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Evaluating the Performance of a Microalgal-Bacterial Nexus for Sustainable Wastewater Treatment

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Abstract

The rising need for sustainable wastewater treatment has prompted the exploration of microalgal-bacterial integrated systems. This study evaluates the treatment efficiency of a treatment system for municipal wastewater collected from Zenin Wastewater Treatment Plant, Giza, Egypt. Experiments were conducted at a range of hydraulic retention time (HRT) from 1.0 d to 2.4 d, to determine the maximum efficiency of the treatment system. The raw wastewater was characterized by total suspended solids (TSS) of 61 mg/L, turbidity of 24 NTU, biochemical oxygen demand (BOD) of 21 mgO_2/L, and a high bacterial load of total coliforms of 9.4×10^6 MPN/100 mL and *E. coli* of 5.4×10^4 MPN/100 mL. Results revealed remarkable pollutant reduction with maximum removal rates of 91.1% for TSS and 94.2% for turbidity at an HRT of 1.7 days. BOD was reduced to 3.2 mgO_2/L, achieving an 85% removal efficiency. Total and fecal coliforms showed reductions of 5.2 and 4 log units, respectively, while *E. coli* was completely removed, meeting Egyptian irrigation water standards. The synergistic interactions between microalgal and bacterial biofilms contributed significantly to pollutant removal, with algal species such as *Chlorella vulgaris* and *Nitzschia linearis* forming dominant biofilms on rotating surfaces, and underscore the potentiality of this system.

Keywords: microalgae; hydraulic retention time; biofilm; pneumatic rotating system; coliform bacteria

1. Introduction

The clean water scarcity issue is congruent with the global demand of water rationalization. The recirculation for the treated wastewater is potential for several applications depending on the treatment efficiency [1]. The convenient wastewater treatment techniques, either biological or non-biological, have their limitations as high operational cost, especially for power consumption, high chemical usage, and the production of toxic pollutants such as sludge [2–4]. For these reasons, bioremediation technologies for wastewater treatment based on employing microbes as biofilters have been applied recently [5,6].

Biofiltration technique is based on the capability of microorganisms to be attached to or colonized on a filter media. These attached microorganisms are responsible for oxidizing the carbon source waste, which serves their food. The adequate supply of carbon enhances the metabolism efficiency; maintaining the microbial population is crucial for achieving efficient contaminants removal [7]. Sonawane *et al.* [8] have reported that the biofilm formation on fixed surfaces enhances the metabolism and the biodegradation more than that found in systems of suspended culture of microorganisms. Also, the material of the filter bed and its roughness play an important role in the attachment of microorganisms [8].

The integration between bacteria and microalgae in bioremediation systems appears to provide bacteria with necessary oxygen for metabolism while generating carbon dioxide required for microalgae's photosynthesis [9]. This symbiotic relationship enhances the attachment of microalgae on the surface of bacterial biofilm [10]. Immobilization of the heterotrophic bacteria on an absorber surface provides them a convenient environment to implement complete degradation of pollutants [11]. This technique was applied for treating the wastewater of fish farms to treat high amounts of ammonia and recorded significant results in wastewater treatment on a pilot scale [12]. Also, it was applied for municipal wastewater treatment with a removal rate of 43 g COD/m^2/d [13,14].

Rotating algal biofilm (RAB) systems have been developed in recent decades to treat industrial and municipal wastewater [15]. In these systems, microalgae and bacteria attach to a flexible belt, forming a biofilm. This belt rotates between the wastewater and the surrounding air, allowing to easily absorb nutrients onto the biofilm surface and facilitating light and gas exchange [16]. Zhao *et al*. [17] reported that RAB system was effective in removing ammonium from municipal wastewater at 32 h hydraulic retention time (HRT). Also, Zhang *et al*. [16] stated that RAB system can achieve removal rates of 25.6 and 28.9 mg/ L·d for ammonium and total organic carbon, respectively. Hu *et al*. [18] demonstrated that RAB showed superior removal for phosphorus (82 %), total nitrogen (91 %) and chemical oxygen demand (92 %) at 2-day HRT.

A remarkable merit of the integrated system techniques is demolishing the risk of secondary pollutants [19]. Noticeably, the microorganism consortium and their ability to treat wastewater differ according to the environmental conditions; however, they are applicable for various toxic pollutants, require less chemical usage, work at room temperature, absence of external

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thermal power, high flow rate, and are cost-effective. Moreover, they have a high degree of efficiency even at low concentrations of contaminants [5,19].

