UNIVERSITI PUTRA MALAYSIA

SAGO EFFLUENT TREATMENT WITH Bacillus spp.

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SAGO EFFLUENT TREATMENT

WITH *Bacillus spp.*

By

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ABSTRACT

Sago effluent treatment by selective bacteria was conducted to study the effect of isolated bacteria in reducing polluting parameter of the effluent. Starch degrading bacteria were isolated from fresh sago effluent. B1 and B2 isolates were found as *Bacillus koreensis* and *Bacillus* spp. based on the 16S rDNA sequence analysis. Both bacteria were found efficient in starch degradation at 1.62 and 0.62 mm diameter of clear zone respectively. Four treatments with three replications were carried out in Completely Randomized Design. Each treatment consisted 1500 mL effluent which was aerated for ten days. Treatment 2, 3 and 4 were pre-set at pH 6.5 to 7.0. Treatment 2 (control) consisted of effluent mixed with 3 mL of NB whilst Treatment 3 and 4 consisted of effluent with 3 mL of NB suspension cultured with *B. koreensis* and *Bacillus* spp. respectively. After 24 hours of treatment, Treatment 3 and 4 showed significant difference in BOD and COD removal. However, reduction of BOD and COD in Treatment 3 was 38 and 25 mg/L or 14.9 and 9.8% higher than in Treatment 4. Reduction of BOD in Treatment 3 was from 255 to 217 mg/L whereas from 255 to 230 mg/L in Treatment 4. For COD removal, as much as 12.7 and 11.3% COD was reduced in Treatment 3 and 4 respectively. The value reduced from 473 to 413 mg/L in Treatment 3 and from 479 to 425 mg/L in Treatment 4. In contrast, TSS and pH were found increased in both treatments. Increment of TSS value in Treatment 3 was from 788 to 930 mg/L (15%) and from 794 to 1453 mg/L (45%) in Treatment 4. The pH value increased from pH 7 to 8. As the conclusion, both *Bacillus* spp. isolated has the ability to reduce sago effluent's physico-chemical characteristics through aeration treatment between 0 to 24 hours with an exception to TSS. This could be attributed to the adaptability of the bacteria to the new environment, lack of dissolved oxygen at optimum level, lack of nutrients to stimulate bacterial metabolism and degradation activity, short treatment period and others.

ABSTRAK

Rawatan sisa cecair sagu oleh bakteria telah dijalankan untuk mengkaji kesan bakteria tersebut dalam mengurangkan tahap pencemaran sisa cecair sagu. Bakteria yang mempunyai keupayaan untuk menguraikan kanji telah dipencil daripada sisa cecair sagu dan dikenalpasti sebagai *Bacillus koreensis* dan *Bacillus* spp. melalui analisis rangkaian 16S rDNA. *Bacillius koreensis* dan *Bacillus* spp. masing-masing menghasilkan zon cerah seluas 1.62 dan 0.62 mm. Empat rawatan dengan tiga replikasi bagi setiap satu rawatan telah dijalankan dalam 'Completely Randomized Design'. Setiap rawatan terdiri daripada 1500 mL sisa cecair sagu yang dirawat selama sepuluh hari. Rawatan 1 dan 2 ialah kawalan. Sisa cecair bagi Rawatan 2, 3 dan 4 telah diseragamkan antara pH 6.5 hingga 7.0. Set eksperimen bagi Rawatan 2, 3 dan 4 masing-masing terdiri daripada 3 mL NB, 3 mL NB yang telah dikulturkan dengan *B. koreensis* dan 3 mL NB yang telah dikulturkan dengan *Bacillus* sp. Keberkesanan tindakan *Bacillus koreensis* dan *Bacillus* spp. dalam mengurangkan parameter pencemaran sisa cecair sagu jelas terbukti selepas 24 jam rawatan. Sebanyak 14.9 dan 9.8% pengurangan BOD dilihat bagi Rawatan 3 dan 4 iaitu pengurangan dari 255 ke 217 mg/L bagi Rawatan 3 dan 255 ke 230 mg/L bagi Rawatan 4. Manakala sebanyak 12.7 dan 11.3% pengurangan COD dilihat bagi Rawatan 3 dan 4 yang mencatat pengurangan dari 473 ke 413 mg/L bagi Rawatan 3 dan 479 ke 425 mg/L bagi Rawatan 4. Nilai TSS bagi kedua – dua rawatan didapati meningkat sedikit demi sedikit hingga ke hari terakhir rawatan. Peningkatan nilai TSS bagi Rawatan 3 meningkat dari 788 ke 930 mg/L (15%) sepanjang tempoh 24 jam rawatan manakala nilai TSS bagi Rawatan 4 meningkat dari 794 ke 1453 mg/L iaitu sebanyak 45%. Nilai pH didapati meningkat dari pH 7 ke pH 8 sepanjang tempoh rawatan. Kesimpulannya, sepanjang sepuluh hari rawatan, keberkesanan kedua – dua bakteria ini hanya dilihat selepas 24 jam rawatan. Kedua – dua bakteria berjaya mengurangkan parameter BOD dan COD, kecuali TSS dan pH. Hal ini berkemungkinan kelemahan bakteria tersebut mengadaptasi pada persekitaran baru, kekurangan oksigen terlarut pada tahap optimum, kekurangan nutrien untuk stimulasi metabolisma dan aktiviti penguraian oleh bakteria, tempoh rawatan yang pendek dan sebagainya.

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APPROVAL

I certify that this research project report entitled "Sago Effluent Treatment with *Bacillus* spp." has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
APHA	American Public Health Association
ATFBR	Anaerobic Tapered Fluidized Bed Reactor
BLAST	Basic Local Alignment Search Tools
BOD	Biological Oxygen Demand
$C_5H_7NO_2$	Glucose – nitrogen derivative
$C_6H_{12}O_6$	Glucose
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
CRD	Completely Randomized Design
DNA	Deoxyribonucleic acid
Eq.	Equation
ETP	Effluent treatment plant
FAS	Ferrous ammonium sulphate
H_20	Water
H_2CO_3	Carbonic acid
H_3O^+	Hydronium ion
HCO_3^-	Bicarbonate ion
HLR	Hydraulic loading rate
NB	Nutrient broth
NCBI	National Centre for Biotechnology Information
NH_3	Ammonia
O_2	Oxygen
OLR	Organic loading rate
PCR	Polymerase Chain Reaction
pH	Potential hydrogen
RBC	Rotating biology contactor
rDNA	Ribosomal deoxyribonucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
TE	Tris-EDTA
TS	Total Solid
TSS	Total Suspended Solid

CHAPTER 1

INTRODUCTION

1.1 Background

Environmental pollution control microbiology is concerned with solving various environmental pollution problems. Among microorganisms, bacteria have attracted the greatest attention in microbiology because they are easy to cultivate and study in pure cultures. The simple characteristics of bacteria have proven to major importance in pollution control microbiology. In fact, the ability of bacteria metabolize different organic compounds provides basis for designing and operating new biological wastewater treatment systems.

Sago palm or its scientific name *Metroxylon sagu* Rottb., grows well with minimum care in swamp and peat areas of Southeast Asia especially in Malaysia, Indonesia and Papua New Guinea. Sago palm has a high starch yield which one palm may yield between 150 to 300 kg of starch. In Malaysia, Sarawak plays an important role as sago starch exporter for Singapore, Japan, Taiwan and other countries (Tie and Lim, 1991). Most of sago factory in Sarawak is located in Mukah, Igan and Oya – Dalat districts of Sibu and in Pusa – Saratok district of Sri Aman. Mukah alone has 42,259 ha of sago plantation over 55,690 ha in whole Sarawak. In 2006, Sarawak exported 42,870.90 t of sago starch which procuring income of RM42,842,666 (Department of Statistics, 2006).

