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Full Length Research Paper

Effects of tourmaline on growth of three kinds of microorganisms

Ying-Liang Zhao[1], Cheng-Bin Li[2], Geng Li[1], Wei-Yao Hu[1], Long Zhu[1], Xing-Lin Wang[1], Bo Zhou[1,2], Jian Liu[2], Xiao-Ying Zeng[1] and Tian-Dong Zhang[1]*

[1]Technology center, Hongyun Honghe Tobacco Group, Kunming, 650202, China.
[2]Yunnan Reascend Tobacco Technology Company, Kunming, 650106, China.

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Tourmaline is a kind of widespread minerals and has been used in many fields of society. Owing to the special electric properties, tourmaline can destroy the hydrogen bond between water molecules. The growth of microorganism may be affected with addition of tourmaline; therefore the effects of tourmaline on three different kinds of microorganisms were studied using microcalorimetric method. The kinetic parameters of growth process were obtained through the thermogenic curves. The results show that high concentration tourmaline inhibited the growth of prokaryotic cells (the growth rate constant of *Escherichia coli* and *Staphylococcus aureus* decreased 68.77 and 35.25% at tourmaline concentration of 160 g/L), while promoted eukaryotic cell (the growth rate constant of *Candida albicans* increased 40.73% at tourmaline concentration of 160 g/L). However, the low concentration tourmaline had complex effects on growth of the studied microorganisms.

Key words: Microcalorimetric, water cluster, growth rate, inhibitory ratio.

INTRODUCTION

Tourmaline is one kind of widespread minerals in nature, which is famous for its electric properties (Guo and Qian, 1997). The general formula of tourmaline may be written as $XY_3Z_6[T_6O_{18}][BO_3]V_3W$, where X = Ca, Na, K, □[vacancy]; Y = Li, Mg, Fe^{2+}, Mn^{2+}, Al, Cr^{3+}, V^{3+}, Fe^{3+}, Ti^{4+}; Z = Mg, Al, Fe^{3+}, V^{3+}, Cr^{3+}; T= Si, Al, (B); B=B, (□); V = OH and W = OH, F (Yavuz et al., 2002). Tourmaline crystals are piezoelectric and often pyroelectric as well. It has the spontaneous and permanent poles, which could produce an electric dipole (Jin et al., 2003; Yamaguchi, 1983; Nakamura et al., 1994). For its special properties, tourmaline has been utilized and applied in various fields, such as the heavy metals adsorption in water system (Jiang et al., 2006; Zheng and Wang, 2010), the decomposition of contamination in waste water (Sun and Misook, 2008). Some research showed that the electric field may have negative or positive effect on the growth and metabolism of organism (Cao et al., 2003; Ellaiah et al., 2003), and the processes could be studied by different methods (Xia et al., 2006; Qiu et al., 2010).

In living systems, various metabolic events occur with the heat production, which is also one of the prominent features of the microbial growth process. Some information of the microorganism metabolism could be obtained by monitoring the heat production (Yang et al., 2005). Microcalorimetry, which is a quantitative, inexpensive, and versatile method, provides a general analytical tool to characterizing the microbial growth process. Besides the thermodynamic information, the kinetic information could also be obtained. It can reveal much temporal details about the organism metabolism (Yan et al., 2008; Liu et al., 1999). Microcalorimetry method has been widely used to study the metabolism process of microorganism and many other cells (Kamiyama et al., 2005; Lisowska et al., 2005; Wang et al., 2010; Chen et al., 2009; Kong et al., 2010; Kong et al., 2011).

Although some researches have been performed to study the effect of tourmaline on some microorganism (Ni et al., 2008), there are fewer studies about whether tourmaline has different effects on different kinds of

*Corresponding author. E-mail: zhangtd1969@163.com. Tel: +86-871-5833412. Fax: +86-871-5811800.

Table 1. The chemical components content of tourmaline.

Chemical component	Mass (%)
C	8.25
O	50.71
Ca	18.89
Al	5.63
Mg	3.61
Fe	1.37
Si	9.3
K	0.29
Na	0.21
Mn	0.11
Sr	0.04
Ni	0.01
Zn	0.003

microorganisms or not. *Candid albicans* (*C. albicans*), *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are perhaps the human pathogenic micro-organisms that have been most extensively studied (Qin et al., 2004). In this paper, the effects of tourmaline particles on growth process of the three kinds of micro-organisms were studied using microcalorimetry method. The purpose of this study was to offer the information about the tourmaline effects on the three kinds of microorganisms, which may be helpful to inhibit the growth of microorganisms by using tourmaline materials.

MATERIALS AND METHODS

Materials

Tourmaline particles (average diameter was 3 mm) were purchased from Zibo Bonatech Co., China. The chemical components analysis was performed on the ZXS100e X-ray fluorescence spectrometer. Table 1 shows the contents of main chemical components.

