Strength properties and reinforcement mechanism of bio-enzyme stabilized marine soils

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Abstract. Bio-enzyme was used to stabilize the marine soils of Ningbo. Strength tests, XRD tests and SEM tests were used to analyze the strength and microstructure properties of bio-enzyme and cement stabilized soil. The results of strength tests show that the bio-enzyme not only has obvious reinforcing effect when added alone, but also can promote the solidification of cement. The results of XRD tests show that there is no new substance formation during the solidification process when only bio-enzyme added, which indicates that the process is a physical effect. The results of SEM tests show that the bio-enzyme can improve the pore arrangement, pore morphology and pore size of the soil, and reduce the directional fractal dimension, probability entropy, porosity fractal dimension and pore shape fractal dimension, which makes the orientation of the pores better, the order of the particles higher, the pore size more uniform, and the degree of particle agglomeration higher, therefore increasing the strength. In all the microstructure parameters, the fractal dimension of pore morphology has the greatest influence on the strength of stabilized soil, that is, promoting the aggregation of soil particles is the main role of bio-enzyme in improving the strength of stabilized soil.

1. Introduction
Bio-enzyme is a kind of protein organic compound extracted from plants and has certain biological activity after fermentation, which has the characteristics of high temperature resistance, non-toxic and non-corrosive. Since the 1990s, it has been gradually applied in road base filling (Scholen 1992; Velasquez et al. 2005; Xie 2006; Chen 2007; Li et al.2009; Malko et al. 2016), contaminated soil remediation (She et al. 2012; Liu et al. 2015), garbage disposal (Qi et al. 2011), petroleum exploitation (Tang et al. 2011) and other fields, and achieved good application results. In recent years, with the development of geotechnical engineering, researchers begun to use bio-enzyme to improve the engineering properties of weak soil foundation (Santoni et al. 2002; Tingle et al. 2003; Naagesh et al. 2010; Sun et al. 2010; Peng et al. 2012; Agarwal et al. 2014; Eujine et al. 2014; Dai et al. 2014; Nandini et al. 2014). Studies have shown that bio-enzyme not only can be used to improve the engineering properties of natural soil alone, but also can be used together with traditional solidifying agents such as cement, lime and fly ash, which may largely reduce the amount of traditional solidifying agents and bring outstanding economic and environmental advantages.

Because of the property difference of various natural soils, the reinforcement effect of bio-enzyme is not only affected by the bio-enzyme itself, but also mainly depends on the engineering properties of natural soils. Only by a thorough understanding of the reinforcement mechanism of bio-enzyme to soil can we better use bio-enzyme in engineering. At present, the study on reinforcement mechanism of different soils is relatively scarce, and little consensus has yet been reached. For example, Velasquez et
al. (2005) believed that bio-enzyme could induce a large number of organic molecules in the soil to combine to form intermediate reactants and change the original soil structure and lead to sealing, thus reducing the ability of soil particles to absorb water, making the soil more compact and improving the strength of the soil; Sun et al. (2010) used bio-enzyme to stabilize the mixture of miscellaneous fill, sand and granite gravel, then studied the compactness and strength of the stabilized soil, and believed that the reinforcement mechanism of bio-enzyme is to adsorb soil, reduce the surface tension of water, and improve the interface structure of aggregate and soil; Peng et al. (2012) used bio-enzyme to improve the pavement performance of tabia, he believed that bio-enzyme could increase the fine particles in the soil, reduce the coarse pores, and enhance the interaction between soil particles so as to improve the strength; Dai et al. (2014) conducted a series of bio-enzyme reinforcement tests on three common soils in Hong Kong, and believed that the reinforcement effect of bio-enzyme was mainly due to the cementation of the interaction between bio-enzyme and clay minerals in the soil. In general, the research on the reinforcement mechanism of bio-enzyme stabilized soil is still very lack, especially for marine soils, which limits the further application of bio-enzyme in geotechnical engineering.

In this paper, three kinds of marine soils in Ningbo area (next to the south of Shanghai, China) were studied. The unconfined compressive strength tests were used to study the strength properties of soil samples mixed with different proportions of bio-enzyme and cement. The microstructure characteristics of stabilized soils were quantitatively analyzed by XRD tests and SEM tests, then the reinforcement mechanism of bio-enzyme is studied to provide reference for further engineering application of bio-enzyme.

