

Urease activity in an autotrophic bacteria *Thiobacillus thiooxidans*

Xiaojuan Liu

Jiangsu Provincial Xuzhou Pharmaceutical Vocational College, Xuzhou, China

Abstract. Urease activity was found in the fermented liquid of *Thiobacillus thiooxidans*. It was found that initial concentration of sulfur and urea, and initial pH influenced urease activity in *Thiobacillus thiooxidans*, and the maximum urease activity was noted after 96h of fermentation with 24g/L initial concentration of sulfur and 0.1g/L of urea, and nature pH 5.82. The experiment found that urease activity showed a steady change which follow along with the growth of *Thiobacillus thiooxidans*. It shows a possibility to determine the *Thiobacillus thiooxidans* attached on the sulfide surface.

1 Introduction

Thiobacillus thiooxidans is a chemolithotrophic acidophilic bacterium that grows on elemental sulfur as energy source and is important in the microbial catalysis of sulfide oxidation. Since it oxidizes both elemental sulfur and sulfide to sulfuric acid, *Thiobacillus thiooxidans* plays a significant role in bioleaching of metals from sulfide ores.

During the bioleaching process, an important fraction of the bacteria attached to the solids which is responsible for the direct attack on the sulfide surface. Practical determination of attached bacteria is not simple. Total protein determination, using the Lowry method, has been used by many authors to evaluate the attachment of bacteria to mineral sulfides. However, the method has a low limit of sensitivity. B. Escobar finds a method based on the determination of the rate of ferrous iron oxidation by attached bacteria on the mineral for evaluating the proportion of free and attached bacteria in the bioleaching, but this method can't apply to *Thiobacillus thiooxidans*.

In our research, we found urease activity in the fermented liquid of *Thiobacillus thiooxidans* when urea was applied as nitrogen source. So the present study was developed to investigate urease activity in *Thiobacillus thiooxidans*, in order to find a new way to determinate the bacteria adhering to solid surfaces.

2 Materials and methods

2.1 Microorganism and media

Thiobacillus thiooxidans was used in this work. The medium contained urea, KH_2PO_4 1.0g/L, MgSO_4 0.5g/L, and sulfur which were dissolved in distilled water. Different concentrations of sulfur, urea, and initial pH were used to determine the effects on *Thiobacillus thiooxidans* (*T.t*).

2.2 Fermentation

After inoculating with the cell sediment of *Thiobacillus thiooxidans*, Erlenmeyer flasks were incubated on rotary shaker of 150 rpm at 37°C. Experiments were undertaken in triplicate to guarantee



the symmetrical nature with respect to space and time for data reproducibility.

2.3 Urease activity assay

After incubation, bacterial cells were harvested by centrifugation (8000rpm, 30min, 4°C), and then fragmented by ultrasonic, after which the sediments would be obtained again by centrifugation (3000 rpm, 30min, 4°C). At the last, the supernatants obtained from the second centrifugation was purified by ethanol .

The hydrolysis of urea was routinely followed by the determination of the ammonia formed. The standard assay system contained 20mL 0.1M phosphate buffer (pH 5.8), 5mL 1M urea, and 1mL enzyme solution in a final volume of 50 ml. The enzyme was replaced by water for the blank. The reaction was carried out at 37°C for 5 min. After incubation, ammonia formed in the reaction mixture was colorimetrically determined with phenol-hypochlorite method. The absorbance of the solution was measured at 697 nm.

One unit of urease activity was defined as the amount that releases 1μmol of ammonia per minute per mL of the enzyme solution.

2.4 Analytical methods

Cell growth was determined by hemacytometer counting method. A colorimetric diacetyl-monoxime's method was used for measuring urea. As urease analyzes urea to ammonia, concentration of ammonia reflects urease activity indirectly. So urease activity was confirmed indirectly by phenol-hypochlorite method. An ion meter was used to measure pH.

3 Results

3.1 Effect of sulfur on *Thiobacillus thiooxidans*

Figure 1 showed the growth pattern of *Thiobacillus thiooxidans* over a period of 144h. Different concentrations of sulfur were used to determine its effect on the cell growth and urea consumption. The maximum cell growth was obtained at 96h in the media of 12g/L, 24g/L, and 28g/L sulfur, while it took 120h to reach a maximum in 16g/L and 20g/L sulfur medium.

