Desalination 250 (2010) 568–572

Contents lists available at ScienceDirect

Desalination

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

The study of pathogenic microbial communities in graywater using membrane bioreactor☆

Jisun Jong, Jaejin Lee, Joonkyu Kim, Kyounghak Hyun, Taemun Hwang, Joonhong Park, Younkyoo Choung *

Department of Civil and Environmental Engineering, College of Engineering, Yonsei University, Seoul (120-749), Republic of Korea

ARTICLE INFO

Available online 30 October 2009

Keywords:
Pathogenic microorganism
Microbial community
Graywater
Reusing
MBR

ABSTRACT

Wastewater originating from any source in the residence except for the toilet is defined as graywater. If graywater is treated appropriately, it can be used as reused water. However, wastewater reclamation carries certain health risks, and hence this study is an analysis of pathogenic microorganism and microbial communities in treated graywater for the reuse of water treated by MBR. To reuse graywater, MBR system of a lab scale was constructed with sediment, anaerobic, anoxic and oxic reactors. In the oxic reactor, a submerged MF (pore size is 0.45 μm) membrane was installed to maintain activated sludge biomass. For the quantification of pathogenic organisms, a standard spread plate method was modified using the selective medium plates. Analysis of 16S rDNA was conducted to detect microbial community. Pathogenic microorganisms such as *Escherichia coli*, *Coliform*, *Staphylococcus aureus* and *Salmonella* were detected in effluent. According to analysis of phylum and class levels, species of microorganism become simplified through membrane. This suggests that the MF membrane in the MBR system could not perfectly remove microorganisms and further research in diverse pathogens is needed for wastewater reclamation.

1. Introduction

While wastewater reclamation secures alternate water sources and increases water usage, it also presents water pollution problems. Internationally, there has been continuous interest and effort in the area of wastewater reclamation, and it is an approach that is used in various areas such as industry, agriculture, gardening and toilet water. However, before wastewater reclamation is used on a widespread basis, not only must the technology be considered but also the hygienic and sanitary aspect of the water must be taken into account [1]. In Korea, with regard to wastewater reclamation, in addition to the physical and chemical indices, *Escherichia coli* (*E. coli*) is also regulated. In this experiment, graywater from household sewage in an apartment complex was used [2]. Graywater includes wastewater from the washing, kitchen, cooking, bathroom and shower, but does not include water from toilets (blackwater). Graywater has less microbes than blackwater and about 90% lower nitrogen levels, so treating both types of water together is needless [3–6]. When graywater is treated according to wastewater reclamation standards, it carries lower contaminants than blackwater, so it can be treated using lower levels of energy. In prior research, the main focus was development methods to secure a usable water source by using graywater, however it was unable to provide information on various microbes. Graywater can be used in various purposes, and as every use has a high potential of human contact there is a need to examine various microbes other than *Coliform*. In this research, numerous pathogenic microorganism, in particular *E. coli*, *Coliform*, *Staphylococcus aureus*, and *Salmonella tyohimurium*, were surveyed. Also, to examine the diverse distribution of microbes after water has been passed through the membrane, the 16S rDNA sequence analysis was conducted to compare microbial communities.

2. Materials and method

2.1. MBR technology

As can be seen in Fig. 1, the MBR (membrane bioreactor) used in this research is an A^2O reactor composed of anaerobic–anoxic–oxic reactors, and it takes on a combined form with the submerged MF membrane (pore size 0.45 μm). The volume of the anaerobic, anoxic, and oxic reactors were 2 L, 2.5 L, and 8 L, respectively. At the end of each reactor was a connecting pump to provide water circulation. In the anaerobic reactor and anoxic reactor, stirrers were installed to induce equalized mixture. In the oxic reactor, diffuser was installed to induce complete mixture within the microorganism mixture in the reactor. A baffle was placed between the oxic reactor and the sediment reactor, and there was some space between the baffle and the oxic reactor so that the mixture from the oxic reactor could flow into the sedimentation reactor, and precipitated sludge from the bottom of the sediment reactor could be conveyed back into the oxic reactor. This reactor has the advantage of correctly identifying the amount of

☆ Presented at the Conference on Membranes in Drinking and Industrial Water Production, 20–24 October 2008, Toulouse, France.

* Corresponding author. Tel.: +82 2 2123 7877; fax: +82 2 365 1965.
E-mail address: choung@yonsei.ac.kr (Y. Choung).

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doi:10.1016/j.desal.2009.09.025

