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Growth study of organic solvent tolerant bacteria (OSTB) in solvent waste mixture of semiconductor industry

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ABSTRACT

Aims: Increasing of organic solvent waste contributed as one of the most critical environmental problems. Huge amount of solvents has been applied in the industrial process, but it is not followed by a good waste treatment. Up to our knowledge only a few studies has been conducted in applying the biological treatment on the solvent waste mixtures specifically by Gram's positive organic solvent tolerant bacteria (OSTB). The study aims to identify the ability of OSTB survival in solvent waste mixture of the semiconductor industry in comparison to synthetic organic solvent by OSTB inoculation.
Methodology and results: Strain of OSTB named as *Bacillus subtilis*, BSIAs was applied in the study. The growth of this OSTB in different concentration of synthetic solvent isopropyl alcohol (IPA) and in actual solvent waste mixture consists of IPA was monitored and measured. There are three different concentrations (v/v) of synthetic solvent IPA was used as a media that are 20%, 10% and 5% for testing the growth of *B. subtilis* BSIAs. The 5% concentration of IPA was suitable for B. *subtilis* BSIAs growth. After 14 h of growth, distillation process was used to separate the remaining solvent from the mixture. It was found that, the volume after biological treatment was 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 h.
Conclusion, significance and impact of study: As a conclusion, the findings reveal that the strain of Gram-positive *B. subtilis,* BSIAs has the ability to utilize synthetic organic solvent (IPA) and the solvent waste mixture from the semiconductor industry as their carbon sources. The selected OSTB can be considered as bio-agents in the industrial waste management pertaining to solvent waste problems thru green technology approaches.

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

INTRODUCTION

Solvent waste management within industrial premises has been classified as one of the most critical and potential environmental problems (Bustard *et al*., 2002). Solvent based industry was the major contributor to the solvent waste either from chemical or cleaning processes and it is mostly contaminated with the unknown substances and in mixed conditions. The generated solvent waste from industry premises has been classified as scheduled waste according to Environmental Quality Act 1974 (EQA 1974) for Malaysia. In year 2008 to compare with, about 250 thousand tons hazardous waste has been produced in Portugal and managed by the Hazardous Industrial Waste (HIW) (Couto *et al*., 2013). Department of Environmental (DOE) Malaysia reported about 1,438,380 tons of scheduled waste has been generated in the same year and about 41,955 tons was the solvent waste. The statistic depicts some others treatment alternative is needed asides from our current disposal methods of scheduled waste that is incineration, secured 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 (Chaniago *et al*., 2014). Organic solvents have been used in the washing and cleaning processes and finally became the solvent waste mixture when it collected before disposal. Solvents are organic compounds that have adverse impacts toward human health and living organisms. 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 (Ruxuan

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et al., 2015). Incineration was used in Malaysia that where solvent waste was transfer for further treatment but this treatment give secondary air pollution (Chang and Lu, 2003).

In addition to the solvent waste incineration, distillation process was applied for solvent recovery. More than 90% solvent is recovered from the process and it is the most common treatment used in the chemical industry (Chaniago *et al*., 2015). On the other hand, biological treatment has been an alternative method for treating the solvent waste by using bacteria. The use of bacteria for breaking down the component structure has been used but their application in solvent waste treatment is limited due to the toxicity effect towards bacteria cells (Sarkar and Ghosh, 2012). There are several types of bacteria that tolerant to the solvent waste and these bacteria can utilize the solvent waste as a substrate and become less toxic product (Torres *et al*., 2011). Bacteria strains reported to grow and utilize saturated concentration of organic solvents such as toluene and transformed resulting a reduction of the pollutants (Sardessai and Bhosle, 2004). Ability of bacteria to utilize high concentration of solvent gives minimum waste treatment process to industry and the methods is effective in converting solvent waste (Bustard *et al*., 2001; Mohammad *et al*., 2006).

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 (Chaniago *et al*., 2015). 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. They are a group of novel microorganisms like bacteria that can adapt with extreme conditions such as high toxicity of the organic solvents (Lăzăroaie, 2009). Most of the bacteria in this group are capable to thrive in the organic solvents condition (Torrer *et al*., 2011). Organic solvent tolerant bacteria (OSTB) that are able to survive and grow in the presence of toxic solvents are the best way to decrease amount of solvent (Heipieper *et al*., 2007). To date, there are lesser previous reports exist concerning using organic solvent tolerant bacteria (OSTB) towards real solvent waste mixture 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 (Weber *et al*., 1996), but there is limited study on the capability of the gram-positive bacteria to adapt well with the solvent (Zahir *et al*., 2005). 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.