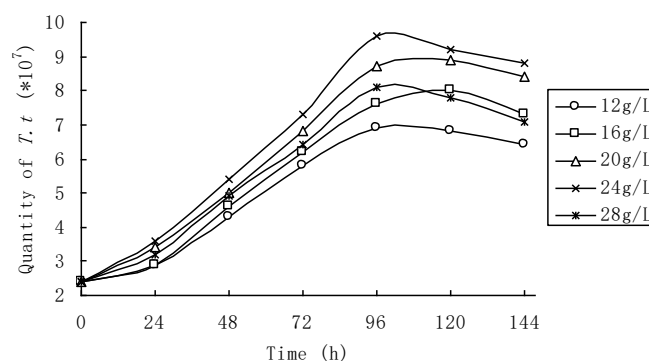


Figure 1. Effect of sulfur on growth pattern of *T.t*

Figure 2 showed the urea consumption of *Thiobacillus thiooxidans*. Similarly as cell growth curve, more than 90% of urea was consumed within 120h when the bacteria were cultivated in 24g/L of sulfur. With other concentrations of sulfur, the consumption of urea was less than 80%.

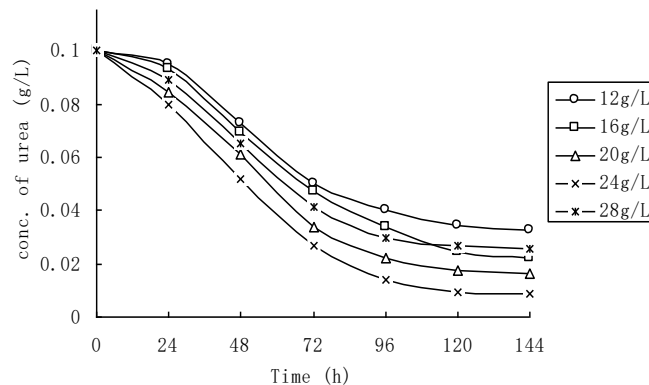


Figure 2. Effect of sulfur on urea consumption of *T. t*

3.2 Effect of urea on *Thiobacillus thiooxidans*

Figure 3 showed urease activity of different addition of urea on *Thiobacillus thiooxidans*. It was found that addition of 0.1g/L initial urea concentration looked suitable for urease production, and high concentrations of urea inhibited cell growth. The initial concentration of urea is 0g/L has no urease activity, which indicated that urease produced by *Thiobacillus thiooxidans* was an induced enzyme and urea is the inducer.

The initial concentration of urea in the culture medium influenced urease activity. Figure 3 also shows urease activity at different time intervals with different urea concentration, and urease activity reached a maximum in the early log phase (48h). The cell growth pattern and urea consumption were similar with Figure 1 and Figure 2. Quantity of *Thiobacillus thiooxidans* attained to maximum value in 96h, and more than 90% of urea was consumed in 120h.

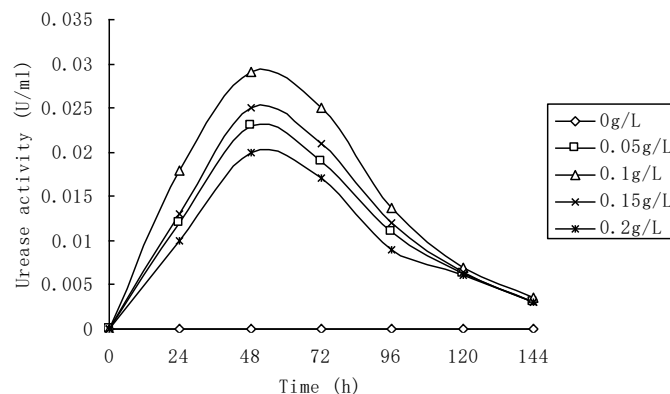


Figure 3. Effect of urea on urease activity of *T. t*

3.3 Effect of initial pH on *Thiobacillus thiooxidans*

Different pH were used to determine its effect on *Thiobacillus thiooxidans*. Nature pH was about 5.82. H_2SO_4 and KOH were used to adjust pH.

