BIOAUGMENTATION OF ACTIVATED SLUDGE WITH SELECTED BACTERIAL STRAINS

THAMARAT SUKJEERADET

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.....
Mr. Thamarat Sukjeeradet
Candidate

.....
Assoc. Prof. Prayad Pokethitiyook, Ph.D.
Major-advisor

.....
Prof. Maleeya Kruatrachue, Ph.D.
Co-advisor

.....
Prof. Banchong Mahaisavariya, M.D., Dip Thai Board of Orthopedics
Dean
Faculty of Graduate Studies
Mahidol University

.....
Assoc. Prof. Prayad Pokethitiyook, Ph.D.
Program Director
Master of Science Program in Environmental Biology
Faculty of Science
Mahidol University

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on
May 12, 2010

.....
Mr. Thamarat Sukjeeradet
Candidate

.....
Mr. Suksun Amornraksa,
Ph.D.
Chair

.....
Assoc. Prof. Prayad Pokethitiyook,
Ph.D.
Member

.....
Mr. Toemthip Poolpak,
Ph.D.
Member

.....
Prof. Maleeya Kruatrachue,
Ph.D.
Member

.....
Prof. Banchong Mahaisavariya,
M.D., Dip Thai Board of Orthopedics
Dean
Faculty of Graduate Studies
Mahidol University

.....
Prof. Skorn Mongkolsuk,
Ph.D.
Dean
Faculty of Science
Mahidol University

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Thamarat Sukjeeradet

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THAMARAT SUKJEERADET 4837482 SCEB/M

M.Sc. (ENVIRONMENTAL BIOLOGY)

THESIS ADVISORY COMMITTEE: PRAYAD POKETHITIYOOK, Ph.D., MALEEYA KRUATRACHUE, Ph.D.

ABTRACT

Many industrial sectors are likely to generate wastewater containing high organic content. Discharge of such wastewater without treatment is known to adversely affect aquatic life, water potability, and agriculture. Thus, wastewater treatment standards are becoming more stringent, and the treatment of industrial wastewater is compulsory in many regions. Wastewater containing high Total Dissolved Solids (TDS) content was obtained from a hydrocarbon processing plant. Under normal operation, the wastewater is diluted by half with raw water prior to treatment. Without dilution, high TDS combined with high organic loading would upset the wastewater treatment plant's operation. Three bacterial strains were isolated using a NaCl-containing medium. Bioaugmentation of activated sludge with these bacterial strains enabled treatment of the wastewater without dilution. Activated sludge bioaugmented with these bacterial strains exhibited many advantages over conventional activated sludge, such as higher treatment performance and higher performance after recovery from shock loading, but direct addition of bacterial cultures to the reactors compromised treatment performance during the early stages. To eliminate the stages of compromised performance, activated sludge was bioaugmented with the selected bacterial strains in a separate reactor. Then, adapted to the wastewater by gradually increasing the concentration, the sludge was added periodically to the experimental reactor. Satisfactory performance was obtained with this new technique without the stages of compromised performance.

KEY WORDS: BIOAUGMENTATION / ACTIVATED SLUDGE / TOTAL DISSOLVED SOLIDS / WASTEWATER TREATMENT

83 pages

การเสริมสมรรถนะแอกทิเวเต็ดสลัดจ์ด้วยแบคทีเรียที่คัดสรรมา

BIOAUGMENTATION OF ACTIVATED SLUDGE WITH SELECTED BACTERIAL STRAINS

ธรรมรัตน์ สุขจีระเดช 4837482 SCEB/M

วท.ม. (ชีววิทยาสภาวะแวดล้อม)

คณะกรรมการที่ปรึกษาวิทยานิพนธ์: ประหยัด โภคฐิติยุกต์, Ph.D., มาลียา เครือตราชู, Ph.D.

บทคัดย่อ

อุตสาหกรรมหลายประเภทก่อให้เกิดน้ำเสียที่มีค่าสารอินทรีย์สูง การปล่อยน้ำเสียดังกล่าวสู่แหล่งน้ำธรรมชาติก่อให้เกิดผลเสียต่อชีวิตสัตว์น้ำ ลดคุณภาพน้ำสำหรับอุปโภคบริโภคและการเกษตร เพราะฉนั้นข้อบังคับและมาตรฐานเกี่ยวกับการบำบัดและปล่อยน้ำเสียจึงเข้มงวดขึ้นในหลายๆพื้นที่ งานวิจัยนี้ได้รับน้ำเสียที่มีค่าสารละลายในน้ำ (Total Dissolved Solids) สูงมาจากกระบวนการผลิตสารไฮโดรคาร์บอน ในการปฏิบัติงานปกตินำ้เสียนี้จะถูกเจือจางหนึ่งเท่าตัวด้วยน้ำดิบ การบำบัดโดยปราศจากการเจือจางทำให้ระบบบำบัดน้ำเสียไม่สามารถดำเนินการอย่างถูกต้องได้ แบคทีเรียสามสายพันธุ์ถูกคัดสรรมาโดยการใช้อาหารเลี้ยงเชื้อที่มี NaCl สูง การเสริมสมรรถนะแอกทิเวเต็ดสลัดจ์ด้วยแบคทีเรียเหล่านี้ทำให้สามารถบำบัดน้ำเสียนี้ได้โดยไม่ต้องเจือจาง แอคทิเวเต็ดสลัดจ์ที่เสริมสมรรถนะด้วยแบคทีเรียเหล่านี้ได้แสดงถึงข้อได้เปรียบหลายด้านเมื่อเทียบกับแอคทิเวเต็ดสลัดจ์ปกติอย่างเช่น สมรรถนะการบำบัดที่สูงกว่าและสมรรถนะที่สูงกว่าหลังการฟื้นตัวจากภาวะช๊อคโหลด (Shock Load) แต่การเติมเชื้อแบคที่เรียสู่แอคทิเวเต็ดสลัดจ์โดยตรงทำให้สมรรถนะการบำบัดลดลงในช่วงแรกๆ การเสริมสมรรถนะแอคทิเวเต็ดสลัดจ์ด้วยแบคทีเรียดังกล่าวในถังปฏิกรณ์ที่แยกออกมาและปรับสภาวะให้มีความเข้มข้นสูงขึ้นอย่างค่อยเป็นค่อยไป แล้วจึงเติมลงไปในถังปฏิกรณ์ที่ใช้ในการทดลอง เทคนิคใหม่นี้ให้ผลการบำบัดน้ำเสียซึ่งเป็นที่น่าพอใจโดยปราศจากช่วงเวลาที่สมรรถนะการบำบัดลดลง

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LIST OF ABBREVIATIONS

°C	=	degree Celsius
BOD	=	Biochemical Oxygen Demand
COD	=	Chemical Oxygen Demand
G	=	gram
HRT	=	Hydraulic retention time
l, L	=	liter
mg	=	milligram
mg/l	=	milligram per liter
ml	=	milliliter
MLSS	=	Mixed liquor suspended solids
MLVSS	=	Mixed liquor volatile suspended solids
M	=	molar
NB	=	nutrient broth
NA	=	nutrient agar
SBR	=	Sequencing batch reactor
SRT	=	Solids retention time
TDS	=	Total dissolved solid

CHAPTER I
INTRODUCTION

Human activities produce both solid and liquid wastes. After passing through variety of usages, the water supply for the activities is contaminated with wastes. Wastewater is removed from residential, commercial and industrial sectors. Accumulation of wastewater leads to decomposition of organic compounds to malodorous gases, and may harbor a number of pathogenic microorganisms. Proper collection, disposal, reuse or treatment of wastewater is essential public health and environmental integrity (Ahmad et al., 2007; Zbontar and Glavic, 2000).

Constituents of wastewater can harm the aquatic environment they are discharged into. The goal of wastewater treatment is to remove these pollutants. Removal of oxygen-demanding materials has been a major focus of wastewater treatment. Most oxygen-demanding materials are organic compounds. These pollutants are consumed by microorganisms, which use oxygen in their metabolism and deplete oxygen from their surroundings. Low dissolved oxygen (DO) concentrations have harmful effects on aquatic life.

Strict regulations, water scarcity and growing environmental awareness have increased the interests in more efficient wastewater treatment system and reuse of wastewater. Most conventional wastewater treatment systems place heavy reliant on biological system. The effective operation of a wastewater treatment system depends highly upon the microorganisms within it. The environmental condition of the wastewater treatment plant provides selection pressure for the microorganisms in the system. Therefore, influent characteristics, mode of operation and design of the treatment system determine which microorganism would survive and persist in the system.

Under changing environmental conditions, microbial population of the system can adjust its composition accordingly. However, they may not adjust in time to prevent the plant's failure to meet the effluent standard. These failures may cause poor COD removal, poor sludge settability, foaming, etc. The operator has several options in dealing with these problems.

The most common approaches involve modification of influent characteristics (e.g. pH, nutrient addition, etc.), modifying the mode of operation (e.g. sludge return rate, sludge removal rate, aeration, etc.), and changing plant configuration (e.g. reorganizing treatment units, build additional capacity). These approaches take in consideration that biological waste treatment systems are dependent on the process variables such as nutrient, dissolved oxygen, sludge retention time (SRT), wastewater characteristics. Managing these variables is a complex and difficult task. In some cases, these approaches may solve the problem. But these approaches involve increased operation cost, which would be too excessive in some cases (Stevens, 1989).

Bioaugmentation offers a potentially powerful and cost-effective solution to these problems. This process attempts to improve the microbial population through direct introduction of selected naturally occurring microorganisms or genetically altered microorganisms to the wastewater treatment system. Bioaugmentation facilitates the establishment of specific population in the microbial community of the wastewater treatment system (Jiang et al., 2007). The superior microorganisms introduced to the system improve several aspects in the wastewater treatment process such as improved flocculation and degradation of recalcitrant compounds (Ivanov et al., 2006).

The present study was presented with a problematic wastewater with high salt content from a hydrocarbon processing plant. The purpose of this study were to screen for salt tolerant microorganisms with ability to remove pollutants from the wastewater, to study the effects of inoculating the selected bacteria into activated sludge, and to find a method for their potential use at larger scales.

CHAPTER II
OBJECTIVES

The objectives of the present study were as follows:

1. To screen for and select bacterial strains with potential for treatment of wastewater with high total dissolved solids ranging from 10,000-20,000 mg/l.

2. To compare treatment performances of the conventional wastewater treatment system to wastewater treatment system bioaugmented with the selected bacterial strains.

3. To improvise a possible method of bioaugmentation of the selected bacterial strains to the actual wastewater treatment system.

CHAPTER III
LITERATURE REVIEW

3.1 Petroleum wastewater

Hydrocarbon processing is a water-intensive sector. The petroleum industry uses and discharges a large volume of wastewater to surface water. Its effluent wastewater may contain a complex combination of contaminative compounds. The composition of the effluent varies according to specific industrial processes and activities. These may include oil, grease, phenols, sulfides, ammonium, and polycyclic aromatic hydrocarbons (PAHs) (Avci et al., 2005; Sheu and Weng, 2001; Hami et al., 2007). Contaminants such as PAHs are known to be carcinogenic. Others are known to be oxidants. The effects of reactive oxygen species produced by stimulation of these contaminants and the oxidative damages they cause in aquatic organisms have been well documented (Avci et al., 2005).

Management of wastewater is carried out through proper collection and treatment. The main aims in management are to protect the environment by reduction of pollutants that may enter local air and water resources consequentially reducing health risks, and to mitigate water scarcity and save freshwater by mobilizing reusable treated wastewater (Ahmad et al., 2007).

The petroleum industry uses combinations of treatment methods to treat the wastewater derived from its processes since the wastewater constituents vary greatly. Various physico-chemical and microbiological treatment methods have been used to treat refinery wastewater. Pretreatments such as gravity separation of oil and Induced Gas Floatation reduce the concentration of oil, grease and suspended solids from the wastewater. The removal of dissolved organics is usually carried out by biological processes (Hami et al., 2007; Sheu and Wang, 2000).

3.2 Aerobic biological wastewater treatment

Biological wastewater treatment utilizes microorganisms for reduction of pollutant. Removal of organic materials by biological oxidation is the core technology in aerobic wastewater treatment process. Generally, wastewater is brought into contact with bacterial biomass. Through their metabolism, soluble organic material is used as energy source, and converted into CO_2cellular material, which is no longer soluble and can be removed through physical means. The key factor in any aerobic biological wastewater treatment system is adequate supply of oxygen. Aerobic treatment of wastewater results in high removal of soluble biodegradable organic material, and the biomass generated is generally well flocculated resulting in low effluent turbidity and suspended solids concentration (Chan et al., 2009). Biological wastewater treatment takes place at ambient temperature like other biological reaction. There is no need to heat or cool the system.

3.3 Activated sludge

Activated sludge is a common wastewater treatment system for municipal and industrial wastewater worldwide. It involves the use of an activated mass of microorganisms for bioconversion of pollutants. Organic waste influent enters the aeration tank and mixes with activated sludge for bioconversion to take place. A mechanical aeration system is provided to keep the environment aerobic, and completely mix the reaction. After the reaction, the mixture from the aeration tank is passed to the settling tank where sedimentation and clarification occur. In the settling tank, microbial mass is separated from treated wastewater. To maintain the concentration of activated sludge in the aeration tank, a portion of the separated microbial mass is recycled back to the aeration tank. The excess microbial mass is removed and disposed.

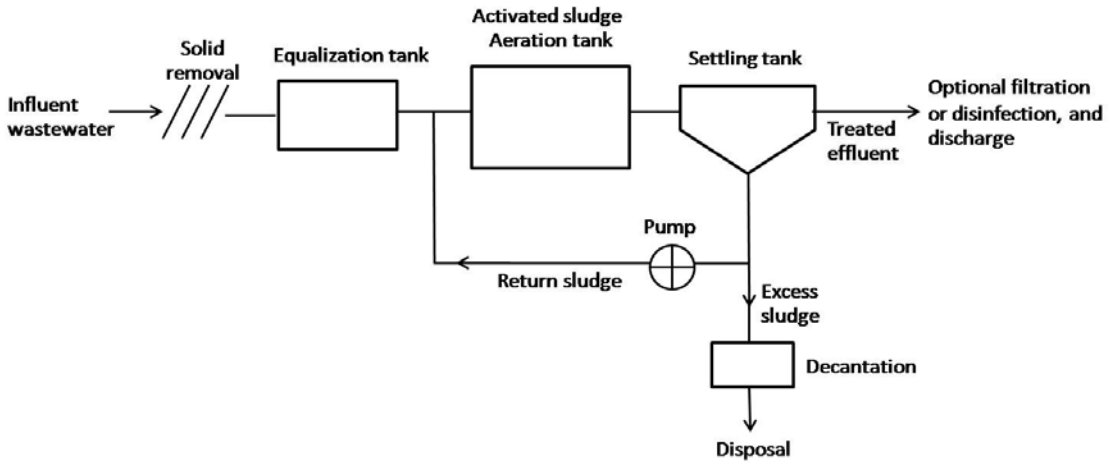

Fig 3-1 Diagram of activated sludge process

A typical flow schematic of municipal or industrial activated sludge wastewater treatment plant is described in Fig 3-1. Influent wastewater generally passes through a pretreatment unit consisting of screens or a settling tank for removal of suspended solids. In wastewater where grease or oil is present, a grease trap or an Induced Gas Floatation (IGF) unit is required to remove these components prior to entering the main treatment units. With the suspended solid removed, the influent wastewater enters the equalization tank. The equalization protects the biological system from abrupt changes in influent characteristics, and flow rate, which may cause washout of biomass or upset the process. Necessary pretreatments such as adjustment of pH occur in the equalization tank prior to entering the aeration tank. The wastewater influent is mixed with the activated sludge biomass in the aeration tank. Turbulence and aeration are provided by turbines to completely mix the liquor and provide sufficient level of dissolved oxygen. In some cases, direct air injection or even injection of oxygen gas is necessary to maintain required dissolved oxygen level. Through their metabolism, activated sludge microorganisms remove the pollutant from the wastewater. Activated sludge microorganisms take up soluble components of wastewater, convert them to energy, and produce more biomass. After leaving the aeration tank, activated sludge biomass and treated wastewater are separated by gravity settling in the settling tank. Well-formed activated sludge flocs settle to the bottom of the settling tank. Addition of flocculants may be necessary for proper

settling characteristics in case of poor settling characteristics. A portion of the activated sludge is returned to the aeration tank via a pump to maintain proper clarified wastewater exits the settling tank for discharge. Filtration or disinfection is sometimes recommended as a downstream process before discharge.

