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Kinetic Properties of Dissolving Phosphorus by the Phosphobacteria 9320-SD

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Abstract

In this study the kinetic properties of dissolving phosphorus by the phosphobacteria 9320-SD is reported using the powdered phosphorite as a reactive material. The samples were treated with H_2SO_4-H_2O_2 for the whole phosphorus assay. The plant-available phosphorus in the samples was determined by a photometer. Living microorganism cells were numbered in colony-forming units by cultivating on solid media. The results indicated that the rate of product forming had a direct ratio relation with the growing rate of phosphobacteria 9320-SD and its cell density. The kinetic equation of dissolving phosphorus by the phosphobacteria 9320-SD is $\frac{dP}{dt} = \alpha\mu X$, and the kinetic equation of transforming plant-nonavailable phosphorus to plant-available phosphorus is $Y = 4.54 \lg x + 11.23$.

Keywords: Phosphobacteria; dissolving Phosphorus; Kinetics

1. Introduction

When chemical fertilizer is used while farming in a large quantity, it brings about the environmental rich nutrition. This case can be improved by using the biological bacteria fertilizer, since the microorganisms in the fertilizer offer nutrition for plants by dissolving mineral materials with plants growing for a long time. Phosphorus nutrition is essential for plant growing and propagating, whereas plants only absorb the dissolved phosphorus (the plant-available phosphorus). The whole phosphorus quantity in the soil is 0.04%~ 0.10% [1], the available phosphorus that plants can absorb is only 0.001%, leaving the plant-nonavailable phosphorus to be 97.50% ~ 99.00% of the whole phosphorus quantity. The plant-nonavailable phosphorus can be turned into available phosphorus by the phosphobacteria, particularly when there are many phosphobacteria around plant roots. Autoum Ham [2] reported that 54% of the bacteria around the plant roots had the ability of dissolving phosphorus.

The characteristics of microorganisms around plants roots were compared with microorganisms in the soil, Sperber [3] discovered that the kind of phosphobacteria is not of the preponderance kind, although they are mostly around plant roots. In state of nature, the existence of phosphobacteria couldn't offer sufficient phosphorus for plants growing, phosphorus nutrition then become an important factor to restrict plants growing, specially, after plants obtain nitrogen nutrition. If the phosphobacteria biofertilizer is supplied to enter soil around the plant roots, the metabolism process of the phosphobacteria around the plant roots can offer a tiny region of supplying enough phosphorus, and plants will grow rapidly because they will obtain a lot of phosphorus. This case has been verified by scientists [4,5]. Additionally, chemical fertilizers has been used for a considerable long time that the environment of farming soil is changed destroying the structure of the soil, and the cultivated land become hard and plants would not grow in such soil. As a result, the agriculture will not develop continually. The quantity of phosphobacteria can be increased by supplying the biofertilijer including phosphobacteria into the farming soil. When the plant-nonavailable phosphorus is reversed into the plant-available phosphorus, the farming soil is restored by microorganisms. It is necessary for the development of agriculture.

2. Materials and Methods

2.1 Strains and powdered phosphorite

Phosphobacteria: 9320-SD; powdered phosphorite was purchased from Wuqing Chemical fertilizer company, Tianjin City.

2.2 Cultural methods

Phosphobacteria were first inoculated onto solid bacteria culture media and is after growing and microscope testing inoculated into a liquid media for 24 hours cultivation. The quantity of bacteria is counted before preparing for the experiment. Culture media: Mingjina floury phosphorite medium [6].

2.3 Method of counting bacteria

The amounting of living cells was numbered in colony quantity by cultivating on the solid media.

The standard: ①the quantity of bacteria is not very different to the sane diluted concentration.

②suitable degree of dilution is that there are 30-300 colonies on dish contained solid medium [7].

2.4 Determination methods of whole phosphorus and plant-available phosphorus

Samples are first treated by H_2SO_4-H_2O_2 via boiling and digesting and at last clarified by laying in silence for a short time. Motikang agent is then added in upper-liquid and the quantity of whole phosphorus can be tested by methods of a colour nalyzing. The lank control does not have any powdered phosphorite.

