Treatment of Oil-containing Wastewater by Immobilized Yeast Cells

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The oil-containing wastewater was treated by the immobilized yeast cells(pellets) and the effects of factors on immobilizations were studied . The pellets were prepared with polyvinylalcohol(PVA)-sodium alginate(SA).The experimental results indicate that the optimized concentration of PVA and SAwas 7% (w/v)and 1.3%(w/v), respectively. Furthermore, the process of preparing pellets was also investigated,and the optimized procedurewas that the pellets were initially treated by $CaCl_2$ solution with concentration of 2% for 6h, then immersed by the saturated solution of boric acid for another 18h. According to the above-mentioned method, pellets have high mechanical strength and mass transfer performance , and the final cell activity is about 80% .

Key words: Yeast; PVA; SA; immobilization; COD removal.

In view of the detrimental impact of oil on oxygen transfer efficiency in aerobic systems[1, 2] and the inhibiting effects of long-chain fatty acids, such as oleic acid, the product of lipid hydrolysis on anaerobic systems[3], traditional biological treatments are not efficient to treat the wastewater containing high concentration of oily contents like salad oil-manufacturing wastewater. To date, physico-chemical treatments, such as acidification, air flotation, coagulation, etc., followed by biological treatment have been mainly utilized for treating this kind of wastewater[4-6]. However, during the physico-chemical treatments, much sludge or scum binding plenty of oil is always produced and it is difficult to deal with.

To overcome the above-mentioned problems of conventional technologies in treating this type of wastewater, yeast instead of bacteria has been given more attention[7-10]. Our previous research has also shown that COD and oil contents in vegetable oil-containing wastewater can be effectively removed in bioreactors inoculated with mixed isolates[11]. Unfortunately, severe filamentous sludge bulking was repeatedly encountered when yeast mixture was applied to treating high-strength salad oil-manufacturing wastewater, leading to the failure of operation[12, 13]. In order to develop a stable system inoculated by yeasts, some measures must be taken to control the transition of yeast morphology.

In the last decades, Cell immobilization technology has been paid more and more attentions which often was used in fermentation industry[14-16] because of its ability of enhanced fermentation productivity, feasibility of continuous processing, cell stability and lower costs of recovery and recycling and downstream processing. The immobilized cells have properties of excellent settle

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ability and metabolic ability which may aid to keep the yeast cells in the reactor. In this study, the optimized conditions of yeast cell immobilization and the effects of cell immobilization on oil-containing wastewater treatment were discussed.

MATERIALS AND METHODS

Yeast Strain and Wastewater

Yeast strain was stored in our laboratory and named as G1. The oil-containing wastewater was collected from a salad oil-manufacturing plant in Ningbo, China.

Preparation of Yeast Cells

One loop of yeast cell was taken into a flask contained 100mL sterilized YPD media from a slant culture. 1L YPD media consist of: yeast extract 5g, peptone 10g, dextrose 10g, deionized water 1L, pH 5.5, autoclaved at 115°C, 25min. The flasks were shaken in vortex at 28°C and 180r/min for 48h. The yeast culture was centrifuged at 4000r/min for 7 min, then the yeast cells was rinsed by deionized water for the further experiments.

Procedures of Yeast Cell Immobilization

PVA and SA were dissolved in deionized water with a certain ratio of them and heated on an oven to make a carrier solution(CA) which further underwent a sterilization. When the CA was cooled to room temperature, a certain of yeast cells was added into it and mixed thoroughly. The mixture was sucked into a syringe and then squeezed out into the immobilization solution. After 24h, the pellets were formed and rinsed by saline water 2-3 times ,then transfer to 0.6% $CaCl_2$ solution and kept at 4°C.

Optimization of Immobilization Time

At room temperature, the pellets were immersed into the CaCl2 solution with a optimized concentration and then dipped into a saturated H_2BO_3 solution for a certain time (Table 1). The mechanical strength and mass transfer performance were evaluated to optimize the immobilization time.

Optimizaiton of Concentration of $CaCl_2$ Solution

Generally, the concentration of calcium ion puts an important influence on the properties of pellets. Under the condition of constant content of H_2BO_3 solution, the $CaCl_2$ content of 0.5% 01%, 2 %, 4% was investigated and the mechanical strength and mass transfer performance were tested.

