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Reduction of organic contaminants and microbial communities in bioecological wastewater treatment system

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Wastewater treatment is a key factor in controlling eutrophication of freshwater bodies. Nutrients discharge in wastewater can cause water quality problems such as eutrophication of freshwater bodies, decreased conservation and recreational value of water systems, and destruction of aquatic life. A pilot scale bioecological wastewater treatment system which consisted of a modified anaerobic\anoxic\oxic (A_2O) system as a biological part and constructed wetland as an ecological part was developed to treat domestic wastewater (sewage). The study was carried out for pollutant removal performance of system and identification of microbial communities present in the system. The system showed excellent removal efficiency for chemical oxygen demand (COD) and organic nutrients such as ammonia, total nitrogen (TN) and total phosphorus (TP). Microbial count and distribution in different units of the bioecological system were dependent on associated factors such as, oxygen level, nutrients concentration and filtration from substrate. The bioecological system was proved to be quite effective in reducing total bacterial count (60%) as well as fecal coliform. The system offers simple operation, low energy consumption and high removal efficiency.

Key words: Wastewater, eutrophication, bioecological, constructed wetland, water quality.

INTRODUCTION

Raw wastewater contains biodegradable organic and inorganic compounds, toxic substances and microbial pathogens. The discharge of untreated wastewater is unsafe, both from health and environmental perspectives (Sehar et al., 2013). For the past several years, wastewater treatment technologies have improved mainly due to the more stringent nutrient discharge limits. Currently, wastewater treatment has become one of the world largest technologies for environmental protection due to increase in industrialization, urbanization and population growth (Nielsen et al., 2010). The practice of wastewater treatment technology is based on several

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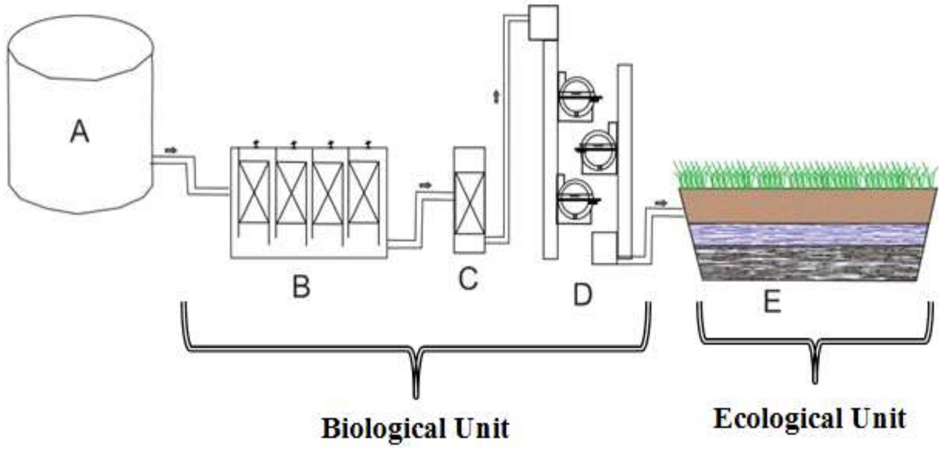

Figure 1. Schematic diagram of the bioecological system; A. Wastewater storage tank; B. Anaerobic baffled reactor; C. anoxic tank; D. Oxic unit (the unit consisted of three sub-units, each unit consisted of a rectangular tank, a rotating disc and a bio-wheel rotating disc); 7. Horizontal flow constructed wetland indicating the directions of wastewater flow

factors, such as origin and composition of wastewater, availability of land, skilled persons, population settlement in the community, etc. (Jhansi and Mishra, 2013). The conventional centralized wastewater treatment technologies are not suitable for the treatment of small populations and wastewater of rural areas because of the scattered and isolated locations which make collection of wastewater difficult; furthermore, high variability in flow rate and nutrients load is also an issue (Zhang et al., 2009). The sustainability of planet earth and reuse of its limited resources is a primary concern. Urban infrastructure and conventional treatment systems are built based on cost, convenience, the technology available and discharge limits for the treated wastewater (Abbasi et al., 2016).

