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Seed yeast cultivation for salad oil manufacturing wastewater treatment

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Abstract: The mixture of five yeast strains obtained from soil could remove about 85% TOC of oil-rich wastewater in batch test. While the highest MLSS was obtained at an N:C of 1:5, the oil removal decreased with the increase of N:C during yeast sludge cultivation. Ammonium chloride was the best nitrogen source for yeast cultivation from the viewpoint of yeast growth and oil utilization. An ammonia concentration of over 1300 mg/L led to mass death of yeast at a pH of 5. The ammonia concentration should be controlled at a level of 1000 mg/L or lower.

Keywords: yeast seed; sludge cultivation; nutrient; ammonia toxicity; wastewater treatment

Introduction

Yeast has been used for production of SCP (single-cell-protein) from wastewater since 1940's(Kou, 1983; Yang, 1992;). Its application in real meaning of wastewater treatment, however, was realized in late 1980's (Chigusa, 1989; 1996; Tosaki, 1991). The yeast wastewater treatment technology demonstrated good performance on such high strength organic wastewaters as food industrial wastewater, fish meal wastewater, oil manufacturing wastewater and so on. The volumetric COD (chemical oxygen demand) loading rate of the process could be 8 to 10 times higher than the conventional activated sludge method. A COD removal of over 93% was obtained even at a COD concentration of 40000 mg/L(Chigusa, 1996). Further, the yeast process has shown a special ability in treating wastewater containing high concentration of oil.

Although the yeast process has been successfully utilized for some industrial wastewater treatment, information about it has been very limited due to lack of basic research on the process. For example, it is still not very clear why yeast have the special ability in decomposing oil of high concentration. The reason of high volumetric COD loading rate was partly attributed to the high concentration of yeast maintained in the system. It is not clear, however, the relationship between the species of yeast and the settling performance. Since facilities utilizing the yeast process have been extremely limited, seed sludge cultivation and supply are also a challenging problem.

In this study, yeast strains screened from salad oil polluted soil were used as seed strains, and the conditions for seed yeast cultivation were thoroughly investigated. The effects of some important parameters, such as the ratio of nitrogen to carbon, the nutrient species, and ammonia concentration, on yeast growth were evaluated in detail.

1 Materials and methods

1.1 Enrichment, isolation and identification

Field soil was collected from several spots contaminated with salad oil wastewater in a salad oil factory near Beijing, China, and enriched according to the method Chigusa *et al*. proposed (Chigusa, 1996). 500 ml Erlenmeyer flasks containing 150 ml of the mixture of soil and salad oil manufacturing wastewater were shaken at a rate of 170 strokes/min on a reciprocal shaker under 25℃. The pH of the solution was adjusted to a range between 5.0 and 5.5, and 60 unites/ml penicillin and 0.25 % sodium propionate were

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added to the solution for preventing the growth of bacteria and mold, respectively. The cultivation was conducted in a fill-and-draw mode for 20 days, and the solution was changed once a day. After cultured in YPD medium (Yeast Extract-Peptone-Dextrose medium) for 2 days, yeast strains were transferred to YPD agar plates for isolation and stored in stock cultures. Microorganisms were identified based on morphological and biochemical characteristics described by Kreger-van Rij (Kreger-van, 1984).

1.2 Wastewater characterization

Wastewater was taken from the same factory where the soil samples were taken. Wastewater characteristics are shown in Table 1. It is apparent that the oil concentration (petroleum ether extract, PEE) was very high and constituted the main part of COD. One important point was that TN (total nitrogen) was very low. Even compared with the ratio of COD:N of 92:1 in the paper of Chigusa *et al*. (Chigusa, 1996), the ratio of COD:N of 873:1 in this sample was extremely low.

