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Comparison of Immobilization Techniques Using *Phanerochaete chrysosporium* for the Treatment of Pulp Waste Effluent

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생물학적 펄프 폐수처리를 위한 *Phanerochaete chrysosporium*의 고정화 방법 비교

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ABSTRACT

Three immobilization techniques and free cell system were tested to determine the most effective technique for the treatment of pulp waste effluent. The tests were conducted using *Phanerochaete chrysosporium* as a biocatalyst in a process designed to treat pulp waste effluent. The results show that Ca-alginate gel was the best immobilization material. The chosen material improved the stability and increased the removal efficiency of the system. The experiment using the chosen material was monitored for 400 hours with no significant changes in the state of the fungus. Common problems with other immobilization materials and free cell system were oxygen transfer resistance caused by air channelling and clogging in the bioreactor.

INTRODUCTION

Recently, because of the growth of factors contributing to an increase in water pollution, the Environmental Protection Agency(EPA) strengthened the restrictions placed on the disposal of pulp companies currently adopt effective treatment processes for the removal of chlorolignin compounds from effluent produced during chlorine bleaching and lignin extraction.

Chlorolignin compounds make up many of the chloro-organic compounds found in pulp waste effluent. chlorolingins are highly toxic to the natural ecosystem and do not decompose in a short period of time. Also, the dark color of the chlorolignins in solution means that a special treatment process must be employed. The prevailing treatment process, the activated sludge method, must be changed in order to comply with the newest EPA restriction.

Generally, a strain of white rot fungus is used as a biocatalyst in the treatment process. *Phanerochaete chrysosporium* is one type of white rot fungus that is commonly used because of its high activity compared with other microorganisms. Until recentlly, most researchers concentrated on free cell systems(1-4). Past investigations focused on various parameters in the free cell

system, like medium composition, pH, temperature, oxygen demand and agitation effects. Only a few immobilization technique studies have been dicussed, recently(5, 6, 7). There are many different method of industrial pulp waste effluent treatments. Precipitation, ultraviolet irradiation, ultrafiltration, and biological treatments are all methods of waste treatment. Among these, biological treatment is known as one of the most economical methods.

Phanerochaete chrysosporium produces oxidative enzymes that interact actively with chlorolignin compounds. The enzymes act as a catalyst to the oxidation of chlorolignin. In the free cell system, accumulation of cells and high biodegradability cannot be expected. This particular fungus consists of many mycelium and will construct large flocs, so it is very difficult to control the fungus and regulate the oxygen transfer to the center of the floc in the free cell system. Therefore, one advantage of the immobilization system over the free cell system is the increased regulation of oxygen transfer. Another benefit of the immobilization system is that bacterial contamination occurs less frequently than it does in free cell systems. The fungus was immobilized to increase the stability and removal efficiency of the system. The experiment consisted of three immobilization supporters which have different natures, and free cell system. The experiment were performed to find the best immobilization supporter for this particular fungus.

EXPERIMENTAL

Fungus and its culture conditions.

Phanerochaete chrysosporium(ATCC 34540) was cultured on malt agar to obtain the conidial inocula. The conidial inocula was transferred from the malt agar to a 5 percent YM broth solution for five days to allow the fungus to grow. After the fungus was grown, the fungus was transferred to the medium solution, which does not contain pulp waste effluent, for two days. In the free cell system, the grown fungus was transferred to the main bioreactor. The growth medium and trace medium compositions are shown in Tables 1 and 2, respectively.

Table 1. The growth media composition used in this research.

Compounds	Concentration(g/ℓ)
Glycerol	1
NH_4NO_3	0.5
Thiamine-HCl	0.0025
Veratryl Alcohol	0.0568
Tween 80	0.1

Table 2. The trace media composition used in this research.