Bioecological wastewater treatment is the combination of biological system and ecological system. For the past several years, anaerobic-anoxic-oxic (A_2O) system and constructed wetland (CW) has been applied to treat a different type of wastewater (Jin et al., 2014). In a bioecological system, various types of microorganisms play a significant role in nutrient removals such as nitrifying and denitrifying bacteria for nitrogen removal and phosphate-accumulating organisms (PAOs) for phosphorus removal (Kim et al., 2013). The function of the bioecological system has been characterized by raw wastewater compositions (chemical oxygen demand, total suspended solids and nutrients) and operational conditions, such as hydraulic retention time (HRT), pH, temperature and dissolved oxygen (DO) (Chan et al., 2009).

Many studies have shown that the impacts of global warming and climatic changes influence the temporal and spatial distribution of precipitation and hydrological cycles subsequent changes in water bodies (Zhang et al., 2010). The bioecological system is an alternative approach to avoid the disadvantages of conventional wastewater systems. Wastewater and its containing nutrients are recognized as a resource (not waste), which should be made available for reuse (Langergraber and Muellegger, 2005). The studied bioecological technology possesses biological unit and an ecological unit. The biological unit is A_2O (anaerobic/anoxic/oxic) and the ecological unit is CW (constructed wetland). The aim of this research was to study the performance of the bioecological system concerning chemical and biological pollutants and identification of microbial flora in different units of the system. The system requires simple construction and is easy for operation and maintenance with little cost. Recovering nutrients, reusing treated wastewater and consumption of low energy make bioecological wastewater treatment more sustainable (Abdel-Raouf et al., 2012).

MATERIALS AND METHODS

Reactor setup

A lab-scale bioecological wastewater treatment system consisted of two parts, a biological unit and an ecological unit (Figure 1).

Biological unit

Biological unit is A_2O system; consisted of an anaerobic baffled reactor (ABR), anoxic tank and oxic part. The anaerobic baffled reactor (ABR) was 1 m long, 0.2 m wide and 0.75 m high with 100 L effective volume. The reactor had five compartments divided by vertical baffles and filled with non-woven cloth. The anoxic tank was 1 m high, 0.2 m long and 0.2 m wide with 32 L effective volume and equipped with outlets at different heights. The oxic unit consisted of three aerobic turntable cells and each cell consisted of a rectangular tank (0.2 × 0.2 × 0.1 m in diameter), a rotating disc and a bio-wheel rotating disc, working on the watermill principle.

Ecological unit

The ecological unit was a horizontal flow constructed wetland (CW) consisting of a rectangular polyvinyl chloride (PVC) container (Length × width × height = 1× 0.2 × 0.6 m). The ecological unit was packed with three layers one over the other (gravel 15 cm as supporting layer, cobblestone (Yao et al., 2013) cm, sand and soil mixture 5 cm). The unit was planted with *Apium graveolens* (celery) because of its economic value in the local market. Horizontal subsurface flow constructed wetlands are widely used for treating domestic wastewater (Vymazal, 1996).

Experimental procedure

The experimental reactor had been running for over four months after system start-up. In the biological unit, ABR startup is a complicated process and need time to maintain full treatment capacity (Yu and Lu, 2014). ABR startup can be affected by many factors such as wastewater concentration and composition, pH, hydraulic retention time (HRT), temperature and reactor size (Hassan et al., 2015). For a start-up, the ABR operated for 50 days with different HRTs. Initial HRT was 72 h for 20 days and gradually reduced to 48 h for 15 days and later 24 h until the COD removal efficiency stabilized at 60%, and the pH stabilized between 7.03 and 7.23. The temperature during the whole study period was 20-32°C. The biological unit directly received wastewater from the storage tank, and after treatment from the biological unit, the effluent water was pumped to the ecological unit. Valves, nozzles and pumps were used to regulate the flow rate of water from one unit to another.

