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To enhance the reproduction of *Phanerochaete chrysosporium* by adding natural lixiviums in liquid medium

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Abstract: Great promotion to the reproduction of white rot fungus *Phanerochaete chrysosporium* by adding natural lixiviums such as from wood, maize core and potato in liquid medium was found in this research. Incubated in the liquid medium contained 10 mg/L glucose as carbon source with natural lixiviums for three days, the production of mycelium pellet reaches more than 80 g/L, which is 5 times more than that of without natural lixiviums. Incubated in the liquid medium contained 5 mg/L glucose as carbon source with natural lixiviums for three days, the production of mycelium pellet can reach 69.5 g/L, while the production in the medium without natural lixiviums is very low. When the liquid medium contained 1—20 g/L glucose as carbon source, the production of mycelium pellet in 3 d can only reach 12.5 g/L to 14.5 g/L. The fungus in the medium with potato lixiviums are easily contaminated by other microorganisms and in the medium with maize core lixiviums are easily bulking, while in the medium with wood lixiviums are neither easily contaminated nor bulking. Medium with wood lixiviums can produce more pellet than other medium, endure contamination and keep better sedimentation capacity. So that, wood lixivium is better additive to the culture of white rot fungi in liquid medium. Addition of the mixture of wood, maize core and potato lixiviums is of advantage to the production of mycelium pellet. The difference of the production in the medium with different amount of wood lixiviums showed little in the first 3 d, while it expanded after 3 d. Wood lixiviums stimulate the growth of *P. chrysosporium* instead of supply organics which fungi need.

Keywords: white rot fungi; medium; additive

Introduction

White rot fungi have been shown to possess biodegradative capabilities for a wide spectrum of recalcitrant organopollutants, including polycyclic aromatic hydrocarbons(PAHs) (Bogan, 1995; Bumpus, 1985; Dhawale, 1992; Hammel, 1992a; 1992b, 1986), chlorophenols(Armenante, 1994; Hammel, 1992b; Valli, 1991; Joshi, 1993), polychlorinated biphenyls(PCBs)(Bumpus, 1985; Thomas, 1992; Yadav, 1995), munitions waste(Bumpus, 1994; Hawari, 1999; Jackson, 1999; Hodgson, 2000), pesticides(Bumpus, 1987), bleach-plant effluent(Michel, 1991), synthetic dyes(Bumpus, 1988; Knapp, 1995; Swamy, 1999; Cripps, 1990; Larking, 1999), synthetic polymers(Deguchi, 1998; Kirbas, 1999; Kennes, 1994) and creosote(Knapp, 1995). Liquid media are conducted in shake flask experiments to research physiological, biochemical characteristics and biological degradability of white rot fungi. Most of researches adopt synthetic media(Eliana, 2001; Srinivasan, 1995). To promote the growth of white rot fungi, many researchers regulate the ingredients of synthetic media(Ilgi, 2000; Arora, 2000; Zhao, 1998). This results the diversity among most of the medium which decreases the comparative of each research. But few researches on the medium for white rot fungi have been reported. Thus, it is important to make further study on the medium to provide a cheaper and simpler culture medium for the research of the white rot fungi.

Media can be divided into two types according to its ingredients: natural and synthetic. Natural media such as peptone and yeast-extract etc. consist of some unknown natural chemical ingredients, are accepted easily, low-cost and in favor of microorganism growth. Synthetic media consist of definite ingredients are synthesized by strict operation to ensure fixed ingredients. But synthetic media are not in favor of the microorganism growth since it create environment the ingredients are different from that of nature condition (Wang, 1999).

White rot fungi can live better in shake bottle than that of in reactors since they can get higher concentration of nutrition, oxygen and larger interface. The purpose of researches on the white rot fungus in shake bottle is to express its virtue on the potential of degradation, while synthesized medium most of researches used can not provide a better nutrition condition and can not reflect its virtue best. So, it is important to compare the effect on the growth between nature medium and synthesized medium.

This paper is to compare the ability of the production of the white rot fungi mycelia pellet between using the medium with natural lixiviums and the one with only synthetic ingredients, so as to provide

reference for the culture of the white rot fungus, to express the potential of degradation and to suggest a better and cheaper additional nutrition in reactors.

1 Materials and methods

1.1 Microorganisms and culture media

The white rot fungus, *Phanerochaete chrysosporium* BMK-F-1767, provided by Fu Shiyu, researcher of Guangzhou Institute of Chemistry, Chinese Academy of Sciences.

