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Comparative evaluation of yeast and bacterial treatment of high salinity wastewater based on biokinetic coefficients

N.P. Dan, C. Visvanathan *, Biswadeep Basu

Urban Environmental Engineering and Management Program, Asian Institute of Technology, P.O. Box 4, Klong Luang, 12120 Pathumthani, Thailand

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Abstract

This paper compares the performance of the aerobic treatment of high organic–high salinity wastewater by yeast and bacterial systems. The biokinetic coefficients for both the systems were determined and used to analyze the behavior of the yeast and bacterial systems under high salinity conditions. It was found that the yeast culture was more efficient compared to the bacterial culture, especially for high salinity conditions that severely inhibit growth and performance of bacterial systems. The values of the biokinetic coefficients obtained from this study are in agreement with the observations. Nutrient removal capacity has also been found to be better for yeast due to higher nutrient uptake in the yeast biomass.

Keywords: Yeast; Respirometric method; Biokinetic coefficients; Saline wastewater treatment

1. Introduction

Vegetable, tanning and seafood processing industries generate large quantities of saline wastewater with high concentration of organic pollutants. In seafood factories located in arid zones, unit processes such as defrosting, butchering and washing produce high organic–high salinity wastewater due to using treated seawater as the raw water source (Woolard and Irvine, 1995). High salt content in wastewater is known (Kargi and Uygur, 1996) to significantly reduce the treatment efficiency of conventional activated sludge, attached-growth, anaerobic, nitrification and denitrification processes. As a result, BOD removal efficiency decreases, effluent turbidity and solid loss increases due to poor solid settling in the secondary sedimentation tanks, and mixed liquor floc protozoan population changes (Dalmacija et al., 1996). Application of salt-tolerant bacteria for biological treatment of wastewater has been found to improve treatment efficiency under such conditions. For example, the use of halophilic microorganisms (e.g. *Halobacter halobium*) in an activated sludge process resulted in better treatment performances at salt contents above 2% (Kargi and Dincer, 2000).

As an alternative to salt-tolerant bacteria, osmotolerant yeast can also be used to treat high organic–high salinity wastewater. Yeast is traditionally used in high substrate concentrations (or high loading) in the fermentation industries such as soy sauce and beer production. Nishihara ESRC (2001) carried out research on treatment of marine products processing wastewater, containing 8.5 g NaCl/l and 5450 mg/l BOD_5, by the yeast cycle system (YCS). BOD_5 and total nitrogen removals obtained were 99% and 86%, respectively. Excess sludge could be reused. A specific yeast strain (*Pichia guilliermondii* A9) has been found (Park and Choi, 1999) to tolerate a very high salt content (up to 100 g NaCl/l) during growth phase. In brine waste treatment this yeast could remove about 90% of the BOD within 24 h. Further, the structure of the yeast flocs facilitates oxygen diffusion, leading to reduction in air requirement and energy consumption.

This study attempted to compare the performance of the yeast and bacterial systems by systematically studying biokinetics coefficients by respirometery. The nature of the reactions as well as the observations made during the course of the study have been explained in light of these biokinetic coefficients.

* Corresponding author. Fax: +66-2-524-5625.
E-mail address: visu@ait.ac.th (C. Visvanathan).

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2. Methods

2.1. Enrichment of seed yeast sludge

Osmotolerant yeast sludge, a mixture of all wild yeast strains, was collected from the bottom sediments of an equalization tank of a fish sauce factory. This tank received wastewater with a high salt and organic content (30.2 g NaCl/l and 800 mg COD/l). The procedure of enrichment for yeast was carried out as presented in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). The propagation of a specific yeast strain based on free competition among different microorganisms in wastewater, (Nishihara ESRC, 2001) was carried out in 2-l containers by the fill-and-draw method. The pH of the yeast culture was adjusted to 3.5 in order to maximize yeast growth and limit bacterial growth (Pelczar and Reid, 1972; Elmaleh et al., 1996). Enrichment comprised of 36 h of aeration followed by approximately 12 h of settling. Thereafter, supernatant was decanted and replaced with fresh wastewater for further enrichment. This procedure was stopped when the yeast biomass exceeded 3000 mg/l.

