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Original Research

Effect of Calcium and Magnesium Ions on Microbial Urease Activity in Landfill Leachate Treatment

Jian Huang[1,2,3], Hongwei Yin[1,2], Hua Zhang[1,2,3]*, Shan-Shan Xi[1,2,3], Tao Luo[1,2], Chun-Hua He[1,2,3], Jiamei Zhang[1,2,3], Jinhua Wang[1,2,3]

[1]School of Environmental and Energy Engineering, Anhui Jianzhu University, Hefei 230601, China
[2]Anhui Provincial Key Laboratory of Environmental Pollution Control and Resource Reuse, Hefei 230601, China
[3]Anhui Research Academy of Ecological Civilization, Hefei 230601, China

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Abstract

Urease is a key enzyme affecting microbial metabolic activity, and its activity is affected by various pollutants,and pollutants have more complex effects on the activity of microbial urease. This study researched the effect of calcium and magnesium ions in landfill leachate on urease activity of anaerobic microorganism according to designing three kinds of calcium and magnesium ion mixtures with different concentration ratios by direct average ray method, investigating the concentration-effect curves of calcium and magnesium ions by BiPhasic and Logistic functions, analyzing the interaction relationship between calcium magnesium ions and microbial urease activity by equivalent line graphic method. The results indicated that with the increase of calcium concentration, the inhibitory effect of urease activity showed a trend of falling first and then rising, and the inhibitory effect of magnesium ions on urease activity increased with the increase of magnesium concentration. The inhibitory effect of calcium magnesium ions on urease activity was obviously time-dependent. The interaction of calcium magnesium ions mixed pollutants on urease activity was synergistic in the 1-4 reaction cycles, and the interaction was antagonistic in the 5 reaction cycles according to the equivalent line diagram. This paper provided support for further study on the action mechanism of pollutants on microbial urease by studying effect of calcium and magnesium ions in landfill leachate on urease .

Keywords: calcium ion, Magnesium ion, urease, interaction

*e-mail: zhanghuapaper@163.com

Introduction

Landfill leachate, as a high-concentration polluted organic wastewater, has extremely complex components. Different landfill leachates have different components, but they all contain different concentrations of calcium and magnesium ions [1]. Calcium and magnesium ions are necessary elements in the biological treatment of landfill leachate, which promote the synthesis of enzymes needed in the process of microbial metabolism [2]. Appropriate amount of calcium and magnesium ions is beneficial to the production of intracellular enzymes in microorganisms, but excessive calcium and magnesium ions interact with related functional groups after entering cells, changing the structure and function of proteins [3] and replacing essential metals at some metabolic sites [4], thereby inhibiting the enzyme activity of microorganisms. Urease, a key restriction enzyme, is widely present in various microorganisms and plays an important role in the nitrogen cycle. Nitrogenous substances in the environment are first decomposed into amino acids and nucleotides, which are further degraded into urea and then hydrolyzed into ammonia by urease before they can be used by plants and soil microorganisms, and is a key enzyme affecting the metabolic activity of microorganisms [5, 6]. Therefore, it is of great significance to study the effect of calcium and magnesium ions on urease activity in anaerobic biological treatment of landfill leachate.

The intracellular urease activity of microorganisms is affected by calcium and magnesium ions in different environments. The results showed that in the treatment of high calcium wastewater, when calcium ions were less than 1320 mg · L^{-1} or 1720 mg · L^{-1}~1920 mg · L^{-1}, calcium ions inhibited urease activity; When the concentration of calcium ions were between 1320 mg L^{-1} and 1720 mg L^{-1}, calcium ions could promote the urease activity of microorganisms to some extent [7]. It was found that when the mass fraction of magnesium ion in soil was 0.6%, the urease activity in soil was the highest, but with the increase of magnesium ion content, the urease activity gradually decreased, indicating that the influence of magnesium ion on urease activity changed with the change of magnesium ion [8]. It can be seen that calcium ions or magnesium ions with different concentrations have different effects on urease activity. However, it is not comprehensive to study the effect of single calcium ion or magnesium ion on urease activity, and the concentration of calcium and magnesium ion is mainly below 2000 mg L^{-1}. Usually, calcium and magnesium ions exist in landfill leachate in a mixture of different concentrations, ranging from several hundred to several thousand mg liters [9]. Studies have shown that mixed pollutants tend to have more complex effects than single pollutants [10-12]. Therefore, it is more practical to study the effect of calcium and magnesium ion mixture on microbial urease activity. However, the effect of calcium and magnesium mixed ions on microbial urease activity in landfill leachate has been rarely reported. Therefore, we should not only pay attention to the influence of single calcium ion or magnesium ion on microbial urease activity, but also pay attention to the influence of calcium and magnesium ion mixture on microbial urease activity and the interaction relationship between calcium and magnesium ions.

