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Comparison of oleaginous microalgal growth and lipid accumulation in saline-alkali leachate: a case from Shandong Province

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ABSTRACT

In this study, the feasibility of using the saline-alkali leachate from Shandong Province to culture three oleaginous microalgae (*Chlorella* sp. HQ, *Chlorella vulgaris*, and *Scenedesmus* sp. LX1) for biomass and lipid production were explored. The results showed that all the three microalgae could grow in the leachates, among which, *Chlorella* sp. HQ kept the maximal density of 1.65 ± 0.05 × 10^7 cells mL^{-1}, largest lipid yield (69.90 ± 6.89 mg L^{-1}) and content (50.53 ± 5.00%) after 25 d of cultivation. During the cultivation, the total organic carbon (TOC), inorganic carbon (IC), total nitrogen, and salinity of the leachates decreased in different degrees. The carbon source utilized by microalgae was mainly IC but not TOC, and the IC removal rate by *Chlorella* sp. HQ was the highest, reaching 89.85 ± 5.48%. The application potential of using saline-alkali leachates for *Chlorella* sp. HQ cultivation was investigated. The results showed that the pH values of five kinds of water [including *L*1 (salinity of 0.18%), *L*2 (salinity of 0.25%), tap water, reclaimed water, and SE standard medium] gradually increased during the cultivation and finally were stable at around eight. After 45 d of cultivation, the order of algal density was *L*2 (19.13 ± 0.70 × 10^7 cells mL^{-1}) > *L*1 (13.55 ± 0.46 × 10^7 cells mL^{-1}) > SE medium (10.99 ± 0.03 × 10^7 cells mL^{-1}) > reclaimed water (5.45 ± 0.46 × 10^7 cells mL^{-1}) > tap water (4.08 ± 0.33 × 10^7 cells mL^{-1}). Compared with SE medium, the lipid yield, and content of *Chlorella* in the saline-alkali leachates were higher, but lower than those of tap water and reclaimed water, which may be due to nutrient deficiency of the latter two. Based on the comprehensive comparison, *Chlorella* sp. HQ was more suitable to cultivate in leachate in the view of not only biomass but also lipid content. Hence, using saline-alkali leachate for the cultivation of oleaginous microalgae is a promising alternative not only to purify leachates but also to serve as the large-scale applications for cost-saving.

Keywords: Oleaginous microalgae, Saline-alkali leachate, Algal density, Biomass, Lipid accumulation

1. Introduction

Soil salinization has existed around the world for many years and has been considered as an environmental problem that needed to be paid attention to and resolved in recent years [1,2]. According to the year 2015 statistics data from the Agriculture Department of China, there were appoximately 340,000 km^2 of saline-alkali soils in China, of which around 124,000 km^2 had the potential for use in agricultural production after remediation [3]. Soil salinization affects soil properties, reduces soil productivity, causes ecosystem changes via plant growth and distribution, and ultimately leads to the consequences of vegetation degradation [4–6].

At present, the main methods of treating the saline-alkali soils mainly include bioremediation technology, chemical modification technology, and water conservancy technology [7]. It is reported that the addition of organic amendments

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to saline-alkali soil made it improved [8]. López-Valdez et al. [9] added sewage sludge to Texcoco soil (an alkaline saline soil) and found that the concentrations of NH_4^+ and NO_3^- decreased, which was beneficial to the vegetation of the Texcoco soil. Wang et al. [10] used jute straw and organic fertilizer to improve the quality of the coastal saline soil, and the results showed that soil enzyme activity and soil microbes increased, soil biological activity, and soil fertility were enhanced. Besides, the use of flue gas desulfurization gypsum for improving saline-alkali soils is deemed promising [11,12]. However, these methods are still far from cost-effectiveness and take a long time to achieve the desired results. In China, the most commonly used technology is a combination of irrigation and drainage to remove salts by leaching, which is also widely used in the world [7]. Whereas, this method requires a large amount of agricultural irrigation water, resulting in the problem of effluents with high salinity and even some organics (called saline-alkali leachate), which requires further treatment with cost increasing.

Microalgae are considered as promising raw materials for biodiesel production owing to their ability to fix CO_2, rapid growth rate, and high lipid productivity [13]. High biomass production is the basis for the practical use of microalgal energy. Some microalgae have high levels of lipids and triacylglycerols (TAGs), which can be used as a kind of ideal raw material for the production of biodiesel. Besides, a large amount of protein, carbohydrates, and other nutrients contained in microalgae can be used as animal feed [14,15]. The researchers have found that most microalgae species can absorb nutrients from wastewater to obtain high biomass while purifying wastewater [16–18]. Gupta et al. [19] found that *C. vulgaris* cultivated in 5 g L^{-1} glucose supplementation of domestic wastewater can obtain high biomass productivity and lipid yield, the total nitrogen (TN) and total phosphate (TP) removal rates were 65.3% and 71.2%, respectively. When *C. vulgaris* was cultured in real centrate wastewater, high lipid content and biomass productivity were obtained, and the removal rates of NH_4^+–N, TN, and TP exceeded 86% [20]. It has been reported that microalgae under stress conditions including nutrient limitations, high oxygen supply, and high salinity can increase the lipid accumulation [14,21,22]. Chokshi et al. [23] cultured *Acutodesmus dimorphus* in BG-11 medium containing NaCl solution and found that the accumulation of lipids increased with the initial salinity of the medium. In view of the above, the saline-alkali leachate contains a large amount of nutrients and trace elements that necessary for the growth of microalgae, and its high salinity may be conducive to the algal lipid accumulation. Therefore, the cultivation of microalgae by using saline-alkali leachate might be a promising method, which could not only reduce the cost of sewage treatment and microalgae cultivation but also improve the utilization efficiency of wastewater.

