## Abstract

Biofilm systems have extensively used for biological treatment of domestic and industrial wastewater. Recently, moving bed biofilm reactor (MBBR) was developed, which combined the principle of suspended activated sludge and biofilm technology with an advantage of retaining biomass on carrier. Sludge recycling system was unnecessary in MBBR system which made the process more cost effective. Carriers are floated and mixed in the MBBR through aeration with rising air bubbles or mechanical force that made no dead space in the reactor. MBBR system also had a better recovery capability from system shock and had potential to replace the old treat system due to less building cost and high efficacy.

A real wastewater was obtained from a secondary-fiber papermaking plant in Taiwan. A Kaldnes MBBR system was used for reducing COD in the papermaking wastewater. The temperature of the influent was respectively controlled at ambient temperature, 40 °C, 50 °C and 60 °C. Efficiency of COD removal from the wastewater by this Kaldnes MBBR system was measured. A thermophilic strain BL11 was added into the system to be the target microorganism for establishing the analysis of microbial community by using molecular approach. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to analyze the microbial community structure of the biofilm. After starting MBBR system (10% of carrier filling fraction without chemical addition), total COD removal efficiency increased from 71% at ambient temperature to 90% at 60 °C. Soluble COD removal efficiency was 37% at ambient temperature and 55% at 60 °C, while the average effluent COD at 60 °C met the discharged limit (<180.0 mg/L).

Chromosomal DNA of the bacterial community in the bioflim was extracted by using traditional phenol-chloroform method and two commercial DNA extraction kits. The results showed that the traditional phenol-chloroform method followed by a DNA purifying step with isopropanol was superior to the commercial kits on extracting DNA from the biofilm. The result of PCR-DGGE showed the strain BL11 was successfully immobilized onto carrier, but disappeared when operation temperature reached 60 °C. For future application, the removal efficiency may be improved with higher carrier filling fraction which retains more biomass in the system. PCR-DGGE analysis can be used to monitor the development of biofilm on the

carrier.

Keywords: Papermaking wastewater, Moving bed biofilm reactors (MBBR), Kaldnes, DGGE, Thermophilic

## Contents

Chapter 1 Introduction	1
1.1 Introduction	1
1.2 Study Objectives	3
Chapter 2 Literatures Review	4
2.1 Secondary-fiber papermaking industry	4
2.2 The moving bed biofilm reactor system (MBBR)	5
2.2.1 Kaldnes MBBR system	7
2.3 Thermophilic treatment system	10
2.4 Microbial community analysis on biofilm	13
Chapter 3 Materials and Methods	18
3.1 Experimental Design	18
3.2 Influent and seed sludge of the MBBR	20
3.3 Inoculum	20
3.4 MBBR reactor	21
3.5 Experiment set-up	26
3.6 Analytical methods	27
3.7 Microbial community analysis of the biofilm	27
3.7.1 DNA extraction methods	27
3.7.1.1 Method I: Traditional phenol-chloroform DNA extraction	29
3.7.1.2 Method II: Viogene Genomic DNA Miniprep Kit for Blood&Tissue	31
3.7.1.3 Method III: UltraClean Soil DNA Isolation kit	32
3.7.2 DNA concentration and purity analysis	33
3.7.3 Polymerase Chain Reaction, PCR	35

3.7.4 Denature Gradient Gel Electrophoresis, DGGE	36
3.7.5 Microbial community similarity analysis	44
Chapter 4 Results and Discussion	45
4.1 The characteristics of raw wastewater	45
4.2 Experiment started-up period	45
4.2.1 COD removal	45
4.2.2 Microbiology of the biofilm on the carrier	47
4.3 COD removal with inoculation of a thermophilic strain BL11	55
4.4 Effect of different operational temperatures on reactor performance	60
4.5 Microbial community analysis of the biofilm with PCR-DGGE	73
4.5.1 Comparison of different DNA extraction methods	73
4.5.2 PCR and nested PCR amplification	75
4.5.2.1 Amplification of 16S rDNA from biofilm with co-culture with strain BL11	75
4.5.2.2 Comparison of different DNA extraction methods	85
Chapter 5 Conclusions and Suggestions	94
5.1 Conclusions	94
5.2 Suggestions	95