The mechanical design of the system is essential for increasing the system efficiency. The main target of this approach is to increase the area applicable for biofilm formation. So, this research article presents a design for the algal-bacterial rotating surfaces (RS) and a treatment system convenient for the municipal wastewater characteristic in Egyptian cities. The rotating surfaces idea was applied in several previous techniques; whereas the presented approach is established based on novel design criteria related to the method of fixing the absorber bed, increasing the area of biofilm, and adjusting the rotating speed to increase the system's capacity via dual optimization of the flow rate and treatment efficiency. The factors affecting the self-attachment of microalgae and the formation of bacteria biofilm are taken into consideration to decrease the cost of harvesting. The quality of the treated water is examined and compared with the Egyptian code of practice for the use of treated municipal wastewater for agricultural purposes ECP 501;2015 [20].

2. Materials and Methods

2.1. Wastewater Sampling

The municipal wastewater was collected after the primary stage from Zenin wastewater treatment plant (WWTP) in Giza governorate, located at 30◦1′57.91″N / 31◦10′53.53″ E in Egypt with seasonal temperature variation range 15 ºC to 40 ºC, and an average daily variation of 10 ºC. Samples were refrigerated and analyzed within 24 h of collection. The physicochemical parameters for the wastewater (total suspended solids TSS, turbidity, BOD_5, and COD) were measured according to APHA (2023) [21]. Also, the density of total coliforms, fecal coliforms, and *E. coli* were detected by employing the most probable number technique (MPN/100 mL) in accordance with standard procedures 9221 B, E, and F, respectively according to APHA (2023) [21].

2.2. System design

The Algal-Bacterial Integrated System (ABIS) was designed of three main ponds as illustrated in Fig. 1. The first pond is covered anaerobic pond (CAP) followed by the algae pond (AP) illuminated 24 h/d by white cool fluorescent of irradiance 95 $\mu mol.m^{-2}.s^{-1}$, followed by settling pond (SP). The volume of the CAP is twice the volume of AP to promote duplicating the hydraulic retention time (HRT) with fixed flow rate, while the volume of SP is half of AP. The used absorber material was granular carbon of pellet size 1mm. The amount of the activated carbon used in system was 5 g; placed in cotton cylinder of 25 mesh pore size and space volume 0.71×10^{-4} m^3. In this system, the total amount of activated carbon is 20 g. The system was operated at different HRT from one day (24 h) to 2.4 days (57.6 h).

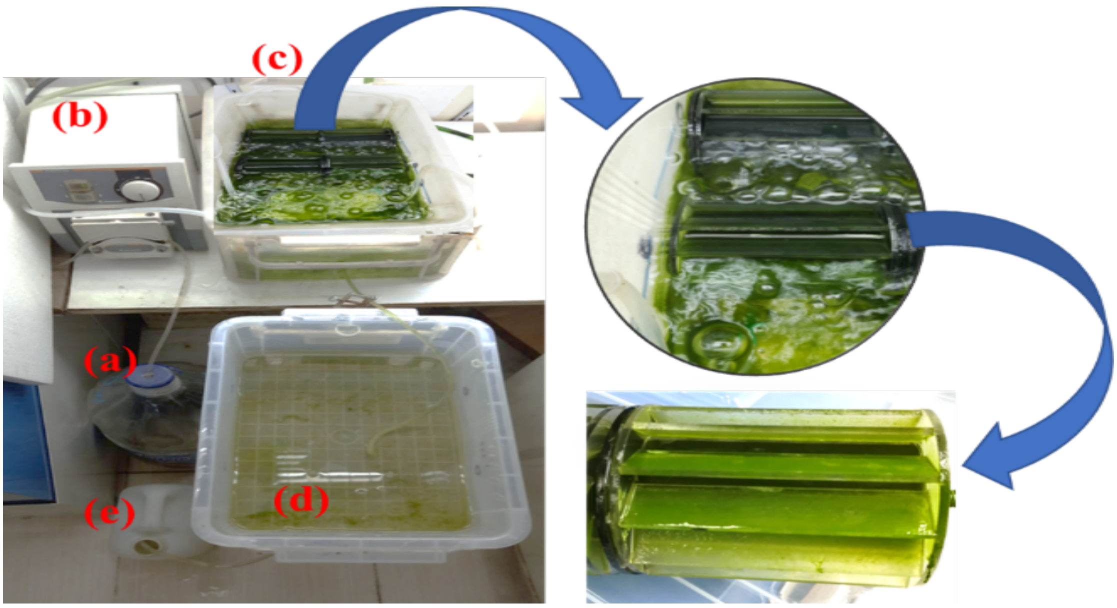

Fig. 1. Algal-Bacterial Integrated System (ABIS). (a) covered anaerobic pond (CAP), (b) dosing pump, (c) algae pond (AP), (d) settling pond (SP), (e) outflow.