Apart of producing sago starch, the factories also discharge large amount of solid sago waste called hampas and sago wastewater (effluent). The solid waste is used as composting material while the untreated effluent is often discharge to nearby rivers. A typical sago mill consumes about 1,000 kg logs per day, generating a minimum of 400 t of slurry effluent which contains about 2 t of solid (Bujang *et al.*, 1996). Thus, this industry is responsible for the production of notable amount of agro-residues.

There are many modernized wastewater treatments carried out by developed countries such as the United States of America. Most widely used treatment is the biological treatment. It is an important aspect of industrial and municipal wastewater treatment. Various principles have been incorporated in order to reduce harms brought by wastewaters. Microbial utilization approach is one of the principles used in treating wastewater. Currently, there is few bacterial treatment of sago effluent studied. However, so far there is no study based on treatment of sago effluent with bacteria isolated from the effluent.

1.2 Problem Statement

Large amount of untreated sago effluent is being discharged daily into nearby rivers. The physico-chemical characteristics of sago effluent show the effluent is highly polluted and do not meet the discharge of effluent Standard B in Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979. Enhancement of selective bacteria is believed to remove much more of organic wastes hazards. Therefore, this study was conducted to utilize bacteria originate from the sago effluent itself in reducing polluting parameter.

1.3 Objectives

The objectives of this study are:

i. To isolate starch degrading bacteria present in fresh sago effluent

ii. To identify the isolated bacteria present in fresh sago effluent

iii. To evaluate the effectiveness of the isolated bacteria in reducing sago effluent polluting parameter

CHAPTER 2

LITERATURE REVIEW

2.1 Sago Palm

Sago palm (*Metroxylon* spp.), under the class of Calamoideae (Saidin, 1993) is one and the only of the few tropical crops that can tolerate wet growing conditions, including peat swamps (Jong, 1995; Ruddle, 1977 ; Morris, 1977).The sago palm is one of the oldest tropical plants exploited by humans for its stem starch. It serves as a staple and cash crop in Sarawak (Mathur *et al.*, 1998). Rauwerdink (1986) has grouped the two species (*Metroxylon sagu* Rottb. and *Metroxylon rumphii* Mart.) into *Metroxylon sagu* and this taxonomy is now widely accepted. Commonly, it is called 'rumbia' in Malays – speaking regions.

The sago palm produces an erect trunk, about 10 m tall and 75 cm thick. It bears a crown of large pinnate leaves 5 m long with short petioles and leaf bases which clasp the stem (Kiew, 1977). The vegetation phase of the sago palm takes about seven to 15 years. Due to its massive size and lengthy vegetative, vast quantities of starch saturated the pith from the base of the stem upwards sometimes almost to the crown (Karim *et al.*, 2008; Lim 1991a, 1991b). The classification of sago palms according to the different physiological growth stages are Plawei (maximum vegetative growth), Bubul (inflorescence developing), Angau Muda (flowering), Angau Tua (fruiting) and Late Angau Tua (Tie *et al.*, 2008). Angau Muda stage has the highest starch content of 41.3 and 41.4% at the base and mid height of the trunk (Flach and Schuiling, 1989). Starch yield ranging from 34 to 975 kg dry starch/palm with an

average of 375 kg/palm (Yamamoto *et al.*, 2007). According to Ishizuka *et al.* (1995), sago palm contains a large amount of starch in its trunk and its productivity was calculated to be four times that of paddy rice.

2.2 Sago Starch Production and Its Use

In the year of 2003, there are eight sago factories operating in Sarawak, seven of which are in the Mukah – Dalat areas, and one is situated in the Igan – Sibu. These factories are fully mechanized using the modern method of starch extraction. During processing, the fibrous pith bark of log sections of about 75 to 90 cm is stripped off. Each of debarked sections is fed into the mechanical rasper (with chrome nails mounted on one face of a disc or a drum) (Manan *et al.*, 2003). Next, starch granules in the debarked pith are washed out using large amount of treated river water (Vikineswary *et al.*, 1994). The resulting starch slurry is made to pass through a series of centrifugal sieves to separate the coarse fibers. Then, it is purified by means of separation in a nozzle separator through sieve bends. Dewatering of starch is carried out using a rotary vacuum drum dryer followed by hot air drying (Azudin and Lim, 1991). The waste products from the sago starch processing are bark, pith residue 'hampas' and sago effluent (Vikineswary *et al.*, 1994). The bark is usually burnt or thrown into the river, while the hampas which contain up to 60% fermentable carbohydrate, may be used as animal feed, compost for mushroom culture, hydrolysis to confectioners' syrup and for particleboard manufacture. The untreated wastewater i di charged directly into the nearby rivers (Phang *et al.*, 2000).

2.3 Sago Effluent

Sago effluent is the wastewater that produced from the extraction of sago starch. Now, almost all sago factories are fully mechanized. With this new development and the ever-increasing productivity of the existing factories, the sago industry has problems in waste disposal. It was estimated that about 300 to 1000 m^3 of wastewater are produced daily within the industry in the Sibu Division (Vikineswary *et al.*, 1994; 1997). In an average factory, the production of wastewater is estimated can be as high as 1000 m^3 per working day (Chew and Shim, 1990). According to Bujang *et al.* (2004), a potential threat of pollution can be hypothesized based on a medium size sago mill which operates at the consumption rate of 600 (standard 1.2 m) logs per day. Each log produces 396.7 L of wastewater containing 11.9 kg of solids with average weight of log at 130 kg. Therefore, at 12 hours per day of sago mill operation would produce approximately 237.6 t of wastewater containing 7.1 t of total solids or hampas (at 3% dry matter). Nearly all the sago factories are situated along the major rivers. Thus, the wastes are pumped indiscrimately into the rivers and results in water pollution and threatening the ecosystem.

2.3.1 Characteristics of Sago Effluent

Sago effluent is normally acidic at pH 3.9 to 4.5 and high in organic load at about 3 to 5% of organic solid such as fibrous pith with some starch residues and glucose (Bujang *et al.*, 2004). It has a high carbon to nitrogen ratio (Phang *et al.*, 2000). Amylolytic and cellulolytic fungi and bacterial are the most abundant microbial found in sago effluent. Sago effluent also has high Biochemical Oxygen Demand

about 1,824 to 6,750 mg/L, Chemical Oxygen Demand (COD) about 5,682 to 16,974 mg/L and Total Suspended Solid (TSS) about 3,913 to 41,230 mg/L. All of these contribute to the polluting load of the wastewater (Chew and Shim, 1993). The large amounts of pith residue and starch in the effluent contribute to the BOD and COD of the wastewater (Vikineswary *et al.*, 1994).

2.3.2 Sago Effluent Treatments

Various treatments of sago effluent have been carried out either for treatment purpose or for production of beneficial compounds. Bujang *et al.* (2004) has worked on aerobic treatment of sago effluent produced by sago factory in Mukah, Sarawak. The agitation treatment of filtered sago effluent enhanced higher COD and TS removal at 96 and 41% respectively, compared to non-filtered sago effluent in the same treatment. This treatment also exhibited highest bacterial growth which may enhances natural degradation of the sago effluent. A laboratory scale anaerobic rotating biological contactor (RBC), for treatment of synthetic sago wastewater was carried out by Sujana and Kamanujam (1997). Optimum COD removal efficiency of 97.2% was obtained at hydraulic loading rate (HLR) of 24 to 48 hours HRT. In the same year, Rangasamy (2007) investigated anaerobic treatability of synthetic sago wastewater. It was carried in a laboratory anaerobic tapered fluidized bed reactor (ATFBR) with a mesoporous granular activated carbon as a support material. The COD removal efficiency was found to be 92% in the reactor. With the increase of organic loading rate (OLR) from 83.7 kg COD/(m^3-d), the COD removal efficiency decreased.

