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Strain Screening and Conditions Optimization in Microalgae-Based Monosodium Glutamate Wastewater (MSGW) Treatment

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Abstract: The wastewater generated from monosodium glutamate production displays distinctive features of elevated salinity, organic content, as well as nitrogen and phosphorus concentrations, and its indiscriminate disposal poses a significant threat to water quality and can cause detrimental impacts on aquatic ecosystems. The application of microalgae for monosodium glutamate wastewater (MSGW) treatment can result in simultaneous wastewater purification and biomass recovery. In this study, the algae species capable of thriving in diluted MSGW were screened, and the wastewater composition and growth conditions were optimized to obtain high algal biomass and nutrient removal rate. Among the tested species, *Chlorella* sp. FACHB-30 demonstrated superior potential for MSGW treatment and achieved a maximum specific growth rate of 0.28 d^{-1} and the highest COD removal rate of 61.50% over a 20-day cultivation period with trace metals supplementation in the wastewater. Moreover, the cultivation of *Chlorella* sp. FACHB-30 yielded considerable reductions in total phosphate (69.09%), total nitrogen (26.93%), and NH_4^+-N (51.91%) levels in the wastewater. The optimum conditions for achieving maximum algal density and highest nutrient removal were determined as light intensity of 150 μmol $m^{-2}s^{-1}$, inoculation concentration of 1×10^5 cells mL^{-1}, and an iron concentration of 10^{-5} mol L^{-1}. Finally, under the optimized conditions, the removal rates of total phosphate, total nitrogen, NH_4^+-N, and COD were determined to be 87.60%, 68.05%, 75.89%, and 77.96%, respectively. The findings of this study highlight the potential for enhancing the nutrient removal efficiency of microalgae-based MSGW treatment through the implementation of a combined approach that involves the selection of tolerant strains, optimization of cultivation conditions, and refinement of wastewater composition.

Keywords: *Chlorella* sp. FACHB-30; monosodium glutamate wastewater; nutrient removal; conditions optimization

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1. Introduction

China produces almost half of the world's monosodium glutamate (MSG) every year [1]. The discharge of MSG wastewater (MSGW) into the environment can pose serious threats to aquatic ecosystems and human health, such as eutrophication, oxygen depletion, toxicity, acidification, and odor emission [2–4]. Various methods have been proposed for the treatment of MSGW, which can be classified into physicochemical and biological methods [5,6]. Physicochemical methods, such as coagulation–flocculation, adsorption, membrane filtration, and microwave catalysis, can remove pollutants rapidly and efficiently under different environmental conditions, but they also entail high capital and operational costs and generate large amounts of sludge [7,8]. Biological methods, such as aerobic

and anerobic treatments, are more environmentally friendly and cost-effective, as they can degrade organic matter and reduce sludge production. However, biological methods are sensitive to various factors that affect the microbial activity, such as pH, temperature, salinity, and oxygen concentration [3,9]. MSGW poses several challenges to traditional microbial purification methods due to its high organic matter and suspended hyphae content, elevated acidity, as well as high levels of ammonia nitrogen and sulfate. These characteristics not only exhibit direct or indirect toxicity towards microorganisms but also contribute to increased purification costs. Therefore, it is urgent to develop an eco-friendly and sustainable biological treatment technology for MSGW.

Compared to traditional microbial wastewater treatment, the use of microalgae in wastewater treatment has many advantages. Microalgae have a short life cycle, no agricultural land occupation, high photosynthesis efficiency accompanied by CO_2 fixation, good tolerance to environmental factors, and accumulation of high-value biomass that can be exploited as biofuels, feeds, health products, cosmetics, and plant fertilizer, etc. [10–12]. However, mass cultivation of microalgae still requires high costs including freshwater and nutrient consumption during cultivation [13]. Microalgae cultivation combined with wastewater treatment can effectively solve the problem. The nitrogen- and phosphorus-rich MSGW does not contain any hazardous or pathogenic substances. Moreover, previous studies have shown that the utilization of diluted MSGW can be advantageous for microalgal growth, while simultaneously reducing the costs associated with nutrient supplementation during cultivation [14,15]. Meanwhile, the wastewater is purified and even recycled, which makes wastewater as a nutrient source for the cultivation of microalgae a potentially economically viable and environmentally friendly method.

Due to the high concentration of ammonia nitrogen and suspended matter, only a few microalgae can survive and thrive in the undiluted MSGW. This is also a key bottleneck in using wastewater as a nutrient source to cultivate microalgae [16]. Most of the tests were conducted in diluted or simulated wastewater to reduce the toxicity of pollutants. The cultivation of *Chlorella* sp. FACHB-31 and *C. vulgaris* FACHB-8 in diluted swine wastewater was found to be feasible, with both species exhibiting high removal rates for total nitrogen (TN), total phosphorus (TP), and NH_4^+-N over a 15-day treatment period [17]. Specifically, *C. vulgaris* FACHB-8 achieved superior removal rates, with 89.85%, 93.68%, and 97.86% for TN, TP, and NH_4^+-N, respectively. When *Scenedesmus obliquus* was used to treat the diluted Agro-Zootechnical digestate for 21 days, the NH_4^+-N removal efficiency reached 83% [18]. Wu et al. treated simulated industrial wastewater with *Chlamydomonas* sp. TAI-2 for 16 days, and the removal rates of NH_4^+-N and PO_4^{-3}-P reached 100% and 33%, respectively [19]. However, excessive dilution of wastewater often increases the cost of wastewater treatment. Therefore, it is necessary to screen the microalgae species that can grow fast in wastewater with a high nutrient level of MSGW. This demand can be attributed to a combined strategy, that is selecting the tolerant strains, improving the wastewater composition, and optimizing the cultivation conditions. However, limited species and strains of microalgae were screened according to this strategy [4]. Liu et al. [4] explored the growth and main metabolites production of *Scenedesmus* sp. SDEC-8, *Golenkinia* sp. SDEC-16, and *C. sorokiniana* SDEC-18 in seawater medium supplemented with MSGW. Yu et al. [20] further optimized the seawater algae *Phaeodactylum tricornutum* and *Nannochloropsis oceanica* in a seawater medium supplemented with MSGW. Other reports mainly concentrate on *C. vulgaris* or *Spirulina subsalsa* cultivated with diluted MSGW [15,21,22]. All these studies focus on either strain selection or wastewater composition and growth condition improvement, the combination of the two aspects is rarely reported.