2.3. Design of rotating surfaces

The algal pond was designed in a rectangular shape to install the rotating surfaces (RS) in it. The RS were designed to rotate freely around the fixed axes arranged in parallel inside the algal pond. The RS were fabricated from acrylic with 12 strips fixed longitudinal with angle 40º on the tangent of the surface as sketched in Fig. 2. The inner cylinder was made of cotton mesh and fixed on the axis tightly; the granular carbon was placed inside the cotton cylinder. Two discs were used to seal both sides of the outer cylinder; these discs have many holes to promote water flow in and out the outer cylinders. The outer cylinders were designed to move by the action of compressed air flew from pipes fixed under the cylinders. The air flow rate used was 2.4 v/v/min.

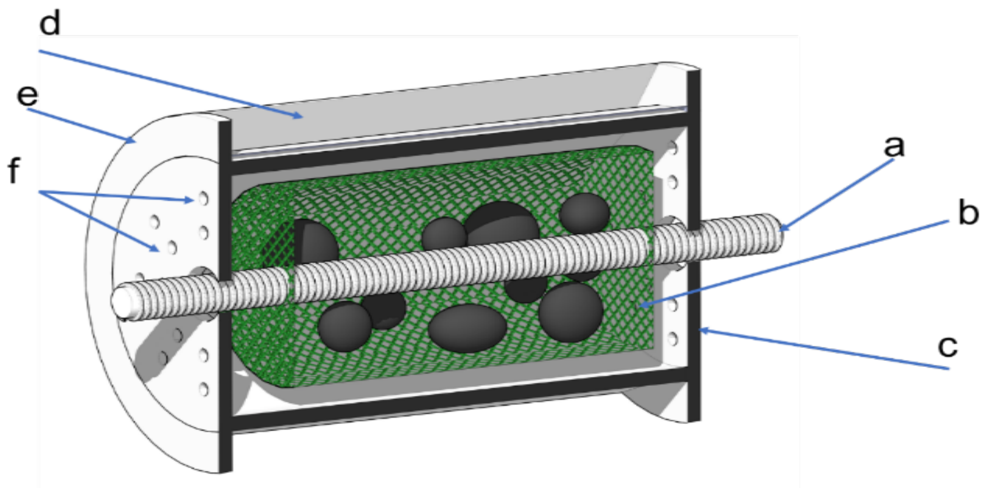

Fig. 2. Detailed sketch of the rotating surface's components a) stationary axis, b) inner cotton cylinder, c) rotating surface, d) strips, e) covering disc, f) holes.

2.4. Treatment efficiency evaluation

The treatment efficiency was assessed by evaluating the reduction in BOD, TSS and turbidity after treatment. Also, it was evaluated by the detection and enumeration of total coliforms, fecal coliforms, and *E. coli* using the most probable number technique (MPN/100 mL) according to standard methods 9221 B, E, and F, respectively [21]. The samples were collected at the start and at the end of each run-in triplicate. Treatment efficiency was calculated according to the following equation:

$$Removal\ (\%) = \frac{C_i - C_f}{C_i} * 100$$

where, C_i represents the bacterial density in the initial wastewater sample (raw), and C_f represents the bacterial density in the treated wastewater samples.

2.5. Analysis of microalgal consortia

Various subsamples were collected from ABIS, left to settle and examined using Sedgewick Rafter cells to identify the composition of the algal community to species level. Microscopical examination was done by Olympus X3 microscope, Olympus Corporation, Tokyo, Japan. The identification process followed the key for freshwater algae respectively [21,22].

2.6. Scanning electron microscopy

The morphology of algal-bacterial biofilm which formed inside the system was scanned using a scanning electron microscope (SEM) model JSM 6360LV, JEOL/Noran, operating at a voltage of 10–15 kV. The samples were coated prior to examination by sputtering them with a thin layer of gold at a low deposition rate, then the samples were cooled and kept for one hour before examination [23].

