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Effectiveness of cyclic treatment of municipal wastewater by *Tetradesmus obliquus* – Loofah biofilm, its internal community changes and potential for resource utilization

Zhongqi He [a], Xu Zhou [a,*], Nannan Mei [a], Wenbiao Jin [a], Jing Sun [b], Shiyu Yin [a], Qilin Wang [c]

[a] State Key Laboratory of Urban Water Resource and Environment, School of Civil and Environmental Engineering, Harbin Institute of Technology (Shenzhen), Shenzhen, Guangdong 518055, China
[b] State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[c] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Ultimo, NSW 2007, Australia

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ABSTRACT

Microalgae biofilm has garnered significant attention from researchers in the field of sewage treatment due to its advantages such as ease of collection and stable sewage treatment capabilities. Using agricultural waste as biofilm carriers has become a hotspot in reducing costs for this method. This study first combined *Tetradesmus obliquus* with loofah to form a microalgae biofilm for the study of periodic nitrogen and phosphorus removal from municipal wastewater. The biofilm could stably treat 7 batches of wastewater within one month. The removal rate of TP almost reached 100 %, while the removal rates of NH_4^+ and TN both reached or exceeded 80 %. The average biomass yield over 25 days was 102.04 mg/L/day. The polysaccharide content increased from 8.61 % to 16.98 % during the cyclic cultivation. The lipid content gradually decreased from 40.91 to 26.1 %. The protein content increased from 32.93 % in the initial stage to 41.18 % and then decreased to 36.31 % in the later stage. During the mid-stage of culturing, the richness of anaerobic bacteria decreased, while the richness of aerobic and facultative bacteria increased, which was conducive to the construction of the microalgae-bacteria symbiotic system and steadily improved the effect of nitrogen and phosphorus removal. As the culturing progressed, the *Rotifers* that emerged during the mid-stage gradually damaged the biofilm over time, leading to a decline in the effectiveness of sewage treatment in the later stages. This study offers technical support for carrier selection in microalgae biofilm methods and for the periodic removal of nitrogen and phosphorus from wastewater.

1. Introduction

It is well known that the freshwater resources of the earth account for only 0.5 % of the earth's water resources, and every year, with the increase in the population and the gradual increase in the demand for industrial capacity, humanity produces a large amount of wastewater, about 380 trillion L/y (Goswami et al., 2021). According to studies, by 2050, more than 50 % of the world's population will face long-term water scarcity, so the treatment of wastewater resources will receive a great deal of attention from countries and scholars around the world (Roshan and Kumar, 2020). Municipal wastewater, as an important part of the water resources cycle, contains a certain amount of nitrogen and phosphorus pollutants and organic matter within it. Sewage discharges from Chinese cities can contain about 0.29 megatonnes of phosphorus per year (Zhou et al., 2017). The removal of ammonia nitrogen and COD from wastewater in municipal wastewater treatment plants is also above 80 % and 88 %, respectively (Zhang et al., 2016). The conventional activated sludge process, on the one hand, generates a large amount of sludge that is difficult to remove after sewage treatment, and on the other hand, the high aeration rate increases the energy consumption of sewage treatment. Currently, some new research, such as culturing denitrifying polyphosphate accumulating organisms (DPAOs), has been used to improve the nitrogen and phosphorus removal capabilities of sewage and reduce aeration energy consumption (Zekker et al., 2021). Moreover, microalgae biorefineries can take advantage of the high potential for bioresource utilization of municipal wastewater as an inexpensive medium for the production of high-value by-products from microalgae, such as biodiesel, proteins, and polysaccharides (He et al.,

* Corresponding author.
E-mail address: zhouxu@hit.edu.cn (X. Zhou).

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2022a).

While traditional suspension culture can effectively remove nitrogen and phosphorus pollutants from wastewater, microalgae need to be harvested by centrifugation and other means at the end of the treatment, which increases the operating cost (He et al., 2022b). Microalgae biofilm as an emerging microalgae bioreaction unit has been gradually applied in municipal wastewater treatment. The biosorption immobilization technique involves the attachment of microorganisms to the surface of