Microalgae are a promising resource for the production of bioactive substances and biofuels [23]. Therefore, treating MSGW with microalgae can achieve both pollution removal and biomass valorization [24]. Instead of regarding MSGW as a waste, it can be considered as a nutrient-rich medium for microalgae cultivation [25]. In this study, we aim to select microalgae strains that can tolerate MSGW and optimize the wastewater composition and growth conditions for maximum biomass production. The findings

will support the economic benefits of MSGW utilization and enhance the feasibility and commercial viability of microalgae-based MSGW treatment.

2. Materials and Methods

2.1. Microalgal Strains, Culture Conditions, and MSGW Preparation

Six distinct strains of single-celled green algae were used in this study. *C. sorokiniana* FACHB-275, *Chlorella* sp. FACHB-3, *Chlorella* sp. FACHB-9, *Chlamydomonas reinhardtii* FACHB-479, and *Tetradesmus obliquus* FACHB-416 strains were acquired from the Freshwater Algae Culture Collection at the Institute of Hydrobiology in China. The strain *C. sorokiniana* GXNN01 was isolated from a wastewater treatment pond of a cassava starch factory in Nanning City, Guangxi province, China [26]. Algal cultures were initially grown in BG-11 medium [27], at 25 ± 1 °C, in a light incubator GDN-260A (Ningbo, China). The light intensity of 90 μmol m^{-2} s^{-1} with a photoperiod cycle of 12:12 h light/dark was provided by LED arrays with 6500 K LED cool daylight above the culture. The culture was manually shaken 2 times a day. At the beginning of experiments, algal cultures in the logarithmic growth phase were collected via centrifugation at 4000× *g* for 10 min and washed with distilled water as the inoculum. Subsequently, the resulting pellets were resuspended in a 250 mL flask with 150 mL modulated medium.

The MSGW was provided by the Linghua Group Co. Ltd., located in Jining City, Shandong Province. The wastewater was allowed to settle for a period of 24 h before undergoing two rounds of centrifugation at 4000× *g* for 5 min each, and the supernatant was then filtered through 0.45 μm membrane and stored at −20 °C for later use. The initial MSGW characteristics were as follows: COD, 347,562.5 ± 11,226.83 mg L^{-1}; TN, 46,526.32 ± 4211.40 mg L^{-1}; TP, 2756.15 ± 163.77 mg L^{-1}; NH_4^+-N, 16,281.75 ± 567.47 mg L^{-1}; and pH, 4.01 ± 0.01.

2.2. Experimental Design

2.2.1. Screening of MSGW Dilution Factor for Promoting Microalgal Growth

C. sorokiniana GXNN01 was selected as the model for MSGW dilution factor optimization. In order to identify the optimal dilution factor for cultivating microalgae, MSGW were prepared at varying dilution multiples (500, 600, 700, 800, 900, and 1000). Following the aforementioned cell collection protocol, the resulting cells were introduced into 250 mL Erlenmeyer flasks containing 150 mL of autoclaved MSGW at an initial density of 5×10^5 cells mL^{-1}. The other conditions were given as mentioned above. Each treatment was repeated four times. Cell growth was monitored every 2 days.

2.2.2. Selection of Microalgae Species Suitable for Cultivation in MSGW

According to the above experimental results, the appropriate dilution factor was initially screened. Thereafter, six algae species were selected for the best growth performance in MSGW of 1000-fold dilution. Three media were used: (1) BBM medium [28] as control, (2) MSGW of 1000-fold dilution (1000MSGW), and (3) MSGW of 1000-fold dilution with BBM trace metals solution (1000MSGW + TMs). The cells were cultured in autoclaved media with an initial density of 1×10^5 cells mL^{-1} and pH adjusted to 7.2. The other conditions were given as mentioned above. For each algal species, four parallel samples were established. Cell growth was monitored every 2 days.

The COD, TN, TP, and NH_4^+-N concentrations were determined before and after the experiments.

2.2.3. Batch Experiments for Optimization of Cultivation Condition

To investigate the optimum cultivation condition for the selected strain suitable for cultivation in MSGW, a series of batch cultivation experiments were carried out. The effect of inoculation densities, initial Fe^{3+} concentrations, and light intensities on microalgae growth and nutrient removal rate in the 1000MSGW + TMs medium was evaluated by performing cultivation tests under different inoculation densities (5×10^6, 1×10^6,

5×10^5, and 1×10^5 cells mL^{-1}), initial Fe^{3+} concentrations (1×10^{-4}, 1×10^{-5}, 1×10^{-6}, and 1×10^{-7} mol L^{-1} in the form of ferric chloride), and light intensities (20, 50, 100, and 150 μmol m^{-2} s^{-1}). In each cultivation test, one parameter was changed while maintaining the other parameters constant. All the experiments were repeated four times.

2.3. Analytical Methods

2.3.1. Determination of Cell Growth

The collected cultures were observed under a microscope using the Neubauer-improved chamber (Marienfeld, Lauda-Konigshofen, Germany), and the cell growth was recorded. The specific growth rate (μ) during the exponential phase was determined using Equation (1).

$$\mu = (\ln D_2 - \ln D_1)/(t_2 - t_1) \quad (1)$$

where D_1 and D_2 represent the algal density at two distinct cultivation timepoints, t_1 and t_2, respectively.

The biomass concentration was determined by subjecting samples of microalgal cells to washing and subsequent drying in an oven set to 105 °C for 3 h.

