Investigating the Bio-corrosion Inhibition Effect of Enzymes in Circulating Cooling Water System

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ABSTRACT

To avoid the secondary pollution that inorganic corrosion inhibitor may cause in circulating cooling water, the bio-corrosion inhibition effect of lysozyme, catalase, lipase, and laccase, as the biologic inhibitors without the above problem, and the increased bio-corrosion inhibition effect of Ca^{+2} and Mg^{+2} are studied. An enzyme with corrosion properties was selected by rotary coupon test. The inhibition rate test and the inhibition rate of ultraviolet test of lysozyme as well as lipase's SEM analysis and elemental analysis were used to explore its inhibition mechanism. Aiming to decrease enzyme's usage cost, the rotary coupon test was performed to study the effect of different ion mass concentrations on enzyme activity; its inhibition effect was first analyzed, and the complex formulation of enzyme with ion and polyaspartate was then investigated. The experiment showed that among all single enzymatic reagents, lysozyme and lipase had the best corrosion inhibition effect, and when Ca^{+2} mass concentration range was 107.75-182.57 mg/L, enzyme activity, microbial resistance, and corrosion inhibition properties were improved; a sample compound showed the best corrosion inhibition effect when 10 mg/L, 50 mg/L, and 50 mg/L of lysozyme, lipase, and poly-aspartic acid respectively were used. When the corrosion speed was controlled at 0.005 mm/a, the inhibition efficiency was above 95%.

Key words: Enzyme, Circulating Cooling Water, Corrosion

INTRODUCTION

Industrial circulating cooling water in production accounts for about 80% of the industrial water. During cycling and enrichment, the minerals in the water content increases, which not only affects the heat transfer efficiency, but also can produce under-deposit corrosion [1]. To solve the problem of scaling, corrosion, and microbial breeding, the traditional chemical treatment method relies on adding a certain amount of chemicals into the circulating cooling water such as nitrite, phosphate and molybdate salt, organic phosphate, and polyols corrosion inhibitors [2-6]. The effect of this approach is obvious, but there are some problems: huge investment, high operation cost, and the difficult management. Also, it is likely to cause corrosion to equipment and change the chemical constituents in the water. Moreover, it is easy to cause secondary pollution to the environment.

Enzymes essence is a kind of protein, which under

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natural conditions, can be degraded without secondary pollution; therefore, it is a good option for a new type corrosion inhibitor. Although the present research on the application of enzyme in circulating water corrosion is limited, the enzyme inhibitor research has gradually come to a boom. The main reason of circulating water pipeline corrosion is electrochemical corrosion and microbial reproduction [7-8]. For this fact, according to the reducibility of enzyme, sterilization or other benefits to slow down the corrosion characteristics of selected lysozyme, lipase, laccase, catalase, and a series of enzymes were studied. Lysozyme is also called murein enzyme or N - acetyl murein chitosan hydrolysis enzyme; it can hydrolyze bacteria of a cell-wall mucopolysaccharides glycosidic bond of alkaline enzyme, and can also lead to dissolving microbial cell death. Lipase is also called the three acyl glycerol acyl hydrolase [9-10], a kind of catalytic synthesis of triglycerides, and it may decompose the floorboard of the enzyme. Widely existing in animals, plants, and microorganisms, it is a specific and efficient biological catalyst. Oil extraction, a refining process of oil spill, and an oil process of waste materials containing fat can use different sources of lipase to be effectively dealt with. Moreover, studies have shown that lipase and other substances have a synergistic effect among them. Laccase is a polyphenol oxidase containing copper, and it can be used for the catalytic oxidation of phenol compounds, it can remove light on the base of electrons or protons form free radicals, and it can lead to phenol and lignin compounds cracking [11-12]. It can also be used for the catalytic oxidation of substrates such as phenol and their derivatives, aromatic amines and their derivatives, acids and their derivatives, biological pigments, metallic organic compounds, other nonphenolic substances etc. Hydrogen peroxide enzyme widely exists in animals, plants, and microorganism oxidase in the body, and its function is to prevent the oxidation. After choosing the enzyme with a good corrosion inhibition effect, in order to improve the corrosion effect of the enzyme and reduce its cost, promotive effect research is necessary. Ca^{+2} and Mg^{+2} in circulating water can be used as a coenzyme or prosthetic group to a variety of enzymes; thus, they improve the stability of enzymes structure. The ions are in natural existence in the circulating water, and by adjusting their concentration, extra inputs can be ignored. Many ions in the water have a negative effect, which will cause the scale formation in circulating water system, and when the ions are used as a prosthetic group to improve the enzyme activity, we may also reduce scaling of the circulating water system. Enzyme composite stabilizer is also a kind of effective means to reduce the cost of an enzyme inhibitor. As to the composite stabilizer, most existing research is about organophosphorus corrosion inhibitors and inorganic corrosion inhibitors remixed with poly-aspartic acid (poly-aspartic acid is a good biodegradable and environmental friendly chemical) as a water treatment agent; furthermore, poly-aspartic acid with a dispersion effect can effectively prevent metal corrosion of the equipment.

EXPERIMENTAL PROCEDURES

Water Quality Analysis

Water experiment is supplied by the recirculating cooling water system of a Qing Dao Refining and Chemical Enterprise. The method of water quality analysis is mentioned in Table 1.

Table 1: Methods for analyzing the quality of circulating cooling water.

Water Quality Index	Experimental Method and Standard
Turbidity (NTU)	Portable Turbidimeter Method (LP2000-11 Type)
pH	pH Indicator Electrode
Ca^{+2} ($mg \cdot L^{-1}$)	EDTA Titration (GB/T 15452-95)
Mg^{+2} ($mg \cdot L^{-1}$)	EDTA Titration (GB/T 15452-95)
Cl^{-1} ($mg \cdot L^{-1}$)	Silver Nitrate Titration (GB/T 15453-95)
Sulfate ($mg \cdot L^{-1}$)	Gravimetric Method (GB/T15893.3-1995)
Total iron ($mg \cdot L^{-1}$)	O-phenanthroline Spectroscopic Analysis Method
Total hydrocarbon ($mg \cdot L^{-1}$)	Chromatography (HJ 604-2011)
Fatty acid ($mg \cdot L^{-1}$)	Gas Chromatography
Total hardness ($CaCO_3$ $mg \cdot L^{-1}$)	EDTA Titration (GB/T 15452-95)
Total alkalinity ($CaCO_3$ $mg \cdot L^{-1}$)	Indicator Method (GB/T 15451-95)

Corrosion Inhibition Rate and Corrosion Rate

Rotary coupon test: Determines the corrosion inhibition using Rotary Coupon Test GB/T 18175-2000 reference. A3 carbon steel is selected as the disorganized object, and RCC-α Type Rotary Coupon Corrosion Test device is used in the corrosion experiment. Enzyme solution needed in the experiment is added into the recirculating water and is treated by well mixing. At the temperature of 40 °C and a rotational speed of 80 rpm (72 hrs of operation), the coupon is washed and weighed before and after washing, and the mass loss is calculated. Meanwhile, a blank test should be performed to calculate the corrosion rate and the corrosion inhibition rate.

