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Full Length Research Paper

Optimal conditions for growth and magnetosome formation of *Acidithiobacillus ferrooxidans*

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Magnetotactic bacteria (MTB), which contain nano-scale membrane bound crystals of magnetic iron minerals, are of great interest in microbiology, biomineralization, advanced magnetic materials and bio-geosciences. However, no application has been exploited on a commercial scale due to the fastidious lifestyle of most cultured MTB. *Acidithiobacillus ferrooxidans* is a facultatively aerobic bacterium which can synthesize intracellular electron dense magnetite magnetosomes. Although the mass cultivation of *A. ferrooxidans* was performed facilely, the optimal conditions for cell growth and magnetosome formation remain unknown. In this study, transmission electronmicroscopy (TEM) and inductively coupled plasma atomic emission spectrometer (ICP-AES) were used to observe magnetosomes and measure iron content of *A. ferrooxidans*, respectively. The effects of ferrous sulfate concentration, ammonium sulfate concentration, oxygen concentration, pH and temperature on the cell growth and magnetosome formation were systematically investigated. Additionally, the orthogonal design of experiments were carried out, and the optimum conditions for cell growth ($FeSO_4$ 120 mM, $(NH_4)_2SO_4$ 3.0 g/L, temperature 30°C, initial pH 1.75, and 20% loadings) and magnetosome formation ($FeSO_4$ 160 mM, $(NH_4)_2SO_4$ 2.4 g/L, temperature 20°C, initial pH 1.75, and 50% loadings) were not identical.

Key words: Magnetotactic bacteria, *Acidithiobacillus ferrooxidans*, magnetosome formation, orthogonal design, transmission electronmicroscopy, optimization.

INTRODUCTION

Magnetotactic bacteria (MTB) are motile Gram-negative prokaryotes that align themselves along magnetic field lines because they have intracellular magnetic nanoparticles, termed as “magnetosomes”, which are membrane-bounded crystals (Bazylinski and Williams, 2007a). The magnetosome is usually arranged in chains inside the cytoplasm, and contains crystals of either an iron oxide magnetite (Fe_3O_4), or an iron sulfide greigite (Fe_3S_4), or a combination of greigite and iron pyrite (FeS_2) (Mann et al., 1990; Pan et al., 2005). MTB are ubiquitous in various environments such as marine, lake, river, pond, marsh, soil and sulphide–rich sediment (Faivre and Schüler, 2008). MTB and their magnetosomes have novel magnetic, physical, and perhaps optical properties that can and have been exploited in a variety of scientific, commercial, and other applications. Potential applications of MTB comprise their use in magnetic separation, magnetic domains analysis, heavy metals and radionuclide recovery (Bazylinski and Schübbe, 2007b). Reported application of bacterial magnetosomes may lie in their potential use as the carriers for enzymes, nucleic acids, antibodies, anticancer drugs, streptavidin, and as a contrast agent for magnetic resonance imaging, and as a hyperthermic thermoseed for magnetic hyperthermia (Faivre and Schüler, 2008). However, their application has not been exploited commercially, which is mainly due to the problems related to mass cultivation of MTB and high yields of magnetosomes (Heyen and Schüler, 2003; Sun et al., 2008).

So far, only a very few MTB are available in pure culture, and most of them are difficult to grow because of their fastidious lifestyle (Bazylinski and Schübbe, 2007b). It has been demonstrated that the cell growth and the

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Table 1. Factors and levels for L16 orthogonal array.

Level	Factors				
	A	B	C	D	E
	$FeSO_4$ concentration (mM)	$(NH_4)_2SO_4$ concentration (g/L)	Temperature (^oC)	pH	Medium loadings (%)
1	80	1.2	20	1.5	20
2	120	1.8	25	1.75	30
3	160	2.4	30	2.0	40
4	200	3.0	35	2.25	50

magnetosome formation are very sensitive to changes in the solution chemistry and physical conditions such as iron concentration, oxygen concentration, nitrogen concentration, pH and temperature (Bazylinski and Schübbe, 2007b). Thus, in order to produce enough cells, magnetosomes for the applications described earlier, and cells must be grown in very large cultures where the conditions for cell growth and magnetosome formation must be optimized.

Acidithiobacillus ferrooxidans, one of the most studied bioleaching bacteria, is a chemolithoautotrophic gram–negative γ–*proteobacterium* that takes in ferrous or reduced inorganic sulfur compounds as energy source (Yan et al., 2010). It has been reported that *A. ferrooxidans* are capable of synthesizing intracellular electron dense magnetite and the magnetosomes were extracted from these bacteria (Xie et al., 2005). Our previous study (Yan et al., 2012) also showed that the magnetosomes were biomineralized in *A. ferrooxidans* and the isolated magnetosomes possess high good biocompatibility. In contrast to fastidious MTB, *A. ferrooxidans* can grow without the drastic regimes of oxidative stress, and the mass cultivation has been easily available. However, several studies concerning the ability of magnetosome formation in *A. ferrooxidans* are available (Liu et al., 2006; Liu et al., 2008; Xie et al., 2005). Poor information about natural habitats and difficulty mimicking them in the laboratory limit efficient cell growth and magnetosome production.

