NITROGEN NUTRITION OF *AGARICUS BISPORUS*

II. UREA AS THE NITROGEN SOURCE OF *AGARICUS BISPORUS*

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Research supported in part by Grant 55/56-MRF-CM-1, JCRR.

ABSTRACT

A comparism of the utilization of nitrogen sources from ammonium sulfate and urea by *Agaricus bisporus* was investigated. Better response in yield of the fungus from urea nitrogen was observed. The pH of the culture solution was also noted. An increase of pH of the culture solution was obtained when the urea served as nitrogen source. In case ammonium sulfate was used, the pH of the culture solution decreased.

INTRODUCTION

Previous paper (Hsu and Hu, 1967) described the utilization of ammonium nitrogen by the cultivated mushroom *Agaricus bisporus*. Two points should be taken into account in further studies. First, the presence of ammonium ions at high concentration affects the growth of mycelium. Second, the utilization of ammonium nitrogen leaves the anion radicals affecting the pH of the culture solution. In many instances, the decrease of pH is sufficient to reduce the growth. This work was done in order to elucidate these difficulties observed during the culture of the common mushroom.

Urea is generally recognized as a utilizable nitrogen source by the fungus possesing the urease enzyme (Cochrane, 1958). The enzyme hydrolyzes urea into ammonia and carbon dioxide. The resulting reaction makes the culture solution has little change of the pH. This paper reported several experiments which were carried out by using urea as the sole source of nitrogen.

MATERIALS AND METHODS

The methods used in this work did not differ materially from those used previously (Hsu and Hu, 1967). The urea solution was sterilized by the bacteriological fritted filter. The required ammount of urea was added to each flask of the other component aseptically. Ammonium sulfate was compared with urea. Measurement of total cellular nitrogen by micro-Kjeldahl method (Strouts *et at.*, 1962) was employed for determination of synthesis of protoplasm in terms of fungus growth. Dry weight of the mycelium was also taken after ovendry (Hsu and Hu, 1967). The pH of the filtrates was noted immediately after removal of the mycelium.

RESULTS

THE EFFECT OF CONCENTRATION OF NITROGEN UPON THE GROWTH OF *AGARICUS BISPORUS* AND PH OF CULTURE FILTRATES:

Economically speaking, the growth of the fungus was effecient when the urea nitrogen was at about 1,000 mg/l (Fig. 1). The concentration of urea nitrogen, up to 2,000 mg/l, gave no significant increase in mycelial production. Similar results were obtained either based on dry weight or determined by mycelial total nitrogen content. The nitrogen requirement from ammonium sulfate for maximum growth was about 800 mg/l. A decrease of fungus growth was observed as the ammonium nitrogen increased beyond 1,000 mg/l. At any given concentration, urea always gives a higher yield as compared with ammonium sulfate.