Calculation and Representation of the Results

Corrosion rate calculation (X_1 (mm/a)) is given by:

$$X_1 = \frac{8760 \times (m - m_0) \times 10}{s \cdot \rho \cdot t} = \frac{87600 \times (m - m_0)}{s \cdot \rho \cdot t} \quad (1)$$

where, m (g) is the mass loss of test piece; m_0 (g) is the average mass loss of test piece in the acidic pickling blank test; s (cm^2) is surface area of test piece; ρ (g/cm^3) stands for the density of test piece; t (hr.) represents the test time of test piece; 8760 (hr/a) and 10 (mm/cm) are the year to hour and centimeter to millimeter conversion factors.

Corrosion inhibition rate calculation [X_2(mass percentage)] is defined by:

$$X_2(\%) = \frac{X_0 - X_1}{X_0} \times 100 \quad (2)$$

where, X_0(mm/a) is corrosion rate of test piece in the blank test without a water treatment agent, and X_1 (mm/a) represents the corrosion rate of test piece in the test with a water treatment agent.

An Inhibitory Rate of Lysozyme

The water sample with a water quality analysis defined above was used as the experimental water, and lysozyme solutions were prepared at the concentrations of 20, 40, 60, 80, and 100 mg/L. The

solution was prepared by having reacted in a water bath at a constant temperature of 35 °C for about 10 min after setting a blank control group. Also, 0.2 mL enzyme solutions of different mass concentrations are added to each solid medium that has been prepared and has solidified. We cultivated them for 24 hrs. There are 3 groups of parallel experiments needed for each concentration gradient: Record the colony, count, and characterize the bacteriostatic and fungi static property of lysozyme by an inhibitory rate given by:

$$Inhibitory\ Rate = \frac{M_0 - M_i}{M_0} \times 100\% \quad (3)$$

where, M_0 is colony count on the plate without an enzyme and M_i is colony count on the plate with an enzyme.

An Inhibitory Rate of Ultraviolet

Using water samples described above as the experiment water, 6 groups of water samples were treated by ultraviolet irradiation at different times, and we adopted the method of calculating an antibacterial rate of each sample. The ultraviolet sterilization of water samples, the sterilization of aseptic rotary coupon corrosion tests, and the calculation of the corrosion rate was performed in a bacteria-free environment.

Effect of Ion Mass Concentration on Lysozyme Activity

With the reference to spectrophotometry GB/T25879-2010 used in the determination of lysozyme activity in egg white, and by replacing the substrate M. Lysodeikticus with chitosan [13], we used the below procedure in the current work:

We prepared lysozyme solution at a concentration of 20 mg/L and Zn^{+2}, Mg^{+2}, and Ca^{+2} solutions (0, 25, 50, 100, 200 mg/L) respectively. Then, 58 mL of a 1.200 g/L chitosan acetate solution (pH=4.5) was reacted with 2.0 mL of the above lysozyme solution at 35 °C for about 60 min, and we took out 6.0 mL solution and poured into a tube with a plug. Next, we added 1.0 mL alkaline potassium ferricyanide solution and shook it up. After 5 min of heating in the boiling water, we took it out, and we added 1.0 mL of 0.05 mol/L high-iron ammonium sulfate to it. The absorbance of the solution is measured at 670 nm using a reagent blank as a reference. Meanwhile, we measured the absorbance of the solution without enzyme as a control experiment. Enzyme activity (U) calculation is given by:

$$U = \frac{100 \times (A - A_0)}{m_1} \quad (4)$$

where, U is enzyme activity, and A is absorbance of the solution with enzyme; A_0 represents absorbance of the solution without enzyme; m_1 (mg) stands for enzyme mass in the solution, and 100 is the proportional coefficient.

Effect of Ion Mass Concentration on Lipase Activity

With the reference to BAI Guangwei's Methods [14] of enzyme activity determination, we prepared a 2 mg/mL lipase solution, and five Mg^{+2} and Ca^{+2} solutions (0, 60, 120, 240, 480 mg/L)respectively. 25 mL ion solution, 25mL phosphate buffer solution, and 1 mL triolein were added to a 100 mL conical flask. They were stirred with a magnetic stirrer for 10-20 min to make an emulsion. The emulsified substrate was divided into 2 groups, and we added 5 mL of the enzyme solution to one of which and 5 mL of deionized water to the other group; furthermore, both groups were placed in a water bath at a constant temperature of 35 °C to react for about 20 min. We took them out then, and used 10 mL, 95% ethanol to terminate reaction. Phenolphthalein (5 drops) was chosen as the

indicator; sodium hydroxide solution was used as the titration agent. We recorded the consumption of *C* and C_0 respectively. Enzyme activity (U) calculation is defined by:

$$U = \frac{100 \times (C - C_0)}{m_2 \times t} \quad (5)$$

where, C (mL) is sodium hydroxide volume consumed in the solution with enzyme, and C_0 (mL) is sodium hydroxide volume consumed in the solution without enzyme; m_2 (mg) is enzyme mass in the solution, and t (min) is the reaction time; 100 is proportional coefficient.

Effect of Ion Mass Concentration on an Enzymatic Corrosion Inhibition Rate

We determined the effect of different ion mass concentrations on lysozyme and lipase corrosion inhibition according to Rotary Coupon Test GB/T 18175-2000. The specific methods used are as follows: We prepare the lysozyme and lipase solutions which are all at the concentration of 20, 70 mg/L respectively, and then we added extra 0, 25, 50, 100, 200 mg/L solutions. We carried out an experiment with the reference to the above experimental procedure, and finally calculated corrosion inhibition rate and corrosion rate.

RESULTS AND DISCUSSION

Result of Water Quality Analysis

The circulating cooling water samples are from a certain refinery in Qingdao, and the water quality analysis is shown in Table 2.

Table 2: Water quality parameters of circulating cooling water.

Water quality parameters	pH	Turbidity (NTU)	Ca^{+2} ($mg \cdot L^{-1}$)	Mg^{+2} ($mg \cdot L^{-1}$)	Cl^{-} ($mg \cdot L^{-1}$)	SO_4^{-2} ($mg \cdot L^{-1}$)	Total iron ($mg \cdot L^{-1}$)	Total hardness ($CaCO_3$ $mg \cdot L^{-1}$)	Total alkalinity ($CaCO_3$ $mg \cdot L^{-1}$)
Value	8.78	5.14	82.57	36.97	302.75	258.78	—	394.11	351.21

Known from the analysis of water quality in Table 2, the circulating cooling water pH is between eight and nine, i.e. the water is alkaline; total hardness and total alkalinity above 300 mg/L is also at a high level.

Biological Enzyme Inhibition Performance Inquiry

To select the enzyme with good corrosion properties, corrosion coupon tests have been carried out on the enzymes that may have the effect of inhibition like lysozyme, lipase, catalase, and laccase in this paper. The results are as shown below.

