Bioresource Technology 157 (2014) 316–326

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

How to increase microbial degradation in constructed wetlands: Influencing factors and improvement measures

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HIGHLIGHTS

- Microbial degradation is critical to pollutants removal in constructed wetlands.
- In this paper, key impact factors related to microbial processes are identified.
- This study reviewed current strategies used to improve microbial degradation.
- Special emphasis is given to the application of bioaugmentation in wetlands.

ARTICLE INFO

Article history:
Received 9 November 2013
Received in revised form 19 January 2014
Accepted 24 January 2014
Available online 4 February 2014

Keywords:
Constructed wetlands
Microorganism
Degradation

ABSTRACT

Microorganisms play a vital role in degradation of multiple pollutants in constructed wetlands (CWs). Thus, the search for methods to improve microbial degradation in CWs is crucial. This study provides a review of critical parameters including availability of organic carbon, redox condition, temperature, pH, presence of plants, media characteristics and their influences on microbial processes. Current strategies focusing on regulation of carbon source, redox condition, and choice of substrates to enhance microbial activity in CWs are also described. A special emphasis is given to the application of bioaugmentation to enhance microbial activities in wetland in future research.

1. Introduction

Compared to conventional wastewater treatment technologies, constructed wetlands (CWs) are economical, environmentally friendly and sustainable engineering systems due to their low cost, easy operation and low maintenance (Puigagut et al., 2008). Over the last few decades, CWs have been widely used for removing pollutants from a wide range of wastewaters such as domestic wastewater, industrial wastewater, agricultural wastewater, acid mine drainage, and even landfill leachate (Saeed and Sun, 2012). Many facilities have been developed and put into operation in Europe, America, Australia, New Zealand, etc. (Knowles et al., 2011).

While the purification performance of CWs is attributed to a variety of removal mechanisms including sedimentation, filtration, precipitation, adsorption, volatilization and plant uptake, it has been recognized that the removal of most pollutants in CWs is due primarily to microbial activity (Wu et al., 2012; Cui et al., 2013). The mineralization of organic compounds is mainly carried out by microorganisms both in anaerobic and aerobic conditions. Nitrogen removal is attributed to microbiological metabolism such as ammonification, nitrification–denitrification and anammox processes. In addition, microbes play a vital role in sulfur transformations, and removal of phosphorous and heavy metal (Faulwetter et al., 2009; Truu et al., 2009; Knowles et al., 2011; Saeed and Sun, 2012).

Currently, a growing number of studies have focused on the design, development, and performance of different types of CWs (Tanner, 2001; Nguyen, 2001; Dulekgurgen et al., 2006; Caselles-Osorio et al., 2007; Vymazal, 2007; Zhao et al., 2011; Saeed and Sun, 2012; Vymazal, 2013). However, little literature focuses on describing the microbial community and critical parameters influencing the microbial degradation ability in CWs (Faulwetter et al., 2009; Truu et al., 2009).

Seeing that organic matter and nitrogen are major target pollutants in most CW systems, this review firstly discuss and categorize the microbial populations critical to organic and nitrogen

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http://dx.doi.org/10.1016/j.biortech.2014.01.095
0960-8524/

removal in brief. The second section is devoted to the review of critical factors and their influences on the degradation capacity of microorganisms. Current and emerging strategies used to improve microbial degradation are also discussed in the following section.

2. Microbial community involved in constructed wetlands

Microbial degradation is an important process in CWs. The biodegradation of chemicals often involves a complex series of biochemical reactions and usually varies with the microorganisms involved. The dominant microbial communities related to removal of organic matter and nitrogen are summarized in detail below.

2.1. Microbial communities related to organic matter removal

Generally, biodegradation of organic matter in CWs is associated primarily with heterotrophic bacteria, autotrophic bacteria, fungi including yeasts and basidiomycetes, and certain specific protozoa (Kadlec and Wallace, 2009). The aerobic heterotrophic bacteria oxidize organics utilizing oxygen as the final electron acceptor and release carbon dioxide and other chemical compounds. The anaerobic heterotrophic bacteria perform the function of degradation in a two-step process: fermentation and methanogenesis. First, acid forming bacteria simplify biopolymers to monomers such as organic acids, monosaccharides, alcohols, and the gases CO_2 and H_2. Next, methane forming bacteria mineralize primary fermentation products to methane and carbon dioxide. In this process, methane oxidizing bacteria (methanotrophs) and methanogenic archaea are the dominant functional bacteria (Faulwetter et al., 2009; Truu et al., 2009).

Methanotrophic bacteria are characterized into three types based on structures of internal membranes and carbon assimilation pathways: Type I (phylogenetically with γ-Proteobacteria, proliferate under low methane conditions and high oxygen), Type II (phylogenetically with α-Proteobacteria, proliferate under high methane and low oxygen conditions), and Type X (phylogenetically with γ-Proteobacteria) clusters (Truu et al., 2009).

