

## A Review on Microalgae for Wastewater Treatment

Kaiwei Xu<sup>1,2,3,4,\*</sup>, Chao Guo<sup>1,2,3,4,a</sup>, Biao Peng<sup>1,2,3,4,b</sup>

<sup>1</sup> Shaanxi Provincial Land Engineering Construction Group Co., Ltd. Xi'an, Shaanxi 710071, China

<sup>2</sup> Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Xi'an, Shaanxi 710071, China

<sup>3</sup> Key Laboratory of Degraded and Unused Land Consolidation Engineering, the Ministry of Natural Resources., Xi'an, Shaanxi 710071, China

<sup>4</sup> Shaanxi Provincial Land Consolidation Engineering Technology Research Center., Xi'an, Shaanxi 710071, China

\*xukaiwei12@163.com, <sup>a</sup>443485346@qq.com, <sup>b</sup>43493022@qq.com

### Abstract

In response to the water pollution and climate change, microalgae have gained increasingly attention as a renewable, alternative and sustainable source biofuel. However, a large of investment in freshwater and nutrient salts during microalgae cultivation can greatly limit the its application. Combine microalgae cultivation with wastewater treatment is a win-win strategy. In this paper, the removal mechanism of pollutants by microalgae is reviewed, and the environmental factors affecting the cultivation of microalgae in wastewater were analyzed. Finally, this paper pointed out the existing problems of the technology, and prospected the focus of the next work, so as to provide a reference for promoting the development of wastewater treatment technology using microalgae.

### Keywords

Microalgae; Wastewater Treatment; Pollutant; Biofuel.

### 1. Introduction

Water is the source of our lives. Although more than 70% of the earth's surface is covered by water, the available for human is very limited. Moreover, the uneven distribution of time and space makes the existing water resources difficult to meet the increasing production and living needs. Therefore, the shortage of water resources has always been the focus of social concern. Meanwhile, with the rapid development of social economy and the continuous increase of the discharge of various pollutants, the pollution carrying capacity of water environment is facing severe challenges.

At present, wastewater treatment plants mostly adopt activated sludge process, which has many problems, such as high energy consumption, large dosage of chemicals, long process flow, complex operation, difficult maintenance of wastewater treatment equipment, serious secondary pollution and insufficient utilization of resources [1-3]. Ecological treatment technology and bio-ecological combined technology have attracted extensive attention due to their advantages such as low construction cost, low energy consumption, high ecological value and high efficiency. This kind of technology includes stable pond system, efficient algal pond, biological purification tank, aquatic plant filter bed, and the composite system of different technical units, which has a good treatment effect on agricultural sewage, domestic sewage and polluted river. Among them, the efficient algal pond system plays a huge role in the cheap

treatment and application of wastewater by taking advantage of the advantages of wide distribution, rapid growth, strong environmental adaptability, high biological value and low system cost [4].

Microalgae is a kind of low organisms, including autotrophic microalgae that can use CO<sub>2</sub> and light for photosynthesis, heterotrophic microalgae that can directly use organic carbon sources and nutrients in the environment, and some mixotrophic microalgae that can both perform photosynthesis and utilize external organic carbon sources [2, 5]. Microalgae can efficiently remove nitrogen and phosphorus efficiently, and can absorb heavy metals by the functional groups on the cell surface. In addition, microalgae biomass can be harvested after wastewater treatment, so as to achieve the resource utilization of sewage.

In this paper, the removal mechanism of pollutants by microalgae is reviewed, and the environmental factors affecting the cultivation of microalgae in wastewater were analyzed. Finally, this paper pointed out the existing problems of the technology, and prospected the focus of the next work, so as to provide a reference for promoting the development of wastewater treatment technology using microalgae.

## 2. Mechanism of Pollutant Removal

### 2.1. Carbon Removal

Carbon accounts for 40% ~ 50% of the dry weight of microalgae cells, and is an important part of the cell structure. The carbon sources used by microalgae can be divided into organic carbon and inorganic carbon. Inorganic carbon is mainly used in the form of CO<sub>2</sub>, bicarbonate and carbonate. Microalgae can use CO<sub>2</sub> in the air as their carbon source through photosynthesis. When the pH value is between 5 and 7, CO<sub>2</sub> is absorbed by the diffusion. When the pH value is higher than 7, the carbonic anhydrase outside microalgae cells will promote the active transport of carbon source into the cells. CO<sub>2</sub> is fixed by Ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCO), and then glucose and other organic substances are synthesized through the Calvin cycle. Some heterotrophic microalgae can use organic carbon such as glucose and sodium acetate as carbon source, and mixotrophic microalgae can use organic and inorganic carbon to grow [6].