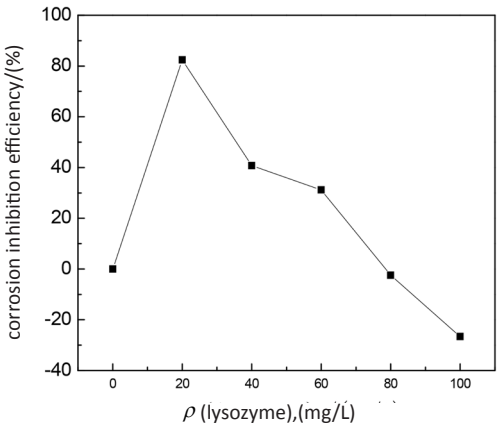

Figure 1: Corrosion inhibition efficiency of lysozyme.

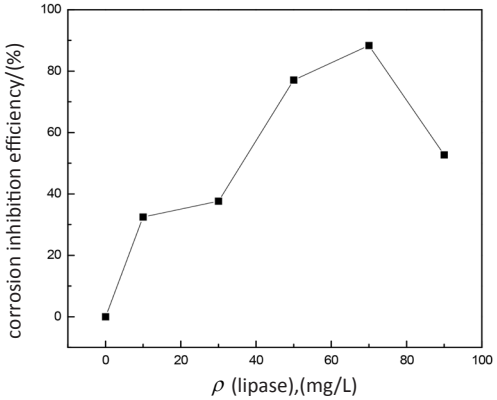

Figure 2: Corrosion inhibition efficiency of lipase.

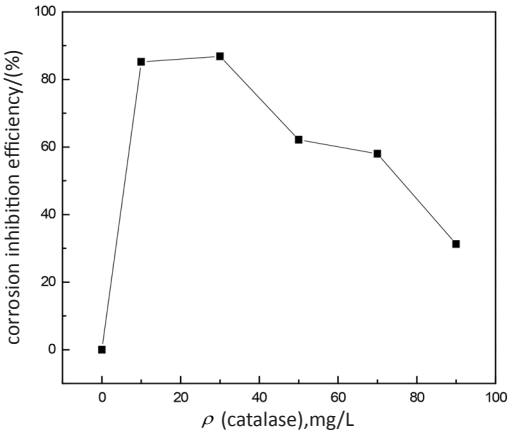

Figure 3: Corrosion inhibition efficiency of catalase.

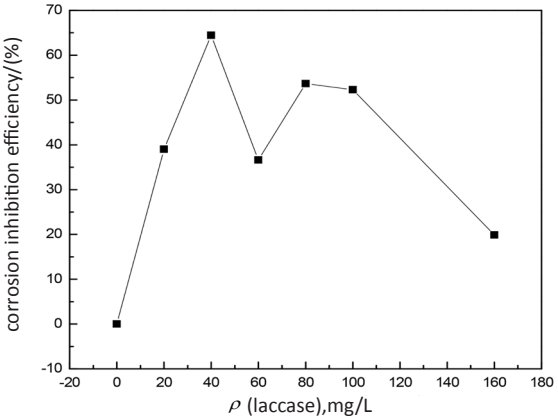

Figure 4: Corrosion inhibition efficiency of laccase.

As can be seen from the figures, lysozyme, lipase, and catalase have a better inhibition effect, whose corrosion inhibition rates are more than 80% when the concentration of the enzyme reaches its optimum concentration; however, the inhibition effect of laccase is not ideal. The maximum corrosion inhibition efficiency is 82% when the mass concentration of lysozyme is 20 mg /L; a lipase mass concentration of 70 mg/L results in the maximum corrosion inhibition rate of 86%; a catalase mass concentration of 30 mg /L leads to the maximum corrosion inhibition efficiency of 90%; a laccase mass concentration of 40 mg/L causes the maximum corrosion rate of 65%. When the mass concentration of laccase is more than 40 mg/L, large fluctuations in corrosion rate appear, which are difficult to control in actual use, and corrosion rate is low compared with the other enzymes.

Considering the inhibition effect, price, storage conditions, and other factors, lysozyme and lipase are selected for further study instead of catalase which is expensive and difficult to store and instead of laccase which has a poor effect.

The Research about the Corrosion Inhibition Efficiency of Lysozyme

The Most Suitable Mass Concentration of Lysozyme

After using the data obtained above in the pre-experiment for the thinning mass concentration ranging from 0 to 30 mg/L, the mass concentration of lysozyme and the corrosion inhibition rate can be obtained. The relation graph of corrosion rate is as follows.

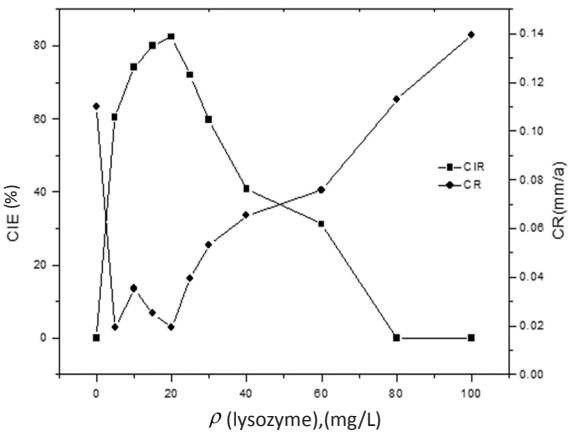

Figure 5: The corrosion inhibition efficiency and corrosion rate of lysozyme at different concentration.

The corrosion inhibition rate of lysozyme increased rapidly when its concentration is between 0-20 mg/L, and reaches the peak of 82% at 20 mg/L. Then, the corrosion inhibition rate decreases with a further increase in the mass concentration of lysozyme, even reducing to 0 when it is more than 80 mg/L. From the corrosion rate, the corrosion status of coupon is more serious compared to the one without an enzyme.

Analysis of Lysozyme Inhibition Mechanism

Circulating water pipeline corrosion is mainly caused by electrochemical and microbial corrosion [15-17]. Also, the majority of microbes resulting in corrosion are the iron bacteria and the mucous membrane-produced bacteria; Iron bacteria can directly give rise to the corrosion of carbon steel piping through its biochemical reaction, and mucous membrane-produced bacteria can form loose biofilms [18] on the inner wall of the pipeline, which plays an important role in constituting the local corrosion cell and the corrosion substance diffusion barriers. With the process of corrosion, loose corrosion product accumulates constantly under the biofilms to form around umbos, which accelerates the corrosion of lysozyme. Lysozyme, which can destroy the structure of cell wall of bacteria and make the microorganisms die, effectively kill the iron bacteria and the mucous membrane-produced bacteria to stop forming bulge. Therefore, the corrosion inhibition efficiency of lysozyme may benefit from its germicidal efficacy.

The fact can be observed intuitively from the following images of coupon test that lysozyme can eliminate the around umbos to some extent, which demonstrates that the corrosion inhibition efficiency of lysozyme is due to its germicidal efficacy.