2.2. Seed bacterial sludge

Bacteria seed sludge was obtained from the activated sludge process of the same fish sauce factory. This plant treats wastewater with a low salt content of 0.72 g NaCl/l.

2.3. Acclimation

After enrichment, the yeast and bacterial sludges were acclimatized to tolerate high salinity wastewater of 32 and 45 g NaCl/l. During the acclimation process, an initial COD concentration of 5000 mg/l for yeast and 1000 mg/l for the bacterial culture were maintained to simulate the actual feeding condition of the YCS. The pH was adjusted to 3.5 and 7.5 for the yeast and bacterial culture respectively (Nishihara ESRC, 2001). Acclimation was conducted in 2-l batch reactors using the fill-and-draw method. After each aeration cycle, the reactor solids were allowed to settle and the COD of the supernatant was determined. Acclimation was continued until the COD removal efficiency exceeded 80%.

2.4. Biokinetic experiments

The kinetic coefficients of the acclimatized yeast and bacterial cultures at varying salt contents were determined using a closed 0.9-l batch respirometer, equipped with a recorder and a dissolved oxygen (DO) meter. Constant temperature was maintained by circulating water through a water jacket enclosing the reactor vessel. Table 1 presents the operating conditions of the experiments. The S_0/X_0 ratio (initial substrate concentration/biomass concentration) that governs the quality of the batch respirometric tests (Cech et al., 1984) was maintained in the range of 0.01–0.35 in order to avoid substrate overloading. Three salt concentrations of 20, 32 and 45 g NaCl/l were studied for biokinetic experiments.

Table 1
Operating conditions of the respirometric experiment of yeast and bacterial cultures

Operating conditions	Yeast culture	Bacterial culture
Initial pH	3.5	7.5
Temperature (°C)	30 ± 0.5	30 ± 0.5
X_0 (mg MLSS/l)	1500	1500 (Cech et al., 1984)
Substrate concentration (mg COD/l)	20–500	20–200
S_0/X_0 ratio	0.01–0.35	0.01–0.15 (Chudoba et al., 1992)
Suppressing nitrification	None	Adding 70 mg N-ammonia/l (Liebeskind, 1999)

The result of the respirometric experiments provided values of the oxygen uptake rates (OUR) that were used for calculating specific growth rates μ. Values of OUR, μ at corresponding substrate concentration (S), maximum oxygen utilization rate (OUR_{max}), observed specific growth rate (μ_{obs}) and half-velocity constant (K_S) at the selected salt concentrations were determined, based on Monod kinetics by regression analysis. By using values of μ_{obs} at various corresponding salt contents (I), the critical inhibitory salt concentration (K_I, above which the reaction ceases) and the maximum specific growth rates μ_{max} were computed according to the Ghose and Tyagi model (Han and Levenspiel, 1988).

2.5. Analytical methods

All chemical analyses were conducted as the *Standard Methods* (APHA, 1995). Potassium dichromate COD was analyzed by the close reflux method with correction for chloride interference.

3. Results and discussion

COD removal efficiency for acclimated yeast and bacterial cultures at 25, 32 and 45 g NaCl/l was studied with respect to the substrate utilization rate U. The results are presented in Fig. 1. It was observed that when salt content increased, U decreased significantly for both yeast and bacteria due to pronounced salt inhibition even though these cultures were acclimated. When salt content was increased from 20 to 45 g/l, U decreased from 3.26 to 0.40 g COD/g MLSS d for the bacterial culture and from 2.65 to 0.88 g COD/g MLSS d for