Based on the hypothesis mentioned above, the growth and lipid production of three oleaginous microalgae (*Chlorella* sp. HQ, *Scenedesmus* sp. LX1, and *C. vulgaris*) cultivated in saline-alkali leachate were explored to evaluate its feasibility for microalgae cultivation. Furthermore, the optimal species were selected to be cultivated in the leachates with two different salinities (*L*1: 0.18% and *L*2: 0.25%) and three controls (including tap water, reclaimed water, and SE standard medium) to characterize its growth and lipid accumulation. This study will provide a theoretical and data basis for future utilization of saline-alkali leachate in coupling microalgae production and post-treatment of irrigation and drainage of saline-alkali soils.

2. Materials and methods

2.1. Microalgae and culture medium

The microalga *Chlorella* sp. HQ (Collection No. GCMCC7601 in the China General Microbiological Culture Collection Center) was used in this study, which was isolated in our previous study. *Scenedesmus* sp. LX1 (Collection No. GCMCC3036 in the China General Microbiological Culture Center) was inoculated by the biology laboratory of College of Environment at Tsinghua University. And *C. vulgaris* was purchased in Freshwater Algae Culture Collection at the Institute of Hydrobiology, FACHB-collection. All the microalgae were cultivated in SE medium for subsequent experiments. The ingredients of the SE medium are 250 mg L^{-1} $NaNO_3$, 75 mg L^{-1} $K_2HPO_4{\cdot}3H_2O$, 75 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 25 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 175 mg L^{-1} KH_2PO_4, 25 mg L^{-1} NaCl, 5 mg L^{-1} $FeCl_3{\cdot}6H_2O$, 0.81 mg L^{-1} $FeCl_3$, 10 mg L^{-1} Na_2EDTA, 2.86 mg L^{-1} H_3BO_3, 1.81 mg L^{-1} $MnCl_2{\cdot}4H_2O$, 0.22 mg L^{-1} $ZnSO_4{\cdot}H_2O$, 0.079 mg L^{-1} $CuSO_4{\cdot}5H_2O$, and 0.039 mg L^{-1} $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$. The experimental conditions of the microalgae cultivation were light intensity of 60 μmol photons m^{-2} s^{-1}, the temperature of 25°C, and the light-dark ratio of 14:10 h.

2.2. Saline-alkali soil samples, reclaimed water sample, and saline-alkali leachate

The saline-alkali soil samples were taken from Dongying, Shandong Province, China. The upper (0–20 cm), middle (20–40 cm), and bottom (40–60 cm) layers of soil were collected and stored in layers. Besides, the reclaimed water was taken from the terminal water of the Beixiaohe Wastewater Treatment Plant in Beijing. The preparation method of saline-alkali leachate was as follows: the bottom, middle, and upper layer of soil samples were placed in plastic pots (100 × 92 × 70 mm) from bottom to top in the order, and each layer was evenly spread. Then, it was rinsed with reclaimed water to obtain the leachate. Finally, the leachate was autoclaved at 121°C for 30 min and cooled to room temperature for *Chlorella* culture. The leachate contains some nitrogen, phosphorus, and trace metal elements (data are illustrated in Table S1).

2.3. Experiment design

2.3.1. Feasibility of cultivating microalgae by saline-alkali leachate

Three microalgae *Chlorella vulgaris*, *Chlorella* sp. HQ, and *Scenedesmus* sp. LX1 were inoculated to the saline-alkali leachate (pH: 7.73; salinity: 0.28%) with an initial density of 2×10^5 cells mL^{-1} for 25 d of cultivation. The algal density was measured every 2 d; the pH, salinity, total organic carbon (TOC), inorganic carbon (IC), and TN of the leachate were

measured every 3 d; the biomass, lipids, and TAGs of the microalgae were measured at days of 25. Each group was conducted in triplicate.

2.3.2. Characterization comparison of Chlorella sp. HQ cultivated in five water samples

Five groups of water samples (tap water, reclaimed water, SE medium, *L*1: salinity 0.18% and *L*2: salinity 0.25%) were set up to culture *Chlorella* sp. HQ with an initial density of 2×10^5 cells mL^{-1} for 45 d. The microalgal density was measured every 2 d; the pH was measured at the initial period, and at days 25 and 45 of the cultivation period; the biomass, lipids, and TAGs of the microalgal cells were measured at days of 45. Each group was conducted in triplicate. The initial pH and salinity of five water samples are shown in Table 1.