In this study, anaerobic sequencing batch activated sludge reactor were constructed to treat landfill leachate. The concentration of calcium ions, magnesium ions and their mixture were changed, to investigate the effects of them on the activity of anaerobic microbial urease and the change law of anaerobic microbial urease activity and reveal the combined effect and interaction relationship among calcium magnesium ions and urease. Direct average ray method [2] was adopted to design calcium and magnesium ion mixtures with different concentration ratio. BiPhasic function [13] and Logistic function [14] were used to nonlinear fit the concentration and its effect, and the concentration-effect curve was obtained to explore the effects of different concentrations of calcium and magnesium on microbial urease activity and its change law. Equivalent line graphic method [15] was used to study the change rule with time of the relation ship between calcium magnesium ion mixture with different concentrations and microbial urease activity, in order to provide support for the study of the mechanism of the influence of calcium magnesium mixed pollutants on urease activity.

Experimental

Materials and Methods

Experimental Setup

Landfill leachate was taken from the regulating tank of a landfill leachate treatment plant in Hefei. Fourteen 500 mL anaerobic sequencing batch activated sludge reactors, operated under the stirring frequency 140 rpm·min^{-1}, the reaction temperature about 35ºC, and the microbial concentration about 13440 mg · L^{-1}, were set up to treat landfill leachate. The reactor cycle was determined to be 36 h, in which the influent time, reaction time, settling time and effluent time were 1, 34.6, 0.2 and 0.2 h, respectively. Calcium magnesium ions and urease activity were detected by inductively coupled plasma emission spectrometry and sodium phenol colorimetry, respectively.

Experimental Methods

Adding calcium magnesium ions and their mixture to the reactor, taking the samples from the reactors every 36 hours to detect the urease activity of anaerobic microorganisms, and calculate the inhibition rates of calcium ions, magnesium ions and their mixture with different concentrations on anaerobic microorganisms. The inhibition rates is calculated by Eq. (1).

$$E = \frac{c - c_0}{c_0} \tag{1}$$

where E is the inhibition rate of calcium ions, magnesium ions and their mixtures with different concentrations on anaerobic microorganisms; C0 is the activity of microbial urease in the blank control group, mg · L^{-1}; C is the activity of microbial urease affected by of calcium ions, magnesium ions and their mixtureswith different concentrations, mg · L^{-1}.

BiPhasic function can be used to fit the nonlinear curve of J-type concentration-effect test data. The concentration-effect data obtained from the experimental results are fitted by BiPhasic function, and then the concentration-effect curve is drawn. The BiPhasic function formula is shown in Eq. (2).

$$E = A_{\min} + \frac{A_{\max 1} - A_{\min}}{1 + 10^{((x - x_{01}) * h_1)}} + \frac{A_{\max 2} - A_{\min}}{1 + 10^{((x_{02} - x) * h_2)}} \tag{2}$$

where E is the inhibition rate; X is the concentration of calcium ion, magnesium ion or mixture, mg L^{-1}; Amin is the lowest inhibition rate (i.e. The lowest point of concentration-effect curve, which is also the maximum promotion effect point); AMAX1 is the highest inhibition rate before Amin; AMAX2 is the highest inhibition rate after Amin; X01, X02, H1, H2 are function parameters.

Logistic function can be used for nonlinear fitting of S-type concentration-effect experimental data. The concentration-effect data obtained from the experimental results are fitted by Logistic function, and then the concentration-effect curve is drawn. The Logistic function formula is shown in Eq. (3).

$$E = \frac{X_{\max} - X_0}{1 + (\frac{c}{c_0})^a} + X_0 \tag{3}$$

where E is the inhibition rate; Xmax is the maximum inhibition rate of calcium and magnesium ions or their mixture with different concentrations on microbial urease activity; X0 is the inhibition rate of blank control group; α is the potential inhibitory capacity constant of the substance; C is the concentration of calcium ion, magnesium ion or mixture of calcium and magnesium ion, mg L-1; C0 is the half effective concentration of calcium ion or magnesium ion, mg · L^{-1}.