MATERIALS AND METHODS

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 (Talib, 2013). The stocks culture were maintained in nutrient broth and kept at 4 °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 250 ml conical flask with 100 ml nutrient broth at 35 °C with shaking inside incubator shaker at 160 rpm. 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 (IPA) 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 10 ml for each vial was then cultivated with 10% fresh culture of OSTB. All vials were incubated inside incubator shaker at 35 °C with rotary speed 160 rpm. The samples were taken every hour to measure the growth of OSTB due to optical density reading at 660 nm. This procedure follows standard methods for examination of water and wastewater, part 9040 sections, Washing and Sterilization (APHA, 2005; Eaton *et al*., 1999). 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 (82 °C) and water (100 °C). To set up the simple distillation unit, the apparatus that been used were round bottom flask (250 mL), stirrer mantle, distilling head, thermometer adapter, thermometer, condenser, vacuum adapter, retort stand and clamp, and boiling chips (Saidur *et al*., 2011). 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

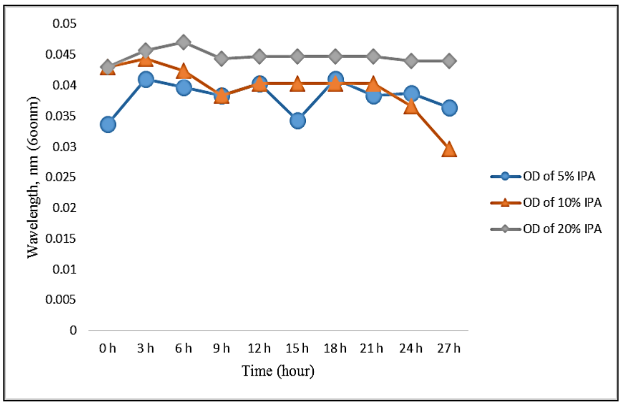

Figure 1: Growth of BSIAs in 20%, 10 % and 5% of IPA.

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 h 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 gives the trend of increasing and decreasing within 15 h and start to decrease again until 27 h 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 (Wypych, 2001).

But, the growth of bacteria inside the 5% of IPA give a small different trend from 10% IPA. The OD (620 nm) of BSIAs inside 5% IPA keep increasing but drop drastically after 15 h and increasing back after 18 h 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 (Skovgaard, 2002). This due to the bacteria that unable to survive and the cells die at a constant rate. Because of this, after 14 h, the distillation process been done to check the remaining solvent after the biological treatment.

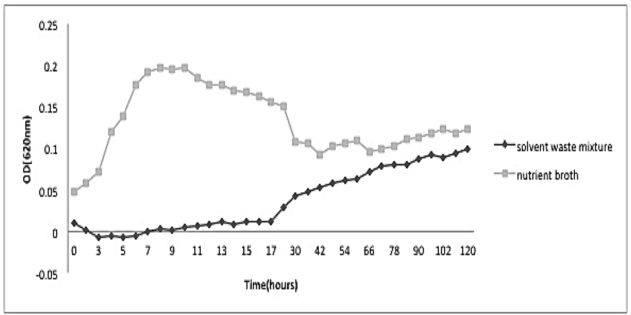

Figure 2: Growth of BSIAs in 1% (v/v) of concentrated solvent waste mixture and nutrient broth (NB).

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 (Margesin and Schinner, 2001; Luengo, 2003). From the results in Figure 2, the 1st 24 h 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 h, the BSIAs started to multiply. Between 78 to 120 h, BSIAs growth rate was increased. At this stage the BSIAs started to utilize the solvent as their carbon sources. It showed that these BSIAs was part of extremophiles microorganisms that tolerant with organic solvents toxicity (Lăzăroaie, 2009). But inside nutrient broth media the growth rate started to decrease after 18 h. 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 (Torres *et al*., 2011).

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 Figure 3, the volume of remaining solvent after 14 h 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 h incubation time was decreasing by 1% after the biological treatment because the initial recovery was 6% while after the biological treatment was 5%. It proved that IPA was used as a carbon sources for this BSIAs to growth.

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 bacterium can tolerant to the organic solvent. So, the BSIAs been used to utilize the 5% of IPA and give the good result

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because there was some reduction of volume after the biological treatment. The percentage of volume recovery for the remaining solvent after 14 h the biological treatment was 5%. This percentage of recovery was reducing by 1% from the initial recovery which is before the biological treatment.

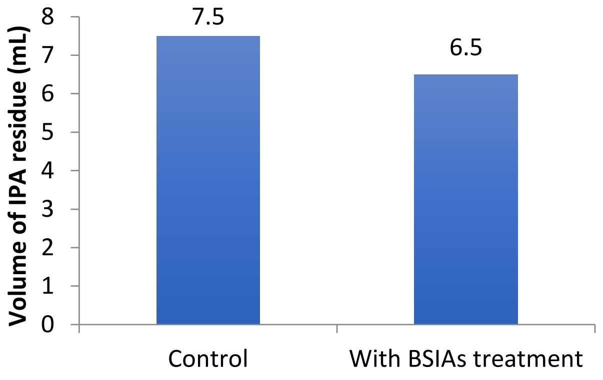

Figure 3: Volume of recovery of IPA before and after biological treatment via distillation process.

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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