2.3.2. Determination of COD, TN, TP, and NH_4^+-N

The cultures (5 mL) were taken at the end of the experiments and centrifuged at 9000× *g* for 10 min. The COD, TN, TP, and NH_4^+-N concentrations of the supernatant were determined according to Chinese national standard, using fast digestion-spectrophotometric method (HJ/T 399-2007), alkaline potassium persulfate digestion UV spectrophotometric method (GB 11894-1989), ammonium molybdate spectrophotometric method (GB 11893-1989), and Nessler's reagent colorimetric method (GB 7479-1987), respectively.

2.4. Statistical Analysis

The mean and standard deviation (SD) of the four replicates were reported as experimental results. The normality and heterogeneity of variances were assessed using the Kolmogorov–Smirnov test and Levene's F-test, respectively. The one-way analysis of variance (ANOVA) was used to determine the significant difference between the means, and then the Duncan multiple-range test was performed at the probability level of $p < 0.05$. All statistical analyses were conducted utilizing IBM SPSS statistics version 20.0 (IBM, Chicago, IL, USA).

3. Results

3.1. Microalgae Species Screening Based on Growth Performance and Removal of Nutrients

The growth curve of *C. sorokiniana* GXNN-01 in diluted MSGW is shown in Figure 1. The results showed that *C. sorokiniana* GXNN-01 grew better with the increasing dilution multiples, and the best growth performance was obtained at the dilution multiples of 1000. At the end of cultivation, the cell density of 1000-dilution multiples increased to 7.78×10^6 cells mL^{-1}, which had no significant difference from that of 900-dilution multiples ($p > 0.05$; F value = 31.262). Moreover, the cell densities of other MSGW dilution multiples were low, which indicated that these dilution multiples containing a high concentration of nutrient salts had a certain inhibition effect on the growth of the alga.

Based on the above findings, the growth performance of six microalgae strains in MSGW of 1000-dilution multiples was further explored (Figure 2). According to the growth performance of six strains in different media, four groups can be divided. Group I including FACHB-9 and FACHB-30 showed a growth trend of 1000MSGW + TMs > 1000MSGW > BBM. Group II including GXNN-01 and FACHB-275 showed a growth trend of 1000MSGW + TMs ≈ 1000MSGW > BBM. Group III represented by FACHB-479 had no significant differences among the three media. Group IV represented by FACHB-416 showed a growth trend of 1000MSGW + TMs > BBM > 1000MSGW. FACHB-9 obtained the highest cell density of 2.42×10^7 cells mL^{-1} after 20 days of culture in the 1000MSGW + TMs. The specific

growth rate (μ) of six strains grown in different media is compared (Table 1). The values of 0.27 d^{-1} and 0.28 d^{-1} appeared in the FACHB-9 and FACHB-30 groups grown in the 1000MSGW + TMs, respectively, which were significantly higher than other groups.

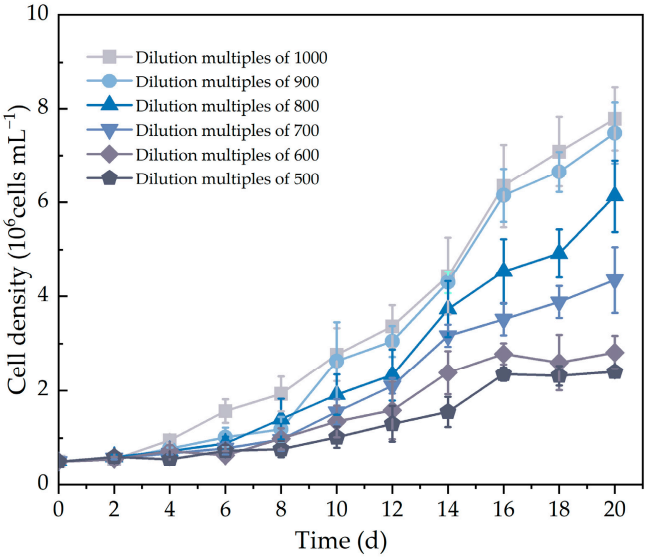

Figure 1. The growth curves of *Chlorella sorokiniana* GXNN-01 at different MSGW dilution multiples.

