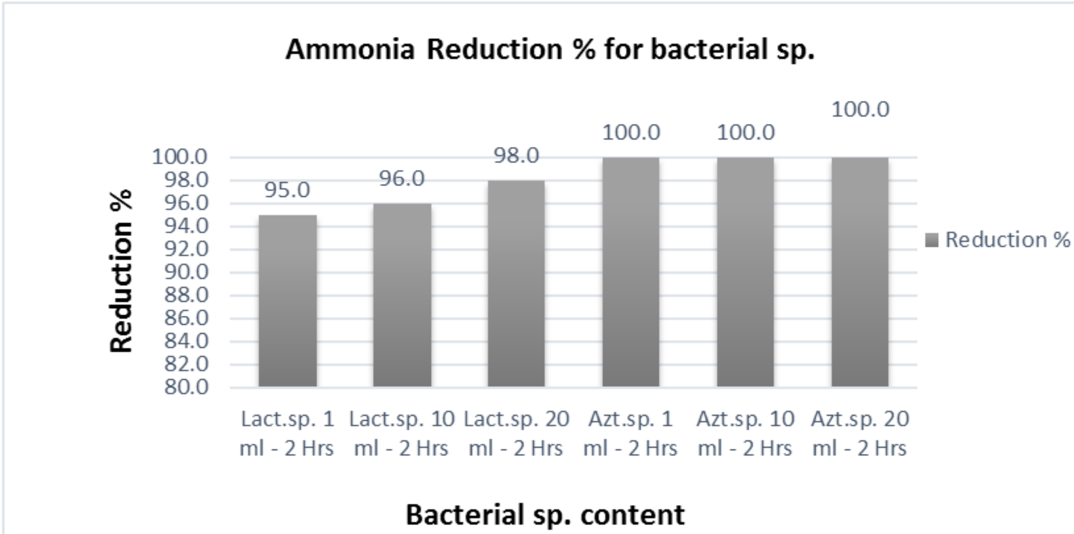

Fig. 8. Ammonia reduction percentage with different bacterial species content

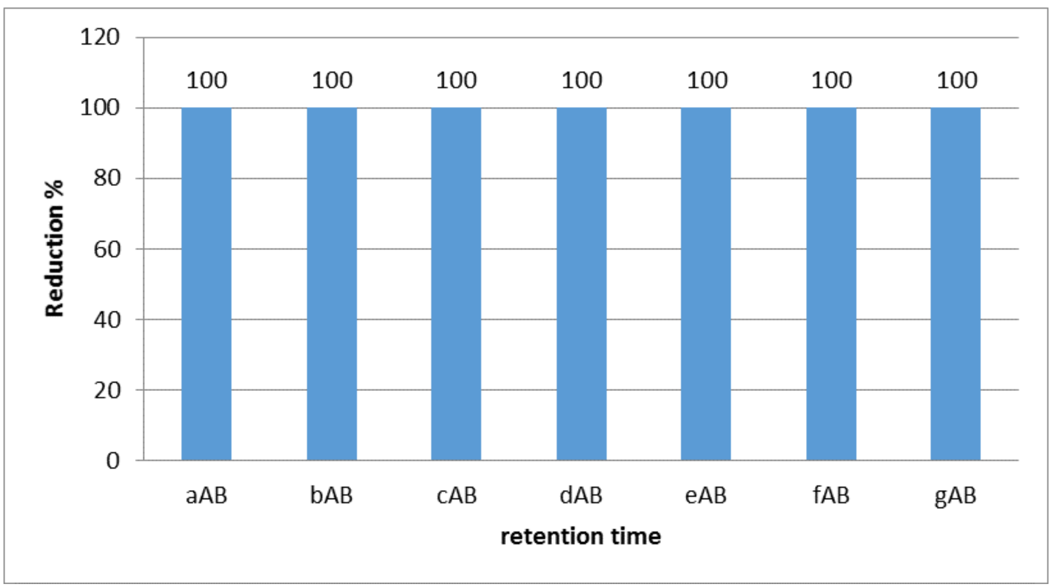

Fig. 9. Average ammonia reduction % for algal & bacterial sp. at different retention time intervals [AB: algal bacterial sp. mixture (*Anabaena* sp. & *Azotobacter* sp.) and retention time, as displayed in Table (3)].

Hence, using samples of mixtures of algal and bacterial sp. enhanced the average reduction efficiency (100%) and retention time (15 min–105 min) for ammonia removal when compared to those for single algal sp. (*Anabaena* sp.) (10–21%), while average reduction efficiency for ammonia is the same when compared to single bacterial sp. (100%), but with an enhancement for retention time interval (Fig. 10)

