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Caprolactam waste liquor degradation by various yeasts

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Waste liquor from caprolactam manufacture contains many mono- and di-carboxylic acids. Of four yeasts tested, *Yarrowia lipolytica* DS-1 was the best at decreasing Chemical Oxygen Demand values, by up to 60% with 50 and 100 g waste liquor/after 48 h. Caproic, butyric and valeric acids were utilized most easily. Adipic acid was not decreased below 13% (w/v).

Key words: Bioremediation, caprolactam, effluent, waste liquor, yeasts.

Caprolactam, produced from benzene, is used for the production of nylon. Gujarat State Fertilizers Co. Ltd (GSFC), Vadodara, is the largest manufacturer of caprolactam in India, producing 70,000 tonnes/year. During its synthesis, a number of mono- and di-carboxylic acids, oxyacids and their polymers and cyclohexyl esters are formed (Mehta *et al.* 1989) and these, on saponification, produce the sodium salts of the acids that are found in the waste liquor (Varshney *et al.* 1989). Some 60 to 70 tonnes/day of waste liquor are presently burnt off in incinerators. Periodically, liquor is drained into an effluent treatment plant. The liquor, with organic acid concentrations as high as 150 to 200 g/l, increases the Chemical Oxygen Demand (COD) in the aeration tank, resulting in loss of microbial viability. A bioprocess for waste liquor disposal requires a consortium of microorganisms capable of degrading it at high concentration.

No information is available on microbial degradation of caprolactam plant effluent, although caprolactam itself is readily degraded by various microorganisms, including algae (Stupina & Lenova 1985; Gvozdyak & Demitrenko 1990). Various yeasts are used in the biodegradation of complex organic compounds, such as polycyclic aromatic hydrocarbons, phenol and lignin, from industrial waste waters (Pavlenko *et al.* 1984; Yukishitsu 1989). The present study was on the degradation of caprolactam waste liquor by various yeast strains. As far as we are aware, this is the first report on degradation of this liquor by yeasts.

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Materials and Methods

Microorganisms and Growth

Yarrowia lipolytica DS-1 (Shah *et al.* 1989), *Y. lipolytica* GSFC-5001, *Candida tropicalis* and an unidentified strain (Y-43) (only the last three strains are isolated from contaminated soil at a local oil refinery), were acclimatized by growing them in shake flasks (200 rev/min. 32 ± 1°C) sequentially in two media labelled WLM-1 (50 g/l waste liquor) and WLM-2 (100 g/l waste liquor) containing (g/l): peptone, 7.0; KH_2PO_4, 6.8; $CaCl_2$, 0.1; $FeSO_4.7H_2O$, 0.02; $MgSO_4.7H_2O$, 0.3; $MnSO_4.H_2O$, 0.02; and $ZnSO_4.7H_2O$, 0.02; at pH 7.0. Acclimatized strains were maintained on WLM-2 agar slants at 4°C.

Analytical Methods

Dry weight of biomass was determined by passing 10 ml of culture broth through pre-weighed 1.0-μm pore filters and washing the filter twice with distilled water before drying them to constant weight at 60°C. COD analysis was by a standard method (Clesceri *et al.* 1989). Concentrations of individual organic acids was determined by HPLC using a refrective index detector, a cation exchange column (SCR-101H; Shimadzu) and 0.005 M H_2SO_4 as eluent at 0.8 ml/min at room temperature. Standard acids used for comparison were obtained from Aldrich.

Results and Discussion

Properties of Caprolactam Waste Liquor

Caprolactam waste liquor is a dark liquor with a pH of 11.0 and contains many organic acids, the major among which are adipic, succinic, butyric, glutaric, valeric and caproic acids. One unknown organic acid, possibly oxycaproic acid, was also detected. The COD was found to be between 800,000 and 1,200,000 p.p.m.

Table 1. Growth and Chemical Oxygen Demand (COD) decrease by various yeast strains after 48 h in WLM-1 and WLM-2.*

Culture	WLM-1		WLM-2	
	Drywt (g/l)	COD after treatment (p.p.m.)	Drywt (g/l)	COD after treatment (p.p.m.)
Strain Y-43	2.8	28,800	1.2	58,600
Candida tropicalis	2.9	30,400	1.4	61,300
Y. lipolytica GSFC-5001	2.9	25,600	4.0	46,400
Y. lipolytica DS-1	3.3	19,200	4.7	32,000
Mixed culture	4.7	14,000	5.2	40,000

* The values are means of three independent experiments. pH varied between 8.0 and 8.7 after 48 h. Initial COD were 48,000 p.p.m. (WLM-1) and 73,600 p.p.m. (WLM-2).

