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ORIGINAL ARTICLE

Estimation of Dominant Bacterial Species in a Bench-Scale Shipboard Sewage Treatment Plant

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Abstract

Recently, an innovative method for wastewater treatment and nutrient removal was developed by combining the sequence batch reactor and membrane bioreactor to overcome pollution caused by shipboard sewage. This system is a modified form of the activated sludge process and involves repeated cycles of mixing and aeration. In the present study, the bacterial diversity and dominant microbial community in this wastewater treatment system were studied using the MACROGEN next generation sequencing technique. A high diversity of bacteria was observed in anaerobic and aerobic bioreactors, with approximately 486 species. Microbial diversity and the presence of beneficial species are crucial for an effective biological shipboard wastewater treatment system. The *Arcobacter* genus was dominant in the anaerobic tank, which mainly contained *Arcobacter lanthieri* (8.24%), followed by *Acinetobacter jahnsonii* (5.81%). However, the dominant bacterial species in the aerobic bioreactor were *Terrimonas lutea* (7.24%) and *Rubrivivax gelatinosus* (4.95%).

Key words : Bench-scale shipboard STP, IMO, MBR, Microorganism, NGS, SBR

1. Introduction

Marine pollution is emerging as a serious threat to the environment globally; hence, there is an urgent need to address this issue, particularly in coastal countries. Shipping is the primary means of transportation worldwide and is involved in approximately 90 percent of global trade (Hanninen and Sassi, 2009). However, ship waste is becoming a huge source of contaminants. Sewage generated on ships cannot be stored on the ship for a very long time and, therefore, has to be discharged into the sea. However, ships are required to treat sewage before discharging it into the sea, and this is most commonly done through a biological method employing aerobic bacteria (Ersu, 2006; Lee, 2013), which requires less space, unlike other methods, as it can be performed in a tank. The biologically treated discharge is considered eco-friendly (Divya et al. 2015).

Biological shipboard sewage treatment involves processes that remove pollutants from wastewater with the help of microorganisms. This treatment system

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Table 1. Characteristics of the raw wastewater

Parameters	Units	Average measured values	MEPC. 227(64) Guidelines
Influent flow rate	L/h	2	
Effluent flow rate	L/h	2	
Temperature	℃	25 ± 3	
pH	-	7.2 ± 0.2	
BOD (conc.)	mg/L	875.5 ± 10.2	≥ 200 mg/L
COD_{Cr} (conc.)	mg/L	1050.4 ± 14.4	
TSS (conc.)	mg/L	1103.4 ± 25.2	≥ 500 mg/L
T-N (conc.)	mg/L	339.78 ± 1.8	
T-P (conc.)	mg/L	48.06 ± 0.5	

BOD: Biological Oxygen Demand; COD: Chemical Oxygen Demand; TSS: Total Suspended Solids; T-N: Total Nitrogen; T-P: Total Phosphorus; MEPC. 227(64): MEPC guidelines for artificial wastewater

mainly includes anaerobic processes (processes which operate in absence of oxygen), aerobic processes (processes which operate in presence of oxygen), or a combination of anaerobic and aerobic processes (Metcalf, 2013). These processes are flexible and may be easily modified, for example, by changing the operating conditions using control systems to optimize existing systems or by developing new systems using new equipment (Yamashita and Yamamoto-Ikemoto, 2014; van der Hoek et al. 2018).

Identifying the species of microorganisms present in an activated sludge system may help determine its properties (Theobald, 2014; Tang, 2017). When using indicator organisms for the biological diagnosis of activated sludge, it should always be kept in mind that their proliferation is affected by both physical and chemical environments (nutrients, water temperature, dissolved oxygen (DO), pH, toxic substances, etc.). The SBR-MBR system is a biological treatment method used to treat shipboard sewage waste. Here, we examined the dominant bacterial species in the aeration tank of a recently developed bench-scale SBR-MBR shipboard sewage treatment system using a Next Generation Sequencing (NGS) technique.

2. Materials and Methods

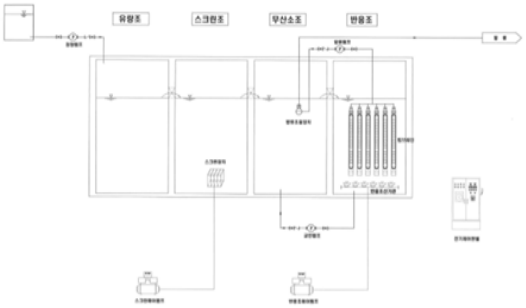
2.1. Wastewater collection and sample properties

The raw wastewater (influent) used in this study was collected from the riverside treatment plant, Busan, Republic of Korea. The average properties of raw wastewater are shown in Table 1. The influent was supplied into the flow metering pump at a constant flow rate. The water quality standards set by MED were satisfied, with BOD_5>200 mg/L and TSS>500 mg/L.

