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Review

Constructed Wetlands: A Review on the Role of Radial Oxygen Loss in the Rhizosphere by Macrophytes

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Received: 8 April 2018; Accepted: 23 May 2018; Published: 24 May 2018

Abstract: Constructed wetlands (CWs) are extensively used as an economical and environmentally friendly sewage treatment under ecological engineering technology. Knowledge of the oxygen in the rhizosphere is of primary importance in understanding the function and regulation of microbial communities and macrophytes. Numerous studies on radial oxygen loss (ROL) have greatly elucidated the mechanism of contaminant removal in CWs. The main sources of oxygen in CWs are atmospheric reoxygenation, macrophyte transmission, and artificial aeration. However, artificial aeration is very expensive, and atmospheric reoxygenation is limited. Therefore, ROL by macrophytes is an essential and economical approach for oxygen input in CWs. In this review, we attempted to study the role of macrophytes in CWs. We described the mechanism of ROL and summarized the methods for determining ROL. We also investigated the role of ROL in contaminant removal in CWs. This review will provide considerable useful information on the oxygen input of CWs.

Keywords: constructed wetlands; radial oxygen loss; rhizosphere; macrophytes; microorganisms

1. Applications and Mechanism of Constructed Wetlands

Constructed wetlands (CWs) are extensively used as an economical and environmentally friendly wastewater treatment method under ecological engineering technology [1,2]. CWs are artificial wastewater treatment systems that can be designed and controlled. It is a useful approach for the removal of abundant contaminants because of the synergistic effects of soil, macrophyte, and microorganism on the physical, chemical, and biological processes in the wetland system [3]. CWs are economical, highly efficient and environmentally friendly when used in wastewater treatment.

CWs are primarily composed of matrices, macrophytes, and microorganisms. Contaminants are degraded or converted under the synergies of the physical, chemical and biological elements in the system [1]. Physical action mainly consists of filtration and settlement. Filtration includes the natural settlement of particulate contaminants and the settlement of colloidal particles to remove some contaminants. Contaminants such as suspended solids can be settled within CWs, including the blocking effect of macrophyte roots and the gravitational interception of solid objects between sand and stone particles. Chemical action mainly consists of adsorption and degradation. Contaminants in ionized states can be removed by adsorption, which describes a chemical bond involving substantial rearrangement of electron density occurring on the macrophyte root surface. Degradation refers to the degradation of contaminants into more unstable compounds by redox reaction. Biological action refers to the plant uptake, the coupling effects between plants and microorganisms, and the decomposition of microorganisms. These processes are closely related to and affect one another. Oxygen is one of the key factors in these biological systems [4,5]. Therefore, the source of oxygen in wetland systems should be investigated. Moreover, CWs can generally be divided into three types, namely: free surface horizontal flow (HF), subsurface HF, and subsurface vertical flow (VF). Each type of wetland has its distinct source of oxygen. Oxygen sources can be divided into natural aeration (macrophyte transmission and atmospheric reoxygenation) and artificial aeration [6]. In natural sources of oxygen, the main oxygen reoxygenation method is atmospheric reoxygenation, because of the larger contact area of free surface HF with air [7]. The surface of the water is lower than the surface of the matrices in subsurface HF, and the oxygen inside the subsurface wetland is mainly from the wetland macrophyte roots. The main approaches to increasing the artificial oxygen in wetlands consist of adding an oxygenator, enhancing aeration, and adding an air compressor [8].

Adding oxygen to CWs can alter the distribution of microorganisms and increase their metabolic efficiency. Thus, this process has a positive significance for the removal of contaminants in sewage [9]. The input of oxygen in wetlands (especially subsurface HF) is one of the key factors in improving the removal of contaminants. However, the enhanced artificial oxygen will result in increasing operational costs, greater difficulty in monitoring, and overgrowth of microorganisms. Thus, the effects of macrophytes are being paid more attention. Augmenting oxygen in CWs by regulating macrophytes is more economical and reasonable as an oxygen input. In this paper, CW oxygen input from macrophytes and its influencing factors, determination, and effects on contaminant treatment are reviewed to better understand the application of oxygen released by the macrophyte root system.