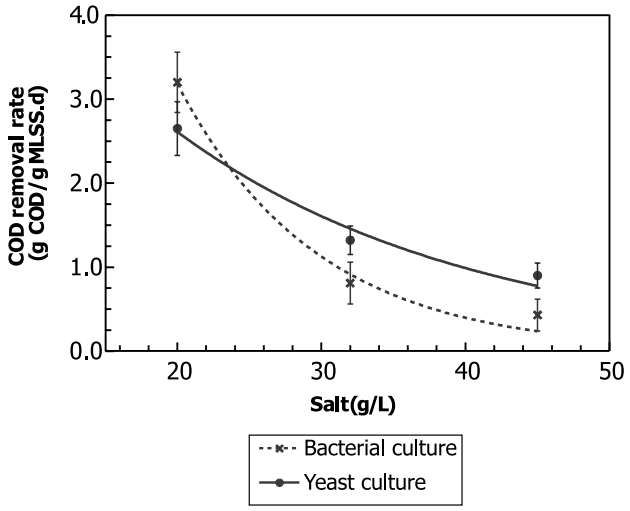

Fig. 1. Variation in COD removal rate as a function of salt contents in yeast and bacterial cultures.

yeast. This showed that the rate of decline for U was higher for the bacterial culture compared to yeast, indicating that bacteria are much more susceptible to changes in the salt concentration. Moreover, it was found that at 45 g NaCl/l, the time required for yeast acclimation was 16 days compared to 26 days for bacterial culture even though the initial F/M ratio of the yeast reactor was 1.12 g COD/g MLSS d to that of 0.5 g COD/g MLSS d for the bacterial reactor, corresponding to organic loadings of 5.0 and 1.0 kg COD/m^3 d respectively. Normally, for aerobic treatment systems higher F/M ratio vis-à-vis higher organic loading places a greater stress on the system, which generally results in low efficiency of substrate removal and low oxygen utilization. In practice the range of F/M is normally controlled between 0.3 and 0.8 kg COD/m^3 d (Metcalf and Eddy, 1991).

These observations indicate that yeast is less vulnerable to high salt concentration as compared to bacteria, allowing yeast to sustain a higher organic loading (higher F/M ratio). High organic loading enables downsizing of reactors and a better rate of acclimation allows for early start-ups and rapid recovery from shock, both being desirable from the practical standpoint.

For anaerobic treatment systems, efficiency in the range of 60–70% is generally obtained for this range of organic loading (1.0 g COD/g MLSS d) as compared to more than 80% (for 45 g NaCl/l) at a HRT of 24 h for yeast. Moreover it was also found (Feijoo et al., 1995) that anaerobic microorganisms were highly susceptible to salt concentration and total inhibition could be noted for some treatment systems for salt content exceeding 20 g/l. Therefore, a yeast based treatment system was found to be better than both aerobic and anaerobic bacterial systems.

It was further observed from Fig. 1 that at salt concentration below 25 g NaCl/l, the bacterial system displayed improved COD removal efficiency. This may be an indication that once the inhibitory effect of salt was reduced, the bacterial system regained its efficiency in the range as is observed for municipal wastewater. However F/M ratio was also an important factor in this case. As is noted earlier, higher F/M ratio for the yeast reactor may be a reason for lower efficiency at lower salt concentration. Further investigation has not been carried out as this study was focused on high organic–high salt concentration.

Specific growth rates (μ) were obtained using the respirometric method at different initial COD of 20–500 mg/l for 25, 32 and 45 g NaCl/l. The variation of DO with time for each substrate concentration generated a respirometric curve. The respiration rate (OUR) at time (t) is given by the slope of the curve obtained. Based on these respirograms, the biokinetic constants: endogenous respiration rate ($OUR_{x,e}$), total respiration rate (OUR_t), net oxygen consumption, yield coefficient (Y) and specific growth rate (μ) were evaluated. Maximum specific growth rate (μ_{obs}) and the half-velocity constant (K_S) were determined from regression analysis. Figs. 2 and 3 show the variation of specific growth rate with substrate concentrations at different salt contents for the yeast and bacterial cultures, respectively. It was generally observed that the growth rate displayed an asymptotic behavior (as predicted by the Monod's model) to reach a quasi-maximum after which the variation of rate was not pronounced. Values of μ_{obs}, K_S, and Y are summarized in Table 2.

