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Magnetization of microorganism cells by sol-gel method

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Microorganism cells could be used as templates during fabrication of magnetic or conductive microstructures in different standard shapes. In this paper, feasibility of magnetizing microorganism cells by sol-gel method, which is to coat cells *of Spirulina* (a type of natural micro-helical microorganism) with the ferrite (a kind of magnetic material), was discussed and investigated. Then the cell form, components and the phase structure were observed and analyzed using various tools including optical microscopy, scanning electron microscopy (SEM), energy dispersive X-ray detector (EDX), transmission electron microscopy (TEM), and X-ray diffraction analysis (XRD). Results showed that *spirulina* cells could be coated with ferrite after the sol-gel process, with the shape of natural helixes well kept, that the components of different sampling points on the surface layer were consistent and the thickness of layer was uniform, and that the type of the surface ferrite layer formed was cubic Fe_3O_4. It was also observed that there were nano-particles yielded in the cells and certain deposit on the walls between cells. The kinetics of the cell magnetization technology by sol-gel was also discussed.

bio-machining, bio-limited machining, hollow micro-helixes, magnetic particles, ferrite

Metallization[1] and magnetization[2] of microorganism cells in various shapes such as sphere and rod-shape have been realized by a bio-limited forming machining method, in which the micro-structures in certain shape were formed by precipitating metal coating onto microorganism cells, the template, with the technique of electroless deposit. Although these functional particles in various shapes have potential application prospect in the field of microwave absorbing, the development was limited by the coating technology since it is hard to coat microorganism cells with ferrite, the most widely applied microwave absorbing material, using the existing electroless deposit technology. In order to improve magnetic properties and enlarge the scale of magnetization of cells, the microorganism cells magnetization technology using sol-gel method was inves-

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tigated in this paper.

In recent years, there has been an increasing interest in research on some application areas of sol-gel method, including mixing sol-gel molecular precursors with organic solvents to form organic-inorganic materials[3], manufacturing mono-dispersed particles[4], and retaining the biological activity[5] of some kind of biomaterials such as proteins, antibodies，and even cells by entrapping them into silicon matrix. But until now there is no research on using this method to form functional micro-structures with microorganism cells in various shapes as templates. As for magnetizing micro-particles using sol-gel method, there is only research on coating cenospheres[6] in limited shapes compared to microorganism cells with ferrite. Research about magnetizing microorganism cells using sol-gel method has not been reported.

1 Materials and methods

1.1 Culture and collection of microorganism cells

Cells of *spirulina platens* (*spirulina platensis*, Nordst. Geitl.), in natural helical shape and blue-green, are used as forming templates. Normally, the width of helix is 26—36 μm, the distance between two coils is 43—57 μm, the helical number is 4—7 (20 at most), the diameter of helix threads made up of multi-cells is 5—8 μm, and the height of each single cell is 4—5 μm. *Spirulina* cells were cultured in an enclosed photobioreactor using strain and culture reagent from the Institute of Hydrophyte, Chinese Academy of Sciences. When grown up, cells were collected using a thin silk sieve with mesh number of 250.

1.2 Cells fixation

The collected *spirulina* cells were fixed with glutaraldehyde before sol-gel process to keep their initial shape during the following process.

1.3 Sol-gel process

First, put 20 mL KOH solution with certain concentration into the beaker; second, add in 20 mL KNO_3 solution with certain concentration and let them mix adequately; third, add in 10 mL $FeSO_4$ solution with certain concentration which has been mixed with some *spirulina* cells. When the gel solution is formed, keep the beaker in water area of 90℃ for enough time.

The entire technology steps include: 1) fixing cells; 2) dipping cells in Fe^{2+} solution; 3) adding cells into the mixture solution of OH^- and NO^{3-}; 4) heating the solution and keeping it at certain temperature for enough time; 5) filtering the solution and washing the cells by distilled water; 6) repeating steps from 2) to 5) to help keep the shape of the micro-structure after it is dried; 7) collecting the micro-structures; 8) dehydrating the micro-structures by ethanol and drying.

1.4 Measurement and equipment

The cell form was observed and studied with XSY-1 optical microscope plus Nikon 4500 photographic camera, Cambridge 250 scanning electron microscope (SEM), and JEM-1230 transmission electron microscope (TEM). The composition analysis and phase structure were researched with Oxford Link 860 energy dispersive spectrometer (EDS) and D/Max 2200 PC automatic X-ray diffraction (XRD), respectively.

