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Effect of Supercritical Fluids on the Biological Activity of *Absidia coerulea* for the Hydroxylation of Reichsterin's Substance Acetate

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Abstract: The viability and biological activity of *Absidia coerulea* in compressed or supercritical CO_2 and C_2H_4 were studied. The specific activity of *Absidia coerulea* in 7.5MPa CO_2 and C_2H_4 at 306K can reach to 23% and 75% respectively, leading to the feasibility of using supercritical C_2H_4 as an alternative to the organic solvent in the hydroxylation of Reichsterin's substance acetate.

Keywords: *Absidia coerulea*, hydroxylation, hydrocortisone, supercritical fluids.

In a number of fermentation processes, organic solvents are often used due to the poor solubility of many substrates and/or products in water. However, the interface between water and organic phases may lead to the diffusion-limited bioconversion. Supercritical fluid technology can be employed to eliminate the mass transfer resistance existed in the biphasic system[1-6].

Hydrocortisone is an effective anti-inflammatory drug and an important precursor of other steroid drugs. The C11β-hydroxylation of Reichsterin's substance acetate (RSA) to β-hydrocortisone (beta-HC) needs both C11β-hydroxylase and its coordination with coenzymes[7]. It is desirable to perform the hydroxylation in supercritical solvents aimed at enhancing the solubility of RSA and the yield of beta-HC. The biological activity of *Absidia coerulea* after being treated by compressed or supercritical CO_2 and C_2H_4 was tested.

Experimental

CO_2 (99.9% purity) and O_2 (99.99% purity) were purchased from North-China Oxygen Company. C_2H_4 (99.0% purity) was supplied by Yanshan Petrochemical Company. RSA, standard hydrocortisone and other reagents were all purchased.

The spores of *A. coerulea*, supplied by Tianjin University of Science and Technology, were gained by washing the slant agar using sterilized water. Then the suspension acquired was transferred into the sterilized liquid medium. The composition of the medium was given elsewhere. The final pH was adjusted to 6.4-6.7 (optimal pH is 6.5) by adding NaOH. The incubation was performed at 28°C on a shaker at 150 rpm

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for 7 hours and then the revolution was changed to 170 rpm for the rest of time. The whole incubation process lasted for about 26 hours. The fermentation would occur after the substrate (RSA dissolved in ethanol) was added.

The experiment was conducted in a 0.5 L autoclave made of stainless steel (rated to 21.6 MPa). A hundred millimeters of *A. coerulea* culture was placed into the autoclave at the beginning of the experiment. The vessel was then enclosed and immersed in the thermostatic water bath at the temperature of interest. Carbon dioxide or ethylene was injected into the autoclave by a syringe pump, which was combined with a pressure reducing regulator and two back-pressure regulators to achieve the steady pressure change in the pressurization process. At last, oxygen was added into the system, the amount of which is proportional to the applied pressure[6]. The exposure of the microorganism to CO_2 or C_2H_4 was performed under 306 K and a variety of pressures. The air inside the autoclave was first purged by low-pressure carbon dioxide or ethylene. Unless otherwise stated, either the pressurized or the depressurized process took about 30 minutes in all experiments.

For determination of the viability of *A. coerulea* spores, about 1×10^7 cells per milliliter were first incubated on the culture disc spread with stock culture for 48 hours, and then the colony units were counted.

The specific activity of *A. coerulea* pellets was calculated according to

$$Specific\ Activity = (Y_P / Y_0)\times 100\% \qquad (1)$$

where Y is the yield of β-hydrocortisone after 24 hours conversion at atmospheric pressure and 28°C. Subscript P stands for the *A. coerulea* treated by compressed or supercritical fluids, and 0 refers to the untreated *A. coerulea*. The product β-hydrocortisone was analyzed by an Agilent 1100 HPLC. The 25 cm long C18 column was used, and the absorption wavelength was chosen at 242 nm.

Results and Discussion

The viability of *A. coerulea* spores, as indicated in **Figure 1**, is not as sensitive to pressure as *Saccharomyces cerevisiae*[6]. Spores are the most resistant form of microorganisms. As reported, the spores of *Aspergillus niger, Bacillus subtilis and Bacillus stearothermophilus* had a higher pressure-resistant ability compared with Baker's yeast and *E. coli*[8]. Obviously, the spores of *A. coerulea* are not an exception. It is worth noting that the yield of beta-HC is significantly reduced while most of the spores are still alive after the treatment of CO_2. The major reason may be due to the change in cell's metabolism. In contrast, the beta-HC yield is quite sensitive to pressure change. It has already been reported that the hypha of *A. coerulea* pellets is largely reduced after being exposed in supercritical CO_2[6].

Figure 2 indicates the optimal initial pH on the viability of *A. coerulea* spores and the yield of beta-HC is changed to about 8 instead of 6.5 after the treatment of supercritical CO_2. Though the increase in viability can be achieved by adjusting the initial pH, it is rather limited. By comparison, about 85% viability of *A. coerulea* spores is achieved after the treatment of supercritical C_2H_4, which is independent on initial pH. The specific activity of *A. coerulea* pellets can reach up to 75% after the treatment of su-

Figure 1 Effect of pressure on the activity of *A. coerulea* pellets and the viability of *A. coerulea* spores at 306.2 K after exposed in compressed and supercritical CO_2 for 30 minutes

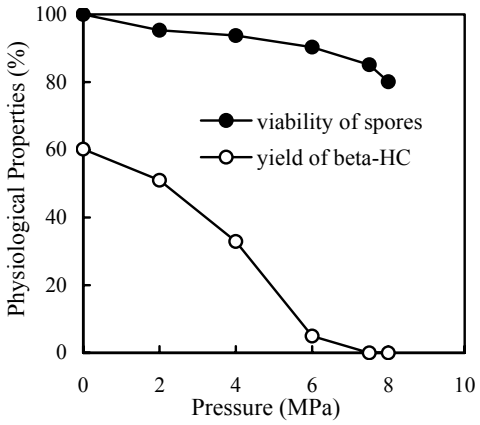
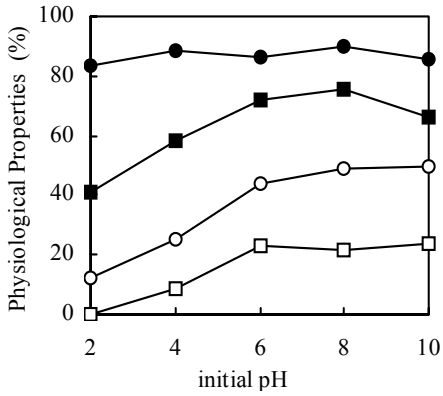


Figure 2 Effect of initial pH on biological activity and spore viability for *A. coerulea* at 7.5 MPa and 306.2 K after 30 minutes exposure



○ viability □ specific activity in CO_2; ● viability ■ specific activity in C_2H_4

percritical C_2H_4. The optimal initial pH does not change much. The micrograph observation indicates the hypha keeps the same after being exposed in supercritical C_2H_4.

The high specific activity of *A. coerulea* after the treatment of supercritical C_2H_4 is significant because the innovative arrangement of operation, such as the cyclic operation between low and high pressure of C_2H_4, can make sure both high solubility of reactants and *in-situ* extraction of products[4].

In summary, experimental investigations on the viability and biological activity of *A. coerulea* under various pH in compressed or supercritical CO_2 and C_2H_4 have been performed for the first time. The high biological activity of *A. coerulea* after being treated in supercritical C_2H_4 leads to the feasibility of using supercritical C_2H_4 as an alternative to the organic solvent in the hydroxylation of RSA.

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