Calcium and Magnesium Ion Concentration Design

The content of calcium and magnesium ions in different landfill leachate is different, and the concentration of calcium and magnesium ions is generally several hundred to several thousand mg liters [8]. In order to more systematic research, three kinds of calcium and magnesium ion mixtures with different concentration ratios (L1, L2, L3) were designed by direct equalization ray method. The specific concentration ratios can be determined as that the calcium ion concentration accounts for 0.27, 0.52 and 0.77 in the mixed pollutants respectively (Fig. 1). 13 groups of calcium and magnesium ion mixtures with different concentration combinations were set on each ray. The dilution factor method was used to design the mixture of calcium and magnesium ions, as shown in Eq. (4).

$$f = (\frac{c_L}{c_H})^{\frac{1}{n-1}} \tag{4}$$

Where: F is the dilution factor; CL is the lowest concentration, mg L^{-1}; CH is the highest concentration, mg L^{-1}; N is the number of test concentration points.

Fourteen groups of calcium and magnesium ion mixtures with different concentrations were designed by dilution factor method, which were 0 mg/L, 8000 mg/L, 6400 mg/L, 5120 mg/L, 4096 mg/L, 3277 mg/L, 2621 mg/L, 2097 mg/L, 1677 mg/L, 1341 mg/L, 1073 mg/L, 858 mg/L, 686 mg/L and 549 mg/L separately.

Analysis of Interaction Relationship between Calcium and Magnesium Ion Mixture

Equivalent line graphic method can be used to judge whether the interaction between calcium magnesium ions with different concentration ratios and urease activity is synergistic, additive, partial additive or antagonistic.

When calcium ions and magnesium ions act lonely to reach a certain same effect, the corresponding concentration values are point A and point B, respectively, connecting point A and point B, which is called AB concentration summation line. The line segment indicates that if the concentration point falls on AB, the interaction between calcium magnesium

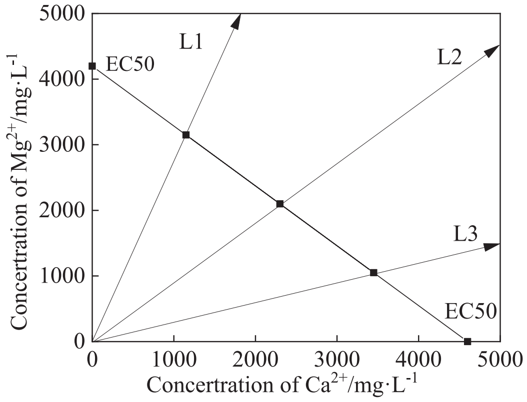

Fig. 1. Design of three kinds of calcium and magnesium ion mixtures by direct equalization ray method.

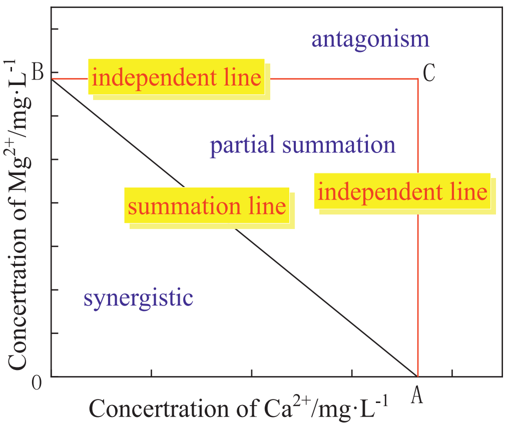

Fig. 2. Schematic diagram of equivalent line diagram.

ions mixed pollutants and urease activity is additive. If the concentration point of the mixture falls below the concentration sum line, it means that the interaction between calcium magnesium ions mixed pollutants and microbial urease activity is synergistic. If the concentration point falls on the two right-angle lines (independent lines) of AC and BC, it shows that the effect of mixed pollutants on urease is only related to the concentration of calcium ions or magnesium ions. If the concentration of the mixture falls below the independent line and above the concentration summation line, it shows that the mixed pollutants of calcium and magnesium ions have synergistic effect; if it is distributed outside the independent line, it is indicated as antagonism.

