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SOIL MICROBIAL POPULATION AND SOIL ENZYME ACTIVITY ON PT BUKIT ASAM VARIOUS RECLAIMED LAND SITES IN SOUTH SUMATRA

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ABSTRACT

Open mining activities result in decreased microbial biomass and negatively impacts soil fertility. Soil microbes play a role in the decomposition of soil organic matter and in nutrient cycles through the process of mineralization by the enzymes they produce. The purpose of this study was to analyse soil fertility levels in PT Bukit Asam's various reclaimed land sites at Muara Enim Regency, South Sumatra, Indonesia, as determined by these areas' microbial populations and soil enzyme activity. The research was conducted by using the explorative method in PT Bukit Asam's various reclaimed land. Soil sample from 7 different reclamation age area were analysed. Our results showed that soil conditions and soil enzyme activity vary by reclamation age. At KTU, a 12-year-old reclaimed land site, urease enzyme activity had a value of 68.83 mg $NH_4^+.g^{-1}dm.h^{-1}$ with a microbial population of 82.64 x 10^4 $CFU.g^{-1}$ soil. The highest phosphatase enzyme activity value of 95.66 mg $pNP.g^{-1}$ $dm.h^{-1}$ was found on the 9-year-old SP702 reclaimed land site, with a soil pH of 5.23. Cellulase enzyme activity on the 21-year-old Udongan reclaimed site had a value of 21.51 mg $GE.g^{-1}dm.h^{-1}$ with a cellulolytic microbial population of 1.9 x 10^4 $CFU.g^{-1}$ soil, higher than on other reclamation sites. Invertase enzyme activity on the 15-year-old Tupak reclaimed land site had a value of 24.37 mg $GE.g^{-1}dm.h^{-1}$. Soil enzyme activity can be an indicator of soil quality and soil microbial activity as it relates to all forms of biochemical transformations occurring in the soil and is highly sensitive to environmental changes.

Keywords: reclamation, soil enzyme activity, soil microbes

INTRODUCTION

Open pit mining results in the degradation of physical, chemical, and biological environments, such as the decline in the soil fertility of microbe-rich top soils (Menta *et al.* 2014; Puspaningsih *et al.* 2010). Efforts to better these environmental conditions are done by improving soil through post-mining reclamation. With time, reclaimed land can function again through forest succession, which is a natural process. Extreme soil conditions on reclaimed land, as a result of mining activities, impact soil biological activity (Asensio *et al.* 2013; Zornoza *et al.* 2015). Microbes in the soil are important components in biogeochemical cycles (Falkowski *et al.* 2008), such as in the decomposition of organic matter and nutrient cycles in the soil.

Microbial activity in nutrient cycles is related to mineralization processes by enzymes that are mostly produced by soil microbes. Soil enzyme activity is closely related to environmental conditions and can serve as an indicator for soil quality (Rietz & Haynes 2003; Yuan *et al.* 2007), growth index, and soil microbial activity (Fornasier *et al.* 2014). Soil enzyme activity, as an indicator of soil quality (which is related to nutrient cycles as well as soil enzyme transformation and activity), is highly sensitive to environmental changes, both naturally occurring and caused by anthropogenic factors

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(Gianfreda *et al.* 2005). The purpose of this study was to analyze soil fertility levels in various reclaimed land areas managed by PT Bukit Asam South Sumatra, as determined by these sites' soil microbial population and soil enzyme activity.

MATERIALS AND METHODS

Soil Sampling

Soil samples were taken from the PT. Bukit Asam, Muara Enim Regency, South Sumatra's (PT. BA) reclaimed land, at the depths of 0-15 cm, based on the reclamation age. The reclaimed areas are East Bangko Barat (reclamation age of 3 years), Mahayung 3 (reclamation age of 6 years), SP702 (reclamation age of 9 years), KTU (reclamation age of 12 years), Tupak (reclamation age of 15 years), Mahayung (reclamation age of 18 years), and Udongan (reclamation age of 21 years) (Fig. 1).

The number of samples taken were determined by following ISO 10381-4 standards. Soil samples were composite samples consisting of several sub-samples taken at random, and the samples were collected based on reclamation age. Soil samples were analyzed at the Environmental Biotechnology Laboratory (EBL) at the Indonesian Center for Biodiversity and Biotechnology (ICBB), Bogor.