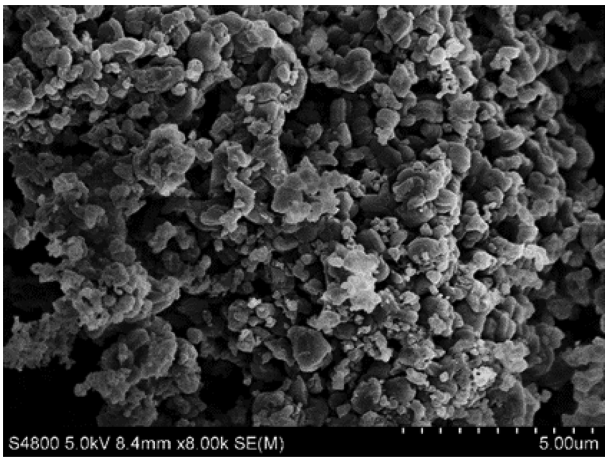

Figure 6: Bolt bulge SEM figure.

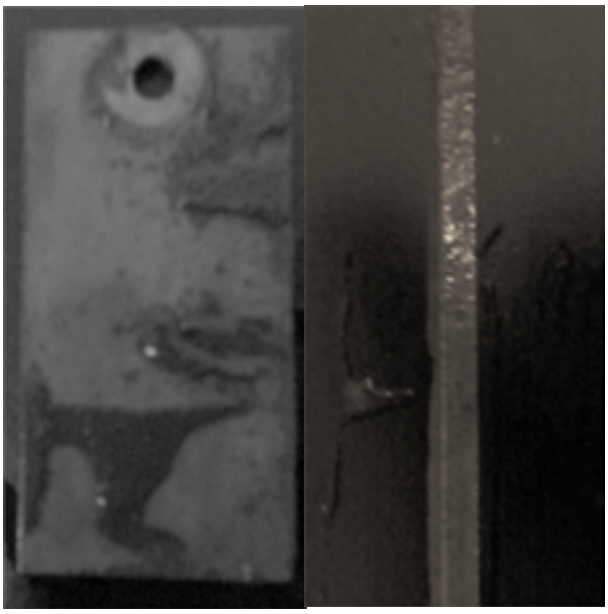

Figure 7: Coupon without lysozyme.

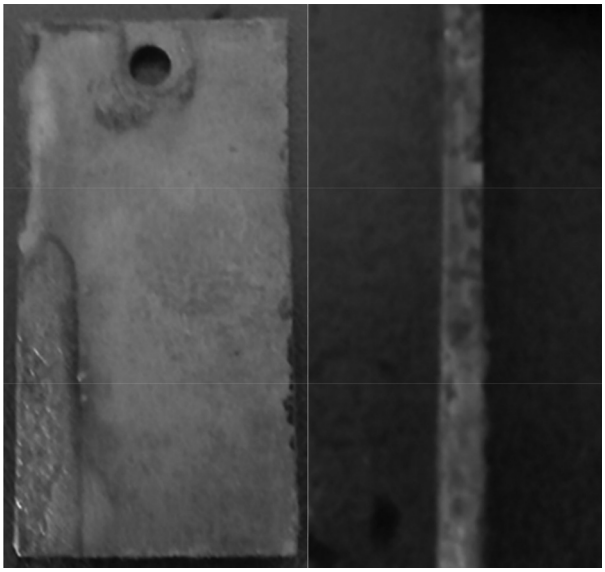

Figure 8: Coupon with lysozyme.

To verify the above conjecture more precisely, we explored the influence of the mass concentration of lysozyme on the total bacteria number in circulating water by the method in described above, and researched the relationship between the circulating water of the inhibitory rate and the corrosion inhibition rate. We created the comparison diagram of the change curve of the total bacteria number in circulating water and that of the inhibitory rate at different mass concentrations of lysozyme (such as Figure 9). Moreover, we created the curve of lysozyme inhibitory rate and corrosion inhibition rate in the ultraviolet to show the experimental results intuitively (such as Figure 9).

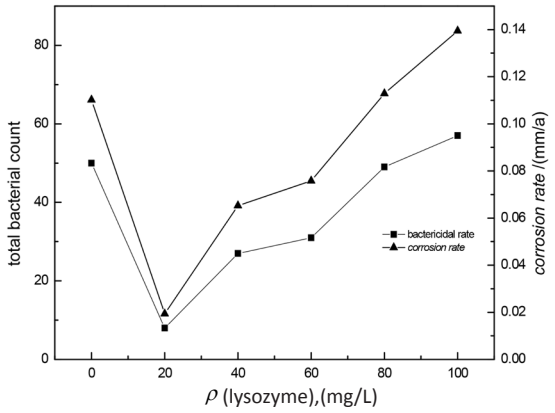

Figure 9: The relation of total number of bacteria and lysozyme concentration.

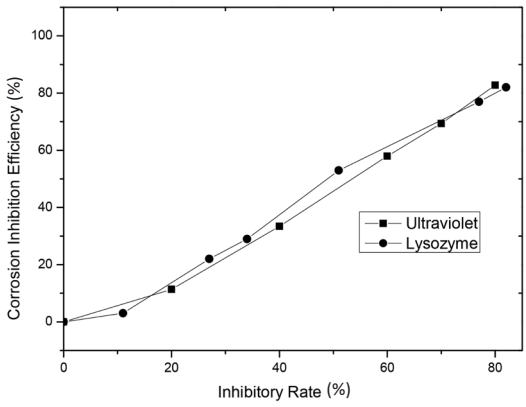

Figure 10: The relation of antibacterial rate and corrosion rate.

As can be seen from Figure 9, the trend of the total bacterial count is basically the same as the inhibition rate. When the mass concentration of lysozyme is above 20 mg/L, Bacteriostatic rate began to decrease, and with the decreasing bacteriostatic rate, the corrosion rate increased. The reason for this change is that the bactericidal effect of lysozyme has relative specificity, so different lysozyme can only specifically inhibit one or several kinds of bacteria; however, for other types of bacteria, inhibition effect is not good. In the water, there are a variety of bacteria that may cause pipeline corrosion at the same time and the majority of these bacteria are in a state of competition under natural conditions. After the lysozyme has inhibited some bacteria, other bacteria are propagated largely because of no competition, which somewhat increases the corrosion of pipelines.

The sterilization of water was used by ultraviolet light taken place of lysozyme in the test, and the curve of the ultraviolet bacteriostatic rate-corrosion inhibition rate was obtained and contrasted with the inhibition rate of lysozyme corrosion rate curve (Figure 10). As can be seen from the figure, the trend of bacteriostatic rate of ultraviolet and lysozyme is basically the same as the corrosion inhibition rate, and their corrosion rate and the bacteriostatic rate have a positive correlation. Combining with the conclusion in Figure 9, it can be proved that the corrosion inhibition of lysozyme and its bactericidal effect are in an inseparable relationship.

Inquiry of the Optimum Mass Concentration of Lysozyme

On the basis of the data obtained above, and by refining the lipase mass concentration in the range of 50-90 mg/L, lipase mass concentration and corrosion rate are obtained as follows:

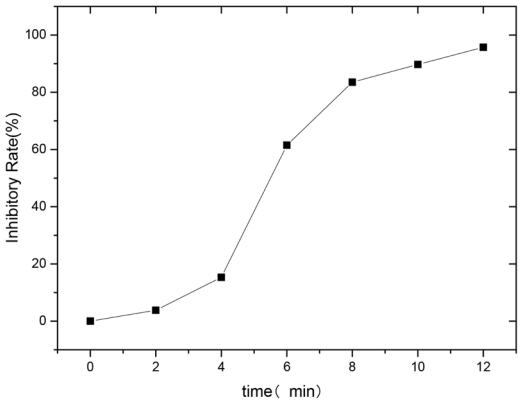

Figure 11: The relation of the time of ultraviolet irradiation and antibacterial rate.

