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Research Paper

Biological treatment of chemical oxygen demand from high saline water by two salt-tolerant strains

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In this study two strain salt-resistant bacterial isolated from soil samples of a sea food industry were used to evaluate their applicability for chemical oxygen demand (COD) removal from high saline water. The effect of major process variables such as feed COD, A/Q ratio, salt concentration, dissolved oxygen and temperature on COD removal efficiency were investigated. The pH was an important parameter effecting on the COD removal efficiency mainly because pH affected bacterial-specific physiology and cellular growth positively correlated with COD removals. The optimal pH was 7 in this study. Experimental results indicate that the COD removal efficiency reached to 90% when the feed COD, A/Q ratio, salt concentration, dissolved oxygen and temperature were kept constant at 4000mg/L, 1500 m^2hm^{-3}, 30g/L, 4mg/L and 25ºC, respectively. Percent COD removal decreased from 93% to 88% and further to 42% when the feed COD increased from 2000mg/L to 4000mg/L and further to 6000mg/L, respectively. COD removal efficiencies increased from 43.1% to 96.7%, while corresponding the A/Q ratio varied from 500 m^2hm^{-3} to 2700 m^2hm^{-3}. Increasing salt concentrations did not affect COD removal considerably resulting in 89% removals for salt concentrations up to 30g/L. However, percent COD significantly dropped to 71% with even high salt concentrations (50g/L) probably because this salt concentration began to inhibit the activity of bacterial and resulted in low COD removals. The results also indicate that dissolved oxygen and temperature had important effect on COD removal efficiencies.

Keywords: Chemical oxygen demand, salt concentration, Biological treatment, bacterial strain, factors.

1. INTRODUCTION

Discharge of food industrial water such as vegetable, tanning, and seafood plants causes significant deterioration of the environment largely because of the presence of organics and other pollutants[1–8]. Performance of conventional biological treatment processes used for this kind of water usually results in low COD removal mainly because high salt concentrations or other components in the water could produce high osmotic pressure on bacteria cells, which causes plasmolysis, dehydration, and disintegration of bacteria cell and loss of cell activity [9–11]. Therefore high salt content are typically desalinated or diluted to an extent that will not impede the microbial activity. Since the desalination by reverse osmosis, ion exchange or electrodialysis is expensive and the dilution is an inefficient use of freshwater resource and further significantly increase wastewater volume, an alternative treatment process should therefore be considered.

Ludzack reported that chloride concentrations of 5–8 g/L did not affect biochemical oxygen demand (BOD) removal but did cause clarification problems in conventional activated sludge treatment processes [12]. Low organic loading and high mixed-liquor volatile suspended solids (MLVSS) concentration increased activated sludge tolerance to chloride changes and high chloride concentrations [12]. In spite of the detrimental effect of salt on microbial activity, moderate acclimation of activated sludge to high salinity is possible. The success of such adaptation depends on several factors, such as the type and growth phase of bacterial, as well as the rapid or gradual increase of salt concentration during acclimation[9]. It has actually been shown that Escherichia coli exhibited the greatest degree of adaptability to NaCl in the early stationary growth phase[13]. Kincannon et al found that switching from a freshwater to a saline influent containing 30 g/L NaCl reduced glucose removal and total suspended solids concentration in a continuous flow reactor[14]. However, subsequent reduction of influent NaCl concentration resulted in increased biomass concentration and improved substrate removal[15]. Some major reports stated that rapid changes in NaCl content (either an increase or decrease) caused severe upsets partly because rapid changes in salt concentration caused immediate release of cellular constituents which resulted in an increase in soluble COD [16,17]. Although the adaptation of activated sludge has already proved to be possible, a major bottleneck is that the proper performance of such salt-adapted systems is usually limited to less than 5% salt [18,19].