Component	Concentration(g/ℓ)
$MgCl_2 \cdot 6H_2O$	2.47
$MnCl_2 \cdot 4H_2O$	2.05
NaCl	1.0
KH_2PO_4	0.2
$CoCl_2 \cdot 6H_2O$	0.1
$ZnSO_4 \cdot 7H_2O$	0.084
$FeCl_2 \cdot 4H_2O$	0.072
$CaCl_2 \cdot 2H_2O$	0.013
H_3BO_3	0.01
$NaMoO_4 \cdot 2H_2O$	0.01
$CuSO_4 \cdot 5H_2O$	0.007
$AlCl_3$	0.003

Pulp waste effluent

The feed solution for the experiment was prepared by mixing the chlorination(C_D) and the extraction(E) stage effluent from a typical pulp bleaching plant. The feed composition is 25% mixed(C_D+E) effluent and 75% mixed(growth and trace) medium. C_D and E mixing ratio of 3:2 is the same as an operating bleaching plant effluent. The pH of the feed was adjusted to pH5.0 prior to use. The types and concentrations of the chlorolignin compounds present in the feed are shown in Table 3. The initial color absorbance of the feed solution was 0.078.

Analytical method

The samples from the experiment were taken from the final effluent. 2ml vials were used to

Table 3. Various chloro-organics included in the feed solutions.

Component	Initial Concentration($\mu g/\ell$)
6-CV	1439.55
35DCC	192.72
45DCG	478.58
TETCG	13.10
456TETCG	7.78
TCS	1.3
TETCV	76.60

Complete Component Names

6CV : 6-Chlorovanillin
35 DCC : 3,5-Dichlorocatechol
45 DCG : 4,5-dichloroguaiacol
TETCG : Tetrachloroguaiacol
456 TETCG : 4,5,6-Trichloroguaiacol
TCS : Trichlorosyringol
TETCV : Tetrachloroveratrol

collect the samples. The samples were centrifuged at 3000rpm for 10 minutes and then the samples were ready for analysis. The chlorinated organic compounds were analyzed by a Varian model 3400 gas chromatography with a 0.25mm DB-1 capillary column. A Varian model 8035 autosampler and an ECD detector were also used. The Gilford model 250 UV spectrophotometer was used to analyze the amount of color removal and the enzyme activity of the effluent. To measure the enzyme activity, Tien and Kirk method(8) was used. the measurement was based on the oxidation of veratryl alcohol to veratraldehyde by lignin peroxidases. Color absorbance was measured using the Sundman and Kirk method(9).

Packed bed reactor

A 2″ ID×10″ glass tube was used as the packed bed reactor, as shown in Fig. 1. The total working volume was maintained at 200ml. Air was supplied through a distributor at rate of 60ml/min. In order to prevent bacterial contamination, the air was filterd by a 0.2μm air filter and a 0.1μm microculture capsule was used to filter the feed. The feed flow rate was 3.0-5.8ml/hr and the retention time was varied from 9.6-16.

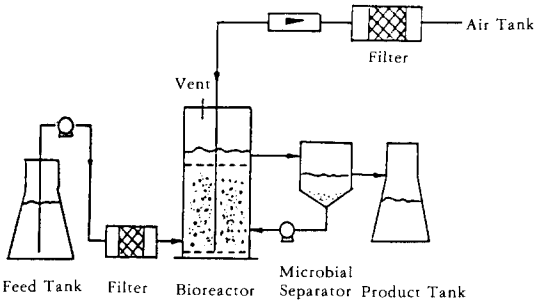

Fig. 1. The simplified bioreactor for evaluating various supporters.

8hr. All retention times werebased on the total reactor volume. The experimental apparatus was enclosed in an insulated incubator to maintain a 33℃ temperature. A separtor was installed following the reactor to prevent the flow of fungus directly to the effluent.

Support media for immobilization

In order to select the most appropriate supporter, three kinds of materials were tested. The properties of each supporter are shown in Table 4. D. Livernoche(10) method was adopted to make Ca-alginate gel beads for fungal immobilization. The fungus was grown for five days in a 500ml shaking flask. The fungus was washed with 0.9% sterile saline solution. The fungus was then centrifuged at 6500rpm for 30 minutes. The fungus was mixed with 2% gel solution and the mixed gel solution was injected slowly through the needle of a syringe into 1% chilled $CaCl_2$ solution. This final solution was transferred to the reactor. The other supporters(volcanic rock, wood chip) were prepared by growing the fungus in a 500ml shaking flask for two days, and then transferring

Table 4. Properties of various support media.

