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Molecular imprinted hydrogel polymer (MIHP) as microbial immobilization media in artificial produced water treatment

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Abstract. Produced water generated during oil and gas exploration and drilling, consists of many chemicals which used in drilling process. The production of produced water is over three fold of the oil production. The water-cut has increased over time and continues to do so because the fraction of oil in the reservoir decreases and it is more difficult to get the oil out from an old oil-field. It therefore requires more sea water to be injected in order to force the oil out; hence more produced water is generated. Produced water can pollute the environment if it is not treated properly. In this research, produced water will be treated biologically using bacterial consortium which is isolated from petroleum processing facility with Molecular Imprinted Hydrogel Polymer (MIHP) for microbial immobilization media. Microbial growth rate is determined by measuring the MLVSS and hydrogel mass, also by SEM-EDS analysis. SEM-EDS analysis is an analysis to evidence the presence of microbe trapped in hydrogel, and also to determine the types and weight of the molecules of hydrogel. From this research, suspended microbial growth rate was found at 0.1532/days and attached microbial growth rate was 0.3322/days. Furthermore, based on SEM analysis, microbe is entrapped inside the hydrogel. Effectiveness of microbial degradation activity was determined by measuring organic materials as COD. Based on COD measurement, degradation rate of organic materials in wastewater is 0.3089/days, with maximum COD removal efficiency of 76.67%.

Keywords: COD, hydrogel, MIHP, produced water, SEM-EDS

1. Introduction
Saline wastes are generated in significant quantities in petrochemical industries such as oil and gas production. These generated wastes contain organic compounds and high concentrations of salt (>1.5%). Generated wastewater is considered as salty water trapped in the reservoir rock and brought up along with oil or gas during production. It subsists under high pressures and temperatures, and usually contains hydrocarbons and metals. Therefore, it must be treated before being discharged to the environment. The discharge of such wastewater containing at the same time of high salinity and high organic content without prior treatment is known to adversely affect the aquatic life, water potability and agriculture [1]. In spite of the detrimental effect of salty produced water on microbial activity, moderate acclimation of activated sludge to high salinity is possible. Acclimation implies the exposure...
of non-salt-adapted micro-organisms to increasing salt concentrations in order to permit the obtention of satisfactory effluent treatment performance at a given salt concentration. The success of such adaptation depends on several factors, such as the type and growth phase of micro-organisms, as well as the rapid or gradual increase of salt concentration during acclimation [1], [2].

Many studies reported that operation of activated sludge process at salt contents higher than 20 g/L is characterized by poor flocculation, high effluent solids, and a severe decrease in substrate utilization rate [2]. Researcher [3] reported that the nature of pollutants and the high salinity (about 29 g/L) of oil-field generated water has an unfavorable effect on the activated sludge process. High hydraulic loadings (above 2.5 m³/m².day) increased the wash-out of the activated sludge from the reactor. The addition of Powdered Activated Carbon improved the sludge volume index and increased the rate of biodegradation. This is due to the ability of biofilm formation on the activated carbon surface. The low settleability of sludge is caused by the salt content in produced water that reduces the population of protozoa, resulting in low settle-ability. Salt content in produced water increases the buoyancy forces, causing low sedimentation efficiencies.

Cell immobilization is an efficient strategy to form an artificial biofilm on a substrate. Biofilm formation in a media/substrate will overcome the buoyancy forces of the biomass in a salty environment and avoid the wash-out of the activated sludge from the treatment plant. The advantages of immobilized technique include easy separation of the cells from the carrier material, greater productivity due to high cell concentrations achieved and the protection of cells against harsh environmental conditions [4], [5], [6]. Studies have shown that polymer types play a dominant role in determining the properties of the microspheres. Examples of synthetic gels include poly(carbamoyl) sulphonate, polyhydroxyethylmethacrylate (polyHEMA) and polyacrylamide and polyvinyl alcohol (PVA). Microspheres are almost exclusively produced using water-soluble polymers which provide a high degree of permeability for nutrient and metabolites with a low molecular weight, thus providing optimal conditions for the functioning of immobilized microbial cells [6], [7].

This study investigated the applicability of an immobilisation technique based on cell entrapment in a Molecular Imprinted Hydrogel Polymer (MIHP), a strategy to increase the ecological competence of degrading microorganisms in treating salty produced water.