### 2.2. Phosphorus Removal

Phosphorus plays an important role in microalgae metabolic processes such as cell energy transfer, photosynthesis, formation of cell membrane and nucleic acid, and has a significant impact on the life activity of microalgae. Phosphorus in wastewater usually exists in the form of inorganic anions. In general, phosphorus removal was attributed to biological phosphorus removal and chemical precipitation. Microalgae cells can convert absorbed phosphate into phospholipids through oxidative phosphorylation, photosynthetic phosphorylation and substrate level phosphorylation. Meanwhile, during the microalgae cultivation, the equilibria of CO<sub>2</sub> (aq)-HCO<sub>3</sub><sup>-</sup>-CO<sub>3</sub><sup>2-</sup> is usually broke down, which results in the increase of pH [7]. When the pH value is above 9.0, chemical precipitation is occurred [8].

### 2.3. Nitrogen Removal

The removal of nitrogen by microalgae mainly depends on the assimilation of cells. Nitrogen in wastewater can be divided into inorganic nitrogen and organic nitrogen. Inorganic nitrogen mainly exists in the form of nitrate, nitrite and ammonia nitrogen. The assimilation effect of microalgae can convert nitrate into nitrite, and then into ammonium salt under the action of nitrate reductase and nitrite reductase respectively, which is then incorporated into the carbon skeleton together with the directly absorbed ammonia nitrogen [9]. The utilization of ammonium depends on the process of glutamate synthase and ATP. Therefore, ammonium can be directly used by microalgae, which consumes the least energy and is most easily absorbed,

followed by nitrite and nitrate. However, excessive ammonium nitrogen can negatively affect the growth of microalgae. For organic nitrogen such as urea and amino acids, microalgae can be directly absorbed by mixotrophy. The indirect removal of nitrogen by microalgae is mainly due to the high pH caused by photosynthesis, which promotes the stripping of ammonia nitrogen. In addition, the appropriate increase of temperature is also beneficial to the stripping of ammonia nitrogen. Some microalgae such as cyanobacteria can also absorb nitrogen from the atmosphere.

#### **2.4. Heavy Metal Removal**

Microalgae have a good enrichment effect on heavy metals, but there is no unified theory on the mechanism of adsorption of heavy metals by microalgae. Many researchers believe that polycomplexes such as polysaccharides, protein and phospholipids on the surface of microalgae cells provide a large number of functional groups that can bind to heavy metal ions. Many common functional groups such as carboxyl (-COOH), hydroxyl (-OH), amino (-NH<sub>2</sub>), sulfhydryl (-SH), carbonyl (-C=O), phosphate (PO<sub>4</sub><sup>3-</sup>), etc. are related to the adsorption of heavy metals in a certain extent [10, 11]. There are two types of enrichment of heavy metals by microalgae: one is adsorption by living microalgae and the other is adsorption by non-living microalgae. It is generally considered that the adsorption mechanism of living microalgae to heavy metals includes two stages: the first stage is independent of metabolism, and the surface functional groups and metal ions can form ligands/complexes by ion exchange, electrostatic attraction, complexation, redox reaction, coordination and microprecipitation. The second stage is biological enrichment, in which the microalgae cells directly transport heavy metal ions to the fine cells for storage through a series of biochemical reactions. The principle of non-living microalgae enrichment is mainly the first stage of living algae adsorption.

### **3. Influence Factor of Wastewater Treatment by Microalgae**

#### **3.1. Microalgae Species**

Different microalgae species have different requirements on nutrition and light, and their ability to adapt to the environment, growth rate and yield are also quite different [12]. Therefore, it is necessary to screen and acclimate microalgae species according to the wastewater characteristics. Studies have found *Spirulina* sp., *Chlorella* sp., *Scenedesmus* sp., *Chlamydomonas reinhardtii* and other microalgae can remove the pollutants in wastewater and harvest microalgae biomass [2, 13, 14]. In addition, the use of local dominant microalgae isolated from wastewater resource has stronger adaptability than single microalgae species or microalgae species selected from microalgae species pools, because it is less affected by changes in local climate and water environment, and local dominant microalgae species often have higher pollutant removal rate and growth rate.

