Mutagenesis_uv

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ENHANCEMENT THE BIODEGRADATION OF BENZENE BY *Pseudomonas aeruginosa* THROUGH ULTRAVIOLET-INDUCED MUTATION

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ABSTRACT

Mutagenesis can increase the capacity of bacterial metabolism to degrade pollutants. Two bacteria mutan strains (ISM1) obtained from the UV mutagenesis of *Pseudomonas aeruginosa* (ISP) is a parental bacteria isolate. Mutants strain isolate were applied to a groundwater microcosm containing a benzene of 125 ppm. For were incubation is carry out an observation includes: analysis of benzene, CO_2, and bacterial population. The result shows that benzene degradation to of 11.82 ppm of 87.8 percent for the ISM1 mutant strain whereas in the presence of parental bacteria a benzene of 38.8 ppm of 60 percent at the initial concentration of 125 ppm in 120 h. Degradation of benzene has followed the increase of the bacterial population and increase of CO_2 produced.

Keywords: biodegradation, benzene, parental, mutant

INTRODUCTION

Benzene is a chemical compound that is often used in the chemical industry and is part of the petroleum revinery of a source of environmental pollution (Dibble and Bartha, 1979). Benzene is dangerous because, while it is difficult to degrade in nature, it is also toxic and carcinogenic; therefore, the allowable upper limit in drinking water is 0.005 mg L^{-1} (Chapelle, 1999).

To solve the problem of benzene pollution using biological methods to thoroughly biodegradation processes using bacterial. Efforts to increase of bacterial capacity for degradation activity can be made through gene mutation. The metabolic capacity of an organism is determined by the genome (Dai and Copley, 2004).

Therefore, bacterial capacity for metabolism can be increased through induction of gene mutations using ultraviolet (UV) irradiation (Ikehata and Ono, 2011). The effect of the mutation, it could result of changes in the genetic code, resulting in improvement bacterial isolate for metabolism of the substrate (Wielgoss et al., 2013). Recently, need to think about strain improvement in the field of environment microbiology to improve the ability of microbes in degradation of pollutants (Kim et al., 2002).

In accordance with the description above, an assay was needed to determine the benzene degradation capacity of mutant strain which produced by UV mutagenesis and to compare it with that of the parental bacteria isolate. The results of mutation induction to obtain bacteria mutant with a high-degradation capacity for the aromatic compound, so that it can serve as an inoculum source to solve the problem of pollution the aromatic hydrocarbon compounds in environment.

MATERIALS AND METHODS

Isolate

Benzene-degrading bacterial isolates are mutant strains (ISM1) obtained from the *Pseudomonas aeruginosa* parental (ISP) has been cultured in the laboratory.

Generation of mutants

UV mutagenesis was performed according to the method of Carlton and Brown with slight modification (Kim et al., 2002). Bacterial cell suspension was transferred to sterile glass petri dish on nutrient agar and exposed to 254-nm UV light. Bacterial colonies that grow after 24 hours incubation are putative mutant bacteria for further assay of benzene degradation ability on the salt mineral media.The results, obtained one mutant isolates the namely ISM1 gave significantly higher capacities of benzene degradation than the parent bacteria isolate.

Application of Mutant Strain in the Microcosm

The study of benzene biodegradation of bacteria mutant strain and parental bacteria was carried out in a groundwater microcosm. The microcosmos was made using a 100 mL flask bottle containing 30 mL of ground water. The nutrients K_2HPO_4 1 g/L and NH_4NO_3 1 g/L were added as phosphorus and nitrogen sources, respectively, and added benzene 125 ppm. The inoculum used was 2 % (v/v) of a suspension of concentration 0.5 McFarland. 1 mL of each sample's headspace was injected for benzene and CO_2 analysis; and bacterial population enumeration.

Analysis of Benzene Concentration

Benzene concentration in the supernatant were measured by gas chromatography Analysis of benzene was performed with a flame ionization detector (FID) (Mottaleb et al., 2003).

CO_2 Concentration Analysis

Headspace CO_2 were monitored by injection of gas samples into a Varian 450-GC gas chromatograph (SRI Instruments, Torrance, Calif) was completed with a thermal conductivity detector (TCD). The CO_2 production rates were calculated by linear regression of CO_2 production vs time.

Bacterial Population Enumeration

Bacterial population were enumerated by employing serial dilution agar plating method. serial dilutions, then are plated onto duplicate sterile petri dishes containing nutrient agar media. Colony counts were made from plates after incubation for 24 h.

RESULTS AND DISCUSSIONS

According to the results of the benzene degradation assay of mutant strain ISM1 (Figure 1) showed the most rapid degradation of benzene which was able to reduce the concentration of 38 percent in 48 hours, then the benzene concentration was reduced to 87.81 percent after a 120 h incubation. The observation of CO_2 in the headspace of the flask produced was 21.20 ppm in 48 h, and after, the amount of CO_2 produced to begin to decline in the late incubation 120 h, followed increased benzene biodegradation activity, except of the last 24–48 h, as well as a decrease in bacterial population. Bacterial growth was followed activation of the benzene degradation too is production of CO_2.