Burnett reported that shock loading with influent containing 32–38 g/L NaCl caused a reduction in BOD removal of up to 73% and elevated levels of suspended solids in the effluent[20]. Freshwater sewage added to a reactor acclimated to seawater sewage also resulted in a drastic reduction in BOD removal efficiency [20]. Kargi reported that the high salt concentration (5%) dramatically inhibit the biological degradation of organics in the wastewater. The removal rate of COD was nearly 370 mg l^{-1} h^{-1} with salt-free wastewater and dropped to a value of 264 mg l^{-1} h^{-1} for a 5% salt concentration, which indicated on approximately 29% reduction.[21] Dincer also reported that percent COD removal decreased from 96 to 32% when salt content increased from 0 to 6%; and COD removals were above 90% for salt contents below 0.5%[22]. Apparently, increases in salt content resulted in significant drops in COD removal efficiency and therefore, effluent COD concentrations increased especially for salt contents above 1%. There have been many studies on the treatment of saline and hypersaline wastewater (>3.5% salt) in constant concentrations or high-polluted groundwater[23]. These authors also conclude that conventional biological processes cannot be used to treat wastewater containing more than 3-5% salt [24,25]. However, the use of specialized organisms (i.e. halophiles) changed this situation and began to be a promising method to enhance the biological treatment of saline wastewater.

Woolard and Irvine showed that biofilms of halophilic bacteria acclimatized in constant concentrations of up to 15% salt have a great capacity for treating hypersaline wastewater. Dincer and Kargi studied the COD removal performance of rotating biological discs system in saline wastewater treatment [26,27][. A salt-tolerant bacterium was added to an activated sludge culture in order to improve the systemic performance.

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As a result, COD removal efficiency was reported to nearly 85% at salt concentration of 5% after Halobacter used [28].

There are many reports on biological treatment of high saline wastewater [29–32]. However, there were few reports about the utilization of pure bacteria in aerobic treatment of high saline wastewater. By considering the potential of two salt-tolerant bacterial in saline wastewater treatment, this study was designed to investigate the COD removal performance of two salt-tolerant bacterial, ST7 and ST31, in the treatment of synthetic saline wastewater by experimental reactor under different operating conditions. The two salt-tolerant bacterial were used throughout the experiment. Feed COD, A/Q ratio, salt concentration, dissolved oxygen and temperature were considered as the major variables impacting the efficiency of COD removal.

2 Materials and Methods

2.1 Experimental Set-up

A schematic diagram of the experimental set-up is depicted in Fig.1. The system was separated into two sections, which including reaction zone and sedimentation zone. The reaction zone had some fillers, which were made of fiber bundle and annuliform plastic disc. Total fillers were immersed in water and covered by biofilm. The liquid volumes in reaction zone and sedimentation zone were Vr=8.15L and Vs =0.85L respectively. The specification of the experimental set-up used is summarized in Table 1 and Figure 1.

The feed wastewater was firstly pumped into the

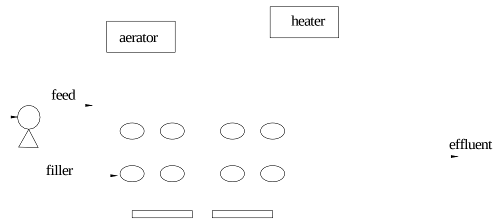

Fig. 1. Schematic diagram of the experimental set-up

Table.1 Summary of experimental set-up

Specification	Unit	Value
Experimental set-up	cm	16.3×25×20.5
Total volume	L	9
Number of stages		2
Number of fillers		16
Total filler surface area	m^2	0.25
Filler spacing (up-down)	cm	3.5
Filler spacing (left-right)	cm	3
Temperature	ºC	25 ±5

reaction zone, then it flowed to sedimentation zone from the foot slot and discharged. The slant of sedimentation zone could conduct sedimentation composition of media to fall. Liquid phase aeration was provided by an aerator. Temperature was controlled with the aid of heating element, cupric tubes and an automatic controller. The pH was controlled manually with the addition of dilute sulphuric acid and NaOH.

2.2 Wastewater Composition

Synthetic wastewaters were used to provide a source of carbon, nitrogen, phosphorus, and trace elements required for biomass growth. The wastewater used was composed of diluted molasses, urea, KH_2PO_4, $MgSO_4$, salt concentrations and trace elements, resulting in COD/N/P =100:10:1 in feed wastewater.

Concentrated molasses had a COD content of approximately 10^6mg/L and was diluted with water to obtain the desired COD. $MgSO_4$ concentration in the feed was 0.05g/L and every 1 L of feed wastewater was added to10 mL of trace elements solution. Table 1 summarizes the compositions of the trace elements solution. Salt concentrations were adjusted by adding required amounts of NaCl into the feed wastewater. The influent was maintained at 4 C before its use in the experimental system.