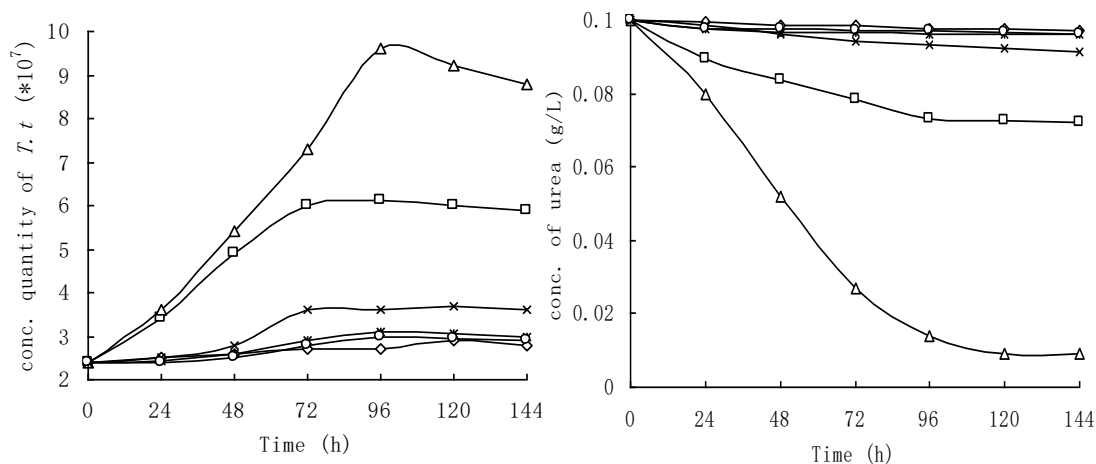


Figure 4. Effect of pH on growth pattern and urea consumption of *T.t*

◇-pH 1.5; □-pH 3; △-nature pH 5.82; ×-pH 7; *-pH 9; ○-pH 11.

Figure 4 and figure 5 show the effect of different initial pH on growth pattern and urea consumption of *Thiobacillus thiooxidans*. It was found that quantity of *Thiobacillus thiooxidans* and urea consumption were similar to Figure 1 and Figure 2 in nature pH 5.82. Others pH expressed a lower growth and urea consumption than nature pH.

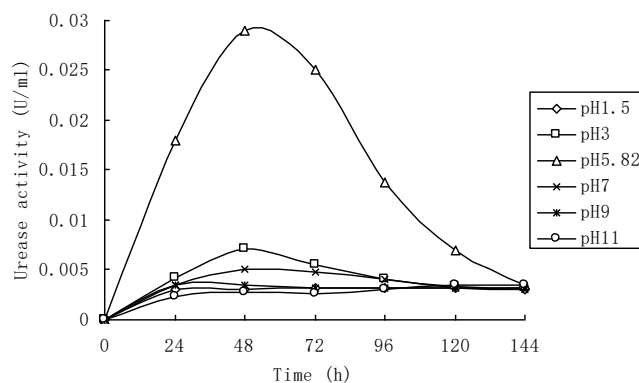


Figure 5. Effect of pH on urease activity of *T.t*

From figure 4 and figure 5, it shows that the best initial pH is nature pH 5.82. Effects of pH on urease activity were apparently different. Nature pH 5.82 displays the best urease activity, and urease activity were much lower than nature pH in other pH conditions.

3.4 Effect of temperature and pH on urease activity

Nine different temperatures from 20 to 55°C were studied to determine the influence of temperature on urease activity. The optimum temperature for urease activity was 37°C with activity almost constant in the range from 37 to 40°C, but it lost activity completely when the reaction was carried out at 55°C.