3. Results and Discussion

3.1. Characterization of raw wastewater

A large number of pollutants are found in wastewater, and it is necessary to remove these pollutants and treat this wastewater to comply with environmental regulations [24]. Total suspended solids (TSS) is one of the significant parameters in controlling the quality of wastewater because its excess in the effluent water causes an increase in the turbidity and a decrease in the dissolved oxygen (DO) [25]. In this study, the average value of total suspended solids (TSS) and turbidity in raw wastewater were 61 mg/L and 24 NTU, respectively. While the average of BOD and COD were 21 mgO_2/L and 101 mgO_2/L, respectively. These average values were calculated according to the difference of municipal wastewater inflow to the WWTP that depends on human activities during 63 days. The average density of total and fecal coliforms was 9.4×10^6 and 3.7×10^6 MPN/100 mL, while it was 5.4×10^4 MPN/100 mL for *E. coli*. The algal consortium exists in raw wastewater consists of *Scenedesmus obliquus, Cyclotella comta, Nitzschia linearis, Oscillatoria limnetica, Ankistrodesmus acicularis, Chlorella vulgaris, Microcystis* sp., *Stephanodiscus* sp., and *Selenastrum* sp.

3.2. Treatment efficiency

Treatment technology using biofilms has gained great attention recently because of its effectiveness, economic feasibility, ease of operation and inexpensiveness [24]. In this study, an algal-bacterial integrated system was constructed to treat municipal wastewater to become comply with environmental regulations and be suitable for unrestricted irrigation of edible crops. The results of treated municipal wastewater using the designed system ABIS elucidate that the maximum removal percentages of TSS is 91.1 % and turbidity is 94.2 % as elucidated in Fig. 3 and Fig. 4. The highest removal rates were achieved starting at HRT 1.7 d. At these conditions of operation, the BOD in treated wastewater was 3.2 mg O_2/L with 85 % removal percentage. The algal- bacterial co-culture system resulted in an increase in removal efficiency of nutrients through the release of growth promoters to each other [26].

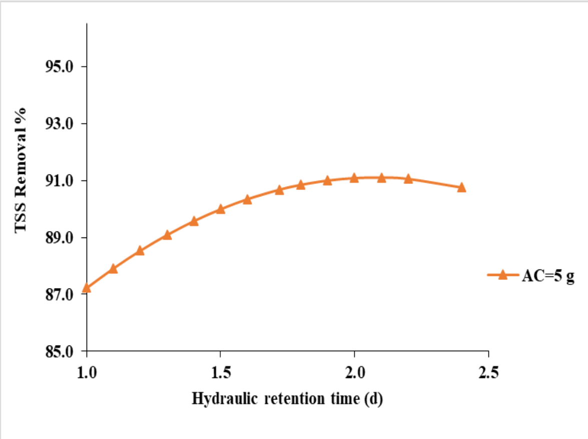

Fig. 3. The effect of hydraulic retention time on total suspended solid removal percentage.

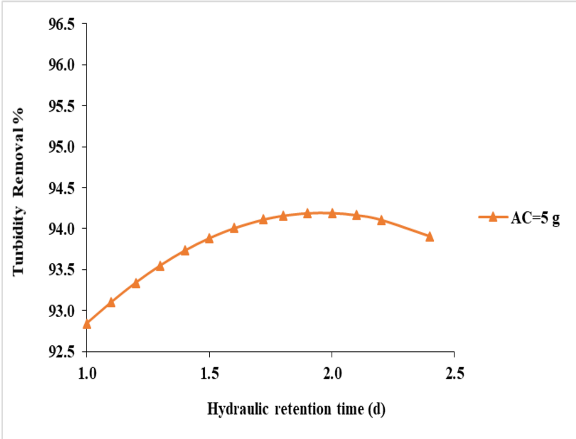

Fig. 4. The effect of hydraulic retention time on turbidity removal percentage.