2. Role of Macrophytes in CWs

Macrophytes are essential components in the design of wetlands. The Root Zone Theory, presented by Seidel and Kickuth in 1972, emphasized the role of macrophytes in the sewage treatment system of wetlands [10] and greatly promoted the study and application of CWs. In general, the role of macrophytes in CWs is divided into the following points.

2.1. Direct Functions

The contaminants can be absorbed directly by plant uptake. However, the removal efficiency of phosphorus and nitrogen through direct absorption is less than 5% and 10%, respectively [11]. Heavy metals in CWs can be removed by macrophytes, called "hyperaccumulators", through immobilization and phytotransformation [12]. Heavy metal content in "hyperaccumulators" comprises more than 0.1–1% of their dry weight [13,14]. Moreover, organic compounds such as phenols can be transported into plant vacuoles and intercellular spaces or transformed into lignin and other components [15].

2.2. Coupling Effects between Plants and Microorganisms

The stems, leaves, and roots of the macrophytes provide attachment sites for microorganisms. More importantly, macrophytes can provide oxygen by ROL and a carbon source from root exudates to the root zone for various processes (shown in Figure 1). Macrophytes transport oxygen obtained from photosynthesis and the atmosphere to the roots by air pressure gradient and diffusion. This phenomenon not only satisfies the respiration of the root, but also releases a part of the oxygen into the rhizosphere. This process is also known as the ROL [14]. ROL provides different aerobic environments for diverse microorganisms in the root system, and effectively plays a key role in microorganisms. This process is highly essential to the removal of contaminants. Root exudates are mainly acquired from the healthy tissues of macrophytes and the decomposition of aging tissue in the root, such as low-molecular-weight organic matter released by the root cell, root products, sticky polymer formed by metabolism, and the decomposition residue of root hair [15]. These root exudates can provide energy and electron donors for root biochemical reactions by releasing inorganic ions and various organic substances [16,17], and carbon sources for heterotrophic microorganisms of roots to promote microbial growth [18,19].

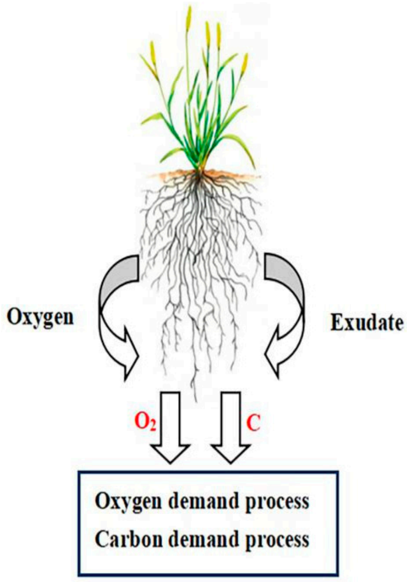

Figure 1. Oxygen supply by ROL and carbon source from root exudates in the root zone of the macrophyte.

In the wetland system, the macrophyte is the first producer, whereas the microorganism is the executor of contaminant removal. Microorganisms convert organic nutrients into inorganic nutrients for the absorption and utilization of macrophytes. Under the ground, the nitrogen-fixing bacteria in the macrophyte roots can affect the migration and transformation of nitrogen elements in the wetland [11]. Moreover, microorganisms promote the growth of macrophytes to some extent. Some rhizosphere microorganisms can live freely in the soil or attach to the roots, stems, and leaves of macrophytes to promote the growth of macrophytes. The main benefits of this relationship depend on the increase of the following: nitrogen content in roots, stems, leaves, and spikes; tiller number, root length, and root area; chlorophyll content and seed germination rate; and improvement in the macrophyte uptake of inorganic elements and water [20–22]. The interaction of macrophytes and microorganism holds or dominates the basic functions of the wetland system [20].

2.3. Other Functions

The macrophyte roots in CWs can enhance the porosity, reduce the sealing ability, and improve the permeability of the matrices. These characteristics strengthen and maintain the water transport in the wetland [23]. Macrophytes are essential in the design of wetlands. On the one hand, energy can be supplied by photosynthesis during sewage purification. On the other hand, macrophytes can improve the landscape environment, maintain moisture, reduce contaminant migration, and perform diffusion [10,24–26].