References		97
References	••••••	9

## List of Tables

Table 2.1	Data of different types of Kaldnes biofilm carrier	9
Table 3.1	Data of K1 type Kaldnes biofilm carrier	25
Table 3.2	Analytical methods used in this study	28
Table 3.3	Main steps of three DNA extraction methods used in this study	34
Table 3.4	Chemical components for PCR with total volume of 50.0 µl	37
Table 3.5	Heating program of PCR in this study	38
Table 3.6	Reagent and concentration of stocked 50X TAE buffer	39
Table 3.7	Components of agarose electrophoresis	40
Table 3.8	Relationship between gel percentage and base pair separation	42
Table 3.9	Reagent and concentration of DGGE	43
Table 4.1	Characteristics of raw wastewater	46
Table 4.2	Mean values and standard deviation of COD, SCOD, TS and MLVSS concentration changes in the MBBR reactor during start-up period at ambient temperature	49
Table 4.3	Results of COD, TS and MLVSS concentration changes from operated at 40 °C, adding thermophilic strain BL11 and controlling DO concentration	57
Table 4.4	Change of COD, SCOD, TS and MLVSS concentration during different operational temperatures: ambient temperature, 40 °C, 50 °C and 60 °C	63
Table 4.5	Comparison of system efficiency of various treating systems for papermaking wastewater	70
Table 4.6	Extracted DNA concentration and purity by using three different extraction methods	74

Table 4.7	Optimal conditions of PCR amplification of bacterial 16S rDNA.	76
Table 4.8	DNA concentrations and their purity of nested PCR products	77
Table 4.9	Similarity analysis of microbial community on the biofilm using the Dice Index (Cs) for PCR-DGGE during different operational conditions	83
Table 4.10	Significance analysis of microbial community on the biofilm using chi-square analysis for PCR-DGGE during different operational conditions	84
Table 4.11	Optimal conditions of PCR amplification of bacterial 16S rDNA.	86
Table 4.12	DNA concentrations and their purity of nested PCR products	87
Table 4.13	Similarity analysis of microbial community on the biofilm using the Dice Index (Cs) for PCR-DGGE using different extraction methods at 50 °C and 60 °C	91
Table 4.14	Significance analysis of microbial community on the biofilm using chi-square analysis for PCR-DGGE using different extraction methods at 50 °C and 60 °C	92

## **List of Figures**

Fig. 2.1.	Different kinds of Kaldnes biofilm carriers	8
Fig. 2.2.	Principle of Kaldnes moving bed biofilm reactor	11
Fig. 2.3.	Principle of denaturing gradient gel electrophoresis	16
Fig. 3.1.	Flow chart of experimental design	19
Fig. 3.2.	Schematic diagram of experimental set-up	22
Fig. 3.3.	Photo of Kaldnes K1 carrier	23
Fig. 4.1.	Change of COD concentration during started-up period at ambient temperature	48
Fig. 4.2.	Photographs of Kaldnes carrier after 1 week of start-up period.	51
Fig. 4.3.	Photographs of Kaldnes carrier after 2 weeks of start-up period	52
Fig. 4.4.	Photographs of Kaldnes carrier after 3 weeks of start-up period.	54
Fig. 4.5.	Change of COD concentration during three different operation periods	56
Fig. 4.6.	Comparison of COD, TS and MLVSS removal efficiency in three different operational conditions	59
Fig. 4.7.	Change of COD concentration at different operational temperatures	61
Fig. 4.8.	Comparison of COD, SCOD, TS and MLVSS removal efficiency with four different operational temperatures: ambient temperature, 40 °C, 50 °C and 60 °C	64
Fig. 4.9.	Removal efficiency versus volume loading rate at different operating temperatures, ambient temperature and 40 °C.	66
Fig. 4.10.	Agarose gel electrophoresis of the nested PCR products	78

Fig. 4.11.	Results of DGGE at different operational temperatures (40 °C, 50 °C and 60 °C) under different denaturant Gradient.	80
Fig. 4.12.	PCR-DGGE profile of microbial community of the biofilm with strain BL11 inoculation at different operational conditions.	81
Fig. 4.13.	Agarose gel electrophoresis of the nested PCR products of the biofilm DNA	88
Fig. 4.14.	PCR-DGGE profile of bacterial 16S rDNA of the biofilm extracted by using different extraction methods	90