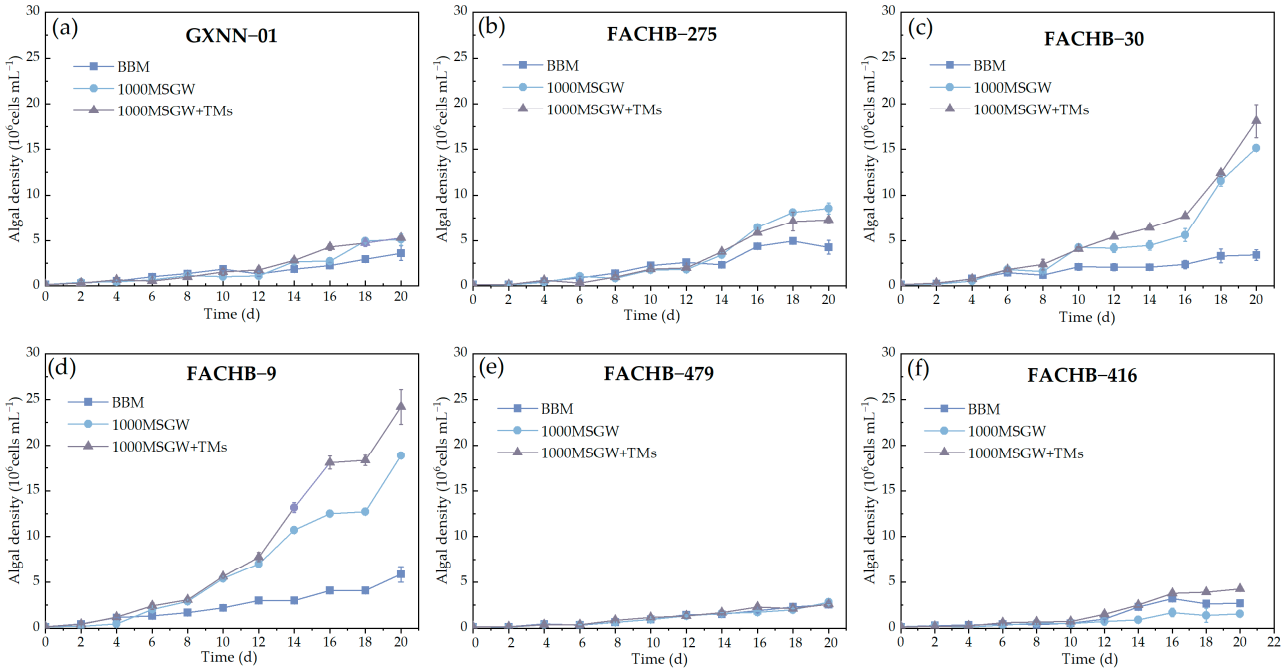

Figure 2. Growth curves of six microalgae cultured in three media during 20 days: (**a**) *Chlorella sorokiniana* GXNN-01, (**b**) *Chlorella sorokiniana* FACHB-275, (**c**) *Chlorella* sp. FACHB-30, (**d**) *Chlorella* sp. FACHB-9, (**e**) *Chlamydomonas reinhardtii* FACHB-479, and (**f**) *Tetradesmus obliquus* FACHB-416.

Table 1. The specific growth rates (d^{-1}) of six microalgae on the 20th day compared with the first day when growing in the BBM, 1000MSGW, and 1000MSGW + TMs. Values (mean ± SD of four replicates) in the same row with different lowercase letters indicate significant differences ($p < 0.05$).

Specific Growth Rate (d^{-1})	Microalgae Species					
	***Chlorella sorokiniana* GXNN-01**	***Chlorella sorokiniana* FACHB-275**	***Chlorella* sp. FACHB-30**	***Chlorella* sp. FACHB-9**	***Chlamydomonas reinhardtii* FACHB-479**	***Tetradesmus obliquus* FACHB-416**
BBM	0.1756 ± 0.0129 bc	0.1852 ± 0.0102 ab	0.1728 ± 0.0099 bcd	0.2031 ± 0.0083 a	0.1572 ± 0.0057 d	0.1604 ± 0.0054 cd
1000MSGW	0.1956 ± 0.0068 d	0.2245 ± 0.0039 c	0.2564 ± 0.0005 b	0.2685 ± 0.0007 a	0.1625 ± 0.0045 e	0.1297 ± 0.0023 f
1000MSGW + TMs	0.1899 ± 0.0071 c	0.2155 ± 0.0019 b	0.2660 ± 0.0046 a	0.2823 ± 0.0035 a	0.1609 ± 0.0138 d	0.1857 ± 0.0028 c

Figure 3a shows that the removal rate of TP by FACHB-30 was significantly higher than those of the other five species. However, the TP removal rate of FACHB-30 without TMs (56.46 ± 2.92%) was significantly lower than that of the TMs group (69.09 ± 2.57%) ($p < 0.05$). The removal efficiencies of TN and NH_4^+-N by FACHB-9 and FACHB-30 with or without TMs were significantly higher than those of the other species ($p < 0.05$). The removal rate of TN by FACHB-9 (30.02 ± 1.12%) was significantly higher than that by FACHB-30 (26.93 ± 0.82%) ($p < 0.05$). In terms of NH_4^+-N, the removal efficiencies of FACHB-9 and FACHB-30 grown in the 1000MSGW + TMs had no significant differences ($p > 0.05$) and were significantly higher than those of the other species ($p < 0.05$). The removal efficiency of COD by FACHB-30 (61.50 ± 3.75%) grown in the 1000MSGW + TMs was significantly higher than those by the other species ($p < 0.05$).

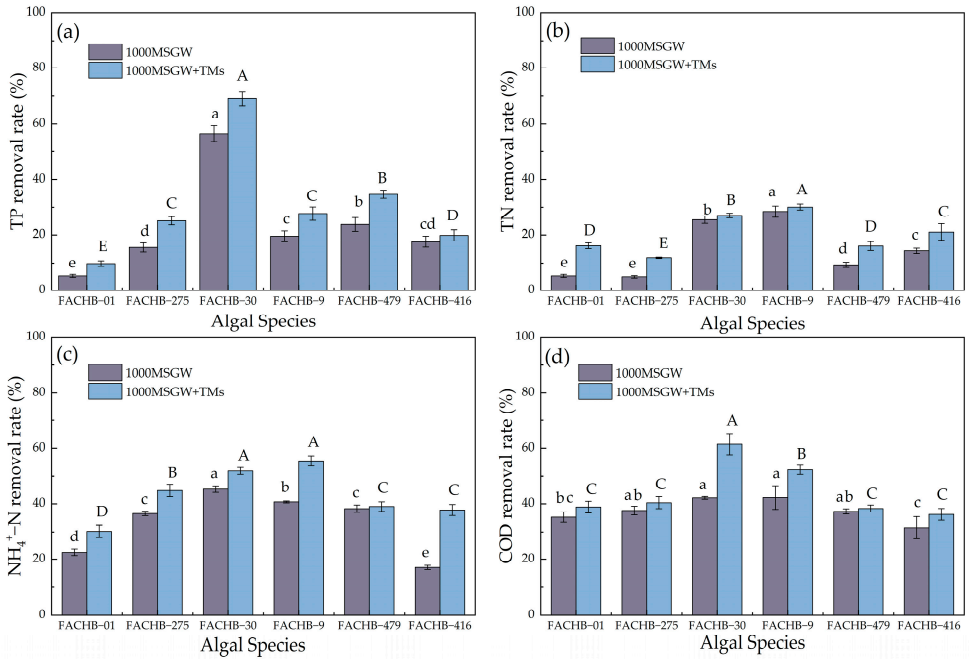

Figure 3. Nutrients removal rates of six microalgae after 20 days of culture: (**a**) total phosphorus, (**b**) total nitrogen, (**c**) ammonia nitrogen, and (**d**) COD. The bars (mean ± SD of four replicates) with different letters (uppercase for 1000MSGW + TMs and lowercase for 1000MSGW) are significantly different ($p < 0.05$).