In this work, TEM were used to examine *A. ferrooxidans*. The effects of various parameters such as ferrous sulfate concentration, ammonium sulfate concentration, oxygen concentration, pH and temperature on the cell growth and magnetosome formation were systematically investigated. Additionally, the conditions for the mass cultivation of *A. ferrooxidans* and magnetosome production were optimized by the orthogonal design of experiments.

MATERIALS AND METHODS

Microorganism and growth conditions

A. ferrooxidans BY-3 (CCTCC-M203071) was isolated from the acidic mine drainage at an abandoned copper mine in Baiyin of Gansu, China. The bacteria were grown aerobically at pH 1.75, 30°C and 120 rpm in a modified 9 K medium which consisted of solutions A and B (Yan et al., 2010). The compositions are as follows; solution A: 3.0 g $(NH_4)_2SO_4$, 0.1 g KCl, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$ and 0.01 g $Ca(NO_3)_2$ in 700 ml of distilled water, and solution B: 33.5 g $FeSO_4 \cdot 7H_2O$ in 300 mL of distilled water, pH adjusted with 1.0 M H_2SO_4 as required and the solution were then autoclaved. Filter sterilized ferrous sulfate solution was added before inoculation [10% (v /v)].

Batch cultivation

All batch experiments were carried out in Erlenmeyer flasks on a horizontal water-bath shaker, operating at 150 rpm. To observe the influence of ferrous sulfate concentration, ammonium sulfate concentration, oxygen concentration, pH and temperature on the cell growth and magnetosome formation, different conditions of ferrous sulfate concentration (40 to 200 mM), ammonium sulfate concentration (1.2 to 3.6 g/L), medium loadings (20 to 70%), pH (1.5 to 2.5), and temperature (20 to 40°C) were evaluated in this study. All experiments were performed in triplicate and the average values were reported.

In order to obtain the optimum conditions for cell growth and magnetosome formation, the experiments were based on a L16 orthogonal array experimental design where the following five variables were analyzed (Table 1): ferrous sulfate concentration (factor A), ammonium sulfate concentration (factor B), temperature (factor C), pH (factor D) and medium loadings (factor E). For each factor, four levels were selected (Table 1). 16 experimental runs were carried out according to the L16 orthogonal array design to complete the optimization process (Tables 2 and 3).

The pH of the solution was adjusted by adding H_2SO_4. After oxidizing 90% of ferrous ions, 200 ml bacterial cultures were centrifuged at 2000 rpm for 10 min to remove the precipitated iron and were centrifuged again at 8500 rpm for 10 min to condense the concentration of bacteria. At last, the cells were washed with dilute sulfuric acid at pH 2.0, the resulting pellets were resuspended in 3 ml iron-free 9 K medium and subjected to measurement of cell growth and magnetism, using dilute sulfuric acid as the blank control.

Analysis

Iron content of *A. ferrooxidans* was determined by an inductively coupled plasma atomic emission spectrometer (IRIS Advantage ER/S, Thermo Jarrell Ash, USA). For iron analysis, *A. ferrooxidans* cultured in 9 K medium, were collected by centrifugation and wash, then dried in an oven at 50°C for 24 h. 20 mg of dry cells were dissolved in 65% (v/v) HNO_3 and analyzed directly.

For electron microscopy, *A. ferrooxidans* cultured in 9 K medium

Table 2. Range analysis of growth of *At. ferrooxidans* through orthogonal experiments.

No.	Factors					OD_{600}
	A	B	C	D	E	
1	80	1.2	20	1.5	20	0.252
2	80	1.8	25	1.75	30	0.268
3	80	2.4	30	2.0	40	0.240
4	80	3.0	35	2.25	50	0.269
5	120	1.2	25	2.0	50	0.248
6	120	1.8	20	2.25	40	0.291
7	120	2.4	35	1.5	30	0.235
8	120	3.0	30	1.75	20	0.352
9	160	1.2	30	2.25	30	0.236
10	160	1.8	35	2.0	20	0.273
11	160	2.4	20	1.75	50	0.246
12	160	3.0	25	1.5	40	0.302
13	200	1.2	35	1.75	40	0.245
14	200	1.8	30	1.5	50	0.278
15	200	2.4	25	2.25	20	0.204
16	200	3.0	20	2.0	30	0.297
T_1	0.257	0.245	0.271	0.267	0.270	
T_2	0.281	0.277	0.256	0.278	0.259	
T_3	0.264	0.231	0.276	0.265	0.269	
T_4	0.256	0.305	0.256	0.250	0.260	
Rj	0.025	0.074	0.020	0.028	0.011	

Table 3. Range analysis of magnetosome formation in *At. ferrooxidans* through orthogonal experiments.

