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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

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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

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* 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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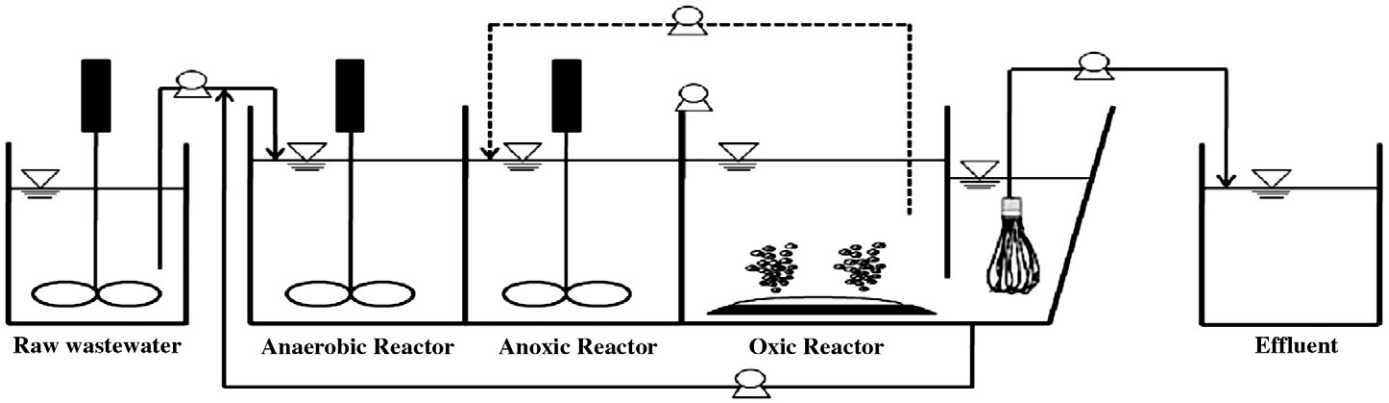

Fig. 1. Schematic diagram of membrane bioreactor system.

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.

Table 3
Standards for graywater usage.

Category	Toilet water	Sprinkler water	Landscape water	Carwash/ cleaning water
Escherichia coli	Must not be detected			
Combined residual chlorine	Over 0.2 ml/L	Over 0.2 ml/L	–	Over 0.2 ml/L
Exterior	User should not feel displeasure			
Turbidity	Under 2NTU			
BOD	Under 10 mg/L			
Odor	Must not give an unpleasant odor			
pH	5.8~8.5			
Color	Under 20	–	–	Under 20
CODmn	Under 20 mg/L			

of 90.6~99.7%. This result was made feasible due to membrane that maintains high MLSS density. The BOD of influent was 23.5~392.4 mg/L, and the average was 8.44 mg/L. The BOD of influent was maintained at less than 7.8 mg/L, and the removal efficiency was 93.7~99.6%.

The SS of influent was 72.5~4250 mg/L, average of 2380 mg/L and the SS of effluent maintained at 0~4.3 mg/L in accordance with MBR system characteristics. The removal efficiency was 98.2~100%. This result was attained because the membrane achieved almost complete solid–liquid separation. The turbidity of influent was 152~4400 NTU and 0~6 NTU in effluent. The removal efficiency was 96.4~100%. The color of influent was 15.8~52, color of effluent 0~4.2 and removal efficiency was 73.4~100%.

Table 3 shows standards based on the purpose of wastewater reclamation in accordance with the 'Enforcement Decree and Regulation of the Water Supply and Waterworks Installation Act' in Korea. The results of the research show that all treated by MBR, water satisfies the standards.

3.2. Pathogenic microorganism

According to the 'Enforcement Decree of the Water Supply and Waterworks Installation Act' the current standards for Korean wastewater reclamation provide only regulations regarding pH, color, turbidity, odor, exterior, COD, BOD, combined chlorine residual and *E. coli.* However, as there is a high possibility of human contact with the wastewater reclamation, there is a need to examine pathogens other than the regulated substances. This research specifically examines *E. coli.*, *Coliform*, *S. aureus* and *S. typhimurium*, and the characteristics of each pathogen are illustrated in Table 4.

