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Research Progress on Nitrogen Removal Performance of Constructed Wetland-Microbial Fuel Cell

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Abstract: Due to their limitations, conventionally constructed wetlands or microbial fuel cells often suffer from some disadvantages such as low denitrification efficiency, high internal resistance, and high activation potential in the process of treating nitrogenous wastewater. In recent years, the emerging constructed wetland-microbial fuel cell (CW-MFC) combines the constructed wetland (CW) and microbial fuel cell (MFC), which is a new bioelectrochemical technology for both electricity production and wastewater treatment, its natural redox gradient, unique cell structure, cathodic reduction characteristics and wetland plants located at the cathode not only provide advantages for nitrogen removal but also enhance the performance of electricity production. As a result of the diversity of wastewater types and their constituents, the effects of nitrogen removal from CW-MFC vary among different components. Most of the existing studies have investigated the effect of nitrogen removal in terms of system structure and composition, this paper reviews the effects of salinity and phosphorus in wastewater components on the denitrification performance of CW-MFC based on the analysis of nitrogen conversion pathways and nitrogen removal principles of CW-MFC, summarizes the problems caused by the limitations of the nitrogen removal process and the effects of salinity and phosphorus concentration, proposes ways as well as directions to strengthen the denitrification performance for the future development of CW-MFC.

Keywords: CW-MFC, Denitrification, Salinity, Phosphorus

1. Introduction

Nitrogen is the main pollutant that causes eutrophication and water bloom in water bodies. It exists in various forms in the water environment and can be transformed into each other under certain conditions. For wastewater denitrification, the conversion of various forms of nitrogen into N_2 and its removal from the water environment utilizing microorganisms and bioelectrochemistry is an important way, the denitrification of wastewater and its discharge to the standard is also one of the important methods to protect the water environment.

CW-MFC is an emerging wastewater treatment system that inherits the advantages and shows the unique performance of CW and MFC. With the in-depth research of CW and MFC, the coupled CW-MFC system has received extensive attention from scholars. This coupled system has the dual functions of CW and MFC, which can treat wastewater and use organic matter in wastewater as raw material for power generation [1]; the system uses microorganisms as biocatalysts to degrade organic substrates and decontaminate wastewater through physical, chemical and biological interactions [2]. Meanwhile, different locations within the CW system have different redox potentials, and the potential difference can be used to generate electricity, the anaerobic environment of the bottom layer provides good environmental conditions for the metabolism of organic matter by electricity-producing microorganisms to generate electrons, so the redox gradient of the natural stratification of the CW system is highly consistent with the operating conditions of MFC, providing the feasibility of coupling CW and MFC systems [3-6].

Compared with conventional CW or MFC, the CW-MFC system can produce electricity and lower cost while purifying wastewater, solving the problems of incomplete nitrification and insufficient carbon source for denitrification in conventional CW technology, and achieving good results for organic matter removal. It can also be applied to recover heavy metal energy and can improve the denitrification performance of the wetland system as a whole, which is environmentally sustainable [7, 8]. Current studies on nitrogen removal from CW-MFC systems have focused on factors such as operating conditions, system structure, and composition, with little research on factors affecting salts and phosphorus. In this paper, the nitrogen removal capacity of CW-MFC was analyzed and compared, the effects of salt and phosphorus on the nitrogen removal performance of CW-MFC were investigated, and the possible directions of optimization of CW-MFC were summarized, to provide more theoretical support for the practical application of CW-MFC.

2. Mechanism of Denitrification by CW-MFC

Nitrogen (N) is a basic constituent element of living organisms [9], and in recent years, due to the extensive use of nitrogen fertilizers, the environment has taken in excessive amounts of nitrogen, and more water bodies have been polluted with nitrogen to varying degrees. There are more sources of nitrogen pollution in water bodies, such as domestic sewage, industrial wastewater, and agricultural drainage [10], which will cause serious effects on human health, plants, animals, and the ecological environment if discharged directly without treatment.

