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Profile of lignocellulose decomposition on excelzyme treated typic haplosaprist

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Abstract The study indicated that application method of the excelzyme did not affect the selected soil chemical properties (SSCP), meanwhile, the dose of excelzyme was significantly affected the SSCP. By the end of the study (12 weeks after the application, WAA), lignin, cellulose, and hemicellulose content were decreased by 31.36%, 4.00%, and 1.58%, respectively, compared to those of control. Also, the soil pH was increased by 0.48 points, and Eh decreased by 7.04 mV compared to the control. The impact of excelzyme application at the soil surface decreased with soil depth but the impact extended to 60 cm. The impact difference among soil layer in the peat profile decreased with time. At the beginning of the study, the difference in lignin, cellulose, and hemicellulose content between the soil surface and 60 cm depth were 25.24%, 1.73%, and 1.26%, respectively, for the lignin, cellulose, and hemicellulose. At the end of the study, the difference in lignocellulose content at the respective depth was 9.72%, 1.49%, and 0.90%, respectively, for the lignin, cellulose, and hemicellulose.

Keywords: Cellulose, Hemicellulose, Lignin, Lignocellulose, Peatland

Introduction

The global peatland area covers about 4.413.500 km^2 or more than 441 million hectares (Mha). At the same time, more than 368 Mha is tropical peatland (Yu *et al*., 2010). Most tropical peatland is in Indonesia, distributed in Kalimantan, Sumatera, Papua, and a few areas in Sulawesi, comprising around 36 % of the world's tropical peatland (Page *et al*., 2011; Ritung, 2011). In Indonesia, peatland covers an area of about 7.4 % of the Indonesian terrestrial area or about 14.95 Mha (Masganti *et al*., 2017). It is potential land for agricultural development due to the lack of available fertile mineral soil, fast

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agricultural land conversion, relatively better topography, and inexpensive preparation for agriculture purposes.

Peatlands are organic soils developed from the imbalance of biomass production and decay, resulting in biomass accumulation because the biomass accumulation rate exceeds its decay rate (Frolking *et al*., 2010; Kurnianto *et al*., 2015). A previous study suggested that the hydrological condition and the plant functional groups affected the rate of peat decomposition (Wiederman *et al.,* 2017). More profound, higher bulk density and more anoxic condition lead to less decomposition rate of peat because an anaerobic breakdown of organic matter yields less energy than aerobic decomposition (Beer *et al*., 2008). In Indonesia and other tropical areas, its slow biomass decomposition rate is usually associated with waterlogging, making it anaerobic. In a few areas, low biomass degradation rates are attributed to low temperatures, such as peat in the mountain area. Besides its plant types and hydrological condition (water table depth), peat thickness also influence its fertility and decomposition rate. Thicker peat has lower fertility (Agus and Subiksa, 2008).

Based on its place development, there are two different peats, i.e., ombrogen and topogen peats. Ombrogen peat is a peat developed in isolated depression areas, where there are no tributaries or water spill into the areas. Peat developed in this condition has low fertility. Topogen peat is developed in depression areas where water from surrounding areas, rivers, and sea spills into the area, bringing and depositing dissolved materials, leading to better fertility of this peat. However, peatland generally considers sub-optimal land characterized by low pH, macro and micronutrient content, low base saturation, low decomposition rate indicated by a high C/N ratio, and hydrophobic characteristics (Banjarnahor *et al*., 2018; Astiani *et al*., 2018, and Agus and Subiksa, 2008).

Enhancement of peat fertility and quality is necessary for agricultural purposes. Improving peatland drainage is compulsory before any treatment. Applying soil ameliorants such as manure, lime, zeolite, and other mineral sources, including volcanic ash, is common practice to improve peat chemical properties. Pamungkas *et al.* (2017) indicated combining trass and volcanic ash on peat soil increases soil pH, NH_4^+, total N, available Na, Zn, and Mn and improves rice growth and yield. A similar result was reported by Banjarnahor *et al.* (2018) that applying a combination of trass and volcanic ash increases peat electric conductivity (EC) and soil pH, and it enhances rice growth and yields in the peat soil of North Sumatera. The effect of volcanic ash application on peat was also studied by Hotes *et al.* (2004), who reported that peat properties such as pH, EC, SiO_2, $SO_4^{=}$, and Na^+ increased due to the volcanic ash application.