3.4 Microbiology of activated sludge

The communities of prokaryotic microorganisms occurring in activated sludge reactors are responsible for most of the carbon and nutrient removal from wastewater, and represent the core component of every biological wastewater treatment plant. In contrary to that fact, the presence of certain bacterial species can contribute to detrimental conditions of the wastewater treatment plant by negatively influencing the settling properties of activated sludge in the settling tank, by formation of foam, or by outcompeting microorganisms required for nutrient removal. The knowledge and thorough understanding of the ecology of the microbial community is required for optimization of biological wastewater treatment plant operation and stability(Wagner and Loy, 2002).

3.4.1 Filamentous bacteria

The occurrence of filamentous bacteria at a certain level is important for proper floc formation, but the excessive growth of filamentous bacteria is detrimental to wastewater treatment system. It causes foaming or settling problems of activated sludge in the settling tank. Many filamentous bacteria have not been isolated and maintained in pure culture, and have only been described by only a few observable characteristics under the microscope. *Microthrix parvicella* is one of the few well known filamentous bacteria in activated sludge. It is well known as a causative agent for foaming and sludge bulking in wastewater treatment plants worldwide (Hwang and Tanaka, 1998). There is yet no reliable strategy for control of this microorganism. *M. parvicella* is able to take up and store long-chain fatty acids under anaerobic conditions and metabolize them under aerobic conditions. This specialized ability offers *M. parvicella* a competitive advantage in nutrient-removal plants with

anaerobic-aerobic cycles over most activated sludge bacteria (Andreasen and Nielsen, 2000).

3.4.2 Nitrogen removal bacteria

Nitrogen compounds in wastewater are removed by conversion to nitrogen gas through nitrification and denitrification in biological wastewater treatment plants. Nitrification, the aerobic oxidation of ammonium to nitrate via nitrite, is carried out by two different groups of autotrophic bacteria—the ammonium oxidizers and nitrite oxidizers. These bacteria are slow-growing. They are the principles of nitrogen removal in many wastewater treatment plants. The causes of failures are not always obvious. Once washout of nitrifiers occur, the recovery process can take very long time due to slow growth of these microorganisms (Campos et al., 2002; Glass and Silverstein, 1999).

3.4.3 Phosphorus removal bacteria

Wastewater treatment plants with enhanced biological phosphorus removal are characterized by cyclic changes between anaerobic and aerobic conditions of the activated sludge. Through the cyclical changes, the growth of polyphosphate-accumulating microorganisms is promoted. Intracellular accumulation of polyphosphate occurs during the aerobic period. Polyphosphate accumulating takes up excess orthophosphate and stores it as polyphosphate. Then phosphorus is removed with the excess sludge from the wastewater treatment plant (Lin-lin et al., 2007). Earlier experiments found *Acinetobacter* sp. to be responsible for phosphorus removal, but further researches have found that certain *Pseudomonas* sp. and *Moraxella* sp. also possess this ability (Lin et al., 2003).

3.5 Operational parameters in activated sludge system

The hydrodynamics of the process have an effect on the rates of pollution removal, kinetics of organic matter degradation and the sludge settling properties. The hydrodynamics of the activated sludge process are mainly dependent on the concentration of its constituents and the geometric characteristics of the reactor, such

as size and aeration dynamics (Tizghadam et al, 2008). Upgrading activated sludge treatment system can be optimized for enhancement of COD removal, nitrification, denitrification and sludge settling properties through modifications of the following parameters:

3.5.1 Mixed liquor suspended solids

Mixed Liquor Suspended Solids (MLSS) is the total amount of organic and minerals suspended solids. MLSS is determined by filtration of an aliquot of sample, drying at 105°C to determine the weight of the solids, and expressed in mg/l of sample.

3.5.2 Mixed liquor volatile suspended solids

Mixed Liquor Volatile Suspended Solids (MLVSS) is the measure of non microbial organic matter, dead and living microorganisms and cellular debris. MLVSS is determined by heating of dried filtered sample at 600-650°C. The volatile portion represents the organic portion of MLSS, which accounts for 65-75% of MLSS.

3.5.3 Food to Microorganisms ratio

Food to Microorganisms ratio (F/M ratio) is the ratio of organic load to total mass of MLSS per day. It is expressed as kg BOD / kg MLSS / day, and calculated using the following equation:

$$F/M = \frac{Q \times BOD}{MLSS \times V}$$

Q – flow rate of wastewater influent (m^3/day)

BOD – Biochemical Oxygen Demand of influent (mg/l)

MLSS – Mixed Liquor Suspended Solid in aeration tank (mg/l)

V – volume of aeration tank (m^3)

F/M is controlled by rate of activated sludge wasting. Low F/M ratio indicates that the microorganisms are under starvation condition. Activated sludge system under starvation will exhibit more efficiency.

3.5.4 Hydraulic retention time

Hydraulic Retention Time (HRT) is the average time that the influent spends in the aeration tank. High HRT means the organic waste is in contact with the microorganisms longer. Thus, it is allowed for a longer reaction time. HRT is calculated using the following equation:

$$HRT = V / Q$$

V – volume of aeration tank (m^3)
Q – rate of wastewater influent (m^3/hour)

3.5.5 Sludge age

Sludge Age is the mean residence time of microorganisms in the system. The importance of sludge age as a control parameter is that it determines the specific growth rate and the physiological state of the microorganisms in the system as well as the settling characteristics. The control of sludge age requires measurements of flow rate and biomass concentration in the aeration tank and settler underflow. Sludge age is an important variable during wastewater treatment plant operation as well as a design consideration. The maintenance of proper sludge age and MLSS in the aeration tank ensures the stability of the overall process (Cakici and Bayramoglu, 1995).

$$\text{Sludge age} = \frac{MLSS \times V}{SS_e \times Q_e + SS_w \times Q_w}$$

SS_e – suspended solids in wastewater effluent (mg/l)
Q_e – quantity of wastewater effluent (m^3/day)
SS_w – suspended solids in wasted sludge (mg/l)
Q_w – quantity of wasted sludge (m^3/day)

3.6 Factors affecting performance of activated sludge

3.6.1 Floc formation and filamentous growth

In healthy activated sludge system, flocculent biomass settles rapidly and compacts properly in the clarifier. The development of flocculent biomass depends on the microbial population. Improper balance of floc-forming and filamentous bacteria leads to settling problem. The stability of activated sludge flocs is important for the solid-liquid separation in wastewater treatment. Reduction of floc stability leads to deflocculation, which increases the turbidity of the effluent, and becomes difficult for sludge decantation leading to disposal problems. Turbidity and suspended solids reduce the quality of the effluent, sometimes to the point of becoming non-compliant for discharge to surface water sources. Filtration may be needed to further purify the effluent. Overgrowth of filamentous bacteria causes filamentous bulking, a condition in which the sludge settles and compacts poorly. This phenomenon reduces the sludge recyclability, and will eventually lead to biomass loss through the effluent. The stability of activated sludge floc follows the rules of colloidal chemistry. Changes in physico-chemical factors such as ionic strength can greatly influence floc stability. Changes in pH and contamination of detergent may also affect floc stability.

The settlability of activated sludge is quantified using Sludge Volume Index (SVI.) It is measured by adding 1 liter of mixed liquor to 1-liter graduated cylinder and allowing the sludge to settle for 30 minutes. At 30 minutes the sludge volume is taken and divided by the initial suspended solid concentration.

When floc stability problems arise, it is usually difficult to make clear conclusions regarding the cause of changes in floc stability. Gradual changes, such as seasonal temperature, occurring over several weeks can cause changes in the microbial community. Changes in influent characteristic or poisoning by toxic substances can quickly alter the physical characteristics of activated sludge flocs. Bacterial metabolism may alter physico-chemical properties of the bulk fluid such as changes in pH, or changes in exopolysaccharide production. Oxygen limitation can cause deflocculation of activated sludge flocs. It is suggested that oxygen limitation causes reduced production of exopolysaccharide by aerobic bacteria, and activity of anaerobic bacteria takes over under the absence of oxygen (Wilen et al., 1999).

Determination of the cause of changes in floc stability may not be timely enough to respond to the problem. When the operator is confronted with problems in sludge settling characteristics, there are a number of short term control measures that may improve the setting characteristics within a time span short enough to prevent wastewater treatment catastrophe. The use of inorganic coagulants, such as aluminum and ferric salts, is a common control procedure, and these chemicals are widely available. Organic polymers are also commercially available for improvement of sludge settling characteristics. These polymers neutralize the charges between colloidal particles and add interparticle bridging. These actions result in denser and larger flocs that settle better (Vanderhasselt and Verstraete, 1998). In an investigation of oil field brine treatment, addition of powdered activated sludge to the bioreactor improved the SVI and lowered the suspended solids concentration of the clarifier effluent. The positive effect of powdered activated sludge addition was explained by adsorption and immobilization of microorganism on powdered activated sludge surface, which helps prevent bacterial wash-out from the bioreactor at high hydraulic loading (Dalmacija et al., 1996).

3.6.2 Solid retention time

Solid Retention Time (SRT) is the average amount of time the solids spend in the system. This value is selected according to specific needs of each operation. In nutrient removal system, longer SRT is required to nitrify nitrogen compounds. For industrial wastewater containing less biodegradable organic matter, higher SRT is needed.

Proper development of sludge floc also depends on SRT. In the complex microbial community of activated sludge, each bacterial species grows at different rates, so proper SRT is needed for balanced growth of floc-forming and filamentous bacteria.

3.6.3 Mixed liquor suspended solids concentration

Successful operation can be operated at a wide range of MLSS concentrations. The performance really is not the effect of MLSS concentration, but rather, the mass of MLSS in the system. After the SRT is determined, the mass of

biomass becomes fixed. Practically, the MLSS concentration is in the range of 2000-5000 mg/l.

3.6.4 Dissolved oxygen

Oxygen is provided by mechanical aerator or compressed air system with bubble diffuser. The mechanical action of these systems also provide mixing and turbulence to keep the contents in aeration tank completely mixed. The mixing action of the system must be sufficient to keep the solids in suspension completely mixed, but not too severe to cause excessive shear force. Disintegration of sludge floc is caused by high shear.

Different Dissolved Oxygen (DO) concentrations promote various biochemical activities. Higher concentrations results in the growth of filamentous bacteria, and may lead to sludge bulking. DO of 2 mg/l is commonly recommended, but not in all cases. Plant conditions and wastewater influent characteristics influence the optimal DO concentration. Oxygen limitation and anaerobic conditions have long been linked to deflocculation of activated sludge. Under these conditions strictly aerobic microorganisms start dying, and overgrowth of anaerobic of facultatively anaerobic bacteria occurs. Flocculation studies suggested that longer the time activated sludge spends under anaerobic conditions results in more deflocculation. Anaerobic conditions suppress the growth of activated sludge eukaryotes, which consume free bacteria, resulting in higher turbidity of the treated effluent. To some extent, deflocculated activated sludge can reflocculate when subjected to aerobic conditions (Wilen et al., 2000). Contrary to deflocculation at low DO concentration, high DO concentrations produce activated sludge flocs with higher compactness and settability (Wilen and Balmer, 1999). In nutrient removal systems, inhibition of denitrification occurs at very low DO concentration (Oh and Silverstien, 1999). Sludge production is also affected by DO concentrations. It is well established that in any activated sludge process, the supply of DO plays an important role in limitation of increased loading rates of the wastewater treatment process. Comparisons between activated sludge system aeration suggest that when an activated sludge system is aerated with purified oxygen, sludge production is reduced by up to 54% as compared

to the conventional air system (Liu and Tay, 2001). To the operator, lowered sludge production translates to lower cost of excess sludge handling and disposal.

3.6.5 Nutrients

Optimal nutrient concentrations need to be achieved for balanced growth of biomass. At low nutrient conditions, outgrowth of filamentous bacteria over floc-forming bacteria will occur causing sludge bulking. Severe nutrient deficiency causes the production of exocellular slime. The resulting sludge settles slowly and poorly compact. In extreme cases, there is no liquid-solid separation in the clarifier.

3.6.6 Temperature

The operated temperature of activated sludge system can affect the microbial activity, oxygen transfer rate and sludge settling characteristics. All of which influence the overall efficiency of the treatment process (Barr et al., 1996). Generally, mesophillic microbial activities increase directly with the temperature up to a maximum around 40°C. Inactivation of mesophillic microorganisms may occur at temperature over 40°C. Several heat sources may alter the temperature of the biological wastewater treatment system. Heat gain may come from biological oxidation, heat of influent wastewater, solar input, and mechanical action from air transfer and mixing equipments. Although heat gain is not usually a concern, the problem can be lessened by proper planning and plant design. Heat loss from the system presents a greater concern due to loss of activities. Ambient temperature varies greatly according to geographic location and seasonal changes. Heat loss can be prevented through proper design and equipment selection. Overall heat loss of submerged aeration system is presumably less than that of an equivalent surface mechanical aeration system. In an experiment on activated sludge treatment of bleached kraft mill effluent, sudden small temperature changes had no detrimental effects on treatment performance. However larger temperature shocks did, more time was needed for the system to recover (Barr et al., 1996).

The effects of low temperature (7°C) caused COD removal and nitrification failure in an experiment with industrial discharge containing an azo dye. The effluent total suspended solids increase by three-fold, the oxygen uptake rated

decreased, and foaming increased (Martin Jr. et al., 2005). Changes in temperature affect the growth and composition of the bacterial community. The imbalance between floc forming and filamentous bacteria prevent the formation of well settling sludge flocs. For efficient and economical wastewater treatment, the settability of activated sludge is very important (Krisna and Loosdrecht, 1999).

Some wastewater treatment systems are maintained at 45-50°C to promote thermophilic population. From wastewater treatment point-of-view, the term "thermophilic" generally refers any process operating at 45°C or higher. In other definitions, it may mean microorganisms that proliferate at temperatures greater than 55-60°C. Advantages of thermophilic wastewater treatment are faster degradation rates, rapid inactivation of pathogenic microorganisms and lower sludge yield. Faster degradation rate is perhaps due to improved dissolution of organic matter at higher temperature (Barr et al., 1996). Higher degradation translate to lower retention time, which requires smaller treatment facility reducing the capital cost of construction. Lower sludge production reduces the cost of sludge disposal (Lapara and Alleman, 1999).

Thermophilic wastewater treatment does present some disadvantages including the higher expense of aeration, poor sludge flocculation characteristics, and foaming problems. Higher degradation rate and lower sludge yield in thermophilic wastewater treatment means that more substrate is used as energy source, and converted to CO_2 instead of biomass. The oxygen requirement for thermopilic system is higher than conventional system, and thus more costly. Thermophilic sludge almost always exhibit poor settling characteristics as an effect of dispersed growth of microorganisms. Therefore, biomass separation from the treated wastewater becomes difficult requiring addition of coagulant (Lapara and Alleman, 1999; Suvilampi et al., 2005).

3.7 Sequencing batch reactor

Sequencing Batch Reactor(SBR) is a type of activated sludge reactor. Unlike conventional activated sludge system, SBR does not contain a settling unit. A batch of wastewater is added to SBR unit, treated to remove undesirable compounds,

and discharged. During the process, equalization, aeration, and clarification all take place in a single unit. Success in using SBR is widespread in both municipal and industrial wastewater treatment.

The unit processes in conventional activated sludge system and SBR are the same. The main differences between these two technologies are that in SBR equalization, biological treatment and clarification occur in a single tank using timed and controlled sequences. In conventional activated sludge system, wastewater continuously flow through multiple treatment units each performing a specific process.

