DECOLORIZATION OF AZO DYES IN ANAEROBIC BAFFLED REACTORS UNDER SULFATE-REDUCING CONDITIONS

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ชลาธิป จันทรศิริ : การกำจัดสีย้อมชนิดอะโซในถังปฏิกรณ์ชนิดแผ่นกั้นไร้อากาศภายใต้สภาวะรีดิวซ์ซัลเฟต. (DECOLORIZATION OF AZO DYES IN ANAEROBIC BAFFLED REACTORS UNDER SULFATE -REDUCING CONDITIONS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก :อ.ดร.เบญจพร สุวรรณศิลป์ 122 หน้า.

งานวิจัยนี้ศึกษาการกำจัดสีย้อมอะโซ 2 ชนิด ในถังปฏิกรณ์ชนิดแผ่นกั้นไร้อากาศแบบ 4 ห้องย่อยภายใต้สภาวะรีดิวซ์ซัลเฟต ดำเนินการทดลองโดยใช้น้ำเสียสังเคราะห์ที่มีสีย้อมอะโซชนิด เอซิดเรด 18 (C.I. Acid Red 18) ที่ความเข้มข้น 200 มก./ล. สำหรับถังปฏิกรณ์ชนิดแผ่นกั้นไร้อากาศA และ เอซิดออเรนจ์ 7 (C.I. Acid Orange 7) ที่ความเข้มข้น 80 มก./ล. สำหรับถังปฏิกรณ์ชนิดแผ่นกั้นไร้อากาศ B ทั้งนี้กรดแลคติกและซัลเฟต ที่มีสัดส่วนซีโอดีต่อซัลเฟต ประมาณ 0.6 ถูกใช้เพื่อกระตุ้นการเจริญเติบโตของแบคทีเรียรีดิวซ์ซัลเฟตในถังปฏิกรณ์ โดยมีซัลเฟตเป็น สารรับอิเล็คตรอนตัวหลักในระบบ ซึ่งทำให้เกิดสภาวะรีดิวซ์ซัลเฟตเกิดขึ้น การกำจัดซีโอดีระหว่างวันที่ 35 ถึง 49 ซึ่งเป็นระยะหลังระยะเริ่มต้น (Start-up)ของระบบและเป็นระยะก่อนเติมสีย้อมชนิดอะโซลงในระบบพบว่า มีการกำจัดซีโอดีได้ใกล้เคียงกันในทั้งสองถังปฏิกรณ์ โดยมีประสิทธิภาพการกำจัดซีโอดีอยู่ที่ 78.6 ± 3.4 % และ 82.3 ± 3.1 % สำหรับถังปฏิกรณ์ A และ B ตามลำดับ อย่างไรก็ตามหลังการเติมสีย้อมชนิดอะโซลงในระบบ พบว่าประสิทธิภาพการกำจัดซีโอดีลดลงในทั้ง 2 ถังปฏิกรณ์ โดยมีค่าเท่ากับ 60.1 ± 3.3 % สำหรับถังปฏิกรณ์ A และ 63.1 ± 4.7 % สำหรับถังปฏิกรณ์ B นอกจากนี้ผลการทดลองพบว่า สีย้อมอะโซชนิด เอซิดเรด 18 และ เอซิดออเรนจ์ 7 ถูกกำจัดในถังปฏิกรณ์ชนิดแผ่นกั้นไร้อากาศภายใต้สภาวะรีดิวซ์ซัลเฟต ได้อย่างมีประสิทธิภาพ โดยมีประสิทธิภาพการกำจัดสีย้อมเท่ากับ 97.8 ± 1.4 % และ98.3 ± 1.7% สำหรับถังปฏิกรณ์ A และ B ตามลำดับ ทั้งนี้การกำจัดสีย้อมชนิดอะโซเกิด ขึ้นในทุกห้องย่อยของถังปฏิกรณ์ โดยห้องย่อยที่ 1 มีประสิทธิภาพในการกำจัดสี ย้อมสูงสุดเมื่อเทียบกับห้องย่อยอื่น การกำจัดสีย้อมจากกระบวนการดูดซับด้วยตะกอนจุลินทรีย์พบว่าเกิดขึ้นน้อย มากจนสามารถละทิ้งได้ นอกจากนี้จากผลของการศึกษาด้วยวิธี 16S rDNA clone library ได้ตรวจพบกลุ่มจุลินทรีย์รีดิวซ์ซัลเฟตและกลุ่มจุลินทรีย์ที่มีความสามารถในการหมักใน ถังปฏิกรณ์B อย่างไรก็ตาม บทบาทของกลุ่มจุลินทรีย์ดังกล่าวในการกำจัดสีย้อมอะโซในถังปฏิกรณ์ชนิดแผ่นกั้นไร้อากาศยังไม่สามารถสรุปได้แน่ชัด

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต .....

ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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CHALATHIP JUNTALASIRI : DECOLORIZATION OF AZO DYES IN ANAEROBIC BAFFLED REACTORS UNDER SULFATE-REDUCING CONDITIONS. ADVISOR : BENJAPORN SUWANNASILP, Ph.D., 122 pp.

This study investigates the decolorization of two mono-azo dyes in two four compartment anaerobic baffled reactors (ABRs) under sulfate-reducing conditions. The azo dyes, 200 mg/L of C.I. Acid Red 18 and 80 mg/L of C.I. Acid Orange 7 were added to the synthetic wastewaters for Reactor A and Reactor B, respectively. Lactic acid and sulfate with the COD:sulfate ratio of ~0.6 were used to stimulate the growth of sulfate-reducing bacteria in the reactors. Sulfate was the main electron acceptor in both reactors and sulfate-reducing conditions were successfully developed. During day 35^{th} until day 49^{th} after the start-up period but before the azo dye addition, the COD removal efficiency in both reactors were rather close in which 78.6 ± 3.4 % and 82.3 ± 3.1 % of COD removal efficiency were achieved for the Reactor A and Reactor B, respectively. However, after the azo dye addition, the COD removal efficiencies in both reactors decreased to 60.1 ± 3.3 % for Reactor A and 63.1 ± 4.7 % for Reactor B. For these two reactors, most of COD removal occurred in the first compartments both before and after the addition of the azo dyes. The results show that both C.I. Acid Red 18 and C.I. Acid Orange 7 were decolorized effectively in the ABRs under sulfate-reducing conditions with the decolorization efficiencies of 97.8 ± 1.4 % and 98.3 ± 1.7%, respectively. In both reactors, azo dye decolorization occurred in all of the compartments but it occurred to the greatest extents in the first compartments. Adsorption of the azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, by inactive biomass from both reactors was negligible. The 16S rDNA clone library results show that sulfate-reducing bacteria and fermentative bacteria were present in Reactor B. Nevertheless, the actual roles of these two groups of microorganisms on azo dye decolorization in anaerobic baffled reactors are still unclear.

Field of Study : Environmental management Student's Signature .....

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CHAPTER I
INTRODUCTION

1.1 Background

Azo dyes, synthetic dyestuffs consist of aromatic rings linked by azo bonds (N=N)are widely used in many industries such as food industry and textile industry. The largest proportion of azo dyes is used in textile production. It has been estimated that during textile manufacturing processes, about 50% of the dyes could be lost in the effluents (Easton 1995). As a result, large amount of azo dyes can be released to the environment via wastewater discharge from textile industry causing adverse impact to the environment. Contamination of azo dyes in surface water is aesthetically displeasing, and it can impede light penetration thereby affecting the photosynthesis process in ecosystems. Furthermore, some metabolites of azo dyes in the forms of aromatic amines, are recognized as carcinogens (Brown and Devito, 1993). For these reasons, the treatment of dye contaminated wastewater is necessary before it is released to the environment.

Several treatment processes have been developed for the treatment of azo dye contaminated wastewater, including physico-chemical and biological approaches. However, biological treatment processes appears to have advantages over physico-chemical processes in terms of operational costs. Although azo dyes are considered as xenobiotic compounds (Seshadri and Bishop, 1994; Dos Santos et al., 2005) that they are generally resistant to degrade biologically (Chen et al., 1999). Previous studies have reported that several microorganisms are capable to partially or even completely degrade the azo dye (Haung et al., 1991).

Azo dyes are generally persistent under aerobic conditions due to the high values of redox potentials; however, they can be decolorized under anaerobic conditions via azo reduction, releasing aromatic amines that can be completely mineralized under aerobic conditions. Several groups of microorganisms, such as fermentative bacteria, methanogens, and sulfate-reducing bacteria, have been reported to be capable of decolorizing azo dyes (Pearce, Lloyd and Guthrie, 2003; Dos Santos

et al., 2006; Yoo et al., 2000). However, the relative importance of these groups of microorganisms on the azo dye decolorization in anaerobic bioreactors is still inconclusive.

During textile processing, sulfate is generally added to dye baths as dye additives. As a result, the textile wastewater typically contains sulfate. Although many studies have investigated the effects of sulfate on the decolorization of azo dyes under anaerobic conditions, the results are still unclear (Carliell et al., 1995; Carliell et al., 1998; Panswad and Luangdilok, 2000; Albuquerque et al., 2005; Cervantes et al., 2006; Cervantes et al., 2007; van der Zee et al., 2003). Moveover, the effects of sulfate on the azo dye decolorization appear to depend strongly on the reactor types of treatment systems. For examples, the increases in sulfate concentrations enhanced the decolorization in the batch experiments (van der Zee et al., 2003; Cervantes et al., 2006) but sulfate addition did not significantly improve the azo dye decolorization in upward flow anaerobic sludge bed reactors (UASBs) (van der Zee et al., 2003). However, until now, the studies on the effect of sulfate on the azo dye decolorization in anerobic baffled reactors (ABRs) is still limited. ABRs provide many advantages (e.g., low cost of construction and operation and low level of maintenance) and are thus an attractive option for the treatment of wastewater from the textile-processing industries. However, information on the azo dye decolorization in ABRs especially under sulfate-reducing conditions is still lacking and should be further investigated.

This study, therefore, aimed to investigate the azo dye decolorization in anaerobic baffled reactors (ABRs) under sulfate-reducing conditions in order to evaluate the roles and capabilities of sulfate-reducing bacteria in azo dye decolorization in ABRs. Lactic acid and sulfate with the COD:sulfate ratio of ~0.6 was supplied to the ABRs to facilitate the enrichment of sulfate-reducing bacteria over fermentative bacteria and methanogens. Two mono-azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, were used as model azo dyes. The results from this study will provide more understanding on the azo dye decolorization in ABRs, which can assist in the improvement of biological treatment processes for azo dye removal in many industries.

1.2 Objectives

1) To investigate the decolorization of azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, in anaerobic baffled reactors under sulfate-reducing conditions.

2) To investigate microbial populations in anaerobic baffled reactors treating azo dyes, C.I. Acid Orange 7 under sulfate-reducing conditions.

1.3 Scope of study

The decolorization of azo dyes was studied in two four-compartment anaerobic baffled reactors. Two mono azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, were selected as the model azo dyes. Azo dye decolorization in anaerobic baffled reactors under sulfate-reducing conditions was investigated. Additionally, the microbial communities in the anaerobic baffled reactor were investigated using 16S rDNA clone libraries.

This study consists of three main parts. The first part was the operation of the anaerobic baffled reactors (ABRs) in order to investigate the decolorization of azo dyes under sulfate- reducing conditions. Two mono azo dyes, C.I. Acid Red 18 at 200 mg/L and C.I. Acid Orange 7 at 80 mg/L were used as model azo dyes in which these two azo dyes were fed into the ABRs, Reactor A, and Reactor B, respectively. Two ABR systems were operated continuously with flow rate of 10 L/d using synthetic wastewater consisting of lactic acid as electron donor, sulfate as electron acceptor and the other necessary elements. The COD:sulfate ratio of the influent synthetic wastewater was approximately 0.6. The second part of this study focused on the adsorption of the azo dyes on inactive biomass. Finally, the microbial communities in anaerobic baffled reactor treating azo dyes, C.I. Acid Orange 7 were investigated using 16S rDNA clone library.

1.4 Expected contributions

This study provides more understanding on the azo dye decolorization in ABRs, which can assist in the improvement of biological treatment processes for azo dye removal in many industries, such as by adjusting the conditions to be suitable for the groups of microorganisms responsible for azo dye decolorization. Also, the obtained results can enhance our understanding on the roles of sulfate-reducing communities in the azo dye decolorization.

1.5 Hypotheses

This experiment based on the hypotheses that lactic acid and excess sulfate concentration can enrich sulfate-reducing bacteria in ABRs, and high decolorization efficiencies can be achieved in the reactors under sulfate-reducing conditions.

CHAPTER II

LITERATURE REVIEW

2.1 Textile manufacturing process and wastewater

Textile manufacturing industry is one of the important industries in Thailand. Recently, the value of this industry has been reported by Thailand textile institute (2010) that it is about 1348.8 million dollar with growing rate at 19.5% compared with the data in 2009.Textile manufacturing is based on conversion of fibre into yarn, fabric, and then textiles. Figure 2.1 shows the most common steps of the manufacturing processes of textiles.

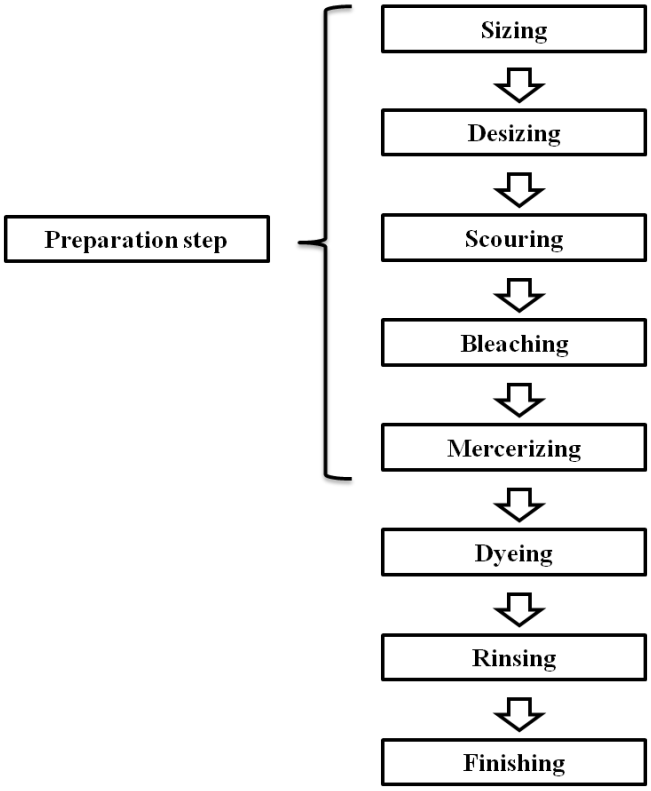


Figure 2.1 Common textile processing steps
(Dos Santos, Cervantes and van Lier, 2007)

Normally, the composition of the textile wastewater in each step of textile manufacturing process is different. For example, the wastewater from the desizing

step usually contains starch, enzyme, sizing agent, etc., and at textile dyeing step, the wastewater usually contains dyes resulting in generation of highly color wastewater. The main pollutants in each step of textile cotton industry is illustrated in Figure 2.2.

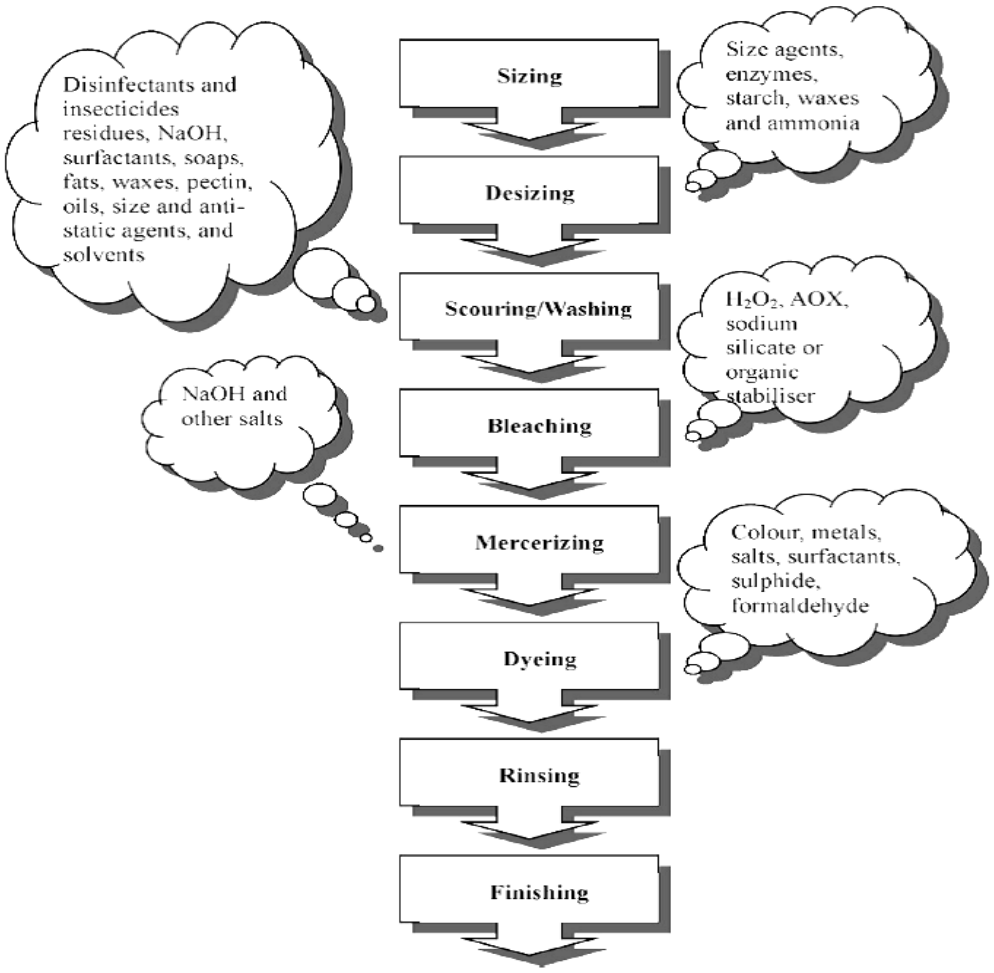


Figure 2.2 The main pollutants in each step of textile cotton industry
(Dos Santos et al. 2007)

Nowadays, textile manufacturing is one of the major industries that has been expanded due to the increases in consumer demands; as a result, dye contaminated wastewater from textile dyeing processes have been increasing proportionally. Generally, the characteristics of the wastewater from textile industry are low COD and BOD values with high concentrations of dyes as shown in Table 2.1.

Table 2.1 Characteristics of dye contaminated wastewater of different types of products

Dyeing products	Characteristics of the influent wastewater					Number of data points
	pH	BOD (mg/l)	COD (mg/l)	MLSS (mg/l)	Color (PtCo)	
Dyeing yarn	8.2	120	300	43	450	13
Dyeing knitted fabric	9.0	110	370	50	570	16
Dyeing weave	8.6	400	1,200	140	670	41
Dyeing yarn and other product	9.1	230	713	65	400	30

Source: San E. 68 consulting engineer Co., Ltd., 2002

Moreover, during textile processing, dye additives including sulfate are generally added to dye baths such as sodium persulfate ($Na_2S_2O_8$) and potassium persulfate ($K_2S_2O_8$). The range of sulfate concentration in from textile processing is 800 to 1300 mg/L or higher (Seif and Moheb, 2001).

The important parameters for evaluation of effluent quality have been reported by the Department of Environmental Quality Promotion under the Ministry of Natural Resources and Environment include pH, Chemical Oxygen Demand (COD), color, and odor. The government standard to control the effluent quality from the textile industry is shown in Table 2.2.

Table 2.2 The effluent standard for textile industry.

Parameter	Standard for the wastewater		Source
	Unit	Values	
1) Chemical oxygen demand (COD)	mg/l	Below 400	Notification of the Ministry of Industry NO.2, B.E.2539(1996)*
2) Color and odor	-	None	-
3) Oil and grease	mg/l	Below 5	Notification Of Industrial Estate Authority of Thailand B.E.2530(1987)
4) Suspended solid(SS)	mg/l	Below 50	Notification of The Ministry Of Industry No 2,B.E.2539(1996)*

*Notification of the Ministry of Industry No.2 B.E. 2539(1996) issue under the Factory Act B.E. 2535(1992).

2.2 Azo dyes

A colored substance, dye, plays an important role in human history since ancient time that it is used in almost commercial products such as food and textile. In the structure of dye, there is a group of atoms called chromophores which it is responsible for the color of dye. Chromophores will absorb the light ranging from 400-700 nm and then reflect the rest of light to human eyes. Moreover, one of the important groups in dye's structure as well as chromophores is an electron withdrawing or donating substituents that cause or intensify the color of the chromophores or increase affinity between dye and substrate, called auxochromes. Example of chromophore and auxochrome of dye will be showed in Table 2.3 and Figure 2.3.

Table 2.3 Example of chromophores and auxochromes (Rangnekar and Singh, 1980)

Structure	Linkage	Name
Chromophore	-N=N-	Azo
	-N=O	Nitroso
	-CH=N-	Azomethine
	>C=O	Carbonyl
	>C=C<	Ethenyl
Auxochrome	$-NH_2$	Amino
	$-NHCH_3$	Methyl amino
	$-SO_3H$	Sulphonic acid
	-COOH	Carboxylic acid
	-OH	Hydroxy

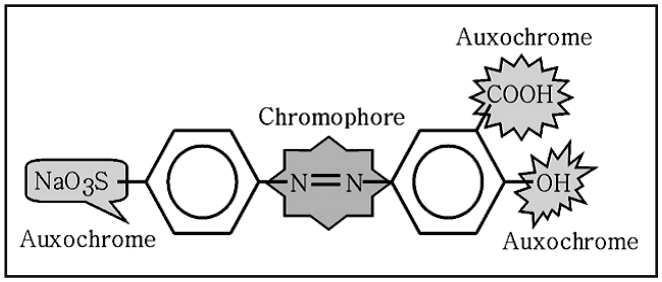


Figure 2.3 Chromophore and auxochrome groups of azo dye mordant yellow 10 (Dos Santos et al., 2007).

Dyes can be typically classified into two groups; natural and synthetic dyes. The uses of synthetic dyes have rapidly replaced traditional natural dyes due to their cost effective and vast ranges of colors. However, the other classification of dye was proposed by Hunger (2003). There are two classification methods; the first one is based on the structure of dyes especially the chromophore groups in the molecule of dyes. The second one is based on the applications of dyes (Figure 2.4) in which this

classification method classifies dyes into 11 groups. 1) Acid dye 2) Basic dye 3) Direct dye 4) Disperse dye 5) Reactive dye 6) Azoic dye 7) Vat dye 8) Mordant dye 9) Ingrain dye 10) Oxidation dye 11) Sulfur dye. Summary of specific properties of 11 groups of dyes are shown in Table 2.4.

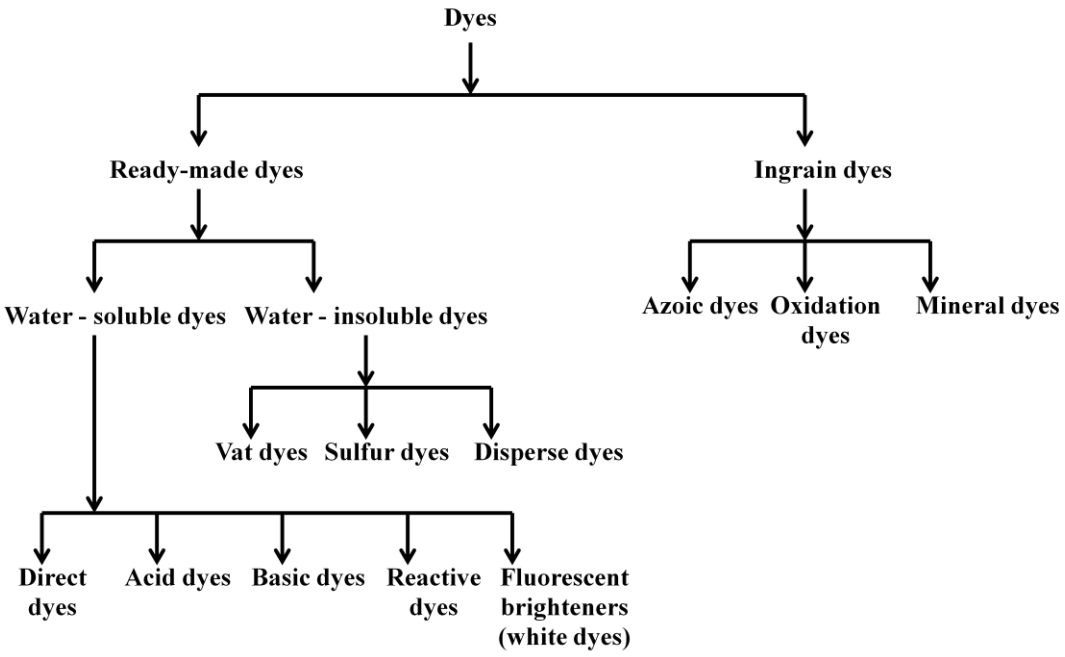


Figure 2.4 Classification of dye based on application method
(Modified from Shenai 1977)

Table 2.4 Summary of specific properties of eleven groups of dye classified by usage

Class	Typical applied	Properties
1) Acid dye	Wool, silk, nylon, paper ink and leather	- Highly water soluble - Anionic compounds
2) Direct dye	Cotton, paper, rayon, leather and nylon	- Highly water soluble - Anionic compounds - Poor wet fastness
3) Basic dye	Leather, silk paper, wool, modified nylon, polyacrylonitrile, polyester, and inks	- Cationic compound - Highly water soluble
4) Disperse dye	Plastics, polyamide, polyester, acetate and acrylic	- Colloidal dispersion - Very low water solubility - Good wet fastness
5) Reactive	Nylon, cotton, wool, and silk	- Anionic compounds; - Highly water soluble and good wet fastness.
6) Azoic dye	Rayon, cotton, polyester and cellulose acetate	- Water-insoluble dye - Apply by treating fibers with coupling compounds. and diazonium salt
7) Vat dye	Wool, cotton and rayon	- Water insoluble dye
8) Mordant dye	Leather, wool and anodized aluminium	- Anionic compounds - Water soluble - Good wet fastness
9) Ingrain dye	Suitable for cotton fabric	- Water-insoluble dye
10) Oxidation dye	Fur and cotton	- Water-insoluble dye
11) Sulfur dye	Rayon and cotton	- Water-insoluble dye

Source: modified from Molen, 2008

Generally, dyes are not recognized as highly toxic compounds However, several raw materials used in dye synthesis have been reported to be highly toxic and carcinogenic to the organisms in the environment, such as 2-napthylamine benzidine (Dos Santos et al., 2007). Dyes are aesthetically displeasing in surface water at even low concentration as 1 mg/L (Bell and Buckley 2003). Moreover, dyes also impede light penetration, thereby, affecting the photosynthesis process in ecosystems.

Azo dyes, synthetic dyestuffs consisting of aromatic rings linked by azo bonds (-N=N-) in their structure (Figure2.5), are synthetic dyes that do not occur naturally. These dyes can have one azo bond called mono azo dye or more than one azo bond, such as diazo (two azo bonds) and triazo (three azo bonds) dyes. Dos Santos et al. (2007) reported that azo dyes account around 70% by weigh of 10^9 kg of all dye produced annually in the world. Azo dyes are widely used in some industries including food and textile industries. However, the largest proportion of azo dyes is used in textile production. Azo dyes have many advantages. For examples, they can offer bright and high intensive complete rainbow of colors and they are cost effective. It has been estimated that during textile manufacturing processes, about 50% of the dyes could be lost in the effluents (Easton 1995). As a result, large amount of azo dyes can be released to the environment via wastewater discharge from textile industry causing adverse impact to the environment. Azo dyes are recognized as the recalcitrant substances that are biologically persistent. Also, the azo dye's metabolic products, aromatic amines, can be carcinogenic and mutagenic to organisms in the environment (Brown and Devito, 1993).

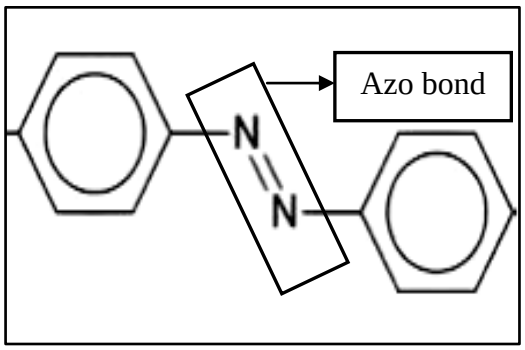


Figure 2.5 Structure of azo dye

2.3 Treatment of dye contaminated wastewater

Recently, several physicochemical processes have been developed and used for the treatment of dye contaminated wastewater. Although physicochemical treatments are capable of treating dye contaminated wastewater, these approaches have several disadvantages (Pearce et al., 2003). For example, physicochemical processes can generate secondary metabolites coming from the chemical usages during the treatment of wastewater. Moreover, physicochemical processes are considered not cost-effective compared with biological treatment processes, which offer lower operational costs. Therefore, biological treatment processes become an attractive alternative for the treatment of azo dye contaminated wastewater due to its several advantages, such as environmental friendly with low cost and low toxic by-products (Robinson et al., 2001). Examples of advantages and disadvantages of physicochemical methods are summarized in Table 2.5.

Table 2.5 Examples of advantages and disadvantages of physicochemical methods

Processes	Advantages	Disadvantages
Oxidation	Rapid process	- Can generate the by-products - High energy costs
Adsorption	Good removal of wide range of dyes	-Absorbent needs to regenerate or disposal
Membrane technologies	Capable to removes all dye types	-Concentrated sludge production
Coagulation/flocculation	Cost effective compared with the other physicochemical treatment	-High sludge production

Source: Pearce et al., 2003

2.4 Treatment of wastewater by using anaerobic baffled reactor (ABR)

Anaerobic baffled reactor is a high rate bioreactor (Bell and Buckley 2003). This system consists of several compartments ranging from 3 to 8 compartments in which the wastewater is controlled to flow upward and downward by several baffles in the system. This flow pattern of wastewater results in well contacts between substrates in wastewater and active biomass in each compartment of the reactors (Wang et al., 2004). Barber and Stuckey (1999) reported that this system can receive the organic loading rate in the range of 10 to 40 kg COD/m^3d. Nevertheless, the recommended value for the initial organic loading rate is approximately 1.2 kg COD/m^3d. ABR has several advantages such as longer biomass retention time (solid retention time-SRT), lower capital and operation costs, lower sludge production, capability of partially separating different groups of microorganisms along the compartments (Bell and Buckley, 2003). Advantages of ABRs have been summarized in Table 2.6.

Table 2.6 Summary of advantages of ABR.

Topic	Advantage
Construction	1) Simple design 2) Low capital cost and operating costs 3) No mechanical mixing
Biomass	1) High sludge retention time 2) Retention of biomass without fixed media or a solid-setting chamber
Operation	1) Long operational time without sludge wasting 2) Capability to partially separating different groups of microorganisms along the compartments

Source: Barber and Stuckey, 1999

2.5 16S rDNA clone library

One of the biomolecular techniques that can use to identify the microbial population is clone library. Clone library is a technique to place the foreign DNA into host cells in order to use the host cell as a gene bank to contain DNA sequences with accompanying information DNA sequences. With these clones, DNA from unknown samples can be identified. There are several steps in the clone library technique. First of all, the DNA samples will be extracted and amplified by using Polymerase chain reaction technique (PCR). The short pieces of DNA (primers) that hybridized to the selected DNA region will cause initiation of DNA synthesis using Taq DNA polymerase. For this reason, DNA will be amplified in each cycle resulting in enough of the DNA templates for further uses. Then, the DNA samples will be run on the gel electrophoresis to check the quantity of the DNA. Next, DNA will be ligated with the plasmid. After that, the plasmid containing DNA samples will be transformed into the host cell (*E. coli*). The microbial colonies containing the DNA samples will be screened by using the selective media. Finally, the DNA sequencing will be

performed to indentify the microbial population. The concept of clone library is illustrated in Figure 2.6.