No.	Factors					C_{mag}
	A	B	C	D	E	
1	80	1.2	20	1.5	20	1.004
2	80	1.8	25	1.75	30	1.003
3	80	2.4	30	2.0	40	1.004
4	80	3.0	35	2.25	50	1.011
5	120	1.2	25	2.0	50	1.061
6	120	1.8	20	2.25	40	1.052
7	120	2.4	35	1.5	30	1.060
8	120	3.0	30	1.75	20	1.048
9	160	1.2	30	2.25	30	1.059
10	160	1.8	35	2.0	20	1.096
11	160	2.4	20	1.75	50	1.135
12	160	3.0	25	1.5	40	1.053
13	200	1.2	35	1.75	40	1.069
14	200	1.8	30	1.5	50	1.062
15	200	2.4	25	2.25	20	1.069
16	200	3.0	20	2.0	30	1.065
T_1	1.005	1.048	1.064	1.045	1.054	
T_2	1.055	1.053	1.046	1.064	1.047	
T_3	1.086	1.067	1.043	1.056	1.044	
T_4	1.066	1.044	1.059	1.048	1.067	
Rj	0.081	0.023	0.021	0.019	0.023	

were harvested and fixed at 4°C in 2.5% (v/v) glutaraldehyde overnight, post fixed in 1% (w/v) OsO_4 for 1 h, dehydrated ethanol

in series, and embedded in Epon 812 resin. Ultrathin sections were cut using an LKB–5 ultramicrotome (LKB), stained with uranyl acetate for 15 min and lead citrate for 3 min. The TEM observation was conducted in a JEM–1230 TEM (JEOL) with an accelerating voltage of 100 kV.

Cell growth and magnetism were measured turbidimetrically, using the optical density at 600 nm (OD_{600}). MTB cells were aligned at different angles relative to the light beam by means of an external magnetic field. Coefficient of magnetism (C_{mag}), the ratio of the maximum (magnetic field parallel to light path) and minimum (magnetic field perpendicular to light path) absorbance, was previously found to be well correlated with the average number of particles per cell and can be used for semi-quantitative estimations of magnetosome content (for practical purposes, C_{mag} = 0 was assumed for non-magnetic cells) (Heyen and Schüler, 2003; Sun et al., 2008). The C_{mag} of *A. ferrooxidans* was measured following the procedure reported by Smith et al. (2006).

For orthogonal experiments, data analysis was carried out through the range analysis to obtain the optimal conditions cell growth and magnetosome formation.

RESULTS

Iron content of *A. ferrooxidans*

A large amount of iron is required for magnetosome formation in MTB. The ICP-AES analysis indicated that the iron concentration of dry *A. ferrooxidans* cells is 12.44 mg/g, a concentration that is 53 times higher than *Escherichia coli* MC4100 (Posey and Gherardini, 2000).

TEM analysis

It has been demonstrated that TEM is a powerful tool to directly characterize the morphological and structural properties of bacterial magnetosomes (Fischer et al., 2008). TEM observation showed that *A. ferrooxidans* contained magnetosomes that were dispersed in cell (Figure 1, white arrows). The number of magnetosome per cell was almost different, ranging between 1 and 3 per cell.

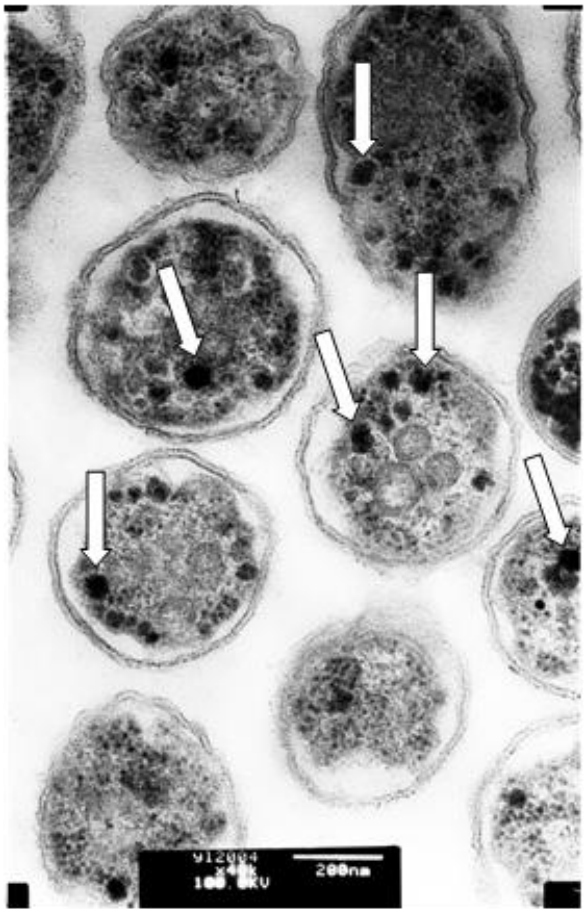

Figure 1. Transmission electron microscopy image of magnetosomes (white arrows) from *A. ferrooxidans.*

Effect of ferrous sulfate concentration

Ferrous sulfate is the main energy source for the growth of *A. ferrooxidans*, which is also essential for the synthesis of magnetosomes. As displayed in Figure 2, in the experimental ferrous sulfate concentration range of40 to 120 mM, the growth of *A. ferrooxidans* increased with the increase of ferrous sulfate concentration. Significant decrease in the growth of *A. ferrooxidans* was observed when the ferrous sulfate concentration was over 120 mM. In the range of sulfate concentrations from40 to 160 mM, the C_{mag} increased with increasing ferrous sulfate concentration. The C_{mag} was found to be 1.000 when the ferrous sulfate concentration was 40 mM. It increased to 1.009 when the ferrous sulfate concentration was 160 nM. It was observed that the C_{mag} value decreased when the ferrous sulfate concentration was over 160 mM (Figure 2). These indicated that the ferrous sulfate concentration have a significant effect on growth of *A. ferrooxidans* and synthesis of magnetosome.