Wastewater treatment should compromise the specific growth rate and nutrient removal rate. According to the above results, FACHB-30 was selected as the dominant algal species in MSGW treatment, which was further investigated in this study.

3.2. Growth of FACHB-30 under Different Light Intensities

The final cell densities of FACHB-30 at 150 and 100 μmol $m^{-2}s^{-1}$ up to 2.07 × 10^7 cells mL^{-1} and 1.95 × 10^7 cells mL^{-1} were significantly higher than those under low light intensities ($p < 0.05$, Figure 4). Meanwhile, it can be seen from Figure 5 that the removal efficiencies of nutrients were correlated with the values of cell intensities. The highest nutrients removal rates in the four experimental groups all appeared in the light intensity of 150 μmol m^{-2} s^{-1}. However, there were no significant differences in the removal rates of TP, TN, and NH_4^+-N between the group of 150 and 100 μmol m^{-2} s^{-1} ($p > 0.05$).

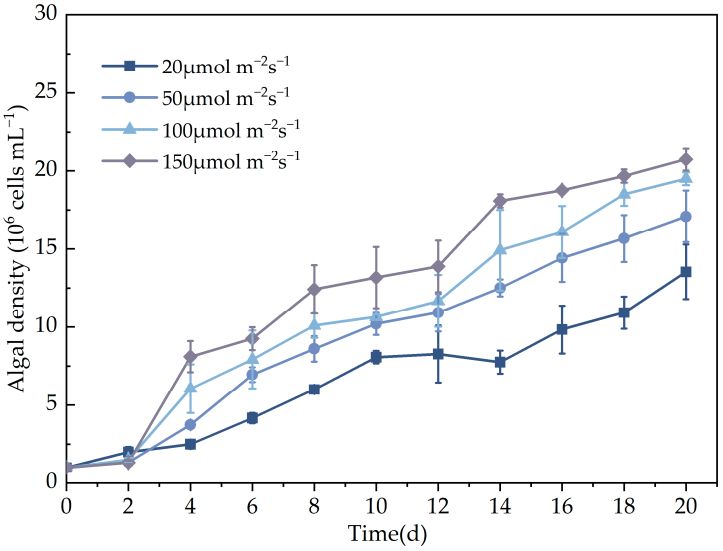

Figure 4. Growth curves during 20 days of culture under different light intensities.

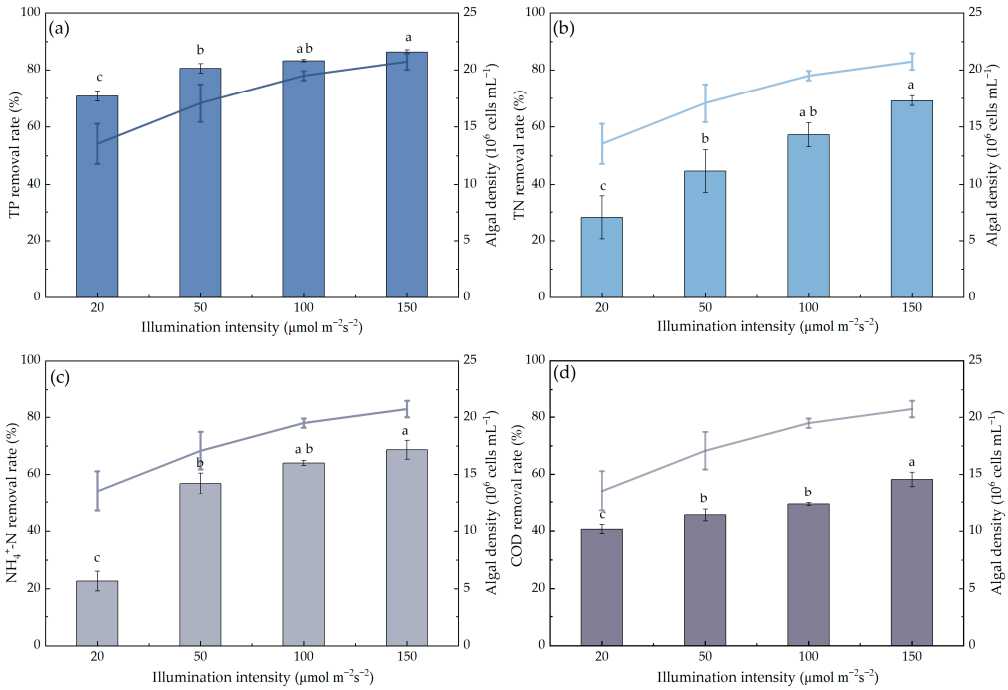

Figure 5. Nutrient removal rates after 20 days of culture under different light intensities: (**a**) total phosphorus, (**b**) total nitrogen, (**c**) ammonia nitrogen, and (**d**) COD. The bars (mean ± SD of four replicates) with different letters are significantly different ($p < 0.05$).

3.3. Growth of FACHB-30 under Different Initial Inoculum Concentrations

There were no significant differences among the final cell densities among the groups of 5×10^5, 1×10^6, and 5×10^6 cells mL^{-1} ($p > 0.05$). However, these three groups were all significantly higher than the group of 1×10^5 ($p < 0.05$, Figure 6a). When compared to the specific growth rate, the group of 1×10^5 was significantly higher than other groups ($p < 0.05$, Figure 6b). The nutrient removal rates did not show a rising trend with increasing initial inoculum concentrations and final cell intensities. The NH_4^+-N and TP removal rates of 5×10^6 were significantly lower than those of other groups ($p < 0.05$, Figure 7a,c).

