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Effect of tungsten concentration on growth of *acetobacter xylinum* as a promising agent for eco-friendly recycling system

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Abstract. Effect of tungsten (W) concentration on *Acetobacter xylinum* growth was studied. In the experimental procedure, concentration of W in the bacterial growth medium containing pineapple peels waste was varied from 0.5 to 50 ppm. To confirm the influence of W, the bacterial incubation process was carried out for 72 hours. Spectrophotometer analysis showed that the growth rate of *Acetobacter xylinum* decreased with increasing concentration of W. The result from fourier transform infra red analysis showed a slightly change on the absorption peak intensities and informing the interaction of W ion and bacteria cell. The result confirmed that *Acetobacter xylinum* was able to uptake W concentration up to 15 ppm, indicating that *Acetobacter xylinum* might act as a promising agent for eco-friendly recycling system.

1. Introduction

Tungsten or Wolfram (W) is one of the rare earth elements and valuable metal groups. The properties of W are high thermo stability, strength, and abrasion resistance. Among metals, W has the highest melting point and the lowest coefficient of thermal expansion. For this reason, W is widely used for electric-lamp filaments, composite materials and polymers, semiconductor components, machine-part cutting tools, thermal shield plates, mill roles, and catalysts for de-nitration of exhaust gases and removal of organic materials.[1]

Due to its excellent performances, the exploitation of W was inevitable. Indeed, because of the demand of W material, waste problems gained from W cannot be avoided. For example, recently Hsu et. al. reported that semiconductor manufacturer waste in Taiwan contains 300 µg/L of W. This result is very high, compared to the world river average concentration of W (less than 0.10 µg/L).[2] Since W is suspected to be toxic (e.g. acute lymphocytic leukemia) and causing environmental damage, [3] finding process for solving this W waste problem is inevitable.

To solve the W waste, physical and chemical removal methods (*e.g.*, ion-exchange[1], solvent extraction[4], and alkaline solution [5]) were studied. However, these methods are economically and technologically difficult. Further, the process associates with the requirement in high cost and high energy, and relates to non eco-friendly waste product.

Biosorption is one of the alternative methods to remove metal from waste water. This method utilizes biomaterials as a main biosorbents. The effectiveness of biosorption has been well-known for the sequestration of heavy metals, rare earth elements, radio nuclides, and metalloids.[6][7]

Malekzadeh et al. reported a biosorption of W by *Bacillus sp.*, as well as the effects of parameters such as pH, initial W and cell concentration, live activity, and addition of other cations on the adsorption capacity.[8] However, the bacteria used in their study is only *Bacillus sp*. And, there is no information regarding transformation of structure and morphology of bacteria during the adsorption process. The mechanism during the interaction process of W with *Bacillus sp*. is also unclear.

Based on our experience in the use of W-related material,[9][10][11][12] the purpose of this study was to investigate the effect of W on the growth of *Acetobacter xylinum*. *Acetobacter xylinum* was used as a bacterial biosorbent model because it is largely available, inexpensive, and harmless. This bacterium also has a rapid cultivation rate. In this study, various W concentrations were tested. The biosorption ability of *Acetobacter xylinum* was also investigated. The results found that W influenced the bacterial growth of *Acetobacter xylinum* was stable when concentration of W of less than 15 ppm. This information indicate that bacterium can uptake W concentration of up to 15 ppm. We believe that *Acetobacter xylinum* might act as a promising agent for eco-friendly recycling system.

2. Experimental method

2.1. Raw materials

Acetobacter xylinum, commercial pure sugar, ammonium sulfate ($(NH_4)_2SO_4$) (Bratachem, Indonesia), ammonium tungstate(VI) pentahydrate (ATP; $(NH_4)_2WO_4.5H_2O$; Wako Chem. Co. Ltd., Japan), and de-ionized water. All purchased raw materials were used without further purifications. Prior to using, pineapple peel waste was washed with de-ionized water, blended, and boiled to get a pineapple peel waste juice under an aseptic condition.