Luria-Bertani (LB) complex medium contained 5 g NaCl, 10 g tryptone, 5 g yeast extract per 1000 ml water solution. The peptone culture medium contained 10 g peptone, 5 g beef extract and 5 g NaCl per 1000 ml water solution. The pH of culture medium was adjusted to 7.00 with 0.01 mol/L NaOH. Then the tourmaline particles and two kinds of medium were sterilized by autoclaving for 20 min at 120°C.

C. albicans was provided by the People's Hospital of Hubei Province, China. *E. coli* (CCTCC AB91112), and *S. aureus* (CCTCC AB910393) were provided by the Chinese Center of Type Culture Collection, Wuhan University, China.

Microcalorimetric measurements

TAM Air (Thermometric AB, Sweden) was used to monitor the continuous heat released of the microorganism growth process and the metabolic growth curves could be obtained. Eight twin-channels were designed in this instrument, in which one was for sample vessel and the other was for a static reference vessel. The temperature of the surrounding was constant. When the heat in sample vessel was released or absorbed, a measurable voltage between the sample and reference would generate and it was proportional to the heat flow. The voltage signal was changed to the power and recorded by the computer (Fan et al., 2008).

First, the three strains were incubated in peptone culture medium at 37°C, with 2×10^6 cells mL^{-1}. These suspensions were used as the inocula for the microcalorimetry assays. 1 ml inocula were homogeneously distributed into LB medium (50 ml) by gentle shaking. Aliquots of 5 ml of the suspensions were added into 20 ml sterilized ampoules containing tourmaline particles with different concentrations. The ampoules were sealed tightly and placed in the calorimeter. The metabolic thermogenic curves of microorganism growth were then recorded continuously.

In the log phase of growth, the cell growth is exponentially (Hall and Hawkins, 1975). If the cell number is n_0 at originate time, and n_t at arbitary time in microorganism growth process, then:

$$n_t = n_0 \exp(kt) \quad (1)$$

Where k is the growth rate constant. If the power output of each cell is w, then;

$$n_t w = n_0 w \exp(kt) \quad (2)$$

$$P_0 = n_0 w \quad \text{and} \quad P_t = n_t w \quad (3)$$

Giving

$$P_t = P_0 \exp(kt)$$

or

$$\ln P_t = \ln P_0 + kt \quad (4)$$

The thermogenic curves of the log phase of growth correspond to Equation 4. Thus, using the data $\ln P_t$ and t taken from the curves to fit a linear equation, the growth rate constant (k) is calculated and the generation time (G) which equals $\ln 2/k$ is also obtained. The inhibitory ratio (I/%) can be defined as:

$$\mathrm{p}\,I = \left[\frac{k_0 - k_c}{k_0}\right] \times 100\% \quad (5)$$

Where, k_0 is the rate constant of the control and k_c is the rate constant of microorganism growth with different concentration of tourmaline.

Effect of tourmaline on the water clusters using ^{17}O NMR method

Tourmaline particles (20 and 50 g/L) were immerged in distilled water respectively. The ^{17}O *NMR* full width at half maximum intensity (FWHM) with different time was determined on Bruker DRX-500 NMR spectrometer.