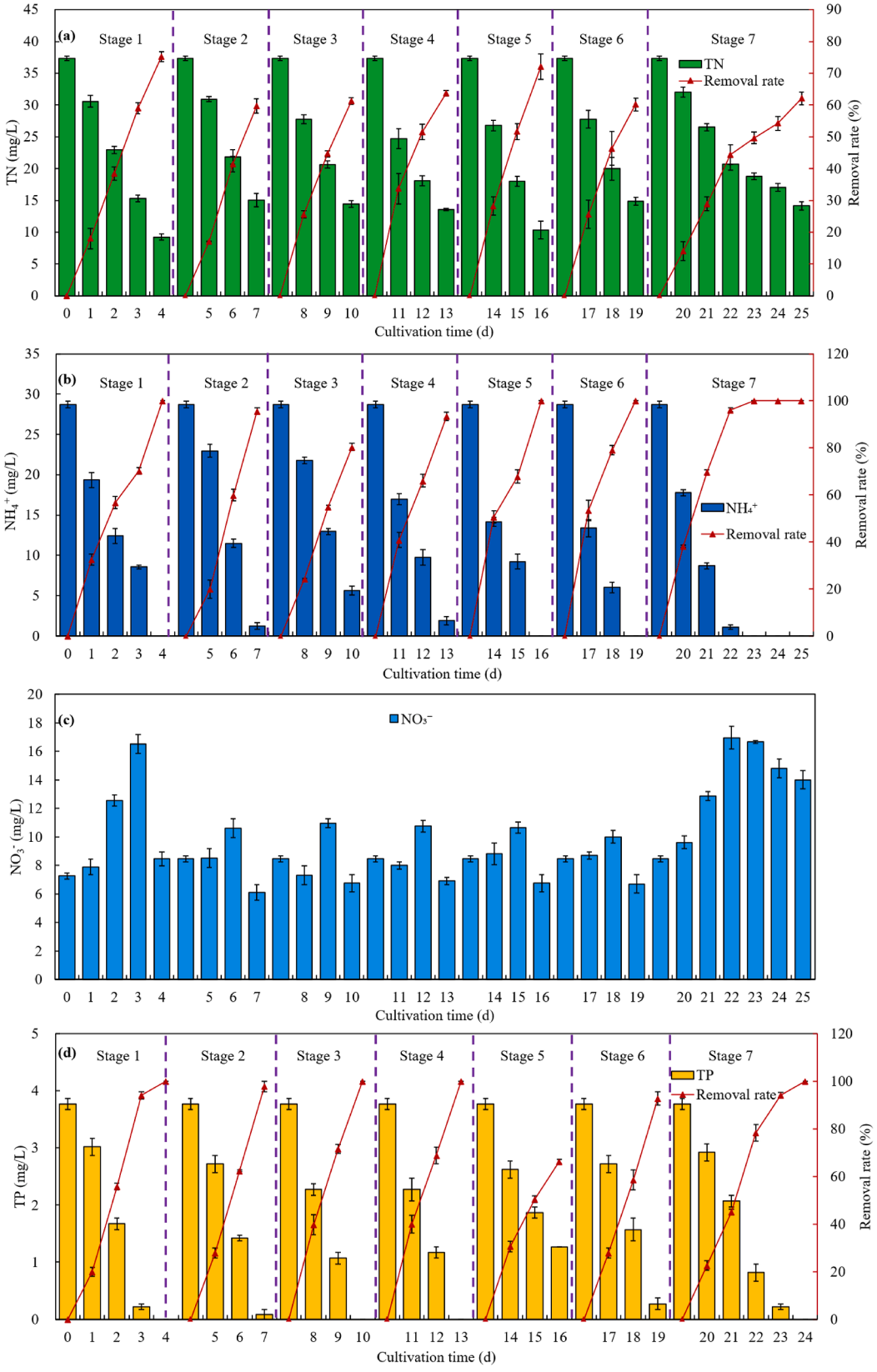

Fig. 1. Cyclic culture of microalgae biofilm for treatment of municipal wastewater. (a) TN, (b) NH_4^+, (c) NO_3^-, (d) TP.

the carrier through intercharge forces such as hydrogen bonding and van der Waals forces (Chen et al., 2016). Compared with suspension culture, microalgae biofilm cannot only effectively increase the culture density as well as the biomass production per unit area, but also simplify the biomass harvesting process, thus expanding the production scale (Han et al., 2023). Li et al. (2022) revealed that the utilization of improved capillary-driven attached biofilm reactors in conjunction with low-concentration phytohormones significantly enhanced biomass production by six-fold compared to traditional reactors. Additionally, Cao et al. (2022) developed an immobilized microalgae membrane bioreactor, which achieved a 4.75-fold increase in the dry weight of microalgae within just 2 days. Furthermore, during the harvest phase, the lipid content per cell increased by 4.5 times.