Table 2. Compositions of Trace Elements Solution

Trace elements solution	Concentration (mg/L)
$MgSO_4 \cdot 7H_2O$	10
$ZnSO_4 \cdot 7H_2O$	50
H_3BO_3	10
$N(CH_3COOH)_3$	4500
$CaCl_2 \cdot 2H_2O$	10
Na_2MO_4	10
$CoCl_2 \cdot 6H_2O$	200
$AlK(SO_4)_2$	10

2.3 Organisms

Two salt-tolerant bacterial were provided by a biological company named with ST7 and ST31. ST7 and ST31 were mixed in 5:1 ratio and used as inoculum.

2.4 Experimental Procedure

Experiments were started batchwise to obtain a dense culture. About 7.5L synthetic wastewater was placed in the reactor, inoculated with two salt-tolerant bacterial. The aeration rate was adjusted to result in desired DO concentration. Withdraw 2L medium from the reactor and pump 2L feed water every two days. Batch wise was operated for about a week. Once the biofilm had formed and the effluent COD was constant (steady state conditions), treatment of the sequence operation was started. The feed wastewater was continuously fed to the reactor. The effluent was discharged from the outlet of tank. Feed COD, A/Q ratio, salt concentration, dissolved oxygen and temperature of the mixed liquor varied throughout the experiment. Once steady state conditions had been reached, testing was carried out for a period of one week for each of the running conditions assayed.

2.5 Analytical Methods

Samples were taken from effluent twice a day and were centrifuged to remove bacterial from the wastewater. Clear supernatants were analyzed for COD, ammonium/nitrate nitrogen and phosphate-P contents. Standard kits (Merck-Spectroquant) and spectrometric methods were used for nitrogen and phosphorous analysis. COD was determined according to Standard Methods [33]. Dissolved oxygen was measured with DO analyzer, DO probe and WTW Multi 350i. The pH was measured with pH analyzer, pH probe and WTW Multi 350i.

3. Results and Discussion

Before starting biological organics removal, an experiment was performed in order to investigate possible physic-chemical organics removal under specific experimental conditions.

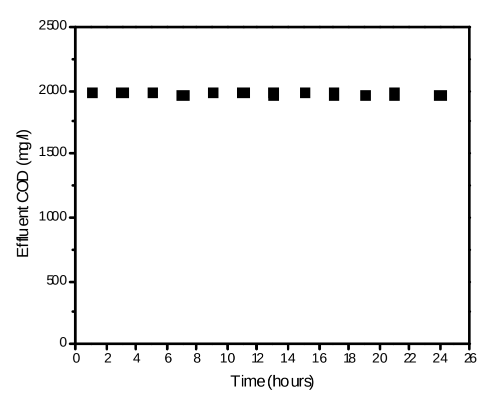

Fig.2. Variation of effluent COD before treatment

For this reason the clean filter (without any bacterial) was used to treat saline wastewater at a concentration of 2000mg/L (COD) and 30g/L (NaCl), under batch operating mode. Mechanical aeration was also provided to the system and for a period of 1-day operation (Fig.2). No substantial change of the concentration of COD was observed, indicating the absence of physico-chemical removal of organics.

3.1 Effect of feed COD

The major objective of this set of experiments was to investigate variations of COD removal efficiencies with feed COD while A/Q ratio, salt concentration, DO and temperature were kept constant at 1500 m^2dm^{-3} , 3%, 4mg/L and 25ºC, respectively. Feed COD varied between 2000mg/L and 6000mg/L.

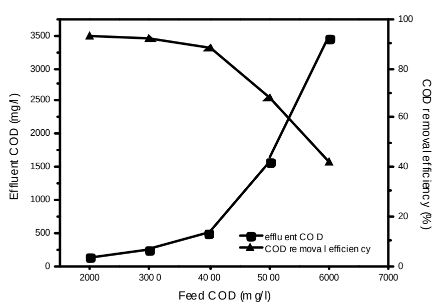

Fig.3. Variation of effluent COD and COD removal efficiency with feed COD

Variation of percent COD removal and the effluent COD with feed COD was depicted in Fig.3. Percent COD removal decreased and the effluent COD increased with increasing feed COD. Percent COD removal decreased from 93% to 88% and further to 42% when the feed COD increased from 2000mg/L to 4000mg/L and further to 6000mg/L, respectively. The increase of feed COD resulted in high COD loading rate, which could impede the bacterial in the reactor, adversely affect the organics removal process and decrease the COD removal efficiencies.