Many countries reuse partially treated or untreated wastewater in agricultural irrigation which exposes crop consumers and farmers to possible health risks [27]. Wastewater could be a harbor for human pathogens like some serotypes of *E. coli*. Total coliforms, fecal coliforms and *E. coli* are common indicator organisms that have been used historically to evaluate the quality of treated wastewater [28,29]. Studies on the assessment of total coliform counts in wastewater are not numerous, as they are not included in the microbiological indicators for wastewater reuse in agriculture. However, these data may be crucial for understanding the microbial impact when heavily polluted water is used for plant irrigation [30]. Also, fecal coliforms are used as an indicator for the removal of pathogens from wastewater after treatment [31]. The density of total coliforms (Fig. 5) in wastewater samples was 6.0×10^6 MPN/100 mL and decreased after treatment at HRT 1.0 d to reach 3.0×10^4 MPN/100 mL (about 2.3 log unit reduction) with removal efficiency 99.5 %. The density of fecal coliforms decreased from 5.8×10^4 to 5.2×10^3 MPN/100 mL (1 log unit reduction) with removal efficiency 91.1 %. While the density of *E. coli* decreased from 1.15×10^3 MPN/100 mL to below the detection level with 100 % removal efficiency. At HRT 1.7 d, the bacterial density decreased from 1.0×10^7 to 1.0×10^6 MPN/100 mL and from 1.0×10^7 to 2.0×10^4 MPN/100 mL for total and fecal coliforms, respectively (Fig. 5). While, a complete removal for *E. coli* was achieved at this HRT with log reduction about 5.2 log unit. At HRT 2.4 d, total coliforms density decreased from 1.0×10^7 to 1.8×10^4 MPN/100 mL and the density of fecal coliforms decreased from 6.0×10^3 to 1.5×10^3 MPN/100 mL and also a complete removal for *E. coli* was achieved (Fig. 5). The system achieved high removal efficiency (> 90 %) for total, fecal coliforms and its removal rate reached about 100 % for *E. coli* at the three HRT. However, HRT 1.7 d showed clear efficiency in removing fecal coliforms and *E. coli* (about 4 and 5 log unit reduction, respectively) when compared with the other HRT, despite the high bacterial load in the inlet samples at this HRT (1.7 d). So, 1.7 d is considered the best HRT that achieved the highest treatment efficiency for wastewater. The removal of *E. coli* could be due to the presence of some mechanisms and interactions which occurred as a result for microalgae-bacteria consortia such as the type of the presented microalgae. Pompei *et al*. [32] reported that the removal of certain pathogens can be influenced by the genus/ species of microalgae. These results prove that there is a great opportunity to use the treated wastewater by the system under study for irrigation of edible crops according to the Egyptian code of practice for the use of treated municipal wastewater for agricultural purposes [20] which stated that *E. coli* level in wastewater must not exceed 100 counts per 100 mL for category B and 1000 counts for category C. The code classified treated wastewater according to the level of treatment into four categories (A, B, C, and D). Each category is specified to irrigate specific types of crops. For instance, wastewater falling into category B is permitted to be used for irrigation of food crops that are processed at high temperatures before eating (such as cooked and processed vegetables, dry grain crops, and medicinal plant crops such as chamomile, anise, and marjoram) and fruit crops that are peeled before eating (such as mangoes, pomegranates, and citrus fruits).

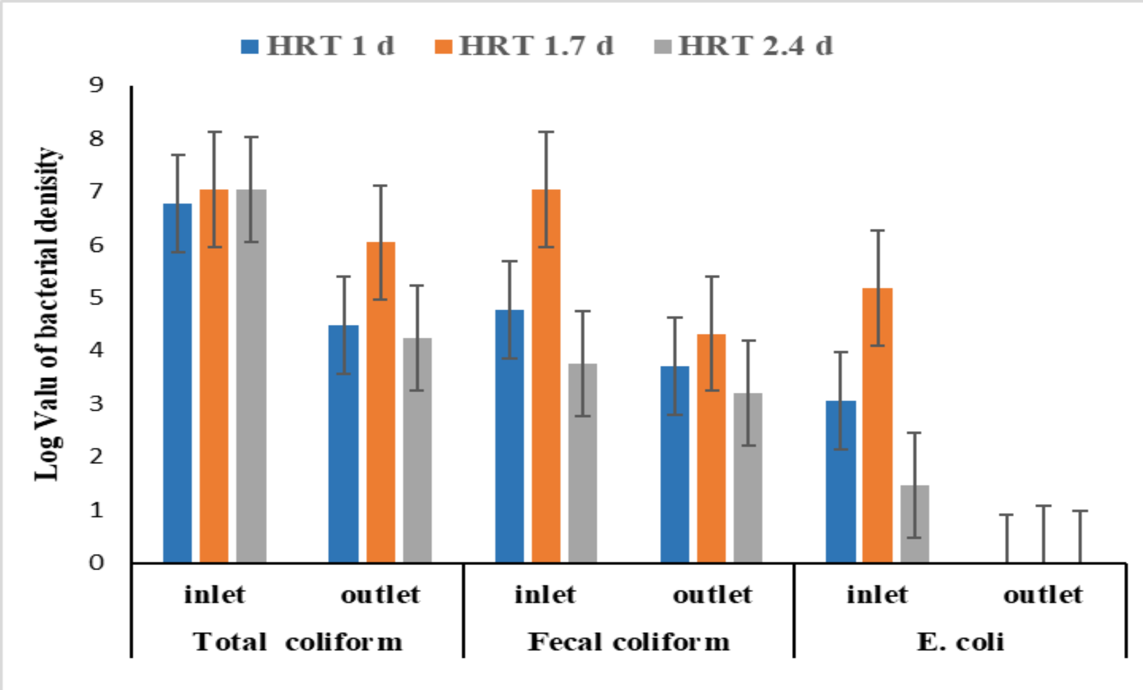

Fig. 5. Log value of the density of total coliform, fecal coliform, and *E. coli* (MPN /100 mL) by multiple tube fermentation technique in wastewater samples before and after treatment.

