DOKUZ EYLÜL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

CONTINUOUS OPERATION OF ANAEROBIC PACKED BED REACTOR FOR COD REMOVAL FROM SALINE WASTEWATER

by

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September, 2006

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CONTINUOUS OPERATION OF ANAEROBIC PACKED BED REACTOR FOR COD REMOVAL FROM SALINE WASTEWATER

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled "**CONTINUOUS OPERATION OF ANAEROBIC PACKED BED REACTOR FOR COD REMOVAL FROM SALINE WASTEWATER**" completed by **Burcu ERTEN** under supervision of **Assoc. Prof. Dr. Ilgi K. KAPDAN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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ABSTRACT

The treatibility of saline wastewater under anaerobic conditions with salt tolerant anaerobic bacteria called *Halanaerobium lacusrosei* was investigated in this study.

The batch flask experiments were carried out to determine the effect of media composition and the salt inhibition model. Experimental results indicated that increasing nitrogen concentration did not significantly improve COD removal, microorganisms need minimum salt in the media but high salt concentrations reduces the COD removal and presence of salt in the media causes competitive inhibition.

An upflow anaerobic packed bed reactor was operated continuously with synthetic saline wastewater at different hydraulic retention times, initial COD and salt concentrations. Under the different operating conditions the organic loading rates were determined. The maximum COD removal efficiency was obtained at low initial COD concentrations. No substrate inhibition effect was observed at high feed CODs. Increasing hydraulic retention time resulted in an improvement in the COD removal. Increasing salt concentration resulted in decreasing in COD removal performance on the system. This result was evaluated as salt inhibition effect on COD removal. 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 calculated.

The results of this study indicated that it is possible to treat high saline wastewater by using anaerobic treatment methods. Inclusion of halophilic anaerobic bacteria into the reactor resulted in high COD removal efficiencies especially at high salt concentration.

Keywords: Saline wastewater; biological treatment; anaerobic; halanaerobium

SÜREKLİ İŞLETİLEN YUKARI AKIŞLI ANAEROBİK DOLGU REAKTÖRDE TUZ İÇEREN SENTETİK ATIKSUDAN KOİ GİDERİMİ

ÖZ

Bu çalışmada, tuzlu atıksuların, anaerobik şartlar altında tuz tolere edebilen anaerobik bakteri türü *Halanaerobium lacusrosei* mikroorganizma kültürü tarafından arıtımı araştırılmıştır.

Besi ortamı ve tuzun engelleyici etkisinin belirlenmesi amacıyla kesikli deneyler yapılmıştır. Deneyler neticesinde azot derişiminin KOİ giderimi üzerine belirgin bir etkisinin olmadığı, mikroorganizmaların büyüme ortamında minimum tuz ihtiyacının olduğu ancak yüksek tuz derişimlerinin KOİ giderimini olumsuz etkilediği ve ‘’rekabetli engellemeye’’ neden olduğu gözlenmiştir.

Yukarı akışlı dolgulu kolon anaerobik reaktör tuz içeren sentetik atıksu ile farklı hidrolik alıkonma sürelerinde, başlangıç KOİ ve tuz derişimlerinde sürekli olarak işletilmiştir. Farklı işletim koşulları altında organik yükleme oranları belirlenmiştir. Maksimum KOİ giderim verimi düşük başlangıç KOİ derişimlerinde elde edilmiştir. Yüksek KOİ değerlerinde substrat engelleme etkisi görülmemiştir. Hidrolik alıkonma süresindeki artış KOİ giderim verimini yükseltmiştir. Tuz derişimindeki artışla, KOİ gideriminde düşüş meydana gelmiş ve bu sonuç tuzun engelleyici etkisi olarak değerlendirilmiştir. Biokinetik katsayıların belirlenmesinde modifiye Stover-Kincannon modeli uygulanmış, doygunluk katsayısı ve maksimum substrat giderim hız sabitleri hesaplanmıştır.

Çalışma sonucunda yüksek tuz içerikli atıksuların anaerobik arıtma metodlarıyla arıtılabilir olduğu ve anaerobik bakteri türünün reaktöre ilavesiyle özellikle yüksek tuz derişimlerinde yüksek KOİ giderimi sağladığı görülmüştür.

Anahtar Kelimeler: Tuzlu atıksu; biyolojik arıtma; anaerobik; halanaerobium

CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 Literature Review

Vegetable, tanning and seafood processing industries generate large quantities of saline wastewater with high concentration of organic pollutants(Woolard & Irvine, 1995). 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(Dinçer, Kargı, 2001). Although, using reverse osmosis or ion exchange technologies for removal of salts from wastewater before biological treatment seems a good alternative, these methods require high capital and operation costs and are not practical. However, some aerobic and anaerobic halophilic microorganisms can grow and remove carbon from wastewater at high salt concentration (1%-10% NaCl). This characteristic of halophilic organisms makes biological systems applicable to treatment of saline industrial effluents. Most of the studies on biological saline wastewater are based on aerobic halophilic organisms and technologies. Anaerobic treatment of salt containing wastewater is rather a new approach and needs detailed studies. The presence of high sodium and/or chloride concentrations has been traditionally considered as inhibitory for anaerobic wastewater treatment. However, isolation of salt tolerant anaerobic bacteria devoted the attentions to anaerobic treatment of saline wastewater.

Characteristic of halophilic organisms makes biological systems applicable to treatment of saline industrial effluents. They offer a multitude of actual or potential applications in various fields of biotechnology. Margesin & Schinner (2001) examined the potential of halophilic and halotolerant microorganisms. Their intracellular salt concentration is low and they maintain an osmotic balance of their cytoplasm with the external medium by accumulation high concentration of various organic osmotic solute.

Their intracellular enzymes have no special salt tolerant. Nonhalophilic microorganisms, able to grow in the absence as well as in the presence of salt, are named halotolerant. They can tolerate nearly 15% salt (2.5 M NaCl). Microorganisms requiring salt for growth are designated halophilic and are further categorized into three groups according to the salinity of their growth optima: slightly halophilic (1–3% w/v), moderately halophilic (3–15% w/v), or extremely halophilic and last group is extreme halophiles (optimal growth at 25% salt concentration). Halophilic Archea, is another type except halophilic and halotolerant, maintain an osmotic balance of their cytoplasm with the hypersaline environment by accumulation high concentration of salt. Their intracelluler enzymes are adaptated and have to function in the presence of salt (Margesin, Schinner, 2001).

Utilization of salt tolerant microorganisms in the wastewater treatment is rather a new subject. Therefore, there are limited number of studies and the subject needs detail investigation and evaluation to be able to develope mathematical models or design equations. Some of these studies have been reviewed in the content of this thesis.

Hamoda and Al-Attar (1995) carried out a study about the effects of high NaCl concentrations on activated sludge process. The results indicated that the activated sludge process was not deteriorated with constant application of NaCl. Presence of salt did not inhibit biomass growth, on the contrary salt appeared to stimulate the aggregation of microbial cells and enhance sludge settling. In addition, the response of the acclimated activated sludge saline-water system was similar to that of the fresh-water system. It was found that kinetic models developed for the fresh wastewater system could be used successfully. This fact most likely related to the growth of halophilic(salt-loving) microorganisms in the system.

Dan, Visvanathan & Basu (2003) studied on the performance of the aerobic treatment of high organic-high salinity wastewater by yeast and bacterial culture. The biokinetic coefficients for both systems were determined and used to analyze the behavior of the

yeast and bacterial systems under high saline condition. They investigated COD removal efficiency for acclimated yeast and bacterial cultures at 25, 32 and 45 g NaCl/L and substrate utilization rates, *U,* were calculated. It was observed that 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 yeast. Nutrient removal capacity has also been found to be better for yeast due to higher nutrient uptake in the yeast biomass. The results showed that the yeast culture was more efficient compared to the bacterial culture. Lower sensitivity of the yeast culture to high salt content may be caused by higher adaptability of some yeast species to high salinity condition. Bacterial cells tend to dehydrate and disintegrate in that situation as a result of a higher osmotic difference between the protoplasm and the ambient high saline situation.

Dinçer & Kargı (2001) studied the performance of rotating biological discs system in saline wastewater for COD removal. They used synthetic saline wastewater containing different concentrations of salt (0-10%). A salt-tolerant bacterium *Halobacterium halobium* was added to an activated sludge culture in order to improve the system's performance. The effects of discs number (or surface area), COD loading rate (or feed COD) and salt concentration were examined. It was shown that COD removal efficiency increased with increasing number of discs (or surface area), but decreases with increasing COD loading rate and salt concentration. COD removal efficiency for 2000 mg/L influent COD concentration was nearly 96%. The efficiency dropped to 43% for the feed COD of 13000 mg/L. COD removal efficiency decreased almost linearly with decreasing number of discs from 88% at 40 discs to 50% at 10 discs. Increasing salt concentration resulted in decreases in COD removal efficiency as a result of disablement of organisms at high salt concentration. The results marked that removal efficiency was higher than 90% for 3% salt concentration. The efficiency fall to 80% and 60% at 5% and 10% salt concentration, respectively, at $(COD)_0$=5000 mg/L and 40 discs.

Another study carried out by Dinçer & Kargi (2001) is about investigation of the influence of salt on nitrification in an activated sludge unit. Initial experiments were

performed with salt-free wastewater and the results were used for determination of the kinetic constant without salt inhibition. Rate and saturation constants were k_0=1.15 $days^{-1}$ and K_{No}=5.14 mg/L. Experiments with 3% salt concentration were performed at different sludge age. Minimum sludge age required for complete nitrification increase from 12 days for salt-free wastewater to 25 days for 3% salt content. The apparent maximum rate and saturation constant values were found to be k_{ap}=1 $days^{-1}$ and K_{Nap}=26 mg/L by using the data obtained with 3% salt, the inhibition constants were found to be K_{T1}=200 g/L and K_{T2}=7.4 g/L. Therefore, salt inhibition was non-competitive type affecting both the maximum rate and the saturation constant. Saturation constant (K_N) was more adversely affected by salt inhibition as compared to the maximum rate constant (k) for nitrification. Further experiments were performed with the synthetic wastewater containing different salt contents between 0-5% to quantify variation of the rate and extent of COD removal with salt concentration. Hydraulic residence time and sludge age were kept constant at θ_H=36 h and θ_c=15 days, respectively. As the salt content increased from 0% to 5% the rate of nitrification dropped from 2.9 mg N/L h to 2.2 mg N/L h. The efficiency dropped from nearly 100% at 0% salt content to E = 96% at 3% salt content and nearly E = 80% for 5% salt content. It was reported that salt contents higher than 3% adversely affects the rate and efficiency of nitrification.

Uygur (2005) studied specific nutrient removal rates in saline wastewater treatment using sequencing batch reactor. Impacts of salt concentration (0–6%, w/v) on specific nutrient removal rates from saline wastewater in a sequencing batch reactor (SBR) were examined. The sequencing batch workings composed of anaerobic, oxic, anoxic and oxic phases with hydraulic residence times (HRT) of 1/3/1/1 h and a settling phase of 3/4 h. Solids retention time (SRT) was kept constant at 10 days in all experiments. Specific nutrient (COD, NH_4-N and PO_4-P) removal rates reduced with growing salt concentration due to adverse effects of salt on microorganisms. A salt tolerant organism, *Halobacter halobium* was added to the activated sludge culture (1/1, v/v) in order to enhance the nutrient removal performance of the SBR. Nutrient removal performances

of Halobacter-free and Halobacter-added activated sludge cultures were compared for all salt contents tested. Specific rates of nutrient removal obtained with the Halobacter- added culture were higher that those of Halobacter-free activated sludge, especially at high salt contents. Specific COD, NH_4-N and PO_4-P removal rates were 10.7 mg COD g biomass^{-1} h^{-1}, 0.80 mg NH_4-N g biomass^{-1} h^{-1} and 0.08 mg PO_4-P g biomass^{-1} h^{-1}, for the Halobacterfree activated sludge culture during those amounts were 31.0 mg COD g biomass^{-1} h^{-1}, 1.45 mg NH_4-N g biomass^{-1} h^{-1}, and 0.18 mg PO_4-P g biomass^{-1} h^{-1} for the Halobacter-added activated sludge. The results indicated clear advantage of inclusion of *Halobacter halobium* to the activated sludge in order to obtain high nutrient removal rates especially at high salt concentrations.

Halophilic/halotolerants can be utilized for hydrocarbon oxidation in the presence of salt. It is useful for the biological treatment of saline ecosystems contaminated with petroleum products. Kuznetsov et al., (1992) found that *Streptomyces albaxialis* was capable to break down crude oil and petroleum product even in the presence of 30% salt.

Woolard & Irvine (1995) demonstrated the applicability of halophilic bacteria for the treatment of hypersaline wastewater. Hypersaline wastewaters were generated during industrial activities that include chemical manufacturing, oil and gas production and waste minimization practices. These wastewater contained organic compounds and high concentrations of salt (>3.5%). It was difficult to treat using conventional microorganisms typically found in wastewater facilities. Biological treatment to remove organics without dilution required the use of halophilic organisms. Studies were conducted with a moderate halophile isolated from the Great Salt Lake, Utah, and U.S.A. The organism was able to degrade phenol in simulated oil field produced water containing 15% salt if iron, nitrogen and phosphorus were added to the medium. This organism was used to develop a halophilic sludge in a sequencing batch reactor (SBR) operated at 15% salt during a 7 month study period. An average phenol removal of over 99.5% was achieved with this reactor and specific substrate removal rates were similar to those reported for more conventional treatment cultures.

Omil, Méndez, & Lema (1995) studied the anaerobic treatment of wastewaters from seafood-processing industry. They used an industrial wastewater-treatment pilot-plant with a 15 m^3 central activity digester (CAD). This technology was conceptually close to the anaerobic contact system. Temperature was maintained in the mesophilic range (37^oC). The effluents have high organic content (10-60 g COD/L), with protein percentages between 25% and 70%, and a salinity similar to sea water: sodium (5-12 g/L), chloride (8-19 g/L) and sulphate (0.6-2.7 g/L). This high concentration of salts, together with the production of sulphide and ammonia due to sulphate reduction and protein breakdown respectively, produced important inhibitor/toxic effects on non-adapted biomass. A mixture of anaerobic sludges from the treatment of non-saline wastewater was used as the inoculum. After an initial start-up procedure, 70-90% organic matter removal was achieved, operating at dissolved sulphide (DS), total ammonia (TA) and sodium concentrations in the ranges of 0.25-0.5 g S-DS/L, 1.0-3.0 g N-TA/L and 6-10 g/L, respectively. The adaptation of the biomass to the salinity and the negative effects on sodium toxicity caused by the presence of other ions made it possible to operate at these high sodium concentrations. The high buffering capacity of the process (3-4 g $CaCO_3$/L) ensured the stability of the pH in the range 7.25-7.6. Due to this factor, pH was maintained above 7.25, maximum free-hydrojen sulphide (FS) concentration of 125 mg S-FS/L, and normally under 100 mg S-FS/L, were expected. These concentrations were not high enough to cause severe problems of inhibition, especially for adapted sludges. However, the control of the influent protein content is necessary, since values higher than 200 mg N-FA/L of free ammonia were shown to be inhibitory for this process.

Guerrero, Omil, Méndez & Lema (1997) investigated anaerobic treatment of the wastewaters from fish meal processing factories. The continuous treatment was carried out in an upflow anaerobic filter (UAF) after a solids removal steps. The reactor was operated over 280 days under different conditions. Temperature was maintained at 37^oC. The maximum applied OLR was 5 kg COD/m^3 day with a salinity content around 7.5 g Cl^-/L. Total and free amonia concentrations were 2.0 g N-TA/L and 0.3 g N-FA/L,

respectively. Recycling ratio (F:R) was a key factor for the performance of the UAF. The change from 1:10 to 1:5 (F:R) caused the high concentrations of VFA, ammonia (up to 6.5 g N-TA/L and 1.3 N-FA/L) and a sharp accumulation of VSS in the effluent. These effects were probably caused by the limitation of mass transfer rate and by a certain acidification in the bottom part of reactor caused by the local increase of substrate concentration. This caused cell lysis and the loss of part of the biomass. However, even under these conditions COD removal efficiency was always higher than 80%. The biomass developed in the reactor was mainly immobilised leaving its specific methanogenic activity higher than 1.0 g CH_4-COD/g VSS day at the end of the operation.

Rovirosa, Sanchez, Cruz, Veiga & Borja (2004) used a laboratory down-flow anaerobic fixed bed reactor (DFAFBR) for treatment of saline wastewater. They evaluated the performance of a down-flow anaerobic fixed bed reactor (DFAFBR) for coliforms removal when treating low-strength saline wastewater at different hydraulic retention times (HRTs). Wastewater was simulated by dilution of piggery manure in a synthetic saline water to obtain a final total COD concentration in the range of 1100–2900 mg/l and a salt concentration of 15 g/l. The DFAFBR was operated at hydraulic retention times (HRT) of 96,48, 24 and 12 h. Total COD, organic nitrogen and total phosphorus removals at steady-state conditions were also determined. Additionally, coliform bacteria were isolated and enumerated at three levels within the reactor, and the concentration of sulphate-reducing bacteria in the raw and digested sludge were also determined. The results showed that at sea salts concentrations in the range from 5 to 15 g/l, total coliform concentration reduction efficiencies higher than 97% were achieved. A decrease in the total and fecal coliform concentration reduction efficiencies from 99.5% to 90.5% and 92.5%, respectively, was followed when the HRT decreased from 96 to 12 h. Enumeration of coliform bacteria isolated from the biofilm in different zones of the reactor showed that more than 94% of the total amount was removed in the upper zone. A HRT of 24 h was required to obtain total COD, organic-N, total-P and fecal coliform concentration reduction efficiencies higher than 72%, 51%, 39% and 98%,

respectively. A concentration of 8.4 g/l for chlorides, 1.25 g/l for sulphates and 4.6 g/l for sodium did not affect the process performance.

Yoshie et al., (2001) studied metallurgic wastewater generated from the processes of recovering valuable metals from industrial wastes contains high concentrations of nitrogen compounds and salts. Biological nitrogen removal from this wastewater was assayed functioning with two series of anaerobic-aerobic circulating bioreactors. The denitrification capability of the system with the anaerobic packed bed was more stable than that of the system with the anaerobic fluidized bed. The NOx removal rate of the anaerobic packed bed was as high as 97%. Microbial community analysis by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16s ribosomal DNA (rDNA) fragments and the cultivation method showed that the community diversity altered in accordance with wastewater composition like the level of salinity and so on. Phylogenetic analysis suggested that the taxonomic affiliation of the dominant species in the anaerobic reactors was to the *y-Proteobacteria* including *Halomonadaceae* species. The PCR-DGGE method as a non-cultivation method was found to be a powerful tool for analysis of the microbial community, because the cultivation method could detect only a fraction of the microbial species present in these systems. The genetic diversity of the isolated bacteria fitting to the *y-Proteobacteria* which reduced both nitrate and nitrite in the anaerobic packed bed was higher than that of the bacteria in the anaerobic fluidized bed. This propose that a genetic standpoint varied microbial community stabilized the denitrifying performance in the anaerobic packed bed.

Lefebvre, Vasudevan, Torrijos, Thanasekaran & Moletta (2005) studied with hypersaline wastewater containing more than 35 g/L total dissolved solids (TDS) which is generated by various industrial activities. Using conventional biological wastewater treatment processes for this wastewater was difficult because of rich in both organic matter and TDS. Tannery wastewater from soak pit was treated in a lab-scale SBR for the removal of organic matter. The effluent, soak liquor is biodegradable, though not easily, and highly variable, depending on the origin and the nature of the hides.

COD was in the range of 1.5–3.6 g/L and TDS was in the range of 21–57 g/L. This soak liquor was biologically treated in an aerobic sequencing batch reactor seeded with halophilic bacteria, and the performance of the system was operated under different conditions with changes in hydraulic retention time, organic loading rate and salt concentration. The changes in salinity appeared to affect the removal of organic matter more than the changes in hydraulic retention time or organic loading rate. In spite of the changings in the attributions of the soak liquor, the reactor obtained proper removal of organic matter, once the adaption of the microorganisms was performed. Optimum removal efficiencies of 95%, 93%, 96% and 92% on COD, $PO_4^{3-}_4$, TKN and SS, respectively, could be reached with 5 days hydraulic retention time (HRT), an organic loading rate (OLR) of 0.6 kg COD $m^3 d^{-1}$ and 34 g NaCl/L. The organisms responsible for nitrogen removal appeared to be the most responsive to the adjustments of these parameters.