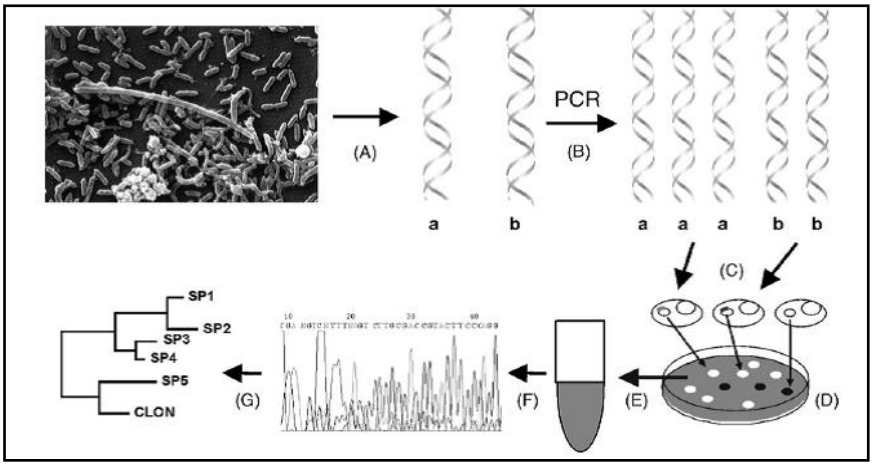


Figure 2.6 Clone library technique (Sanz and Köchling, 2007). (A) DNA extraction; (B) PCR amplification; (C) DNA ligation and transformation; (D) Screening of transformants; (E) DNA amplification from selected clones; (F) DNA sequencing (G) Drawing the phylogenetic tree

2.6 Sulfate-reducing bacteria (SRB)

Sulfate-reducing bacteria (SRB) is a group of bacteria involving in organic compound degradation in sulfate containing wastewater under anaerobic conditions. This group of bacteria is obligate anaerobic bacteria and chemoheterotrophs living and growing by acquiring energy from chemical reactions from organic material degradation. The specific characteristics of sulfate-reducing bacteria are the ability to reduce sulfate and change it to hydrogen sulfide. Sulfate-reducing bacteria use sulfate as a terminal electron acceptor to oxidize organic compounds such as organic acids, volatile fatty acids and alcohols.

Sulfate-reducing bacteria (SRB) can be divided into 4 main groups; Gram-negative mesophilic SRB, Gram-positive spore forming SRB, thermophilic bacterial SRB and thermophilic sulfate-reducing archaea (Castro, Williams and Ogram, 2000). However, sulfate-reducing bacteria can be classified into two main groups based on

organic degradation criteria (Munsin tuntoolvest, 2003). 1) Incompletely oxidizing sulfate-reducing bacteria; I-SRB. The substrate for I-SRB such as lactic acid, pyruvic acid and ethanol. The product from organic degradation is acetate. I-SRB such as *Desulfovibrio* sp., *Desulfomonas* sp., and *Desulfotomaculum* sp. 2) Completely oxidizing sulfate-reducing bacteria; C-SRB. The substrate for C-SRB is fatty acid especially acetic acid. The organic substrate will be completely degraded and changed to carbon dioxide (CO_2). C-SRB such as *Desulfococcus* sp. and *Desulfosarcrina* sp.

2.7 Biological azo dye decolorization

Several physico-chemical processes have been developed and used for the treatment of dye contaminated wastewater. However, physico-chemical processes are considered not cost-effective compared with biological treatment processes, which offer lower operational costs. Biological treatment processes become an attractive alternative for the treatment of azo dye contaminated wastewater due to its several advantages, such as environmental friendly with low cost and low toxic by-products. Microorganisms including, bacteria, fungi, and yeast, have been reported to be capable of decolorizing azo dyes (Chen et al., 1999; Meehan et al., 2000; Adosinda, et al., 2001; Khera, et al., 2005a; Khera, et al., 2005b; Yang et al., 2005; Gou, et al., 2009). Nevertheless, among these three groups of microorganisms, bacteria are considered to play important roles in decolorization of azo dyes in industrial wastewater treatment systems. Decolorization of azo dyes by bacteria, therefore, have been investigated in many studies (Wong and Yuen 1998; Asad et al.,2007; Barraga´n et al.,2007; Elisangela et al., 2009). Azo dye decolorization mechanisms have been proposed by van der Zee and Villa verde (2005), in which the azo bond is reduced under anaerobic conditions, resulting in the formation of colorless aromatic amines ($R\text{-}NH_2$). Then the aromatic amines are completely mineralized into water, ammonia (NH_3) and carbon dioxide (CO_2) under aerobic condition. The proposed azo dye decolorization mechanisms are illustrated in Figure 2.7.

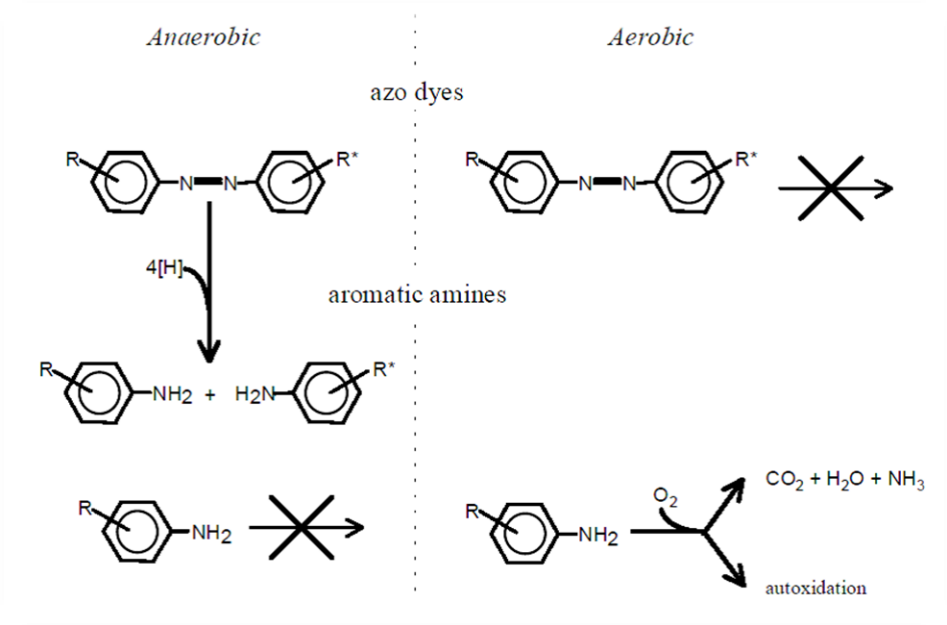


Figure 2.7 Azo dye decolorization mechanisms under anaerobic and aerobic conditions (van der Zee, 2002)

In the review of Saratale et al. (2011), it has been reported that under aerobic conditions, oxygen can inhibit the reduction of the azo bonds because respiration under aerobic conditions may dominate utilization of NADH, resulting in the inhibition of electron transfers from NADH to azo bonds. Similarly, Haung et al. (1991) and Gou et al. (2009) also reported that due to high redox potentials of azo bonds, decolorization of azo dyes under aerobic conditions are typically not feasible. In contrast, anaerobic treatment is known to be capable of decolorizing azo dyes. A number of previous studies have investigated the decolorization of azo dye under anaerobic conditions (Chinwetkitvanich, et al. 2000; Plumb, et al., 2001; Diniz, et al., 2002; Kim et al., 2008.) Decolorization of azo dyes under anaerobic conditions can occur via azo reduction by several mechanisms as shown in Figure 2.8, including 1) direct enzymatic azo reduction, 2) azo reduction via redox mediators such as quinones, and 3) azo reduction by chemical reductants such as dithionite or by biogenic reductants such as sulfide (Davis, et al. 1993; Weber, et al., 1995; Beydilli et al., 2000; Yoo, et al., 2000; van der Zee, 2002; Albuquerque et al., 2005). Nevertheless, the relative importance of these mechanisms on azo dye reduction under anaerobic conditions still remains unclear.

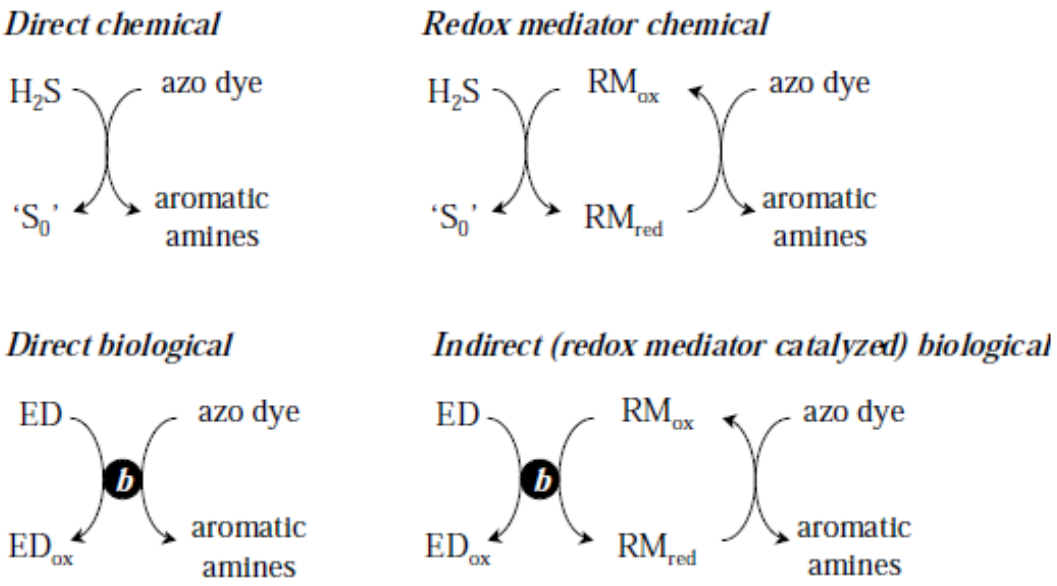


Figure 2.8 Mechanisms of azo dye decolorization (van der Zee et al., 2003)

Several studies have investigated azo dye decolorization under anaerobic conditions using pure cultures of bacteria. In the study performed by Yoo et al. (2000), the pure culture of sulfate-reducing bacteria (SRB), *Desulfovibrio desulficans*, was capable of decolorizing azo dye C.I. Reactive Orange 96 (RO96) and C.I. Reactive Red 120 (RR 120). Semde´et al. (1998) reported that the pure culture of *Clostridium perfringens*, fermentative bacteria, was capable of reducing several azo dyes under anaerobic conditions. In the experiment performed by Dos Santos et al. (2006), two methanogens; *Methanothermobacter* - related strain NJ1 and *Methanothermobacter thermoautotrophicus* ΔH have been shown to decolorize azo dye Reactive Red 2 (RR2) in the presence of ribloflavin. Previous studies using microbial pure cultures for azo dye decolorization are summarized in Table 2.7

Table 2.7 Summary of several studies using microbial pure culture

Pure culture of microorganisms	Dyes	Reference
Desulfovibrio desulfuricans	C.I.Reactive Orange 96 C.I. Reactive Red 120	Yoo et al. (2000)
Clostridium perfringens	Methyl orange, Amaranth	Semde´ et al. (1998)*
Methanothermobacter thermoautotrophicus ΔH and *Methanothermobacter* - related strain NJ1	Reactive Red 2, Reactive Red 4 and Reactive Orange 14	Dos Santos et al. (2006)
Lactobacillus casei	Tartrazine	Perez-Diaz and Mcfeeters 2008
Bacteriodes fragilis	Amaranth, Orange 2,tartrazine	Bragger et al.1997*
Stenotrophomonas maltophilia	Methylene blue, Congo red	Galai et al.2009
Escherichia coli	Reactive Red 22	Chang and Lin (2001)**
	Ethyl Red, Methyl Red	Nakanishi et al. (2001)**

Source: *Pearce et al. (2003), **Forgacs et al. (2004)

Although pure cultures of bacteria have been shown to be capable of decolorizing azo dyes in laboratory studies, their uses in actual industrial wastewater treatment systems for the treatment of azo dyes are unfeasible. It is considered impossible to maintain the pure cultures in full-scale wastewater treatment systems. In contrast, microbial communities are the key to decolorization of azo dyes in real wastewater treatment systems. Khera et al. (2005a, 2005b) reported three-fold increase in decolorization efficiency of AR-88 at 20 mg/l by using developed

consortium HM-4 consisting four bacterial strains as compared to the individual strains. Moreover, faster rates of decolorization of various azo dyes , such as AR-88, AR-119 with diverse and complex structures were observed in the consortium compared with the individual strains. Similarly, Cui et al. (2012) also found that the bacterial consortium AN were capable of completely decolorizing azo dye, Methyl Red at 100 mg/L within 10 hours. However, only 78% of Methyl Red was decolorized after 10 hours by the isolated individual strain Y3. Moreover, Saratale et al. (2009) conducted the experiment by using consortium GR to decolorize Scarlet R. The result showed that six times faster decolorization rate were achieved using the consortium compared with the individual strain.

Previous studies have investigated microbial populations in dye contaminated treatment systems under anaerobic conditions. In the study of Plumb et al. (2001), the fluorescent in situ hybridization (FISH) technique with 16S ribosomal DNA (rDNA) was used to characterize microbial populations in an anaerobic baffled reactor treating food dye wastewater containing several different azo dyes and other dye compounds. The results showed that a diverse group of bacteria and archaea, including sulfate-reducing bacteria and methanogens, was observed in the system. Khelifi et al. (2009) have used polymerase chain reaction-single stranded conformation polymorphism (PCR-SSCP) to investigate microbial communities in the bioreactor treating Indigo dye. The results showed that fermentative bacteria, *Clostridium* sp., and sulfate-reducing bacteria, *Desulfovibrio* spp., were dominant members of the bacterial community in the bioreactor. However, the findings may be not applicable to the cases of azo dye due to the differences between indigo dye and azo dye.

Furthermore, Dafale et al. (2010) conducted the experiment by using an anoxic–oxic bioreactor and investigated the bacterial community in the reactor using 16S rDNA clone library. The results revealed that the biodiversity in the anoxic reactor consisted of the genera of *Clostridium* sp., *Klebsiella* sp. and *Bacteroides* bacterium. Unidentified Gamma-proteobacteria were also found in this study. Information on microbial populations in azo-dye contaminated wastewater treatment

systems is still lacking but required to improve the efficiency and reliability of the biological treatment systems for azo dye decolorization.

In addition, several researchers have investigated microorganisms that are capable of decolorizing azo dyes in the environment. In the study of Chen et al. (2010), halotolerance bacteria capable of decolorizing several azo dyes such as Reactive Blue 160 and Reactive Black 5 effectively were identified by using SDS–PAGE followed by 16S rRNA analyses on nucleotide sequences. The results showedthat the halotorelance bacteria found in this study were *Exiguobacterium acetylicum* NIU-K2, *Staphylococcus gallinarum* NIU-K1 and *Exiguobacterium indicum* NIU-K4. Zhang et al. (2010) have investigated the indigenous bacteria obtained from the environment that were capable of decolorizing azo dyes such as Reactive blue 160 and Direct orange 39. These bacteria were characterized using morphological and biochemical tests followed by 16S rRNA gene sequencing. The results showed that the bacteria found in this study were *Klebsiella pneumoniae*, *Aeromonas hydrophila*,*Proteus hauseri*, *Acinetobacter johnsonii* and *Enterobacter cancerogenus*. Similarly, Khalid et al. (2012) have also investigated the bacteria obtained from seawater that were capable ofdecolorizing 100 mg/L of three different reactive azo dyes such as Reactive Blue BRS, Reactive Black 5, and Reactive Golden Ovifix at high salinity. The obtained results from 16S rRNA gene sequence showed that these bacteria were closely related with the bacteria in the genus of *Psychrobacter* sp. and *Staphylococcus* sp.

Furthermore, the microbial diversity in microbial fuel cells using anaerobic sludge as an inoculum that can generate the electricity and decolorize azo dye, Congo Red was investigated in the study of Hou et al. (2012). The results revealed that sulfate-reducing bacteria in the phylum of Gamma Proteobacteria and fermentative bacteria in the phylum of *Firmicutes* and *Bacteroidetes* were found in the biocathode of the microbial fuel cell. Nevertheless, it should be noted that the mechanisms of azo dye decolorization in microbial fuel cells were likely to be different from those in anaerobic bioreactors.

Several studies have reported microorganisms involved in decolorization, including fermentative bacteria (Chinwetkitvanich, et al. 2000), sulfate-reducing bacteria (SRB) (Plumb, et al. 2001; Yoo, et al., 2001; Diniz, et al., 2002; Libra, et al., 2004; Albuquerque, et al., 2005) and methanogens (Bra´s et al., 2001; Dos Santos et al., 2006). Yoo et al. (2001) investigated the roles of microbial groups on the decolorization of azo dye by using the specific inhibitors, molybdate and BES, to inhibit SRB and methanogens, respectively. The results suggested that SRB played important roles in azo dye decolorization. The results from Yoo et al. (2001) agreed well with the study by Albuquerque et al. (2005), which found that the inhibition of SRB resulted in the dramatically decrease in system's decolorization capacity. In the study of Yadav et al. (2012), the constructed wetland was performed in order to treat the azo dye, Methyl Orange. The set of the experiment that contained sulfate-reducing bacteria enrichment in the constructed wetland had highest decolorization efficiency compared with the normal constructed wetland. As a result, biological treatment by sulfate-reducing bacteria could play a significant role on reduction of azo dye together with constructed wetland. Chinwetkitvanich et al. (2000) suggested that SRB were not likely to play an important role in the azo dye decolorization, but acid forming bacteria were likely to be responsible for the azo dye decolorization. Until now, it is still inconclusive on which groups of microorganisms play important roles in the decolorization of azo dyes in wastewater treatment systems.

Previous studies have reported the anaerobic treatability of dye contaminated wastewater in several types of bioreactors, including fluidized bed reactor (Sen and Demirer 2003), upflow anaerobic sludge bed reactor (van der Zee et al. 2001) and anaerobic baffled reactors (Barber and Stuckey 1999; Bell and Buckley 2003). Bell and Buckley (2003) investigated the decolorization of the azo dye C.I. Reactive Red 141 in 8 compartments ABR. The results showed that the color removal was efficient with an average color removal of 86 %. Moreover, COD removal efficiency of the system was greater than 90%. Naimabadi et al. (2009) conducted the experiment in an anaerobic/aerobic sequential process using laboratory scale anaerobic baffled reactor (ABR) together with fixed activated sludge reactor (FAS) in order to treat azo dye Reactive Red 2. Under anaerobic condition in ABR, the average color removal of azo

dye Reactive Red 2 was 89.5% and the average COD removal was 54.5%. Under aerobic fix activated sludge reactor, COD removal was 69% with only slight removal of color. This finding indicates that the decolorization under anaerobic condition in ABR has higher efficiency than under aerobic fix activated sludge reactor. From the previous research, ABR has been shown to be capable of treating azo dye contaminated wastewater effectively.

One of the important factors affecting the efficiencies of azo dye reduction is the COD:sulfate ratio in which it can influence the microbial community in the system. Sulfate may have two important roles on azo dye decolorization (van der Zee et al. 2003). The first role is to be an electron acceptor competing for electrons with the azo dyes. The second role of sulfate in azo dye decolorization is that it is directly involved with sulfide production under anaerobic conditions in which sulfide can abiotically reduce azo dyes. Although the effects of sulfate on the decolorization of azo dyes have been investigated by several researchers, the conflicting results on this issue still remained.

No effect of sulfate on azo dye decolorization have been reported in many studies (Carliell et al., 1995; Carliell et al., 1998; Panswad and Luangdilok, 2000; van der Zee et al., 2003; Cervantes et al., 2007). Carliell et al. (1995) and Carliell et al. (1998) conducted batch scale experiments to investigate the redox potential effects of sulfate on azo dye decolorization in the period of 13 hours. The result revealed that no significant effect of sulfate on the reduction of azo dye was observed. However, this experiment was performed in short period of time; therefore, the microbial communities may be not significantly change within this period. Moreover, Panswad and Luangdilok (2000) have investigated the effect of sulfate on the azo dye decolorization in anaerobic-aerobic sequencing batch reactors. The results showed that there was no significant effect of sulfate on azo dye decolorization in the anaerobic-aerobic sequencing batch reactors. Similarly, van der Zee et al. (2003) found that sulfate has no significant effect on reduction of azo dye, Reactive Red 2, in upward-flow anaerobic sludge bed reactors (UASBs). Furthermore, Cervantes et al. (2007) showed that little or no effect of sulfate (5-10 g sulfate/L) on the rate of

decolorization of Reactive Orange 14, Direct Blue 53, and Direct Blue 71 was observed when no external redox mediator was provided. the presence of sulphate, also a normal constituent. Dafale et al. (2010) reported that sulfate had no significant effect of azo dye decolorization capacity of an anoxic–oxic bioreactor. Two sets of bioassay experiment was conducted in this study, the first set was azo dye, RB-5 containing sulfate (up to 20 mM) and the second set was a control that had no sulfate. The results showed that sulfate ions did not affect the reduction of azo bond of RB-5 dye.

On the other hand, several researchers found that sulfate can affect the decolorization of the azo dyes (Albuquerque et al., 2005; van der Zee et al., 2003; Cervantes et al., 2006). Albuquerque et al. (2005) conducted the experiment in anaerobic-aerobic sequencing batch reactors similar to Panswad and Luangdilok (2000). However, Albuquerque et al. 2005 reported that azo dye decolorization was improved due to sulfate reduction. Nevertheless, using of anaerobic-aerobic sequencing batch reactors did not appear to be suitable for maintaining anaerobic microorganisms, including sulfate-reducing bacteria. As a result, the results from the studies by Panswad and Luangdilok 2000 and Albuquerque et al. 2005 may not be applicable to continuous anaerobic bioreactors. The results of batch assays performed by van der Zee et al. (2003) were also different from the results obtained in UASBs. The results showed that in batch assays azo dye decolorization was influenced by sulfate due to biogenic sulfide formation from sulfate reduction. In agreement with van der Zee et al. (2003), Cervantes et al. (2006), also found that decolorization of Reactive Orange 14 in batch studies with anaerobic sludge was stimulated by increasing sulfate concentration. As a result, the questions on the effects of sulfate on azo dye decolorization are still awaiting for further investigation. Types of reactors and scales of experiment might also influence the effects of sulfate on azo dye decolorization. Table 2.8 shows the summary of several studies on the effect of sulfate on azo dyes decolorization.

Recently, Wangsaviboon (2011) has studied the effects of sulfate on the decolorization of azo dye, Acid Red 18, in four-compartment anaerobic baffled

reactors that had hydraulic retention time of 24 hours. Three anaerobic baffled reactors operated with three different sulfate concentrations, no sulfate, COD:SO_4^{2-} of 4, and COD:SO_4^{2-} of 0.6. Sucrose was used as an electron donor source at the COD concentrations of 500 mg/L in the influent. The results showed that the supplement of sulfate enhanced the decolorization efficiencies of anaerobic baffled reactors. However, the microbial populations of these three reactors remain unclear. Moreover, since the substrate used in the study by Wangsaviboon et al. (2011) was sucrose, it was likely that fermentative bacteria co-existed in the system. The roles of each group of microorganisms in azo dye decolorization were still unclear. In order to understand more on the roles of sulfate-reducing bacteria in azo dye decolorization in anaerobic baffled reactors, this study will therefore investigate azo dye decolorization in anaerobic baffled reactors under sulfate-reducing conditions which would be developed by using lactic acid as an electron donor to enrich sulfate-reducing communities in the reactors. The microbial populations and decolorization efficiencies of these systems will be investigated.

Table 2.8 Summary of several studies on effect of sulfate on decolorization of azo dyes

Effect of sulfate	Azo dye	Scale of experiment	Reference
No effect of sulfate on azo dye decolorization	Reactive Red 141	Batch scale. Use only short periods of time at 13 hours.	Carliell et al. 1995 and Carliell et al., 1998
	Reactive Black 5, Reactive Blue 19 Reactive Blue 5 and Reactive Blue 198	anaerobic-aerobic sequencing batch reactors	Panswad and Luangdilok 2000
	Reactive Red 2	Upward-flow anaerobic sludge bed reactors(UASB)	van der Zee et al., 2003
	Reactive Blue 5	Batch scale.	Dafale et al. 2010
	Reactive Orange 14, Direct Blue 53, and Direct Blue 71	Batch scale	Cervantes et al., 2007
Sulfate increased the azo dye decolorization	Acid orange 7	Anaerobic-aerobic sequencing batch reactors	Albuquerque et al. 2005
	Reactive Red 2	Batch scale	van der Zee et al., 2003
	Reactive Orange 14	Batch scale	Cervantes et al., 2006

CHAPTER III
METHODOLOGY

3.1 Scope of the study

This study consists of three parts of laboratory experiments. The first part was the investigation of azo dye decolorization under sulfate-reducing conditions in the anaerobic baffled reactors (ABRs). Two mono azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, were fed into each ABRs system, Reactor A and Reactor B, respectively. Both reactors were operated continuously with the flow rate of 10 Liter/day using synthetic wastewater consisting of lactic acid as electron donor, sulfate as electron acceptor and the other necessary elements. The COD and sulfate concentration in reactor A and reactor B were identical with the COD:sulfate ratio of approximately 0.6. The operation of ABR systems can be divided into two phases. The first phase was the start-up period of the reactor. The second phase was the azo dye addition phase using two azo dyes, C.I. Acid Red 18 at 200 mg/L and C.I. Acid Orange 7 at 80 mg/L for reactor A and reactor B, respectively. The second part of this study was focused on adsorption of the azo dye by inactive biomass. Finally, in the third part of this study, the microbial community in the anaerobic baffled reactor treating C.I. Acid Orange 7 was investigated using 16S rDNA clone library.

3.2 Materials, chemicals, and laboratory equipment

Materials

1) Four-compartment anaerobic baffled reactors
2) Influent and effluent storage tanks
3) Peristaltic pumps
4) Silicone and Masterflex® tubes
5) Glassware e.g. flasks, beakers, pipettes

Chemicals

1) Sodium sulfate anhydrous (Na_2SO_4)
2) Sodium bicarbonate ($NaHCO_3$)
3) Azo dyes (Table 3.5)
4) Lactic acid
5) Chemicals for COD measurement: potassium dichromate, sulfuric acid, ferroin indicator, silver sulfate and ferrous ammonium sulfate
6) Chemicals for sulfate measurement: barium chloride and conditioning reagent
7) Chemicals for alkalinity measurement: sulfuric acid
8) Chemicals for preparation of synthetic wastewater: lactic acid, sodium sulfate, sodium bicarbonate, macronutrients and micronutrients
9) Chemicals for 16S rDNA clone library experiment: LB medium. SOC medium, X-Gal, IPTG, agarose gel and etc.

Laboratory equipment

1) pH and ORP meter(Hach® session 2, USA)
2) Turbidity meter (WTW®, Germany)
3) Sulfide probe(WTW®, Germany)
4) Magnetic stirrer
5) Spectrophotometer (Genesys®, USA)
6) PCR machine
7) Horizontal gel electrophoresis system
8) Gel Documentation system
9) Incubator and shaker

3.3 Experimental methods

3.3.1 Investigation of azo dyes decolorization under sulfate-reducing conditions in anaerobic baffled reactors (ABRs)

This experiment aims to investigate the decolorization of two mono azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, in two four-compartment ABRs. Synthetic wastewater, consisting of lactic acid as an electron donor, sulfate as an electron acceptor and other necessary elements, were fed into these two ABRs. At first, both reactors were operated without the addition of azo dyes (Start-up phase). After steady states were achieved in both ABRs; then, C.I. Acid Red 18 and C.I. Acid Orange 7 were introduced into the ABRs called Reactor A and Reactor B, respectively. The experimental procedure for the investigation of the azo dye decolorization under sulfate-reducing conditions in ABRs is illustrated in Figure 3.1.The operational conditions of both ABRs were summarized in Table 3.1. The azo dye decolorization and other parameters shown in Table 3.2 were monitored throughout the experiment. The analytical methods used for parameter measurements are summarized in Table 3.2.

Table 3.1 Operational conditions of the ABRs, Reactor A and Reactor B

Constant parameter	Value in experiment
COD: sulfate ratio	0.6
Electron donor source	Lactic acid
Volume of reactor	10 Liter
Hydraulic retention time	1 day
Flow rate	10 Liter/day

Source: modified from Bell and Buckley (2003) and Krishna et al. (2009)

Table 3.2 Analytical methods for parameter measurement

Parameters	**Method/Experimental equipments**
Oxidation-Reduction potential	Electrometric method (2580*)
pH	Electrometric Method (4500B*)
COD	Close reflux, Titrimetric Method (5220C*)
Sulfate	Turbidimetric method (4500E*)
Sulfide	Potentiometric method
Alkalinity	Titration Method (2320B*)
MLSS	Total Suspended Solids Dried at 103–105°C (2540D*)
Absorbance of color (Azo dye addition phase)	Spectrophotometric Method (2120C*)

(*APHA, 2005)

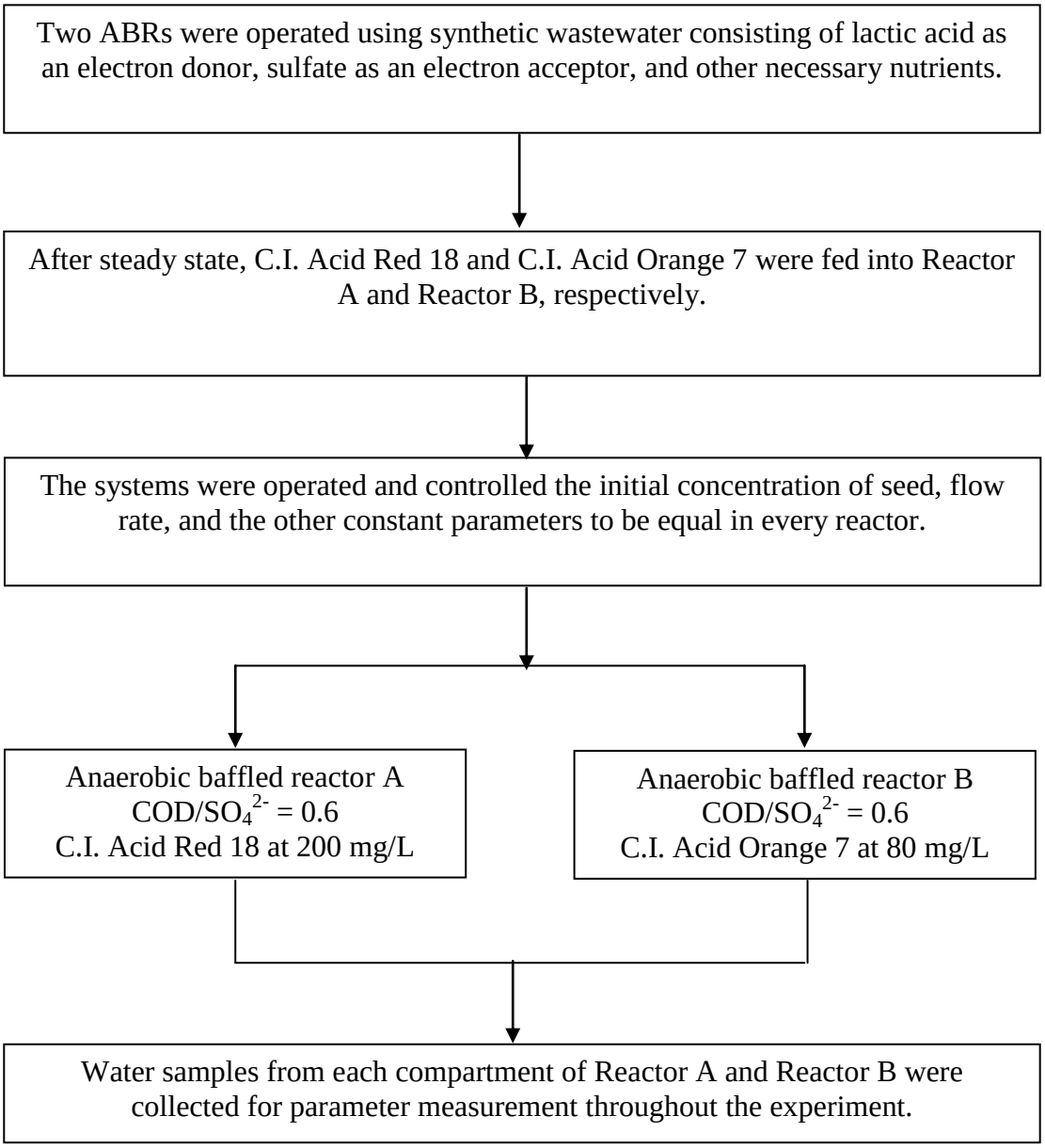


Figure 3.1 Experimental procedure for the investigation of azo dye decolorization under sulfate-reducing conditions in ABRs

3.3.1.1 Synthetic wastewater preparation

Synthetic wastewater used in this experiment was prepared from deionizing water with lactic acid and sulfate at the COD: sulfate ratio of approximately 0.6 throughout the experiment. Lactic acid was added to be electron donor source to enrich sulfate-reducing bacteria (Suzuki et al., 2007). Sulfate (833 mg/l) was added in the form of sodium sulfate (Na_2SO_4). Sodium bicarbonate of 2,520 mg/l (30 mM) was added in the synthetic wastewater serving as a pH buffer (Munsin tuntoolvest, 2003). After the systems approached steady states, azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, were added into the synthetic wastewaters fed into Reactor A and Reactor B, respectively. Synthetic wastewater composition for each reactor was summarized in Table 3.3 and Table 3.4.

