UTILIZATION OF SOLVENT WASTE MIXTURE FROM SEMICONDUCTOR INDUSTRY AS A CARBON SOURCES FOR ORGANIC SOLVENT TOLERANT BACTERIA (OSTB)

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ABSTRACT

Increasing of organic solvent waste contributed as one of the most critical environmental problems. Huge amount of solvents have been applied in the industrial process, but it is not followed by a good waste treatment. Up to our knowledge there is less effort that has been conducted in exploring the solvent waste mixtures treatment by biological process approaches specifically by applying gram's positive organic solvent tolerant bacteria (OSTB). This research has ventured a solvent waste mixture. The study aims to identify the ability of OSTB survival within organic solvent and solvent waste mixture by OSTB inoculation. Strain of OSTB named as Bacillus subtilis, BSIAs was applied in the study. The growth of this OSTB in different concentration of synthetic solvent waste mixture and in real solvent waste mixture was monitored and measured. There are three different concentrations of synthetic solvent was used as a media which are 20%, 10% and 5% of isopropyl alcohol (IPA) for testing the growth of Bacillus subtilis BSIAs. The 5% isopropyl alcohol is suitable for Bacillus subtilis BSIAs growth. After 14 hours of growth, distillation process is used to separate the remaining solvent from the mixture. It was found that, the volume after biological treatment is reduced by 1 mL from the initial volume of solvent before the biological treatment. This OSTB also utilized solvent in 1% concentration of real solvent waste mixture within 120 hours. The findings reveal gram-positive Bacillus subtilis, BSIAs has the ability to utilized synthetic solvent and solvent waste mixture as their carbon source.

Keywords: *Bacillus subtilis*, Gram-positive, Organic solvent tolerant bacteria (OSTB), Solvent waste mixture.

INTRODUCTION

Solvent waste came from the chemical or cleaning processes from certain industry and it mostly contaminated with the unknown substances and in mixed condition. This waste had been classified as scheduled waste in the book of Environmental Quality Act 1974 (EQA 1974) and had its own regulation for managing and handling this hazardous waste. In year 2008, about 250 thousand tons hazardous waste had been produced at Portugal and managed by the Hazardous Industrial Waste (HIW) [1]. Department of Environmental (DOE) Malaysia reported about 1,438,380 tons of scheduled waste been generated at the same year including the solvent waste that contribute about 41,955 tons. These statistics show that the disposals of scheduled waste are high especially in Malaysia even this waste has been manage by several facilities like incineration, landfill, solidification and physico-chemical.

Semiconductor industry have disposed their waste that been classified as scheduled waste consists of various type of organic solvents involving from their manufacturing highly complex process [2]. Organic solvents have been used in the washing and cleaning processes and finally became the solvent waste mixture when it collected before disposal. This solvents give adverse effects to environment because it's can cause various types of pollution. It was characterized as hazardous waste because they are very toxic, high volatility and ignitability. Furthermore, solvent waste contains a lot of compound after using it's as a cleaning agent. Besides, this solvent waste can migrate into the soil and groundwater rather than polluted into the air [3]. Incineration was used in Malaysia that where solvent waste was transfer for further treatment but this treatment give secondary air pollution [4].

Besides sending the solvent waste for incineration, distillation process was applied to recover the solvent back. More than 90% solvent recovered from this process and it is the most common treatment used in the chemical industry [5]. On the other hand, biological treatment has been alternative method for treating the solvent waste by using bacteria. There are several types of bacteria that that tolerant to the solvent waste and these bacteria can utilize the solvent waste as a substrate and become less toxic product [6]. Ability of bacteria to utilize high concentration of solvent gives cheaper and smaller waste treatment process to industry and these methods is an effective technique of converting solvent waste [7,8].

In previous study, there are a lot of recovery had been done to recover this valuable compound rather than just disposed it to the environment by using distillation process. Based on the theory, the distillation process can recovery about 90% and above from its sources [5]. But, it's normally done on the higher volume of solvent inside the solvent waste with the lower volume of water. While, recovery on the high volume of water than solvent inside one mixture and also recovery the solvent waste mixture that contain a lot of compound inside it not really efficient if using the distillation process.

Introducing solvent waste to bacteria as their carbon sources is one of the objectives of this study. Organic solvent tolerant bacteria (OSTB) were in the group of extremophilic microorganisms. Most of the bacteria in this group are capable

to thrive in the organic solvents condition [6]. No previous reports exist concerning using organic solvent tolerant bacteria (OSTB) towards real solvent waste mixture to date in the literature. A recent study shows some of the bacteria adapted well with the solvent that called as extremophilic bacteria. Gram-negative bacteria are already known for its capabilities to adapt in the solvent waste [9], but there is limited study on the capability of the gram-positive bacteria to adapt well with the solvent [10]. In this study, we report the growth of organic solvent tolerant bacteria with gram-positive inside real solvent waste and synthetic solvent waste as their carbon source.

