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Growth optimization of a thermophilic strain geobacillus caldoxylosilyticus utm6 isolated from selayang hot spring

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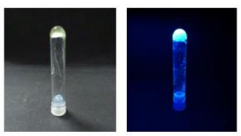
GRAPHICAL ABSTRACT



(a) Inverted test flow of $\mathbf{BTA_{1+2}}$ organogel and (b) $\mathbf{BTA_{1+2}}$ organogel observed under hand-held UV lamp 365 nm

ABSTRACT

A thermophilic strain, *Geobacillus caldoxylosilyticus* UTM6 has been reported to reduce toxic chromium (VI) to non-harmful of chromium (III). However, growth conditions of this thermophile has not been reported. Thus, this study was carried out to optimize the growth conditions of *G. caldoxylosilyticus* UTM6. Factors affecting growth of *G. caldoxylosilyticus* UTM6 notably effect of glucose, temperature and pH were evaluated using one-variable-at-a-time (OVAT) strategy in shake flask. The growth of the *G. caldoxylosilyticus* UTM6 for 24 h was quantified by measuring the turbidity of the culture using UV-Vis spectrophotometer. Cultivation of *G. caldoxylosilyticus* UTM6 in 250 ppm glucose, 55 ºC and pH 6.5 gave highest OD_{600} reading of 0.910. This is the first report on optimization of the growth conditions for *G. caldoxylosilyticus* UTM6.

Keywords: Thermophiles, glucose, temperature, optimization, geobacillus, OVAT.

1. INTRODUCTION

The environmental problems like global warming, acid rain, air pollutions, ozone layer depletion and water pollution are rising with the increase of development requirements. Three main developments that cause environmental crises are rapid growth of population, industrialization and urbanization [1]. Water pollution is one of the serious problems that affect the environment as whole. There are many different sources of water pollution either natural or human made. Industrial waste water is one of the main sources of water pollution [2]. Industrial waste water contains high concentration level of toxic heavy metals. Chromium, cadmium, zinc, copper, nickel, lead and mercury are often detected in industrial wastewater, which originated from metal plating, mining activities, smelting, battery manufacturing, tanneries, petroleum refining, paint manufacturing, pesticides, pigment manufacturing, printing and photographic industries [3].

A variety of bioremediation methods have been developed to support and increase the degradative activities of native microbial populations (natural attenuation), thus allowing a reduction in time required and subsequent savings in cost. The two main approaches to bioremediation are; (a) environmental bio stimulation involving the addition of mainly oxygen and/or mineral nutrients (usually combination of nitrogen, phosphorus and trace metals) and (b) bio augmentation through the direct application of selected degrader microorganisms to the site [4].

Thermophilic biological methods offers considerable potential as means of treating such wastes. Their likely benefits include rapid biodegradation rates, low sludge yields, process stability and a significantly improved ability to destroy pathogens [5-6]. Thermophilic hydrogen degraders, predominantly bacilli, have been of special interest for the bioremediation of polluted soils mainly in naturally hot environments such as desert soils [7] or in composting as a bioremediation process [8]. However, it has recently been shown that many extremely thermophilic bacilli, reclassified into the new genus *Geobacillus* [8-10], exist in cool soil environments [11-12] and are capable of degrading alkanes when the temperature is raised above 40 ºC [13]. However, the degradation of such wastes by *Geobacillus caldoxylosilyticus* UTM6 could be performed well under suitable growth conditions. Thus, present study was carried out to study the optimal growth parameters of the thermophile.

2. EXPERIMENTAL

2.1 Effect of Glucose Concentration

Active culture of thermophile was prepared by inoculating a loopful of 24 h *G. caldoxylosilyticus* UTM6 into a series of 250 mL Erlenmeyer flask containing 25 mL NB and incubated for 24 h at 200 rpm (Certomat-R, Germany). Then, 10 % (v/v) inoculum was transferred into a series of 500 mL Erlenmeyer flasks containing 50 mL of NB and (a) 250 ppm, 500 ppm and 1000 ppm glucose. The flasks were incubated at 53 ºC (Memmert, United Kingdom) for 24 h and at 200 rpm (Certomat-R, Germany). The cell turbidity was measured every 4 h using UV-Vis spectrophotometer (D5000 Hach, United States) and graph was plotted.