3.2 Effect of A/Q ratio

In this experiment the effects of different A/Q ratio on percent COD removal by two bacterial was evaluated when feed COD, salt concentration, DO and temperature were kept constant at 4000mg/L, 30g/L, 4mg/L and 25ºC, respectively.

The A/Q ratio varied from 500 m^2hm^{-3} to 2700 m^2hm^{-3}. Fig.4 depicts the variation of effluent COD and COD removal efficiency with A/Q ratio. The experimental results showed that the effluent COD decreased and percent COD removal increased as the increase of A/Q ratio. There was a significant decrease in effluent COD while A/Q ratio increased between 500 m^2hm^{-3} to 1500 m^2hm^{-3}. This sharp decrease in effluent COD with increasing A/Q ratio is a result of high biomass intensity on filler surfaces at high A/Q values [22].

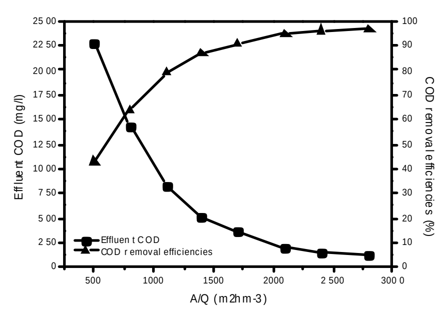

Fig.4. Variation of effluent COD and COD removal efficiency (E) with A/Q ratio

Percent COD removal increased from 43.1% to 96.7%, while corresponding the A/Q ratio varied from 500 m^2hm^{-3} to 2700 m^2hm^{-3}. These observations are in good agreement with the report of Kargi and Uygur who found that the organics removal was not effective by activated sludge culture added into halophilic organisms when A/Q ratio value was lower than 1600 m^2hm^3. The A/Q ratio values should be larger than 1800 m^2hm^3 in order to keep the COD removal efficiency larger than 90%.

3.3 Effect of salt concentration

In this set of experiments, the salt concentration was changed between 0mg/L and 50mg/L when feed COD, A/Q ratio value, DO and temperature were kept constant at 4000mg/L, 1500 m^2dm^3, 4mg/L and 25ºC, respectively. Variations of effluent COD concentrations and COD removal efficiencies (percent COD removal) with salt content are depicted in Fig. 5.

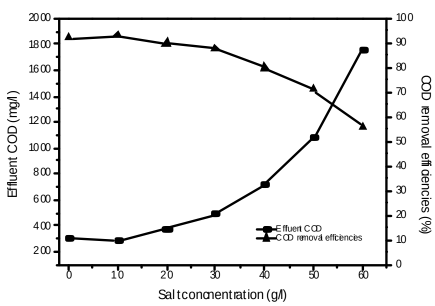

Fig.5. Variation of effluent COD and COD removal efficiency (E) with salt concentration

The results showed that salt concentrations from 0 to 10mg/L had slightly improved the percent COD removal. However, percent COD removal decreased from 93 to 56% when salt content increased from 10 to 60g/L. COD removals were above 90% for salt contents below 3%. Effluent COD concentration increased from 285 to 1760 mg/L when salt content was raised from 10 to 60g/l. Apparently, increases in salt content from 10mg/L to 60mg/L resulted in drops in COD removal efficiency and therefore, effluent COD concentrations increased especially for salt contents above 3%. The faster organic uptake rate of the bacterial suggested that low NaCl concentrations of up to 10 g/L could stimulate the uptake of substrate by bacterial. These observations are in good agreement with those reported by Winslow and Dolloff (1928) who found that the growth of Escherichia coli was stimulated at NaCl concentration of 0.005–0.3 M, and with those of Winslow and Harywood (1931), who reported that NaCl concentration ranging from 0.005 to 0.25 M stimulated the viability of Escherichia coli. The effluent COD increased and percent COD removal decreased when salt concentration varied from 10g/l to 60g/l. It has been known that high levels of salt content could produce high osmotic pressure even on halophilic bacteria, which causes plasmolysis, dehydration, and disintegration of bacteria cell and loss of cell activity, thereby resulting in low organic removal efficiencies. (Dan et al. 2003).