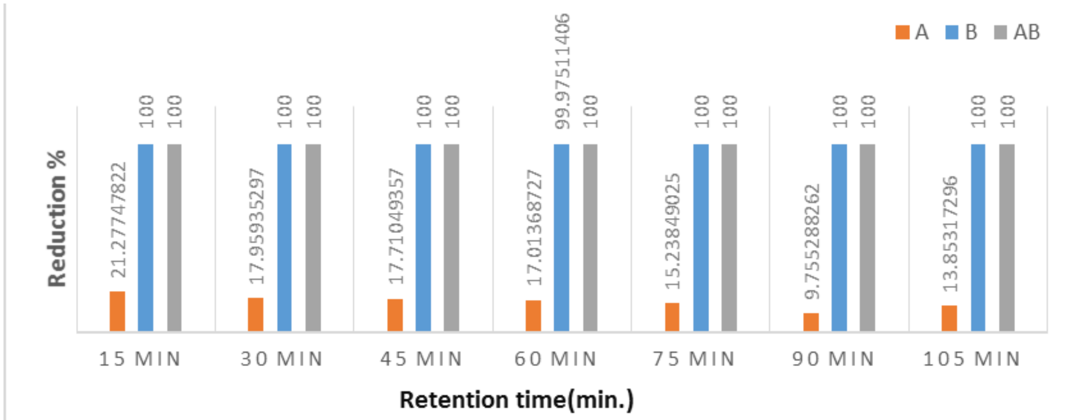

Fig. 10. Average ammonia reduction % for algal sp., bacterial sp., and mixture of both at different retention time intervals [A: Algal sp. (*Anabaena* sp.), B: Bacterial sp. (*Azotobacter* sp.)].

5. Effect of continuous operation in ammonia removal using MBBR system

This was investigated for algal and bacterial sp. (*Anabaena* sp. and *Azotobacter* sp.) in addition to a mixture for both algal and bacterial sp. for different retention time intervals ranging from 9 to 48 hrs. As shown in Fig. (11), *Anabaena* sp. samples showed slight ammonia reduction efficiency (10–15%), with an average maximum reduction at 9-hours retention time interval. A single bacterial sp. (*Azotobacter* sp.) showed an average maximum reduction efficiency of 81– 100% for ammonia, with the highest reduction at 27 hours. While, mixed algal and bacterial samples showed an average maximum reduction efficiency of 74–96% for ammonia, with the highest reduction at 48 hours.

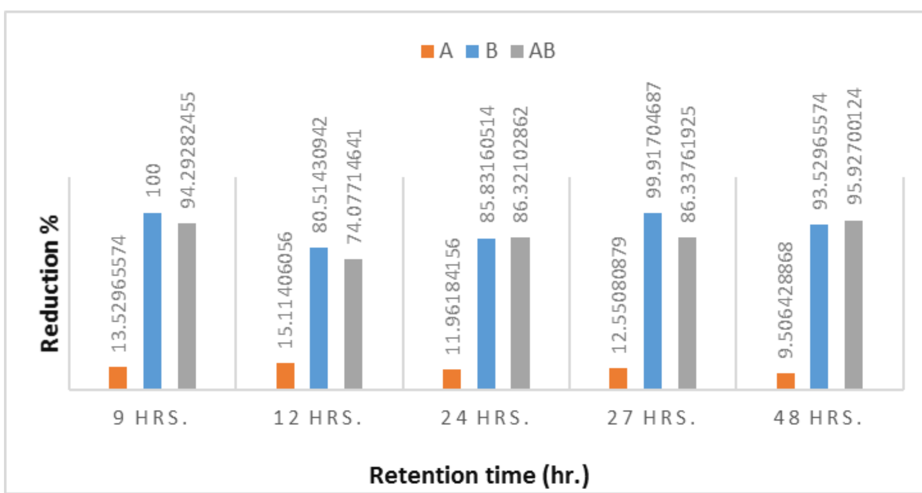

Fig. 11. Average ammonia reduction % for algal sp., algal and bacterial sp., and mixture of both for continuous operation at different retention time intervals [A: Algal sp. (*Anabaena* sp.), B: bacterial sp. (*Azotobacter* Sp.), AB: mixture of algal and bacterial species].

Previous studies have also investigated the use of algal-bacterial systems for wastewater treatment with emphasis on nutrient and micropollutant removal. **Oruganti *et al.* (2022)** reported that, nutrient removal in algal-bacterial consortia is superior in comparison to algal and conventional systems due to multiple pathways available via assimilation, stripping, nitrification-denitrification, oxidation of organic carbon to carbon dioxide and adsorption. Additionally, the SRT (solids retention time) is a key operational parameter that allows controlling the nutrient removal processes and growth of microalgal-bacterial systems **(Rada-Ariza *et al.*, 2019)**.

For algal and combined algal and bacterial samples, the average maximum reduction percent of 100 % was recorded at lower retention time intervals (from 15 to 105 min) and then decreased for higher retention time intervals (from 12 to 48hr). This may be attributed to the change in the surrounding environment for algal and bacterial sp. due to their transfer from growth media (rich in nutrients) to water media (poor in nutrients), which affect the growth of organisms and requires longer time for adaptation to new media **(Morgan-Kiss *et al.* 2006; Palacios *et al.* 2022)**.

CONCLUSION AND RECOMMENDATIONS

Biological treatment methods can be employed to mitigate ammonia concentration in raw water for water treatment plants. This approach leverages natural processes and microorganism metabolic activities to effectively reduce ammonia levels. Therefore, the current study assessed the efficiency of using moving bed bioreactor carrier (MBBRC) hybrid with algae and bacterial strains for ammonia removal in samples of water from Rosetta branch of the River Nile. The results showed that, using MBBR system hybrid with algal and bacterial species, mixed algal and bacterial sp. enhanced the average reduction efficiency and retention time for ammonia removal compared to single algal species.

Therefore, utilizing MBBR system hybrid with different algal and bacterial species is recommended for ammonia reduction in water samples. This will contribute to the development of sustainable and efficient biological treatment approaches for water quality improvement, particularly in scenarios of low water demand. Further research and optimization of these biological treatment methods are needed to enhance their practical application in water treatment plants and environmental management.

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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.

ETHICAL APPROVAL

This paper does not contain any studies with human participants or animals performed by any of the authors.

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