2.2 Effect of Temperature

Active culture was prepared by inoculating a loopful of 24 h *G. caldoxylosilyticus* UTM6 into a series of 250 mL Erlenmeyer flask containing 25 mL NB and incubated for 24 h at 200 rpm (Certomat-R, Germany). Then, 10 % (v/v) inoculum was transferred into a series of 500 mL Erlenmeyer flasks containing 50 mL of NB and 250 ppm of glucose varying temperature of 45 ºC, 50 ºC, 55 ºC, 60 ºC, 75 ºC and 80 ºC. The flasks were incubated for 24 h at 200 rpm (Ceromat-R, Germany). The cell turbidity was measured every 4 h using UV-Vis spectrophotometer (D5000 Hach, United States) and graph was plotted.

2.3 Effect of pH Medium

Active culture was prepared by inoculating a loopful of 24 hours *Geobacillus caldoxylosilyticus* UTM6 into a series of 250 mL Erlenmeyer flask containing 25 mL NB and incubated for 24 h at 200 rpm; (Certomat-R, Germany). Then, 10% (v/v) inoculum was transferred into a series of 500 mL Erlenmeyer flasks containing 50 mL of NB and 250 ppm of glucose varying pH of pH 5.0, pH 6.5, pH 7.0 and pH 9.0. The flasks were incubated for 24 h at 200 rpm (Ceromat-R, Germany). The cell turbidity was measured every 4 h using UV-Vis spectrophotometer (D5000 Hach, United States) and graph was plotted.

3. RESULTS AND DISCUSSION

3.1. Effect of Glucose Concentration in *G. caldoxylosilyticus* UTM6 Growth

In this study, the effect of glucose concentration in *Geobacillus caldoxylosilyticus* UTM6 growth was examined. It was reported that several trials of carbon sources, such as glucose, lactose and sucrose, were tested on the thermophiles. Glucose was found to have greater overall and maximum growth biomass than other trials [59]. Therefore, glucose was selected for this study to assess their role as carbon sources for growing the thermophile.

From Figure 4.2, the OD_{600} for all conical flasks increased after 12 h of incubation. 250 ppm of glucose gave highest bacterial growth at 0.712 while glucose concentration of 500 ppm and 1000 ppm gave 0.416 and 0.328, respectively. There was not much difference in lag phase of 250 ppm, 500 ppm and 1000 ppm of glucose. This indicate during the lag phase (0-12 h), *G. caldoxylosilyticus* UTM6 is adapting and exploiting to the new environment conditions [60]. However, during the exponential phase (12-17 h), 250 ppm glucose showed drastic increase as compared to others.

According to Wali et. al [60], addition of glucose was markedly increase the growth rate possibly associated with increased levels of the enzymes of the glycolytic as well as Krebs cycle pathways [61]. In the experiment, observed that higher glucose concentration gave lower growth of *G. caldoxylosilyticus* UTM6. The use of high concentrations of glucose and carbon source can lead to higher consumption of glucose which eventually caused the accumulation of by-products, such as acetic acids as well as the decline of other food resources in the cultures contribute to the findings [62].

The inhibitory effect suggested may be caused by the inhibition of catabolic enzymes involved in the breakdown of proteinaceous molecules, which would otherwise be incorporated for continued cell growth of strain. Previous research has supported the inhibition effect of glucose on bacteria growth [62-64]. For example, the concentrated sugar solutions have not only been shown to reduce microbial growth when used as a food preservative, but they have also been applied to wounds to create an unfavorable environment for bacterial agents [63]. Thus, present study showed that 250 ppm glucose gave highest growth of *G. caldoxylosilyticus* UTM6.