2.3.3 Sago Effluent Treatment by Bacteria

Based on the physico-chemical constituents, several enzymatic or microbial utilization and treatment strategies can be proposed to treat sago effluent (Vikineswary *et al.*, 1994). This is achieved by Vikineswary *et al.* in 1997 when sago effluent was treated by growing an indigenous isolate of phototrophic bacteria, *Rhodopseudomonas palustris* strain B1 isolated from a rice noodle factory wastewater. The growth was set up in anaerobic light culture system and enhanced by the use of tungsten lamps at 4000 lux, stimulated by the addition of 0.1% yeast extract. About 77% of COD removal resulted after four days of treatment (Vikineswary *et al.*, 1997).

The latest treatment of sago effluent by is carried out by Ayyasamy *et al.* (2008). It is the treatment of sago effluent by aerobic bacterial consortium. The starch degrading bacteria were isolated from sago effluent. The genera, *Alcaligenes*, *Bacillus* and *Corynebacterium* were found efficient in starch degradation in sago effluent. The physico-chemical properties such as electrical conductivity, TS, TSS, dissolved solids, BOD, COD, nitrogen and phosphate were found decreased in effluent after 72 hours. The pH of the effluent was relatively increased from 3 to 6.7. The amount of BOD and COD were decreased from 2,400 to 1,010 mg/L and 3,600 to 916 mg/L respectively. Correspondingly, the amount of the total solids from 15,100 to 1,600 mg/L, nitrogen from 76 to 12 mg/L, phosphate from 56 to 18 mg/L and starch from 0.8 to 0.3 mg/L were also decreased after 72 hours. However, the amount of Total Solids (15,780 mg/L) and Suspended Solids (14,600 mg/L) were found to be higher. This was mainly due to higher concentration of bacterial biomass in the effluent.

2.4 Degradation of Organic Wastewater by Bacteria

2.4.1 Bacterial Metabolism

Bacteria metabolize nutrients to remain alive and produce new cells. The organic metabolizing bacteria are known as heterotrophic. Each group of bacteria has their own specific metabolic characteristics that determine their ability to survive in different environments. Bacteria are able to thrive and adapt themselves to break down polysaccharides and nitrogenous compounds (Davis, 1971). Enzymes mediate bacteria metabolism and have been called organic catalysts since they increase the rate of reaction without becoming part of the final products. The bacterial oxidation reactions normally occur in a water environment with oxygen being supplied as dissolved oxygen in the water. These reactions with dissolved oxygen are aerobic reactions. Aerobic metabolism is very important in the environment with the production of the most oxidized form of carbon and hydrogen, as well as yielding the most energy for the energy (McKinney, 2004). According to Maier *et al.* (2009), the complete oxidation of a substrate under aerobic conditions is represented by the mass balance equation:

$$a(C_6H_{12}O_6) + b(NH_3) + c(O_2) \rightarrow d(C_5H_7NO_2) + e(CO_2) + f(H_20) \qquad \text{Eq. (2.4.1)}$$

In Eq. (2.4.1), the substrate is a carbohydrate such as glucose, which represented as the formula $C_6H_{12}O_6$ with a, b, c, d, e and f represent mole numbers. The mass balance equation has a number of practical applications which it can be used to estimate the amount of oxygen and nitrogen required for growth and utilization of a particular substrate. This is useful for wastewater treatment (Maier *et al.*, 2009). The

carbon dioxide produced as an end product will depress the environment pH slightly unless as excess of alkalinity is available to keep the pH from dropping (Muyima *et al.*, 1997).

2.4.2 Heterotrophic Bacteria in Organic Wastewater

In wastewater treatment, bacteria are the one responsible for degradation of organic matters (Liu, 2007). The primary consumers of organic waste are the heterotrophic bacteria since organic carbon is the most important energy source for them (Ritmann and McCarty, 2001). The heterotrophic bacteria dominate the biocenosis (mixed culture of organisms) and make up more than 98% of the active organism mass in the biological treatment of wastewater system (Wentzel *et al.*, 2003; Pike and Curds, 1971). These organisms respire in the aerobic conditions and break down the waste into minerals, gases and water. For example, ammonia is converted into nitrates (Williams and Paul, 2002). Important genera of heterotrophic bacteria include *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Citromonas*, *Flavobacterium*, *Pseudomonas* and *Zooglea*. The ultimate success of wastewater treatment depends upon the selection of metabolically capable microorganisms and the efficient separation of those organisms from the treated effluent (Ritmann and McCarty, 2001).

2.5 Detection and Identification of Bacteria

2.5.1 Polymerase Chain Reaction (PCR)

The double-stranded sequence of the complementary nature of DNA is the basis for the methodology known as polymerase chain reaction or PCR. PCR can be used to amplify bacterial rRNA allowing specific detection and identification at the species level and in some cases to the strain or biovar level. PCR amplification of DNA and RNA (Mullis *et al.*, 1994) has become a key protocol in many biological laboratories. This DNA polymerase catalyzed reaction allows repeated synthesis of specific DNA sequences. A typical cycle involves denaturing the double stranded DNA into single strand, annealing short oligonucleotide primers to the single strands and extending the primer sequences using a DNA polymerase to complete the synthesis strands complimentary to the original single strands. This cycling is repeated to obtain an exponential increase in the copies of the original DNA strand (Pepper and Gerba, 2005).

i. Denaturation

Denaturation involves separation of the two strands by heating at 95 °C to 100 °C, breaking the hydrogen bonds which bind the two strands of DNA together.

ii. Primer Annealing

The strands will once again hybridize by forming hydrogen bonds between the two strands at 55 °C.

iii. Extension

The Taq polymerase recognizes the primed strands and attach to the double stranded portion. The optimum temperature for the Taq polymerase is at 72 °C. At this temperature the Taq polymerase start constructing the complementary strands using the four deoxynucleotides (deoxyadenosine 5'-triphosphate (dATP), deoxycytosine 5'-triphosphate (dCTP), deoxyguanosine 5'-triphosphate (dGTP), and deoxythymidine 5'-triphosphate (dTTP) which are also present in the solution.

2.5.2 Sequence Analysis

In recent years identification of unknown bacterial isolates has been enhanced via PCR amplification of 16S rDNA gene sequencing and subsequent sequence analysis. Portions of the 16S rDNA gene sequences within bacterial are identical in all known bacteria. Conversely, between these holomogous regions there are unique sequences of DNA associated with specific bacteria. Universal primers have been designed that anneal to the conserved rDNA gene sequences, allowing amplification of the unique sequences within the conserved regions. Aliquots of amplified gene that result from PCR with these universal primers can be sequenced inexpensively in commercial laboratories. Once the sequence of analysis is known, it can be used to identify the original bacterial source of DNA at the genus or species level. Computerized sequence databases have been compiles on large numbers of bacterial species. This allows for a comparison of an unknown rDNA gene sequence product with the known bacterial sequences that exist in the data base. Several computer software sequence analysis programs are available to aid in sequence searches. One such data

base is the Basic Local Alignment Search Tool (BLAST) which is provided by the National Centre for Biotechnology Information (NCBI) and is available on the internet; this program allows researches to perform sequence comparisons between known rDNA sequences and the amplified rDNA sequence of interest (Pepper and Gerba, 2005).

CHAPTER 3
METHODOLOGY

3.1 Sago Effluent Collection

Effluent sampling took place in a commercial sago mill, Nitsei Sago Industries Pte. Ltd. in Mukah, Sarawak. Five hundred mL raw sago effluent was collected from an open drain, 1 m from the decanters. Another 50 L raw sago effluent was collected in a sterile Scotch bottle (modified from Apun *et al.*, 2000).