Central Complex Design Method for Optimization of Content of PVA/SA

The content of PVA/SA is a key parameter of wastewater treatment using immobilized yeast cells. By selecting PVA as x1,SA as x2, the COD removal as the response, the experiment with two factors and five levels was designed (Table 2). According to Table 2, The PVA/SA solution with the content of 4 g/100mL of yeast cell was dropped into a 2% $CaCl_2$ solution . The pellets with a diameter of 3-5 milimeter were formed. After 8h of immobilization, the pellets were taken out and rinsed by saline water 2-3 times, then immersed into the saturated H_2BO_3 solution for 16h. After rinsed by saline water, the pellets underwent the determination of the mechanical strength and mass transfer performance, then were used to treat the oil-containing wastewater.

Optimization of Content of Yeast Cell

With the optimized ration of PVA to SA, the content of yeast cells at 4.3, 6.1 , 7.1, 8.3, 9.5 g/ 100mL was added to the PVA/SA solution. The pellets were produced according to the above-mentioned procedure and rinsed by saline water 2-3times. A certain of pellets were introduced into an SBR reactor to degrade the wastewater. The COD removal was used as an indicator to evaluate the efficiency of wastewater treatment.

Determination of Mechanical Strength of Pellets

Fifty pellets with the same size were selected and put in a small Erlenmeyer flask with 40mL saline water. Three glass beads were added into the flask which further underwent the vortex at 300 r/min for 24h. The number of damaged pellets was counted and the mechanical strength (MS) was calculated by the following equation.

$$MS= (N/50) \times 100\%$$

N: the number of pellets without damage

Determination of mass transfer performance of pellets

Ten pellets with the same size were selected and placed in a beaker. 100 mL saline water and two drops of methylene blue solution was added. 20 min later, the pellet was split into two pieces with a knife. The mass transfer performance of pellets was assessed in term of the extent of staining of pellet.

Efficiency of Oil-containing Wastewater by Pellets

A certain of pellets formed in the optimized conditions was put into an SBR reactor. Refill and discharge, adjustment of pH were carried out daily. The ratio of influent to effluent is 1/2. The SBR was operated for 4 days. For comparison, equal quality of free yeast cells was added into another SBR run under the same conditions. The effluent COD was measured every day by a COD apparatus. The activity (A) of yeast cells was calculated by the following equation.

$$A=((COD_{im}-COD_{fr})/COD_{fr}))\times 100\%$$

COD_{im}: COD of effluent of SBR with immobilized yeast
COD_{fr}: COD of effluent of SBR with free yeast cells

RESULTS AND DISCUSSION

Effects of Immobilization Mode on Properties of Pellets

Once CA solution was pinched by a syringe into $CaCl_2$ solution, pellets with smooth surface were produced. Then,the pellets expanded to a certain extent. When the pellets were taken out and immersed into H_2BO_3 solution, the surface became coarse. However, a few minutes later, the surface turned into smooth and the volume of pellets contracted. The result showed in Table 3. It's reasonable to conclude that the expansion of pellet in $CaCl_2$ solution results in good mass transfer performance. On the other hand, the mechanical strength of pellet increased with the immobilization time in H_2BO_3 solution.

Table 1. Optimization of immobilization time

Immobilizationmode	Immobilization time (in $CaCl_2$ solution)(h)	Immobilization time (in H_2BO_3 solution)(h)
1	0	24
2	6	18
3	12	12
4	18	6

Table 2. Central complex design method: factors and levels

Factors	Levels				
	-1.67	-1	0	1	1.67
x1	5.7	7	9	11	12.3
x2	0.5	0.7	1	1.3	1.5

Table 3. Effects of immobilization mode on properties of pellets

Immobilization mode	Mechanical strength(%)	Mass transfer performance
1	100	+
2	98	+++
3	96	+++
4	96	+++

Note + represents average, ++ represents good, +++ represents very good.

Effects of the Concentration of $CaCl_2$ Solution on the Properties of Pellets

The properties of pellets were listed in Table 4 under the condition of different concentration of $CaCl_2$ solution when the CA solution comprises of 7% PVA and 1% SA. With the increase of the concentration of $CaCl_2$ solution, the mechanical strength and mass transfer performance were elevated but this trend was stopped when the concentration was increased > 2%. Therefore, the optimized concentration of $CaCl_2$ solution is 2%.