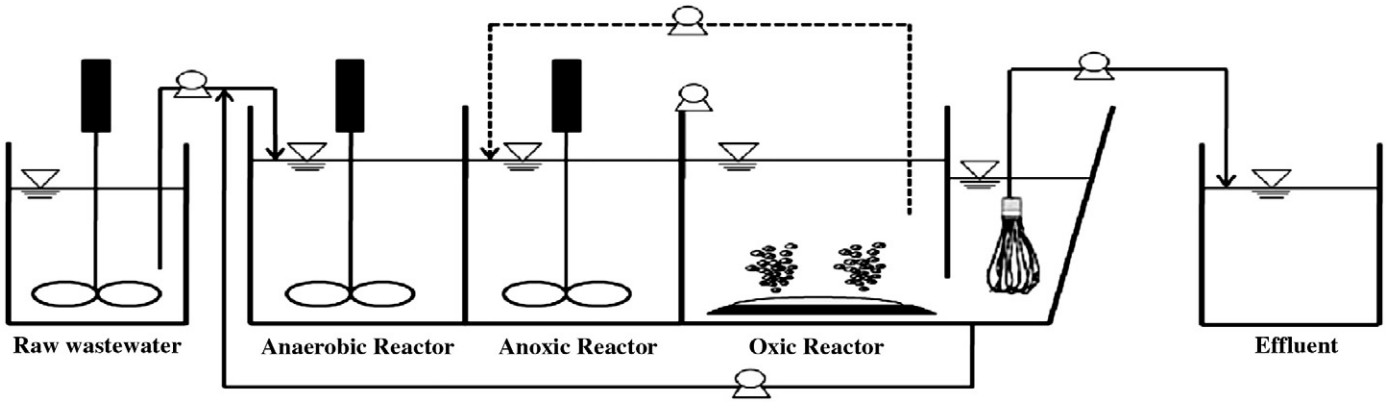

Fig. 1. Schematic diagram of membrane bioreactor system.

suspended solid substance within the reactor [7]. The temperature of the experiment equipment was maintained at 25 ± 3 °C, and the rate of internal recycle was sustained to Q, solid retention time limited to 10 days, and the amount of MLSS (mixed liquid suspended solids) was restricted to 6500–7000 mg/L.

Table 1 shows the characteristics of the submerged membrane used in MBR process. The MBR reactor has the advantage of preventing active microorganisms from out-flowing, thus maintaining the high density of microorganisms and increasing the removal efficiency of organisms [8].

2.2. Sample collection

Sample was collected from apartment located in Gyeonggi-do. Used water from households, such as water from washing, kitchen, cooking, bathroom and shower, but excluding water from toilets, was used. To maintain the equalization of the samples, graywater was stored in the equalization tank, and a sample was collected every week.

2.3. Physicochemical content analysis

Three times per week, CODcr, BOD, and SS values of influent treated water from each reactor and effluent were analyzed using a standard method [9]. Turbidity and color were measured three times a week using DR4000 (HACH, UV–VIS spectrophotometer, USA). And pH, DO and MLSS in the oxic reactor were measured everyday to examine the conditions in the reactor.

2.4. Measurement method for pathogens

The *E.coli.*, *Coliform*, *S. aureus*, and *S. tyohimurium* were measured through culture test. For *E.coli* and *Coliform*, CHROMagar™ ECC (CHROMagara, France) medium, for *S. tyohimurium*, CHROMagar™ *Salmonella* (CHROMagar, France) medium, and for *S. aureus*, Mannitol Salt Agar(Difco™) medium were used to detect each pathogenic microorganism. Every sample was spread on a plate, and the count of colonies were measured after 24~48 h of incubation [10,11].

2.5. Microorganism colony examination method by 16S rDNA

Samples collected from influent and effluent were filtered (Whatman, membrane filter, USA). After obtaining solid substances the DNA within the samples was collected through UltraClean™ Soil Isolation Kit (MO BIO Laboratories, Inc. USA), and was stored at −20 °C refrigeration. Used bacterial universal primer 27f-FAM [Flourescence labeled] (AGAGT TTGAR CATGG CTCAG) and 1492r (TACGG TTACC TTGTTA CGACTT) for 16S rDNA gene amplification from DNA extraction. Amplification of PCR (Polymerase chain reaction) was conducted for 3 min at 94 °C, 1 min at 94 °C, 30 s at 55 °C, 2 min at 72 °C for 30 cycles, and was finally set for reaction for 5 min [12]. PCR product joined pGEM-T easy vector, then culture using of the transformed cells LB (Luria-Bertani, Miller, Difco™) medium to which 50 μg/ml of Ampicillin was added were cultivated, and the conjugation colonies were collected and the 16S rDNA sequence was analyzed [13,14]. The analyzed sequence was compared to the registered database in NCBI Gene Bank (National Center for Biotechnology Information: www.ncbi.nim.hig.gov) [15].

3. Results and discussion

3.1. Results of physicochemical

Table 2 shows the summary of results from MBR. To maintain equalization, the samples were collected from the equalization tank, but there was an enormous density difference in terms of physicochemical contents between influent water.

The COD of influent was 119~3740 mg/L and average was 807.7 mg/L. The COD of influent was stably maintained at less than 7.85 mg/L. In terms of removal efficiency, regardless of the enormous change in influent, the efficiency was maintained to a satisfactory level

Table 1
Characteristic of membrane.

Item		SuperMAK®
Module type		Hollow fiber
Operation type		Submerged
Pore size		0.4 μm
Specification	Model	SuperMAK
	Fitting size	1/2 in. PT
Material	Membrane	PVDF (Poly vinyl difluoride)
	Head	ABS
	Core tube	PVC
	Bonding	Urethane and epoxy resin

Table 2
Characteristic of the influent and effluent.

Item	Influent	Effluent	Removal eff (%)
pH	7.02~7.86 (7.35)	7.12~7.85 (7.43)	–
DO	8.06~8.87 (8.44)	7.15~8.33 (7.74)	–
CODcr (mg/L)	119~3740 (807.7)	3~18 (6.57)	90.6~99.7
BOD (mg/L)	23.5~392.4 (254.6)	1.2~7.8 (93.17)	93.7~99.6
SS (mg/L)	72.5~4250 (2180)	0~4.3 (1.22)	98.2~100
Turbidity (NTU)	152~4400 (2131)	0~6 (1.63)	96.4~100
Color	15.8~52 (43.42)	0~4.2 (1.73)	73.4~100

Note: values inside parentheses are the average.