ISSN : 0974 - 7435 Volume 6 Issue 6

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 6(6), 2012 [190-194]

Growth and sporulation of *Bacillus mucilaginosus* in a pressure oscillating, solid-state fermentation using peat as an inert support

Jiang Qi-Pei[1]*, Li Zuo-Hu[2]

[1]Institute of Environment Protection of Baoding, Hebei. Baoding, 071051, (P.R.CHINA)

[2]Institute of Process Engineering, Chinese Academy of Sciences, Beijing, 100080, (P.R.CHINA)

E-mail : cellulose420@163.com

Received: 29th July, 2012 ; Accepted: 25th August, 2012

ABSTRACT

Bacillus mucilaginosus was cultured on peat for biofertilizer production in a pressure oscillating, solid-state fermentation. The effects of pressure amplitude, oscillation frequency and moisture content were studied in detail. At desirable culture condition such as 2 atm pressure amplitude, 15 min oscillation frequency and initial moisture content 83.3 %, viable spores of *Bacillus mucilaginosus* reached 1.7×10^8 c.f.u. g^{-1} (Chinese standard: 1×10^8 c.f.u. g^{-1}).

KEYWORDS

Bacillus mucilaginosus; Pressure oscillating solid-state fermentation; Biofertilizer.

INTRODUCTION

Biofertilizers, a cheap source of nurtients, can be used to supplement chemical fertilizer and increase the organic content in the soil[1]. *Bacillus mucilaginosus*, usually called silicate bacteria or K-bacteria in China, is one of the largest output biofertilizers in the world. More than 100 thousands tons per year are produced in China and 80 % of the biofertilizers in China are silicate bacteria[14]. Silicate bacteria, a type of PGPR (plant growth promoting rhizobacteria)[10]. has many advantages over other microbes as a biofertilizer. Silicate bacteria are an excellent N-fixer, K-solubilizer and also can produce many kinds of phytohormones to promote crop growth[12]. Another reason it is so popular in China is that its spore form, has a greater survival rate than other species when mixed with the chemical fertilizer.

Solid-state fermentation (SSF) is microbial growth on or within particles of a solid matrix where the water content is sufficient to assure growth of cells. There has been a resurgence of interest in solid-state culture due to several advantages over submerged culture, such as high yield, low energy consumption and low environmental impact of the process. However, the existence of steep gas concentration and thermal gradients in conventional SSF has hindered further applications in bacteria culture[4]. Recently, it was reported[2,4]. that the pressure oscillation coupled with forced aeration in SSFcould minimize these gradients to a large extent, but mainly in filamentous fungi fermentation. In conventional silicate bacterial production, submerged fermentation (SmF) was usually has been employed, so little information is available on the production of *B. mucilaginosus* by SSF. Therefore, this work was undertaken to evaluate SSF production of *B. mucilaginosus*. To compensate for water loss due to vaporization during periodic pressure oscillation coupled with forced aeration SSF, peat was used as an ideal

solid support for microbial processes due to its high water retention capacity. The purpose of this article was to investigate using peat as an inert carrier for silicate bacteria production in a pressure oscillating, solid-state culture.

MATERIALS AND METHODS

Pressure oscillation

A stainless steel cylindrical vessel 20 cm long × 5.6 cm diameter with total capacity of 0.5 L was used as the bioreactor for these studies. Air pressure amplitude was regulated by modification of a pressure meter fitted on the exhaust gas line of the fermenter. Oscillation of the pressure was manipulated by periodical on-off action of inlet and outlet valves by a controller. A representative profile of oscillating pressure between normal atmospheric pressure (1 atm) and 2.5 atm is shown in Figure 1, where the duration of the high pressure (t_1) was 0 min, whereas and the duration of the low pressure (t_2) was varied.

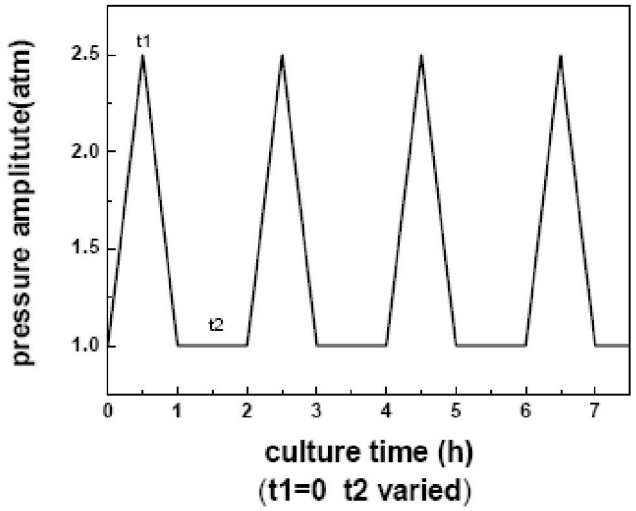

Figure 1: Representative oscillating air pressure from normal atmospheric (1 atm) to 2.5 atm

