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Short communication

# Anaerobic treatment of saline wastewater by *Halanaerobium lacusrosei*

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**Abstract**

An upflow anaerobic packed bed reactor was operated continuously with synthetic saline wastewater at different initial COD concentrations ($COD_0$ = 1900–6300 mg/L), salt concentrations (0–5%, w/v) and hydraulic retention times ($\theta_H$ = 11–30 h) to investigate the effect of those operating parameters on COD removal from saline synthetic wastewater. Anaerobic salt tolerant bacteria, *Halanaerobium lacusrosei*, were used as dominant microbial culture in the process. The percent COD removal reached up to 94% at $COD_0$ = 1900 mg/L, 19 h hydraulic retention time and 3% salt concentration. No substrate inhibition effect was observed at high feed CODs. Increasing hydraulic retention time from 11 h to 30 h resulted in a substantial improvement in the COD removal from 60% to 84% at around $COD_0$ = 3400 mg/L and 3% salt concentration. Salt inhibition effect on COD utilization was observed at above 3% salt concentration. Modified Stover–Kincannon model was applied to the experimental data to determine the biokinetic coefficients. Saturation value constant, and maximum utilization rate constant of Stover–Kincannon model for COD were determined as $K_B$ = 5.3 g/L day, $U_{max}$ = 7.05 g/L day, respectively.


*Keywords:* Anaerobic; Saline wastewater; *Halanaerobium*; Packed bed

## 1. Introduction

Conventional biological treatment systems are known as insufficient in removal of COD from saline wastewaters because of the adverse effects of salt on microbial flora. The reason for this is loss of activity of organisms in biological wastewater treatment operation due to plasmolysis in the presence of salt. Microorganisms requiring salt for growth are designated halophilic. The intracellular salt concentration of halophilic and halotolerant microorganisms is low and they maintain an osmotic balance of their cytoplasm with the external medium by accumulating high concentration of various organic osmotic solutes [1]. Therefore, utilization of salt tolerant microorganisms in biological wastewater treatment systems could be a solution for COD removal from saline wastewater.

Most of the studies on biological saline wastewater treatment are based on use of aerobic halophilic organisms and technologies. Dincer and Kargi [2,3] studied the performance of rotating biological discs system in saline wastewater for COD removal. A salt-tolerant bacterium *Halobacterium halobium* was added to an activated sludge culture in order to improve the system's performance. Nitrification/denitrifcation and nutrient removal in saline wastewater in different bioprocess technologies have also been carried out [4,5].

Anaerobic treatment of salt containing wastewater is rather a new approach and needs detailed studies. The experimental studies were carried out under various environmental conditions and bioprocess configurations with acclimatized anaerobic sludge [6–9]. However, different anaerobic halophilic organisms have been isolated so far. For example, *Haloanaerobacter chitinovorans* which was isolated from a saltern in California [10], *Haloanaerobium congolense* from an African oil field [11], *Halanaerobium lacusrosei* from sediment of hypersaline lake [12]. *Haloanaerobium praevalens* [13] and *Haloanaerobium alcaliphilum* [14], from Great Salt Lake sediment. These organisms were also identified in canned food [15]. *Haloanaerobium praevalens* was reported as effective in carbon removal in Great Salt Lake [16]. The potential utilization of these cultures in anaerobic treatment of saline wastewater needs detailed investigation.

By considering the potential of salt tolerant anaerobic organisms in saline wastewater treatment, this study was designed to investigate the COD removal performance of anaerobic salt tolerant organisms, *H. lacusrosei*, from saline synthetic wastewater in a continuously operated upflow

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anaerobic packed bed reactor under different operating conditions and to determine the biokinetic coefficients by using Stover–Kincannon model. To the best of our knowledge, this is the first report about the utilization of *H. lacusrosei* in anaerobic treatment of saline wastewater.

## 2. Materials and methods

### 2.1. Microbial culture

Anaerobic halophilic, *H. lacusrosei*; microbial culture was obtained from DSZM (10165), Germany as pure culture. The culture was cultivated under aseptic conditions in the laboratory of Dokuz Eylül University.