Table 3.3 The composition of synthetic wastewater

Reactor / Parameter	Anaerobic baffled reactor A	Anaerobic baffled reactor B
COD: sulfate ratio	~0.6	~0.6
Sulfate concentration (mg/L)	833	833
Sodium bicarbonate (mg/L)	2,520	2,520
Lactic acid concentration(mg/L)	469.17	469.17
Azo dye added	C.I. Acid Red 18	C.I. Acid Orange 7
Concentration of azo dye (mg/L)	200	80

In previous study by Wangsaviboon (2011), sucrose was used as an electron donor in the ABR system for azo dye decolorization; however, sucrose cannot be used directly by sulfate-reducing bacteria. The presence of fermentative bacteria was required to transform sucrose into small volatile fatty acids that sulfate-reducing bacteria can further utilize. Therefore, in this study, lactic acid was selected instead of sucrose in order to favor the growth of the sulfate-reducing bacteria directly. Lactic acid is the substrate commonly used for the enrichment of sulfate-reducing bacteria. Moreover, very low COD: sulfate ratio equal to 0.6 were used in this study to favor the growth of sulfate-reducing bacteria over methanogens that might coexist in the reactors

Furthermore, these two azo dyes were added into synthetic wastewaters at different concentrations because different types of azo dyes contribute to different levels of absorbance. Different amount of azo dyes was used to achieve approximately the same absorbance (equal to 3) in order to observe clearly about the reduction of C.I. Acid Red 18 and C.I. Acid Orange 7 from approximately the same levels of absorbance.

Table 3.4 Nutrients and elements in synthetic wastewater

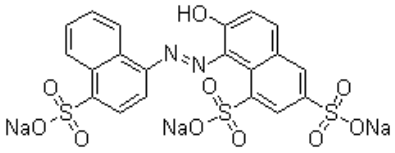
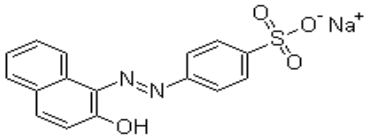
Nutrients and elements	Concentration (mg/L)	Chemical form
Macronutrients		
Nitrogen	248	NH_4Cl
Phosphorus	63.1	$NaH_2PO_4.2H_2O$
Sulfur	61.4	$MgSO_4.7H_2O$
Micronutrients		
Iron	35.70	$FeCl_2.4H_2O$
Cobalt	0.093	$CoCl_2.6H_2O$
Nickel	0.097	$NiCl_2.6H_2O$
Zinc	0.083	$ZnCl_2$
Copper	0.051	$CuCl_2$ anhydrous
Manganese	0.165	$MnCl_2.4H_2O$
Boron	0.137	H_3BO_4
Common cation		
Potassium	763	KCl

Source: modified from Rittman and MacCarty (2001)

3.3.1.2 Azo dyes used in the experiment

In the azo dye addition phase, two mono azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, were added to the synthetic wastewater continuously. The azo dyes used in this study were purchased from P.K.S. Chemicals Co., Ltd., Bangkok, Thailand. Table 3.5 shows information of the two azo dyes used in this study.

Table 3.5 Information of two azo dyes used in this study

Azo dye Information	C.I. Acid Red 18	C.I. Acid Orange 7
Synonym	C.I. 16255; Cochineal Red A; New Coccine; Trisodium 1-(1-naphthylazo)-2-hydroxynaphthalene-4',6,8-trisulphonate	C.I. 15510; Orange II sodium salt; 4-(2-Hydroxy-1-naphthylazo)benzenesulfonic acid sodium salt; Sodium 4-[(2-hydroxy-1-naphthyl)azo benzenesulphonate
Molecular structure		
Molecular formula	$C_{20}H_{11}N_2Na_3O_{10}S_3$	$C_{16}H_{11}N_2NaO_4S$
Molecular weight	604.47	350.32

Source: modified from Mozia et al. (2007) and Daneshvar et al. (2007)

These two azo dyes were selected to use in this study because of their mono azo bond structures. The reduction of these azo dyes can occur atonly one azo bond position, which made the decolorization of these two azo dyes easy to be observed at one specific wavelength. On the other hand, using of azo dyes having more than one azo bond can make the observation of azo dye decolorization more complicated due to the shifts in colors since the azo reduction can occur atmore than one azo bond positions.

3.3.1.3 Reactor configurations

Anaerobic baffled reactor used in this study are rectangle shape consisting of four compartments with the size of 0.3x 0.1x 0.4 m (height x width x length) and the water depth of 0.25 m. The total and application volumes of the reactor are 12 L and 10 L, respectively. Several verticals baffles are installed inside the reactors to control the downward and upward flow of water. The width ratio between these two parts is 1:3. This configuration can improve the biomass retention in the ABRs (Dama et al., 2002). Moreover, 45 degree of the curve at the end of the baffle can help to provide good contacts between biomass and wastewater (Bachmann et al., 1985). The anaerobic baffled reactor used in this study is shown in Figure 3.2

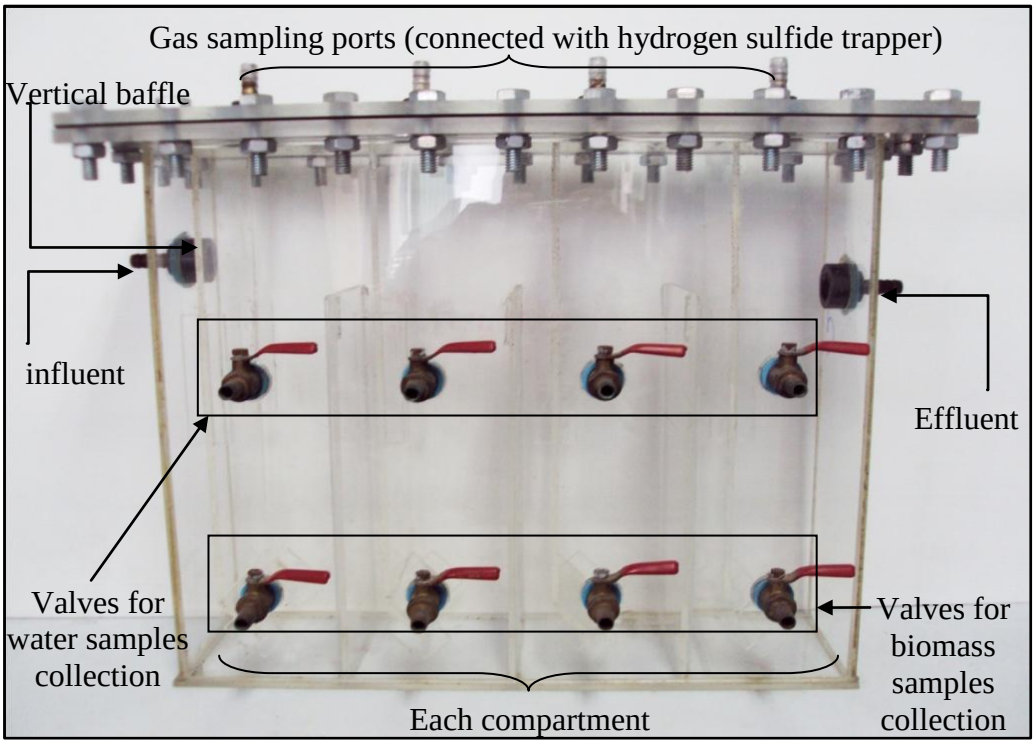


Figure 3.2 Anaerobic baffled reactor (ABR) used in this study

3.3.1.4 Installation and operation of the system

The system consists of an ABR, an influent tank, an effluent tank, a peristaltic pump, and a H_2S trapping system. Microbial seed inoculums used in this study were obtained from an anaerobic digester seeded with swine manure and operated until a steady state was reached (San E. 68 Lab Co., Ltd, Thailand). The ABRs were seeded 17% by volume with the microbial seed inoculums. The synthetic wastewater from influent tank was fed continuously into the reactor with the flow rate 10 L/day by peristaltic pump to the reactor. The reactors were continuously operated at room temperature. The synthetic wastewater inside the reactor flowed downward through biomass and then flowed to the next compartment of the reactor. Furthermore, there were gas sampling ports on the top of the reactor that they normally connected with the tube to trap hydrogen sulfide gas from the system by using zinc acetate in order to prevent the unpleasant smell. During the operation of the systems, the reactors were covered with black plastic bag to prevent the exposure of light. The schematic setup of the anaerobic baffled reactor (ABR) is shown in Figure 3.3 and 3.4.

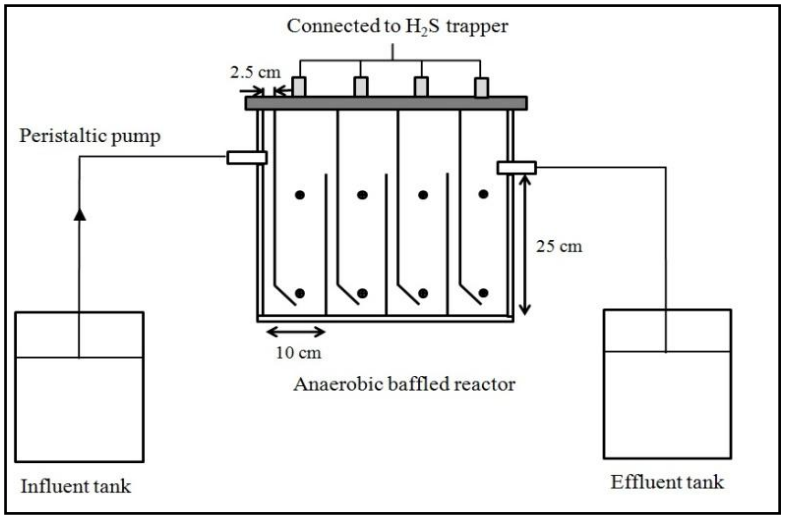


Figure 3.3 Schematic setup of the anaerobic baffled reactor (ABR)

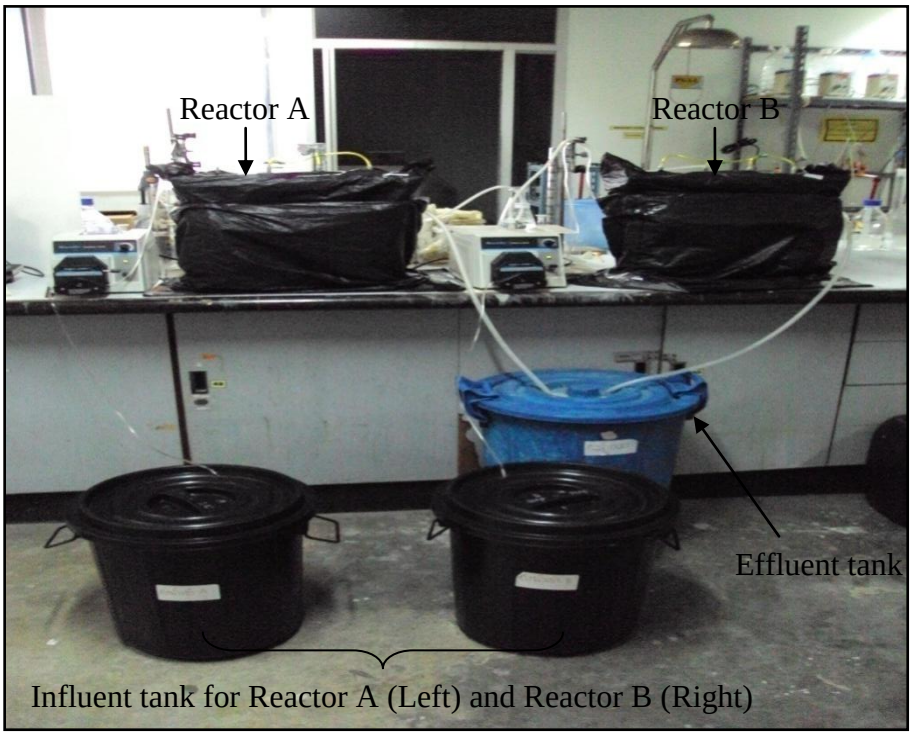


Figure 3.4 Setup of the anaerobic baffled reactors (ABRs) in laboratory

3.3.1.5 Sample collection from anaerobic baffled reactors and parameter measurements

Water and biomass samples from the ABR systems were collected as follows:

- Water samples were collected from five locations as following; influent tank, compartment 1, 2, 3 and 4 of ABR system.

- Biomass samples were collected from the clear water zone of the compartment 4 of ABR systems to determine the biomass loss from the systems during the operation.

The sampling locations and the sampling frequencies of each parameter are summarized in the Table 3.6.

Table 3.6 Measurement of parameters, sampling locations and frequencies

Parameters	Sampling locations	Frequencies
1) ORP	Four compartments for each reactor	2 times/week
2) pH	Four compartments for each reactor	2 times/week
3) COD	Influent tank and four compartments for each reactor	2 times/week
4) Absorbance of color	Influent tank and four compartments for each reactor	2 times/week
5) Sulfate	Influent tank and four compartments for each reactor	2 times/week
6) Alkalinity	Four compartments for each reactor	1 time/week
7) MLSS	-The initial seed before the operation of ABRs -The fourth compartment of each reactor during the operation period -Four compartments for each reactor after the operation of ABRs	-Before the operation period -1 time/week -After the operation period

3.3.1.6 Absorbance of azo dye

The azo dyes used in this study are mono-azo dyes, consisting of only one azo bond in their structures. Thus, these azo dyes have only one maximum absorbance at a specific wavelength. Spectrophotometer was used to scan the azo dye samples between the wavelength of 400 to 700 nm to determine the maximum absorbance of C.I. Acid Red 18, and C.I. Acid Orange 7. The scanning results of the azo dyes, C.I. Acid Red 18, and C.I. Acid Orange 7 are shown in Figure 3.5.

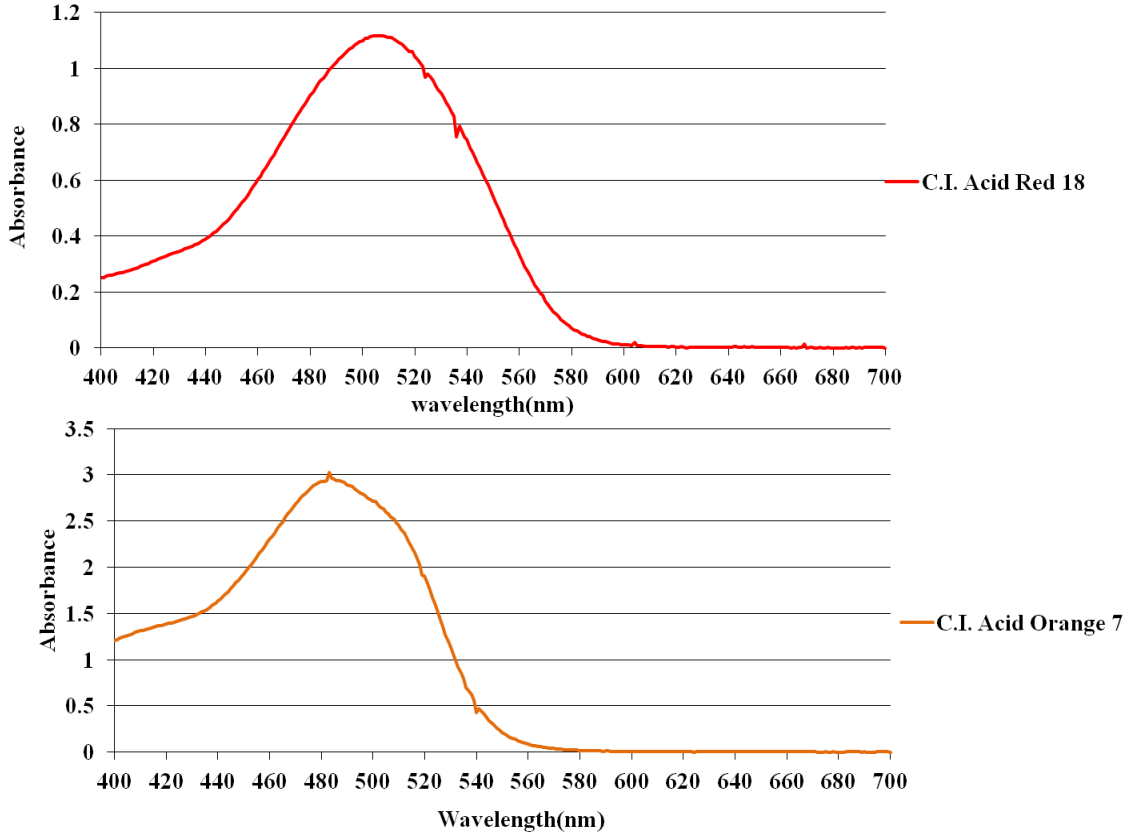


Figure 3.5 Absorbance scans at wavelength 400-700 nm: C.I. Acid Red 18 (above) and C.I. Acid Orange 7 (below)

From the scanning results, the maximum absorbance for C.I. Acid Red 18 and C.I. Acid Orange 7 are 508 nm and 483 nm, respectively. Thus, the azo dye decolorization of C.I. Acid Red 18 and C.I. Acid Orange 7 was monitored using the absorbance at 508 nm and 483 nm, respectively. Prior to the decolorization measurement, all the water samples from the anaerobic baffled reactors were filtered using 0.45 µm membrane filters.

3.3.2 Adsorption of the azo dyes by inactive biomasses

After the operation of the ABR systems, the mixed liquors from the first compartment of reactor A and B were collected to investigate the adsorption of azo dyes by inactive biomasses in the system. The biomass from the first compartment of both ABRs was selected to use in this experiment because of its highest decolorization efficiencies compared to other compartments.

First of all, the mixed liquors were centrifuged and washed using deionized water three times. Then the resuspension with deionized water was added to the three replicate serum bottles at the MLSS concentrations equal to that in the first compartment of each reactor. Then the biomasses were autoclaved to inhibit the microbial activity. After that, the azo dyes were added to each of the three replicate serum bottles to obtain the concentration at 200 mg/L and 80 mg/L for C.I. Acid Red 18 and C.I. Acid Orange 7, respectively. Azo dyes decolorization was then monitored over time using spectrophotometer. The procedure of this experiment is illustrated in Figure 3.6.

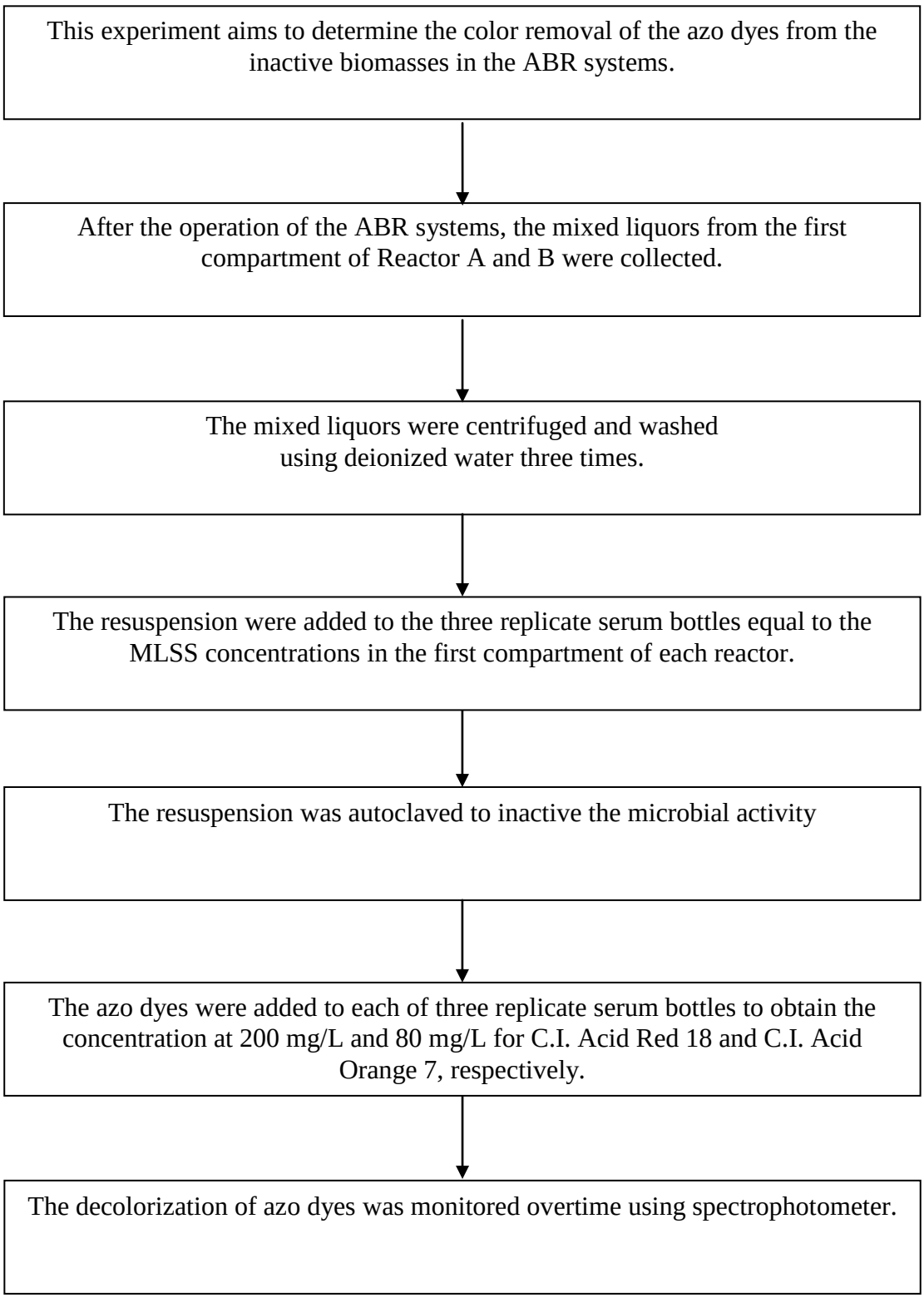


Figure 3.6 The experimental procedure to investigate the adsorption of the azo dyes by inactive biomasses

3.3.3 Microbial community using 16S rDNA clone library

At the end of the anaerobic baffled reactor operation, the biomass sample in the first compartment of the Reactor B were collected to investigate the microbial community using 16S rDNA clone library technique. The experimental procedure of this experiment is shown in Figure 3.7.

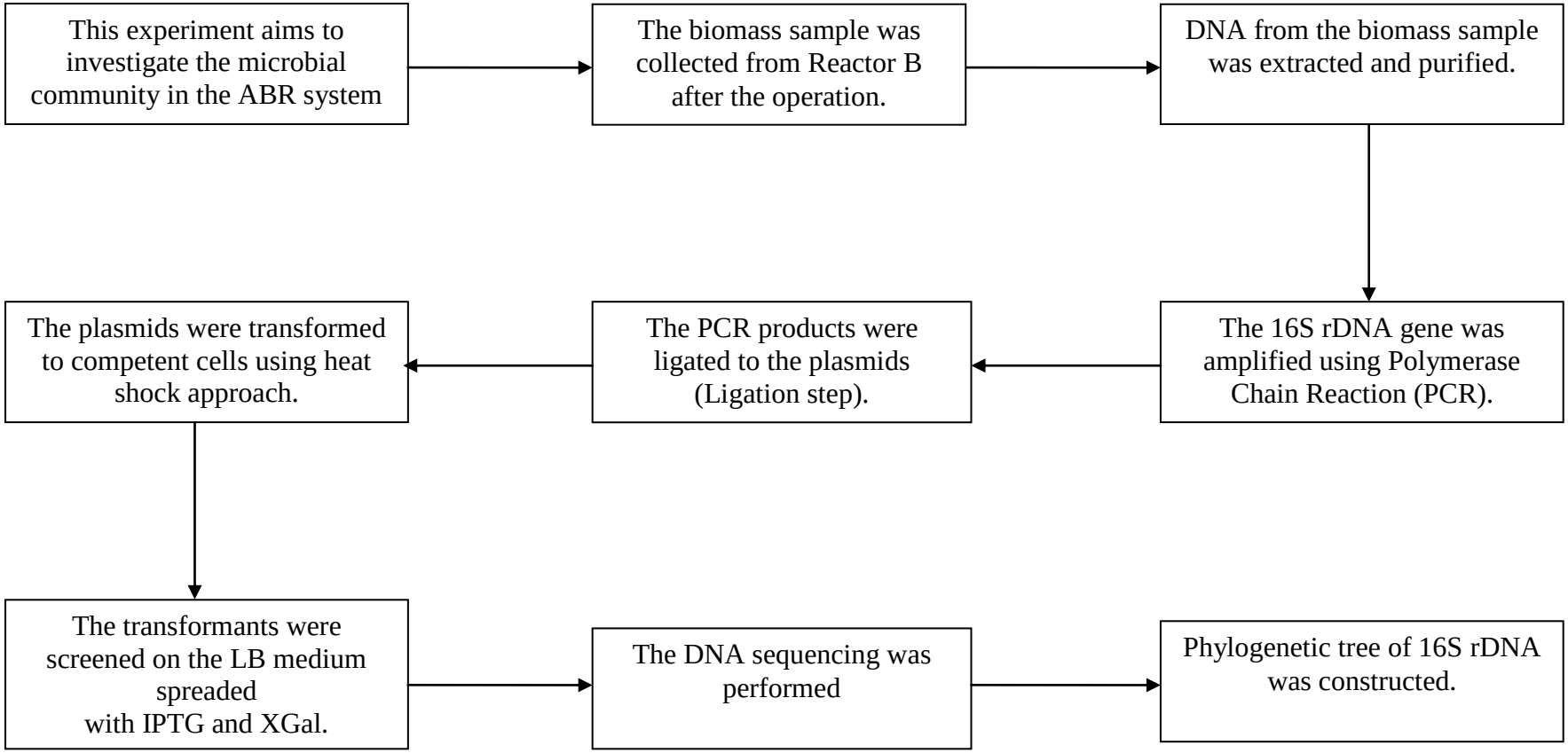


Figure 3.7 The experimental procedure for the construction of 16S rDNA clone library

There are five main steps in the construction of 16S rDNA clone libraries described as follows:

1) DNA extraction

First, DNA was extracted from biomass samples using DNA extraction kits (MP biomedical, USA). The quality of the DNA was identified by measuring the OD260/280 ratio (CIMMYT, 2005).

2) Polymerase Chain Reaction (PCR) of 16S rDNA

Polymerase Chain Reaction (PCR) was used to amplify the 16S rDNA from the extracted DNA using universal primers for bacteria, 8f (5'-AGAGTTTGATCCTGGCTCAG)and 1492r (5'-GGTTACCTTGTTACGACTT).The PCR mixture consists of 500 ng of DNA templates, 2.5 mM of dNTPs, 10 µm of primers, and 1 unit of Taq DNA polymerase (Takara, Japan). The PCR was performed for 35 cycles with the following steps:

-Denaturation 94 °C for 0.45 minutes

-Annealing 52 °C for 0.30 minutes

-Elongation 72 °C for 1.30 minutes

-Final Elongation 72 °C for 10 minutes

The PCR products of 16S rDNA were confirmed using the horizontal gel electrophoresis. The PCR products were then purified using a gel extraction kit, Nucleospin extract II (Machery-nagel, Germany).

3) DNA cloning

This step was performed using pGem-T® Easy vector systems (Promega, USA). The detail of pGem-T® Easy was shown in the Appendix D. PCR products were ligated with the plasmid pGEM-T and transformed to competent cell, *E. coli* JM109 (Promega, USA). At the screening step, the competent cells were spreaded on the on the LB (Luria Bertani) plate with 100 µg/ml of amplicilin, 100 mM of IPTG (Isopropyl-beta-thiogalactopyranoside) and 50 mg/ml of XGal (5-Bromo-4-chloro-Indoly-β-D-Galactosidase). The clones containing PCR products were appeared in white colonies. The appearance of white colonies was due to the interruption of the

coding sequence of β-galactosidase in the plasmid by inserted DNA. Therefore, β-galactosidase enzyme was not produced resulting in no utilization of X-Gal in the medium. On the other hand, the clones without PCR products that had only plasmid were appeared in blue colonies because the β-galactosidase gene in the plasmid was encoded the β-galactosidase enzyme to utilize X-Gal in the LB medium.

4) Screening of the transformants containing inserted DNA

The white colonies were selected to grow on the LB medium consisting of ampicilin, IPTG and Xgal again. After that the white colonies were inoculated to 5 ml of LB broth containing 5 µl of 100 µg/ml ampicilin. Then the plasmid in the tranformants cell growing on LB broth was extracted by using plasmid extraction kits (Geneaid, Taiwan). The inserted DNA in the plasmids was cut out by EcoRI restriction enzyme (Thermo scientific, Lithuania). Finally, the inserted DNA was confirmed using the horizontal gel electrophoresis.