Microorganism

Bacillus mucilaginosus was obtained from the Institute of Soil and Fertilizer, Chinese Academy of Agricultural Sciences (Beijing China). The organism was cultured at 30 °C for 12h in Erlenmeyer flasks on the Alexandrov medium[12]. containing starch 5 g, sucrose 2 g, $MgSO_4$ 2 g, K_2HPO_4 2 g,$(NH_4)_2SO_4$ 0.5 g, yeast extract 1 g, $FeCl_3$ 0.02 g and per 1000 mL tap water.

Pretreatment of peat and solid state fermentation

Peat was allowed to dry at 105 °C for 3 h, ground to about 2 mm particle size, then autoclaved at 121 °C for 60 min. The resultant peat was mixed thoroughly with the medium at the given ratio. The contents were sterilized for 30 min at 0.1 MPa pressure, then cooled and inoculated with the culture prepared previously, seed charge 10 %, then followed by the incubation at 32 °C for 72 h. Viable cells enumerated in a given time. Pressure oscillation started at beginning of fermentation.

Biomass measurements——viable count method

Viable bacterial counts was estimated using standard plate count technique. Fermented mass, 1 g, was periodically withdrawn from the fermentor and soaked in 100 ml sterile tap water, then shaken vigorously for 30 min with the electronic shaker. The liquor was serially diluted and then plated. The number of viable cells was express as c.f.u. (colony forming units) per gram dry rot (c.f.u. g^{-1}) after applying a correction for moisture in the original sample. Triplicate sample were set up for each experimental variation. Sporulation and growth of microbes were examined by microscope at a given time. Sporulation rate was the ratio of final spores, count to maximum viable cells in 48 h during the fermentation of *B. mucilaginosus.*

RESULTS AND DISCUSSIONS

Pressure amplitude

Unlike other microorganisms as biofertilizer, final fermenting product of *B. mucilaginosus* was spore other than microbe itself. Spore yield was the important index to the silicate bacteria SSF process. As shown in Figure 2, the viable cells of silicate bacteria increase obviously under various pressure amplitudes in all cases, compared to those obtained in a conventional static system (pressure amplitude=1 atm). The higher pressure amplitude was given, the higher maximum viable cells was obtained. However, sporulation did not present the same situation, higher initial growth of silicate bacteria produced by high pressure amplitude can not result in high spore yield. As indicate in Figure 2, the viable cells increased rapidly in 48 h and then declined. The maximal viable spores, 1.66×10^8

FULL PAPER

c.f.u. g^{-1} (SDM), were obtained at the end of sporulation when 2 atm pressure amplitude was selected. Autolyzed cells appeared in the high pressure amplitude fermentation. It was observed by microscope that sporulation started at 54 h, then completely form at 72 h at 2 atm pressure amplitute, comparable to conventional static system sporulation starting 60 h and about 60 % spores in 72 h.

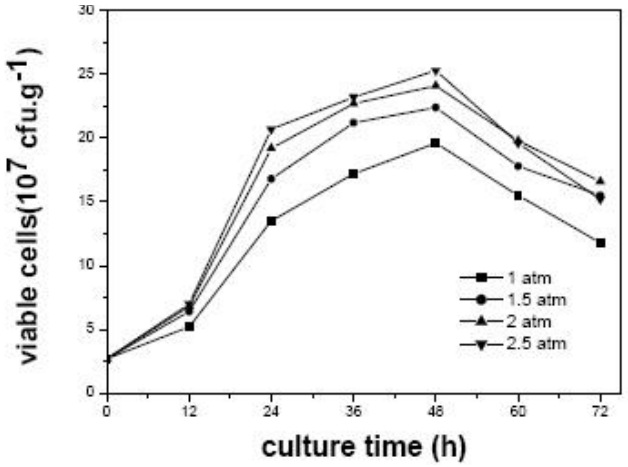

Figure 2 : Viable cells of *Bacillus mucilaginosus* at 83.3 % initial moisture content, 15 min duration of low pressure and various pressure amplitude

Oscillation frequency

Oscillation frequency refers to duration of low pressure in this paper, namely, high oscillation frequency or high airflow rate means short duration of low pressure. As indicates in Figure 2, the viable cells increased with the decrease the duration of low pressure. Evidently, high frequency of pressure oscillation was found to be helpful to increase viable cells at the beginning of fermentation. However, it presents another situation after 48 h. It was observed by microscope that high autolyzed cell number appeared in the high oscillation frequency. The maximum spores yield, 1.66×10^8 c.f.u.g^{-1}, was obtained when the duration of low pressure was 15 min. Growth of silicate bacteria was stimulated under the condition of intense changing of pressure, at the same time, viable cells was easy to decline and autolyze in the high pressure amplitude and airflow rate.

