The Ability of Bacterial Isolates of Actinobacillus sp. in Degrading Pollutants p-Cresols and Sunset Yellow

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Abstract

A gram-negative bacterium Actinobacillus sp. have been isolated from kefir grains which proved capable of degrading phenol to a concentration of 8000 ppm. The aim of this study is to know the ability of the bacterial isolates to degrade p-cresol and sunset yellow dye that might be pollutants in the aquatic environment. Biodegradation test conducted on samples of p-cresol 40 ppm and 70 ppm sunset yellow in M9 medium, using a bacterial inoculum in the growth phase and stationary phase. Changes in the concentration of p-cresol and sunset yellow were measured using a spectrophotometer at a wavelength of 276 and 482 nm respectively. The results showed that the bacterial isolates of Actinobacillus sp. proved able to degrade p-cresol and sunset yellow. Growth phase inoculum of Actinobacillus sp. (6 hours old) is more effective in degrading p-cresol (56.76%) compared to the stationary phase inoculum (20 hours old) (36%). Meanwhile stationary phase inoculum of Actinobacillus sp. is more effective in degrading sunset yellow (11.88%) compared to the growth phase inoculum (2.02%).

Keywords: p-cresol biodegradation, sunset yellow biodegradation, Actinobacillus sp.

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Introduction

From kefir grains have been isolated a gram-negative bacterium of Actinobacillus sp. which have been proven capable of degrading phenol to concentration of 8000 ppm (Muntholib, et al., 2011). According to Khleifat(2007), bacterial isolates of Actinobacillus sp. able to take advantage of the high concentration of phenol as carbon and energy source.

Phenol is a chemical compound that is harmful to the body because it can cause damage to the blood forming system of the bone marrow. Symptoms of acute poisoning by phenol are characterized by decreasing levels of erythrocytes and leukocytes in the blood (Mahawati, et al., 2006). Most of the phenolic compounds also are carcinogenic and harmful to living things, one example is cresol.

Cresol is a trivial name for methylphenol organic compounds, with molecular formula of C7H8OH, and has isomers as ortho, meta and para. The melting point of cresol is almost close to the room temperature so generally cresol be a liquid or solid depending on the temperature of their surroundings. Cresol oxidized slowly when exposed to the air so its purity decreases. This is indicated by the colour change from yellow to brownish red (AQD_ICA, 2008).

Some studies showed that the m/p-cresol can cause irritation to the skin, blindness, damage to the mucous membranes until death. p-cresol compound reported more dangerous, these compounds are carcinogenic, triggers the formation of cancer cells in the body (GDCh, 2003).

Pollution degradation of phenolic compounds can be done in various ways such as chlorination, ozonation and carbon sequestration. Those ways, however, are relatively more expensive and cause other environmental pollution.

Bioremediation is more desirable because it is more effective and environmentally friendly. Biodegradation in bioremediation were performed by using enzymatic processes (Bhattacharya, et al., 2012). One source of enzymes for bioremediation purposes is Actinobacillus sp. which has been shown capable to degrade phenol. On the other hand, the similarity in structure between p-cresol and phenol, particularly the presence of conjugated double bonds in the p-cresol, allow p-cresol degradation by Actinobacillus sp. isolates.

On other hand, one compound having an aromatic ring is sunset yellow, which is an azo compound and has been used as food synthetic dyes. The main component of sunset yellow compound is disodium-2-hydroxy-1-(4-sulfonatofenilazo) naphthalene-6-sulfonic acid. These compounds provide the colour
yellow to orange in food. Using the sunset yellow as food dye set by JECFA

Since 1982 and SCF since 1984. Based on ADI (Acceptable Daily Intake), consumption of food dyes is limited to only about 2.5 mg per kilogram of body weight. These compounds are carcinogenic if use in excessive (EFSA, 2009). Based on the structure, sunset yellow is one polynucleated aromatic hydrocarbons. These compounds are classified as derivatives of naphthalene. Biodegradation of phenolic compounds by bacteria is likely to occur due to an overhaul of the conjugated double bond or aromatic ring. Sunset yellow which has three aromatic group (benzene) is likely can be degraded by Actinobacillus sp.

![Figure 1. Structure of (a) Phenol, (b) p-Cresol, and (c) Sunset Yellow](image)

The aim of this study is to know the ability of bacterial isolates of Actinobacillus sp. to degrade p-cresol and sunset yellow by using inoculum bacteria in the growth phase and stationary phase.

