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Investigation on the Physicochemical Conditions Affecting the Growth of Mycelial Pellet of White-rot Fungus *(Phanerochaete chrysosporium*)

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The present paper deals with the studies on the influence of various physicochemical conditions on the mycelial growth of white rot fungus (*Phanerochaete chrysosporium*). The factors include glucose concentration, rice hull lixivium in the synthetic medium, temperature, pH, rotational speed and cultivation time in the prevailing environmental conditions. Effect of synchronous factors has been investigated. The best growth of the fungus in terms of dry weight was obtained with the rotation speed of 160 r/min, a temperature of 30°C, rice hull lixivium 0.419 ml/ml, glucose concentration 35 g/L and a pH of 3.5.

Key words: Environment conditions, Culture medium, White-rot fungus, Infection.

The white-rot fungus (*Phanerochaete chrysosporium*) is a kind of saprophytic fungus on wood or timber. The name of white-rot fungus is coming from its action causing the wood rotted with white color. Besides decomposing the lignin, the white-rot fungus has a very strong decomposing ability to xenobiontics that determines its important position and role in environmental science[1]. Therefore, the profound and systematic study on the biological characteristics of the white-rot fungi and the influence of the external environment upon its growth can lay a good foundation for the white-rot fungus widely applied in the environmental protection domain.

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MATERIAL AND METHODS

Instrument

The rotary bottle tank at constant temperature and adjustable speed (HYG-II), made in Shanghai Xinlei Automation equipment factory; Cell counting instrument, made in Korea; microscope(CX31-12C02), made in OLYMPUS.

Culture

The culture from the Institute of Microbiology, Chinese Academy of Sciences

Culture medium

1. Synthetic medium: KH_2PO_4 0.2g/L, $MgSO_4$ 0.5g/L, $CaCl_2$ 0.1g/L, glucose 10g/L, $C_4H_5O_4Na$ 10mol/L, thiamin 0.001 g/L, $NH_4H_2PO_4$ 0.25 g/L, Tween80 0.5mL/L,$CuSO_4 \cdot 5H_2O$ 7mg/L; $ZnSO_4 \cdot 7H_2O$ 0.7mg/L; $FeSO_4 \cdot 7H_2O$ 7mg/L, pH natural[2].

2. Soybean sprout culture medium: Soybean sprout 100g/L, glucose 50g/L, pH natural[3];
3. Zha's culture medium: $NaNO_3$ 2g/L, $FeSO_4$ 0.01g/L, KH_2PO_4 1g/L, cane sugar30g/L, KCI 0.5g/L, $MgSO_4$ 0.5g/L, pH natural
4. PDA culture medium[4].

Spore suspension

After the confected physiological saline sterilized under high pressure, put the white-rot fungus spore in the sterile room to inoculate in physiological saline, counting the spore with the cell calculating instrument to guarantee about 6 million /mL. Shake it uniform and reserve for next using[5,6].

RESULTS AND DISCUSSION

The influence of a single factor of the environmental condition upon the growth of the white-rot fungus.

This experiment has mainly taken the white-rot fungus as research object and researched the environmental conditions that can foster the white-rot fungus to form the ball. In order to enhance the efficiency of the experiment and reduce experimental error, we first studied the influence of the single factor fostering condition upon the forming ball of the white-rot fungus, including the type of culture medium and composition of basic medium, and various environmental factors. According to the existing conditions of the laboratory, it is decided to select six factors such as, the type of culture medium, the glucose concentration, the temperature, pH, the rotational speed, the fostering time for research so as to choose the best condition.

Choice of basic medium

We select four medium. The confected isometric spore suspension 10 mL inoculates into different mediums in the sterile room. Set up 30°(Celsius), pH natural, 140r/min and shake the mediums. The dry weight of pellet can be determined after five day fostering (Fig. 1). The pellets output of the four different mediums in the rotary bottles after 3, 4 and 5 day fostering are shown in Fig. 1. It can be seen that the four kinds of different mediums all can produce the mycelium pellet, and the pellet output of the synthetic medium is higher than other mediums under the same fostering time. This shows that the synthetic medium is more suitable for growing the white-rot fungus. Therefore, the following experiments all select the synthetic medium for fostering test of the white-rot fungus.

Glucose concentration influence

Adjust the glucose concentration as 0, 5, 10, 15, 20g/L in the confected synthetic medium. And inoculate the confected isometric spore suspension into the mediums in sterile room with equal amount of 10 mL. Set the rotary bottle at 30°(Celsius), pH natural, the 140r/min and measure the dry weight of the pellet after five days respectively. (Fig. 2).