2 Experiment results

2.1 Results of single-time sol-gel process

After single-time sol-gel process the cells changed from green semi-transparent (see Figure 1(a)) into black semi-transparent (see Figure 1(b)), and the black matter on the cell surface combined with cells firmly. When magnetic field was applied using NdFeB magnet, *spirulina* cells could rotate with the changing of direction of the magnetic field, which meant the cells had been magnetized by the single sol-gel process. The composition analysis showed that the cell coating was composed of Fe (accounting for 44.19 atom %), O (accounting for 55.81 atom %) and a little of S (accounting for less than 1 atom%), which meant that main composition of the cell coating was iron oxide. It also could be found that the cells were not strong enough to preserve their shapes and easily became anamorphic and were broken after dehydrating by ethanol and drying at 60℃ (see Figure 1(c)).

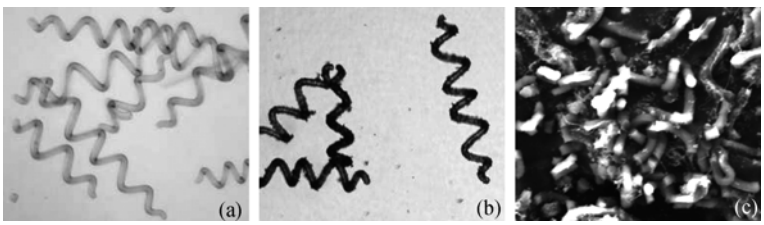

Figure 1 Photos of *spirulina* cells in different steps (×300). (a) Optical photo of green semi-diaphanous *spirulina* cells before sol-gel process; (b) optical photo of black semi-transparent *spirulina* cells after single-time sol-gel process; (c) SEM photo of anamorphic and broken *spirulina* cells after drying.

2.2 Results of multi-time sol-gel process

In order to solve the problem that the shape of cells could not be kept well after single-time sol-gel process, the process procedure was repeated several times. After that the cells became non-transparent (see Figure 2(a)), the magnetism of cells was strengthened, and the shape could be kept well after drying (see Figure 2(b)).

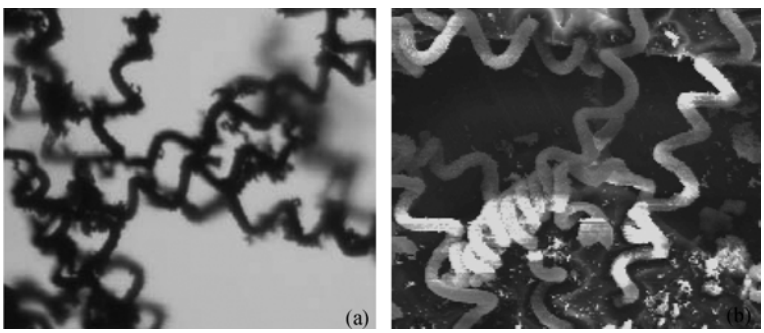

Figure 2 Photos of *spirulina* cells after multi-time sol-gel process (×300). (a) Optical photo of black non-transparent *spirulina* cells after multi-time sol-gel process; (b) SEM photo of undeformed and completed *spirulina* cells after drying.

When observing samples of the ultrathin section with transmission electron microscope, it could be found that the black coating of *spirulina* cells was basically continuous and the thickness of the coating was uniform on the whole (see Figure 3(a), in which the thickness of the coating is around 0.3 μm). It also could be found that there were nano-particles yielded in cells

(see Figures 3(a), (b), and (c)), some of which were assembled, and some deposit yielded on the walls between single cells. The composition analysis showed that the surface coating of cells was still mainly of Fe and O, and the composition ratios of different points were basically uniform (see Table 1). As shown in Figure 4 and Table 2, except for a few impurities (there are no theoretical values on peak Nos. 1, 3, 5, 10, and 13), the composition of the cell coating is cubic crystal Fe_3O_4 because the measured values of crystal face distance are basically consistent with the theoretical values of Fe_3O_4 (see Table 2).

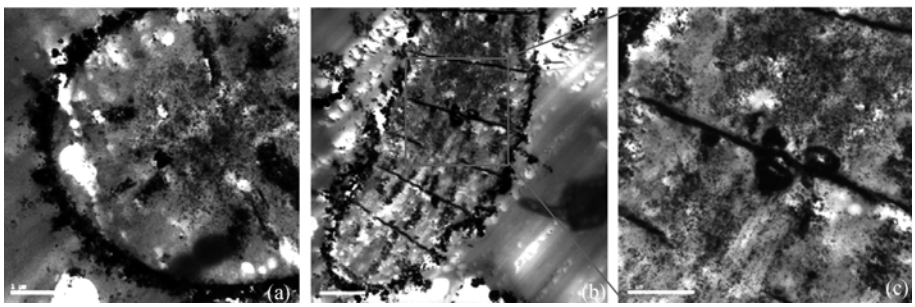

Figure 3 TEM photos of *spirulina* cells after multi-time sol-gel process. (a) Cell form of coating layer; (b) photo of walls between single cells; (c) enlarged photo of walls between single cells.