The principle of nitrogen removal in CW-MFC is based on the traditional biological denitrification theory - nitrification and denitrification processes and has been developed on this basis, the denitrification process of CW-MFC can occur in the anode and cathode chambers separately [11]. Xu et al. [12] used multiple biological cathodes to construct a CW-MFC system in an attempt to achieve higher nitrogen removal rates by obtaining higher power. When the number of biocathodes was increased from 1 to 3, the maximum power density of the system increased from 12.56 mW/m^2 to 26.16 mW/m^2. Due to the effect of bioelectrically derived interactions between power generation and system nitrification rate (r_{Ni}) and denitrification rate (r_{De}), there was a significant increase in r_{Ni} and r_{De} for all three biocathode systems, thus that the enhanced electrical performance promoted the nitrification rate and denitrification rate. Cui et al. [13] constructed a dual-cathode, three-chamber MFC system to achieve simultaneous nitrification-denitrification and carbon removal power production function. The experimental results showed that the denitrification was strongly influenced by the initial concentrations of $NO_3^- - N$ and $NH_4^+ - N$ in the influent water. With the increase of cathodic $NO_3^- - N$ concentration, the removal effect on aerobic cathode $NH_4^+ - N$ did not fluctuate much, but the removal rate was always high and remained above 95%; the removal rate of anoxic cathode on $NO_3^- - N$ showed a decrease, from 40% to about 26%. Zhao et al. [14] in MFC with $NO_2^- - N$ as the electron acceptor, and investigated the effects of influent nitrite concentration and external resistance on the denitrification performance of MFC. As the influent $NO_2^- - N$ concentration increased from 60.11 mg/L to 188.12 mg/L, the TN removal rate increased significantly from 26.91 $\pm$ 1.72 $gm^{-3}d^{-1}$ to $54.80\pm0.01 gm^{-3}d^{-1}$, while the nitrification rate increased from 0.33% to 26.33%, the MFC operated better at a high influent $NO_2^- - N$ concentration of 188 mg/L. Thus, the cathode exhibited significant $NO_2^- - N$ nitrification under the condition of strict maintenance of cathode anoxia, and the nitrification characteristics increased with increasing $NO_2^- - N$ concentration. Nitrogen in untreated wastewater exists in the form of nitrogen-containing compounds, mainly inorganic and organic nitrogen. Inorganic forms of nitrogen include ammonia nitrogen ($NH_4^+ - N$), nitrate-nitrogen ($NO_3^- - N$), nitrite-nitrogen ($NO_2^- - N$), etc. Organic forms of nitrogen include amino acids, proteins, urea, etc. The removal of nitrogenous compounds in CW-MFC is mainly based on biological denitrification, and the microorganisms for biological denitrification are widely present in the wetland fuel cell system and have various forms of nitrogen conversion. The main processes based on conventional denitrification and new denitrification include nitrification, denitrification, short-range nitrification-denitrification, synchronous nitrification-denitrification, anaerobic ammonia oxidation, and ammonification. Figure 1 shows the nitrogen conversion processes in CW-MFC and the corresponding microorganisms.