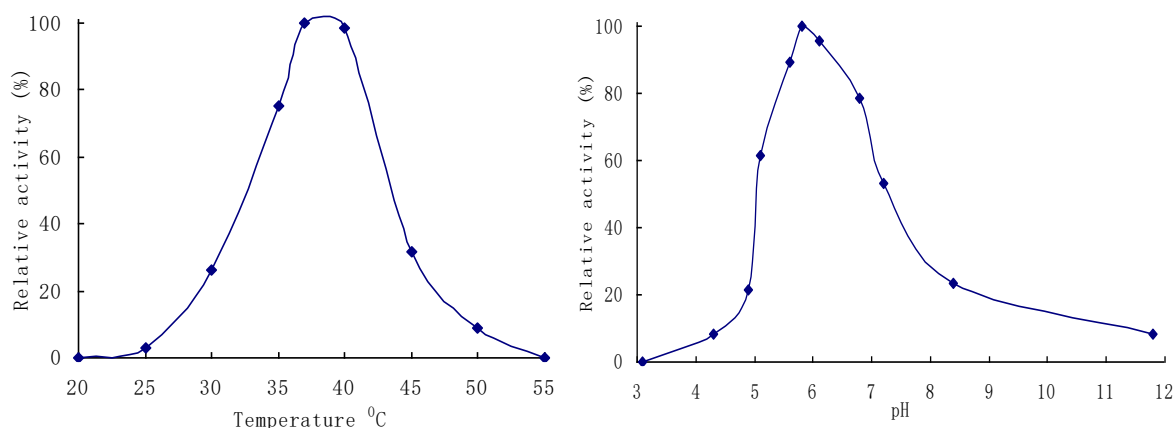


Figure 6. Effect of temperature and pH on urease activity

To monitor the effect of pH on urease activity, the activity of the enzyme was studied different pH from 3.0 to 12.0. Maximum urease activity was obtained at pH 5.8. Optimum urease activity was observed in a pH range of 5.5–6.5, and more than 80% of activity was lost below pH 5 and above pH 8.5.

4 Discussion and conclusion

Most known bacterial and plant ureases show an optimum pH for enzyme activity at neutral and slightly alkaline conditions according to the results, but we found that urease from *Thiobacillus thiooxidans* shows an optimum pH at acidic environment.

In many bacterial leaching experiments, an important fraction of the bacteria remains attached on the sulfide surface. However, the method used to determine the evolution of attached bacteria requires the use of more sophisticated instruments that sometimes may not be readily available. From the experiment, we concluded that following along with the growth of *Thiobacillus thiooxidans*, urease activity shows a steady change. So urease activity shows a possibility to determine the *Thiobacillus thiooxidans* attached on the sulfide surface.

Acknowledgements

This work was financially supported by grants from Fundamental Research Funds (No. 2013XK05) for the Central Universities, Jiangsu Ordinary University Graduate Innovative Research Programs.

References

- [1] A. Anita., C. A. Sastry., M. A. Hashim. Urease immobilized on nylon: preparation and properties. *Bioprocess Engineering*, 1997, 17: 241-245.
- [2] A. Anita., C. A. Sastry., M. A. Hashim. Immobilization of urease on vermiculite. *Bioprocess Engineering*, 1997, 16: 375-380.
- [3] B. Escobar., E. Jedlicki., J. Wiertz., T. Vargas. A method for evaluating the proportion of free and attached bacteria in the bioleaching of chalcopyrite with *Thiobacillus ferrooxidans*. *Hydrometallurgy*, 1996, 40: 1-10.
- [4] Cristian Follmer., German E. Wassermann., Célia R. Carlini. Separation of jack bean (*Canavalia ensiformis*) urease isoforms by immobilized metal affinity chromatography and characterization of insecticidal properties unrelated to ureolytic activity. *Plant Science*, 2004, 167: 241-246.
- [5] D. Olivera-Severo., G.E. Wassermann., C.R. Carlini. *Bacillus pasteurii* urease shares with plant ureases the ability to induce aggregation of blood platelets. *Archives of Biochemistry and Biophysics*, 2006, 452: 149-155.
- [6] E. Benitez., R. Nogales., G. Masciandaro., B. Ceccanti. Isolation by isoelectric focusing of humic-urease complexes from earthworm (*Eisenia fetida*)-processed sewage sludges. *Biol Fertil Soils*, 2000, 31: 489-493.

- [7] Elmar Rokita., Athanasios Makristathis., Alexander M. Hirschl., Manfred L. Rotter. Purification of surface-associated urease from *Helicobacter pylori*. Journal of Chromatography B, 2000, 737: 203-212.
- [8] Gulay Bayramoglu., Haydar Altinok., Adnan Bulut., Adil Denizli., M. Yakup Arica. Preparation and application of spacer-arm-attached poly (hydroxyethyl methacrylate co-glycidyl methacrylate) films for urease immobilization. Functional Polymers, 2003, 56: 111-121.
- [9] Hsuan-Liang Liu., Bor-Yann Chen., Yann-Wen Lan., Yang-Chu Cheng. Biosorption of Zn(II) and Cu(II) by the indigenous *Thiobacillus thiooxidans*. Chemical Engineering Journal, 2004, 97: 195-201.
- [10] Hsuan-Liang Liu., Yann-Wen Lan., Yang-Chu Chen. Optimal production of sulphuric acid by *Thiobacillus thiooxidans* using response surface methodology. Process Biochemistry, 2004, 39: 1953-1961.
- [11] Rachana Sahney., S. Anand., B. K. Puri., A. K. Srivastava. A comparative study of immobilization techniques for urease on glass-pH-electrode and its application in urea detection in blood serum. Analytica Chimica Acta, 2006, 578: 156-161.