Bioresource Technology 166 (2014) 132–141

Contents lists available at ScienceDirect

Bioresource Technology

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

Antibacterial and enzymatic activity of microbial community during wastewater treatment by pilot scale vermifiltration system

Sudipti Arora [a,*], Ankur Rajpal [a], Renu Bhargava [a,1], Vikas Pruthi [b], Akansha Bhatia [a], A.A. Kazmi [a]

[a] Department of Civil Engineering, Indian Institute of Technology (IIT) Roorkee, Roorkee 247667, India
[b] Department of Biotechnology, IIT Roorkee, Roorkee 247667, India

HIGHLIGHTS

- The activity of earthworms enhances the BOD, COD and pathogens removal efficacy.
- Vermifilter showed more diverse and complex microbial community than geofilter.
- The antibacterial activity of microorganisms is accountable for pathogen removal.
- Enzymatic activity of microorganisms promotes the biodegradation of organic matter.

ARTICLE INFO

Article history:
Received 8 March 2014
Received in revised form 5 May 2014
Accepted 7 May 2014
Available online 21 May 2014

Keywords:
Wastewater treatment
Vermifilter
Pathogens
Antibacterial activity
Enzymatic activity

ABSTRACT

The present study investigated microbial community diversity and antibacterial and enzymatic properties of microorganisms in a pilot-scale vermifiltration system during domestic wastewater treatment. The study included isolation and identification of diverse microbial community by culture-dependent method from a vermifilter (VF) with earthworms and a conventional geofilter (GF) without earthworms. The results of the four months study revealed that presence of earthworms in VF could efficiently remove biochemical oxygen demand (BOD), chemical oxygen demand (COD), total and fecal coliforms, fecal streptococci and other pathogens. Furthermore, the burrowing activity of earthworms promoted the aeration conditions in VF which led to the predominance of the aerobic microorganisms, accounting for complex microbial community diversity. Antibacterial activity of the isolated microorganisms revealed the mechanism behind the removal of pathogens, which is reported for the first time. Specifically, cellulase, amylase and protease activity is responsible for biodegradation and stabilization of organic matter.

1. Introduction

The main source of pollution in water bodies is the discharge of sewage. Untreated sewage encompasses a large portion of biodegradable organic matter and numerous disease causing pathogenic organisms that causes detrimental effects on human health. Various technologies for wastewater treatment have been extensively investigated. Many on-site treatment technologies, such as septic tanks, aerobic biological treatment units, fixed-activated sludge treatment, membrane bioreactors, recirculating sand filter, trickling filters and constructed wetlands are functioning and effective in removing the pollutants (Xing et al., 2011). However, these technologies are restricted to use because at times, these are prone to failure due to high capital, operations and maintenance costs that are not affordable in developing countries (Muga and Mihelcic, 2008). Therefore, it is necessary to seek an alternative onsite wastewater treatment process that is economically affordable, environmentally sustainable, and socially acceptable (Wu et al., 2013).

Vermifiltration is a novel technology for wastewater treatment, which adopts modern concept of ecological design and extends the existing chain of microbial metabolism by introducing earthworms (Yang et al., 2013). It is a bio-oxidative process in which

Abbreviations: BOD, biochemical oxygen demand; CFU, colony forming unit; COD, chemical oxygen demand; DO, dissolved oxygen; EPS, extracellular polymeric substances; GF, geofilter; HLR, hydraulic loading rate; HRT, hydraulic retention time; FC, fecal coliforms; FS, fecal streptococci; MPN, most probable number; MR-VP, methyl red-voges-proskauer; OD, optical density; SEM, scanning electron microscope; SPC, standard plate count; TSI, triple sugar iron; TC, total coliforms; VF, vermifilter.

* Corresponding author. Tel.: +91 9690013440.
E-mail address: sudiptiarora@gmail.com (S. Arora).

[1] Deceased.

http://dx.doi.org/10.1016/j.biortech.2014.05.041

earthworms interact intensively with microorganisms within the decomposer community, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties (Liu et al., 2012). The inoculation of earthworms in conventional geofilter (GF), termed vermifilter (VF), has been widely used to treat municipal and industrial sewage (Wang et al., 2011, 2013; Xing et al., 2010, 2011) and sludge reduction and stabilization (Zhao et al., 2010; Liu et al., 2012; Yang et al., 2013). Microorganisms (bacteria and fungi) that populate in the filter bed along with earthworms reflect the functioning and performance of vermifiltration process (Wang et al., 2013). Earthworms achieve greater utilization of organic matter by the enzymatic activity of microorganisms in its filter bed (Sinha et al., 2008). Enzymes are biological catalysts giving pace and rapidity to biochemical reactions. Cellulases, amylases and proteases are a consortium of most important free enzymes that causes the hydrolysis of cellulose to glucose, starch molecules to finer products such as dextrins and proteins to amino acids (Gautam et al., 2012; Gupta et al., 2003). Organic matter is basically composed of cellulose, starch, sugars, proteins and lipids, which constitute the biodegradable portion of wastewater and these enzymes, have the potential to biodegrade the substrate proficiently.