### 2.2. Wastewater composition

Synthetic wastewater used throughout the studies was composed of 1 g/L $NH_4Cl$, 0.3 g/L $KH_2PO_4$, 2 g/L $MgCl \cdot 6H_2O$, 0.2 g/L $CaCl_2 \cdot 2H_2O$, 1 g/L $C_2H_3NaO_2 \cdot 3H_2O$, and various concentrations of salt (0–5% NaCl). Glucose was used as carbon source. Trace elements were composed of 3 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L $MnSO_4 \cdot 2H_2O$, 1 g/L NaCl, 0.1 g/L $FeSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 0.180 g/L $ZnSO_4 \cdot 7H_2O$, 0.01 g/L $CuSO_4 \cdot 5H_2O$, 0.01 g/L $H_3BO_3$, 0.01 g/L $Na_2MoO_4 \cdot 2H_2O$, 0.25 g/L $NiCl_2 \cdot 6H_2O$. One milliliter of stock trace element solution was added for 1 L synthetic feed wastewater. 2.5 grams per liter $NaHCO_3$ was provided to improve the buffering capacity of this wastewater (3–4.5 g $CaCO_3$/L). pH was adjusted to pH 7 by adding 5% phosphoric acid and 5% KOH.

### 2.3. Experimental set-up

The continuous treatment of synthetic saline wastewater was performed in a 3 L upflow anaerobic packed bed reactor made of chrome-nickel stainless steel. Plastic non-commercial support materials were used for the immobilization of the organisms. The system was feed from the bottom with synthetic wastewater via a peristaltic pump. Temperature was maintained at 37 °C by using a heating jacket.

Anaerobic reactor was inoculated with 2.5 g/L *Halanaerobium lacusrosei* at the beginning of the study. The system was operated with effluent recycle at 2% salt concentration and 2000 mg/L COD concentration for 30 days to allow the immobilization of the culture on the support materials. The liquid phase of the system was refreshed with new synthetic media every week to provide nutrients. After immobilization of microorganisms, continuous operation was started. System was loaded with fresh media at the beginning of each experimental condition and it was operated at least 15 days until the system reached to almost steady state conditions which was determined as obtaining the almost the same effluent COD concentration (standard deviation less than 10%) for the last five consecutive operation days. pH, temperature, initial and effluent COD concentrations of daily samples were monitored.

### 2.4. Experimental conditions

The salt concentration was gradually increased from 0% to 5%. The initial COD concentration ($S_0$) was 3300 ± 67 mg COD/L and hydraulic retention time (HRT) was $\theta_H = 30$ h in the first part of the experiments. Initial $COD_0$ concentration was increased from around 1914 ± 35 mg/L to 6272 ± 37 mg/L in the second part of the study. The salt concentration and hydraulic retention time were kept constant at $[NaCl]_0 = 3\%$ and $\theta_H = 19$ h, respectively. Finally, the hydraulic retention time was varied between 11 h and 30 h at $S_0 = 3332 \pm 180$ mg COD/L and salt concentration of 3%.

### 2.5. Analytical methods

Samples were removed from the effluent once a day and centrifuged at 5000–6000 rpm for 0.5 h. COD analyses were carried out on clear supernatant according to *Standard Methods* [17].

## 3. Results and discussion

### 3.1. Effect of salt concentration on COD removal performance of the system

In order to determine the maximum tolerated salt concentration and the effect of salt concentration on the COD removal performance of these organisms, initial salt concentration was gradually increased from 0% to 5%.

The effect of salt concentration on the effluent COD concentration ($S_e$) and COD removal efficiency ($E_{COD}$) is summarized in Fig. 1. The final COD concentration was determined by calculating mean and standard deviation of the last 5 days COD concentrations. *H. lacusrosei* was able to decrease the COD concentration from $S_0 = 3212 \pm 4$ mg/L to $S_e = 1536 \pm 22$ mg/L in the salt free synthetic wastewater. Effluent COD concentration decreased to around 1153 ± 84 mg/L for 1% salt concentration with $E_{COD} = 63\%$. The maximum removal efficiency was observed as $E_{COD} = 82 \pm 2\%$ at 3% salt concentration. The higher salt concentration ($[NaCl]_0 > 3\%$) caused decreasing in COD removal performance of the culture. The effluent COD concentration reached to 1884 ± 65 mg/L resulting in 43% removal efficiency.