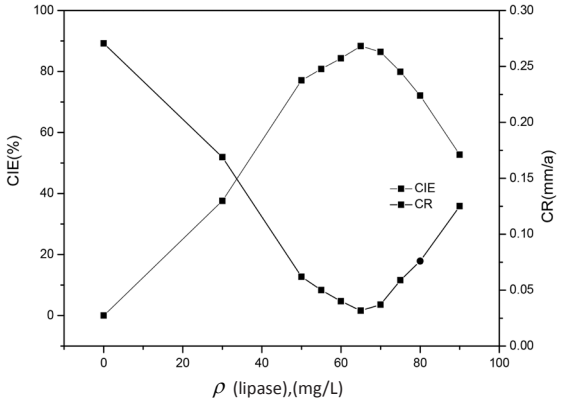

Figure 12: The corrosion inhibition efficiency and corrosion rate of lipase at different concentrations.

The corrosion rate of lipase in the mass concentration of 65 mg/L was the largest, which was 88.76% (larger than that of lysozyme). In 0-65 mg/L concentration range, inhibition rate increases with increasing the concentration of enzyme. At the mass concentrations greater than 65 mg / L, inhibition rate decreased with increasing the concentration of enzyme, but there are still certain inhibition effects.

Analysis of Inhibition Mechanism of Lipase

Lipase is mostly used in food industry and has not yet been covered in the inhibition field. Analysis shows that a layer of black film is formed on the surface of coupon and attached to it after adding the lipase. The surface of coupon is smooth and has no significant corrosion point after the black substance is scraped off; therefore, it is guessed that an inhibition effect of lipase comes from the layer of black substance (a is in the presence of lipase, while b is in the absence of lipase).

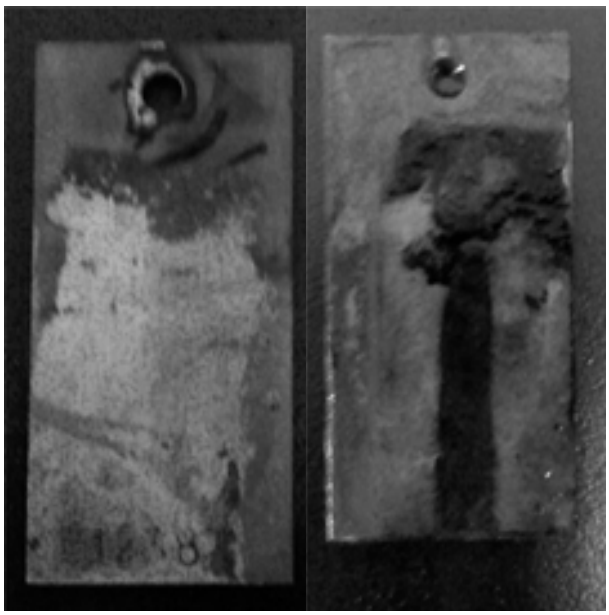

Figure 13: Black substance after adding the lipase.

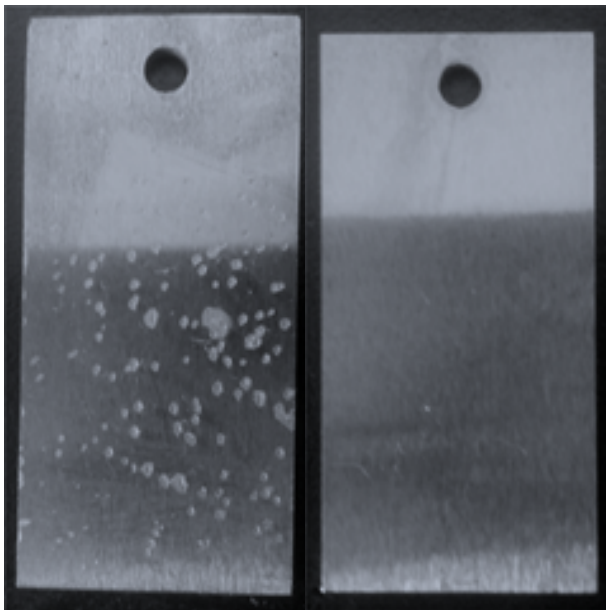

Figure 14: Surface of the bolt after the corrosion.

By the analysis of the main ingredients of ferro ferric oxide [19-20], the lipase on the surface of coupon form a layer of protective film, protecting the coupon. Below are the SEM images, which are

produced by lipase on the surface of the coupon. Figure 15 shows the surface of the black material, and it obviously has the characteristics of film forming. Figure 16 shows the surface of coupon without adding the lipase. Moreover, the surface of the Figure 16 renders fragmentation. Finally, the experimental results support our previous guesses.

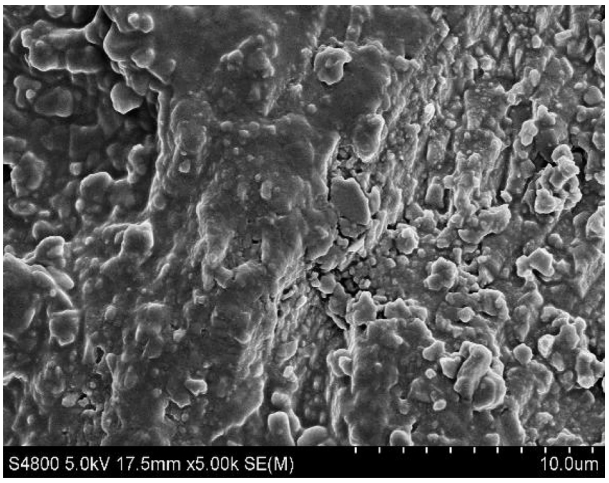

Figure 15: Coupon surface with lipase.

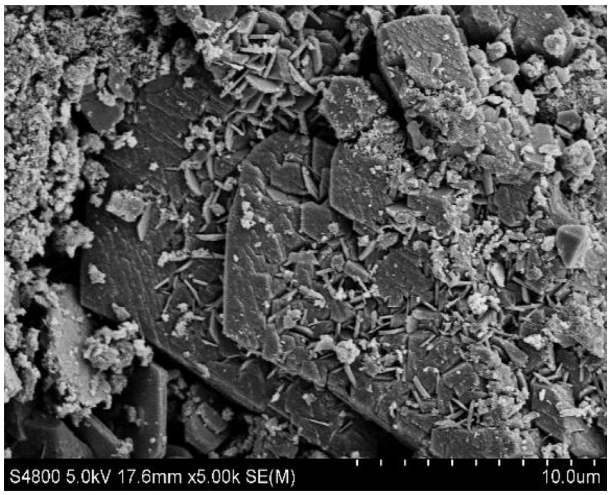

Figure 16: Coupon surface without lipase.