2. Strength tests

2.1. Test materials
(1) Bio-enzyme: Perma zyme 11X produced by International Enzymes, Inc. was used, which has a brownish viscous liquid appearance, aromatic smell, very soluble in water, density of 1.08 kg/L, boiling point of 100°C, PH value is around 5.
(2) Soils: three kinds of marine soils from Ningbo area, namely silt, silty clay and clay, the basic physical properties of undisturbed soil samples as shown in Table 1.
(3) Cement: P.O. 32.5 common Portland cement produced by Jiaxing Dongjin Company.

| Physical property index | silt | silty clay | clay |
|-------------------------|------|------------|------|
| Water content (%)       | 32.1 | 31.2       | 91.2 |
| density (g/cm³)         | 1.83 | 1.87       | 1.50 |
| Specific Weight         | 2.70 | 2.72       | 2.76 |
| Void ratio              | 0.95 | 0.91       | 2.52 |
| Plastic limit(%)        | 24.7 | 18.6       | 26.8 |
| Liquid limit(%)         | 34.0 | 33.2       | 56.9 |
| Plastic index           | 9.3  | 14.6       | 30.1 |
| Optimum moisture content(%) | 24.3 | 19.2 | 25.5 |

2.2. Samples preparation
Because it is difficult to control the quality of soil samples when preparing bio-enzyme stabilized soil samples using undisturbed soil, the remolded soil was used to prepare the samples. Firstly, the soils were dried and crushed, and then prepared to stabilized soil samples with various proportion of solidifying agents at the optimal water content. In order to study the reinforcement effect of bio-enzyme and bio-enzyme combined with cement, three kinds of Perma zyme concentration and three kinds of cement
content were selected to prepare the samples of stabilized soil, as shown in Table 2. In sample preparation, the soil mixture is added into a cylinder mold with a diameter of 50mm and a height of 50mm, and six parallel specimens were prepared for each group. The samples were sealed and cured in a curing room with a temperature of 20°C and a relative humidity of 53% to the period of 7d, 28d and 90d.

Table 2. Bio-enzyme concentration and cement content of stabilized soil

| Concentration of bio-enzyme | Cement content (%) |
|-----------------------------|-------------------|
|                             | 0     | 3    | 6    |
| 0:3000                      | P0C0  | P0C3 | P0C6 |
| 1:1000                      | P1000C0 | P1000C3 | P1000C6 |
| 1:500                       | P500C0 | P500C3 | P500C6 |

2.3. Test results

Unconfined compressive strength tests were carried out on samples cured to the specified age, the results are shown in Fig.1~Fig.3.

(a)Cement content 0%

(b)Cement content 3%

(c)Cement content 6%

Fig.1 Strength of stabilized silt with different proportions
Fig. 2 Strength of stabilized silty clay with different proportions

(a) Cement content 0%

(b) Cement content 3%

(c) Cement content 6%
An analysis of Fig. 1~Fig. 3 shows the following laws:

(1) Comparing with the soil samples without solidifying agent, the unconfined compressive strength of all the soil samples mixed with Perma zyme increased obviously. Taking the soil samples cured for 90 days as an example, the strength of silt increased by 10.2% ~ 33.1%, the strength of silty clay increased by 37.6% ~ 80.2%, and the strength of clay increased by 20.5% ~ 37.9%. For different soils, there is an optimum concentration for the mix proportion of Perma zyme, in the range of this paper, for silt the optimum concentration is 1:3000, for silty clay is 1:1000, and for clay is 1:500. It can be preliminarily determined that the optimum concentration of the Perma zyme is related to the plasticity index of the soil when only the Perma zyme is added, the larger the plasticity index, the larger the optimum concentration.

(2) For the soil samples only mixed with Perma zyme, the strength of 7 days age generally reaches 80% ~ 95% of the strength of 28 days age, while the strength increases very little when it is until 90 days age. Meanwhile, it can be found that the increasing rate of early age strength of the soil samples stabilized by Perma zyme is much higher than that of the soil samples only mixed with cement, which indicates that the effect of Perma zyme on the soils is very rapid, which is of great significance for the stabilized soils with early strength requirements.

(3) Comparing with the soil samples only mixed with cement, the unconfined compressive strength of the soil samples mixed with both Perma and cement increased obviously. When the cement content is 3%, the strength increased magnitudes of silt, silty clay and clay are 12.9%, 17.1% and 33.7%, respectively. When the cement content is 6%, the magnitudes are 16.3%, 27.7% and 39.4%, respectively. Thus it can be seen that the addition of Perma zyme can greatly enhance the solidification effect of cement on soil.