At high salt contents, the specific growth rate of yeast was found (Table 2) to be higher than that of bacteria, while it was lower than that of bacteria at lower salt

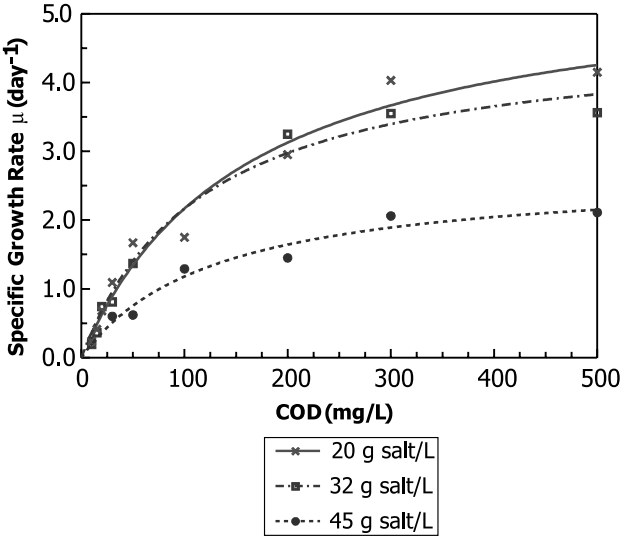

Fig. 2. Variation in specific growth rate of the yeast culture as a function of substrate concentration at various salt concentrations.

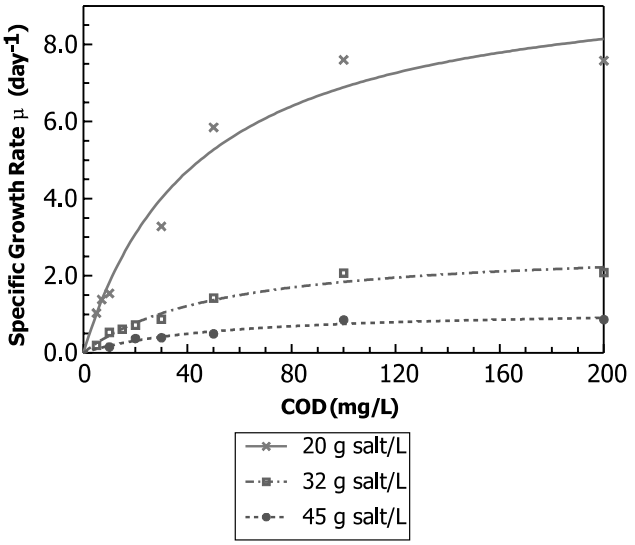

Fig. 3. Variation in specific growth rate of the bacterial culture as a function of substrate concentration at various salt concentrations.

contents. This indicated that the inhibitory effect of salt was less on the yeast system, which allowed for higher growth rate of yeast that resulted in higher COD removal rate. The reverse phenomenon was observed at low salt content.

K_S values for the bacterial culture (45–53 mg COD/l) at high salt contents were found to be higher than the K_S value of normal activated sludge (5–30 mg/l). This indicated that heterotrophic aerobic microorganisms living in high salinity condition showed lower affinity for the substrate than those at low salinity. This was probably due to reduced functioning and reduced growth. K_S values of yeast were found to be 5–6 times higher than those of bacteria in the range of 130–170 mg/l. Thus the maximum growth rate of the yeast culture was obtained at high organic substrate concentrations. One of the probable reasons for higher growth rate in the yeast culture was the presence of halophilic species of yeast. Previous researchers (Kargi and Dincer, 2000; Park and Choi, 1999) showed that halophilic organisms were usually more osmotolerant. Kushner and Kamckura (1988) found that the primary protein structure of these organisms adapted to the high saline environment by releasing halophyte enzymes that functioned better in high salinity conditions and sustained the growth rate of these organisms. On the other hand, several bacterial species tend to dehydrate and disintegrate due to the higher osmotic pressure difference between the protoplasm and the ambient high saline condition that leads to severe inhibition of growth rate for bacteria.