**Methodology**

The study begins with the making of the growth curve of Actinobacillus sp. by culturing the bacteria in NC medium (peptone, beef extract) for 32 hours. From the growth curves, can be obtained the growth phase and stationary phase of bacteria to reproduce the bacteria for biodegradation assay.

Previously, it was also conducted the confirmatory test of the phenol biodegradation in concentration of 4000 ppm. During the biodegradation, the bacterial growth was measured by measuring the Optical Density (OD) at a 600 nm, whereas decrease of phenol, p-cresol, and sunset yellow was measured at a wavelength of 235, 276 and 482 nm respectively.

In addition, to confirm the compounds changes in the solutions were measured a wavelength range of 245-350 for phenol and p-cresol and 440-580 for sunset yellow.

**Results and Discussion**

**Bacterial Growth Curve of Actinobacillus sp.**

The growth curve of bacterial isolates of Actinobacillus sp. can be seen in Figure 2.

![Figure 2. Growth Curve of Bacterial Isolates of Actinobacillus sp.](image)

The curve results showed that the bacteria have no adaptation phase. This might be due to the bacteria was cultured in liquid media previously. The growth phase occurs to 25 hours old. In this phase, it occurs the increasing of bacterial growth activity. The next phase is a stationary phase that can be seen when the inoculum was 25 to 30 hours old. When the inoculum was more than 30 hours, it occurs the decreasing of the amount of bacteria cells that have entered to the death phase.

**Biodegradation of p-Cresols and Sunset Yellow by Bacterial Isolates of Actinobacillus sp.**

Biodegradation conducted on two samples, namely 40 ppm of p-cresol and 70 ppm of sunset yellow, using inoculum of growth phase (6 hours old) and stationary phase (20 hours old). The results showed that the bacterial isolates capable of degrading p-cresol as indicated by increasing of turbidity and decreasing of absorbance at wavelength of 276 nm. The increasing of turbidity is defined as an increasing of the number of bacterial cells, whereas decreasing of absorbance at 276 nm is due to decreasing of the p-cresol concentration. As well as phenol, p-cresol also has a conjugated double bond, so can absorb wavelengths at the UV region (276 nm).

Figure 3 shows that the decreasing of p-cresol concentration using growth phase inoculum is sharper than stationary phase inoculum. This is in line with the increasing of the number of the cell, when using the same inoculums as can be shown at Figure 4.

The faster of the growth, the more nutrients are needed so that p-cresol as the source of carbon and energy for bacteria isolates of Actinobacillus sp. more degraded by the growth phase inoculum than the stationary phase inoculum. It can be said that the growth phase inoculum is more effective in degrading p-cresol compared to tationary phase inoculum.

Bacterial isolates of Actinobacillus sp. also able to degrade sunset yellow. It is observed by increasing turbidity of bacterial cultures to degrade and decreasing concentration of sunset yellow. However, in contrast to the degradation of p-cresol, the growth
phase inoculum are less able to degrade sunset yellow than stationary phase one, as shown at Figure 5.

Thus, it can be interpreted that sunset yellow as the only of carbon source can be degraded by bacterial isolates of *Actinobacillus sp.* for their survival.

According to Figure 5, it seems that the stationary phase inoculum is more effective in degrading sunset yellow compared to the growth phase inoculum. This means that the degradation enzymes were produced in the stationary phase are more suitable and more degrading sunset yellow than in the growth phase. On the other hand, as shown at Figure 6, the growth phase inoculum was faster than the stationary phase inoculum.

The number of sunset yellow degraded is smaller than p-cresol. It can be caused by conjugated double bond at sunset yellow is more complex than in the p-cresol. In addition, and the greater molecular weight of sunset yellow cause more difficult to degrade by *Actinobacillus sp.*

Changes in the composition of the compounds before and after biodegradation by *Actinobacillus sp.* can be observed from the change in absorption spectrum, as shown at Figure 7 to Figure 10.
The differences of absorption spectrum of p-cresol and sunset yellow, before and after degradation by Actinobacillus sp indicated the formation of new compounds. To know the structure of new compounds, however, further study is needed.

Conclusions

The results showed that the bacterial isolates of Actinobacillus sp. proved able to degrade p-cresol and sunset yellow. Growth phase inoculum of Actinobacillus sp. (6 hours old) is more effective in degrading p-cresol (56.76%) compared to the stationary phase inoculum (20 hours old) (36%). While stationary phase inoculum of Actinobacillus sp. is more effective in degrading sunset yellow (11.88%) compared to the growth phase inoculum (2.02%).

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