2.2. Bacterial growth conditions

To prepare a medium, the pineapple peel waste juice was put into the aqueous solution (containing 7.50 %w/v of sugar and 0.50 %w/v of ammonium sulfate), mixed, and boiled. Then, the medium was cooled to room temperature and left for about 24 h to make sure all chemicals dissolved completely. Subsequently, *Acetobacter xylinum* (10%v/v) was inoculated into 250 mL Erlenmeyer flask containing 100 mL of the medium. Then, the flask was sealed with an absorbent cotton cap and incubated for 72 h under room temperature.

2.3. Preparation of W solution

A metal solution containing W ions was prepared by dissolving a known weight of ATP in de-ionized water. The concentration of W was varied from 0.5 to 50 ppm.

2.4. Sorption of W by Acetobacter xylinum

In a typical sorption experiment, W solution was added to the medium containing *Acetobacter xylinum.* The experiment was conducted at room temperature for 72 h. To follow the time course of the microbial W adsorption, aliquots of the mixture were periodically withdrawn and the concentration of bacteria in the solution was determined.

2.5. Characterization

The growth of *Acetobacter xylinum* with addition of various W concentrations was determined by an UV-visible spectrophotometer (UV-Vis; Shimadzu Corp., Japan). The morphology and structure of the samples were characterized by a transmission electron microscope (TEM; JEM-1400, JEOL Ltd., Japan; operating at 20 kV). Elemental and chemical compositions of the samples were evaluated by a fourier transform infrared spectroscopy analysis (FTIR, Shimadzu-8400, Shimadzu Corp., Japan).

3. Results and Discussion

Figure 1 shows the effects of various W concentrations on the bacterial growth rate. The concentration of bacteria in the medium without addition of W solution increased from 20 to 65 h. With addition of W concentration of up to 5 ppm, the bacterial growth pattern remained the same. In the sample with W concentrations of 10 and 15 ppm, the bacterial growth curve almost stable. However, the concentration decreased after 65 h. Regarding samples with W concentrations of more than 25 ppm, the bacterial growth rate decreased when the addition of W concentration was altered from 25 to 50 ppm. This results confirmed that the W concentration higher than 15 ppm must be considered the maximum adsorption of *Acetobacter xylinum*.