An economical, environmentally friendly carrier with a high adsorption effect is necessary to enhance the cost-effectiveness of immobilized cultured microalgae. As an agricultural waste, loofah has good biocompatibility with a large specific surface area and a complex network of 3D hollow fibers that can rapidly bind to microalgae to form microalgal biofilms (Chen et al., 2018; Saeed and Iqbal, 2013). Also, its advantages are low production cost, large specific surface area, good biosorption, high tensile strength as well as biodegradability, which makes it an excellent fibrous biocomposite for the immobilization of microalgal biofilms (Akhtar et al., 2008; D'Almeida et al., 2005; Shazryenna et al., 2015). Currently, most research focuses on the immobilization of loofah in microalgal biofilms and its application as a biosorbent, such as its combination with *Chlamydomonas reinhardtii* (OR242521) to adsorb methylene blue in wastewater (Moghazy and Mahmoud, 2023) or its use for heavy metal adsorption. Additionally, some algal-bacterial biofilms using loofah as a carrier had effectively removed nitrogen and phosphorus from rare earth element tailings wastewater (Liu et al., 2023). In these cases, the loofah not only served as a support material but also enhanced system performance by acting as a carbon source. Less research has been done on the stability of the cyclic treatment of municipal wastewater with loofah microalgae biofilms and the changes in biorefining during the process.

In this study, the first cyclic treatment of municipal wastewater by using the loofah as a carrier for the biofilm of *Tetradesmus obliquus* was carried out, focusing on the stabilizing effect of the biofilm in removing monomers and phosphorus in one month. The potential of biorefining resources was analyzed according to the internal composition changes of biomass in different culture stages. The reasons for the change in water quality treatment were analyzed by analyzing community composition. This study provides technical support for the subsequent industrialization research on the treatment of municipal wastewater by adsorption and immobilized microalgae.

2. Results and discussion

2.1. Cyclic treatment of municipal wastewater with microalgae biofilms

The group performed heat treatment modification of the loofah to prepare adsorption-immobilized microalgal biofilms with better growth conditions. The biofilm had reached a steady state by the time it was put into the experiment. The chlorophyll-a content in the suspension liquid was less than 1 mg/L. In this study, we investigated the cyclic nitrogen and phosphorus removal from municipal wastewater by microalgal biofilms over nearly one month, and the experimental results are shown in Fig. 1. When the nitrogen and phosphorus index reached the discharge standard, the municipal wastewater would be added again. The microalgae biofilm could treat 7 batches of municipal wastewater within 25 days, and the culture time of each batch was 4 days, 3 days, 3 days, 3 days, 3 days, 3 days and 7 days. The microalgal biofilm had a stable effect on nitrogen and phosphorus removal from municipal wastewater for almost one month. The treatment efficiency of microalgae biofilm for municipal wastewater showed an increasing, then stabilizing, then decreasing trend throughout the operation cycle. TP achieved almost 100 % removal in each culture cycle. Ammonia removal was in the range of 80–85 %. TN removal was largely maintained above 80 %. Combined with Fig. 1(b) and (c), it could seen that the nitrate nitrogen content in the wastewater mainly constrained the removal efficiency of total nitrogen. The nitrate nitrogen content fluctuated during the cultivation cycle, but this was because some of the ammonia nitrogen in the wastewater was converted to nitrate nitrogen by aeration or by ammonia-oxidizing bacteria and nitrite-oxidizing bacteria within the microalgal biofilm (Kang et al., 2018). At the end of the first six cycles, the support capacity of microalgal biofilm with loofah as the carrier decreased, resulting in a buildup of the carrier material on top of the culture unit due to air flotation. Furthermore, due to the aging of the biofilm, some parts of it might detach and fall into the cultivation tubes. At this time, the light transmission rate of sewage inside the culture device, nitrogen and phosphorus mass transfer and so on were affected, which in turn affected the effect of water quality treatment.

2.2. Analysis of biofilm composition and resource utilization potential

The dry weight of the biofilm and the percentage of the composition of the biofilm at various periods of the microalgal biofilm treatment of municipal wastewater are shown in Fig. 2(a). The initial (A1), medium (A2), and late (A3) stages were the 2nd, 13th and 25th days, respectively, when the microalgal biofilm was put into the culture device. The initial microalgal biofilm dosing was approximately 1.20 ± 0.03 g/L. The dry weights of their microalgal biofilms were 1.25 ± 0.03 g/L, 2.75 ± 0.07 g/L, and 3.75 ± 0.11 g/L for A1, A2, and A3, respectively. The biomass yield was 124.33 mg/L/d during A1 and A2 and 77.31 mg/L/d during A2 and A3, with an average biomass yield of 102.04 mg/L/d over the 25-day cycle. As can be seen from the experimental results, the biomass growth of microalgal biofilm was significantly higher in the early stage than in the later stage. The polysaccharide content within the microalgae increased from 8.61 % to 16.98 % throughout the culture ($p < 0.05$). The protein content increased from 32.93 % in A1 to 41.18 % in A2 and then decreased to 36.61 % in A3 ($p < 0.05$). The lipid content gradually decreased from 40.91 in A1 to 26.1 % in A3 ($p < 0.05$). The high initial lipid content might be due to the severe deprivation of nitrogen and phosphorus within the medium at the time of biofilm formation, resulting in excessive lipid content within the microalgae (Mou et al., 2022). This was accompanied by frequent changes in wastewater during the cultivation cycle, which led to a reduction in nitrogen and phosphorus deficiencies. Moreover, in the later stage, the lipid content in the microalgae body decreased, and the proportion of polysaccharides and proteins was relatively high. From the experimental results, the biofilm at the early stage of culture was more suitable for biodiesel extraction, while the biofilm at the later stage of culture was more suitable for protein and polysaccharide utilization.