Effect of ammonium sulfate concentration

The influences of ammonium sulfate concentration on growth and magnetosome formation of *A. ferrooxidans* were investigated by using different ammonium sulfate concentrations in the range of 1.2 to 3.6 g/L (Figure 3). As the ammonium sulfate concentration increased from 1.2 to 3.0 g/L, the OD_{600} increased from 0.605 to 0.636. However, it was observed that when the concentration exceeded 3.0 g/L, the OD_{600} no longer increased. It can be seen that the C_{mag} was fluctuating irregularly in the experimental ammonium sulfate concentration range of 1.2 to 3.6 g/L, and the maximum C_{mag} value was found to be 1.019 when the ammonium sulfate concentration was 2.4 g/L.

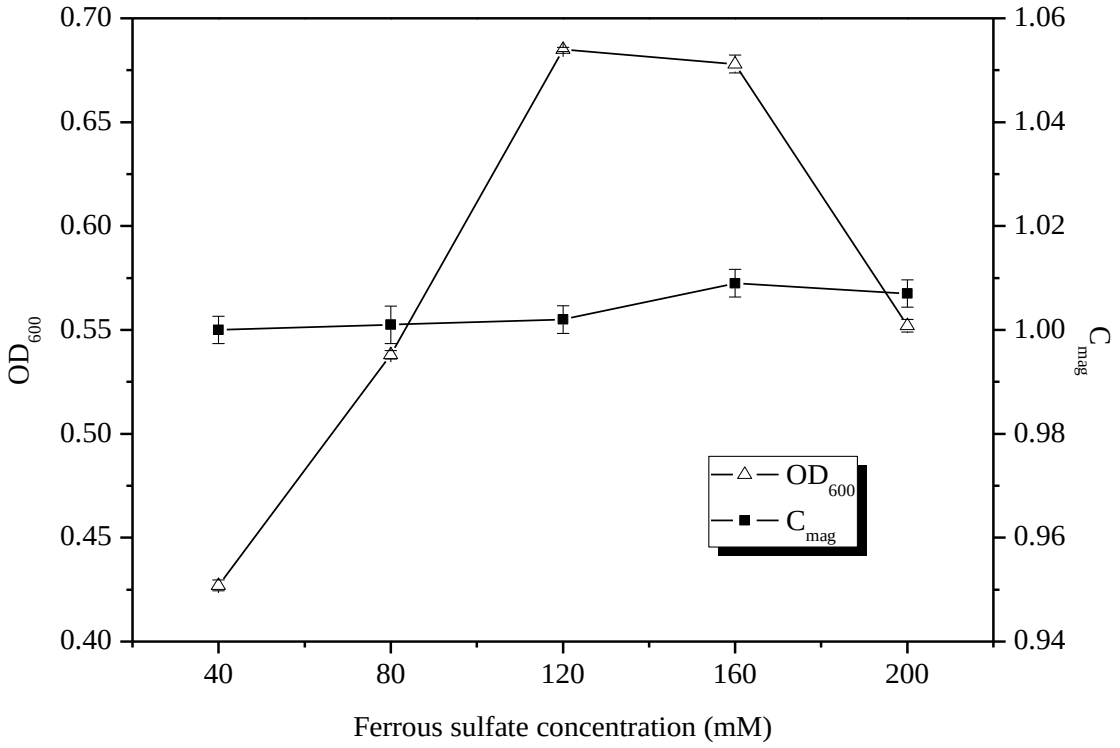

Figure 2. Effect of ferrous sulfate concentration on growth and magnetosome formation of *A. ferrooxidans*.

