SOIL MICROBIAL POPULATION AND SOIL ENZYME ACTIVITY ON VARIOUS RECLAMATION AREA OF PT BUKIT ASAM IN SOUTH SUMATERA

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ABSTRACT

Open mining activities result in a decrease in microbial biomass and a negative impact on soil fertility. Soil microbes play a role in the decomposition of soil organic matter and nutrient cycles through the process of mineralization by enzyme they produce. The purpose of this research is to analyze levels of soil fertility in various land reclamations of PT Bukit Asam South Sumatra determined by microbial population and soil enzyme activity. The results show that soil conditions and soil enzyme activity vary by reclamation age. Urease enzyme activity at KTU with the reclamation age of 12 years is 68.83 mg NH_4^+.g^{-1}dm.h^{-1} and the microbial population is 82.64 x 10^4 CFU.g^{-1}. The highest phosphatase enzyme activity on the 9-year SP702 land reclamation is 95.66 mg pNP.g^{-1} dm.h^{-1} and soil pH 5.23. The activity of the cellulase enzyme on the 21 years old reclamation field is 21.51 mg GE.g^{-1}dm.h^{-1} with the cellulolytic microbial population 1.9 x 10^4 CFU.g^{-1} which is higher than other reclamation sites. Activity of invertase enzyme on Tupak land reclamation with the reclamation age of 15 years is 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 transformation occurring in the soil and has high sensitivity to environmental changes.

Keywords: soil enzyme activity, soil microbes, reclamation

INTRODUCTION

Open pit mining results in degradation in physical, chemical, and biological environments, such as a decrease in soil fertility in microbe-rich top soils (Menta *et al.* 2014; Puspaningsih *et al.* 2010). Efforts to improve the condition are done by land improvement through post-mining reclamation. As time goes by the 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 have an impact on 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 decomposition of organic matter and nutrient cycles in the soil.

Microbial activity in the nutrient cycle is related to the mineralization process 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 a nutrient cycle as well as transformation and activity of soil enzyme has high sensitivity to environmental changes both naturally occurring and by anthropogenic factors (Gianfreda *et al.* 2005) . The purpose of this study is to analyze soil

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fertility levels in various reclaimed lands at the area of PT Bukit Asam South Sumatra determined by soil microbial population and soil enzyme activity.

MATERIALS AND METHODS

Soil Sampling

Soil samples were taken from the reclaimed land of PT Bukit Asam South Sumatra, based on the reclamation age at 0-15 cm depth. The reclamation 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) (Figure 1).

Determination of the number of samples follows the standard of ISO norm 10381-4. The soil sample is a composite sample consisting of several sub-samples taken at random, and the samples were collected based on age of reclamation. Analysis of soil samples was conducted at the Environmental Biotechnology Laboratory (EBL) of the Indonesian Center for Biodiversity and Biotechnology (ICBB), Bogor.

Urease Enzyme Activity

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

Phosphatase Enzyme Activity

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

Activity of Cellulase and Invertase Enzymes

The cellulase activity was measured by using CMC 0.7% as substrate and it was incubated for 24 hours at 50°C, and the invertase activity was measured by using sucrose 6% as substrate and it was incubated for 3 hours at 50°C, and reducing sugar was determined by using Schinner and Mersi's (1990) method.