The number of colonies resulting from the cultivation in influent, oxic reactor, effluent is shown in Table 4. To compare the total number of microorganisms and pathogens, Plate count Agar (Difco™, USA) was used to measure the number of microorganisms in influent water, oxic reactor water and effluent water [16–18].

As shown in Table 5, microorganisms were detected in all effluent water passed through the membrane. In the case of the *E. coli* of 0.5*1.5 μm and the *S. aureus* of 1 μm, they were all thought to be bigger than the pore size of 0.45 μm in, the membrane used in MBR process. And, the experiment results comparing influent and effluent show that the microorganism decreases, but the *E. coli* did not satisfy wastewater reclamation standards. Also, other pathogenic microorganism experiment results show that the total number of pathogens did decrease compared to influent, but they were still detected in effluent.

Table 4
Characteristics of pathogens.

Name	Characteristic
E. coli.	Diarrhea, abdominal cramps, low-grade fever, vomit
Coliform	Safety of water and food
Staphylococcus aureus	Food poisoning
Salmonella tyohimurium	Typhoid or typhoid-like fever in humans

Table 5
Detection of microorganisms from influent, oxic reactor, effluent (unit: CFU/mL).

	Total microorganism	*E. coli*	*Coliform*	*Staphylococcus aureus*	*Salmonella tyohimurium*
Influent	5832	40	12	18	54
Oxic reactor	5800	32	9	17	51
Effluent	5630	13	7	13	43

3.3. Microorganism colony comparison results

To compare the microbial communities in influent and effluent, 16S rDNA sequence was compared. Among the kingdom > phylum > class > order > family, colonies in the phylum and class levels were compared. Fig. 2 and Fig. 3 show the phylum and class levels of microorganisms in oxic reactor and effluent. In both samples *Proteobacteria* and *Betaproteobacteria* are dominant at phylum (oxic reactor: 64.3%, effluent: 69.2%) and class (oxic reactor:50%, effluent: 61.5%) level, which corresponds to major nitro-organisms such as *Nitrosomona* and *Nitrosococcus* and the pathogens examined in this research, *E. coli.*,*Coliform*, *S. aureus* and *S. tyohimurium.* In effluent water that has passed through the membrane, the diversity in phylum level increases, and there is no change in the dominant proteobacteria. However, the rate of bacteroidete decreases, while tm7, actinobacteria and verrucomicrobia are newly detected.

Fig. 4 shows a systematic analysis of the microorganisms, which was conducted to examine the variety of species in the influent and

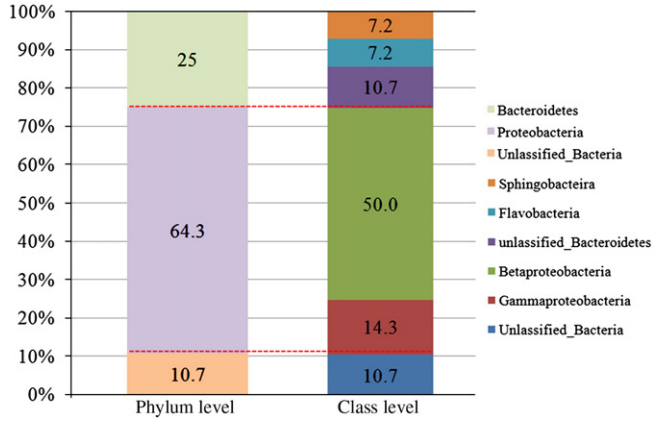

Fig. 2. Phylum and class level of oxic reactor.