In this article, the design system ABIS is described in detail, highlighting its engineering criteria and advantages over other wastewater treatment (WWT) systems. ABIS is designed to optimize treatment efficiency and upgrade the treated wastewater to be suitable for unrestricted irrigation according to the Egyptian specifications. The key design considerations include the positioning of inflow and outflow streams in the three ponds to avoid turbulence, the fixed internal cylinder containing the absorber to minimize the weight and the friction forces, and slow rotation of the RS to facilitate attraction between microalgae and the RS. The absorber material is distributed along the internal cylinder to prevent accumulation and provide more space for bacterial biofilm formation. In the process of microbial adhesion to the granular activated carbon, the properties of charge and the mode of action are influenced by several factors, including physicochemical properties of the carbon, the bacteria, and the surrounding environment. As the system is divided into three ponds the pH of the inflow stream is decreased according to the bacterial existence in the first pond, the culture's pH in the second pond that contains the rotating surfaces is in the range of 7-8 even at low flow rates. The point of zero charge of the activated carbon is 8, resulting in a positive charge of its surface. The bacterial cells are generally negatively charged due to the presence of phospholipids, proteins, and lipopolysaccharides. In general, it is worth to mention the ionic strength of the water molecules can screen the electrostatic interaction between the negatively bacterial cells and the granular activated carbon cells. This is elucidated by the physical adsorption of the porous structure of the 1 mm sizes of activated carbon as a mode of action that enthusiasm the physical adhering of bacteria to be trapped in the pores. The rotation of the RS is achieved pneumatically, reducing power consumption and providing the culture with carbon dioxide. Microalgae harvesting is conducted weekly by scratching the strips of the RS and using suction pump to harvest the algae in the pond.

This ABIS is potential for highly efficient WWT comparing to other previous WWT systems based on growing algae such as the belt rotating surface performed by Zhou et al. [33] which performed 90% nickel removal from industrial wastewater through the biofilm formed on the rotating belt exposed to fluorescent light. Marella et al. [34] used Nualgi containing Si, Fe, and metal ions to enhance the diatom growing performing 51% BOD reduction from the urban wastewater in eutrophic Hussain Sagar Lake. While Johnson et al. [12,35] used the microbial communities in biofilm growing on rotating surfaces for ammonia removal; the feedstocks contained high total ammonia and relatively low levels of dissolved inorganic carbon. The system of Johnson et al. [12,35] depends on the biofilm on their sunlit exterior surfaces. The ABIS differs from these systems in its design focus on efficient feedstock treatment for producing water suitable to unrestricted irrigation according to the Egyptian specifications.

3.3. Algal community structure

The optimal operating condition for the treatment process is a hydraulic retention time (HRT) of 1.7 days. Under these conditions, various microalgal species were observed in the raw wastewater. Among the green microalgae, *Chlorella vulgaris*, *Scenedesmus obliquus*, *Selenastrum* sp., and *Ankistrodesmus acicularis* were identified. The diatom group included *Cyclotella comta*, *Stephanodiscus* sp., and *Nitzschia linearis*. The blue green algal group was represented by *Oscillatoria limnetica* and *Microcystis* sp.

In the algal pond (AP), *Chlorella vulgaris* dominated, forming biofilms on the pond walls and the rotating surface (RS). In contrast, the raw wastewater contained only a small amount of *C. vulgaris*, as shown in Fig. 6. *Oscillatoria limnetica* was predominantly represented in raw wastewater, which proliferated significantly and formed biofilms on the pond walls and RS. Substantial quantities of *Nitzschia linearis* were found in the pond, contributing to the biofilm on the walls and RS, as well as being abundant within the RS. *Scenedesmus obliquus*, *Selenastrum* sp., and *Ankistrodesmus acicularis* were present in smaller quantities in both the pond and the raw wastewater. In contrast, *Cyclotella comta*, *Stephanodiscus* sp., and *Microcystis* sp. were more prevalent throughout the pond. Fig. 7 presents a scanning electron microscopy (SEM) analysis of the biofilm formed within the RS, confirming that *Nitzschia linearis* was abundant, while *Cyclotella comta* was found in lower numbers.