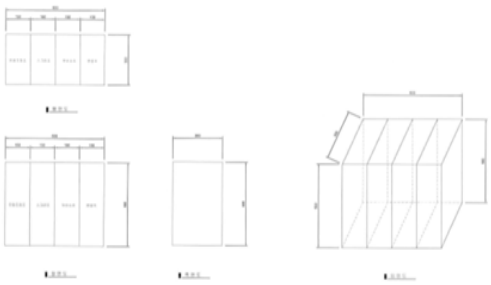
2.2. Experimental apparatus

The device used in this experiment was designed to be of a minimum size to ensure smooth operation of the bench-scale SBR process. The device specifications are (L) 567 mm × (W) 300 mm × (H) 502 mm, and the flow rate of raw wastewater was 2 L/h for each experimental condition. The developed device consists of a down flow anaerobic reactor, a screen, an aerobic bioreactor, and a membrane reactor, and a constant level of raw wastewater was maintained through a pump to check runoff. A specific cover for the bioreactor was made to conveniently inspect the equipment, replace any broken parts, and prevent bad odors during the operation. The device and its piping and valves were made of lightweight and corrosion

(A) Front view of the Bench-scale shipboard advanced wastewater treatment plant



(B) Scheme of shipboard STP



(C) A drawing of shipboard STP for this experiment

Fig. 1. Experimental apparatus used in this study.

-resistant acrylic material. The developed device is shown in Fig. 1(A) and the schematic diagram of the shipboard STP is shown in Fig. 1(B) and Fig. 1(C).

Raw wastewater was supplied directly into the anaerobic bioreactor and through a mesh material screen into the aerobic bioreactor; filtered suspended solids (SS) were stocked at the bottom of the device to form the anaerobic tank. The reaction was carried out at a bench-scale and involved merged SBR and MBR processes. The aeration time in the MBR separator (a hollow fiber membrane) was set at 7 min operating time and 3 min stop time, and treated water was collected through a pump.

2.3. Operating conditions of bench-scale SBR system

The existing SBR method involves the following steps: aeration → precipitation → mixing → precipitation. However, the precipitation time is reduced in the bench-scale SBR-MBR process. This study was performed using repetitive operation of mixing and aeration processes. The C: N: P ratio of raw wastewater was adjusted at 10:3:1 and the removal efficiencies of T-N, T-P, and COD_{cr} from the final effluent were measured. The device was designed to use the bench-scale SBR method to maximize efficiency, while occupying a limited space on a ship.

According to the preliminary studies, the optimum operating conditions for the effective treatment of raw wastewater in a sewage treatment plant were found to be 70 min of mixing and 50 min of aeration. Thus the dominant microbial species were only examined under the optimal operating conditions. These operating conditions are presented in Table 2.

2.4. Microbial Analysis

In this study, the dominant species of microorganisms in the anaerobic and aerobic tanks were analyzed under optimal operating conditions for

Table 2. Operating conditions of the Bench-scale shipboard STP

Parameter			Unit	Conditions
Anaerobic tank	Drain flow		L/min	Auto
	Screen	Aeration	sec	11
		Air flow	L/min	121
		No-aeration	min	5
SBR reactor	MLSS	-	mg/L	2,000
	Mixing	-	min	70
	Aeration	-	min	50
MBR (only aeration period)	Drain flow	-	L/h	2
	Drain (Off/On)	-	min	7/3
HRT			min	360

sewage treatment using a recently developed bench -scale SBR-MBR wastewater treatment facility. The NGS system developed by MACROGEN Co. Ltd., Seoul, Republic of Korea, was used to analyze the microbial community in the wastewater and the species it included. The NGS experimental method involved four steps: 1) DNA/RNA extraction 2) Library construction 3) Sequencing and 4) Bioinformatical analysis. The experimental procedure is described in Fig. 2.

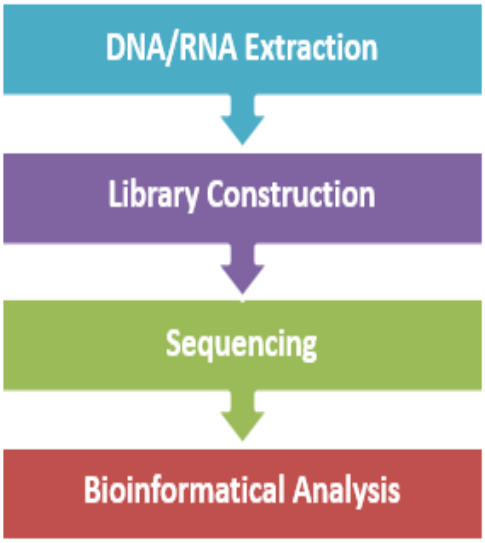


Fig. 2. Overview of NGS experimental process.