Table 2. Organic acid concentration (g/l) in WLM-1 and WLM-2 with different yeast strains.*

Organic acid	WLM-1					WLM-2				
	At 0 h	After 48 h				At 0 h	After 48 h			
		Y-43	C.t.	5001	DS-1		Y-43	C.t.	5001	DS-1
Succinic	0.4	0.2	0.1	0.1	0.1	0.7	0.3	0.3	0.5	0.5
Glutaric	0.5	0.2	0.1	0.1	0.2	1.0	0.6	0.4	0.5	0.3
Adipic	3.3	2.3	1.6	1.1	1.2	6.6	5.5	5.9	6.6	5.7
Unknown†	3.9	3.6	1.8	1.7	1.3	7.8	7.0	7.8	7.8	4.3
Butyric	1.2	0.0	0.5	0.2	0.0	2.5	2.0	1.4	0.8	0.6
Valeric	3.4	0.0	0.3	0.3	0.2	6.8	5.2	2.5	0.3	0.3
Caproic	0.9	0.0	0.0	0.0	0.0	1.9	0.7	0.3	0.0	0.2
Total	13.6	6.3	4.4	3.5	3.0	27.3	21.3	18.6	16.5	11.9
Decrease		7.3	9.2	10.1	10.6		6.0	8.7	10.8	15.4

* Values are means of three independent experiments.
† Detected by HPLC, probably oxycaproic.
Y-43—Unidentified isolate; C.t.—*Candida tropicalis*; 5001—*Yarrowia lipolytica* GSFC-5001; DS-1—*Yarrowia lipolytica* DS-1.

Acclimatization of Yeast Strains

All four yeast strains took 7 to 8 days in WLM-1 to reach 3×10^8 to 4×10^8 cells/ml. Three serial transfers were required before cells reached 1×10^9 cells/ml in 48 h indicating that the cultures had acclimatized in WLM-1. These adapted cells were used as inocula for further acclimatization in WLM-2 media.

Waste Liquor Degradation Studies

Since normal residence time for effluent in the aeration tank is between 48 and 72 h, growth and COD reduction profiles of the yeasts were checked in both media after 48 h (Table 1). While Y-43 and *C. tropicalis* grew less in WLM-2 than in WLM-1, the other two strains grew well and consequently decreased the COD to a greater extent. *Y. lipolytica* DS-1 decreased COD by as much as 57% (by 41,600 p.p.m.) even at the high initial COD load of 73,600 p.p.m. (WLM-2; Table 1): A mixed culture of all four strains decreased the COD value of WLM-1 and WLM-2 by about the same amount (34,000 p.p.m.) (Table 1), indicating that such a consortium may not yield significantly better results than DS-1 alone.

Monocarboxylic acids were more readily utilized by all four strains than dicarboxylic acids in both media (Table 2). Strains Y-43 and *C. tropicalis*, which did not grow well in WLM-2 (Table 1), failed to decrease the concentrations of most of the organic acids to below 35% (w/v) and may not therefore be suitable for biodegradation of caprolactam waste liquor.

Y. lipolytica GSFC-5001 generally utilized all organic acids better and decreased total organic acids more than Y-43 and *C. tropicalis*. However, the decreases in the total concentrations of organic acids in the two media were similar (10.8 and 10.1 g/l; Table 2). At the higher concentration of liquor the concentration of the unknown and adipic acids were not reduced, showing that this strain preferentially utilized other acids.

Y. lipolytica DS-1 gave the greatest degradation, utilizing all acids to below 35% (w/v) and caproic, valeric and butyric acids almost totally (Table 2). Even at the higher

liquor concentration, it decreased the latter three acids by 80 to 95%. Glutaric acid, the unknown acid and succinic acid were decreased to 36%, 55% and 66%, respectively. However, adipic acid did not fall below 87%. Total organic acid concentrations in both media were decreased more by this strain than the three others. Only this strain utilized more total organic acids in WLM-2 than in WLM-1, indicating that it is probably the best of the strains for further biodegradation studies.

Closure of the incinerators in GSFC's caprolactam plant results in drainage of waste liquor into the effluent treatment plant, taking the final concentration of organic acids up to 200 g/l. If these acids are to be completely degraded the search for organisms capable of dealing with such concentrations must continue. It may be possible to continue the acclimatization programme with *Y. lipolytica* DS-1 to achieve this goal.

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