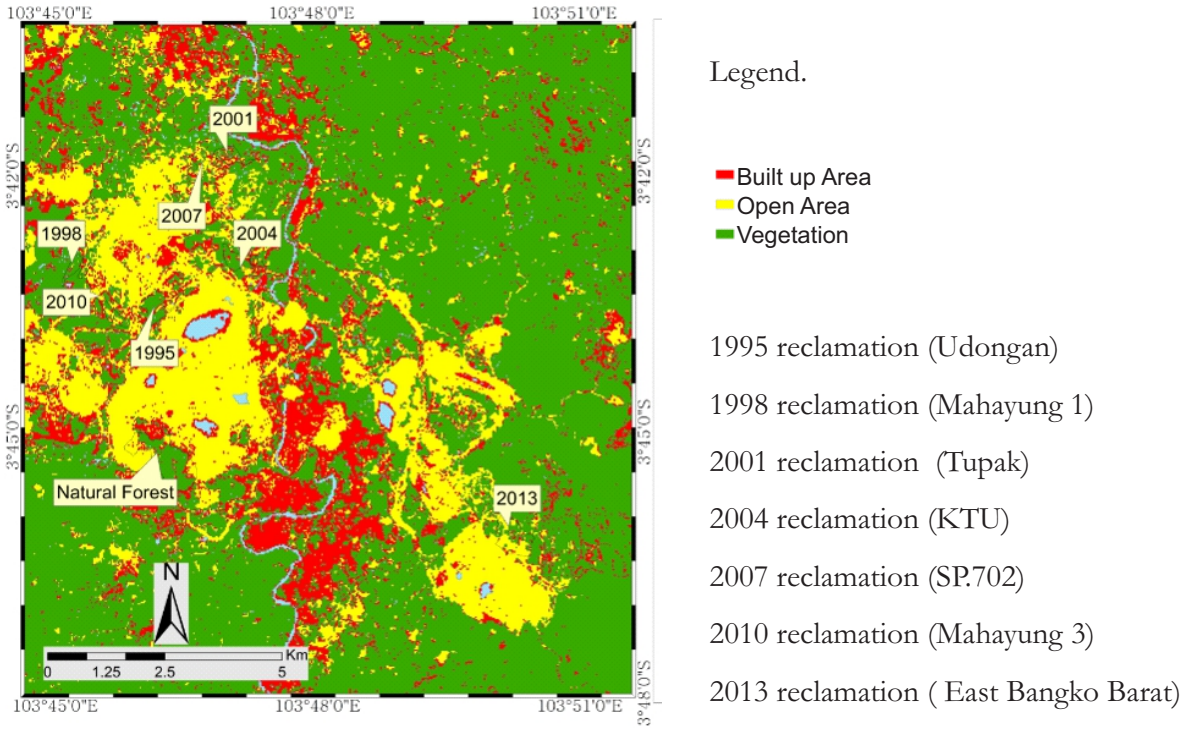

Figure 1. Location of soil sample within reclamation area

Organic Matter Content

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

RESULTS AND DISCUSSION

Soil Conditions and Vegetation cover

Soil conditions and land cover on the reclaimed land of PT Bukit Asam South Sumatra, based on reclamation age, show different conditions (Table 1). The reclaimed land of Udongan has been reclaimed for 21 years and East Bangko Barat for 3 years which is the youngest in reclamation age, and both are objects of this research (Table 1). The soil pH conditions range from 3.86 to 5.23. The highest soil pH is found on reclamation site of SP702 on the 9-year reclamation age, and the highest level of acidity, e.g. 3.86, is found on Mahayung 3 on the 6-year reclamation age.

Water content ranges from 7.59% to 19.31%, and the highest humidity condition is in the reclamation field of Udongan with moisture content of 19.31%. The lowest water content, e.g. 7.59, is found in Mahayung 3 reclamation field. The condition of the reclamation land of Mahayung 3, which is a 6-year-old reclamation land, is eroded. This happens because the soil used in the reclamation was an overbunden soil. An overbunden soil has low water holding capacity and has no nutrients (Chaubey *et al.* 2012).

The highest microbial population is found on KTU reclamation field which is 82.64 x 10^4 CFU.g^{-1}, while the lowest soil microbial population is found on Mahayung 3 reclamation field which is 5.42 x 10^4 CFU.g^{-1}. The highest cellulolitic microbial population is found on Udongan land reclamation, e.g. 18.74 x 10^3 CFU.g^{-1} with moisture content of 19.31%, while the lowest cellulolitic microbial population is found on Mahayung 3 land reclamation, e.g. 7.85 x 10^3 CFU.g^{-1}. 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 an indicator 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 research on urease enzyme activity on various land reclamations in PT Bukit Asam South Sumatera show that the highest enzyme activity, e.g. 64.83 μg NH_4^+.g^{-1}dm.h^{-1}, is on the land reclamation of KTU which is a 12-year-old land reclamation, while urease enzyme activity on Udongan which is a 21-year-old land reclamation is 50.74 μg NH_4^+.g^{-1}dm.h^{-1}. The lowest urease enzyme activity, e.g. 3.83 μg NH_4^+.g^{-1}dm.h^{-1}, is found on Mahayung 3 (Table 3). The condition of KTU which is a 12-year-old land reclamation is characterized by well grown vegetation (Figure 2).