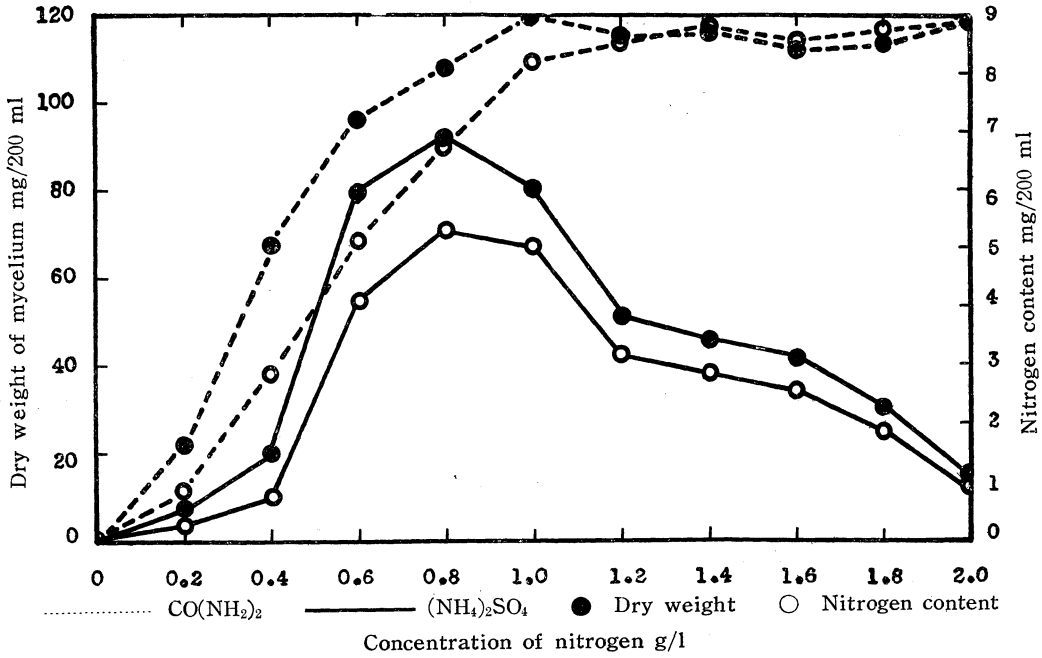

Fig. 1. The effect of the concentration of nitrogen on the growth of *A. bisporus*

The pH of the culture solution at the end of the growing period was also noticed. In all cases when the ammonium sulfate was used, the solution became acidic. The pH of the solution dropped to 3.4–3.6. In case where urea was used, the pH generally became slightly basic or neutral. When smaller amount of urea was used, the final pH was more or less neutral. As the urea nitrogen increased in the medium, the final pH increased.

THE EFFECT OF NITROGEN ASSIMILATION UPON THE GROWTH OF *AGARICUS BISPORUS* AND PH OF THE MEDIUM:

The growth of the fungus in the synthetic solution at 800 mg/l nitrogen as affected in the lapse of the time for incubation of cultures is illustrated in Fig. 2-A. Either based on mycelial dry weight or its nitrogen content, the maximum yield of the fungus was obtained at the fourteenth day when the ammonium

sulfate was used as nitrogen source and then followed by a decrease of the growth. In case urea was used, the maximum growth was observed at the sixteenth day. A very slight decrease or not at all was noticed until to the end of the culture. At the beginning of the culture the growth of the fungus was faster when ammonium sulfate was used as nitrogen source. The growth of the fungus gradually became more active in the case where urea was supplied as nitrogen source.

Changes of pH of culture solution affected by the assimilation of nitrogen in the medium was also noted (Fig. 2-B). In the case where ammonium sulfate

