Optimization of Trichoderma Fermentation Medium

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Abstract. In this paper, the initial growth curve of Trichoderma spp. Was used to determine the stable growth period of Trichoderma spp. And the best age of Trichoderma spp. Then, using the dry weight of Trichoderma as an indicator, the single factor analysis the carbon source, nitrogen source and inorganic salt ion were determined respectively, and the best fermentation medium was screened out. To provide a theoretical basis for liquid fermentation of Trichoderma.

1. Introduction
At present, bio-control technology is widely used in China. Trichoderma resources are abundant, various types and effects are different. At the same time, various preparations of Trichoderma are gradually complete, and its significant role in the prevention and control of plant diseases is increasingly recognized. The main experimental contents of this thesis are as follows: Firstly, the biological characteristics of Trichoderma were determined. [1-3] the dry weight of the fungus was used as the index, and the best carbon source, nitrogen source and inorganic salt ion were screened by single factor analysis. The best Trichoderma fermentation medium was obtained.

2. Medium preparation
Solid medium: The peeled potatoes were added to sterile water at a ratio of 200 g/L, heated and boiled, and continuously stirred to form a paste, which was filtered with eight layers of gauze, and glucose was added to the filtrate at a ratio of 20 g/L. Agar was added in a ratio of 15 g/L, and after it was dissolved, it was cooled to room temperature and then brought to an appropriate volume. It was then placed in a steam sterilizer and sterilized at 120 ° C for 30 minutes. After removal, place the plate in a sterile environment in a clean bench.

Liquid medium 1: The peeled potatoes were added to sterile water at a ratio of 200 g/L, heated and boiled, and continuously stirred to form a paste, which was filtered with eight layers of gauze and added to the filtrate at a ratio of 20 g/L. Glucose, after it is dissolved, is cooled to room temperature and then brought to the appropriate volume. It was then placed in a steam sterilizer and sterilized at 120 ° C for 30 minutes.
3. Determination of the initial growth curve of Trichoderma

In a sterile environment, the spores on the activated solid medium scraped off with an inoculating loop are added to an appropriate amount of sterile water, and dispersed and mixed as a seed liquid by aseptic operation.

Under aseptic conditions, 1 mL of the seed liquid was pipetted into 16 bottles of liquid medium 1, and the inoculated liquid medium was placed in an environment of 28 °C, 180 r/min for cultivation. Trichoderma biomass was measured every twelve hours.

The mass of the centrifuge tube was weighed by an analytical balance, and the whole fermentation broth in the flask was centrifuged at 5000 r/min for 20 min, and the supernatant was aspirated. The precipitated cells were further added with deionized water, centrifuged again, and the supernatant was aspirated, and the above operation was repeated twice. The precipitating bacteria were placed in a digital blast drying oven, dried at 80 °C to constant weight, and the analytical balance was weighed to obtain G2, and the biomass of the cells was calculated.

\[ G = G_2 - G_1 \]

The bacterial biomass is the ratio of dry weight G to sample volume.

4. Drawing of Trichoderma growth curve

The dry weight of Trichoderma spp. was sampled every 12 hours and plotted as a growth curve, Figure 1 below. It can be seen from Fig. 1 that at 0-84 hours, the mycelium of the Trichoderma grows very fast, the lag period is short and almost no, and it quickly enters the logarithmic growth phase. The Trichoderma mycelium is a soft spherical shape of different sizes, and the Trichoderma culture medium is relatively clear at this stage. During the period of 84-144 hours, the dry weight of Trichoderma mycelium was basically stable and was in a stable period. Due to the continuous consumption of nutrients in the medium, the accumulation of Trichoderma metabolites appeared, and the liquid medium appeared cloudy and viscous. During 144-192 hours, the dry weight of Trichoderma mycelium decreased continuously, the liquid medium was brown and viscous, and the Trichoderma cells adhering to the wall of the conical flask showed green spores. Under the biomass of Trichoderma, the nutrient consumption in the fermentation broth and the metabolites are not conducive to the growth of Trichoderma, and the autolysis of the bacteria occurs. It can be seen from Figure 1 that the stable period of Trichoderma is between 84 and 144 hours. When screening the best carbon source, nitrogen source, inorganic salt, the fermented biomass of Trichoderma should be selected between 84-144 hours, so the fifth day is preferred.