The Fig. 2 shows that when the glucose concentration in the medium is of 0g/L, the mycelial Pellet hardly appears. This indicates that the glucose is the important nutrient for growing the white-rot fungus. When the glucose concentration is 5g/L, the pellet starts to grow maintaining at certain quantity. When the glucose concentration increases up to 10g/L, the growth

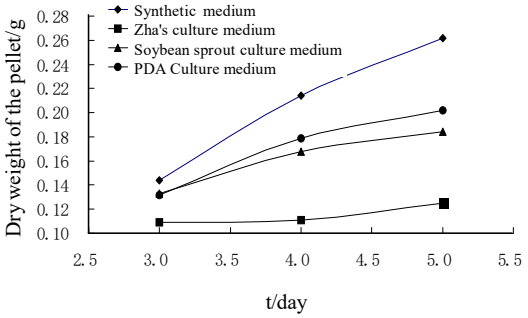

Fig. 1. The influence of four kinds of basic culture medium on the growth of the white-rot fungus

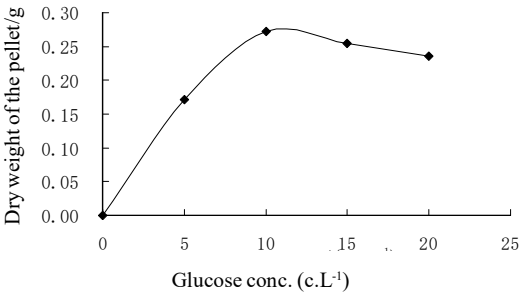

Fig. 2. The glucose density the influence on the growth of the white-rot fungus

quantity of the white-rot fungus appears the maximum value. If we continue to increase the glucose density, its growth quality starts to drop down. Therefore, 10g/L of glucose concentration is selected for the following tests.

Temperature influence

The temperature of the synthetic medium is set at 20, 25, 30, 35, 40°(Celsius) respectively and the confected isometric spore suspension is inoculated into the different mediums in sterile room at the equal amount of 10mL. Measure the dry weight of the pellet after five day fostering in the rotary bottle under the conditions: glucose concentration 10g/L, pH natural, 140r/min Fig. 3.

Fig. 3 shows that when the ambient temperature increases gradually from 20 to 40°(Celsius), the curve of the whole appears M shape. When the temperature is at 20°(Celsius), the growth quantity of the white-rot fungi is the smallest. When the temperature rises up to 25°(Celsius) it grows better. When the temperature is up to 35°(Celsius), the dry weight of the pellets is the maximum. This indicates that this temperature is the most suitable for growing the white-rot fungus. When the temperature continues to increase up to 40°(Celsius), the dry weight of the pellets drops obviously. Therefore, 35°(Celsius) is the best temperature condition for fostering the white-rot fungus.

pH Influence

The pH value of culture medium solution will be adjusted with the solutions of 0.1mol/L NaOH and 0.2mol/L HCl to 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 respectively. Each triangle bottle is loaded with the basic medium of 125mL and the confected isometric spore suspension is inoculated into the different culture mediums in sterile room with an equal amount of 10mL. Set

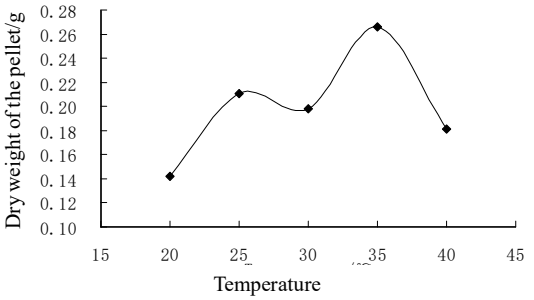

Fig. 3. The ambient temperature the influence on the growth of the white-rot fungus

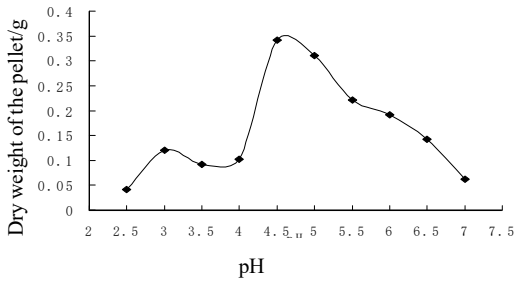

Fig. 4. pH influence on the growth to the white-rot fungus

the rocker machine at 35°(Celsius), glucose concentration at 10g/L, rotational speed at140r/min and measure the dry weight of the pellet after five day fostering respectively (Fig. 4).