2.4. Analytical methods

2.4.1. Microalgal growth analysis

Microalgal density was measured by counting the number of cells using the hemocytometer. Algal biomass was determined as follows: 20 mL of algae solution was taken and filtered using a sand core filter equipped with a pre-weighed 0.45 μm membrane. After filtration, the membrane was dried in an oven (MOV-112F, SANYO, Japan) at 110°C for 24 h and weighed again, and then the microalgal biomass (dry weight) was calculated.

2.4.2. Lipid extraction and TAGs analysis

The determination of lipid and TAGs were performed using the chloroform-methanol extraction method [24] and the enzyme colorimetric kit method (A110-1 GPO-PAP M Enzyme Method Single Reagent Type), respectively. The detailed steps were as follows: the tested samples were placed in 50 mL centrifuge tubes, the operating conditions of the high-speed refrigerated centrifuge (CR22G, HITACHI, Japan) were adjusted to the rotation speed of 12,000 rpm and a temperature of 4°C for 10 min, and the mixture was concentrated to 0.8 mL. Then, 2 mL of chloroform, 2 mL of methanol, and 1 mL of distilled water were added for extraction. After the extraction, centrifugation was again carried out, and the mixture was divided into three layers. The bottom layer (chloroform layer) was transferred to a pre-weighed glass tube and blown to a constant weight with a nitrogen blower (DC-12, ANPEL, China). Finally, the microalgal lipid was quantified gravimetrically. When the measurement of algal lipid was completed, 0.4 mL of isopropyl alcohol was added into the glass tube, and the TAGs were measured by using the enzymatic method [25].

Table 1
The initial pH and salinity values of five water samples

Parameters	Water samples				
	Reclaimed water	Tap water	*L*1	*L*2	SE medium
pH	7.18	7.07	7.74	7.48	6.17
Salinity	0.02%	0.02%	0.18%	0.25%	0.04%

Note: *L*1: salinity of 0.18%; *L*2: salinity of 0.25%

2.4.3. Chemical analysis

The pH was measured using a pH acidometer (PB-10, Sartorius, Germany). Salinity was measured using a conductivity meter (Raymond DDS-307 conductivity meter, INESA Scientific Instrument Co., China). The algal liquid needs to be pretreated before the measurement of TN, TOC, and IC as described below. First, 20 mL of the culture solution was placed in an Erlenmeyer flask, and the supernatant was obtained by freeze centrifugation (4°C, 12,000 rpm × 10 min), and then filtered through a 0.45 μm water-based filter. Finally, the clear filtrate was obtained for TN, TOC, and IC analysis. Total organic carbon analyzer (TOC-VCPH, SHIMADZU, Japan) was used to determine the concentrations of TN, TOC, and IC. Total carbon (TC) was calculated by adding TOC and IC. TP was determined by Chinese standard testing methods [26]. The soluble sulfate ion and chloride ion were analyzed using a Dionex-4500i ion chromatogram, and several metal contents were determined by an inductively coupled plasma optical emission spectrometry system (Optima 5300DV, PerkinElmer, USA).

3. Results and discussion

3.1. Three oleaginous microalgal growth characteristics in saline-alkali leachate

Three oleaginous microalgae *Chlorella* sp. HQ, *C. vulgaris*, and *Scenedesmus* sp. LX1 were cultivated in saline-alkali leachate, and their growth curves are shown in Fig. 1. It can be seen that all the densities of three microalgae were increased during the cultivation period. The growth rates of *Chlorella* sp. HQ and *C. vulgaris* in the early cultivation period were relatively fast, and at days of 11 the algal density reached $1.43 \pm 0.01 \times 10^7$ and $1.30 \pm 0.23 \times 10^7$ cells mL^{-1},

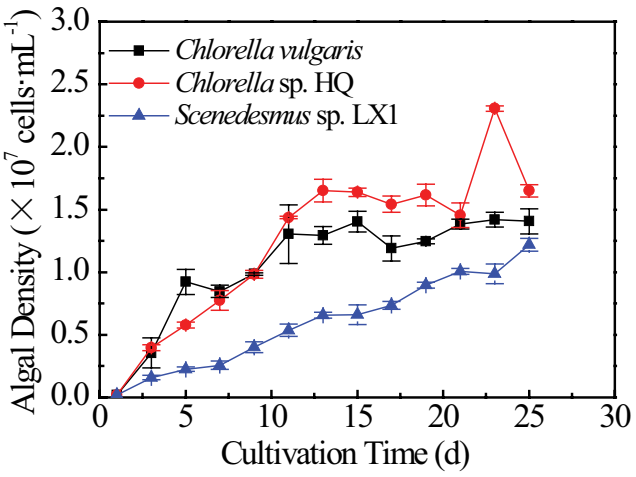

Fig. 1. Growth curves of three microalgae in the saline-alkali leachate.

