Microbial Activity in Peat Soil Treated With Ordinary Portland Cement (OPC) and Coal Ashes

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Abstract. Peat soil is a cumulative of decayed plant fragment which developed as a result of microbial activity. The microbes degrade the organic matter in the peat soils by the production of hydrolysis enzyme. The least decomposed peat, known as fibric peat has big particles and retain lots of water. This made peat having high moisture content, up to 1500 %. The most decomposed peat known as sapric peat having fines particles and less void ratio. The present study aimed to understand the effects of solidification process on the bacterial growth and cellulase (CMCase) enzyme activity. Two types of mixing were designed for fibric, hemic and sapric peats; (i) Ordinary Portland cement (OPC) at an equal amount of dry peat, with 25 % of fly ash (FA) and total of coarse particle, a combination of bottom ash and fibre of 22 – 34 %, (ii) fibric peat was using water-to-binder ratio (w/b) = 1, 50% OPC, 25 % bottom ash (BA) and 25 % FA. For hemic and sapric peat, w/b=3 with 50 % OPC and 50 % BA were used. All samples were prepared triplicates, and were cured for 7, 14, 28 and 56 days in a closed container at room temperature. The results revealed that the first mix design giving a continuous strength development. However, the second mix design shows a decreased in strength pattern after day 28. The influence of the environment factors such as alkaline pH, reduction of the water content and peat temperature has no significant on the reduction amount of native microbes in the peat. The microbes survived in the solidified peat but the amount of microbes were found reduced for all types of mixing Fibric Mixed 1 (FM1), Hemic Mixed 1(HM1) and Sapric Mixed 1 (SM1) were having good strength increment for about 330 – 1427 % with enzymatic activity recorded even after D56. Nevertheless, with increase in the strength development through curing days, the enzymatic activities were reduced. For the time being, it can be concluded that the microbes have the ability to adapt with new environment. The reactivity of the microbes relates with the strength of solidified peat.

1. Introduction

The solidification of peat soils has been investigated for decades since early 1980’s due to the poor engineering properties of the peat soils. The main challenge of the peat is the high water content, which reach to 1800 % as well as organic matter which induce the microbial activity that made peat easily subsidence and having high compressibility. Therefore, the successful solidification process is that has the ability to reduce these parameters before any engineering work could be carried out in peat area [1].
Peat is developed by decomposing of decay plant as a result of microbial activity. The presence of *Thiobacillus* sp., *Pythium* spp, *Rhizobium rhizogenes*, *Streptomyces griseoviridis*, and *Bacillus mucilaginosus* have been demonstrated by the researchers in the literature [2-4]. The microbe diversity is different geographically and seasonally [5, 6].

The solidification process aims to increase the peat strength by more than 2400%. This process might be performed by adding of cement, sand, coal ashes, sodium bentanoite and ground-granulated blast-furnace slag [7-9]. These additives act by elevating pH of the peat to pH 8-10 which in role inhibit microbial activity and thus degrading of the peat components to be stable for long period [10]. However, Mitchell and Santamarina [11] stated that many of the microbes in the peat soil exhibit high resistance for the inhibitors and they can adapt to survive under the hard environment by the natural selection process, which allows for microbes to be reactivated in the solidified peat after sometime. Therefore, most of the solidification processes for peat soils which have investigated by the researcher exhibit some of efficiency for increasing the peat soil strength but to limited times. The reasons for problem lies in the absence the deep understanding for the microbial activity in solidified peat in which microbe degrade the organic content in the solidified soil and reduce the peat strength. The microbes degrade the organic matter in the peat soils by the production of hydrolysis enzyme mainly the cellulolytic enzymes that use to degrade the cellulolytic materials which represent more than 90% of the peat soil. This process take place by using three types of cellulase enzymes included endo-β-1-4-glucanases (EC 3.2.1.4) (CMCase), exoglucanases (EC 3.2.1.74), and β-glucosidases (EC 3.2.1.21). Among these enzymes the CMCase represent the key enzyme for the degradation of cellulose compounds (non-soluble compound) into di and mono-saccharides (soluble substances) [12]. Therefore, the present study aimed to determine the role of ordinary Portland cement (OPC) used for the solidification process in the inactivation of bacterial enzymes in three types of the peat soils included fibric, hemic and sapric and its relation with its strength.