Fig.4 shows that the pH value obviously affects the growth of the white-rot fungus. When pH is 2.5~4.0, the growth of the white-rot fungus has small fluctuation. When pH is 4.0, the growth of the pellets starts changing better. Until pH is 4.5, the growth is at the highest point. That is the most suitable growth's pH value is 4.5. And then the increase of the pH value causes the decrease of the dry weight of the white-rot fungus pellet. When the pH value approaches the neutral, the white-rot fungus nearly stops growing. This shows that the acidic environment is suitable for the white-rot fungus to grow.

According to this experiment, we select pH 4.5 as the best pH condition to foster white-rot fungus.

Rotational speed influence

The rotational speed of the synthetic medium is adjusted at 100, 110, 120, 130, 140, 150r/min respectively. Each triangle bottle is loaded with 125mL of the basic medium, and the confected isometric spore suspension will be inoculated into the different culture mediums in sterile room with an equal amount of 10mL. Set the glucose concentration as 10g/L, pH 4.5, temperature at 35°(Celsius), measure the dry weight of the pellet after five day fostering respectively (Fig. 5).

Fig. 5 shows the influence of the rotational speed to the growth of the white-rot

fungus. When the rotational speed is 100-130r/min, the white-rot fungus's growth appears the increasing tendency and when the rotational speed is 130r/min, the white-rot fungus's growth quantity is the most (maximizing). That is the best rotational speed of the white-rot fungus's growth is 130r/min. And when the speed continues to increase from 130r/min to 150r/min, the white-rot fungus's growth quantity drops obviously.

According to this experiment, the rotational speed of 130r/min is the best condition to be selected for fostering the white-rot fungus.

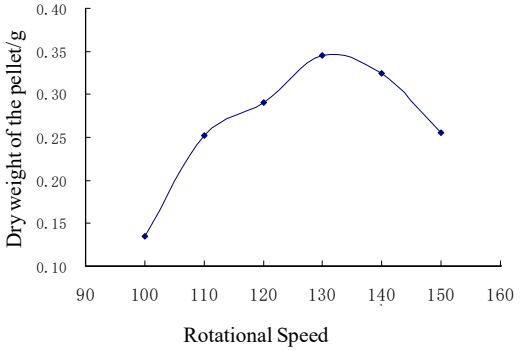

Fig. 5. Rotational speed the influence which grows to the white-rot fungus

Fostering time influence

The fostering time of the synthetic medium is adjusted respectively as 3, 4, 5, 6, 7 and 8 days. Each triangle bottle is loaded with the basic medium of 125mL and the confected isometric spore suspension is inoculated into the triangle bottle in sterile room with an equal amount of 10mL. Set the glucose concentration as 10g/L, pH 4.5, temperature at 35°(Celsius), the rotational speed 130r/min. After five days measure the dry weight of pellet (See Fig. 6).

Fig. 6 shows that along with the increase

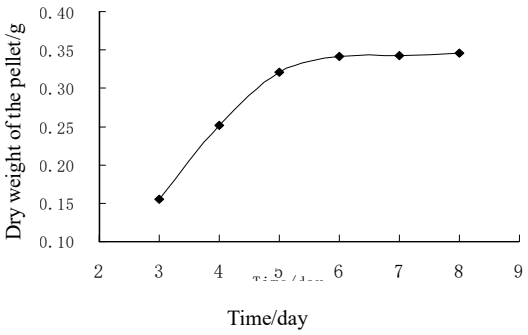

Fig. 6. The influence of Culture time on the growth of the white-rot fungus

of fostering time, the white-rot fungus is growing in quantity. Especially during the 3rd to 6th day the growth is obviously increase. By the Sixth day, the growth is up to the maximum value. And after the 6th day, the white-rot fungus nearly does not grow in quantity.

Therefore 6 days is the best fostering time upon the growth of the white-rot fungus.

Influence of adding the different allogenic material into the medium

Take the lumber, the corncob, the rice hull, the bran, bamboo, each for 20g Respectively, add the distilled water of 500mL, and boil 30min on the electric stove, filtrate with 8 layer pledget, take supernatant(fluid) waiting for use.

Adjust the synthetic medium, separately increase about 25mL of the lixivium of the lumber, the corncob, the rice hull, the bran, the bamboo and inoculate the confected isometric spore suspension in the Triangle bottles in sterile room with an equal amount of 10mL respectively. Set the glucose concentration as 10g/L, pH 4.5, at temperature 35!(Celsius), the fostering time of 6 days, the rotational speed 130r/min. After six days measure the dry weight of pellet (See Fig. 7).