respectively, while the growth of *Scenedesmus* sp. LX1 was relatively slow. After 25 d of cultivation, the algal density of *Chlorella* sp. HQ, *C. vulgaris*, and *Scenedesmus* sp. LX1 were 1.65 ± 0.05 × 10^7 , 1.41 ± 0.10 × 10^7, and 1.22 ± 0.05 × 10^7 cells mL^{-1}, respectively. However, during the experiment, it was found that *Scenedesmus* sp. LX1 appeared pale yellow and became aggregated to settle on the bottom of the Erlenmeyer flask (shown in Fig. S1), which may be caused by excessive salinity of the leachate. Oxidative stress caused by salinity has been found to reduce the chlorophyll content [27,28]. Therefore, when the salinity of the growth medium exceeds the optimum value of the *Scenedesmus* sp. LX1, the chlorophyll content may be reduced and appeared yellow.

The changing curves of salinity and TN in the saline-alkali leachate are illustrated in Fig. 2. As shown in Fig. 2a, in the first 15 d, the salinity of the culture medium of three microalgae had a different degree of decline. The results showed that the three microalgae could absorb the nutrients from the leachate for their own growth. From the days of 15, the salinity of the three microalgal culture medium increased slightly. This may be due to the decomposition of organic matter in the leachate, especially the carbonate and bicarbonate ions produced by a carbon-fixed process. At the end of cultivation, the salinity of *Scenedesmus* sp. LX1 medium decreased from 0.28% to 0.24%, while the salinities of *C. vulgaris* and *Chlorella* sp. HQ were close to their initial values. As shown in Fig. 2b, the concentrations of TN in the culture medium of three microalgae decreased with the culture time. The TN removal rates of *C. vulgaris*, *Chlorella* sp. HQ and *Scenedesmus* sp. LX1 were 48.42 ± 1.21%, 52.72 ± 0.54%, and 55.44 ± 0.69%, respectively. In summary, each microalgae strain can adapt to its own salinity conditions and utilize nutrients from the saline-alkali leachate to grow.

3.2. The utilization of carbon sources by three microalgae in saline-alkali leachate

Organic carbon is an important nutrient source for cell build-up, which is found in saline-alkali leachate [29]. IC can be used to indicate the balance between photosynthesis and respiration of microalgae [30]. The changes of TOC and IC content in saline-alkali leachate during microalgae cultivation are shown in Fig. 3. The results showed that the carbon

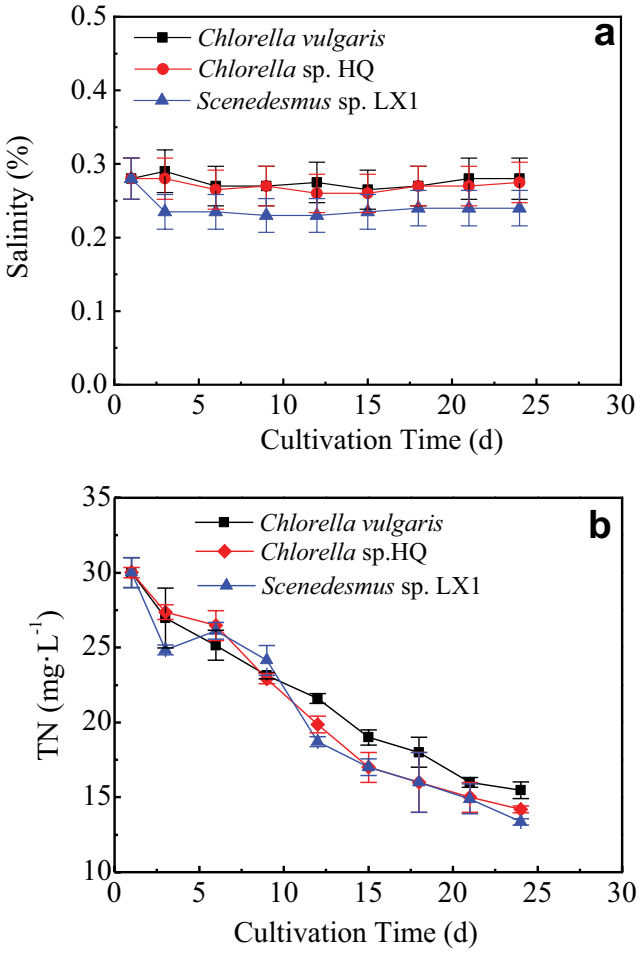

Fig. 2. The changing curves of salinity and TN in the saline-alkali leachate with microalgae cultivation [(a) salinity and (b) TN].