Effect of tourmaline on pH of LB medium

Different concentrations of tourmaline (0, 20, 40, 60, 80 and 100

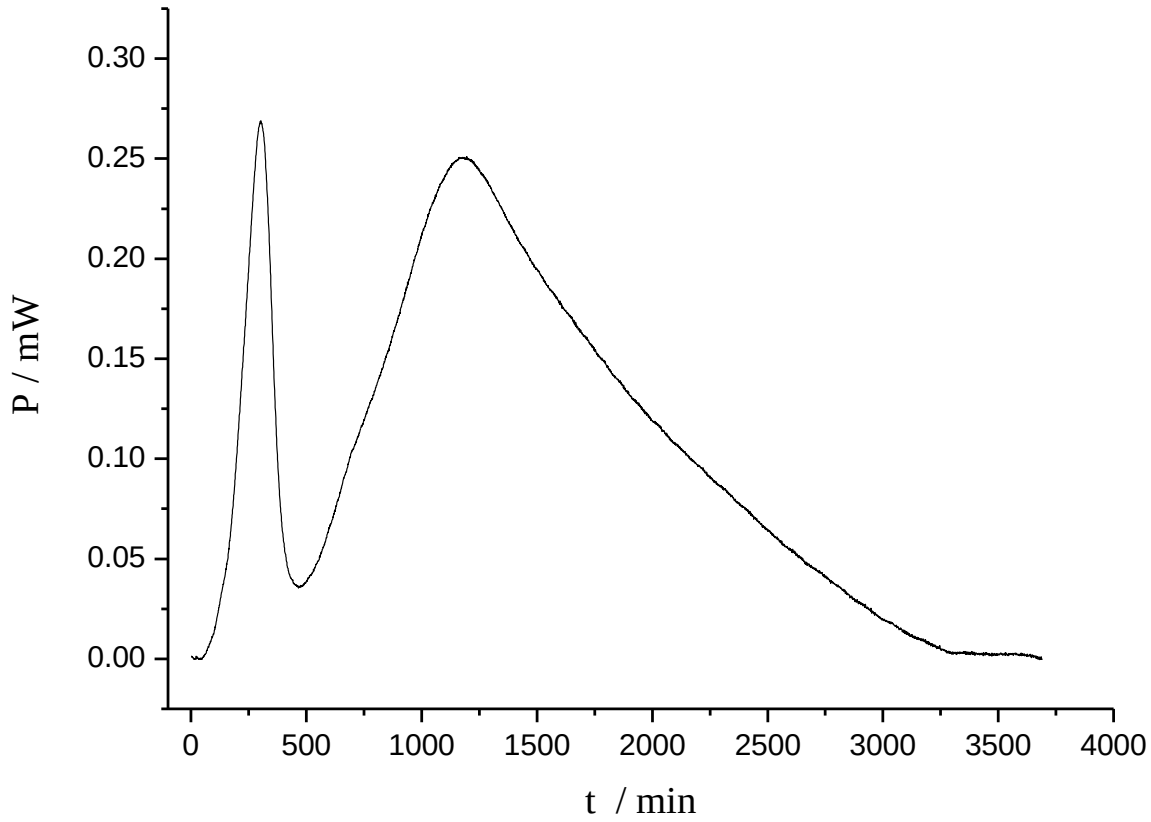

Figure 1. The metabolic thermogenic curve (power-time curve) of *C. albicans* growth.

g/L) were immerged into LB medium. Mettler SevenMulti pH meter (S40) was used to determine the pH value of LB medium with different immerging time.

RESULTS AND DISCUSSION

The metabolic thermogenic curve (power-time curve) of *C. albicans* growth without addition of tourmaline was shown in Figure 1. There were two exothermic peaks in *C. albicans* growth process.

The first peaks in *C. albicans* growth process with different concentrations of tourmaline are shown in Figure 2. Table 2 shows the kinetic parameters of *C. albicans* growth processes. When tourmaline concentrations were lower than 40 g/L, the growth rate constants were lower than the sample without addition of tourmaline (control). The value of *k* was close to control at the concentration of 40 g/L, and gradually increased along with tourmaline concentration increasing (above 40 g/L). The inhibitory ratio was -40.73% at the concentration of 160 g/L, which means that the growth of *C. albicans* was promoted obviously. Generation time had the contrary changing trend with growth rate constant. Therefore, it could be concluded that low concentration tourmaline inhibited the growth of *C. albicans*, but high concentration tourmaline had a promoting action on the growth of *C. albicans*.

Normally, it was considered that the log phase appeared at the beginning of the microorganism growth, so it was not meaningful to calculate the growth rate constant of the second growth period.

Tourmaline effect on the growth of *E. coli*

The metabolic thermogenic curves of *E. coli* growth with different concentrations of tourmaline are shown in Figure 3. There was only one exothermic peak in the *E. coli* growth process. From the parameters of *E. coli* growth process shown in Table 3, it is seen that the *E. coli* growth was promoted at low concentration of 10 g/L and inhibited when the concentrations were higher than 10 g/L. The *k* value decreased with the increasing of tourmaline concentration and the inhibitory ration reached 68.77% at the concentration of 160 g/L.

Tourmaline effect on the growth of *S. aureus*

The metabolic thermogenic curves of *S. aureus* growth with different concentrations of tourmaline are shown in Figure 4. There was also only one exothermic peak in *S. aureus* growth process. As shown in Table 4, the growth