Payne and Blackford (2005) reported that applying volcanic ash on peat did not affect decomposition and humification after six years of application.

Besides soil amendment, biofertilizers, effective microorganisms, and extracellular enzymes have been reported (Wulandari, 2021; Prawito *et al*., 2023; Liu *et al*., 2021). Wulandari (2021) reported that applying Excelzyme (a cocktail of extracellular enzymes) on peat soil increased the rate of peat decomposition, indicated by decreasing lignocellulose content up to 5 months after application, and it was suspected that the effect of the applied enzymes continues to go on. However, it attracts controversial issues, especially those concerned with peat conservation. Due to its fast decomposition rate, applying such an enzyme may destroy treated peat. Using such an enzyme must be very prudent if the concern is valid.

Excelzyme is a consortium enzyme consisting of hemicellulose, cellulase, and ligninase, which is a local enzyme isolated from a hot spring in Gunung Pancar, Bogor, Indonesia (Ekwanda *et al.,* 2023; Puspaningsih *et al.,* 2018). The enzyme has a significant role in the degradation of hemicellulose, cellulose, and lignin commonly conceived in agricultural waste and peat soil. Ekwanda *et al.* (2023) reported that the 1:1:1 ratio of hemicellulase:cellulase: and ligninase is the best treatment for decomposing oil palm empty fruit bunch (OPEFB) with an optimum time of 6 hours. It can decrease the cellulose and lignin content of OPEFB from 30,9% to 25.94 % and 29.51% to 22.67%, respectively. This study also reported that Scanning Electron Microscope (SEM) proofed that applying Excelzyme on OPEFB caused breaking of the lignocellulose structure.

Regardless of its essential role in agricultural development, utilizing peat land for agriculture creates pros and cons in society, including utilizing various enzymes to improve peat decomposition. Therefore, utilizing soil amendments to peatland, such as enzymes, must be carefully investigated to minimize its negative impact.

The purposes of this study were to determine the effect of the Excelzyme application methods and doses on the selected soil chemical properties (SSCP) including soil pH, EH, lignocellulose (lignin, cellulose, and hemicellulose) and to determine the effect of the Excelzyme applied to the lignocellulosic profile.

Materials and methods

The study was conducted at The University of Bengkulu Integrated Agricultural Research Station, situated at the latitude of 3^{o}45’06” South and longitude of 102^{o}17’01” East, from October 2021 to January 2022. The site of this study was a depression area covering up to 2 – 3 ha, about 50% of the whole research area. During the rainy season, the area was cropped to rice, while during the dry season, the area was left as bare land.

The land preparation was initiated by applying herbicide on a selected location approximately two weeks before the soil tillage. After the weeds had dried, the soil was tilled, let to dry for a week, and re-tilled while removing undecayed biomass. The study site was divided into two main plots, separated by a one-m-wide ditch. Each main plot was split into 4 blocks, each consisting of 4 subplots. Each subplot was 2 m wide and 3 m long. A ditch 50 cm wide and 30 cm depth was prepared among the subplots, and the soil was homogeneously spread over the beds for higher beds to prevent flooding.

Figure 1. Study site indicating the depression areas surrounded by sloping mineral soil

The experimental design was a Randomized Complete Block Design with two factors, arranged in split plots. The main plot was the methods of excelzyme application i.e. A1 - direct application and A2 – gradual application. The subplot was the dosage of applied Excelzyme consisting of D0 – without excelzyme, D1 – 1 l excelzyme plot^{-1}, D2 – 1.5 l excelzyme plot^{-1}, and D3 – 2 l excelzyme plot^{-1}. Each treatment combination was repeated 4 times. A fence was established to the surrounding experimental site, and treatment combination labels were put on each plot accordingly.

Excelzyme was applied to all plots according to the designated treatment. Each dose of Excelzyme was diluted to 20 l with water for direct application, and it was homogenously decanted onto the assigned plot. In the gradual application, each dose of excelzyme was divided into four applications; a quarter of each excelzyme dose was diluted with water to 20 l and applied by homogenously pouring it down onto the assigned plots. The application was repeated every two weeks until the remaining Excelzyme was applied.