Table 4. Effects of the concentration of $CaCl_2$ solution on the properties of pellets

$CaCl_2$ concentration	Mechanical strength (%)	Mass transfer performance
0.5%	96	++
1.0%	96	+++
2.0%	98	+++
4.0%	98	+++

Table 5. Effects of the ratio of PVA/SA on the properties of pellets

No.	Factors		Index		
	x1	x2	Settleability	Strength(%)	Mass transfer
1	-1.67	0	+	92	+++
2	-1.67	1	+	92	+++
3	-1.67	1.67	+	94	++
4	-1	-1	++	92	++
5	-1	0	++	94	+++
6	-1	1	+++	98	+++
7	0	-1	++	96	+++
8	0	0	+++	96	++
9	1	-1.67	+++	96	++
10	1.67	-1.67	+++	96	+

Effects of the Ratio of PVA/SA on Properties of Pellets

PVA and SV are both good carrier for cell immobilization. PVA has the advantage of weak bio-toxicity, physical and chemical stability, low price and difficulty to biodegradation. However, its mass transfer performance was inferior to ones of SA. In contrast, SA is a natural carrier with good properties of weak bio-toxicity, convenience to solid and good cell enrichment. Nevertheless, SA has a poor stability and weak mechanical strength[17]. Therefore, many researches[18] have focused on the use of mixture of PVA and SA as a carrier.

According to Table 5, as the concentration of PVA grew, the mechanical strength and settleability of pellets increased, but the difficulty of forming the pellet increased also. SA was often used as a additive. The higher concentration of SA usually results in the difficulty to form pellets. In terms of difficulty to form pellets, settle ability, mechanical strength and mass transfer performance, the No.6 in Table 5 is a good ratio of PVA/SA. The concentration of PVA and SV is 7% and 1.3%, respectively.

Further, the obtained pellets were applied to treat oil-containing wastewater for 4 days. When the concentration of yeast cells of 4g/100mL, the activity of yeast cell was shown in Fig.1.

Effects of Concentration of Yeast cell on the Activity of Yeast Cells

From Fig.2, when the concentration of yeast cells is between 4.3% and 8.3%, the activity increase quickly. However, when the concentration is more than 8.3%, there is not obvious increase in

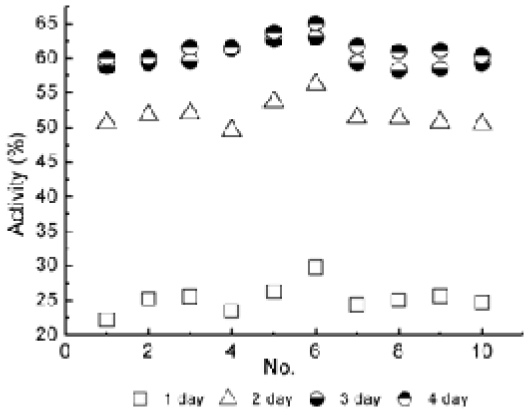

Fig. 1. The activity of immobilized yeast cells with different ratio of PVA/SA

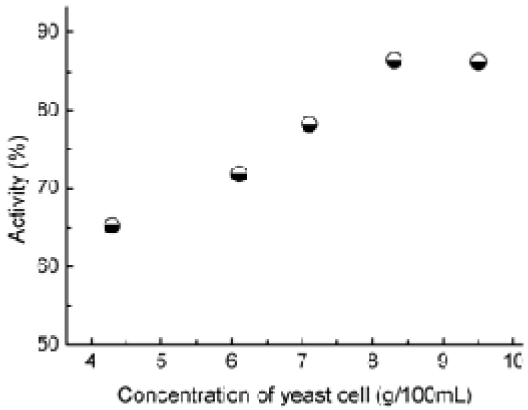

Fig. 2. Effects of concentration of yeast cells on the activity of pellets

the activity. The results can be interpreted by the following reason. At beginning, the amounts of immobilized cells is relative small, the biomass increase is the predominant role on the activity; while the amounts of immobilized cells reach a certain value, the competition of yeast cells for oxygen maybe confined the activity. Therefore, the optimized concentration of yeast cells is 8.3%. Fig.3 shows pellets formed under the optimized conditions. The pellets is round and with a good settle ability.

Fig. 3. Pellets formed under the optimized conditions

CONCLUSION

1) The optimized immobilization condition is to immerse into 2% $CaCl_2$ solution for 6hÿthen transfer to saturated H_2BO_3 solution for 18h.
2) Pellets have good mechanical strength, mass transfer performance and high activity when the concentration of PVA and SV is 7% and 1.3%, respectively.
3) The activity of immobilized yeast cells can achieve 80%. The immobilized yeast cells can overcome the filamentous bulking which result from the conversion of morphology of yeast cells.

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