Table 1 Soil Conditions

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

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

Tabel 2. Vegetation condition of reclamation area in PT. Bukit Asam, Sumatera Selatan

Location	Species
East Bangko Barat	*Cassia siamea Lamk, Melaleuca leucadendra, Falcataria m oluccana*
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*

Table 3 Results of Analysis of Soil Enzyme Activity

Locations	Soil enzyme activity			
	Urease (µg 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

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

Phosphatase Enzyme Activity

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

Cellulase Enzyme Activity

Cellulase is a group of complex enzymes composed of endoglucanase, exoglucanase, and glucosidase. The cellulase enzyme plays a role in hydrolysis of cellulose in the process of degradation of organic matter. The research results show that the highest cellulase enzyme activity is on Udongan land reclamation, e.g. 1.10 mg GE.g^{-1}dm.h^{-1}, and the lowest is in the reclamation area of East Bangko Barat, e.g. 0.28 mg GE.g^{-1}dm.h^{-1}. High cellulase activity on reclaimed land of Udongan is influenced by high population of cellulolytic microbes in the land reclamation. The population of cellulolytic microbes on Udongan is 1.9 x 10^5 CFU.g^{-1} which is 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 the reclaimed land. In addition, cellulase activity in the soil is affected by several factors, such as temperature, pH, moisture, soil aeration, and chemical structure of organic matter (Deng & Tabatabai 1994; Alf & Nannipieri 1995; Steinberger & Whitford 1988). The cellulase activity on a land indicates the natural metabolism that exists in that land (Kanazawa & Miyashita 1987). The cellulase enzyme has low activity at the value of pH of less than 5 and is optimum at pH 5-6 at 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 that is widely found in plants (Frankenberger & Johanson 1983). Results of research of invertase enzyme activity on various reclamation fields that exist in PT Bukit Asam South Sumatra show that the highest invertase enzyme activity is on Tupak, e.g. 24.37 mg GE.g^{-1}dm.h^{-1}, whereas the value on Udongan is 21.51 mg GE.g^{-1}dm.h^{-1}, and the lowest is on Mahayung 3, e.g. 9.82 mg GE.g^{-1}dm.h^{-1} (Table 3).

Tupak is a land reclamation with reclamation age of 15 years. The reclaimed land has been used by surrounding community for agriculture, plantation, and pasture. Kandeler *et al.* (1999) states 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 the fraction of the soil particle with the size of <63 mm (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

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

Soil enzyme activity on reclaimed land of PT Bukit Asam South Sumatra does not show a significant correlation ($p < 0.05$ and $p < 0.01$) with reclamation age. The soil that has been polluted by heavy metals affects microbial biomass through inhibition of microbial activity (Utobo & Tewari 2015). Renella *et al.* (2011) and Yan *et al.* (2013) state that concentration of metal contaminants in the soil can decrease soil enzyme activity. In addition, soil conditions, vegetation cover, microbial diversity biomass, and natural succession of vegetation cover have effects on soil enzyme activity (Taylor *et al.* 2002).

Urease enzyme activity on various reclamed lands in PT Bukit Asam varies (Table 3) based on the age of reclamation. Urease enzyme activity on the land shows a significant correlation ($p<0.05$ and $p<0.01$) with soil pH and soil microbial population (Table 4). The urease hydrolyzes urea into ammonia so that the soil pH increases and promotes the growth of urease-producing microbes (Fisher *et al.* 2017). Urease activity in the soil happens in horizon A layer (Fisher *et al.* 2017). The urease activity is not directly related with soil organic matter (Saha *et al.* 2008).

Table 4 Coefficient of Correlation 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

Phosphatase enzyme activity on reclamed land of PT Bukit Asam South Sumatra shows a significant correlation ($p < 0.05$ and $p < 0.01$) with pH and moisture content (Table 4). Phosphatase activity becomes high in soils that have one type of vegetation with low acidity, rich in organic matter and soil moisture (Rahmansya 2009, Zhang *et al.* 2010). The activity of phosphatase enzymes in soil is related to the pattern of soil biological activity in the process of degradation of 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 a unique characteristic because they can only work on a particular substrate (Burns 1982). Soil enzyme activity in the soil 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).

CONCLUSIONS

Activity of soil enzyme and microbial population at various reclaimed lands in PT Bukit Asam South Sumatra show different results based on reclamation age. Different soil conditions on every reclamed land of PT Bukit Asam South Sumatra have effects on 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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REFERENCES

Alexander M. 1961. Soil Microbiology. New York: John Wiley & Sons, Inc.

Alf K, Nannipieri P. 1995. Cellulase activity, methods in applied soil microbiology and biochemistry. London: Academic.

Al-Reza,D.D, L.B. Prasetyo, R. Hermawan. 2016. Biodiversity of Plants and Birds in Reclamation Area of PT Bukit Asam, Muara Enim, South Sumatera. Jurnal Manajemen Hutan Tropika. Vol. 22, (3): 158-168.

Asensio V., Covelo E.F., Kandeler E. 2013. Soil management of copper mine tailing soils–sludge amendment and tree vegetation could improve biological soil quality. Sci Total Environ. 456(457):82–90.