Substrate removal rates (COD removal rate) were calculated as $R_s = Q(S_0 - S_e)/V$, where $R_s$ represents substrate removal rate (mg/L day), $Q$ the operation flowrate (L/day), $V$ the liquid volume in the reactor (L), $S_0$ the initial COD concentration and $S_e$ is the effluent COD concentration at steady state (mg/L). The maximum substrate removal rate was obtained as $R_s = 2.40$ g/L day at 3% salt concentration. Higher salinities caused decreasing in the rate to the level of $R_s = 1.13$ g/L day indicating salt inhibition effect on COD removal. In addition, low COD removal efficiency and removal rates at salt free environment can be explained that these organisms require minimum amount of salt for metabolic activity. In summary, the maximum salt concentration should be 3% for efficient COD removal with *H. lacusrosei* under anaerobic conditions.

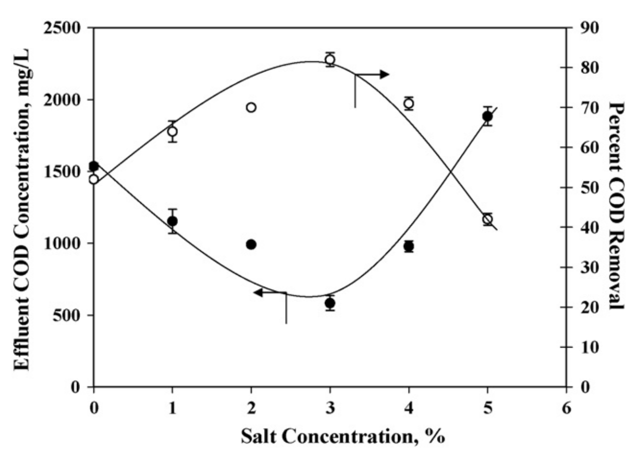


Fig. 1. Effect of salt concentration on effluent COD concentration and COD removal efficiency ((●) effluent COD concentration and (○) COD removal efficiency).

### 3.2. Effect of initial COD concentration and hydraulic retention time on COD removal

Table 1 depicts the effect of initial COD concentration on effluent COD concentration and COD removal efficiency. The effluent COD concentration increased from $S_e = 113 \pm 11$ mg/L to $S_e = 996 \pm 1.0$ mg/L with respect to increase in initial COD concentrations from $S_0 = 1914 \pm 35$ mg/L to $S_0 = 3445 \pm 1.0$ mg/L. Depending on that variation in effluent COD, removal efficiency decreased from 94% to 71%, respectively. The percent COD removal did not vary significantly for the higher COD concentrations ($COD_0 > 3445 \pm 1$ mg/L) and remained constant around 55%. These results indicate that, anaerobic salt tolerant *H. lacusrosei* microbial culture can efficiently remove low COD concentrations with higher than 94% efficiency. However, 55% removal efficiency around 6272 mg/L initial COD concentration can be evaluated as high COD removal performance for the anaerobic conditions at $[NaCl]_0 = 3\%$ salt concentrations. This efficient COD removal should be attributed to the salt tolerant anaerobic organism *H. lacusrosei*.

As seen from the table, substrate removal rate increases with increasing initial COD concentration or organic loading rate ($L_o = S_0Q/V$). It was around $R_s = 2.2$ g/L day for the effluent COD concentration of $S_e = 113$ mg/L and increased up to $R_s = 4.24$ g/L day with the increase in effluent COD concentrations around $S_e = 2790$ mg/L at organic loading rate of $L_o = 7.7$ g/L day. Since, there was no decreases in the substrate removal rate, it can be concluded that no substrate inhibition effect occurs up to $6272 \pm 37$ mg/L initial COD concentrations. The maximum substrate removal rate was determined as $R_s = 4.24$ g/L day at quite high COD concentration around 6272 mg/L which point outs that *H. lacusrosei* can tolerate high CODs at $[NaCl]_0 = 3\%$ salt concentrations.