Feijoo, Soto, Méndez & Lema (1995) studied anaerobic digestion which become one of the most interesting treatment methods for highly organic polluted wastewaters. Inhibition and toxicity problems in the methanization process with the presence of sodium salts may have negative effects for the application of this technique to the treatment of a wide range of wastewaters. The effect of sodium on the methanization of volatile fatty acid (VFA) mixtures was evaluated by the researchers. Three different anaerobic sludges were used to test their tolerance to sodium. The first sample was taken from effluent of a lab-scale anaerobic filter reactor (AF sludge) after treating mussel processing wastewater for more than 2 years. The second sample came from the suspended biomass of a central activity digester (CAD) pilot plant, which is a modified contact system with an internal settler, after operating for 1 year with a mixture of seafood processing wastewater. Both AF and CAD sludges were acclimatized to high sodium concentrations, ranging from 5 to 10 g/L. The third sludge came from a UASB reactor treating potato processing wastewater. The results indicated that those three sludges had very different tolerance to sodium toxicity. Sodium concentrations causing 50% inhibition were 3.0, 6.8 and 16.2 g Na^+/L in the for the granuler, CAD and AF

sludges, respectively. Sludge pregrown in the presence of high sodium concentrations showed a higher tolerance to sodium. This reality was regarded to be a consequence of sludge adaptation to sodium. Sodium in sea-water environments causes a lower inhibition effect than sodium in NaCl solutions. Nutrients also influenced the sodium toxicity. The effect of sodium in each step of the anaerobic digestion process appeared to be different depending on the sludge.

A number of obligate anaerobic chemoorganotrophic moderately halophilic bacteria have been isolated from the bottom sediments of the Dead Sea and the Great Salt Lake, Utah. This study was performed by Oren (1986). (1) *Halobacteroides halobius*, a long motile rod from the Dead Sea, fermenting sugars to ethanol, acetate, H_2 and CO_2; (2) *Clostridium lortetii*, a rod-shaped bacterium from the Dead Sea, producing endospores with attached gas vacuoles; (3) a spore-forming motile rod-shaped bacterium, fermenting sugars, isolated from the Dead Sea; (4) *Haloanaerobium praevalens*, isolated from the Great Salt Lake, fermenting carbohydrates, peptides, amino acids and pectin to acetate, propionate, butyrate, H_2 and CO_2. Analysis of their 16S rRNA shows that these organisms are related to each other, but unrelated to any of the other subgroups of the eubacterial kingdom, to which they belong. *H. praevalens* and *H. halobius* regulate their internal osmotic pressure by the accumulation of salt (Na^+, K^+, Cl^-) rather than by organic osmotic solutes.

The other approaches for saline wastewater treatment includes natural treatment systmes and physicochemical methods. A series investigation demonstrated that constructed wetlands were an option for removing pollutants from saline wastewater. Those series experiments were performed by Nitisoravut & Klomjek (2005). Eight emergent plants; cattail, sedge, water grass, Asia crabgrass, salt meadow cordgrass, kallar grass, vetiver gras and Amazon, were planted in experimental plots. After plant acclimation, all plots were fed with municipal wastewater spiked with NaCl to simulate the saline condition of 14-16 mS/cm electrical conductivity (EC). Treatment performance of planted units were found to be 72.4-78.9% for BOD_5, 43.2-56% for SS,

67.4-76.5% for NH_3-N and 28.9-44.9% for TP. All macrophytes were found tolerant under the test condition except Amazon and vetiver grass. The results showed that cattail and Asia crabgrass were clearly superior for nitrogen uptake as well as for BOD_5 removal. Both were evaluated again in a continuous flow constructed wetland system receiving saline feed processing wastewater. A high removal rate regularly occurred in long-term operating conditions. The reduction in BOD_5, SS, NH_3-N and TP was in the range of 44.4-67.9%, 41.4-70.4%, 18-65.3% and 12.2-40.5%, respectively. The influences of higher saline concentration on treatment wetlands are under investigation.

Aquaculture systems have also been used for saline wastewater. Brown, Glenn, Fitzsimmons & Smith (1999) determined the feasibility of using salt-tolerant plants (halophytes) as biofilters to remove nutrients from saline aquaculture wastewater. *Suaeda esteroa*, *Salicornia bigelovii* and *Atriplex barclayana* (Chenopodiaceae) were grown in sand in draining containers (lysimeters) in a greenhouse experiments. They were irrigated to meet evapotranspiration demand and produce a 0.3 leaching fraction, using aquaculture effluent generated from an intensive tilapia culture system. The effluent salinity was increased with NaCl to make salinity treatments of 0.5, 10 and 35 ppt. The plant-soil system removed 98% and 94% of the applied total and inorganic nitrogen, respectively. It removed 99% and 97% of the applied total and soluble reactive phosphorus, respectively. High removal rates occurred despite the high leaching fraction. Salt inhibited the grow rate, nutrient removal, and volume of water that all three plant species could process. *Suaeda* and *Salicornia*, which were succulent salt marsh species, performed better than the desert saltbush, *Atriplex*, at the higher salinity.

Treatment of saline wastewaters with using electrochemical method was investigated by Lin, Shyu & Sun (1998). They examined several aspects, such as wastewater conductivity (or salinity), pH and current density, temperature and initial phenol concentration, to determine their effect on the treatment efficiency. It was found that the saline wastewater provided conductivity sufficient for efficient operation of the electrochemical treatment because of the presence of anions and cations in the aqueous

solution. Addition of a small amount of hydrogen peroxide (H_2O_2) up to 60 mg/L was highly conductive to the present electrochemical treatment in enhancing both the COD removal and settling of flocks. For maximum COD removal, a best pH of around 3 was recommended. It was observed that at low or medium salinity, phenol removal was dominated by direct oxidation at electrode surface. The results showed that electrochemical method was quite effective for dealing with the saline wastewaters with salinity of up to 3.5%.

A laboratory-scale electrochemical (EC) disinfector was used for the disinfection of various wastewater effluents, including saline primary effluent, saline secondary effluent, and freshwater secondary effluent by Li, Ding, Lo & Sing (2002). Such EC disinfection was highly effective for saline effluent with a salinity content of around 8parts per thousand. A killing efficiency of 99.9% on total coliform bacteria was achieved for saline secondary effluent with a contact time of less than 10 s and a power consumption rate no more than 0.006 k Wh/m^3. For primarily treated saline effluent, the same degree of disinfection was obtained with a contact time of less than 20 s and a power consumption of lower than 0.08 k Wh/m^3. The efficiency of EC disinfection was regulated by both the contact time (t) and current density (I-d) applied, and a kinetic function in terms of survival ratio (N/N-0) was developed for the saline secondary effluent, i.e., log(N/N-0)= -0.01(I(d)t)(1.87). While EC disinfection is highly applicable for saline effluent, it did not exhibit a similar degree of effectiveness for freshwater sewage effluent, even with a longer contact time and higher power input. Based on the results of the EC disinfection and comparative direct chlorination experiments, it is argued that the main disinfecting action of the EC process may not be electrochlorination. The EC process could produce other short-lived, more powerful germicidal substances that exert the strong killing function within a short contact time.

Tanaka, Feng, Sugiura & Maekawa (2004) studied on electrochemical treatment of the saline wastewater of Japanese pickle production processes (brown mustard and Japanese radish). The ammonium ion (18 and 8 mg/L) was almost completely removed

after 1h of treatment in the presence of NaCl (3%). The removal rate of total organic carbon (TOC) exceeded 90% for 7 mg/L and 60% for 352 mg/L, indicating that electrochemical treatment is useful in removing ammonium ions and organic substances from high-salinity wastewater.

1.2 Objective and Scope

Early studies with saline wastewater revealed that the overall efficiency of biological treatment increase by using of halophilic organisms. Despite of isolation for anaerobic halophilic organisms, performances of these organisms in the wastewater treatment have not been explored, so practical use of these organisms in wastewater treatment is quite a new idea. Use of aerobic halophilic bacteria have been widely investigated for COD and nutrient removal from wastewaters under different operating conditions and reactor configurations. The main objective of this thesis is to investigate the COD removal performance of halophilic anaerobic bacteria called *Halanaerobium lacusrosei* from salt (NaCl) containing synthetic wastewater in a continuously operated up flow anaerobic packed bed reactor.

In the content of that objective, the scope of the study can be summarized as follows;

1. to investigate the effects of initial COD concentration on COD removal performance of *Halanaerobium lacusrosei*.
2. to determine the optimum hydraulic retention time (HRT).
3. to investigate inhibition effect of salt on COD removal by *Halanaerobium lacusrosei.*
4. to determine biokinetic coefficients for the continuous operation by using different models.
5. to discuss the possible application of this wastewater treatment approach to industrial saline effluents.

CHAPTER TWO
MATERIALS AND METHODS

2.1 Microbial Culture

Anaerobic halophilic microbial culture, *Halanaerobium lacusrosei* was obtained from DSZM, Germany as pure culture. The culture was cultivated under aseptic conditions in the laboratory of Environmental Engineering Department in Dokuz Eylül University.

2.2 Wastewater Composition

Synthetic wastewater used during the studies was composed of 1 g/L NH_4Cl, 0.3 g/L KH_2PO_4, 2 g/L $MgCl.6H_2O$, 0.2 g/L $CaCl_2.2H_2O$, 1 g/L $C_2H_3NaO_2.3H_2O$, 2.5 g/L $NaHCO_3$ and various concentrations of salt (1-5% NaCl). Glucose was used as carbon source and ranged between 1500-6000 mg/L. 1 ml trace element solution was added for 1 L synthetic feed wastewater. Trace elements was composed of 3 g/L $MgSO_4.7H_2O$, 0.5 g/L $MnSO_4.2H_2O$, 1 g/L NaCl, 0.1 g/L $FeSO_4.7H_2O$, 0.1 g/L $CaCl_2.2H_2O$, 0.180 g/L $ZnSO_4.7H_2O$, 0.01 g/L $CuSO_45H_2O$, 0.01 g/L H_3BO_3, 0.01 g/L $Na_2MoO_4.2H_2O$, 0.25 g/L $NiCl_2.6H_2O$. pH was adjusted to pH=7 by adding 5% H_3P0_4 or 10% KOH when necessary.

2.3 Experiments

2.3.1 Batch Experiments

Batch experiments were made in 80 ml serum bottles and in a temperature controlled incubator shaker at 37 ^{0} C and 100 rpm. Initial nitrogen, COD, salt and biomass concentrations were changed in the experiments. Initial nitrogen concentration was varied between 250 mg/L and 3100 mg/L. The salt, biomass and COD concentration in this set of experiment were 3%, 500 mg/L and 1427±24 mg/L, respectively. Biomass concentrations were changed between 400 mg/L and 1000

mg/L in the serum bottles. Salinity and COD concentration were 3% salinity and 1588±85 mg/L. Salt inhibition effect was determined by changing both salt percentage and COD concentration in the serum bottles. Salinities of the synthetic media were 0%, 1%, 2%, 3% and COD concentrations were between 500-5000 mg/L. Biomass concentration and salinity was 500 mg/L.

2.3.2 Reactor Experiments

2.3.2.1 Experimental Set-up

The continuous treatment of synthetic saline wastewater was performed in an upflow anaerobic packed bed reactor. The reactor was made up of chrome-nickel with dimensions of 60 cm height and 8 cm diameter. The liquid volume in the reactor was 3000 ml. There were 3 sampling ports along the packed column height. The system was feed from the bottom with synthetic wastewater by a pump and effluent was given to drainage canal. Plastic non-commercial support materials were used for the immobilization of the organisms. The total surface area of the support materials was 8608 cm^2. Temperature was maintained at 37^oC with a heating jacket. Schematic diagram of the reactor is given in Figure 2.1.

Anaerobic reactor was inoculated with dens culture of *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 5 days. After immobilization of microorganisms, the continuous experiments were started.

2.3.2.2 Continuous Experiments

The anaerobic reactor was operated continuously with synthetic saline wastewater at different initial hydraulic retention times (HRT), COD and salt concentrations. System was loaded with fresh media at the beginning of each experimental condition and it was operated approximately 15 days until the system reached to almost steady

state conditions. pH, temperature, initial and effluent COD concentrations of daily samples were monitored.

Effect of initial COD concentration was investigated by changing the glucose concentration which is the sole carbon source in the synthetic wastewater. System was operated at 6 different initial COD concentrations between COD_0=1500-6300 mg/L. In this set of experiment, salt concentration and HRT were kept constant at [NaCl]=3% and θ_H=19 h.

The hydraulic retention time was increased from 11 h to 30 h in the second part of the experiments. Initial COD concentration was around 3332±180 mg/L and salt concentration was [NaCl]=3 %.

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 feed COD concentration was 3300±67 mg/L and HRT was θ_H=30 h..

2.4 Analytical Methods

2.4.1 Sampling

Samples were removed from the effluent once a day and centrifuged at 8000-10000 rpm for 0.15 h. COD analyses were carried out on clear supernatant. Samples were preserved at fridge with +4 ^{0}C for further analyses.

2.4.2 Chemical Oxygen Demand (COD) Analysis

Closed reflux colorimetric method was used for COD measurements. Digestion solution was prepared by adding 10.216 g $K_2Cr_2O_7$, 167 ml conc. H_2SO_4 and 33.3 g $HgSO_4$ into distilled water to be 1000 ml and the solution was cooled to room temperature. Sulfuric acid reagent was prepared as in open reflux method. Potassium

hydrogen phthalate (KHP) standard was used to obtain COD concentration-absorbance calibration curve. KHP was lightly crushed and then dried to constant weight at 120 ^{0}C. Then different initial KHP concentrations were dissolved in distilled water to obtain different COD concentrations. KHP solution had a theoretical COD of 900 mg/L for 0.765 g KHP/L. More than five standards of KHP were prepared to obtain COD concentrations of between 50 to 900 mg COD/L. COD content of the samples were determined by using absorbance concentration calibration curve (Greenberg A.E, 1989, pp. 5,9-10). Novaspec II, (Pharmacia Biotech) visible spectrophotometer was used to measure the absorbance of the color developed at 600 nm after 2 hours of reaction at 140 ^{0}C was completed. The samples were diluted prior to measurements to reduce the concentration between 50-900 mg/L if necessary. Excess amount of $HgSO_4$ were added into the samples to keep the interference of Cl^-ion to COD measurements..

2.4.3 Salinity and pH measurement

pH was measured by using 720 WTW(inolab) pH meter. Calibration of pH meter was carried out with standard solutions. Salinity control was made by using YSI model(33) S-C-T meters.

2.4.4 NH_4-N measurements

Ammonium-nitrogen analyses were carried out in clear supernatant by colorimetric method according to phenate with standard deviations of ± 0.025. Merck 1.14752.0001 NH_4-N kits and Merck-NOVAspec 60 photometer were used in the analysis.

2.4.5 Biomass measurement

Initial biomass concentration for batch experiments were determined by filtration of pure culture on pre-weighted filter (Whatman GF/C) which was dried at 105 ^{0}C for 24 h and then weighed after cooled to room temperature in a desicator.

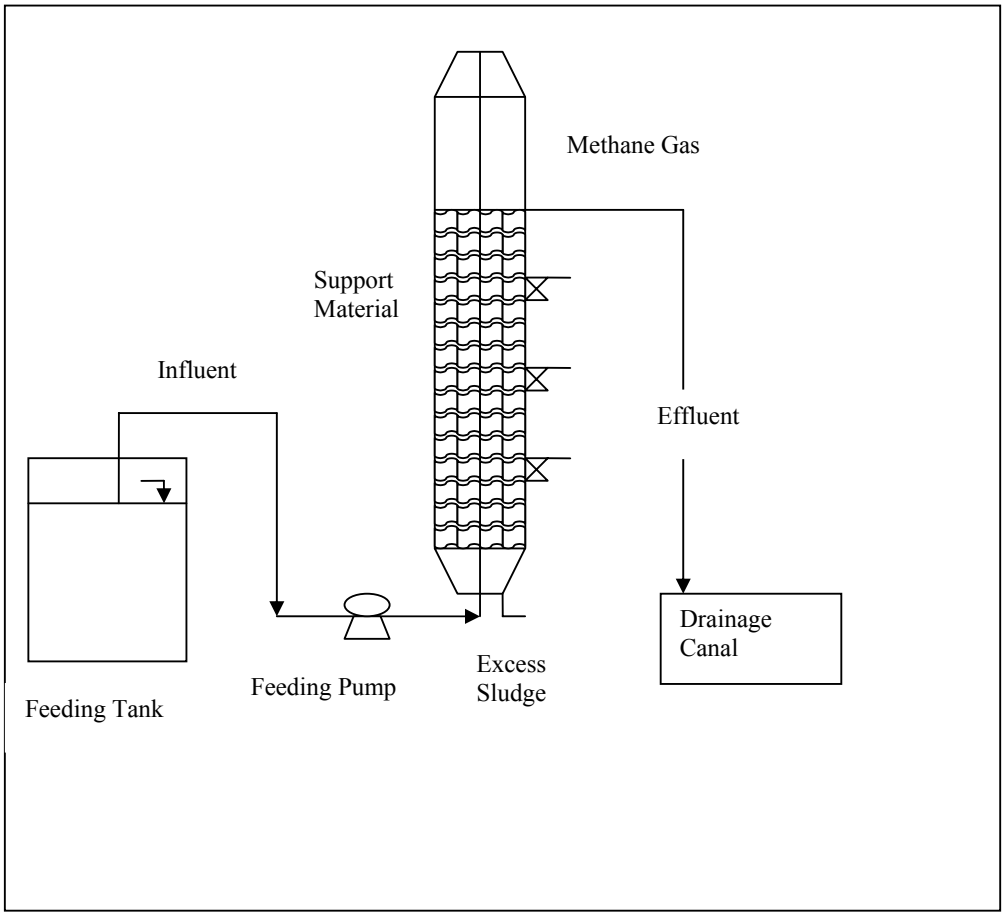

Figure 2.1 Schematic diagram of upflow anaerobic packed bed reactor.

CHAPTER THREE
RESULTS AND DISCUSSIONS

The study has two parts as batch flask experiments and reactor experiments. An anaerobic salt tolerant bacteria, *Halanaerobium lacusrosei*, was used as dominant microbial culture in the studies. Batch experiments were conducted to investigate the effect of media composition and to determine the salt inhibition kinetics. The reactor experiments contain continuous operation of an upflow anaerobic packed bed reactor with synthetic saline wastewater at different initial COD concentrations, salt concentrations and hydraulic retention times. The effect of those parameters on COD removal in saline wastewater were evaluated, optimum operating conditions were determined and kinetic coefficients of COD removal were calculated by using Stover–Kinconnon mathematical model.

3.1 Batch Studies

3.1.1 Effect of Nitrogen Concentration

Different NH_4-N concentrations were studied to determine the minimum nitrogen requirement for efficient COD removal. Initial nitrogen concentration (N_0) was varied between 250-3100 mg/L. Salinity and initial COD concentration were 3% and 1427±24 mg/L, respectively.

Figure 3.1 depicts variation of effluent COD concentration and COD removal efficiency with initial nitrogen concentration at COD_0=1427±24 mg/L, $[NaCl]_0$=3%. Effluent COD concentration gradually decreased to about COD_e=365-410 mg/L as the initial nitrogen concentration was increased from 250 mg/L to 3100 mg/L. As a result, COD removal efficiency varied between 70% - 75%.

Figure 3.2 shows the effect of initial nitrogen concentration on nitrogen removal from saline wastewater under anaerobic condition. Effluent nitrogen concentration increased from 120 mg/L to 2400 mg/L when initial nitrogen concentration was

increased from 250 mg/L to 3100 mg/L. Maximum nitrogen removal efficiency was obtained as 69% at 325 mg/L initial nitrogen concentration. However, nitrogen removal performance of the *H. lacusrosei* significantly affect for N_0 > 1200 mg/L and the nitrogen removal decreased to 22% with final nitrogen concentration of 2400 mg/L.