In addition, the fatty acid composition was analyzed to determine whether the internal lipids of microalgae could be used for biodiesel. The components of the fatty acid of biofilms of municipal wastewater cultured *Tetradesmus obliquus* are shown in Table 2. The percentage of saturated, monounsaturated and polyunsaturated fatty acid content at different stages of cyclic culture is shown in Fig. 2(b). The percentage of C16:00, C18:2n-6t, C18:3n-6 and C20:00 was high throughout the cultivation stage. C16:00 decreased from 19.19 % in A1 to 13.15 % in A3. C18:2n-6t increased from 13.86 % in A1 to 15.33 % in A3. C18:3n-6 decreased first from 22.19 % in A1 to 18.86 % in A2 and then increased to 19.51 % in A3. C20:00 had a small difference of 10.57 % and 10.1 % in A1 and A3, respectively, but rose to 15.19 % in A2. In terms of the degree of saturation of fatty acids, the percentage of saturated and unsaturated fatty acids was stable throughout the stages of cultivation, with saturated fatty acids decreasing from 35.36 % in A1 to 33.88 % in A3. Unsaturated fatty acids increased from 64.64 % in A1 to 66.12 % in A3. The fatty acid components were mainly medium and long-chain fatty acids (C16–C20) suitable for use as feedstock in the preparation of biodiesel (Huang et al., 2010).

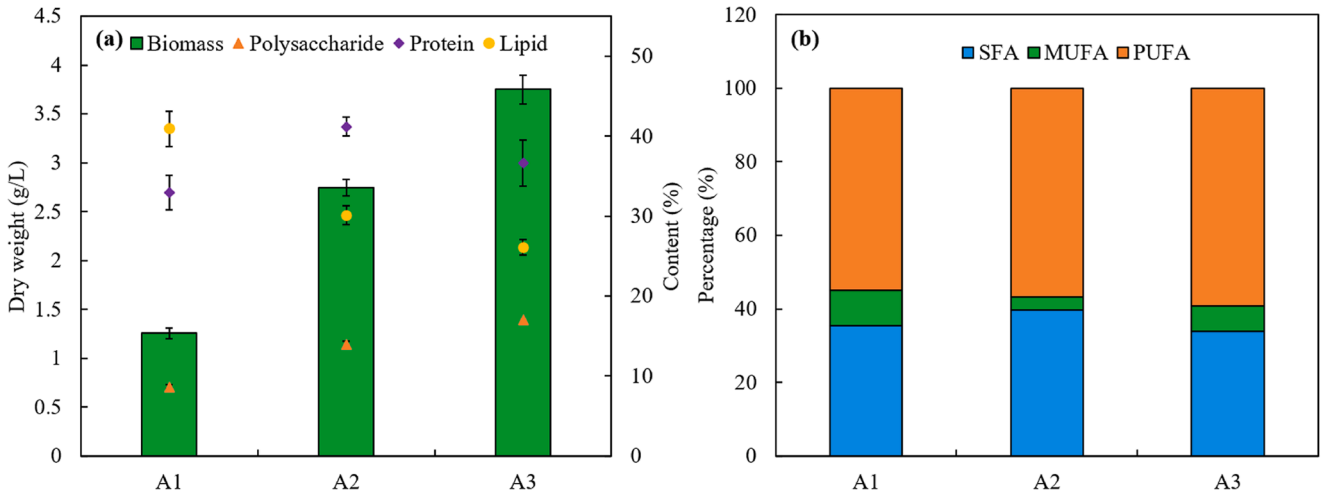

Fig. 2. Biomass composition of microalgal biofilms at different cyclic municipal wastewater treatment stages. (a) Biomass and high-value by-products, (b) Fatty acid saturation ratio.

2.3. *Microbial community analysis*

In this study, we characterized the biodiversity changes of microalgal biofilms during one month of operation in municipal wastewater by examining the operational taxonomic unit (OTU) (Geng et al., 2016), Shannon and Simpson in alpha diversity index (SureshKumar and Thomas, 2019), and species relative abundance (Hu et al., 2023) of microalgal biofilms in different stages of cultivation. Thus, the reasons for fluctuations in the quality of water treated by biofilms during the operating cycle are analyzed from the point of view of species diversity.