The effect of hydraulic retention time on effluent COD concentration and COD removal efficiency was given in Table 2. The final COD concentration was between 1328 mg/L and 1161 mg/L for the hydraulic retention times $\theta_H = 11$ h and 14 h, respectively. However, increasing hydraulic retention time up to 30 h provided significant improvement in COD removal. The effluent COD concentration decreased to around 550 mg/L resulting in 84% COD removal. These results indicates that, for the efficient COD removal at COD concentrations such as 3400 mg/L and 3% salt concentration with *H. lacusrosei*, the hydraulic retention time should be higher than 25 h.

There are limited number of studies on anaerobic treatment of saline wastewater. Omil et al. [6] obtained 70–90% organic matter removal from seafood-processing industry which has salinity similar to sea water. Guerrero et al. [7] investigated anaerobic treatment of the wastewaters from fish meal processing factories after a previous solids removal steps and over 80% COD removal efficiency was observed at salinity content around 7.5 g $Cl^-$/L. Similarly, the anaerobic treatment of fishery wastewater by marine sediment inoculum was reported by Aspé et al. [8]. Effect of hydraulic retention time on metanogenic activity at 18 °C and 37 °C was studied in a continuously stirred tank reactor and kinetic coefficients were determined by using different mathematical models. It was concluded that, fishery effluent can be anaerobically treated. Rovirosa et al. [9] studied treatment of saline wastewater by using a laboratory down-flow anaerobic fixed bed reactor (DFAFBR). The results showed that a HRT of 24 h was required to obtain total COD, organic-N, total-P and feacal coliform concentration reduction efficiencies higher than 72%, 51%, 39% and 98%, respectively, at sea salts concentrations in the range from 5 g/L to 15 g/L. The recent publication about treatment of saline wastewater of tannery soak liquor in an upflow anaerobic sludge blanket reactor (UASB) showed that 78% COD removal can be obtained at 5 days HRT and 71 g/L total dissolved solids (TDS) as salinity [18]. Gebauer studied on anaerobic treatment of sludge from saline fish farm effluents and percent COD removal in undiluted sludge with 35‰ salinity varied between 40% and 54% depending on the operating condition [19].

Table 1
Effect of initial COD concentration on COD removal

| $\theta_H$ (h) | $COD_0$ (mg/L) | $COD_e$ (mg/L) | $L_o$ (g/L day) | $E_{COD}$ (%) | Rate (g/L day) |
|---|---|---|---|---|---|
| 19 | 1914 ± 35 | 113 ± 11 | 2.31 ± 0.03 | 94.0 ± 0.1 | 2.18 ± 0.03 |
| 19 | 3445 ± 1 | 996 ± 1.0 | 4.27 ± 0.06 | 71.1 ± 0.28 | 3.04 ± 0.04 |
| 19 | 4274 ± 5 | 1808 ± 72 | 5.61 ± 0.09 | 54.3 ± 3.7 | 3.04 ± 0.22 |
| 19 | 5235 ± 58 | 2332 ± 40 | 6.32 ± 0.07 | 56.4 ± 2.0 | 3.57 ± 0.14 |
| 19 | 6272 ± 37 | 2790 ± 23 | 7.69 ± 0.06 | 55.2 ± 0.80 | 4.24 ± 0.06 |

Table 2
Effect of hydraulic retention time on COD removal

| $\theta_H$ (h) | $COD_0$ (mg/L) | $COD_e$ (mg/L) | $L_o$ (g/L day) | $E_{COD}$ (%) | Rate (g/L day) |
|---|---|---|---|---|---|
| 11 | 3338 ± 12 | 1328 ± 17 | 7.4 ± 0.05 | 60.2 ± 0.4 | 4.44 ± 0.03 |
| 14 | 3379 ± 68 | 1161 ± 42 | 5.6 ± 0.11 | 65.7 ± 3 | 3.70 ± 0.15 |
| 25 | 3151 ± 43 | 555 ± 37 | 3.0 ± 0.01 | 82.0 ± 1.5 | 2.71 ± 0.29 |
| 30 | 3420 ± 61 | 546 ± 3.6 | 2.7 ± 0.05 | 84.0 ± 0.28 | 2.30 ± 0.05 |