3. Role of ROL of Macrophytes

As an important part of CWs, macrophytes primarily transport oxygen to the roots through well-developed aeration tissues to adapt to the long-term flooding environment. Oxygen in wetlands is mainly derived from atmospheric reoxygenation, ROL, and influent reoxygenation. ROL is the main method of oxygen input in subsurface CWs [27,28]. Macrophyte root oxygen secretion can affect the distribution of dissolved oxygen and microorganism in the root micro-environment. This process has a positive effect on the removal of contaminants in CWs.

Macrophytes can be divided into emerging, floating, and submerged plants, depending on the different depths of water in the growth environment. The ROL rates of different macrophytes are shown in Table 1. Emerging plants, such as *Phragmites australis*, have an ROL rate of approximately 105.71–253.72 μmol O_2 d^{-1} g^{-1} DW_{root}. Floating plants, such as the *Arrowhead*, have a maximum ROL rate of approximately 126.64 μmol O_2 d^{-1} g^{-1} DW_{root}. Submerged plants, such as *Hornwort*, have an ROL rate of approximately 367.88 μmol O_2 d^{-1} g^{-1} DW_{root} in the light saturated condition. The oxygen contribution of the *Phragmites australis* root to CWs cannot be ignored, even during harvest in winter [29]. Therefore, ROL has gradually become one of the hot spots in the study of the decontamination mechanisms of CWs.

Table 1. ROL rate of different macrophytes.

Type	Macrophytes	ROL Rate (μmol O_2 d^{-1} g^{-1} DW_{root}) [1]	Ref.
Emerging plant	*Bog rush*	177.87 ± 10.02	[30]
	Cattail	124.82 ± 26.97	[30]
	Bamboo reed	84.13 ± 5.99	[30]
	Zizania aquatica	50.98–95.89	[31,32]
	Phragmites australis	105.71–253.72	[32]
	Calamus	117.46 ± 17.35	[33]
	Canna	120.54 ± 1.52	[30]
Floating plant	*Arrowhead*	126.64	[31]
	Calla	103.05	[31]
	Water hyacinth	19.8–67.15	[34]
Submerged plant [2]	*Eel grass*	190.66	[35,36]
	Hornwort	367.88	[35,36]
	Black algae	320.96	[35,36]
	Myriophyllum verticillatum	263.26	[35,36]
	Water caltrop	332.91	[35,36]

[1] Presents the amount of O_2 realised from plant per day per root biomass. [2] Measured in the light saturated condition.

3.1. Definition and Theory of ROL

Macrophytes deliver the oxygen by means of baric gradient and spread. This oxygen originates from the atmosphere and photosynthesis to the root system to adapt to the anoxic environment produced by water flooding [37]. The oxygen in the atmosphere can enter the interior of the macrophyte by thermo-osmosis and humidity-induced convection through the stomata.

One part of the oxygen transported to the roots is used for respiration, while the other part is released into the root micro-environment through root tips and lateral roots [38]. These parts have essential effects on various physical and chemical reactions and biological processes. The specific mechanism of this oxygen input is shown in Figure 2.

3.2. Factors that Affect ROL

3.2.1. Perspectives on the Input of Oxygen

The oxygen produced by photosynthesis is the main source of oxygen for ROL. Therefore, the factors that influence photosynthesis will also influence ROL. Environmental factors and the characteristics of macrophytes can affect photosynthesis [39]. Environmental factors, such as light intensity, light time, and temperature, and the characteristics of macrophytes, such as leaf area, net photosynthetic rate, and stomatal conductance, can indirectly influence the oxygen source of ROL by affecting the photosynthesis intensity of the macrophytes [40]. With enhancing photosynthesis, the concentration of dissolved oxygen in the root micro-environment and the rate of ROL increase [39]. The atmosphere is another source of oxygen. This oxygen mainly enters the pores of macrophytes through thermal infiltration and convection. Therefore, temperature and humidity are the two main factors that affect the entry of oxygen to the macrophyte [41].