#### **3.2. Light Intensity and Photoperiod**

Light provide energy for photoautotrophic/mixotrophic microalgae cells, and the increase of light intensity will enhance the removal efficiency of pollutants in a certain extent. Under a suitable growth environment, the number of microalgae can double in a short time, but if the light intensity is not controlled, the rapid increase in the concentration of microalgae cells will reduce the penetration of light in suspension, which will reduce the photosynthetic efficiency and growth of microalgae. In addition, the photoperiod can also make a difference in the treatment effect. For example, under the light-dark ratio of 12 h: 12 h, the total organic carbon removal rate by *Parachlorella kessleri* was slightly higher than that under the continuous light condition, which was 88.1% and 86.4%, respectively [15].

### 3.3. Temperature

Generally, the microalgae culture temperature should be maintained at 20-30 °C. The growth of microalgae increases exponentially with slightly increasing temperature, and the efficiency of microalgae wastewater treatment is usually significantly reduced at low temperature. The changes of temperature can affect the activities of related enzymes in microalgae cells. With the increase of temperature, the metabolic activities of microalgae cells increase. Munoz et al. [16] observed that when the temperature rose from 26.5°C to 31.5°C, the removal efficiency of sodium salicylate increased from 14 mg L<sup>-1</sup> h<sup>-1</sup> to 27 mg L<sup>-1</sup> h<sup>-1</sup> by the aggregates composed of *Chlorella sorokiniana* and *Ralstonia basilensis*. However, if the temperature is higher than the optimum, the growth of microalgae will stop, and then the biomass will decrease. This is because excessive temperature can inactivate metabolic enzymes in microalgae cells [17].

### 3.4. pH

The optimal pH range for microalgae growth is 6.0 – 8.0. During cultivation, the pH value of the system is generally difficult to stabilize due to the influence of CO<sub>2</sub> balance, but most microalgae species have a wide range of pH tolerance. Posadas et al. [18] evaluated the influence of pH (7, 8 and 9) on the performance of secondary domestic wastewater treatment and biomass productivity/composition in outdoors pilot raceways, and found that the influence of pH on wastewater treatment was negligible. It should be noted that drastic changes in pH will affect the concentration of nutrients in the substrate. For example, high pH values can lead to NH<sub>3</sub> stripping and phosphorus precipitate.

## 4. Problems in the Application of Microalgae in Wastewater Treatment

### 4.1. Wastewater Composition

The wastewater composition varies greatly depending on the source, making the cultivation of microalgae challenging. In the microalgae cultivation stage, the N/P ratio of wastewater should be fully considered to select the appropriate microalgae strain, and extra nutrients should be avoided. In addition, various types of wastewaters may contain toxic and harmful chemicals that inhibit the growth of microalgae. For example, excessive phenols, aldehydes, antibiotics and heavy metals may exist in industrial wastewater. Colored wastewater containing a large number of suspended solids, organic molecules, humus or melanin will affect the transmission of sunlight and reduce the rate of photosynthesis. Therefore, it is particularly important to pretreat wastewater before cultivating microalgae. Commonly, pretreatment methods include filtration, autoclaving, UV sterilization and dilution. Some studies have shown that certain chemical agents such as sodium hypochlorite (NaClO) can be used to disinfect wastewater or reduce turbidity. Filtration is more economical and reliable for large-scale microalgae cultivation systems. Sahu et al. [19] treated municipal wastewater by flocculation and anthracite to increase the light transmission rate to 85%, which is more conducive to the growth of microalgae.

### 4.2. Microalgae Separation

Separation is an urgent problem to be solved in the process of microalgae cultivation. Due to their small cell size, density close to water, and good suspension stability, microalgae separation often requires one or more separation steps, leading to difficulties in separation and utilization. It was reported that microalgae separation process could account for 20%-30% of the total cost of microalgae product. At present, no universal technology has been established for efficient harvesting of microalgae [20]. Table 1 summarizes the advantages and disadvantages of the commonly used separation methods for microalgae. In the future, it is necessary to combine the characteristics of microalgae, fully consider the species, density,

particle shape and size, electrical property of cell wall, and the value of goal products, and further develop efficient concentration and separation technology of microalgae.