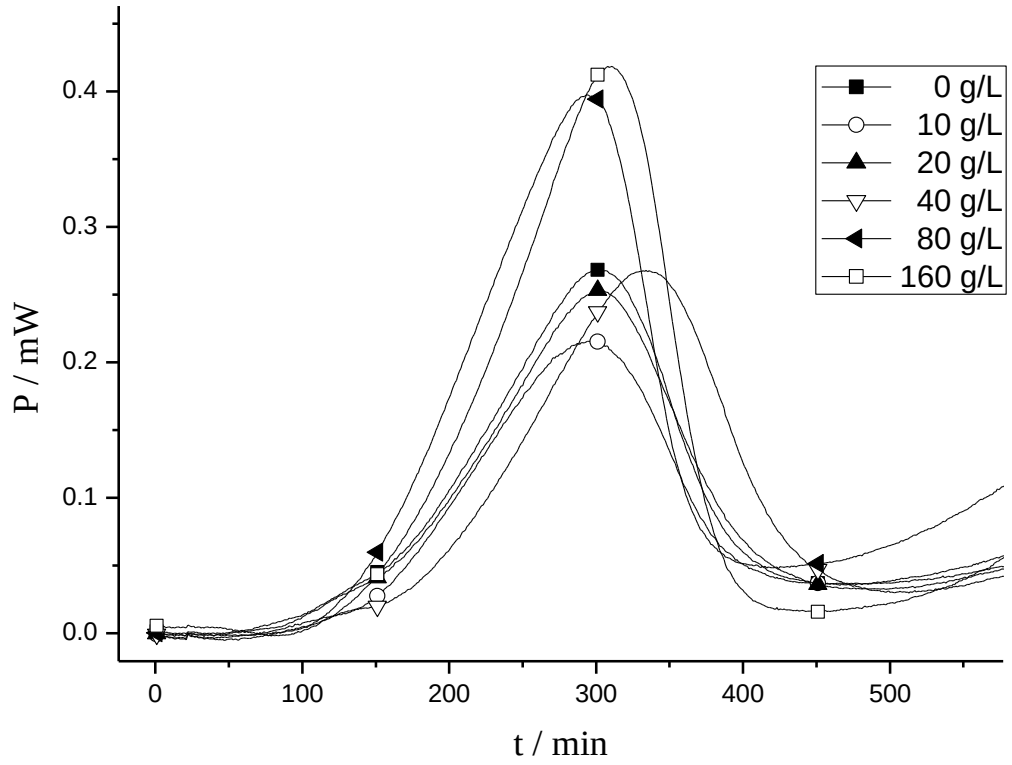

Figure 2. The first peaks of metabolic thermogenic curves (power-time curve) of *C. albicans* growth in the presence of tourmaline (0, 10, 20, 40, 80, 160 g/L).

Table 2. Kinetic parameters of the *C. albicans* growth processes.

Concentration of tourmaline (g/L)	k [a] (min^{-1})	R [b]	t_G [c] (min)	I (%)
0	0.00496	0.99832	139.7	0
10	0.00483	0.98868	143.5	2.62
20	0.00472	0.99305	146.8	4.83
40	0.00495	0.99836	140.0	0.20
80	0.00672	0.99208	103.1	-35.48
160	0.00698	0.99856	99.3	-40.73

[a]Growth rate constant; [b]correlation coefficient; [c]generation time.

rate constants were all lower than the sample without addition of tourmaline (control), which implied that the growth of *S. aureus* was inhibited in the studied concentration range. The maximum inhibitory ratio (60.31%) appeared at the concentration of 10 g/L. However, the relativity between *k* and tourmaline concen-tration could not be found.

Effect of tourmaline addition on ^{17}O *NMR* FWHM for distilled water

The ^{17}O *NMR* method was a very useful method to probe water structure and investigate the water clusters size. Figure 5 shows the ^{17}O *NMR* spectra of distilled water. FWHM was used to detect the average cluster size in liquid water (Martin, 1995; Li et al., 2004); larger value of FWHM meant bigger average size of water cluster. From Figure 6, it was obviously seen that the average size of water cluster reduced when immerged with tourmaline and in a very short time period the size of water cluster could reach a minimum value.

Effect of tourmaline addition on pH of LB medium

Table 5 shows that the pH of LB medium changed when treated with tourmaline. When tourmaline was in a low