5) DNA sequencing and phylogenetic tree

The plasmids containing inserted DNA were sent for DNA sequencing using primer M13F (5'-GTTTTCCCAGTCACGACGTTGTA-3') and M13R (5' CAGGAA ACAGCTATGACC 3') (Macrogen, Korea). The remaining portion of plasmid and low quality sequences were trimmed off using CodonCode Aligner software. The resulting sequences were also assembled using CodonCode Aligner software. The short sequences that cannot be assembled were discarded. Finally, the resulting sequences of the 16S rDNA werecompared to the GenBank database using BLASTn search (National Center for Biotechnology Information, U.S.). The 16S rDNA sequences of the clones together with their closely related species were used to construct the phylogenetic tree using MEGA5 softwarewith the neighbor joining algorithm and 500 bootstrapped replicates.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Investigation of azo dyes decolorization under sulfate-reducing conditions in anaerobic baffled reactors (ABRs)

The anaerobic baffled reactors were continuously operated at room temperature (30 ± 2 ^{o}C). The synthetic wastewater having COD:sulfate ratio of ~0.6 was fed into Reactor A and Reactor B. The reactors were started up and operated without the addition of azo dyes for 53 days. After 34 days of experiments, the pH of the influent synthetic wastewaters was adjusted to 7.2 ± 0.1. Then after 53 days of experiments, 200 mg/L of C.I. Acid Red 18 and 80 mg/L of C.I. Acid Orange 7 were added to the synthetic wastewaters for Reactor A and Reactor B, respectively. The ABRs were continuously operated until day 114th.

4.1.1 COD removal efficiencies in the ABRs

The COD concentrations in each compartment of Reactor A and Reactor B are shown in Figure 4.1. During day 35th until day 49th after the start-up period but before the azo dye addition, the average influent COD of Reactor A and Reactor B were 437.63 ± 50.74 mg/L and 438.13 ± 64.19 mg/L, respectively. The average COD in the last compartment were 92.57 ± 7.46 mg/L and 76.82 ± 11.87 mg/L for Reactor A and Reactor B, respectively. The COD removal efficiencies in both reactors were rather close in which 78.6 ± 3.4 % and 82.3 ± 3.1% of average COD removal efficiencies for Reactor A and Reactor B were achieved. For both reactors, most of COD removal occurred in the first compartments in which the average COD removal efficiency were 62.48 ± 7.20% and 68.76 ± 2.90% for Reactor A and Reactor B, respectively.

After 53 days of the experiment in the azo dye addition period, the average influent COD were increased due to the COD of the azo dyes (89.3mg/l for C.I. Acid Red 18 and 88.36 mg/l for C.I. Acid Orange 7). The average influent COD

concentrations from day 54th to day 114th of Reactor A and Reactor B were 526.38 ± 54.41 mg/L and 517.54 ± 59.60 mg/L, respectively. The average COD in the last compartment were 208.60 ± 11.99 mg/L and 189.37± 19.24 mg/L for Reactor A and Reactor B, respectively. The average COD removal efficiencies in both reactors were rather close in which the average COD removal efficiencies were 60.1 ± 3.3 % and 63.1 ± 4.7 % for Reactor A and Reactor B, respectively. For both reactors, most of COD removal occurred in the first compartments in which the average COD removal efficiency were 52.90 ± 4.88% and 51.22 ± 6.15% for Reactor A and Reactor B, respectively.

The results show that the COD removal efficiencies for both reactors were rather close in which the COD removal efficiency were higher than 75 % after the startup period. However, after the azo dye addition, the COD removal efficiencies in both reactors decreased. Most of COD removal occurred in the first compartments for both reactors. The COD removal efficiency before the azo dye addition in this study are in agreement with the results obtained from the study by Krishna et al. (2008) using 8-compartment ABRs treating low strength wastewater (500 mg/L of COD) at hydraulic retention time of 20, 15, 10, 8 and 6 hours. The study by Krishna et al. (2008) showed that COD removal efficiencies in the ABRs were higher than 80% for all of the hydraulic retention time tested.

After the azo dye addition, the decreases of COD removal efficiencies were observed for both reactors. However, the COD removal efficiencies in this study were in agreement with the study by Naimabadi et al. (2009) using 5-compartment ABR followed by fixed activated sludge to treat azo dye, C.I. Reactive Red 2, in synthetic wastewater with the COD concentration of 4,000 mg/L. The results from Naimabadi et al. (2009) revealed that 54.5 % of the average COD removal efficiency was obtained. Bell et al. (2000) also found that 8-compartment ABR treating various dye including azo dye were capable of treating the COD with the efficiency of 50-60%.

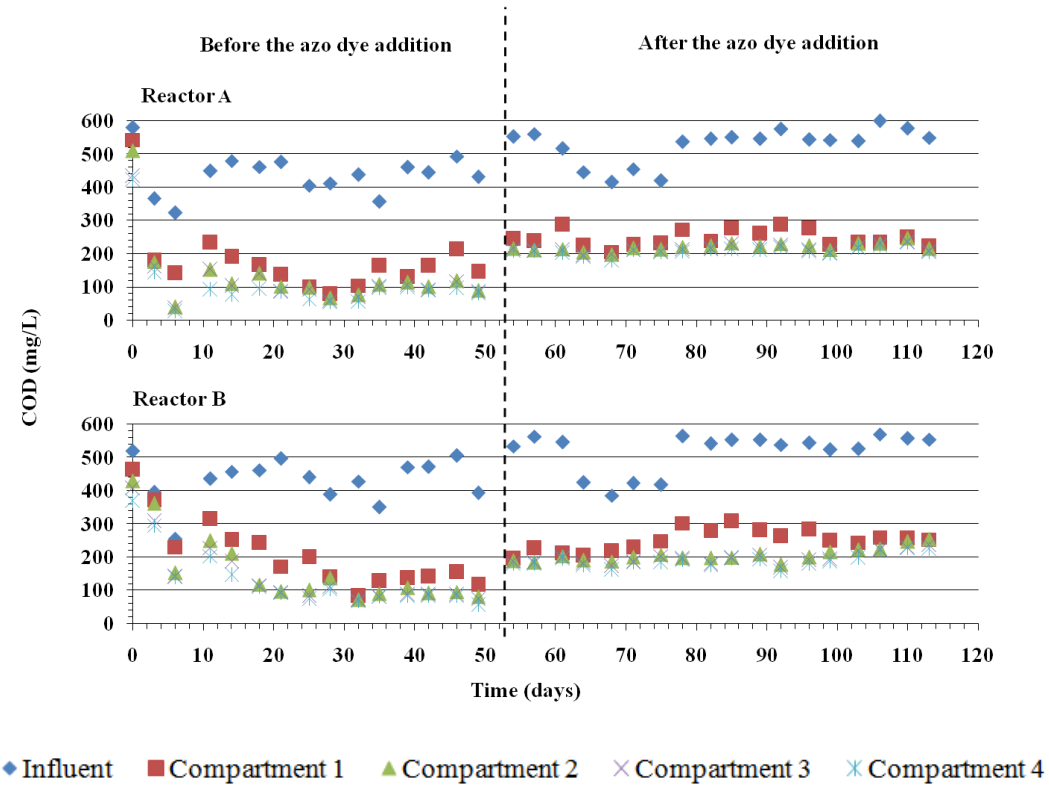


Figure 4.1 COD concentrations (mg/L) in each compartment of Reactor A and Reactor B, (– – –) line for separation between before and after the azo dye addition on day 53th

4.1.2 Sulfate removal efficiencies in the ABRs

Figure 4.2 shows the sulfate concentrations in each compartment of Reactor A and Reactor B. During day 35th until day 49th after the start-up period but before the azo dye addition the average influent sulfate concentrations of Reactor A and Reactor B were 852.99 ± 11.42 mg/L and 859.16 ± 7.51 mg/L, respectively. The average sulfate concentrations in the last compartment were 507.35 ± 31.87 mg/L and 545.74 ± 26.73 mg/L for Reactor A and Reactor B, respectively. The sulfate removal efficiencies in both reactors were rather close in which 40.5 ± 3.5% and 36.5 ± 2.7% of average sulfate removal efficiencies for Reactor A and Reactor B were achieved. For both reactors, most of sulfate removal occurred in the first compartments in which

average sulfate removal efficiency were 36.3 ± 2.6% and 31.4 ± 3.2% for Reactor A and Reactor B, respectively.

After the addition of C.I. Acid Red 18 into Reactor A, the average influent sulfate concentration of Reactor A increased to 890 ± 15 mg/L due to the impurities in the C.I. Acid Red 18 used in this study. Nevertheless, the average influent sulfate concentrations of Reactor B did not significantly change (865 ± 15 mg/L) after the addition of C.I. Acid Orange 7. The addition of 200 mg/l of C.I. Acid Red 18 contributed to the increases in sulfate concentrations of 58.3 ± 7.9 mg/l in the influent. On the other hand, the addition of 80 mg/l of C.I. Acid Orange 7 increased the sulfate concentrations approximately 11.6 ± 3.7 mg/l in the influent.

Sulfate removal in both reactors gradually increased over time during the course of the experiment in which the average sulfate concentrations in the last compartment for the last 8 days were 234.58 ± 12.71 mg/L and 229.79 ± 19.23 mg/L for Reactor A and Reactor B, respectively. The average sulfate removal efficiencies in both reactors were rather close in which the average sulfate removal efficiencies for the last 8 days of the experiment were 73.9 ± 1.8% and 73.5 ± 2.5 % for Reactor A and Reactor B, respectively. Similarly to the COD removal, most of sulfate removal occurred in the first compartments for both reactors in which the average sulfate removal efficiency were 72.81 ± 2.2% and 69.40 ± 4.3% (average over the last 8 days) for Reactor A and Reactor B, respectively.

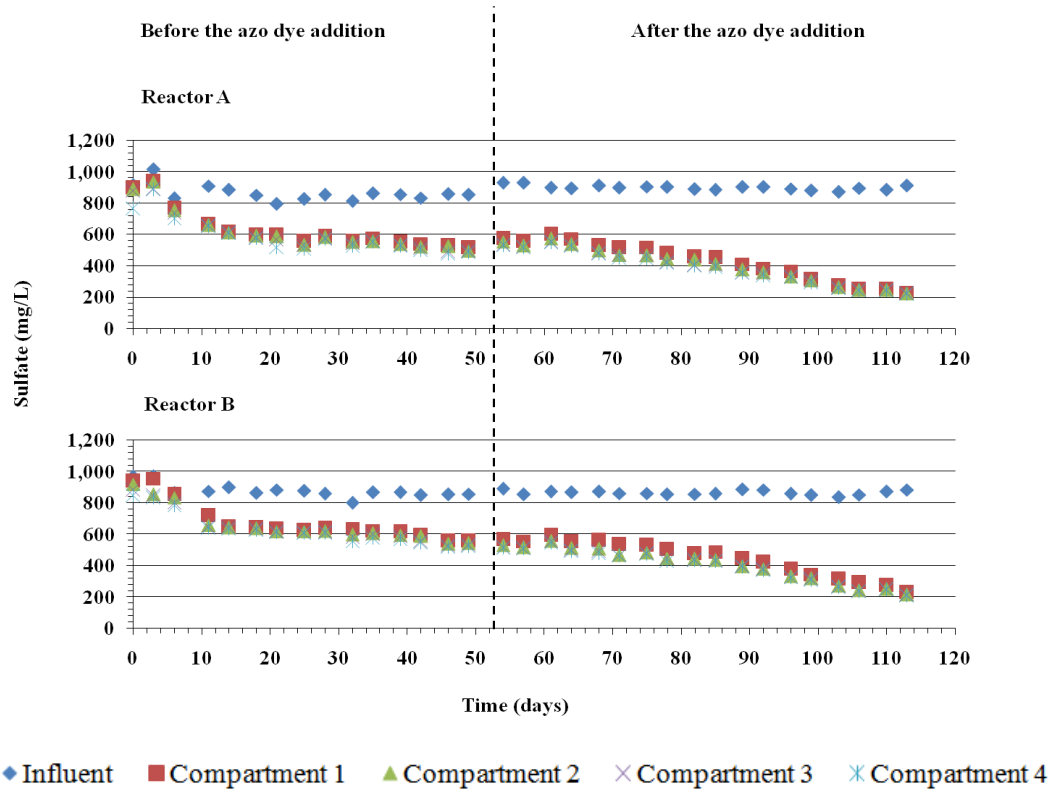


Figure 4.2 Sulfate concentrations (mg/L) in each compartment of Reactor A and Reactor B, (– – –) line for separation between before and after the azo dye addition on day 53th

4.1.3 Sulfide production in the ABRs

Sulfide concentrations within each compartment of Reactor A and Reactor B are shown in Figure 4.3. During day 35th until day 49th after the start-up period but before the azo dye addition, the sulfide measurement results show that average sulfide concentrations in the last compartment of Reactor A and Reactor B were 32.60 ± 5.25 mg/L and 23.94 ± 1.37 mg/L, respectively. However, after the azo dye addition, the average sulfide concentrations (over the last 8 days) in the last compartment of Reactor A and Reactor B increased to 198.11 ± 43.80 mg/L and 175.82 ± 25.12 mg/L, respectively. The increases in sulfide production in the ABRs followed the same trend as the increases in sulfate reduction after the azo dye addition.

Nevertheless, the results obtained from sulfide measurement throughout the experimental period revealed that the sulfide concentrations observed were lower than the theoretical concentrations expected from the sulfate reduction. It is possible that the sulfide produced was lost from the systems via gaseous H_2S; while a fraction of the H_2S might be oxidized to elemental sulfur (S^o) by trace oxygen in the systems.

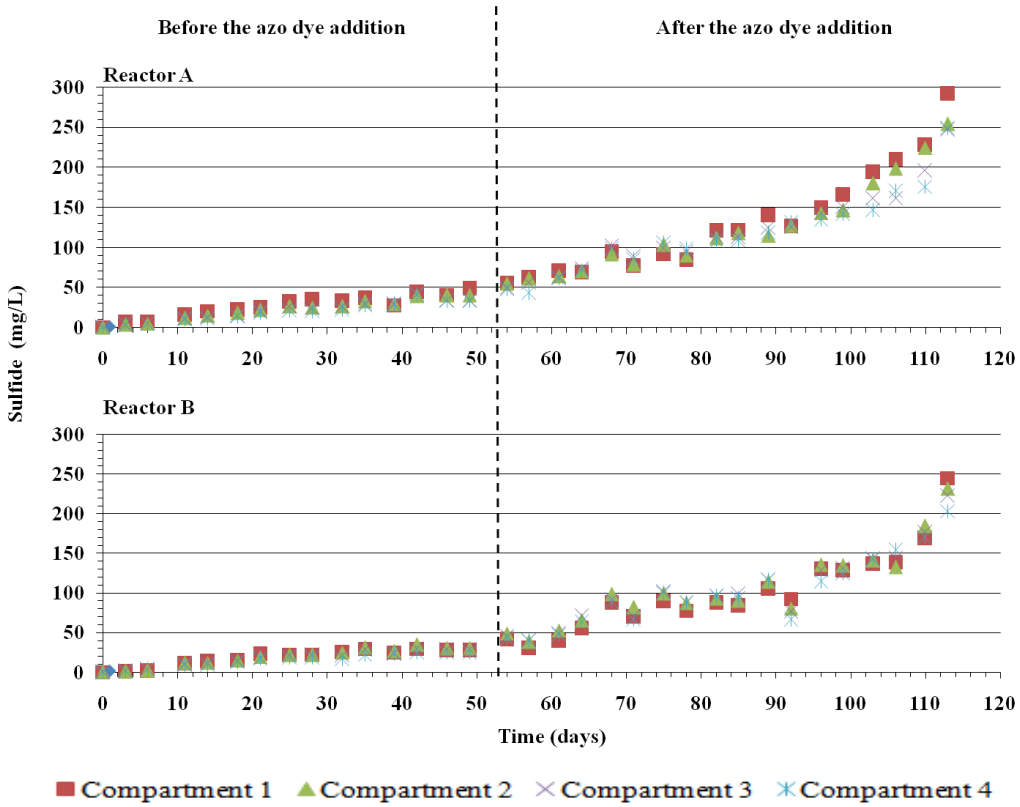


Figure 4.3 Sulfide concentrations (mg/L) in each compartment of Reactor A and Reactor B, (– – –) line for separation between before and after the azo dye addition on day 53th

4.1.4 pH values in the ABRs

The pH values within each compartment of Reactor A and Reactor B are shown in Figure 4.4. Before the pH of the influent synthetic wastewaters was adjusted on day 35th, the range of pH values in all compartments of Reactor A and Reactor B were in the range of 7.31 – 7.72 and 7.37 – 7.81, respectively. After the pH adjustment on day 35th, the range of pH values in all compartments were in the range

of 7.03 – 7.2 for Reactor A and 6.97 – 7.36 for Reactor B, respectively. For both reactors, the pH values slightly increased along the compartments. During day35th until day 49th after the start-up period but before the azo dye addition, the average pH in the last compartment was 7.13 ± 0.04 and 7.15 ± 0.04 for Reactor A and Reactor B, respectively. After azo dye addition, the average pH in the last compartment was 7.15 ± 0.02 for Reactor A and 7.15 ± 0.06 for Reactor B, respectively.

In both reactors, the pH values slightly increased along the compartments. The pH increases could be due to the production of alkalinity during sulfate reduction process and the degradation of volatile fatty acids along the compartments (Krishna et al. 2008). The addition of the azo dyes to the ABRs did not result in any significantly changes in the pH values in both reactors. The pH values obtained in this study were in the range commonly observed in previous studies. For example, the pH range of 6.4 – 7.6 was observed in the study by Bell et al. (2000) using 8-compartment ABR to treat the dyes including azo dyes. The pH in the range of 6.0-8.0 were observed in the study by Naimabadi et al. (2009) using 5-compartment ABR together with fix activated sludge reactor to treat the azo dye, Reactive Red 2.

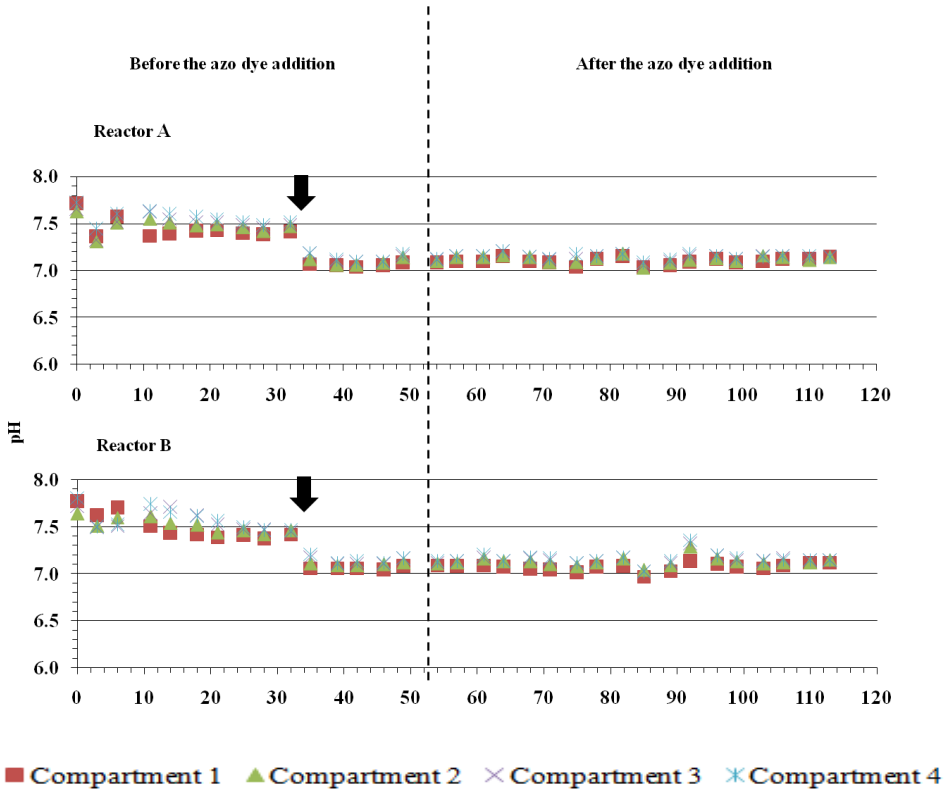


Figure 4.4 pH values in each compartment of Reactor A and Reactor B: (⬇) after 34 days of the experiment, the pH in synthetic wastewater was adjusted to pH ~7, (– – –) line for separation between before and after the azo dye addition on day 53th

4.1.5 Oxidation Reduction potentials (ORPs) in the ABRs

The oxidation reduction potential within each compartment of Reactor A and Reactor B were showed in Figure 4.5. The oxidation reduction potentials (ORPs) in all compartments of Reactor A and Reactor B were always below -244 mV, indicating that anaerobic conditions were maintained throughout the experiment. The values obtained from this study conformed with the ORP values suggested to be suitable for supporting biological sulfate reduction which is below -200 mV (Munsintuntoolvest, 2003).

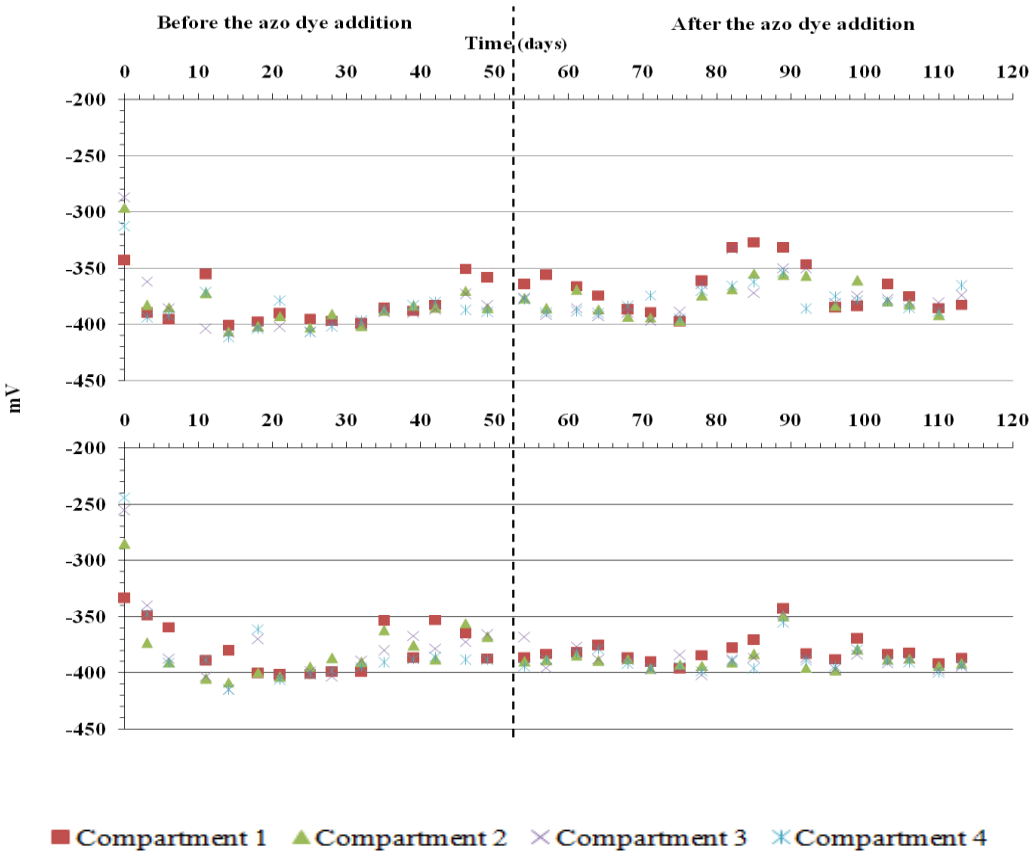


Figure 4.5 ORP values in each compartment of Reactor A and Reactor B, (– – –) line for separation between before and after the azo dye addition on day 53th

4.1.6 Alkalinity in the ABRs

In this study, alkalinity was measured in terms of calcium carbonate ($CaCO_3$) throughout the experimental period. Figure 4.6 shows alkalinity values within each compartment of Reactor A and Reactor B. The alkalinity values during day 35th until day 49th after the start-up period but before the azo dye addition were in the range of 869 – 919 mg/L for Reactor A and 913 – 857 mg/L for Reactor B, respectively. In both reactors, the alkalinity values slightly increased along the compartments. The highest alkalinity values were achieved in the last compartment at 908 ± 13 mg/L for Reactor A and 892 ± 19 mg/L for Reactor B in both reactors. The alkalinity production is considered the common characteristics of the biological sulfate reduction processes.

After the azo dye addition, the alkalinity values were in the range of 881 – 1,081 mg/L for Reactor A and 863 – 1,063 mg/L for Reactor B, respectively. The highest alkalinity values were achieved in the last compartment at 1,003 ± 54 mg/L for Reactor A and 972 ± 64 mg/L for Reactor B in both reactors. The results show that the alkalinity in both reactors were sufficient throughout the experiment, which can be observed by the stable pH in the systems.

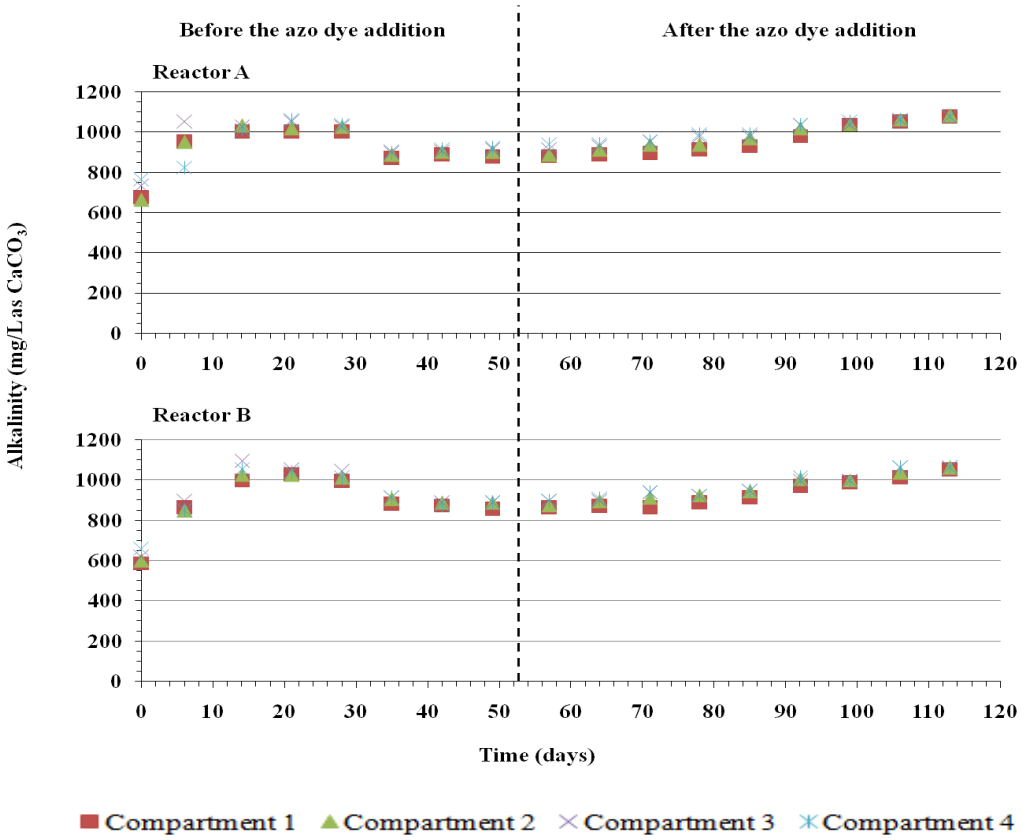


Figure 4.6 Total alkalinity within each compartment of Reactor A and Reactor B, (– – –) line for separation between before and after the azo dye addition on day 53th

4.1.7 Mix liquor suspended solids (MLSS) in the ABRs

Both Reactor A and Reactor B were initially seeded 17% by volume with the sludge obtained from an anaerobic digester with the MLSS of 54,307 ± 2,318 mg/L into each compartment of the reactors. As a result, the initial MLSS concentrations within each compartment of both reactors were approximately 9,232 mg/L. During the ABR operation, the MLSS loss from the systems was monitored over time via the measurement of the MLSS in the clear water zone of the last compartment of the reactors as shown in Figure 4.7. The MLSS values in the clear water zone of the last compartment of both reactors were generally low with the values of 0.47 ± 1.12 mg/L for Reactor A and 4.65 ± 9.25 mg/L in Reactor B, respectively. The results suggested that only small proportion of biomass was lost from the systems for both reactors. Finally, the final concentrations of the total MLSS after the operation of the reactors was measured. Each compartment of the reactors was well mixed before the sampling of MLSS. The final concentrations of MLSS in each compartment of both reactors were summarized in Table 4.1.The results indicated that the biomass in the systems significantly increased during the operation of the ABRs. The greatest amount of biomass was achieved in the first compartment of both reactors with the average MLSS concentration of 21,300 ± 212.13 mg/L and 19,700 ± 2,828.43 mg/L in the first compartment of Reactor A and Reactor B, respectively.

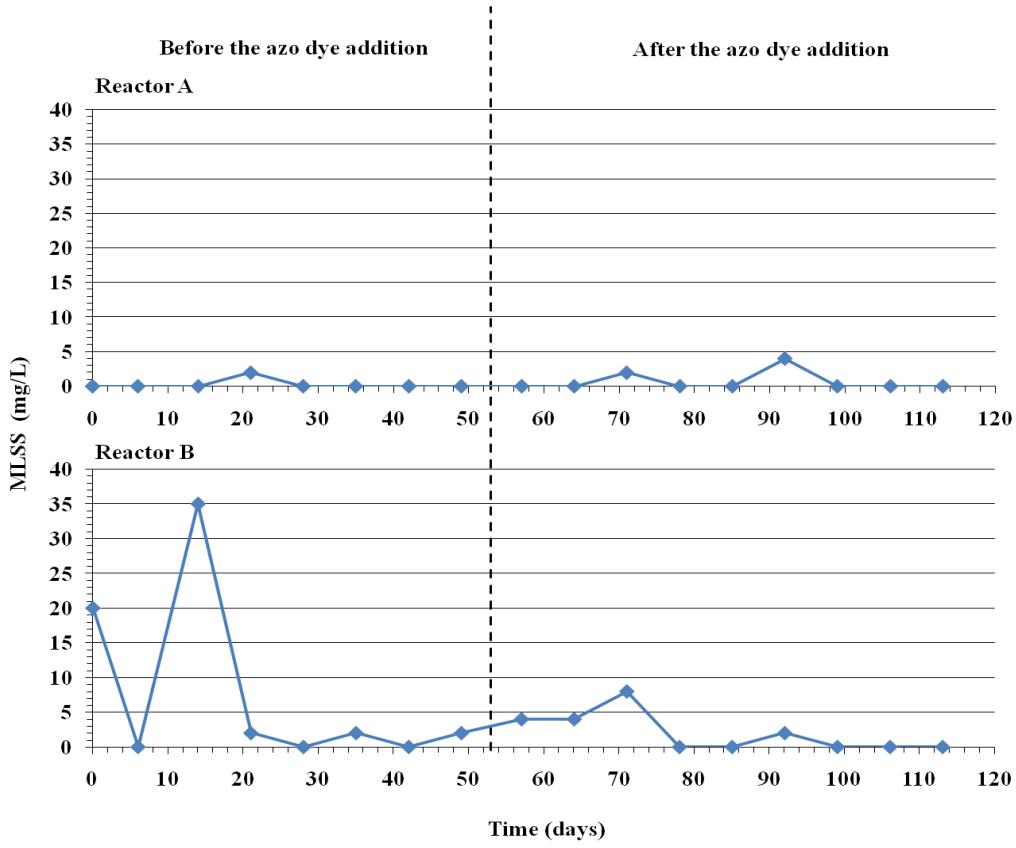


Figure 4.7 Mix liquor suspended solid (MLSS) concentrations in clear water zone within the fourth compartment of Reactor A and Reactor B, (– – –) line for separation between before and after the azo dye addition on day 53th

Table 4.1 The final concentrations of MLSS in each compartment of both reactors

Reactor / Compartment	Reactor A	Reactor B
Compartment 1	21,300 ± 212.13	19,700 ± 2,828.43
Compartment 2	11,725 ± 530.33	16,650 ± 1,202.10
Compartment 3	14,750	13,025 ±1,803.122
Compartment 4	14,625 ± 2,298.10	9,375 ± 1,308.15

4.1.8 Decolorization of azo dyes

After 53 days of experiment, two mono-azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, were added into Reactor A and Reactor B, respectively. Figure 4.8 shows absorbance values at 508 nm for Reactor A and at 483 nm for Reactor B. The average absorbances at the azo dyes's maximum wavelengths in the influents of Reactor A and Reactor B were 2.817 ± 0.042 and 2.954 ± 0.028, respectively. The average decolorization efficiencies of the C.I. Acid Red 18 in Reactor A was 97.8 ± 1.4%; while the average decolorization efficiencies of the C.I. Acid Orange 7 in Reactor B was 98.3 ± 1.7%. The decolorization efficiencies of Reactor A treating C.I. Acid Red 18 and Reactor B treating C.I. Acid Orange 7 were rather close. Furthermore, similar trends of azo dye decolorization were observed in both reactors. In both reactors, azo dye decolorization occurred in all of the compartments but it occurred to the greatest extents in the first compartments.