The basic composition of the liquid media is as follows (Arora, 2000): glucose 10 g/L; KH_2PO_4 2 g/L; ammonium tartrate 0.2 g/L; $CaCl_2$ 0.1 g/L; $MgSO_4$ 0.25 g/L; vitamin B_1 5 mg/L; trace elements 1.0 ml/L. The trace elements contained: $CuSO_4 \cdot 7H_2O$ 0.08 g/L; H_2MoO_4 0.05 g/L; $MnSO_4 \cdot 7H_2O$ 0.07 g/L; $ZnSO_4 \cdot 7H_2O$ 0.043 g/L; $Fe_2(SO_4)_3$ 0.05 g/L.

Additive, (1) Potato lixivium: 30 g fresh potato was cut into 1 cm^3 cubes and put into a baker. Adding about 100 ml deionized water into it. It boiled in the electric cooker for twenty minutes, and then filtrated with mesh filter with 40 meshes. The residue was discarded and 1 share of potato lixivium was got. (2) Wood lixivium: 5 g wood was cut into 1 cm^3 cubes and put into a baker. Adding about 100 ml deionized water into it. It boiled in the electric cooker for twenty minutes, and then filtrated with mesh filter with 40 meshes and fast filter paper. The residue was discarded and 1 share of wood lixivium was got. (3) Maize core lixivium: 7.5 g maize core was cut into 1 cm^3 cubes and put into a baker. Adding about 100 ml deionized water into it. It boiled in the electric cooker for twenty minutes, and then filtrated with mesh filter with 40 meshes and fast filter paper. The residue was discarded and 1 share of maize core lixivium was got.

1.2 Apparatus

100A-electronic scale, Denver Instrument Company, U. S. A.

YXQ-SG-280S-portable electric stainless steel steam sterilizer, medical treatment apparatus factory of Shanghai Boxun Industry Inc.

TZ – 2DH constant temperature oscillator, Beijing Wode Electric Experiment Apparatus Factory.

HPS-280 biochemistry incubator, Harbin Donglian Electric Technology Inc.

202-Jinyu brand oven, Jiangsu Dongtai Electric Equipment Factory.

1.3 Experimental methods

On each experiment, each medium ingredients were added into 250 ml flask, and then deionized water were added to make the liquid volume of 200 ml, the flask was sealed with 8 layers of gauze and sterilized at 121℃ for 10 min, cooling to room temperature, *P. chrysosporium* was inoculated into flask and was cultured in the sterilized medium at 30℃, on the 150 r/min shaker. Pellet was collected by filtering the cultured fungus with a 40 mesh net and then weighed

1.3.1 Effects of adding different natural lixiviums on the production of the white rot fungus *P. chrysosporium*

The basic medium with 10 g/L glucose and with adding a share of potato, wood, maize core lixiviums separately. The pellet was filtered and weighed, and the wet weight is conversed into the weight of each liter media can produce.

1.3.2 Effects of concentrations of glucose in different media on the production of the white rot fungus *P. chrysosporium*

Most of the researchers added 10—20 g/L glucose in the liquid medium to produce white rot fungus pellet. But high concentration of carbon source will increase the cost highly in the practice. Thus, it is significant to decrease the concentration of carbon source.

Wood lixiviums, maize core lixiviums and potato lixiviums were separately added into a series of basic liquid media. Glucose concentrations were 0, 1, 2, 5, 10 and 20 g/L separately, incubated for 3 d.

To realize the production rule of the white rot fungus at lower glucose concentration media, this experiment added 5 g/L glucose to realize the 5 d production of the white rot fungus.

Then prolong the culture time to measure the production changing at 1, 2, 5, 10 and 20 g/L glucose concentrations in basic media.

1.3.3 Effects of the mixing additives on the production of the white rot fungus *P. chrysosporium*

Mixtures of wood, maize core, potato lixiviums were added into the basic media. As listed in Table

1, natural additives were added separately into 250 ml flasks, glucose concentration was 5 g/L, other ingredients were added according to the prescription of the basic media, and then, deionized water were added to make the liquid volume of 200 ml. At 1, 2, 3, 4 and 5 d, the pellet was filtered and weighed and the wet weight is conversed into the weight of each liter media can produce.