Figure 3.3 indicates the variation of COD and nitrogen removal rates with initial nitrogen concentration. Initial removal rates were calculated as given in equation 1;

$$R_i = -(S_0 - S_t)/(t_0 - t_t) \quad \text{eq.1.}$$

where, R_i represents initial substrate removal rate, S_0 is initial COD or nitrogen concentration, S_t is the final COD or nitrogen concentration at time "t". Specific substrate removal rates were determined by dividing the substrate removal rates to initial biomass concentration. COD removal rate was not significantly affected with initial nitrogen concentration. The rate varied between 211-227 mg/L d for the the nitrogen concentrations of 250 mg/L and 3100 mg/L, respectively. However, nitrogen removal rate increased from 0.026 mg/ L d to 0.139 mg/ L d for the studied nitrogen concentrations. These results indicated that the minimum nitrogen concentration necessary for the efficient COD removal was 250 mg/L. Higher nitrogen concentrations did not provide significant improvement in the COD removal performance of the *H. lacusrosei*.

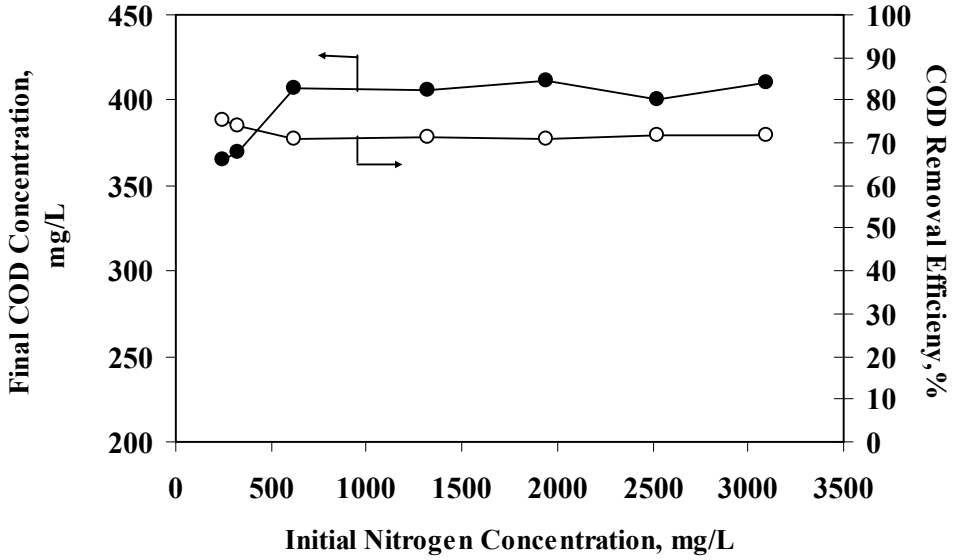

Figure 3.1 Variation of final COD concentration and COD removal efficiency with initial nitrogen concentration at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$=3% (—●—; final COD concentration, —○—; COD removal efficiency, %).

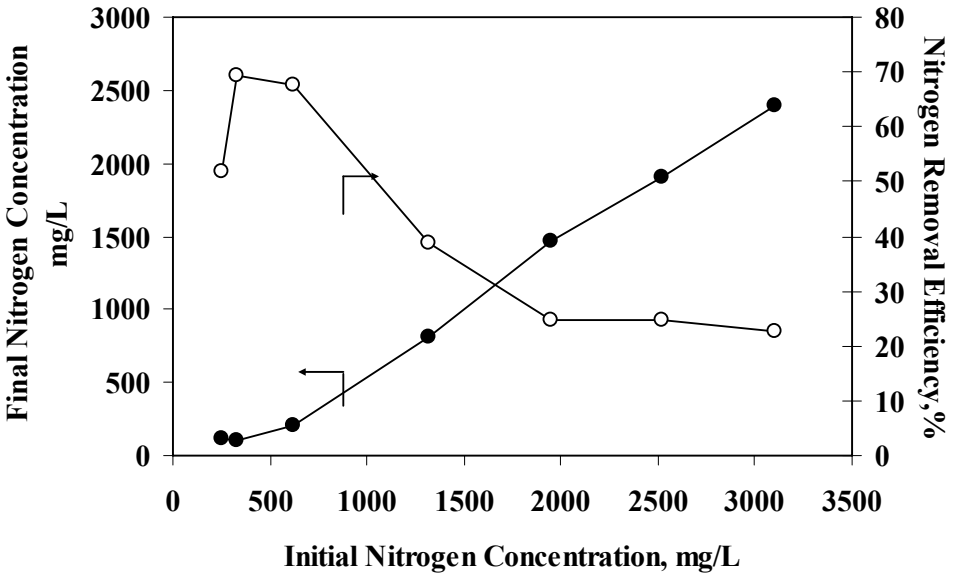

Figure 3.2 Variation of final nitrogen concentration and nitrogen removal efficiency with initial nitrogen concentration at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$= 3% (—●—; final Nitrogen concentration, —○— ; Nitrogen removal efficiency, %).

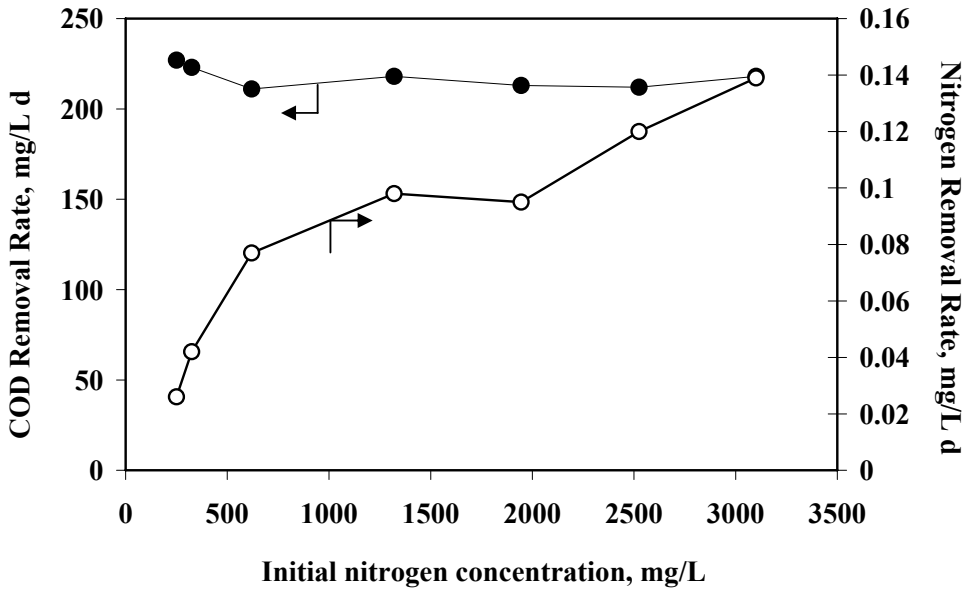

Figure 3.3 Variation of COD and nitrogen removal rate with initial nitrogen concentration at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$=3% (—●—; COD removal rate, —○—; Nitrogen removal rate)

3.1.2 Effect of Initial Biomass Concentration

The biomass concentration is an important parameter in biological wastewater treatment processes. Therefore, the effect of biomass concentration on COD and nitrogen removal under anaerobic conditions were investigated with batch flask test in this set of experiement. The biomass concentrations (X_0), were 0.4, 0.5, 0.6, 0.8, 1.0 g/L at the beginning of experiments. Initial COD and salt concentrations were around 1400 mg/L and 3%, respectively.

Figure 3.4 summarizes the effect of initial biomass concentrations on final COD concentration and percent COD removal. COD concentration increased from 365 mg/L to 876 mg/L with respect to increase in initial biomass concentrations from 0.4 g/L to 0.8 g/L. As a result, COD removal efficiency decreased from 75% to 54%, respectively.

Initial removal rate (Rs) and specific COD removal rate (Rx) at different biomass concentrations were calculated and the results were given in Figure 3.5. The initial COD removal rate did not vary significantly with the biomass concentration. It was between 227 mg/L d and 222 mg/L d for the all studied biomass concentrations. However, specific COD removal rate decreased from 568 mg/gX d to 222 mg/gX d as the biomass concentration was increased from 0.4 g/L to 1 g/L, respectively.

Figure 3.6 and 3.7 indicate the effect of biomass concentration on nitrogen removal from saline wastewater by *H. lacusrosei*. The nitrogen removal (R_N) efficiency decreased from 52% to 35% as biomass concentration was increased from 0.4 g/L to 1.0 g/L. The highest nitrogen removal rate obtained as 29 mg/Ld at 0.6 g/L biomass concentration. However, the increase in the initial biomass concentration caused a decrease in specific nitrogen removal rate (Rx_N) from 65 mg/gX d to 19 mg/gX d.

The decrease in the specific COD and nitrogen removal rates indicate that biomass concentration significantly affects the COD and nitrogen removal performance of the anaerobic organism. The maximum biomass concentration should be 0.6 g/L for efficient COD and nitrogen removal at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$=3% .

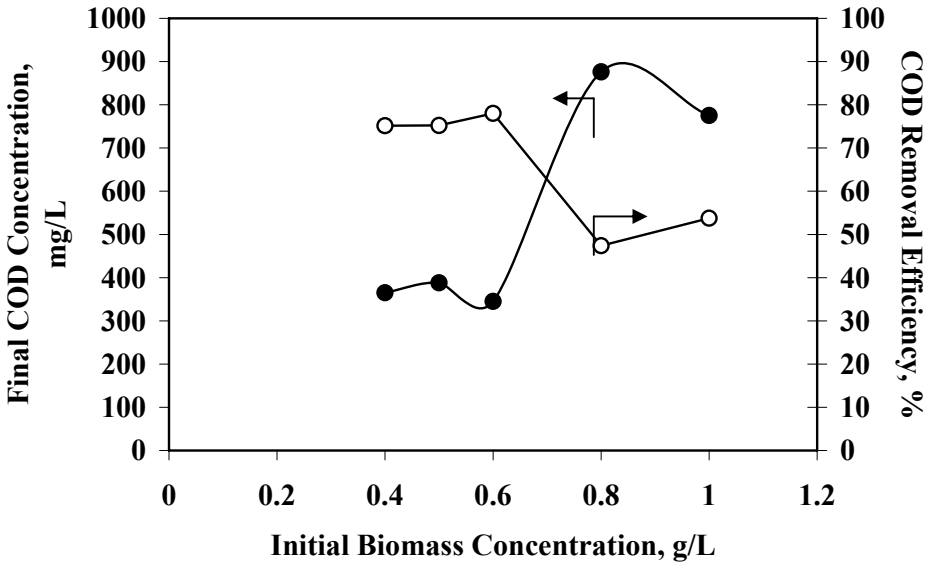

Figure 3.4 Variation of effluent COD concentration and percent COD removal with initial biomass concentration at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$=3% (—●—; final COD concentration, —○—; COD removal efficiency).

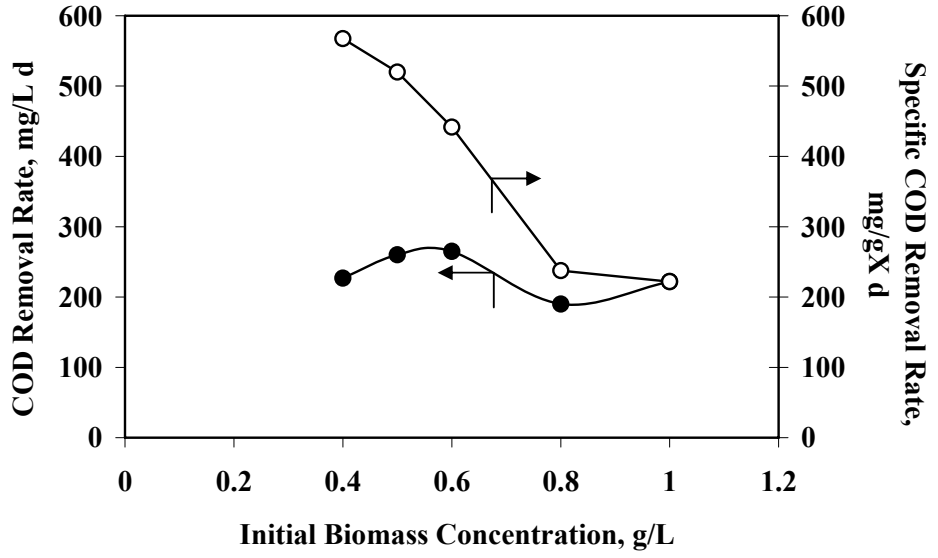

Figure 3.5 Variation of COD removal rate and specific COD removal rate with initial biomass concentration at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$=3% (—●—; COD removal rate, —○—; Specific COD removal rate)

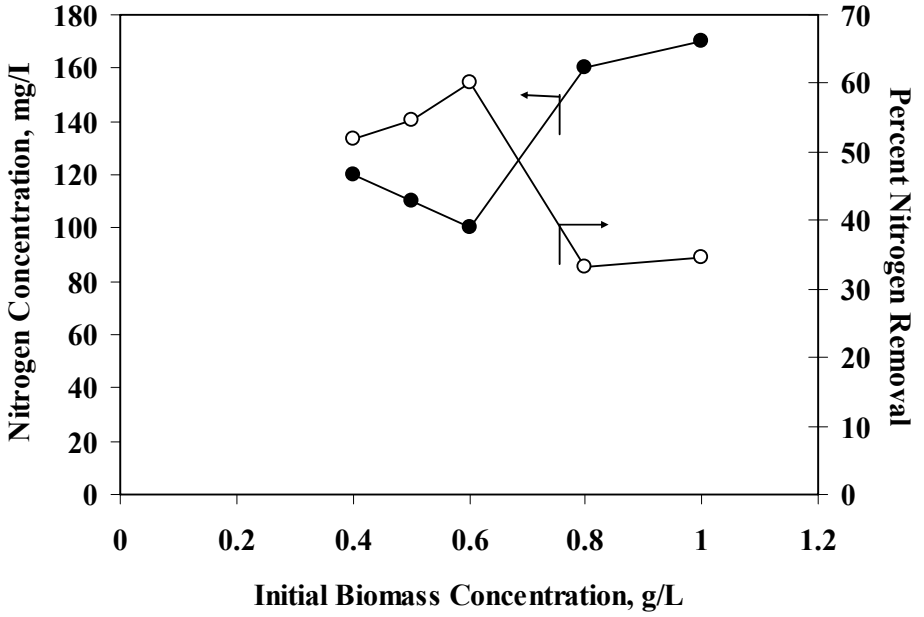

Figure 3.6 Variation of effluent nitrogen concentration and percent nitrogen removal with initial biomass concentration at $COD_0 \cong 1400$ mg/L, $[NaCl]_0 = 3\%$ (—●—; final nitrogen concentration, —○—; Nitrogen removal efficiency).

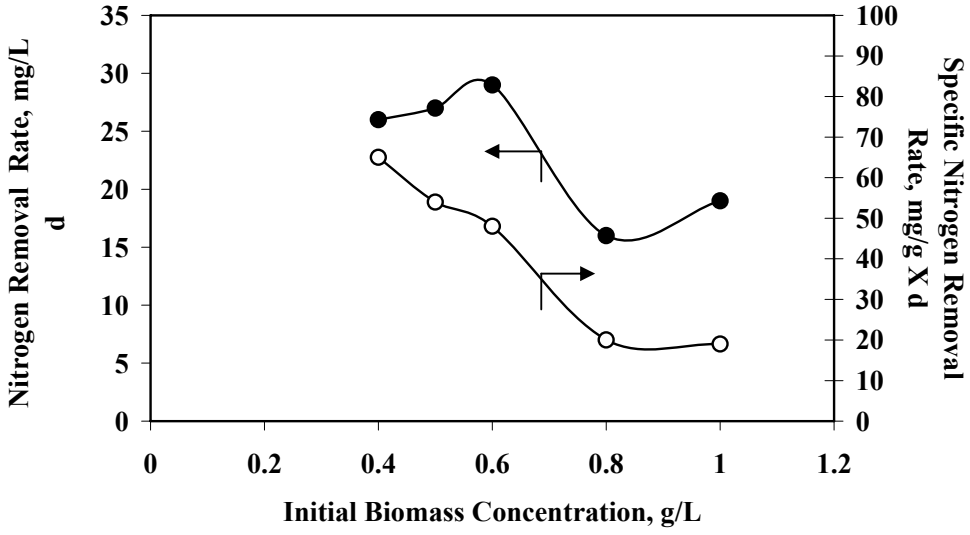

Figure 3.7 Variation of nitrogen removal rate and specific nitrogen removal rate with initial biomass concentration at $COD_0 \cong 1400$ mg/L, $[NaCl]_0=3\%$ (—●—; Nitrogen removal rate, —○—; Specific Nitrogen removal rate)

3.1.3 Effect of Salt Concentration on COD Removal

In order to investigate the effect of salt concentration on the COD removal performance of these organisms, initial salt concentration was varied between 0% and 3%. Initial COD_0 concentrations gradually increased from around 500 mg/L to 5000 g/L at each salt concentrations to determine the salt inhibition kinetics in batch experiments.

Variation of final COD concentration and percent COD removal at different initial COD concentrations and salt concentrations were given in Figure 3.8 as a; 0% NaCl, b; 1% NaCl, c; 2% NaCl, d; 3% NaCl. At the beginning of this part of experiment, performance of *H. lacusrosei* in salt free wastewater was examined. The percent COD removal was in the range of 40% and 64% for the initial COD concentrations of 1215 mg/L - 4253 mg/L (Figure 3.8 a). Increasing salt concentration to 1% did not significantly affect the COD removal. The efficiency was almost the same as salt free experiments. There was a slight improvement in COD removal at 2% salt concentration especially at low initial COD concentrations around 1000 mg/L (Figure 3.8.c). Percent COD removal increased to around 60% while it was only 40% for the salt free and 1% salt concentrations. However, the microorganisms were quite affected with 3% salt concentration. Percent COD removal decreased to less than 40% for the all COD concentrations studied.

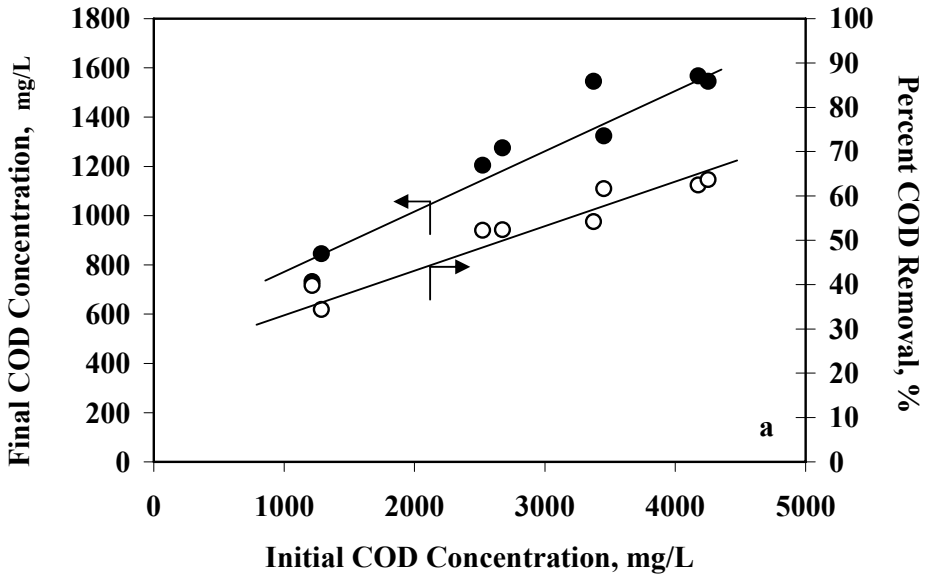

Figure 3.8a Variation of final COD concentration and percent COD removal with different initial COD concentrations and salt concentration at $[NaCl]_0$=0% (—●—; final COD concentration, —○—; COD removal efficiency).