METHODOLOGY

Solvent Waste Mixture

Samples of solvent waste mixture used in this experiment were obtained from manufacturing process in semiconductor industry, Silterra Sdn. Bhd. Solvents that had been chosen were isopropyl alcohol (IPA) in this study.

Organic Solvent Tolerant Bacteria (OSTB)

Bacillus subtilis BSIAs that was isolated from industrial area in Penang, and have been identified in the laboratory was used in this study. Details regarding isolation and general characteristics of this OSTB have been discussed previously [11]. The stocks culture were maintained in nutrient broth and kept at 4 º C.

Medium

Three types of media were used in this study. First media is nutrient broth (NB) consists of peptone, beef extract and sodium chloride. Nutrient broth (NB) was prepared as a culture medium for these bacteria. Each strain inoculum was prepared in 250ml conical flask with 100ml nutrient broth at 35 º C with shaking inside incubator shaker at 160rpm. Second media is synthetic organic solvents, which is isopropyl alcohol (IPA) media. For this media IPA was chosen because it was one of the major components inside real solvent waste mixture. Third is real solvent waste mixture media. This real solvent waste mixture was prepared in 1% (v/v) concentration with sterile distilled water.

Culture media and inoculum preparation

The selected OSTB, BSIAs was cultivated into different concentration (%) of synthetic organic solvent 5%, 10% and 20% v/v (IPA). Growth of OSTB in different concentration of synthetic solvents was monitored. Then the OSTB was cultivated inside NB media and in concentration 1% (v/v) real solvent waste mixture media. The liquid media with working volume 10ml for each vial was then cultivated with 10% fresh culture of OSTB. All vials were incubated inside incubator shaker at 35º C with rotary speed 160rpm. The samples were taken every hour to measure the growth of OSTB due to optical density reading at 660nm. This procedure follow standard methods for examination of water and wastewater, part 9040 sections, Washing and Sterilization [12]. This experiment had been repeated for three times.

Distillation process for recovering IPA

Distillation process that been used was simple distillation because the boiling point for IPA (82ºC) and water (100ºC). To set up the simple distillation unit, the apparatus that been used were round bottom flask (250ml), stirrer mantle, distilling head, thermometer adapter, thermometer, condenser, vacuum adapter, retort stand and clamp, and boiling chips [14]. After the set up completed, the water tap was opened and let the water running in and out the condenser. Then, the thermometer adapter was removed and the funnel was put at distilling head. After that, the sample was poured pour into the round bottom flask that has a few of boiling chips inside there. Then, the thermometer adapter with the thermometer was placed back to its original place and the distillation process was started when the heating sources is on.

RESULTS AND DISCUSSION

Growth of OSTB

Figure 1 showed the graph of growth of BSIAs inside 20%, 10% and 5% of IPA. The growth of bacteria inside 20% of IPA gives the trend of decreasing for 27 hours of experiment. And the growth of BSIAs inside the 10% of IPA also gives the same trend like growth of BSIAs inside 20% of IPA. But, inside the 5% IPA the growth of BSIAs give the trend of increasing and decreasing within 15 hour and start to decrease again until 27 hours of experiment. This was because alcohol has been historically a kill bacterium especially when the alcohols have higher concentration. Higher concentration removed the important enzymes and give effect to the bacteria's metabolic processes and this also affecting the integrity of the bacteria's cell wall and cell membrane [15].

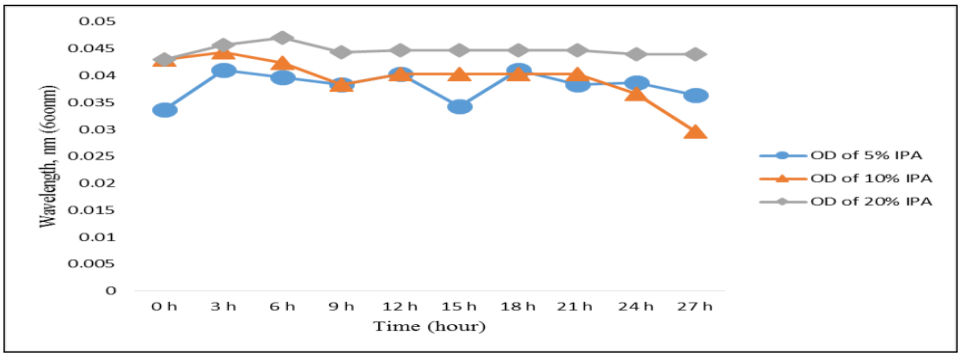

Figure 1 Growth of BSIAs inside 20%, 10 % and 5% of IPA

But, the growth of bacteria inside the 5% of IPA give a small different trend from 10% IPA. The OD (620nm) of BSIAs inside 5% IPA keep increasing but drop drastically after 15 hours and increasing back after 18 hours and keep decreasing for the next hour. Based on the bacterial growth curve, when the OD was decreasing, the graph shows the death phase of bacteria [16]. This due to he bacteria that unable to survive and the cells die at a constant rate. Because of this, after 14 hour, the distillation process been done to check the remaining solvent after the biological treatment.