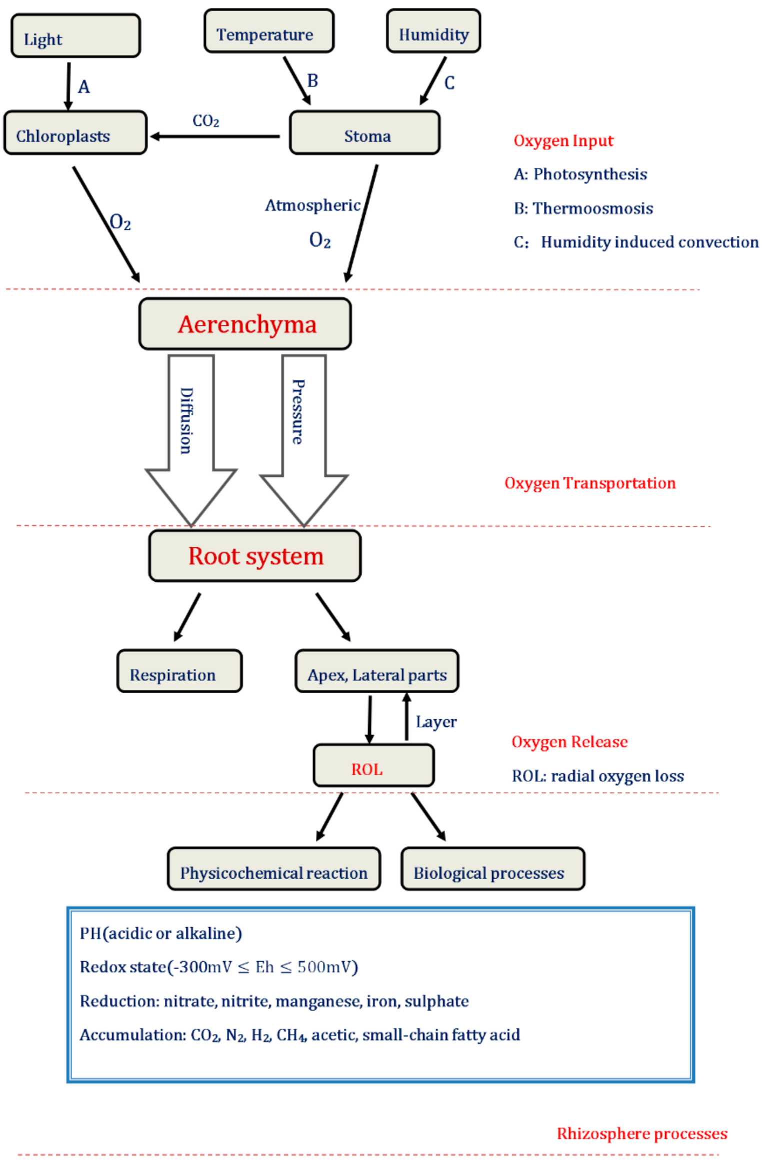

Figure 2. Schematic diagram of the ROL.

3.2.2. Perspectives on the Transportation of Oxygen

Oxygen can be transported because of the presence of aerenchyma in macrophytes [39]. Aerenchyma is the main channel by which oxygen is delivered in macrophytes. Given the varied proportions of aerenchyma in the different macrophytes, macrophytes also have distinct oxygen delivery capacities. An obvious positive correlation is observed between the ROL and the structure area of the root aeration tissue. The more developed the aerenchyma is, the more prosperous ROL will be [42]. Oxygen is transported within the aerenchyma by spreading and baric gradient. The diffusion of oxygen in the aerenchyma is affected by the metabolic activity of the root system. If the metabolic activities of the root cells consume abundant oxygen, oxygen will be difficult to transport to the rhizosphere [43,44]. Moreover, the respiration of macrophyte roots and oxygen content in the soil can regulate the oxygen delivery in the macrophytes through the baric gradient. Thus, the transport of oxygen in the aerenchyma should be further evaluated.

3.2.3. Perspectives on the Release of Oxygen

Numerous factors, which may be internal or external, affect the release of oxygen in the root system. From the source of oxygen, photosynthesis plays an important role in the root system oxygen secretion [39,45]. Therefore, external factors, such as temperature, humidity, light intensity, and atmospheric oxygen [40,46], can indirectly affect oxygen release by affecting photosynthesis.