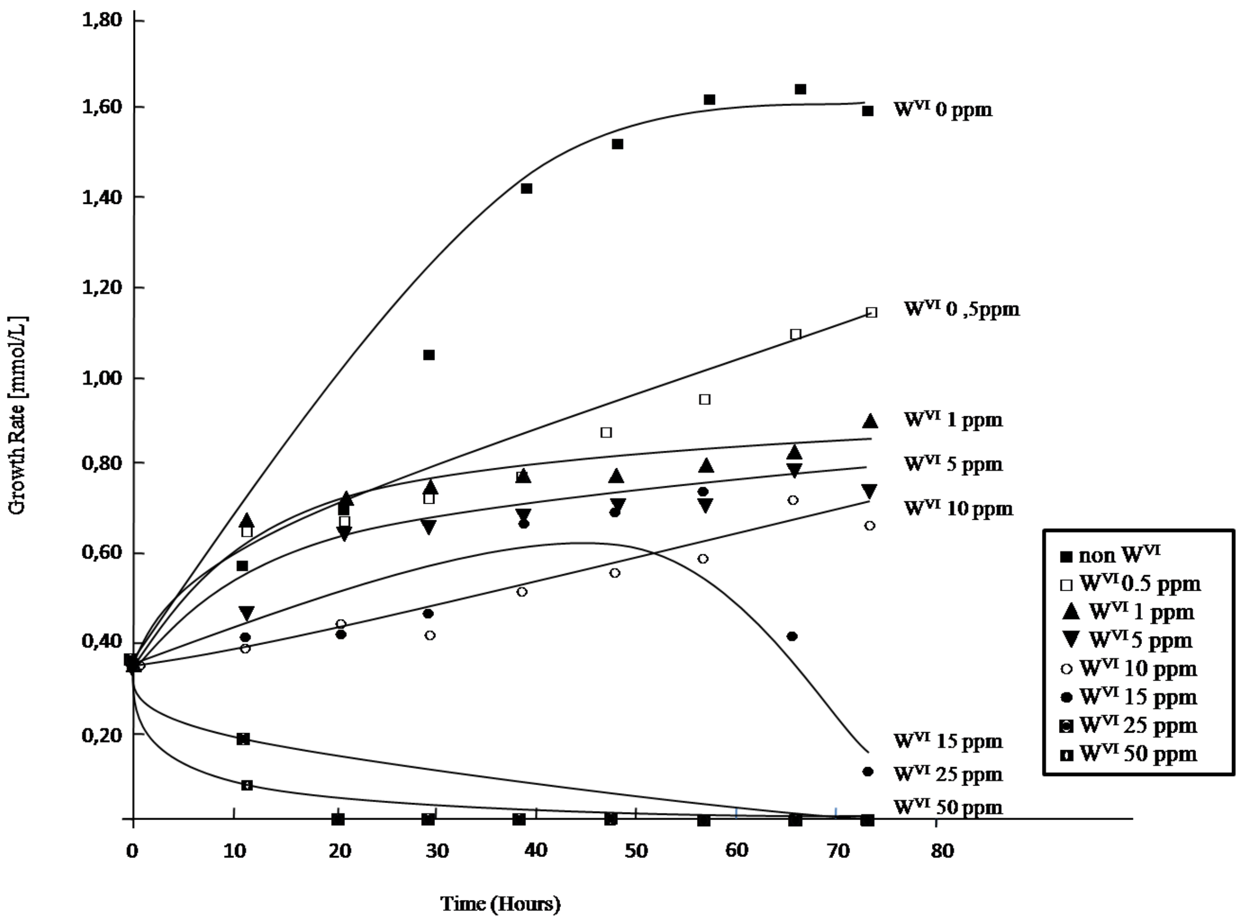

Figure 1. The curve of *Acetobacter xylinum* growth rate in the medium with various W concentrations.

Figures 2a and 2b shows the TEM images of samples before and after additional 50 ppm of W solution in the medium, respectively. TEM images indicated that the samples contained cell with bacillus structure. The sample with additional W solution had W-related particle (about 100 nm) on the outside of the body of the bacteria (**Figure 2b**), while these particles did not appear in the sample without W solution (**Figure 2a**). Based on these results, the main reason of the formation of particles can be described in the following: First, with the existence of W ion in the solution, bacteria use this ion for reduction and oxidation reactions.[12] The result of the reduction and oxidation reaction is the W-related particle. Some of the particles are adsorbed on the body, whereas the other is released to the medium. In addition, the possibility of the complete adsorption of W on the bacteria body depends on the charge of bacteria themselves.[13] Therefore, zeta potential analysis must be considered and will be done in our future study.

Figure 2c presents the FTIR analysis results of samples before and after additional 50 ppm of W solution. For the sample bacteria in the medium with addition of W solution, peaks at 3423, 2923, 1654, 1542, 1379, 1280, and 1068 cm^{-1}were detected. The detection of peaks for this sample is the same with that for sample bacteria in the medium without addition of W solution. There was only a slightly change on the absorption peaks intensity, which belonged to several functional groups of W-related component, such as carboxyl and phosphate groups (COOH and PO_4^{3-}).

