

The Second International Conference on Genetic Resources and Biotechnology

Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Bogor, Indonesia • 24–25 May 2021

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021 virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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The Potential Use of Zeolite and Exopolysaccharide Bacteria for Reduction of Degradation and Carbon Emission on Oil Palm Plantation in Tropical Peatland

Laksmi P. Santi^{a)}, Haryo T. Prakoso and Donny N. Kalbuadi

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Abstract. Zeolites are aluminosilicates widely used as ameliorant in oil palm plantation especially on peatland. The zeolite mineral is known to have an excellent molecular sieve property which enables it to hold organic carbon compounds, CO₂, and water leading to its potential use for carbon sequestration. Moreover, bioactivated zeolite with bacterium producing exopolysaccharides could promote the formation of peat molecular structure and their configurations that related to water and nutrient supply for plant. The objective of this study is to characterize natural zeolite clinoptilolite type from Bayah, Banten as exopolysaccharide bacterial carrier for peat ameliorant and reduction of carbon emission. The physical-chemical characteristics of 60–80, 80–100, and 100–150 particles mesh of zeolite were determined using standard analytical procedures. Furthermore, separation capability of a zeolites sample for carbon dioxide (CO₂), NH₃, and N₂ was measured by temperature programmed desorption (TPD). The Brunauer-Emmett-Teller (BET) technique was used in order to estimate the specific surface area of zeolite while the relative crystallinity of the samples was estimated by comparing their X-ray diffraction (XRD) pattern. The morphology of the samples was investigated by field emission scanning electron microscopy. Total exopolysaccharide bacteria in the matrix of zeolite were counted using total plate count in ATCC 14 media. The results indicated that 100–150 particle mesh of natural clinoptilolite Bayah zeolite has excellent properties of porous structure which contribute to carbon and N₂ adsorption, water retention, and bacterial carrier.

INTRODUCTION

Peatlands cover an estimate of 400 million hectares or equivalent to 3% of Earth's land surface. Tropical peatland is in the range of 30–45 million ha, which is 10–12% of the global peatland resource. Most of them are used for cultivation, especially for oil palm plantation in Indonesia, although the yield potential of oil palm planted on these areas has always been a controversial subject [1]. The long-term cultivation use of peatlands has an impact on peatlands environment such as decrease of groundwater level, changes of aerobic conditions, changes in rhizosphere communities, and root exudates of cultivated plants as well as degradation and mineralization of peat [2]. Furthermore, the natural structure and organic molecule configuration of peats are closely related to their water retention capacity [3]. The dominance of organic materials in peats tends to be water saturated makes it impossible to form complex organic bonding. Meanwhile, on a dry peat, water and nutrient retention capacity are considerably low. To obtain maximum productivity, an effort has been conducted to stabilize a relatively unstable peat material by implementing soil amelioration approach [4].

Peat physical properties will changed due to addition of zeolites in the large-scale test, which led to increase in the soil water retention capacity and also decrease the percolation [5]. Zeolite not only increases the water holding capacity but also increase the effect of mineral fertilizers [6]. Zeolites use in acid soil has beneficial effect on improving the nitrogen, phosphorous, and potassium uptake [7]. Unlike other soil amendment such as lime, the zeolite does not break down over time but it stays in the soil for improving nutrient retention. The natural zeolites have porous structure which helps to keep the soil aerated and moist as well as active for long period of time. Zeolite

is not acidic in nature. In fact, it is marginally alkaline and if amended with fertilizers can help buffer soil pH levels and may reduce the use of lime.

Data regarding mechanism of microbial interaction in relation to the formation of complex organic bonding and nutrient availability for plantation crops planted on peatland are somewhat limited. Interaction of microbes with host has very important role to the crop plant to be able to adapt to environmental changes [8]. There are some mechanisms of microbial interaction that promote plant growth, i.e. increase nutrient availability; improve soil structure; induce crop tolerance against pathogen via producing antibiotic, antifungal compounds, or enzyme; fix biological N₂; produce phytohormone (auxin, cytokinin, and gibberellin) [9]. Some researchers also reported that a bacterium of *Burkholderia* genera, particularly *B. cenocepacia* has high potential to inhibit the growth of *Ganoderma boninense* [10, 11]. Interaction between exopolysaccharide-producing microbe with host plant especially oil palm seedlings has been studied by [12, 13]. Consistency of positive impacts due to polysaccharide-producing endophytic microbe application on plant growth has been reported by [14, 15]. This research was carried out to study the characteristic physico-chemical of zeolite originating from Bayah and its potential use as CO₂, NH₃, and N₂ sorption and carrier material for exopolysaccharide bacteria.