Initial moisture content

The variation of the spores yield of silicate bacteria at different initial moisture content was indictated in Figure 4. The maximal viable spores, was obtained at 72 h

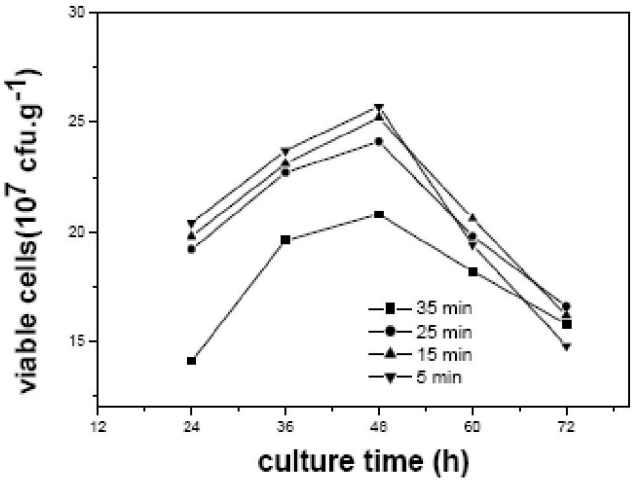

Figure 3 : Viable cells of *Bacillus mucilaginosus* at 2 atm pressure amplitude, 83.3 % initial moisture content and various duration of low pressure

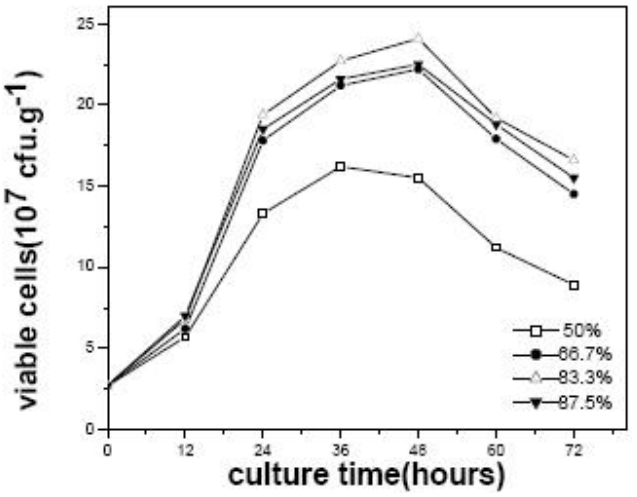

Figure 4 : Viable cells of *Bacillus mucilaginosus* at 2 atm pressure amplitude, 15 min duration of low pressure and various initial moisture content Comparison among peat, wheat straw, corn straw and technical lignin as inert supports

when 1:5 the initial moisture content was chosen. It was well known that water activity can interact with various physical and chemical factors to affect microorganism growth and sporulation in SSF process[8], especially in pressure oscillation SSF. Gas exhaust in pressure oscillation result in much lost of water content. Therefore, high initial moisture content of solid substrate was needed. However, cultivation of microorganism at high initial moisture content levels frequently resulted in substrate particles sticking together. Furthermore, the spaces between the particles were filled by water at higher moisture content, which limits gas exchange. Clearly, the presence of a critical initial moisture con-

tent above which the performance of the pressure oscillation fermentation was indicated. In this experiment, the suitable initial moisture content was 83.3 % and final moisture content of substrate was 71.3 % due to water losses.

Wheat straw, corn straw and technical lignin[3,4]. had been reported as inert support for biofertilizer production. In this experiment, a trial that compared four differents inert support on silicate bacteria production was made.

TABLE 1 Effects of the four different inert support on the viable spores of silicate bacteria at desirable cultural condition.