Nitrification is a key part of the traditional nitrogen cycle and consists of aerobic ammonia oxidation (nitrosative phase) and nitrite oxidation (nitrification phase) [15]. Ammonia nitrogen is oxidized to nitrite nitrogen by ammonia-oxidizing bacteria (AOB) under aerobic conditions in the nitrification phase and then oxidized to nitrate nitrogen by nitrite-oxidizing bacteria (NOB) in the nitrification phase [16]. Denitrification is microbial respiration under lower dissolved oxygen conditions that uses autotrophic denitrifying bacteria (ADB) to convert $NO_3^- - N$ to N_2 and N_2O for eventual removal from the ecosystem [17], nitrification is usually coupled with the denitrification process to produce N_2 to achieve nitrogen removal. The principle of short-range nitrification-denitrification reaction is to make AOB the dominant flora by controlling the reaction conditions while inhibiting the action of NOB, which leads to the accumulation of nitrite nitrogen, and completing the denitrification process by reducing nitrite-nitrogen to N_2 using ADB. Short-range nitrification-denitrification biological denitrification technology is gaining popular attention because of its advantages such as reducing energy consumption and saving carbon sources [18]. When nitrification and denitrification processes are carried out in a single reactor, this phenomenon is known as synchronous nitrification-denitrification (SND) [19]. Anaerobic ammonia oxidation is the process by which $NH_4^+ - N$ and $NO_2^- - N$ are converted to N_2 by the action of anaerobic ammonia-oxidizing bacteria (AnAOB) in an anaerobic environment. This reaction process does not require an additional carbon source and aeration, which is considered one of the most promising processes in current denitrification processes [20]. Ammonification, also known as deamination, is the initial step of denitrification, a process in which ammonia-oxidizing bacteria decompose and convert organic nitrogen into ammoniacal nitrogen (NH_3, NH_4^+), deamination is divided into aerobic and parthenogenic, anaerobic types depending on the type of bacteria.

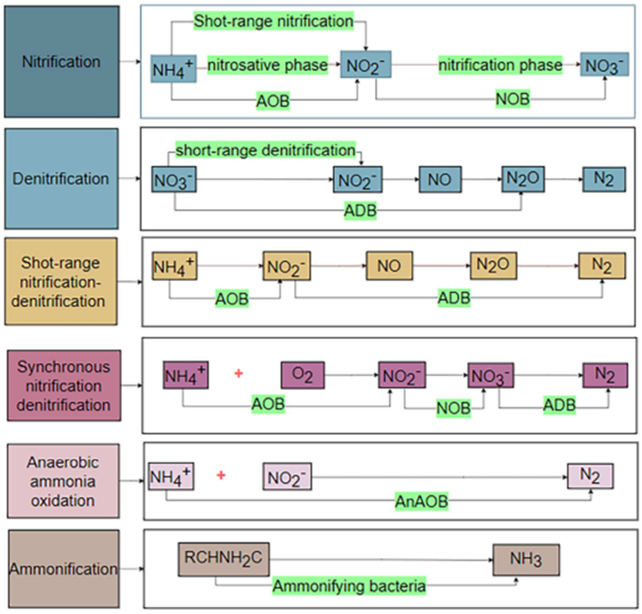

Figure 1. *CW-MFC nitrogen conversion process.*

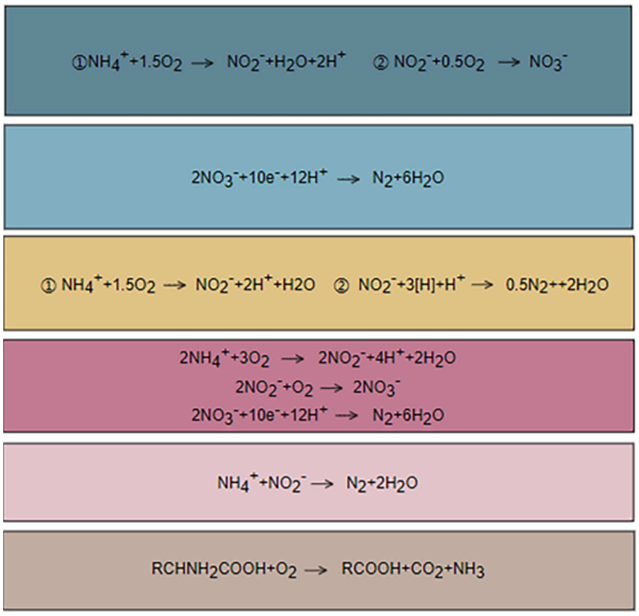

Figure 2. *Nitrogen conversion reaction formula.*

With the continuous research, many new denitrification processes and combinations emerged on the basis of the nitrogen conversion process described above, such as short-course nitrification-anaerobic ammonia oxidation, short-course denitrification-anaerobic ammonia oxidation, simultaneous denitrification by heterotrophic nitrification-aerobic denitrification [21], simultaneous anaerobic ammonia oxidation-denitrification by partial nitrification [22], etc. It can be found that although the process is constantly updated and developed, it is always developed and innovated around the principles of nitrification, denitrification and ammonification.