Urease Enzyme Activity

Urease activity was measured using urea 720 mM as substrate. The amount of ammonium released was determined through Nessler's reagent (Vogel 1990) and it was incubated at 37°C for 2 hours. Measurement of released ammonium was done using Schinner *et al.*'s method (1996).

Phosphatase Enzyme Activity

Phosphatase activity was measured using p-Nitrophenyl Phosphate 115 mM as substrate and it was incubated at 37°C for 1 hour using tris buffer (hydroxymethyl) aminomethane, pH 6.5. The release of p-nitrophenol (pNP) was measured using Eivazi and Tabatabai's method (1977).

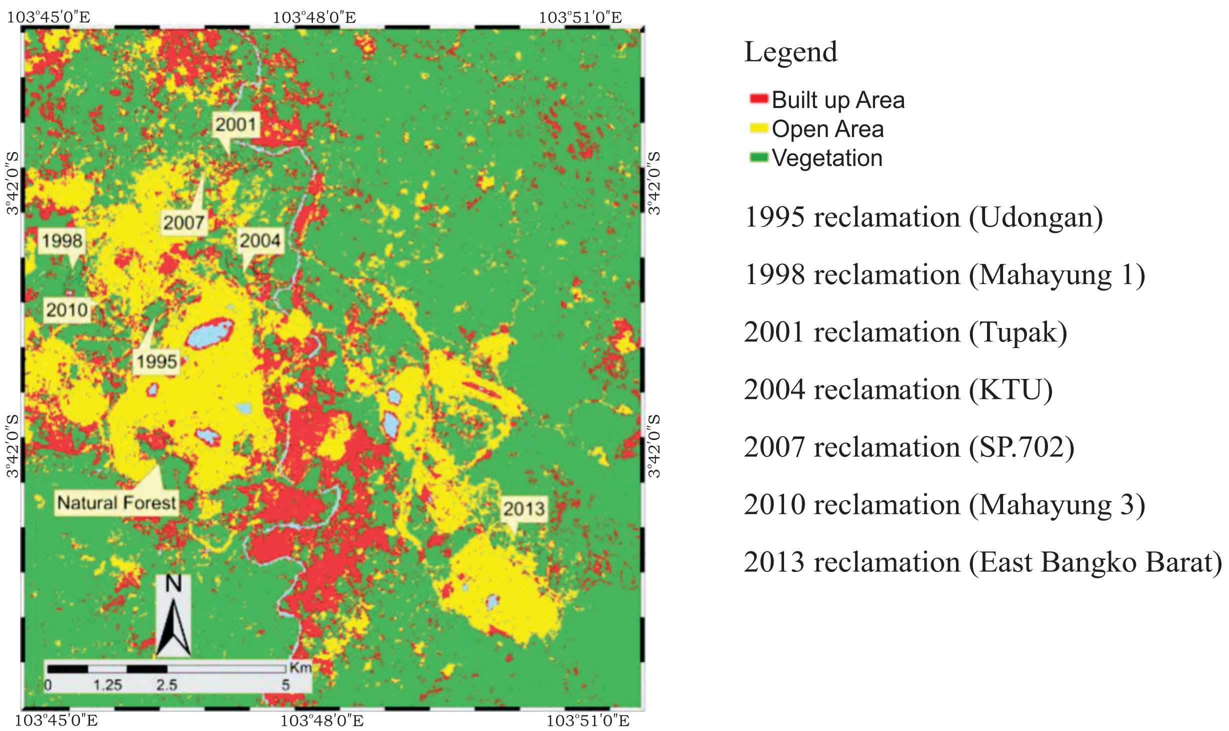

Figure 1 Soil sample locations within reclaimed areas

Activity of Cellulase and Invertase Enzymes

Cellulase activity was measured using CMC 0.7% as substrate and it was incubated for 24 hours at 50°C. Invertase activity was measured using sucrose 6% as substrate and it was incubated for 3 hours at 50°C. Sugar reduction was determined using Schinner and Mersi's method (1990).

Organic Matter Content

Determination of organic matter content can be done by determining the soil organic carbon content multiplied by the van Bemmelen correction factor, assuming that the soil organic matter contained 58% soil organic-C. Determination of organic-C content was done through colorimetry, using Walkley and Black's method (1934).