Sewage characteristics

The raw sewage for this study was obtained from the campus of the Southeast University at Wuxi. The average pH of raw sewage was 7.06, chemical oxygen demand (COD) 258.4 mg/L, TN 33.8 mg/L, NH_4^+-N 25.6 mg/L, TP 4.3 mg/L and TSS 276 mg/L. The wastewater was generated from dormitories, laboratories and restaurants on the University Campus.

Analytical methods

Chemical oxygen demand (COD), ammonia (NH_4^+-N), total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS) were analyzed according to standard methods (Federation and Association, 2005). Dissolved oxygen (DO) and pH were analyzed by DO200 and PH100 probes (YSI), respectively.

Bacterial profiling and microbiological analysis

Bacterial diversity colonizing and microbiological analysis in the bioecological unit were studied. For this purpose, 50 ml of the water sample from the effluent of each part of the bioecological unit was collected and serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}) were prepared in phosphate buffer saline (PBS) by serial dilution method (Holt, 1994). The appropriate dilution was selected and 1 ml water was taken by pipette and poured on nutrient agar (NA) plates. The water was spread on NA agar plates by using a sterile spreader and the plates were incubated at 37°C for 24 h. After incubation, colonies were distinguished by morphology, size and color.

For bacterial profiling and to obtain pure cultures, different colonies were further sub-cultured on *Salmonella-Shigella* agar (SSA), eosinmethylene blue agar (EMB), *Pseudomonas* cetrimide agar (PCA), mannitol salt agar (MSA), MacConkey's agar (MacA) and blood agar (BA). These plates were again incubated for 24 h at 37°C. On the basis of morphology, microscopy and biochemical characteristics, sub-cultured organisms were identified.

A microbial analysis was carried out by most probable number technique (MPN index) and colony forming unit (CFU/mL). The number of colonies was counted by placing the NA plate under colony counter and calculation was made according to the following formula:

CFU/mL = number of colonies × dilution factor/inoculum size

For the investigation of coliform, fecal coliforms and pathogens (*Escherichia coli*, *Shigella, Salmonella*, Klebsiella sp., *Citrobacter* and *Enterobacter*) samples were incubated in MacConkey's broth for 24-48 h at 42.2°C. Positive tubes were sub-cultured on NA, MacAand MSA plates and incubated for 24-48 h at 37 ± 2°C. Gram staining and light microscopy were done for the positive cultures to differentiate the Gram positive and Gram negative microorganisms.

Data analysis

SPSS version-18.0 (SPSS incorporation Chicago, Illinois, USA) and MS-excel programs were used for data analysis and presentation.

RESULTS AND DISCUSSION

Bioecological wastewater treatment system holds lots of attraction since is based on natural cycles instead of chemical and mechanical systems to treat wastewater (de-Bashan and Bashan, 2004).

Removal of organic pollutants and nutrients

Organic material and nutrients are crucial components of municipal wastewater which are responsible for eutrophication (Shon et al., 2006). The average influent concentration, effluent concentration and removal efficiencies of COD, ammonia, TN and TP during the study period are shown in Table 1. Influent concentrations of pollutants were between 180 to 321, 22 to 42, 17 to 36 and 3.2 to 5.5 mg/l for COD, TN, ammonia and TP, respectively. The variation in the influent concentration is because of the instable and discontinuous quantity and quality of the sewage water entering the system and impact of rainwater that diluted the raw sewage. The average effluent concentrations was 19.6 ± 4.6, 7.96 ± 1.84, 2.35 ± 0.99 and 0.36 ± 0.10 mg/l with the removal efficiency of 92, 78, 92 and 92% for COD, TN, ammonia and TP, respectively. The bioecological system showed highly significant effect ($P < 0.001$) for COD, TN, ammonia and TP.