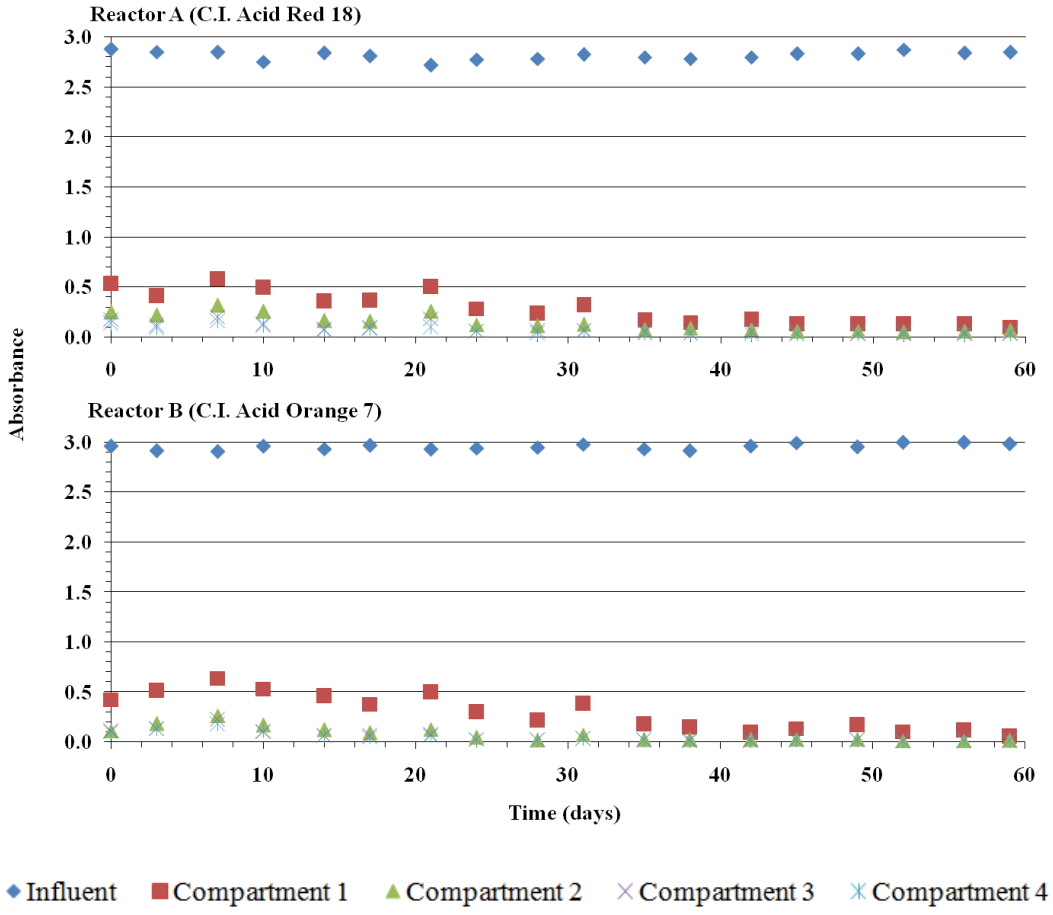


Figure 4.8 Absorbance values at 508 nm for Reactor A and at 483 nm for Reactor B

Figure 4.9 shows the absorbance scanning results of samples obtained from all compartments of both reactors on day 31th after the azo dye addition. The result confirms the azo dye decolorization in both reactors. The absorbance peaks of both azo dyes did not shift to other wavelengths, suggesting that azo dye decolorization truly occurred in these reactors. Therefore, it could be expected that the azo bonds responsible for the azo dye appearance were broken, resulting in the azo dye decolorization. Aromatic amines were likely to be produced in these systems; however, their production were not monitored nor confirmed.

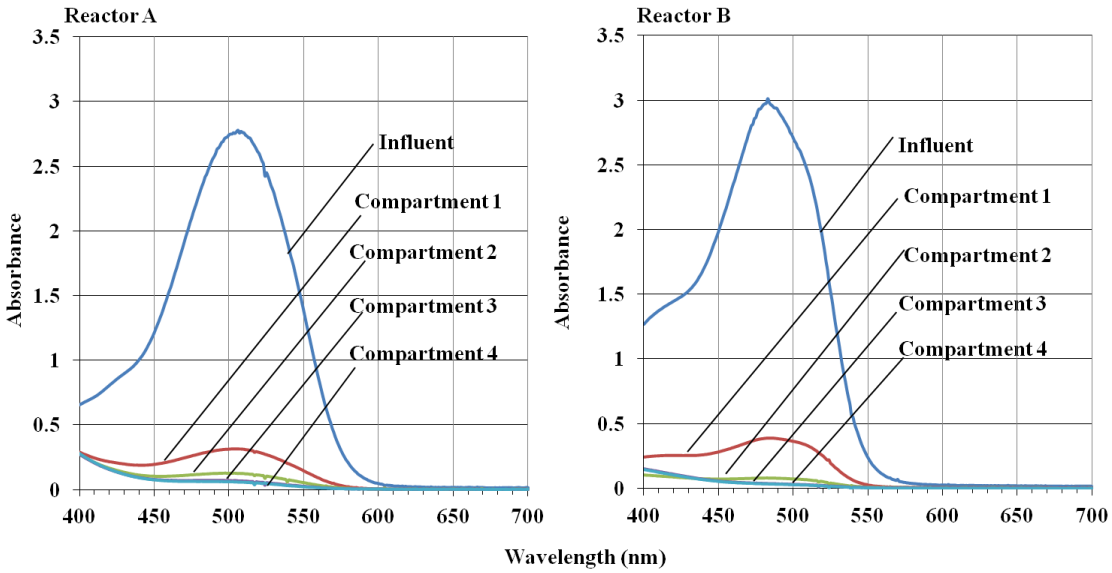


Figure 4.9 Absorbance scans at wavelength 400-700 nm for samples obtained on day 31th after the azo dye addition.

In fact, the percentages of electron equivalents from COD removal transferring to sulfate reduction in Reactor A and Reactor B were 98.1 ± 14.5% and 93.1 ± 20.0%, respectively, during the period of azo dye addition. This estimation of electron equivalents indicates the sulfate-reducing conditions in both reactors, in which C.I. Acid Red 18 and C.I. Acid Orange 7 can be effectively decolorized. Figure 4.10 and 4.11 shows azo dye decolorization in the reactors and in the test tubes after filtered through 0.45 µm membrane on day 31th.

Azo dye decolorization under anaerobic conditions has been found to occur via azo reduction (Stolz 2001; Pearce et al., 2003; Dos Santos et al., 2007). Azo dyes can serve as electron acceptors under anaerobic conditions although energy may not be gained by the microorganisms. However, in our systems sulfate is present at high concentrations and serves as the main electron acceptor. Despite the presence of both electron acceptors, sulfate and azo dyes, sulfate reduction and azo dye decolorization still occurred concomitantly in this study. High sulfate concentrations did not appear to prevent the azo dye decolorization. This observation agrees well with the studies by Cervantes et al (2006) and van der Zee et al (2003) that reported the concurrent azo

reduction and sulfate reduction. In addition, one of the mechanisms of azo dye decolorization proposed by van der Zee (2003) was chemical reduction by reductants such as biogenic hydrogen sulfide. Therefore, it could be expected that sulfide formation from sulfate reduction in this study could also contribute to the reduction of azo dyes in anaerobic baffled reactors.

Although our study did not have a reactor fed with synthetic wastewater without sulfate to serve as a negative control; the study by Wangsaviboon (2011) might help explain the effects of sulfate on azo dye decolorization in anaerobic baffled reactors. In the study of Wangsaviboon (2011), the anaerobic baffled reactors that had COD: sulfate ratio of 0.6 and 4 had higher azo dye decolorization efficiencies than the reactor without sulfate supplement ;therefore, Wangsaviboon (2011) concluded that the supplements of sulfate enhanced the azo dye decolorization efficiencies in anaerobic baffled reactors. However, it should be noted that the substrate used in the study of Wangsaviboon (2011) was sucrose, which was different from in this study in which lactic acid was used.

High azo dye decolorization efficiencies observed in current study in the presence of high sulfate are in agreement with the study by van der Zee et al. (2003) and Cervantas et al. (2006) that suggested the positive effects of sulfate on azo dye decolorization. In the study of van der Zee et al. (2003) revealed that reduction of Reactive Red 2 was faster in presence of sulfate due to biogenic sulfide formation indicating that sulfate influence the reduction of the azo dye. Cervantes et al. (2006) also found that the decolorization of Reactive Orange 14 in batch studies with anaerobic sludge was stimulated by increasing sulfate concentrations.

In contrast, the results of this study did not agree with the study by Carliell et al. (1995) and Carliell et al. (1998) in which no effects of sulfate on azo dye decolorization was observed. The experiments in these studies (Carliell et al, 1995; Carliell et al., 1998) were conducted in batch scale to investigate the redox potential effects of sulfate on azo dye decolorization in the period of only 13 hours. These experiments were performed in short period of time; therefore, the microbial

communities were not likely to change within this period. The differences in time scales of the experiments might help in explaining the conflicting results with this current study.

In addition, Panswad and Luangdilok (2000) have also investigated the effect of sulfate on the azo dye decolorization in anaerobic-aerobic sequencing batch reactors. The results showed no effect of sulfate on azo dye decolorization. However, anaerobic-aerobic sequencing batch reactors may not be capable to maintain anaerobic microorganisms, such as sulfate-reducing bacteria.

Moreover, in the study of van der Zee et al. (2003), sulfate has no significant effect on reduction of azo dye, Reactive Red 2, in upward-flow anaerobic sludge bed reactors (UASBs). The results by van der Zee et al. (2003) are conflicting with this current study. This could be due to the differences in biomass characteristics in UASBs and anaerobic baffled reactors. For examples, the microorganisms in anaerobic baffled reactors were not in the form of granules like in UASBs. The natural selection of the microorganisms within these two systems could be different, which may result in the microbial community within these two reactors. Furthermore, the difference in biomass concentrations could be an important factor on the effects of sulfate on azo dye decolorization as suggested by van der Zee et al. (2003) that biogenic sulfide may have more effect on azo dye decolorization with lower amount of biomass concentration below 35 g/L of VSS. In this study, the amount of biomass in the anaerobic baffled reactors was below 21,300 mg/L for every compartment in both reactors indicating that the formation of biogenic sulfide from sulfate reduction in this study could have more effects on azo dye reduction compared with the study of van der Zee et al. (2003).

The effect of sulfate on decolorization of the other azo dye also found in the other study. In the study of van der Zee et al. (2003) revealed that in batch studies, the reduction of Reactive Red 2 was faster in presence of sulfate due to biogenic sulfide formation indicating that sulfate influence the reduction of the azo dye.

Cervantes et al. (2006), found that decolorization of Reactive Orange 14 in batch studies with anaerobic sludge was stimulated by increasing sulfate concentration.

Table 4.2 summarizes the result of COD removals, sulfate removals and decolorization efficiencies in each compartment of Reactor A and Reactor B. The result revealed that most of COD removal, sulfate removal and decolorization efficiency occurred in the first compartment. This due to the higher biomass, COD sulfate and azo dyes in the first compartment compared to other compartments in the reactors.

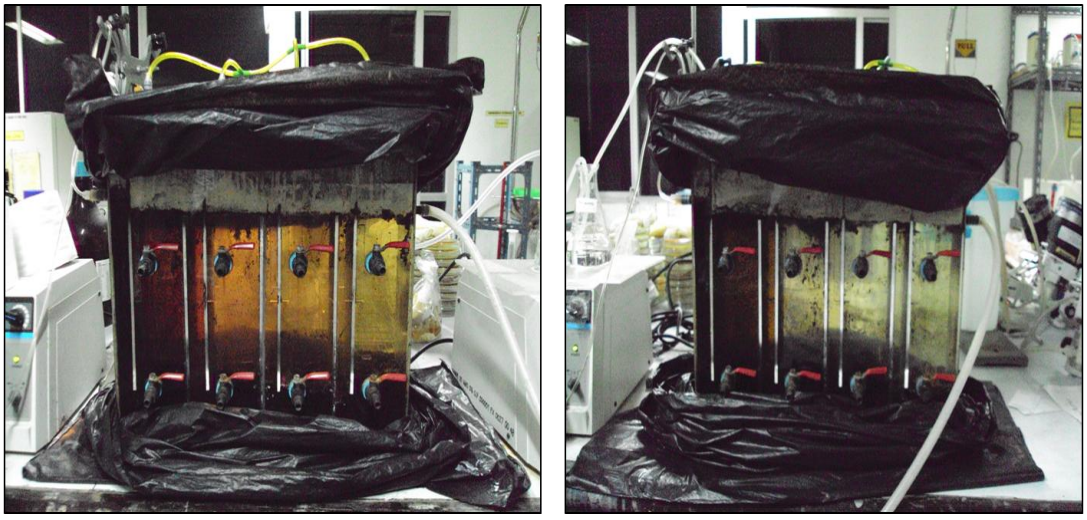


Figure 4.10 The reduction of azo dye, C.I. Acid Red 18 at 200 mg/L and C.I. Acid Orange 7 at 80 mg/L in the ABRs on day 31th:
(Left) Reactor A, (Right) Reactor B

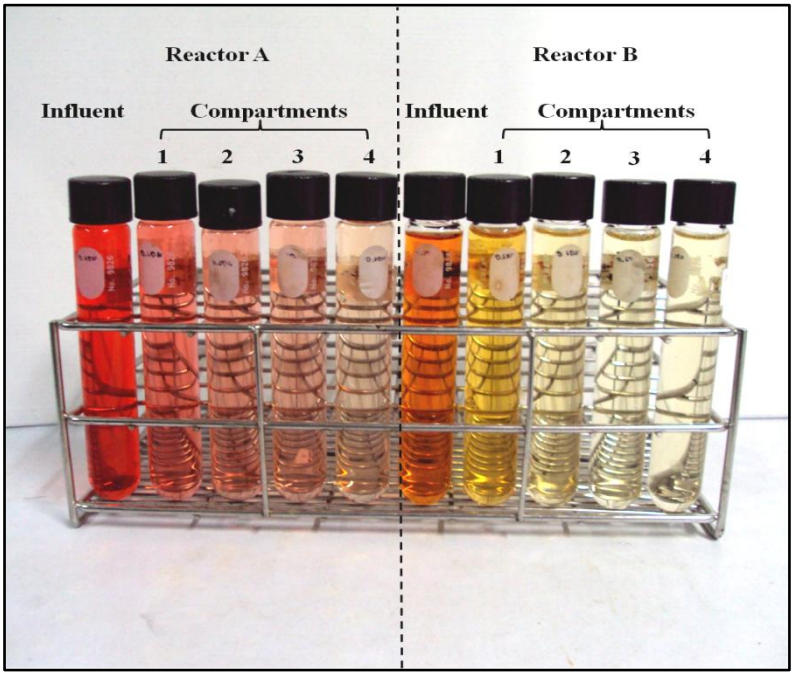


Figure 4.11 The reduction of azo dyes, C.I. Acid Red 18 at 200 mg/L and C.I. Acid Orange 7 at 80 mg/L in the synthetic wastewater collected from the Reactor A and Reactor B, respectively, and filtered through 0.45 µm membrane on day 31^{t}

Table 4.2 COD removals, sulfate removals and decolorization efficiencies in each compartment of Reactor A and Reactor B.

Reactor	Compartment	During day35th until day 49th after the start-up period but before the azo dyes addition		Azo dyes addition		
		COD removal efficiency	Sulfate removal efficiency	COD removal efficiency	Sulfate removal efficiency	Color removal efficiency
Reactor A	Compartment 1	62.48 ± 7.20 %	36.31 ± 2.57 %	52.90 ± 4.88 %	72.81 ± 2.22%	89.81 ± 5.76 %
	Compartment 2	13.11 ± 6.01 %	1.90 ± 0.70 %	4.96 ± 3.98 %	0.67 ± 0.62 %	5.21 ± 2.83 %
	Compartment 3	1.34 ± 0.74 %	1.37 ± 1.47 %	1.10 ± 0.80 %	0.20 ± 0.14 %	2.08 ± 1.27 %
	Compartment 4	1.66 ± 1.47 %	0.94 ±0.71 %	1.13 ± 1.02 %	0.18 ± 0.04 %	0.64 ± 0.80 %
Reactor B	Compartment 1	68.76 ± 2.90 %	31.31 ± 3.22 %	51.22 ± 6.15 %	69.40 ± 4.34 %	89.93 ± 6.27 %
	Compartment 2	9.73 ± 2.70 %	1.69 ± 0.78 %	8.58 ± 6.20 %	3.39 ± 2.42 %	7.44 ± 3.99 %
	Compartment 3	2.15 ± 1.19 %	1.92 ± 1.36 %	1.68 ± 1.39 %	0.28 ± 0.22 %	0.82 ± 0.75 %
	Compartment 4	1.65 ± 1.81%	1.51 ± 0.72 %	1.61 ± 1.18%	0.48 ± 0.12 %	0.15 ± 0.37 %

4.1.9 Rate of azo dye decolorization in ABRs

With the assumption that each compartment of the ABRs was completely mixed, the rate of azo dye decolorization can be calculated as follows:

$$-r_n = (A_{n-1} - A_n)/HRT$$

$-r_n$ = Rate of azo dyes decolorization in compartment n (hr^{-1})
A_n = Absorbance of azo dyes in compartment n
A_{n-1} = Absorbance of azo dyes in compartment n-1
A_0 = Absorbance of azo dyes in the influent
HRT = Hydraulic retention time in each compartment (6 hrs)
(Boonchayaanant et al., 2012, in press)

Even though completely mixed assumption might not be true in terms of biomass mixing, the azo dye was assumed to be completely mixed as homogenous color can be observed within each compartment of the reactors.

In this study, the absorbance of azo dyes that used to calculate the rate of azo dye decolorization were measured at 508 nm and 483 nm for C.I. Acid Red 18 and C.I. Acid Orange 7, respectively. The decolorization rate in each compartment of ABRs was estimated as summarized in Table 4.3.

Table 4.3 Rates of azo dye decolorization in ABRs

Reactor	Compartment	Rate of azo dye decolorization (hr^{-1})
Reactor A	Compartment 1	0.422 ± 0.029
	Compartment 2	0.024 ± 0.013
	Compartment 3	0.010 ± 0.006
	Compartment 4	0.003 ± 0.004
Reactor B	Compartment 1	0.443 ± 0.033
	Compartment 2	0.036 ± 0.019
	Compartment 3	0.004 ± 0.004
	Compartment 4	0.001 ± 0.002

The results revealed that the highest rate of azo dye decolorization occurred in the first compartment in both reactors. This was likely due to the higher biomass and azo dye concentrations in the first compartment compared to other compartments in the reactors. It should be noted that the rates of azo dye decolorization in Reactor A and Reactor B appear to be close although different azo dyes were treated.

4.2 Adsorption of the azo dyes by inactive biomasses

Adsorption of azo dyes by inactive biomasses was investigated using the biomasses obtained from the first compartment of both reactors after the operation of the ABRs. The biomasses were washed to remove other compounds and autoclaved. The initial MLSS concentrations used for the adsorption experiment were set to be to the same as the final MLSS concentrations in the first compartment of each reactor after the operation, which were 21,300 ± 212 mg/L and 19,700 ± 2,828 mg/L for Reactor A and Reactor B, respectively. The azo dyes removal by inactive biomasses was monitored over time. Figure 4.12 shows the average absorbance values of triplicates of inactive biomasses obtained from Reactor A (C.I. Acid Red 18) and

Reactor B (C.I. Acid Orange 7) at wavelength 508 nm and 483 nm, the wavelengths with maximum absorbance of the C.I. Acid Red 18 and C.I. Acid Orange 7, respectively. The azo dye decolorization efficiencies by inactive biomasses from Reactor A and Reactor B were 3.24 ± 0.053% and 3.83 ± 0.17%, respectively, over 15 days. The results indicated that the adsorption of azo dyes by inactive biomasses from both reactors were negligible, especially when compared with the time scale of the hydraulic retention time (1 day) of the ABRs used in this study. The results were in agreement with the previous study by van der Zee et al. (2007) that investigated the decolorization of the azo dye, RO14, DB53, and DB71, using autoclaved sludge. Their study shows that the decolorization of azo dyes by inactive biomass was negligible (<5%). In fact, Bell and Buckley (2003) also commented that the autoclave process may have increased the surface area of adsorption onto biomass; therefore, the adsorption on autoclaved biomass could overestimate the adsorption by untreated biomass.

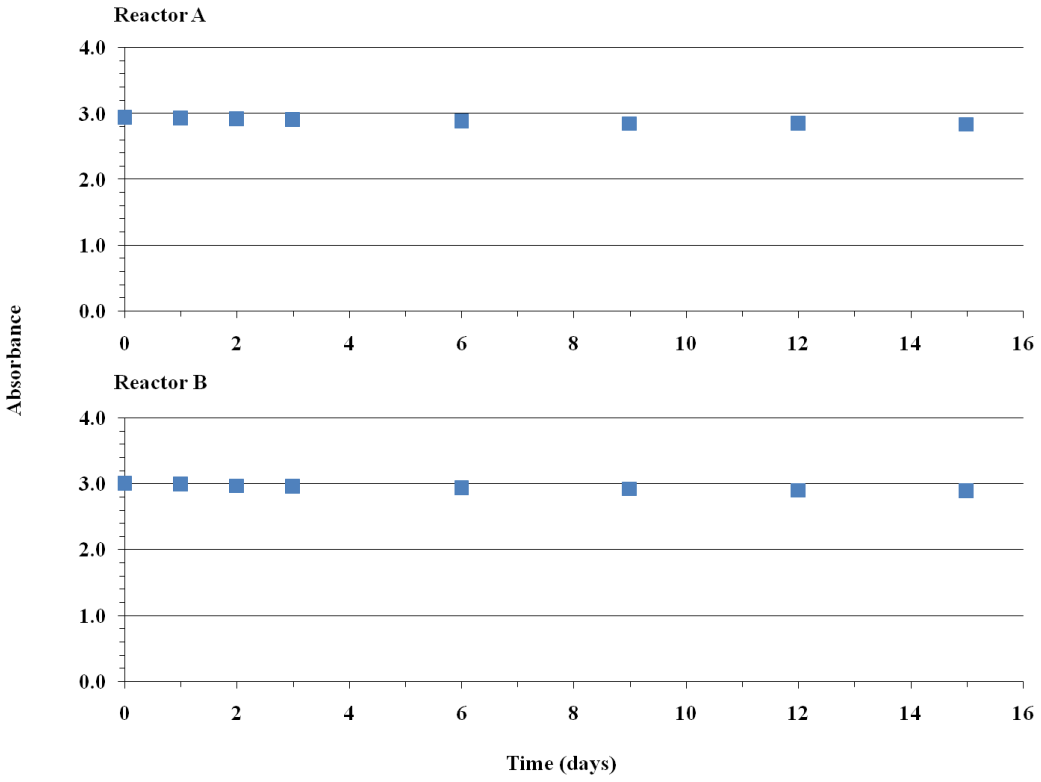


Figure 4.12 Average absorbance values of triplicates of inactive biomass obtained from Reactor A (C.I. Acid Red 18) and Reactor B (C.I. Acid Orange 7) at wavelength 508 nm and 483 nm, the wavelengths with maximum absorbance of the C.I. Acid Red 18 and C.I. Acid Orange 7, respectively

4.3 Microbial community by 16S rDNA clone library

After the operation of the anaerobic baffled reactors, the microbial community in the biomass sample from the first compartment of Reactor B treating C.I. Acid Orange 7 was studied. The biomass sample from the first compartment was selected to use in this experiment because of its highest azo dye decolorization efficiencies. The phylogenetic tree of the 16S rDNA clone library of the biomass sample from Reactor B was shown in Figure 4.13. Closely related species and two well-known sulfate-reducing bacteria, *Desulfovibrio desulfuricans* and *Desulfobacter postgatei* strain 2ac9 were included into the phylogenetic tree for comparison. In this study 18 clones were achieved in which 44.4% of all clones were closely related to

sulfate-reducing bacteria. Most of the sulfate-reducing bacteria found in Reactor B were closely related to *Desulfobacca acetoxidans* strain ASRB2 based on the 16S rDNA sequences, which accounts around 75% of all sulfate-reducing bacteria. In addition, the species closely related to fermentative bacteria, such as *Clostridium ganghwense* strain HY-42-06 and *Clostridium butyricum* strain VPI3266 were also observed. Other microorganisms closely related to *Anaeroarcus burkinensis* strain DSM 6283 and *Alkaliflexus imshenetskii* DSM 15055 strain were also observed. *Anaeroarcus burkinensis* strain DSM 6283 and *Alkaliflexus imshenetskii* DSM 15055 are bacteria that can grow anaerobically.

The results of this study are in agreement with the study by Plumb et al. (2008) using Fluorescence In Situ Hybridization (FISH) to investigate the microbial population in anaerobic baffled reactor treating food dyes including the azo dye, such as C.I. Red 9 and C.I. Yellow 3. Plumb et al. (2008) reported that sulfate-reducing bacteria was predominant in the reactor together with the bacteria in the members of the gamma subclass of the class *Proteobacteria*. Nevertheless, sulfate concentrations were not reported in the study by Plumb et al (2008).

The results in this study revealed the presence of sulfate-reducing bacteria and fermentative bacteria in the anaerobic baffled reactor treating C.I. Acid Orange 7. However, in this study, 16S rDNA was used to investigate the bacterial community. The observation of fermentative bacteria in 16S rDNA clone library did not necessary suggest that they are active in the system nor responsible for the reduction of azo dyes. It might be possible that fermentative bacteria that are capable of forming spores can tolerate unsuitable conditions and remain in the system without their activities. In contrast, the activity of sulfate-reducing bacteria in the system was clearly observed from sulfate reduction in the reactor.

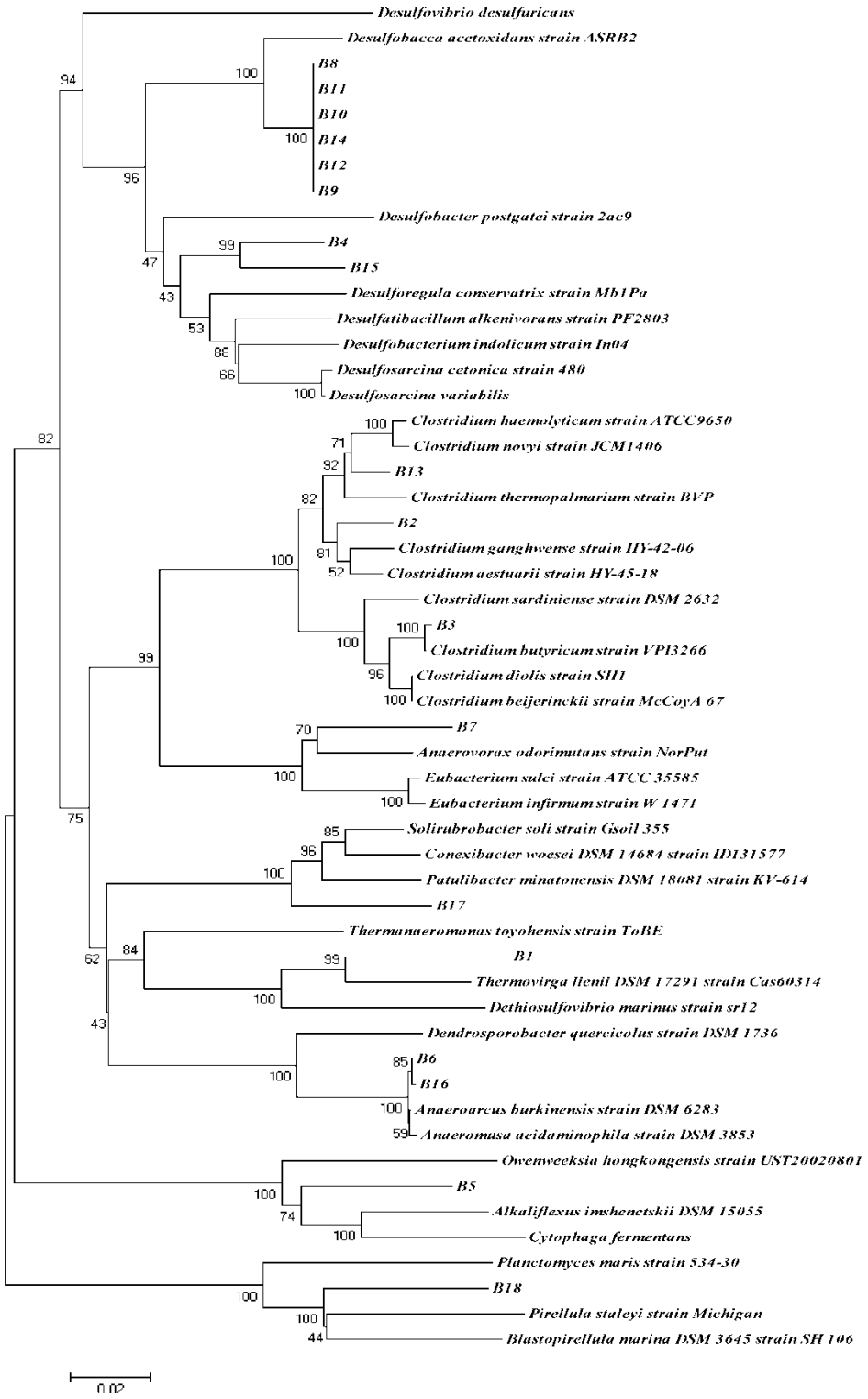


Figure 4.13 The phylogenetic tree of the 16S rDNA clone library of the biomass sample from the first compartment of Reactor B

Chapter V
CONCLUSIONS AND SUGGESTIONS

Conclusions

In this study, two four compartments ABRs operated with the HRT of 1 day were used to investigate the decolorization of two mono azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7, under sulfate-reducing conditions. The synthetic wastewaters fed to the ABRs consisted of lactic acid serving as the electron donor (COD of ~500 mg/L), and sulfate serving as the electron acceptor. The resulting medium has the COD:sulfate ratio of ~0.6, in which sulfate was present slightly in excess compared to the COD. The result showed clearly that two mono-azo dyes, C.I. Acid Red 18 and C.I. Acid Orange 7 can be decolorized effectively in ABRs under sulfate-reducing conditions in which sulfate serves as the main electron acceptor. The decolorization efficiencies of the ABRs treating C.I. Acid Red 18 and C.I. Acid Orange 7 under sulfate-reducing conditions were rather close in which 97.8 ± 1.4 % and 98.3 ± 1.7%, of decolorization efficiency were achieved for Reactor A and Reactor B, respectively. Sulfate reduction and azo dye decolorization occurred concomitantly in this study. High sulfate concentrations did not appear to prevent the azo dye decolorization. Moreover, the decolorization of azo dyes by inactive biomasses was negligible (3.24 ± 0.053 % and 3.83 ± 0.17 % for biomass from Reactor A and Reactor B, respectively), suggesting that adsorption onto biomass was not the primary mechanisms for azo dye decolorization in this study.

