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Organic Pollutant Biodegradation by an Immobilized Mixed Bacterial Culture

Biodegradasi Polutan Organik oleh Kultur Bakteri Campuran Amobil

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Abstract. Pollution of organic compounds in rivers is generally contributed by wastewater discharge from human activities e.g. untreated black- & greywater, livestock farming, and industry. Without proper control of this waste generation, the river ecosystem will suffer if just rely on its self-purification and dilution by natural runoff to reduce the downstream effect. This research aimed to treat organic wastewater with an immobilized mixed bacterial culture. Immobilization was carried out using calcium alginate in a form of macrocapsule/beads, allowing the degrader microbe to be entrapped in its matrix thus preventing it from washout from the system. 3% (w/v) sodium alginate was mixed with 5 mg (dry) mixed culture and dropwise on 4% (w/v) calcium chloride solution. The results showed that immobilized microbes were still able to grow despite being entangled in a calcium alginate matrix with an initial growth of 6.1 x 10^5 to 7.4 x 10^7 colonies/grams and reduced COD content up to 68.3%. Degrader microbe immobilization offers considerable advantages compared to free/ suspended cell system due to the influence of river water flow.

Keywords: Biodegradation, Organic, Pollutant, Immobilized, Bacteria

Abstrak. *Pencemaran senyawa organik di sungai umumnya disumbang oleh pembuangan air limbah dari aktivitas manusia, seperti blackwater & greywater yang tidak diolah, peternakan, dan industri. Tanpa pengendalian yang tepat dari timbulan limbah ini, ekosistem sungai akan menderita jika hanya mengandalkan pemurnian diri dan pengenceran oleh limpasan alami untuk mengurangi efek di bagian hilir. Penelitian ini bertujuan untuk mengolah air limbah organik dengan kultur bakteri campuran amobil. Imobilisasi dilakukan dengan menggunakan kalsium alginat dalam bentuk mikrokapsul/bead, sehingga mikroba pengurai dapat terperangkap dalam matriksnya sehingga mencegah terjadinya washout dari sistem. 3% (b/v) natrium alginat dicampur dengan kultur campuran 5 mg (kering) dan diteteskan pada larutan kalsium klorida 4% (b/v). Hasil penelitian menunjukkan bahwa mikroba amobil masih dapat tumbuh meskipun telah terjerat dalam matriks kalsium alginat dengan pertumbuhan awal 6,1 x* 10^5 *menjadi 7,4 x* 10^7 *koloni/gram dan menurunkan kadar COD hingga 68,3%. Imobilisasi mikroba pengurai menawarkan keuntungan yang cukup besar dibandingkan dengan sistem sel mikroba tersuspensi karena pengaruh aliran air sungai.*

Kata kunci: Biodegradasi, Organik, Polutan, Imobilisasi, Bakteri

INTRODUCTION

Sustainable development is the process of meeting the demands of the present generation without compromising the ability of future generations to meet their own. One of the elements that must be addressed in order to achieve sustainable development is how to reduce environmental degradation without jeopardizing the need for economic growth and social fairness. Environmental sustainability places a strong emphasis on environmental cleanup. To restore polluted areas to its natural state, they must be cleaned of contaminants resulting from residential or industrial operations, the use of pesticides and fertilizers, and the emission of other pollutants. Remediation of the environment in many environmental media, including groundwater, surface water, soil, sediment, and air. Remediation of water is the removal of pollutants from water. Surface water in ponds, lakes, and rivers can be contaminated immediately by pollutants dumped directly into the water or runoff water. As hydro-ecological, economic, and social systems, rivers play crucial roles in water supply, flood control, drought management, transportation, microclimate, ecosystem health, green lines, and education, among others. The contamination of river water bodies with contaminants arising from industrial, agricultural, animal, and residential activities has resulted in a considerable decline in water quality. More than seventy-four percent of Java's major rivers do not match the Class II Water Criteria. This suggests that the river has been exploited as a dumping ground for numerous types of waste, resulting in a considerable decline in water quality. In light of these conditions, continual technological efforts to prevent and control water pollution are required in the framework of regulating and preventing water pollution.

Conventional remediation involves transporting contaminated water to another location and treating it at a treatment plant. Bioremediation is a more promising and cost-effective method for cleaning contaminated water and some soil than conventional methods. To purify contaminated water, bioremediation employs biological agents, particularly microorganisms such as yeast, fungi, and bacteria. Microbes play a crucial part in the natural cycle and are the primary boosters in bioremediation of polluted environments. The development of microbial groups can be accomplished in a number of methods, including by stimulating growth through the addition of nutrients, by introducing terminal electron acceptors, and by managing humidity and temperature conditions, to name a few. Microorganisms utilize pollutants as a source of nourishment or energy during bioremediation. Bioremediation employs low-cost, low-tech approaches that are often well-accepted by the public and can be performed on-site. Due to the narrow spectrum of contaminants, the timescale involved is very lengthy, and the level of residual contaminants may not always be enough (Cheng, 2014)

Nevertheless, bioremediation can only be effective where environmental circumstances allow for microbial growth and activity. When environmental conditions are not conducive to their growth, environmental parameters are altered to promote microbial growth and accelerate decomposition. Utilizing microorganisms that naturally exist in nature minimizes any environmental damage. Occasionally, they are native to a contaminated area; otherwise, they must be transported to the site. Immobilizing bacterial cells improves the survival and retention of bioremediation agents in polluted settings. When employed for multi-enzyme systems, the immobilization of entire cells has been developed as biocatalysts for environmental pollutions (Bayat et al., 2015).