**Table 1.** Advantages and disadvantages of different microalgae harvesting techniques.

Harvesting techniques	Advantages	Disadvantages
Filtration	<ul style="list-style-type: none"> <li>● High harvesting efficiency</li> <li>● No chemicals required</li> <li>● Low energy consumption (natural and pressure filter)</li> </ul>	<ul style="list-style-type: none"> <li>● Time-consuming, pressure or vacuum required</li> <li>● Unsuitable for small microalgae</li> <li>● Membrane fouling or/and clogging</li> </ul>
Centrifugation	<ul style="list-style-type: none"> <li>● High harvesting efficiency</li> <li>● Species independent</li> <li>● Preferred for small scale and laboratory</li> </ul>	<ul style="list-style-type: none"> <li>● High energy consumption</li> <li>● High operation and maintenance costs</li> <li>● Risk of cell destruction</li> </ul>
Sedimentation	<ul style="list-style-type: none"> <li>● Easy to operate</li> <li>● Low-cost and efficient technique</li> </ul>	<ul style="list-style-type: none"> <li>● Time-consuming</li> <li>● Limited to low density microalgae</li> </ul>
Flocculation	<ul style="list-style-type: none"> <li>● Efficient and easy technique</li> <li>● Less energy requirements</li> <li>● Easy to scale up</li> <li>● Applied to vast range of species</li> </ul>	<ul style="list-style-type: none"> <li>● Flocculants may be expensive</li> <li>● Highly pH dependent</li> <li>● Toxic materials will affect the quality of the downstream products</li> <li>● Culture medium recycling is limited</li> </ul>
Flotation	<ul style="list-style-type: none"> <li>● Time-saving and efficient technique</li> <li>● Low space requirement</li> <li>● Suitable for large scale</li> </ul>	<ul style="list-style-type: none"> <li>● Surfactant required</li> <li>● Oversized bubbles break up the floc</li> <li>● High energy consumption</li> </ul>

### 4.3. Downstream Processing

Another major purpose of microalgae wastewater treatment is to convert microalgae biomass into renewable energy such as biofuel. Due to the differences in the structural composition of different microalgae strains, some intracellular substances of microalgae may be easy to obtain, while some microalgae have hard cell walls and need to be extracted by relevant process. The lipid extraction method should be rapid and effective and not harmful to the biorefinery. At present, the main extraction methods of microalgae lipids include cell drying, cell lysis and solvent extraction. Among them, solvent extraction is the most commonly used. Zou et al. [21] found that the optimum lipid yield of 18.75 wt% was obtained at exposure time of 13.05 min, hexane: isopropanol ratio of 4.04 v/v, solid concentration of solid concentration of 1.24 w/v% and transducer power of 38%. Subhash and Mohan [22] studied an integrated biorefinery approach where microalgae cakes after lipid extraction were used as substrates to produce biohydrogen with the assistance of selectively enriched acidogenic consortia as biocatalysts. To further verify a certain microalgae biorefinery approach, the necessities reside in the detailed analysis of energy consumption and cost-effectiveness under different product price scenarios.

## 5. Conclusion and Suggestion

Microalgae is cultured in wastewater, which is a win-win strategy. This can not remove the pollutants from wastewater, but also obtain the microalgae biomass, and obtained microalgae biomass can be converted into biofuels, high-value chemicals, and so on. Although much attention has been paid to the ability and mechanism of microalgae to absorb nutrients from



the wastewater, large-scale cultivation of microalgae is still not feasible for various reasons. At present, most research reports are carried out in the lab-scale or pilot scale. Therefore, in order to more effectively remove pollutants and produce microalgae biomass, a complete microalgae wastewater cultivation system should be established as soon as possible in combination with the environmental impact factors of microalgae wastewater cultivation, and by referring to or integrating other industrial wastewater and domestic wastewater treatment technologies.