2.4.1. DNA/RNA extraction

Genomic DNA and RNA were extracted using following the manufacturer's instructions with a slight modification. The quality and quantity of DNA and RNA were checked after extraction.

2.4.2. Library construction

The sequencing library was prepared by random fragmentation of DNA and cDNA samples by 5′ and 3′ adapter binding. The tagmentation reaction was used to combine fragments and link reactions in a single step, which greatly enhanced the efficiency of library preparation. The adapter and associated fragments were amplified by polymerase chain reaction and purified by gel electrophoresis.

2.4.3. Sequencing

For cluster creation, the library was stored in a cell, capturing fragments from surface-bonded oligos complementary to the library adapter. Each fragment was, then, amplified into colonial clusters distinguished by bridge amplification. Once cluster creation was complete, the templates were sequenced using the Illumina Sequencing By Synthesis (SBS) technique.

The SBS technology detects a single base bound to a DNA template strand using a unique, gauge terminator-based method. Four reversible inversions

are possible; thus, a terminator-coupled deoxy nucleoside triphosphate (dNTP) is required during each sequencing cycle. Natural competition minimizes bias and reduces error rates as compared to other technologies, resulting in accurate base sequencing that virtually eliminates sequence – context - specific errors, including repetitive sequence regions and homopolymers.

2.4.4. Bioinformatical analysis

Sequencing data obtained through Illumina SBS analysis were converted into raw data for further analysis. For metagenomics profiling, reads containing low-quality or ambiguous bases were discarded. Taxonomic assignment was performed by comparing sequencing reads using BLAST-based searches and additional pairwise similarity comparisons.

3. Results and Discussion

Previously, we modified the activated sludge system by combining the SBR and MBR processes to create an innovative shipboard sewage treatment system (Choi et al., 2018). The performance of this system was, then, analyzed by changing different factors. In this study, at the optimum operating condition (70 min mixing and 50 min aeration), contents of the anaerobic tank and the aerobic bioreactor were analyzed for the identification of dominant species using the MACROGEN NGS technique. Illumina sequencing revealed great bacterial diversity in the tested samples. The results showed that the bacterial community of the bench-scale SBR-MBR system included 486 species and the top 10 microorganisms adapted to the anaerobic tank and aerobic bioreactor are listed in Tables 3 and 4.

The most abundant microorganism in the anaerobic tank was *Arcobacter lanthieri* (8.24%), followed by *Acinetobacter jahnsonii* (5.81%); the bacterium *A. lanthieriis* known to reduce nitrate to nitrite (Pérez -Cataluñaet al., 2018). The most abundant microorganism in the aerobic bioreactor was *Terrimonas lutea* (7.24%), which is a gram-negative aerobic bacterium. *Dechloromonas hortensis* is an anaerobic bacterium and has the ability to reduce chlorate (Lindqvist et al., 2012). The presence of *D. hortensis* in the bench-scale SBR-MBR system indicates that a large proportion of oxygen remains in the aeration tank and oxygen is not properly transferred to all parts of the reactor during the aeration process.

Table 3. Dominant microorganisms found in the anaerobic tanks of Bench-scale SBR process

Dominant microorganisms	Anaerobic tank(%)
Arcobacter lanthieri	8.24
Acinetobacter johnsonii	5.81
Dechloromonas hortensis	5.24
Aquabacterium parvum	3.08
Comamonas granuli	2.05
Hydromonas duriensis	1.95
Owenweeksia hongkongensis	1.83
Terrimonas lutea	1.68
Marinimicrobium koreense	1.66
Byssovorax cruenta	1.64

Table 4. Dominant microorganisms found in the aerobic tanks of Bench-scale SBR process

Dominant microorganisms	Aerobic tank(%)
Terrimonas lutea	7.24
Rubrivivax gelatinosus	4.95
Byssovorax cruenta	3.79
Dechloromonas hortensis	3.73
Mangrovitalea sediminis	2.88
Racemicystis persica	2.29
Aggregicoccus edonensis	1.88
Nannocystis pusilla	1.81
Kofleria flava	1.56
Aquabacterium parvum	1.42

Biological wastewater treatment systems are mostly designed from an engineering perspective; however, the ecological and dynamic aspects of microbial communities involved in these systems should also be taken into consideration (Cydzik-Kwiatkowska and Zielińska, 2016). Understanding the microbial communities in biological wastewater treatment systems is essential for predicting the possible variations in their function and structure in response to various conditions. The stability of a biological treatment system is affected by the diversity and dynamics of its microbial community (Kim et al., 2010; Kim, 2017). The dynamics of microbial communities are influenced by deterministic (competition and niche-specific variables) and stochastic (microbial dispersion) factors (Ofiţeru et al., 2010).

