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Short communication

Effects of tourmaline addition on the dehydrogenase activity of *Rhodopseudomonas palustris*

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Abstract

The structural change of water clusters of distilled water induced by tourmaline addition was observed and a bacterium *Rhodopseudomonas palustris* was used to investigate the effects of tourmaline addition on the dehydrogenase activity of the microorganism. The results showed that tourmaline reduced ^{17}O NMR full width at half maximum intensity (FWHM) for distilled water and the volume of clusters of water molecules. The dehydrogenase activity was improved and remained stable within the pH range from 5 to 11 when tourmaline was added. The results showed that tourmaline controlled the pH of different initial values between 5 and 11 all to about 8.0. Besides the structural change of water clusters, the promoted activity of dehydrogenase observed in this study may be caused by pH self-control by tourmaline. This study provided useful information for the application of tourmaline addition in biological processes such as wastewater treatment.

Keywords: Tourmaline; Dehydrogenase; *Rhodopseudomonas palustris*; Water clusters; Enzyme activity; pH

1. Introduction

There have been numerous studies on the effects of electric fields on living organisms. Although some of them show a negative effect, reports are also available showing the significant and positive effects of electric field on the growth and metabolism of organism [1,2].

Tourmaline is a kind of complex borosilicate mineral belonging to the trigonal space group C_{3v}^{5}-R_{3m} [3]. The general chemical formula can be written as $XY_3Z_6Si_6O_{18}(BO_3)_3W_4$, where X is Na, Ca, K or vacancies; Y is Mg^{2+}, Fe^{2+}, Mn^{2+}, Al, Fe^{3+}, Mn^{3+}, Li; Z is Al, Fe^{3+}, Cr^{3+}, Mg; W is OH, F, O [4]. The most important feature among the electric properties of the tourmaline is the possession of spontaneous and permanent poles, which would produce an electric dipole, especially in a small granule with a diameter of several microns or less [5–7]. Therefore, a strong electric field exists on the surface of a tourmaline granule [6,8,9]. The electric field effect of tourmaline particles can be utilized and applied in various fields.

Jin et al. [10] showed that tourmaline stimulated the proliferation of vascular endothelial cells (ECV-304) using co-culture method. Their experiment was performed using a millicell membrane culture dish between ECV-304 and tourmaline, so tourmaline was not directly contacted with ECV-304 and the culture medium was circulated through millicell membrane. Xia et al. [11] found that the growth of caco-2 cells was promoted and the activity of alkaline phosphatase was improved when tourmaline-treated-Dulbecco's Modified Eagle Medium was used to incubate the cell. Those reports indicated that the effects of tourmaline on cultured cells may be mediated via the culture medium. However, the reason of this effect has not been understood yet.

It is clear that life on Earth depends on the unusual structure and anomalous nature of liquid water. Water molecules form an extended and dynamic hydrogen-bonded network [12,13]. The structural change of water clusters can lead to multiple biological effects [14,15]. It is well known that electric fields affect liquid water cluster structure. In the present study, we investigated the effect of tourmaline on water clustering of liquid water. Since there have been many studies on the application of purple nonsulfur bacterium in biological wastewater treatment and the dehydrogenase activity was used as an index for active bacterial populations and overall microbial activity [16,17], we also used *Rhodopseudomonas palustris* to investigate the effects of tourmaline on the activity of dehydrogenase.

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2. Materials and methods

2.1. Materials

The tourmaline samples with purity of larger than 85% were schorl ores from the east area of Inner Mongolia Autonomous Region, China. The particle size was less than 1 μm. The chemical compositions of tourmaline were as follows (%, by dry weight): SiO_2 46.03, Al_2O_3 16.71, B_2O_3 10.24, FeO 0.92, Fe_2O_3 18.24, TiO_2 0.45, Na_2O 1.12, K_2O 0.06, MgO 2.28, CaO 3.95. The strain used in this study was *R. palustris,* a purple nonsulfur bacterium. Culture medium was the same as reported previously [18].

2.2. Effect of tourmaline addition on the water clusters of liquid water by ^{17}O NMR method

Varying amounts of tourmaline were dispersed in distilled water for 10 min (or for varying time with constant amounts of tourmaline). The supernatant was filtered. ^{17}O NMR full width at half maximum intensity (FWHM) of the supernatant was measured by a Bruker DMX 500 NMR spectrometer operating at 67.7 MHz.

2.3. Effect of tourmaline addition on dehydrogenase activity of *Rhodopseudomonas palustris*

Varying amounts of tourmaline were added to the bacterial suspensions at 37 °C and pH 8.4 for 12 h (or for varying time with constant amounts of tourmaline). Samples were removed and measured for dehydrogenase activity. For the tourmaline addition concentration trial, the enzyme activity of tourmaline-free control at 12 h was defined as 100%. For the varying time trial, the enzyme activity of tourmaline-free control at 0 h was defined as 100%. The relative activity (%) of the tested treatment was calculated.