Results and Discussion

Results and Discussion

The Regularity of Inhibitory Effect of Calcium Ions with Different Concentrations on Urease Activity Varying with Cycles

The inhibitory effect of calcium ions with different concentrations on anaerobic microbial urease in landfill leachate is shown in Fig. 3. As can be seen from Fig. 3, the inhibition effect of calcium ions on urease showed a trend of first decreasing, then increasing and finally tending to be flat, and fitted the J-typecurve with the increase of calcium ions concentration. Calcium ions promoted the activity of urease to some extent at the 800 ~ 1100 mg L^{-1} calcium ions concentration. The growth rate of inhibition effect was gradually flat at more than 3300 mg • L^{-1} calcium ions concentration. The maximum inhibition effect of calcium ion on urease activity reached 90%with the concentration of calcium ions reaching 8000 mg • L^{-1}. Fig. 3 also showed that the inhibition effect of calcium ions increased with the increase of reaction period at the same calcium ions concentration, indicating that the extension of reaction time improved the inhibition effect of calcium ions, and the promotion effect of calcium ions on urease activity gradually changed into inhibition effect.

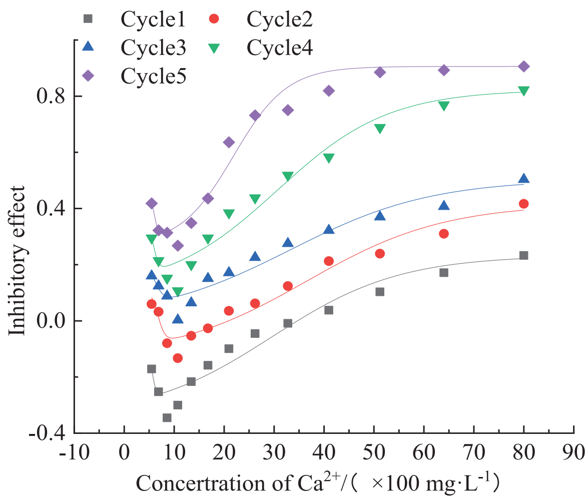

Fig. 3. Inhibitory effect of calcium ion on urease activity.

The Regularity of Inhibitory Effect of Magnesium Ions with Different Concentrations on Urease Activity Varying with Cycles

The inhibitory effect of magnesium ions with different concentrations on anaerobic microbial urease in landfill leachate is shown in Fig. 4. It can be seen from Fig. 4 that the inhibitory effect of magnesium ions on urease activity showed an increasing trend with the extension of reaction periodunder certain concentration conditions.The inhibitory effect of magnesium ionson urease was smallat the 500~1000 mg L^{-1} magnesium ions concentration, but the inhibitory effect of magnesium ions on urease activity increases obviously with the increase of magnesium ions concentration, which indicated that high concentration of magnesium

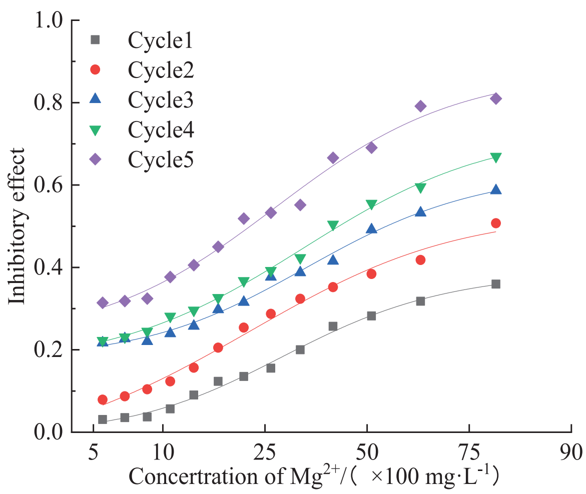

Fig. 4. Inhibitory effect of magnesium ion on urease activity.

ions had obvious inhibitory effect on urease activity. The inhibition rate of magnesium ions on urease activity increased from 36% in the first cycle to over 80% in the fifth cycle at the 8000 mg L^{-1} magnesium ions concentration, which indicated that the inhibition effect of magnesium ions on microorganismsurease activity of increased obviously with the increase of reaction cycle at the same concentration, and the inhibition effect of magnesium ions on microorganismsurease activity of had obvious time dependence.