On a substrate, cell immobilization is an effective method for forming an artificial biofilm. Biofilm deposition on a media/substrate will counteract the buoyant forces of the biomass in a salty environment and prevent the washout of the degrader microbe. The benefits of immobilized cells include the provision of a high biomass, the avoidance of cell washout issues at high dilution rates, and protection from harsh environmental conditions (Kardena et al., 2018).

In this investigation, a bacterial consortia capable of efficiently degrading organic compounds was utilized, which had been identified in a prior study. The bacterial consortium was immobilized using macrocapsule-sized calcium alginate.

MATERIALS AND METHODS

Preparation of calcium alginate microcapsule

Sodium alginate extra pure was purchased from Nacalai tesque, inc. Nutrient Broth, Calcium chloride was purchased from sigma-aldrich, Polyvinyl alcohol/PVA was purchased from Merck. Degrader microbe consortium was obtained from environmental laboratory, bioscience and biotechnology research center, Institute of Technology Bandung, Indonesia. In nutrient broth, a bacterial consortium was cultivated to the late exponential growth phase. After 24 hours, the medium was centrifuged at 6000 g for 15 minutes to remove the supernatant, and the cell pellet was resuspended in sterile deionized water.

To make a 2 percent (w/v) solution, 100 mL of boiling distilled water was mixed with sodium alginate. The cell pellet was extensively mixed with Sodium Alginate solution after being resuspended in deionized water. In sterilized deionized water, a 4 percent w/v solution of calcium chloride was created. A micropipette was used to drop the sodium alginate mixture into the calcium chloride solution. During this technique, almost spherical beads were created. These beads were then dipped in deionized water and magnetically agitated to remove any excess calcium chloride (Khalid et al., 2018). The macrocapsule of immobilized cells was preserved at 4 °C for further research after numerous washes and dryings of the beads.

Preparation of artificial domestic wastewater

The making of artificial waste was carried out in the laboratory with a composition $(NH_4)_2SO_4$ 0.01 g, sucrose 0.5 g, KH_2PO_4 0.01 g, K_2HPO_4 0.03 gr, $MnSO_4$ 0.007 gr, and $MgSO_4$ 0.03 gr in one liter of aquades (Xiao et al., 2011).

Organics degradation assay

A preliminary biodegradation assay was established and set up to determine the effectiveness of immobilized consortium in degrading domestic wastewater:
• Control reactor (C): alginate beads without addition of consortium degrader.
• Reactor (R): alginate beads with addition of consortium degrader (immobilized cell).
Chemical oxygen demands (COD) concentration and growth of consortium degrader were observed on certain time.

RESULTS AND DISCUSSION

Macrocapsule formation and viability assay

The gel formation from alginate was started by combining sodium alginate with consortium pellet. The gelling process begins when a drop of alginate + pellet mixture comes into contact with calcium chloride solution. Calcium acts as a chemical crosslinking agent for sodium alginate, resulting in the formation of small macrocapsules (**Figure 1**).

Figure 1. Visual image of alginate bead containing consortium of degrader microbe (immobilized cell)

In the viability assay, immobilized cells were immersed in COD solution of 1000 mg/l at room temperature and observed its growth. The results of the viability test (**Figure 2**) showed that at the beginning of the measurement, microbial consortium concentration was 6.1 x 10^5 CFU/ml, it increased at week 1 to 7.4 x 10^7 CFU / ml. It was observed a decrease in COD concentration of 68.3% in the first week of incubation time. From the third week to the sixth week of the incubation time, the COD concentration has been reduced ranging from 80-87.5 % respectively. It was noticed that consortium of the degrader microbe stable and survive until the sixth week of incubation time.

The phenomenon that occurs when alginate bead is immersed in a phosphate buffer pH 7.4 is a swelling of the bead. This occurs because alginate beads tend to be unstable in phosphate buffers so that water can enter the bead and increase its mass as shown in **Figure 3**. The highest swelling of calcium alginate beads occurs at 15 hours incubation time with a swelling of 34.3% by weight of initial bead. To avoid further bead damage due to this swelling process, modifications were made to strengthening the structure of alginate bead with PVA. Calcium alginate beads with the addition of PVA were able to reduce the swelling process to 12.6% after reaching an incubation time of 53 hours.