MATERIALS AND METHODS

Physico-Chemical Analyses of Zeolite

A 60–80, 80–100, and 100–150 mesh of particle zeolite originating from Bayah, Banten was used in this experiment and subjected to physico-chemical analyses. Physical analyses such as pore structure measurements, specific surface area, pore-volume, and average pore diameter based on N₂-sorption method were done [16]. Chemical analyses were performed, including pH, cation exchange capacity (CEC), silica, aluminum, calcium, sodium, potassium elemental analyses, and water retention capacity. Both physical and chemical analyses were performed using standard laboratory analyses outlined by Indonesian national standardization [17] and as mentioned by Klute [18], respectively.

Mineralogical Characteristics

Mineralogical composition of zeolite powder was determined by using X-ray diffraction (XRD) analysis by running it at 0–30° 2-theta with Cu K α at the Indonesian Institute of Sciences (Lembaga Ilmu Pengetahuan Indonesia/LIPI), Serpong. Identification of the mineral present was carried out by using major intensive peak that characterize the mineral [19].

Electron Microscopy

The surface morphology of the zeolites was observed by scanning electron microscope (SEM) (Jeol JSM-5310LV). The electron beam is accelerated through a high voltage 20 kV and pass through a system of apertures and electromagnetic lenses to produce a thin beam of electrons [20]. In the early stages a material sample leveled with a special tool. After sputter coating the cast with 35 nm of gold-palladium (Au-Pd), electron micrographs were recorded.

Adsorption Capacity

Adsorption capacity of the Bayah zeolite were performed using CO₂, N₂, and NH₃ adsorption analyses at Chemical Engineering Department, LIPI. The CO₂ temperature-programmed desorption (TPD) performance of the N-FGO was evaluated in a modified setup of TG/DTA 6300 at atmospheric pressure. A certain amount of an adsorbent was placed in a small aluminum pan and was loaded inside the TGA. Initially, it was heated at 120°C for 30 min to remove moisture content, then a continuous flow of N₂ gas was set until the system was stable. Afterwards, the N₂ gas was stopped and pure CO₂ gas was flowed into the system for 180 min to achieve complete saturation. The weight increase after the adsorption process indicated as the adsorption capacity of the adsorbent.

The specific surface area of each sample was determined using the Brunauer-Emmett-Teller (BET) method [21], which was denoted as SBET. The total pore volume (V_t) of the adsorbent from the adsorption amount of nitrogen

gas at P/Po ~0.99 was obtained. The micropore volume (<2 nm, Vmi), ultra-micropore volume (<0.7 nm, Vultra), and mesopore volume (2~50 nm, Vme) were calculated from the density functional theory (DFT) method.

Exopolysaccharide Bacteria

The exopolysaccharide bacteria used as active ingredient of a molecule sieve material is *B. cenocepacia*. The bacteria produce exopolysaccharide in average of 5.03 mg/ml. Moreover, *B. cenocepacia* also has potential in fixing atmospheric N₂ non-symbiotically measured with acetylene reduction analysis (ARA) with the value of 0.73 μmol/g. Also, it can produce indole acetic acid (IAA) hormone of 78.9 ppm and can survive on pH 3–7 for 6–12 months [22]. Total population of exopolysaccharide bacteria in the zeolite matrix were estimated using total plate count method in ATCC 14 media.

Formulation Zeolite and Exopolysaccharide Bacteria as Peat Ameliorant

A newly-constructed material sieve molecule ameliorant containing *B. cenocepacia* was prepared by the following steps: (i) inoculation of the bacteria into 500 ml shake flasks containing 150 ml pf ATCC No. 14 medium which incubated on orbital shaker at 150 rpm, 30°C for 7 days. Following incubation, the total cell count of exopolysaccharide bacteria was 10⁸ cell/ml. The American Type Culture Collection (ATCC) No. 14 medium consists of (L⁻¹): 0.2 g KH₂PO₄, 0.8 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 0.1 g CaSO₄·2H₂O, 2.0 mg FeCl₃, Na₂MoO₄·2H₂O (*trace*), 0.5 g yeast extract, 20 g sucrose, 15 g bacto agar, and final pH 7.2; (ii) incorporation of the cultured microbe onto a pasteurized zeolite; (iii) packaging [8].