Composite soil samples (taken from 4 different points in each plot) were collected from 0 – 15 cm, 15 – 30 cm, and 30 – 60 cm depth at 2, 4, 6, and 12 weeks after the application (WAA) of the enzyme. Soil samples were air-dried, ground, and sieved for laboratory analyses. The SSCP consisting of lignin, cellulose, hemicellulose, pH, and EH was determined.

Statistical analyses of variance were carried out at P value <0.05. Excelzyme dosage treatment means were separated using Duncan's Multiple Range Test (DMRT).

Results

The soil of the study site was organic soil or peat, with 0.5 m depth in the perimeter and ranging from 2 to 3 m depth in the inner part of the depression. The peat was dominated by Oa horizon (sapric material) to a depth of more than 1 m, indicating that the peat has an advanced decomposition stage. The soil has no specific properties and fulfills the great group of Haplosaprist and the sub group of Typic Haplosaprist. The area was sub-optimal land, indicated by low content of N, P, K, and Ca (Table 1).

Table 1. Characterization of peat in the study site and the reference of eutrophic and oligotrophic peats

Peat properties	Eutrophic peat#	Mesotrophic Peat#	Oligotrophic peat#	Study Site$
Nitrogen (%)	2.50	2.00	0,80	1.92
K_2O (%)	0.10	0.10	0.03	0.06
P_2O_5 (%)	0.25	0.20	0.05	0.14
CaO (%)	4.00	1.00	0.25	0.86
Ash (%)	10.00	5.00	2.00	3.50

Criteria according to Driessen and Supraptoharjo (1974). $ Result of composite sample analyzed in the Soil Science Laboratory University of Bengkulu.

Result indicated that the peat fertility in this site is less than mesotrophic peat and only slightly better than or comparable with the oligotrophic peat, the least fertile peat (Table 1).

In general, the result of statistical analyses showed no significant effect of excelzyme application methods on the SSCP regardless of the time duration after application (2, 4, 6, and 12 WAA) (Table 2). In contrast, the excelzyme dose significantly affected the decomposition of treated peat, as indicated by decreasing lignocellulose (lignin, cellulose, and hemicellulose) content. The dose treatment also significantly affected soil pH and Eh. The effect of excelzyme is more determined by its activity (concentration) regardless of how it was applied. The effect of excelzyme doses was consistently observed from 2 WAA to 12 WAA, indicating that the Excelzyme was continuously active during that period or longer. There were also consistently found interactions between the application methods and the dose of Excellzymes, except for the soil pH and Eh.

Table 2. The analysis of variance of excelzyme effect on SSCP of Typic Haplosaprist at a depth of 0 – 15 cm at 2, 4, 6, and 12 WAA

Selected Soil Chemical Properties (SSCP)	**Calculated-F**			**CV (%)**
	Application methods (A)	**Excelzyme dosages (D)**	**Interaction (AxD)**	
2 Weeks After Application (WAA)				
Lignin	1.28^{ns}	71.86*	5.51*	4.79
Cellulose	0.31^{ns}	52.68*	14.30*	5.50
Hemicellulose	6.80^{ns}	70.18*	5.29*	7.59
Soil pH	0.16^{ns}	4.85*	0.22^{ns}	4.71
Soil Eh	0.59^{ns}	33.76*	0.29^{ns}	1.69
4 WAA				
Lignin	5.48^{ns}	791.56*	16.61*	2.66
Cellulose	0.33^{ns}	70.06*	14.19*	5.32
Hemicellulose	25.91*	92.06*	4.55*	8.02
Soil pH	0.39^{ns}	13.35*	1.2^{ns}	3.27
Soil Eh	0.28^{ns}	2.99*	0.96^{ns}	1.49
6 WAA				
Lignin	0.14^{ns}	356.12*	8.16*	3.29
Cellulose	1.60^{ns}	78.87*	13.71*	22.42
Hemicellulose	30.76*	100.67*	4.06*	8.44
Soil pH	0.51^{ns}	19.29*	2.26^{ns}	2.97
Soil Eh	6.10^{ns}	18.46*	1.76^{ns}	1.69
12 WAA				
Lignin	0.19^{ns}	1379.85*	33.72*	2.40
Cellulose	4.34^{ns}	87.89*	13.10*	5.31
Hemicellulose	35.03*	107.94*	3.48*	8.98
Soil pH	0.61 ^{ns}	22.19*	3.13 ^{ns}	3.04
Soil Eh	0.01^{ns}	9.25*	2.29^{ns}	2.10