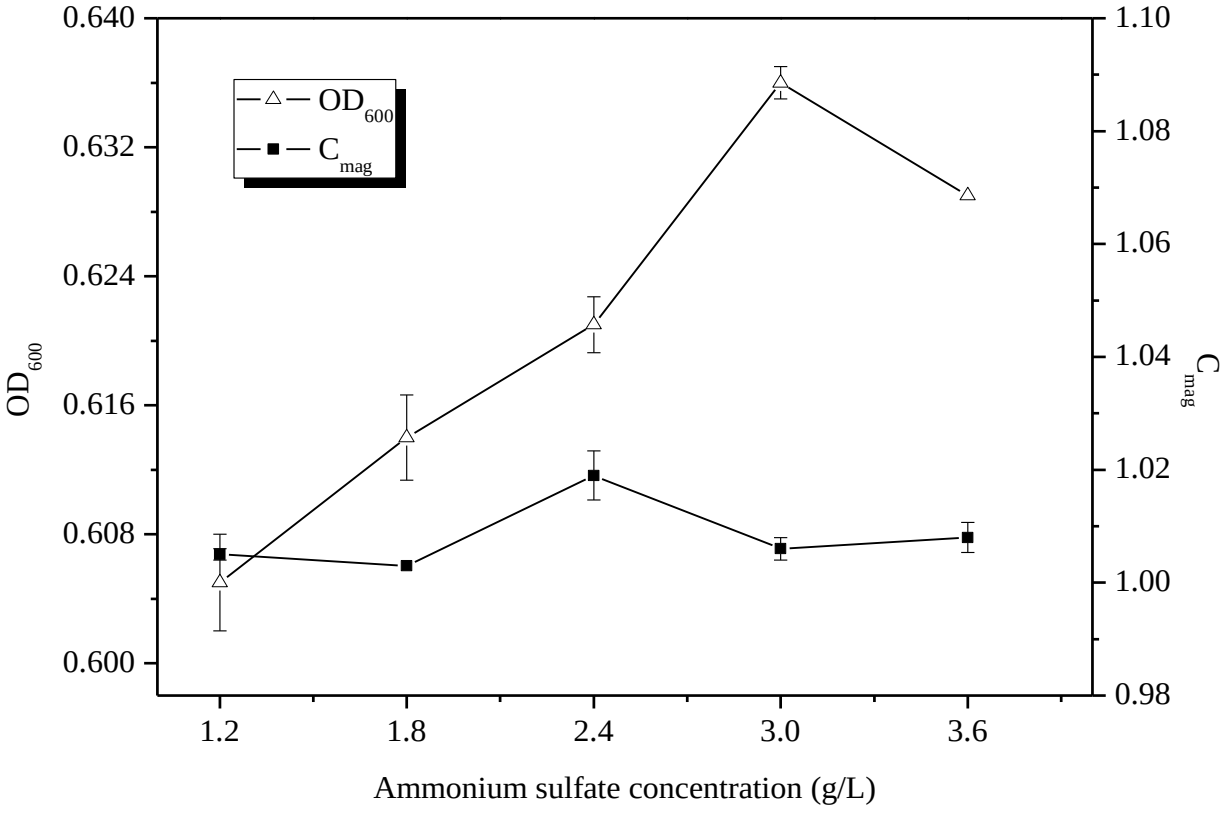

Figure 3. Effect of ammonium sulfate concentration on growth and magnetosome formation of *A. ferrooxidans*.

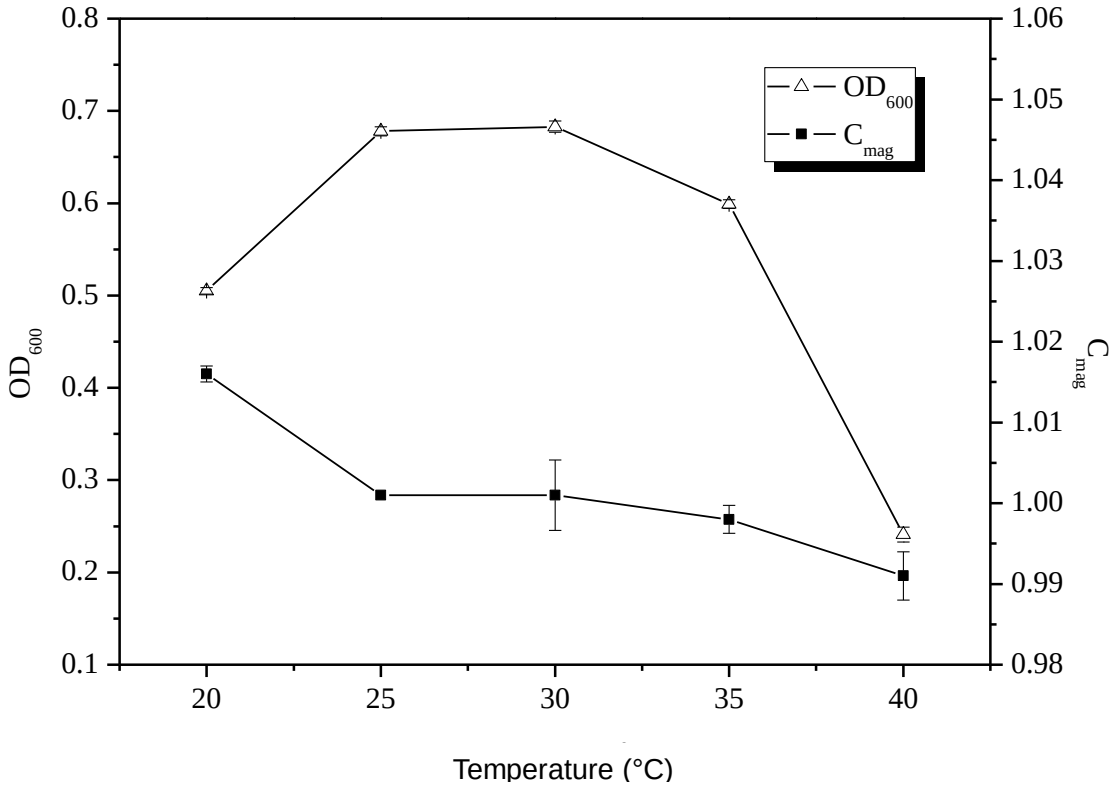

Figure 4. Effect of temperature on growth and magnetosome formation of *A. ferrooxidans*.

Effect of temperature

The effects of temperature on growth and magnetosome formation of *A. ferrooxidans* were graphically presented in Figure 4. The data showed that, the OD_{600} increased from 0.505 to 0.683 with the increase of temperature range from 20 to 30°C, but it decreased significantly when the temperature was above 30°C. It can be seen from the figures that the C_{mag} decreased with increasing temperature, and the maximum C_{mag} value was found to be 1.016 at 20°C.

Effect of pH

A. ferrooxidans are extremely acidophilic bacteria, which can grow at external pH values of 1.0 to 3.5. Figure 5 shows the effect of different initial pH on growth and magnetosome formation of *A. ferrooxidans*. The OD_{600} and C_{mag} increased as the medium pH increased up to 1.75, and then decreased. It can be found that the maximum values were 0.598 and 1.007 for OD_{600} and C_{mag}, respectively.