Table 1 Composition analysis of surface layer (atom fraction (%))

Number of sampling points	1	2	3	4
O	57.00	55.57	50.19	56.09
Fe	43.00	44.22	49.38	43.08
S	0	0.21	0.43	0.83

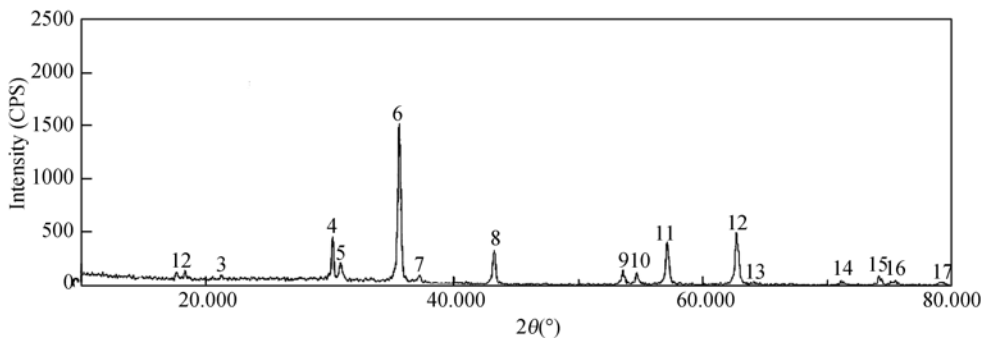

Figure 4 XRD analysis.

Table 2 Comparison of XRD data and theoretical data of Fe_3O_4 of crystal face distance

Peak No.	XRD data (d1/nm)	Theoretical data (d/nm)	Crystal face (*hlk*)	Peak No.	XRD data (d1/nm)	Theoretical data (d/nm)	Crystal face (*hlk*)
2	4.8282	4.8520	111	11	1.6109	1.6158	511
4	2.9578	2.9670	220	12	1.4797	1.4845	440
6	2.5205	2.5320	311	14	1.3245	1.3277	620
7	2.4150	2.4243	222	15	1.2773	1.2807	533
8	2.0920	2.0993	400	16	1.2590	1.2659	622
9	1.7081	1.7146	422	17	1.2092	1.2119	444

Data in column of Peak No. in this table are consistent with the peak numbers in Figure 4.

3 Mechanism analysis

3.1 Producing of Fe_3O_4 particles.

By mixing $FeSO_4$ solution and KOH solution, $Fe(OH)_2$ colloid particles are generated and further gel forms. With the KNO_3, a type of tender oxidant, there will be many tiny Fe_3O_4 particles yielded. And because of the existing of gel net, these particles could not be observed obviously and will not aggregate or fall down easily. When the number of particles becomes as big as enough, some of the gel net will dissolve and particles will assemble and form masses immediately. Within the mass there are certain number of first-level particles, some of which act as the cores of second-level particles and the others adjacent to cores will accumulate around cores and then form the second-level particles. At the same time, the rest of gel net will prevent the second-level particles from assembling or falling down, so the system of mono-dispersed spherical powders forms[7]. The reaction formulas are shown as the following.

$$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2\downarrow \quad (1)$$

$$3Fe(OH)_2 + NO_3^- \rightarrow (FeO\cdot Fe_2O_3)\downarrow + NO_2^- + 3H_2O \quad (2)$$

Just because of the existing of gel net, the size of particles generated by sol-gel method is basically uniform (refer to Figure 5. The diameter of the spherical particles is 50—60 nm, which are fabricated by the sol-gel method with the same reagent ratio as that mentioned in section 1.3).

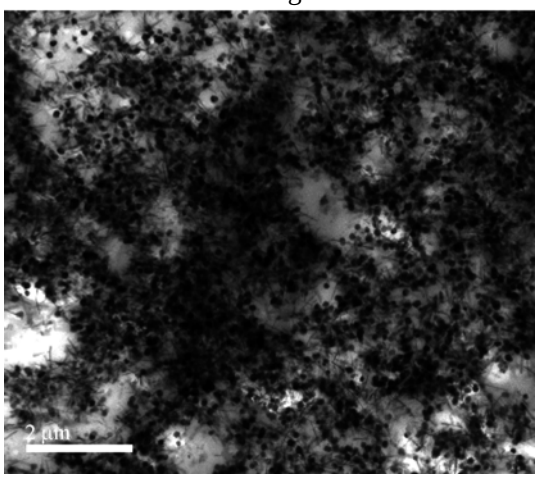

Figure 5 SEM photo of Fe_3O_4 particles fabricated by sol-gel method.