*: Significantly different at $P < 0.05$, ns: non-significantly different, CV: Coefficients of variance

Effect of application methods on SSCP

The study resulted that the application method of excelzyme generally did not significantly affect the lignocellulose degradation, presented by the content of lignocellulose in the peat soil, except the hemicellulose monitored at 4, 6, and 12 WAA (Table 2). It showed the collected data of lignocellulose content based on the application methods and the observation of 2, 4, 6, and 12 WAA (Table 3). The average of SSCP also showed no consistent trend between the application methods. Result indicated that the decomposition of lignocellulose in the peat soil was determined by the excelzyme activity (concentration) regardless of when the excelzyme was applied. It suggested that the applied enzyme roles immediately after the application and continuously react with the available substrate to 12 WAA or could be more resulted.

Table 3. The SSCP of 0 -15 cm peat soil depth treated with different-application methods of excelzyme

SSCP	2 WAA	4 WAA	6 WAA	12 WAA	Average#
Direct Application					
Lignin	21.34	61.98	56.99	52.87	48.30
Cellulose	12.64	11.36	11.70	11.39	11.77
Hemicellulose	3.89	3.35	3.37	3.19	3.45
Soil pH	3.49	3.58	3.59	3.63	3.55
Soil EH	175.39	166.59	162.74	157.79	165.63
Gradual Application					
Lignin	67.40	61.98	59.26	56.55	61.30
Cellulose	11.99	11.36	11.04	10.73	11.28
Hemicellulose	3.71	3.35	3.17	3.00	3.31
Soil pH	3.49	3.58	3.59	3.63	3.57
Soil EH	180.73	169.41	163.74	158.08	167.99

SSCP: Selected soil chemical properties; WAA: weeks after treatment; # Average from 4 observations and 4 replications

Effect of dose of excelzyme on SSCP

Result showed the result of treatment separation analyses using DMRT at a significant $P < 0.05$. The data was collected at a 0 – 15 cm depth and 4 sampling times, i.e., 2, 4, 6, and 12 WAA (Table 4). Regardless of the sampling time, a higher dose of excelzyme exhibited more degraded lignocellulose. It likewise increased the dose of excelzyme, followed by an increase in soil pH. In contrast, soil Eh was consistently decreased with increasing doses of excelzyme since the early study (2 WAA) to the end of the study (12 WAA), from the maximum Eh of 172.82 mV (at the 2 WAA of untreated peat) to the minimum Eh of 136.96 mV (at 12 WAA of 2 l Exelzyme plot^{-1}).

A consistent increase in the dose of excelzyme with increasing lignocellulose decomposition in the treated peat was indicated by decreasing lignin, cellulose, and hemicellulose content in the peat, regardless of the observed time after application. It suggested that the application of this consortium of extracellular enzymes in the field was determined by the activity (concentration) of the applied enzyme. The excelzyme 2 l plot^{-1} showed the lowest lignocellulosic content, indicating that the dose can increase lignocellulosic decomposition to smaller or less complex compounds.

Table 4. The effect of applicated dose of excelzyme on SSCP of the treated peat at a depth of 0 – 15 cm

Excelzyme Dose (l plot^{-1})	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Soil pH	Soil Eh
			2 WAA		
0.0	66.69^{a}	12.05^{a}	3.07^{a}	3.55^{b}	172.82^{a}
1.0	55.79^{b}	11.24^{b}	2.93^{a}	3.71^{ab}	167.46^{b}
1.5	52.55^{c}	10.52^{c}	2.32^{b}	3.80^{ab}	163.85^{c}
2.0	47.85^{d}	8.56^{d}	1.84^{c}	3.87^{a}	159.11^{d}
			4 WAA		
0.0	65.29^{a}	11.71^{a}	2.98^{a}	3.68^{c}	158.41^{a}
1.0	51.69^{b}	10.72^{b}	2.72^{a}	3.83^{b}	155.34^{b}
1.5	44.88^{c}	9.92^{c}	2.07^{c}	3.88^{b}	152.10^{c}
2.0	39.69^{d}	7.96^{d}	1.57^{d}	4.07^{a}	148.04^{d}
			6 WAA		
0.0	63.9^{a}	11.37^{a}	2.89^{a}	3.74^{c}	151.20^{a}
1.0	47.60^{b}	10.20^{b}	2.51^{b}	3.89^{b}	149.27^{a}
1.5	37.21^{c}	9.32^{c}	1.83^{c}	3.91^{b}	146.22^{b}
2.0	35.62^{d}	7.67^{d}	1.44^{d}	4.18^{a}	142.50^{c}
			12 WAA		
0.0	63.9^{a}	11.37^{a}	2.89^{a}	3.80^{c}	144.00^{a}
1.0	47.60^{b}	10.20^{b}	2.51^{b}	3.95^{b}	143.21^{ab}
1.5	37.21^{c}	9.32^{c}	1.83^{c}	3.95^{b}	140.34^{b}
2.0	31.54^{d}	7.37^{d}	1.31 ^{d}	4.28^{a}	136.96^{c}