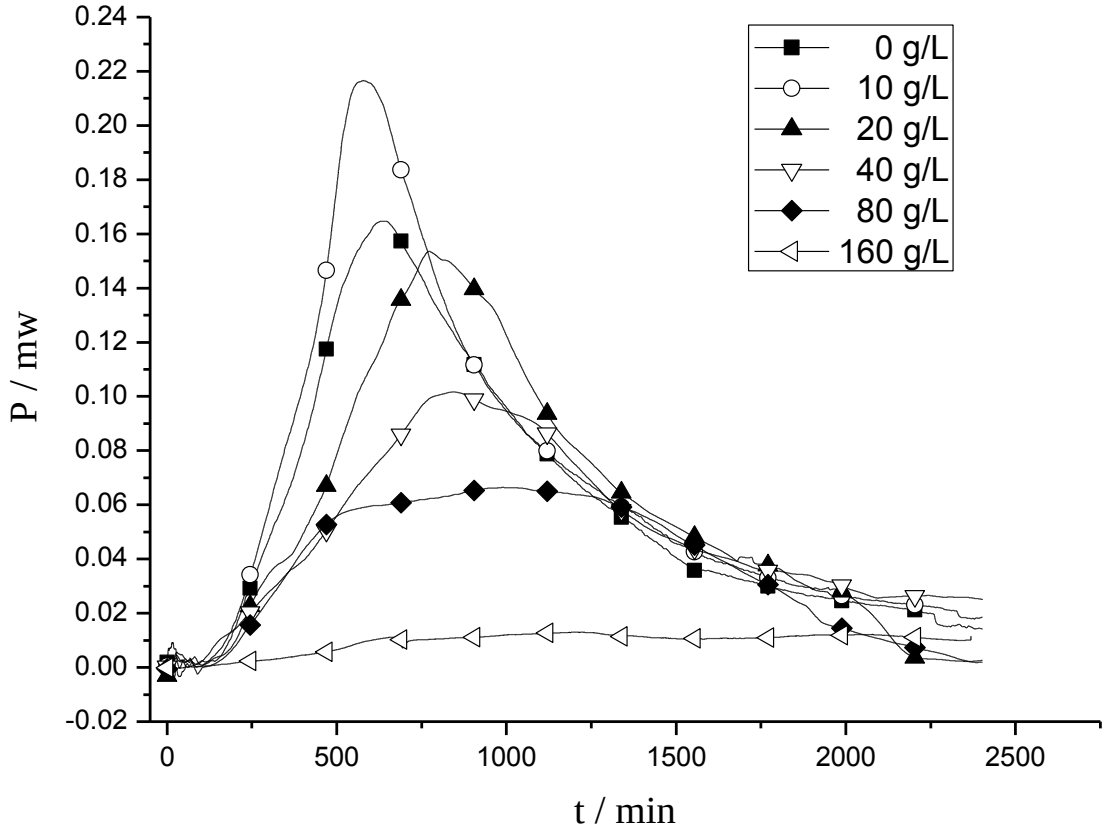

Figure 3. The metabolic thermogenic curves (power-time curve) of *E. Coli* growth in the presence of tourmaline (0, 10, 20, 40, 80, 160 g/L).

Table 3. Parameters of the *E. coli* growth processes.

Concentration of tourmaline (g/L)	k (min^{-1})	R	t_G (min)	I (%)
0	0.01761	0.99598	39.4	0
10	0.01812	0.98987	38.3	-2.90
20	0.01688	0.99342	41.1	4.14
40	0.01175	0.99885	60.0	33.27
80	0.01096	0.99465	63.2	37.76
160	0.00550	0.99036	126.0	68.77

concentration range (10 and 20 g/L), the pH values changed slightly. The pH values increased when tourmaline concentration was relatively high, especially at the concentration 160 g/L, and the pH value of LB medium increased quickly in short time. However, the pH value changed no more than 0.3.

The hydrogen bonds appeared between water molecules in a series of clusters, and the hydrogen-bonded network was continuously being formed and destroyed (Peeters, 1995). The structure of water clusters have been thought to play an important role in chemical, biochemical and physiological reactions in biological systems (Lo et al., 2000; Liu et al., 1996). It was reported that the activity of water was increased when the water cluster size reduced, and it was reported that small cluster water had numerous useful characteristics by providing accelerated absorption of drugs and food through the digestive tract; it also affects the metabolism and growth of cells (Xia et al., 2006; Peeters, 1995; Zhang et al., 2004).

Electric fields, in general, tend to be the reason for the weakening or destruction of the hydrogen-bonding network of water. 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. Therefore, after immerging with