The quantity of plant-available phosphorus in which liquid cultivating media with added Motikang agent is directly tested with photometer model: vis-723, with the resulting wave-length as 700 nm. The lank control is a media adding powdered phosphorite and no-inoculating bacteria

3. Results and discussion

3.1 The dynamics concerning the time of the growth of phosphobacteria and the products of plant-available phosphorus

The 50ml liquid media with 0.5g powdered phosphorite was loaded into 100ml bottles. 1ml liquid cultured phosphobacteria was then inoculated into each of the 24 bottles. The bottles were then cultivated in rocking culture box at 30℃.

Table 1. The quantity of phosphobacteria 9320-SD in liquid medium.

Time /d	The quantity of phosphobacteria 9320-SD (cells/ml)		
0	1.26×10^3	1.05×10^3	1.02×10^3
	3.40×10^3	4.20×10^3	2.5×10^3
	1.00×10^3	3.00×10^3	3.00×10^3
1	1.67×10^3	1.23×10^3	1.40×10^3
	8.20×10^3	7.40×10^3	6.30×10^3
	7.00×10^3	1.20×10^4	1.80×10^4
2	1.58×10^5	1.91×10^5	3.38×10^5
	1.39×10^6	8.40×10^5	1.02×10^6
	5.10×10^6	6.40×10^6	7.00×10^6
3	8.00×10^6	8.90×10^6	8.40×10^6
	6.00×10^7	5.90×10^7	5.10×10^7
	7.60×10^8	7.20×10^8	8.40×10^8
4	9.82×10^7	1.23×10^8	7.45×10^7
	1.81×10^8	1.21×10^8	2.41×10^8
	2.70×10^8	3.11×10^8	1.70×10^8
5	1.74×10^9	1.71×10^9	1.76×10^9
	4.53×10^9	6.10×10^9	5.39×10^9
	1.18×10^{10}	9.00×10^9	9.60×10^9
6	5.71×10^8	4.13×10^8	4.87×10^8
	8.50×10^8	7.80×10^8	1.02×10^8
	2.90×10^9	4.70×10^9	n/a
7	1.79×10^7	1.57×10^7	2.85×10^7
	5.20×10^8	5.72×10^8	3.97×10^8
	2.57×10^9	3.18×10^9	3.70×10^9

Three bottles were taken every 24 hours with the aim of testing the quantity of bacteria by numbering colony-forming unites on solid media for 8 days. The testing results of each sample of three bottles were then averaged. Table 1 indicates this outcome. This experiment was repeated three times.

At the same time, by testing the concentration of plant-available phosphorus in cultivated media and using the cultivated media as the blank control, in which no phosphobacteria were inoculated but there was powdered phosphorite. The abscissa indicates the growing time of bacteria and the ordinate exhibits concentration of plant-available phosphorus in cultivated media. The kinetic curve of phospho-bacteria dissolving plant-nonavailable phosphorus is shown in Figure 1. According to literature [7] and the computation principle of the method of counting bacteria on solid media, the abscissa is the time of bacteria growth and the ordinate is the logarithm of quantity of bacteria and this is shown in Figure 2.

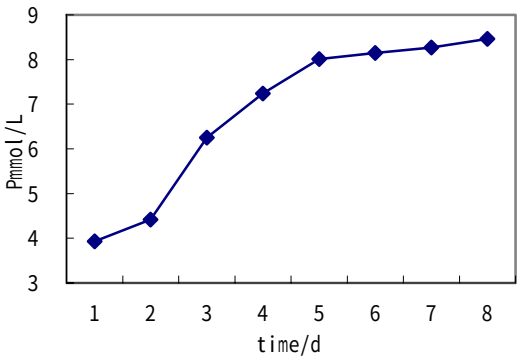

Figure 1. The Kinetics curve of phosphorus dissolving by phosphobacteria 9320-SD.

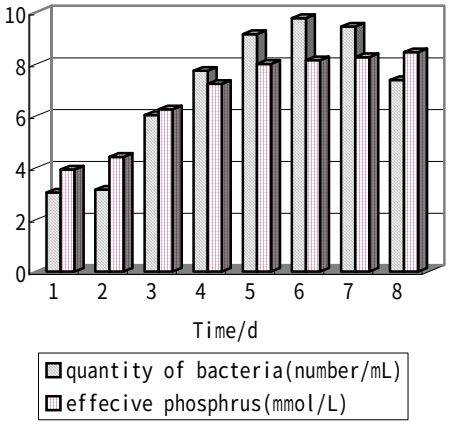

Figure 2. Comparing the quantity of phosphobacteria with the quantity of available phosphorus.