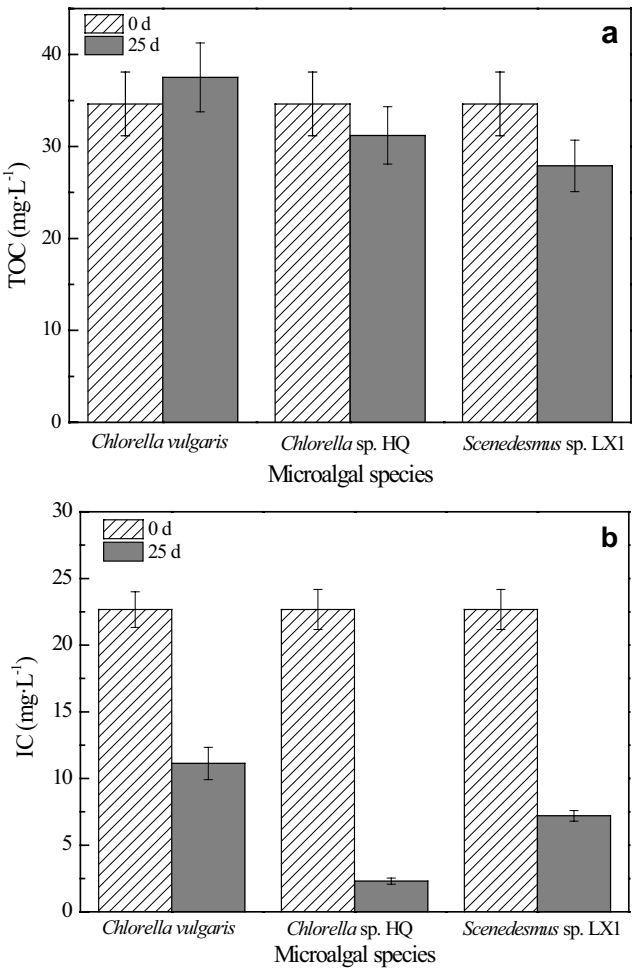

Fig. 3. The changing content of TOC and IC in the saline-alkali leachate with microalgae cultivation [(a) TOC and (b) IC].

source utilized by the three microalgae from saline-alkali leachate was mainly IC. When *C. vulgaris*, *Chlorella* sp. HQ, and *Scenedesmus* sp. LX1 were cultured for 25 d, the IC decreased from 22.67 ± 1.50 mg L^{-1} to 11.13 ± 1.21, 2.30 ± 0.23, and 7.20 ± 0.40 mg L^{-1}, and the corresponding removal rates were 50.90 ± 1.32, 89.85 ± 0.57, and 68.24 ± 0.84%, respectively. As shown in Fig. 3a, after 25 d cultivation of *Chlorella* sp. HQ and *Scenedesmus* sp. LX1 in saline-alkali leachate, the TOC decreased from 34.62 ± 2.93 mg L^{-1} to 31.21 ± 2.90 and 24.55 ± 1.48 mg L^{-1}, respectively. Cao et al. [31] used the acidification of swine wastewater to culture *C. vulgaris* and found that the IC removal efficiency from swine wastewater was higher than that of TOC, indicating that the growth of *C. vulgaris* mainly consumed IC. It is consistent with the results we obtained in the present study. This part of the carbon source is utilized by *C. vulgaris* through obligate photoautotrophic action and is used in the form of soluble carbonate for algal cell growth. Meanwhile, the carbonate is simultaneously converted to free carbon dioxide by direct uptake or by the hydrogen carbonate activity of *Chlorella* [32].

3.3. Microalgal lipid accumulation characteristics in saline-alkali leachate

In the present study, the biomass, lipid, and TAGs accumulation characteristics of three microalgae at the end of cultivation are illustrated in Fig. 4. Of the freshwater algae tested, the maximum biomass was *Scenedesmus* sp. LX1 (165.78 ± 1.49 mg L^{-1}), and the biomass of *Chlorella* sp. HQ was relatively low at 138.34 ± 2.12 mg L^{-1} after 25 d of cultivation. Generally, biomass productivity is rarely related to lipid content, and due to lower growth rates or smaller cell sizes, the biomass productivity is lower even though their lipid content is high [33,34].

In the current study, the maximum lipid yield and content were 69.90 ± 6.89 mg L^{-1} and 50.53% ± 5.00% attained by *Chlorella* sp. HQ, respectively, which were about twice of those attained by *C. vulgaris* and *Scenedesmus* sp. LX1. However, the TAGs yields and contents of the three microalgae were lower, with *Chlorella* sp. HQ being the lowest. This indicates that it may be not conducive to the accumulation of neutral lipids at this salinity. It has been reported that the ability of the microalgae to survive in a salt environment under osmotic stress affected cell growth and lipid formation [35]. Srivastava et al. [28] investigated that salts such as NaCl, KCl, $MgCl_2$, and $CaCl_2$ could improve lipid accumulation of *Chlorella sorokiniana CG12* and *Desmodesmus GS12*, and KCl and $MgCl_2$ contribute to the induction of polar lipid, while NaCl and $CaCl_2$ contribute to neutral lipid production.