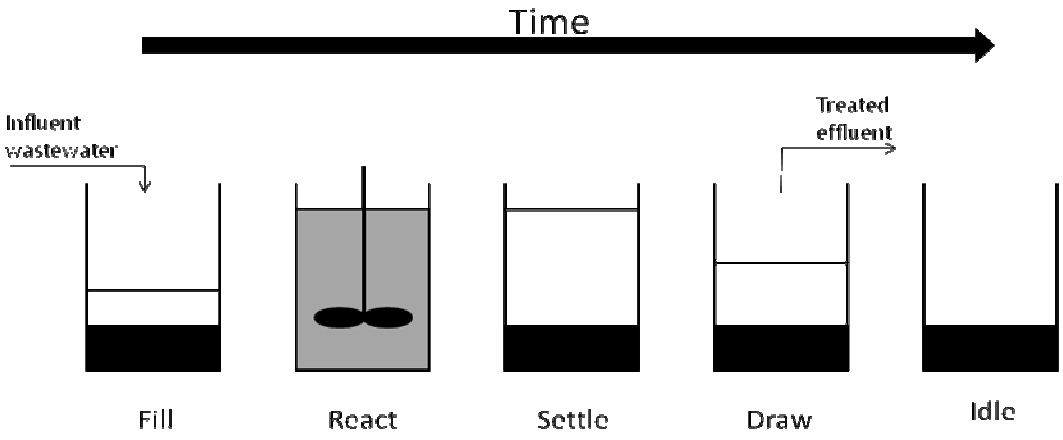

Fig 3-2 Sequence of operation in one SBR cycle

In a typical wastewater treatment plant using SBR, wastewater passes through screens for removal of solids and suspended matter. Then, the wastewater enters the SBR reactor containing activated biomass. This biomass is already acclimatized to the components of wastewater during the previous cycles. When the reactor is full, biological treatment occurs through aeration and mixing. During the reaction, SBR performs like the aeration unit of conventional activated sludge system, but without continuous influent and effluent flow. When the reaction is complete, aeration and mixing are stopped. The sludge is allowed to settle, and the treated supernatant is removed. Excess biomass can be wasted any time during the cycle to maintain nearly constant biomass to substrate ratio from cycle to cycle. In a continuous flow system, the biomass to substrate ratio is controlled by adjusting return sludge flow rate to match continually changing influent flow rates, characteristics, and

sludge concentration at the bottom of the settling tank. SBR is a much less sophisticated system. In some cases after SBR, the batch of wastewater is filtered to remove solids or disinfected.

3.8 Bioaugmentation

Effective operation of a biological wastewater treatment plant depends highly upon the microorganisms within it. Selection pressure provided by the environmental conditions of the system dictates the microorganisms that are present. Only those able to survive would persist in the system. The existing selection pressures are determined by the influent characteristics, the mode of operation and design of the treatment system.

For example, a toxic compound in the influent could suppress the growth of certain microorganisms, or the lack of certain nutrient may favor the growth of one group of microorganisms over others. In the mode of operation, an activated sludge system may suffer insufficient ammonia removal if the mode of operation requires higher sludge wastage. The nitrifying bacteria responsible for ammonia removal, which are slow growing, are lost from the system. Lastly, the design of the wastewater treatment can have great selection pressures on the existing microorganisms. The environmental conditions of an activated sludge system are very different from a trickling filter. The composition of the microbial population between the two treatment systems would also differ as the result of the design.

It is well known that under selection pressure bacterial communities are able to adjust their composition correspondingly. Despite this ability, many microbial communities cannot adjust in time to prevent plant's failure to meet the effluent standard. These failures may result in poor COD removal, poor sludge settlement, etc. In order to cope with the problems, plant operators have several options:

1. Modification of influent characteristics such as pH, nutrient, etc.
2. Modification of mode of operation such as aeration, sludge wastage, etc.
3. Changing plant design, reorganizing treatment units, or altering volumes
4. Alteration of the microbial community

a. Addition of sludge from another plant
b. Bioaugmentation

The most common and immediate actions in response to treatment plant failure involve modification of influent characteristics and modification of the mode of operation. These approaches are quick, but do not always solve the problems and increase the operational cost. Changing plant design can be costly, and requires additional space, which may not be available due to density of the area.

Bioaugmentation presents an attractive alternative to the other options. The addition of specialized bacterial strains was originally intended to solve problems such as shock loading in wastewater treatment plant. Results from shock loading may hamper the operation of a biological system. Activated sludge microorganisms may die.

Further studies on bioaugmentation examined many benefits of addition of indigenous, wild-type or genetically modified microorganisms to wastewater treatment bioreactors. Bioaugmentation facilitates the development of specific functional population in the microbial community. Progress in bioaugmentation studies has moved on from focusing on maintenance of treatment plant process stability upon receiving variable influent characteristics. The addition of specialized strains has become a powerful tool in improving several aspects of wastewater treatment system. Bacterial strains have been added to activated sludge in order to remove recalcitrant or undesired compounds. In some cases, efficient flocculation was achieved as a result from bioaugmentation of floc-forming bacteria. Microorganisms obtained from saline environments accelerated the removal of pollutants of salty wastewater. In general, bioaugmentation provides these benefits to the wastewater treatment system:

1. Higher growth rate
2. Increase tolerance to toxicity and environmental conditions
3. Increase the diversity of bacteria available for natural selection
4. Increase the number of specific population in order to respond to specific substrate

3.9 Treatment of saline wastewater

Saline wastewater is generated by various industries including agro-food, petroleum and leather industries. In this type of wastewater, high concentration of salt is usually accompanied by high organic content. The release of this wastewater to natural surface water source would adversely affect the aquatic life, usability in agriculture and potability for human consumption. Treatment of such wastewater can be broadly divided into two categories: physico-chemical treatment and biological treatment.

3.9.1 Physico-chemical treatment of saline wastewater

Physico-chemical treatment can remove salt as well as organic matter from wastewater. Physico-chemical treatment techniques such as evaporation, coagulation-flocculation, and membrane techniques have previously been investigated.

3.9.1.1 Thermal techniques

Solar evaporation has been used for many centuries as a desalination process for production of freshwater. Recently, the use of solar evaporation pans as a treatment method for saline wastewater was investigated. This technique has low operating cost and low energy requirement, but requires very large area of land. Therefore, it is not suitable for densely populated industrial estates, where land cost is high. The salt recovered from this process is not reusable due to high impurity (Lefebvre et al., 2005).

3.9.1.2 Coagulation-flocculation

Coagulation-flocculation can be used as a pretreatment for saline effluents to remove the colloidal COD, but is not effective for salt removal. Aluminum sulfate and ferric chloride effectively clarify tannery wastewater. The reduction in COD and SS could lead to lower cost of wastewater disposal (Song et al., 2003). In another experiment, 90% COD removal from food industry effluent by coagulation-flocculation method using aluminum sulfate was reported (Ellouze et al., 2003).

3.9.1.3 Membrane techniques

Interest in membrane processes in wastewater treatment is continuously growing. Ultrafiltration can be used to remove suspended solids and

colloidal COD from saline waste stream. The use of ultrafiltration enables removal of petroleum derivatives from oily wastewater. The basis of membrane technique is the transfer of selected molecules under the influence of concentration or pressure gradient. Ultrafiltration has been successfully used to concentrate and recycle protein in wastewater from a seafood processor as well as reduction in effluent COD (Afonso and Borquez, 2002). Membrane distillation has also been applied to saline wastewater. In this process, the volatile components of the feed evaporate through the membrane. When membrane distillation is used to treat saline wastewater, the process separates the feed into two parts: pure water and the concentrate containing the pollutants in the feed. This process enables the concentration of solutions up to the supersaturated state followed by crystallization of salts (Gryta et al., 2006).

3.9.2 Biological treatment of saline wastewater

Saline wastewater is rich in both organic matter and total dissolved solids. It is difficult to treat using conventional biological wastewater treatment process. Salt is known to have adverse affects on biological wastewater treatment system. It has been demonstrated in many studies that COD removal rate of biological wastewater treatment systems decrease with increasing salt concentration. Plasmolysis of the organisms resulting in loss of metabolic activity or release of cellular material at high salt concentration is considered to be the reason for the decrease in COD removal efficiency. Other than decrease in COD removal efficiency, high salt concentrations also adversely affect nitrogen and phosphorus removal from wastewater. Increasing salt concentration causes poor sludge settling characteristics resulting in high effluent suspended solids and turbidity, and complicates the operation of the wastewater treatment plant (Uygur et al., 2004; Campos et al., 2001). To migitate the effects high salt concentration on biological wastewater treatment system, a common and simple solution is dilution with freshwater to lower the salt concentration. This process requires a large amount of water, and larger aeration tank, which leads to higher operating cost (Kubo et al., 2000).

3.9.3 Acclimatization of microorganism to saline wastewater

Despite the detrimental effect that salt has on biological wastewater treatment system, acclimatization of activated sludge is possible to a certain degree. Acclimatization is a process where non-salt-adapted microorganisms are exposed to gradually increasing salt concentration in order to perform satisfactory treatment of wastewater at a given salt concentration. The success of the adaptation process depends on several factors such as the type of microorganisms and the rate of increase of salt concentration of acclimatization (Oren et al., 1992). Some reports indicate that rapid shifts in salt concentration have adverse effects on the performance of the wastewater treatment system. As a result, temporary drop in treatment performance have been reported especially when shifts in salt concentration is accompanied by high organic loading. The explanation for this response is that plasmolysis of cell and release of cellular material to the bulk fluid result in an increase of soluble COD (Kincannon and Guady, 1968; Uygur et al, 2004; Campos et al., 2001). Although acclimatization of activated sludge is possible, there are limitations and disadvantages to this method of acclimatization. Satisfactory result is limited to less than 5% salt (Wong, 1992; Kargi and Dincer, 1997; Dincer and Kargi 2001). This adaptation to higher salt concentration is quickly lost if the influent salt concentration drops, which is a likely scenario due to the highly variable characteristics of industrial wastewater (Kincannon and Gaudy, 1968). Major shortcomings in biological treatment of saline wastewater can be summarized into four categories (Kargi and Dincer, 1996):

1. Limited extent of adaptation: conventional activated sludge cannot be used to treat saline wastewaters with salt concentration greater than 3-5% effectively. Salt-adapted activated sludge easily loses the acquired characteristic when subjected to salt-free influent.
2. Sensitivity to changes in ionic strength: shifts in salt concentration from 0.5-2% may cause significant disruptions in system performance. Even with salt-adapted microorganisms, satisfactory performance requires constant ionic composition. Rapid changes in salt concentration causes more adverse affects than gradual change. Adjustment to constant salt concentration is necessary before treatment of saline wastewater.

3. Reduction in degradation kinetics: biodegradation of organic pollutants decreases as salt concentration increases. It is usually required that saline wastewater be treated at lower F/M ratio.
4. High effluent suspended solids concentration: changes in ionic strength alters physico-chemical properties of activated sludge flocs, which may lead to decrease floc stability resulting in high effluent turbidity and suspended solids.

3.9.4 Bioaugmentation in treatement of saline wastewater

Many attempts to adapt conventional microorganisms to treat saline wastewater have shown its limitations and advantages. The use of halophilic inoculums has been proposed as a way to improve treatment efficiency of saline wastewater. In a Japanese investigation, salt-tolerant microorganisms *Staphylococcus* sp. and *Bacillus cereus* were isolated using neutralized high salinity wastewater from pickled plum manufacturer. Satisfactory treatment of this wastewater could not be achieved with conventional activated sludge without dilution (20 to 50 times) of the waste stream with freshwater. In a batch experiment, co-culture of the two bacteria achieved about 90% COD removal of undiluted wastewater (Kubo et al., 2000). Exploring a similar concept, a euryhaline *Halobacter* strain significantly improved the performance of activated sludge (Kargi and Dincer , 1996). The addition of *Halobacter halobium* to activated sludge in an aerated rotating biodisc contactor resulted in higher COD removal efficiencies from a synthetic wastewater especially at salt concentration higher than 3% (Kargi and Dincer, 1998). The addition of *H. halobium* to activated sludge led to improved reactor performance, particularly in the higher salt concentrations. Using the same *H. halobium,* saline effluent from pickling industry was successfully treated by enrichment of activated sludge with the bacterium. COD removal exceeded 95% (Kargi et al., 2000). Applying the same technique, a highly saline wastewater generated by pickled plum industry, which was previously unable to treat without dilution, was successfully treated. Growth of microorganism was strongly inhibited by its high salt concentration. Inoculation of halo-tolerant *Staphylococcus* sp. and *B. cereus* to a sequencing batch reactor achieved

COD removal efficiency of 90% from the highly saline wastewater without any dilution (Kubo, et al., 2001). Another effective strategy involves inoculation of a mixture of halophilic microorganisms from diverse saline environment, such as estuarine or marine sediments. Introduction of such community will add microorganisms that are able to withstand high salt concentration and treat the wastewater at the same time. This technique was employed to treat a saline tannery soak liquor using SBR. Microorganisms were harvested from various salt-rich environments. The reactor was inoculated four times to accelerate the establishment of halophilic population and increase the microbial diversity (Lefebvre et al., 2005).

CHAPTER IV
MATERIALS AND METHODS

4.1 Materials

4.1.1 Chemicals

The list of chemicals used in this study is shown in Table 4-1.

Table 4-1 List of chemicals used in this study

Chemical name	Chemical formula	Source
Ammonium chloride	NH_4Cl	Sigma
Bacto agar	-	Difco
Bacto peptone	-	Difco
Beef extract	-	Difco
Calcium chloride	$CaCl_2$	Merck
Disodium hydrogen phosphate	Na_2HPO_4	Sigma
Ferric chloride	$FeCl_3 \cdot 6H_2O$	Sigma
Hydrochloric acid	HCl	Merck
Magnesium chloride	$MgCl_2 \cdot 6H_2O$	Merck
Sodium chloride	NaCl	Sigma
Sodium hydroxide	NaOH	Merck
Sucrose	$C_{12}H_{22}O_{11}$	Mitrphol
Yeast extract	-	Difco

4.1.2 Instruments

The instruments used in this study were as follows:

1. Autoclave: Isuzu, model 20-5030/5040
2. Electronic balance: Satorius model BA310S
3. UV-VIS Spectrophotometer: Hach DR-5000
4. Hot plate, magnetic stirrer: Yhana model HMS-10
5. Incubator: Memmert
6. Laminar flow hood: Issco model Bvt 124
7. pH meter: Hanna instruments (pH211) Microprocessor pH meter
8. Vacuum pump: Millipor model DOA-V130-BN
9. Vortex: Genie model K-550-GE

4.2 Media

4.2.1 Nutrient Broth

Beef extract	3 g
Bacto peptone	5 g
Yeast extract	5 g

Deionized water added t up to 1,000 ml

The medium was sterilized by autoclaving at 121°C, 15 psi for 15 minutes

4.2.2 Nutrient Agar

Beef extract	3 g
Bacto peptone	5 g
Yeast extract	5 g
Bacto agar	15 g

Deionized water added up to 1,000 ml.

The medium was sterilized by autoclaving at 121°C, 15 psi for 15 minutes

4.2.3 Modified Artificial Sewage

Sucrose	5 g
Na_2HPO_4	4.64 g
NH_4Cl	0.77 g
$MgCl_2 \cdot 6H_2O$	0.2 g
$FeCl_3 \cdot 6H_2O$	0.1 g
NaCl	0.02 g
$CaCl_2$	0.01 g

Deionized water was added up to 1,000 ml.

Modified from formula given by Wantanabe et al., 1996.

4.3 Wastewater

Wastewater used in all experiments was collected from an olefins processing plant. The source of this wastewater was a mixture of neutralized spent caustics and dilution steam from hydrocarbon processing. Oil was removed from the wastewater by an induced gas floatation unit. Basic physico-chemical characteristics of the wastewater are shown in Table 4-2.

Table 4-2 Physico-chemical characteristics of collected wastewater

Parameter	Value
COD	1500-3000 mg/l
TDS	10000-14000 mg/l
pH	~8.0

Before entering the activated sludge treatment system, the wastewater arrived in the equalization basin, where it was equalized and diluted by approximately one-fold with raw water. Without dilution, full strength wastewater would upset the wastewater treatment plant operation resulting in poor effluent quality, sludge bulking, and in worse cases, sludge washout leading to mandatory shut down of upstream processes and loss of plant's revenue.

4.4 Activated sludge

Activated sludge used in all experiments was obtained from the aeration basin of a wastewater treatment plant receiving wastewater from an olefins processor, the same wastewater in section 4.3. Design of the wastewater treatment plant was based on activated sludge system with sludge recycling system. Aeration and turbulence was provided by impellers and high pressure air injection. Before entering the system, wastewater was diluted by approximately 1 fold to reduce the concentration in the equalization basin. Physico-chemical properties of the aeration basin under normal operation are listed in Table 4-3.

Table 4-3 Physico-chemical properties of the aeration basin under normal operation

Parameter	Value
$COD_{influent}$	400-900 mg/l
TDS	4000-6000 mg/l
MLSS	4000-5000 mg/l
pH	~8.0

4.5 Screening and isolation of salt-tolerant microorganisms

4.5.1 Screening of microorganisms from various sources

Water samples were taken from equalization basin, aeration basin and return sludge of the wastewater treatment plant, and sediment samples were taken from estuarine sediments from Khao Yee San Temple, Samutsakorn province. 5 ml of each water sample and 5 g of each sediment sample were transferred to NB and incubated on a rotary shaker for 3 days at 200 rpm. After 3 days, 5 ml of culture medium was transferred to NB + 0.35M NaCl at pH 8.0 containing 5% autoclaved wastewater, and incubated on a rotary shaker for 3 days at 200 rpm. After incubation, 0.1 ml of culture medium was serially diluted and spread on NA plates and incubated at 30°C for 3-7 days.