Figure 2 shows the removal rate of pollutants in different units of the bioecological system. The system had an anaerobic, anoxic and oxic phase with a CW. The most effective COD removal took place in the anaerobic unit and it was supposed that the heterotrophic bacteria were responsible for chief quantity of organic matter removal (Yao et al., 2013). The average removal rate of

Table 1. Statistical analysis of pollutants.

	Influent (mg/L)			Effluent (mg/L)			Efficiency (%)		
	Min	**Max**	**Mean ± sd****	**Min**	**Max**	**Mean ± sd****	**Min**	**Max**	**Mean**
COD	185	321	268.2 ± 30.75	10	28	19.6 ± 4.6	90	95	92
TN	22	42	36.01 ± 4.59	3.6	11.2	7.96 ± 1.84	71	84	78
Ammonia	17	36	29.9 ± 4.42	0.5	4.6	2.35 ± 0.99	86	97	92
TP	3.2	5.7	4.7 ± 0.59	0.2	0.58	0.36 ± 0.10	87	95	92

COD stand for chemical oxygen demand, whereas TN is total nitrogen and TP represents total phosphorus. **is the probability value > 0.001.

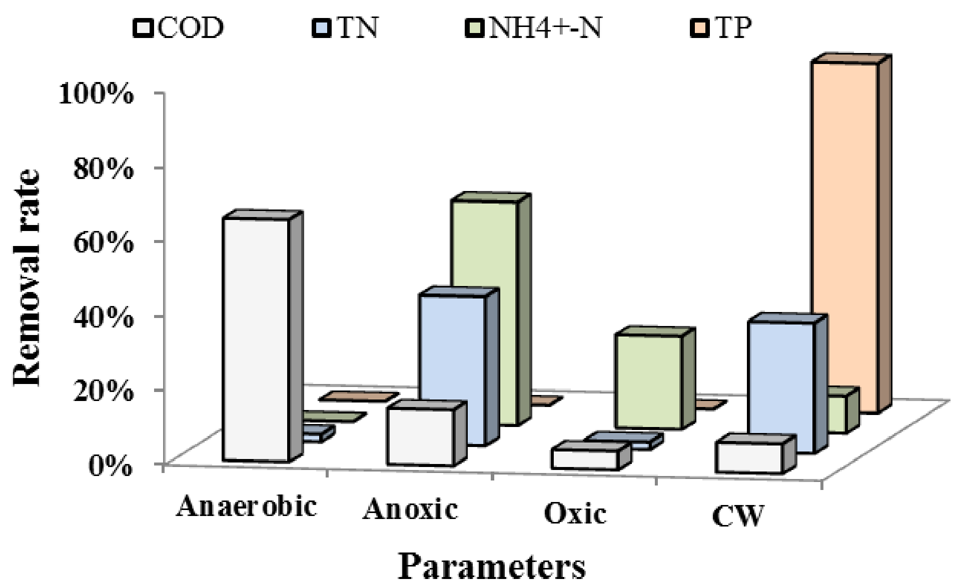

Figure 2. Removal efficiency of pollutants in different units of bioecological system.

TN in the anoxic tank and CW was 40 and 35%. Nitrogen removal occurred through nitrification, denitrification, volatilization and by plant uptake (Ye and Li, 2009). One of the primary functions of CW is nitrogen uptake by plants (Tunçsiper, 2009). The main part of TP was removed by the CW and adsorption, biological oxidation and plant uptake were the main processes for TP removal (Mina et al., 2011).