Types of inert supports	Viable cells ($\times 10^7$ c.f.u.g^{-1})			Viable spores*	Sporulation rate (%)
	24 h	48 h	72 h		
Peat	19.4	25.2	16.8	16.6	65.9
Corn straw	17.2	21.2	15.1	15.1	71.2
Wheat straw	15.5	19.6	14.3	14.3	73.1
Technical lignin	6.8	10.6	8.4	8.4	79.2

Viable spores* ($\times 10^7$ c.f.u.g^{-1})

The variation of the viable cells on different supports was summarized in TABLE 1. Silicate bacteria havest different spore yields in different inert supports. It was obvious in TABLE 1 that peat had been demnonstrated as the best inert support for silicate bacteria production. Adversely, high sporulation rate occurs in the lower spore yield support, such as technical lignin and wheat straw ect. As an alternative, corn straw and wheat straw also can function as inert support for the purpose.

CONCLUSIONS

In conventional aerobic solid-state fermentation systems, interaction of mass tranfer effects with bioreaction plays an important role on the yields and productives of the bioreactor. Although all oxygen concentration are uniform in the SSF systems at the beginning, oxygen concentration gradients develop in the fermentation process owing to mass transfer resistances, which will adversely affect productivity in terms of biomass[4]. However, these gradients might be minimized to a large extent by periodic pressure oscillation in a pressure oscillation solid state fermentation. The forced aeration can increase substrate porosity, which enlarges the total surface of diffusion. Air can be uptaken into and expelled from the intraparticle of the medium. Thus, efficient aeration and heat removal are obtained, which, at last, enhance biomass productivity.

Growth and sporulation of silicate bacteria did not present the same law in the pressure oscillating bioreactor. High pressure amplitude and oscillation frequency can obtain high viable cells number but not high spore yields. Water content of substrate played a important role on growth and sporulation of microorganism, expecially in pressure oscillating reactor. However, 12 % water loss in the course of fermentation can not result in metabolizing deviation but be conducive to sporulation of silicate bacteria. As a conclusion, compared to conventional static fermentation, higher yield was obtained in *B. mucilaginosus* production in the pressure oscillating SSF process.

REFERENCES

[1] S.C.Bhandari, L.L.Somani; Production and quality control in biofertilizers. In: L.L.Somani, S.C.Bhandari, S.N.Saxena, K.K.Vyas, (Eds); Biofertilizers. India: Pawan Kumar Scientific Publishers, 345-353 **(1988)**.

[2] Xiaoyong Zhang, Haitao Mo; A solid-state bioreactor coupled with forced aeration and pressure oscillation, Biotechnology Letters, **25**, 417-420 **(2003)**.

[3] Xiaoyong Zhang, Hua Zhao; Growth of *Azotobacter vinelandii* in a solid-state fermentation of technical lignin, Bioresource Technology, **95**, 31-33 **(2004)**.

[4] Hua Zhao, Xiaoyong Zhang; Growth of *Rhizobium leguminosarum* in a periodic pressure oscillating, solid-state fermentation of wheat straw, Biotechnology Letters, **23**, 827-829 **(2001)**.

[5] Blas Dorta, Jorge Arcas; Sporulation of *Metarhizium anisopliae* in solid-state fermentation with forced aeration, Enzyme and Microbial Technology, **23**, 501-505 **(1998)**.

[6] Blas Dorta, J.Rodolfo; Characterization of growth and sporulation of *Metarhizium anisopliae* in solid-substrate fermentation, Enzyme and Microbial Technology, **19**, 434-439 **(1996)**.

[7] Claudia Lareo, F.Andrea; Characteriztion of growth and sporulation of *Mucor bacilliformis* in solid stater fermentation of an inert support, Enzyme and Microbial Technology, **38**, 391-399 **(2006)**.

[8] T.Arzumanov, N.Jenkins; Effect of aeration and substrate moisture content on sporulation of *Metarhizium anisopliae* var. *acridum*, Process Biochemistry, **40**, 1037-1042 **(2005)**.

[9] Haitao Mo, Xiaoyong Zhang; Control of gas phase for enhance cellulase production by *Penicillium decumbens* in solid culture, Process Biochemistry, **39**, 1293-1297 **(2004)**.

[10] J.Kevin Vessey; Plant growth promoting rhizobacteria as biofertilizers, Plant and Soil, **255**, 571-586 **(2003)**.

[11] S.C.Wu, Z.H.Cao; Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial, Geoderma., **125**, 155-166 **(2005)**.

[12] Alexandrov; Silicate Bacteria (in chinese) [M], Science Press, **(1955)**.

[13] C.P.Sharma, J.Verma; Performance of lignite based carriers on the survival of Rhizobia, Science and Culture, **45**, 493-494 **(1979)**.

[14] Tingwei Chen; Classification and characteristic of *Bacillus mucilaginosus*, Chinese J.of Soil and Fertilizer, **4**, **(2002)**.