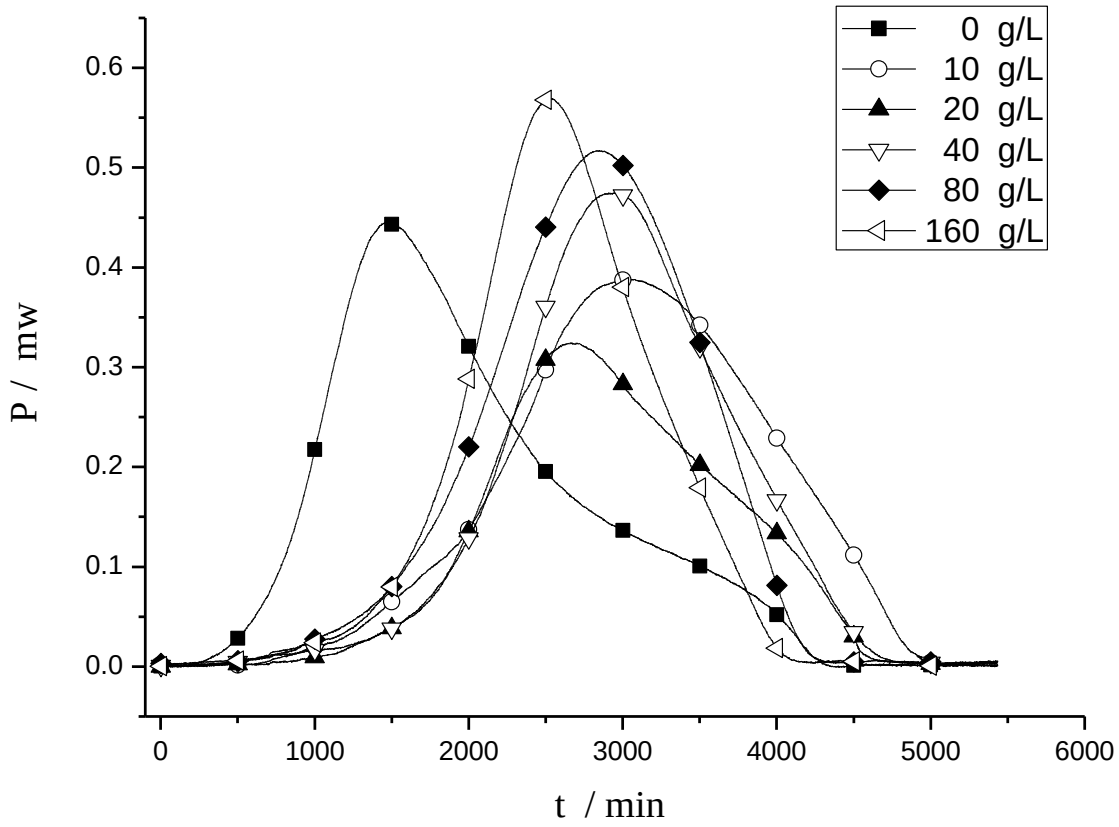

Figure 4. The metabolic thermogenic curves (power-time curve) of *S. aureus* growth in the presence of tourmaline (0, 10, 20, 40, 80, 160 g/L).

Table 4. Parameters of the *S. aureus* growth processes.

Concentration of tourmaline (g/L)	k (min^{-1})	R	t_G (min)	I (%)
0	0.00383	0.99441	181.0	0
10	0.00152	0.99627	456.0	60.31
20	0.00244	0.99387	284.1	36.29
40	0.00228	0.9994	304.0	40.47
80	0.00200	0.99922	346.6	47.78
160	0.00248	0.9997	279.5	35.25

k, Growth rate constant; G, generation time; I, inhibitory ratio.

tourmaline, the size of water clusters reduced as shown by ^{17}O *NMR* results. The change of water clusters induced by tourmaline accounted for some the growth change of microorganisms.

The three microorganisms studied in this paper belonged to different kind of cells. *S. aureus* is a typical Gram-positive bacterium, the cell wall of which is fully composed of peptide polyglycogen. *E. coli* is a typical Gram-negative bacterium, the cell wall of which is made up of a thin-layer peptide polyglycogen and an outer lipopolysaccharide layer.

Unlike the prokaryotic cells (*S. aureus* and *E. coli*), *C. albicans* is a kind of eukaryotic cell (fungus). The cell wall of *C. albicans* is composed of lipid bilayer in which embedded and attached proteins perform many of its functional roles.

Figure 7 shows the relationship between *k* and concentration of tourmaline. In the studied concentration range, tourmaline had different effects on growth of the three different kinds of microorganisms. For *C. albicans*, the values of *k* first decreased and then increased corresponding to the increasing of tourmaline concentration. For *E. coli*, the values of *k* first increased and then decreased corresponding to the increasing of

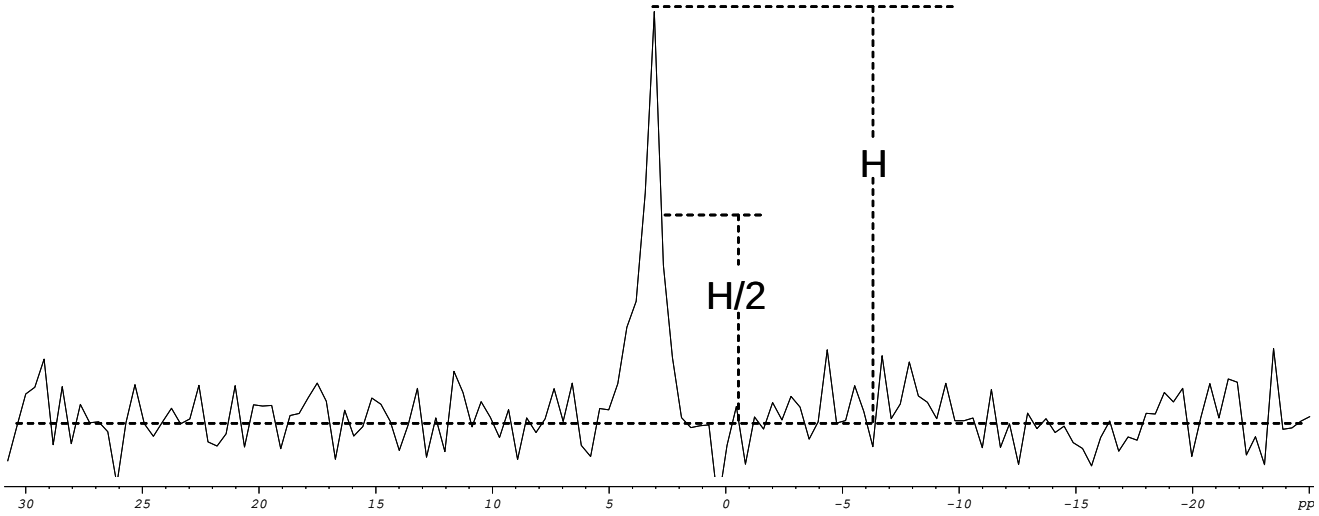

Figure 5. ^{17}O *NMR* spectra of distilled water.