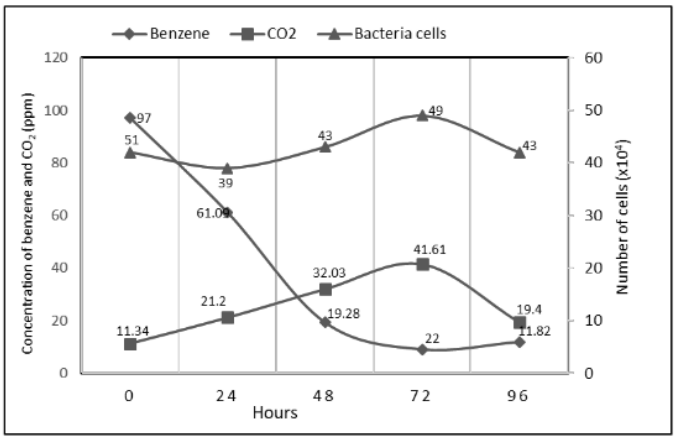

Figure 1. Benzene concentration, number of bacterial cells and CO_2 production of biodegradation process by mutant strain ISM1

If the mutant strains ISM1 were compared with ISP parental bacteria (Figure 2) as the comparison control, benzene degradation occurred very slowly since the beginning of the incubation to incubation of 72 h. At the 48 h, the benzene concentration was only decreased of 42.33 percent. As a whole, from the beginning to the end of incubation degradation has occurred reaching 60 percent, its associated with of growth of the bacteria population was also relatively slow, although from 72 h there was an increase the bacterial population to the end of the incubation.

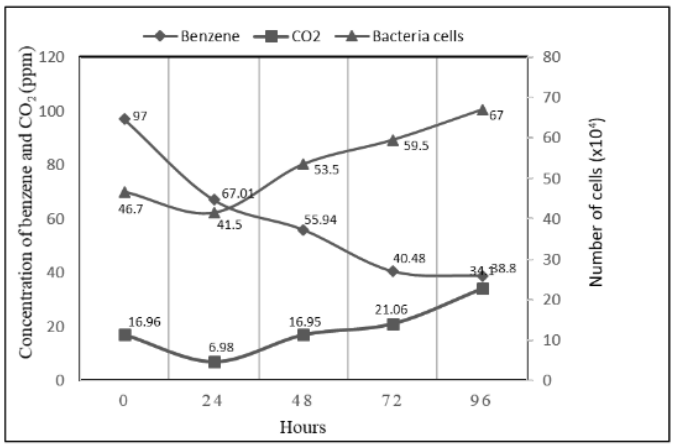

Figure 2. Benzene concentration, number of bacterial cells and CO_2 production of biodegradation process by parental isolate ISP

The present result shows that ISM1 mutant strain had a faster of degradation, especially at 48 h. Then, if the mutant strains are compared with the parental bacteria, both ISM1 mutant strains were proven to have an increased capacity to degrade benzene. The degradation ability was significant for the ISM1 mutant strain, which could reduce the benzene concentration of 80.41 percent at 48 h.

The invesitigate bacterial population, growth of bacteria cell decreased at the beginning of the incubation, evidence that microbes need a long time in the adaptation phase when

using benzene as a carbon and energy source (Yoshikawa et al., 2017) is called lag phase. It appeared especially for the ISP parental bacteria, whereas for both mutant strains have the lag phase was relatively more quickly.

Degradation of benzene was not followed by an increase in bacteria cell growth and consequently CO_2 production also decreases, this is related to the intermediate compounds formed in the degradation of benzene compounds which inhibit the increase of cell growth. Bacterial growth is not always followed by benzene degradation, because cell metabolic processes will produce organic acids that can be used by microbes as a carbon and energy source while benzene is used for growth (Pettigrew et al., 1991).

According to the above description, UV-induced mutations can change the metabolic capacity of bacteria. The relevance of this phenomenon is that mutation induction can increase the capacity of bacteria to generate a metabolic product. Mutation can be used to create microbial genetic variation to increase the biodegradation of pollutant compounds in the environment (Dai and Copley, 2004; Wielgoss et al., 2013).

Mutation induction can produce strain improvement without having to go through foreign gene insertion. Mutants have the advantages of specific degradation and characterization that are appropriate to be requested, and can even be increased in capacity several times over the potential of wildtype isolates (Sadhu et al., 2014). Data demonstrated that the ability of *Rhodococcus sp*. strain DK180 to grow on benzene resulted from a mutation in the gene encoding the meta-cleavage dioxygenase, attempts were made to identify the corresponding metabolite in the benzene catabolic pathway (Kim et al, 2002).

CONCLUSION

Pseudomonas aeruginosa mutant strains showed higher ability to benzene degradation compared to parental bacteria isolate, this is evident when two mutant are applied to a groundwater microcosm containing benzene of 125 ppm, result is most able to degradation of benzene is the ISM1 mutant strain is degrading benzene of 87.81 percent, whereas in the ISP parental isolate as control is degrading benzene of 60 percent in 120 h.

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