As can be seen in Fig. 3(a), the OTU of prokaryotic on the microalgal biofilm was sampled in A1, A2, and A3 as 1385, 1342, and 1393, respectively, during nearly one month of cultivation. Of these, 710 were common to all three biofilm samples. In addition, 181 OTUs were common to A1 and A2, 141 OTUs were common to A1 and A3, and 301 OTUs were common to A2 and A3. The number of OTUs shared by A2 and A3 was higher than the other combinations, indicating that the prokaryotic microbial OTUs of A2 and A3 were more similar among the three samples. Shannon for prokaryotes on biofilms increased from 3.89 in A1 to 5.23 in A2. Moreover, Simpson decreased from 0.101 in A1 to 0.016 in A2. By comparing the alpha diversity indices of the three different culture stages, it could be found that both Shannon and Simpson show a gradual decreasing trend, while the total number of species of prokaryotes was basically the same. According to Fig. 3(b), biofilm samples A2 and A3 were located in the same branch of the clustering tree, indicating that the structure and abundance of their prokaryotic communities were relatively similar. A1 was located in a separate unit from A2 and A3, suggesting that the structure and abundance of the prokaryotic communities in samples A2 and A3 differed significantly from that of A1. This was due to the differences that occurred between biofilm populations as the cultivation time increased, resulting in differences in community structure. As can be seen in Fig. 3 (c), microalgal biofilms from stages A1, A2 and A3 were dominated by *Proteobacteria, Bacteroidia* and *Chloroflexi* at the Phylum level. At the Class level, this mainly included prokaryotes such as *Bacteroidia, Gammaproteobacteria, Alphaproteobacteria* and *Anaerolineae* (Krohn-Molt

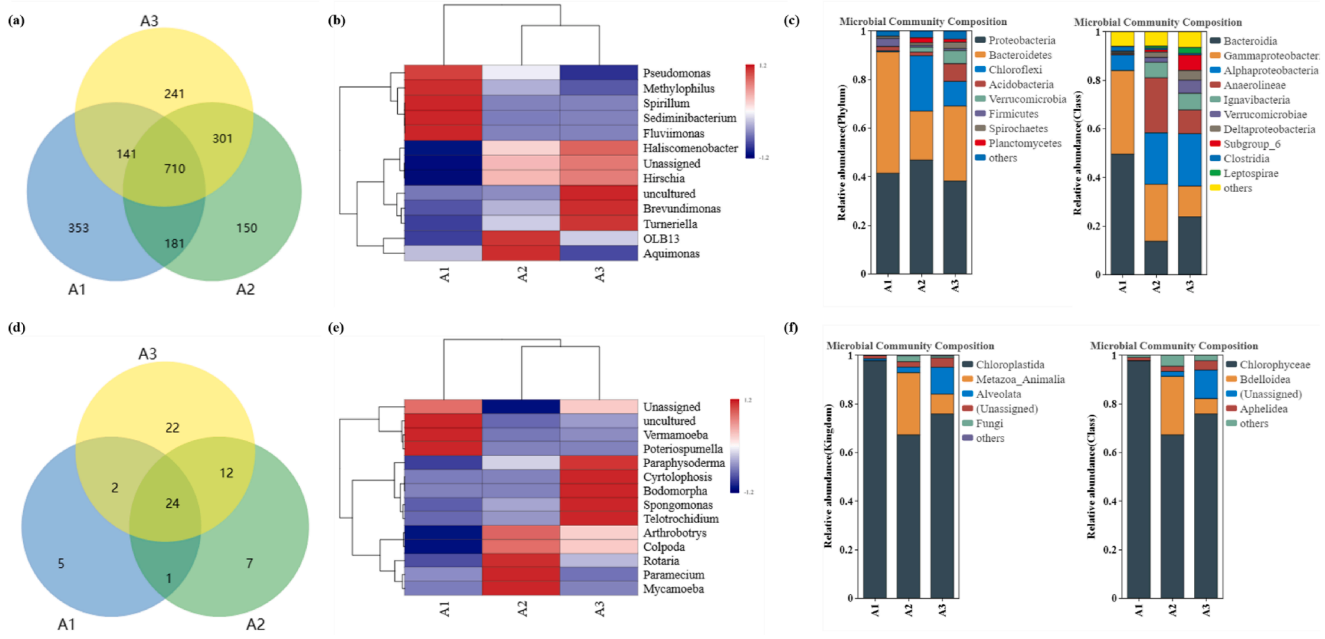

Fig. 3. Community analysis of microalgal biofilms at different cyclic municipal wastewater treatment stages. (a)-(b) OTUs and heat maps of prokaryotes, (c) Community composition of prokaryotes, (d)-(e) OTUs and heat maps of eukaryotes, (f) Community composition of eukaryotes.