Sulfate reducing bacteria (SRB) also contribute to organic material removal in CWs. SRB are commonly anaerobes utilizing sulfate as a terminal electron acceptor. However, some species that persist in oxic conditions and survive extended periods of oxygen exposure have been discovered. According to the reports, 25% of the carbon removal was attributed to the SRB community (Faulwetter et al., 2009).

2.2. Microbial communities related to nitrogen removal

Due to variable nitrogen forms existing in CWs, phylogenetic groups involved in nitrogen removal are diverse. In general, the dominant microbial communities related to nitrogen removal include ammonia-oxidizing microbial communities, denitrifying microbial communities and anammox microbial communities.

2.2.1. Ammonia-oxidizing microbial communities

Ammonia oxidation is the first and rate-limiting step of nitrification that converts ammonium to nitrite in CWs (Pester et al., 2012). Ammonia-oxidizing organisms include ammonia-oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA).

AOB are aerobic chemolithoautotrophic microbes, and specific molecular markers for AOB are functional gene carrying α subunit of the ammonia monooxygenase (amoA) gene and CTO region of 16S rDNA (Truu et al., 2009). Based on CTO region of the 16rRNA gene, a total of around 15 different AOB populations can be distinguished. AOB belong to two phylogenetic groups: β-Proteobacteria (*Nitrosomonas* and *Nitrosospira* spp.) and γ-Proteobacteria (*Nitrosococcus oceani* and *Nitrosococcus halophilus*) (Faulwetter et al., 2009).

AOA is a new group of microorganisms which has been discovered and confirmed recently. Similar to AOB, AOA also have the functional amoA encoding the a-subunit of ammonia monooxygenase (AMO), which catalyzes the first step in ammonia oxidation. Based on ammonia monooxygenase subunit A (amoA) genes sequences, AOA were diversified into five major clusters: Nitrosopumilis cluster, Nitrososphaera cluster, Nitrosocaldus cluster, Nitrosotalea cluster and Nitrososphaera sister cluster (Pester et al., 2012; Munz et al., 2012).

2.2.2. Denitrifying microbial communities

Denitrifying microorganisms involve a wide range of bacterial groups, such as Bacillus, Enterobacter, Micrococcus, Pseudomonas, Spirillum, Proteus, Aerobacter, and Flavobacterium. Most denitrifying bacteria are facultative anaerobic chemoheterotrophs using organic compounds as electron donors and a source of cellular carbon and using nitrate as terminal electron acceptors (Vymazal, 2007). Denitrification is a four-reaction process converting nitrate to nitrogen gas in an anaerobic environment via intermediaries (Saeed and Sun, 2012). Specific PCR primers for nirK, nirS, and nosZ have been used in several studies to explore the genetic diversity of denitrifiers in CWs (Faulwetter et al., 2009).

2.2.3. Anammox microbial communities

Anammox is an anaerobic ammonium oxidation reaction, where ammonium is directly converted to nitrogen gas by nitrite (Faulwetter et al., 2009). Compared with nitrification and denitrification processes, the anammox process has the advantage of not needing external carbon sources. The dominant microbial population to perform anammox is planctomycete-like bacteria *Candidatus Brocadia anammoxidans* in wastewater treatment and *Candidatus Kuenenia stuttgartiensis* in bacterial biofilms. Anammox bacteria have relatively lower growth rate (0.04–0.06 d^{-1} at 35 °C), and biomass yield (Faulwetter et al., 2009).

3. Critical factors and their influences on microbial degradation

A multitude of different external and internal factors affect microbial processes in CWs, which could be generalized as several aspects: availability of organic matter, redox condition, temperature, pH, presence of plants and media characteristics. A brief discussion on the interactions between these factors and microbial degradation is presented below. In addition, this section also tries to discuss several mathematical models used to better understand microbial transformation and the degradation processes in CWs.

3.1. Availability of organic matter

Studies have shown that the quantity and quality of carbon supply in wastewater are essential for microorganisms to carry out a variety of removal processes. Generally, autotrophic nitrification is limited by inorganic carbon supply (Paredes et al., 2007), while denitrification depends on organic carbon (especially the easily degradable fraction) present in CWs. It was found that 2.86 g BOD is needed for entire denitrification of 1 g NO_3-N to be N_2 (Ye and Li, 2009). Additionally, Wu et al. (2009) concluded that the optimal operating condition of COD/N ratio was 5 to achieve most effective removal of nitrogen in a surface flow (SF) system. Zhao et al. (2011) observed highest TN removal rates at C/N ratio 5–10 in a two stage VF wetland planted with *A. calamus*. Fan et al. (2013) obtained high removals of COD (96%), ammonia nitrogen (99%) and total nitrogen (90%) at COD/N ratio of 10 in intermittently aerated vertical flow (VF) CWs. Due to uneven distribution