Effect of oxygen concentration

Oxygen concentration, one of the most important environmental factors, not only influences the formation of magnetosomes of cultured MTB, but also affects the growth of these MTB. The different oxygen concentrations were established by different percent loadings of medium in this experiment. The effects of oxygen concentration on growth and magnetosome formation of *A. ferrooxidans* were investigated by using different percent loadings of medium in the range of 20 to 70% (Figure 6). It has been observed that OD_{600} decreased with increasing percent loading, and the maximum OD_{600} was 0.595 when the percent loading was 20%. It can be found that the C_{mag} was fluctuating irregularly in the percent loadings of medium in the range of 20 to 70%, and the maximum C_{mag} value was found to be 1.016 when the percent loading was 50%.

Optimized conditions for cell growth and magnetosome formation

Range analysis of growth and magnetosome formation of *A. ferrooxidans* through orthogonal experiments are displayed in Tables 2 and 3, respectively. The sum of each experimental factor at same level is T, and the range is Rj. It can be found from Table 2 that the value of T_2 was the maximum among all T_n value of A factor, implying that the optimal level was A_2 (Ferrous sulfate concentration: 120 mM). In the same way, the optimal

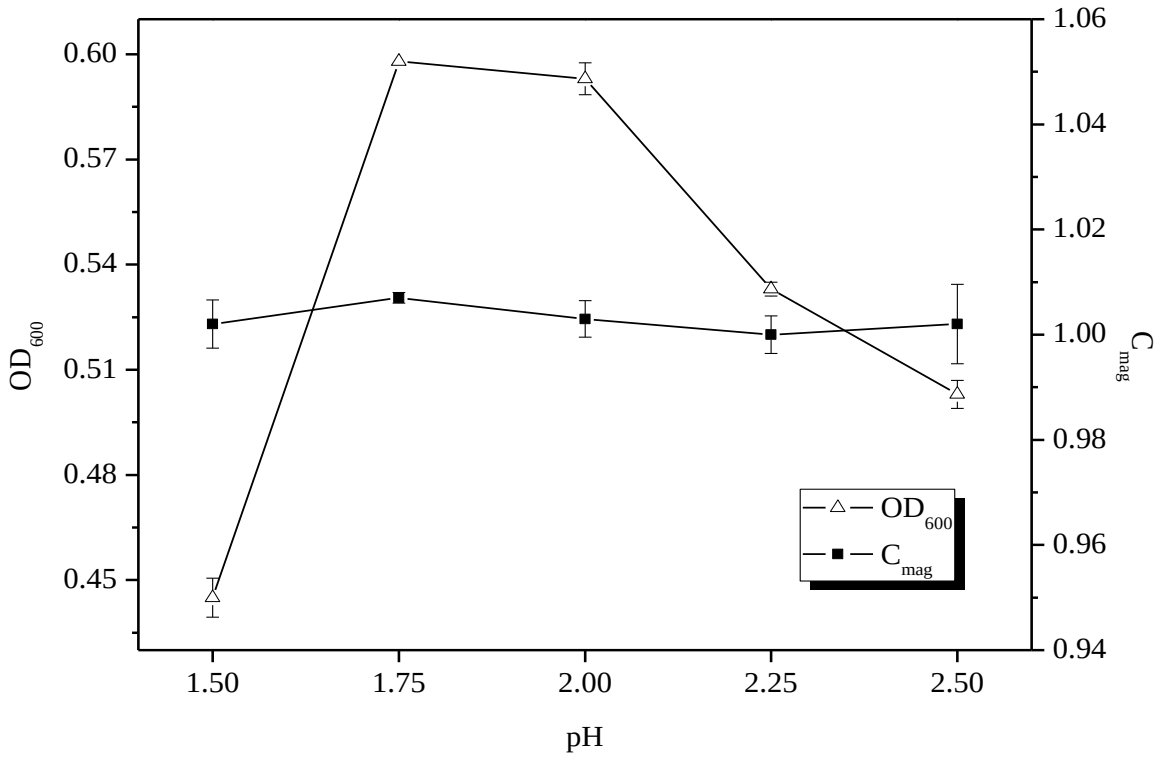

Figure 5. Effect of pH on growth and magnetosome formation of *A. ferrooxidans*.