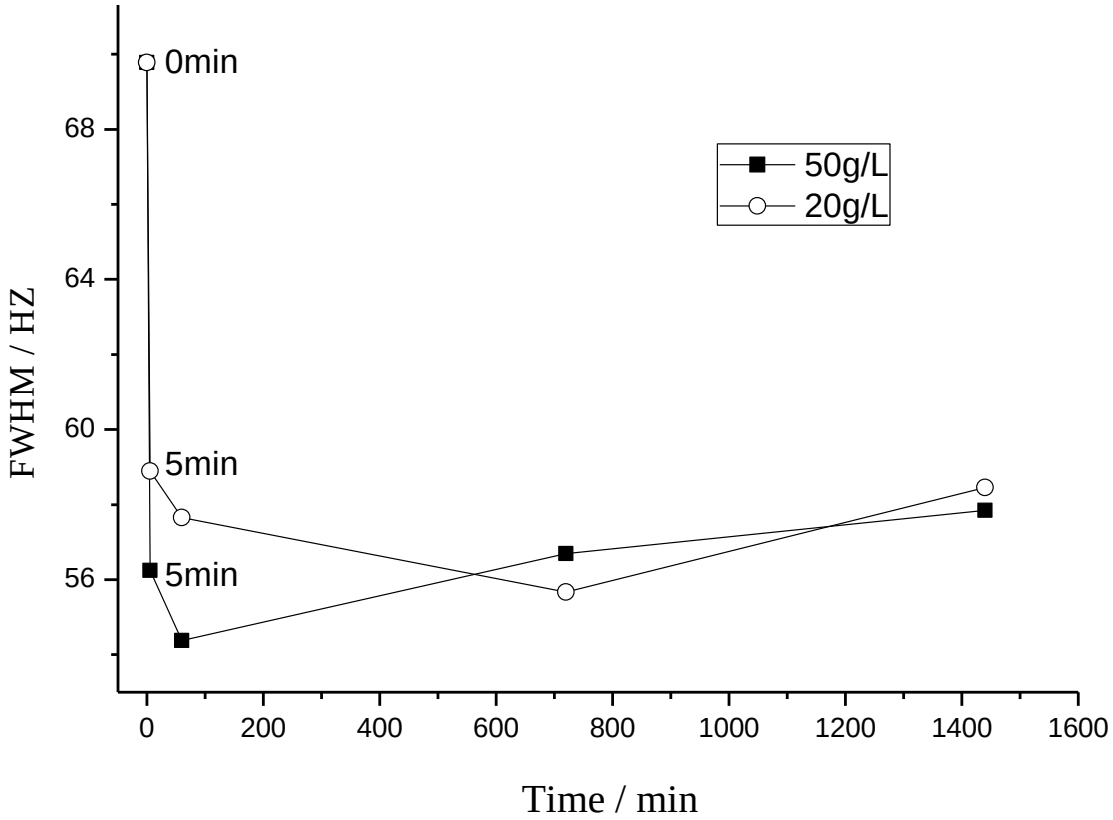

Figure 6. The full width at half maximum intensity (FWHM) change plots according to the immerging time with different concentrations of tourmaline.

tourmaline concentration. However, for *S. aureus*, the discipline between *k* and tourmaline concentration could not be found. In the studied concentration range, the values of *k* were all lower than the sample without tourmaline.

As reported by Coates et al. (2002), multiplying and non-multiplying metabolisms of *E. coli* exist simul-taneously and two exothermic peaks would appear and the curves had been reported by Kong et al. (2011). In TAM air experiment, some oxygen also was sealed in the

Table 5. The pH value of LB medium immerged with tourmaline.

Tourmaline concentration (g/L)	0	10 h	20 h	40 h	80 h	160 h
0	7.00	7.00	7.00	7.00	7.00	7.00
2	7.03	7.06	7.08	7.06	7.09	7.12
5	7.05	7.09	7.06	7.04	7.11	7.16
10	7.08	7.08	7.06	7.08	7.09	7.10
30	7.03	7.03	7.04	7.05	7.18	7.17
60	6.99	7.01	7.09	7.12	7.14	7.21

LB, Luria-Bertani.