4.5.2 Isolation of microorganisms

After 3-7 days of incubation, the NA plates were observed under a stereomicroscope. Single colonies of ten most dominant microorganisms were selected and transferred to NB + 0.35M NaCl at pH 8.0 containing 5% autoclaved wastewater. The cultures were incubated on a rotary shaker at 200 rpm at room temperature.

4.5.3 Testing activity of microorganisms in wastewater

Microorganisms were cultivated in NB + 0.35M NaCl at pH 8.0 containing 5% autoclaved wastewater for 48 hours. After 48 hours, 15 ml of culture medium was added to 285 ml wastewater and incubated for 3 days on a rotary shaker at 200 rpm. At the end of three days, samples were taken and analyzed for COD removal performance. Each sample was filtered through 0.45 μm membrane filter to remove bacterial cells before analysis.

4.6 Bioaugmentation of activated sludge

4.6.1 Preparation of selected bacteria stock

Stocks of selected bacteria strains were made by inoculating one loop of selected bacteria into 25 ml NB and incubated overnight at 30°C on a rotary shaker at 150 rpm. Then, 10 ml of bacterial cultures were inoculated into 500 ml NB, and incubated at 30°C on a rotary shaker at 150 rpm for 48 hours. After 48 hours, the bacterial cultures were kept refrigerated at 4°C for further use.

4.6.2 Cultivation of selected bacteria

The selected bacteria were cultivated by inoculating 50 ml stock culture into 1000 ml of modified artificial sewage. Aeration was provided for 48 hours by bubbling through 5 mm silicon tubing with no diffuser. After 48 hours, the bacterial cultures were ready for bioaugmentation.

4.6.3 Reactor set-up

All following experiments were performed using sequencing batch reactors. The laboratory scale sequencing batch reactors consisted of an aeration tank with 800 ml working volume. At the beginning of the experiment, 300 ml of activated sludge was added to the reactor, and the volume was made up to 800 ml by addition of 500 ml of wastewater.

4.6.3.1 Sequencing batch reactor operations

All sequencing batch reactors were run with one cycle per day as shown in Fig 4-1. Each cycle was composed of four different stages: dump filling of 500 ml, an aerobic phase of 20 hours for bioconversion of pollutants, settling for 2 hours, and 2 hours for drawing 500 ml of supernatant.

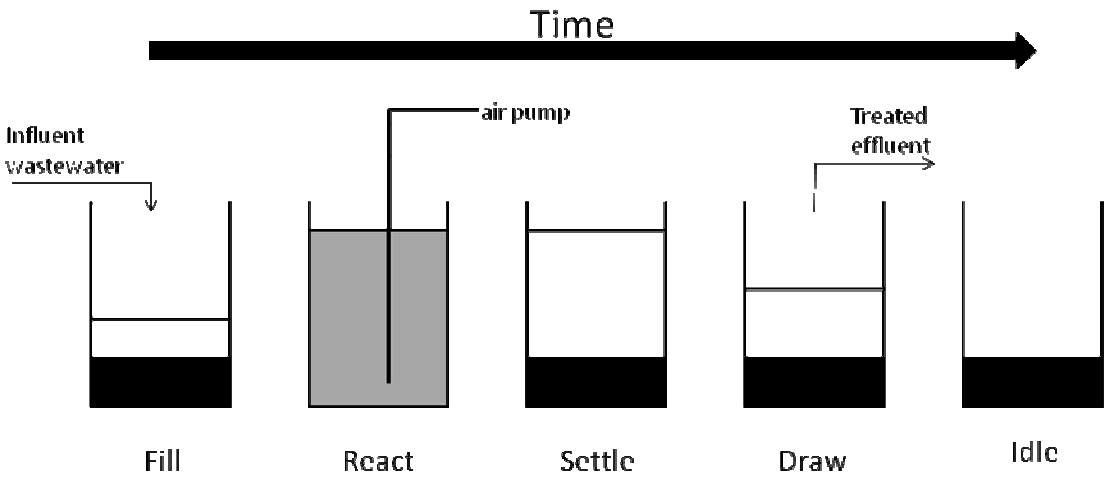

Fig 4-1 One SBR cycle

At the beginning of each cycle, 500 ml of wastewater was prepared and added to decanted activated sludge. Then, aeration was turned on for 20 hours. Air was supplied through 5 mm silicon tubing with no diffuser. A sample for initial COD concentration was collected after the air has been turned on for five minutes.

After aeration for 20 hours, the air was turned off to allow sedimentation of reactor's solids for 2 hours. After clear separation of the content, the supernatant was removed via a siphon. A sample of supernatant was collected for

analysis of final COD concentration. 500 ml of wastewater was added to the remainder of the reactor to begin a new cycle.

4.6.3.2 Preparation of bioaugmented reactor

For preparation of bioaugmented reactors, 300 ml of activated sludge was added to the reactor as shown in Fig 4-2. Equal amount of selected bacterial cultures were combined to form a total volume of 500 ml and added to the reactor containing activated sludge. Aeration was provided overnight, and stopped to allow sedimentation of the biomass. The supernatant was removed. The remaining sludge was bioaugmented and ready for further experimentation.

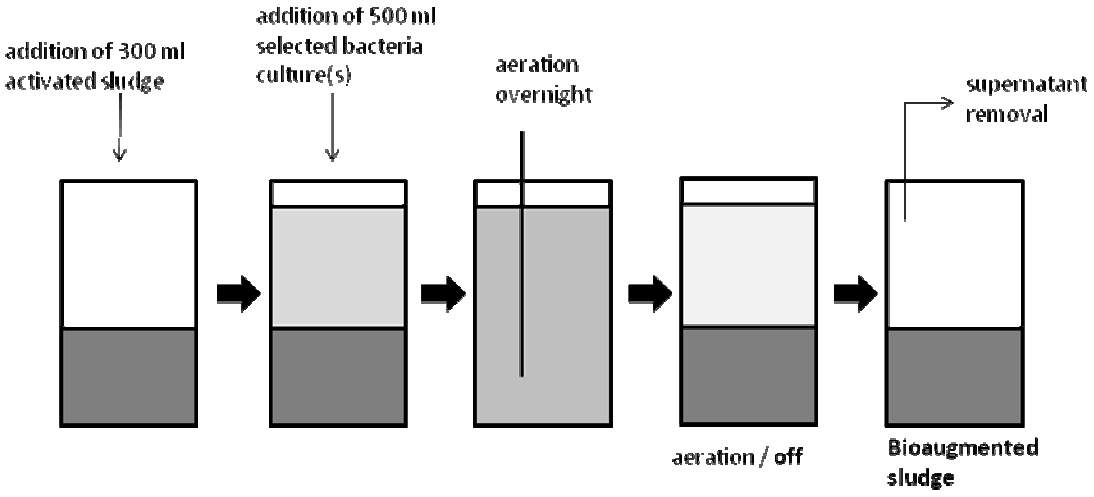

Fig 4-2 Bioaugmentation of SBR

4.6.4 Comparison of non-bioaugmented and bioaugmented reactors

Two sequencing batch reactors were set up. The first was non-bioaugmented, and prepared using the method described in section 4.6.3. The other was bioaugmented with all three selected bacterial strains, and prepared using the methods described in section 4.6.3.2. Wastewater was added to the reactors with step-wise increase in concentration for each cycle. The performances of the two reactors were compared. The proportions of wastewater and raw water added to the reactors are shown in Table 4-4 and Fig 4-3.

Table 4-4 Step-wise increase in wastewater concentration by reduction of dilution

Cycle	$V_{wastewater}$*	$V_{raw\ water}$*	% Dilution
1	167	333	66.7
2	333	167	33.3
3	415	85	16.7
4	500	0	0
5	500	0	0
6	500	0	0
7	500	0	0

*ml

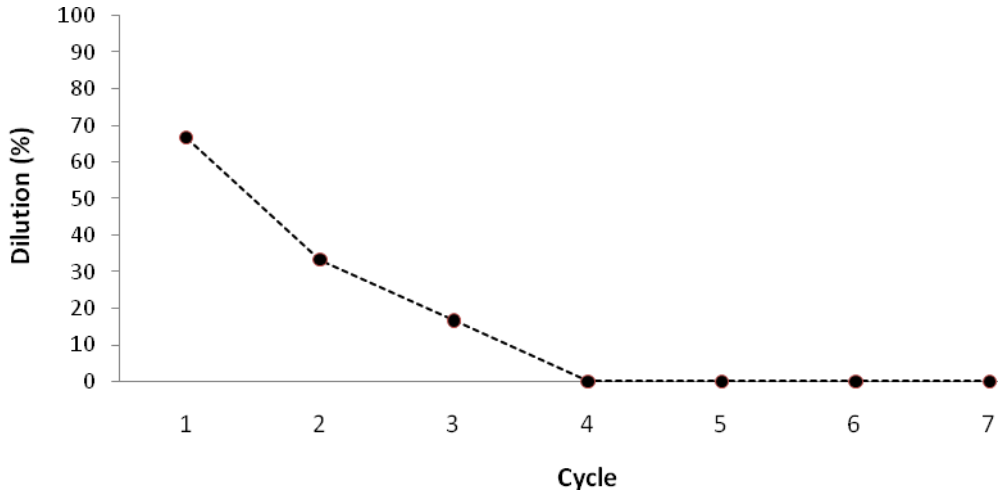

Fig 4-3 Step-wise increase in concentration by reduction of dilution

4.6.5 Effect of shock loading

Two sequencing batch reactors were set up. The first was non-bioaugmented, and prepared using the method described in section 4.6.3. The other was bioaugmented with all three selected bacterial strains, and prepared using the methods described in section 4.6.3.2. Full strength wastewater was added to the reactors for every cycle. The performances of the two reactors were compared.

4.6.6 Effect of influent pH on bioaugmented reactor

4.6.6.1 Influent pH adjustment

At the beginning of each cycle, 2,000 ml of influent was prepared to the specified concentration by dilution with raw water. Then, it was divided into 4 portions of 500 ml each. The pH of each portion was adjusted to 7.5, 8.0, 8.5 and 9.0.

4.6.6.2 Effect of influent pH on bioaugmented reactors

Four sequencing batch reactors were set up according to the method described in section 4.6.3.2. Each reactor was fed with influent of different pH prepared in section 4.6.6.1 for seven cycles. The performances of bioaugmented reactors at different influent pH were compared.

4.6.7 Bioaugmentation with individual selected bacterial strain

Each bacterial strain was cultivated according to method described in section 4.6.2. Then, three sequencing batch reactors were set up by adding 300 ml of activated sludge to the reactor. 500 ml of individual bacterial culture were added to their respective reactor. Aeration was provided overnight, and stopped to allow sedimentation of the biomass. The supernatant was removed. Wastewater was added to the reactors with step-wise increase in concentration for each cycle. The performances of reactors bioaugmented with individual selected bacterial strains were compared.

4.7 Lab-scale simulation of bioaugmentation

4.7.1 Preparation of adapted seed sludge

Each selected bacterial strain was cultivated according to method described in section 4.6.2. After cultivation was completed, equal volumes of each bacterial culture were combined to form a total volume of 1,000 ml. A 2 l reactor was prepared by the addition of 600 ml activated sludge, and the combined selected bacterial culture was added. Aeration was provided overnight, and stopped to allow

sedimentation of the biomass. The supernatant was removed via a siphon. 200 ml of wastewater was added to the remaining biomass, and aeration was turned on. For the next 6 days, 200 ml of wastewater was added to the reactor every day to gradually increase the concentrations. After that, aeration was stopped to allow sedimentation of biomass. The supernatant was removed, and wastewater was added making up to working volume of the reactor (1,600 ml). Aeration was provided overnight, and stopped to allow sedimentation of the biomass. The supernatant was removed, and the remaining sludge, which was adapted to the wastewater, was preserved for further experimentation. The steps in adapted seed sludge production is shown in Fig 4-4.

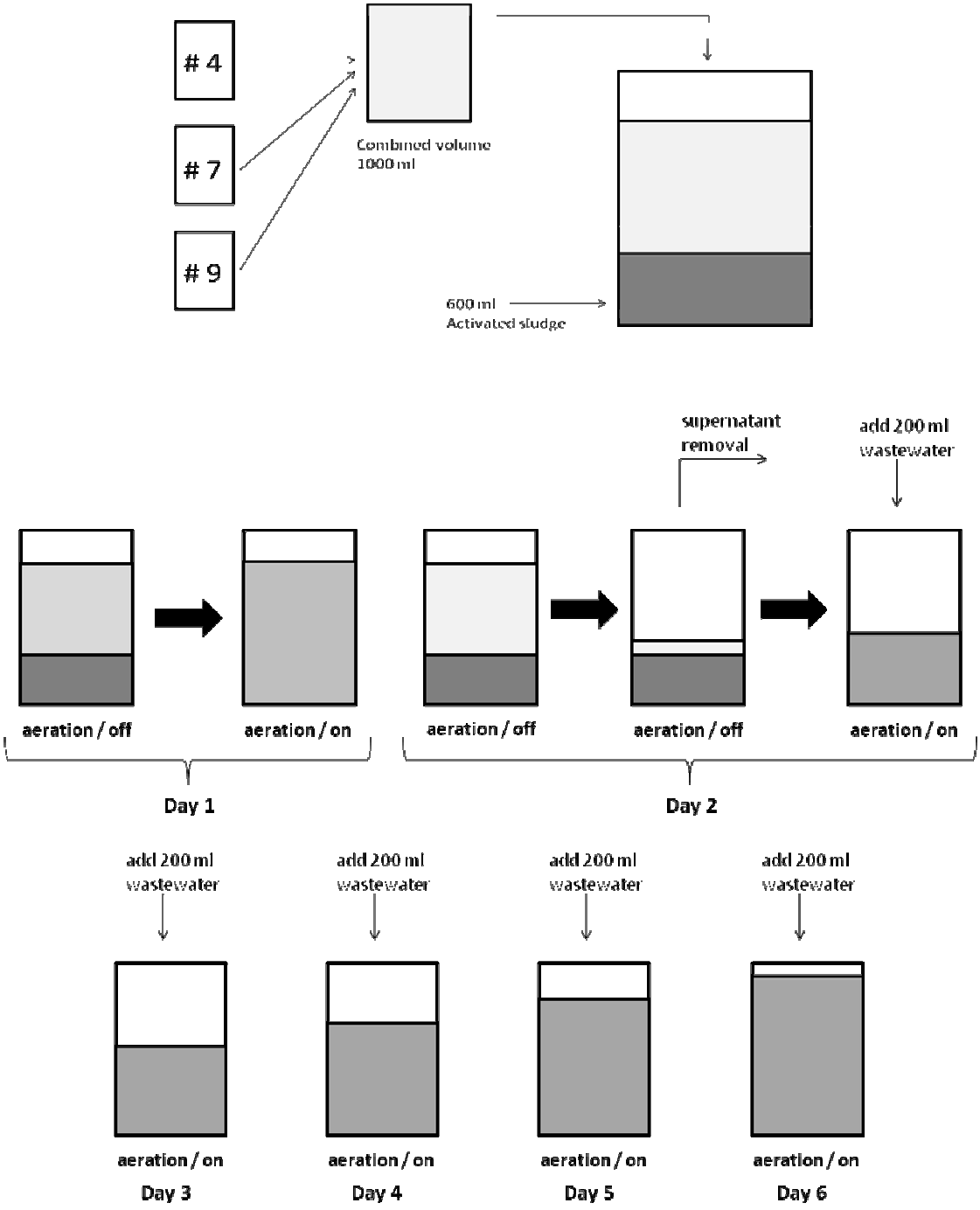

Fig 4-4 Production of adapted seed sludge

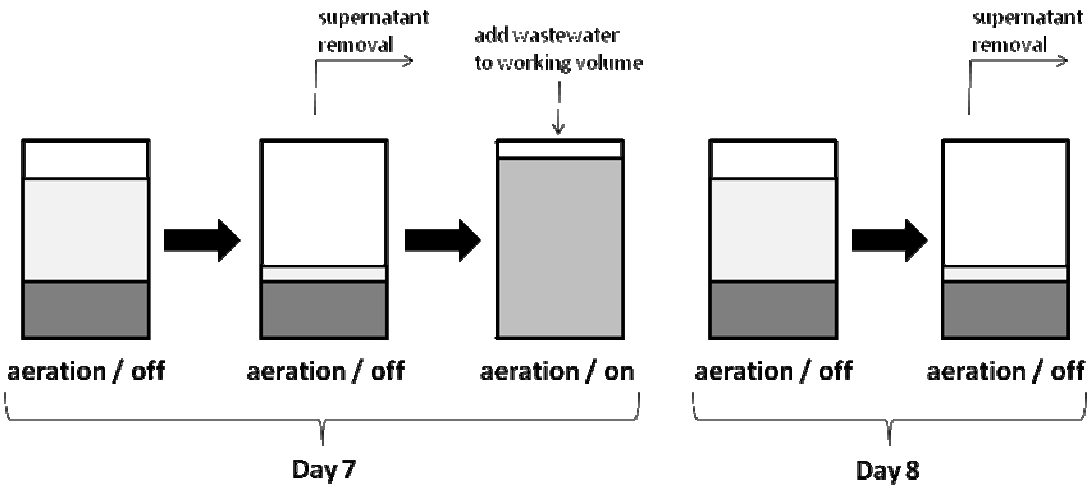

Fig 4-4 Production of adapted seed sludge

4.7.2 Bioaugmentation with adapted seed sludge

Two sequencing batch reactors were set up by adding 300 ml of activated sludge to the reactor. The first reactor served as the control. The second was bioaugmented with adapted seed sludge. At the beginning of each cycle, 5% of the reactor's content was removed, and replaced with equal volume of adapted seed sludge. Both reactors were fed with an influent with similar concentration of the actual wastewater treatment plant, which was the wastewater diluted by one-fold with raw water. The reactors were operated in parallel for 14 cycles, and the COD removal performances were compared.