2. Materials and Methods

2.1 Sampling and solidification process

All three types of peat soil which are fibric, hemic and sapric samples were excavated from Pontian, Johor. The characteristics of peat soil were tested for water content, fiber content, pH, loss on ignition (LOI), unconfined compressive stress (UCS), bacterial load as well as presence of CMCase enzyme in the isolated bacteria before and after the solidification process (curing period of 7, 14, 28 and 56 days). The raw peat soil was subjected for the solidification process as described in previous work [9] with some modifications as following; in the previous study, Abd Rahman [9] stress out that two type of phenomena were recorded for peat solidification process. First mixing was showing a good continues increasing strength pattern while the second mixing shows a decrease in strength after curing days of 56. Therefore, in this study, both mixing were used to find the different in the bacterial growth and enzyme activity between these two patterns. First mixed design was used ordinary Portland cement (OPC) at an equal amount of dry peat, with 25% of fly ash and total of coarse particle, a combination of bottom ash and fibre of 22–34%. The samples were labeled as Fibric Mixed 1 (FM1), Hemic Mixed 1(HM1) and Sapric Mixed 1 (SM1). Second mixing design for fibric peat was using water-to-binder ratio, water/binder (w/b) = 1, 50% OPC, 25% BA and 25% FA. For hemic and sapric peat, w/b=3 with 50% OPC and 50% BA was used. The samples were labeled as Fibric Mixed 2 (FM2), Hemic Mixed 2 (HM2) and Sapric Mixed 2 (SM2). The isolation of bacteria from the peat soil was conducted by a spread plate technique with serial dilutions (10⁻¹ to 10⁻⁵), while the bioassay for CMCase was carried out according to Al-Gheethi [12].
3. Results and discussion

3.1 Characteristics of peat soil

The characteristics of the peat are illustrated in Table 1. The results of LOI revealed that the carbon is the dominant element in all types of the peat which is coming from cellulose and other decay plants [13]. The pH value was acidic (3.3 to 4.2). The peat moisture content is varies from one place to another. However, in this study, the moisture content of fibric peat was less than that for hemic and sapric peat. This is because the fibric peat is located at the first layer of peat soil and expose directly to the sunlight which lead to evaporate the water content, while hemic and sapric peat layers in deeper location as describe by Mutalib [14]. Moreover, the water contents recorded in this study was low compared to the previous studies [7, 8], probable because the study was carried out on April which is a dry season in Malaysia [15]. The amount of fiber in the fibric peat was more than that determined in hemic and sapric peat (68.8 % vs. 56 % and 14 % respectively). This might be due to the low microbial activity and degradation process in this layer (due to the low moisture content) compared to hemic and sapric soils. Husnain et al. [16] specified that soil should be moist enough to enhance the aerobic microbial activities thus support the previous hypothesis for fibric peat with low moisture content. The strength for raw peat was recorded as 4, 8 and 12 kPa for fibric, hemic and sapric peat respectively which indicate very low in strength, while the minimum ‘allowable bearing’ of a soil for road construction must exceed 100 kPa [17].

| Characteristics  | Fibric        | Hemic         | Sapric        |
|------------------|---------------|---------------|---------------|
| Moisture content (%) | 171.90 ±5.73  | 651.00 ± 11.40 | 759.72 ± 15.89 |
| Fiber content (%)  | 68.80 ± 1.82  | 56.48 ± 1.71  | 14.62 ± 0.75  |
| pH               | 3.30 ± 0.08   | 3.8 ± 0.07    | 4.20 ± 0.11   |
| LOI (%)          | 97 ± 0.34     | 97 ± 0.55     | 97 ± 0.45     |
| UCS (kPa)        | 4± 0.02       | 8± 0.02       | 12± 0.01      |

The concentrations of bacterial cells in the investigated peat soil are presented in Table 2. It was noted that Johor peat soils have high load of bacteria, ranged from $3.5 \times 10^{13}$ to $2.8 \times 10^{15}$ CFU/g. This finding is not common as most researcher [18, 19, 20] found the bacteria in their peat samples to be around $10^6 - 10^{10}$ CFU/g. However the concentrations of the bacterial loads have differed significantly ($p<0.05$) among the peat soils, the maximum concentrations were determined in the sapric soil due to the high moisture content as determined by a significant positive correlation between bacterial cell concentrations and moisture content as well as with the pH, where high concentrations were determined in the sapric soil with pH 4.2, while less concentrations were detected in fibric with pH 3.3. The primary identification of the isolated bacteria by using Gram Staining and biochemical testses indicated that the bacteria is belonged to *Bacillus* spp., which has also reported in the peat soil by the previous studies [21]. The *Bacillus* spp. are anaerobic [22] therefore, it was noted that the CMCase activity in the fibric soils was more than that in hemic and sapric soils (Table 3).
### Table 2. Microbes’ count for raw peat

| Peat type | Bacteria Count Test (CFU/g) | Average | P-value (ANOVA) | Significance |
|-----------|-----------------------------|---------|-----------------|--------------|
|           | Run 1 | Run 2 | Run 3 |              |              |
| Fibric    | $4.1 \times 10^{13}$ | $3.5 \times 10^{13}$ | $4.4 \times 10^{13}$ | $4.0 \times 10^{13}$ | 0.0082 | Significant |
| Hemic     | $9.3 \times 10^{13}$ | $1.8 \times 10^{14}$ | $2.4 \times 10^{14}$ | $1.72 \times 10^{14}$ |              |              |
| Sapric    | $1.0 \times 10^{15}$ | $2.8 \times 10^{15}$ | $1.8 \times 10^{15}$ | $1.89 \times 10^{15}$ |              |              |

### Table 3. Enzyme activities in peat

| Halo zones | Fibrice | Hemic | Sapric |
|------------|---------|-------|--------|
| +          | ++      | +     |        |

Negative (-), positive (+), high production (++)