3.2. Effect of Temperature in *G. caldoxylosilyticus* UTM6 Growth

The effect of different temperatures in *G. caldoxylosilyticus* UTM6 was studied. Temperature is a cardinal factor controlling the growth rate of microbial populations [63]. Based on Figure 4.3, it was evident that the growth of *G. caldoxylosilyticus* UTM6 was highest at 55 ºC with OD_{600} of 0.910 as compared to other temperatures after 24 h of incubation. However, at 75 ºC and 80 ºC, the highest reading OD_{600} were 0.138 and 0.140 respectively.

Thermophiles are able to withstand to extreme temperatures due to the chemical stability of lipids. Because of ether bonds of archaeal lipids are the most part not broken down under conditions in which ester linkages are completely methanolyzed, it is generally believed that the archaeal ether lipids are thermotolerant or heat resistant [64].

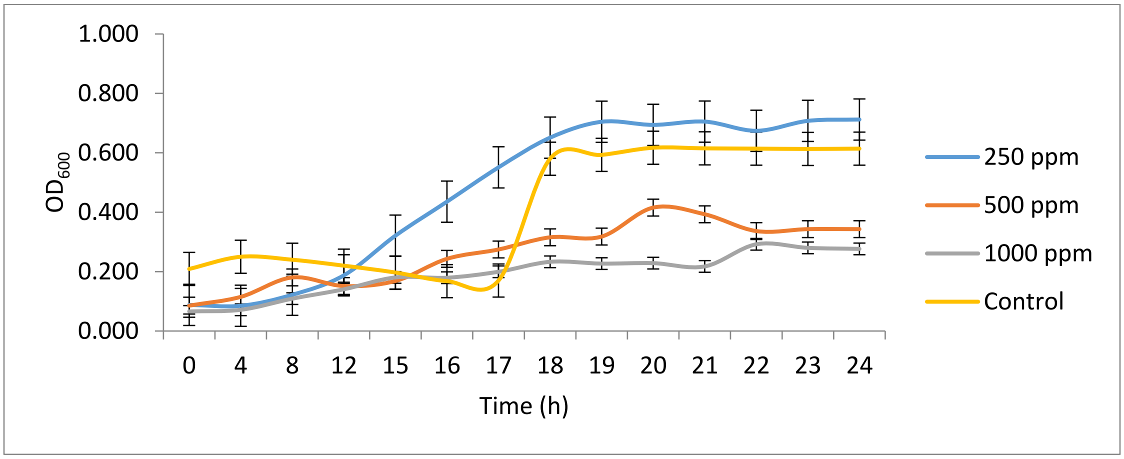


Figure 1: Growth profile of G. caldoxylosilyticus UTM6 in different concentration of glucose.

This heat tolerance property of thermophiles, i.e. *G. caldoxylosilyticus* UTM6, enable them to change their membrane lipids compositions. They still contain phosphate groups and long fatty acid tails, but they also contain ether linkages instead of ester linkages. The ether linkages make the bonds between phosphate groups and hydrocarbons more stable because the carbon connecting the phosphate group and glycerol molecules is more electron-rich than it would be in an ester, making that carbon less electrophilic and therefore less chemically reactive. This allows the ether-linked phospholipids to be more stable and less susceptible to breakdown from large amounts of increase thermal energy. This contributes to the archaea's stability to live such extreme environments [64].

Although *G. caldoxylosilyticus* UTM6 is heat tolerant, bacteria are unable to withstand extreme temperatures, including 75 ºC and 80 ºC. In this study, growth of *Geobacillus caldoxylosilyticus* UTM6 was observed to be lower as compare to 55 ºC. This may due to nature of *G. caldoxylosilyticus* UTM6 isolated from Selayang Hot Spring. This is due to the denature of protein in *G. caldoxylosilyticus* UTM6. Protein stability is determined by the large number of interaction that contribute of the native-folded state. [65]. The destabilization of protein could be caused by the breaking of the hydrogen bonding [66]. Thus, present study depicted that the optimal temperature of *G. caldoxylosilyticus* is 55 ºC.