3. Effect of Salt on Denitrification

In saline wastewater, the main ions are chloride (Cl^-), sodium (Na^+), sulfate (SO_4^{2-}), and magnesium (Mg^{2+}) [23], if the nitrogenous substances in it get discharged in large quantities, it will not only cause eutrophication of water bodies and damage ecology but also affect human production and life [24]. Salinity also affects the effect of wastewater denitrification by affecting osmotic pressure, microorganisms, and plants [25], so the treatment of saline wastewater is more complicated than that of ordinary wastewater.

Regarding the study of the effect of salinity on nitrification and denitrification performance, Lu et al. [26] studied the effect of seawater salt wastewater on synchronous nitrification-denitrification, and the results show that increasing salinity when the proportion of nearshore seawater does not exceed 50%, $NH_4^+ - N$ removal rate remained above 97% and total nitrogen removal rate did not exceed 70%, indicating the salinity directly affected the denitrification and had little effect on nitrification since NOB is more sensitive than AOB, which is in agreement with the results of Dinçer [27], Liu [28], Mosquera-Corral [29] and Wang [30]. In studying the effect of salt on denitrification, Mariangel [31] increased the concentration of NaCl from 0.47 g/L to 100 g/L, and the reduction rates of nitrate and nitrite were increasingly inhibited, with a threefold decrease in the nitrate reduction rate and a decrease in the nitrite reduction rate from 19.54 to 9.26 mg $N(g\ VSS\ h)^{-1}$, indicating that the effect of NaCl on the nitrate reduction rate was greater than that on the nitrite reduction rate.

Table 1 compares the effect of partial salt concentration on the denitrification rate. Lu et al. [32] used the CW-MFC system for the treatment of high-salt wastewater and found that the CW-MFC system had a stable output voltage and could continuously provide electron donors for the cathodic denitrification process. The CW-MFC system $NH_4^+ - N$ removal rate decreased from 38.5% to 24.1% and the TN removal rate decreased from 66.88% to 63.08% when the salinity increased from 1% to 3%, indicating that the increase of salinity inhibited the denitrification effect of CW-MFC. Zhang et al. [33] investigated the effect of five groups of high salt wastewater on nitrogen removal from a single chamber microbial fuel cell (SCMFC) with five reactor salinities of 18.89 g/L, 18.96 g/L, 19.06 g/L, 19.18 g/L, and 19.36 g/L, respectively, and operated for ten cycles to ensure system stability when a stable voltage output was achieved. The experiments measured that the $NH_4^+ - N$ removal rate was 58.22%, 96.55%, 89.07%, 80.26%, and 21.29%, and the TN removal rate was 56.09%, 96.17%, 84.85%, 77.78% and 22.01%, in which the short duration of power generation in the first group of reactors thus affected the nitrification rate and reduced the nitrogen removal rate, in the remaining groups, the nitrogen removal rate decreased gradually with the increase of salinity. When the salinity was increased from 0g/L to 5g/L, Xu et al. [34] and Ouyang Delong [35] gave consistent conclusions. Xu compared CW-MFC treatment of saline wastewater and ordinary wastewater, and the experimental results showed that the removal rates of

