Washout of a yeast population during continuous treatment of salad-oil-manufacturing wastewater

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Abstract

During continuous treatment of salad-oil-manufacturing wastewater using yeast isolates, a large loss of biomass was observed, which subsequently reduced the treatment efficiency. The correlation between biomass washout and loss of species was monitored using the microbiological characteristics of the effluent and the aeration tank. Of the five yeast species, only *Candida tropicalis* ultimately remained in the aeration tank possibly because it had the best settleability. Addition of nitrogen to the final effluent stimulated the activity of this yeast, which resulted in a treatment efficiency similar to that of the mixed yeast system.

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1. Introduction

Recently, several reports have demonstrated the efficient treatment of high-strength organic wastewaters using yeast in open continuous treatment system (Chigusa et al., 1996; Zheng et al., 2001, 2002). However, few studies have investigated the development of isolates in a reactor, the effect of biomass washout on degradation efficiency, or strategies to restore degradation profiles in detail during the transformation from batch enrichment culture to continuous running. Therefore, using five yeast species obtained from previous studies (Zheng et al., 2001, 2002), we investigated the effect of population washout on the running performance during the treatment of salad-oil-manufacturing wastewater (SOMW).

2. Methods

2.1. Yeast strains and wastewater

The five yeast species, *Rhodotorula rubra*, *Candida tropicalis*, *Candida utilis*, *Candida boidinii* and *Trichosporon cutaneum*, and wastewater used in this study were described in previous studies (Zheng et al., 2001). The wastewater analyses followed standard methods (Committee of SEPA, 1998).

2.2. Continuous operation in a bench-scale system

The continuous treatment system used in this study was similar to that used for the classic conventional activated sludge process. The seed sludge preparation and the startup of the continuous system were reported in previous studies (Zheng et al., 2001). Diluted SOMW, containing approximately 1400–2200 mg l⁻¹ of oil and 4100–7400 mg l⁻¹ COD, was continuously fed into the system. The recycle rate was arbitrarily adjusted to ensure that the settled sludge could be returned effectively. The yeast cultures in the aeration tank sampled on day 10 were cultured for 2 days in batch mode, and the cells were collected and introduced into the aeration tank on day 12.

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2.3. Characteristics of yeast species

Each yeast strain cultured for 3 days at 25 °C (Zheng et al., 2002) was inoculated into 10-fold diluted SOMW in batch tests. At two-day intervals, cells were collected by centrifugation and added to the next batch of freshly diluted wastewater. The maximum specific growth rate of each yeast species was determined during the first two cycles. In the third cycle, the settling and oil removal rates of each species were examined. The settling fraction $x(-)$ was calculated using:

$$x = (1 - C_s/C_t)100$$

where, C_s (mg l^{-1}) is the supernatant MLSS value after settling for 30 min and C_t (mg l^{-1}) is the total MLSS value.

2.4. Retreating effluents after population washout

The final effluent was retreated in batch mode with (a) mixed cultures of five yeast species ('mixed yeast cultures'), (b) cells left in the aeration tank at the end of continuous run with the addition of 500 mg l^{-1} total ammonia ('nitrogen addition'), and (c) cells left in the aeration tank at the end of continuous run without added ammonia, as a control.

3. Results and discussion

Initially, high-strength influent, designed to give a load of 1.5–2.5 $\text{kg COD kg}^{-1} \text{MLSS d}^{-1}$ according to Chigusa et al. (1996), was introduced into the continuous system. Owing to the rapid decrease in MLSS concentration in the aeration tank and the high MLSS level in the effluent, the initial loading was increased to 5 $\text{kg COD kg}^{-1} \text{MLSS d}^{-1}$ (Fig. 1). Three yeast species, *T. cutaneum*, *C. tropicalis*, *C. boidinii*, were found in the effluent on day 10, with one third as many viable cells as in the aeration tank.

Although fresh yeast cells from batch cultures were added to the aeration tank on day 12, the biomass washout continued until the MLSS levels in the effluent were reduced to a minimum of below 100 mg l^{-1} (Fig. 1). At that time, only *C. tropicalis* was detected in the aeration tank.

Of the five yeast species, *C. utilis* and *C. boidinii* had the greatest degradation capability, *C. tropicalis* settled the most effectively, and *R. rubra* and *C. utilis* had the highest specific growth rates, approximately double those of the other strains (Table 1). Obviously, settleability was the most important factor in the treatment system; since *C. tropicalis*, which had the best settleability, was the only remaining yeast in the aeration tank at the end of the run.

Although the influent dilution rate (0.1–0.11) and the total dilution rate including the influent and return flow

Fig. 1. Change in parameters during the continuous treatment of SOMW during 22 days: (a) ●: MLSS in aeration tank, ○: effluent MLSS (mg l^{-1}); (b) ▲: COD removal rate, △: oil removal rate (%); (c) △: COD loading rate ($\text{kg COD kg}^{-1} \text{MLSS d}^{-1}$), ●: oil loading rate ($\text{kg oil kg}^{-1} \text{MLSS d}^{-1}$); (d) ◆: influent flow velocity, ◇: return flow velocity (ml min^{-1}). ↓: addition of yeast culture.

(0.22–0.59) resulted in a suitable influent COD load, most of the biomass was washed out of the aeration tank. Only cells that settled to the bottom of the sedimentation column were returned to the aeration tank. Biomass washout often plays different roles during the transformation from batch culture to a continuous one (Chigusa et al., 1996; Klecka and Maier, 1985). In this study, this loss of species might have affected the treatment efficiency of oil and COD toward the end of run.

The effects of mixed yeast culture and nitrogen addition on retreating the effluent were compared in batch mode. The addition of nitrogen obviously stimulated the growth of yeast resulting in treatment efficiency similar to

Table 1

The oil removal rate, settling fraction, and specific growth rates of five yeast species in 10-fold diluted SOMW at 25 °C

Species	<i>R. rubra</i>	<i>C. tropicalis</i>	<i>C. utilis</i>	<i>C. boidinii</i>	<i>T. cutaneum</i>
Oil removal rate (kg oil kg ⁻¹ MLSS d ⁻¹)	0.64	0.71	0.96	0.94	0.62
Settling fraction (%)	0	76	0	44	49
$\mu_{\max}$ (h ⁻¹)	0.24	0.13	0.25	0.14	0.11

Table 2

Comparison of the effects of mixed yeast culture treatment and the addition of nitrogen for retreating effluent^a

Treatments	MLSS		Final value (mg l ⁻¹)		Removal (%)		Removal speed ^b	
	Initial	Final	Oil	COD	Oil	COD	Oil	COD
Mixed yeast cultures	313 ± 15	903 ± 40	72 ± 3	999 ± 45	85	68	0.70	3.52
Nitrogen addition	250 ± 10	1237 ± 100	78 ± 4	915 ± 45	84	71	0.56	2.99
Control	227 ± 13	227 ± 9	318 ± 12	3071 ± 135	36	2	0.78	0.29

^a Values are the means ± standard errors of three determinations.^b kg oil kg⁻¹ MLSS d⁻¹ for oil, kg COD kg⁻¹ MLSS d⁻¹ for COD.

that of the mixed yeast system (Table 2). The final MLSS values were about three times higher than the initial one with mixed culture addition, and five times higher with nitrogen addition. Compared to other wastewater from an oil-processing factory (Chigusa et al., 1996), SOMW in this study had a far higher ratio of COD to nitrogen. Therefore, ammonia should be added to counteract the reduction in performance due to the loss of species.

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