4. Conclusion

To improve a wastewater treatment technology, an in depth understanding of its microbial community and how this community is affected by the treatment system is crucial. In this study, we analyzed the dominant species of microorganisms in a bench-scale SBR-MBR sewage treatment system under optimal operating conditions using the MACROGEN NGS technique. *Arcobacter lanthieri* was found to be dominant in the anaerobic bioreactor, and *Terrimonas lutea* was found to be the dominant species in the aerobic bioreactor. In the aerobic bioreactor, the SBR process alternates between mixing and aeration, and a large proportion of oxygen remains in the aeration tank indicates that oxygen is not properly transferred to all parts of the reactor during the aeration process. It is, thus, necessary to find a way to distribute oxygen thoroughly in the aerobic bioreactor during the aeration process. A comprehensive study is required to further understand the precise role of each microorganism involved in this bench-scale SBR -MBR wastewater treatment system.

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References

Choi, Y. I., Shin, D. Y., Mansoor, S., Kwon, M. J., Jung, J. H., Jung, B. G., 2018, A Study on microorganism dominant species in Bench-scale shipboard STP using combined SBR and MBR process, Journal of the Korean Society for Environmental Technology, 19, 550-555.

Cydzik-Kwiatkowska, A., Zielińska, M., 2016, Bacterial communities in full-scale wastewater treatment systems. World Journal of Microbiology and Biotech - nology, 32, 66.

Divya, M., Aanand, S., Srinivasan, A., Ahilan, B.. 2015, Bioremediation - an eco-friendly tool for effluent treatment: A review, International Journal of Applied Research, 1, 530-537

Ersu, C. B.. 2006, Biological nutrient removal in Bench-scale membrane bioreactor and full-scale sequencing batch reactor under various configurations and conditions. Retrospective Theses and Dissertations, 1254, Iowa State University, IA, USA.

Hanninen, S., Sassi, J., 2009, Estimated nutrient load from waste waters originating from ships in the Baltic area, Research report VTT-R-07396-08, VTT Technical Research Center of Finland, Finland.

Kim, M. K., 2017, Rhodopsin reconstruction reveals the presence of Bacteroidetesderived rhodopsin from fresh water environment. Master's dissertation, Sogang University, Seoul, Republic of Korea.

Kim, T. S., Kwon, S. D., Kim, H. S., Lee, S. Y., Hwang, S. H., Park, H. D., 2010, Analysis of bacterial community composition and dynamics in an activated sludge wastewater treatment plant, Korean Society on Water Environment, 2010:85-86.

Lee, D. H., 2013, Development of MBR system for treatment of RO concentrated water generated from sewage reuse process. Master's dissertation, Myongji University, Seoul, Republic of Korea.

Lindqvist, M. H., Johansson, N., Nilsson, T., Rova, M., 2012, Expression of chlorite dismutase and chlorate

reductase in the presence of oxygen and/or chlorate as the terminal electron acceptor in Ideonella dechloratans. Applied and Environmental Microbiology, 78, 4380 -4385.

Metcalf, E.. 2013, Wastewater engineering: Treatment and resource recovery, McGraw-Hill Education, New York.

Ofiţeru, I. D., Lunn, M., Curtis, T. P., Wells, G. F., Criddle, C. S., Francis, C. A., Sloan, W. T., 2010, Combined niche and neutral effects in a microbial wastewater treatment community, Proceedings of the National Academy of Sciences of the United States of America, 107, 15345-15350.

Pérez-Cataluña, A., Salas-Massó, N., Diéguez, A. L., Balboa, S., Lema, A., Romalde, J. L., Figueras, M. J.. Revisiting the taxonomy of the genus Arcobactor: getting order from the Chaos, Frontiers in Microbiology, 9, 2077.

Tang, K., 2017, The microorganisms found in sewage, Sciencing.

Theobald, D.. 2014, Microorganisms in activated sludge, Water Technology Solutions for Industrial Water Management.

Van, der H. J., Duijff, R., Reinstra, O., 2018, Nitrogen recovery from wastewater: possibilities, competition with other resources, and adaptation pathways, Sustainability, 10, 4605.

Yamashita, T., Yamamoto-Ikemoto, Y.. 2014, Nitrogen and phosphorus removal from wastewater treatment plant effluent via bacterial sulfate reduction in an anoxic bioreactor packed with wood and iron, International Journal of Environmental Research and Public Health, 11, 9835-9853.

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