The Regularity of Inhibitory Effect of Calcium and Magnesium Ions with Different Concentrations Ratios on Urease Activity Varying with Cycles

Fig. 5 showed the inhibitory effect of calcium and magnesium ions with different concentrations ratios on urease activity. (a), (b) and (c) showed that the ratios of calcium and magnesium ions were 0.2, 0.5 and 0.8, respectively. It can be seen from Fig. 5 that the concentration-effect curves of the three mixed pollutants of calcium and magnesium ions with different concentrations ratios on urease action were all J-shaped, that is, the inhibition effect of urease activity showed a trend of first decreasing, then increasing and finally tending to be flat with the increase of concentration. The inhibitory effect of mixed pollutants decreased with the increase of reaction period at the less than 680 mg • L^{-1} mixed pollutants concentration. The inhibitory effect of mixed pollutants was the lowest at the 680 mg • L^{-1} mixed pollutants concentration. The inhibitory effect of mixed pollutants gradually increased and then tends to be flat with the increase of reaction period at the more than 680 mg • L^{-1} mixed pollutants concentration. The mixed pollutants with low concentration of calcium and magnesium ions promoted urease activity in the first cycle by comparing the figures (a), (b) and (c). The inhibitory effect of mixed pollutants was increasing with the increase of reaction period. In the same cycle, the inhibitory effect of mixed pollutants on urease activity was more obvious than the other two ratios at the 0.5 calcium ionsproportionin mixed pollutantsunder the same concentration of mixed pollutants [16].

Inhibitory Effect and Interaction of Calcium Magnesium Ions Mixed Pollutants on Urease Activity

The regularityof the interaction relationship of calcium magnesium ions mixed pollutants with three different concentration ratios varying with cyclesby equivalent line diagram method is shown in Fig. 6. In the first reactioncycle, it can be seen from Fig. 6 (a, b) that when the inhibition effect of calcium magnesium ions and their mixed pollutants on urease was 10% and 20%, the concentration points of the mixed pollutants with three different concentration ratios dropped below the concentration sum line, which indicated that the inhibition effect of calcium magnesium ions on urease activity was synergistic [17]. In the 2^{nd}~4^{th} reaction cycle, it can be seen from Fig. 6 (c-g) that the concentration points of mixed pollutants all fell below the concentration summation line, which indicated that the interaction of the mixed pollutants of calcium magnesium ions with three concentration ratios on urease activity was synergistic in three reaction cycles. As can be seen from Fig. 6 (h, i), in the fifth reactioncycle, when the inhibitory effect of mixed pollutants on urease was 70% and 80%, and the proportion of calcium ions in mixed pollutants was 0.2 and 0.5, the inhibitory effect of calcium magnesium ions on urease activity was synergistic [18]. The concentration of mixed pollutants fell outside the independent line at the 0.8 calcium ionsratio, which indicatedthat the inhibition effect of mixed pollutants of calcium magnesium ions on urease activity was antagonistic [19].

Comparing the interaction of calcium magnesium ions mixed pollutants with different ratios on urease activity, it can be seen that when the proportion of calcium ions in mixed pollutants is 0.2 and 0.5, (i.e.