Table 1 Characteristics of salad oil manufacturing wastewater

Items and concentrations			
COD, mg/L	134380	TP, mg/L	6495
PEE, mg/L	89687	TN, mg/L	154
SS, mg/L	9600	pH	8.5 – 9.0

1.3 Preparation of yeast inoculum

All of the yeast strains obtained were inoculated into a 500 ml Erlenmeyer flask containing 150 ml YPD medium, and cultured at 180 strokes/min under sterile condition for 2 – 3 days. Then, the mixture was centrifuged and washed by aseptic physiological saline three times so as to remove the organic matters absorbed on yeasts. The MLSS of the mixture was 692 mg/L. The yeast mixture was preserved with aseptic physiological saline at 4℃, and used as the inoculum in follow-up tests.

1.4 Performance of the yeast strains for oil removal

The inoculum was added into a 500 ml Erlenmeyer flask containing 150 ml of diluted wastewater to obtain an initial MLSS (mixed liquor suspended solids) of 550 mg/L. The mixture was adjusted to a pH of 4.5 and then cultured at 160 strokes/min. Sampling interval was 8 – 10 hours.

1.5 Nitrogen effects on yeast growth and oil removal

0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot H_2O$, 0.01% $CaCl_2 \cdot 2H_2O$, and 0.001% $MnSO_4 \cdot 4H_2O$ were added into 500 ml Erlenmeyer flasks containing 150 ml of diluted wastewater with a TOC of 1650 mg/L. The N:C of the mixture was varied from 1:3, 1:4, 1:4.5, 1:5, 1:6, 1:7.5 to 1:10 by adding different amount of ammonium sulfate. The mixture was adjusted to a pH of 5 after inoculation, and cultured at 25℃ on a shaker at 175 strokes/min.

1.6 Comparison of different nitrogen sources

0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot H_2O$, 0.01% $CaCl_2 \cdot 2H_2O$ and 0.001% $MnSO_4 \cdot 4H_2O$ were added into 500 ml Erlenmeyer flasks containing 150 ml of diluted wastewater with a TOC of 1050 mg/L. The N:C of the mixture was fixed at 1:5. NH_4Cl, $(NH_4)_2SO_4$, $(NH_2)_2CO$, and $NaNO_3$ were used respectively as nitrogen sources to evaluate the effect of nitrogen source. The mixture was adjusted to a pH of 5 after inoculation, and cultured at 25℃ on a shaker at 175 strokes/min.

1.7 Toxicity of ammonium to yeast strains

One ml of the inoculum was inoculated in 150 ml Erlenmeyer flasks containing 50 ml of aseptic filtrated saline to which ammonium chloride corresponding to 0, 800, 1000, 1200, 1300 and 1400 mg/L of ammonium was respectively added. The pH of the mixture was adjusted to 5. After shaking at 25℃ and 170 r/min for 10 hours, the concentration of live yeasts was counted by plating cultures. The whole manipulation is aseptic.

1.8 Seed sludge cultivation

All of the strains acquired through the above enrichment and isolation procedure were inoculated to the

seed tank containing 30 liters of diluted wastewater, and the mixture was aerated at pH 5—6. Everyday after settling for one hour, 15 liters of supernatant was replaced by the new diluted wastewater into which 1L raw wastewater, 0.25% sodium propionate, 0.3% ammonium chloride and 0.04% KH_2PO_4 (N:P, 5:1) were added. The ammonia concentration was controlled at a constant concentration of 500 mg/L.

1.9 Analytical methods

A rapid COD analyzer (CTL-12, Huatong Ltd., China) and TOC analyzer (TOC-500, Shimazdu Corp., Japan) were respectively used for COD and TOC measurement. Other analyses were carried out in accordance with standard methods (Committee of SEPA, 1998). During MLSS measurement, the filter papers were rinsed with petroleum ether for 3 times to remove residual oil after filtration. Oil content was determined by separation with petroleum ether.