Table 1 Additive ingredient of the mixing media

Media	Additive ingredient(share)		
	Wood lixivium	Maize core lixivium	Potato lixivium
Media with mixing wood, maize core and potato lixiviums	1/3	1/3	1/3
Media with mixing wood and maize core lixiviums	1/2	1/2	0
Media with mixing wood and potato lixiviums	1/2	0	1/2
Media with mixing maize core and potato lixiviums	0	1/2	1/2
Media with wood lixiviums	1	0	0
Media with maize core lixiviums	0	1	0
Media with potato lixiviums	0	0	1
Basic media	0	0	0

1.3.4 Effect of different quantity of wood lixivium on the production of the white rot fungus *P. chrysosporium*

To understand the effect of different quantity of wood lixivium on the production of the white rot fungus, 0, 0.125, 0.25, 0.5 and 1 share of wood lixiviums were added into the basic liquid media containing 5 g/L glucose. At 1, 2, 3, 4 and 5 d, the pellet was filtered and weighed and the wet weight is conversed into the weight of each liter media can produce.

2 Results and discussion

2.1 Effects of adding different natural lixiviums on the production of the white rot fungus *P. chrysosporium*

Fig.1 shows the effects on the production of the white rot fungus *P. chrysosporium* by adding different natural lixiviums. The result was the production of the white rot fungus in the basic media could only get 14.5 g/L, while the production of the white rot fungus in the nature media could get more than 80 g/L, which is 5 times more than that of basic media. The potato media are easily contaminated by bacteria and became turbid, but this phenomenon did not occur in the wood media. The pellet bulked in maize core media after 7 d culture, but it never occurred in the wood media. So that, when glucose concentration is 10 g/L, the medium with wood lixiviums can best activate the production of the white rot fungus *P. chrysosporium*.

Most of the white rot fungi lives in the forest soil or on the rot wood and get necessary nutrition they need. So that they can get capacity of antagonism to other microorganisms. Maybe this is the reason why the wood media can resist contamination.

This result accords with the growth pattern of the white rot fungus in the natural conditions. The natural growth pattern is that the spore sprouts to mycelium invading into the wood. They grow slowly without optimum environmental condition, and boom very fast while in the proper environmental conditions (Zhao, 1998). White rot fungi are higher fungi which need special complex nutrition, especially organic substances. Synthetic media cannot provide necessary nutrition the white rot fungi need. It is the reason why little natural additive can promote the growth of white rot fungi greatly.

It shows that the medium with natural complex compounds enhance the production of white rot fungus *P. chrysosporium* greatly. This means that white rot fungus lives in synthetic media under ecological stress because of lacking unknown growth factor. So that, it is not a reasonable method by applying synthetic media with simple ingredients to study on the biodegradability of white rot fungi to different organics.

2.2 Effect of glucose concentration in different nature media on the production of the white rot fungus *P. chrysosporium*

Fig.2 shows the effect of different media with 5 g/L glucose on the production of the white rot fungus in 3 d. The production of medium without natural lixiviums is very low. When the basic liquid medium contained 1 g/L between 20 g/L glucose as carbon source, the production of mycelium pellet in 3 d can only reach 12.5 g/L to 14.5 g/L. It can be concluded that the production of the white rot fungus in the

wood media increased greatly when the glucose concentration varies from 0 g/L to 10 g/L, while it changed little when the glucose concentration varied from 10—20 g/L. The production of the white rot fungus in the maize core media increased slowly when the glucose concentration varied from 0—20 g/L. The production of pellet in this medium with 0 g/L glucose concentration could even reach 9.55 g/L. This maybe because of high concentration of glucide substance in maize core provided the carbon source for white rot fungus. The production of the white rot fungus in the potato media increased obviously when the glucose concentration varies from 0—5 g/L, and changed little when the glucose concentration varies from 5—20 g/L.

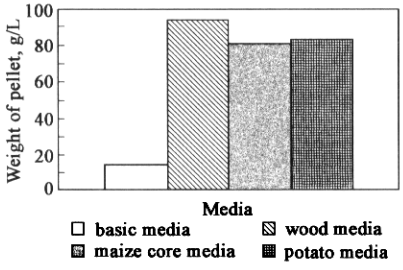

Fig.1 Production of the white rot fungus pellet in basic media and in that with different natural lixiviums with 10 g/L glucose

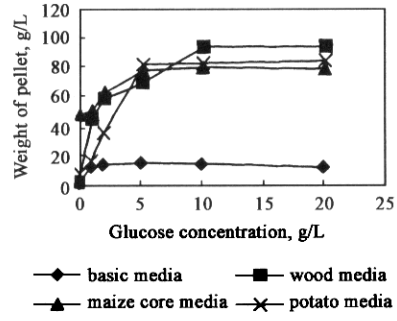

Fig.2 Effect of the glucose concentration in the natural media on the production of the white rot fungus in 3 d

We can see that the production of the white rot fungus increased rapidly when the glucose was lower than 5 g/L, then increased slowly, and changed little later. So that, it is necessary to understand the growth curve in the basic media and natural media with 5 g/L glucose.