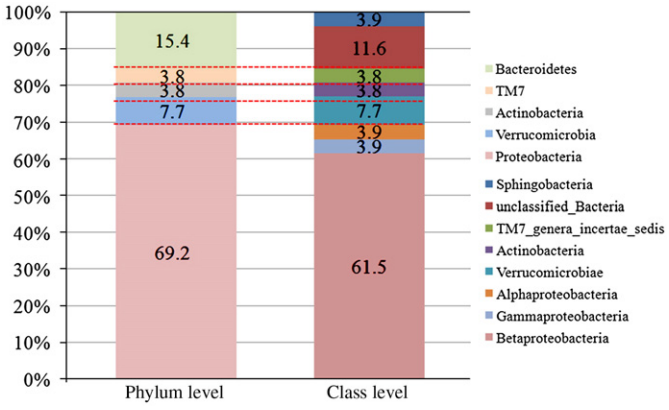

Fig. 3. Phylum and class level of effluent.

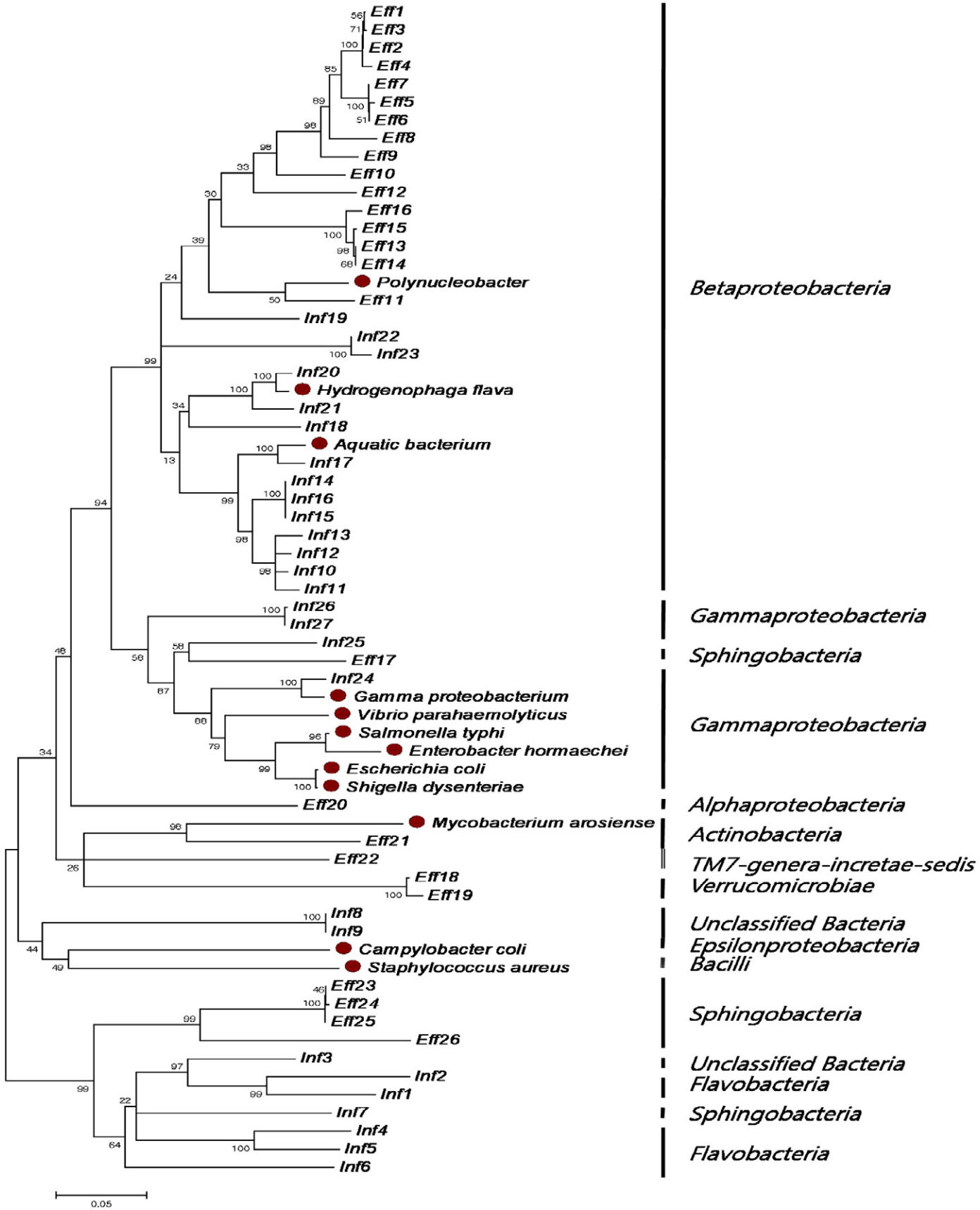

Fig. 4. Phylogenetic relationship of the oxic reactor and effluent. The evolutionary tree was constructed by the neighbor-joining method, and drawn using theMEGA program. (Inf: influent, Eff: effluent, ●: pathogenic microorganism).

effluent. Food poisoning bacteria expect *Hydrogenophage flava* (AJ420328), *Aquaticbacterium (*AB195755) and *Gammaproteobacteria F8* (AY077611) in Oxic reactor and expect *Polynucleobacter sp.* (AJ879783) in effluent. As seen in the figure, in influent and effluent the microorganisms show no close relationship, which means that there is no similarity between the two samples in terms of the microorganisms. In effluent, there is a systematic difference in microorganisms compared to influent, as some microorganisms could not pass through the membrane in the MBR process.

4. Conclusion

In this research, wastewater was collected from households and treated through MBR process to be used as graywater. Through physicochemical analysis, microorganism survey and molecular biology methods, the treated water was examined to confirm whether it satisfied graywater standards. In addition, the diversity of species of microorganisms between influent water and effluent water was examined. The results are as follows.

The physicochemical contents of treated graywater via the MBR process satisfied all wastewater reclamation standards. The membrane showed a high level of floating particle eradication, and it also increased the removal rate of organisms by maintaining MLSS, however it was not effective in removing microorganisms that were larger than the pore size. The colony comparison that examined the diversity of species showed that there was a definite difference of