2. Research Method

2.1. Bacterial and growth condition

Mixed culture of degrading bacteria was obtained from culture collection of Environmental Laboratory, Bioscience and Biotechnology Research Center, Institut Teknologi Bandung, Indonesia. All culture were previously isolated from petroleum-contaminated soils by an enrichment culture technique using crude oil as the sole source of carbon and energy. The culture was maintained at 4°C on crude oil basal medium.

The synthetic oilfield-produced water was used throughout the study to determine the capability of strains of bacteria to degrade organic compounds. The synthetic oilfield-produced water was prepared by mixing crude oil (1.5 mL/L) and salt water (sea water and tap water), as described in the literature [8]. The C/N/P ratio of the medium was adjusted to approximately 100/5/1 by adding appropriate concentrations of (NH₄)₂SO₄ and KH₂PO₄ to the synthetic oilfield-produced water. This synthetic oilfield-produced water was used throughout our study to determine the capability of immobilized cell of bacteria to degrade organic compounds. The characteristics of the real and synthetic oilfield-produced water samples are listed in Table 1.
2.2. Molecular Imprinted Hydrogel Polymer (MIHP) Synthesis

Reagent used in MIHP synthesis are bis-acrylamide, N-N’methylenbisacrylamide, NaOH 4N, potassium persulfate, tetramethyletilendiamine (TEMED), and buffer pH 6. All chemical are obtained from Merck, Sigma-Aldrich and JT Baker. MIHP produced in the form of dry gel Bis-acrylamide and N-N’methylenbisacrylamide used to synthesize acrylamide-bis 40%, which functions as crosslinker. MIHP synthesized by adding a buffer solution of pH 6 into acrylamide-bis 40%. After homogenized, add potassium persulfate (as initiator) and TEMED (as catalyst) to speed up the polymerization process. The solution was left overnight until a hydrogel is formed. Hydrogel formed are washed by demineralized water until it reaches neutral pH, then dried in the oven with temperature of 110°C. The hydrogel are used as immobilized media and attached media for microbes growth throughout the experiment in the synthetic oilfield-produced water treatment. SEM-EDS analysis was used to determine the hydrogel structure and microbial attachment both in outer and inner of the hydrogel.

2.3. Produced water biodegradation by immobilized cells

In order to assess the biodegradation potential of oilfield-produced water by the immobilized degrader, the bacteria were acclimated first to the intended environment. Acclimation implies the exposure of non-salt-adapted microorganisms to increasing salt concentrations substrate until it reached the desired performance. The degradation potential was based on the growth rate (µ) and chemical oxygen demand (COD) removal efficiency. The microbial growth was obtained by measured the MLVSS over the observation time (t). The initial and final CODs were measured using standard methods (DR-2800, Hach Company). Reactor-1 arrangement (PW+Cell) is produced water + immobilized cell, while reactor-2 (PW-Cell) is a control reactor contains produced water without immobilized cell addition.

3. Results and Discussion

3.1. Cell immobilization

This research focuses on the synthesis of molecular imprinted hydrogel polymer as microbial immobilization medium. The polymer used is non-template polymer, the cavity formed in hydrogel is a cavity of water molecules that evaporate due to heating process. Polyacrylamide is synthesized from bis-acrylamide 40%, which contains a monomer of acrylamide and bis-acrylamide as crosslinker. To initiate polymerization, an initiator is required. The initiator is a material that can immediately turn into free radicals when heated or irradiated by electromagnetic radiation. Initiator and catalyst used in MIHP polymerization is 10% potassium persulfate and tetraethylmethylendiamine (TEMED). Excessive or too low concentrations of ammonium persulfate will not produce MIHP, whereas TEMED has an effect on polymerization time. The ratio between bis-acrylamide 40%, buffer pH 6, initiator, and catalyst must be precise, otherwise polymerization will be difficult and hydrogel is hard to form. The hydrogel structure can be seen in Figure 1.

![Figure 1. Wet hydrogel structure (left), and SEM image with 1000x magnification (right).](image_url)
Immobilized cells have advantages of higher activity, higher cell density and longer stability than free cells. Hence, the immobilized cells have the potential to degrade toxic chemicals at higher concentration as compared to the suspended cells. Requirement for an effective and safer carrier for resting microorganisms should be nontoxic and non-polluting, consistent in quality, have a long shelf life, allow sufficient cell activity and cell density [6], [9]. MIHP hydrogel was introduced to the microbial culture suspension in order to imbedded the cell into the hydrogel structure by using a shrink/swell procedure. The cell suspension will enter and entrapped into the hydrogel pore, also attached on the surface of the hydrogel structure (Figure 2).