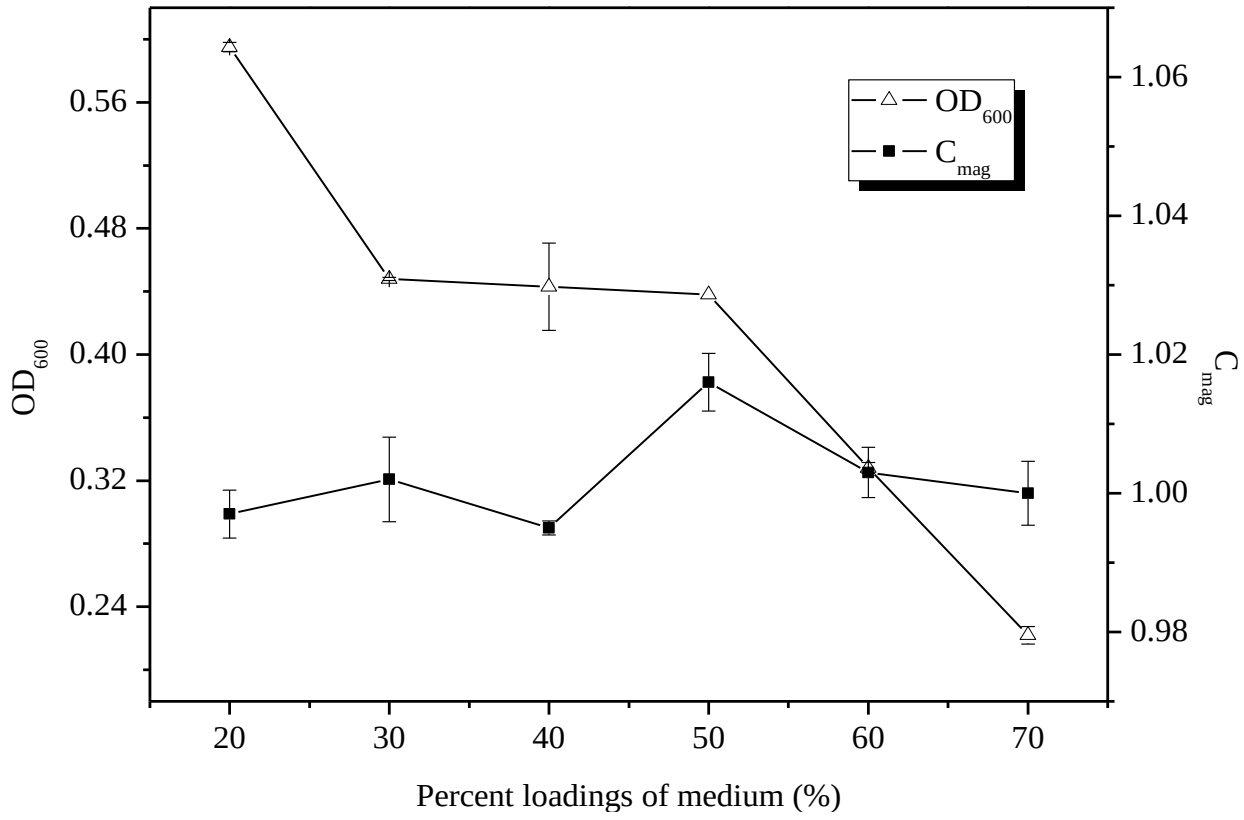

Figure 6. Effect of percent loadings of medium on growth and magnetosome formation of *A. ferrooxidans*.

level of B factor was B_4 (Ammonium sulfate concentration: 3.0 g/L), the optimal level of C factor was C_3 (Temperature: 30°C), the optimal level of D factor was D_2 (pH: 1.75), the optimal level of E factor was E_1 (Medium loadings: 20%).

It can be seen from Table 3 that the value of T_3 was the maximum among all T_n value of A factor. This indicated that the optimal level was A_3 (Ferrous sulfate concentration: 160 mM). In the same way, the optimal level of B factor was B_3 (Ammonium sulfate concentration: 2.4 g/L), the optimal level of C factor was C_1 (Temperature: 20°C), the optimal level of D factor was D_2 (pH: 1.75), the optimal level of E factor was E_4 (Medium loadings: 50%).

DISCUSSION

A. ferrooxidans is an acidophilic chemolithoautotrophic bacterium that can grow in the presence of either the weak reductant Fe^{2+}, or reducing sulfur compounds. It can be found that there are many similar characteristics between *A. ferrooxidans* and MTB. These properties include morphological types (bacillus), gram stain (gram–negative), motility (by means of flagella), ecological distribution (waters), medium composition (contains iron and/or sulfur element), and culture temperature (normal temperature) (Arakaki et al., 2008; Bazylinski and Williams, 2007a; Xie et al., 2005). TEM analysis revealed that the bacteria cultured in 9 K medium are able to synthesize magnetosome. It can also be found that the magnetosomes are not arranged in chains but distributed irregularly in cell (Figure 1). These indicated that the magnetotaxis and magnetism are very weak. Similar results have been observed in a number of studies reported previously (Liu et al., 2006). In most cultured MTB, magnetosomes are arranged in a chain or chains, resulting in a cell magnetic dipole that is the sum of the individual magnetosome dipoles (Bazylinski and Schübbe, 2007b). In order to biomineralize magnetite, MTB require several orders of magnitude more iron than nonmagnetotactic bacteria, reaching up to 4.0% of their cell dry weight (Schüler and Baeuerlein, 1996). The ICP-AES analysis indicated that *A. ferrooxidans* contain a large amount of iron, and the sufficient iron is potential raw material for the formation of magnetosomes.

A. ferrooxidans grows in a high iron concentration environment; the number of the magnetosomes synthesized is very small, so large amount of biomass must be obtained at first to get more magnetosomes. However, the growth conditions of cell are not completely consistent with the conditions for magnetosomes synthesis. In view of this, the effects of different physical and chemical factors on the growth and magnetosomes synthesis of *A. ferrooxidans* were investigated in our study. The main inorganic component of magnetosomes is iron compound, so the magnetosomes synthesis must be affected by the iron source concentration significantly, besides, the growth of cell would be influenced by the iron in the environments. Ferrous ion cannot be autooxidized in extreme acidophilic environment, but can be oxidized facilely by *A. ferrooxidans*. In this case, iron uptake is an energy-independent and regulated process (Yang et al., 2001). Additionally, ferrous iron is not only the energy source for *A. ferrooxidans* growth, but is the source for synthesis of magnetosomes. Results showed that growth of *A. ferrooxidans* and magnetosomes synthesis is correlated with ferrous sulfate concentration, but not strictly positive (Figure 2). It has been demonstrated that the adequate iron was conducive to synthesize magnetosomes, but too high concentration would also inhibit the growth of biomass (Yang et al., 2001).