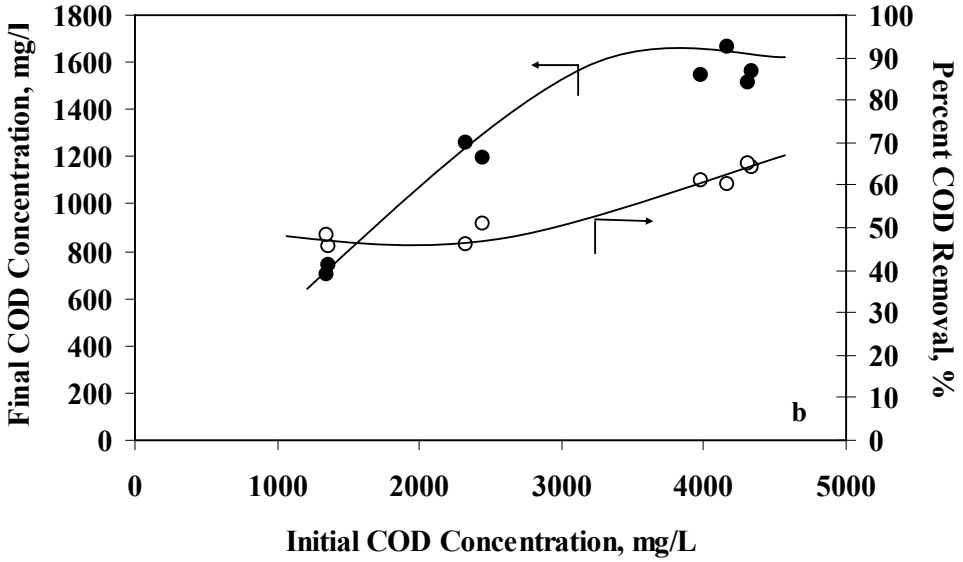

Figure 3.8b Variation of final COD concentration and percent COD removal with different initial COD concentrations and salt concentration at $[NaCl]_0$=1% (—●—; final COD concentration, —○—; COD removal efficiency).

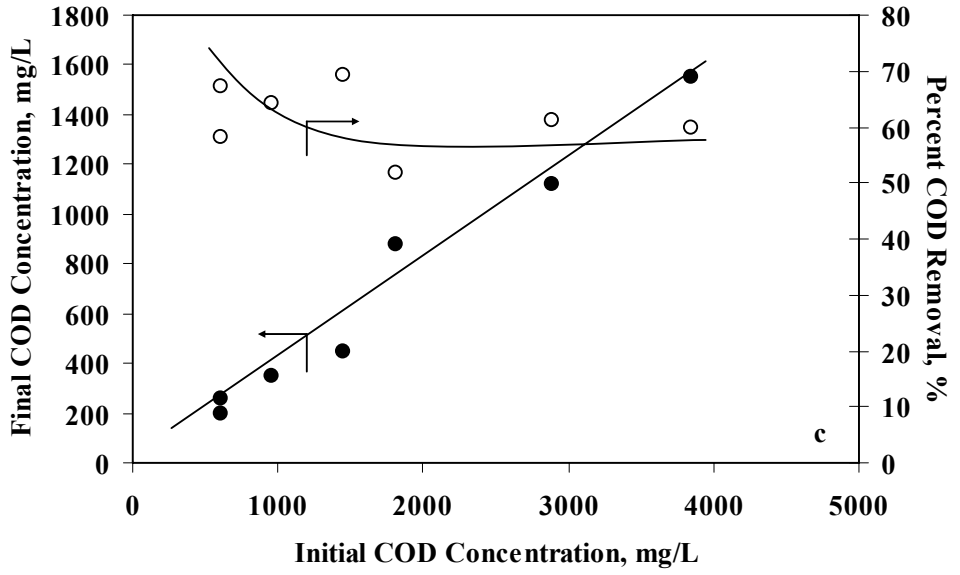

Figure 3.8c Variation of final COD concentration and percent COD removal with different initial COD concentrations and salt concentration at $[NaCl]_0$=2% (—●—; final COD concentration, —○—; COD removal efficiency).

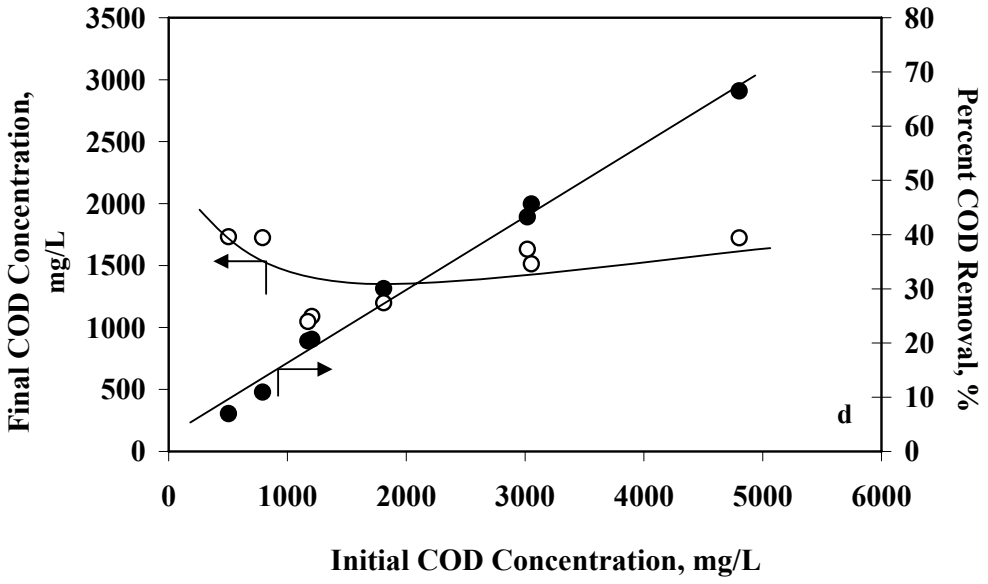

Figure 3.8d Variation of final COD concentration and percent COD removal with different initial COD concentrations and salt concentration at $[NaCl]_0$=3% (—●—; final COD concentration, —○—; COD removal efficiency).

3.1.4 Determination of Salt Inhibition Kinetics

Michaelis-Menten kinetic relationship was used in determination of kinetic coefficients, K_m, saturation constant and R_m, maximum substrate removal rate. In batch kinetics, initial substrate removal rates, R_i, are used to determine the coefficients. Initial COD removal rate is calculated as equation 2;

$$R_i = - (S_0 - S_t)/(t_0 - t_t) \qquad \text{eq.2.}$$

where, R_i represents initial rate, S_0 is initial COD concentration, S_t is the effluent COD concentration at time "t". The slope of time vs effluent COD concentration, gives the initial substrate removal rate. R_i vs S_0 plot results in general batch substrate removal relationship as in Figure 3.9 if there is no substrate inhibition.

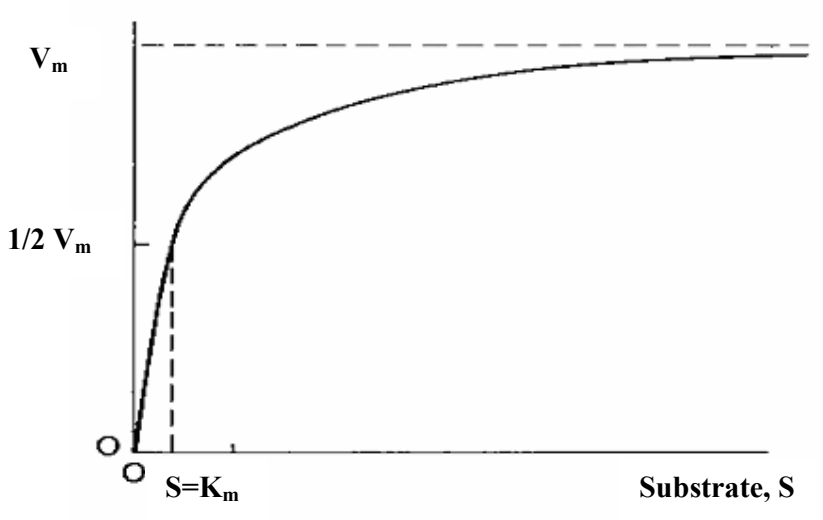

Figure 3.9 The Michealis-Menten relationships between initial substrate concentration and reaction rate.

The substrate concentration corresponds to half reaction rate gives the saturation constant. But accurate determination of K_m from this plot is very difficult. Therefore, general Michaelis Menten saturation kinetic equation is used (eq 3).

$$R = \frac{R_{max} * S}{K_m + S} \qquad \text{eq.3}$$

where, R_{max} is maximum substrate removal rate, S is the final substrate concentration. Since initial substrate concentrations and initial substrate removal rates are considered in batch kinetic, the eq 3 takes the following form;

$$R_i = \frac{R_{max} * S_0}{K_m + S_0} \qquad \text{eq.4}$$

The specific rate of substrate removal (R_{xi}) is determined by dividing the initial rates (R_i) to initial biomass concentration as given in eq 5.

$$R_{xi} = \frac{R_i}{X_0} = \frac{k * S_0}{K_m + S_0} \qquad \text{eq.5}$$

If the equation 5 is linearized in double reciprocal form as in eq. 6 and a plot of $1/R_{Xi}$ versus $1/S_0$ yields a linear line with a slope of K_m/k and y-axis intercept of 1/k.

$$\frac{1}{R_{Xi}} = \frac{1}{k} + \frac{K_m}{k}\,\frac{1}{S_o} \qquad \text{eq.6}$$

The presence of certain compounds in the media may effect the growth rate, enzyme activity or substrate removal rate. These compounds are known as inhibitors.

The effect of inhibitors is analyzed by using inhibition kinetic models. These models are classified according to the effect of inhibitor on reaction rate and saturation constant.

Inhibition models

Competitive inhibition can be defined as in equation 7;

$$R = \frac{R_m[S]}{K'_{m,app} + [S]} \qquad \text{eq.7}$$

where $K'_{m,app} = K'_m\left(1 + \frac{[I]}{K_1}\right)$

The net effect of competitive inhibition is an increased value of $K'_{m,app}$ and, therefore, reduced reaction rate. Competitive inhibition can be overcome by high concentration of substrate.

Non competitive inhibition can be defined as in equation 8;

$$R = \frac{R_{m,app}}{\left(1 + \frac{K'_m}{[S]}\right)} \qquad \text{eq.8}$$

where $R_{m,app} = \frac{R_m}{\left(1 + \frac{[I]}{K_1}\right)}$

The net effect of noncompetitive inhibition is a reduction in R_m. High substrate concentrations would not overcome noncompetitive inhibition.

Uncompetitive inhibition can be defined as in equation 9;

$$R = \frac{\frac{R_m}{\left(1+\frac{[I]}{K_1}\right)}[S]}{\frac{K_m^{'}}{\left(1+\frac{[I]}{K_1}\right)}+[S]} \qquad \text{eq.9}$$

The net effect of uncompetitive inhibition is a reduction in both R_m and K_m values (Kargı, Shuler, 2002).

The specific substrate removal rates at different salt and COD concentrations were calculated according to equation 5 and linearized as given in equation 6. Double reciprocal plots of substrate removal rates at different salt concentrations were depicted in Figure 3.10. These figures were combined in Figure 3.11 to determine the inhibition model. As seen from the figure the same intercept point with varing slope was obtained for the 1%, 2%, 3% salinity. This results indicate a competitive inhibition model. The coefficients of the competitive inhibition is given in Table 3.1.

Table 3.1 The kinetic coefficients of competitive inhibition

Constant	**0%**	**1%**	**2%**	**3%**
k (d^{-1})	6213	7863	7680	7462
K_m (mg/L)	11687	-	-	-
$K_{m,app}$ (mg/L)	-	13292	15354	25687

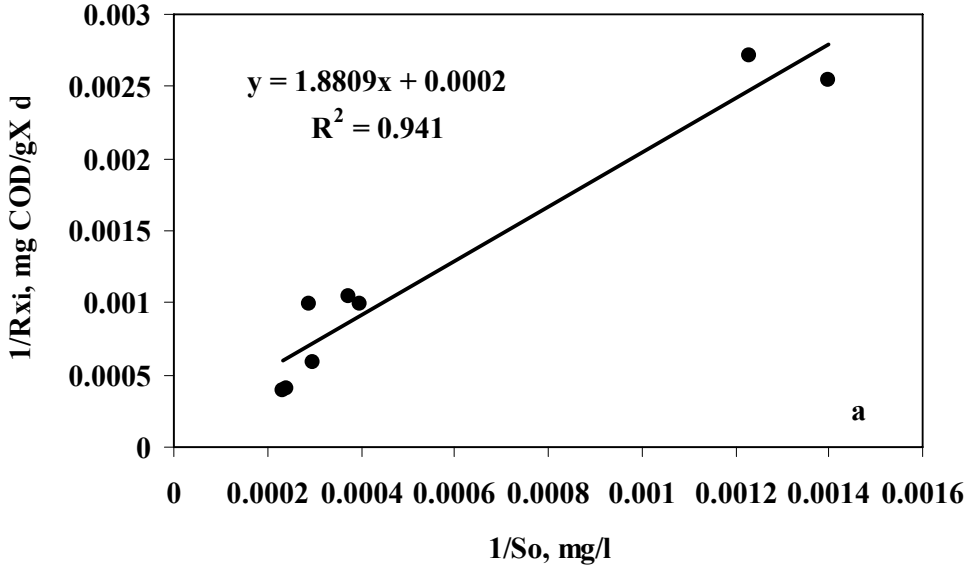

Figure 3.10a Double reciprocal plot of specific substrate utilization rate and initial substrate concentration at $[NaCl]_0$=0%

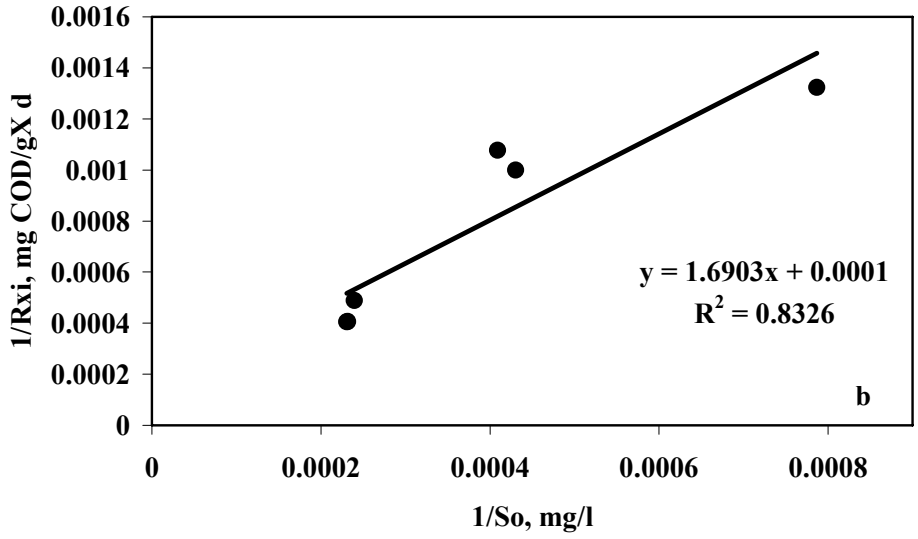

Figure 3.10b Double reciprocal plot of specific substrate utilization rate and initial substrate concentration at $[NaCl]_0$=1%

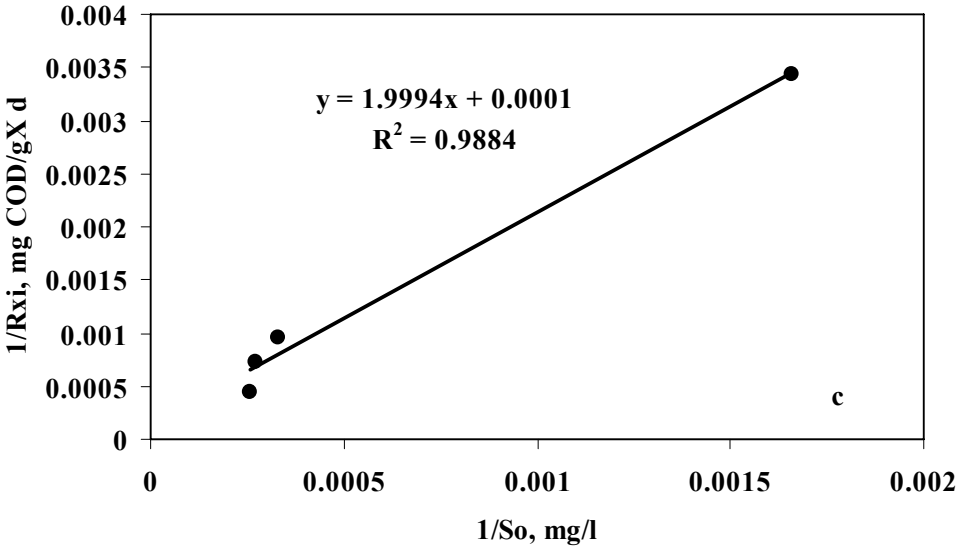

Figure 3.10c Double reciprocal plot of specific substrate utilization rate and initial substrate concentration at $[NaCl]_0$=2%

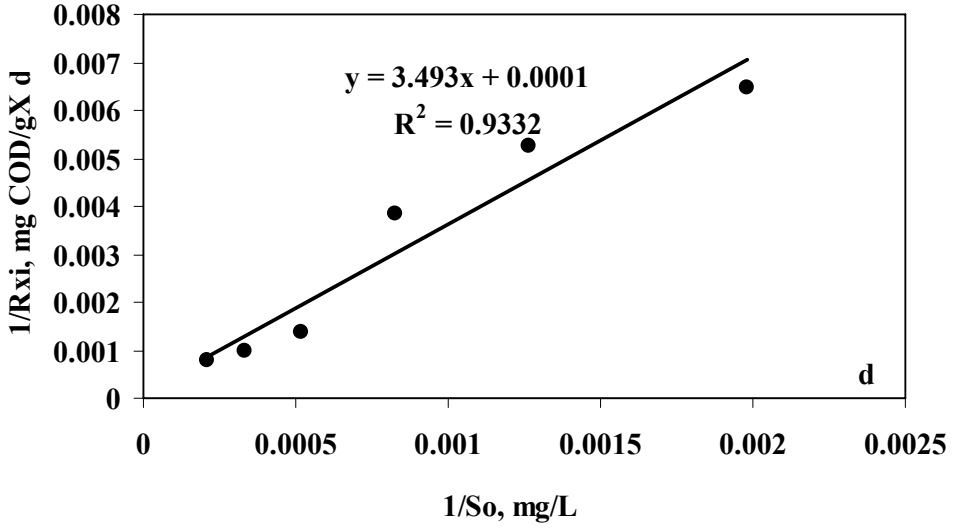

Figure 3.10d Double reciprocal plot of specific substrate utilization rate and initial substrate concentration at $[NaCl]_0$=3%

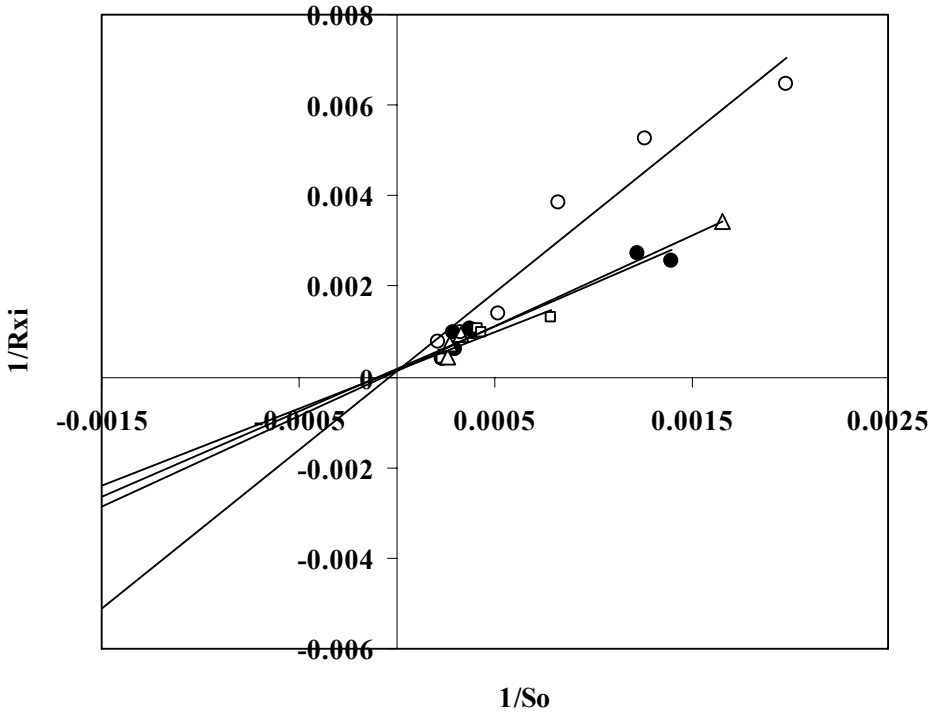

Figure 3.11 Determination of salt inhibition kinetics (—●—; $[NaCl]_0$=0%, —□—; $[NaCl]_0$=1%, —Δ—; $[NaCl]_0$=2%, —○—; $[NaCl]_0$=3%)

3.2 Continuous Operation of Anaeroobic Packed Bed Reactor for Treatment of Saline Wastewater

3.2.1 Effect of Initial COD Concentration on COD Removal Performance of the System

Initial COD_0 concentration was increased from around 1900 mg/L to 6300 mg/L in the first part of the experiments. Initial salt concentration and HRT were kept constant at $[NaCl]_0$=3% and θ_H=19 h, respectively. COD analyses were carried out on daily samples taken from influent and effluent wastewater of the system.