Internal causes are mainly related to the metabolic rate of macrophytes and the physiological characteristics of macrophyte roots. The oxygen release rate of macrophytes at the growth stage is higher than that at other periods, and this is related to the concentration of oxygen that reaches the root system [47]. Moreover, the release of oxygen is limited by the root barrier, which is affected by the environment in which the root system is located [48]. When the oxygen is transported to the root, the oxygen is first used for respiration, and the remaining oxygen is released through the intercellular space of the root tip and the lateral root [49]. Therefore, during oxygen transport, the release rate of the rhizosphere is affected by the presence of the rhizosphere pressure, which is essential in relation to the number, length, and porosity of the root system [50,51]. To some extent, the more porous the root of the macrophyte is, the less the shielding effect of the ROL will be, which is the same as for the length and quantity of the root [46]. This phenomenon is found in the aeration of many different macrophytes.

4. Measurement of ROL

Several methods have been used to measure the ROL, and each method presents several advantages and potential disadvantages (listed in Table 2). Soaking of the root solution is one of the most common methods, in which the roots of the macrophytes are soaked in an oxygen-consuming medium that is separated from the overlying water [52]. The ROL is measured by determining the net absorption of oxygen. This method can be used to measure the whole root and to reduce root damage. However, the measured value is low, because oxygen is easily absorbed in the solution, and the macrophyte reabsorbs oxygen.

Table 2. The advantages and disadvantages of the methods of measuring ROL.

Measurement of ROL	Advantages	Disadvantages
The soaking method of root solution	Measure whole macrophyte, low damage	Low measured value
Titrimetric method	Analogy the conditions of the redox potential in the soil	Higher measurement of oxygen, less accurate
Oxygen micro-electrode method	Able to simulate soil conditions from the day to night	More expensive, fragile electrode
Sediment redox potential	Actual time determination	Easily influenced by environmental factors
Mathematical model method	Provide a theoretical basis	Easily limited by the data and assumptions
Measurement of rhizoplane	Well studied the root/soil interface soil conditions, easy to control	Different characteristics of the root system compared with non-interfering soil macrophytes

The titrimetric method is another method for measuring ROL. Utilizing the reducibility of Ti^{3+} results in the oxidation of Ti by the oxygen in the rhizosphere, this process is recorded by a spectrophotometer [53]. This method can show the conditions of the redox potential in the soil. However, the oxygen concentration cannot be detected anymore when all of the Ti^{3+} is completely oxidized. This method is not suitable for the overall measurement of macrophytes with high oxygen release (*Scirpus validus*, *Acorus calamus*).

The oxygen micro-electrode method is also a common method for measuring ROL. In this process, the oxygen released by the single root is measured by the anion and anode, and the natural environment is simulated. However, this method has several shortcomings, such as fragile electrodes and high cost [54].

Sediment redox potential is another method for measuring ROL. The amount of oxygen produced by the root system is obtained indirectly by analyzing the degree of redox of the sediments [55]. The potential can be measured in real-time, and continuous data can be obtained. However, this method is vulnerable to environmental factors, such as oxygen in the atmosphere, soil pH and redox potential (Eh) values, etc.

The mathematical model is not frequently used in the desired measurement. This process explains the actual problems by calculating the results and accepts the actual test to establish the whole process of the mathematical model. This technique facilitates a better understanding of the ROL by providing a theoretical basis for this parameter. The scale of the model should be determined according to the size of the measured sample. However, the establishment of the model is easily influenced by environmental factors, which are difficult to measure accurately [55].

5. Role of ROL in Wastewater Treatment

5.1. Effects on Organic Matter Removal

ROL has direct and indirect effects on organic matter removal. The direct effect of ROL on organic matter is that oxygen serves as the terminal electron acceptor during aerobic degradation [11]. The effect is achieved through the cation radicals of organic matter resulting from electron transfer from the organic matter to oxygen on the excitation of the charge transfer [56]. This process causes the alkalization of organic cations.