et al., 2013; Shao et al., 2021). At the Class level, the relative abundance of *Bacteroidia* was 49.61 %, 13.72 % and 23.81 % for A1, A2 and A3, respectively. The relative abundance of *Gammaproteobacteria* was 34.39 %, 23.47 % and 12.66 %, respectively. The relative abundance of *Alphaproteobacteria* was 6.43 %, 21.14 % and 21.55 %, respectively. The relative abundance of *Anaerolineae* was 0.41 %, 22.66 % and 9.75 %. The biofilm growth was accompanied by a significant decrease in the proportion of anaerobic bacteria (*Bacteroidia* and *Gammaproteobacteria*). Whereas the proportion of aerobic and parthenogenetic anaerobic bacteria (*Alphaproteobacteria, Ignavibacteria, Verrucomicrobiae* and *Deltaproteobacteria*) increased significantly. This suggested that the initial anaerobic bacterial flora dominated in municipal wastewater, while the proportion of dissolved oxygen in the water increased along with the incorporation of microalgal biofilms, leading to an increase in the proportion of aerobic and parthenogenetic anaerobic bacterial flora. However, due to the multilayer structure during microalgal biofilm cultivation, the local biofilm space was under anaerobic conditions, increasing the proportion of some anaerobic bacteria (*Anaerolineae*) (Zhang et al., 2023).

As shown in Fig. 3(d), after one month of cyclic cultivation, the eukaryotes of the microalgal biofilm had 33, 44 and 60 different OTUs in A1, A2 and A3, respectively. Twenty-four of all OTUs were common to all three biofilm samples. One OTU was common to A1 and A2, two OTUs were common to A1 and A3, and 12 OTUs were common to A2 and A3. The number of OTUs shared by A2 and A3 of the biofilm was significantly higher than the other combinations, indicating that the eukaryotic microbial OTUs of A2 and A3 were more similar among the three samples. Shannon for biofilm prokaryotes showed an increasing and then decreasing trend from 0.141 in A1 to 0.962 in A3, while Simpson showed a decreasing and then increasing trend from 0.956 in A1 to 0.952 in A3. According to Fig. 3(e), biofilm samples A2 and A3 were located in the same branch of the clustering tree, suggesting that their eukaryotic community structure and abundance were relatively similar. A1 was located in a separate branch outside of A2 and A3, suggesting that the structure and abundance of eukaryotic communities in samples A2 and A3 were significantly different from that of A1. As can be seen in Fig. 3(f), the microalgal biofilms of the three stages A1, A2 and A3 were dominated by *Chloroplastida, Metazoa-Animalia* and *Alvolata* at the Kingdom level. At the Class level, it mainly included eukaryotes such as *Chlorophyceae, Bdelloidea* and *Aphelidea*. Throughout the culture stage, *Chlorophyceae* dominated the eukaryotic fraction, with relative abundances of 97.74 %, 67.24 % and 75.86 % in A1, A2 and A3. In contrast, the percentage of *Bdelloidea* rase significantly in A2, accompanied by the emergence of metazoa such as *Rotaria, Colpoda, Paramecium* and *Mycamoeba* (Kanavillil and Kurissery, 2013). *Aphelidea* had a small increase at the end of cultivation. The presence of these organisms, such as *Rotaria*, which would prey on microalgae caused the microalgal biofilm to be damaged to some extent at the later stage, which affected the water quality treatment effect (Rego et al., 2015; Subhash et al., 2019).

2.4. Outlook

As an economical and environmentally friendly method, microalgae-based wastewater treatment has many advantages in pollutant removal, resource recovery, CO_2 capture, etc., and it will be a powerful competitor in the field of wastewater treatment in the future (Ma et al., 2023). At present, the most successful commercialized microalgae cultivation application system is the open microalgae cultivation system, but its disadvantages of large floor area and low microalgae density limit its wide application (Huang et al., 2023). Although the column photobioreactor greatly saves the floor area and increases the biomass of microalgae, the difficulties in harvesting brought about by suspension culture and the energy consumption required for circulation and stirring restrict its application (Wang et al., 2022). Compared to suspension culture, the microalgae biofilm method significantly reduces the energy consumption caused by mechanical stirring and later centrifugation harvesting. Moreover, its biomass productivity and wastewater treatment efficiency are far superior to traditional suspension culture. However, the support materials used in previous studies are mostly organic composites, which, although non-toxic to organisms, are mostly non-degradable, causing considerable trouble for subsequent treatment (You et al., 2022).