Inquiry on Ions of Enzyme Inhibition Effect

Inquiry on Calcium Ion of Enzyme Inhibition Effect

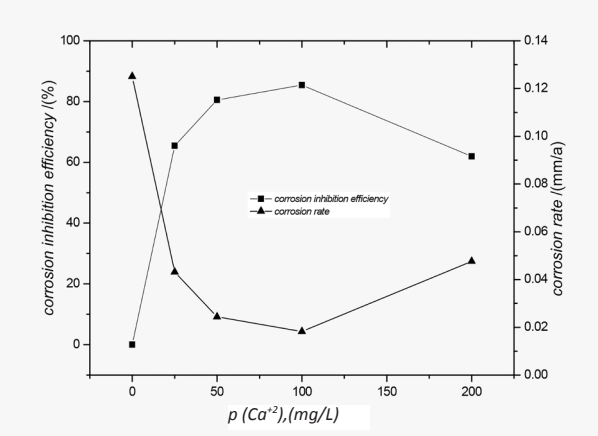

Figure 17: The corrosion inhibition efficiency and corrosion rate of lysozyme at different Ca^{+2} concentrations.

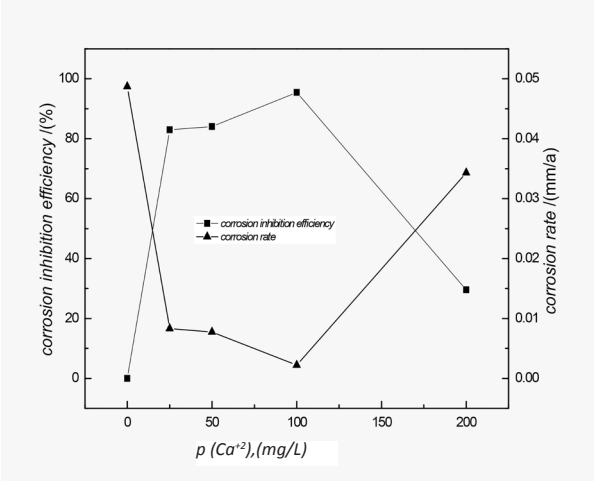

Figure 18: The corrosion inhibition efficiency and corrosion rate of lipase at different Ca^{+2}concentrations.

Analysis obtained from Figure 17 is that at the calcium ion mass concentration of 107.57 mg/L, the function of lysozyme is very good, and the corrosion rate is larger than 90% .At an ion mass concentration of 182.57 mg/L, the corrosion inhibition rate is the largest; at 99.18%, the corrosion rate is 0.0022 mm/a. The effective concentration of calcium ions is in a large range, and the calcium ion concentration is in the range of 107.57-182.57 mg/L from the data in the figure. Besides, the corrosion rate has improved greatly compared to the inhibition rate by only adding enzyme. This is because calcium is a good ion stabilizer, which can stabilize the enzyme protein structure to improve the activity.

It can be seen from the analysis of Figure 16 that when calcium ion mass concentration reaches 107.57 mg/L, the corrosion effect decreased compared to when only lipase is added; when calcium ion mass concentration reaches the range of 132.57-182.57 mg/L, the corrosion rate difference is very small compared to the case of separate dosing lipase. When the ion mass concentration is 182.57 mg/L, the corrosion-inhibition rate reaches the largest value at 92.12%, and the corrosion rate is 0.0183 mm/a. When the mass concentration of calcium ions is larger than 200 mg/L, the corrosion-inhibition rate of lipase falls, and the corrosion-inhibition rate decreases compared to when lipase is added. It is analyzed that calcium improves the hardness of water, and chloride ion corrosion effect could thus be enhanced [21] in high hardness water. Moreover, calcium itself can cause fouling and increase the corrosion, and the corrosion inhibition effect of lipase will be offset partly. Thus, the improvement of lipase corrosion inhibition was not expected to be good in the presence of calcium ions in the actual experiments.

The effect of ions on the rate of enzyme inhibition is that of the enzyme activity in essence. The following figures show the influence of Ca^{+2} concentration on the enzyme activity of lysozyme and lipase.

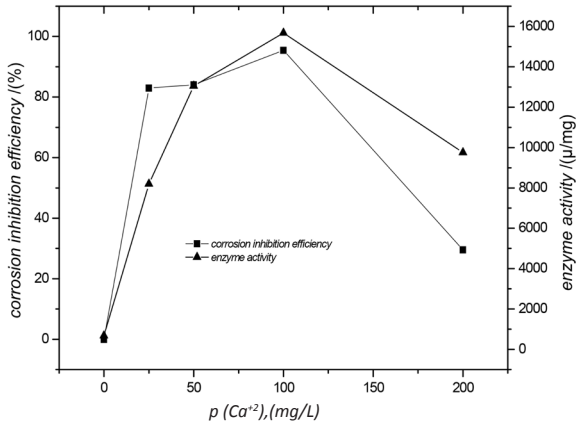

Figure 19: The relation of Ca^{+2} concentration and lysozyme activity.

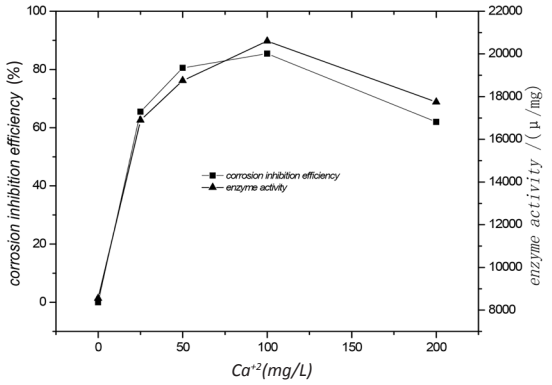

Figure 20: The relation of Ca^{+2} concentration and lipase activity.

When calcium ion concentration is 182.57 mg/L, the lysozyme and lipase activity reached their maximum values respectively at 15675 μ/mg and 20600 μ/mg, which is close to ion concentration when corrosion rate is maximized and the tendency of its curve is at a consistent variation with the curve of the inhibition rate. It may indicate that in some indication, the rate of increase in enzyme inhibition is due to the fact that the ion is improving the activity of the biological enzyme.

In summary, the control of calcium ion concentration in a reasonable range can effectively improve the corrosion inhibition effect of enzyme inhibitor.

Explore Magnesium Ions on Enzyme Inhibition Effect

Based on the analysis above, first the effect of Mg^{+2} concentration on the activity of lysozyme and lipase is studied.

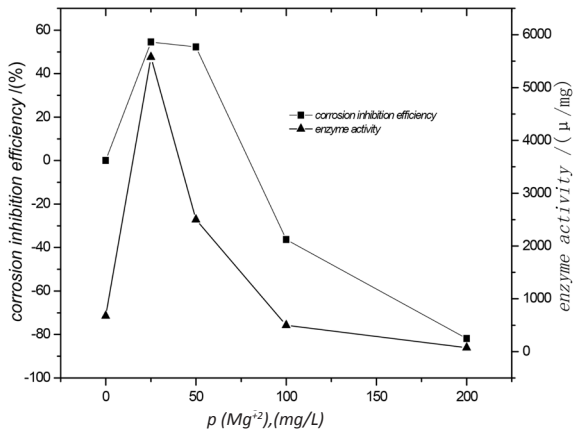

Figure 21: The relation of Mg^{+2} concentration on lysozyme activity.