Figure 3.12 depicts the variation of effluent COD concentration and COD removal efficiency with time at COD_0=1818±100 mg/L initial COD concentration. COD concentration gradually decreased to about COD_e=650 mg/L at the end of 5 days

continuous operation. A further decrease in COD concentration was observed in the system and final COD oncentration was obtained as 105 mg/L. The resulting COD removal efficiency was 94% .

Variation of effluent COD concentration and COD removal efficiency with time at COD_0=2393± 171 mg/L initial COD concentration are shown in Figure 3.13. No significant COD removal was observed until 6th day of continuous operation. The effluent COD concentration decreased to 1490 mg/L with 36% removal efficiency. Fortunately, COD decreased to around 917 mg/L at the end of 6th day of continuous operation. Then it remained constant around 1065 mg/L in average resulting in about 55% COD removal efficiency.

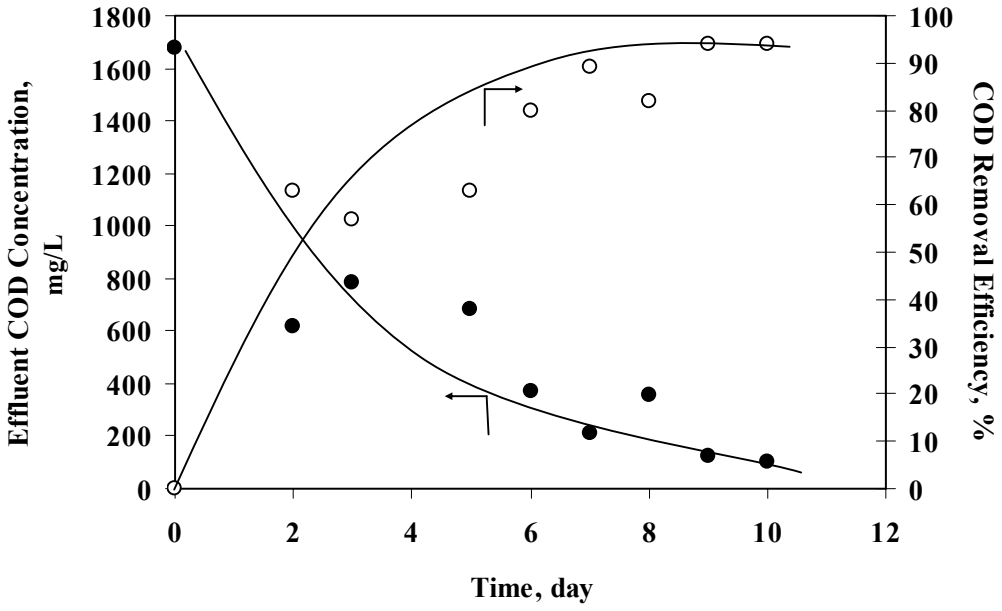

Figure 3.12 Variation of effluent COD concentration and removal efficiency with time at COD_0= 1818±100 mg/L, $[NaCl]_0$=3% and θ_H=19 h (—●—; effluent COD concentration, —○—; COD removal efficiency).

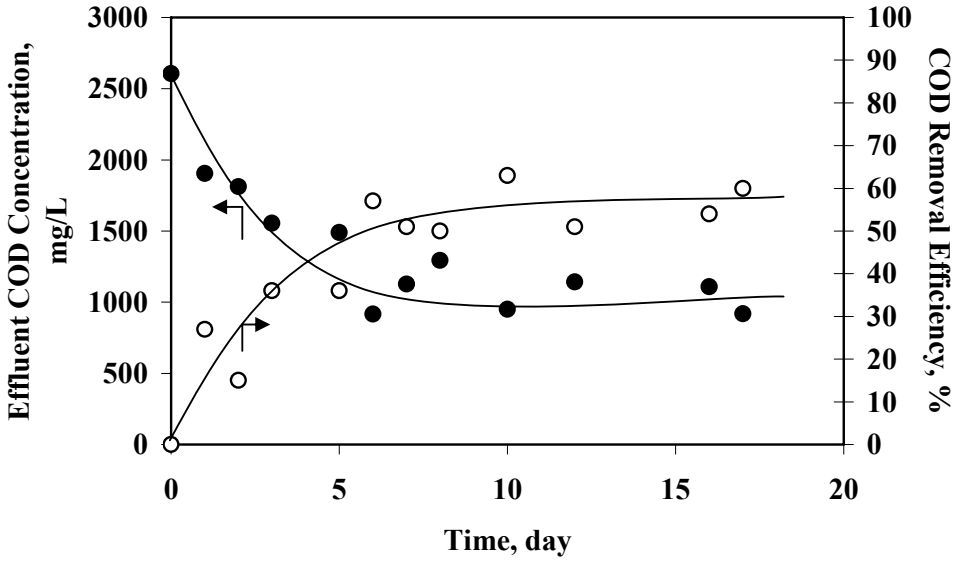

Figure 3.13 Variation of effluent COD concentration and removal efficiency with time at COD_0=2393±171 mg/L (—●—; effluent COD concentration, —○—; COD removal efficiency).

Figure 3.14 shows the variation of effluent COD concentration and removal efficiency with time at COD_0= 3263± 65 mg/L $[NaCl]_0$=3% and θ_H=19 h. There was no significant variation in the effluent COD concentration from start-up to final days of the continuous operation. The effluent COD concentration was around 1890 mg/L for the first 3 days operation and slightly decreased to 1436 mg/L at the end of 15th days continuous operation. As a result COD removal efficiency was 57 %.

Figure 3.15 indicates the variation of effluent COD concentration and removal efficiency with time at COD_0= 4278 ±111mg/L, $[NaCl]_0$=3%, θ_H =19 h. For the first 4 days, there was no significant reduction in COD concentration. The percent removal was only 30%. However, the system performance slightly improved from day 6. The removal efficiency increased from 30 % to 56 % and remained almost constant until the end of experiment. The resulting COD removal efficiency was 53 % with 2010 mg/L effluent COD concentration.

Initial COD_0 concentration was further increased to 5269±45 mg/L. Figure 3.16 depicts the variation of effluent COD concentration and removal efficiency with time. At the beginning of operations, there was a sudden decrease in COD to 2223 mg/L resulting in 56 % for the first days. There were no significant decrease in COD concentration for the rest of continuous operation. The final effluent COD concentration was 2364 mg/L at the end of the experiment.

The highest initial COD concentration examined in this set of experiment was 6208±69 mg/L. As seen from Figure 3.17 there was a gradual decrease of COD concentration to about COD_e=2785 mg/L. Depending on this decrease in COD concentration, the removal efficiency varied between 13% - 56%.

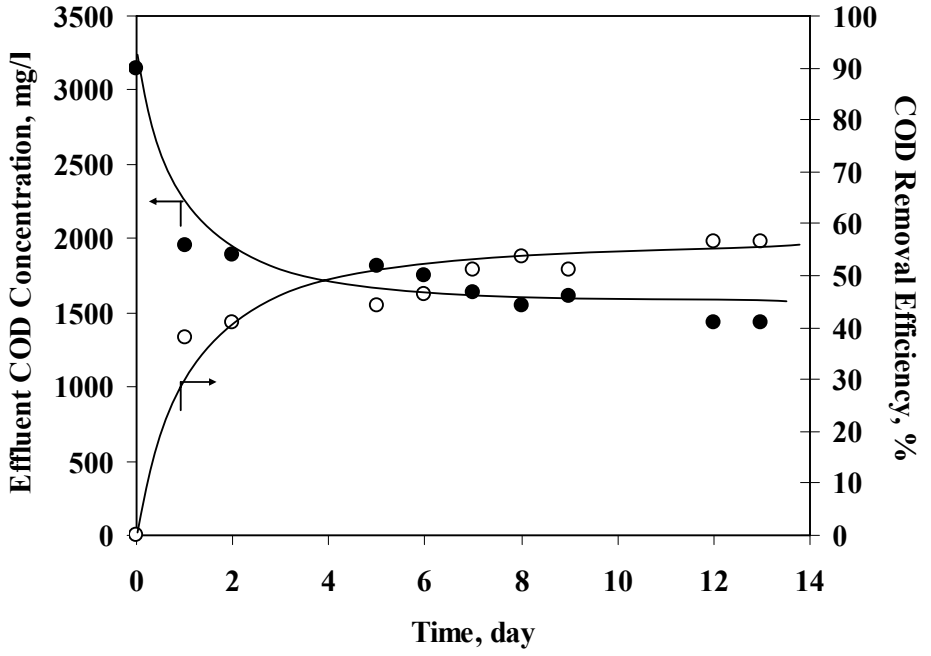

Figure 3.14 Variation of effluent COD concentration and removal efficiency with time at COD_0=3263±65 mg/L θ_H=19 (—●—; effluent COD concentration, —○—; COD removal efficiency).

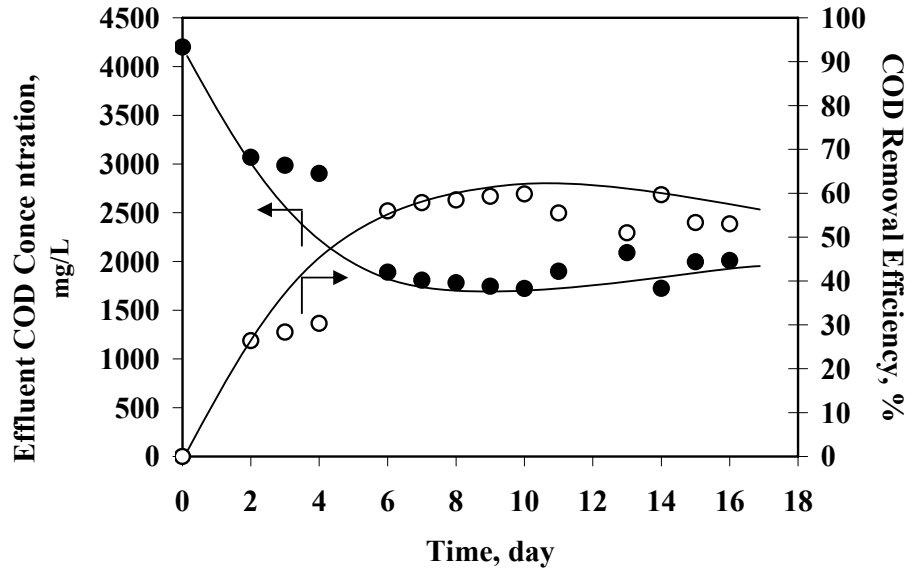

Figure 3.15 Variation of effluent COD concentration and removal efficiency with time at COD_0= 4278±11 mg/L θ_H=19 (—●—; effluent COD concentration, —○—; COD removal efficiency).

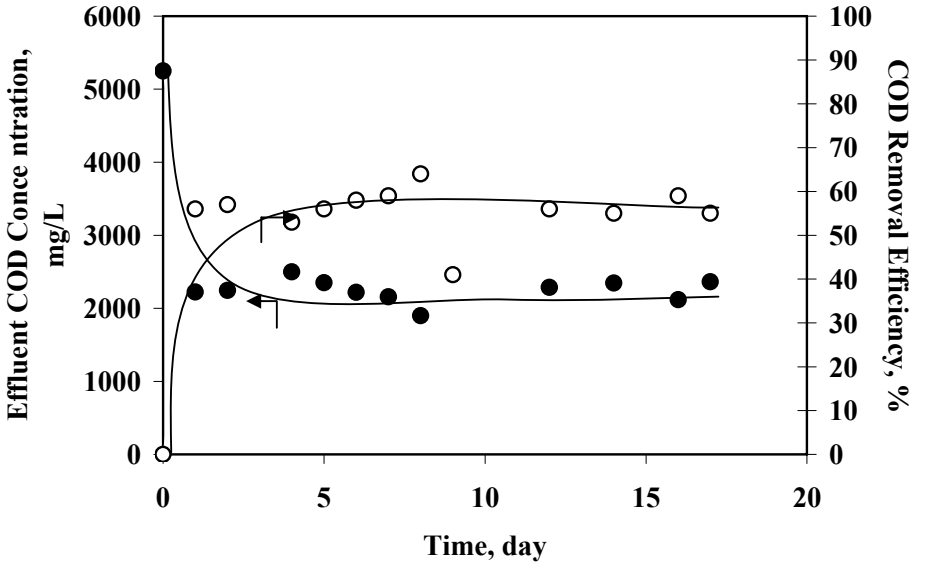

Figure 3.16 Variation of effluent COD concentration and removal efficiency with time at COD_0=5269±45 mg/L θ_H=19 (—●—; effluent COD concentration, —○—; COD removal efficiency).

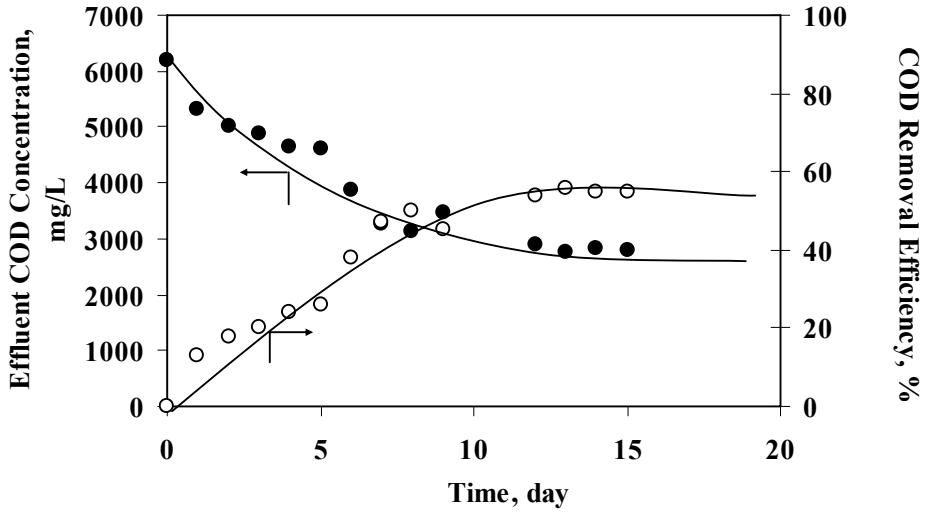

Figure 3.17 Variation of effluent COD concentration and removal efficiency with time at COD_0= 6208±69 mg/L θ_H=19 (—●—; effluent COD concentration, —○—; COD removal efficiency).

Figure 3.18 shows the effect of initial COD concentration on effluent COD concentration and COD removal efficiency. Effluent COD concentration increase from 105 mg/L to 950 mg/L with respect to increase at initial COD concentrations from 1879 mg/L to 2574 mg/L. Depending on that variation in effluent COD concentration, removal efficiency decreases from 94% to 55%, respectively. These results indicate that, anaerobic salt tolerant *H. lacusrosei* microbial culture can efficiently remove low initial COD concentrations ($COD_0 \leq 2000$ mg/L) with COD removal efficiency of 94%. However, there was no significant difference in COD removal perfomance of the *H. lacusrosei* between 2500 and 6300 mg/L initial COD concentration. The removal efficiency remained almost constant around 56% at $[NaCl]_0$=3% salt concentration and 19 h HRT. The decrease in COD removal at high initial COD concentrations could be explained as substrate inhibition on the organisms. It can be summarized that *H. lacusrosei* containing anaerobic packed bed reactor can be operated continuously at 2000 mg/L initial COD concentration in order to obtain higher that 90% removal efficiency. However, 55% removal efficiency around 6000 mg/L initial COD concentration can be evaluated as high COD removal performance for the anaerobic conditions at $[NaCl]_0$=3% salt

concentrations. This high COD removal performance can be explained as presence of salt tolerant anaerobic organism *H. lacusrosei*.

Figure 3.19 shows the variation of substrate removal rates with effluent COD concentrations. Substrate removal rates were calculated as $R_i=Q(S_0- S_t)/(t_0-t_t)$, where, R_i represents initial rate, Q operation flowrate, S_0 is initial COD concentration, S_t is the effluent COD concentration at time "t". As seen from the Figure the substrate removal rate increases from 1.56 g/L d to 4.34 g/L d with increasing effluent COD concentrations between CODe=105-2800 mg/L. The maximum rate was determined as R_i= 4.34 g/L d at quite high COD concentration around 6315 mg/L which supports that *H. lacusrosei* can tolerate high substrate concentrations with efficient COD removal at $[NaCl]_0$=3% salt concentrations.

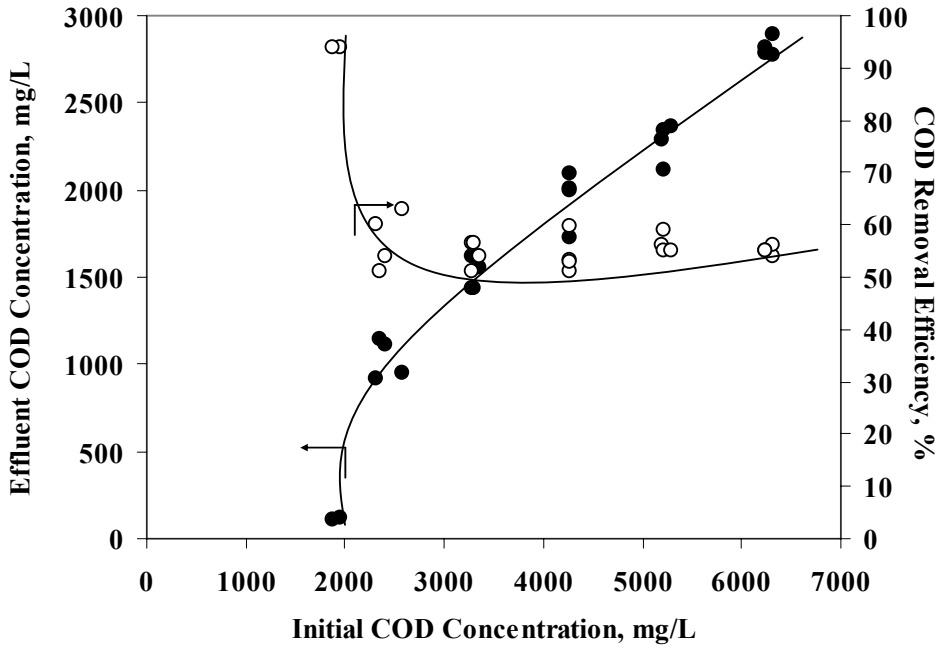

Figure 3.18 Effect of initial COD concentration on effluent COD concentration and COD removal efficiency (—●— ; effluent COD concentration, —○—; COD removal efficiency).