3.2 Isolation of Bacteria from Sago Effluent

3.2.1 Serial Dilution

Ten of 100 mL of fresh sago effluent was put into a test tube. This test tube served as the undiluted sample. By using sterilized 10 mL pipette, 1 mL of the sago effluent from the undiluted sample was transferred into a test tube. Nine mL of sterilized distilled water was added to make up the volume to 10 mL. The test tube was shaken to mix them well. The test tube was the 10^{-1} dilution factor. This step was continued until dilution factor 10^{-10} is obtained (Cappucino and Sherman, 1987).

3.2.2 Cultivation of bacteria

By using 1 mL sterilized pipette, 0.1 mL of the each dilution was pipette out and it was spread evenly onto nutrient agar. Three replications were prepared. All the nutrient agar plates were incubated for 24 hours at room temperature. Streak plates

were prepared based on number of colonies present after the incubation period. The plates were incubated for 24 hours at room temperature (Cappucino and Sherman, 1987).

3.2.3 Starch Degradation Test

Eleven isolates were single – streaked individually in starch agar plates. The plates were incubated for three days at room temperature. Observation on starch hydrolysis was carried out from the first day of incubation. The plates were flooded with iodine solution. Clear zone formed around the single streaked colony was measured using dissecting microscope. Strain showing starch hydrolysis activity was subjected to identification steps (Cappucino and Sherman, 1987).

3.3 Identification of Starch Degrading Bacteria

3.3.1 DNA Extraction

Two selected isolates labeled as B1 and B2 were cultured individually in nutrient broths which were shaken overnight in waterbath. Each cultured nutrient broth was poured into 1.5 µL tube and centrifuged at 8,000 g for 3 minutes. The supernatant was discarded. The cell pellet formed at the base of the tube was washed with 400 µL TE solution and centrifuged at 8,000 g for 3 minutes. Another 200 µL of TE solution added into the tube and it was vortexed for few seconds until the cell pellet dissolved. Then, 200 µL of phenol added into the tube and it was vortexed for 60 seconds followed by centrifugation at 13,000 g for 5 minutes. Top aqueous formed was slowly transferred into a new 1.5 mL Eppendorf tube. Two hundred µL of

chloroform was added into the tube and vortexed for 60 seconds followed by centrifugation at 13,000 g for 5 minutes. The aqueous phase formed contained extracted DNA of the isolate was transferred into a new 1.5 mL Eppendorf tube. Ten µL of the extracted DNA was mixed slowly with 3 µL of six times loading dye and the mixture was dripped into 1% of agarose gel. Gel electrophoresis was carried out at 100 V for 60 minutes. Bands produced were observed using gel imaging machine (modified from Pepper and Gerba, 2005).

3.3.2 Polymerase Chain Reaction (PCR)

DNA extracts left was scaled up to the amount of 50 µL based on volumes of components of master mix provided by PCR Protocol (Table 1). The master mix was prepared for three aliquots of negative control, B1and B2 strains. The total volume of master mix which was 129 µL was transferred into three 0.2 µL tubes. Therefore, there was 43 µL of master mix in each tube. Then, 7 µL of sterilized distilled water, DNA extracts of B1 and DNA extracts of B2 was transferred into their respective tubes labeled earlier. Each tube contained 50 µL of aliquot. The tubes were placed in a PCR machine (XP Thermal Cycler). The PCR operated for 2 hours and 30 minutes. The aliquots produced are called PCR products. Ten µL of each aliquot were slowly mixed well with 3 µL of six times loading dye. The mixture was loaded into an agarose gel. Gel electrophoresis was carried out as foresaid. Bands produced in the gel were observed using gel imaging machine (modified from Pepper and Gerba, 2005).

Table 1: PCR Protocol for master mix preparation

Component	Volume (µL)	
	Standard	3X
dH_20	26.75	80.25
10X PCR Buffer	5	15
25 mM $MgCl_2$	5	15
F primer	2.5	7.5
R primer	2.5	7.5
dNTP	1	3
Taq polymerase	0.25	0.75
Template DNA	7	21
	Master mix: 50	Master mix: 150

*Note: Each standard volume of master mix was prepared for three-timed of its volume

3.3.3 DNA Purification

Forty µL of PCR products left in each tube was transferred into a 1.5 mL tube. Four µL of sodium acetate and 100 µL of absolute ethanol were added into each tube. The tubes were invertly shaked and kept in ice flakes for 30 minutes. Then, the tubes were centrifuged at 13,000 g for 15 minutes at 4 °C. The supernatant was discarded. Two hundred µL of 70% ethanol was added into each tube and centrifuged at 13,000 g for 5 minutes at 4 °C. Next, the supernatant was discarded and the tube left air-dried. After that, 15 µL sterilized distilled water was added into each tube, tapped and short spin. Five µL of the mixture was mixed slowly with 3 µL of six times loading dye. Gel electrophoresis and imaging of bands produced were carried as steps foresaid (modified from Pepper and Gerba, 2005).

3.3.4 DNA Sequencing

B1 and B2's 10 µL of mixture left was sent to 1st BASE Laboratories Sdn. Bhd. in Kuala Lumpur for DNA sequencing (modified from Pepper and Gerba, 2005).

3.4 Sago Effluent Treatment

3.4.1 Preparation of Inoculums

A loop of identified culture was inoculated individually in sterilized 100 mL of nutrient broth. The flask was kept in a shaker at 120 rpm for 24 hours at room temperature. One percent of each suspension was used as inoculums for the sago effluent treatment (modified from Ayyasamy *et al.*, 2008).

3.4.2 Aerobic Treatment

One thousand five hundred mL sago effluent was poured into four sterilized 2000 mL beakers. There were four sets of treatment system (Table 2) with three replications for each treatment. The treatment progressed for ten days at room temperature. 250 mL of treated sago effluent was sampled on the first, fourth, seventh and eleventh day of treatment to analyze its parameter (modified from Ayyasamy *et al.*, 2008).

Table 2: Experimental set-up for aeration treatment of sago effluent with selected bacteria

Treatment	1	2	3	4
Sago effluent (mL)	1500	1500	1500	1500
Treatment	Aeration	Aeration	Aeration	Aeration
Pre-aerated (min)	-	30	30	30
Pre-set pH	-	6.5 – 7.0	6.5 – 7.0	6.5 – 7.0
Inoculation of bacteria suspension	-	3 ml of sterilized NB	3 ml of NB cultured with B1	3 ml of NB cultured with B2

3.5 Analytical Method

Effluent samples collected were analyzed for parameters such as pH, BOD, COD and TSS to investigate the effectiveness of degradation of sago effluent by selected bacteria. These analyses were based on APHA (2005).

3.5.1pH

Effluent samples' pH were measured using Lp 115 Mettler Toledo pH meter.