The results from 16S rDNA clone library of the microbial community in the first compartment of Reactor B revealed that 44.4% of 19 clones were closely related to sulfate-reducing bacteria. However, species closely related to fermentative bacteria were also observed.

Suggestions

This study has demonstrated that ABRs can be used effectively for the treatment of azo dyes under sulfate-reducing conditions. It also provides better understanding on the azo dye decolorization in ABRs. ABRs are alternative anaerobic bioreactors that can be applied to the treatment of industrial wastewater due to several advantages, such as, low costs of construction and operations, and low maintenance.

However, further studies are still needed in order to clarify the questions remained in this study, such as the roles of sulfate-reducing bacteria and fermentative bacteria on azo dye decolorization in ABRs, the roles of biogenic sulfide on azo dye decolorization in ABRs, the effects of reactor operations (hydraulic retention time, azo dye concentrations, and COD and sulfate concentrations) on azo dye decolorization in ABRs. Applications of specific inhibitors of sulfate-reducing bacteria might be used to further investigate their roles in azo dye decolorization in the ABRs. Azo dye decolorization in ABRs using actual textile wastewaters should also be further investigated since there might be possible inhibition of azo dye decolorization by other constituents in actual textile wastewater..

In addition, there are also a few cautions regarding the uses of anaerobic bioreactors under sulfate-reducing conditions, such as, hydrogen sulfide odor and corrosion problems. The presence of sulfate can also decrease the yield of methane in anaerobic treatment systems. Nevertheless, textile wastewaters are usually not suitable for methane production due to their low COD. All of these issues should be further addressed before applying this knowledge to actual industrial applications.

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APPENDICES

APPENDIX A

Table A-1 COD concentrations (mg/L) within influents and each compartment of anaerobic baffled reactors

Date	Days	Reactor A				
		Influent	Compartments			
			1	2	3	4
30/6/2554	0	580.56	542.80	509.76	434.24	421.97
3/7/2554	3	366.91	178.75	175.93	161.82	143.00
6/7/2554	6	323.19	141.25	40.70	35.91	26.33
11/7/2554	11	448.60	233.00	151.92	151.92	91.34
14/7/2554	14	479.81	190.94	109.55	103.43	76.5
18/7/2554	18	462.00	165.86	141.83	138.60	94.71
21/7/2554	21	476.33	137.37	101.69	88.31	85.63
25/7/2554	25	405.25	102.05	98.11	92.19	63.60
28/7/2554	28	412.44	77.58	67.76	57.94	53.03
1/8/2554	32	438.37	103.81	76.26	68.88	57.07
4/8/2554	35	356.70	163.70	106.84	100.38	96.93
8/8/2554	39	460.68	129.80	114.40	105.60	98.12
11/8/2554	42	445.56	163.09	100.11	92.83	90.71
15/8/2554	46	493.50	213.85	117.97	117.50	96.82
18/8/2554	49	431.73	144.95	90.09	84.74	80.28
23/8/2554	54	552.35	245.03	215.26	215.26	208.85
26/8/2554	57	560.69	238.35	211.11	209.75	208.84
30/8/2554	61	516.47	286.78	214.08	210.07	201.15
2/9/2554	64	446.03	224.48	203.50	196.18	190.32
6/9/2554	68	415.48	205.39	197.40	197.40	180.48
9/9/2554	71	454.72	227.36	218.08	212.05	212.05
13/9/2554	75	420.38	230.61	212.51	207.41	204.16
16/9/2554	78	536.74	272.60	220.90	214.32	207.27
20/9/2554	82	545.69	239.15	223.70	215.28	213.41
23/9/2554	85	550.30	276.54	232.46	228.75	212.51
27/9/2554	89	546.92	259.90	221.48	219.67	210.18
30/9/2554	92	575.92	287.50	230.00	224.48	221.26
4/10/2554	96	544.64	275.54	224.48	210.22	207.00
7/10/2554	99	542.96	227.48	212.08	201.96	198.88
11/10/255	103	538.56	233.20	231.88	218.68	217.36
14/10/255	106	599.99	233.66	231.71	231.22	224.39
18/10/255	110	577.60	249.38	247.00	236.08	232.75
21/10/255	113	549.30	222.49	215.57	210.03	204.03

Table A-2 COD concentrations (mg/L) within influents and each compartment of anaerobic baffled reactors

Date	Days	Reactor B				
		Influent	Compartments			
			1	2	3	4
30/6/2554	0	519.20	462.56	429.52	405.92	368.16
3/7/2554	3	395.14	370.68	361.27	307.64	295.41
6/7/2554	6	253.29	230.30	152.74	141.25	138.37
11/7/2554	11	436.18	315.02	249.78	228.03	203.18
14/7/2554	14	457.16	252.14	210.53	186.05	146.27
18/7/2554	18	462.00	242.55	117.81	115.5	110.42
21/7/2554	21	496.398	171.71	96.78	95.00	92.77
25/7/2554	25	439.756	199.17	100.57	83.32	73.46
28/7/2554	28	387.89	142.39	137.48	109.49	104.09
1/8/2554	32	428.04	82.66	71.83	71.34	67.90
4/8/2554	35	349.81	127.09	89.18	81.85	79.70
8/8/2554	39	470.80	137.28	107.80	88.00	82.72
11/8/2554	42	471.41	141.94	92.59	86.95	82.25
15/8/2554	46	505.25	155.57	95.41	86.95	83.66
18/8/2554	49	393.37	117.30	81.17	74.93	55.75
23/8/2554	54	534.03	196.02	187.78	185.95	180.91
26/8/2554	57	562.96	226.09	183.87	183.87	180.24
30/8/2554	61	546.35	211.85	203.38	199.81	194.46
2/9/2554	64	424.56	203.50	191.30	183.00	174.70
6/9/2554	68	385.40	217.14	187.53	172.49	160.74
9/9/2554	71	422.24	228.29	200.45	187.46	185.14
13/9/2554	75	417.60	248.24	207.41	203.23	184.67
16/9/2554	78	564.00	302.21	197.87	195.52	190.82
20/9/2554	82	541.01	278.93	197.96	180.65	174.56
23/9/2554	85	552.16	307.63	198.59	197.66	194.88
27/9/2554	89	552.34	280.69	207.92	203.85	194.36
30/9/2554	92	538.20	262.20	177.10	168.36	157.76
4/10/2554	96	544.64	282.90	200.10	190.90	179.40
7/10/2554	99	523.60	248.60	215.16	193.16	186.56
11/10/255	103	525.36	243.76	221.76	217.80	198.00
14/10/255	106	569.75	259.51	224.39	221.95	220.49
18/10/255	110	557.65	255.55	247.00	230.38	227.05
21/10/255	113	553.92	260.34	253.88	236.34	223.88

Table A-3 Sulfate concentrations (mg/L) within influents and each compartment of anaerobic baffled reactors

Date	Days	Reactor A				
		Influent	Compartments			
			1	2	3	4
30/6/2554	0	919.14	898.00	890.23	875.88	764.00
3/7/2554	3	1016.39	940.29	936.78	889.64	884.35
6/7/2554	6	831.26	766.58	754.68	738.55	702.94
11/7/2554	11	909.63	663.03	659.54	654.46	650.74
14/7/2554	14	885.67	615.51	612.45	610.33	606.63
18/7/2554	18	851.75	596.02	591.80	577.63	576.45
21/7/2554	21	795.19	603.37	587.30	565.07	518.62
25/7/2554	25	825.27	555.85	534.12	530.75	506.68
28/7/2554	28	854.27	588.95	583.34	575.11	572.19
1/8/2554	32	813.11	558.16	552.21	545.81	525.62
4/8/2554	35	863.65	576.93	556.11	552.55	553.78
8/8/2554	39	856.44	553.65	537.35	531.21	524.14
11/8/2554	42	833.75	540.63	522.58	510.03	499.18
15/8/2554	46	858.08	529.63	523.68	490.50	475.20
18/8/2554	49	853.05	515.46	495.55	492.72	484.43
23/8/2554	54	928.84	576.34	554.35	536.08	528.18
26/8/2554	57	930.90	557.95	531.06	517.51	517.42
30/8/2554	61	899.50	601.22	575.72	551.99	547.29
2/9/2554	64	894.04	572.27	538.87	531.56	525.77
6/9/2554	68	911.77	530.37	498.16	479.20	475.10
9/9/2554	71	899.09	515.10	468.83	467.11	448.63
13/9/2554	75	905.46	518.20	466.86	466.35	445.37
16/9/2554	78	901.80	481.53	444.22	422.92	419.41
20/9/2554	82	890.11	458.98	441.03	403.19	397.97
23/9/2554	85	886.16	454.28	413.76	405.64	390.04
27/9/2554	89	904.08	410.46	377.51	355.92	355.87
30/9/2554	92	904.76	383.56	360.24	350.51	336.37
4/10/2554	96	891.66	365.92	331.35	329.35	328.64
7/10/2554	99	879.37	315.15	307.28	305.75	292.36
11/10/255	103	870.19	272.79	264.56	261.17	257.68
14/10/255	106	896.02	257.86	245.56	242.38	240.82
18/10/255	110	886.54	249.53	245.67	244.25	242.96
21/10/255	113	912.10	224.77	222.90	222.08	219.96

Table A-4 Sulfate concentrations (mg/L) within influents and each compartment of anaerobic baffled reactors

Date	Days	Reactor B				
		Influent	Compartments			
			1	2	3	4
30/6/2554	0	966.13	939.59	920.06	882.12	844.35
3/7/2554	3	973.97	955.86	854.93	845.05	831.05
6/7/2554	6	862.09	854.97	834.49	803.54	784.37
11/7/2554	11	874.86	720.26	659.45	648.10	637.15
14/7/2554	14	900.71	646.58	644.82	644.56	636.95
18/7/2554	18	865.20	644.85	639.08	634.59	625.65
21/7/2554	21	883.43	640.81	618.87	615.17	613.92
25/7/2554	25	878.15	626.18	617.98	609.56	607.38
28/7/2554	28	860.19	640.37	621.43	610.70	606.43
1/8/2554	32	801.93	636.44	597.20	580.40	553.32
4/8/2554	35	867.31	623.33	605.89	599.95	577.87
8/8/2554	39	866.67	616.00	595.88	584.13	567.40
11/8/2554	42	850.64	593.89	590.73	554.69	543.70
15/8/2554	46	854.08	558.87	540.80	524.69	517.20
18/8/2554	49	857.10	556.78	542.61	530.19	522.55
23/8/2554	54	890.19	568.01	530.00	512.39	511.31
26/8/2554	57	855.83	555.65	517.91	510.50	509.83
30/8/2554	61	874.04	592.61	556.20	551.96	546.97
2/9/2554	64	867.86	554.89	513.60	501.98	490.60
6/9/2554	68	873.26	560.72	507.47	490.27	476.40
9/9/2554	71	861.45	535.67	467.16	465.90	465.65
13/9/2554	75	862.03	530.00	483.95	476.30	471.83
16/9/2554	78	853.11	503.23	447.26	445.59	429.33
20/9/2554	82	856.82	476.03	446.06	435.63	432.86
23/9/2554	85	860.19	488.28	435.39	434.15	428.92
27/9/2554	89	886.46	446.69	394.30	394.16	392.48
30/9/2554	92	883.94	422.97	379.52	379.04	370.48
4/10/2554	96	858.63	376.91	333.94	326.28	326.07
7/10/2554	99	848.59	343.85	320.87	315.83	307.09
11/10/255	103	837.19	314.78	271.84	264.63	260.44
14/10/255	106	851.99	293.15	241.27	240.17	235.08
18/10/255	110	871.42	274.73	251.56	250.06	245.83
21/10/255	113	884.44	228.82	216.29	211.54	208.47

Table A-5 Sulfide concentrations (mg/L) within each compartment of anaerobic baffled reactors

Date	Days	Reactor A			
		Compartments			
		1	2	3	4
30/6/2554	0	0.44	0.19	0.16	0.17
3/7/2554	3	6.30	3.36	3.09	4.52
6/7/2554	6	7.76	4.91	4.62	4.55
11/7/2554	11	15.39	12.08	10.97	10.23
14/7/2554	14	20.44	15.08	13.22	11.35
18/7/2554	18	21.91	18.56	15.08	13.40
21/7/2554	21	24.81	21.91	20.59	18.05
25/7/2554	25	32.05	26.59	25.69	21.31
28/7/2554	28	34.35	25.16	23.80	20.16
1/8/2554	32	32.73	26.59	25.69	22.06
4/8/2554	35	36.56	32.73	29.50	27.15
8/8/2554	39	27.91	28.89	28.11	29.10
11/8/2554	42	43.46	39.18	41.41	40.84
15/8/2554	46	40.56	40.28	34.11	32.95
18/8/2554	49	48.22	40.28	34.11	32.95
23/8/2554	54	56.15	54.61	49.57	47.55
26/8/2554	57	61.86	61.01	55.76	43.16
30/8/2554	61	70.06	63.59	62.29	61.43
2/9/2554	64	68.15	70.06	73.03	70.55
6/9/2554	68	93.69	91.14	101.10	97.67
9/9/2554	71	76.66	78.81	88.65	85.04
13/9/2554	75	91.77	103.22	98.34	104.66
16/9/2554	78	83.87	89.88	93.69	97.67
20/9/2554	82	120.20	111.39	113.72	107.60
23/9/2554	85	121.87	117.73	112.16	109.10
27/9/2554	89	139.96	114.51	124.43	117.73
30/9/2554	92	126.16	127.04	125.29	130.61
4/10/2554	96	148.96	142.90	142.90	134.27
7/10/2554	99	165.25	145.90	149.99	141.91
11/10/255	103	193.76	179.56	160.74	145.90
14/10/255	106	209.08	197.82	160.74	169.89
18/10/255	110	227.19	224.06	195.10	175.87
21/10/255	113	291.45	253.78	246.86	248.57

Table A-6 Sulfide concentrations (mg/L) within each compartment of anaerobic baffled reactors

Date	Days	Reactor B			
		Compartments			
		1	2	3	4
30/6/2554	0	0.02	0.07	0.06	0.05
3/7/2554	3	0.51	1.01	1.40	1.72
6/7/2554	6	1.54	2.49	3.34	3.45
11/7/2554	11	10.30	11.59	9.68	8.79
14/7/2554	14	13.69	11.92	11.92	8.85
18/7/2554	18	15.72	14.67	14.67	12.17
21/7/2554	21	22.52	19.34	16.73	17.44
25/7/2554	25	22.06	21.61	20.87	18.30
28/7/2554	28	22.06	21.76	21.61	17.80
1/8/2554	32	24.30	24.81	21.31	16.27
4/8/2554	35	29.50	30.96	27.91	22.21
8/8/2554	39	24.81	26.59	23.80	22.68
11/8/2554	42	29.50	34.59	29.30	24.81
15/8/2554	46	26.96	30.33	28.11	24.99
18/8/2554	49	26.96	30.33	28.11	24.99
23/8/2554	54	41.12	48.89	44.99	41.99
26/8/2554	57	30.12	38.11	40.56	39.45
30/8/2554	61	39.45	52.76	48.55	46.90
2/9/2554	64	54.99	65.38	71.04	63.16
6/9/2554	68	87.43	98.34	91.14	88.65
9/9/2554	71	70.06	81.58	69.58	66.29
13/9/2554	75	89.26	99.71	101.10	99.71
16/9/2554	78	77.73	87.43	87.43	87.43
20/9/2554	82	87.43	93.05	95.66	95.66
23/9/2554	85	83.87	89.88	98.34	94.34
27/9/2554	89	104.66	114.51	116.11	116.11
30/9/2554	92	92.41	80.46	76.13	65.83
4/10/2554	96	129.70	135.20	127.92	114.51
7/10/2554	99	127.92	134.27	130.61	124.43
11/10/255	103	136.14	140.93	142.90	141.91
14/10/255	106	138.04	132.43	143.89	154.20
18/10/255	110	168.72	184.60	175.87	169.89
21/10/255	113	245.15	231.95	222.52	203.37

Table A-7 pH values within each compartment of anaerobic baffled reactors

Date	Days	Reactor A			
		Compartments			
		1	2	3	4
30/6/2554	0	7.71	7.63	7.65	7.72
3/7/2554	3	7.36	7.31	7.32	7.44
6/7/2554	6	7.57	7.51	7.52	7.6
11/7/2554	11	7.37	7.55	7.62	7.63
14/7/2554	14	7.39	7.51	7.55	7.6
18/7/2554	18	7.42	7.48	7.52	7.57
21/7/2554	21	7.43	7.49	7.51	7.55
25/7/2554	25	7.4	7.46	7.49	7.52
28/7/2554	28	7.38	7.42	7.45	7.48
1/8/2554	32	7.41	7.47	7.48	7.52
4/8/2554	35	7.07	7.12	7.18	7.19
8/8/2554	39	7.05	7.06	7.1	7.11
11/8/2554	42	7.04	7.06	7.09	7.09
15/8/2554	46	7.05	7.08	7.09	7.1
18/8/2554	49	7.08	7.14	7.15	7.17
23/8/2554	54	7.08	7.1	7.11	7.12
26/8/2554	57	7.1	7.14	7.16	7.16
30/8/2554	61	7.1	7.14	7.16	7.16
2/9/2554	64	7.15	7.16	7.2	7.2
6/9/2554	68	7.1	7.14	7.14	7.14
9/9/2554	71	7.08	7.09	7.11	7.13
13/9/2554	75	7.04	7.09	7.12	7.17
16/9/2554	78	7.12	7.13	7.15	7.15
20/9/2554	82	7.15	7.18	7.17	7.17
23/9/2554	85	7.03	7.03	7.05	7.08
27/9/2554	89	7.05	7.08	7.1	7.11
30/9/2554	92	7.09	7.11	7.15	7.17
4/10/2554	96	7.12	7.13	7.16	7.16
7/10/2554	99	7.08	7.1	7.12	7.13
11/10/255	103	7.1	7.16	7.16	7.14
14/10/255	106	7.12	7.14	7.16	7.16
18/10/255	110	7.12	7.11	7.13	7.16
21/10/255	113	7.14	7.14	7.14	7.14

Table A-8 pH values within each compartment of anaerobic baffled reactors

Date	Days	Reactor B			
		Compartments			
		1	2	3	4
30/6/2554	0	7.77	7.64	7.72	7.81
3/7/2554	3	7.62	7.51	7.49	7.5
6/7/2554	6	7.7	7.6	7.51	7.52
11/7/2554	11	7.51	7.61	7.64	7.74
14/7/2554	14	7.43	7.54	7.71	7.66
18/7/2554	18	7.42	7.52	7.62	7.61
21/7/2554	21	7.39	7.44	7.52	7.56
25/7/2554	25	7.41	7.46	7.47	7.49
28/7/2554	28	7.37	7.42	7.47	7.46
1/8/2554	32	7.42	7.46	7.45	7.46
4/8/2554	35	7.06	7.11	7.18	7.21
8/8/2554	39	7.06	7.09	7.1	7.11
11/8/2554	42	7.06	7.09	7.1	7.13
15/8/2554	46	7.04	7.1	7.1	7.12
18/8/2554	49	7.08	7.12	7.16	7.16
23/8/2554	54	7.09	7.11	7.12	7.13
26/8/2554	57	7.08	7.12	7.13	7.13
30/8/2554	61	7.09	7.16	7.17	7.2
2/9/2554	64	7.07	7.13	7.13	7.13
6/9/2554	68	7.05	7.13	7.16	7.18
9/9/2554	71	7.04	7.1	7.15	7.16
13/9/2554	75	7.01	7.08	7.11	7.12
16/9/2554	78	7.07	7.12	7.13	7.13
20/9/2554	82	7.08	7.16	7.17	7.17
23/9/2554	85	6.97	7.04	7.03	7.02
27/9/2554	89	7.03	7.09	7.12	7.13
30/9/2554	92	7.13	7.29	7.32	7.36
4/10/2554	96	7.1	7.16	7.19	7.19
7/10/2554	99	7.07	7.13	7.15	7.16
11/10/255	103	7.06	7.11	7.14	7.13
14/10/255	106	7.09	7.12	7.16	7.15
18/10/255	110	7.12	7.12	7.14	7.15
21/10/255	113	7.12	7.15	7.15	7.14

Table A-9 ORP values (unit of mV) within each compartment of anaerobic baffled reactors

Date	Days	Reactor A			
		Compartments			
		1	2	3	4
30/6/2554	0	-343.30	-296.10	-286.80	-313.10
3/7/2554	3	-390.00	-382.80	-361.90	-393.90
6/7/2554	6	-395.70	-384.80	-385.50	-391.80
11/7/2554	11	-355.70	-372.20	-404.00	-371.70
14/7/2554	14	-401.00	-406.10	-407.60	-411.70
18/7/2554	18	-397.20	-401.80	-402.00	-403.70
21/7/2554	21	-390.50	-392.60	-402.20	-379.30
25/7/2554	25	-395.00	-402.60	-406.70	-406.90
28/7/2554	28	-396.70	-391.00	-397.20	-402.10
1/8/2554	32	-399.20	-401.40	-400.30	-397.00
4/8/2554	35	-385.60	-387.70	-389.10	-386.80
8/8/2554	39	-387.90	-383.20	-389.80	-383.30
11/8/2554	42	-382.50	-385.10	-386.50	-380.40
15/8/2554	46	-350.60	-370.00	-373.30	-387.40
18/8/2554	49	-358.60	-385.50	-383.50	-389.40
23/8/2554	54	-364.60	-376.70	-376.90	-378.10
26/8/2554	57	-356.30	-385.60	-391.40	-389.00
30/8/2554	61	-365.80	-369.00	-385.50	-388.80
2/9/2554	64	-374.20	-386.60	-392.50	-390.40
6/9/2554	68	-386.80	-393.10	-389.80	-383.80
9/9/2554	71	-388.90	-393.80	-396.70	-374.30
13/9/2554	75	-397.70	-396.70	-389.40	-395.30
16/9/2554	78	-361.40	-374.10	-364.40	-370.50
20/9/2554	82	-331.80	-368.60	-332.80	-365.40
23/9/2554	85	-326.90	-354.80	-371.90	-362.80
27/9/2554	89	-331.80	-355.90	-350.00	-353.60
30/9/2554	92	-346.40	-356.80	-349.90	-386.40
4/10/2554	96	-385.20	-383.50	-380.70	-374.90
7/10/2554	99	-384.00	-361.00	-375.00	-378.30
11/10/255	103	-364.50	-379.40	-378.10	-379.40
14/10/255	106	-374.90	-382.30	-380.40	-385.50
18/10/255	110	-385.40	-391.60	-380.90	-389.20
21/10/255	113	-382.40	-383.50	-374.40	-365.70

Table A-10 ORP values (unit of mV) within each compartment of anaerobic baffled reactors

Date	Days	Reactor B			
		Compartments			
		1	2	3	4
30/6/2554	0	-333.60	-285.10	-255.70	-244.00
3/7/2554	3	-348.80	-373.30	-339.70	-347.50
6/7/2554	6	-359.20	-390.70	-387.10	-390.60
11/7/2554	11	-389.10	-405.20	-403.10	-388.20
14/7/2554	14	-380.30	-408.30	-415.20	-415.10
18/7/2554	18	-400.30	-399.50	-370.20	-361.20
21/7/2554	21	-401.70	-403.50	-402.60	-406.20
25/7/2554	25	-400.60	-394.10	-398.40	-401.30
28/7/2554	28	-399.30	-386.80	-403.10	-398.50
1/8/2554	32	-399.20	-390.50	-388.80	-394.80
4/8/2554	35	-353.80	-362.10	-380.20	-391.00
8/8/2554	39	-386.90	-375.50	-367.00	-387.80
11/8/2554	42	-352.80	-388.10	-378.30	-386.20
15/8/2554	46	-364.90	-355.70	-372.30	-388.40
18/8/2554	49	-387.00	-367.90	-366.00	-388.70
23/8/2554	54	-386.80	-389.80	-368.50	-394.50
26/8/2554	57	-383.00	-388.60	-395.40	-387.80
30/8/2554	61	-382.20	-384.60	-376.50	-382.30
2/9/2554	64	-375.40	-389.20	-387.70	-379.00
6/9/2554	68	-386.90	-387.70	-388.50	-392.20
9/9/2554	71	-390.50	-396.30	-396.50	-396.20
13/9/2554	75	-396.30	-392.30	-384.30	-394.30
16/9/2554	78	-384.20	-393.70	-402.30	-398.40
20/9/2554	82	-378.00	-390.60	-388.50	-389.80
23/9/2554	85	-370.70	-383.20	-386.40	-396.40
27/9/2554	89	-342.90	-349.60	-347.20	-355.10
30/9/2554	92	-383.40	-395.40	-386.90	-389.10
4/10/2554	96	-387.70	-397.70	-396.80	-394.60
7/10/2554	99	-369.60	-379.10	-383.50	-378.80
11/10/2554	103	-383.00	-388.00	-391.20	-389.30
14/10/2554	106	-382.70	-387.20	-387.20	-391.10
18/10/2554	110	-391.20	-393.70	-397.60	-399.60
21/10/2554	113	-386.60	-391.50	-394.30	-393.40

Table A-11 Alkalinity values (unit of mg/L as $CaCO_3$) within each compartment of anaerobic baffled reactors

Date	Days	Reactor A			
		Compartments			
		1	2	3	4
30/6/2554	0	675.00	662.50	731.25	762.50
6/7/2554	6	950.00	950.00	1050.00	825.00
14/7/2554	14	1000.00	1031.25	1025.00	1012.50
21/7/2554	21	1000.00	1018.75	1050.00	1056.25
28/7/2554	28	1000.00	1025.00	1025.00	1037.50
4/8/2554	35	868.75	887.50	900.00	893.75
11/8/2554	42	887.50	900.00	906.25	912.50
18/8/2554	49	875.00	900.00	912.50	918.75
26/8/2554	57	881.25	887.50	912.50	937.50
2/9/2554	64	887.50	912.50	931.25	937.50
9/9/2554	71	893.75	937.50	950.00	956.25
16/9/2554	78	912.50	937.50	975.00	987.50
23/9/2554	85	931.25	968.75	975.00	987.50
30/9/2554	92	981.25	1018.75	1037.50	1037.50
7/10/2554	99	1037.50	1037.50	1037.50	1050.00
14/10/2554	106	1050.00	1062.50	1062.50	1062.50
21/10/2554	113	1075.00	1081.25	1075.00	1075.00

Table A-12 Alkalinity values (unit of mg/L as $CaCO_3$) within each compartment of anaerobic baffled reactors

Date	Days	Reactor B			
		Compartments			
		1	2	3	4
30/6/2554	0	587.50	600.00	618.75	656.25
6/7/2554	6	862.50	850.00	893.75	850.00
14/7/2554	14	1000.00	1025.00	1093.75	1056.25
21/7/2554	21	1025.00	1025.00	1050.00	1037.50
28/7/2554	28	993.75	1012.50	1043.75	1012.50
4/8/2554	35	881.25	906.25	912.50	912.50
11/8/2554	42	875.00	887.50	887.50	875.00
18/8/2554	49	856.25	887.50	887.50	887.50
26/8/2554	57	862.50	875.00	900.00	900.00
2/9/2554	64	868.75	893.75	900.00	906.25
9/9/2554	71	862.50	912.50	937.50	937.50
16/9/2554	78	887.50	925.00	925.00	925.00
23/9/2554	85	912.50	943.75	950.00	943.75
30/9/2554	92	968.75	1006.25	1000.00	1012.50
7/10/2554	99	987.50	1000.00	1000.00	1000.00
14/10/2554	106	1012.50	1037.50	1062.50	1062.50
21/10/2554	113	1050.00	1062.50	1062.50	1062.50

Table A-13 MLSS concentrations in the clear zone of the last compartments of anaerobic baffled reactors

Date	Days	The fourth compartment of Reactor A				The fourth compartment of Reactor B			
		A	B	C	D	A	B	C	D
30/6/2554	0	0.2744	0.2744	0.00	0.00	0.2453	0.2454	0.00	20.00
6/7/2554	6	0.2749	0.2749	0.00	0.00	0.2457	0.2457	0.00	0.00
14/7/2554	14	0.2705	0.2705	0.00	0.00	0.2700	0.2707	0.00	35.00
21/7/2554	21	0.2554	0.2555	0.00	2.00	0.2433	0.2434	0.00	2.00
28/7/2554	28	0.2342	0.2342	0.00	0.00	0.2273	0.2273	0.00	0.00
4/8/2554	35	0.2456	0.2456	0.00	0.00	0.2280	0.2281	0.00	2.00
11/8/2554	42	0.2448	0.2448	0.00	0.00	0.2272	0.2272	0.00	0.00
18/8/2554	49	0.2541	0.2541	0.00	0.00	0.2696	0.2697	0.00	2.00
26/8/2554	57	0.2445	0.2445	0.00	0.00	0.2692	0.2694	0.00	4.00
2/9/2554	64	0.2446	0.2446	0.00	0.00	0.2744	0.2746	0.00	4.00
9/9/2554	71	0.2511	0.2512	0.00	2.00	0.2757	0.2761	0.00	8.00
16/9/2554	78	0.2399	0.2399	0.00	0.00	0.2754	0.2754	0.00	0.00
23/9/2554	85	0.2471	0.2471	0.00	0.00	0.2719	0.2719	0.00	0.00
30/9/2554	92	0.2498	0.2500	0.00	4.00	0.2743	0.2744	0.00	2.00
7/10/2554	99	0.2451	0.2451	0.00	0.00	0.2739	0.2739	0.00	0.00
14/10/2554	106	0.2735	0.2735	0.00	0.00	0.2533	0.2533	0.00	0.00
21/10/2554	113	0.2549	0.2549	0.00	0.00	0.2753	0.2753	0.00	0.00

A = Weight of membrane filters before filtration of the water samples in the fourth of anaerobic baffled reactors
B = Weight of membrane filters after filtration of the water samples in the fourth of anaerobic baffled reactors and dry at 105 °C.
C = Difference between A and B
D = MLSS concentration (mg/L)

Table A-14 Absorbance values within each compartment of anaerobic baffled reactors

Date	Days	Reactor A				
		Influent	Compartments			
			1	2	3	4
23/8/2554	0	2.881	0.531	0.250	0.176	0.132
26/8/2554	3	2.852	0.411	0.217	0.113	0.089
30/8/2554	7	2.846	0.575	0.317	0.198	0.156
3/9/2554	10	2.754	0.494	0.258	0.125	0.114
6/9/2554	14	2.841	0.354	0.165	0.072	0.069
9/9/2554	17	2.813	0.366	0.160	0.086	0.080
13/9/2554	21	2.718	0.498	0.256	0.185	0.095
16/9/2554	24	2.777	0.284	0.123	0.062	0.062
20/9/2554	28	2.784	0.235	0.112	0.060	0.038
23/9/2554	31	2.824	0.317	0.127	0.069	0.059
27/9/2554	35	2.799	0.166	0.076	0.050	0.033
30/9/2554	38	2.784	0.145	0.087	0.040	0.039
4/10/2554	42	2.799	0.174	0.077	0.050	0.032
7/10/2554	45	2.832	0.128	0.058	0.030	0.027
11/10/2554	49	2.835	0.129	0.062	0.039	0.026
14/10/2554	52	2.875	0.127	0.048	0.035	0.030
18/10/2554	56	2.840	0.125	0.061	0.035	0.031
21/10/2554	59	2.846	0.101	0.066	0.040	0.029