The method of measuring dehydrogenase activity was based on using a colorless artificial electron acceptor which, when reduced, develops a color that can be measured spectrophotometrically. The used artificial electron acceptor was triphenyltetrazolium chlorlde (TIC). The detailed test procedure has been described earlier [17]. One unit of dehydrogenases is equilalent to 1 μg of triphenyl formazan produced per hour.

2.4. Effect of tourmaline addition on the thermal and pH stability of dehydrogenase activity

Two grams of tourmaline were added with 100 ml of bacterial suspensions. In the first set of experiments, the temperature was changed between 20 and 60 °C while the initial pH of the medium was 8.4. Samples were removed after 30 min and measured for dehydrogenase activity. In the second set of experiments, the initial pH was changed between 5.0 and 11.0, whereas the temperature was constant at 37 °C. Samples were removed after 24 h and measured for dehydrogenase activity. The enzyme activity of tourmaline-free control at 37 °C and pH 8.4 was defined as 100%. The relative activity (%) of the tested treatment was calculated.

2.5. Effect of tourmaline on the pH value of the bacterial suspensions

Two grams of tourmaline were added with 100 ml of bacterial suspensions. The initial pH of the medium was changed between 5.0 and 11.0. The pH of the

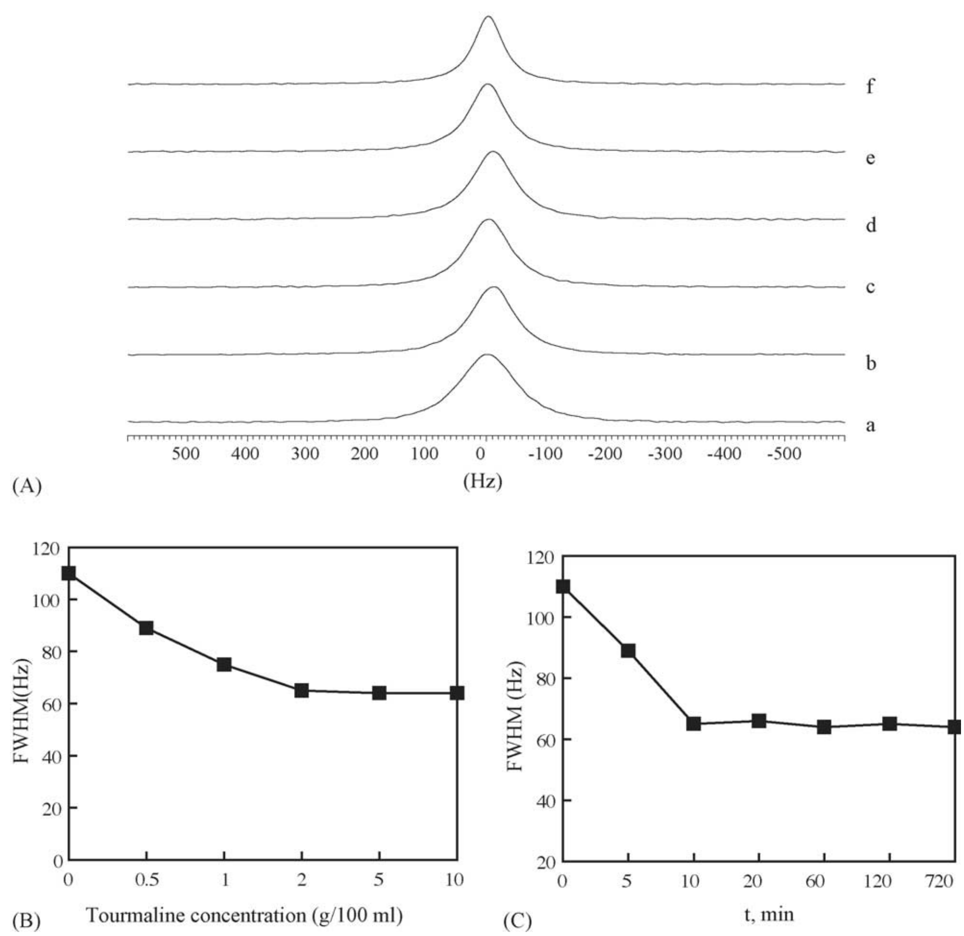

Fig. 1. Effect of tourmaline addition on ^{17}O NMR full width at half maximum intensity (FWHM) of distilled water. (A) ^{17}O NMR spectra of distilled water induced by different addition concentration of tourmaline for 10 min. a, b, c, d, e, f represent the addition concentration of 0, 0.5, 1, 2, 5, 10 g/100 ml. (B) ^{17}O NMR FWHM of distilled water induced by tourmaline with the addition concentration range from 0 to 10 g/100 ml for 10 min. (C) ^{17}O NMR FWHM of distilled water induced by tourmaline addition of 2 g/100 ml within 0–720 min.