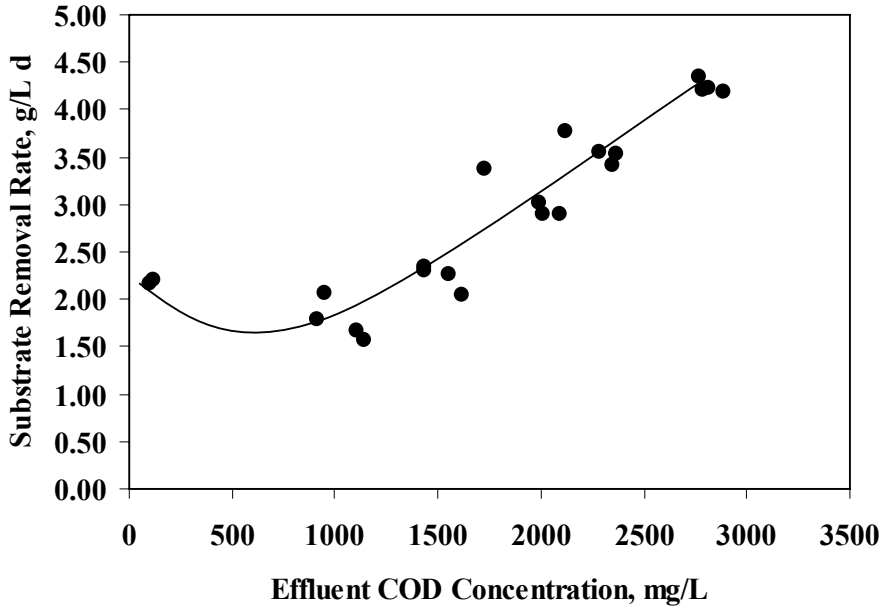

Figure 3.19 Effect of effluent COD concentration on substrat removal rate

3.2.2 Effect of Hydraulic Retention Time on COD Removal Performance of the System

The hydraulic retention time was varied between 11 hours and 30 hours. The initial COD_0 concentration was around 3100-3500 mg/L and salt concentration was kept constant at 3%.

Variation of effluent COD concentration and COD removal efficiency at 3% salt concentration and 11 hours HRT is shown in Figure 3.20. At the beginning of operation effluent COD dropped to 1216 mg/L from 3343±35 mg/L resulting in about 64 % COD removal efficiency. The effluent COD concentration did not vary much with time for the rest of experiment and remained constant around 1300 mg/L. The resulting COD removal efficiency at 11 h HRT was determined as 60%.

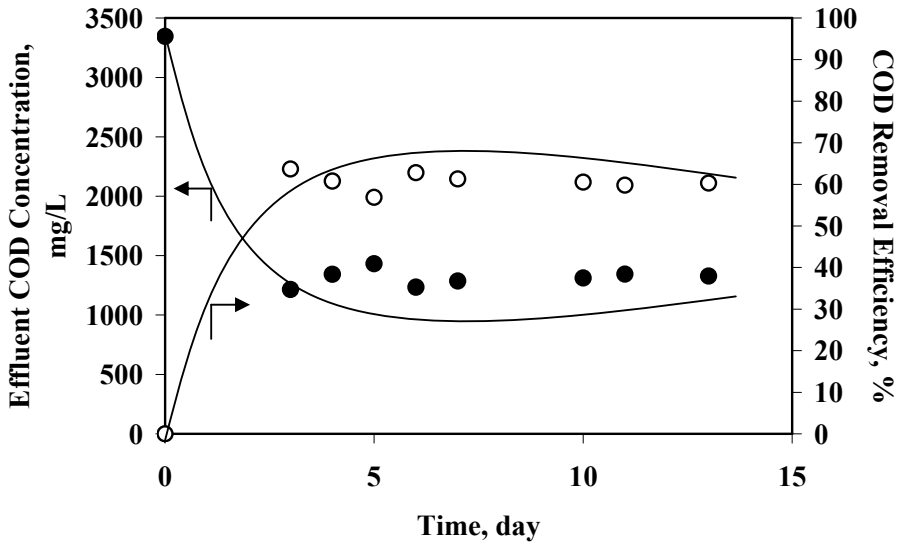

Figure 3.20 Variation of effluent COD concentration and removal efficiency with time at COD_0= 3343±35 mg/L, $[NaCl]_0$=3% and θ_H=11 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency).

Figure 3.21 shows the variation of effluent COD concentration and removal efficiency with time at COD_0=3444±79 mg/L, $[NaCl]_0$=3%, θ_H=14 h. At the beginning of operations, there was a sharp decrease in COD to about 717 mg/L. Removal efficiency was 80% for the first days. However, effluent COD concentration slightly increased to 1000 mg/L with 70% COD removal at 11th day of continuous operation. Finally, COD decreased to 890 mg/L at the end of 14 days resulting in 74 % COD removal efficiency.

Figure 3.22 shows variation of effluent COD concentration and removal efficiency with time for $[NaCl]_0$=3% and θ_H=20 h. The lowest COD removal efficiency was obtained as 65% at the first days of experiments. Minimum COD concentration observed during the operations was 990 mg/L. At the 3rd day of continuous operation, removal efficiency increased to 70 %. After the operations for 15 days, 71 % removal efficiency and 1010 mg/L effluent COD concentration were obtained.

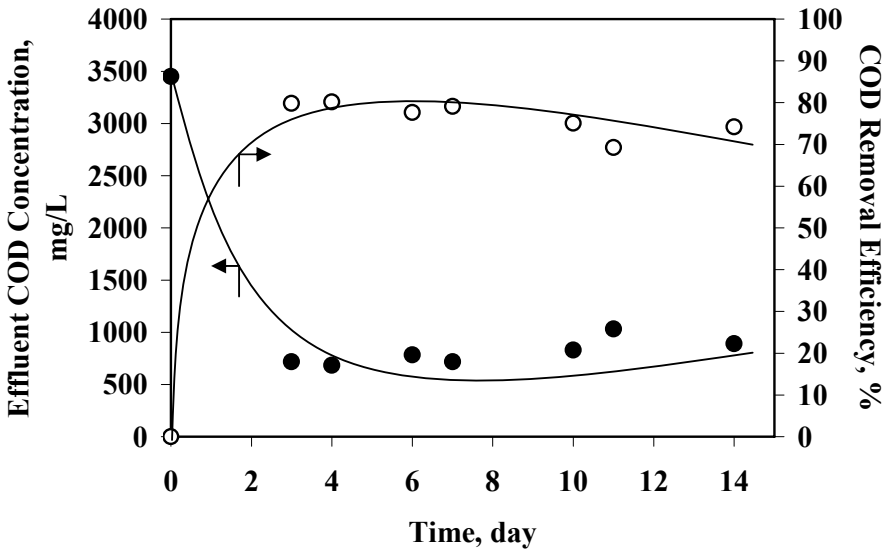

Figure 3.21 Variation of effluent COD concentration and removal efficiency with time at COD_0= 3444±79 mg/L, $[NaCl]_0$=3% and θ_H=14 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency)

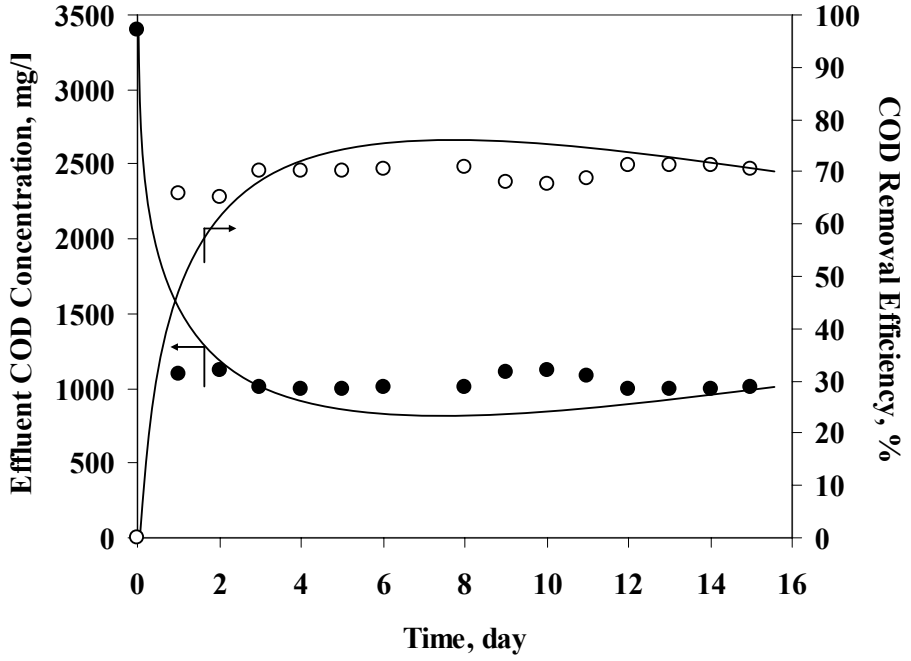

Figure 3.22 Variation of effluent COD concentration and removal efficiency with time at COD_0=3391±82 mg/L, $[NaCl]_0$ = 3% and θ_H=20 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency).

Figure 3.23 depicts the variation of effluent COD concentration and removal efficiency with time at θ_H=25 h. Initial COD_0 concentration was adjusted to at about 3180±44 mg/L. There was a sharp decrease in COD concentration to about 945 mg/L after 6 days of continuous operation and then it decreased to 514 mg/L after 7th days of operations. As a result, removal efficiency increased from 71% to 84%. Percent removal and effluent COD concentration did not change significantly for the rest of operation.

Figure 3.24 shows the variation of effluent COD concentration and removal efficiency with initial COD_0 concentration at about 3445±83 mg/L and 30 hours HRT. The effluent COD concentration dropped to 815 mg/L for the first days of continuous operation resulting in 77 % COD removal. As seen from figure there was a gradual decrease of COD concentration. At the end of 13 days operation period, effluent COD concentration was 545 mg/L and percent removal was 84%.

The effect of hydraulic retention time on effluent COD concentration and COD removal efficiency was given in Figure 3.25. The final COD concentration was between 1328 mg/L and 1161 mg/L for the hydraulic retention times θ_H=11-14 h. 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 indicate that, for the efficient COD removal at COD concentrations such as 3500 mg/L with *H. lacusrosei,* the hydraulic retention time should be higher than 20 hours.

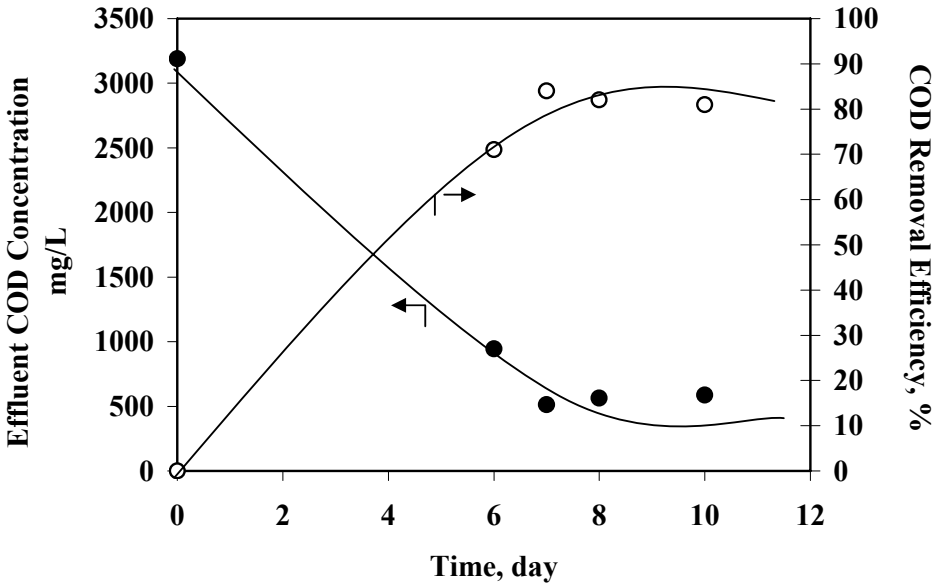

Figure 3.23 Variation of effluent COD concentration and removal efficiency with time at COD_0= 3180±44 mg/L, $[NaCl]_0$=3% and θ_H=25 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency).

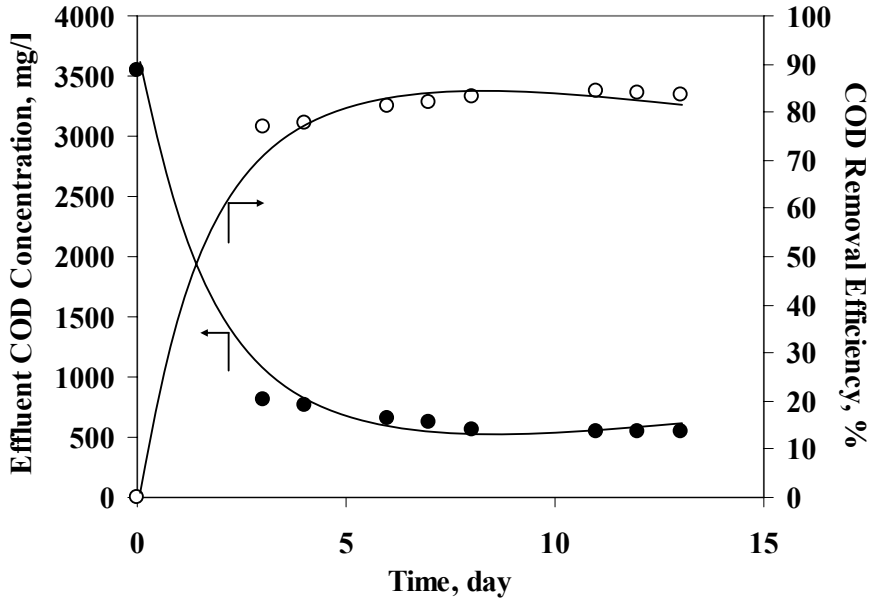

Figure 3.24 Variation of effluent COD concentration and removal efficiency with time at COD_0=3445±80 mg/L, $[NaCl]_0$=3% and θ_H=30 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency).

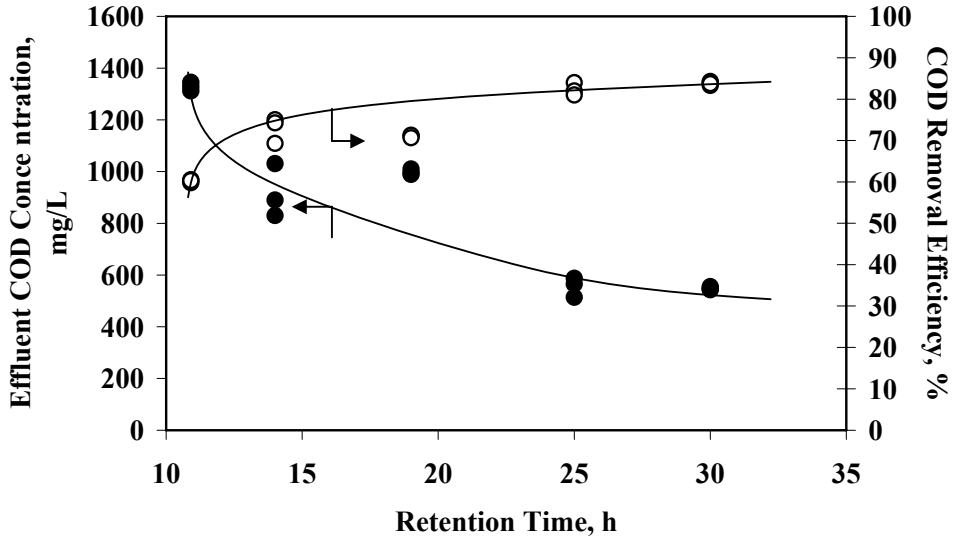

Figure 3.25 Effect of hydraulic retention time on effluent COD concentration and COD removal efficiency. (—●—; effluent COD concentration, —○—; COD removal efficiency).

3.2.3 Effect of Salt Concentration on COD Removal Performance of the System

Isolation studies about *H. lacusrosei* indicated that this culture can tolerate up to 20% salt concentration. However, these studies are carried out under sterile and well controlled condition. In order to determine the maximum tolerated salt concentration and the effect of salt concentration on the COD removal performance of these organisms in a heterogenous condition as in wastewater treatment plant, initial salt concentration was gradually increased from 0% to 5%. Although maximum COD removal efficiency was obtained at around COD_0=1850 mg/L with the 94% removal efficiency, the initial COD concentration was adjusted to COD_0=3300±67 mg/L to evaluate the system performance at relatively higher CODs. The hydraulic retention time was 30 h. The variation of COD removal with time at different salt concentrations were given through Figures 3.26-3.31, and results were summarized in Figures 3.32-3.33.

Variation of effluent COD concentration and COD removal efficiency at salt free synthetic media is shown in Figure 3.26. Initial COD concentration was adjusted to 3205 ±26 mg/L. The COD removal efficiency was around 53% with 1500 mg/l effluent concentration. Halophilic microorganisms require salt for growth. Because of this reason, the organisms showed low COD removal in salt free media.

Effluent COD concentration and COD removal efficiency for 1% salt concentration are given in Figure 3.27. At the beginning of operation, effluent COD concentration decreased from 3215±15 mg/L to the 1010 mg/L. There was no significant difference in effluent COD concentration during the whole operation period. The steady state COD concentration was determined as 1150 mg/L resulting in 62 % removal efficiency.

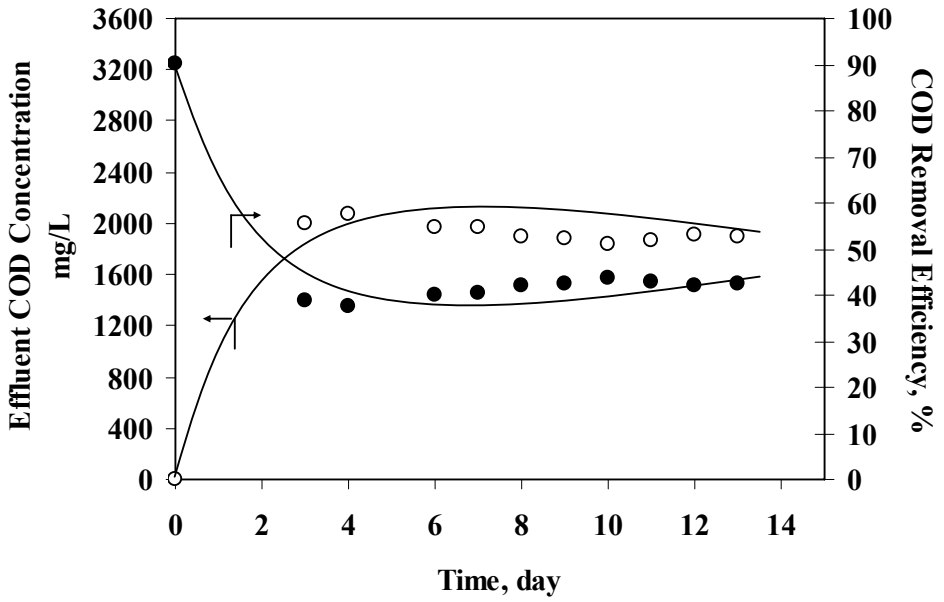

Figure 3.26 Variation of effluent COD concentration and removal efficiency with time at COD_0=3205±26 mg/L, $[NaCl]_0$ = 0% and θ_H=30 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency).

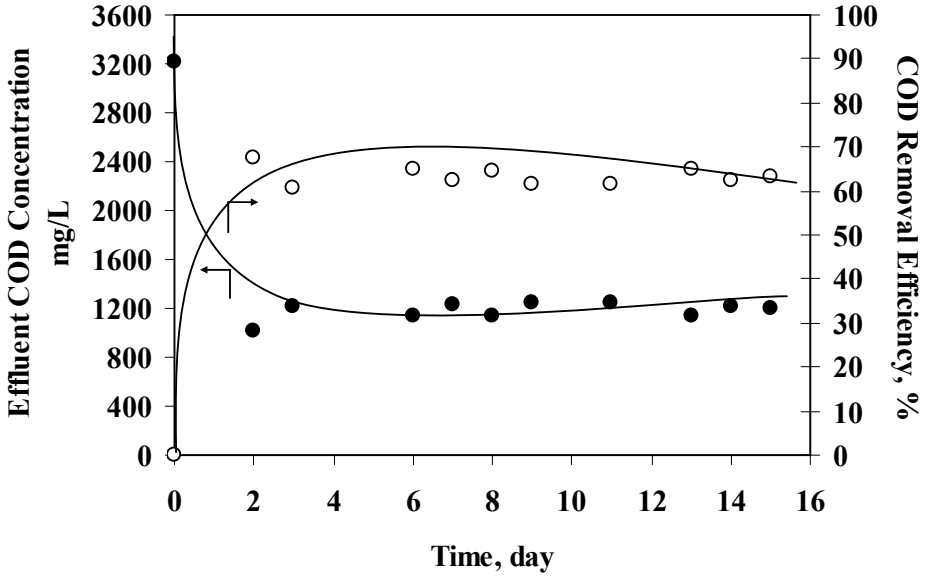

Figure 3.27 Variation of effluent COD concentration and removal efficiency with time at COD_0= 3215±15 mg/L, $[NaCl]_0$=1% and Qh=30 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency).