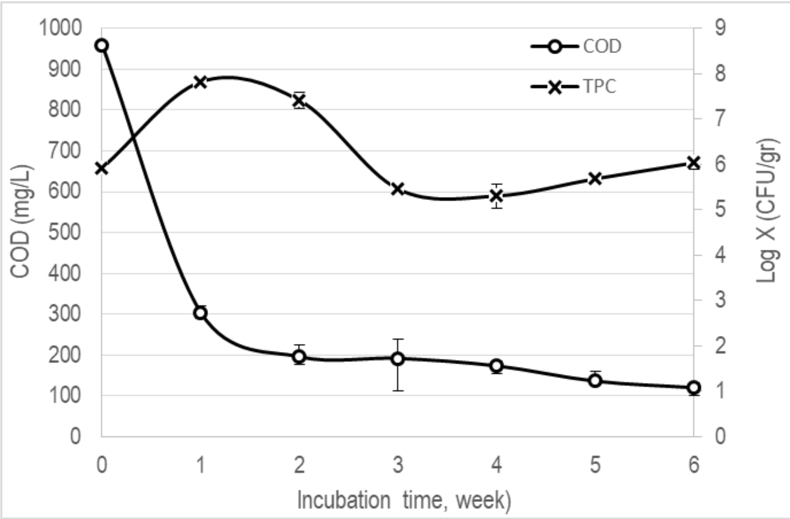

Figure 2. Viability assay of immobilized cell

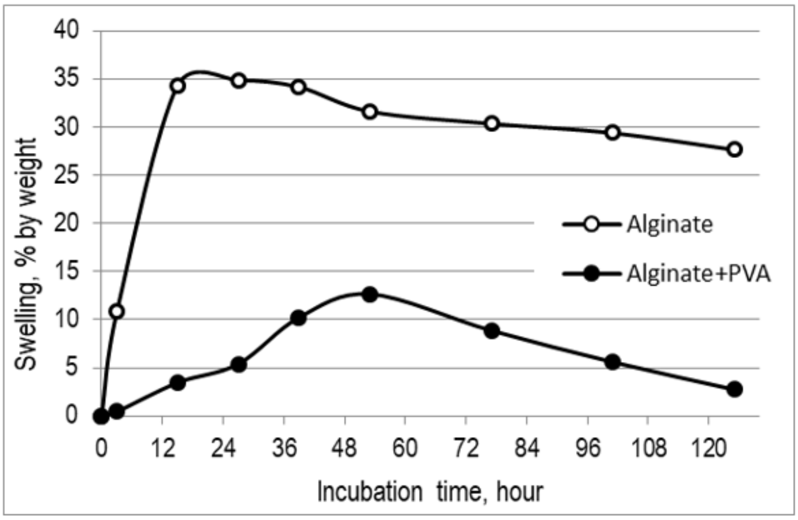

Figure 3. Swelling of calcium alginate and calcium alginate + PVA beads

Two causes can cause alginate gel swelling in phosphate buffer: 1) reversible swelling due to fluid diffusion into the gel matrix; and 2) irreversible swelling due to partial disintegration of the connecting zone caused by Ca^{2+} ion exchange with Na^{+} (Levic et al., 2015). PVA was used to coat alginate beads in order to protect them from severe environments such as stream flow, turbulence, and shear stress (Kim et al., 2015). PVA improves mechanical strength, but larger polymer concentrations may result in a more compact structure, which limits substrate and other essential nutrient transport into the matrix (Chen et al., 2008). Pore wall thickness and stability rose as polymer concentration increased, but pore number and size dropped (Ha et al., 2009; Khalid et al., 2018).

Biodegradation assay

The biodegradation potential of a microbial consortia after immobilization in macrocapsules (calcium alginate beads) was examined using artificial domestic wastewater. **Figure 4** depicts the effectiveness of organics removal and the time required for degradation.

Figure 4. Organics removal efficiency

COD estimation is a measure of the overall extent of mineralization product. Experiments were carried out using artificial domestic wastewater with an initial concentration of 700 mg/l and incubated at room temperature (30 ºC) to determine the performance of the microbial consortia immobilized in macrocapsules in the breakdown of organic compounds. Initially, COD in the medium was decreased reaching 34.7% removal efficiency after 12 hour of incubation time. Maximum COD removal efficiency (80.6%) was obtained after 78 hour incubation time. Compared with Control reactor, there are no significant accumulation of organics compound was observed, suggested that biodegradation of organics was occured or conversion of organics into microbial biomass. Results indicate that microbial consortium immobilized in alginate macrocapsule has effectively degraded organics compound.

CONCLUSION

Calcium alginate beads/macrocapsules with a membrane layer of Polyvinyl alcohol/PVA could be utilized to immobilize microorganisms. The PVA covering prevented the microbial consortium from leaking out of the macrocapsule, shielding it from physically hostile surroundings (shear stress, and turbulence of stream flow). The results show that a microbial consortia immobilized in alginate beads degrades organics from wastewater effectively (up to 80 percent removal efficiency). These findings may aid in the understanding and management of residential wastewater treatment in the context of river restoration or clean-up efforts. The benefits of immobilized cells include high biomass, avoidance of cell washout difficulties at high dilution rates, and protection from harsh environmental conditions in water bodies.

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