RESULTS AND DISCUSSION

Soil Conditions and Vegetation Cover

Soil conditions and land cover on the reclaimed land of PT. BA, based on reclamation age, show different circumstances (Table 1). Udongan was reclaimed 21 years ago and East Bangko Barat—the youngest in reclamation age—3 years ago, and both were the objects of this research (Table 1). Soil pH ranged from 3.86 to 5.23. The highest soil pH was found on the 9-year-old SP702 reclamation site, while the highest level of acidity, i.e., 3.86, was found on the 6-year-old Mahayung 3 reclamation site.

Water contents ranged from 7.59% to 19.31%, and the highest humidity was found in the reclaimed site of Udongan which had a moisture content of 19.31%. The lowest water content, i.e., 7.59, was found on the Mahayung 3 reclaimed site. Mahayung 3, a 6-year-old reclaimed site, was eroded because overburden soil was used in the reclamation. Overburden soil has low water holding capacity and has no nutrients (Chaubey *et al.* 2012).

The highest microbial population was found on the KTU reclaimed site, at 82.64 x 10^4 CFU.g^{-1} soil, while the lowest soil microbial population was found on the Mahayung 3 reclaimed site, at 5.42 x 10^4 CFU.g^{-1} soil. The highest cellulolitic microbial population was found on the Udongan reclaimed site, at 18.74 x 10^3 CFU.g^{-1} soil with a moisture content of 19.31%, while the lowest cellulolitic microbial population was found on the Mahayung 3 reclaimed site, at 7.85 x 10^3 CFU.g^{-1} soil. Soil microbial population and diversity are influenced by environmental factors, such as humidity, temperature, organic matter, acidity, and nutrient content (Alexander 1961) and can be used as indicators to determine soil productivity (Zornoza *et al.* 2009).

Urease Enzyme Activity

Urease is an enzyme produced by both plants and microbes but mostly produced by soil microbes, such as *Lactobacillus ruminis*, *Lactobacillus fermentum*, *Lactobacillus reuteri,* and *Klebsiella aerogenes* (Kakimoto *et al.* 1989; Mulrooney *et al.* 2005). The enzyme plays a role in hydrolyzing urea into ammonia and carbon dioxide (Krajewska *et al.* 2012; Banerjee & Aggarwal 2012). Results of the research on urease enzyme activity on various reclaimed land sites in PT. BA show that KTU, a 12-year-old reclaimed site, had the highest enzyme activity value, i.e., 64.83 μg NH_4^+.g^{-1} dm.h^{-1}, while Udongan, a 21-year-old reclaimed site, had an urease enzyme activity value of 50.74 μg NH_4^+.g^{-1}dm.h^{-1}. The lowest urease enzyme activity, i.e., 3.83 μg NH_4^+.g^{-1} dm.h^{-1}, was found on Mahayung 3 (Table 3). KTU, 12-year-old reclaimed site, is characterized by well-grown vegetation (Fig. 2). The difference of urease enzyme activity among the location is due to the variation of soil properties.

Age of reclamation influences the vegetation cover of a reclamation area. At the beginning of reclamation, these reclaimed areas were planted by fast growing species. After years of reclamation and succession, Udongan has the highest species diversity, followed by Mahayung 3, Tupak, KTU and East Bangko Barat. Meanwhile, SP702 and Mahayung 3 have the lowest plant biodiversity (Al-reza *et al.* 2016) (Table 2).

Table 1 Soil conditions on PT BA's reclaimed land sites

Location	Reclamation Age (year)	pH	Water Content (%)	Soil Microbial Population (CFU.g^{-1})	Organic Matter Content (%)	Cellulolitic Microbe (CFU.g^{-1})
East Bangko Barat	3	4.19	15.23	1.4 x 10^5	2.70	9.07 x 10^3
Mahayung 3	6	3.86	7.59	5.42 x 10^4	3.96	7.85 x 10^3
SP702	9	5.23	16.05	34.77 x 10^4	27.72	1.6 x 10^4
KTU	12	5.13	15.32	82.64 x 10^4	8.00	8.76 x 10^3
Tupak	15	4.78	17.94	26.96 x 10^4	5.10	8.54 x 10^3
Mahayung 1	18	4.51	14.88	25.83 x 10^4	10.69	17.52 x 10^3
Udongan	21	4.94	19.31	3.9 x 10^5	7.33	1.9 x 10^4