RESULTS AND DISCUSSION

The analysis showed that the zeolites have alkaline pH (7.7). The CEC values that meet the SNI 13-7168-2006 standards are zeolites with a size of 100–150 mesh (≥100 cmol₍₊₎/kg). Moreover, in terms of the effects of microbiological analysis, the zeolite material has the minimum technical requirements standards of *E. coli* and *Salmonella* (pathogenic bacteria) with the value of below 3 MPN/gram, and heavy metal levels are overall below the permissible threshold (Table 1).

TABLE 1. Physicochemical characteristics of the Bayah zeolite.

| Type of analysis | Zeolite 60–80 mesh | Zeolite 80–100 mesh | Zeolite 100–150 mesh | Kepmentan 261/KPTS/SR.310/M/4/2019 | Unit | Method |
|-------------------------------|--------------------|---------------------|----------------------|------------------------------------|-------------------------|-------------------|
| pH | 7.8 | 7.2 | 8.2 | 7–12 | - | pH meter |
| Organic carbon | 0.2 | 0.1 | 0.7 | - | % | Spectrophotometer |
| N | 0.013 | 0.012 | 0.013 | - | % | Kjedahl |
| P ₂ O ₅ | 27.9 | 36.1 | 89.92 | - | ppm | Spectrophotometer |
| K ₂ O | 1.54 | 1.58 | 1.75 | - | % | ICP-AES |
| CEC | 70.35 | 86.97 | 126.45 | Min. 60 | cmol ₍₊₎ /kg | Titration |
| <i>Escherichia coli</i> | <3 | <3 | <3 | <3 | MPN/g | TPC |
| <i>Salmonella</i> | <3 | <3 | <3 | <3 | MPN/g | TPC |
| As | nd | nd | nd | 10 | ppm | ICP |
| Hg | nd | nd | nd | 1 | ppm | ICP |
| Cd | nd | nd | nd | 2 | ppm | ICP |
| Pb | 3.48 | 3.34 | 3.38 | 50 | ppm | ICP |
| Fe | 88.45 | 93.24 | 105.34 | Min. 7 | mg/100 g | ICP |
| Mn | 8.96 | 10.52 | 30.38 | - | ppm | ICP |
| Zn | 1.34 | 1.5 | 2.06 | - | mg/100 g | ICP |
| Ni | nd | nd | nd | Max. 50 | ppm | ICP |
| Cr | 0.20 | 0.29 | 0.32 | Max. 180 | ppm | ICP |
| WHC | 43.59 | 49.22 | 60.50 | - | % | |

CEC = cation-exchange capacity, ICP-AES = inductively coupled plasma-atomic emission spectroscopy, TPC = total plate count, WHC = water-holding capacity, nd = not detected.

Based on these characteristics, the new molecule sieve material formulated from zeolite, both at laboratory and scale-up production processes, conform to quality standard requirements enforced by the local authority for commercial uses. Furthermore, according on XRD analysis, the Bayah zeolite consists of clinoptilolite-Na formula ($[\text{NaKCa}]_5\text{Al}_6\text{Si}_{30}\text{O}_{72}\cdot 18\text{H}_2\text{O}$) with other minerals such as mordenite and quarts present as accessory minerals (Fig. 1). Most application of this mineral in Indonesia was reported focused on agricultural sector especially in soil amendment and fertilizer industries. Based on these characteristics, this mineral has a great potential as an adsorbent [24] of greenhouse gas sequestration at peat land area [25] especially for oil palm cultivation.