WAA: weeks after application; numbers followed by the same letter in the same column indicated no significant difference at DMRT at $P < 0.05$.

Profile of lignocellulose in the peat

The lignocellulose profile is the lignin, cellulose, and hemicellulose distribution with soil depth. The lignocellulose profile was observed if the applied enzyme had any affected on the soil surfaces to the deeper depth. It showed that after 4 WAA, the enzyme applied at the soil surface (0 – 15 cm) affected the peat depth of 60 cm, suggested by decreasing the lignin, cellulose, and hemicellulose from 68.37%, 12.32%, and 68% at 2 WAA to 60.94%, 11.62%, and 3.44% at 4 WAA, respectively (Figure 2). The effect of surface application of the excelzyme continuously took place up to 12 WAA which was showed by decreasing lignocellulose content to 54,78%, 11.05%, and 3.09% for the lignin, cellulose, and hemicellulose, respectively. The difference in the lignocellulose content among the soil depth was more significant in the early period (2 WAA) and gradually decreased with time (12 WAA). For example, at the beginning of the study (2 WAA), the difference in lignin content between the 0 – 15 cm depth and those of the 15 – 30 cm depth and 30 – 60 cm depth were 11,91% and 25,24%, respectively. However, at the end of this study (12 WAA), the difference in lignin content at the respective depth was 4,54% and 9,72%.

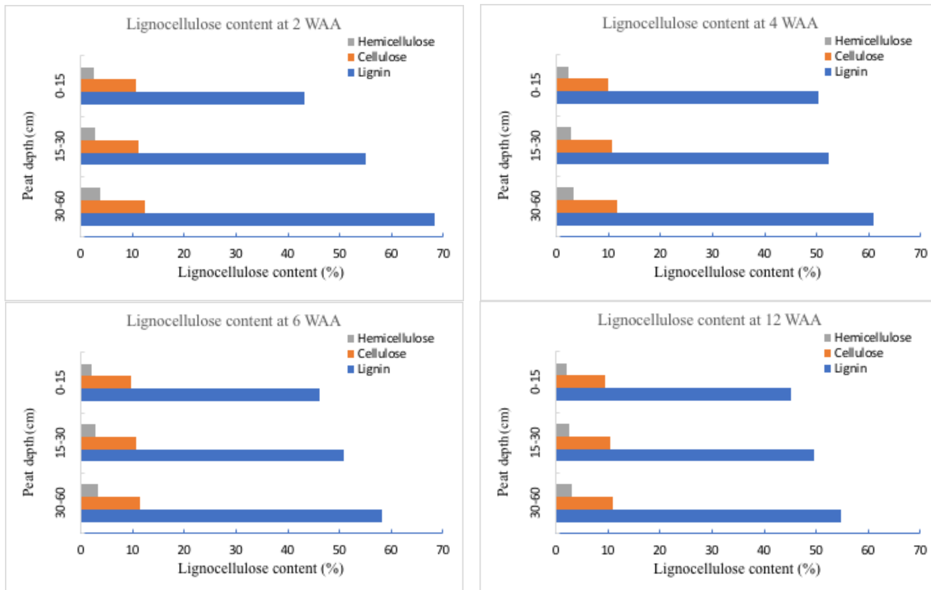

Figure 2. Average lignocellulose of the excellzyme treated peat distribution with depth at 2, 4, 6, and 12 WAA

Discussion

The soil of the site study is classified into the subgroup of Typic Haplosaprist (SSS. 2014), indicating that the soil has no specific character in the subgroup. Based on the site position, the peat should have higher fertility, that usually considered eutrophic peat. The soil analysis result, however, indicated that the soil was comparable with oligotrophic peat, the lowest fertility peat, except for the nitrogen content, which is comparable with mesotrophic peat. Even though the site was a small depression surrounded by sloping mineral soil, the surrounding soil was degraded with low fertility status, and it was less likely to contribute significant fertility to the peat in this area. The relatively high N content in this soil peat may have resulted from N fertilizer applied during the last couple of years when the site was used for rice cultivation.