3.5.2 Biological Oxygen Demand (BOD)

Two mL effluent sample was diluted with distilled water up to 100 mL and slowly poured into a BOD bottle. Three mL-sized BOD nutrient buffer pillow was diluted with distilled water to 3 L in volume. Then, the solution was poured into the BOD bottle until the water level reached the bottle lid. The mixture was aerated using BOD aerator probe (YSL 5905) for 15 minutes and the initial dissolved oxygen was measured using DO meter (YSL 52). The BOD bottle was placed in an incubator for five days at 20 °C. After five days, the final DO was measured again. BOD value was calculated as formula below:

$$BOD_5\,(mg/L) = (DO_0 - DO_5) / P \qquad \text{(Eq. 3.5.2)}$$

3.5.3 Chemical Oxygen Demand (COD)

Twenty mL effluent sample was added with 10 mL 0.00417 M potassium dichromate, 30 mL concentrated sulphuric acid containing silver sulphate and several bits of anti bumping agent into Erlenmeyer flask and mixed well. The flask was attached to the condenser, refluxed for 2 hours and let cooled for a while after reflux. Then, the mixture was diluted with distilled water to 150 mL in volume. Three drops of ferroin was added to the diluted mixture until the colour changes sharply from blue-green to reddish-brown. Finally, the mixture was titrated with 0.25 M FAS until the mixture colour change back to blue-green. The COD value of each effluent sample was calculated as formula below:

$$\text{COD (mg/L)} = [(a-b) \times c \times 8000] / v \qquad \text{Eq. (3.5.3)}$$

3.5.4 Total Suspended Solid (TSS)

A glass fiber filter paper was weighed for its initial weight. One hundred mL of effluent sample was passed through a glass fiber filter paper using a vacuum filtration. Then, the filter paper was oven dried at 103 °C for 2 hours. Drying was continued until the weights of filter papers are constant. TSS value was calculated as formula below:

$$\text{TSS (mg/L)} = [b - a / v] \times [1000 \text{ (mg)}/1 \text{ (g)}] \times [1000 \text{ (mL)}/1 \text{ (L)}] \qquad \text{Eq. (3.5.4)}$$

3.6 Statistical Analysis

The experimental design used for this study is Completely Randomized Design (CRD). All data were subjected to Analysis of Variance (ANOVA). The effects of interaction between period of treatment and treatment types were analyzed using factorial in CRD.

CHAPTER 4

RESULTS

4.1 Isolation of Starch Hydrolyzing Bacteria

Two of the 11 bacteria strains isolated from sago effluent showed starch hydrolyzing activity. The two isolates were labeled as B1 and B2. B1 isolate recorded clear zone at mean of 1.62±0.04 mm whereas B2 produced clear zone at mean of 0.62±0.20 mm in diameter. Next, B1 and B2 were subjected for identification of genus and species.

4.2 Identification of Starch Hydrolyzing Bacteria

4.2.1 Polymerase Chain Reaction (PCR)

B1 and B2 DNAs were extracted by mean of phenol/chloroform method. Observation of bands produced by these isolates was carried out by running the PCR products on 1% agarose gel for 60 minutes (Figure 1 and Figure 2).

Figure 1: A band of B1 isolate formed in PCR. (-ve) served as control

Figure 2: A band of B2 isolate formed in PCR. (-ve) served as control

4.1.1 DNA Purification

10 µL of 50 µL scaled up PCR products of B1 and B2 were dropped into one percent agarose wells. The DNA purification was carried out by electrolysis mean at 100 V for 60 minutes.

Figure 3: Bands of B1 and B2 isolates formed in DNA purification step. (X) = unknown strains

4.2.3 DNA Sequencing

DNA concentrations (ng/μL) of B1 and B2 were calculated (Table 3). 10 μL pure DNA of each isolate and the data of DNA concentration were sent to 1st BASE Laboratories Sdn. Bhd. in Kuala Lumpur for DNA sequencing. 16S rDNA sequences obtained from the company were uploaded in Applied Biosystem Sequence Scanner Vol. 0.1.

Table 3: DNA concentrations of B1 and B2 isolates

Sample	ng/5 μL	ng/ μL
B1	244.04	48.808
B2	196.42	39.284

Table 4 shows 16S rDNA nucleotide sequences obtained from BLAST program. There are 914 and 844 letters of nucleotide sequences of B1 and B2 respectively.

Table 4: 16S rDNA nucleotide sequences

Isolate	Nucleotide sequences
B1	GCGGCTGGCTCCTTACGGTTACCCCACCGACTTCGGGTGTTACAAA CTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCAT GTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAATGATTTTATG AGATTAGCTTCACCTCGCGGCTTCGCAACCCTTTGTATCATCCATT GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGAC GTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAG TGCCCAACTTAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTG CGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCAT GCACCACCTGTCACTTTGTCCCCCGAAGGGGAAAGCTCTATCTCTA GAGTGGTCAAAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCT TCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAAT TCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCT TAATGCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACT TAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTG TTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAA AGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACC GCTACACGTGGAATTCCGCTTTCCTCTTCTGCACTCAAGTTCCCCA GTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGA CTTAAGGAACCGCCTGCGCGCGCTTTACGCCCAATAATTCCGGA
B2	GCTAGCTCCTTACGGTTACTCCACCGACTTCGGGTGTTACAAACTC TCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCA CCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTA GGCGAGTTGCAGCCTACAATCCGAACTGAGAATGGTTTTATGGGA TTGGCTTGACCTCGCGGTCTTGCAGCCCTTTGTACCATCCATTGTAG CACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCAT CCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCC AACTAAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGG ACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCAC CACCTGTCACTCTGTCCCCCGAAGGGGAACGCTCTATCTCTAGAGT TGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGA ATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCT TTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAAT GCGTTAGCTGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGC ACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTG CTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAAAAAGCC GCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTA CACGTGGAATTCCGCTTTTCTCTTCTGCACTCAAGTTCCCCAGTTTC CAATGACCCTCCACGGTT

B1 sequences were found to be having 100% significant alignments with *Bacillus koreensis* AY667496 at 1782 score bits while B2 sequences were found to have 100% significant alignments with *Bacillus megaterium*, *Bacillus subtilis* and *Bacillus flexus* at 1673 score bits.

4.3 Effect of *Bacillus* spp. on Sago Effluent Treatment

Statistical analysis showed there was an interaction of treatments and period of treatments for each parameter tested. There were significant differences between days of treatment at $p \leq 0.05$, Tukey's Test. At least one treatment showed significant difference within days of treatments for each parameter. Treatment 3 and 4 were compared in order to evaluate their efficiency in reducing parameter tested. Reduction of value indicates removal of the parameter.

4.3.1 Biological Oxygen Demand (BOD)

For determination of BOD of treated effluent, there was a significant difference between Treatment 3 and 4 within first day of treatment. Treatment 3 showed the lowest BOD value at 217 mg/L within one day of treatment indicating 38.00 mg/L of BOD removal after 24 hours treatment whereas Treatment 4 only showed 25.00 mg/L of BOD removal (Table 5).

Table 5: Biological Oxygen Demand of treated sago effluent

Period	Treatment			
	T1	T2	T3	T4
D0	270.00±15.00^{a}	261.67±2.57^{a}	255.00±1.50^{a}	255.00±5.10^{a}
D1	265.00±5.00^{a}	252.50±2.50^{ab}	217.00±1.32^{c}	230.18±5.26^{b}
D4	263.00±10.40^{a}	249.68±4.54^{ab}	225.18±5.00^{b}	235.83±5.53^{b}
D7	258.00±6.20^{a}	246.50±6.06^{b}	225.00±5.00^{b}	238.50±5.06^{b}
D11	255.17±5.26^{a}	246.18±6.33^{b}	223.33±3.06^{b}	235.33±6.03^{b}

*Note: D = Day. (Means within rows with the same alphabets are not significant different at 0.05 level of probability, Tukey's Test)

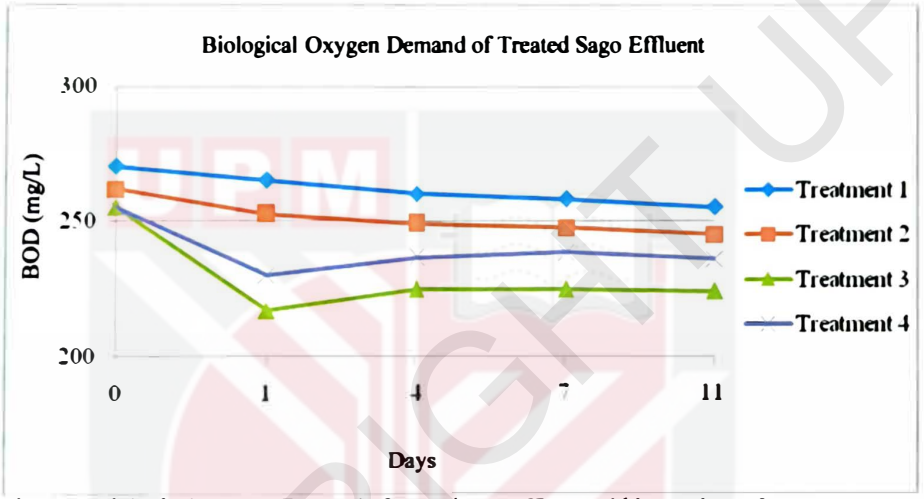

Figure 4: Biological Oxygen Demand of treated sago effluent within ten days of treatment

4.3.2 Chemical Oxygen Demand (COD)

There was no significant difference between Treatment 3 and 4 within ten days of treatment. However, Treatment 3 showed the lowest COD value at 413.67 mg/L within one day of treatment compared to Treatment 4, indicating 42.00 mg/L of COD removal after 24 hours treatment whereas Treatment 4 only showed 30.00 mg/L of COD removal (Table 6).