4.7.3 Simulation of possible scale-up scenario

Three scale-up scenarios were designed in order to determine implementation feasibility at actual industrial scale without severe reduction in COD removal efficiency, which would cause a catastrophic event leading to shut down of many plant's operations and major loss in revenue. Engineering and geometric considerations at the actual scale were taken into the design of these lab-tested scenarios.

4.7.3.1 Scenario 1

The feeding regime for this scenario was to gradually increase the concentration of wastewater influent. For the cycles 1-3, the wastewater was diluted by one-fold with raw water, and added to the reactor. For each successive cycle, the volume of raw water used decreased by 5%. For reactor set up, on the first cycle, 300 ml of activated sludge was added to the reactor. The prepared wastewater influent was added to the reactor, and aeration was provided to mix the content of the reactor. After mixing, 5% of the reactor's content was removed, and replaced with the same volume of adapted seed sludge. The same procedure was performed at the beginning of each cycle in order to accumulate the adapted seed sludge as the influent wastewater concentration increased in strength.

4.7.3.2 Scenario 2

The feeding regime for this scenario was to increase the concentration of wastewater influent more rapidly than Scenario 1. For the cycles 1-3, the wastewater was diluted by one-fold with raw water, and added to the reactor. For each successive cycle, the volume of raw water used to dilute the wastewater was reduced by 10%. Reactor set up and bioaugmentation regime followed the same procedure as Scenario 1. 300 ml of activated sludge was added to the reactor. The prepared wastewater influent was added to the reactor, and aeration was provided to mix the content of the reactor. After mixing, 5% of the reactor's content was removed, and replaced with the same volume of adapted seed sludge. The same procedure was performed at the beginning of each cycle in order to accumulate the adapted seed sludge as the influent wastewater concentration increased in strength.

4.7.3.3 Scenario 3

The feeding regime of this scenario followed the same procedure as Scenario 1. For the first cycle, the wastewater was diluted by one-fold with raw water, and added to the reactor. For each successive cycle, the volume of raw water used decreased by 5%. For reactor set-up, on the first cycle, 300 ml of activated sludge was added to the reactor. The prepared wastewater influent was added to the reactor, and aeration was provided to mix the content of the reactor. After mixing, 30% of the reactor's content was removed, and replaced with the same volume of adapted seed sludge. For the beginning of each successive cycle, 5% of

reactor's content was removed and replaced with the same volume of adapted seed sludge. This bioaugmentation regime allowed more rapid accumulation of adapted seed sludge as the influent wastewater concentration increased in strength.

4.8 Analytical methods

4.8.1 Determination of Total Dissolved Solids (TDS)

An evaporation dish was weighed to the nearest 0.1 mg. Sample was filtered through 1.2 µm glassfiber microfilter to remove suspended solids. 25 ml of filtrate was added to the evaporation dish, and put in a hot air oven at 105°C until all the water evaporated. Then, the evaporation dish was cooled in a desiccator overnight. The weight of the evaporation dish and remaining solid was measured and recorded for calculation.

$$\text{mg TDS / L} = \frac{(W_a - W_b) \times 1000}{V_s}$$

W_a – dry weight of evaporation dish and remaining solids, mg
W_b – dry weight of evaporation dish
V_s – sample volume

4.8.2 Determination of Mixed Liquor Suspended Solids (MLSS)

A standard glassfiber microfilter was weighed to the nearest 0.1 mg. 25 ml of well-mixed activated sludge sample was filtered through the filter. The filter and solids that it retained was dried in a hot air oven at 105°C for 2 hours, and cooled in a desiccator. The weight of dried filter and retained solid was measured and recorded for calculation.

$$\text{mg MLSS / L} = \frac{(W_a - W_b) \times 1000}{V_s}$$

W_a – dry weight of filter and retained solids, mg

W_b – dry weight of filter, mg

V_s – sample volume, ml

4.8.3 Determination of Chemical Oxygen Demand (COD)

All COD measurements were performed with USEPA compliant system from Hach Company. The system was based on the Dichromate Chemical Oxygen Demand test, which measured the oxygen equivalent of the organic matter oxidizable by potassium dichromate in a 50% sulfuric acid solution. After oxidation was complete, the amount of dichromate consumed is determined colorimetrically with Hach's DR 5000 UV-VIS spectrophotometer.

2.0 ml of sample was homogenized and added to COD digestion reagent vial of appropriate range. The vial was capped and inverted several times to mix the content thoroughly. Then, the vial was inserted into a preheated COD reactor and heated for 2 hours. After 2 hours, the reactor was turned off and allowed to cool. When cooled, colorimetric measurement for COD was taken with Hach's DR 5000 UV-VIS spectrophotometer.

CHAPTER V
RESULTS

5.1 Screening of microorganisms

Microorganisms from wastewater treatment system and estuarine sediments were cultured in nutrient broth containing 0.35 M NaCl and wastewater. Then, the cultures were spread on nutrient agar. After 3-7 days of incubation, the microbial colonies were visually observed. Ten dominant microorganisms were selected and culture in NB + 0.35M NaCl. Then, the pure cultures were spread on nutrient agar. The colony morphology of each microorganism grown on nutrient agar was observed under a stereomicroscope. The characteristics of each microbe are described in Table 5-1. The ten selected microorganisms were preserved for further experimentation.

Table 5-1 Colony morphology of isolated microorganisms

Strain	Colony morphology
# 1	Pink, circular shape, dull wrinkled surface
# 2	Light yellow, circular shape, smooth surface, entire margin
# 3	Creamy white, circular shape, dull wrinkled surface, undulate margin
# 4	Creamy white, circular shape, wrinkled surface
# 5	Yellow, circular shape, shiny surface, smooth clear margin
# 6	Creamy white, circular, clear margin, milky area surrounding colony
# 7	Pink, circular shape, smooth shiny surface, smooth margin
# 8	Pink, circular shape, shiny surface, clear smooth margin
# 9	Dark yellow, circular shape, shiny surface, smooth margin
# 10	White, circular shape, smooth shiny surface, smooth margin

5.2 Selection of microorganisms

After isolation and purification, each microorganism was cultivated, and tested by inoculation into wastewater. Samples were tested after 3 days of incubation for analysis of COD. The samples were filtered through 0.45 µm membrane filter before analysis. The COD removal performances of each bacterial strain are shown in Fig 5-1. Bacterial strains # 9, # 7 and # 4 exhibited the three highest percentages of COD removal. Bacterial strains # 9 and #7 were identified as *Brevibacterium luteolum* and *Brevundimonus diminuta*, respectively. Strain # 4 was not identified.

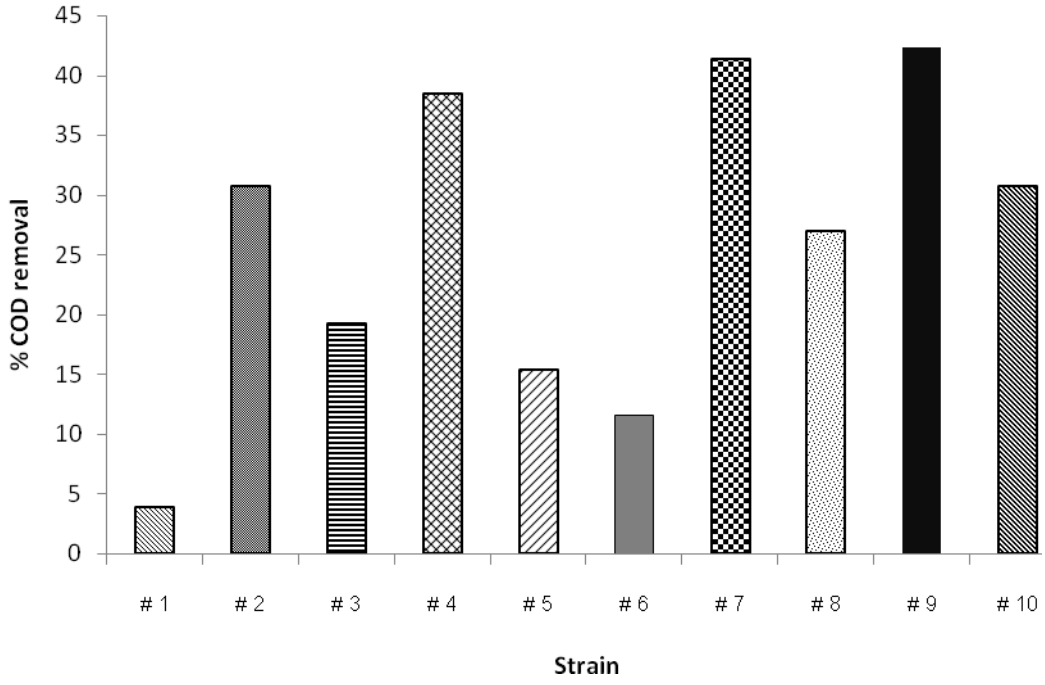

Fig 5-1 COD removal performance of each bacterial strain

5.3 Bioaugmentation of activated sludge with selected bacterial strains

5.3.1 Performance of bioaugmented vs. non-bioaugmented reactor

Performance of bioaugmented and non-bioaugmented reactors were compared. Two sequencing batch reactors were set up with activated sludge from the actual wastewater treatment plant. Selected microorganisms were inoculated into the bioaugmented reactor. Wastewater was added to the reactors at the beginning of each sequencing batch cycle. Diluted wastewater was added on cycle 1, and the concentration increased on each following cycle until no dilution was achieved on cycle 4 and maintained through cycle 7.

During the first three cycles in both bioaugmented and non-bioaugmented treatments, the percentage of COD reduction were unsteady with high drops in cycles 2 and 3. During this period, the concentration of influent was increasing with each successive cycle. Then, performance was recovered in cycle 4. The value of percentage of COD reduction stabilized from cycle 4 through the end of the experiment. At that point on, it is assumed that the reactors have achieved steady-state. When compared to non-bioaugmented treatment, the percentage of COD reduction is much higher throughout steady-state for the bioaugmented treatment. The steady-state COD removal percentage ranges from 28.7-47.5% and 70.7-81.4% in non-bioaugmented and bioaugmented reactor, respectively as shown in Table 5-2 and Fig 5-2.

Table 5-2 COD removal performance of non-bioaugmented vs. bioaugmented reactor

Cycle	Non-bioaugmented*	Bioaugmented*
1	82.33	48.25
2	73.34	-23.67
3	-63.51	2.09
4	47.47	70.65
5	28.73	76.52
6	45.64	81.14
7	34.81	81.42

*%

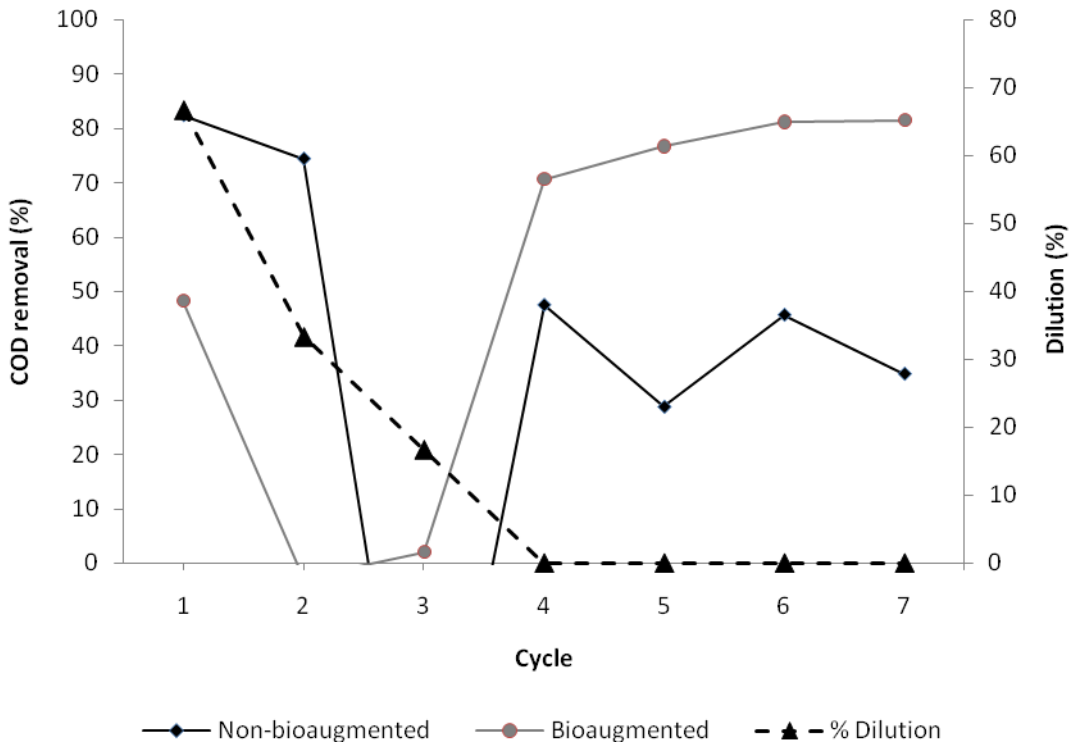

Fig 5-2 Performance of non-bioaugmented reactor vs. bioaugmented reactor

5.3.2 Effect of shock loading

Performances of bioaugmented and non-bioaugmented reactors under shock loading condition were investigated. Two sequencing batch reactors were set up with activated sludge for actual wastewater treatment plant. The bioaugmented reactor was inoculated with selected microorganisms. Undiluted wastewater was added to the reactor for 7 cycles.

At the end of each cycle, samples of the effluent was taken, and analyzed for COD. The results of non-bioaugmented and bioaugemented reactors are shown in Fig 5-3 and Table 5-3.

During the first three cycles in both bioaugmented and non-bioaugmented treatments, the percentage of COD reduction were unsteady with sharp reduction in performance. This value stabilized from cycle 4 through the end of the experiment. At that point on, it is assumed that the reactor has achieved steady-state. When compared to non-bioaugmented treatment, the percentage of COD reduction of the bioaugmented treatment was much higher throughout steady-state. During the steady-state, COD removal percentage ranges from 25.4-35.5% and 70.8-78.5% in non-bioaugmented and bioaugmented reactor, respectively as shown in Table 5-3 and Fig 5-3.