$NH_4^+ - N$ and TN were around 60% at 0 g/L concentration, and the removal rates of both increased to 79.67% and 70.86% at 5 g/L, respectively, indicating that the increase of low salt concentration played a facilitating role in nitrogen removal. Ouyang Delong similarly tested the denitrification efficiency of CW-MFC for saline wastewater with a gradual increase in salinity from 0 g/L to 5 g/L. As shown in Table 1, both $NH_4^+ - N$ and TN removal rates increased steadily in the concentration range of 0 g/L to 5 g/L. The same experimental results were also given by Yoshie et al. [36], they compared the denitrification efficiency at 10% and 2% salinity and studied the characteristics of the strains isolated from the denitrification system, the results showed that the denitrification efficiency was higher at high salinity. The reason for this is that the sludge used in this experiment was domesticated for a long time and therefore the microorganisms were better adapted to the saline conditions. The strains isolated from the denitrification system were identified and most of the isolates belonged to the genus Halomonas and could grow at 10% NaCl, so the denitrification efficiency was enhanced.

Table 1. *Effect of salinity on TN and* $NH_4^+ - N$.

Auth.	Xu et al.	Ouyang	Zhang et al.	Lu et al.
Reactor	CWMFC	CWMFC	SCMFC	CWMFC
Salinity (max)	5 g/L	5 g/L	19.36 g/L	30 g/L
Nitrogen removal rate	TN	TN	TN	TN
	70.86%	71.30%	22.01%	63.08%
	NH_4^+-N	NH_4^+-N	NH_4^+-N	NH_4^+-N
	79.67%	79.91%	21.29%	24.1%
Salinity (min)	0 g/L	0 g/L	18.89 g/L	10 g/L
Nitrogen removal rate	TN	TN	TN	TN
	62.0%	61.87%	56.09%	66.88%
	NH_4^+-N	NH_4^+-N	NH_4^+-N	NH_4^+-N
	62.0%	61.37%	58.22%	38.5%
removal rate change	TN	TN	TN	TN
	+8.86%	+9.43%	-34.08%	-3.8%
	NH_4^+-N	NH_4^+-N	NH_4^+-N	NH_4^+-N
	+17.67%	+18.54%	-36.93%	-14.4%
Ref.	[34]	[35]	[33]	[32]

4. Effect of Phosphorus on Denitrification

Phosphorus, like nitrogen, is a significant factor causing eutrophication in water bodies. With the continuous development of industrialization in China, the total phosphorus content of wastewater is increasing, which decreases the self-purification capacity of water bodies [37], and the corresponding demand for phosphorus removal is gradually increasing. Many wastewater treatments process complete denitrification and phosphorus removal simultaneously, so there will be conflicts such as the effect of nitrate on phosphorus release, different sludge ages of nitrifying and phosphorus-polymerizing bacteria, and differences in nutrient and survival condition requirements of mixed microbial cultures [38], which will also make the carbon source requirements of the system more complex, for example, phosphorus-polymerization and nitrification are aerobic processes with low carbon source requirements, while denitrification requires an additional carbon source and nitrification wastewater reflux to denitrify, so the reflux of nitrification solution will inhibit the anaerobic phosphorus release process. There are also treatment processes that separate the phosphorus removal process from the nitrogen removal process to exclude phosphorus and nitrogen from interfering with each other [39], but they also cause an increase in the complexity of the separated anaerobic, anoxic, and aerobic phases, this conventional wastewater treatment process is often cost-intensive and time-consuming [40]. In recent years, domestic and foreign research scholars have studied the theory of biological denitrification and phosphorus removal different from the traditional ones, such as some strains have both denitrification and phosphorus removal based on nitrogen removal, so phosphorus concentration is a factor limiting the efficiency of nitrogen removal has attracted the author's interest. Existing studies have mainly focused on nitrogen removal and phosphorus removal, while the interaction between the two has been little studied.