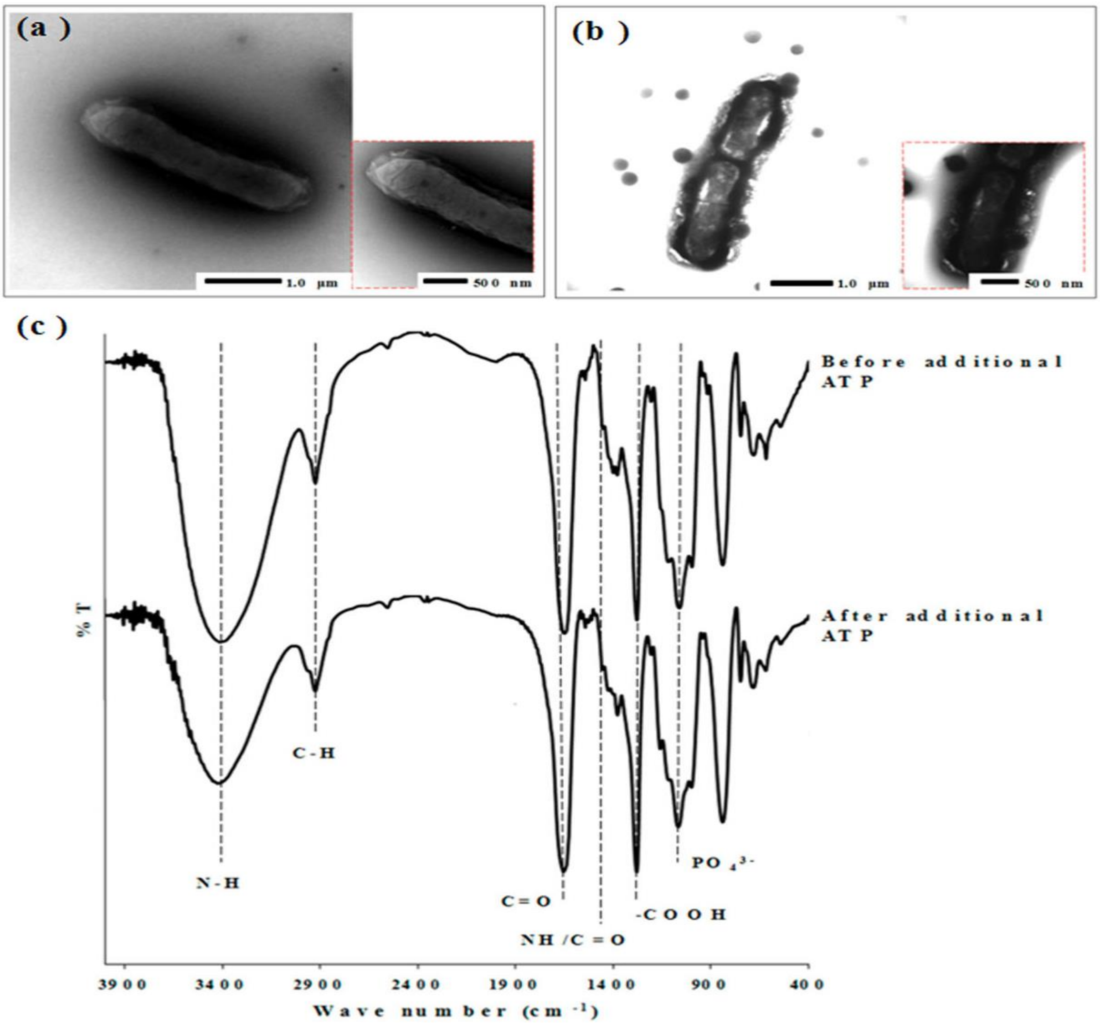

Figure 2. The physicochemical properties of samples Figure (a) is the TEM image of bacteria without addition W solution in the medium, whereas Figure (b) is the TEM image of bacteria with addition W concentration of 50 ppm in the medium. Figure (c) is the FTIR analysis results of samples.

4. Conclusions

We successfully investigated the effects of various W concentrations on the growth of *Acetobacter xylinum*. The *Acetobacter xylinum* was able to uptake W concentration of up to 15 ppm. The result from this study indicates that *Acetobacter xylinum* can be promising as an agent for eco-friendly recycling system.

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