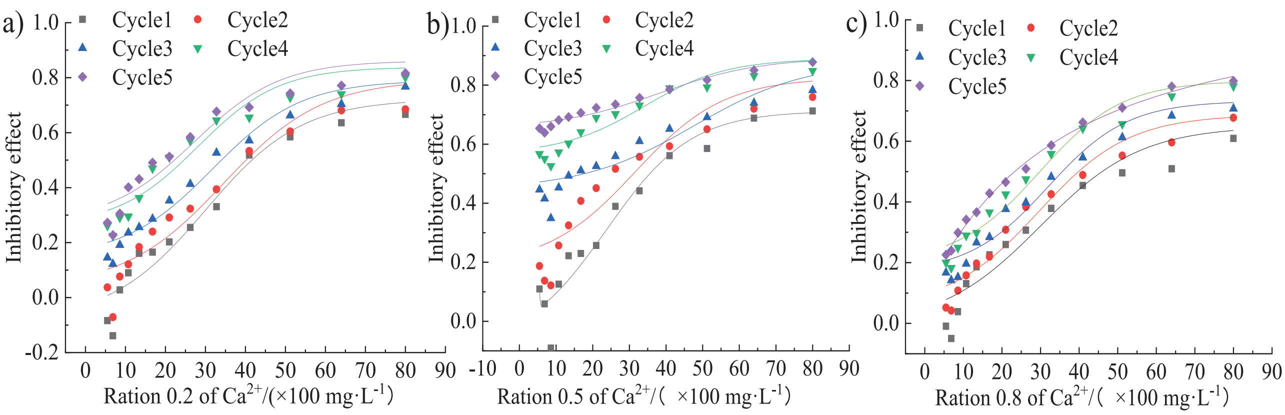

Fig. 5. Inhibitory effect of calcium and magnesium ions mixed pollutants on urease activity: a) when the proportion of calcium ions in mixed pollutants is 0.2, b) when the proportion of calcium ions in mixed pollutants is 0.5, c) when the proportion of calcium ions in mixed pollutants is 0.8.