MPN Index of wastewater

Human excreta in municipal wastewater contain pathogenic organisms and consequently may be hazardous. Figure 3a and b show the variation of colony-forming unit (CFU) and MPN index different units of the bioecological system. Microbiological existence, survival and distribution are affected by the type of wastewater treatment units and associated factors (Cabral, 2010). The bacterial count for raw sewage was 9.3×10^7 CFU/mL. It shows an increasing trend of microbial count in ABR compartment I, II and III. This was possibly the result of elevated substrate concentration. Therefore, presence of conditions and nutrients for anaerobic microbial growth resulted in increased microbial number (Movahedyan et al., 2007). The compartment IV and V show low number of CFU and MPN, possibly because the less number of available nutrients for anaerobic microbial growth, as most of the nutrients, were consumed in first three compartments. In the whole bioecological system, the oxic unit shows highest number of bacterial count. Similar observations were found by Kim et al. (2013) for A_2O process and obtain 1,546, 2,158 and 3,743 reads in anaerobic, anoxic and oxic chambers, respectively. Although, there were increasing trend in bacterial count from ABR to oxic unit because of functionality and atmospheric conditions (Liu et al., 2007), however effluent from the final stage of bioecological system which was CW shows the lowest number of bacterial count. The sand bed present in CW successfully decreases bacterial count in wastewater after treatment from the bioecological system (Guchi, 2015).

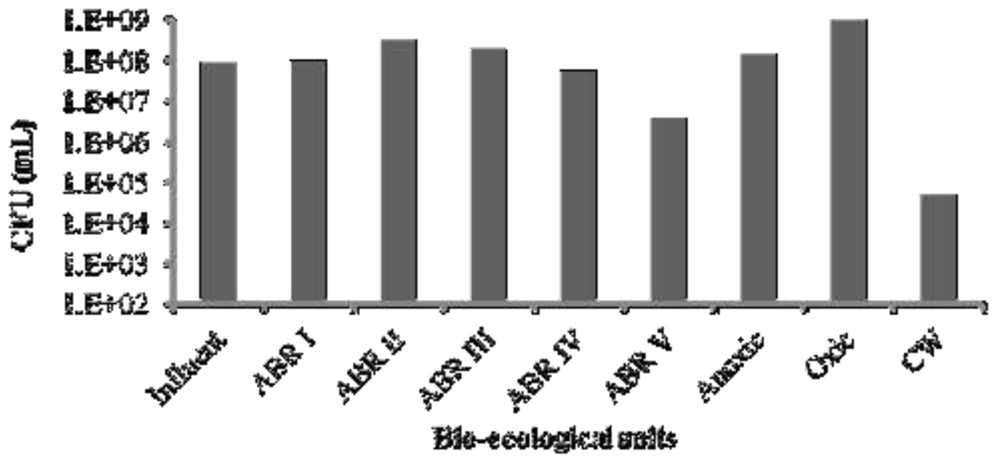

Figure 3a. Estimation of colony-forming unit (CFU) in different units of bioecological system.

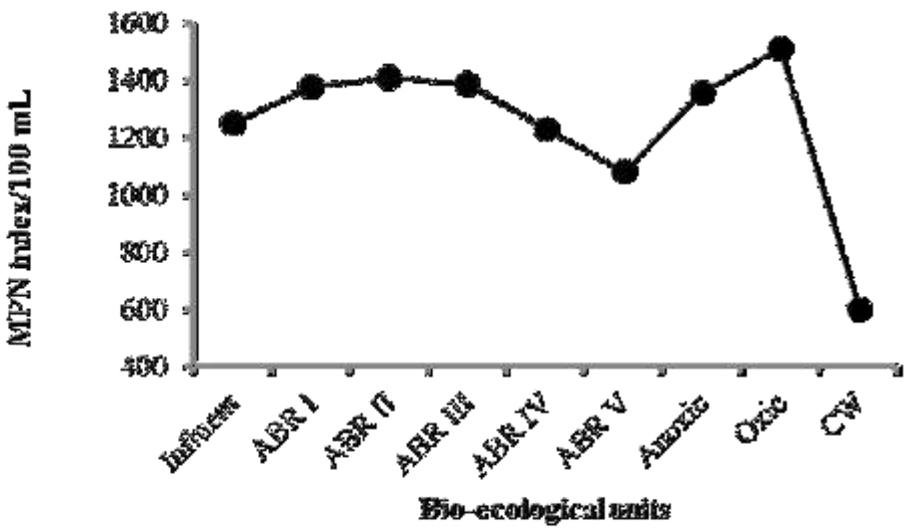

Figure 3b. Estimation of most probable number (MPN) in different units of bioecological system.