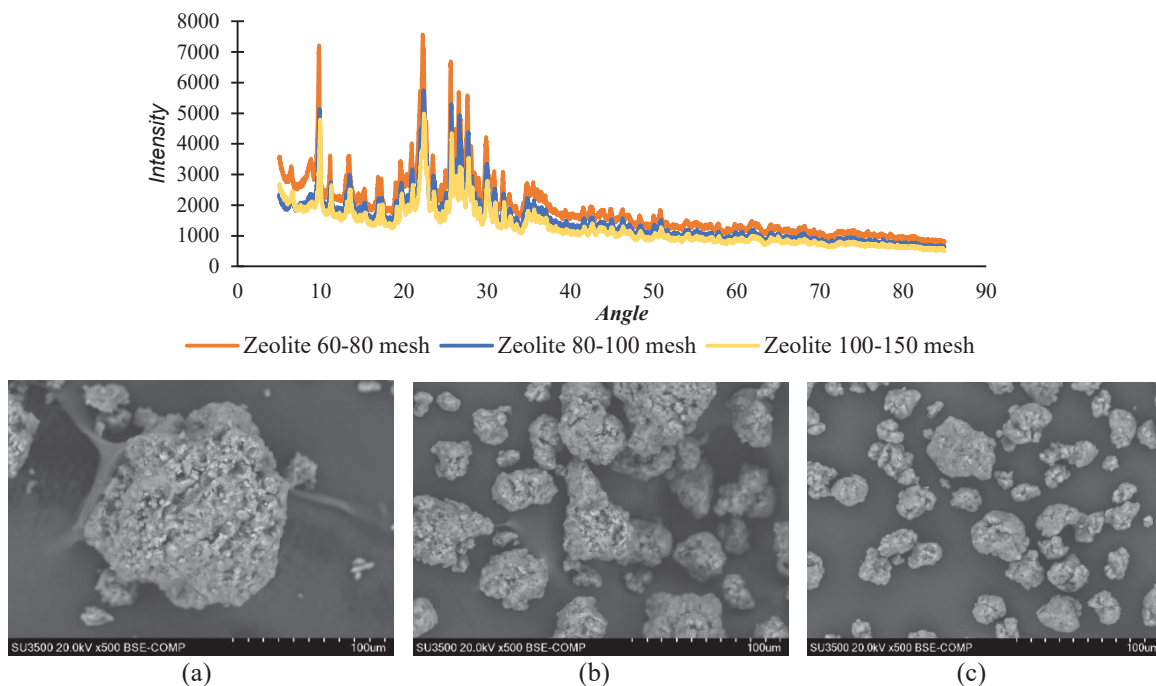


FIGURE 1. X-ray diffractogram (top) and scanning electron microscopes (bottom) of Bayah zeolite indicating the particle size of zeolite of 60–80 mesh (a), 80–100 mesh (b), and 100–150 mesh (c). The smaller of zeolite particle size would increase water-holding capacity and enhance the efficiency of water use.

SEM analysis showed that zeolite is a crystalline alumina-silicate mineral. It has high porosity and a large surface area suited for microbial carrier, especially exopolysaccharide bacteria on peat application. The exopolysaccharide-producing bacteria could help form structure and peat molecule configuration related to water and nutrient supply to the plants. Exopolysaccharide bacteria analysis in this study confirmed that the population was high, averaged in 10^7 – 10^8 CFU/gram of the product until 12 months observation under ambient temperature and about 60% of relative humidity. Furthermore, zeolite absorbs and releases water without changing its crystal structure according to the molecular sieve material to minimize fires in peatlands. The zeolite-clinoptilolite material from Bayah also has water absorption characteristics that can increase the water-holding capacity of the peat. Zeolites with 100–150 mesh of particle size have a higher water-holding capacity (60.50%) than 60–80 mesh (43.59%) and 80–100 mesh (49.22%) (Table 1).

Improving energy production and consumption in plantation can increase the number of reactive N_2 . This accumulation of N_2 can alter the natural ecosystem and composition of various species, biodiversity, and ecosystem functions and affect nutritional imbalances. It is estimated that 60% of global NH_3 -N emissions are produced from anthropogenic activity [26]. It needs to get special attention because it can cause problems in the future, such as damage to the plant leaves, increase sensitivity to drought checks, decrease biodiversity, increase environmental risk, eutrophication, and health. Reduce ammonia accumulation in an ecosystem environment is required.

The analysis of the adsorption-desorption potential of the molecular sieve material against NH_3 used the Temperature Programmed Desorption (TPD) method. The smaller the size of zeolite particles (100–150 mesh), the higher the CO_2 and NH_3 adsorption capacity. A high specific surface area combined with high pore volume appears as a determining factor for achieving high adsorption capacity (Table 2).