Fig. 7 shows the influence of adding different lixivium into the culture medium upon

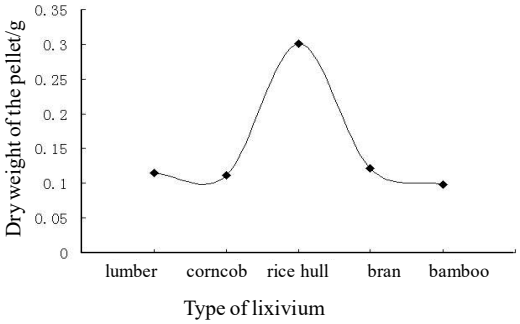

Fig. 7. The influence of different matteron the growth of the white-rot fungus

the growth of the white-rot fungus. The first adding the rice hull lixivium into the culture medium the white-rot fungus grows best, obviously higher than other four kinds and the lixivium of lumber and bran are next. The lixivium of corncob and bamboo is worst to

Table1. Factor and level of orthogonal experiment

S. No.	A Rotational speed (r/min)	B Lixivium (rice hull)mL/mL	C Glucose conc. (g/L)	D pH	E Temperature (°(Celsius))
1	140	0.359	25	4	38
2	120	0.286	15	4.5	30
3	80	0.419	5	3.5	35
4	160	0.468	35	3	26

Table 2. Intiutive analysis of orthogonal experiment $L_{16}(4^5)$

	A	B	C	D	E	Experimental result
Experimental No.	Rotational spped	Lixivium (rice hull)	Glucose conc.	pH	Temperature (Celsius)	dry weight of pellet (g)
1	1(140r/min)	1(0.359mL/mL)	1(25g/)	1.(4.0)	1(38)	0.2830
2	1	2(0.286mL/mL)	2(15g/)	2(4.5)	2(30)	0.1050
3	1	3(0.419mL/mL)	3(5g/)	3(3.5)	3(35)	0.3285
4	1	4(0.468mL/mL)	4(35g/)	4(3.0)	4(26)	0.1944
5	2(120r/min)	1	2	3	4	0.1388
6	2	2	1	4	3	0.1462
7	2	3	4	1	2	0.8186
8	2	1	3	4	2	0.3114
9	3(80r/min)	1	3	4	2	0.1662
10	3	2	4	1	2	0.2355
11	3	3	1	2	4	0.1747
12	3	4	2	1	3	0.2221
13	4(160r/min)	1	4	2	3	0.6112
14	4	2	3	1	4	0.1692
15	4	3	2	4	1	0.5318
16	4	4	1	3	2	0.8136
K1	0.9109	1.1992	1.4175	1.4929	1.3617	
K2	1.415	0.6559	0.9977	1.2023	1.9034	
K3	0.7895	1.8536	0.9753	1.5164	1.308	
K4	2.1258	1.5415	1.8597	1.0386	0.6771	
1	0.228	0.300	0.354	0.373	0.340	
2	0.354	0.164	0.249	0.301	0.476	
3	0.200	0.436	0.244	0.379	0.327	
4	0.531	0.385	0.465	0.260	0.169	
Range(R)	1.3273	1.1977	0.892	0.4778	0.169	
Primary→Secondary			AEBCD			

Note: The solution volume in the triangle bottle is 200mL.

influence the white-rot fungus growing. Therefore, the following experiments we select the lixivium of rice hull as the allogenic material of basic medium.

The influence of multi- factors of synthesis conditions on the growth of the white-rot fungus

According to the analysis on the single factor, we decided to select the synthetic medium for the basic experiment. Separately select the rotational speed, the lixivium capacity, the temperature, pH, the glucose concentration as 5 factors at 4 levels, to carry on the orthogonal experiments on the white-rot fungus (Table 1).

Table 2 shows the data obtained in 16 groups of orthogonal experiments. This experiment took the dry weight of the pellet as the target. According to the calculation of the K value and the range difference R, the best plan is A4E2B3C4D3. that is, when the rotational speed 160r/min, the temperature at 30°(Celsius)the rice hull lixivium is 0.419mL/mL, glucose concentration 35g/L, the pH 3.5, the growing quantity is the biggest.

According to the intiutive analysis on the data of orthogonal experiments, we carried out the variance analysis and obtained the F critical value and significance(Table 3).

The Table 3 shows that the rotational speed is extremely remarkable to influence the mycelium pellet output. It is the primary factors to affect the mycelium pellet output.

According to the intiutive analysis on the data of the orthogonal experiments, draw up the diagram effect curves of orthogonal experiments (Fig. 8). The diagram of effect curves shows that the temperature, the rice hull lixivium, pH have obtained the best value and the rotational speed, the glucose concentration may continue to be tested.