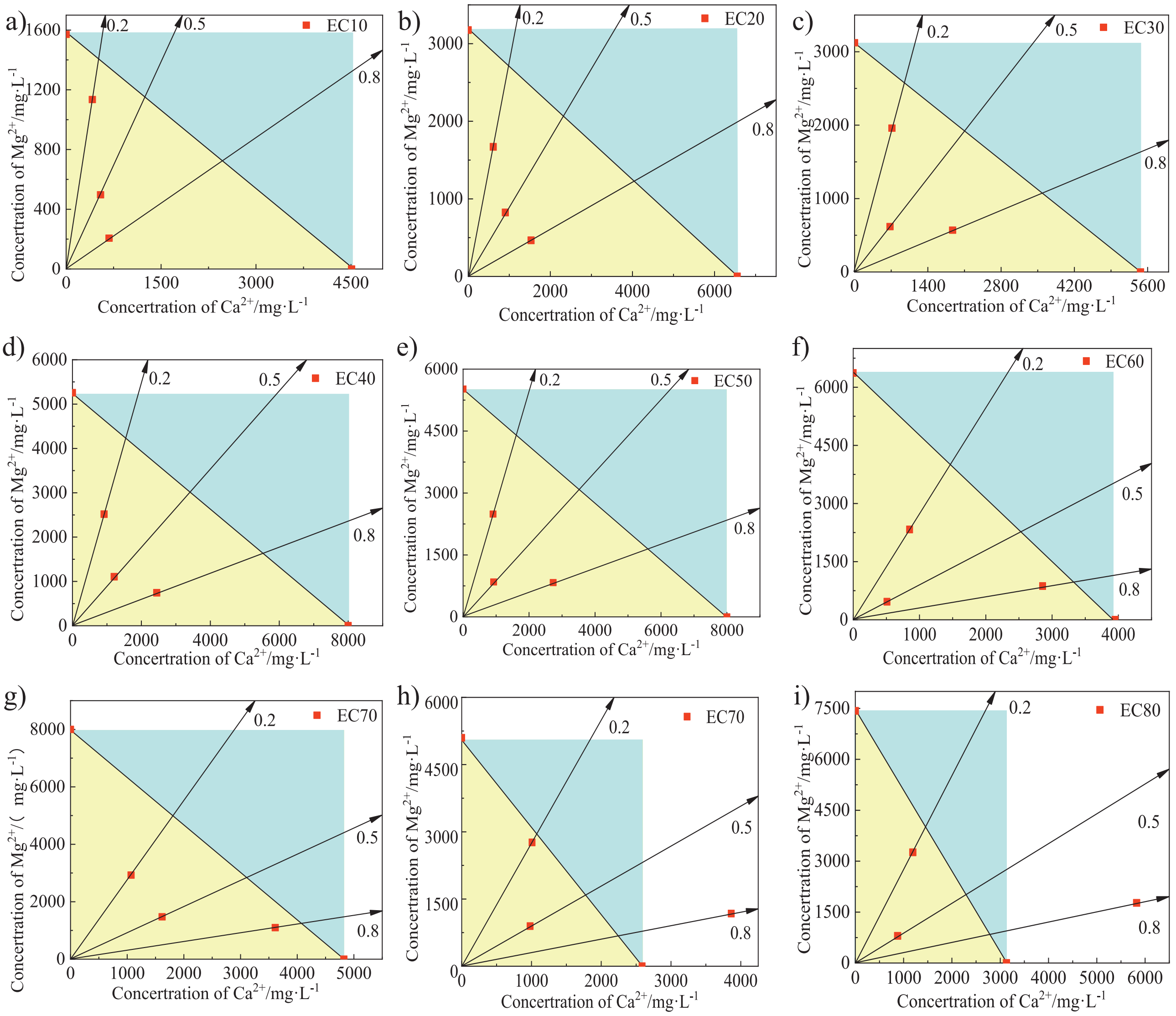

Fig. 6. Interaction between calcium and magnesium ions mixed pollutants with different ratios and urease activity: a) Cycle1-EC10, b) Cycle 1-EC 20, c) Cycle 2-EC 30, d) Cycle 2-EC 40, e) Cycle 3-EC 50, f) Cycle 4-EC 60, g) Cycle 4-EC 70, h) Cycle 5-EC70, i) Cycle 5-EC80.

low the proportion of calcium ions) magnesium ions react with negatively charged functional groups on the surface of microorganisms [20, 21], calcium ions react with carbon dioxide or carbonate ions in wastewater to form precipitates on the surface of microorganisms, which together inhibit the metabolic activities of microorganisms, and the interaction between the two ions is synergistic. When the concentration of calcium ions in mixed pollutants is increasing, more calcium carbonate crystals will be generated, while the existence of magnesium ions will hinder some calcium ions from precipitating, and the synergistic effect between the two ions will decrease. With the increase of the proportion of calcium ions, the antagonism between calcium and magnesium ions will become stronger and stronger.

Conclusions

(1) The inhibitory effect of single calcium ions on urease activity first decreased and then increased with the increase of reaction cycle. The inhibitory effect of single magnesium ions on microbial urease activity increased gradually with the extension of time, and the dependence on time was obvious. The inhibitory effect of calcium magnesium ions on microbial urease activity was obviously time-dependent.

(2) The inhibition curves of the mixed pollutants on microbial urease activity all showed J-shaped curves, showing a trend of first increasing, then decreasing and finally tending to be flat at three different ratios calcium magnesium ions. The inhibitory effect of calcium and magnesium ions mixed pollutants with different ratios was enhanced with the increase of the concentration of mixed pollutants. At the same concentration,

the longer the reaction time, the stronger the inhibitory effect of mixed pollutants, and the inhibitory effect of mixed pollutants on microbial urease activity was most obvious at the 0.5 calcium ions ratio. The inhibitory effect of mixed pollutants on microbial urease activity was obviously time-dependent.