Figure 3.28 depicts variation of effluent COD concentration and COD removal efficiency with time at 2% salt concentration. For the first days of continuous operation, COD decreased from 3275±39 mg/L to 1125 mg/L with about 65% removal efficiency. After six days of operation COD gradually decreased to 995 mg/L. Depending on this decrease in concentration, COD removal efficiency reached to 70 % .

Figure 3.29 shows variation of effluent COD concentration and COD removal efficiency at 3% salt concentration, 30 hours hydraulic retention time and 3455±74 mg/L initial COD concentration. For the first 4 days COD gradually decreased from 3500 mg/L to 765 mg/L with about 78% removal efficiency. Better removal efficiency was observed between 5-12th days and percent removal increased to 84 % with effluent COD concentration of 550 mg/L.

Salt concentration was increased to 4% and the results were given in Figure 3.30. Initial COD_0 concentration was 3372±57 mg/L and effluent COD concentration

dropped to 982 mg/L (E=70%) for the first days. A slight decrease in the concentration to 846 mg/L (E=75%) was observed at the end of 8 days of continuous operation. However, effluent COD gradually increased from 846 mg/L to 1015 mg/L resulting in 69% removal efficiency at the end of the 12^{th} days.

Finally salt concentration was increased to 5%. As it is seen from Figure 3.31, this salt concentration significantly affects the system performance. COD concentration reduced from 3300±33 mg/L to 1890 mg/L at the end of 19 days continuous operation. As a result, COD removal efficiency was only around 43% at 5% salt concentration This result indicates that *H. lacusrosei* can not tolerate 5% salt concentration during carbon removal from the synthetic wastewater.

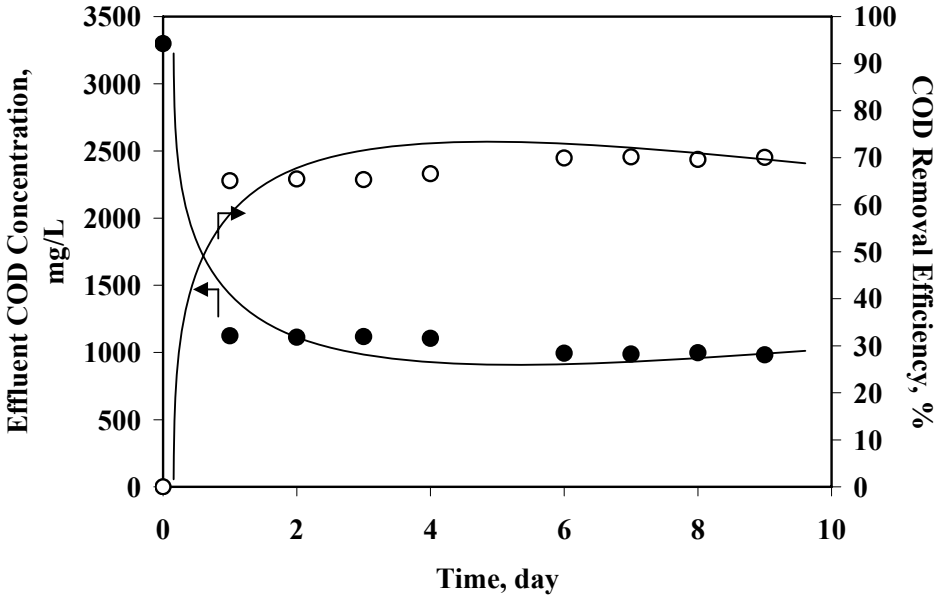

Figure 3.28 Variation of effluent COD concentration and removal efficiency with time at COD_0=3275±39 mg/L, $[NaCl]_0$=2% and Qh=30 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency).

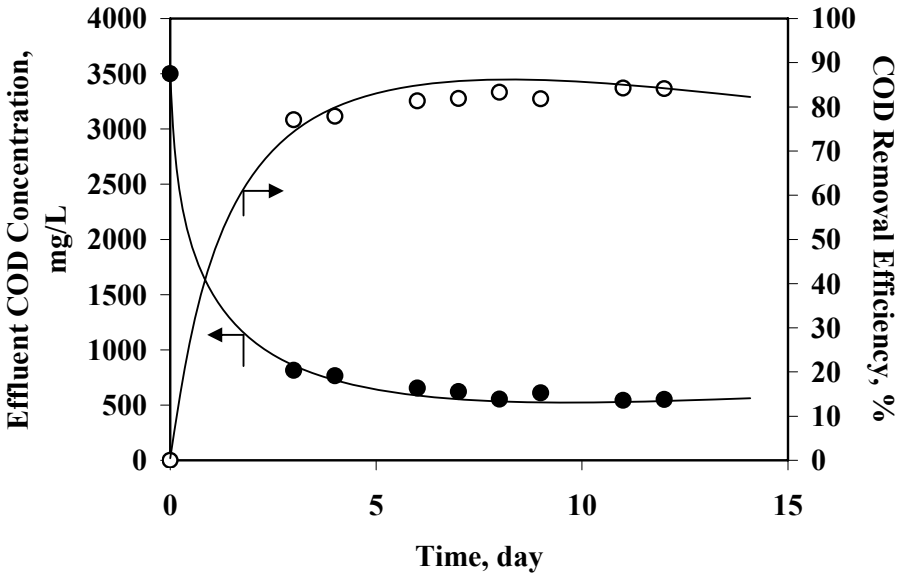

Figure 3.29 Variation of effluent COD concentration and removal efficiency with time at COD_0= 3455±74 mg/L, $[NaCl]_0$=3% and Qh=30 h retention time (—●—; effluent COD concentration, —○—; COD removal efficiency).

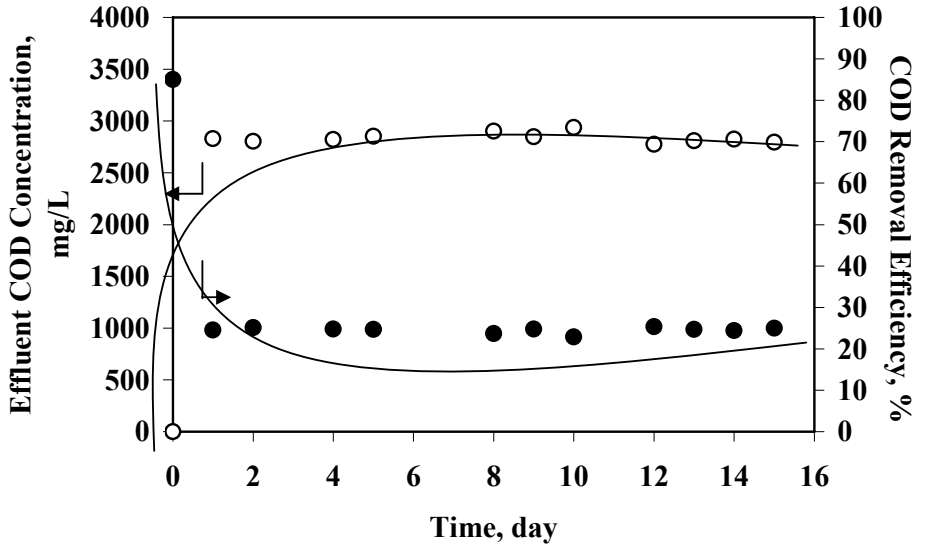

Figure 3.30 Variation of effluent COD concentration and removal efficiency with time at COD_0= 3372±57 mg/L, $[NaCl]_0$=4%, θ_H=30 h (—●—; effluent COD concentration, —○—; COD removal efficiency).

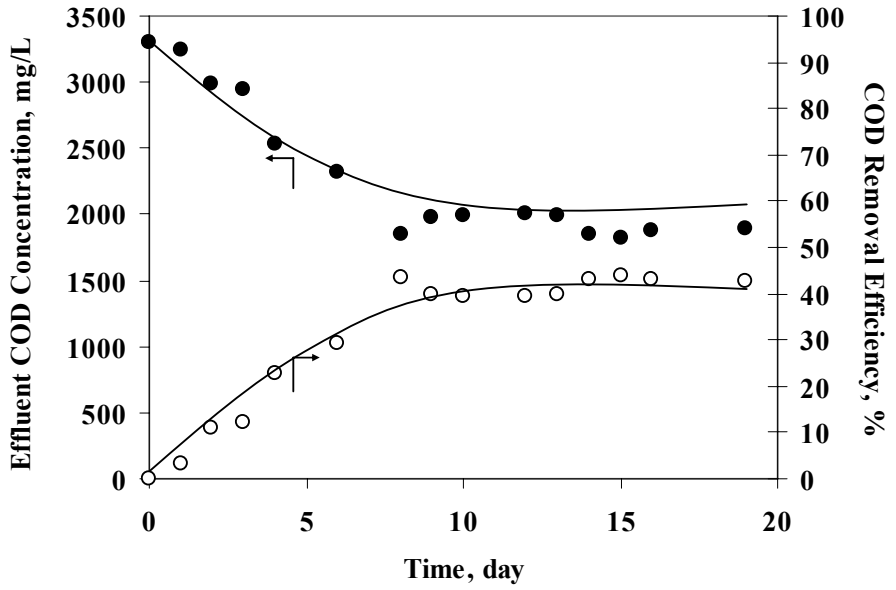

Figure 3.31 Variation of effluent COD concentration and removal efficiency with time at COD_0= 3300±33 mg/L, $[NaCl]_0$=5% θ_H=30 h (—●—; effluent COD concentration, —○—; COD removal efficiency).

The effect of salt concentration on the COD removal is summarized in Figure 3.32. Effluent COD concentration was decreased from around 3215 mg/L to 1546 mg/L for the salt-free (0%) wastewater and 53% COD removal was achived. Effluent COD concentration decreased to around 1200 mg/L for 1% salt concentration with 63% COD removal efficiency. Better COD removal was observed for 2% salt concentration with 70% COD removal efficiency. The maximum COD removal performance was observed at 3% with more than 80% efficiency. However, higher salt concentration, $[NaCl]_0$=5%, significantly affected system performance. Efficiency decreased to 43% with more than 1875 mg/L effluent COD concentration. These results indicate that, for the efficient COD removal at COD concentrations such as 3300 mg/L with *H. lacusrosei,* the maximum salt concentration should be less than 3%. High salt concentrations inhibit carbon removal.

Initial COD removal rates of continuous operation for different salt concentrations were computed as stated before and the results were given in Figure 3.33. The rate decreases with increasing salt concentration. The maximum and minimum COD removal rate was around 1.34 mg/L d for 0 % salt concentration and 2.40 mg/L d for 3 % salt concentration, respectively. Higher salinities (NaCl › 3%) caused decreasing in the rate to the level of 1.13 g/L d indicating salt inhibition effect on COD removal. In addition, low COD removal efficiency and rate at salt free environment can be explained as these organisms need 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.

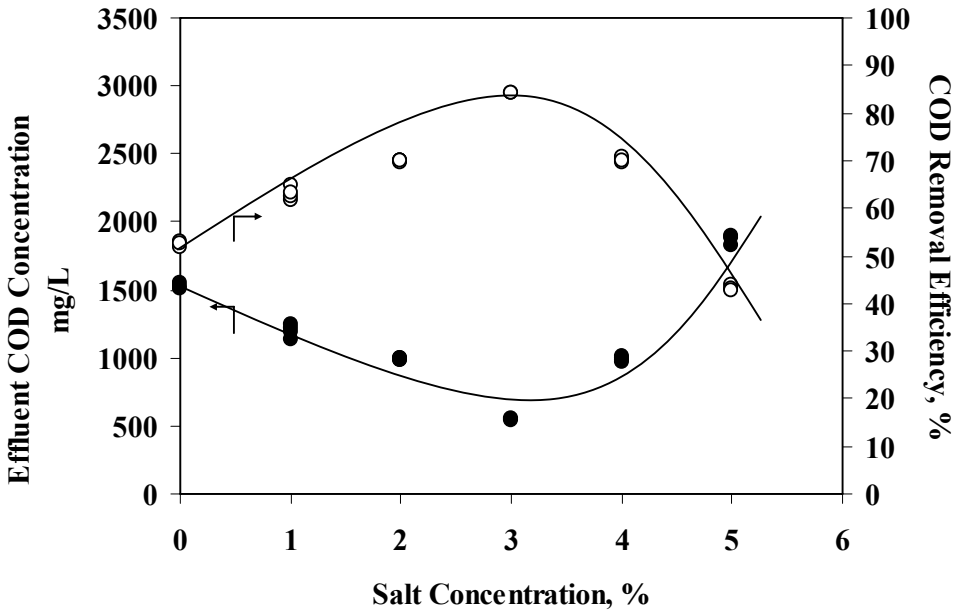

Figure 3.32 Effect of initial COD concentration on effluent COD concentration and COD removal efficiency. (—●—; effluent COD concentration, —○—; COD removal efficiency).

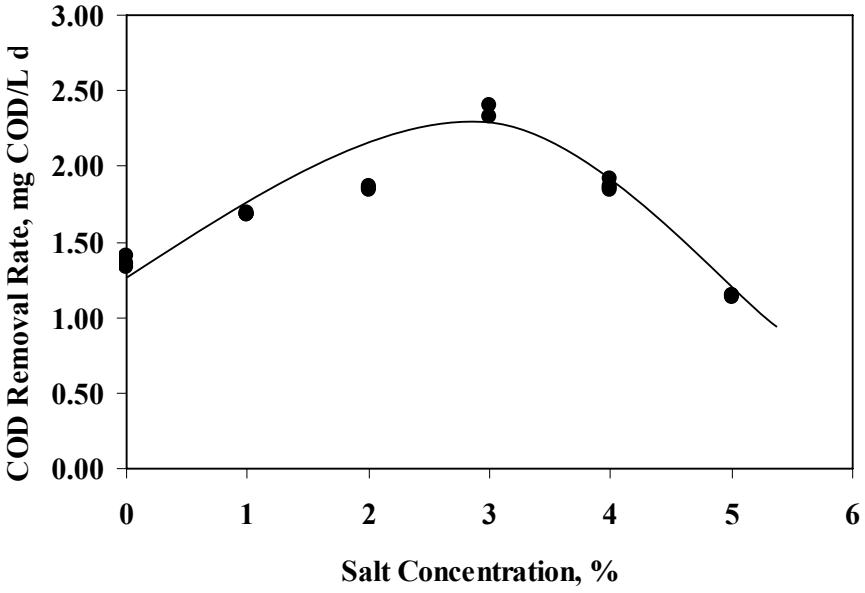

Figure 3.33 Variation of COD removal rates with initial salt concentration

3.3 Determination of Kinetic Coefficients

Monod type kinetic analysis based on substrate and cell mass is used to determine the biokinetic coefficients for the substance. Substrate or specific substrate utilization rates are the rate expressions used in the Monod kinetic. The later one gives more accurate coeffcient determination. Therefore, biomass concentration is continuously monitored in a biological system to be able to calculate specific substrate utilization rate. However, it is very diffucult to have a certain biomass concentration in an immobilized system. The biofilm thickness and coverage on the support particle may change throughout the system. Stover-Kincannon is one of the most widely used mathematical model to determine the kinetic constants in immobilized systems. The model have been applied to continuously operated mesophilic and thermophilic upflow anaerobic filters for treatment of paper-pulp liquors (Ahn JH., Forster CF. , 2002) and simulated starch wastewater (Ahn JH, Forster CF, 2000), anaerobic filter for soybean wastewater (Yu H, Wilson F, Tay J, 1998), wastewater treatment and anaerobic hybrid reactor

(Buyukkamaci N, Filibeli A. 2002) and also aerobic treatment of synthetic dairy wastewater in trickling filters (Raj SA, Murthy DVS ,1999), municipal wastewater treatment in submerged biofilters (Martinez SG, Lippert- Heredia E, Hernandez Esparza M, Doria-Serrano C. 2000) and determination of decolorization kinetic constants (Kapdan KI, 2005). Stover- Kincannon model was used for the kinetic analysis of COD removal for saline wastewater in an upflow anaerobic packed bed reactor in this study.

Stover-Kincannon model deals with the organic substance removal rate as a function of organic loading rate at steady state as in Eq. 10.

$$\frac{dS}{dt} = \frac{Q}{V}(S_0\text{-}Se) \qquad \text{eq. 10}$$

The original Stover Kincannon model for rotating biofilm reactor is described as in Eq. 11.

$$\frac{dS}{dt} = \frac{Q(S_0\text{-}S_e)}{V} = \frac{U_{max}(QS_0/A)}{K_B + (QS_0/A)} \qquad \text{eq. 11}$$

Where, A; represents the total disc surface area whereby total biomass concentration immobilized on discs. The suspended biomass concentration is assumed to be negligible compare to that of attached biomass. The simple modification of original Stover –Kincannon model is the introduction of total organic loading rate, QS_0/V into the eq.10 instead of QS_0/A , resulting in eq.12. Since void space has a significant importance in obtaining high removal efficiency in anaerobic filters. The Modified Stover Kincannon model has been applied to anaerobic filters and fixed bed region of hybrid reactors (Yu H, Wilson F, Tay J, 1998; Buyukkamaci N, Filibeli A, 2002)

$$\frac{dS}{dt} = \frac{Q(S_0 - S_e)}{V} = \frac{U_{max}(QS_0/V)}{K_B + (QS_0/V)} \qquad \text{eq. 12}$$

Linearization of Eq. 12 gives the relationship

$$\frac{dt}{dS} = \frac{V}{Q(S_0 - S_e)} = \frac{K_B}{U_{max}} * \frac{V}{QS_0} + \frac{1}{U_{max}} \qquad \text{eq. 13}$$

Where dS/dt is the substrate removal rate (g/Ld); S is substrate concentration in the reactor (g/L); U_{max} is the maximum substrate removal rate constant (g/Ld) and K_B is a saturation value constant (g/Ld). The plot of $V/[Q(S_0-S_e)]$, inverse of the loading removal rate, vs $V/(QS_0)$, 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.