Support media	Size	Shape
Volcanic rock	(3-5mm)	Crushed
wood chip	(6-8mm)	Chip
Ca-alginate gel	(4-5mm)	Bead

the fungus to each supporter. The fungus was allowed to attach on the surface of the supporter, and then the solution was transferred to the reactor.

RESULTS AND DISCUSSION

All experimental data were obtained after the reactor system reached a steady state condition. At first, the system was operated as batch reactor. After steady state was reached, the operation of the system was changed to a continuous reactor. Once the continuous process was fully established, samples were taken from the final effluent solution. The feed rate was changed according to the appropriate retention time. During the experiment, the retention time was changed from 9.6hr to 16.8hr. By measuring the chlorophenolic compound removal and color removal, the steady state condition can be determined. After steady state was reached, samples were taken and then the feed rate was changed. During the entire experiment, the initial medium and feed solution concentrations were held constant.

Color removal

The color removal percentage for the various supporters are shown in Fig. 2 and 3. Generally, the color removal rate increased as the retention time increased. The Ca-alginate system exhibited especially high color removal ability. The color removal rate for this system was about 55% at the retention time of 16.8hr, as shown in Fig. 2. In the volcanic rock system, the fungal condition was stable until the system was run for more than 400 hours. Because of this stability, the color removal rate was found to be a high uniform level. After 400hours, the color removal rate dropped markedly despite a long retention time, as shown in Fig. 3. This dramatic decrease in color removal rate might be caused by poor oxygen transfer, which could lead to a low oxygen level and substrate to decrease the fungus viability in the system. Also, in the full-grown fungal state, the dispersion of air was not uniform and

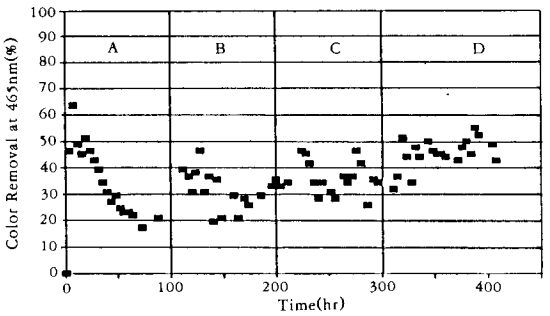

Fig. 2. Color removal percentage vs. time in the Ca-alginate gel system.
Parameter: Retention time(hr)
A. 9.6hours C. 14.4hours
B. 12hours D. 16.8hours

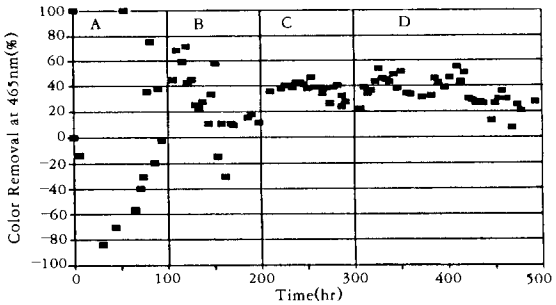

Fig. 3. Color removal percentage vs. time in the volcanic rock system.
Parameter: Retention time(hr)
A. 9.6hours C. 14.4hours
B. 12hours D. 16.8hours

air chaneling was observed. The Air channeling restricts the mass transfer in the system.

On the other hand, the fungus in the wood chip system would not even grow. This inability to grow fungus makes the wood chip a poor supporter. The effect of stability on color removal can be illustrated by studying the free cell system shown in Fig. 4. At a retention time of 12hr, the color removal rate was found to be around 60% in the early steady state condition. After 100hours, the stability of the fungus decreased rapidly. As a result of this loss in stability, the solution color actually changed to a color darker than the initial feed color. In the free cell system, the mycelium

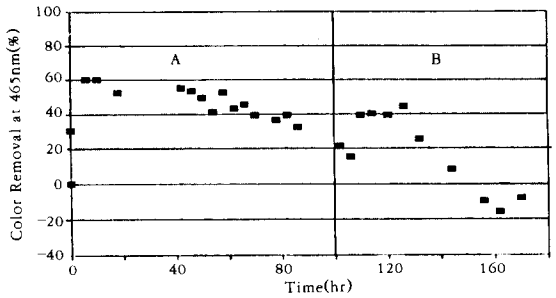

Fig. 4. Color removal percentage vs. time in the free cell system.
Parameter: Retention time
A. 12hours B. 14.4hours

grew in big pellets, having a diameter of about 2–3cm.