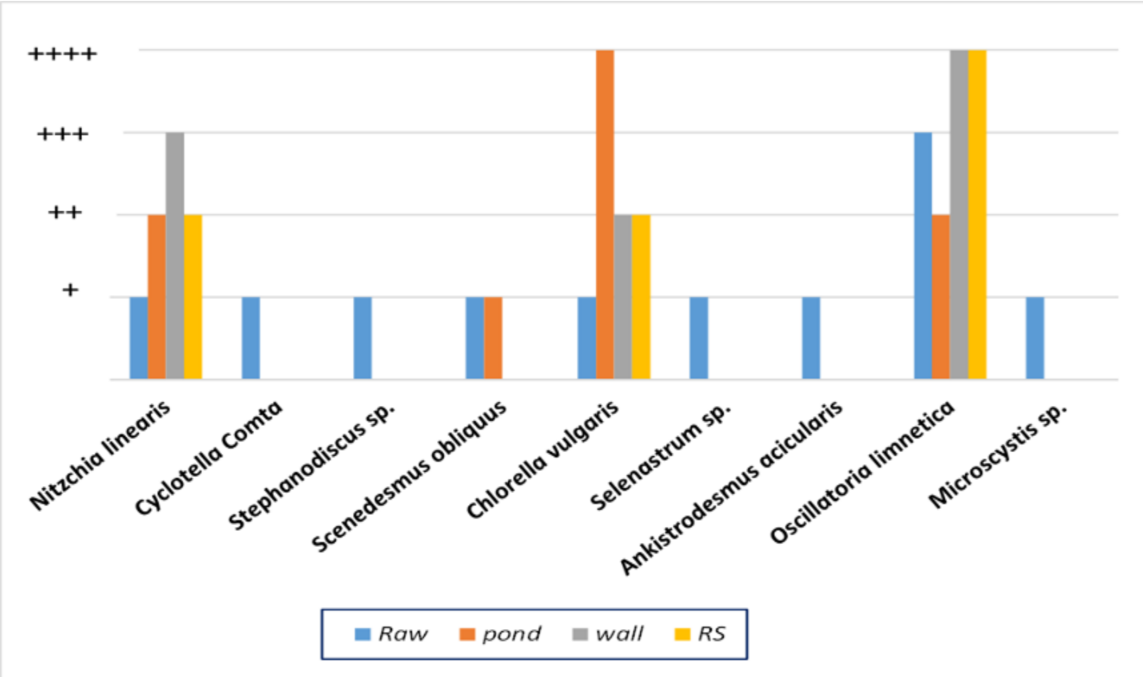

Fig. 6. Algal strains distribution, (++++) Dominant; (+++) Abundance; (++) Many; (+) Appreciable

3.4. Algal-bacterial biofilm

Scanning electron microscopy (SEM) imaging conducted on the dried biomass of biofilm formed inside the RS is exhibited in Fig. 6. Eukaryotes such as *Nitzschia linearis* and *Cyclotella Comta* could be clearly distinguished. Interestingly, the biofilm that formed is characterized by the presence of a huge density of coccoid shaped bacteria along with different types of algae, especially diatoms as clearly indicated in Fig. 6-C. Previous research articles have indicated that Proteobacteria and Bacteroidetes are the main heterotrophic bacterial phyla that associated with the presence of diatoms. Among these phyla, *Alteromonas, Roseobacter, Flavobacterium,* and *Sulfitobacter* appear to have a significant relationship with diatoms, as evidenced by their frequent appearance in various studies [36–40].

The interaction between microalgae and bacteria occurs through a variety of interfaces, including the bilateral transfer of dissolved organic compounds, and the physical contact between cells [41]. These microscopic interactions take place in an extracellular region around the algal colony or single cell known as the phycosphere. In the phycosphere, the diffusion of organic and inorganic materials through the algal cells provides bacteria with their mandatory nutrients. For this reason, bacterial communities are attracted to the phycosphere [41]. The microorganisms excrete extracellular polymeric substances (EPS) which agglomerate the microalgae and bacteria and improve their stability [42,43]. Also, cell to cell communication occur by other compounds such as indole-3-acetic acid (phytohormone) that enhance the interaction between microalgae and bacteria, stimulating the metabolism of microalgae. Likewise, heterotrophic bacteria provide the essential micronutrient vitamin B_{12} to microalgae, which in turn uses the oxygen produced by microalgal photosynthesis to oxidize organic carbon [43]. Nitrifying bacteria (NB) can use this oxygen for the oxidation of ammonium into NO_3^-. In the meantime, microalgae utilize CO_2 generated by heterotrophic bacteria as a source of carbon [26,43]. Treatment systems based on the integration of microalgae and bacteria have the potential for bioremediation due to the self-sufficient oxygen production provided by microalgae during photosynthesis [43].