TABLE 2. The CO₂ volume adsorbed by zeolite.

| Particle size distribution of zeolite (mesh) | mol CO ₂ (mmol) | Basicity (mmol/g) CO ₂ | mol NH ₃ (mmol) | Acidity (mmol/g) NH ₃ |
|--|----------------------------|-----------------------------------|----------------------------|----------------------------------|
| 60–80 | 0.03355735 6 | 1.0586 | 0.02572543 6 | 0.8380 |
| 80–100 | 0.05392426 6 | 1.7856 | 0.02932856 6 | 0.8729 |
| 100–150 | 0.07211457 4 | 2.4038 | 0.03261893 7 | 1.0290 |

A very significant source of greenhouse gas emissions comes from the manufacture of synthetic nitrogen (N) fertilizers, which are widely used in agricultural and plantation crop fertilization activities. It is commonly reported that the application of this N fertilizer dramatically contributes to the cause of N₂O emissions from the soil. In modern agricultural and plantation cultivation technology, various efforts have been made to reduce greenhouse gas emissions, including reducing the dosage of fertilizers and utilizing soil microorganisms to increase fertility and land productivity. Based on research of Kusin *et al.* [27] stated that for each release of N₂O between 19.11–22.17 kg N₂O-N/ha, an equivalent emission would be 1052.26–1209.51 kg CO₂-eq/ha. According to the Intergovernmental Panel on Climate Change [28], one percent of N₂O-N emission is generated from the total N given during the application of fertilization. Based on the above research, a further study is needed on the impact of using N fertilizers and practical efforts to reduce the rate of N₂O emissions.

The potential use of zeolite for molecular sieve material to adsorb N₂ gas has been studied in this research. Zeolites with 100–150 mesh of particle sizes have a higher adsorption capacity (75.6 cm³/g STP) of N₂ at relative pressure (0.989 P/Po) and absolute pressure (752.2 mmHg) compared to 80–100 mesh (71.7 cm³/g STP) and 60–80 mesh (67.7 cm³/g STP) (Fig. 2).

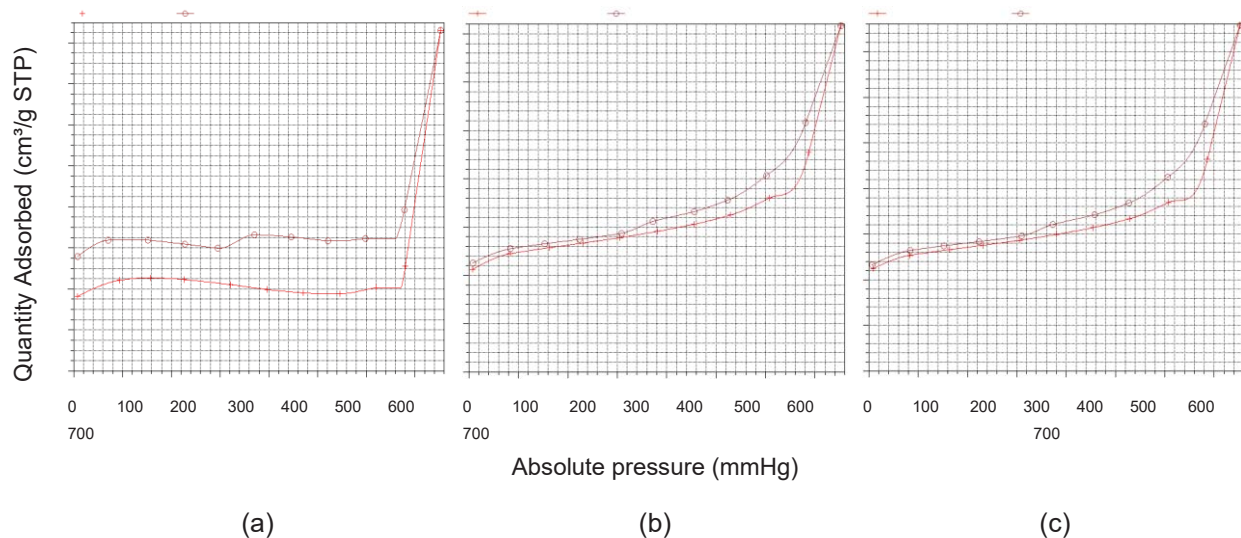


FIGURE 2. Graphics of the adsorption pattern (+) and desorption pattern (-) N₂ from zeolite 60–80 mesh (a), 80–100 mesh (b), and 100–150 mesh (c).

CONCLUSIONS

Natural clinoptilolite zeolite of Bayah origin in 100–150 mesh particle offers a reliable solution to minimize degradation of peat soils via its cation exchange, CO₂, N₂, NH₃ adsorbent, and have high water holding capacity functions. The viability of exopolysaccharide bacteria in the zeolite was high until 12 months. Thus, zeolite was appropriate as an inoculation carrier, especially under field conditions.

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