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Preliminary Study on Zeolite 13X as a Potential Carrier for Algal Immobilization

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ABSTRACT

Immobilization of microalgae in polymers can overcome problems associated with biomass harvesting from suspended free cells cultivated in wastewater. Although various carriers have been applied for microalgae immobilization (e.g. natural such as alginate and synthetic such as polyacrylamide), problems such as low stability, toxicity and high cost still remain a challenge for the method to be commercialized. In the present study, an effective carrier (zeolite molecular sieves 13X) has been used for the immobilization of green microalgae, Chlorella vulgaris. The immobilization was done by suspending microalgae in a culture medium with different pHs (ranging from 5-9) along with zeolite 13X. Scanning electron microscope (SEM) was used to observe the morphology of the cells adsorbed onto the carrier after the immobilization process. It was found that higher microalgal immobilization occurred in the medium with an acidic condition (pH=5) compared to other pHs. This indicates that zeolite 13X is capable to be a potential support for the immobilization of Chlorella vulgaris. Furthermore, zeolite-immobilized Chlorella can be applied in different applications such as wastewater treatment and biofuel production.

1. Introduction

The role of algae in water stream process is not hidden to anyone. Algae are not only suitable to treat wastewater but also the system produces algal biomass for the production of valuable chemicals such as biofuel and pharmaceuticals [1]. However, the algae-wastewater system faces some difficulties including harvesting, dewatering and processing of biomass [1]. Immobilization of algae can be a good solution to overcome these drawbacks. Also, it provides higher cell density and contains high-value compounds such as lipid, chlorophyll and protein than the suspended one [2-4]. Various materials have been used as a support for algal immobilization including natural (e.g. alginate) and synthetic (e.g. polyacrylamide)[5]. The natural carriers have low stability [6] while synthetic ones have

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toxicity and high cost [5] hence making them less preferable to be used in the system. The ideal carrier for algal immobilization has to fulfill some characteristics including nontoxic, cheap, reusability and high stability. One of the suitable carriers can be proposed for algal immobilization is zeolite. They are inorganic materials that are cost-effective, resistant to microbial degradation and also they have good thermostability performance [7]. Up to now, the researchers used them as a carrier for the immobilization of biomass such as bacteria [8-11] and yeast [12]. In this study, immobilization of microalgae, *Chlorella vulgaris*, onto zeolite molecular sieves 13X was investigated.

2. Material and Methods

2.1 Preparation of Carrier

Zeolite molecular sieves (type 13X) was purchased from Sigma Aldrich, Germany [13]. It was powdered and washed twice with deionized water. Then, it was dried and activated at 105^{o}C and 250 ^{o}C both for 3 hours, respectively [10,13]. Then it was slowly cooled down in a closed system (desiccator) [13] and kept until further use. The prepared support was applied for immobilization of *Chlorella vulgaris*.

2.2 Cultivation of Microalgae

The microalgae species used was *Chlorella vulgaris* obtained from Australian National Algae Culture Collection at CSIRO, Australia [14]. Sterilized MLA solution was used as a culture medium [14], [15]. The cultivation of *Chlorella* was done under white fluorescent lights (12:12 h light / dark cycle) at around 20 ^{o}C with the inoculum ratio of 1:10 [14]. The culture was mixed by a magnetic stirrer at 400 rpm. Monitoring cell growth was carried out by the measurement of optical density (OD) at the wavelength of 680 nm by a spectrophotometer. Biomass concentration was determined based on the calibration curve prepared by plotting dry weight of microalgae versus optical density at 680 nm.

2.3 Immobilization of Microalgae onto the Carrier

Immobilization of microalgae was done by centrifugation (4000 rpm, 20 min) of 200 ml of algal suspension in its exponential growth phase. Then, the sediment volume was adjusted to 20 ml. This concentrated algal suspension had the biomass concentration of 2.40 g/l with 15.6 × 10^{7} cells/ml. Immobilization was conducted by suspending the concentrated biomass in MLA medium in an amount of 10% (v/v) with different pH (5-9) along with powdered zeolite 13X in an amount of 2% (w/v). In order to adjust the value of pH, NaOH and HCl (both 1 M) were added to the solution. All flasks were put on a shaker (150 rpm, 1 day) to let sufficient attachment of *Chlorella* cells to the carrier surface. After the immobilization, all bioparticles were centrifuged (1200 rpm, 5 min) and the sediment was twice washed with sterilized distilled water and prepared for the analysis by drying in an oven at 60 ^{o}C for 1 night.

2.4 Scanning Electron Microscopy (SEM)

The dried samples were coated with an ultra-thin film of gold by a sputter coater. Then, the morphology of the samples was studied by scanning electron microscopy (SEM).