The excelzyme application methods involved direct application, which applied all doses of excelzyme at the beginning of this study. Furthermore, the gradual application was applying a quarter of the excelzyme dose at the beginning of the study, followed by applying another quarter every two weeks until all excelzyme was applied. A gradual application method was just delaying the addition of the enzyme to the peat. Finally, all the added enzymes will react with the available lignin, cellulose, and hemicellulose in the peat, regardless of when the enzyme was added. This enzymatic reaction immediately took place after the application of the enzyme. A laboratory study indicated that the reaction started immediately (after the addition of the enzyme to the substrate), and the optimum incubation time of the excelzyme added to material containing high lignocellulose (oil palm empty fruit bunch) was 6 hours (Ekwanda *et al*., 2023). A laboratory study by Bisswanger (2014) confirms that the immediate enzymatic reaction takes about 10 s at the early stage (following an exponential pattern), followed by the linear state to 100 s or even up to 17 min, and then followed by very low reaction rate until the rate is not detected. A previous study indicated that after the enzymatic reaction occurred and the substrate broke down, the enzyme remained in the solution to react with the available substrate or was inactive due to the solution/environmental condition, such as temperature, pH, salt content, and other (Nisha *et al.,* 2012). Wulandari (2021) reported that field application of Excelzyme on peat soil affected lignocellulose decomposition for up to 4 months or more.

The application methods did not affect lignocellulose degradation. This result suggests that the reaction of the added excelzyme to the peat was determined by the concentration (activity) of the enzyme and the available substrates (lignin, cellulose, and hemicellulose) in the treated peat. Bisswanger (2014) concluded that the reaction velocity directly correlated to enzyme concentration, following a linear pattern. In contrast, the enzymatic reaction followed a hyperbolic correlation pattern with the substrate. The lignocellulose content in the peat was in abundance containing 66.09%, 12.05%, and 3,07% for the lignin, cellulose, and hemicellulose content, respectively, for the untreated peat at the beginning of the study. These conditions represent the available substrate before the enzyme was applied. Comparing this lignocellulose content (66.09% lignin, 12.05% cellulose, and 3.07% hemicellulose) to the added excelzyme into the peat 1.0 l plot^{-1}, 1.5 l plot^{-1}, and 2.0 l plot^{-1} (which is equivalent with 5.0%, 7.5% and 10.0% of excelzyme to the dry peat weight), the added enzyme was much lower than the available substrate in the peat. The ratio between the substrate (lignin) and the added enzyme ranged from 66: 1 (for the lowest dose) to 13.2: 1 (for the highest dose). A previous study reported by Ekwanda *et al*. (2023) showed that the optimum ratio between the substrate (hemicellulose) and the added enzyme (hemicellulase) was 1:1, and the increasing ratio of the substrate to the enzyme (4:1) decreased the production of the enzymatic reaction shown by the lower reduced sugar.

The profile of lignocellulose in the peat shows consistently increasing lignocellulose content with depth from the beginning (2 WAA) to the end of the study (12 WAA). It means that the decomposition of lignocellulose in the peat due to the excelzyme application has the most significant effect on the surface soil and decreases with depth. However, definitely, the effect reaches 60 cm depth. It is definitely due to applying the enzyme on the soil surface (0 – 15 cm).