3.4. Effect of DO and Temperature

DO and Temperature were varied from 1mg/L to 8mg/L and 5ºC to 40ºC in this set of experiments while feed COD, A/Q ratio value and salt concentration were constant at 4000mg/L, 1500 $m^2\cdot h/m^3$ and 30g/l, respectively. Variations of effluent COD with different DO and temperature were depicted in Fig.6. The effluent COD decreased significantly while dissolved oxygen varied from 1mg/L to 4mg/L, but maintained at about 500mg/L for further increase of dissolved oxygen. The experimental results showed that the ability of bacterial was affected by DO. A further decrease of DO adversely affected the COD removal efficiency

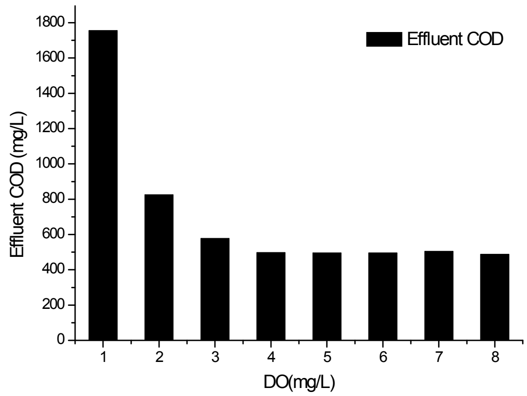

Fig.6. Variation of effluent COD with dissolved oxygen

when dissolved oxygen was lower than 4mg/L. It is enough for general aerobic to maintain dissolved oxygen at 2mg/L. However, more oxygen was needed to satisfy two salt-tolerant bacterial. So that the dissolved oxygen should be larger than 4mg/L to obtain reasonable COD removal efficiencies.

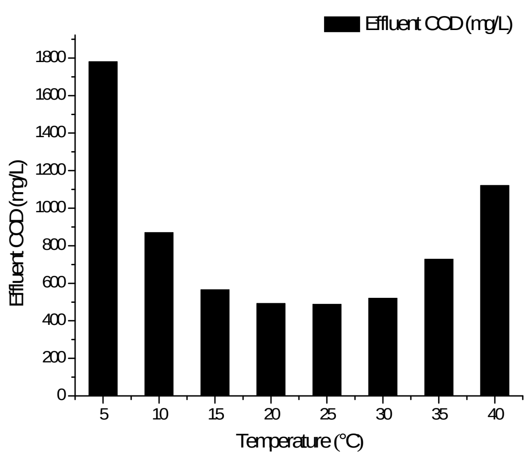

Fig.7. Variation of effluent COD with temperature

The effect of temperature on COD removal was noted in Fig.7. The effluent COD maintained at about 500mg/L when temperature varied from 20 ºC to 30ºC. However, a sharp increase in effluent COD appeared when the temperature was lower than 15ºC or higher than 30ºC. The range of optimum temperature for bacterial is approximately from 20ºC to 30ºC. The reason for this observation could be that high temperature had adverse effect on enzyme activity and cell grow, even made enzyme denature and cell die while low temperature could decrease enzyme activity and present bacterial growth resulting in low COD removal. So temperature should be maintained from 15ºC to 30ºC in order to get a high COD removal efficiency.

5. Conclusions

In this study, two salt-resistant bacterial were isolated from high salt environment, and applicability of organics removal from high saline wastewater was evaluated at a laboratory scale under different experimental conditions. Variables considered were feed COD, A/Q ratio, salt concentration, DO and temperature.

(1) COD removal efficiency decreased with increasing feed COD, and this decrease was steeper especially at high feed COD. The increase of feed COD resulted in high COD loading rate, which could impact the bacterial in the reactor, adversely affect the organics removal process and decrease the COD removal efficiencies.

(2) A increasing A/Q ratio values could improve the COD removal efficiency, which increased from 43.1% at A/Q ratio of 500 m^2hm^3 to 96.7% at A/Q ratio of 2700 m^2hm^3.

(3) Increasing salt concentrations resulted in decreases in COD removal efficiency as a result of inactivation of organisms at high salt concentrations.

(4) Dissolved oxygen and temperature were also the important factors, which affected the ability of bacterial.

(5) The optimal pH was about 7, and pH was an important parameter effecting on the COD removal efficiency mainly because pH affected microorganism-specific physiology and cellular growth positively correlated with COD removals.

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