Banerjee S., Aggarwal A. 2012. Isolation, partial purification, characterization, and inhibition of urease (E.C. 3.5.1.5) enzyme from the Cajanus cajan seeds. Asian Journal of Bio Science. 7: 203–209.

Burns RG. 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol Biochem 14:423-427.

Chaubey O.P., Bohre P., Singhal P.K. 2012. Impact of Bio-reclamation of coal mine spoil on nutritional and microbial characteristics a case study. Int J Bio Sci Bio Technol. 4: 69-79.

Deng S.P., Tabatabai, M.A. 1994. Cellulase Activity of Soil. Soil Biol Biochem. 26:1347-1354.

Doyle J., Pavel R., Barness G., Steinberger Y. 2006. Cellulase dynamics in a desert soil. Soil Biol Biochem. 38:371-376.

Eivazi F., Tabatabai M.A. 1977. Phosphatases in soils. Soil Biol Biochem. 9:167-172.

Falkowski P.G., Fenchel T., Delong E.F. 2008.The microbial engines that drive Earth's biogeochemical cycles. Science 320:1034-1039.

Fisher K.A., Yarwood S.A., James B.R. 2107. Soil urease activity and bacterial ureC gene copy numbers: Effect of pH. Geoderma. 285:1–8.

Fornasier F., Ascher J., Ceccherini M.T., Tomat, E., Pietramellara, G. 2014. A simplified rapid, low-cost and versatile DNA-based assessment of soil microbial biomass. Ecol Indic. 45:75–82.

Frankenberger W.T., Johanson J.B. 1983. Factors affecting invertase activity in soils. Plant and Soil. 74:313-323.

Gianfreda L., Rao M.A., Piotrowska A., Palumbo G., Colombo C. 2005. Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Sci Total Environ. 341:265–279.

Hu C., Cao Z. 2007. Size and activity of the microbial biomass and soil enzyme activity in long-term field experiment. World Journal of Agriculture Sciences 3(1): 63-70.

Hussain A., Rashid M.H., Perveen R., Ashraf M. 2009. Purification, kinetic and thermodynamic characterization of soluble acid invertase from

sugarcane (Saccharum officinarum L.). Plant Phys Biochem. 47(3):188-194.

Isgitani M,, Kabirun S., Siradz S.A. 2005. Effect of Bacterial Inoculation of Phosphate Solvent on the growth of sorghum on various P soil contents. JITL. 5(1):48-54.

Joner E.J., Aarle I.M., Vosatka. 2000. Phosphatase activity of extraradical arbuscular mycorrhiza hyphae: a review. Plant soil. 226:199-210.

Kakimoto S., Sumino Y., Akiyama S., Nakao Y. 1989. Purification and characterization of acid urease from *Lactobacillus reureri*. Agric Biol Chem. 53:1119-1125.

Kanazawa S., & Miyashita K. 1987. Cellulase Activity in Forest Soils. Soil Science and Plant Nutrition. 33(3):399-406.

Kandeler E., Palli S., Stemmer M., Gerzabek M.H. 1999. Tillage changes microbial biomass and enzyme activities in particle size fractions. Soil Biol Biochem. 31:1253-1264.

Krajewska B., Van-Eldik R., Brindell M. 2012. Temperature and pressure dependent stopped flow kinetic studies of Jack Bean urease. Implications for the catalytic mechanism. Biological Inorganic Chemistry. 17:1123-1134.

Kshattriya S., Sharma G.D., Mishra R.R. 1992. Enzyme Activities Related to Litter Decomposition in Forests of Different Age and Altitude in North East India. Soil Biol Biochem. 24:265-270.

Martinez-Pagan P., Faz A., Acosta J.A., Carmona D.M., Martinez-Martinez S. 2011. A multidisciplinary study formining landscape reclamation: a study case on two tailing ponds in the Region of Murcia (SE Spain). Phys Chem Earth 36:1331–1344.

Menta, C. F.D., Conti, S., Pinto, A., Leoni, C., Lozano-Fondon. 2014. Monitoring soil restoration in an open pit mine in northern Italy. Appl Soil Ecology. 83:22–29.

Mulrooney S.B., Ward S.K., Hausinger R.P. 2005. Purification and properties of the Klebsiella aerogenes UreE metal-binding domain, a functional metallochaperone of urease. J Bacterio. l10:3581-3585.

Oyedeji O., Bakare M.K., Adewale I.O., Olutiola P.O., Omoboye O.O. 2017. Optimized production and characterization of thermostable invertase from Aspergillus niger IBK1, using pineapple peel as alternate substrate. Biocatalysis and Agricultural Biotechnology. 9:218–223.