3. Microstructure tests

3.1. XRD tests

In order to analyze the reinforcement mechanism of bio-enzyme preliminary, the XRD tests were carried out to find out the change of mineral composition of stabilized clay. Four kinds of proportion such as P0C0, P0C6, P1000C0 and P1000C6 were selected in the tests, and the cured ages were all 14 days. The Rigaku D/max-ultima IV diffractometer was used. The results of the XRD tests are shown in Figure 4.
Comparing with Fig. 4 (a) and Fig. 4 (c), it is found that the diffraction patterns of the samples with and without Perma zyme are almost the same, the peak intensity of the diffraction patterns is unchanged, the main crystal phase of the samples is quartz, and there are a small amount of primary minerals such as muscovite and anorthite. It can be concluded that there is no chemical reaction in the solidification process of clay when only Perma zyme is added, and the effect of Perma zyme on soil is a physical action. Compared with figure 4 (b) and Figure 4 (d), it is known that the reaction products of soils mixed with cement alone and mixed with both cement and Perma zyme are basically unchanged, the difference is that the peak value of quartz decreases from 1100 to 590 after adding Perma zyme, which indicates that the Perma zyme accelerated the cementation of cement, and accelerated the generation of cementitious substances.

3.2. SEM tests
In order to study the reinforcement mechanism of bio-enzyme stabilized soil from the microstructure level, the SEM tests were carried out to find out the change of microstructure of stabilized clay. Five kinds of proportion such as P0C0, P0C6, P3000C0, P1000C0 and P1000C6 were selected in the tests, and the cured ages were all 14 days. The SIRION-200 thermal field emission scanning electron microscope was used. The SEM image of each soil sample is shown in Figure 5.

Fig.5 (a) shows that the grain shape of sample P0C0 is granular, and the basic structural unit of microstructure is a more dispersed structure formed by granular superposition. These granules are irregular in shape, disorderly in arrangement, incomplete in contact, and there are many small and medium pores between the granules. There is no obvious orientation of the microstructure and the
distribution of pores. Fig.5 (b) shows that when cement is added, the stabilized soil has obvious lumpy appearance, and the fine particles become larger, the layered structure is more obvious, and the integrity is stronger. Fig.5 (c) shows that the overall framework of the sample doped with 1:3000 Perma zyme has not changed much compared with that of Fig.5 (a). The main part is still a soil skeleton composed of granular superimposed aggregates through surface-to-surface contact. The pores between particles are large and the orientation is poor. Fig.5 (d) shows that the contact between particles is surface-to-surface contact, the small particles begin to polymerize, and the layered structure becomes obvious and tends to be flattened. It can be seen from Fig.5 (e) that the pores between the particles are further reduced and the joints are tighter when 1:1000 Perma zyme and 6% cement are mixed at the same time. From the qualitative analysis of microstructure, it is consistent with the results of unconfined compressive strength tests.
In order to further study the reinforcement mechanism of bio-enzyme, this paper quantitatively analyzes the microstructure changes of stabilized soils from three aspects: pore arrangement characteristics, pore morphology characteristics and pore size characteristics.

The characteristics of pore arrangement can be characterized by fractal dimension of pore direction $D_d$ and probability entropy $H_m$. The two expressions are as follows (Wen et al. 2011):

$$D_d = \frac{\sum_{i=1}^{n} m_i \ln(M/m_i)}{M \ln \alpha}$$

(1)

$$H_m = \frac{\sum_{i=1}^{n} m_i \ln(M/m_i)}{M \ln n}$$

(2)

where, $\alpha$ denotes the change of pore distribution intensity in a certain direction, and 0~180° is divided into $n$ intervals, each interval represents an angle range of $\alpha = 180°/n$, the value of $\alpha$ is usually 5°, 10° or 20°, in this paper, the value of $\alpha$ is 5°; $m_i$ is the number of pore orientation angles in the $i$th interval; $M$ is the total number of pores.

The directional fractal dimension $D_d$ indicates the orientation of pores, the smaller the $D_d$ is, the better the orientation of pores, and the worse the contrary; the probability entropy $H_m$ indicates the orderliness of pore arrangement in microstructure, and the larger the value of $H_m$ is, the more disorderly the pore arrangement and the lower the orderliness is.

The characteristics of pores morphology can be characterized by fractal dimension $D_m$ (Wen et al. 2011). The equivalent area $A$ and circumference $P$ of each pore can be calculated and plotted in the double logarithmic coordinate system ($\lg A ~ \lg P$). If the data points can be fitted into a straight line, the pore morphology is fractal, and two times of the slope of the fitted line is the fractal dimension of pore morphology as follows:

$$D_m = 2 \frac{d(\lg P)}{d(\lg A)}$$

(3)

The larger the fractal dimension of pore morphology is, the more complex the pore structure is, and the lower the degree of particle aggregate is.