In a study by Zhang et al. [41] on the effect of C/N on simultaneous denitrification and phosphorus removal in an oxidation ditch process, it was observed that the removal of both TN and TP was affected by the carbon source. When C/N$<$9, both denitrification and phosphorus release were inadequate due to the competition for carbon sources, and both removal efficiencies were low; with the increase of C/N, when C/N$>$11, the TN removal rate increased to 93.48% and the TP removal rate was close to 100%, which accomplished good simultaneous denitrification and phosphorus removal. The correlation coefficient of the linear regression curve obtained reached 0.985, indicating that the correlation between the two is high. Yang et al. [42] isolated a novel strain of NP5 with efficient heterotrophic nitrification, aerobic denitrification, and phosphorus accumulation, and NP5 was able to achieve simultaneous nitrification-denitrification and phosphorus removal (SNDPR) in a single reactor. In the analysis of the heterotrophic nitrification performance, it was found that this performance was influenced not only by organic carbon but also by the amount of P. The P/N ratio significantly affected the proliferation and nutrient removal of NP5 ($P<0.05$). The removal of $NH_4^+ - N$ increased with the increase of the P/N ratio; the removal efficiency of $NH_4^+ - N$ was higher when the P/N ratio was greater than 0.2, indicating that P is essential in bacterial growth, and the lack of P in the medium leads to the deficiency of NP5 for heterotrophic nitrification-aerobic denitrification. The removal efficiency of $PO_4^{3-} - P$ and $NH_4^+ - N$ decreased as P increased from an initial concentration of 20 mg/L to 50 mg/L and 100 mg/L, which may be due to insufficient reaction time and carbon source during incubation. Therefore, it can be inferred that although P facilitates cell growth and heterotrophic ammonium oxidation in strain NP5, a suitable P/N ratio is always required to achieve effective SNDPR. Chu et al. [43] investigated the effect of different potassium phosphate concentrations in MFC by adding them to the MFC for denitrification. Without the addition of potassium phosphate, the nitrate degradation was more complete and the nitrate exit mass concentration in the cathode chamber decreased from 100.00 mg/L to 8.15 mg/L. When the initial nitrogen to phosphorus mass concentration ratio increased to 1:2, the nitrate exit mass concentration in the cathode chamber became 27.56 mg/L, so it was concluded that the degradation of nitrate decreases with the increasing addition of potassium phosphate. The possible reasons for this are that under anoxic conditions, the addition of potassium phosphate increases the osmotic pressure in the cathode chamber reaction solution inhibiting microbial metabolism, and because potassium phosphate is converted to metaphosphate, which reduces microbial activity, resulting in a lower nitrate degradation rate. Rout et al. [44] evaluated the growth and removal capacity of strain GS-5 in the presence of ammonium, nitrate, and nitrite nitrogen, respectively. The phosphate removal efficiency was only 37% at 93% ammonium removal; 69.5% phosphate removal at 98.7% nitrate removal. The relatively low amount of nitrite and phosphate removal under nitrite nitrogen conditions compared to nitrate nitrogen may be due to the lower growth rate of microorganisms induced by nitrite toxicity.

5. Problems

CW forms CW-MFC by coupling with the MFC system, the decontamination ability is improved to a certain extent, and it has good potential to produce electricity and control cost at the same time, which has become one of the hot spots of research in recent years.

(1) The nitrogen removal principle of CW-MFC is based on the traditional biological denitrification theory and developed based on it. The main processes include nitrification, denitrification, short-range nitrification-denitrification, synchronous nitrification-denitrification, anaerobic ammonia oxidation, ammonification, etc. The combined processes include short-range nitrification-anaerobic ammonia oxidation, short-range denitrification-anaerobic ammonia oxidation, simultaneous denitrification by heterotrophic nitrification-aerobic denitrification, simultaneous anaerobic ammonia oxidation-partial nitrosation, etc. Although the processes are constantly updated and developed, they are always developed and innovated around the principles of nitrification, denitrification as well as ammonification, and the problems of wide footprint and carbon source dependence have never been better solved.