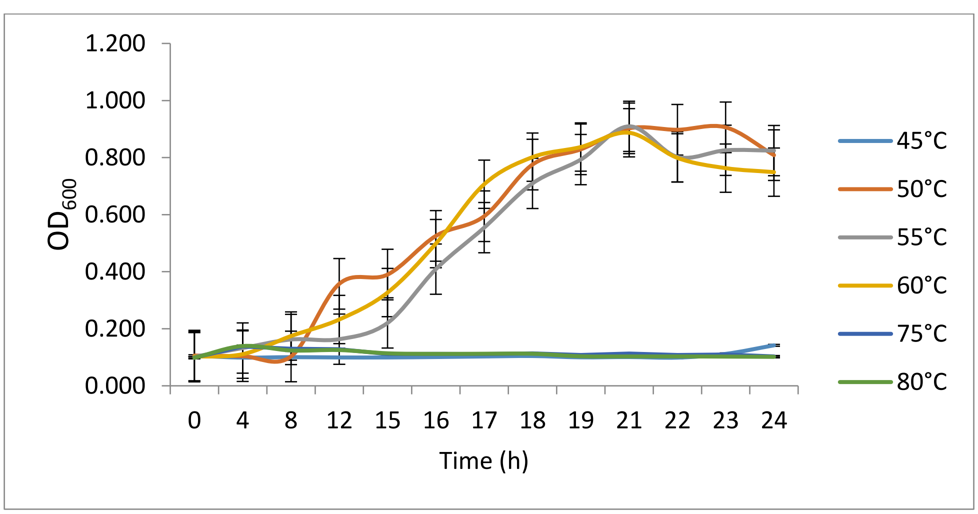


Figure 2: Growth profile of G. caldoxylosilyticus UTM6 bacteria in different temperatures.

3.3. Effect of Different pH Medium in *G. caldoxylosilyticus* UTM6 Growth

In this study, the effect of different pH medium in *G. caldoxylosilyticus* UTM6 growth was examined. Figure 4.4, the optimal growth of *G. caldoxylosilyticus* UTM6 was observed at pH 6.5 with OD_{600} reading of 0.910 while for pH 5.0, pH 6.5, pH 7.0 and pH 9.0 were 0.790, 0.672 and 0.078 respectively after 24 h of incubation.

Environmental pH is important for the pathogenesis of related *Bacillus* species, such as the food-borne pathogen *Bacillus cereus*, which encounters acidic environments in the gastrointestinal tract and in food products where organic acids are used as preservatives [67-68]. Under acidic (pH 5.0) and alkaline (pH 9.0) conditions, the growth rate of *G. caldoxylosilyticus* UTM6 were considerably reduced. At pH 9.0 the microbial activity is stagnant as earlier as 8 h of incubation. The disturbance of the system through addition of sodium hydroxide is one possible explanation for the observed reduced activity.

At pH 5.0, the OD_{600} was still lower than the control which is pH 6.5 in our preliminary study. This is because a thermophlic bacterial community does not tolerate to acids. Choi and Park [65] showed that the growth of thermophilic bacteria in food waste compost was stimulated by the addition of yeast that eliminated the organic acids. The accumulation of the organic acids in the growth medium causes a rapid fall in pH that inhibits further growth of the cell [66].