The comprehensive comparison suggested that all three microalgae could grow in the leachate and accumulate a certain amount of biomass and lipid, which proved that it is feasible to use saline-alkali leachate for cultivating three microalgae. Besides, though the biomass of *Chlorella* sp. HQ was slightly lower than that of the other two species, its total lipid yield and content were higher. Therefore, according to the growth status and the accumulation of lipids, it is concluded that *Chlorella* sp. HQ has a more significant potential for comprehensive utilization when coupling with purifying saline-alkali leachate.

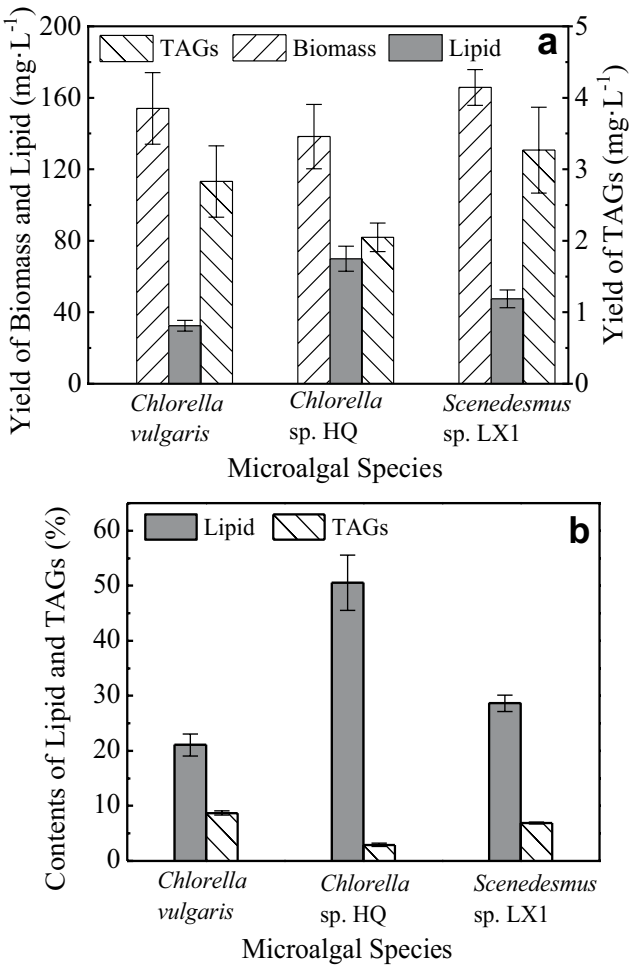

Fig. 4. Biomass, lipid, and TAGs accumulation characteristics of three microalgae cultivated in the saline-alkali leachate [(a) yield of biomass, lipid, and TAGs and (b) contents of lipid and TAGs].

3.4. Comparison of growth and lipid accumulation characteristics of Chlorella sp. HQ in different water samples

In order to further explore the advantages of cultivating microalgae by saline-alkali leachate, the growth, and lipid accumulation of *Chlorella* sp. HQ in tap water, reclaimed water, SE medium, and different salinity leachates were compared. The growth curves of *Chlorella* sp. HQ in various water samples are shown in Fig. 5. As can be seen from the figure, the algal density of *Chlorella* sp. HQ in five water samples increased with the cultivation time and reached the maximum at days of 35. Densities of *Chlorella* sp. HQ increased continuously at a steady rate in all five water samples from days of 11 to 35 and gradually entered the stable phase after 35 d. By comparing the maximum algal density of *Chlorella* sp. HQ under five culture conditions, it was found that the algal density in *L*2 and *L*1 was higher than that of the other three water samples, reaching 22.69 ± 1.75 × 10^7 and 15.94 ± 1.49 × 10^7 cells mL^{-1}, which was 2.05 and 1.44 times that of SE medium, respectively, indicating that saline-alkali leachate has potential as a medium of *Chlorella* sp. HQ.

The changes in pH after 1, 25, and 45 d of *Chlorella* sp. HQ cultivation in five various water samples is shown in

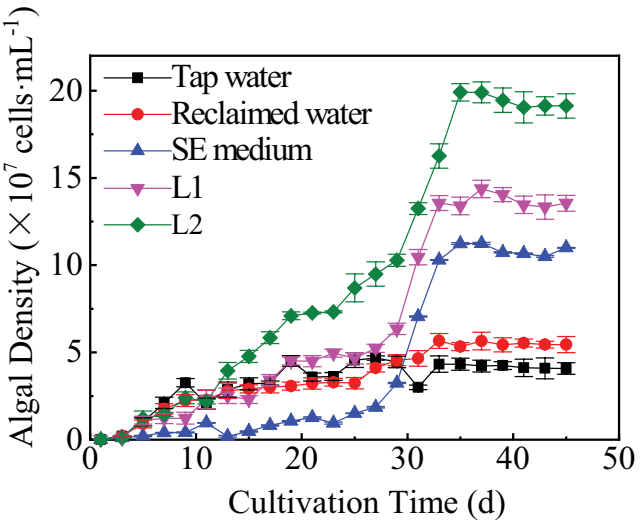

Fig. 5. The growth curves of *Chlorella* sp. HQ cultured in different water samples (*L*1: salinity of 0.18% and *L*2: salinity of 0.25%).