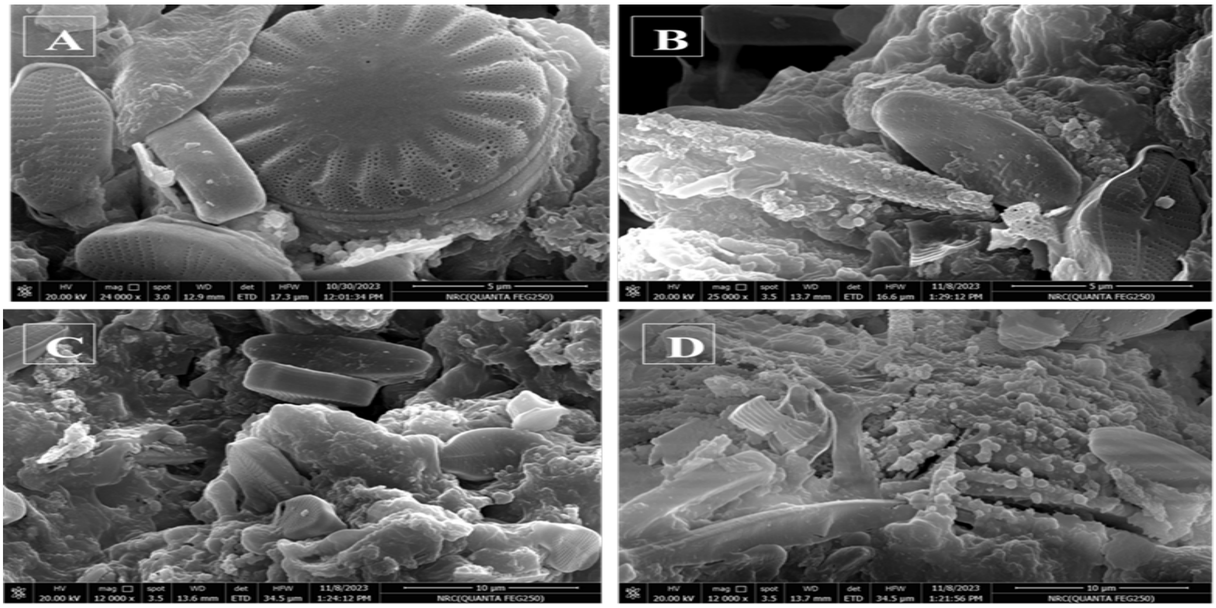

Fig. 7. Scanning electron microscopic images of biofilm formed inside the RS: (A) *Nitzschia linearis* and *Cyclotella Comta* at 24.000 x; (B) Bacterial colonies and *Nitzschia linearis* at 25.000 x; (C) *Nitzschia linearis* and biofilm at 12.000 x; (D) Bacterial colonies at 12.000 x.

4. Conclusion

This study demonstrated the successful integration of algae and bacteria in a promising wastewater treatment system with a notable level of treatment efficiency. This technique presents a green eco-friendly and cost-effective alternative to conventional wastewater treatment methods. The design of rotating surfaces for the Algal-Bacterial Integrated System (ABIS) helped in the formation of algal-bacterial biofilm. The synergistic interaction between bacterial cells, algal cells, and the surface is responsible for the initiation and the growth of the biofilm. Our findings indicated that this system can achieve over 90 % removal efficiency for TSS and turbidity. Also, it achieved good reduction for COD and BOD levels by comparing to the initial samples. This system showed a notable efficiency for removing fecal coliforms and *E. coli* reached 4 and 5 log unit reduction, respectively at HRT 1.7 d with using 5 g of granular carbon. SEM analysis of the biofilm within the RS confirmed the abundance of diatoms, particularly *Nitzschia Linearis*. These results increase the possibility of using treated wastewater for irrigation of edible crops according to the Egyptian Code of Practice for the Use of Treated Municipal Wastewater for Agricultural Purposes. However, the results highlight the importance of further research to optimize the parameters of operation to achieve higher treatment efficiency.

Conflicts of interest

There are no conflicts to declare.

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