Table A-15 Absorbance values within each compartment of anaerobic baffled reactors

Date	Days	Reactor B				
		Influent	Compartments			
			1	2	3	4
23/8/2554	0	2.960	0.424	0.113	0.109	0.105
26/8/2554	3	2.916	0.512	0.186	0.135	0.129
30/8/2554	7	2.909	0.638	0.265	0.223	0.178
3/9/2554	10	2.957	0.532	0.170	0.105	0.113
6/9/2554	14	2.932	0.461	0.124	0.067	0.064
9/9/2554	17	2.968	0.379	0.093	0.061	0.052
13/9/2554	21	2.931	0.497	0.127	0.070	0.067
16/9/2554	24	2.940	0.299	0.047	0.028	0.028
20/9/2554	28	2.949	0.214	0.021	0.016	0.016
23/9/2554	31	2.978	0.385	0.074	0.032	0.030
27/9/2554	35	2.933	0.178	0.024	0.015	0.015
30/9/2554	38	2.913	0.147	0.025	0.019	0.013
4/10/2554	42	2.962	0.093	0.024	0.014	0.013
7/10/2554	45	2.994	0.135	0.029	0.017	0.014
11/10/2554	49	2.952	0.169	0.029	0.015	0.014
14/10/2554	52	2.996	0.105	0.012	0.008	0.008
18/10/2554	56	2.995	0.115	0.013	0.009	0.008
21/10/2554	59	2.980	0.055	0.015	0.011	0.010

Appendix B

Table B-1 Absorbance values of triplicates of adsorption experiments by inactive biomass obtained from the first compartment of Reactor A and Reactor B

Date	Days	Reactor A				Reactor B			
		Replicate			Average	Replicate			Average
		1	2	3		1	2	3	
17/1/2555	0	2.914	2.950	2.944	2.936 (± 0.019)	3.005	2.999	2.995	3.000 (± 0.005)
18/1/2555	1	2.908	2.939	2.933	2.927 (± 0.016)	2.996	2.989	2.987	2.991 (± 0.005)
19/1/2555	2	2.893	2.924	2.919	2.912 (± 0.017)	2.981	2.967	2.969	2.972 (± 0.008)
20/1/2555	3	2.882	2.913	2.906	2.900 (± 0.016)	2.966	2.955	2.953	2.958 (± 0.007)
23/1/2555	6	2.854	2.900	2.885	2.880 (± 0.023)	2.941	2.933	2.930	2.935 (± 0.006)
26/1/2554	9	2.828	2.871	2.859	2.853 (± 0.022)	2.938	2.923	2.921	2.927 (± 0.009)
29/1/2555	12	2.820	2.861	2.852	2.844 (± 0.022)	2.914	2.902	2.898	2.905 (± 0.008)
1/9/2554	15	2.818	2.855	2.850	2.841 (± 0.020)	2.895	2.884	2.875	2.885 (± 0.010)

Appendix C

Table C-1 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B

Clone number	Sequence
1	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCNNNACAT GCAAGTCGTACGGTTCATGTATCTTTCAAGCTTGCTTGCTGGTACATG GATAGTGGCGGACGGGTGAGTAACGCGTGAATATCTATCCATTGGAG GGGGACAACCCGTCGAAAGGCGGGCTAATACCGCGTAGGCCATTTTT TATTGGTGAAAGGTCACGTTTTGTGGCTGCCGATGGATGAGTTTGCGT CCTATCAGCTTGTTGGTGGGGTAAGGGCCTACCAAGGCTAAGACGGG TAGCCGGTCTGAGAGGATGGCCGGTCACACTGGAACTGAGATACGGT CCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGGGCAATGGGG GTAACCCTGACCCAGCGACGCCGCGTGAGGGATGAATGTTTTCGGAT TGTAAACCTCTGTTGTACGGGAAGAAGAGCGTGACGGTACCGTACGA GTAAGCCCCGGCAAACTACGTGCCAGCAGCCGCGGTAACACGTAGG GGGCGAGCGTTGTCCGGAATTACTGGGCGTAAAGGGCGCGCAGGCG GTTATGTTAGTCTGCTGTGAAAAGCATCGGCTTAACCGGTGTCTTGCG GCGGATACTGCATAGCTTGAGTACTGGAGAGGGAAGTGGAATTCCCG GTGTAGCGGTGAAATGCGTAGATATCGGGAGGAACACCGGTGGCGA AGGCGGCTTTCTGGCCAGTTACTGACGCTCATGCGCGAGAGCTGGG TTAGCGAACCGGATTAGATACCCGGGTAGTCCCAGCCGTAAACGCTG AATGCTAGGTGTGGGTGTCGCAGGGCATCCGTGCCGCAGTTAACGCG TTAAGCATTCCGCCTGGGGAGTACGATCGCAAGATTGAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGTGGAGCACGTGGTTTAATTCG ATGCAAACCGAAGAACCTTACCTGGGTTTGACATGTACGTATTAGGG GCATGAAAGTGTTCTGACCCTATTTTCGGATGGGGAGCGTACACAGG TGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC CCGCAACGAGCGCAACCCCTGCATTCAGTTGCCATCGGTATGGCCGG GCACTCTGGATGGACTGCCGGCGACAAGCCGGAGGAGGGTGGGGAT GACGTCAAGTCATCATGGCCCTTATGCCCAGGGCGACACACGTGCTA CAATGGCCGGCACAGCGGGAAGCGAAGGGGCGACTCTAAGCGGATC CCTCAAAGCCGGTCCCAGTTCGGATTGCAGTCTGCAACTCGACTGCA TGAAGTCGGAATCGCTAGTAATCGTGGATCAGCCAAGCCGCGGTGAA TACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACCCGAGTTG GGTGCACCCGAAGCCGGTGGCCGAACCTGTAAAGGACGGAGCCGTCT AAGGTGTGCCTGGTAAGGGGGGTGAAGTCGTANCAAGGTAACC

Table C-2 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
2	GGTTACCTNNNACGACTTCACCCCAATCGCTGACCCTACCTTAGGAC GCTTCCTCCCTTGCGGGTTAGATCACGTACTTTGGGTATTGCCAACTC TCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACC GCGACATGCTGATTCGCGATTACTAGCAACTCCAGCTTCATGTAGGC GAGTTTCAGCCTACAATCCGAACTGAGATCGGTTTTCCAGTTTTGCTC CCCCTCGCGGGCTTGCGTCTTATTGTACCGGCCATTGTAGCACGTGTG TAGCCCTAGACATAAGGGGCATGATGATTTGACGTCATCCCCACCTT CCTCCTGGTTAACCCAGGCAGTCTCGCTAGAGTGCTCAACCTAATGG TAGCAACTAACGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC ATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCTTCCTG TCCCCGAAGGGACTTCCCCGATTAAGGGTAATTCAGGAGATGTCAAG TCTAGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCGC TGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACC GTACTCCCCCAGGCGGAATACTTATTGCGTTTGCTGCGGCACCGAGG GTGGTACCCCCCGACACCTAGTATTCATCGTTTACGGCGTGGACTACC AGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTATCTCAGCGTCA GTTACAGTCCAGAAAGTCGCCTTCGCCACTGGTGTTCTTCCTAATCTC TACGCATTTCACCGCTACACTAGGAATTCCACTTTCCTCTCCTGCACT CTAGACATCCAGTATCAAATGCAGCTCCCAGGTTAAGCCCGGGAA TTTCACATCTGACTTAAATATCCGCCTACATACTCTTTACGCCCAGTA AATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCAC GTAGTTAGCCGTGGCTTCCTCCTCAGGTACCGTCATTATCGTCCCTGA AGACAGAGTTTTACAATCCGAAGACCTTCATCACTCACGCGGCGTTG CTGCGTCAGGGTTTCCCCCATTGCGCAATATTCCCCACTGCTGCCTCC CGTAGGAGTCTGGACCGTGTCTCAGTTCCAATGTGGCCGATCACCCT CTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCA ACTAGCTAATGCGCCGCGGGCCCATCTCAAAGCGATAAATCTTTAAT GAAGGTACCATGCGATTCCTTCATGTTATGCGGTATTAATCCTCCTTT CGGAGGGCTATTCCCCACTTTGAGGCAGGTTGCCCACGTGTTACTCA CCCGTCCGCCGCTAATCCACTCCCGAAGGGGCTTCATCGCTCGACTTG CATGTGTTAGGCACGCCGCCAGCGTTCGTCCTGAGCCAGGATCAAAC TCT

Table C-3 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
3	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTNAACAC ATGCAAGCCGAGCGATGAGGCTCCTTCGGGAGTGGATTAGCGGCGG ACGGGTGAGTAACACGTGGGTAACCTGCCTCATAGAGGGGAATAGC CTTTCGAAAGGAAGATTAATACCGCATAAGATTGTAGTACCGCATGG TACAGCAATTAAAGGAGTAATCCGCTATGAGATGGACCCGCGTCGCA TTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGC CGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCCCA GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGGGAA ACCCTGATGCAGCAACGCCGCGTGAGTGATGACGGTCTTCGGATTGT AAAGCTCTGTCTTTAGGGACGATAATGACGGTACCTAAGGAGGAAGC CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG CGTTGTCCGGATTTACTGGGCGTAAAGGGAGCGTAGGTGGATATTTA AGTGGGATGTGAAATACCCGGGCTTAACCTGGGTGCTGCATTCCAAA CTGGATATCTAGAGTGCAGGAGAGGAAAGGAGAATTCCTAGTGTAG CGGTGAAATGCGTAGAGATTAGGAAGAATACCAGTGGCGAAGGCGC CTTTCTGGACTGTAACTGACACTGAGGCTCGAAAGCGTGGGGAGCAA ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATACTA GGTGTAGGGGTTGTCATGACCTCTGTGCCGCCGCTAACGCATTAAGT ATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTG ACGGGGGCCCGCACAAGCGGCGGAGCATGTGGTTTAATTCGAAGCA ACGCGAAGAACCTTACCTAGACTTGACATCTCCTGAATTACTCTGTA ATGGAGGAAGCCACTTCGGTGGCAGGAAGACAGGTGGTGCATGGTT GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC GCAACCCTTATTGTTAGTTGCTACCATTTAGTTGAGCACTCTAGCGAG ACTGCCCGGGTTAACCGGGAGGAAGGTGGGAATGACGTCAAATCAT CATGCCCCTTATGTCTAGGGCTACACACGTGCTACAATGGTCGGTAC AATGAGATGCAACCTCGCGAGAGTGAGCAAAACTATAAAACCGATC TCAGTTCGGATTGTAGGCTGAAACTCGCCTACATGAAGCTGGAGTTG CTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCT TGTACACACCGCCCGTCACACCATGAGAGTTGGCAATACCCAAAGT TCGTGAGCTAACCGCANGGAGGCAGCGACCTAAGGTAGGGTCAGCG ATTGGGGTGAAGTCGTANNNNAACC

Table C-4 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
4	GGTTACCTNNNCGACTTCACCCTAATTATCAGCCATACCTTAGGTGCC TGCACCCCCGAAAGGTTTGCCTAGCAACTTCTGGTACAACCAACTCT CATGGTGTGACGGGCGNNNTACAAGGCCCGGGAACGTATTCACCGC GGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATNNNCGAGT TGCAGACTCCAATCCGAACTTGGGACGGGTTTCTGGGATTGGCTCCA CCTTGCGATATCGCTACCCTTTGTTCCGTCCATTGTAGCACGTGTGTA GCCCTGGGCATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCC TCCCCGTTAACCGAGGCGGTATCTTTAGAGTCCCCAACCGAATCGCT GGCAACTAAAGATAGGGGTTGCGCTCGTTGCGGGACTTAACCCAACA TCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTTGGGGC TCCCCGAAGGGCACTTCGCACTTTCATGCAAATCCCCCAGATGTCAA GCCCAGGTAAGGTTCTTCGCGTTGCGTCGAATTAAACCACATGCTCC ACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTTGCGG CCGTACTCNCCAGGCGGTTCACTTAATGCGTTAGCTGCGGCACAGCA GGGGTCAATACCCGCTACACCTAGTGAACAACGTTTACTGCGTGGAC TACCAGGGTATCTAATCCTGTTTGCTCCCCACGCCTTCGCGCCTCAGC GTCAGTATTGGTCCAGAAAGTCGCCTTCGCCACCGGTGTTCCTCCTGA TATCTACGAATTTCACCTCTACACCAGGAATTCCACTTTCCTCTCCCA TACTCAAGCCCAACAGTATCAAATGCACTTCCGGGGTTGAGCCCCGG GCTTTCACACCTGACTGATCGGGCCGCCTGCGCGCCCTTTACGCCCAA TGATTCCGAGTAACGCTTGCACCCCCCGTATTACCGCGGCTGCTGGC ACGGAGTTAGCCGGTGCTTCCTTCAGTGGTACCGTCAACATACATGG ATATTAACCATGCACGACTTCTTCCCACTTGACAGAGCTTTACGACCC GAAAGCCTTCATCACTCACGCGGCGTTGCTGCGTCAGGGTTCCCCCC ATTGCGCAAAATTCCTCACTGCTGCCTCCCGTAGGAGTCTGGACCGT GTACCAGTTCCAGTGTGGCTGACCATCCTCTCAGACCAGCTACCCATC GTCGCCTTGGTAGGCCATTACCCCACCAACAAGCTAATGGGACGCGG GCTCATCTCCAAACAATAGCTTTCATGAAGAGGCCATCTTTGATCATA AACCCCAAAGAATCTATAATATCATCCGGTATTAGCATCACTTTCGC AATGTTATCCCCAATTCGGAGGTAGATTACCCACGTGTTACTCACCCG TGCGCCACTTTACTCGCCTCTGCAAGCAGAAGCTTTCTCGTTCGACTT GCATGTGTTAAGCACGCCGCCAGCGTTCACTCTGAGCCAGGATCAAA NNNN

Table C-5 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
5	GGTTACCTTGTTACGACTTAGCCCCAGTCACTGGTTTTACCCTAGGCC GCTCCTTGCGGTTACGGACTTCAGGTACCCCCAACTTCCATGGCTTGA CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGCCATGGCT GATGCGCGATTACTAGCGAATCCAGCTTCACGGAGTCGAGTTGCAGA CTCCGATCCGAACTGAGACCGGCTTTCGAGATTCGCACTGCCTCACA GCATAGCTGCCCTCTGTACCGGCCATTGTAACACGTGTGTAGCCCTG GACATAAGGGCCGTGCTGATTTGACGTCATCCCCACCTTCCTCTCGGT TTACACCGGCAGTTTCGCTAGAGTTCCCGGCATTACCCGCTGGCAACT AACGATAAGGGTTGCGCTCGTTATGGGACTTAACCCGACACCTCACG GCACGAGCTGACGACAACCATGCAGCACCTTGCAGAAAGTTCCGAA GAAAATCCCGGTTTCCCGAGAATGCACTCTGCATTTAAGCCCAGGTA AGGTTCCTCGCGTATCATCGAATTAAACCACATGTTCCTCCGCTTGTG CGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCC CAGGTGGATCACTTAATGCTTTTGCTTAGCCACTGACTGTATATCGCC AACAGCGAGTGATCATCGTTTACGGCGTGGACTACCAGGGTATCTAA TCCTGTTTGATCCCCACGCTTTCGTGCATCAGCGTCAGTAATGGCCCA GTAAGCTGCCTTCGCAATCGGTGTTCTGTGTAATATCTAAGCATTTCA CCGCTACATTACACATTCCGCCTACCTTGACCATACTCAAGACTAACA GTATCAACGGCAAGTCTACAGTTAAGCTGTAGGTTTTCACCACTGAC TTATTAACCCGCCTACGCACCCTTTAAACCCAATAAATCCGGATAAC GCTCGCATCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGA TGCTTATTCCCAGGGTACCGTCAAATCCCGATACATCGGAAAGTTTCT CCCCCTGGAAAAGCAGTTTACAACCCATAGGGCCGTCTTCCTGCACG CGGGATGGCTGGTTCAGGCTTCCGCCCATTGACCAATATTCCTCACTG CTGCCTCCCGTAGGAGTCTGGTCCGTGTCTCAGTACCAGTGTGGGGG ATCACCCTCTCAGGTCCCCTAAACATCGTCGCCTTGGTAGGCCGTTAC CCTGCCAACTAGCTAATGTTCCGCATGCCCATCCCGTACCGCCTCAGC TTTAAATATCACAAGGATGCCCTTGAATATAATTATGGGGTATTAGTC CGAATTTCTCCGGGTTATCCCCCTGTACAGGGTAGGTTGCATACGTGT TACTCACCCGTGCGCCGGTCGCCATCAAAGTATTGCTACTCTATGCTG CCCCTCGACTTGCATGTGTTAAGCCTCCCGCTAGCGTTCATCCTGAGC CAGGATCAAACTCT

Table C-6 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
6	GGTTACCTNNNTACGACTTCACCCCAATCATCGACTCCACCTTCGACG GCTGGCTCCTTACGGTTACCTCACCGGCTTCGGNNGTCTCCAACTCTC GTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGC AGTATGCTGACCTGCGATTACTAGCGATTCCGACTTCATGCAGGCGA GTTGCAGCCTGCAATCCGAACTGGGACCGGGTTTTTGAGATTCGCTTC ACCTCGCGGCTTCGCTGCCCTCTGTTACCGGCCATTGTAGTACGTGTG TAGCCCAGGACATAAGGGGCATGATGACTTGACGTCATCCCCGCCTT CCTCCGTCTTGTCGACGGCAGTCTCCCATGAGTTCCCGACTTTACTCG CTGGCAACATAGGATAGGGGTTGCGCTCGTTGCGGGACTTAACCCAA CATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTTTTTGT GTCCCCGAAGGGAGGGACCTATCTCTAGGTCTTTCACTCAATGTCAA GCCCTGGTAAGGTTCTTCGCGTTGCGTCGAATTAAACCACATACTCCA CCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGC CGTACTCCCCAGGCGGGGTACTTATTGCGTTAACTCCGGCACAGGAG GGGTCGATACCCCCTACACCTAGTACCCATCGTTTACGGCTAGGACT ACCGGGGTATCTAATCCCGTTCGCTCCCCTAGCTTTCGCGCCTCAGCG TCAGACATCGTCCAGAAAGTCGCCTTCGCCACTGGTGTTCTTCCAAAT CTCTACGCATTTCACCGCTACACTTGGAATTCCACTTTCCTCTCCGAC ACTCAAGAATACCAGTTTCTGTCCCCTCACGAGGTTGAGCCTCGCACT TTTAAGACAGACTTGATATCCCGCCTGCGCGCGCTTTACGCCCAATA ATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCAC GTAGTTAGCCGTGGCTTCTTATTCAGGTACCGTCACTATCTCTCATTA TTTACAAAAAATACGTTCGTCCCTGACGAAAGAGCTTTACGATCCGA AAACCTTCTTCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATT GCGGAAGATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTC TCAGTCCCAGTGTGGCCGTTCATCCTCTCAGACCGGCTACTGATCGAA GCCTTGGTGGGCCGTTACCCCTCCAACTAGCTAATCAGACGCAGGCT CATCTTCTAGCGGTAGCATATTCAGAGGCCACCTTTAGTAGCATCTCC ATGCGGAAATACTACAACATTCGGTATTAGCACCCCTTTCGGGATGT TGTCCCCATCTAGAAGGCAGATTGCCTACGCGTTACTCACCCGTTCGC CACTAAGATCCATTGCTGAATCTCCGTTCGACTTGCATGTGTTAAGCA CGCCGCCAGCGTTCGTCCTGAGCCAGGATCAAACTCT

Table C-7 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
7	AGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCNNGCCTAACAC ATGCAAGTCGAGCGAGAAACCTGCAATTGAAACTTCGGTAGATATTG AAGGCGGAAAGCGGCGGACGGGTGAGTAACGCGTAGGCAACCTGCC CTTTGCAGAGGGATAGCAACGGGAAACTGTTATTAAAACCTCATAAT ACCGACATGTCAAATGACAAGACGGTCAAAGATTTATCGGCAAGGG ATGGGCCTGCGTCTGATTAGTTAGTTGGTAGGGTAACGGCCTACCAA GGCAGCGATCAGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGA ACTGAGACACGGTCCAAACTCCTACGGGAGGCAGCAGTGGGGAATA TTGCACAATGGGCGAAAGCCTGATGCAGCAACGCCGCGTGAAGGAT GAAGGCCTTTGGGTCGTAAACTTCTGTCCTTGGGGAAGAAAAAAATG ACGGTACCCCTGGAGGAAGTCCCGGCTAACTACGTGCCAGCAGCCGC GGTAATACGTAGGGGGCGAGCGTTATCCGGAATTATTGGGCGTAAAG AGTACGTAGGTGGTTTTTTAAGCGCAGGGTTTAAGGCGATGGCTCAA CTGTCGTTCGCCCTGTGAACTGGAAGACTTGAGTACAGGAGAGGAAA GCGGAATTCCTAGTGTAGCGGTGAAATGCATAGATATTAGGAGGAAC ACCAGTGGCGAAGGCGGCTTTCTGGACTGTAACTGACACTGAGGTAC GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC TGTAAACGATGAGCACTAGGTGTCGGGGTCGCAAGATCTCGGTGCCG CAGTTAACGCATTAANNGCTCCGCCTGGGGAGTACGCACGCAAGTGT GAAACTCAAAGGAATTGACGGGGACCCGCACAAGCAGCGGAGCATG TGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGAACTTGACATC CTCCTGACAGGCCTTTAATCGGGCCCTTCTTCGGACAGGAGAGACAG GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT CCCGCAACGAGCGCAACCCTTGTCATTAGTTGCTAACATTAAGTTGA GGACTCTAATGAGACTGCCGGGGACAACTCGGAGGAAGGTGGGGAT GACGTCAAATCATCATGCCCCTTATGTTCTGGGCTACACACGTGCTAC AATGGCCGGTACAACGAGAAGCGAAGTCGCAAGGCGGAGCAAACCT CAAAAACCGGTCCCAGTTCGGATTGCAGGCTGAAACCCGCCTGCATG AAGCCGGAGTTGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATGC GTTCCCGGGTCTTGTACACACCGCCCGTCACACCATGGAAGTTGGGG GCGCCCGAAGTTGGTCAAATAATAGATTACCTAAGGCGAAATCAATG ACTGGGGTGAAGTCGTAACAAGGTAACC

Table C-8 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
8	AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCCTAACAC ATGCAAGTCGAACGAGAAAGCCTGGCTTGCCAGGCAAGTAAAGTGG CGCACGGGTGAGTAACGCNNGGGTAATCTACCTTTGTCTGGGGGATA ACATACCGAAAGGTGTGCTAATACCGCATAACGTGATCGGGTTGCGA CTTGGTCACCAAAGAAGGCCTCTCCCTGGAAGCTTTCGGGTAAAGAT GAGCCCGCGTCCCATTAGCTAGTTGGTAGGGTAAAAGCCTACCAAGG CTACGATGGGTAGCTGGTCTGAGAGGATGGCCAGCCACACTGGAACT GGAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATCTTG CGCAATGGGGGCAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAA GGCCTTCGGGTCGTAAAGCTCTGTCAAGTGGGAAGAATAACCTTGAG ACGAATAATCTCGAGGTGTGACGGTACCACTGGAGGAAGCACCGGCT AACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTATT CGGAATTATTGGGCGTAAAGGGCGTGTAGGCGGCCAGATAAGTCAG ATGTGAAAGCCCGGGGCTCAACCCCGGAAGTGCATTTGAAACTATTT GGCTTGAGTGCGGGAGAGGAAAGCGGAATTCCCCAGTGTAGAGGTG AAATTCGTAGATATTGGGAGGAACACCGGTGGCGAAGGCGGCTTTCT GGACCGACACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGCACTAGGTGT AGGAGGTTTTTAACCCTCCTGTGCCGTAGCTAACGCATTAAGTGCCCC GCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGG GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGCG CAGAACCTTACCTGGGCTTGACATCTGGAGAACCCTTTGGAAACAAG GGGGTGCGTAGCAATACGAACTCCAAGACAGGTGCTGCATGGCTGTC GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA ACCCTTGTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAGAGACT GCCGGTGTTAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATG GCCTTTATGTCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAG GGTTGCCAACCCGCGAGGGTCAGCTAATCCCAAAAAACCGACCTCAG TTCGGATTGGAGTCTGCAACTCGACTCCATGAAGTTGGAATCGCTAG TAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCACGAAAGCCTGTTGTACCAGAAGTCGCTG GCCTAACCCTTCGGGGAAGGAGGCGCCTAAGGTATGGCCGGTGATTG GGGTGAAGTCGTANCAAGGTAACC

Table C-9 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
9	AGAGTTTGATCCTGGCTCAGAATGAACGNNGGCGGCNNGCCTAACAC ATGCAAGTCGAACGAGAAAGCCTGGCTTGCCAGGCAAGTAAAGTGG CGCACGGGTGAGTAACGCGTGGGTAATCTACCTTTGTCTGGGGGATA ACATACCGAAAGGTGTGCTAATACCGCATAACGTGATCGGGTTGCGA CTTGGTCACCAAAGAAGGCCTCTCCCTGGAAGCTTTCGGGTAAAGAT GAGCCCGCGTCCCATTAGCTAGTTGGTAGGGTAAAAGCCTACCAAGG CTACGATGGGTAGCTGGTCTGAGAGGATGGCCAGCCACACTGGAACT GGAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATCTTG CGCAATGGGGGCAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAA GGCCTTCGGGTCGTAAAGCTCTGTCAAGTGGGAAGAATAACCTTGAG ACGAATAATCTCGAGGTGTGACGGTACCACTGGAGGAAGCACCGGCT AACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTATT CGGAATTATTGGGCGTAAAGGGCGTGTAGGCGGCCANATAAGTCAG NTNTGAAAGCNCGGGGCTCAACCCCGGAAGTGCATTTGAAACTATTT GGCTTGAGTGCGGGAGAGGAAAGCGGAATTCCCAGTGTAGAGGTGA AATTCGTAGATATTGGGAGGAACACCGGTGGCGAAGGCGGCTTTCTG GACCGACACTGACGCTGAGGCGCGANAGCGTGGGGAGCAAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGCACTAGGTGTA GGAGGTTTTTAACCCTCCTGTGCCGTAGCTAACGCATTAAGTGCCCCG CCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGG GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGCGC AGAACCTTACCTGGGCTTGACATCTGGAGAACCCTTTGGAAACAAGG GGGTGCGTAGCAATACGAACTCCAAGACAGGTGCTGCATGGCTGTCG TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA CCCTTGTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAGAGACTG CCGGTGTTAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGG CCTTTATGTCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGG GTTGCCAACCCGCGAGGGTCAGCTAATCCCAAAAAACCGACCTCAGT TCGGATTGGAGTCTGCAACTCGACTCCATGAAGTTGGAATCGCTAGT AATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTAC ACACCGCCCGTCACACCACGAAAGCCTGTTGTACCAGAAGTCGCTGG CCTAACCCTTCGGGGAAGGAGGCGCCTAAGGTATGGCCGGTGATTGG GGTGAAGTCGTAACAAGGTAACC

Table C-10 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
10	AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCCTAACAC ATGCAAGTCGAACGAGAAAGCCTGGCTTGCCAGGCAAGTAAAGTGG CGCACGGNNNAGTAACGCGTGGGTAATCTACCTTTGTCTGGGGGATA ACATACCGAAAGGTGTGCTAATACCGCATAACGTGATCGGGTTGCGA CTTGGTCACCAAAGAAGGCCTCTCCCTGGAAGCTTTCGGGTAAAGAT GAGCCCGCGTCCCATTAGCTAGTTGGTAGGGTAAAAGCCTACCAAGG CTACGATGGGTAGCTGGTCTGAGAGGATGGCCAGCCACACTGGAACT GGAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATCTTG CGCAATGGGGGCAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAA GGCCTTCGGGTCGTAAAGCTCTGTCAAGTGGGAAGAATAACCTTGAG ACGAATAATCTCGAGGTGTGACGGTACCACTGGAGGAAGCACCGGCT AACTCCGTGCCAGCAGCCGCGGTAATACGGNNGGTGCAAGCGTTATT CGGAATTATTGNGCGTAAAGGGCGTGTAGGCGGCCAGATAAGTCAG ATGTGANAGCNCGGNGCTCAANNNCNGAAGTGCATTTGAAACTATTT NGCTTGAGTGNNNNNGANGANAGCGGNNNTCCCAGTGTAGAGGTGA AATTCGTAGATATTGGGAGGAACACCGGTGGCGAAGGCGGCTTTCTG GACCGACACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGCACTAGGTGTA GGAGGTTTTTAACCCTCCTGTGCCGTAGCTAACGCATTAAGTGCCCCG CCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGG GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGCGC AGAACCTTACCTGGGCTTGACATCTGGAGAACCCTTTGGAAACAAGG GGGTGCGTAGCAATACGAACTCCAAGACAGGTGCTGCATGGCTGTCG TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA CCCTTGTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAGAGACTG CCGGTGTTAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGG CCTTTATGTCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGG GTTGCCAACCCGCGAGGGTCAGCTAATCCCAAAAAACCGACCTCAGT TCGGATTGGAGTCTGCAACTCGACTCCATGAAGTTGGAATCGCTAGT AATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTAC ACACCGCCCGTCACACCACGAAAGCCTGTTGTACCAGAAGTCGCTGG CCTAACCCTTCGGGGAAGGAGGCGCCTAAGGTATGGCCGGTGATTGG GGTGAAGTCGTANCAAGGTAACC

Table C-11 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
11	AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCGTGCCTAACAC ATGCAAGTCGAACGAGAAAGCCTGGCTTGCCAGGCAAGTAAAGTGG CGCACGGNNNAGTAACGCGTGGGTAATCTACCTTTGTCTGGGGGATA ACATACCGAAAGGTGTGCTAATACCGCATAACGTGATCGGGTTGCGA CTTGGTCACCAAAGAAGGCCTCTCCCTGGAAGCTTTCGGGTAAAGAT GAGCCCGCGTCCCATTAGCTAGTTGGTAGGGTAAAAGCCTACCAAGG CTACGATGGGTAGCTGGTCTGAGAGGATGGCCAGCCACACTGGAACT GGAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATCTTG CGCAATGGGGGCAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAA GGCCTTCGGGTCGTAAAGCTCTGTCAAGTGGGAAGAATAACCTTGAG ACGAATAATCTCGAGGTGTGACGGTACCACTGGAGGAAGCACCGGCT AACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTATT CGGAATTATTGGGCGTAAAGGGCGTGTAGGCGGCCAGATAAGTCAG ATGTGAAAGCCCGGGGCTCAACCCCGGAAGTGCATTTGAAACTATTT GGCTTGAGTGCGGGAGAGGAAAGCGGAATTCCCAGTGTAGAGGTGA AATTCGTAGATATTGGGAGGAACACCGGTGGCGAAGGCGGCTTTCTG GACCGACACTGACGCTGAGGCGNNAAAGCGTGGGGAGCAAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGCACTAGGTGTA GGAGGTTTTTAACCCNNNNTGTNNCGTAGCTAACGCATTAAGTGCCC CGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACG GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGC GCAGAACCTTACCTGGGCTTGACATCTGGAGAACCCTTTGGAAACAA GGGGGTGCGTAGCAATACGAACTCCAAGACAGGTGCTGCATGGCTGT CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC AACCCTTGTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAGAGAC TGCCGGTGTTAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCAT GGCCTTTATGTCCAGGGCTACACACGTGCTACAATGGTCGGTACAAA GGGTTGCCAACCCGCGAGGGTCAGCTAATCCCAAAAAACCGACCTCA GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGTTGGAATCGCTA GTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGT ACACACCGCCCGTCACACCACGAAAGCCTGTTGTACCAGAAGTCGC TGGCCTAACCCTTCGGGGAAGGAGGCGCCTAAGGTATGGCCGGTGAT TGGGGTGAAGTCGTANCAAGGTAACC