(3) When the reaction was in 1~4 cycles, the combined effects of three mixed pollutants of calcium magnesium ions with different concentrations ratios on microbial urease activity were synergistic. In the fifth cycle of the reaction, When the concentration of calcium ions in the mixed pollutants was 0.8, the combined effect of calcium magnesium ions on microbial urease activity showed antagonism at the 0.8 calcium ions ratio. It shows that the synergistic inhibition effect of calcium magnesium ions on mixed pollutants will gradually weaken with the increase of the proportion of calcium ions, and finally the synergistic effect of calcium magnesium ions will become antagonistic effect.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. NAVEEN B.P., MAHAPATRA D.M., SITHARAM T.G., SIVAPULLAIAH P.V., RAMACHANDRA T.V. Physical-chemical and biological character of urban functional landfill leachate [J]. Environmental Collection, **220** (220), 1, **2016**.
2. ZHANG L., ZHANG M.Z., HANG Y.N. Application of equivalent line graphic method in pharmacodynamic interaction research [J]. International Journal of Anesthesiology and Resuscitation, **28** (05), 472, **2007**.
3. ZHOU C.Q., KONG X.Q., CHEN L., GAO M. Effect of PESA on enzyme activity of sludge in high calcium wastewater [J]. Journal of Environmental Engineering, **11** (04), 2212, **2017**.
4. WEI L.L., LI Y., NOGUERA D.R., ZHAO N.B., SONG Y., DING J., ZHAO Q.L., CUI F.Y. Adsorption of Cu^{2+} and Zn^{2+} by extracellular polymeric substances (EPS) in different sludges: effect of EPS fractional polarity on binding mechanism [J]. Journal of Hazardous Materials, **321**, 473, **2017**.
5. JIA F.X., LIU Y.J., YU X.H., CAI W.W., XIN W. Research progress on biological treatment technology principle and process of urea wastewater [J]. Journal of Environmental Sciences of China, **40** (12), 5270, **2020**.
6. MAPHUHLA N.G. The effects of physicochemical parameters on analyzed soil enzyme activity from alice landfill site [J]. International Journal of Environmental Research and Public Health, **18** (1), 221, **2020**.
7. ZHOU C.Q. Changes of microbial community and biological enzymes during biological treatment of high calcium wastewater [D]. Lanzhou: Lanzhou University of Technology, 21, **2016**.
8. NIU M.F., LI P.J. Effect of excessive Mg^{2+} on enzyme activity in soil [J]. Journal of Shenyang Jianzhu University: Natural Science Edition, **25** (03), 552, **2009**.
9. LIU P.R. Effects of Ca^{2+} and Mg^{2+} on Anaerobic Biological Treatment of Landfill Leachate and Microbial Community [D]. Anhui: Anhui Jianzhu University, 8, **2017**.
10. CHANG M. Research progress on calendula officinalis Pb/CD compound policy [J]. Journal of Physics: Conference Series. **1865** (2), 022023, **2021**.
11. ZHOU C.R., MA Q., LI S.L., ZHU M.M., XIA Z.Q., YU W.T. Toxicological effects of single and joint sulfamethazine and cadmium stress in soil on pakchoi (*Brassica chinensis* L). Chemosphere, **263**, 128296, **2021**
12. HE C.F., ZHOU J.N., YANG C., SONG Z.Y., HE J.H., HUANG Z.Q., DENG Y.R., WANG J.L., YU X., ZHI D. Effects of tetracycline and cadmium combined pollution on rice roots [J]. Journal of Environmental Sciences, **41** (4), 1518, **2021**.
13. HOU L.R., FU Y.H., ZHAO C. Research progress on mixed toxicity of veterinary drugs and antibiotics to ecological environment [J]. Environmental Chemistry, **40** (01), 55, **2021**.
14. MO L.Y., ZHAO D.N., QIN M., QIN L.T., ZENG H.H., LIANG Y.P. Joint Toxicity of Six Common heavy metals to chlorella pyrenoidosa [J]. Environmental Science and Survey Research, **26** (30), 30554, **2019**.
15. LIU S.S., LIU F., LIU H.L. Toxic effects of 20 water-soluble organic solvents on luminescent bacteria [J]. Environmental Sciences of China, **2007** (03), 371, **2007**.
16. SUN X.H., MIAO L.C., WU L.Y., WANG C.C. Study of magnesium precipitation based on biocementation [J]. Marine Georesources & Geotechnology, **37** (10), **2019**.
17. MOOSAZADEH R., TABANDEH F., KALANTARY F., GAN J.N., FALLAH H., LOTFABAD T.B., YAZDIAN F. Mitigation of the liquefaction potential of soil by Ca-carbonate precipitation induced by indigenous urease-producing Staphylococcus [J]. International Journal of Environmental Science and Technology, **16** (7), **2019**.
18. HU Wenle, WEN C.C., WEN S.J., KE Y. Revealing the Enhancement and Degradation Mechanisms Affecting the Performance of Carbonate Precipitation in EICP Process [J]. Frontiers in Bioengineering and Biotechnology, **9**, **2021**.
19. BENSLIMANE S., BOUHIDEL K.E., FERFACHE A., SOUHILA F. 2020. Mechanistic study of the synergetic inhibiting effects of Zn^{2+}, Cu^{2+} and Mg^{2+} ions on calendar carbonate prevention [J]. Water Research, **186**, 116323, **2020**.
20. MA X.C., FEI H. Application of metal coordination in polypeptide and protein research [J]. Advances in Chemistry, **28** (Z2), 184, **2016**.
21. LI Y.Y. Experimental study on mechanical properties and microstructure of microbial cured calcareous sand based on particle gradation [D]. Guangxi: Guangxi University, **2022**.