Nitrogen source is an additional factor for growth of MTB and magnetite magnetosome biomineralization. Results revealed that $(NH_4)_2SO_4$ concentration had no significant effect on growth of *A. ferrooxidans* (Figure 3), indicating that cell could grow well in a wide range of ammonium nitrogen concentration have a nonlinear effect on sulfate concentration. It has been suggested the presence of that nitrogen source significantly increased magnetite formation (Jiang et al., 2002). Magnetite formation depends on the nitrogen source concentrations and the increase of nitrogen source concentrations result in reduced magnetite magnetosome formation (Matsunaga et al., 2000) It was shown in our study that magnetosomes synthesis of *A. ferrooxidans*, indicating that the utilization of nitrogen for magnetosomes synthesis of *A. ferrooxidans* was different from other MTB.

A. ferrooxidans could grow in a wide temperature range, growth of *A. ferrooxidans* and magnetosomes synthesis in the temperature range of 20 to 40°C were investigated. Results indicated that *A. ferrooxidans* could grow well in the temperature range of 20 to 35°C, the maximum biomass obtained at 30°C (Figure 4), which agreed with the previous study (Ni et al., 2008). The optimum temperature for magnetosomes synthesis was 20°C, this was attributed to the energy-independent process of iron uptake (Yang et al., 2001). However, the growth of bacteria is an energy-dependent process which needs heat to maintain the growth. There are large quantities of ferrous and ferric ions at the exponential growth phase, and ferric ions cannot be taken up directly. With the growth of microorganism, the pH increased and ferric ions decreased; at this time, *A. ferrooxidans* absorbed iron to synthesize the magnetosomes without additional energy, which was also a way of iron source storage in cell (Liu et al., 2006).

Most of the reported MTB grow at neutral pH (Heyen and Schüler, 2003; Popa et al., 2009; Yang et al., 2001); *A. ferrooxidans* could grow in a pH range of 1.0 to 3.5. At neutral pH, iron is generally available as insoluble ferric forms in the presence of oxygen. Microbes overcome this

insolubility by two ways. One is biosynthesis and excretion of iron chelators. The iron loaded chelators are then taken up into the cell by way of specific siderophore receptors in an active energy dependent process. The other way depends on the process of reducing the ferric iron to more soluble ferrous forms, and transport into the cell (Yang et al., 2001). *A. ferrooxidans* grows at low pH, iron in the medium was almost in a soluble state; so, low pH was helpful for the ferrous ion utilization of *A. ferrooxidans* and magnetosomes synthesis. Too high or too low pH would both inhibit growth of *A. ferrooxidans*, results showed that pH did not affect significantly the magnetosome formation, and the optimum pH for magnetosomes synthesis and growth of microorganisms was the same (Figure 5).

Oxygen is known to be required for magnetite formation and growth of MTB. It has been demonstrated that magnetite formation in MTB occurred only in a narrow range of low oxygen concentration (Heyen and Schüler, 2003; Popa et al., 2009; Sun et al., 2008; Yang et al., 2001). During growth of *A. ferrooxidans*, oxygen which was as an electron acceptor received electron from ferrous ion, resulting in energy for growth of microorganism (Valdés et al., 2008). The effects of oxygen concentration on growth of *A. ferrooxidans* and magnetosomes synthesis were investigated in this study. Results showed that the higher the oxygen concentration, the better the growth of microorganism (Figure 6), which agreed with the aerobic property of *A. ferrooxidans*. The C_{mag} changed with oxygen concentration, the maximum value reached when the percent loading of medium was 50%. The lower the percent loadings of medium, the higher the oxygen concentrations. The high oxygen concentration can promote the growth of *A. ferrooxidans*, and also can generate autooxidation of ferrous ion in solution. Therefore, the ferrous ion concentration sharply decreased, resulting in the reduction of ferrous ion which was used to synthesize the magnetosomes. The high percent loadings, that is, the low oxygen concentration, can inhibit the growth of *A. ferrooxidans*. Thus, a limited number of cells may only produce a small amount of magnetosomes. In brief, only the oxygen concentration was maintained at an optimal level, *A. ferrooxidans* could grow well, and not too fast, to ensure that there are a large number of biomass for the synthesis of magnetosomes.

In order to obtain more *A. ferrooxidans* contained magnetosomes, the effect of various physical and chemical factors on growth and magnetosomes synthesis of *A. ferrooxidans* must be investigated comprehensively to get the optimal conditions for magnetosomes synthesis. In this study, orthogonal tests in five factors and four-level are designed to optimize the conditions for cell growth and magnetosomes formation (Tables 2 and 3). The results showed that the optimal conditions for cell growth and magnetosome synthesis are different, implying that cell growth and magnetosomes synthesis are out of step. Furthermore, it can be concluded that magnetosome formation was not essential for cell growth, but serve as a way for iron storage.

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