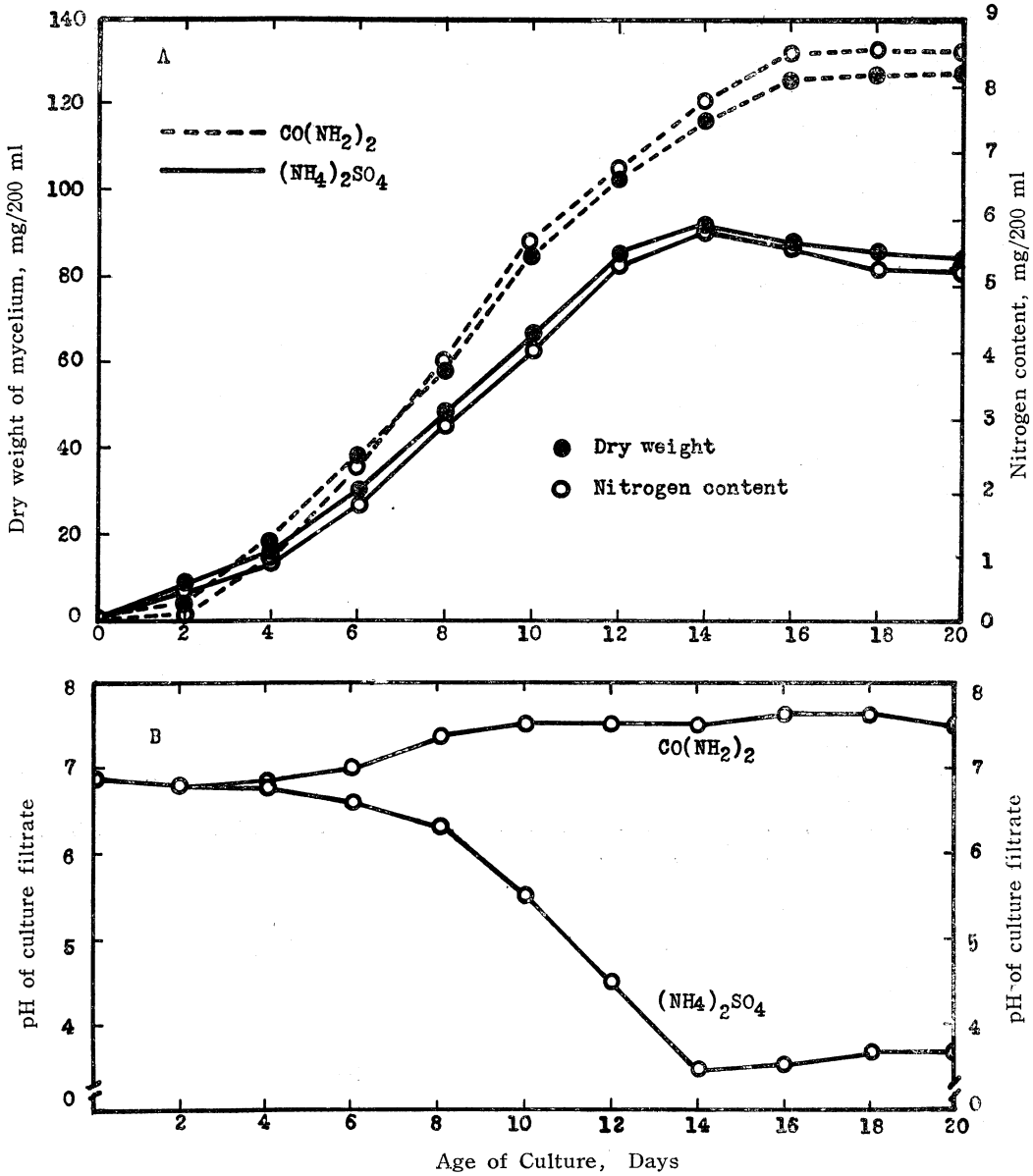

Fig. 2. (A) Growth of *A. bisporus* in a synthetic medium and (B) changes of pH of culture solution in the lapse of time.

was used, the pH of the culture solution began to drop on the sixth day. The pH dropped to about 4.0 at the fourteenth day and remained at about 3.5 up to the end of the culture. Contrary, the assimilation of urea nitrogen slowly caused the increase of pH at the first eight days and maintained at about 7.5–7.8 up to the end of the experiment.

DISCUSSION

Urea is considered as a utilizable nitrogen source by the fungus in general (Cochrane, 1958). *A. bisporus* grows extensively in the synthetic medium supplied with urea as sole nitrogen source. Optimal growth of the fungus was obtained at 1,000 mg/l urea nitrogen as measured on dry weight. If fungus growth was determined based on its total cellular nitrogen content, the optimal concentration for the growth was at 1,400 mg/l urea nitrogen. No significant increase in mycelial dry weight was observed when the urea nitrogen concentration increased beyond 1,000 mg/l. This is probably due to being exhaustive of available carbon source in the medium. Little changes in the rise of pH was observed throughout the period of the cultures. The change of pH in the medium is affected by the rate of change of urea to ammonia and the rate of absorption of ammonia by the fungus.

Final pH of the culture solution decreased when the ammonium sulfate was used. Ammonium enters the cell by passive diffusion of the undissociated NH_3 molecule, and its absorption is far greater than the uptake of anions. The ammonium salt of the strong inorganic aicd finally makes the culture filtrate extreamly acidic as the ammonia assimilated. The acidic reaction further retards the utilization of ammonium ions by the fungus and finally stops the growth of the fungus. Decreases in mycelial yield and rises in pH of culture filtrate at later stage of culture are probably due to the autolysis of the fungus.

LITERATURE CITED

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洋菇之氮素營養

II. 尿素的利用

徐惠廸　胡開仁

1. 本文詳述尿素及硫酸銨對洋菇生長之比較。
2. 尿素係供洋菇生長之良好氮素源。
3. 尿素對洋菇之生長比硫酸銨更佳。
4. 洋菇生長於含尿素之合成培養基時，培養液逐漸呈微鹼性或中性。
5. 洋菇生長於含硫酸銨之合成培養基時，培養液逐漸呈酸性。