Table 6: Chemical Oxygen Demand of five days of treated sago effluent

Period	Treatment			
	T1	T2	T3	T4
D0	465.00±5.00[a]	461.38±0.78[a]	473.17±5.22[a]	479.00±5.00[a]
D1	460.17±5.26[a]	445.83±10.0[b]	413.67±1.75[b]	425.17±5.25[b]
D4	468.33±3.25[a]	452.33±0.23[ab]	420.17±3.25[b]	430.17±5.00[b]
D7	467.33±7.09[a]	454.50±6.50[ab]	425.00±5.00[b]	435.33±4.75[b]
D11	465.17±5.25[a]	450.18±2.75[ab]	420.00±6.00[b]	436.50±3.00[b]

*Note: D = Day. (Means within rows with the same alphabets are not significant different at 0.05 level of probability, Tukey's Test)

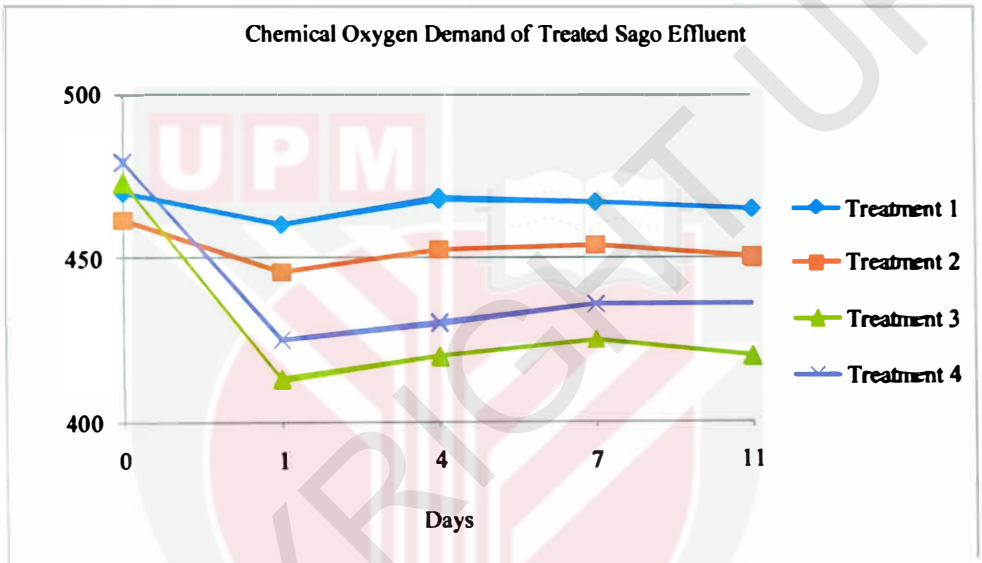

Figure 5: Chemical Oxygen Demand of treated sago effluent within ten days of treatment

4.3.2 Total Suspended Solid (TSS)

There is significant difference between Treatment 3 and 4 within ten days of treatments except on the second, fourth and tenth days. TSS values for both treatments gradually increase until the last of treatment (Table 7).

Table 7: Total Suspended Solid of treated sago effluent

Period	Treatment			
	T1	T2	T3	T4
D0	177.45±2.81^{e}	788.33±2.825^{e}	788.23±6.04^{c}	794.48±2.00^{e}
D1	825.22±4.90^{b}	1898.67±15.05^{a}	930.67±3.02^{bc}	1453.16±1.65^{b}
D4	706.73±1.20^{d}	1048.00±7.915^{d}	1004.57±12.5^{ab}	1595.67±3.96^{b}
D7	763.80±2.88^{c}	1327.33±100.2^{c}	1100.55±3.88^{a}	1569.60±95.6^{c}
D11	861.54±1.23^{a}	1621.33±100.2^{b}	1150.27±1.99^{a}	1628.87±19.5^{a}

*Note: D = Day. (Means within rows with the same alphabets are not significant different at 0.05 level of probability, Tukey's Test)

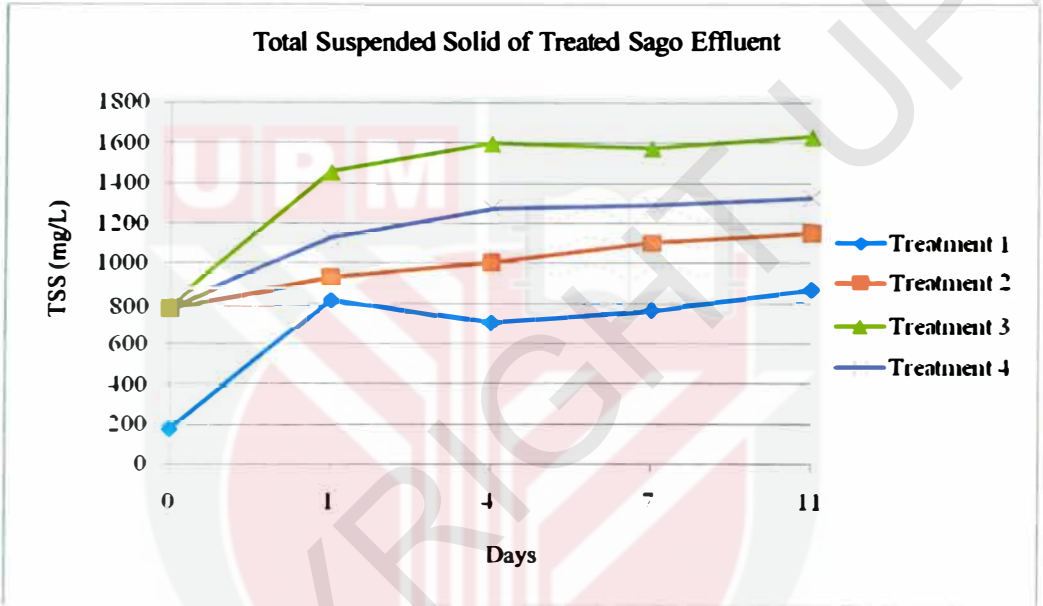

Figure 6: Total Suspended Solid of treated sago effluent within ten days of treatment

4.3.2 pH

There was no significant difference in pH value between Treatment 3 and 4 within the treatment period. For both treatments, the pH values showed gradual increment until the last day of treatment except on the fourth day which values showed some decrease (Table 8).