3. Results and Discussion

In this research, immobilization of *Chlorella vulgaris* onto zeolite 13X was done based on adsorption via physicochemical interactions. Physical interaction happens by an electrostatic attraction between positively charged zeolite surface (related to Na^+) [10] and negatively charged *Chlorella* surface (related to uronic acid and/or sulfate groups) [1]. Also, chemical interaction occurs by acid-base binding between zeolite surface as a Lewis acid with the active site of sodium [10], and some functional groups on *Chlorella* such as sulfate and hydroxyl as Lewis bases. Hence, the immobilization can happen between sodium ions onto the zeolite surface and some functional groups on the surface of *Chlorella vulgaris*. Fig. 1.a and b show SEM images of microalgae and powdered zeolite 13X, respectively.

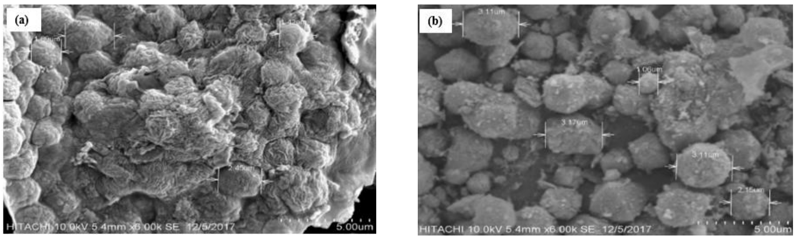

Fig. 1. SEM images of (a) microalgae and (b) powdered zeolite 13X

Fig. 2. shows SEM images of immobilized *Chlorella vulgaris* cells onto the surface of zeolite 13X in a medium with pH=9, 8, 6 and 5. According to the images, it was observed that more immobilization happened in a medium with acidic pH. The trend of immobilization shows that from pH=9 to pH=5, the immobilization is increasing. This is probably due to the fact that in an alkali medium, there is a competition between negative functional groups on the surface of *Chlorella* and hydroxyl groups in the medium to attach to the sodium ions on the surface of carrier, whereas in contrast, in an acidic medium, there is a less competition resulted in more binding of microalgae to the surface of support.

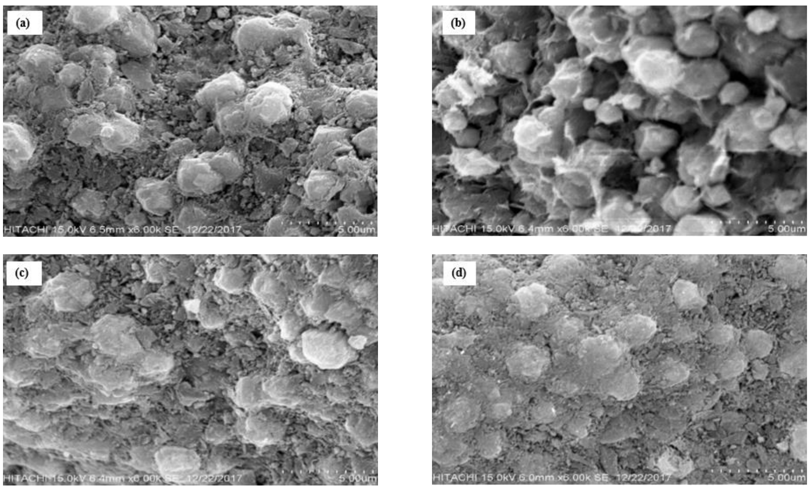

Fig. 2. SEM images of immobilized *C. vulgaris* cells onto the surface of zeolite 13X in a medium with (a) pH=9, (b) pH=8, (c) pH=6, (d) pH=5

Fig. 3. compares SEM images of powdered zeolite 13X and zeolite-immobilized microalgae in a medium with pH=5. According to the images, microalgae has almost covered all the carrier surface.

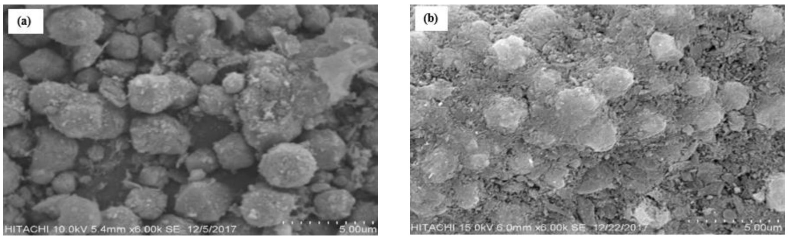

Fig.3. SEM images of (a) powdered zeolite 13X and (b) zeolite-immobilized microalgae in a medium with pH=5

4. Conclusions

In the current research, immobilization of *Chlorella vulgaris* onto powdered zeolite molecular sieves 13X was investigated. This type of zeolite includes a basic structure with exchangeable sodium ions. SEM images revealed that the maximum immobilization of *Chlorella* onto the powdered zeolite occurred in a medium with pH=5 after 1 day. The result showed that the zeolite 13X could be applied as an efficient carrier for immobilization of *Chlorella vulgaris*. However, further investigation on different experimental conditions needs to be done to verify on the algal immobilization. This new biomaterial can be applied to various applications such as water stream treatment, biofuel production and etc.

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