Bacterial profiling

The faces of a single individual may contain at least 300 different species of bacteria. Most of these bacterial species are strict anaerobes and remaining facultative anaerobes. *E. coli* is a common facultative anaerobe in feces. Bacteria from human excreta and other sources enter into the influent wastewater treatment system. Bacterial profiling of bioecological wastewater treatment system was performed in different units independently. The bioecological system serves as an important reservoir for accumulation of various types of microorganism groups. Morphological, microscopic and biochemical analysis were performed to isolate and identify the bacterial strains by following the protocols of Bergey's Manual of Determinative Bacteriology (Vos et al., 2011) from the water samples of bioecological systems (Table 2).

According to these findings influent sample was positive for *Staphylococcus aureus*, *Proteus* species, *Klebsiella spesies, Alcaligenes faecalis, Salmonella* species, *Escherichia coli, Shigella* species, *Bacillus* species, *Pseudomonas* species, *Enterobacter* species and *Micrococcus* species. In ABR, *Enterobacter, Shigella, Pseudomonas, Proteus, Shigella, Klebsiella* and *E. coli* species were positive in all compartments of ABR (ABR I, II, III, IV and V), whereas *Staphylococcus, Micrococcus, Bacillus* and *Alcaligenes* species was present only in ABR I. The presence of aerobic microflora in ABR I was found because it was positioned just next to

Table 2. Diversity of bacterial species in different units of bioecological system.

Species	Influent	ABR					Anoxic	Oxic	CW
		I	II	III	IV	V			
Staphylococcus aureus	+	+	-	-	-	-	+	+	+
Micrococcus species	+	+	-	-	-	-	-	+	+
Bacillus species	+	+	-	-	-	-	-	+	-
Enterobacter species	+	+	+	+	+	+	-	-	-
Shigella species	+	+	+	+	+	+	-	-	+
Pseudomonas species	+	+	+	+	+	+	+	+	+
Proteus species	+	+	+	+	+	+	+	+	+
Shigella species	+	+	+	+	+	+	+	-	+
Klebsiella species	+	+	+	+	+	+	-	-	-
Escherichia coli	+	+	+	+	+	+	+	+	-
Alcaligenes faecalis	+	+	-	-	-	-	-	+	-

storage tank, as the anaerobic conditions linger in other compartments of ABR (II, III, IV and V) the aerobic bacteria were unable to survive in absence of molecular O_2 (Kato et al., 1997).

The anoxic tank was positive for *S. aureus, Proteus* species, *Pseudomonas* species, *Klebsiella* species and *Escherichia coli. Bacillus* species, *Micrococcus* species, *Staphylococcus aureus, Pseudomonas* species, *Proteus* species, *Escherichia coli* and *Alcaligenes* species were isolated from the oxic unit. Whereas, the water sample of CW was positive for *Pseudomonas*, *Shigella*, *Proteus*, *Salmonella, S. aureus* and *Micrococcus* species.

Conclusion

Based on the conducted study, the main findings are as followings: The bioecological system performed very well for COD, TN, ammonia and TP removal: 92, 96.7, 83.6 and 95.3% removal efficiency, respectively. Microbial count, distribution and survival in different units were dependent on associated factors such as, oxygen level, nutrients concentration and filtration from substrate. The bioecological system was proved to be quite effective in reducing bacterial count as well as fecal coliform. The system is a low cost, energy saving, an alternative and appropriate technology to wastewater treatment, in particular for the rural regions.

Conflict of interests

The authors have not declared any conflict of interests.

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