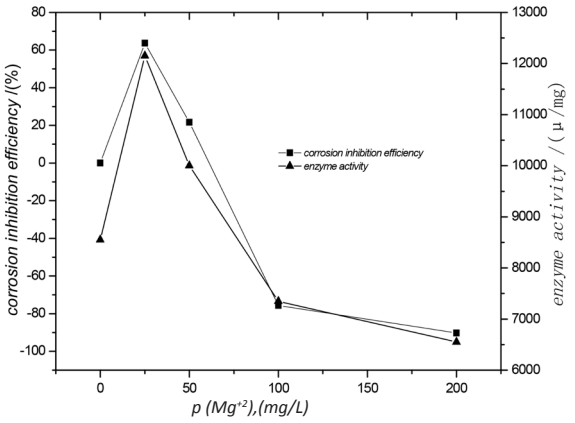

Figure 22: The relation of Mg^{+2} concentration on lipase activity.

As can be seen from the figure, Mg^{+2} can improve the activity of lysozyme and lipase to a certain extent. However, the effect is not significant on the improvement of enzyme activity compared to the previous study of Ca^{+2}, and when the mass concentration of Mg^{+2} is more than 100 mg/L, the activity of enzyme could be reduced. This coincides with the research results of LiuHui [22].

It can be speculated that Mg^{+2} is not much helpful in increasing lysozyme and lipase inhibition rate, but Mg^{+2} will reduce the enzyme inhibition rate when it reaches a certain concentration.

The actual results of the experiment are as follows:

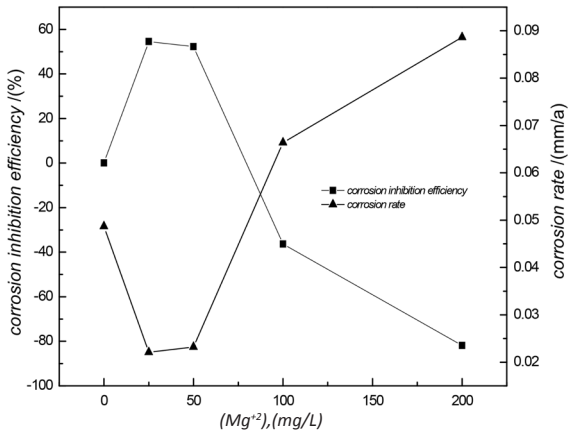

Figure 23: The corrosion inhibition efficiency and corrosion rate of lysozyme at different Mg^{+2} concentrations.

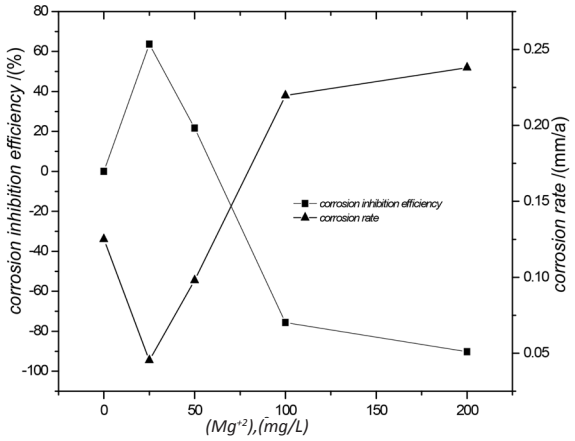

Figure 24: The corrosion inhibition efficiency and corrosion rate of lipase at different Mg^{+2} concentrations.

As can be seen from Figure 23, when Mg^{+2} mass concentration reaches 61.97 mg/L, lysozyme maximum corrosion inhibition rate is at 91.82%, and the corrosion rate is 0.0221 mm/a. Hence, lysozyme maximum corrosion inhibition rate has increased only by 11.4% compared to lysozyme corrosion rate (82.40%) with no additional Mg^{+2}. Moreover, the corrosion is enhanced when ion mass concentration is larger than 136.97 mg/L. In Figure 24, within the scope of the whole experiment magnesium ion mass concentration, lipase corrosion rate decreases with an increase in the mass concentration of Mg^{+2}. The experimental results and expected results are basically identical. The main reason for this is that the corrosion inhibition of Mg^{+2} promoting enzyme activity may not be enough to offset the corrosion effect caused by Cl^- in high salinity cases.

To sum up, in order to make the corrosion inhibition of lysozyme exert, the mass concentration of magnesium ions in water should be strictly controlled; where possible, one should try to make the quality of magnesium ion concentration reach the lowest level [23-24].

Effect of Enzyme on Calcium and Magnesium Ions

The calcium and magnesium ions in water are the main source of water hardness, whose corresponding salt can cause surface deposition and scaling in pipes and result in corrosion under the scale as one of the main reasons for circulating water pipeline corrosion. We performed an elemental analysis before and after the enzymatic composition of corrosion products on the surface of the bolt, as shown in Table 3.

Table 3: Content of calcium and magnesium(%).

Element	With enzymes	Without enzymes
Ca	2.91	7.58
Mg	6.43	6.61

Table 3 shows an analysis of the ingredients of corrosion product on coupon surface. After adding the enzyme, the content of the calcium salt (mainly calcium carbonate) from the surface corrosion products declined obviously; moreover, the content of magnesium salts (mainly magnesium hydroxide and magnesium carbonate) shows a little change. The results show that the calcium ion improves the activity of enzyme; also, enzyme also decreased calcium salt deposition on the surface of the bolt, so controlling the mass concentration of the calcium ion can significantly improve the effect of enzyme inhibitor. Furthermore, enzyme cannot effectively prevent the magnesium salt scaling; also, magnesium ions do not effectively improve the activity of enzyme, and the content of magnesium ions in the water should be reduced. The conclusions are consistent with previous conclusions.

Complex Formulation

Complex Formulation with Ca^{+2}

According to the result of the single factor test, lysozyme (A), lipase (B), and calcium (C) are the design factors; also, it was studied that each factor of level was respectively chosen by orthogonal test, using inhibition efficiency as the index. In the case of three chosen levels, using an orthogonal L9 (34) table, the experimental design can be summarized in Table 4.

Table 4: Experimental factor levels.

Factors / Value	A (mg/L)	Blank	B (mg/L)	C (mg/L)
1	10	0	30	107.57
2	20	0	50	132.57
3	30	0	70	157.57

Based on the levels chosen from the experiments respectively and the order of orthogonal Table L9 (34), nine groups of experiments were considered as follows. The test results as well as intuitive analysis can be seen from Table 5.

Table 5: Inhibition rate indicators of the orthogonal intuitive analysis.