Table 5-3 COD removal performance of non-bioaugmented vs. bioaugmented reactors under shock load

Cycle	Non-bioaugmented*	Bioaugmented*
1	31.21	49.40
2	9.30	-30.85
3	-15.82	11.88
4	25.66	75.59
5	25.43	70.80
6	35.53	78.53
7	30.09	77.91

*%

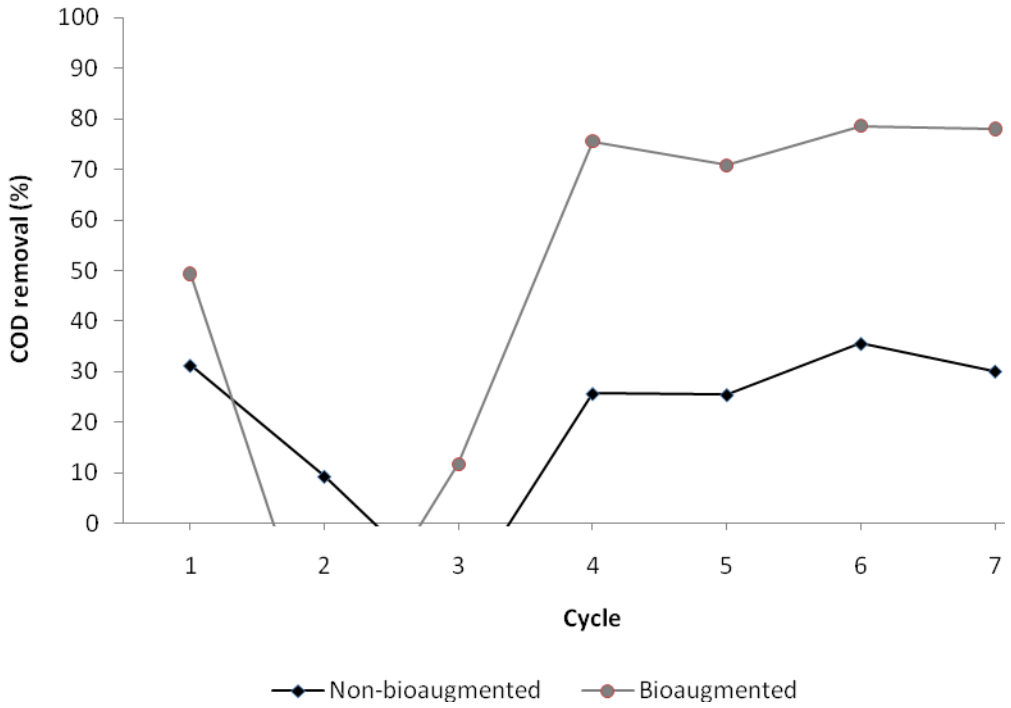

Fig 5-3 Performance of non-bioaugmented vs. bioaugmented reactor under shock load

5.3.3 Effect of influent pH on bioaugmented reactor

Effects of pH of influent wastewater were investigated for bioaugmented reactors. Four sequencing batch reactors were set up with activated sludge from actual wastewater treatment plant and inoculated with selected microorganisms. Wastewater of pH 7.5, 8.0, 8.5 and 9.0 were prepared by adjustment with 6.0 N HCl or 10% NaOH. Wastewater of various pH was added to their respective reactor for 7 cycles.

At the end of each cycle, sample of the effluent was taken and analyzed for COD. The performances of bioaugmented reactors at various pH are shown in Fig 5-4 and Table 5-4.

Performances of the reactors were monitored for 7 cycles. Performances of bioaugmented reactors were similar throughout the selected pH range. Percentage of COD reduction dropped during the first three cycles, and rose up on the fourth cycle

and maintained high performance for the remainder of the experiment. From the fourth cycle on, the reactors were assumed to have reached the steady-state. During this period, percentages of COD reduction of all reactors were ranged between 75 to 80%.

Table 5-4 COD removal performances of bioaugmented reactors at various pH

Cycle	pH 7.5*	pH 8.0*	pH 8.5*	pH 9.0*
1	51.75	48.25	76.06	68.15
2	34.67	-2.67	5.58	29.71
3	-4.66	2.09	-72.31	20.00
4	77.25	70.65	79.96	72.35
5	74.94	76.62	76.03	75.86
6	79.90	81.14	80.69	79.02
7	80.19	81.42	79.85	75.68

*%

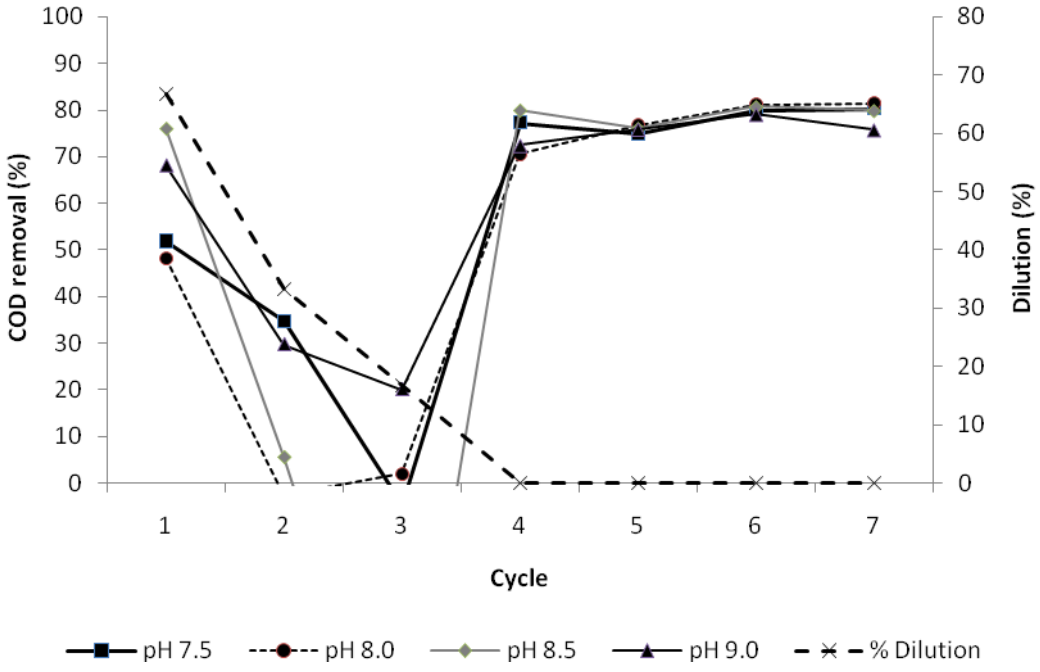

Fig 5-4 Performance of bioaugmented reactor at various influent pH

5.3.4 Bioaugmentation with individual bacterial strain

The effects of each bacterial strain on the sequencing batch reactor were studied. Three sequencing batch reactors were set up with activated sludge from actual wastewater treatment plant. Each selected microorganism was cultivated and inoculated to their respective reactor. Diluted wastewater was added on cycle 1, and the concentration increased on each following cycle until no dilution was achieved on cycle 4 and maintained through cycle 7.

The results for non-bioaugmented reactor is shown in Fig 5-10. COD removal performance for non-bioaugmented reactor and reactors bioaugmented with individual selected bacterial strain are shown in Fig 5-5 and Table 5-5.

Performance of each reactor was monitored for 7 cycles. All reactors followed a similar trend. During the first 3 cycles, percentage of COD reduction was unstable with sharp reduction in COD removal efficiency. After cycle 1, the

performance of all reactors dropped in cycle 2 and 3. Then, performance was regained in cycle 4 and rose steadily for the remain of the experiment.

Table 5-5 COD removal performance of reactor bioaugmented with individual bacterial strain

Cycle	Non-bioaugmented*	#4*	#7*	#9*
1	82.33	54.26	64.81	58.90
2	73.34	32.83	-4.94	-125.70
3	-63.51	-1.59	5.97	-25.77
4	47.47	25.66	69.47	70.61
5	28.73	72.65	71.93	70.88
6	45.64	79.70	83.09	79.65
7	34.81	77.98	82.94	79.65

*%

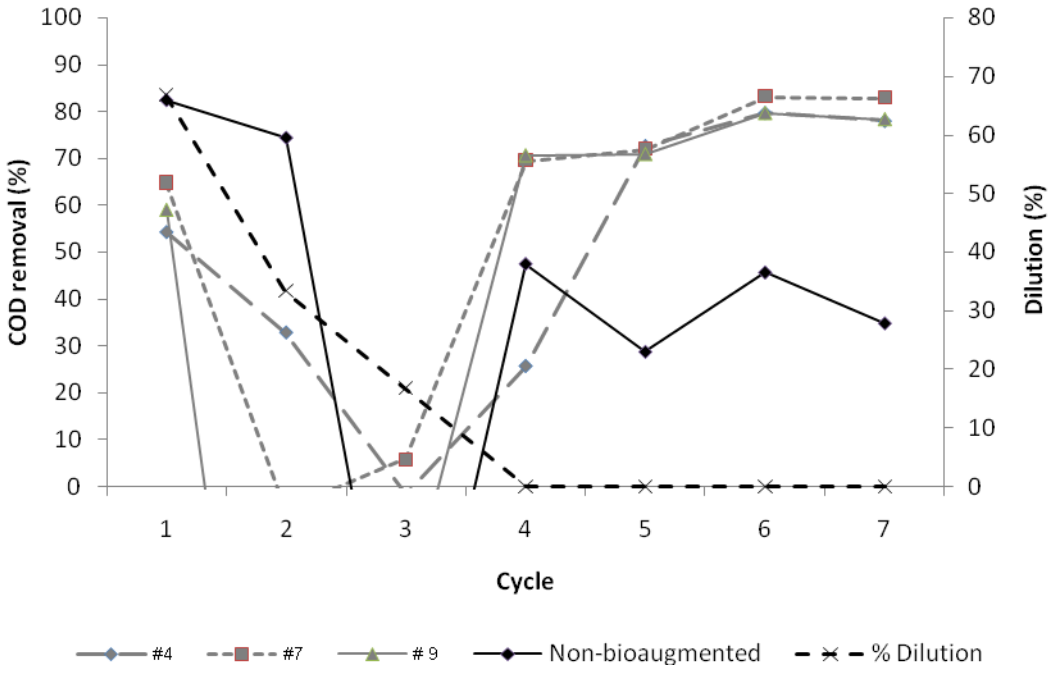

Fig 5-5 Performances of reactors bioaugmented with individual bacterial strain

5.4 Bioaugmentation with adapted seed sludge

Adapted seed sludge was produced with method described in section 4.8.1. Two sequencing batch reactors were seeded with activated sludge. The first reactor was not bioaugmented. 5% of the second reactor's content was removed and replaced by equal volume of adapted seed sludge on every cycle. Both reactors were operated in parallel, and received the same influent, which was the wastewater diluted by one-fold with raw water. COD removal performances of both reactors were monitored for 14 cycles.

The COD removal performances of the non-bioaugmented and bioaugmented reactors are shown in Table 5-6 and Fig 5-6. The percentages of COD removal of non-bioaugmented and bioaugmented reactors ranged from 29.0-73.8% and 71.5-86.1%, respectively.

Table 5-6 COD removal performances of non-bioaugmented vs. bioaugmented reactor

Cycle	Non-bioaugmented*	Bioaugmented*	TDS**
1	59.8	78.5	5440
2	66.6	78.1	5440
3	71.9	83.6	5440
4	54.6	73.8	5440
5	70.4	71.5	5440
6	71.7	84.1	5440
7	29.0	74.5	5440
8	68.4	86.1	5440
9	59.6	80.3	5440
10	73.8	86.1	5440
11	62.5	74.1	5440
12	66.0	80.5	5440
13	64.8	76.9	5440
14	65.3	76.4	5440

* %; **mg/l

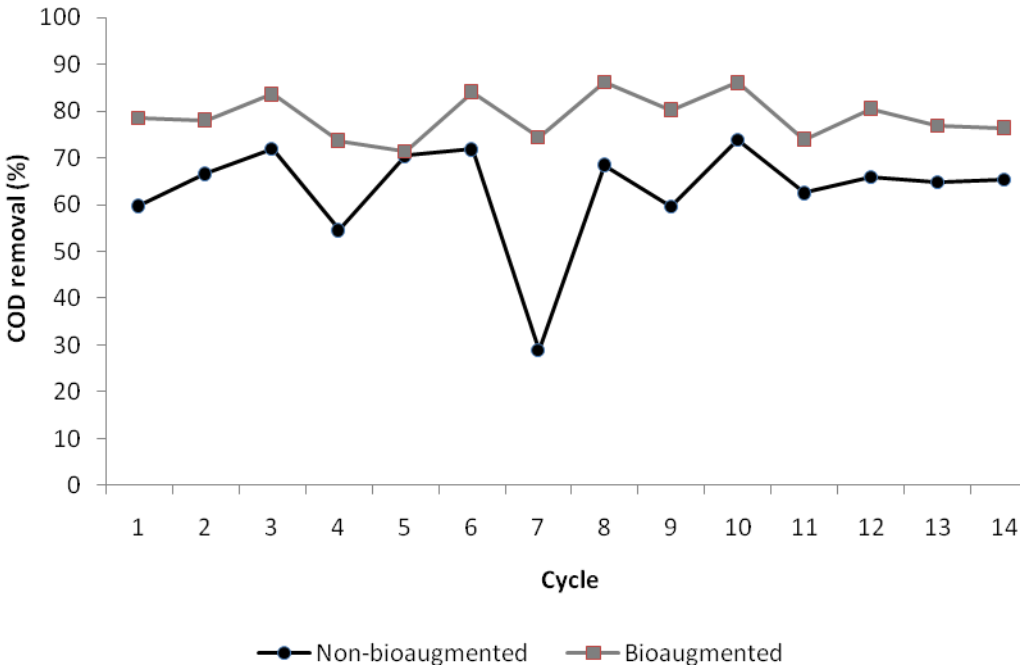

Fig 5-6 Performance of non-bioaugmented vs. bioaugmented reactor

5.5 Simulations of possible scale-up scenario

5.5.1 Scenario 1

During the course of this experiment, there was removal of 5% reactor's content, and replacement with equal volume of adapted seed sludge on every cycle to gradually change the microbial community composition. For cycles 1-3, the influent concentration remained constant at one-fold dilution of wastewater with raw water. From cycle 4 onward, the raw water used for dilution was reduced by 5% for each successive cycle. Reductions of dilution caused increases of influent strength and TDS. The result from Scenario1 is shown in Table 5-7 and Fig 5-7. The percentages of COD removal ranged from 71.0-86.5%, while TDS increased from 5440 to 7503 mg/l.

Table 5-7 COD removal performance of bioaugmented reactor in Scenario 1

Cycle	% COD removal	TDS*
1	78.1	5440
2	73.0	5440
3	83.4	5440
4	71.0	5579
5	77.3	5726
6	84.6	5581
7	74.2	6044
8	86.5	6217
9	81.7	6400
10	86.1	6594
11	69.3	6800
12	79.6	7019
13	80.4	7253
14	78.1	7503

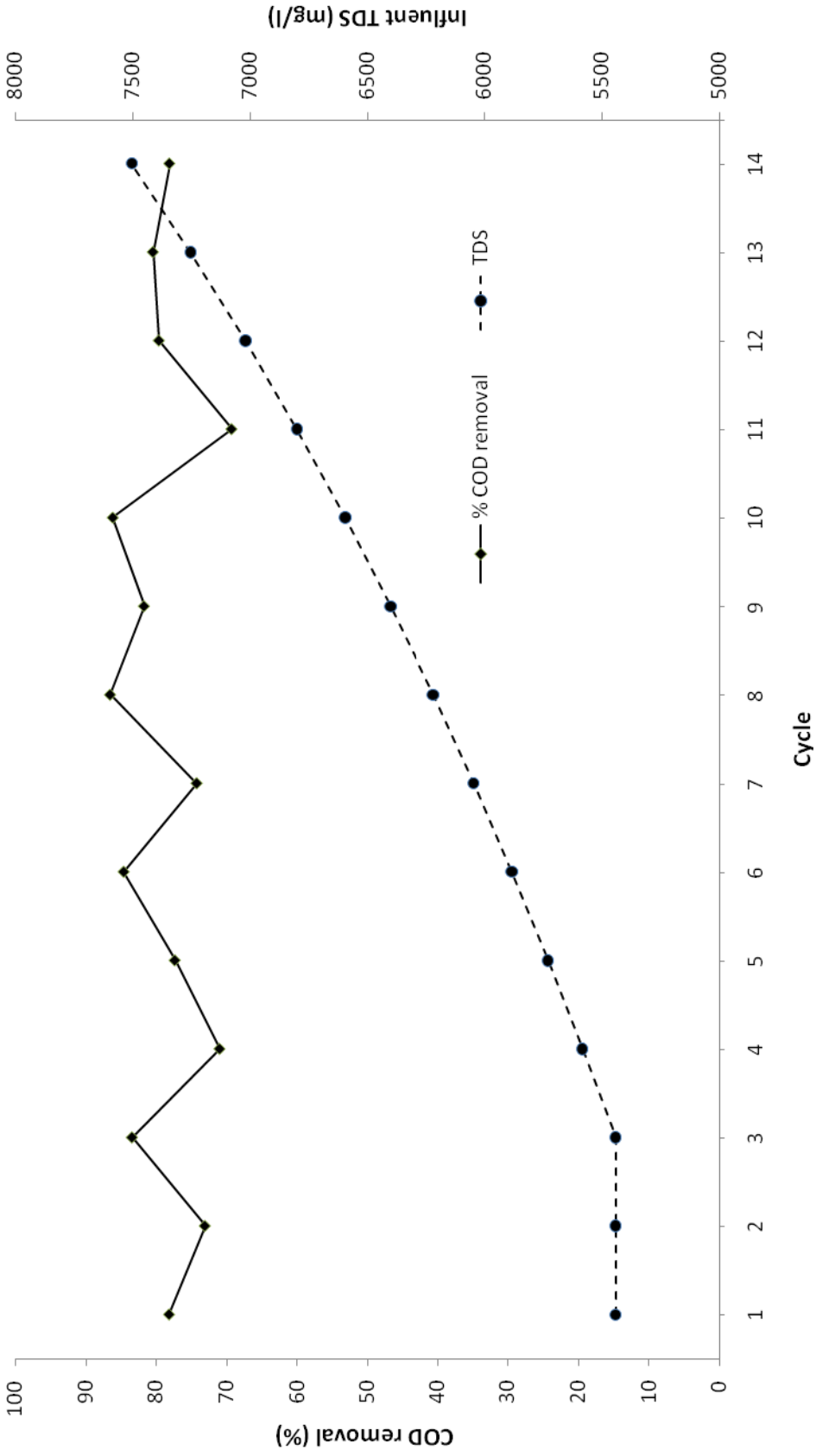

Fig 5-7 Performance of reactor bioaugmented with adapted sludge in Scenario 1

5.5.2 Scenario 2

During the course of this experiment, there was removal of 5% reactor's content, and replacement with equal volume of adapted seed sludge on every cycle to gradually change the microbial community composition. For cycles 1-3, the influent concentration remained constant at one-fold dilution of wastewater with raw water. From cycle 4 onward, the raw water used for dilution was reduced by 10% for each successive cycle until no dilution was achieved in cycle 13 and 14. Higher rate of reductions of dilution caused more rapid increases of influent strength and TDS than Scenario 1. The result from Scenario 2 is shown in Table 5-8 and Fig 5-8. The percentages of COD removal ranged from 70.5-86.6%, while TDS increased from 5440 to 10880 mg/l.