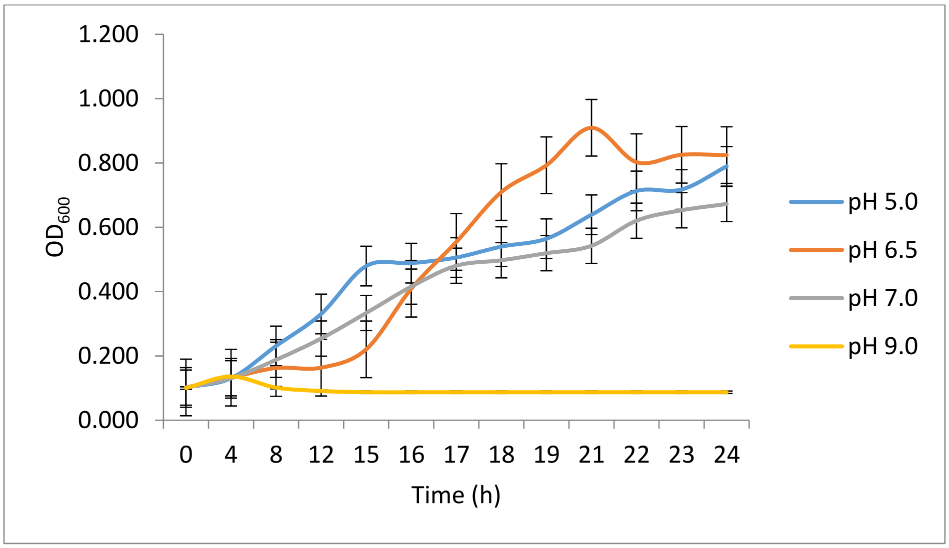


Figure 3: Growth profile of G. caldoxylosilyticus UTM6 bacteria in different pH of the medium.

4. CONCLUSION

As a conclusion, the optimum growth parameters in this study increased the growth rate of *G. caldoxylosilyticus* UTM6. From the results obtained, a strain of thermophilic bacteria, identified as *G. caldoxylosilyticus* UTM6 was found to grow optimally at 250 ppm of glucose, 55 ºC and pH 6.5 with highest reading OD_{600} of 0.910.

REFERENCES

[1] Jorgenson A.K, Burns T.J, 2007. Effects of Rural and Urban Population Dynamics and National Development on Deforestation in Less-Developed Countries, 1990-2000. *Sociological Inquiry*. 77, 460-482.

[2] AlOtaibi, 2009. Bacteriological Assessment of Urban Water Sources in Khamis Mushait Governorate, *Southwestern Saudi Arabia. International Journal of Health Geographics*, 2009. 8,16.

[3] Yin Gang, Wang Soug-You, Xu Ming, Ghen Liang-Yao. Theroretical Calculation of The Optical Properties of Gold Nanoparticles. *Journal of the Korean Physical Society*, 2006. 49, 2108-2111.

[4] Korda, A., Santas, P., Tenente, A., Santas, R., 1997. Petroleum hydrocarbon bioremediation: sampling and analytical techniques, in situ treatments and commercial microorganisms currently used. *Appl. Microbiol. Biotechnol*. 48, 677-686.

[5] LaPara TM, Alleman JE (1999) Thermophilic aerobic biological wastewater treatment. *Water Res*. 33: 895-908.

[6] Sorkhoh, N.A., Ibrahim, A.S., Ghannoum, M.A., Radwan, S.S., 1993. High-temperature hydrocarbon degradation by *Bacillus stearothermophilus* from oil-polluted Kuwaiti desert. *Appl. Microbiol. Biotechnol*. 39, 123-126.

[7] Beaudin, N., Caron, R.F., Legros, R., Ramsay, J., Ramsay, B., 1999. Identification of the key factors affecting composting of a weathered hydrocarbon-contaminated soil. *Biodegradation* 10,127-133.

[8] Nazina, T.N., Tourova, T.P., Poltaraus, A.B., Novikova, E.V., Grigoryan, A.A., Ivanova, A.E., Lysenko, A.M Petrunyaka, V.V., Osipov, G.A., Belyaev, S.S., Ivanov, M.V., 2001. Taxonomic study of aerobic thermophilic bacilli: desccriptions of *Geobacillus subterraneus* gen. nov., sp. Nov. and *Geobacillus uzenensis* sp. Nov from petroleum reservoirs and transfer of *Bacillus strearothemophilus, Bacillus thermocatenulatus, Bacillus thermoleovorans, Bacillus kaustophilus, Bacillus thermoglucosidasius and Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. strearothermophilus, G. thermocatenulatus, G. thermoleovorans, G. kaustophilus, G. thermoglucosidasius and G. thermodenitrificans*. *Int. J. Syst. Evol. Microbiol*. 51, 433-446.

[9] Banat, I.M., Marchant, R., Rahman, T.J., 2004. *Geobacillus debilis* sp. Nov., a novel obligately thermophilic bacterium isolated from a cool soil environement, and reassignment of *Bacillus pallidus* to *Geobacillus pallidus* comb. nov. *Int. J. Syst. Evol. Microbiol*. 54, 2197-2201.