Fig.3 shows that when the glucose concentration was 5 g/L, the production of the white rot fungus in the basic media was very low and increased slowly. The production maintained at about 14.5 g/L from 3 to 5 d. The production of the white rot fungus in the natural media could reach 69.5 g/L. The production in the wood media and the maize core media increased persistently in 5 d, while the production in the potato media decreased after 3 d.

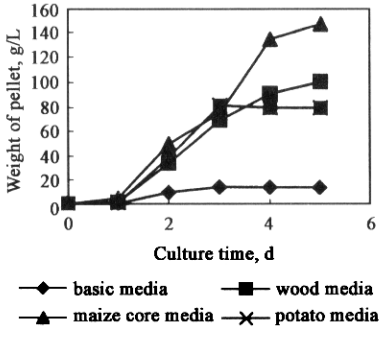

Fig. 3 The effect of different media with 5 g/L glucose on the production of the white rot fungus

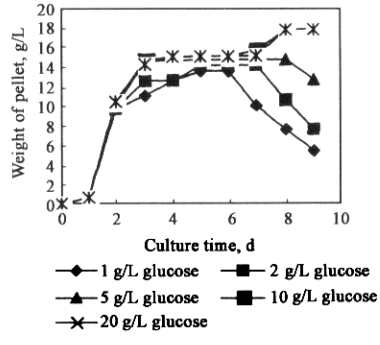

Fig.4 The production of the white rot fungus in the basic media with different glucose concentration varied with culture time

Fig. 4 shows the production of the white rot fungus in the basic media with different glucose concentration varied with culture time. Little difference came into being in the first 6 d. The production of the white rot fungus in the basic media with 1 g/L glucose began to decline at the sixth day, while the production of the white rot fungus in the basic media with 2 g/L glucose began to decline at the seventh day, and the production of the white rot fungus in the basic media with 5 g/L glucose began to decline at the eighth day, and the production of the white rot fungus in the basic media with 10 g/L and 20 g/L glucose went on to increase in the 9 d. So that, we can conclude that the production declined on account

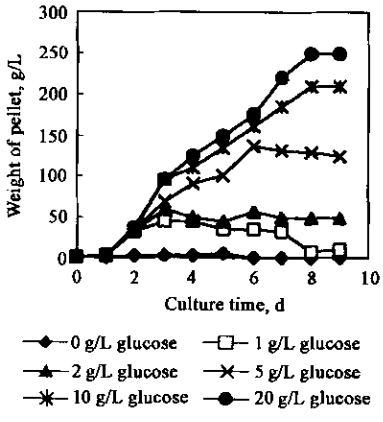

Fig.5 The production of the white rot fungus in the wood media with different glucose concentrations varied with culture time

of carbon source exhausted.

Fig.5 shows the production of the white rot fungus in the wood media with different glucose concentration varied with culture time. It is obvious that the glucose concentration greatly affected the production of the pellet. The production of the white rot fungus in the 1 g/L glucose concentration reached maximum at the third day and declined at 7 d, the production of the white rot fungus in the 2 g/L glucose concentration reached maximum at the third day and declined at 9 d, the production of the white rot fungus in the medium with 5 g/L glucose reached maximum at the sixth day and declined later, and the production of the white rot fungus in the medium with 10 g/L and 20 g/L glucose reached maximum at the eighth day.

In a word, the production of the white rot fungus in wood media increased and not declined for a long time. The glucose concentration affected the production intensively. Wood lixiviums can promote the production of the white rot fungus with every unit of carbon source.

Fig.5 can also shows that the production of the white rot fungus in the wood media without glucose as carbon source reached only 0 g/L. This result showed that the wood lixiviums stimulate the growth instead of providing carbon source it needed.

2.3 Effect of the mixing additives on the production of the white rot fungus *P. chrysosporium*

Fig.6 shows the effect of the mixing additives on the production of the white rot fungus *P. chrysosporium* in three days. The production of the white rot fungus in the medium with all three lixiviums can reach 135.5 g/L, while the production of the white rot fungus in the medium with two lixiviums can reach 99.8 g/L averagely, and the production of the white rot fungus in the medium with one lixiviums can reach 75.4 g/L in average.