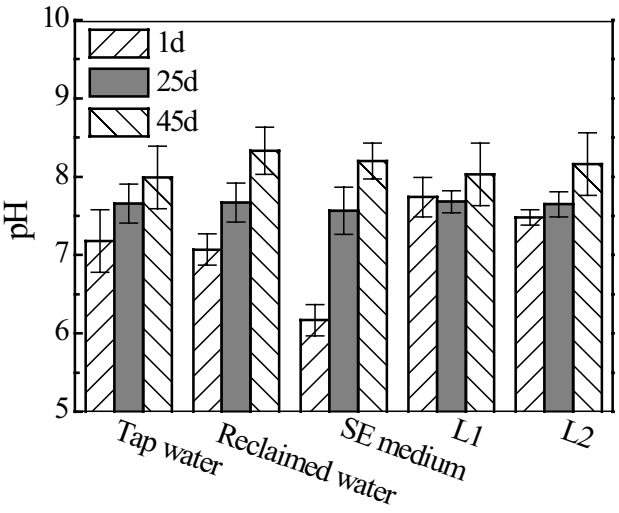

Fig. 6. The pH values of different water samples cultured with *Chlorella* sp. HQ during cultivation (*L*1: salinity of 0.18%, *L*2: salinity of 0.25%).

Fig. 6. After *Chlorella* was cultured for 45 d in tap water, reclaimed water, SE medium, *L*1, and *L*2, the pH values ended in a range of 7.99–8.16. According to previous studies, *Chlorella* sp. HQ were cultured for 30 d at different initial pH of 5.00, 7.00, 9.00, 10.00, 11.00, and the pH values showed a change tendency toward neutrality and found that *Chlorella* had a good ability to adjust the pH values to fit for cell growth [36]. In the current study, the pH value of the leachate could be maintained within the range suitable for the growth of the *Chlorella* sp. HQ, which was beneficial to the continuous growth of the microalgae, and further illustrated the feasibility and superiority of the cultivation of the microalgae in the leachate. Many previous studies have shown that salinity stress had an adverse effect on the growth of microalgae, while a small number of studies had found that the elevated salinity in appropriate range can promote microalgae growth. According to Yao et al. [37], *Tetraselmis subcordiformis* gained larger biomass under 5.4 g L^{-1} NaCl than that under 67.5 g L^{-1} [37]. In the current study, the algal biomass of *Chlorella* sp. HQ in *L*1 and *L*2 were both higher than that of other water samples (Fig. 7a). After 45 d of cultivation, the maximum algal biomass in *L*2 and *L*1 was 409.08 ± 15.12 and 388.09 ± 35.67 mg L^{-1}, which was 1.21 and 1.15 times that of SE, respectively, indicating that it is beneficial to the accumulation of biomass under suitable salinity conditions.

The algal biomass of the tap water and reclaimed water groups was lower, but more lipids can be accumulated. Among them, the maximal total lipid yield in the tap water was 266.45 ± 21.53 mg L^{-1}, and the maximal TAGs yield was 225.69 ± 18.25 mg L^{-1}, which may be due to fewer nutrients in the tap water and the nutrient stress contributing to the lipid accumulation [34]. In addition, it has been proved that salinity stress is beneficial for lipid accumulation in microalgae

Table 2
Lipid content of *Chlorella* comparisons from various types of wastewater

Algal species	Types of wastewater	Cultivation conditions			Lipid content	References
		Light intensity	Light/Dark	Temperature		
Chlorella sp. Wu G23	Textile wastewater (adding extra K_2HPO_4 8 mg/L)	4,300 lx	24:0	Room temperature	16.60%	[40]
Chlorella sp. Wu G23	Textile wastewater (adding extra K_2HPO_4 4 mg/L)	4,300 lx	24:0	Room temperature	6.30%	[40]
Chlorella vulgaris	Diluted tannery effluents (Tannery effluent:Sewage = 20:80)	35 μmol $m^{-2}s^{-1}$	24:0	27°C ± 1°C	9.30%	[41]
Chlorella sp.	Digested dairy manure	200 μmol m^{-2} s^{-1}	24:0	25°C ± 2°C	13.60%	[42]
Chlorella saccharophila	Carpet mill	75–80 μmol m^{-2} s^{-1}	24:0	25°C ± 1°C	18.10%	[43]
Chlorella sp. HQ	Saline-alkali leachate (salinity: 0.18%)	60 μmol m^{-2} s^{-1}	14:10	25°C	54.11%	This study
Chlorella sp. HQ	Saline-alkali leachate (salinity: 0.25%)	60 μmol m^{-2} s^{-1}	14:10	25°C	58.67%	This study