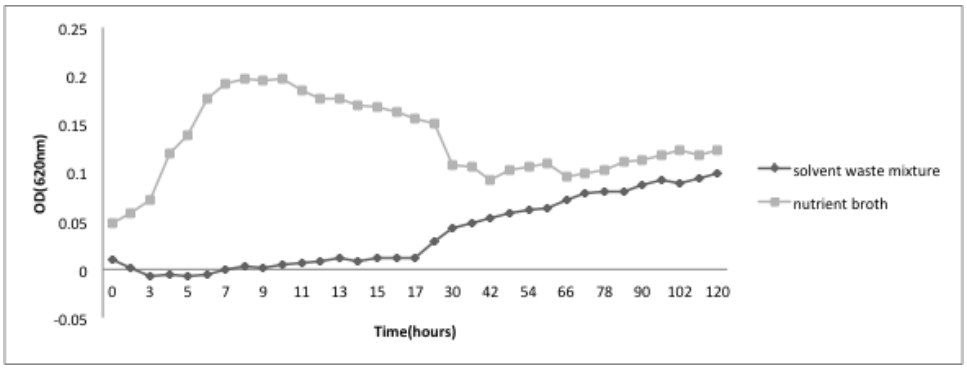

Figure 2 Growth of BSIAs inside concentration 1% (v/v) real solvent waste mixture and inside nutrient broth (NB)

This Figure 2 shows the comparison growth of OSTB in selective media (NB) and in real wastewater solvent that was used as a media. Result exhibits that this BSIAs has the ability to degrade or used the solvent as their substrate [17, 18]. From the results in Figure 2, the 1st 24 hours has been shown to be the acclimatization period for BSIAs in solvent waste mixture compared to BSIAs inside nutrient broth. This is the acclimatization period for BSIAs in real wastewater. After 24 hours, the BSIAs started to multiply. Between 78 to 120 hours, BSIAs growth rate was increased. At this stage the BSIAs started to utilize the solvent as their carbon sources. It showed that these BSIAs were part of extremophiles microorganisms that tolerant with organic solvents toxicity [19]. But inside nutrient broth media the growth rate started to decreased after18 hours. At this death phase stage there are no more nutrients for them to utilize. Their enzyme can degrade a variety of natural substrates that contributes for their nutrient cycling. So, they use their enzyme to utilize the solvent for their nutrient and continue to life inside the extreme condition. This gram-positive bacterium can tolerant with organic solvent depend on the toxicity of the solvent [6].

Recovery of IPA after biological treatment

After 5% concentration of IPA been chosen as a medium for BSIAs to growth and utilize the solvent, the volume of solvent after the biological treatment been measured by doing the distillation process. From Fig. 3, the volume of remaining solvent after 14 hour is 8 mL. The volume is lowered by 1 mL from 9 mL of volume of solvent before the biological treatment. From this, the BSIAs have done their work to utilize the solvent become another product not harmful to human and environment. The percentage of volume recovery of solvent after 14 hours incubation time was decreasing by 1% after the biological treatment because the initial recovery was 6% while after the biological treatment was 5%.

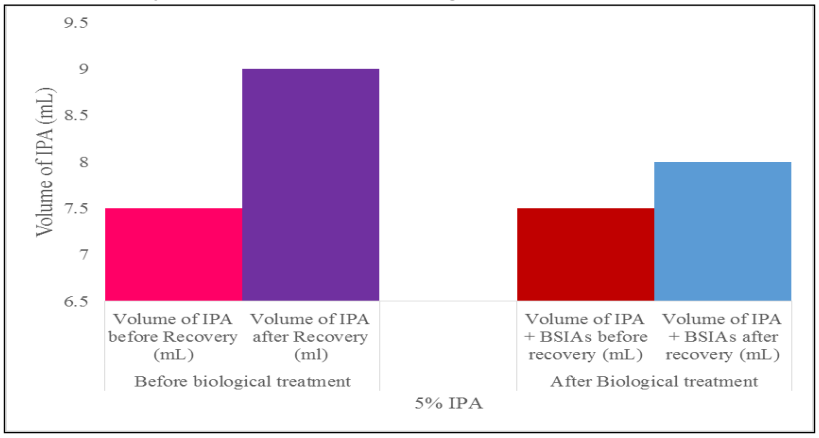

Figure 3 Volume of recovery of IPA before and after biological treatment using distillation process

Biological treatment been introduced to the solvent waste which is rarely to be found because bacteria cannot live in the extreme condition. But, this gram-positive bacteria can tolerant to the organic solvent. So, the BSIAs been used to utilize the 5% of IPA and give the good result because there were some reduction of volume after the biological treatment. The percentage of volume recovery for the remaining solvent after 14 hours the biological treatment was 5%. This percentage of recovery was reducing by 1% from the initial recovery which is before the biological treatment.

CONCLUSION

The gram-positive OSTB BSIAs could utilized the solvent as their carbon sources in lower concentration of solvents. This indicates that this OSTB may have potential to treat lower concentration of solvent waste in the future.

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