### 3.2 Characteristics of solidified peat

Solidified peat for all mixing formulation has been tested with UCS to measure its strength. Two patterns have been recorded as shown in Fig. 1. The first mixing (FM1, HM1 and SM1) has giving a continuous strength increment until day 56. The second mixing (FM2, HM2 and SM2) shows a decrease in strength on day 56. The presence of such pattern is expected due to the microbial activity in the solidified peat. Pichan and O’Kelly [23] expecting that microbial activity in solidified peat might be freeze due to change of properties of the living medium. However, the finding of this study has reject the hypothesis as bacteria was found survived in solidified peat. Fig. 2 shows the microbes’ count in solidified peat for FM1, HM1 and SM1. The number of colonies in FM1 and HM1 at D7 was found higher compared to the original peat soil. This is align with the theory made by Bárcenas- Moreno et al. [24] where bacteria in peat is often adjust themselves in new environment. pH of peat was initially acidic, when peat was mixed with OPC and coal ashes, the pH had adjusted to alkaline (pH 8-11). This reaction is an exothermal process where heat was release during mixing. The water content in solidified peat also reduced dramatically. The water was consumed by OPC through hydration process. However, beside all these inherent, the microbes were found to be survived at all type of mixing and curing days. Both FM1 and HM1 shows a negative correlation. It is shown that with the increase of the peat strength, the amount of colony reduced. The same conclusion can also be used for SM1 but the different pattern was found in D28. During cementation process, bacteria could successfully survive in solidified peat. Kalantari and Prasad [7] stated that the cementation process takes place immediately until 7 days. The days after that will be pozzalanic activity where the soil skeleton is strengthen over time. Therefore, it can be indicated that Bacillus spp. is reactive during cementation process and deteriorate during pozzalanic reaction.
Figure 1. Strength over curing days of solidified peat

For mixing 2 (FM2, HM2 and SM2), different trend was shown in Fig. 3. FM2 had achieved to highest strength of all types of mixtures on D28. However, the strength has decrease drastically on D56. Number of bacteria in Mixing 2 (FM2, HM2, SM2) was found less than in Mixing 1 (FM1, HM1, SM1). Surprisingly, the pattern of the microbes’ colony in FM2 shows an inverted pattern of the UCS of solidified peat. The microbes in solidified peat started to increase in number, proportionate with the decrease of the peat strength. The growth of microbes is very much dependent on the availability of food and the environment. [25] Through this finding, the possible conclusion is that Bacillus spp. can adapt to the new environment when solidification reaction has stopped at D28. The microbes is then started to consume the fiber in the solidified peat thus, making the strength of the solidified peat decreases. Nonetheless, this preliminary conclusion should be supported with chemical analysis and morphology of the sample to understand the changes between Mixing 1 and Mixing 2 samples.
FM2 = Fibric Mixed 2; HM2 = Hemic Mixed 2; SM2 = Sapric Mixed 2

Figure 3. Correlation between colony in peat and UCS – Mixing 2

The enzymatic observation could be a guide in determining the activity of the microbes. Table 4 show the enzymatic observation of all samples. FM1 which having a good continuously increasing strength pattern has no enzymatic activity. As the number of microbes was decreased, this observation was in a good agreement with the enzymatic pattern. The rest of the observation has given a prompt conclusion which is; the number of bacteria is not associated with its activity. However, samples with decreasing amount of bacteria were found having less enzymatic activity. Samples with decreasing strength were seen to be having microbes which secretes enzyme. Thus, these findings support the preliminary conclusion that saying microbes might be one of the contributors to the decreasing of strength pattern own by Mixing 2. One of the solutions suggested to keep the strength of the peat soil is the using of urease producing bacteria which react with calcium sources, mainly from OPC or FA and produce calcite precipitation that will strengthen concrete material [26]. According to Ismail et al. [27], Bacillus spp. has the ability to precipitate silica. As silica is commonly found in OPC and coal ashes, the use of these admixtures could work well with the presence of native microbes in peat soil. Bacillus spp. is also used in concrete study as reported by Irwan et al. [28]. This theory could be used in further peat solidification study.

Table 4. Enzymatic activities observed in solidified peat

| Halo zone | D7  | D14 | D28 | D56 |
|-----------|-----|-----|-----|-----|
| FM1       | -   |     |     |     |
| HM1       | ++  | ++  | ++  | -   |
| SM1       | ++  | ++  | +   | -   |
| FM2       | +   | -   | -   | +   |
| HM2       | +   | -   | -   | +   |
| SM2       | +   | +   | -   | +   |

Negative (-), positive (+), high production (++)

4. Conclusion
The uses of OPC and coal ashes confirmed successfully increase the strength of peat soil. However, an adequate quantity is important to ensure the strength of the solidified peat remains for long period.
Microbes in peat, specifically Bacillus spp. was found survived in solidified peat. The number of microbe’s colony in solidified peat in both mixing decrease with the increase of the solidified peat strength. Bacillus spp. is expected reactivate after cementation process (hydration reaction) is completed. The microbes secrete its enzyme to breakdown the cellulose in the peat thus increase the moisture content and reduced the strength of solidified peat.

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