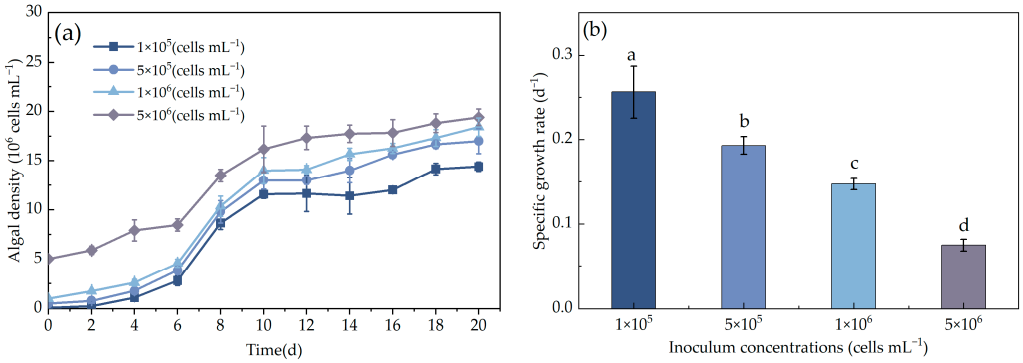

Figure 6. (**a**) Growth curves during 20 days of culture under different inoculum concentrations and (**b**) specific growth rates on the 20th day compared with the first day with different inoculum concentrations. The bars (mean ± SD of four replicates) with different letters are significantly different ($p < 0.05$).

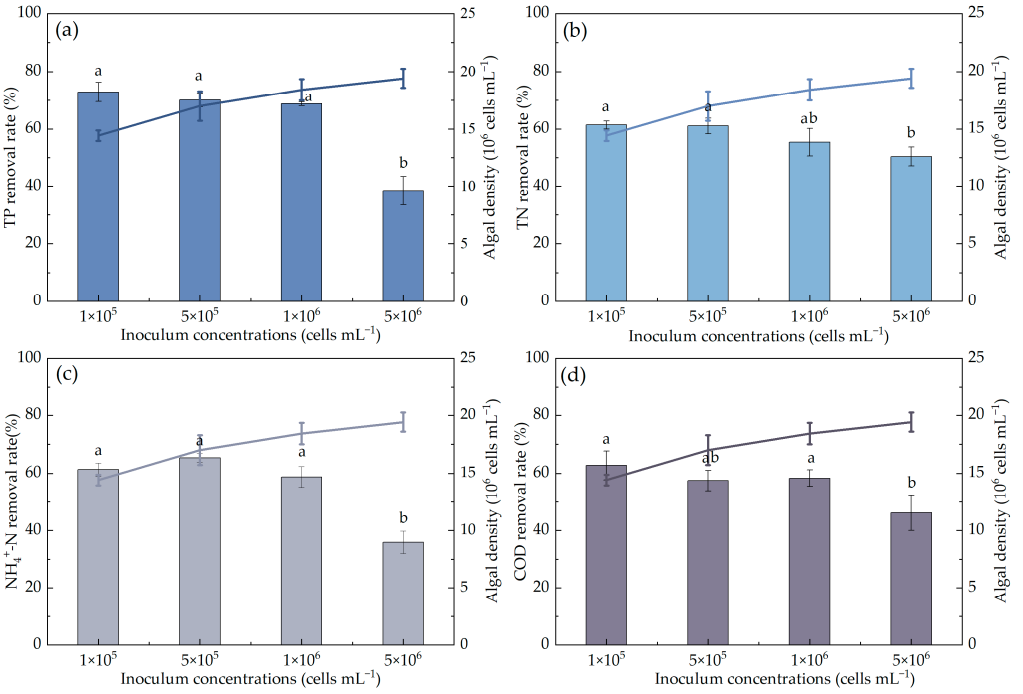

Figure 7. Nutrients removal rates under different initial inoculum conditions: (**a**) total phosphorus, (**b**) total nitrogen, (**c**) ammonia nitrogen, and (**d**) COD. The bars (mean ± SD of four replicate) with different letters are significantly different ($p < 0.05$).

3.4. Growth of FACHB-30 under Different Fe^{3+} Concentrations

As indicated in Figure 8, the growth curves of FACHB-30 were affected by the Fe^{3+} concentrations in MSGW. The optimum Fe^{3+} concentration for cell growth was

10^{-5} mol L^{-1}. The nutrients removal rates coincided well with the change in final cell densities (Figure 9). However, only NH_4^+-N removal rate of 10^{-5} mol L^{-1} was significantly higher than those of other concentrations ($p < 0.05$).

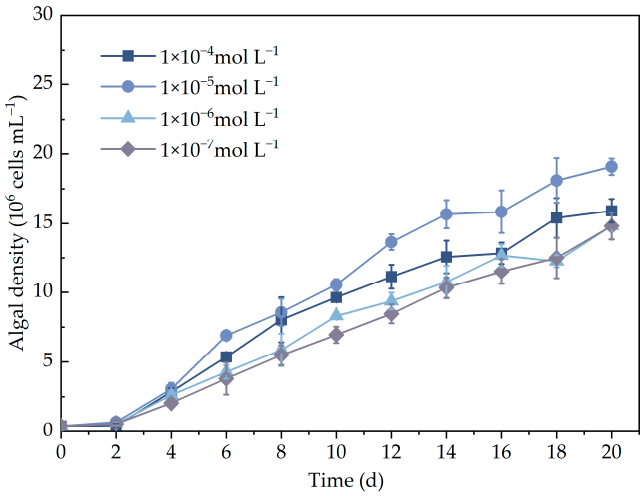

Figure 8. Growth curves during 20 days of culture under different Fe^{3+} concentrations.