(2) Salt is a stressor for nitrification and denitrification processes, mainly causing microbial cells to dehydrate and separate cellular protoplasm in a saline environment to affect denitrification performance, so the denitrification efficiency tends to decrease when CW-MFC treats saline wastewater compared to ordinary wastewater. The effect of salt concentration on the inhibition of microorganisms in the anode and cathode zones is different, so the effect on the nitrification process and denitrification process is also different. From the results of each experiment, the effect of salinity on denitrification is greater than that on nitrification, because the C/N ratio in most saline wastewater is low, and the lack of carbon source in the denitrification process leads to insufficient nutrients for the growth of denitrifying bacteria and poor denitrification effect. At lower salinities, nitrogen removal rates appear to increase with salinity, which is associated with increased power production, increased cathodic electron donor, and enhanced microbial activity. When the salinity is too high, it not only disrupts the growth rate of wetland plants, reduces the nutrient uptake capacity, root mass, and root activity, but also shows microbial inhibition in the anodic zone and affects the activity and abundance of microbial flora, which in turn directly affects the denitrification performance of denitrification, so the nitrogen removal rate decreases with increasing salinity.

(3) The process of simultaneous denitrification and phosphorus removal from wastewater can affect each other due to the influence of nitrate on phosphorus release, the different ages of nitrifying bacteria and phosphorus-polymerizing bacteria mud, the differences in nutrient and survival conditions required for mixed microbial culture, and the competing demands of the system for carbon sources; separate treatment processes can exclude mutual interference between phosphorus removal and denitrification processes, but they can also cause constraints such as increased complexity and cost-intensive and time-consuming. Some strains have denitrification and phosphorus removal in addition to nitrogen removal, so their nitrogen removal rate is also related to phosphorus content in addition to the carbon source. Phosphorus is an essential element in bacterial growth, and a moderate amount of phosphorus can promote nitrogen removal efficiency. With the increasing phosphorus content in the wastewater, the removal efficiency of both $PO_4^{3-} - P$ and $NH_4^+ - N$ will decrease due to constraints such as reaction time and carbon source. In addition, if there is the presence of phosphate, phosphate can use nitrate and nitrite as electron acceptors and make all three removal rates increase, ammonia as an electron donor can not be

completed, but phosphate will also be converted to metaphosphate, which weakens microbial activity, resulting in lower denitrification rate.

6. Summary and Outlook

Compared with the stand-alone CW and MFC systems, CW-MFC can significantly improve the decontamination capacity and power production performance, reduce costs, and have great potential for environmental protection applications. The current research on the factors affecting the denitrification performance of CW-MFC mainly focuses on device structure, electrode materials, substrates and wetland plants, etc. In this paper, we discuss the denitrification mechanism, salinity, phosphorus, and other factors to provide more ideas for improving the performance of CW-MFC and provide references for promoting CW-MFC to practical applications. Among the measures to enhance the performance of nitrification-denitrification, measures can be taken to improve the electrical performance, simplify the denitrification process, and maintain the $NO_3^- - N$ concentration and $NO_2^- - N$ concentration in a moderate concentration range, while it should be noted that the $NO_3^- - N$ removal rate decreases with increasing $NH_4^+ - N$ concentration and too high $NH_4^+ - N$ concentration is not conducive to denitrification to proceed; in enhancing the microbial for denitrification at high salt concentration, long-term domesticated sludge, selection or cultivation of salt-tolerant microorganisms, and pre-desalination treatment of wastewater can be used; when treating phosphorus-containing wastewater, the C: N: P ratio in the wastewater can be regulated to keep the microorganisms in the best active state, and the reaction conditions should be controlled to avoid the generation of metaphosphoric acid. Although CW-MFC has many advantages, the real application to production life still has many shortcomings, in addition to the above-mentioned limitations, there are complex sewage composition, the impact on microorganisms and plants, internal resistance, high initial construction costs, and difficulty to promote and other problems. Therefore, the research on CW-MFC to improve the performance in denitrification and efficiently treat high-salt and phosphorus-containing wastewater is still one of the hot contents to be explored in the future.

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