Table C-12 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
12	AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCNNGCCTAACAC ATGCAAGTCGAACGAGAAAGCCTGGCTTGCCAGGCAAGTAAAGTGG CGCACGGGTGAGTAACGCGTGGGTAATCTACCTTTGTCTGGGGGATA ACATACCGAAAGGTGTGCTAATACCGCATAACGTGATCGGGTTGCGA CTTGGTCACCAAAGAAGGCCTCTCCCTGGAAGCTTTCGGGTAAAGAT GAGCCCGCGTCCCATTAGCTAGTTGGTAGGGTAAAAGCCTACCAAGG CTACGATGGGTAGCTGGTCTGAGAGGATGGCCAGCCACACTGGAACT GGAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATCTTG CGCAATGGGGGCAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAA GGCCTTCGGGTCGTAAAGCTCTGTCAAGTGGGAAGAATAACCTTGAG ACGAATAATCTCGAGGTGTGACGGTACCACTGGAGGAAGCACCGGCT AACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTATT CGGAATTATTGGGCGTAAAGGGCGTGTAGGCGGCCAGATAAGTCAG ATGTGAAAGCCCGGNGCTCAACCCCGGAAGTGCATTTGAAACTATTT GGCTTGAGTGCGGGAGAGGAAAGCGGAATTCCCAGTGTAGAGGTGA AATTCGTAGATATTGGGAGGAACACCGGTGGCGAAGGCGGCTTTCTG GACCGACACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGCACTAGGTGTA GGAGGTTTTTAACCCTCCTGTGCCGTAGCTAACGCATTAAGTGCCCCG CCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGG GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGCGC AGAACCTTACCTGGGCTTGACATCTGGAGAACCCTTTGGAAACAAGG GGGTGCGTAGCAATACGAACTCCAAGACAGGTGCTGCATGGCTGTCG TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA CCCTTGTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAGAGACTG CCGGTGTTAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGG CCTTTATGTCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGG GTTGCCAACCCGCGAGGGTCAGCTAATCCCAAAAAACCGACCTCAGT TCGGATTGGAGTCTGCAACTCGACTCCATGAAGTTGGAATCGCTAGT AATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTAC ACACCGCCCGTCACACCACGAAAGCCTGTTGTACCAGAAGTCGCTGG CCTAACCCTTCGGGGAAGGAGGCGCCTAAGGTATGGCCGGTGATTGG GGTGAAGTCGTANCAAGGTANCC

Table C-13 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
13	GGTTACCTNNNTACGACTTCACCCCAATCACCAATCCCACCTTCGACT GCTGGCTCCTTGCGGTTACCTCACAGGCTTCGGGTGTTACCGGCTCTC ATGGTGTGACGGGCGGNNTGTACAAGACCCGGGAACGTATTCACCGC GACATTCTGATTCGCGATTACTAGCAACTCCAACTTCATGTAGGCGA GTTTCAGCCTACAATCCGAACTGGGACCGGTTTTTTAGTTTGGCTCCC CCTCACGGGTTTGCTTCTCGTTGTACCGGCCATTGTAGCACGTGTGTA GCCCTAGACATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCC TCCTGGTTAACCCAGGCAGTCTCGCTAGAGTGCTCAACTTAATGGTA GCAACTAACGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACAT CTCACGACACGAGCTGACGACAACCATGCACCACCTGTCTTCCTGCC CCGAAGGGCTTCCTCTGTTACGAGTAATTCAGGAGATGTCAAGTCTA GGTAAGGTTCTTCGCGTTGCTTCGAATTAAGCCACATGCTCCGCTGCT TGTGCGGGTCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTAC TCCCCAGGCGGAATACTTATTGCGTTTGCTGCGGCACCGAGGGTGGT ACCCCCCGACACCTAGTATTCATCGTTTACGGCGTGGACTACCAGGG TATCTAATCCTGTTTGCTCCCCACGCTTTCGTATCTCAGCGTCAGTTA CGGTCCAGAAAGTCGCCTTCGCCACTGGTGTTCTTCCTAATCTCTACG CATTTCACCGCTACACTAGGAATTCCACTTTCCTCTCCCGCACTCTAG ATACCCAGTTTCAAATGCAGCGCCCAGGTTAAGCCCGGGAATTTCAC ATCTGACTTAAATATCCGCCTACATACTCTTTACGCCCAGTAAATCCG GACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTT AGCCGTGGCTTCCTCCTTGGGTACCGTCATTATCGTCCCCAAAGACAG AGCTTTACAATCCGAAGACCTTCATCACTCACGCGGCGTTGCTGCGTC AGGGTTTCCCCCATTGCGCAATATTCCCCACTGCTGCCTCCCGTAGGA GTCTGGACCGTGTCTCAGTTCCAATGTGGCCGATCACCCTCTCAGGTC GGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTA ATGCGCCGCGGGTCCATCTCAAAGCGGATTGCTCCTTTGATTATCTTC TCATGCGAGAAAATAATGTTATGCGGTATTAATCTCCCTTTCGGGAG GCTATCCCCCTCTTTGAGGCAGGTTACCCACGTGTTACTCACCCGTCC GCCGCTAATCCGTTCCCGAAGGAACTTCATCGCTCGACTTGCATGTGT TAGGCACGCCGCCAGCGTTCGTCCTGAGCCAGGATCAAACTCT

Table C-14 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
14	AGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCNNGCCTAACAC ATGCAAGTCGAACGAGAAAGCCTGGCTTGCCAGGCAAGTAAAGTGG CGCACGGGNNAGTAACGCGTGGGTAATCTACCTTTGTCTGGGGGATA ACATACCGAAAGGTGTGCTAATACCGCATAACGTGATCGGGTTGCGA CTTGGTCACCAAAGAAGGCCTCTCCCTGGAAGCTTTCGGGTAAAGAT GAGCCCGCGTCCCATTAGCTAGTTGGTAGGGTAAAAGCCTACCAAGG CTACGATGGGTAGCTGGTCTGAGAGGATGGCCAGCCACACTGGAACT GGAACACGGTCCAGACTCCTACGGGAGGCAGCAGTGAGGAATCTTG CGCAATGGGGGCAACCCTGACGCAGCAACGCCGCGTGAGTGAAGAA GGCCTTCGGGTCGTAAAGCTCTGTCAAGTGGGAAGAATAACCTTGAG ACGAATAATCTCGAGGTGTGACGGTACCACTGGAGGAAGCACCGGCT AACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTATT CGGAATTATTGGGCGTAAAGGGCGTGTAGGCGGCCAGATAAGTCAG ATGTGAAAGCCCGGGGCTCAACCCCGGAAGTGCATTTGAAACTATTT GGCTTGAGTGCGGGAGAGGAAAGCGGAATTCCCAGTGTAGAGGTGA AATTCGTAGATATTGGGAGGAACACCGGTGGCGAAGGCGGCTTTCTG GACCGACACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGATGGGCACTAGGTGTA GGAGGTTTTTAACCCTCCTNNNCCGTAGCTAACGCATTAAGTGCCCC GCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGG GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGCG CAGAACCTTACCTGGGCTTGACATCTGGAGAACCCTTTGGAAACAAG GGGGTGCGTAGCAATACGAACTCCAAGACAGGTGCTGCATGGCTGTC GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA ACCCTTGTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAGAGACT GCCGGTGTTAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATG GCCTTTATGTCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAG GGTTGCCAACCCGCGAGGGTCAGCTAATCCCAAAAAACCGACCTCAG TTCGGATTGGAGTCTGCAACTCGACTCCATGAAGTTGGAATCGCTAG TAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCGGGCCTTGTA CACACCGCCCGTCACACCACGAAAGCCTGTTGTACCAGAAGTCGCTG GCCTAACCCTTCGGGGAAGGAGGCGCCTAAGGTATGGCCGGTGATTG GGGTGAAGTCGTAACAAGGTAACC

Table C-15 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
15	GGTTACCTTGNTACGACTTCACCCTAATTATCAGCCATACCTTAGGTG CCTGCACCCCCGAAAGGTTTGCCTAGCAACTTCTGGTACAACCAACT CTCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCAC CGCGGCATGCTGATCCGCGATTACTAGCGATTCCAACTTCATGGAGT CGAGTTGCAGACTCCAATCCGAACTTGGGACGGGTTTCTGGGATTGG CTCCACCTTGCGATATCGCTACCCTTTGTTCCGTCCATTGTAGCACGT GTGTAGCCCTGGGCATAAGGGCCATGAGGACTTGACGTCATCCCCAC CTTCCTCCCCGTTAACCGAGGCGGTATCTTTAGAGTCCCCAACCGAAT CGCTGGCAACTAAAGATAGGGGTTGCGCTCGTTGCGGGACTTAACCC AACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTTG GGGCTCCCCGAAGGGCACTTCGCACTTTCATGCAAATCCCCCAGATG TCAAGCCCAGGTAAGGTTCTTCGCGTTGCGTCGAATTAAACCACATG CTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTTAGCCTT GCGGCCGTACTCCCCAGGCGGTTCACTTAATGCGTTAGCTGCGGCAC AGCAGGGGTCAATACCCGCTACACCTAGTGAACAACGTTTACTGCGT GGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCCTTCGCGCCT CAGCGTCAGTATTGGTCCAGAAAGTCGCCTTCGCCACCGGTGTTCCTC CTGATATCTACGAATTTCACCTCTACACCAGGAATTCCACTTTCCTCT CCCATACTCAAGCCCAACAGTATCAAATGCACTTCCGGGGTTGAGCC CCGGGCTTTCACACCTGACTGATCGGGCCGCCTGCGCGCCCTTTACGC CCAATGATTCCGAGTAACGCTTGCACCCCCCGTATTACCGCGGCTGCT GGCACGTAGTTAGCCGGGGCTTCCTCCAAGGGTACCGTCATTTGTTTC TTCCCCAAGGGCAGAGCTTTACGACCCAAAGGCCTTCATCGCTCACG CGGCGTTGCTGCATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTG CTGCCTCCCGTAGGAGTTTGGACCGTGTCTCAGTTCCAATGTGGCCGA TTACCCTCTCAGGTCGGCTACTGATCGTCGCCTTGGTGAGCCGTTACC TCACCAACTAGCTAATCAGACGCAGGCCCATCCCATACCGATGAATC TTTGGCGAGAAGTCCATGTGAACTCCTCGCGTTATGAGGTTTTAATCC CGGTTTCCCGAGGCTATCCCTCTGTATGGGGCAGGTTGCCTACGCGTT ACTCACCCGTCCGCCGCTCTCTATGGCTTCACTCGACCGAAGTTTCAT TCCACCATATACCGCTCGACTTGCATGTGTTAGGCACGCCGCCAGCG TTCATCCTGAGCCAGGATCAAACTCT

Table C-16 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
16	GGTTACCTTGTTACGACTTCACCCCAATCATCGACTCCACCTTCGACG GCTGGCTCCTTACGGTTACCTCACCGGCTTCGGGTGTCTCCAACTCTC GTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGC AGTATGCTGACCTGCGATTACTAGCGATTCCGACTTCATGCAGGCGA GTTGCAGCCTGCAATCCGAACTGGGACCGGGTTTTTGAGATTCGCTTC ACCTCGCGGCTTCGCTGCCCTCTGTTACCGGCCATTGTAGTACGTGTG TAGCCCAGGACATAAGGGGCATGATGACTTGACGTCATCCCCGCCTT CCTCCGTCTTGTCGACGGCAGTCTCCCATGAGTTCCCGACTTTACTCG CTGGCAACATAGGATAGGGGTTGCGCTCGTTGCGGGACTTAACCCAA CATCTCACGACACGAGCTGACGACAGCCATGCACCACCTGTTTTTGT GTCCCCGAAGGGAGGGACCTATCTCTAGGTCTTTCACTCAATGTCAA GCCCTGGTAAGGTTCTTCGCGTTGCGTCGAATTAAACCACATACTCCA CCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGC CGTACTCCCCAGGCGGGGTACTTATTGCGTTAACTCCGGCACAGGAG GGGTCGATACCCCCTACACCTAGTACCCATCGTTTACGGCTAGGACT ACCGGGGTATCTAATCCCGTTCGCTCCCCTAGCTTTCGCGCCTCAGCG TCAGACATCGTCCAGAAAGTCGCCTTCGCCACTGGTGTTCTTCCAAAT CTCTACGCATTTCACCGCTACACTTGGAATTCCACTTTCCTCTCCGAC ACTCAAGAATACCAGTTTCTGTCCCCTCACGAGGTTGAGCCTCGCACT TTTAAGACAGACTTGATATCCCGCCTGCGCGCGCTTTACGCCCAAT AATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGTTGGCA CGTAGTTAGCCGTGGCTTCTTATTCAGGTACCGTCACTATCTCTCATT ATTTACAAGAAATACGTTCGTCCCTGACGAAAGAGCTTTACGATCCG AAAACCTTCTTCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCAT TGCGGAAGATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGT CTCAGTCCCAGTGTGGCCGTTCATCCTCTCAGACCGGCTACTGATCGA AGCCTTGGTGGGCCGTTACCCCTCCAACTAGCTAATCAGACGCAGGC TCATCTTCTAGCGGTAGCATGTTCAGAGGCCACCTTTAGTAACAACTT CATGCGAAGTCGCTACAACATTCGGTATTAGCACCCCTTTCGGGATG TTGTCCCCATCTAGAAGGCAGATTGCCTACGCGTTACTCACCCGTTCG CCACTAAGATCCATTGCTGAATCTCCGTTCGACTTGCATGTGTTAAGC ACGCCGCCAGCGTTCGTCCTGAGCCAGGATCAAACTCT

Table C-17 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

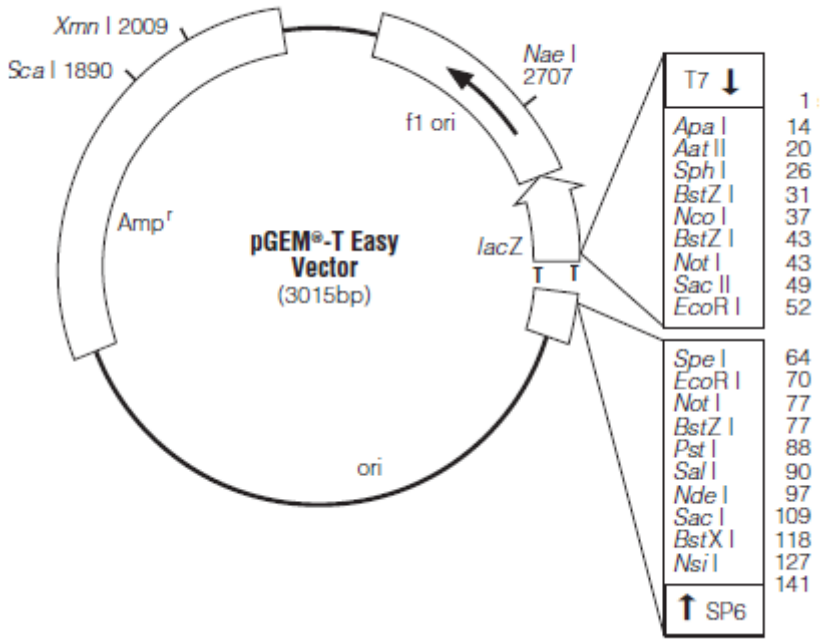
Clone number	Sequence
17	AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGTGTGCTTAACAC ATGCAAGTGGAGCGACGAACCAGGGCTTGCCCTGGGGCAGAGCCGC GAACGGGTGAGTAACACGTGGGTAATCTACCCCGATGACCGGGACA ACCCGAGGAAACTCGGGCTAATACCGGATGTGCACGCGACTCACGA GAGTTGCGTACAAAGGAAGCTTCGGCCTCCGCATCGGGAGGGGCCCG CGGCCCATTAGCTCGTTGGTGGGGTAACGACCTACCAAGGCTGCGAT GGGTAGCTGGTCTGAGAGGACGATCAGCCACACTGGGACTGAGACA CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGCGCAATG CGCGAAAGCGTGACGCAGCAACACCGCGTGAGGGAAGACGGCCTTC GGGTTGTAAACCTCTTTCAGGAGGGACGAAGGTCGGCCGGTTAACAG CCGTGCCGGCTGACGGTACCTCCAGAAGAAGCTCCGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGGAGCGAGCGTTGTCCGGAATC ATTGGGCGTAAAGCGCGCGTAGGCGGCCCGATAAGTCCGCTGTGAAA GTCAACGGCTCAACTGTTGAAAGCCGGTGGATACTGTCGGGCTGGAG TACGGAAGAGGCAAGTGGAATTCCTGGTGTAGCGGTGGAATGCGCA GATATCAGGAGGAACACCTATAGCGAAGGCAGCTTGCTGGGACGTTA CTGACGCTAAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC CCTGGTAGTCCACGCCGTAAACGATGAGAACTAGGTGTGGGAGGTGT CGACTCCTCCCGTGCCGTCGCTAACGCATTAAGTTCTCCGCCTGGGGA GTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCA CAAGCAGCGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTT ACCTGGGTTTGAAATGTTGGTGACCGGTGTGGAAACACACCTTTCCTT CGGGACACCATCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGT GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTGTCCTATGT TGCCAGCATTCAGTTGGGGACTCATGGGATACTGCCGGTGACAAACC GGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCCAG GGCTACACACGTGCTACATTGGCGCGCACAGAGGGAAGCAATGCCGT GAGGTGGAGCAAATCCCACAAAGCGCGTCCCGGTTCGGATTGGAGG CTGCAATTCGCCTCCATGAAGTTGGAGTTGCTAGTAATCGCGGATCA GCAACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGT CACACCACGAAAGCAGGTAACACCCGAAGCCGGTGAGCTAACCCTTT TGGGAGGCAGCCGTCGAAGGTGGGACTTGTGATTGGGGTGAAGTCGT AACAAGGTAACC

Table C-18 16S rDNA sequence from each clones in the biomass sample from the first compartment of Reactor B (continued)

Clone number	Sequence
18	GGTTACCTTGTTACGACTTAGTCCCAATCGCGGNNNTCATCTTCGGCG CTTGCTTCCCTTGCGGGTTGGCACGGCGACTTCGGATGCCCCCCACTT TCGTGGCTTGACGGGCGGTGTGTACANNGCTCAGGAACACATTCACC GCGGTATGCTGACCCGCGATTACTAGCGATTCCGGCTTCATGCAGGC GAGTTGCAGCCTGCAATCCGAACTGGGGCGCGTTTTTTGGGATTTGCT TGCTCTCGCGAGTTTGCATCCCTTTGTGCGCGCCATTGTAGGACGTGT GCAGCCCTANNCATAAGGGCCATGAGGACTTGACGTCATCCCCACCT TCCTCCGGTTTAACACCGGCAGTCTCACTAGAGTCCCCGACATGACTC GCTGGCAACTAGTGACAAGGGTTTCGCTCGTTAAGGGACTTAACCCG ACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGCACG TTCCACCCGAAGNCGTGGCCCGGCTTTCACCGGGTTAATCCGTGCAT GTCAAGACTAGGATANNNNNCTTNNCGTAGCCTNNAATTAAGCCACA TCCTCCACCGCTTGTGTGAGCCCCCGTCAATTCCTTTGAGTTTCAGCC TTGCGACCATACTCCCCAGGCGGAGCACTTAACACTTTCGCTTCGGCT GGAAGGCTATGAGGGACCCTCCAACNCAGTGCTCATCGTTTACGGCT AGGACTACCGNGGTATCTAATCCCGTTTGCTCCCCTAGCTNTCGTGCA TCAGCGTCAGTAGAGACCCAGTGAGCCGCTTTCGCCACTGGTGTTCC TTAGGATATCAACGCATTTCACCGCTCCACCCTAAGTTCCGCTCACCT CTGTCTCCCTCAAGCACGGCAGTTTTGAGCGCAGTTCCTCGGTTGAGC CGAGGGATTTCACACCCAACTTTCCGCGCCGCCTACGCACCCTTTAA GCCCAGTGATTCCGAATAACGTTTGTACGGTCCGTATTACCGCGGCT GCTGGCACGAACTTAGCCCGTACTTCCTCTGCGGATAGGTCAAACAT GGGGGAGTACCCCCATGCATTTCCTCCCCACTGACAGCGGTTTACAA CCCGAAGGCCTTCATCCCGCACGCGGCGTCGCTCGGTCAGGCTTGCG CCCATTGCCGAAGATCCTCGACTGCAGCCACCCGTAGGTGTCTGGGC AGTGNNNCAGTCCCAGTGACGCGGGTCGTGCTCTCACACCCGCTACC CATCATTGCCTTGGTAGGCCATTACCCTACCAACTAGCTAATAGGAC GCAGACCGATCCCCGGGCGGAGTCTCACCTTTGGTNCGGANACGTTA TCCCGTATTACCTGCAGTTTCCCGCNNNTATCCGAGANCCGGGGGTA CGTATCNNNNTGTTCCTGTCCCTTTCGCCGCTTTCTGNNTCCCGAAGG NNNCTTCGCGCTCGANTTGCATGCCNAATCNNNGCCGCCAACGTTCA TTCTGAGCCAGGNNNAANNNNN

APPENDIX D

Information of pGEM®-T Vector Sequence (Promega, USA) used in the clone library technique



T7 RNA Polymerase transcription initiation site 1

multiple cloning region 10–128

SP6 RNA Polymerase promoter (–17 to +3) 139–158

SP6 RNA Polymerase transcription initiation site 141

pUC/M13 Reverse Sequencing Primer binding site 176–197

lacZ start codon 180

lac operator 200–216

ß-lactamase coding region 1337–2197

phage f1 region 2380–2835

lac operon sequences 2836–2996, 166–395

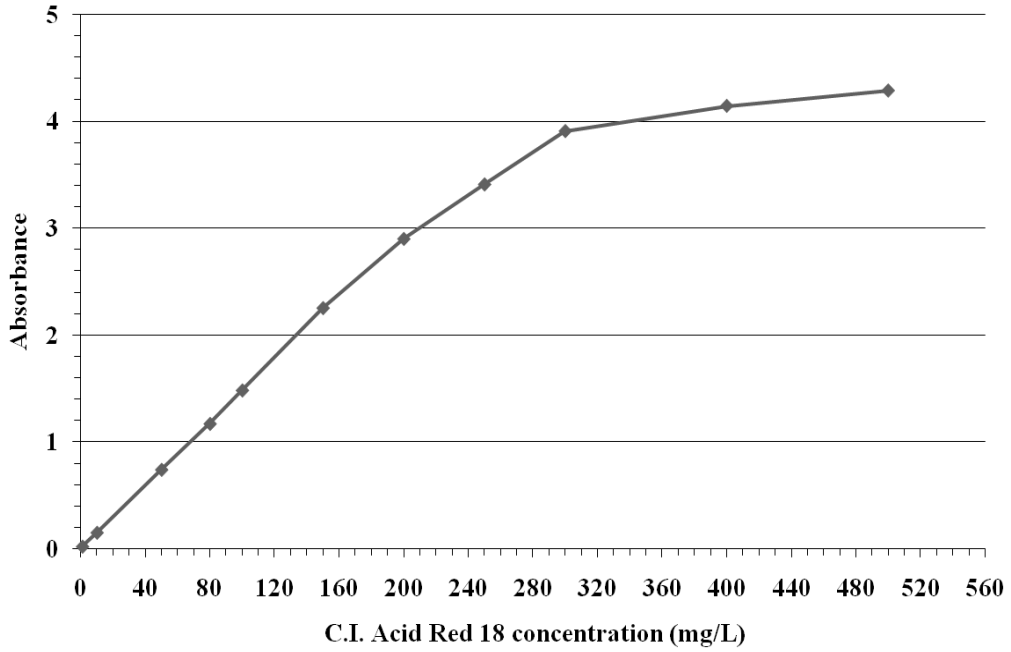
pUC/M13 Forward Sequencing Primer binding site 2949–2972

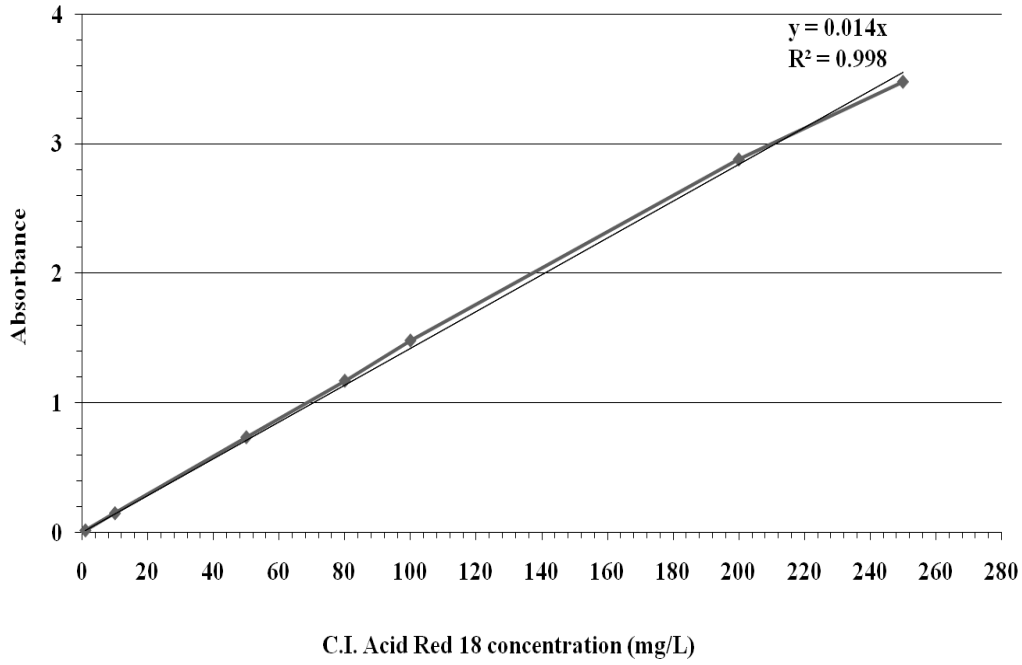
T7 RNA Polymerase promoter (–17 to +3) 2999-3

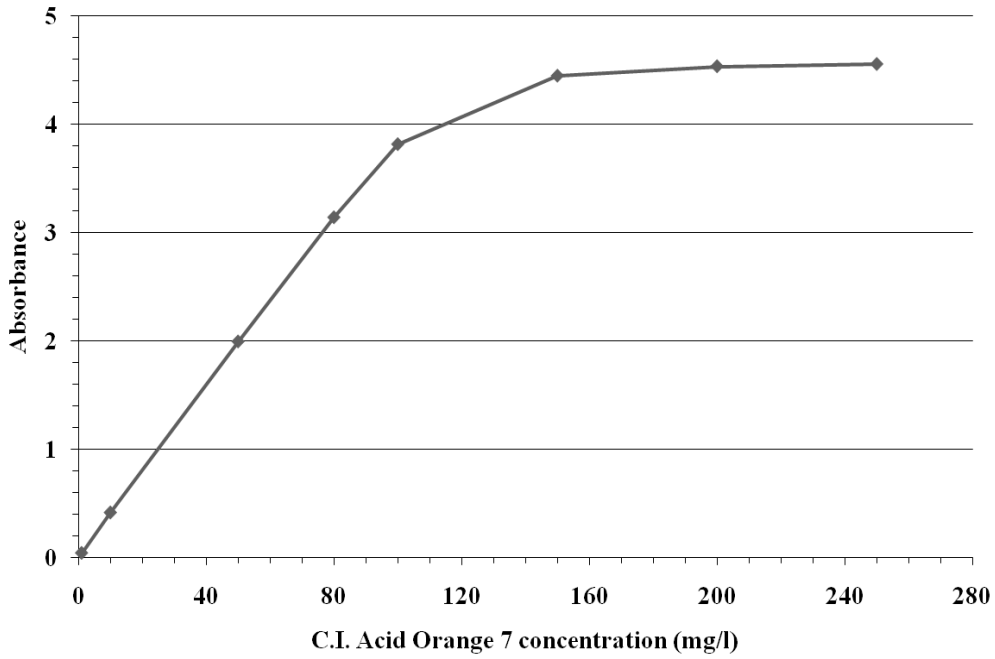
APPENDIX E

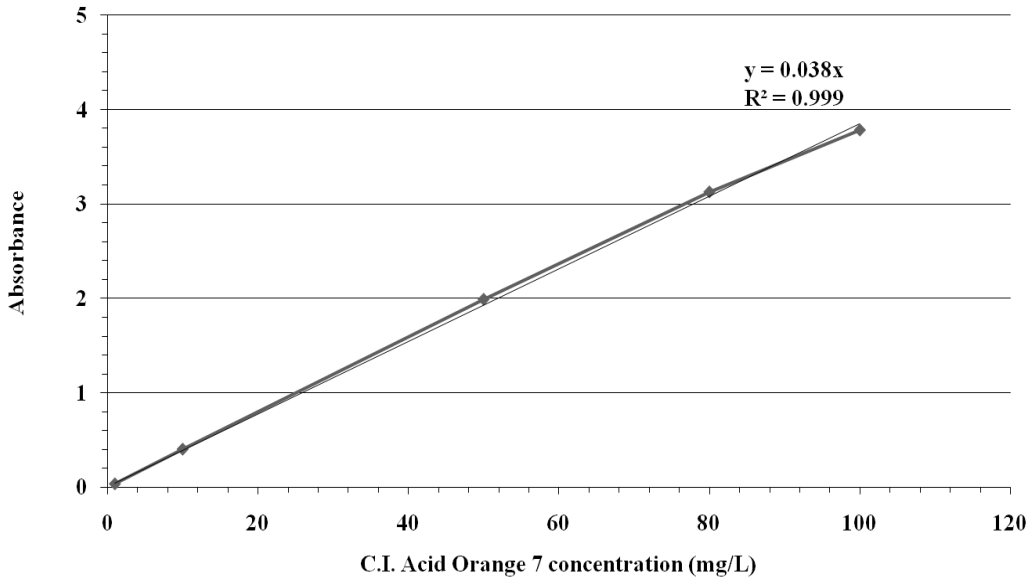
Azo dyes standard curve

The standard curve was plotted between azo dyes concentration in mg/L and absorbance values obtained from spectrophotometer.






Absorbance
0
1
2
3
4
5
0
40
80
120
160
200
240
280
C.I. Acid Orange 7 concentration (mg/l)


y = 0.038x
R² = 0.999
Absorbance
0
1
2
3
4
5
0
20
40
60
80
100
120
C.I. Acid Orange 7 concentration (mg/L)

BIOGRAPHY

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Conference Paper Publication

Chalathip Juntalasiri and Benjaporn Boonchayaanant (2012). Decolorization of Azo Dyes in Anaerobic Baffled Reactors under Sulfate-Reducing Conditions**.** 1st Annual International Conference on Sustainable Energy and Environmental Sciences (SEES 2012), Singapore, 13th- 14th Feb 2012.

Reward from the conference

Best student paper award from 1st Annual International Conference on Sustainable Energy and Environmental Sciences (SEES 2012), Singapore, 13th- 14th Feb 2012.