When phosphobacteria was inoculated into the media in which powdered phosphorite was the only phosphorus source, Figure 2 shows that there was a small portion of available phosphorus. Although the quantity of available phosphorus is so smaller, it can

stimulate phosphobacteria growing in the new environment. After the adaptation period, the phosphobacteria grew rapidly and while they grew metabolized plant-available phosphorus from the powdered phosphorite was produced in large scale and the quantity of plant-available phosphorus is as illustrated and it indicates that the plant-available phosphorus was increased with time.

Comparing growth quantity of the phosphobacteria to the molar quantity of available phosphorus, the result of Figure 2 indicates that the growth quantity of bacteria by time is positive related to the molar quantity of produced plant-available phosphorus. Because the both quantities mentioned above are in direct proportion relation, the type of fermenting kinetics is growth being interrelated. According to empirical mathematical model [8], the kinetic equation of speed on forming products and bacteria dissolving phosphorus is:

$$\frac{dP}{dt} = \alpha\mu X$$

where α is a constant of growth relation. μ is the bacterial growth speed and X is the growth quantity of bacteria.

If α represents ratio of forming products, P is the molar quantity of products and X is the growth quantity of bacteria, and

$$\alpha = \frac{P}{x}$$

so the quantity proportion of the forming products (Q_P) is:

$$Q_p = \frac{dP}{Xdt} = \alpha\mu$$

This equation indicates that the ratio of the forming products is in direct proportion to the ratio of bacteria growing and the quantity of bacteria. The quantity proportion of the forming products is only in direct proportion to the ratio of bacteria growing.

Therefore, kinetics of phosphobacterial fertilizer must follow the facts. First, the speed of dissolving phosphorus can be enhanced by increasing the quantity of phosphobacteria. The quantity of phosphobacteria in bacterial biofertilizer is 10^9 per gram biofertilizer. Consequently, in order to increase the quantity of phosphobacteria in rhizosphere soil, using phosphobacteria biofertiliger is a powerful strategy for the purpose of increasing plant-available phosphorus in farming soil. Secondly, the speed of dissolving phosphorus can be improved by choosing the phosphobacteria with the higher ability of dissolving phosphorus.

Microbiological ecologists thought the micro-organisms in soil are commonly in a hungry state. After the phosphobacteria in biofertilizer domicile around the plant roots, nutrient condition around plant roots is the main factor of limiting the growth of bacteria. When the phosphobacteria from biofertilizer are released into rhizosphere soil, the excreting and shedding materials from plants, such as dead root cells will fall off the plant roots and could offer nutrient to phosphobacteria resulting in a better growth of the bacteria and will dissolve more powdered phosphorite. Therefore, if farmers want to obtain more plant-available phosphorus in farming soil by using phosphobacteria biofertilizer, it is essential that the phosphobacteria biofertilizer is correctly used.

3.2 Kinetics of transforming plant-nonavailable phosphorus into available

50ml media is poured into 100ml glass bottles with a total of 14. Powdered phosphorite is added separately with the quantities 0.063, 0.125, 0.250, 0.500, 1.000, 2.000 gram per bottle with each dosage taking up two bottles. Phosphobacteria is then inoculated into the bottles, cultivating in oscillator at 30℃. The experimental process is repeated. After seven days the plant-available phosphorus is mensurated and the blank control is a media of containing powdered phosphorite and no inoculating phosphobacteria. The results are shown in Table 2.

Table 2. The transformation results of plant-nonavailable phosphorus.

Powdered phosphorite(g)	0.063	0.125	0.250	0.500	1.000	2.000
dissolving phosphorus (mmol/L)	4.8	7.2	8.4	10.1	10.7	11.1
	5.4	7.1	8.3	10.2	11.8	12.9
	4.8	7.0	8.0	11.1	12.1	13.2
	5.4	7.3	11.0	9.3	10.3	10.8
Average (mmol/L)	5.1	7.1	8.9	10.2	11.2	12.0

The abscissa exhibits the quantity of plant-nonavailable phosphorus (powdered phosphorite) and the ordinate exhibits the molar quantity of plant-available phosphorus in cultivated media. The outcome is shown in Figure 3. Kinetic curve of transforming phosphorus is drawn in Figure 3A.