The enzyme's downward movement was believed to follow the movement of gravitational water or the water table movement. The downward movement is slow because of the site position in the depression, where the water table is only about 20 cm and could be shallower during a rainy day. It is the reason the lignin content at 60 cm depth (68.37%) was significantly higher than that of the surface (43.17%) at 2 WAA. In contrast, after 12 WAA, the lignin content at the 60 cm depth (54.78%) slightly differed from that of the surface (45,06%). The Excelzyme remains active up to the 12 WAA despite its slow-down movement. A previous study involving excelzyme on peat decomposition reported that the decomposition of lignocellulose still takes place up to 16 WAA (Wulandari, 2021). The result showed that Excelzyme is capable of withstanding the field condition. However, Bisswanger (2014) and Nisha *et al*. (2012) warn that enzymes must be carefully kept to avoid harsh conditions such as high temperature, high pressure, low pH, and the presence of salt and other contaminants. Laras *et al*. (2017) explained that excelzyme is a consortium enzyme consisting of local isolates taken from Gunung Pongkor Hot Spring, Bogor, Indonesia. The isolate was tested in the temperature range from 20-90°C, and pH of 3-10, with an optimum temperature, was 60-70°C and optimum pH of 5-6. Even though the enzymatic reaction in the field condition did not proceed at the optimum rate, the reaction still occurred.

Soil pH increased with increasing the dose of excelzyme and time. The breakdown of lignocellulose eventually will release nutrients and humic substances, particularly humic and fulvic acids, to the system. These acids reach organic functional groups such as carboxyl, phenolic, and others (Spark, 2003). Peat soil has a high amount of iron even though most are in chelate form, and only 4-5% of total iron is in soluble and exchangeable forms (Fahmi *et al*., 2010). Another study was confirmed that peat soil contained Fe, Zn, Mn, Pb, Cr, Ni, and Cu (Zamri *et al*., 2021). Functional groups, mainly carboxyl and phenolic groups, covalently bond to metals to form insoluble organic-metal complexes (Huang *et al*., 2022; Fahmi *et al*., 2021; Wan *et al*., 2018; Sposito, 2008). The formation of organic-metal complexes will eventually reduce proton (H^+) production from metal hydrolysis, increasing soil pH. Rahman *et al.* (2010) suggested that the complexation of Fe and Cd with humic acid increases soil pH. The increase in soil pH with time is associated with the enzymatic reaction proceeds, increasing the production of humic substances during the process. Likewise, the rise of soil pH with the increase in excelzyme doses is attributed to the intensity of the enzymatic reaction.

Besides soil pH, another SSCP is a soil redox potential, Eh. Redox potential measures the intensity of reduction or oxidation in wet soil. Generally, soil Eh ranges from -300 to +900 mV, whereas waterlogged soil has Eh of less than +

350 to + 250 mV (Pezeshki, 2001). The measured Eh in this site was between 136,96 mV to 172,82 mV. The lowest value was found at the end of the study with the highest excelzyme dosage, while the highest was found in the early study with no excelzyme application. It means that the more the excelzyme is added and the time of reaction go on, the more the reduction level of the peat. One of the increasing reduction levels in waterlogged soil was when there was an available organic matter for microbial activity (Snakin *et al.,* 2001). It may occur when a complex substrate such as lignocellulose is broken down in an enzymatic reaction, producing a simpler compound available for microbial degradation. The enzymatic reaction between excelzyme and lignocellulose may be followed by microbial degradation of a simpler compound in the treated peat. It is a challenging issue if the following enzyme-treated peat produces simpler compounds that may make available for microbial degradation. Regardless of its Eh differences in the soil profile, the Eh value is in the same category, moderately reduced, between 100 – 400 mV (Pezeshki, 2001; Kaurichev and Sishova, 1967).

The study can be concluded that the excelzyme application methods did not affect SSCP. The dose of excelzyme affected the lignocellulose decomposition; increasing the dose of excelzyme is followed by increasing lignocellulose degradation. By the end of the study (12 WAA), the lignocellulose decomposition was expressed by lignin, cellulose, and hemicellulose decreased by 31.36%, 4.00%, and 1.58%, respectively, compared with those of control. Meanwhile, the soil pH was increased by 0.48 points, and EH decreased by 7.04 mV compared to the peat control. The impact of excelzyme application (at the soil surface) decreased with soil depth, extending to 60 cm, and the difference decreased with time. At the beginning of the study, the difference in lignin, cellulose, and hemicellulose content between the soil surface and the 60 cm depth were 25.24%, 1.73%, and 1.26%, respectively for the lignin, cellulose, and hemicellulose. At the end of the study, the difference in lignocellulose content at the respective depth was 9.72%, 1.49%, and 0.90%, respectively, for the lignin, cellulose, and hemicellulose.

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