The characteristics of pore size can be characterized by porosity fractal dimension $D_s$ (Wen et al. 2011). For a given pore diameter $r$, the number of pores $N(r)$ larger than the pore diameter can be calculated, and the plot $\lg N(r) ~ \lg r$ can be drawn. If the data points can be fitted into a straight line, the porosity is fractal, and the slope of the fitted line is the fractal dimension of porosity as follows:

$$D_s = -\frac{d[\lg N(r)]}{d[\lg r]}$$

(4)

Porosity fractal dimension $D_s$ denotes the degree of pore homogenization, that is, the difference of pore size. The smaller the value is, the smaller the difference of pore size is, and vice versa.

In this paper, the image processing software ImageJ is used to binarize the SEM images of each sample, and the geometric parameters of the pores of each sample are counted. The microstructure parameters of each sample are calculated according to Equation (1) ~ Equation (4) as shown in Table 3.
Table 3. Microstructural parameters of each sample

| Microstructural parameters | P0C0 | P0C6 | P1000C0 | P3000C0 |
|---------------------------|------|------|---------|---------|
| $D_d$                     | 0.86 | 0.82 | 0.76    | 0.74    |
| $H_m$                     | 0.58 | 0.56 | 0.52    | 0.51    |
| $D_m$                     | 2.77 | 1.43 | 1.44    | 1.44    |
| $D_s$                     | 1.94 | 1.89 | 1.88    | 1.83    |

As can be seen from Table 3, the value of all parameters of the sample without solidifying agent (P0C0) is much higher, indicating that the sample has poor pore orientation, poor pore arrangement orderliness, low degree of granulation, and inhomogeneous pore size, which leads to the low strength. When the solidifying agent added, all the parameters are reduced, and the microstructure of the samples is improved in all aspects. In terms of reduction, $D_d$, $H_m$ and $D_s$ decreased 3%~14%, and $D_m$ decreased by about 50%. When only cement added (P0C6), $D_d$, $H_m$ and $D_s$ decreased slightly, and $D_m$ decreased greatly; when only Perma zyme added (P1000C0, P3000C0), $D_d$ and $H_m$ decreased significantly, $D_m$ decreased greatly, $D_s$ decreased slightly; when both cement and Perma zyme added (P3000C6), the value of $D_d$ and $H_m$ is between P0C6 and P1000C0, $D_m$ and $D_s$ decreased to the minimum.

According to the results of Fig. 3, the strength of each sample in turn from big to small is P3000C6, P0C6, P3000C0, P1000C0 and P0C0, combined with Table 3 it can be considered that $D_m$ is the main factor affecting the strength of the sample under the premise of the same mineral composition. The smaller the $D_m$ is, the greater the strength is. $D_d$, $H_m$ and $D_s$ also affect the strength of the samples, the smaller the value is, the greater the strength is, but the influence is relatively small. Therefore, the main role of bio-enzyme in soil should be to enhance the directionality and orderliness of soil particles, increase the aggregation degree of particles and the homogenization degree of pores, which can make the soil compact and enhance the cementation between particles. When cement is added, it can also enhance the connection between particles and cementitious substances, thereby improving the soil strength.

4. Conclusions
(1) Bio-enzyme has obvious solidification effect on silt, silty clay and clay of this paper, and the solidification reaction is fast. For different soils, the optimum concentration of bio-enzyme is different, and the greater the plasticity index, the greater the optimum concentration. At the same time, when both bio-enzyme and cement added, bio-enzyme can also significantly promote the solidification of cement, which can greatly reduce the amount of cement.

(2) XRD tests showed that no new minerals were found in the soil when bio-enzyme was added alone, indicating that bio-enzyme reinforcement of soil was a physical action. At the same time, when both bio-enzyme and cement were added, the bio-enzyme can accelerate the hydration of cement and accelerate the generation of cementitious substances.

(3) Qualitative and quantitative analysis of SEM tests showed that bio-enzyme could improve pore arrangement, pore morphology and pore size. The directional fractal dimension of pore direction, probability entropy and fractal dimension of porosity decreased obviously after bio-enzyme mixed, and the fractal dimension of pore morphology decreased greatly, which showed that the pore orientation of soil was better, the orderliness of pore arrangement was improved, the pore size became uniform, the degree of granulation was increased, therefore the strength of soil was improved. In all the microstructure parameters, the fractal dimension of pore morphology has the greatest influence on the
strength of stabilized soil, that is, promoting the aggregation of soil particles is the main role of bio-enzyme in improving the strength of stabilized soil.

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