Puspaningsih N., Murtilaksono K., Sinukaban N., Jaya I.N.S., Setiadi Y. 2010. Reforestation achievement monitoring at mining area through soil index model. JMHT. 15(2):53-62.

Rahmansyah S.M. 2009. Phosphatase Activity in Soil Belongs to Natural and Non-natural Forest Landscape. Berita Biologi 9(6):783-792.

Rashad M., Mahmoud A.E.E., Desouky, M.A., Nooman, M.A. 2006. Purification and characterization of extra and intracellular beta-fructofuranosidase from Saccharomyces cerevisiae growing on Eichhornia crassipes leaf extract. Deutsche Lebensmittel – R Rundschau. 102:157-166.

Renella G., Zornoza R., Landi L., Mench M., Nannipieri P. 2011. Arylesterase activity in trace element contaminated soils. Europan Journal Soil Science. 62:590–597.

Rietz D.N., Haynes R.J. 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol Biochem. 35:845–854.

Saha S., Prakash V., Kundu S., Kumar N., Mina B.L. 2008. Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean-wheat system in N-W Himalaya. European Journal of Soil Biology. 44: 309-315.

Schinner F., Kandeler E., Ohlinger R., Margesin. 1996. Methods in Soil. Boilogy. German:Spinger

Schinner F., Mersi W von. 1990. Xylanase, CM-cellulase and invertase activity in soil, an improved method. Soil Biol Biochem 22:511-515.

Skujins J. 1978. History of abiontic soil enzymes research: Soil enzymes. (1stedn). New York (USA): Academic Press.

Steinberger Y., Whitford W.G. 1988. Decomposition processes in the Negev ecosystem. Oecologia (Berlin). 75:61–66.

Stemmer M., Gerzabek M.H., Kandeler E. 1999. Invertase and xylanase activity of bulk soil and particle-sizefractions during maize straw decomposition. Soil Biolo Biochem. 31:9-18.

Tabatabai, M.A. 1994. Soil enzymes. In: Weaver RW, Angle JS, Bottomley PS (eds.). Methods of soil analysis, part 2. Microbiological and biochemical properties. SSSA Book Series No. 5. Soil Science Soc. Amerika, pp. 775-833.

Taylor J.P., Wilson B., Mills M.S., Burns R.G. 2002. Comparison of Microbial Numbers and Microbial Activities in Surface Soils and Sub Soils Using Various Techniques. Soil Biol Biochem. 34:387- 401.

Trasar-Cepeda C., Leiros M.C., Gil-Sotres F. 2008. Hydrolytic enzyme activities in agricultural and forest soils. Some implications for their use as indicators of soil quality. Soil Biol Biochem. 40:2146–2155.

Utobo E.B., Tewari L. 2015. Soil enzymes as bioindicators of soil ecosystem status. *Appl. Ecol. Environ. Re*s. 13:147–399.

Vogel. 1990. Buku Teks Analisis Anorganik Kualitatif Makro dan Semimikro. Edisi ke-5. Penerjemah Setiono dan A. Hadyana Pudjaatmaka. Jakarta (ID): Kalman Media Pustaka.

Walkley A., Black C.A. 1934. An Examination of the Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method. Soil Sci. 37: 29-38.

Yan J., Quan G., Ding C. 2013. Effects of the combined pollution of lead and cadmium on soil urease activity and nitrification. Procedia Environmental Sciences. 18:78–83.

Yuan B.C., Li Z.Z., Liu H., Gao M., Zhang Y.Y. 2007. Microbial biomass and activity in salt affected soils under arid conditions. Appl Soil Ecol. 35: 319–328.

Zanuzzi A., Arocena J.M., van Mourik J.M., Faz A. 2009. Amendments with organic andindustrial wastes stimulate soil formation in mine tailings as revealed by micromorphology.*Geoderma*. 154:69–75.

Zhang Y.L., Chen L.J., Sun C.X., Wu Z.J., Chen Z.H., Dong G.H. 2010. Soil hydrolase activities and kinetic properties as affected by wheat cropping systems of Northeastern China. Plant Soil and Environ. 56: 526–532.

Zornoza R., Landi L., Nannipieri P., Renella G. 2009. A protocol for the assay of arylesterase activity in soil. Soil Biol Biochem. 41:659–666.

Zornoza R., Acosta J.A., Martínez-Martinez S., Faz A., Baath E. 2015. Main factors controlling microbial community structure and function after reclamation of a tailing pond with aided phytostabilization. Geoderma. 245-246:1–10.