Table 3. Variance analysis of orthogonal experiment

Factor	Sum of squres	Degree of freedom	F/	F(Critical value)	Significance
Rotate speed	0.274	3	1.450	9.280	***
Lixivium(rice hull)	0.197	3	1.042	9.280	
Glucose conc.	0.131	3	0.693	9.280	
pH	0.040	3	0.212	9.280	
Temperature	0.189	3	1.000	9.280	
Error	0.19	3			

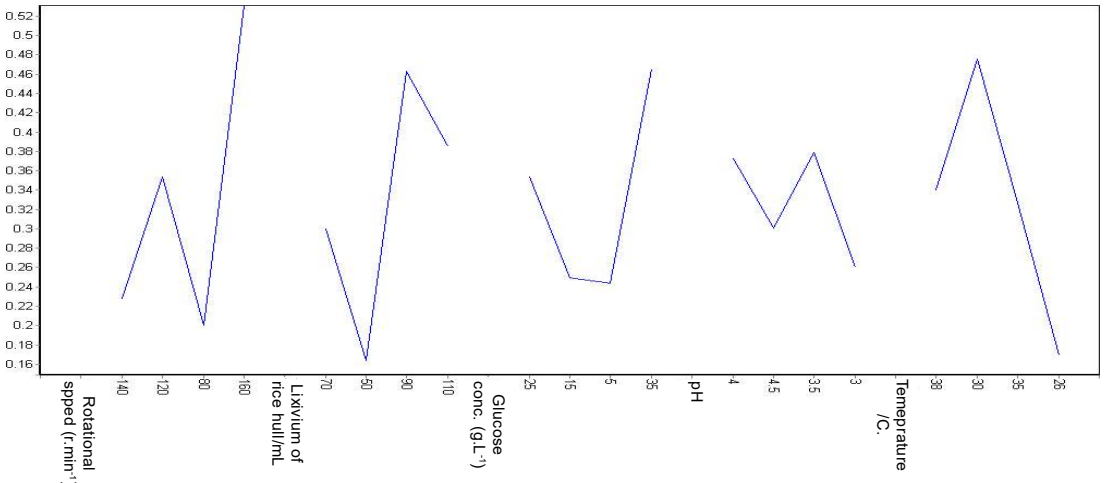

Fig. 8. Trend map of orthogonal experiment

Table 4. Interaction of temperature and pH

pHTemperature	4	4.5	3.5	3
38	0.283	0.311	0.235	0.532
30	0.819	0.105	0.814	0.166
35	0.222	0.611	0.329	0.146
26	0.169	0.175	0.139	0.194

The solution volume is 200mL in the triangle bottle.

Fig. 9. Shape of white-rot fungus mycelial pellet

According to the best plan obtained, set up rotational speed 160r/min, the temperature 30°(Celsius), rice hull lixivium 0.419mL/mL, glucose concentration 35g/L, the pH value 3.5 for the confirmatory test. The obtained result of pellet dry weight is 0.9125g(4.563g/L). The output is the highest in orthogonal experiment groups. This indicates that the result obtained by the orthogonal experiment is correct.

At the same time, we have analyzed the diagram of effect curves and researched the interaction with two factors of temperature and pH (Table 4).

Table 4 shows the influence upon the growing quantity of the mycelium pellet when the temperature and pH are used as two interaction factors. The research found that the interaction is obvious when the temperature at 30°(Celsius), pH4.0. The pellet dry weight up to 0.819g(4.095g/L).

CONCLUSION

1. Through the experiment of single factor influence, we found that the type of culture medium, different lixivium, glucose concentration, pH, temperature, rotational speed and fostering time are the primary factors affecting the growth of the white-rot fungus (Fig. 9).
2. Through multi-factors synthesis influence experiment, we have obtained that the best plan is rotational speed: 160r/min, the temperature: 30°(Celsius), rice hull lixivium: 0.419mL/mL, glucose concentration: 35g/L, pH 3.5.
3. Through the confirmatory test, we validated the best plan of the orthogonal experiment and obtained mycelium pellet 0.9125g(viz. 4.563g(dry weight)/L). The output is the highest in orthogonal experiment groups. This has indicated that the result obtained by orthogonal experiment is correct.
4. Through the variance analysis, we discovered that the rotational speed is extremely remarkable to influence the mycelium pellet output. It is the main factor to affect the mycelium pellet output. The temperature is remarkable to influence the mycelium pellet output.
5. Through the diagram of effect curves, we discovered that the temperature, the rice hull lixivium, pH have the best effect and the rotational speed, the glucose concentration may continue to be tested.
6. The temperature and pH have reciprocal effect. When 30°(Celsius), pH4.0 the interaction is obvious.

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