species in influent and effluent water, which was due to the size of microorganisms passing through the filter system in the membrane and the dominance of the microorganism. And the microbial community and 16S rRNA gene phylogenetic analysis showed that microbes in the effluent (after filtration) were significantly different from those in the oxic reactor (before filtration). This suggests a strong population selection power by the MF membrane. However, MF membrane could not remove to pathogenic microorganism perfectly. To use wastewater reclamation and reuse treated by MBR technology, disinfection system is proposed.

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References

[1] K. Jung, Korean Society of Water and Wastewater (2004) 111–120.
[2] G. Ramona, Desalination (2004) 241–250.
[3] A.C. Hrulimann, Desalination (2006) 167–177.
[4] M. Emaculate, Physics and Chemistry of the Earth 32 (2007) 1231–1236.
[5] W. Guo, Desalination (2008) 305–313.
[6] F. Fatone, Desalination (2008) 72–84.
[7] N. Kim, Korean Environmental Engineering Research 29 (2007) 204–210.
[8] B. Lim, J. Korean Society of Environmental Engineers (2002) 2101–2109.
[9] Standard Methods for the Examination of Water and wastewater, 20th ed., APHA, AWWA, WEF, 1998.
[10] L. Guardabassi, Water Research 36 (2002) 1955–1964.
[11] V. Schönenbrücher, International Journal of Food Microbiology 123 (2008) 61–66.
[12] D. Ki, KSCE Journal of Civil Engineering 27 (2007) 703–710.
[13] A. Hiraishi, Journal of Bioscience and Bioengineering 90 (2000) 148–156.
[14] N. pavese, Applied Soil Ecology 31 (2006) 251–266.
[15] Erick Cardenas, Environmental Engineering Research 14 (2009) 3–9.
[16] T. Sainz, International Journal of Food Microbiology 105 (2005) 357–367.
[17] J. Ottoson, Water Research 40 (2006) 1449–1457.
[18] M. Salgot, Desalination 187 (2006) 29–40.