3.2 Forming mechanism of Fe_3O_4 coating on microorganism cells

When microorganism cells are mixed with $FeSO_4$ solution and churned up, Fe^{2+} will be distributed uniformly around cells. Then after adding the admixture of cells and $FeSO_4$ into the mixed solution of KOH and KNO_3, $FeSO_4$ and KOH will react immediately and yield $Fe(OH)_2$ colloid particles. Because Fe^{2+} have been distributed uniformly around cells and $Fe(OH)_2$ colloid particles are in nano-scale size with large ratio value of surface to volume, $Fe(OH)_2$ colloid particles are easy to attach to cells with bigger surface. And because cells are light enough, they are not easy to fall down in gel net, thus, churning should be avoided in order to prevent the cells from leaving the gel net at this time. After that, while $Fe(OH)_2$ colloid particles form the gel net, the cells are fixed in the gel net and the existing gel net will help cells contact adequately with the reactants around them.

Meanwhile, under the oxidizing function of KNO_3, the reactants near the cells will react on the cell surface and become the forming cores of the cell coating. The forming procedure of cores on the cell surface is heterogeneous.

Then the forming cores will attract the atoms around them and grow up, and while growing there are new forming cores generated and growing on the cell surface. The first-level and second-level particles generated in gel net are easy to attach to cells because of their small size. The crystals on the cell surface grow up gradually and the particles around cells will accumulate and attach to cells more and more and continue to grow up. When the crystals adjacent to the cell surface contact each other, they will no longer grow up in the parallel direction but spread in the vertical direction until all the crystallization process completes. Then the continuous coating forms. The mechanism sketch is shown in Figure 6.

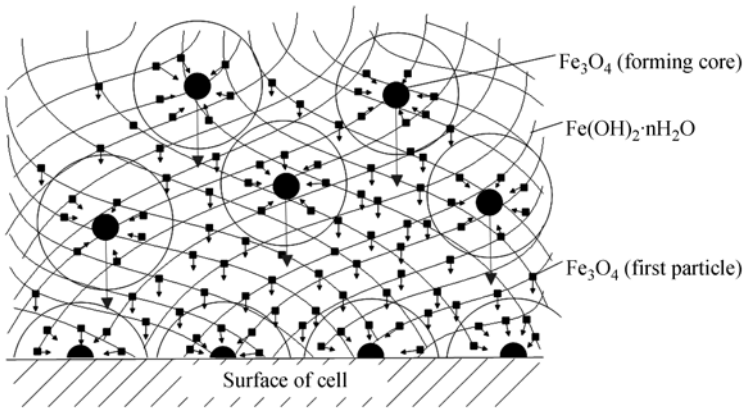

Figure 6 Sketch of how microorganism cells are coated with Fe_3O_4 layers.

In general, the forming procedure of the Fe_3O_4 coating on the cell surface could be divided into three steps. (1) Gel net forms around cells. (2) Heterogeneous forming procedure of Fe_3O_4 crystal occurs on the cell surfaces and the first-level particles form in the outer space around cells. (3) The crystals on the cell surface center on crystal cores and grow up, then the cells attract the first and second level particles yielded around them, and finally, these particles and crystal cores grow up on the cell surface together.

After multi-time sol-gel process, the thickness of the crystal coating of cells increases to facilitate keeping the shape of cells.

4 Conclusions and development

(1) Microorganism cells can be covered with magnetic ferrite material on the surface by sol-gel method.

(2) It is difficult to keep shape of cells after drying through single-time sol-gel process. By multi-time sol-gel process the problem is solved. The thickness and composition of the formed magnetic cell coating are uniform, and there are some nano-particles yielded in cells and some deposit on the walls between single cells.

(3) With the experiment conditions used in this paper, the ferrite coating of cells is cubic crystal Fe_3O_4.

(4) The technology mentioned in this paper has high possibility to be used on other microorganism cells in different shapes and characters. Besides, microorganism cells could be covered

with more kinds of ferrite materials using similar technology or mechanism.

Naturally, microorganism cells in various shapes could not be fabricated by any traditional manufacturing methods. If certain special shapes could be combined with appropriate materials and form absorbers, good microwave absorbing effect would be obtained. But both experimental and theoretic researches on this topic are absent and need to be developed. The research in this paper is of good prospect and research value, because it provides not only a new method to improve microwave absorbing function of absorbers, but also a new way to research the theory on how shape of absorbers affects the absorbing effects.

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