Table 2 Vegetation conditions on PT. BA's reclaimed land sites

Location	Species
East Bangko Barat	*Cassia siamea Lamk, Melaleuca leucadendra, Falcataria moluccana*
Mahayung 3	*Cassia siamea Lamk*
SP702	*Acacia auriculiformis*
KTU	*Acacia auriculiformis*, *Acacia mangium*, *Melaleuca cajuputi*
Tupak	*Pterocarpus indicus, Acacia,auriculiformis, Acacia mangium*
Mahayung 1	*Acacia auriculiformis, Paraserianthes falcataria, Vitex pinnata*
Udongan	*Scleria sumatrana, Tetracera indica*, *Saccharum spontaneum*, *Diplazium proliferum*, *Dicranopteris linearis*, *Urena lobata*

Phosphatase Enzyme Activity

Soil phosphatase enzyme is an extracellular enzyme that plays a role in the mineralization of P-organic into P-inorganic, which can then be absorbed and metabolized by plant root cells and microbes (Burns 1982; Isgitani *et al.* 2005). The results of this research show that enzyme phosphatase activity on SP702, i.e., 95.66 mg pNP.g^{-1}dm.h^{-1}, was higher than that of Udongan, i.e., 83.36 mg pNP.g^{-1}dm.h^{-1}. The 6-year-old Mahayung 3 reclaimed site had the lowest phosphatase activity, i.e., 18.84 mg pNP.g^{-1}dm.h^{-1}. SP702, which was reclaimed 9 years ago, is now completely covered by grass vegetation. Trasar-Cepeda *et al.* (2008) stated that phosphatase activity in the P-cycle on land with grass vegetation cover is higher than that of land with tree vegetation. Phosphatase in soil is produced by plants and microbes on soils with low phosphate availability (Joner *et al.* 2000).