Previous studies on vermifiltration only investigated the treatment efficacy on water quality. However information on the microbial community dynamics is also important to maximize the treatment capacity of the system. Fewer studies have reported the communities of ammonia-oxidizing bacteria in relation with removal of ammonia nitrogen (Wang et al., 2013) and evaluated microbial community diversity and structure in a tower VF, and their relationship with the removal of excess nutrients from wastewater (Wang et al., 2011). Earlier studies have reported bacterial removal rate constant and pathogen removal efficiency in conventional wastewater treatment system (Kadam et al., 2008). However, understanding the pathogen removal efficacy of vermifiltration is still a major research gap. No attention has been paid so far to the effect of earthworms on the antibacterial and enzymatic properties of the microbial community diversity and its role in VF. Thus, investigation of the microbial consortium and its functionality can help in understanding and controlling the vermifiltration process. Assessment of enzymatic activity of the isolated microflora brings an insight to the organic matter biodegradation during vermifiltration. To the best of author's knowledge, pathogen removal efficacy and its underlying mechanism is reported for the first time during vermifiltration.

Hence, the objective of the present study is to compare the treatment efficacy of a vermifilter (VF) with geofilter (GF) as a control by continuous monitoring of physico-chemical and microbiological parameters. The study was carried out in following steps: (1) to evaluate the removal rate of biochemical oxygen demand (BOD), chemical oxygen demand (COD), total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS) and enteric pathogens *Escherichia coli* and *Salmonella* from synthetic domestic wastewater; (2) to isolate, identify and characterize the population of total indigenous heterotrophic bacteria, total fungus and actinomycetes from VF and GF; (3) to evaluate antibacterial activity of isolated microflora against the tested microorganisms (gram-positive *Staphylococcus aureus* and gram-negative *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*); (4) to investigate the enzymatic activity (cellulase, amylase and protease) of the isolated microorganisms.

2. Methods

2.1. Experimental set-up and operation

The study was carried out in Environmental Engineering Laboratory, Department of Civil Engineering at Indian Institute of Technology Roorkee (IITR), India. A pilot scale reactor made up of perspex sheet, was set up as shown in Fig. 1. The reactor was divided into two chambers; one is vermifilter (VF) with earthworms and another is geofilter (GF), which is devoid of earthworms. It consisted of filter bed material, wastewater storage tank, wastewater distribution system and effluent collection system. Both the chambers were 80 cm long and 40 cm wide with a depth of 80 cm, and have 65 cm of packed bedding of different materials. An empty space or free board of around 15 cm is kept at the top for aeration purpose. The filter bed consisted of 4 layers. The description of filter bed layers is illustrated in Table 1. The pump and constant head tank were installed to collect and transfer the influent (wastewater) to the reactors. Wastewater passed through different layers in sequence by gravity flow. The experiments were performed during the months of June 2013 to September 2013 (120 days), when the average extreme air temperature was 30 °C (ranged 25–30 °C), which is the optimum temperature range for earthworm species (Tripathi and Bhardwaj, 2004).

2.2. Design parameters for pilot scale vermi-reactor

The design parameters of vermifiltration include hydraulic loading rate (HLR), hydraulic retention time (HRT), stocking density of earthworms, and the filter media. The volume and amount of wastewater that a vermifiltration system can reasonably treat in a given time is the HLR of the vermifilter system. It can be defined as the volume of wastewater applied, per unit area of vermifilter bed per unit time (Sinha et al., 2008). High HLR leads to reduced HRT and could reduce the treatment efficiency (Sinha et al., 2008). Hence, the HLR of the reactor was kept constant at $1.0\ m^3\ m^{-2}\ day^{-1}$ (320 L per day each for VF and GF) during all experiments (Li et al., 2009). Based on the flow rate and reactor configuration, the HRT was found to be 7.8 h. The performance of the system is measured in terms of BOD, COD, indicator organisms like TC, FC, FS and pathogens like *Salmonella*, *E. coli* removal efficiencies. The VF was inoculated with earthworm species *Eisenia fetida* at an initial earthworm stocking density of 10,000 worms/m^3 (Wang et al., 2013). There are reports about 8000–10,000 numbers of earthworms per cubic meter of the filter bed for optimal function (Sinha et al., 2008). *E. fetida* is readily available in the institute culture laboratory, where it has been cultured from last 10 years. During experiments, the natural habitat of the earthworms was not disturbed. They were maintained in suitable environmental conditions inside VF and were not harmed or killed during the study. Continuous feeding to earthworms and the earthworm activity ensured that the microbial-earthworm ecofilter was not in a state of inundation. This reasoning holds equally well for the use of *E. fetida* in the present study. The moisture content in VF during the study period was observed to be 85–90% throughout, which is an optimum range for earthworm growth and activity (Gunadi and Edwards, 2003).

2.3. Wastewater composition and characteristics

During the study, synthetic wastewater was used as the influent and was prepared in the laboratory by dissolving molasses, urea and KH_2PO_4 to give the ratio of COD/N/P as 300/30/1 (Seetha et al., 2010) such that it simulates actual domestic wastewater of medium strength. The influent characteristics are: pH 7.9 ± 0.1, temperature 27.4 ± 1.5 °C, dissolved oxygen (DO) 0.35 ± 0.3 mg/L, BOD 242 ± 30 mg/L, COD 456 ± 32, coliforms ranging 10^6–10^8 MPN/100 mL, *Salmonella* and *E. coli* ranging 10^6 CFU/mL, Total heterotrophic bacteria, total fungi and actinomycetes in the range 10^5–10^9 CFU/mL. The reason behind using synthetic wastewater was that a continuous study requires large amount of sewage, (approx. 650 L per day) which was not available near the study