![Figure 1. Trichoderma initial growth curve](image-url)
5. Effect of Different Carbon Sources on Fermentation of Trichoderma

The culture medium to which different carbon sources were added was cultured at 28 °C, 180 r/min for five days, and the dry weight of Trichoderma mycelium was measured. The results are shown in Table 1 below. A bar chart is drawn from the data in the table, as shown in Figure 2. It can be seen from Fig. 2 that the dry weight of the mycelium of Trichoderma is not much different. Compared with the blank control group, the utilization rate of the six carbon sources of Trichoderma is very strong, and both the monosaccharide and the disaccharide and the polysaccharide can satisfy the wood. Mildew needs for carbon sources. Among them, maltose is more favorable to the mycelial growth of Trichoderma than other carbon sources, followed by sucrose, glucose, glycerol and lactose.

Table 1. Biomass of Trichoderma under different carbon sources

| Carbon source | Concentration | Trichoderma dry weight g/100mL |
|---------------|---------------|-------------------------------|
| glucose       | 20g/L         | 0.86                          |
| lactose       | 20g/L         | 0.79                          |
| maltose       | 20g/L         | 1.03                          |
| sucrose       | 20g/L         | 0.96                          |
| glycerin      | 2mL/100mL     | 0.83                          |
| blank         | 0g/L          | 0.27                          |

![Figure 2: Effect of Different Carbon Sources on Fermentation of Trichoderma](image)

6. Effect of Different Nitrogen Sources on Fermentation of Trichoderma

The culture medium to which different nitrogen sources were added was cultured at 28 °C, 180 r/min for five days, and the dry weight of Trichoderma mycelium was measured. The results are shown in Table 2 below. A bar chart is drawn from the data in the table, as shown in Figure 3. It can be seen from Fig. 3 that the dry weight of Trichoderma mycelium differs greatly with different nitrogen sources, and the organic nitrogen source is obviously more favorable than the inorganic nitrogen source for the fermentation of Trichoderma. Among the three organic nitrogen sources, peptone Yeast extract is most beneficial to the fermentation of Trichoderma bacteria.
Table 2. Biomass of Trichoderma under Different Nitrogen Sources

| Nitrogen source          | concentration | Trichoderma dry weight g/100mL |
|------------------------|---------------|-------------------------------|
| Potassium nitrate      | 3g/L          | 0.28                          |
| Urea                   | 3g/L          | 0.11                          |
| Ammonium sulfate       | 3g/L          | 0.17                          |
| Beef cream             | 30g/L         | 0.62                          |
| Peptone                | 30g/L         | 0.89                          |
| Yeast extract          | 30g/L         | 0.83                          |
| blank                  | 0g/L          | 0.25                          |

Figure 3. Effect of Different Nitrogen Sources on Fermentation of Trichoderma

7. Effect of Inorganic Salt Ions on the Growth of Trichoderma Strains

The culture medium to which different inorganic salts were added was cultured at 28 °C, 180 r/min for five days, and the dry weight of Trichoderma mycelium was measured. The results are shown in Table 3 below. A bar chart is drawn from the data in the table, as shown in Figure 4. As can be seen from Figure 4, magnesium ions can significantly promote the growth of Trichoderma hyphae, and the dry weight of Trichoderma mycelium is more than twice as high as that of the blank group. It is possible that the growth of Trichoderma has a greater demand for magnesium ions. Calcium chloride can also increase the dry weight of the fungus of Trichoderma, which is less effective than magnesium sulfate. Secondly, ferrous sulfate and sodium chloride also have some promoting effects on the growth of Trichoderma. Manganese sulfate and potassium chloride have no significant effect on the growth of Trichoderma.

Table 3. Biomass of Trichoderma under Different Inorganic Salt Ions

| Inorganic salt         | concentration | Trichoderma dry weight g/100mL |
|-----------------------|---------------|-------------------------------|
| Magnesium sulfate     | 2g/L          | 1.05                          |
| Ferrous sulfide       | 2g/L          | 0.7                           |
| Potassium chloride    | 2g/L          | 0.49                          |
| Calcium chloride      | 2g/L          | 0.82                          |
| Sodium chloride       | 2g/L          | 0.72                          |
| Manganese sulfate     | 2g/L          | 0.45                          |
| Blank                 | 0g/L          | 0.49                          |
Trichoderma belongs to the genus of fungi. In agriculture, it not only plays a role in biocontrol and promotes the growth of crops, but also greatly reduces the use of pesticides, thereby improving soil quality and reducing environmental pollution. In industrial production, Trichoderma can antagonize a variety of fungi, and it is not easy to introduce bacteria to reduce the yield during the fermentation process. At the same time, the metabolism of Trichoderma is fast, and the yield is also increased. Combining the many advantages of Trichoderma, Trichoderma has diverse development prospects in the future.

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