Table 5-8 COD removal performance of bioaugmented reactor in Scenario 2

Cycle	% COD removal	TDS*
1	78.1	5440
2	76.3	5440
3	84.2	5440
4	73.8	5726
5	79.6	6044
6	81.0	6400
7	79.9	6800
8	73.4	7253
9	83.9	7771
10	86.6	8369
11	70.5	9067
12	83.5	9891
13	75.3	10880
14	78.0	10880

*mg/l

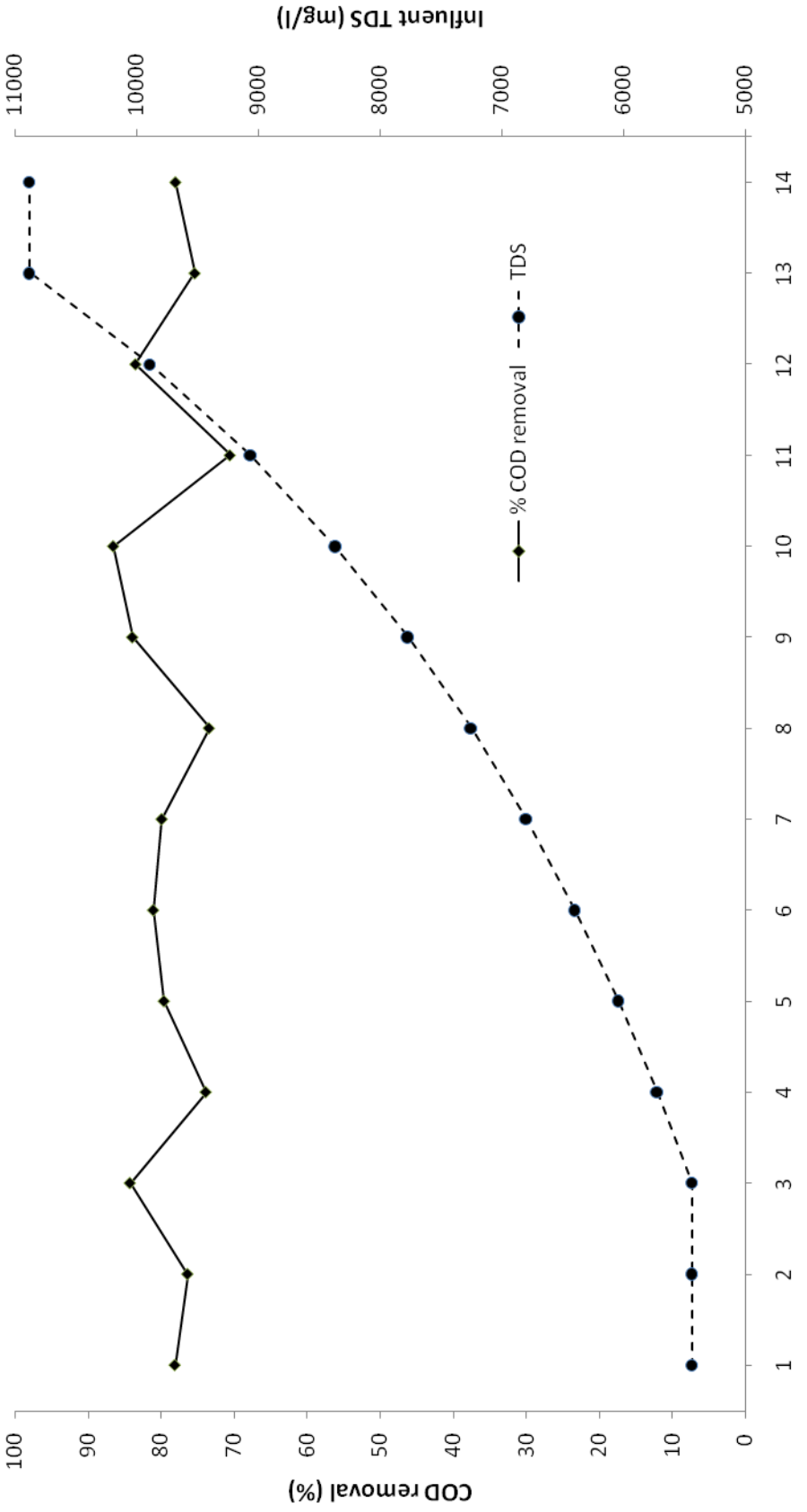

Fig 5-8 Performance of reactor bioaugmented with adapted sludge in Scenario 2

5.5.3 Scenario 3

Scenario 3 took a different approach from Scenario 1 and 2. On the initiation of cycle 1, 30% of reactor's content was removed and replaced with equal volume of adapted seed sludge. For each successive cycle, 5% of reactor's content was removed and replaced with adapted seed sludge. The influent for cycle 1 was wastewater diluted by one-fold with raw water. For each successive cycle, the raw water used for dilution was reduced by 5%. The large amount of adapted seed sludge on cycle 1 would facilitate an accelerated change in microbial community composition. The result from Scenario 3 is shown in Table 5-9 and Fig 5-9. The percentages of COD removal ranged from 72.9-84.6%, while TDS increased from 5440 to 8059 mg/l.

Table 5-9 COD removal performance of bioaugmented reactor in Scenario 3

Cycle	% COD removal	TDS*
1	77.9	5440
2	75.2	5579
3	84.6	5726
4	72.9	5881
5	81.5	6044
6	83.2	6217
7	78.5	6400
8	80.3	6594
9	83.0	6800
10	84.4	7019
11	78.2	7253
12	83.2	7503
13	73.7	7771
14	78.0	8059

*mg/l

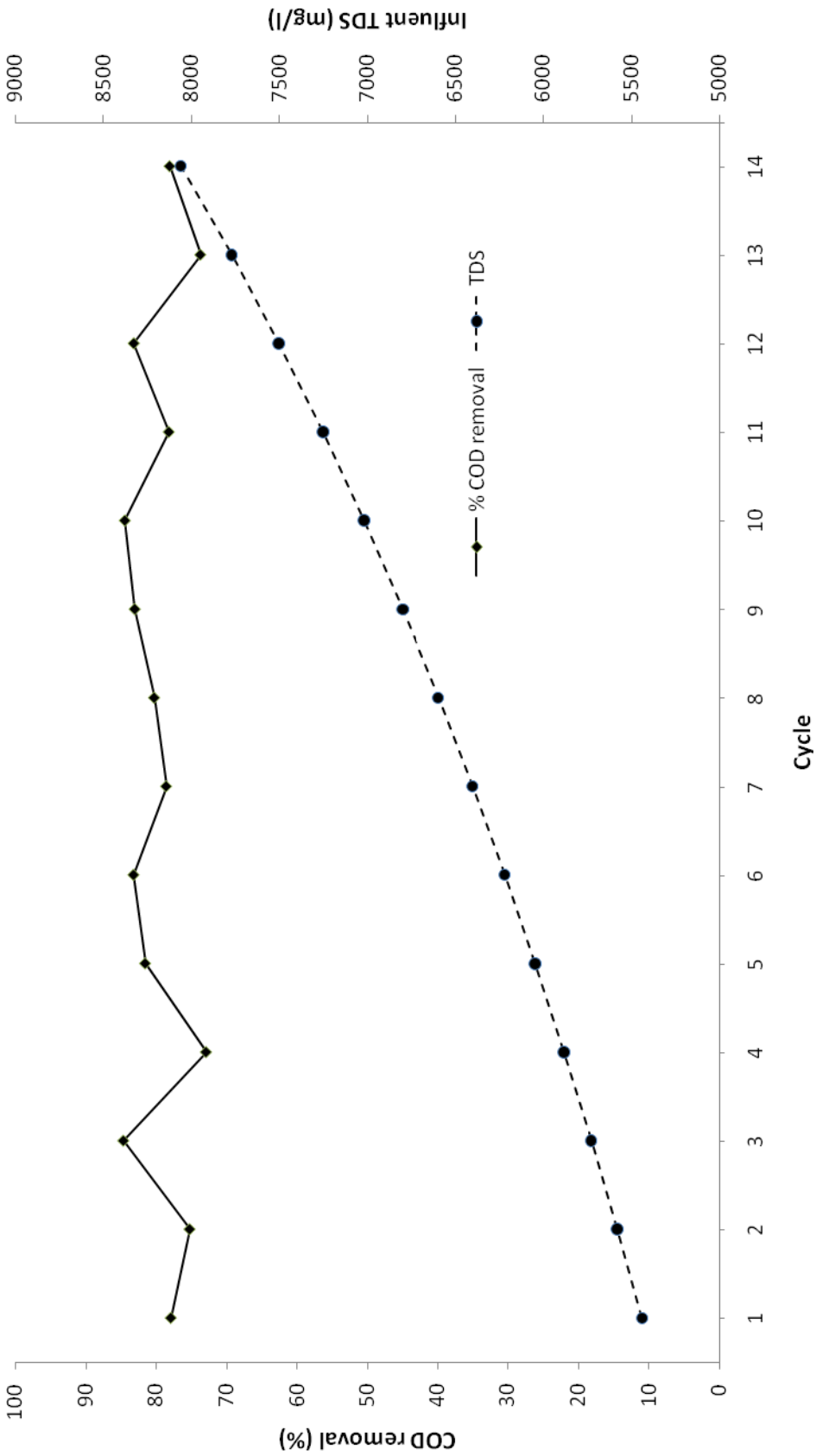

Fig 5-9 Performance of reactor bioaugmented with adapted sludge in Scenario 3

CHAPTER VI
DISCUSSION

6.1 Screening and selection of microorganisms

Halophilic microorganisms have been found to have potential in biological treatement of saline wastewater. Halophilic inoculums can be used to to improve the performance of aerobic treatment processes (Kargi and Dincer, 1996). Researchers have focused on estuarine environment as a source of halotolerant microorganisms that are able to stand high salt concentrations and treat the pollution at the same time. Estuarine sediments from Samutsakorn and activated sludge samples from petroleum processing plant were inoculated into saline selective medium to isolate capable microorganisms.

Ten salt-tolerant bacterial strains with potential to treat high TDS wastewater in the range of 10,000-20,000 mg/l were selected from the initial screening with saline selective medium containing 0.35 M NaCl, which is equivalent to approximately 20,000 mg/l of TDS. These bacterial strains were further tested for COD removal performance in the wastewater from hydrocarbon processing plant. The wastewater is a combination of neutralized spent caustic and dilution steam from a hydrocarbon processing plant. The spent caustic is responsible for high TDS concentrations. Neutralization of this highly basic waste with concentrated acid produces a variety of salts, including sulfate, carbonate and chloride salts, in various concentrations. In the hydrocarbon processing plant, a variety of chemicals and additives are added to both the caustic tower and quench column to protect from corrosion, foaming, fouling, and to improve the overall efficiency. One group of the chemical additives is amines, which protects the system against acid attacks and corrosion. Amines and amine derivatives are also known to be inhibitory for microbes as it is commonly used as disinfectant (Hernadez et al., 2005). Any amines left over from the process would find its way into the wastewater treatment system, thus reducing the performance of the biological wastewater treatment system. In order to

effectively treating the concerned wastewater, the selected bacteria must be able to tolerate both high TDS and existing inhibitory compounds. Three bacterial strains with highest performances were selected and preserved for further experimentation.

Two of the bacterial strains selected were identified as *Brevundimonas diminuta* and *Brevibacterium leoteolum*. *Brevundimonas diminuta*, as the name indicates, is a very small bacterium. It is commonly used to test the effectiveness of filters and membrane systems (Reis and Sydney, 2001). No literature concerning the use of these bacteria in wastewater treatment was found.

6.2 Bioaugmentation of activate sludge with selected bacterial strains

The results from all treatments were compared. A similar finding was noticed in all treatments. Cycles 1-3 of all treatments showed instability in treatment performance. In the more extreme cases, the final COD of the wastewater was higher than the initial COD reducing the COD removal performance to below 0%. Similar phenomenon has been observed and explained in earlier investigations(Lefebvre & Molleta, 2006; Kargi & Dincer, 1996). When rapid changes in salinity are combined with higher organic loading, reductions in performance was observed. One response to rapid increase in salt concentration is the release of cellular material to the surrounding, resulting in an increase of soluble COD (Kincannon and Guady, 1968). Then, from cycle 4 to the end of the experiment, the COD removal performance of the reactors recovered and stabilized. During this period, the systems were assumed to have reached steady-state. In some cases, a period of up to 10 days was required for an activated sludge system to reach steady-state (Watanabe, et al., 1996; Boon, et al., 2000).

When the steady-state performances of bioaugmented and non-bioaugmented reactors were compared, the bioaugmented system was superior to the non-bioaugmented system. Even though high percentages of salt are known to hinder the correct operation of conventional aerobic wastewater treatment (Ludzack and Noran, 1965), the bioaugmented reactor yielded satisfactory performance throughout the steady-state. Using step-wise increase in wastewater concentration allowed the microbial community to be exposed to gradually increasing wastewater

concentrations. The bioaugmented system successfully acclimatized to the wastewater at full strength. The changes in environmental conditions favored the growth of specific group of microorganisms. Microorganisms that could not withstand the increasing concentrations were inhibited. After acclimatization, the non-bioaugmented system was not satisfactory in performance. Bioaugmentation of bacterial strains specifically screened for their ability to tolerate and perform at higher TDS increased the adaptability of the bioaugmented system due to the increase in microbial diversity and the presence of microorganisms with specialized function.

The novel characteristics of the selected bacterial strains were even more pronounced in the examination of the effect of shock loading. In the non-bioaugmented system, the sudden change in environmental condition strongly inhibited the microorganisms. The performance was lower when compared with the non-bioaugmented system receiving step-wise increase in wastewater concentration. It could be speculated that the sudden increase in wastewater concentrations killed some of that microorganisms that would acclimatize and survive if exposed to gradual increase in wastewater concentrations. In the bioaugmented reactor, the treatment performance recovered satisfactorily after exposure to the sudden increase in wastewater concentration. This reinforces the beneficial characteristics of the selected bacterial strains.