The results showed that the complex organic materials do well to the growth of white rot fungus which is consistent with its saprobic characteristic, and the media with simple compounds are limited to its growth.

So that, although it is difficult to rebuild the natural nutrition environment for the white rot fungus, we can add complex natural lixiviums to provide the necessary nutritional elements for it.

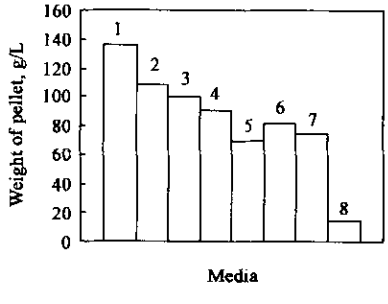

Fig.6 Effect of the mixing additives on the production of the white rot fungus *P. chrysosporium* in 3 d
1. medium with wood, maize core, potato lixiviums; 2. medium with wood, maize core lixiviums; 3. medium with wood and potato lixiviums; 4. medium with maize core and potato lixiviums; 5. medium with wood lixiviums; 6. medium with potato lixiviums; 7. medium with maize core lixiviums; 8. basic medium

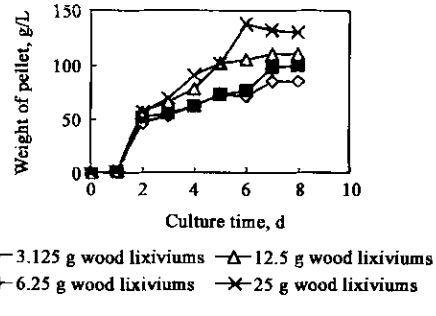

Fig.7 The effects on the production of the white rot fungus *P. chrysosporium* in the media with 5 g/L glucose and different wood lixiviums

2.4 Effects of the medium with different concentration of wood lixiviums on the production of the white rot fungus *P. chrysosporium*

Fig.7 shows the effects on the production of the white rot fungus *P. chrysosporium* of the medium with 5 g/L glucose and different wood lixiviums. From the figure we can see that amount doubled wood lixiviums did not result in the doubling of the pellet production. Especially in the early phase, the

production shows little difference. It can be concluded that minimum complex ingredients can stimulate the growth of the white rot fungus greatly.

3 Conclusions

This experiment shows that great promotion to the production of white rot fungus *P. chrysosporium* by adding natural lixiviums such as from wood, maize core and potato in liquid medium.

(1) Cultured in the liquid medium contained 10 mg/L glucose as carbon source with natural lixiviums for three days, the production of mycelium pellet reaches more than 80 g/L, which is 5 times more than in that of without natural lixiviums.

(2) When the basic medium contained 1—20 g/L glucose as carbon source, the production of mycelium pellet in 3 d can only reach 12.5 g/L to 14.5 g/L. The production of mycelium pellet in the wood medium contained 0—20 g/L glucose can reach 0—94 g/L. The production in 3 d increased little when the glucose concentration increased from 10 g/L to 20 g/L.

(3) Incubated in the liquid medium contained 5 mg/L glucose as carbon source with natural lixiviums for three days, the production of mycelium pellet can reach 69.5 g/L, while the production of the medium without natural lixiviums can only reached only 12.5—14.5 g/L.

(4) High concentration of glucose can prolong life time of the white rot fungus.

(5) The fungus in the medium with potato lixiviums are easily contaminated by other microorganisms and in the medium with maize core lixiviums are easily bulking, while in the medium with wood lixiviums are neither easily contaminated nor bulking. Medium with wood lixiviums can produce more pellet than other medium, has better capacity of resistance to contamination and keeps better sedimentation capacity. So that, wood lixivium is a better additive to the culture of white rot fungi in liquid medium. The difference of the white rot fungus production in the wood medium with different glucose concentration increased with culture time. The wood lixiviums stimulate the growth of the white rot fungus instead of providing carbon source for it.

(6) Addition of the mixture of wood, maize core and potato lixiviums is of advantage to the production of mycelium pellet. The production of the white rot fungus in the medium with three kinds of lixiviums can reach 135.5 g/L, while the production of the white rot fungus in the medium with two lixiviums can reach 99.8 g/L averagely, and the production of the white rot fungus in the medium with one lixiviums can reach 75.4 g/L averagely.

(7) The difference of the production in the medium with different amount of wood lixiviums showed little in the first 3 d, while it expanded after 3 d.

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