Figure 2. Apparent structure of hydrogel: (left) internal structure of hydrogel indicating an immobilized cells; (middle) cells attached on the surface of hydrogel after 3 days incubation; (right) cells attached on the surface after 5 days incubation.

3.2. Biodegradation of oilfield-produced water by immobilized cells
Performance of immobilized cell in the biodegradation of synthetic oilfield-produced water was studied in the 2L reactor volume that contain initial concentration of 868 mg/L MLVSS degrader microbe. The growth and performance of the immobilized cell in the treatment of oilfield-produced water can be seen in Figure 3. Characteristics of the synthetic oilfield-produced water used in this study and its comparison with typical produced water is described in Table 1.

Table 1. Characteristics of produced water (PW).

| No. | Parameter        | Synthetic PW | Typical PW* |
|-----|------------------|--------------|-------------|
| 1   | COD, mg/L        | 1,200        | 100-4000    |
| 2   | Oil&Grease, mg/L | -            | 2-500       |
| 3   | TSS, mg/L        | -            | 10-200      |
| 4   | pH               | 6.89         | 4-10        |
| 5   | Conductivity, µS/cm | 13,080      | 4,000-58,000 |
| 6   | Salinity, %      | 0.73         | 0-2.5       |
| 7   | Phenols, mg/L    | 0.472        | 0-23        |

*adapted from [8], [10], [11]

The growth of degrader microbe in the reactor (PW+Cell) tends to grow and reach the exponential phase without showing any lag phase. This indicates that the strains of bacteria can consume organic compounds in the oilfield-produced water as a carbon source as soon as they come into contact with the wastewater. The specific growth rate (µ) and COD removal of the strains of bacteria tended to increase over time showing the efficacy of the degrader microbe. As observed in Figure 3, the COD removal rate was increased significantly after 2 days of incubation time. On the other side, the control reactor (PW-Cell) where there is no augmented degrader bacteria that added in the system shows a considerable increase in COD removal for up to 33.3% efficiency after 5 days of incubation time. Even without addition of degrader microbe, degradation process will occurred because of the oxidation from aeration process in the reactor.
The performance of the activated sludge in the treatment period can be seen in Figure 3. It was observed that, from the beginning of lag phase, the concentration of MLVSS increased. However, reactor without addition of microbial starter (PW-Cell) reached a steady-state condition after day 1, while reactor with addition of microbial starter (PW+Cell) continue to increase and became more adapted to the new environment as a function of time. In the fifth days of incubation period, the highest MLVSS concentration was observed in PW+Cell reactor (2.532 mg/L), while the lowest concentration was found in PW-Cell reactor (385 mg/L). COD removal efficiency in the PW+Cell compared to PW-Cell reactor within 5 days incubation time was 76.6% to 33.3%, respectively. As can be seen in Figure 4, attached growth rate is higher than in suspended state in the PW+Cell reactor, indicating that microbe able to attached in the hydrogel and utilized it as a growth support media.

4. Conclusion
The produced water discharged from both onshore and offshore platforms is typically treated by use of chemical and physical systems due to its simple operations. These conventional technologies, however, does not remove the organics such as suspended oil particles and other dissolved compounds. Some chemical treatment technologies also produce hazardous sludge and the cost of those operational processes can be significant. Biological treatment of produced water can be a cost-effective and environmental friendly method, but could be a problem on offshore installation due to the space limitations on the installations. To overcome the space limitation, a compact biological
process is needed such as microbial entrapment or immobilization methods can be used. High concentration of microbe/MLVSS can be achieved in a compact reactor compare to conventional suspended activated sludge units that require larger space to achieve the same cell density. Molecular Imprinted Hydrogel Polymer (MIHP) from Polyacrylamide can be used as a support medium for microbial immobilization. This indicated by the SEM analysis, which shows that on the fifth days of incubation, the internal surface of the hydrogel is covered by microbes. The rate of microbial growth in wastewater suspension is 0.1532/day and the rate of microbial growth in hydrogel is 0.3322/day. The rate of microbial growth in hydrogels is higher than in suspended state. This shows that microbes are able to utilized hydrogel as an attached growth media. The advantage of this method is the amount of biomass required in the synthetic oilfield-produced water treatment process fulfilled rapidly, because the microbe grows faster with hydrogel supporting media.

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