Table 8: pH of treated sago effluent

Period	Treatment			
	T1	T2	T3	T4
D0	3.93±0.70^{d}	6.40±0.48^{e}	6.42±0.57^{e}	6.40±0.73^{e}
D1	3.86±0.70^{e}	7.22±0.48^{b}	7.27±0.57^{c}	7.27±0.73^{c}
D4	4.17±0.70^{c}	6.84±0.48^{d}	6.93±0.57^{d}	6.98±0.73^{d}
D7	5.00±0.70^{b}	7.15±0.48^{c}	7.52±0.57^{b}	7.73±0.73^{b}
D11	5.71±0.70^{a}	7.82±0.48^{a}	8.05±0.57^{a}	8.50±0.73^{a}

*Note: D = Day. (Means within rows with the same alphabets are not significant different at 0.05 level of probability, Tukey's Test)

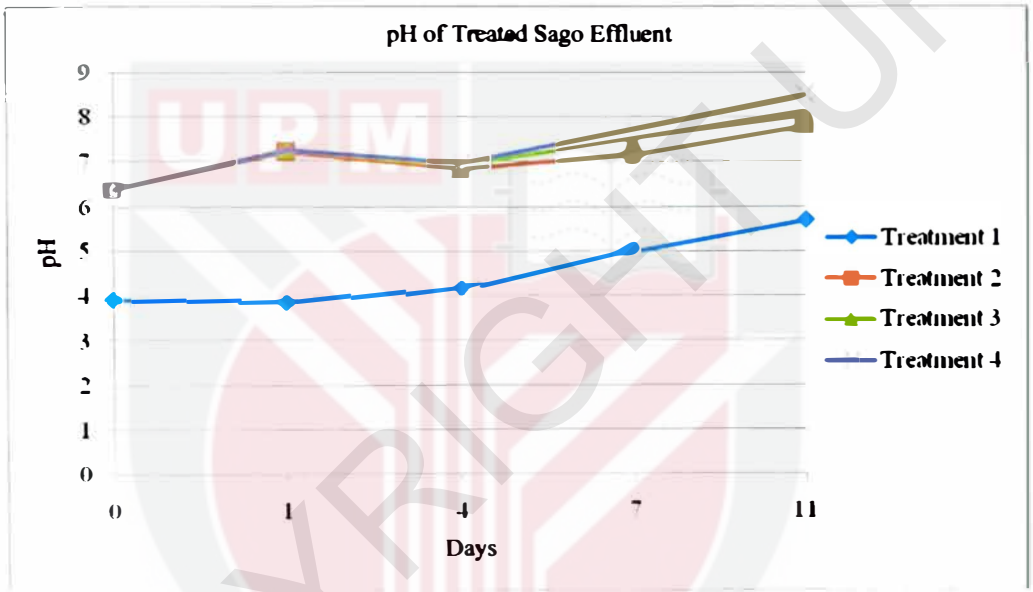

Figure 7: pH of treated sago effluent within ten days of treatment

CHAPTER 5

DISCUSSION

5.1 Isolation and Identification of Bacteria

Two isolates from sago effluent were identified as *Bacillus* spp. Comparative 16S rDNA gene sequence analysis (BLAST) showed that both isolates formed a distinct phyletic line within the genus *Bacillus*. B1 strain shared 100% identity with *Bacillus koreensis* AY667496 at 1782 score bits and 92.76% identity with *B. subtilis* and *B. megaterium*. On the other hand, B2 strain was found to have 100% identity with *B. megaterium*, *B. subtilis*, and *B. flexus* at 1673 score bits. Therefore, B1 isolate was known to be *B. koreensis* whereas B2 isolate could be *B. megaterium*, *B. subtilis* or *B. flexus*.

B1 and B2 strains showed positive result in starch hydrolyzing activity at 1.62 and 0.62 mm of clear zone respectively. These results were similar to Apun *et al.* (2000) which reported isolation of *B. amyloliquefaciens* from sago effluent. *Bacillus amyloliquefaciens* showed positive results in starch, catalase and citrate utilization biochemical test. *Bacillus* was one of the five genera found in sago effluent derived from tapioca processing factory (Ayyasamy *et al.*, 2008). The isolate utilized starch and grown well on starch agar and produced 20 mm diameter zone around the colonies. This is due the presence of either alpha or beta amylase enzymes.

According to Muyima *et al.* (1997), *Bacillus* is classified under family Bacillacea and can be aerobic, facultative aerobic and anaerobic. Therefore, *Bacillus* spp. has

the ability to survive in conditions different levels of oxygen. It synchronizes with sago effluent treatment which was conducted in aerobic condition.

Dawkar *et al.* (2008) isolated and identified *Bacillus* spp. from textile effluent contaminated soil which has a potential in biodegradation of disperse textile dye Brown 3REL. Similar to present study, bacterial identification was carried out via 16S rDNA analysis too. Sukumar (2007) also reported *Bacillus* spp. isolates found in textile dye and the bacteria showed maximum decolourization of dyes. The same result was reported by Pourbabaee (2007) whom carried out aerobic decolourization and detoxification of dispersed textile dyes by *Bacillus* spp. isolated from the effluent. On the basis of 16S rRNA gene sequencing, *Bacillus* spp. was found to be present in effluent treatments plants (ETP) of pesticides and pharmaceutical industries. It is believed can be exploited for efficient and functioning and maintenance of ETPs (Rani *et al.*, 2008).

5.2 Effect of *Bacillus* spp. in Sago Effluent Treatment

5.2.1 Biological Oxygen Demand (BOD)

Table 7 shows the comparison of percentage BOD removal after treatment of sago effluent. BOD of both treatments decreased within one day of treatment. After one day of treatment, the value increased gradually. Overall after treatment, Treatment 1 showed the highest percentage removal of BOD about 14.9% BOD removal compared with Treatment 2 at 9.8%.

This is attributed to high activity of *B. koreensis* to degrade organic matter which only effective in 24 hours. Furthermore, *B. koreensis* showed larger clear zone diameter of starch degradation in biochemical test. Thus, its ability to degrade starch may contribute to its efficiency in reducing BOD of treated sago effluent. Presence of starch residues and high carbon to nitrogen ratio in sago effluent may stimulate the growth of bacterial species and influences the degradation of sago effluent. Ayyasamy *et al.* (2008) reported 58% of BOD removal in treated sago effluent derived from tapioca processing factory. The effluent was treated with bacterial consortium of *Alcaligenes*, *Bacillus* and *Corynebacterium* showed reduction amount of BOD from 2,400 to 1,010 mg/L.

Table 9: Comparison of percentage of BOD removal after one day of treatment

Treatment	% BOD removal
T3	14.90
T4	9.80

*Note: T3 = *B. koreensis*, T4 = *Bacillus* spp.

5.2.2 Chemical Oxygen Demand (COD)

Results showed 12.68 and 11.27% COD removal in Treatment 3 and 4 respectively (Table 10). Similar to effects on BOD removal, Treatment 3 showed higher removal of COD compared to Treatment 4. This is due to *B. koreensis* has better ability in biologically and chemically oxidized organic matter. Higher COD removals were reported in previous study. In aeration treatment of filtered sago effluent, about 96% COD reduction was reported (Bujang *et al.*, 2004). Compared to present study which treatment carried out ten days, the aeration treatment of filtered sago effluent was

carried out within 35 days. Therefore, much higher COD removal is recorded. It could be attributed to the amount of dissolved oxygen present in treatment tanks. The more the dissolved oxygen, the higher the degradation rates thus the higher the COD being reduced. This principle implies to BOD removal too. According to Ayyasamy *et al.* (2008), 73% COD removal was recorded. The COD value was reduced from 3, 600 to 916 mg/L for tapioca derived-sago effluent treatment by bacterial consortium. There was 97.04% COD removal recorded in sago effluent treatment with 0.3 mg/L Bak-Wira MP300, a commercial microbial amendment which consists of Class 1 bacteria (mainly *Bacillus* strains) and enzymes (Jampang, 2005). Higher COD removals in these studies were due to higher and better degradation by mixtures of high capability bacteria in degrading sago effluent as the ultimate success of wastewater treatment depends upon the selection of metabolically capable microorganisms and the efficient separation of those organisms from the treated effluent (Muyima *et al.*, 1997). Treatment of sago effluent by *Rhodopseudomonas palustris* B1 showed 77% of COD removal. Higher COD removal compared to present study was due to lacking of stimulators for enhancing bacterial metabolism since 0.1% of yeast extract was added into treatment tank to enhance degradation processes (Vikineswary *et al.*, 1997).