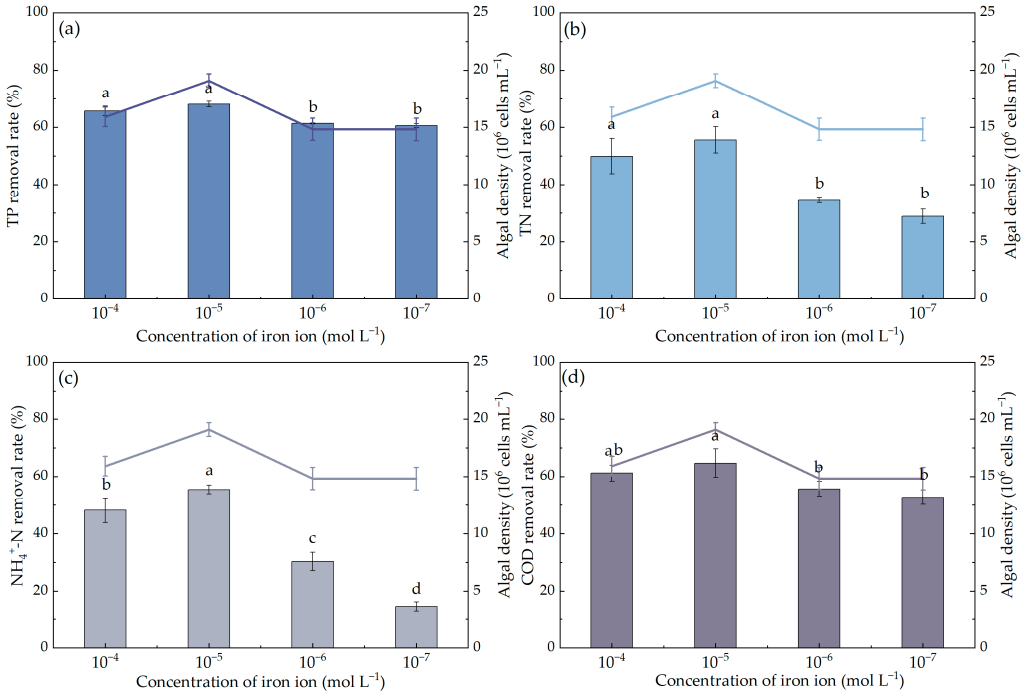

Figure 9. Nutrients removal rates under different Fe^{3+} concentration conditions: (**a**) total phosphorus, (**b**) total nitrogen, (**c**) ammonia nitrogen, and (**d**) COD. The bars (mean ± SD of four replicates) with different letters are significantly different ($p < 0.05$).

3.5. Comparison of the Nutrient Removal Rate and Biomass Production before and after Optimization

After the optimization of light intensity, iron concentration, and initial inoculum, the removal rates of TP, TN, NH_4^+-N, and COD were significantly increased from 69.10 ± 2.57% to 87.60 ± 1.50%, 26.93 ± 0.82% to 68.05 ± 3.89%, 51.91 ± 1.28% to 75.89 ± 1.82%, and 61.50 ± 3.75% to 77.96 ± 2.95% ($p < 0.01$, Table 2), respectively. The removal rate of TP after optimization was still the highest among the four parameters. However, the highest increasing rate of TN removal rate up to 152.69% was observed

among the nutrients determined. The final biomass concentration was also significantly increased by 18.98% ($p < 0.05$) and reached 1.423 ± 0.018 g L^{-1}.

Table 2. Comparison of removal rate and biomass concentration before and after optimization. Values (mean ± SD of four replicates) within the same row with asterisk labeled are significantly different (the Student *t* test, ** $p < 0.01$; * $p < 0.05$).

Parameters	Before Optimization	After Optimization	Increasing Rate
The removal rate of TP (%)	69.10 ± 2.57 **	87.60 ± 1.50 **	26.77%
The removal rate of TN (%)	26.93 ± 0.82 **	68.05 ± 3.89 **	152.69%
The removal rate of NH_4^+-N (%)	51.91 ± 1.28 **	75.89 ± 1.82 **	46.19%
The removal rate of COD (%)	61.50 ± 3.75 **	77.96 ± 2.95 **	26.76%
Biomass concentration (g L^{-1})	1.196 ± 0.087 *	1.423 ± 0.018 *	18.98%

4. Discussion

4.1. The Selecting of the Tolerant Strains Grown in the MSGW

Microalgae can grow rapidly under favorable culture conditions with sufficient nutrients. As shown in Figure 1, MSGW contains macronutrients such as nitrogen and phosphorus that are essential for algal growth; however, high levels of nutrient salts in the MSGW diluted 500–800 times prevented cell reproduction. It is likely due to the high concentration of wastewater containing high levels of organic or inorganic compounds, which were toxic to microorganisms [29,30]. Jiang et al. [14] speculated that the stress caused by the high initial concentration of ammonia (≥20 mg L^{-1} in MSGW diluted 500–800 times in the present study) may be the main reason for the cell growth inhibition [22]. The final cell density of the 1000-times dilution group was higher than that of the 900-times dilution group, although there were no significant differences, indicating that both dilution factors were suitable for successive optimization. For ease of operation, a 1000-times dilution was selected for the screening of the tolerant strains grown in the MSGW.

Different levels of tolerance to the MSGW were observed among the six strains. The growth rates of most *Chlorella* strains were higher than *Chlamydomonas* and *Tetradesmus* strains (Table 1). *Chlorella* is commonly used for microalgae-based wastewater treatment due to its high adaptability to different types of wastewaters [31–33]. Furthermore, *Chlorella* sp. FACHB-9 and FACHB-30 performed best among the *Chlorella* strains. Liu et al. [34] also reported the different growth performances of *Chlorella* strains in swine wastewater. One possible reason is that the traditional *Chlorella* taxa are dispersed over two classes of chlorophytes, the *Trebouxiophyceae* and the *Chlorophyceae*, and certain heterogeneity is observed in the rRNA similarity and biochemical and physiological properties even in the same species [35].

The MSGW plus TMs medium resulted in better growth performance than the MGSW or BBM medium in FACHB-9 and FACHB-30 (Figure 2), which suggested the diluted MSGW did not have enough TMs for the algae to grow. Even though trace metal elements of various kinds are present in MSGW, the concentration of cobalt (Co^{2+}), molybdenum (Mo^{2+}), and manganese (Mn^{2+}) in MSGW diluted 1000 times would be very low, up to 10^{-9} mg L^{-1} [36]. However, Co^{2+} and Mo^{2+} at a concentration of 10^{-3} mg L^{-1} and Mn^{2+} at a concentration of 10^{-2} mg L^{-1} were found to be necessary for the best growth of algae [37,38]. Other reports about wastewater treatment with microalgae also emphasized the importance of the TMs addition to the medium [39,40].