2 Results and discussion

2.1 Isolation and characteristics of oil decomposing yeasts

According to colonies' characteristics of stock cultures, 12 strains of yeasts isolated from enrichment cultures were chosen for identification. The identification results indicated that 5 strains, namely, *Rhodotorula rubra*, *Candida tropicalis*, *Candida utilis*, *Candida boidinii*, *Trichosporon cutaneum*, were included in the yeast isolated. 3 of the 5 strains belonged to *Candida* genus, which was reasonable since *Candida* genus has been considered to be preponderant in soil (Mingxia, 1983). According to Chigusa *et al*. (Chigusa, 1996), 5 of the 8 strains they obtained from similar samples also belonged to *Candida* genus. It is interesting that all of the strains obtained here were different from those obtained by Chigusa *et al*. except from *Candida tropicalis*.

2.2 Performance of the yeast strains for oil removal

A batch test was conducted to evaluate the performance of mixed yeast strains on oil removal. The initial MLSS was 550 mg/L. As shown in Fig. 1, the TOC was removed from 2500 mg/L to 370 mg/L in 24h. Since the main organic composition was oil, the result indicated that the yeast strains obtained had relatively good oil removal ability. The settleability of the yeast mixture was very good. Although the yeast strains were much different from those obtained by Chigusa *et al*. (1996), they demonstrated similarity in the aspects of oil removal performance and settleability.

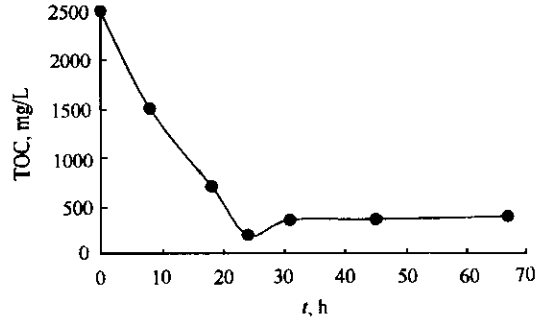

Fig. 1 Treatment of wastewater by yeast

2.3 Nitrogen effects on yeast growth and oil removal

It has been a normal practice to supplement the necessary amount of nitrogen needed for enhancing the growth of microorganisms. Fig. 2 shows the effects of N:C on MLSS and PEE removal rate, respectively. A N:C of 1:5, which was near the N:C ratio of yeast cell (Wuxi University of Light Industry, 1990), was the optimum value for yeast growth. That is to say, a proper N:C was necessary to ensure efficient yeast cultivation. Fig. 2b shows, however, that the PEE utilization decreased with the increase of N:C, especially at an N:C higher than 1:5. Maybe the yeast strains from an environment heavily deficient in nitrogen did not need much nitrogen for removal of organic matters. Even for yeast cultivation, it is also required that the oil removal of the cultivation medium is as high as possible since the used medium will be discharged as wastewater. According to Fig. 2, the highest yeast yield was obtained at the N:C of 1:5, where the oil removal was also relatively high. So, the N:C ratio of 1:5 was used for the followed sludge

cultivation.