In this study, the heat-modified loofah used as the support material for the microalgae biofilm showed a good adsorption effect on *Tetradesmus obliquus*, reaching 93.48 %. Compared to suspension culture, the biomass increased by 63.2 %. In one month, the treated water quality was good during the process of nitrogen and phosphorus removal from sewage, and 7 batches of municipal wastewater could be treated. The support effect remained stable, and there were no crushing or breaking phenomena during harvesting. Using economical and environmentally friendly agricultural waste as a carrier for biofilms to reduce the cost of biofilm treatment for water pollution has become a research hotspot. The loofah-microalgae biofilm not only saves the cost of harvesting microalgae using previous methods such as centrifugation, but also reduces the energy consumption of aeration in traditional sludge treatment of wastewater. As an agricultural waste and renewable resource, it reduces the cost of biofilm carriers while mitigating the risk of subsequent environmental pollution. This study explored the nitrogen and phosphorus removal effect of municipal wastewater using *Tetradesmus obliquus*-loofah biofilm over a period of one month, providing data support for the subsequent industrial application of this method. Additionally, the analysis of changes in the biological community and the composition of various high-value by-products of microalgae provides technical support for subsequent technological regulation and resource utilization.

3. Conclusion

In the initial and middle stages of the whole seven batches of culture, the treatment effect of nitrogen and phosphorus in municipal wastewater was stable, and the discharge standard could be reached within 3 days. From the biomass fractions at different stages, biofilms at the early stage of culture were more suitable for biodiesel extraction, while biofilms at the later stage of culture were more suitable for protein and polysaccharide utilization. While in the middle of culture, the percentage of *Bdelloidea* increased significantly, accompanied by the emergence of *Rotaria, Colpoda, Paramecium* and *Mycamoeba*, which gradually damaged the microalgal biofilm, leading to the decrease of the efficiency of microalgal biofilm in treating wastewater in the late stage.

4. Materials and methods

4.1. Microalgae strain and wastewater used in the experiment

The microalgae used in this experiment were screened by *Tetradesmus obliquus* (276) purchased from the Institute of Hydrobiology, Wuhan, China, which was suitable for growth in municipal wastewater after ARTP mutagenesis. Microalgae were pre-preserved in a light incubator using BG11 medium in 250 ml conical flasks and shaken three times a day. The microalgae were cultivated using a light/dark ratio of 12:12 and a light intensity of 4000 $\pm$ 300 lux. The incubator temperature was set at 24 °C $\pm$ 0.5 °C year round.

The municipal wastewater used for the experiments was obtained from the interior of the university city of Shenzhen, Guangdong Province, China. The concentrations of ammonia, nitrate nitrogen, total nitrogen, and total phosphorus ranged from 32 to 42, 1–5, 40–55, and 3.2–4.8 mg/L. The wastewater used for the experiments was the supernatant of the effluent that was settled for 1 h and filtered through a 200- mesh screen. None of the experimental water was sterilized.

4.2. Adsorption immobilized carrier and pre-treatment conditions

Adsorption-immobilized carriers were selected from the loofah. Each loofah was cut into cylinders of 4 ± 0.5 cm. 4–5 pieces of material were evenly placed in each culture tube to ensure that it had a carrier fill rate of about 5 %. According to previous experimental studies, loofah needed to be modified by heat treatment before conducting experiments. The cut loofahs were washed and soaked in deionized water for 24 h. The loofahs were then dried and dried at 0.125 MPa and 130 °C. The surface was washed with deionized water and placed in an oven at 120 °C for 12 h, then washed again and dried in an oven at 60 °C until constant weight.

4.3. Experimental design

The modified microalgae biofilm was placed in a column photobioreactor made of transparent glass, and the volume required for the actual experiment was 800 mL, with a biofilm filling rate of 5 %. The experimental setup is schematically shown in Fig. 4. The experimental temperature was controlled at 25 ± 2 °C, the light/dark ratio was 12 h:12 h, the light intensity was 4500 Lux, and the operation cycle was nearly one month. The effect of adsorption-immobilized microalgae biofilm on municipal wastewater nitrogen and phosphorus removal was investigated during the operation cycle. The resource potential of the biofilm was assessed by examining its biomass content and changes in the composition of each high-value by-product (polysaccharides, lipids and proteins). The causes of fluctuations in biofilm treatment of municipal wastewater were analyzed from a microscopic perspective by examining the community succession of biofilms during the operating cycle.

4.4. Analytical methods

4.4.1. Determination of microalgal growth and composition

The growth of microalgae in this study was characterized by measuring dry weight (Cheng et al., 2020). During the A1, A2 and A3 of microalgae biofilm cultivation, a piece of loofah-based biofilm was collected separately and repeatedly rinsed to ensure that no microalgae remained on the filler. The rinsed microalgae were then filtered through a membrane and dried at 80 °C for approximately 24 h to calculate the dry weight of the microalgae. The total lipids of microalgae were determined using the pre-modified chloroform-methanol method of the group (He et al., 2022c). The protein and polysaccharide yields were determined by the rapid Lowry method and the phenol sulfate method, respectively (Noreen et al., 2021; Wu et al., 2022).

4.4.2. Measurement of water quality indicators

In this study, Standard Analysis Methods were used to determine the content of TN, TP, ammonia nitrogen, and nitrate nitrogen in wastewater and their removal was used to represent the effectiveness of microalgae in removing nitrogen and phosphorus indicators from wastewater (Nepa, 2012).