4.2. Optimizing the Conditions for the Maximum Biomass Production and Nutrient Removal Rates

4.2.1. Light Intensity

Light intensity and biomass increase had a positive correlation in FACHB-30 (Figure 4). Generally, there is a certain level of light that is best for microalgae to grow [41]. The optimal light level for microalgae growth can vary in wastewater since it has low light penetration and harmful substances. Kiran and Mohan [42] reported an optimum light

intensity of 100 μmol $m^{-2}s^{-1}$ when using *C. sorokiniana* to treat dairy wastewater, which was similar to the present study of 100–150 μmol $m^{-2}s^{-1}$. However, Qu et al. [43] found that the best light intensity for growing *Parachlorella kessleri* in real swine wastewater was 600 μmol $m^{-2}s^{-1}$.

There was also a positive correlation between light intensity and nutrient removal rate. However, low light intensity (20 μmol m^{-2} s^{-1}) reduced the removal rates of TN and NH_4^+-N more than those of TP and COD compared to high light intensity (Figure 5). The reason may be that high light intensity induced photosynthesis-related protein synthesis, and more nitrogen is required under high light [44,45].

4.2.2. Initial Inoculum Concentration

Although the groups with high inoculum concentration had the advantage in the final cell density, the inoculum concentration of 1×10^5 cells mL^{-1} was the highest from the perspective of specific growth rate (Figure 6), which may be due to the competition between light and nutrients among cells with the increase in inoculum concentration. Studies have shown that the biomass tended to decline when the inoculum concentration exceeded a certain value [46]. In this study, it was also observed that the inoculum of 5×10^6 cells mL^{-1} had yellowing and precipitation in the process of culture. In terms of nutrient removal rate, when the inoculum concentration reached 5×10^6 cells mL^{-1}, the removal rate did not increase significantly with the increase in the inoculum concentration (Figure 7). A high inoculum concentration may shorten the lag phase and accelerate the growth of microalgae in MSGW, but it may also reduce light availability and photosynthesis due to cell shading. This is consistent with the nutrient removal rate that depends on light intensity as indicated by Figure 5. Wang et al. [47] and Lao et al. [48] also reported that the nutrient removal rate did not increase with the increasing inoculum concentration in the wastewater.

4.2.3. Fe^{3+} Concentrations

Iron plays an important role in regulating the growth and community composition of phytoplankton. It is ferric iron that is absorbed and utilized by algae. In the laboratory culture experiment, Park et al. found that iron could promote the growth rate of marine *Chlorella*, and the content of intracellular chlorophyll was also increased. Studies have shown that with the increase in iron concentration, algae will show a rapid growth trend and also increase the absorption of N and P [49]. High iron concentration enhances algal photosynthesis by increasing the synthesis of proteins and enzymes related to the light reaction and Calvin cycle, which results in more ATP and NADPH production and a higher carbon fixation rate. However, compared with the Fe^{3+} concentration of 10^{-4} mol L^{-1}, FACHB-30 at 10^{-5} mol L^{-1} had the highest final cell density and nutrient removal rate among the four groups (Figures 8 and 9), probably because excessive iron would produce a large amount of reactive oxygen species [50], which caused oxidative damage to the photosynthetic membrane of the cells [51,52]. The optimal Fe^{3+} concentration for maximizing biomass production of *C. pyrenoidosa* during municipal wastewater treatment was determined to be 2.88×10^{-5} mol L^{-1} [53]. The expression levels of accD and rbcL genes, encoding acetyl-CoA carboxylase (ACC) and ribulose bisphosphate carboxylase large chain (RuBisCO), respectively, reached the highest level in *C. vulgaris* under the iron concentration of 9×10^{-5} mol L^{-1} [54].

The nutrient removal rate also correlated well with the iron concentration. However, low iron concentration (10^{-7} mol L^{-1}) reduced the removal rates of TN and NH_4^+-N more than those of TP and COD compared to high iron concentration (Figure 9). The reason may be the same as the light intensity as mentioned above. Because high iron concentration also induces photosynthesis-related protein synthesis [54], more nitrogen is required for the operation of photosynthesis.

4.3. Comparison of Other Research for the MSGW Treatment

The optimization of light intensity, iron concentration, and initial inoculum resulted in a 27% to 153% increase in the removal rates of all nutrients, as illustrated in Table 2. The most significant improvement after optimization was observed in the TN removal rate, with the optimization of light conditions being the primary contributing factor (Figure 5). This finding is consistent with the report of Rani and Maróti [55], who demonstrated that the nitrate removal of green microalgae from synthetic wastewater was dependent on light color as well as light intensity. After optimization, the maximum biomass could reach 1.423 g L^{-1}, which is comparable to the highest biomass reported in many studies about the MSGW treatment. The cultivation of *P. tricornutum* with MSGW mixed in seawater resulted in the highest biomass of 0.93 g L^{-1} [20]. Jiang et al. [22] achieved the highest biomass of 2.862 g L^{-1} when treating MSGW with *S. subsalsa*, while they reported a maximum biomass of 1.46 g L^{-1} when using *C. vulgaris* in a separate study [21]. In short, further optimization of conditions still has the potential to increase the current biomass production.

5. Conclusions

Based on the criteria of growth and nutrient removal, *Chlorella* sp. FACHB-30 was identified as the most suitable strain for the MSGW treatment among the screened candidates. The optimal MSGW purification effect of microalgae was observed when the light intensity reached 150 μmol m^{-2} s^{-1}, the inoculum concentration started at 1×10^5 cells mL^{-1}, and the initial Fe^{3+} concentration in the wastewater was 10^{-5} mol L^{-1}. Through conditions optimization, the removal rates of TP, TN, NH_4^+-N, and COD increased from 69.10% to 87.60%, 26.93% to 68.05%, 51.91% to 75.89%, and 61.50% to 77.96%, respectively. The present study demonstrates that the nutrient removal efficiency of microalgae-based wastewater treatment can be significantly improved by applying a combined strategy of selecting tolerant strains, improving wastewater composition, and optimizing cultivation conditions. The findings of this study can serve as a valuable reference for future research and development aimed at enhancing the efficacy of microalgae-based treatment of MSGW.

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