When inhibitors are present in the wastewater, the Ghose and Tyagi model (Han and Levenspiel, 1988) is probably more suitable because it considers the inhibition effects. The model can be written as follows:

$$\mu = \mu_m \left(1 - \frac{I}{K_I}\right) \frac{S}{S + K_S} = \mu_{obs} \frac{S}{S + K_S} \quad (1)$$

$$\mu_{obs} = \mu_m \left(1 - \frac{I}{K_I}\right) \quad (2)$$

where I is the salt content (mg/l); K_I is the critical salt content above which reaction stops (mg/l); μ_{obs} is the observed maximum specific growth rate at certain salt content (d^{-1}); μ_m is the maximum specific growth rate in salt free solution (d^{-1}).

The inhibitory effects of high salt on yeasts and bacteria, based on the Ghose and Tyagi model, are presented in Fig. 4. The salt inhibition constant (K_I) is

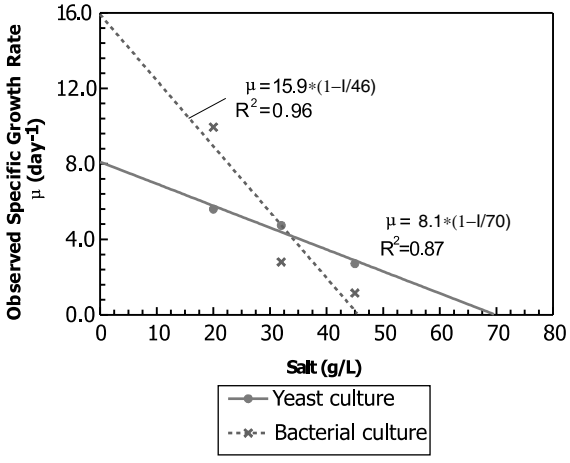

Fig. 4. Inhibition effect of salt content on the yeast and bacterial cultures.

Table 2
Biokinetic coefficients of the yeast and bacterial cultures at high salt contents

S (g salt/l)	Substrate	μ_{obs} (d^{-1})		Y (g VSS/g COD)		K_S (mg COD/l)	
		Yeasts	Bacteria	Yeasts	Bacteria	Yeasts	Bacteria
20	Glucose	5.60	9.95	0.46	0.57	158	45
32	Glucose	4.74	2.80	0.48	0.58	118	55
45	Glucose	2.70	1.15	0.41	0.53	130	53
<1.0	Domestic wastewater (Henze et al., 1997)		4–8		0.5–0.7		5–30

determined by the slope of the relationship between μ and the salt content, I. K_I was found to be 70 g/l for yeast and 46 g/l for the bacterial culture. These findings indicated that the inhibitory salt limit for the bacterial culture was lower than for the yeast, which supports the analysis done so far for different observations of this study. Earlier research on osmotolerant microorganisms suggested that the actual critical salt limits might be higher than the values found from the Ghose and Tyagi model. Park and Choi (1999) reported that growth of the yeast strain *Pichia guilliermondii* on *kimchi* brine waste was sustained at up to 120 g salt/l while Kargi and Dincer (2000) reported that the bacterial strain *Halobacter* in activated sludge culture could continue COD removal at 50 g salt/l. Therefore, the limits obtained in this study (using the Ghose and Tyagi model) were indicative, however, and useful in comparing the relative performances of the two systems.