To examine the benefits of the individual selected bacterial strains, each bacterial culture was used to bioaugment an SBR unit. The results showed that bioaugmentation of activated sludge with any of the three selected bacterial strains provided similarly satisfactory performance after acclimatization. Previous researches report similar findings. The use of halophilic inoculums is the best way to improve the performance of aerobic treatment process under halophilic condition. Inoculation of *Halobacter halobium* to activated sludge improved the treatment performance significantly (Kargi and Dincer, 1996). Later with the same microbial strain, over 95% COD removal from an effluent generated by pickling industry with activated sludge was achieved (Kargi et al., 2000). The examination of COD removal performance of each selected bacterial strains confirmed that the additions of any of the three selected bacterial strains improves the performance of activated sludge against the increase in wastewater concentrations. Even though the addition of only

one selected bacterial strain would improve the treatment performance, it is not an effective strategy. Addition of all three selected bacterial strains would improve the robustness of the system against environmental changes. In the actual application, environmental conditions of wastewater treatment plant and influent characteristics change with upstream activities. The increase in diversity of the microbial community would provide more options as the selection pressure changes. Such strategy has been described in previous experiment by inoculating a mixture of halophilic microorganisms collected from estuarine environment (Lefebvre, 2004). The use of halophilic sludge enabled biological treatment of hypersaline wastewater to achieve treatment efficiency similar to the treatment of fresh wastewater.

During normal operation, the pH of the actual wastewater is adjusted to approximately 8.0, but fluctuates slightly. Fluctuations in pH in the influent and the resulting changes in reactor pH hamper the operation of a biological treatment plant. In general, microorganisms in activated sludge function in an optimal pH range of 6.5 to 8.0. Activated sludge microorganisms begin to lose their function when pH fluctuates out of that range. In an experiment comparing performances of conventional and bioaugmented activated sludge reactors against pulse shocks of pH 12 in the influent, treatment efficiencies of both reactors declined when the pH value went above 9, and both reactors eventually failed because activated sludge wash out. Washing out is a common phenomenon resulting from the death of activated sludge microorganisms causing improper sedimentation and separation of treated wastewater (Chong, et al., 1997). Bioaugmentation is not a solution for guarding against extreme pH. Control of pH is necessary for correct functioning of biological wastewater treatment plant. To establish a working pH for further experiment, performance of activated sludge bioaugmented with the selected bacterial strains was examined with wastewater influent pH at 7.5, 8.0, 8.5 and 9.0. The bioaugmented reactors performed satisfactorily within the selected pH range. The pH of the actual wastewater treatment process is adjusted to approximately 8.0 and would not fluctuate out of this range since control of pH is easily conducted by addition of acid or base. Therefore, pH 8.0 was selected for further experiment, because the wastewater was already adjusted. There would be no need for unnecessary pH adjustment.

6.3 Lab-scale simulation of bioaugmentation

The previous experiments in the present study had displayed the benefits of enriching activated sludge with the selected bacterial strains. Employing the same strategy to a real wastewater treatment system is not feasible due to the size of the system. It has been reported that initial survival of the newly introduced strain depends highly on the size of the inoculums which determines cell density in the system (Limbergen, et al., 1998). Cultivation of selected bacteria in volume sufficient enough to inoculate a large industrial wastewater treatment plant, and achieving high enough cell density in order to survive is not possible by any economical or engineering point of view. A method was devised in order to overcome the obstacles in scaling up.

It has been known that non-salt-adapted microorganisms can be acclimatized to high salinity and still maintain satisfactory treatment performance. Acclimatization has been achieved by exposure to gradual increase in the salt concentration. Although acclimatization of microorganisms is possible, proper performance of adapting microorganism to higher salt concentration is limited to less than 5% salt (Kargi and Dincer, 1997; Dincer and Kargi, 2001). It was established earlier that the activated sludge at hand was incapable of performing at higher TDS and organic loading. But in this study, this concept was applied along with bioaugmentation of the selected bacterial strains. Activated sludge was enriched with the selected bacterial strains, and was subjected to gradually increasing TDS by addition of wastewater. By the end of this process, the resulting activated sludge was capable of sufficient treatment of the wastewater, which has been referred to as adapted seed sludge in the earlier sections of this report. During this process, the gradual increase in wastewater concentration slowly selected out the microorganisms incapable of survival under the changing environmental condition, and favored the growth of microorganisms fitted to survive including the selected bacterial strains. The time more than seven days to complete this process allowed enough generation of the selected microorganisms to impact changes in the microbial community.

Comparison between an SBR bioaugmented with adapted seed sludge and non-bioaugmented reactor showed that adapted seed sludge improve the performance

of activated sludge even when the conditions were held constant at one fold dilution of the wastewater. The finding showed that the microbial community of the adapted seed sludge responded to the specific substrates present in the wastewater with higher activity. Bioaugmentation with adapted seed sludge would improve the treatment performance even if the influent characteristics of the wastewater treatment plant were held at the present conditions.

In planning for scale-up of the inoculation process using adapted seed sludge, three scenarios varying in inoculation frequency and volumes, and rate of TDS increase were simulated at the lab-scale. It would be costly if plant's performance fails to meet the effluent standard in an actual scale up operation. Engineering feasibilities were taken into consideration for all scenarios.

The satisfactory results from all three scenarios showed that the reactors enriched with adapted seed sludge responded well to the changes in TDS and the increasing concentrations of toxic compounds present in the wastewater. These scenarios were designed with differences in rates of adapted seed sludge addition and changes on wastewater concentrations, so upon their success or failures, the management team may have options to choose from according to the schedule or budget. Since all three scenarios were successful, it is worth to pursue at a larger scale depending on the level of risk one is willing to take. However, at an actual wastewater treatment plant, failure is costly, and may lead to shut down of upstream processes. Scenario 1 takes the most gradual approach thus it has the least risk of failure. Compared to Scenario 2, the wastewater concentration in Scenario 1 increased more gradually. Gradual changes allows more time for microbial community to adapt. Scenario 3 took a much less economical approach. The cost of equipments and logistics required for production and bioaugmentation of such large volume of adapted seed sludge would make the more gradual and less risky of Scenario 1 more justifiable in terms of economics.

CHAPTER VII
CONCLUSIONS

Human activities, whether domestic, municipal, or industrial, produce liquid waste. The polluting contaminants must be removed before discharging to surface water to prevent polluting precious water sources. Nowadays, strict regulations are placed on placed on pollution control. In more populous regions, the scarcity of water calls for better management and reuse of treated water.

In this study, samples from saline environments and industrial wastewater treatment were screened for salt-tolerant microorganisms. Ten microorganisms were isolated and purified. They were tested for potential use in treatment of an industrial wastewater with high TDS. Three microorganisms were selected for their COD removal performance. Two were identified as *Brevundemonas diminuta* and *Brevibacterium luteolum*. The three microorganisms were cultivated and inoculated into reactors containing activated sludge. The bioaugemented reactors were tested under various conditions, and compared against non-bioaugmented reactors. Bioaugmentation with the selected bacterial strains proved to provide many advantages over the non-bioaugmented systems. But not without technical concerns, before satisfactory performance was obtained, reduction in performance was observed in all experiments. If similar event occurred in a real wastewater treatment system, the non-conforming effluent would cause mandatory shut down of many of the plant's processes resulting in loss of revenue.

With the concern of temporary reduction in performance, a method was devised to eliminate the period of lowered performance. By inoculation of the selected bacteria strains to the activated sludge in a separate reactor rather than the target reactor, and acclimatizing the microbial community to the wastewater, the period of lowered performance could be bypassed. At the lab-scale, the production of adapted seed sludge provided a safe method for bioaugmentation of the biological

wastewater treatment system with the benefits of the selected bacterial strains, and without any indication of lowered performance.

Future study

With the findings and methods in this study, future study should be performed in conventional activated sludge system. The SBR used in this study could not operate at higher MLSS concentrations. A lab-scale conventional activated sludge system with a clarifier unit dedicated to separation of treated wastewater from the biomass could perform at lower F/M ratio, which would be more representative of the actual wastewater treatment system and provide higher efficiency. The bioaugmentation methods in this study should be performed at pilot-scale to confirm its effectiveness at larger scale before any attempts on an actual wastewater treatment scale to reduce the chances of costly failures.

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APPENDICES

APPENDIX A
SCREENING AND ISOLATION OF SALT-TOLERANT MICROORGANISMS

Table A-1 COD removal performance of each bacterial strain

Strain	Initial COD*	Final COD*	% COD removal
# 1	2080	2000	3.85
# 2	2080	1440	30.77
# 3	2080	1680	19.23
# 4	2080	1280	38.46
# 5	2080	1760	15.38
# 6	2080	1840	11.54
# 7	2080	1220	41.35
# 8	2080	1520	26.92
# 9	2080	1200	42.31
# 10	2080	1440	30.77

*mg/l

APPENDIX B
PERFORMANCE OF BIOAUGMENTED VS NON-BIOAUGMENTED REACTORS

Table B-1 Performance of non-bioaugmented reactor

Cycle	Initial COD*	Final COD*	% COD removal
1	1986	351	82.33
2	1068	274	73.34
3	1310	2142	-63.51
4	1835	964	47.47
5	1876	1337	28.73
6	2456	1335	45.64
7	2106	1373	34.81

*mg/l

Table B-2 Performance of bioaugmented reactor

Cycle	Initial COD*	Final COD*	% COD removal
1	3942	2040	48.25
2	1087	1116	-2.67
3	1197	1172	2.09
4	1540	452	70.65
5	1724	403	76.52
6	1872	353	81.14
7	1502	279	81.42

*mg/l

APPENDIX C
EFFECT OF SHOCK LOADING

Table C-1 Effect of shock loading on non-bioaugmented reactor

Cycle	Initial COD*	Final COD*	% COD removal
1	2358	1622	31.21
2	1903	1726	9.30
3	1884	2182	-15.82
4	2050	1524	25.66
5	2088	1557	25.43
6	2544	1640	35.53
7	2034	1422	30.09

*mg/l

Table C-2 Effect of shock loading on bioaugmented reactor

Cycle	Initial COD*	Final COD*	% COD removal
1	3316	1678	49.40
2	1637	2142	-30.85
3	1482	1306	11.88
4	1639	400	75.59
5	1548	452	70.80
6	1928	414	78.53
7	1548	342	77.91

*mg/l

APPENDIX D
EFFECT OF INFLUENT pH ON BIOAUGMENTED REACTORS

Table D-1 Performance of bioaugmented reactor at influent pH 7.5

Cycle	Initial COD*	Final COD*	% COD removal
1	3254	1570	51.75
2	1096	716	34.67
3	1244	1302	-4.66
4	1530	348	77.25
5	1548	388	74.94
6	1876	377	79.90
7	1550	307	80.19

*mg/l

Table D-2 Performance of bioaugmented reactor at influent pH 8.0

Cycle	Initial COD*	Final COD*	% COD removal
1	3942	2040	48.25
2	1087	1116	-2.67
3	1197	1172	2.09
4	1540	452	70.65
5	1724	403	76.62
6	1872	353	81.14
7	1502	279	81.42

*mg/l

Table D-3 Performance of bioaugmented reactor at influent pH 8.5

Cycle	Initial COD*	Final COD*	% COD removal
1	2970	711	76.06
2	1076	1016	5.58
3	650	1120	-72.31
4	1477	296	79.96
5	1556	373	76.03
6	1844	356	80.69
7	1648	332	79.85

*mg/l

Table D-4 Performance of bioaugmented reactor at influent pH 9.0

Cycle	Initial COD*	Final COD*	% COD removal
1	3548	1130	68.15
2	1121	788	29.71
3	1260	1008	20.00
4	1548	428	72.35
5	1520	367	75.86
6	1916	402	79.02
7	1612	392	75.68

*mg/l

APPENDIX E
PERFORMANCE OF ACTIVATED SLUDGE BIOAUGMENTED WITH INDIVIDUAL BACTERIAL STRAIN

Table E-1 Performance of activated sludge bioaugmented with # 4

Cycle	Initial COD*	Final COD*	% COD removal
1	3308	1513	54.26
2	1051	706	32.83
3	1197	1216	-1.59
4	2050	1524	25.66
5	1572	430	72.65
6	1916	389	79.70
7	1558	343	77.98

*mg/l

Table E-2 Performance of activated sludge bioaugmented with # 7

Cycle	Initial COD*	Final COD*	% COD removal
1	3106	1093	64.81
2	1174	1232	-4.94
3	1311	1234	5.87
4	1546	472	69.47
5	1596	448	71.93
6	1892	320	83.09
7	1606	274	82.94

*mg/l

Table E-3 Performance of activated sludge bioaugmented with # 9

Cycle	Initial COD*	Final COD*	% COD removal
1	3752	1542	58.90
2	1043	2354	-125.70
3	1172	1474	-25.77
4	1538	452	70.61
5	1576	459	70.88
6	1823	371	70.88
7	1534	371	79.65

*mg/l

APPENDIX F

BIOAUGMENTATION WITH ADAPTED SEED SLUDGE

Table F-1 Performance of non-bioaugmented reactor

Cycle	Initial COD*	Final COD*	% COD removal	TDS**
1	1258	506	59.8	5440
2	875	292	66.6	5440
3	825	232	71.9	5440
4	570	259	54.6	5440
5	758	224	70.4	5440
6	722	204	71.7	5440
7	700	497	29.0	5440
8	792	250	68.4	5440
9	900	364	59.6	5440
10	869	228	73.8	5440
11	738	277	62.5	5440
12	846	288	66.0	5440
13	793	279	64.8	5440
14	776	269	65.3	5440

*mg/l

**influent TDS; mg/l

Table F-2 Performance of reactor bioaugmented with adapted seed sludge

Cycle	Initial COD*	Final COD*	% COD removal	TDS**
1	1246	268	78.5	5440
2	749	164	78.1	5440
3	682	112	83.6	5440
4	480	126	73.8	5440
5	635	181	71.5	5440
6	653	104	84.1	5440
7	642	164	74.5	5440
8	634	88	86.1	5440
9	674	133	80.3	5440
10	717	100	86.1	5440
11	613	159	74.1	5440
12	668	130	80.5	5440
13	624	144	76.9	5440
14	679	160	76.4	5440

*mg/l

**influent TDS; mg/l

APPENDIX G

DETERMINATION OF POSSIBLE SCALE-UP SCENARIO

Table G-1 Scenario 1

Cycle	Initial COD*	Final COD*	% COD removal	TDS**
1	1252	274	78.1	5440
2	742	200	73.0	5440
3	674	112	83.4	5440
4	520	151	71.0	5579
5	670	152	77.3	5726
6	654	101	84.6	5581
7	690	178	74.2	6044
8	592	80	86.5	6217
9	786	144	81.7	6400
10	806	112	86.1	6594
11	732	225	69.3	6800
12	876	179	79.6	7019
13	878	172	80.4	7253
14	926	203	78.1	7503

*mg/l

**influent TDS; mg/l

Table G-2 Scenario 2

Cycle	Initial COD*	Final COD*	% COD removal	TDS**
1	1255	275	78.1	5440
2	727	172	76.3	5440
3	660	104	84.2	5440
4	470	123	73.8	5726
5	695	142	79.6	6044
6	675	128	81.0	6400
7	795	160	79.9	6800
8	436	116	73.4	7253
9	932	150	83.9	7771
10	883	119	86.6	8369
11	994	293	70.5	9067
12	1210	200	83.5	9891
13	1237	305	75.3	10880
14	1272	280	78.0	10880

*mg/l

**influent TDS; mg/l

Table G-3 Scenario 3

Cycle	Initial COD*	Final COD*	% COD removal	TDS**
1	1216	269	77.9	5440
2	871	216	75.2	5579
3	703	108	84.6	5726
4	490	133	72.9	5881
5	731	135	81.5	6044
6	684	115	83.2	6217
7	741	159	78.5	6400
8	476	94	80.3	6594
9	836	142	83.0	6800
10	841	131	84.4	7019
11	806	176	78.2	7253
12	1104	186	83.2	7503
13	900	237	73.7	7771
14	1002	220	78.0	8059

*mg/l

**influent TDS; mg/l

BIOGRAPHY

NAME	Mr. Thamarat Sukjeeradet
DATE OF BIRTH	July 20, 1980
PLACE OF BIRTH	Chiang Mai, Thailand
INSTITUTIONS ATTENDED	Assumption University, 2000-2004 Bachelor of Science (Food Technology) Mahidol University, 2005-2010 Master of Science (Environmental Biology)
Research Grants	Center for Environmental Health, Toxicology and Management of Chemical under Science & Technology Postgraduate Education and Research Development Office (PERDO)
Home Address	49/23 Changpuek Rd. Sriphume, Muang Chiang Mai E-mail: totns@yahoo.com