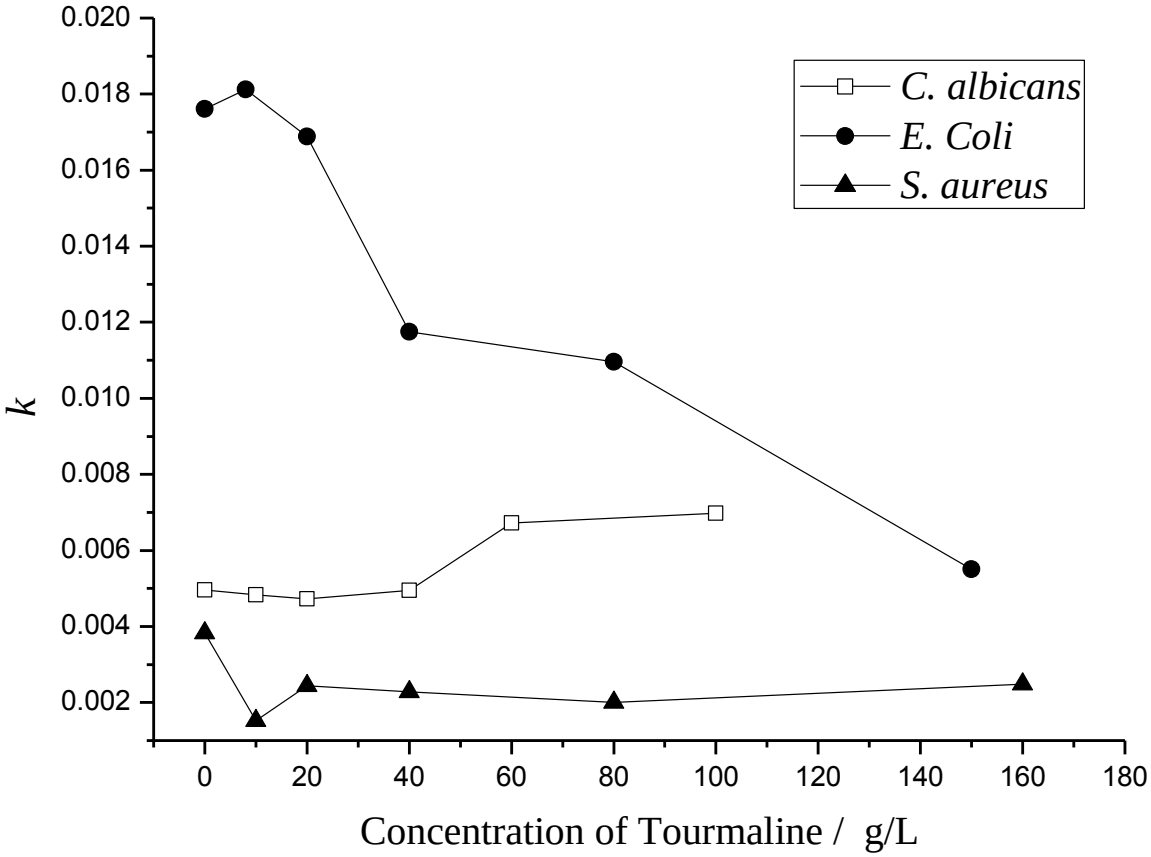

Figure 7. Plots of *k* for the three microorganisms vs. concentration of tourmaline.

ampoule bottle, so the multiplying metabolism process could be more easily preceded and the main heat may be released. Additionally, the different initial cell density and testing conditions may cause different metabolism process. In this paper, only one peak was detected, which may be caused by the cell density. The instrument did not distinguish the two peaks and only one peak was obtained. Therefore, for each serial experiment, a control sample must be performed in order to avoid the error caused by external factors.

Tourmaline effect on microorganism was a very complex process, and it may have different effects on the growth of different kinds of microorganisms. The hydrogen bonds were broken down when treated with tourmaline, so the pH of solution could be changed. The pH adjustment was one obvious property of tourmaline (Nishi et al., 1996). It could adjust the pH value of acidic and alkaline solution. However, the initial pH value of LB medium in this study was 7.00, so the pH change was not obvious. Therefore, in this study pH change may have

little effect on the microorganism growth.

Conclusion

As a widespread mineral with special electric properties, tourmaline could reduce the size of water cluster, which could affect the growth of microorganisms. In this paper, *C. albicans, E. coli* and *S. aureus* were introduced to study the microorganism growth effected by tourmaline. The results showed that high concentration tourmaline inhibited the growth of prokaryotic cells, while promoted the growth of eukaryotic cell. In the meantime, the low concentration tourmaline had complex effects on growth of the studied microorganisms which is still unclear so far. To sum up, this research offered a feasible and facile method to study the effect of minerals on growth of microorganisms. The affect mechanism of tourmaline on microorganism could be clarified with the further study and the help of other methods.

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