Considering the above results, it appears that the Ca-alginate gel is the best supporter since the Ca-alginate gel system retains good color removal ability even after long reaction time. In the cases of the volcanic rock system and the free cell system, the floc grew continuously as reaction time increased. As the feed rate was almost constant in making color removal runs, the levels of oxygen and substrate for the fungus became relatively low as the floc grew. Therefore the fungus eventually died and the color removal rate decreased rapidly due to lack of oxygen and substrates. On the other hand the size of the Ca-alginate was almost constant in operation since the growing mycellium on the surface of the gel was cut off by the motion of bubble and the mixing. The detached mycellium was then washed out from the reactor. Out of the four waste effluent treatment systems, only the operation of Ca-alginate gel system could be well controlled in levels of oxygen, substrates and the size of the floc.

Enzyme activity

The enzyme activities for the various supporters are shown in Fig. 5, 6 and 7. The Ca-alginate system exhibited relatively higher enzyme activities than the other immobilization supporters. In the other systems, the color removal rate was high despite low enzyme activity. The increased color removal rate could be due to adsorption into the supporter surface.

Chloro-organic removal

In Fig. 8–11, typical chloro-organic removals are shown for the Ca-alginate system of the organics were reduced by 95%. But in the case of tetrachloroveratrol(TETCV) and tetrachloroguaiacol(TETCG), the removal rate dropped to 90%.

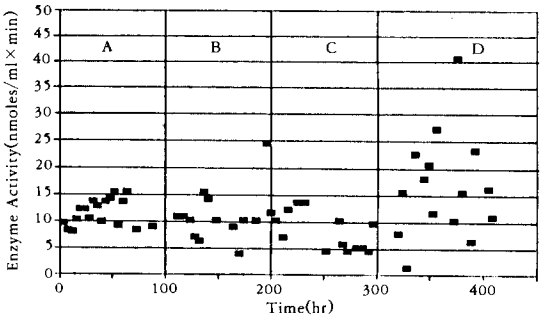

Fig. 5. Enzyme activity v*s. time in the Ca-alginate gel system.
Parameter: Retention time(hr)
A. 9.6hours C. 14.4hours
B. 12hours D. 16.8hours

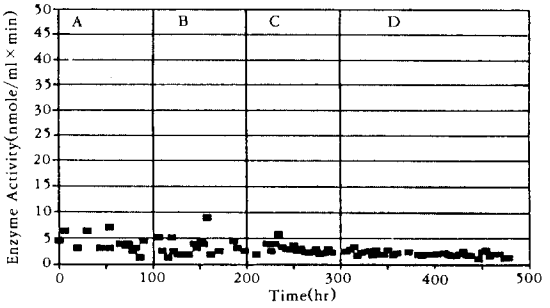

Fig. 6. Enzyme activity vs. time in the volcanic rock system.
Parameter: Retention time(hr)
A. 9.6hours C. 14.4hours
B. 12hours D. 16.8hours

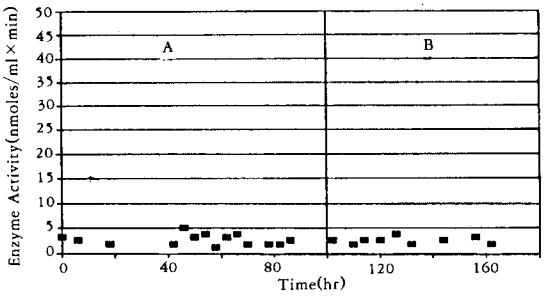

Fig. 7. Enzyme activity vs. time in the free cell system.
Parameter: Retention time(hr)
A. 12hours B. 14.4hours