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## References

- [1] K. Li, Q. Liu, F. Fang, R. Luo, Q. Lu, W. Zhou, S. Huo, P. Cheng, J. Liu, M. Addy, Microalgae-based wastewater treatment for nutrients recovery: A review, *Bioresource technology*, 291 (2019) 121934.
- [2] Y. Wang, S.-H. Ho, C.-L. Cheng, W.-Q. Guo, D. Nagarajan, N.-Q. Ren, D.-J. Lee, J.-S. Chang, Perspectives on the feasibility of using microalgae for industrial wastewater treatment, *Bioresource Technology*, 222 (2016) 485-497.
- [3] W. Mo, Q. Zhang, Can municipal wastewater treatment systems be carbon neutral?, *Journal of environmental management*, 112 (2012) 360-367.
- [4] D.L. Sutherland, S. Heubeck, J. Park, M.H. Turnbull, R.J. Craggs, Seasonal performance of a full-scale wastewater treatment enhanced pond system, *Water research*, 136 (2018) 150-159.
- [5] K.W. Chew, S.R. Chia, P.L. Show, Y.J. Yap, T.C. Ling, J.-S. Chang, Effects of water culture medium, cultivation systems and growth modes for microalgae cultivation: A review, *Journal of the Taiwan Institute of Chemical Engineers*, 91 (2018) 332-344.
- [6] M. Mubashar, Z. Ahmad, C. Li, H. Zhang, C. Xu, G. Wang, D. Qiu, L. Song, X. Zhang, Carbon-negative and high-rate nutrient removal using mixotrophic microalgae, *Bioresource Technology*, 340 (2021) 125731.
- [7] V.C. Eze, S.B. Velasquez-Orta, A. Hernández-García, I. Monje-Ramírez, M.T. Orta-Ledesma, Kinetic modelling of microalgae cultivation for wastewater treatment and carbon dioxide sequestration, *Algal Research*, 32 (2018) 131-141.
- [8] K. Xu, X. Zou, H. Wen, Y. Xue, Y. Qu, Y. Li, Effects of multi-temperature regimes on cultivation of microalgae in municipal wastewater to simultaneously remove nutrients and produce biomass, *Applied microbiology and biotechnology*, 103 (2019) 8255-8265.
- [9] T. Cai, S.Y. Park, Y. Li, Nutrient recovery from wastewater streams by microalgae: status and prospects, *Renewable and Sustainable Energy Reviews*, 19 (2013) 360-369.
- [10] K.S. Kumar, H.-U. Dahms, E.-J. Won, J.-S. Lee, K.-H. Shin, Microalgae—a promising tool for heavy metal remediation, *Ecotoxicology and environmental safety*, 113 (2015) 329-352.
- [11] Y.K. Leong, J.-S. Chang, Bioremediation of heavy metals using microalgae: Recent advances and mechanisms, *Bioresource technology*, 303 (2020) 122886.
- [12] K. Xu, X. Zou, Y. Xue, Y. Qu, Y. Li, The impact of seasonal variations about temperature and photoperiod on the treatment of municipal wastewater by algae-bacteria system in lab-scale, *Algal Research*, 54 (2021) 102175.
- [13] S.F. Ahmed, M. Mofijur, T.A. Parisa, N. Islam, F. Kusumo, A. Inayat, I.A. Badruddin, T.Y. Khan, H.C. Ong, Progress and challenges of contaminate removal from wastewater using microalgae biomass, *Chemosphere*, 286 (2022) 131656.
- [14] D. Nagarajan, A. Kusmayadi, H.-W. Yen, C.-D. Dong, D.-J. Lee, J.-S. Chang, Current advances in biological swine wastewater treatment using microalgae-based processes, *Bioresource technology*, 289 (2019) 121718.

- [15] K. Lee, C.-G. Lee, Effect of light/dark cycles on wastewater treatments by microalgae, *Biotechnology and Bioprocess Engineering*, 6 (2001) 194-199.
- [16] R. Muñoz, C. Köllner, B. Guieysse, B. Mattiasson, Photosynthetically oxygenated salicylate biodegradation in a continuous stirred tank photobioreactor, *Biotechnology and bioengineering*, 87 (2004) 797-803.
- [17] H. Shin, S.-J. Hong, C. Yoo, M. Han, H. Lee, H.-K. Choi, S. Cho, C.-G. Lee, B.-K. Cho, Genome-wide transcriptome analysis revealed organelle specific responses to temperature variations in algae, *Scientific reports*, 6 (2016) 1-11.
- [18] E. Posadas, M. del Mar Morales, C. Gomez, F.G. Acién, R. Muñoz, Influence of pH and CO<sub>2</sub> source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways, *Chemical Engineering Journal*, 265 (2015) 239-248.
- [19] A.K. Sahu, J. Siljudalen, T. Trydal, B. Rusten, Utilisation of wastewater nutrients for microalgae growth for anaerobic co-digestion, *Journal of environmental management*, 122 (2013) 113-120.
- [20] K. Xu, X. Zou, W. Chang, Y. Qu, Y. Li, Microalgae harvesting technique using ballasted flotation: A review, *Separation and Purification Technology*, 276 (2021) 119439.
- [21] X. Zou, K. Xu, W. Chang, Y. Qu, Y. Li, Rapid extraction of lipid from wet microalgae biomass by a novel buoyant beads and ultrasound assisted solvent extraction method, *Algal Research*, 58 (2021) 102431.
- [22] G.V. Subhash, S.V. Mohan, Deoiled algal cake as feedstock for dark fermentative biohydrogen production: an integrated biorefinery approach, *International journal of hydrogen energy*, 39 (2014) 9573-9579.