Factors / Value	A	Blank	B	C	CIE (mm/a)	CR (%)
Exp.1	1	1	1	1	0.0996	63.19
Exp.2	1	2	2	2	0.0642	76.28
Exp.3	1	3	3	3	0.0144	94.68
Exp.4	2	1	2	3	0.0011	99.59
Exp.5	2	2	3	1	0.0310	88.55
Exp.6	2	3	1	2	0.0487	82.00
Exp.7	3	1	3	2	0.0255	90.59
Exp.8	3	2	1	3	0.0177	93.46
Exp.9	3	3	2	1	0.0382	85.89
k_1	78.05	84.46	79.55	79.21		
k_2	90.05	86.09	87.25	82.96		
k_3	89.98	87.53	91.27	95.91		
R	12.00	3.07	11.72	16.70		

From the inhibition efficiency of the orthogonal reagent in Table 5, the inhibition efficiency in 5th, 6th, and 9th experiments is above 80% basically, and that is very stable with an inhibition efficiency above 90% in 3nd, 4th, and 7th experiments; however, the corrosion rate of carbon steel can be controlled within a low level. The intuitive analysis shows that the result of experimental 4th is the most ideal because of the 99.59% inhibition efficiency and good general inhibition effect.

After analyzing the data of Table 3, the range of calcium reaches the top of 16.70. The next ranges of calcium belong to lysozyme at 12.00 and then to lipase at 11.72, while that of the blank group is only 3.07. The corresponding factor A column is: $k_2>k_3>k_1$; Factor B column is: $k_3>k_2>k_1$; Factor C column is: $k_3>k_2>k_1$. The optimal plan determined is $A_2B_3C_3$, which is lysozyme at a mass concentration of 20 mg/L, lipase at a mass concentration of 70 mg/L, and calcium at a mass concentration of 157.57mg/L. The experiment shows that the carbon steel corrosion rate of this proposed scheme measured in circulating water is 0.0007 mm/a, and the inhibition efficiency is 99.79%.

By the orthogonal test results, we can see that, when compound biological enzyme inhibitor is added, the effect is more significant. Also, it explains well that calcium has obvious synergism with biological enzyme inhibitors.

Complex Formulation with Polyaspartic Acid (PASP)

According to the result of single factor test, lysozyme (A), lipase (B), and poly-aspartic acid (C) were used as the design factors; it was studied that each factor of level was respectively chosen by orthogonal test, using inhibition efficiency as the index. In the case of three chosen levels, using an orthogonal L9 (34) table, the experimental design can be summarized in, Table 6.

Table 6: Experimental factor levels.

Factors / Value	A (mg/L)	Blank	B (mg/L)	C (mg/L)
1	10	0	30	20
2	20	0	50	30
3	40	0	70	50

Based on the levels chosen from the experiments respectively and the order of the orthogonal table L9 (34), nine groups of experiment were considered as follows. The test results as well as intuitive analysis can be seen in Table 7.

Table 7: Inhibition rate indicators of the orthogonal intuitive analysis.

Factors / Value	A	Blank	B	C	CIE (mm/a)	CR (%)
Exp.1	1	1	1	1	0.0443	83.64
Exp.2	1	2	2	2	0.0310	88.55
Exp.3	1	3	3	3	0.0055	97.96
Exp.4	2	1	2	3	0.0232	91.41
Exp.5	2	2	3	1	0.0304	88.75
Exp.6	2	3	1	2	0.1090	59.71
Exp.7	3	1	3	2	0.0625	76.89
Exp.8	3	2	1	3	0.0459	83.03
Exp.9	3	3	2	1	0.0133	95.09
k_1	90.05	83.98	75.46	89.16		
k_2	79.96	86.78	91.68	75.05		
k_3	85.00	84.25	87.87	90.80		
R	10.09	2.79	16.22	15.75		

From the inhibition efficiency of the orthogonal reagent in Table 6, the inhibition efficiency in 1st, 2nd, 5th, and 8th experiments is above 80% basically, and that is very stable with the inhibition efficiency above 90% in 3nd, 4th, and 9th experiments, while the corrosion rate of carbon steel can be controlled within a low level. The intuitive analysis shows that the result of the 3th experiment is the most ideal because of the 97% inhibition efficiency. The other test groups also keep inhibition efficiency above 80% and corrosion rate below 0.05 mm/a, and have a good general inhibition effect.

After analyzing the data of Table 6, the range of lipase reaches the top of 16.22. Next come poly-aspartic acid and lysozyme, which shows that lipase plays a leading role in the compound; however, the effect of lysozyme and poly-aspartic acid in circulating water is also considerable. The corresponding factor A column is: $k_1>k_3>k_2$; Factor B column is: $k_2>k_3>k_1$; Factor C column is: $k_3>k_1>k_2$. The optimal plan is determined to be A1B2C3. The experiment shows that the carbon steel corrosion rate of this proposed scheme measured in circulating water can be controlled at a level lower than 0.005 mm/a, and the inhibition efficiency can reach as high as 99%. This proposed scheme makes the advantage of lysozyme and lipase assisted by the efficient compound agent of poly-aspartic acid and shows a far better effect than a single enzyme system.

Single PASP with an inhibition efficiency of 60%-70% is not really a good inhibitor [25]. However, when used as a compound agent, it leads to a good effect and can improve other inhibitors inhibition efficiency greatly. The other two inhibitors chosen in this experiment, namely lysozyme and lipase, are of importance in the compounded system. Lysozyme can contribute to bacteriolysis, so it can protect the tablets. Its inhibition effect is very considerable and can reach quite a high level (>85%). Meanwhile, the amount of 10 mg/L cannot cause water eutrophication. Hence, in this way, it develops its advantages, and avoids disadvantages. The function of lipase is degrading de-lipid in the circulating water. On the one hand, it decreases the nutrient of microbes in water and has a synergistic effect with lysozyme. On the other hand, it avoids the preventing influence that de-lipid has on other inhibitors and protects these inhibitors.

CONCLUSIONS

According to the results obtained, the following conclusions can be drawn:

1. Lysozyme, lipase, and catalase have a good inhibition effect. The optimal concentration and the best inhibition efficiency are achieved as follows: an inhibition rate of 82% at a lysozyme concentration of 20 mg/L, an inhibition rate of 88% at a lipase concentration of 65 mg/L, and an inhibition rate of 90% at a catalase concentration of 30 mg/L. Laccase inhibition efficiency is not stable and it has a poor effect.

2. When the concentration of calcium ranges from 107.57-182.57 mg/L, there is a good synergism with both lysozyme and lipase. To help the biological enzyme inhibitors such as lysozyme and lipase to have the best effect, we can limit calcium concentration within that range in the practical production.

3. Magnesium has an inhibitory effect on lysozyme and lipase to some extent. If lysozyme and lipase are used in production, magnesium should be removed as much as possible.

4. Complex formulation with calcium manifests that calcium optimal concentration is 157.57 mg/L and different from that of the before test under the condition that lysozyme and lipase coexist.

5. Complex formulation with PASP indicates that when the inhibitor is composed of 10 mg/L lysozyme, 50 mg/L lipase and 30 mg/L poly-aspartic acid, the inhibition efficiency is optimum and can be above 99%.

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