The indirect effect of ROL on organic matters is that it can form aerobic, facultative anaerobic, and anaerobic regions in the rhizosphere, providing suitable habitats for different microorganisms [57,58]. Aerobic microorganisms can consume oxygen to decompose organic matter to CO_2 and H_2O, providing energy and cell substances for microorganisms [59]. Anaerobic bacteria can break down organic matter to produce methane for energy and nutrition [60]. Rhizosphere oxygen plays an essential role in the degradation of organism transformation and mineralized microorganisms.

5.2. Effects on Nitrogen Removal

Nitrification and denitrification are primary processes for removing nitrogen from wastewater. These processes require aerobic and anoxic conditions, respectively [61]. Oxygen secretion in the macrophyte roots could remarkably increase the number of aerobic and facultative aerobic bacteria in the root micro-environment. According to the Root Zone Theory, ROL provides continuous aerobic, anoxic, and anaerobic conditions in soil, allowing simultaneous nitrification and denitrification [62]. The nitrifying bacteria and ammonia-oxidizing bacteria can use oxygen produced by ROL to transform ammonia into nitrate and nitrite, whereas denitrifying bacteria transform nitrate into nitrogen in the anoxic zone far from the roots [63,64]. In addition, ROL exerts a positive effect on the removal of nitrification-inhibiting substances, such as organic matter and heavy metals. Therefore, nitrogen removal efficiency is usually higher in constructed wetlands compared with general sewage treatment systems.

5.3. Effects on Heavy Metal Uptake

The ROL capacity of macrophytes is considered to be one of the most important biological factors in controlling heavy metal uptake. ROL can directly affect some metal mobility-regulating soil factors, such as Eh and pH. Previous studies showed that ROL increases Eh and decreases pH in the rhizosphere, concurrently with reductive substance oxidization [65]. Reductive substances in the rhizosphere, such as Fe^{2+} and Mn^{2+}, are oxidized to form a red-brown film called Ferromanganese oxide film, which is wrapped in the root system surface [66,67]. A thick Ferromanganese oxide film can become a barrier and enriched library for heavy metals and metalloid contaminants, promoting their migration and transformation in the medium [68]. In addition, H^+ produced by ionic oxidization, such as Fe^{2+}, was consumed by carbonates. Thus, heavy metals in carbonate form dissolve [65], and those in less available forms transform into a more easily available form.

6. Concluding Remarks

In this review, the key role of macrophytes and the specific mechanisms, determination methods, and functions of ROL in CWs were expounded in detail. The factors that affect ROL were summarized according to input, transportation, and release. The release of oxygen from the root system is influenced by many external and internal factors, regulated by environmental factors and macrophyte physiological characteristics. Bearing in mind the importance of ROL, many studies on the effects of the combination of environmental factors and physiological characteristics on the removal of pollutants can be safely ignored. Measuring the real-time distribution oxygen concentration in the rhizosphere enables the understanding of where and when the roots are active in respect to root respiration and how the soil responds to this process. The techniques for ROL determination were also summarized in this review. Numerous techniques, such as the titrimetric method, oxygen micro-electrode method, and soaking method of root solution were applied in measuring the ROL. However, these methods have several limitations in terms of cost, accuracy, and real-time determination. The interactions between oxygen secretion and the microorganism are of great significance for contaminant removal. Knowledge of oxygen concentration gradients is of primary importance in understanding the function and regulation of microbial communities and macrophytes. However, the specific coupling mechanism between the ROL and microorganism remains ambiguous. The mechanism of ROL and microorganism interactions should be examined further. Broad investigations on the enhancement of ROL could lay a foundation for optimizing CWs.

Author Contributions: Q.W. and Y.H. wrote, coordinated and reviewed the paper and finalized the data collection. H.X. and Z.Y. contributed to refining the paper structure and to improving the scientific aspects.

Acknowledgments: This work was supported by the National Science Foundation of China (Nos. 51608315 and 51708340), Promotional Research Fund for Excellent Young and Middle-aged Scientists of Shandong Province (Nos. ZR2016CB18 and BS2014HZ019).

Conflicts of Interest: The authors declare no conflict of interest.

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