Kinetic equation is: $Y=a\lg x+b$ ……①

Y: quantity of effective phosphorus
X: bacterial concentration
a: constant of transforming
b: characteristic constant

The following expressions according to equation ①:

$$\begin{cases} 11.23 = a\lg 1 + b \\ 7.13 = a\lg 0.125 + b \end{cases} \quad \text{……②}$$

The results according to equation ②:

a = 4.54 , b = 11.23

And the kinetic equation accuracy is verified by simulating the curve:

Y_1=4.54 $\log_{10}$0.06 +11.23 = 5.68
Y_2=4.54 $\log_{10}$0.12 +11.23 = 7.05
Y_3=4.54 $\log_{10}$0.25 +11.23 = 8.50
Y_4=4.54 $\log_{10}$0.50 +11.23 = 9.86
Y_5=4.54 $\log_{10}$0.75 +11.23 = 10.66
Y_6=4.54 $\log_{10}$1.00 +11.23 = 11.23
Y_7=4.54 $\log_{10}$1.25 +11.23 = 11.67
Y_8=4.54 $\log_{10}$1.50 +11.23 = 12.03
Y_9=4.54 $\log_{10}$1.75 +11.23 = 12.33
Y_{10}=4.54 $\log_{10}$2.00 +11.23 = 12.59

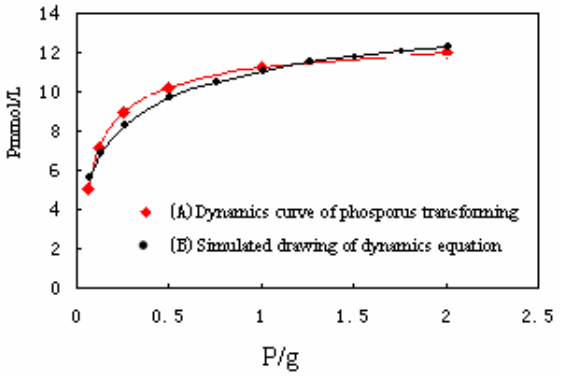

Figure 3. Simulated drawing of kinetics equation via mathematics course.

The mathematical course of fitting kinetic equation is good as fig 3(b) shows. Therefore, kinetic equation of transforming plant-nonavailable phosphorus (Powdered phosphorite) into plant-available phosphorus is: Y =4.54lgx+11.23.

This research has proved a lot of results: Activity and community structure of the microorganism of root border has a tremendous influence on the phosphorus nutrition of the plant [9] [11]. The quantity of the phosphobacteria subsisting in the root border influence the phosphorus nutrition supply of the root border since the phosphobacteria can transform the invalid phosphorus into the plant-available phosphorus via phosphorbacterial metabolism. With increased phosphobacteria quantity, it is promoted that plant-nonavailable phosphorus is transformed into plant-available phosphorus via dissolving powdered phosphorite in farming soil. On the other hand, using the chemical fertilizer for a long time, the cultivated soil will turn hard and change its soil structure but by using the phosphobacteria biofertilizer for a period, the cultivated soil will be restored again. The author has worked with the screening of phosphobacteria, fungi and applying phosphobacteria biofertilizer to soil in laboratory and field, cultivating plants for a long time. We can affirm that widely using the biology fertilizer containing active microorganism could overcome the environmental pollution problem which was caused by use of a large amount chemical fertilizer. The environment of cultivated soil would be improved by the repairing function of the microorganism, it will bring about an advance in lasting agriculture.

4. Conclusions

Because cultivet phosphobacteria experimentation was processed in an environment of limited nutrition with time the growth of phosphobacteria went into the decline period. However when the phosphobacteria biofertilizer was supplied into the farming soil, the plants could offer excreting and shedding materials to phosphobacteria for their growth to continue. The metabolism of phospho-bacteria could produces a large number of dissolving phosphorus and plants can obtain surplus available phosphorus produced by phosphobacteria. It can stimulate the plants to grow rapidly. When the plant-available phosphorus produced by dissolving phosphorus is absorbed unceasingly into plants, the balance point of dissolving-phosphorus kinetics is then moved in the direction of producing plant-available phosphorus, in a tiny ecosystem around the roots. So the phosphobacterial biofertiliter can offer nutrition to plant growth continuously while it is applied into farming soil.

Phosphobacteria can transform plant-nonavailable phosphorus into available. The kinetic equation of transforming plant-nonavailable phosphorus into plant-available phosphorus is: Y=4.54lgx+11.23 and quantity of available phosphorus (Y) is only relationship with bacterial concentration (X) while the phosphobateria growth environment is well.

Acknowledgements

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