4.4.3. Community analysis

The composition of microalgal biofilm communities at different stages of cyclic cultivation from municipal wastewater was analyzed. High-throughput sequencing was used to determine 16S rDNA and 18S rDNA of biofilms. The sequencing regions and amplification primers selected for this experiment are shown in Table 1.

4.4.4. Data processing

In this research, all measurements were taken in triplicate. The data were presented in graphical and tabular form and filed as mean ± standard deviation. SPSS software was used to perform statistical analysis of the results.

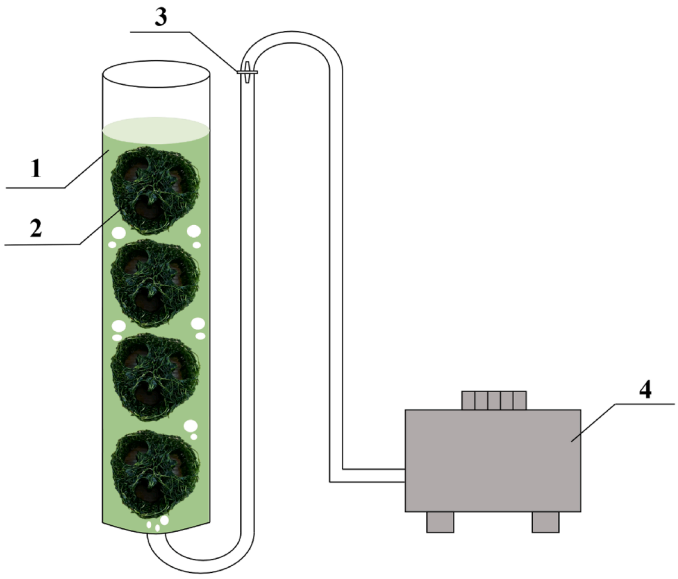

Fig. 4. Experimental setup.

Table 1
PCR amplification primer information sheet.

Amplified regions	Primer name	Primer pairs
Bacteria V3V4-1	338F	ACTCCTACGGGAGGCAGCA
	806R	GGACTACHVGGGTWTCTAAT
Fungi 18SV4-1	538F	GCGGTAATTCCAGCTCCAA
	New 706R	AATCCRAGAATTTCACCTCT

Table 2
Changes in the fatty acid composition of loofah- microalgae biofilm at different culture stages.

Fatty acids	Fatty acid composition (%)		
	A1	A2	A3
15:01	0.45 ± 0.04	0.92 ± 0.02	0.88 ± 0.11
16:00	19.19 ± 0.35	15.45 ± 2.01	13.15 ± 0.34
16:01	1.24 ± 0.03	1.00 ± 0.2	0.78 ± 0.14
17:00	1.42 ± 0.31	1.91 ± 0.1	0.57 ± 0.07
17:01	8.08 ± 0.23	1.75 ± 0.5	5.19 ± 2.51
18:00	4.16 ± 0.41	5.47 ± 0.96	6.91 ± 0.32
18:1n-9t	7.27 ± 0.19	8.44 ± 0.56	6.05 ± 0.83
18:1n-9c	4.00 ± 0.2	3.32 ± 0.21	3.83 ± 0.49
18:2n-6t	13.86 ± 0.32	13.37 ± 0.97	15.33 ± 2.35
20:00	10.57 ± 0.16	15.16 ± 2.04	10.10 ± 1.2
18:3n-6	22.19 ± 1.01	18.86 ± 1.22	19.51 ± 2.72
21:00	0.02 ± 0.00	1.60 ± 0.5	3.15 ± 0.35
20:02	5.00 ± 0.99	8.20 ± 1.6	11.68 ± 0.91
22:5n-3	2.55 ± 0.3	4.56 ± 0.79	2.88 ± 0.96
∑C16	20.43 ± 0.32	16.45 ± 2.2	13.93 ± 0.2
∑C18	51.49 ± 0.93	49.45 ± 1.56	51.63 ± 0.63
Saturated fatty acid (SFA)	35.36 ± 0.26	39.58 ± 0.21	33.88 ± 1.5
Mono-unsaturated fatty acid (MUFA)	9.76 ± 0.29	3.67 ± 0.92	6.84 ± 2.76
Polyunsaturated fatty acid (PUFA)	54.87 ± 3.01	56.75 ± 5.35	59.27 ± 8.26

CRediT authorship contribution statement

Zhongqi He: Writing – original draft, Investigation. **Xu Zhou:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Nannan Mei:** Investigation, Formal analysis. **Wenbiao Jin:** Writing – review & editing. **Jing Sun:** Writing – review & editing. **Shiyu Yin:** Writing – review & editing. **Qilin Wang:** Writing – review & editing.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

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