Table 10: Comparison of percentage of COD removal after one day treatment

Treatment	% COD removal
T3	12.68
T4	11.27

*Note: T3 = *B. koreensis*, T4 = *Bacillus* spp.

5.2.2 Total Suspended Solid (TSS)

TSS in Treatment 3 and 4 increased as much as 15 and 45% within 24 hours of treatment. This is due to rapid replications of bacteria and other microorganisms present in both treatment tanks. There was no reduction in TSS value up to the tenth days of treatment. Similar to results reported by Ayyasamy *et al.* (2008), the TSS value was found to increase from 4,700 to 14,600 mg/L after 72 hours of treatment. The high amount of TSS is due to high bacterial biomass and formation sludge as by-product of bacterial degradation. In contrast, Jampang (2005) reported 62.3% TSS reduction in sago effluent treated with 0.3 mg/L Bak-Wira MP300 in ten days of treatment. Meanwhile, Bujang *et al.* (2004) recorded 41% TSS reduction in aeration treatment of filtered sago effluent.

Table 11: Comparison of TSS percentage after one day of treatment

Treatment	% TSS Increment
T3	15
T4	45

*Note: T3 = *B. koreensis*, T4 = *Bacillus* spp.

5.2.3 pH

For Treatment 3 and 4, the pH value did not show significant difference. Bacteria started to oxidize the organic matter in sago effluent and converted it to CO_2 and H_2O. The CO_2 produced as an end product will depress the environment pH slightly

and the effluent tends to be alkaline. This is because the dissolved CO_2 will form carbonic acid, H_2CO_3 which reacts slightly and reversibly in effluent to form a hydronium ion (H_3O^+) and an alkaline bicarbonate ion (HCO_3^-) aids to alkalinity of effluent at the end of treatment period (Gustafson *et al.*, 2002).

CHAPTER 6

CONCLUSION

Two starch degrading bacteria isolated from fresh sago effluent were identified as *Bacillus koreensis* and the other isolate could be *B. megaterium*, *B. subtilis* or *B. flexus*. From the study, results have shown that it is possible for aeration treatment of filtered sago effluent to undergo degradation process assisted by *B. koreensis* and *Bacillus* spp. isolated from the sago effluent itself. Sago effluent with *B. koreensis* showed higher BOD and COD removal compared to the effluent treated with *Bacillus* spp. at 14.90 and 12.68% respectively within 24 hours of treatment. This is attributed to *B. koreensis* in degrading starch better than *Bacillus* spp. There was no reduction in TSS value and pH value increased that the effluent was alkaline at pH 8. This could be attributed to the adaptability of the bacteria to the new environment, lack of dissolved oxygen at optimal level, lack of nutrients to stimulate bacterial metabolism and degradation activity, unknown bacterial concentration in inoculums, short treatment period and others. The treated sago effluent still does not meet the requirements of standard discharge effluent Standard B in Environmental Quality (Sewage and Industrial Effluent) Regulations, 1979. Sludge formed at the end of treatment period can be used as composting materials to produce a stable compound called humus.

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APPENDICES

Eq. (3.5.2) : $BOD_5\ (mg/L) = (DO_0 - DO_5) / P$

where;

BOD_5 = Biological Oxygen Demand for 5 days

DO_0 = initial dissolved oxygen (mg/L)

DO_5 = final dissolved oxygen (mg/L)

P = decimal fraction of sample used

Eq. (3.5.3) : $COD\ (mg/L) = [(a - b) \times c \times 8000] / v$

where;

a = FAS (mL) used for blank

b = FAS (mL) used for sample

c = molarity of FAS

v = volume of sample (mL)

Eq. (3.5.4) :

$$TSS\ (mg/L) = [b - a / v] \times [1000\ (mg)/1\ (g)] \times [1000\ (mL)/1\ (L)]$$

where;

b = final weight of filter paper (g)

a = initial weight of filter paper (g)

v = volume of sample (mL)

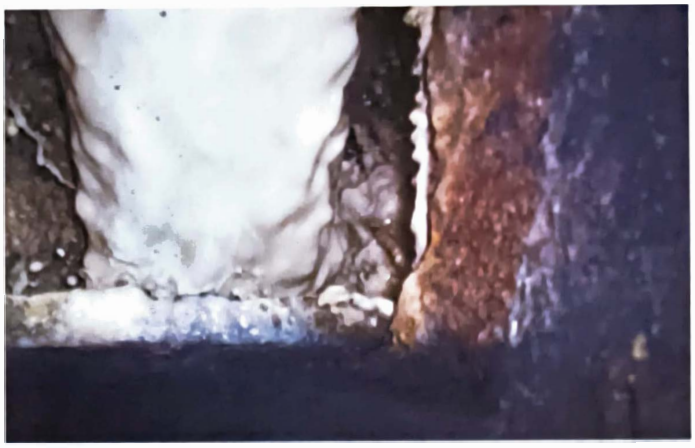

Plate 1: Open drain releasing sago effluent from the decanters to the river

Plate 2: Experimental set-up of sago effluent treatment

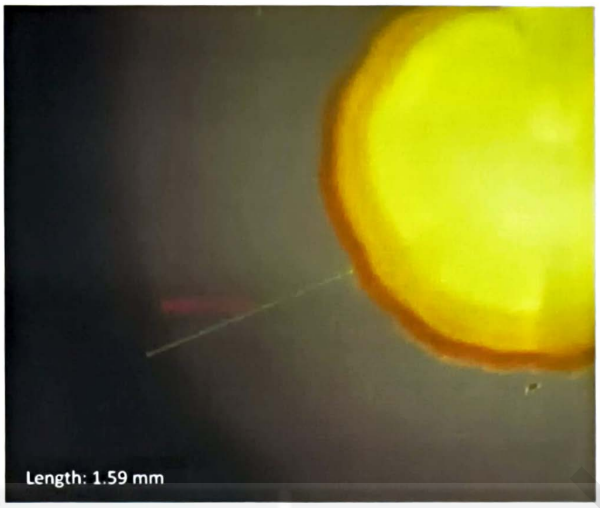

Figure 8: B1R1 (*Bacillus koreensis*) showing clear zone

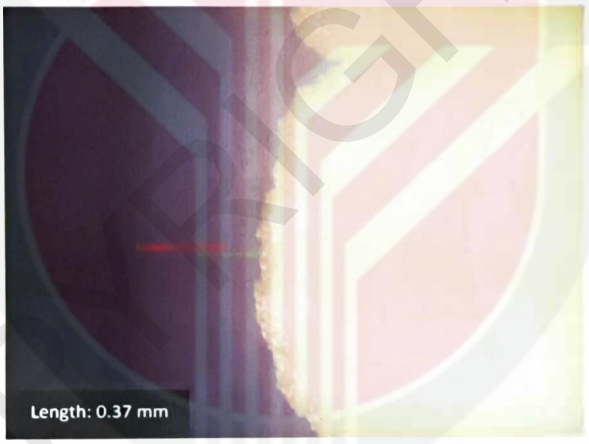

Figure 9: B2R3 (*Bacillus* sp.) showing clear zone

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "**Sago Effluent Treatment with *Bacillus* spp.**" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.

Nurul Wahida Binti Hani

Date: 4 May 2009