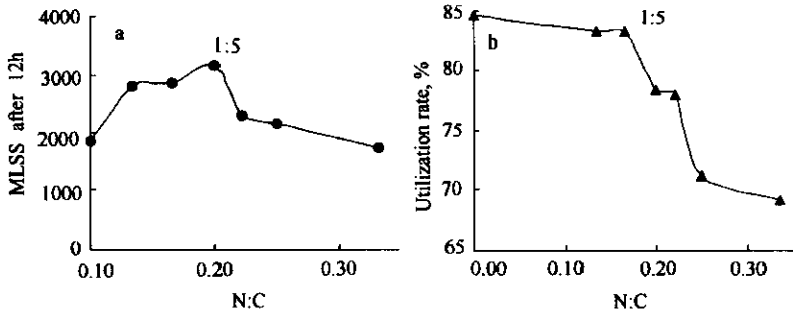

Fig. 2 (a) Effect of N:C on yeast growth; (b) Effect of N:C on removal of PEE

2.4 Comparison of different nitrogen source

In order to find out what kind of nitrogen source was suitable for yeast cultivation, NH_4Cl, $(NH_4)_2SO_4$, $(NH_2)_2CO$, and $NaNO_3$ were used as a nitrogen source, respectively. Table 2 shows the MLSS and oil removal for each nitrogen source after 24 hour's cultivation. In consideration of yeast yield, NH_4Cl and $(NH_4)_2SO_4$ had similar effects. Koh (Koh, 1983) have reported that $(NH_4)_2SO_4$ was the most effective nitrogen source to stimulate the growth of yeasts though the disparity between ammonium sulfate and ammonium chloride was very small. $(NH_2)_2CO$ and $NaNO_3$ were clearly not proper for stimulating yeast growth. From the viewpoint of oil utilization rate, however, NH_4Cl was apparently better than $(NH_4)_2SO_4$, NH_4Cl was the most effective nitrogen source in the meaning of ensuring both the yeast yield and oil removal rate. NH_4Cl was used as the sole nitrogen source in the following test.

Table 2 Effects of nitrogen source on yeast growth and oil removal

Nitrogen source	MLSS, mg/L	PEE removal, %
NH_4Cl	1208	79.68
$(NH_4)_2SO_4$	1196	71.51
$(NH_2)_2CO$	643	71.31
$NaNO_3$	740	37.65

2.5 Toxicity of ammonia to yeast strains

During yeast cultivation, the dilution rate of wastewater was gradually decreased in order to raise yeast concentration. It was found, however, that the MLSS began to decrease and dyeing tests evinced the mass death of yeasts when the concentration of COD and MLSS arrived at 20000 mg/L and 10000 mg/L respectively. Ammonia concentration increased with increasing COD, and was 1250 mg/L at the time. It was speculated that the relatively high ammonia concentration caused the failure of yeast cultivation. So, the toxicity of ammonia to yeast was verified, and the results are shown in Table 3. It was obvious that the number of live yeast decreased significantly at an ammonia concentration of 1300 mg/L or higher. So, the ammonia should be controlled to be lower than 1200 mg/L. The permitted ammonia concentration would be lower at higher pH since the ratio of free ammonia increases with rising pH.

Table 3 Toxicity of NH_3 to yeast at pH 5.0

Concentration of NH_3, mg/L	No addition	800	1000	1200	1300	1400
Count of live yeast, 5×10^{11}/ml	136	143	254	141	61	29

2.6 Preparation of high-concentration yeast sludge

Since the main reason of the failure of yeast cultivation was clarified, preparation of seed yeast sludge

was tried again. In this case, the COD concentration was increased from 5000 to 20000 mg/L step by step, and ammonia was kept at a concentration of slightly lower than 1000 mg/L(Fig. 3). The concentration of MLSS increased smoothly from the initial 76 mg/L to near 15000 mg/L, while the SVI (sludge volume index) was maintained to be below 40. The results demonstrated that high concentration of yeast sludge with a significantly good settleability could be acquired within a very short period under a controlled cultivation condition.

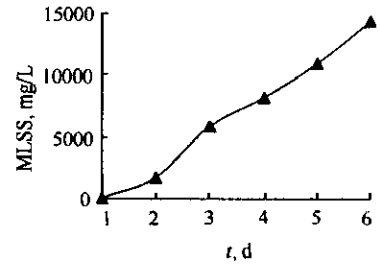

Fig.3 Variation of MLSS in cultivation tank

3 Conclusion

Yeast strains isolated from soil of a salad oil factory were applied for salad oil wastewater treatment, and the cultural conditions of seed sludge were specially investigated from several aspects. The following results were obtained: (1) The mixture of five yeast strains obtained from soil could remove about 85% TOC of oil-rich wastewater in batch test. (2) While the highest MLSS was obtained at a N:C of 1:5, the oil removal decreased with the increase of N:C during yeast sludge cultivation. (3) Ammonium chloride was the best nitrogen source for yeast cultivation from the viewpoint of yeast growth and oil utilization. (4) An ammonia concentration of over 1300 mg/L led to mass death of yeast at a pH of 5. The ammonia concentration should be controlled at a level of 1000 mg/L or lower. (5) The concentration of MLSS increased smoothly from the initial 76 mg/L to near 15000 mg/L and the SVI was maintained to be below 40 when the addition of ammonia was controlled.

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