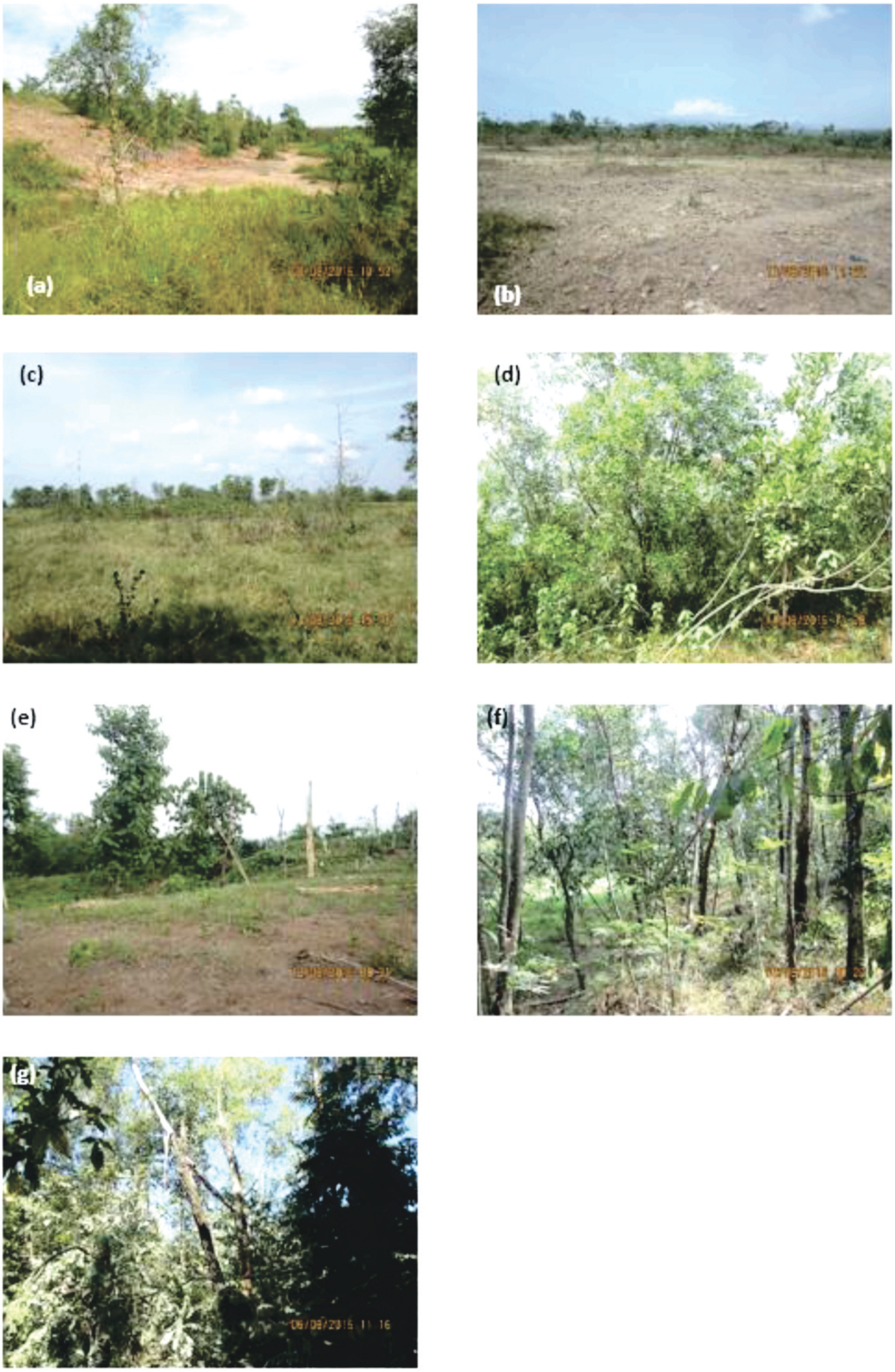

Figure 2 Land cover condition of reclaimed land sites: (a) East Bangko Barat, (b) Mahayung 3, (c) SP702, (d) KTU, (e) Tupak, (f) Mahayung 1, (g) Udongan

Table 3 Results of analysis of soil enzyme activity

Locations	Soil enzyme activity			
	Urease ($\mu g\ NH_4^+.g^{-1}dm.h^{-1}$)	Phosphatase ($mg\ pNP.g^{-1}dm.h^{-1}$)	Cellulase ($mg\ GE.g^{-1}dm.h^{-1}$)	Invertase ($mg\ GE.g^{-1}dm.h^{-1}$)
East Bangko Barat	13.13	47.21	0.28	9.82
Mahayung 3	3.83	18.84	0.29	8.95
SP702	49.51	95.66	0.92	14.24
KTU	64.83	67.55	0.33	10.29
Tupak	50.41	77.58	0.36	24.37
Mahayung	38.63	63.73	0.63	12.13
Udongan	50.74	83.36	1.10	21.51

Cellulase Enzyme Activity

Cellulase is a group of complex enzymes composed of endoglucanase, exoglucanase, and glucosidase. The cellulase enzyme plays a role in the hydrolysis of cellulose in the degradation process of organic matter. Results showed the Udongan reclaimed site to have the highest cellulase enzyme activity, i.e., 1.10 mg $GE.g^{-1}dm.h^{-1}$, and the East Bangko Barat reclaimed site to have the lowest, i.e., 0.28 mg $GE.g^{-1}dm.h^{-1}$. High cellulase activity on the Udongan site is influenced by a high population of cellulolytic microbes on the reclaimed land. Udongan's cellulolytic microbe population was found to be at 1.9 x 10^5 $CFU.g^{-1}$ soil, higher than that of other reclamation sites (Table 1).

This shows that there is a relationship between cellulolytic microbial population and cellulase enzyme activity on reclaimed land. In addition, cellulase activity in the soil is affected by several factors, such as temperature, pH, moisture, soil aeration, and the chemical structure of organic matter (Deng & Tabatabai 1994; Alf & Nannipieri 1995; Steinberger & Whitford 1988). Cellulase activity on a land site indicates the site's natural metabolism (Kanazawa & Miyashita 1987). The cellulase enzyme has low activity at a pH value of less than 5 and optimum activity at pH 5-6, at a temperature of 30-50°C (Doyle *et al.* 2006).

Invertase Enzyme Activity

The invertase enzyme was once used as one of the criteria for classifying soil fertility (Skujins 1978). Invertase activity in the soil is very important because it is associated with the degradation of sucrose widely found in plants (Frankenberger & Johanson 1983). Results for invertase enzyme activity on various reclaimed sites showed that Tupak had the highest invertase enzyme activity, i.e., 24.37 mg $GE.g^{-1}dm.h^{-1}$, whereas Udongan had an invertase enzyme activity value of 21.51 mg $GE.g^{-1}dm.h^{-1}$, and Mahayung 3 the lowest with a value of 9.82 mg $GE.g^{-1}dm.h^{-1}$ (Table 3).