owing to its vital role in causing changes in fatty acid metabolism [38]. Xia et al. [39] found that the presence of NaCl was a potent inducer of lipid accumulation in *Desmodesmus* and the highest lipid content of 34.59% obtained when treated with 20 g L^{-1} NaCl [39]. In this study, comparing *L*1 and *L*2, it was found that the lipid yield and content of *Chlorella* in *L*2 were higher than *L*1 (Fig. 7). It indicated that biomass and total lipid accumulation of *Chlorella* sp. HQ all increased with the increase of salinity under the appropriate conditions. Compared with SE medium, the lipid yield and content of *Chlorella* in saline-alkali leachate were higher. The peak in microalga lipid content of 58.67% in this study was achieved in *L*2, which was higher than that in other wastewaters (Table 2). Therefore, saline-alkali leachate had a promoting effect on the growth of *Chlorella* sp. HQ and was beneficial to the accumulation of biomass and lipid to a certain extent. However, the yield and content of TAGs in *L*1 and *L*2 were lower which may be related to the distributional flow relationship among chlorophyll, carbohydrates, proteins, and lipids in microalgal cells.

4. Conclusion

The cultivation of oleaginous microalgae *Chlorella* sp. HQ, *C. vulgaris*, and *Scenedesmus* sp. LX1 using saline-alkali leachate proved to be feasible. The growth of three microalgae all mainly consumed IC in saline-alkali leachate, and *Chlorella* sp. HQ had the highest algal density and total lipid production, and the total lipid content per unit biomass reached 50.53% ± 5.00%. By comparing the growth of *Chlorella* sp. HQ in tap water, reclaimed water, SE medium, *L*1, and *L*2, it was found that saline-alkali leachate promoted the growth of *Chlorella* sp. HQ and was beneficial to the accumulation of biomass and lipid. Therefore, the saline-alkali leachate has more advantages in culturing microalgae than the SE medium and has the potential as a culture medium of *Chlorella* sp. HQ. Besides, the stress caused by insufficient nutrients in tap water was also beneficial to the accumulation of lipids. The high lipid in microalgae is of great significance for the production of biodiesel. In conclusion, *Chlorella* sp. HQ was more suitable for cultivation in saline-alkali leachate, the coupling technique of using leachate for microalgae cultivation and leachate purification by microalgae has the application potential to realize wastewater treatment, resource utilization, and low-cost cultivation of microalgae.

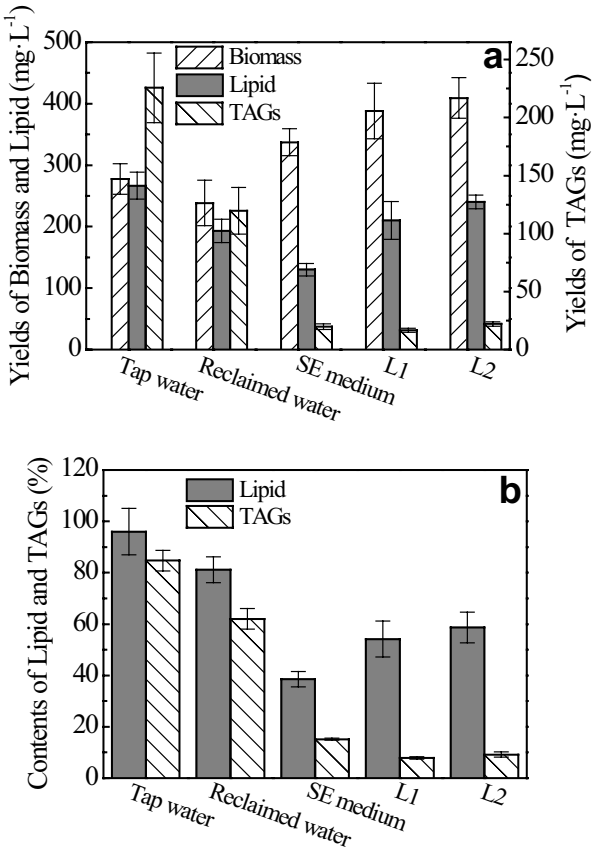

Fig. 7. Biomass, lipid, and TAGs accumulation characteristics of *Chlorella* sp. HQ cultivated in different water samples [(a): yields of biomass, lipid, and TAGs and (b) contents of lipid and TAGs; *L*1: salinity of 0.18%, and *L*2: salinity of 0.25%].

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Supplementary information:

Fig. S1. Color comparison of three algae liquids at the end of cultivation with saline-alkali leachate (cultivation conditions: salinity of 0.28%, initial density of 2×10^5 cells mL^{-1}, light intensity of 60 μmol m^{-2} s^{-1}, temperature of 25°C, and light-dark ratio of 14:10 h).

Table S1
Components of the saline-alkali leachate (salinity of 0.28%)

Composition	Concentration (mg L^{-1})	Composition	Concentration (mg L^{-1})
Cl^-	556.26	SO_4^{2-}	127.91
Ca^{2+}	474.63	Mg^{2+}	118.70
Na^+	2,270.09	K^+	332.17
Zn^{2+}	0.80	Cu^{2+}	0.40
Mn^{2+}	0.80	TP	2.63
TC	164.60	TN	47.77