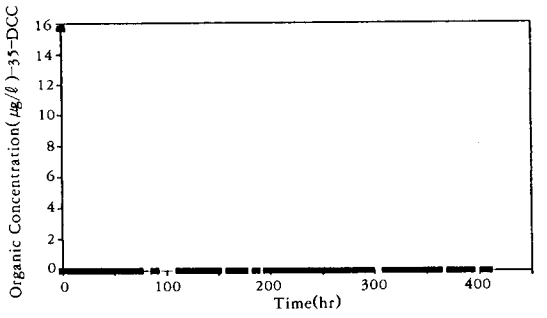

Fig. 8. Organic(3-5 DCC) concentration vs. time in the Ca-alginate gel system.

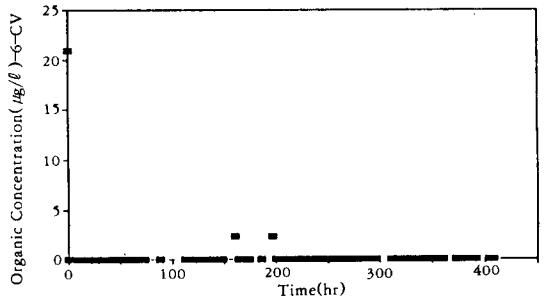

Fig. 9. Organic(6CV) concentration vs. time in the Ca-alginate gel system.

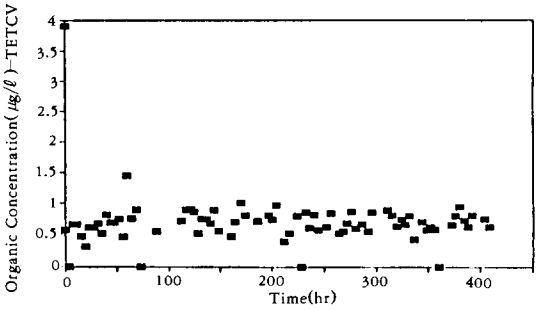

Fig. 10. Organic(TETCV) concentration vs. time in the Ca-alginate gel system.

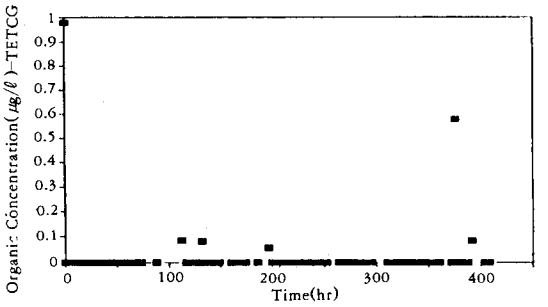

Fig. 11. Orginic(TETCG) concentration vs. time in the Ca-alginate gel system.

CONCLUSION

Ca-alginate was found to be a good material to use for the immobilization of *P. chrysosporium* in the treatment of pulp bleaching plant waste water. This material exhibited extremely high stability and the system was able to support and abundance of fungus. Air channeling and clogging in the actual system were considerably less with this supporter compared with other supporters. The color removal rate and enzyme activities were greater than the other systems. In the case of Ca-alginate, the color removal rate was 55%, the chlorophenolic removal was 95% and 25nmoles/ml · min enzyme activity was obtained at a retention time of 16.8hr.

요 약

펄프 폐수처리를 위한 효과적인 생물학적 처리법으로서 세 가지 고정화 방법과 자유균체를 이용한 방법을 사용하여 실험하였다. 균체로서는 *Phanerochaete chrysosporium*을 이용하였다.

고정화방법 중 Ca-alginate gel을 담체로 사용한 방법이 가장 우수하였다. 이 방법은 안정성과 처리효율면에서 좋은 결과를 보였으며, 약 400시간 동안 균체의 커다란 상태변화없이 반응시킬 수 있었다.

다른 고정화 방법들과 자유균체를 이용한 방법들의 가장 큰 문제점은 생물 반응기 안에서의 공기 channelling 현상에 의한 산소전달 저항과 균체끼리의 clogging으로 인한 반응기 내부의 제어가 힘들게 된 것이었다.

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