Tupak is a reclaimed land site with a reclamation age of 15 years. The reclaimed land has been used by surrounding communities for agriculture, plantation, and pasture. Kandeler *et al.* (1999) stated that land management may affect activity of invertase enzymes in the soil. Invertase activity generally takes place in the humus layer (Kshattriya *et al.* 1992) and about 80% takes place on a fraction of soil particles <63 mm in size (Stemmer *et al.* 1999). Invertase is an enzyme produced by plants and microbes both intracellularly and extracellularly (Rashad *et al.* 2006; Hussain *et al.* 2009) and is stable in acidic pH (Oyedeji *et al.* 2017).

Correlation between Soil Enzyme Activity and Soil Condition

PT Bukit Asam South Sumatra applied an open pit mining system. The main issue with reclaiming land on open pit mining is the formation of acid mine drainage (AMD), which results in decreased pH. Low pH, high metal concentrations, and low quantities of organic matter are a major problem in post-mining reclaimed land (Zanuzzi *et al.* 2009; Martinez-Pagan *et al.* 2011).

Soil enzyme activity on PT Bukit Asam South Sumatra's reclaimed land does not show a significant correlation ($p<0.05$ and $p<0.01$) with reclamation age. Soil that has been polluted by heavy metals affects microbial biomass through the inhibition of microbial activity (Utobo & Tewari 2015). Renella *et al.* (2011) and Yan *et al.* (2013) state that a concentration of metal contaminants in the soil can decrease soil enzyme activity. In addition, soil condition,

Table 4 Correlation coefficient of soil enzyme activity

Parameter	Soil Enzyme			
	Urease	Phosphatase	Cellulase	Invertase
Reclamation age	0.64	0.55	0.59	0.65
Soil pH	0.94[ab]	0.92[ab]	0.55	0.43
Water content	0.72	0.86[ab]	0.53	0.53
Soil microbial population	0.84[a]	0.48	0.11	0.03
Organic matter	0.39	0.64	0.59	0.01

Note: Correlation is significant at [a]p $p<0.05$ and [ab]p $p<0.01$

vegetation cover, microbial diversity biomass, and the natural succession of vegetation cover impact soil enzyme activity (Taylor *et al.* 2002).

Urease enzyme activity on various reclaimed land sites in PT. BA varied (Table 3) based on reclamation age. Urease enzyme activity on these reclaimed sites showed a significant correlation ($p<0.05$ and $p<0.01$) with soil pH and soil microbial population (Table 4). Urease hydrolyzes urea into ammonia so that soil pH increases, promoting the growth of urease-producing microbes (Fisher *et al.* 2017). Urease activity in the soil happens in the horizon A layer (Fisher *et al.* 2017). Urease activity is not directly related with soil organic matter (Saha *et al.* 2008).

Phosphatase enzyme activity on PT. BA's reclaimed land sites shows significant correlation ($p<0.05$ and $p<0.01$) with pH and moisture content (Table 4). Phosphatase activity becomes high in soils with one type of vegetation, low acidity, and that are rich in organic matter and moisture (Rahmansyah 2009; Zhang *et al.* 2010). Phosphatase enzymes activity in the soil is related to the pattern of the soil's biological activity in the process of degrading organic matter (Hu & Cao 2007).

Soil enzymes are a group of enzymes produced by soil organisms but are mostly produced by soil microbes and play a role in maintaining soil ecology, soil physical and chemical properties, soil fertility, and soil health (Zornoza *et al.* 2009) and have unique characteristics because they can only work on a particular substrate (Burns 1982). Soil enzyme activity becomes an indicator for assessing the quality of the soil and its microbial activity (Fornasier *et al.* 2014) because all forms of biochemical transformation occurring in the soil are related to soil enzymes (Tabatabai 1994).

CONCLUSION

Soil enzyme and microbial population activity in PT. BA's various reclaimed land sites varies based on reclamation age. Different soil conditions such as pH, water content, and organic matter content on all reclaimed land sites impact soil enzyme activity and soil microbial population. Soil enzyme activity can be an indicator for soil quality and soil microbial activity.

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