### 3.3. Determination of kinetic coefficients

Stover–Kincannon is one of the most widely used mathematical models to determine the kinetic constants for immobilized systems. The model have been applied to

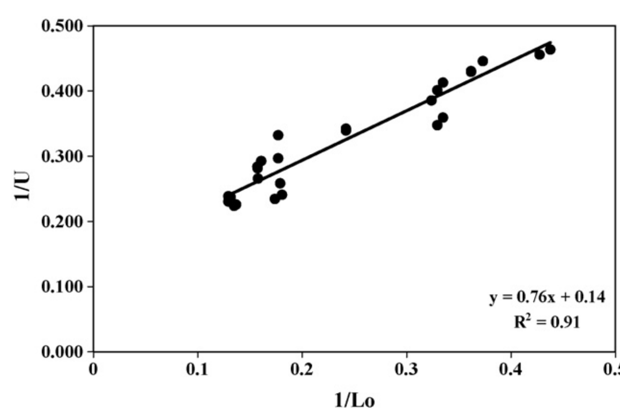


Fig. 2. Stover–Kincannon model plot for COD removal.

continuously operated mesophilic and thermophilic upflow anaerobic filters for treatment of paper-pulp liquors [20], simulated starch wastewater [21], anaerobic filter for soybean wastewater treatment [22], anaerobic hybrid reactor [23], and also determination of decolorization kinetic constants in packed bed reactor [24]. The modified Stover–Kincannon model considers the organic substance removal rate as a function of organic loading rate at steady state as described in Eq. (1):

$$\frac{dS}{dt} = \frac{Q(S_0 - S_e)}{V} = \frac{U_{max}(QS_0/V)}{K_B + (QS_0/V)} \quad (1)$$

where $dS/dt$ is the substrate removal rate (g/L day), $S_0$ and $S_e$ the substrate concentrations in the feed and in the reactor (g/L), respectively, $Q$ the flow rate, $U_{max}$ the maximum substrate removal rate (g/L day) and $K_B$ is a saturation value constant (g/L day).

Linearization of Eq. (1) gives the following relationship:

$$\frac{dt}{dS} = \frac{V}{Q(S_0 - S_e)} = \frac{K_B}{U_{max}} \frac{V}{QS_0} + \frac{1}{U_{max}} \quad (2)$$

The plot of $V/[Q(S_0 - S_e)]$, inverse of the removal rate, versus $V/(QS_0)$, inverse of the total loading rate will result in a straight line. Intercept and slope of the line results $1/U_{max}$ and $K_B/U_{max}$, respectively.

Modified Stover–Kincannon model was applied to experimental results and kinetic constants for COD removal were determined. COD loading rates and removal rates were calculated at different hydraulic retention times and initial COD concentrations (Tables 1 and 2). $K_B$ and $U_{max}$ values were calculated as 5.3 g/L day and 7.05 g/L day, respectively, from $1/U$ versus $1/L_o$ plot as given in Fig. 2. Therefore, the rate expression for COD removal takes the following form:

$$\frac{Q(S_0 - S_e)}{V} = \frac{7.05(QS_0/V)}{5.3 + (QS_0/V)} \quad (3)$$

And effluent COD concentration can be predicted by using Eq. (4):

$$S_e = S_0 - \frac{7.05 S_0}{5.3 + (QS_0/V)} \quad (4)$$

## 4. Conclusions

The results of this study indicated that utilization of salt tolerant *H. lacusrosei* enhances COD removal from the saline wastewater under anaerobic conditions. The culture is able to remove up to 3445 mg COD/L with over 70% efficiency at 3% salt concentration and 19 h of hydraulic retention time. COD removal efficiency can be increased to 84% by extending the hydraulic retention time to 30 h for the same synthetic wastewater composition. The substrate removal rate and effluent wastewater quality decreases when the salt concentration is over 3%. More detailed studies should be conducted to understand the possibility of using salt tolerant organisms in treatment of real saline industrial effluents.

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