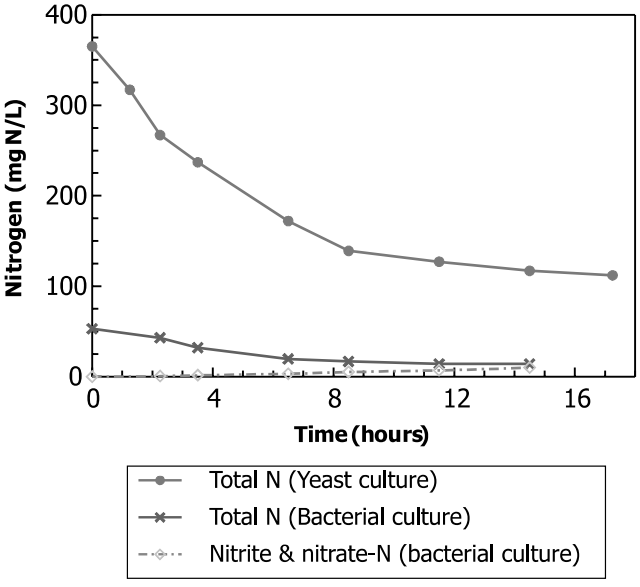

Fig. 5. Variation of nitrogen concentrations in the yeast and bacterial cultures at 32 g salt/l.

It can be also observed from Table 2 that irrespective of the salt content, the yield (Y) of the yeast culture is lower than that of the bacterial culture. This indicates that lower excess sludge would be produced from the yeast system compared to the bacterial system for the same substrate quantity. This was due to higher specific utilization rate of the yeast culture, which has been discussed earlier. Reduction in the sludge volume would reduce the problems with sludge handling, treatment and disposal.

The efficiency of nitrogen removal from wastewater depends directly on the extent of nitrification. Nitrogen removal was found to be higher for the yeast system compared to the bacterial culture (Fig. 5). A possible reason for better nitrogen removal was higher nutrient (nitrogen and phosphorous) uptake in the yeast cells during cell metabolism compared to bacterial cells. The amount of nitrogen in the yeast biomass was found to be 6.75% on an average as compared to 3.16% in the case of the bacterial culture (Table 3). Therefore, the uptake of nitrogen in the biomass vis-à-vis nitrogen removal efficiency, was found to be higher in the yeast culture, and the residual nitrite and nitrate lower than in the bacterial culture.

4. Conclusions

The results obtained from this study indicated that the yeast culture was more efficient in treating high organic–high salinity wastewater compared to bacterial cultures. Values of biokinetic coefficients showed that substrate utilization rate, maximum specific growth rate and half-velocity constant were higher for the yeast culture at high salt content, which explained the higher COD removal rate for the yeast culture. Performance of the yeast culture was sustained even at higher organic loading vis-à-vis F/M ratio.

Lower susceptibility of the yeast culture to high salt content may be due to the higher adaptability of some yeast species to high salinity condition. These organisms

Table 3
Composition of bacterial and yeast sludge at high salt contents

Microorganisms	Volatile solid (%)	COD:N:P	Nitrogen content (% of dried weight)	Phosphorous content (% of dried weight)
Yeast culture:				
20 g NaCl/l	94.1	100:4.7:1.8	5.79	2.23
32 g NaCl/l	92.3	100:5.9:1.5	7.18	1.79
45 g NaCl/l	95.0	100:7.2:1.7	7.29	1.70
Average	93.8	100:5.9:1.7	6.75	1.91
Bacterial culture:				
20 g NaCl/l	89.5	100:2.4:0.5	2.70	0.61
32 g NaCl/l	92.0	100:3.0:0.8	3.05	0.86
45 g NaCl/l	94.0	100:4.3:0.6	3.74	0.90
Average	91.8	100:3.7:0.6	3.16	0.79

adapted to the high saline environment by releasing halophyte enzymes that sustained the growth rate of these organisms. Bacterial cells tend to dehydrate and disintegrate in this situation due to a higher osmotic differential between the protoplasm and the ambient high saline condition. The critical inhibitory salt limit as was obtained from the Ghosh and Tyagi model was found to be higher for the yeast culture confirming the earlier results.

Specific nutrient uptake for the yeast cells was also found to be higher than that of the bacterial cells: that resulted in higher nitrogen and phosphorous removal rates from the wastewater. However, the study also indicated that with reduction in the salt content, bacterial cultures tended to perform better. At this stage, it can be concluded that a yeast system may be a better form of treatment for high organic–high salinity wastewater. Thus, using yeast for primary treatment of high organic–high salinity wastewater followed by a secondary treatment with bacteria (as is done in the YCS) may render a higher degree of performance and may be a possible method to treat high organic–high salinity wastewater.

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