The substrate balance for the reactor can be written as follows

$$QS_0 = QS_e + V(dS/dt) \qquad \text{eq. 14}$$

Substitution of eq. 12 to eq. 14 results in

$$QS_0 = QS_e + \frac{U_{max}(QS_0/V)}{K_B + (QS_0/V)} * V \qquad \text{eq. 15}$$

This expression can then be solved for either the effluent substrate concentration (eq.16) or the required volume of the anaerobic filter (eq. 17) by substituting kinetic constants U_{max} and K_B.

$$S_e = S_0 - \frac{U_{max}S_0}{K_B + (QS_0/V)} \qquad \text{eq. 16}$$

$$V = \frac{QS_0}{(U_{max}S_0/S_0\text{-}S_e)\text{-}K_B} \qquad \text{eq. 17}$$

Modified Stover-Kincannon model was applied to experimental results of continuously operated up flow anaerobic packed bed reactor for COD removal from saline wastewater 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 (Table A.23 and Table A.24). Figure 3.34 indicates the plot of COD loading rate, $V/(QS_0)$, versus removal rate, $V/[Q(S_0\text{-}S_e)]$. K_B and U_{max} values were calculated as 5.3 g/L day and 7.05 g/L day, respectively, from 1/U vs $1/L_0$ plot as given in Figure 3.35. Therefore the rate expression for COD removal takes the following form,

$$\frac{Q\,(S_0\text{-}S_e)}{V} = \frac{7.05\;(QS_0/V)}{5.3 + (QS_0/V)} \qquad \text{eq. 18}$$

And effluent COD concentration can be predicted by using the equation 18.

$$S_e = S_0 - \frac{7.05\,S_0}{5.3 + (QS_0/V)} \qquad \text{eq. 19}$$

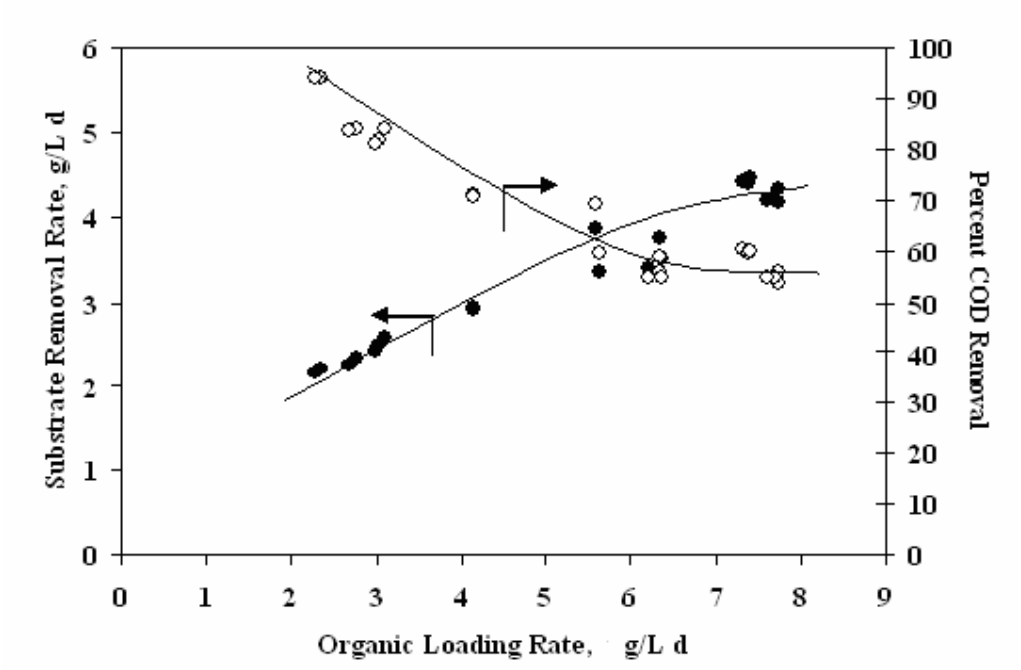

Figure 3.34 Substrate removal rates and percent COD removal at different organic loading rates. (—●—; COD removal rate, —○—; COD removal efficiency).

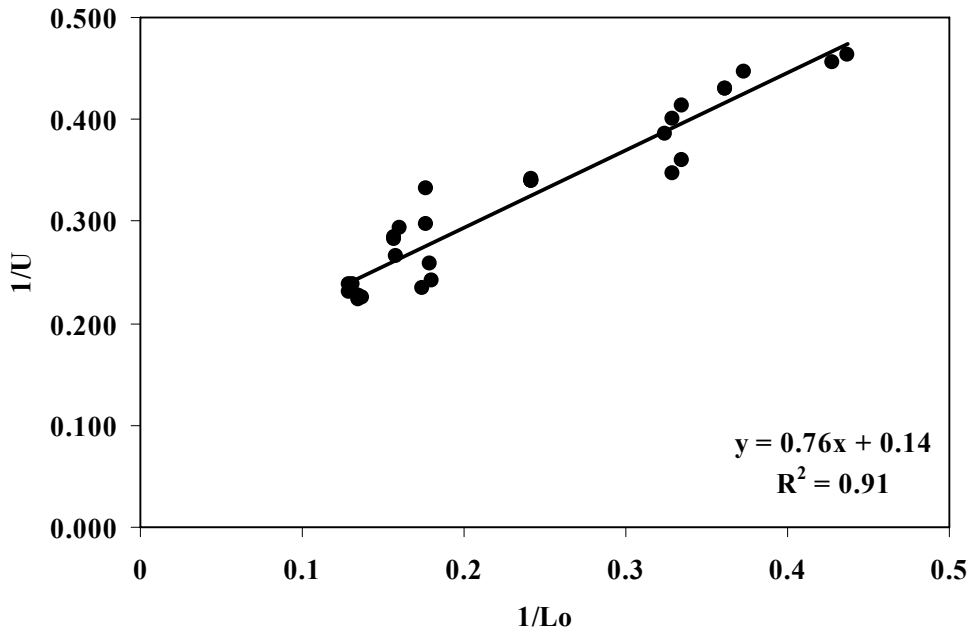

Figure 3.35 Stover-Kinconnon model plot for COD removal

CHAPTER FOUR
CONCLUSIONS

There are numerous studies on application of anaerobic treatment technologies to wastewater (Saravanane. Murthy, 2000). 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. For example, Omil**,** Méndez and Lema (1995) studied the anaerobic treatment of wastewaters from seafood-processing industry. Salinity was similar to sea water and after an initial start-up procedure, 70-90% organic matter removal was achieved. Guerrero, Omil, Mendez and Lema (1997), 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, Marti and Roeckel, (1997). Effect of hydraulic retention time on metanogenic activity at 18 ^{0}C and 37 ^{0}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, Sánchez, Cruz, Veiga and Borja (2004), studied treatment of saline wastewater by using a laboratory down-flow anaerobic fixed bed reactor (DFAFBR). Their results showed that at sea salts concentrations in the range from 5 to 15 g/L, total coliform concentration reduction efficiencies higher than 97% were achieved. A HRT of 24 h was required to obtain total COD, organic-N, total-P and faecal coliform concentration reduction efficiencies higher than 72%, 51%, 39% and 98%, respectively.

However, different anaerobic halophilic organisms have been isolated so far. For example, *Haloanaerobacter chitinovorans* which was isolated from a saltern in California (Liaw, HJ, Mah RA, 1992), *Haloanaerobium congolense* from an African oil field (Ravot G, Magot M, Ollivier B, Patel BKC, Ageron E, Grimont PAD, Thomas P, Garcia J-L, 1997), *Halanaerobium lacusrosei* from sediment of

hypersaline lake (Cayol J-L, Ollivier B, Patel BKC, Ageron E, Grimont PAD, Prensier J, Garcia J-L, 1995). *Haloanaerobium praevalens* (Zeikus JG, Hegge PW, Thompson TE, Phelps TJ, Langworthy TA, 1983) and *Haloanaerobium alcaliphilum* (Tsai C-R, Garcia J-L, Patel BKC, Cayol J-L, Baresi L, Mah RA., 1995), from Great Salt Lake sediment. These organisms were also identified in canned food (Kobayashi T, Kimura B, Fujii T., 2000). *Haloanaerobium praevalens* was reported as effective in carbon removal in Great Salt Lake (Oren A, Weisburg WG, Kessel M, Woese CR., 1984). 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, *Halanaerobium lacusrosei*, from saline synthetic wastewater in a continuously operated upflow 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.

The preliminary studies were the batch experiments to understand the responds of the organism to COD removal. *H. lacusrosei* was not effective in COD removal in salt free wastewater as excepted. The organism need minimum amount of salt to regulate its metabolism and survive. The minimum amount of salt requirement was determined as 2 % in the batch experiments. Higher concentrations caused decreasing in the COD removal. That might be because of the loss of the culture by plasmolysis. Since the *H. lacusrosei* isolation studies pointed out that these types of cultures can tolerate up to 20% salt concentrations, 2% salt tolerance looks unfortunate for this study. However, to exist, to survive or to be active in an environment are different concepts. The culture may survive in 20% salt concentration but may not be active to remove a substrate. Therefore, this study revealed that the salt tolerance of this organism to be able to remove a substrate efficiently in a batch operation is around 2%. In addition, the competitive inhibition

effect of salt was determined as a result of experimental studies. The competitive inhibition occurs when there is a substrate analog in the media. In other words, salt and organic substance competes with each other for the active site of the enzyme which is catalyzing the glucose, for this study. However, the salt can not be glucose analog. The possible explanation for this result is that, salt may deteriorate the three dimensional shape of the active site, then enzymatic conversion of glucose is inhibited. The nitrogen requirement of the culture to remove COD was quite low. This is the general characteristics of the anaerobic organisms. High nitrogen concentrations might be inhibitory on the organisms. That inhibitory effect was not observed in this study. Increasing nitrogen concentration did not provide any improvement or adverse effect on COD removal by *H. lacusrosei.* This can be evaluated as an advantageous especially for the wastewater which has high COD and nitrogen content. One of the most complicated results in the batch experiments was the lower COD removal efficiency and rate at higher biomass concentrations. The general idea in wastewater treatment is the higher the biomass, better the effluent water quality. This was not valid for our study. The possible explanations for this result that the culture was not active during the experiment, or insufficient mixing might have caused agglomeration of the biomass which caused limitation in the diffusion of substrate to the microorganisms pellets.

Continuously operated bioreactors represent the real wastewater treatment conditions more. As a result, more realistic information about the applicability of the treatment approach to an industrial effluent can be obtained. The results of this study indicated that utilization of *Halanaerobium lacusrosei* enhances organic substance removal from the saline wastewater. Almost complete COD removal can be obtained when the COD concentration is around 1900 mg/L at 3 % salt concentration and 19 hours of hydraulic retention time. Although, it seems that the COD removal decreases at high initial COD concentrations, 70% removal efficiency at 3300 mg COD /L can be evaluated efficient treatment especially at high salt concentration of 3 % and relatively short HRT of 19 for an anaerobic process. In addition, percent COD removal can be increased to 84% by extending the retention time to 30 h under the same media composition. The effect of salt concentration in the continuous

operation was almost the same as in the batch experiments. The substrate removal rate and effluent wastewater quality decreases when the salt concentration is over 3%. This effect of high salt concentration can be evaluated as salt inhibition on COD removal. Moreover, this result confirms that the culture can only tolerate up to 3 % salt concentrations, plasmolysis effect is possible at higher salt concentrations.

The experimental results showed that it is possible to treat high saline wastewater by using anaerobic treatment methods. Inclusion of halophilic anaerobic bacteria into the reactor resulted in high COD removal efficiencies especially at high salt concentration. However, more detailed studies should be carried out to determine the full scale application of this system to industrial effluents.

CHAPTER FIVE
RECOMMENDATIONS

Treatment of saline wastewater under anaerobic condition with salt tolerant organisms is rather a new application compare to treatment of saline wastewater under aerobic conditions. Becouse of the new research area , the studies carried out in the content of this thesis can be considered as the first step on this subject. The following items can be recommended as further studies that should be conducted to highlight the possible full scale application of anaerobic saline wastewater treatment to industrial effluents.

1. Methane production have to be investigated for saline wastewaters,
2. Different anaerobic halophilic microorganisms apart from *H. lacusrosei* can be used and the organism which is capable of removing higher COD and producing methane can be determined,
3. Different support material types and media compositions in upflow anaerobic filter can be used,
4. The performance of different reactor configurations to remove saline wastewaters such as UASB, hybrid etc can be investigated,
5. The system can be operated with real industrial wastewater.

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APPENDICES

RAW DATA OF EXPERIMENTAL STUDIES

Table A.1 Raw data of the batch experiments at different initial nitrogen concentrations and at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$=3%

CODo (mg/L)	CODe (mg/L)	E_{COD} (%)	No (mg/L)	Ne (mg/L)	E_N (%)	R_S mg/Ld	R_N mg/Ld
1467	365	75	250	120	52	227	0.026
1419	370	74	325	100	69	223	0.042
1399	407	71	620	200	68	211	0.077
1415	406	71	1320	810	39	218	0.098
1420	411	71	1945	1465	25	213	0.095
1416	400	72	2525	1900	25	212	0.12
1454	410	72	3100	2400	23	218	0.139

Table A.2 Raw data of the batch experiments at different biomass concentrations at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$=3%

Xo (g/L)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)	No (mg/L)	Ne (mg/L)	E_N (%)
0.4	1467	365	75	250	120	52
0.5	1564	388	75	243	110	55
0.6	1568	345	78	250	100	60
0.8	1664	876	47	240	160	33
1.0	1675	775	54	260	170	35

Table A.3 Raw data of substrate removal rates at different biomass concentrations at $COD_0 \cong 1400$ mg/L, $[NaCl]_0$=3%

Xo(g/L)	R_S (mg/Ld)	R_X (mg/gXd)	R_N (mg/Ld)	Rx_N (mg/gXd)
0.4	227	568	26	65
0.5	260	520	27	54
0.6	265	442	29	48
0.8	190	238	16	20
1	222	222	19	19

Table A.4 Raw data of batch experiment at different initial COD and salt concentrations

Salinity (%)	CODo(mg/L)	CODe(mg/L)	E_{COD}(%)
0 %	1215	732	40
	1288	845	34
	2524	1205	52
	2675	1275	52
	3453	1324	62
	3373	1545	54
	4176	1567	62
	4253	1545	64
1 %	1369	744	46
	1347	698	48
	2444	1196	51
	2323	1256	46
	4172	1663	60
	3983	1545	61
	4335	1558	64
	4315	1512	65
2 %	603	198	67
	615	258	58
	965	345	64
	1447	446	69
	1819	876	52
	2890	1123	61
	3850	1550	60
3 %	505	305	40
	791	479	39
	1205	905	25
	1173	892	24
	1810	1314	27
	3053	1997	35
	3019	1893	37
	4802	2910	39

Table A.5 Raw data of salt inhibition rates at different initial COD concentrations and salt concentrations

Salinity(%)	R_i(mg/Ld)	Rxi(mgCOD/gXd)	So(mg/L)	1/Rxi	1/So
0 %	196.5	393	715	0.0025	0.0014
	184	368	813	0.0027	0.0012
	504	1008	2524	0.0010	0.0004
	478.5	957	2675	0.0010	0.0004
	504	1008	3453	0.0010	0.0003
	849	1698	3373	0.0006	0.0003
	1245	2490	4176	0.0004	0.0002
	1271	2542	4253	0.0004	0.0002
1 %	378	756	1271	0.0013	0.0008
	464	928	2444	0.0011	0.0004
	500	1000	2323	0.0010	0.0004
	1024.5	2049	4172	0.0005	0.0002
	1017.5	2035	3985	0.0005	0.0003
	1235	2470	4335	0.0004	0.0002
	1232.5	2465	4315	0.0004	0.0002
2 %	144	291	603	0.0025	0.0014
	521.5	1053.5	3010	0.0004	0.0002
	1097	2216	3850	0.0013	0.0008
	681.5	1377	3675	0.0011	0.0004
3 %	77.5	155	505	0.0065	0.0020
	95	190	791	0.0053	0.0013
	129.5	259	1205	0.0039	0.0008
	362	724	1937	0.0014	0.0005
	502	1004	3019	0.0010	0.0003
	651	1302	4802	0.0008	0.0002

Table A.6 Raw data of continuous operation of packed bed reactor at COD_0=1818±100 mg/L, 3% salt concentration and θ_H=19 h

Time (day)	CODo (mg/lt)	CODe (mg/lt)	E_{COD} (%)
0	1679	1679	0
2	1679	620	63
3	1808	782	57
5	1857	684	63
6	1857	369	80
7	1857	210	89
8	1950	358	82
9	1950	121	94
10	1879	105	94

Table A.7 Raw data of continuous operation of packed bed reactor at COD_0=2393±1714 mg/L, 3% salt concentration and θ_H=19 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	2605	2605	0
1	2605	1904	27
2	2128	1812	15
3	2415	1555	36
5	2318	1490	36
6	2125	917	57
7	2304	1128	51
8	2574	1293	50
10	2574	950	63
12	2348	1141	51
16	2417	1109	54
17	2308	918	60

Table A.8 Raw data of continuous operation of packed bed reactor at COD_0=3263±65 mg/ L, 3% salt concentration and θ_H=19 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3150	3150	0
1	3150	1950	38
2	3215	1895	41
5	3260	1815	44
6	3260	1750	46
7	3355	1640	51
8	3355	1550	55
9	3290	1615	51
12	3290	1433	56
13	3304	1436	57

Table A.9 Raw data of continuous operation of packed bed reactor at COD_0=4278±11 mg/L, 3% salt concentration and θ_H=19 h

Time (day)	CODo (mg/L)	CODe (mgL)	E_{COD} (%)
0	4200	4200	0
2	4167	3068	26
3	4167	2986	28
4	4167	2904	30
6	4293	1890	56
7	4293	1809	58
8	4293	1782	59
9	4293	1747	59
10	4293	1724	60
11	4268	1899	56
13	4268	2091	51
14	4277	1725	60
15	4277	1996	53
16	4277	2010	53

TableA.10 Raw data of continuous operation of packed bed reactor COD_0=5269±45 mg/L, 3% salt concentration and θ_H=19 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	5250	5250	0
1	5257	2223	56
2	5257	2245	57
4	5315	2500	53
5	5315	2350	56
6	5315	2220	58
7	5287	2157	59
8	5287	1899	64
12	5190	2287	56
14	5214	2346	55
16	5214	2118	59
17	5301	2364	55

Table A.11 Raw data of continous operation of packed bed reactor COD_0=6208±69 mg/L, 3% salt concentration and θ_H=19 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	6200	6200	0
1	6115	5312	13
2	6115	5023	18
3	6128	4880	20
4	6128	4650	24
5	6213	4613	26
6	6213	3882	38
7	6213	3266	47
8	6225	3118	50
9	6225	3118	50
12	6315	2890	54
13	6315	2770	56
14	6250	2815	55
15	6250	2785	55

Table A.12 Raw data of continous operation of packed bed reactor at COD_0=3343±35 mg/L, 3% salt concentration and θ_H=11 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3345	3345	0
3	3346	1216	64
4	3425	1343	61
5	3321	1432	57
6	3320	1235	63
7	3324	1287	61
10	3324	1312	61
11	3345	1345	60
13	3345	1328	60

Table A.13 Raw data of continous operation of packed bed reactor COD_0=3444±79 mg/L, 3% salt concentration and θ_H=14 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3450	3450	0
3	3553	717	80
4	3455	685	80
6	3510	785	78
7	3432	717	79
10	3325	830	75
11	3357	1031	69
14	3455	890	74

Table A.14 Raw data of continous operation of packed bed reactor COD_0=3391±82 mg/L, 3% salt concentration and θ_H=20 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3400	3400	0
1	3215	1098	66
2	3215	1121	65
3	3345	1004	70
4	3345	996	70
5	3345	998	70
6	3445	1010	71
8	3445	1002	71
9	3445	1103	68
10	3445	1115	68
11	3445	1079	69
12	3445	990	71
13	3445	995	71
14	3445	989	71
15	3445	1010	71

Table A.15 Raw data of continous operation of packed bed reactor at COD_0=3180±44 mg/L, 3% salt concentration and θ_H=25 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3190	3190	0
6	3215	945	71
7	3215	514	84
8	3161	565	82
10	3110	587	81

Table A.16 Raw data of continuous operation of packed bed reactor at COD_0=3445±83 mg/L, 3% salt concentration and θ_H=30 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3550	3550	0
3	3553	815	77
4	3455	765	78
6	3510	655	81
7	3432	623	82
8	3325	555	83
11	3455	543	84
12	3455	550	84
13	3350	545	84

Table A.17 Raw data of continuous operation of packed bed reactor at COD_0=3205±26 mg/L, 0% salt concentration and θ_H=30 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3250	3250	0
3	3167	1403	56
4	3188	1356	55
6	3188	1443	55
7	3188	1448	56
8	3208	1520	53
9	3208	1534	52
10	3208	1567	51
11	3215	1546	52
12	3215	1512	53
13	3215	1521	53

Table A.18 Raw data of continuous operation of packed bed reactor at COD_0=3215±15 mg/L, 1% salt concentration and θ_H=30 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3220	3220	0
2	3100	1010	67
3	3100	1213	61
6	3250	1143	65
7	3250	1225	62
8	3220	1141	65
9	3220	1242	61
11	3243	1245	62
13	3243	1136	65
14	3243	1220	62

Table A.19 Raw data of continuous operation of packed bed reactor at COD_0=3275± 39 mg/L, 2% salt concentration and θ_H=30 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3300	3300	0
1	3224	1125	65
2	3224	1114	65
3	3224	1118	65
4	3310	1106	67
6	3310	995	70
7	3310	988	70
8	3286	998	70
9	3286	983	70

Table A.20 Raw data of continuous operation of packed bed reactor at COD_0=3455±74 mg/L, 3% salt concentration and θ_H=30 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3500	3500	0
3	3553	815	77
4	3455	765	78
6	3510	655	81
7	3432	623	82
8	3325	555	83
9	3357	610	82
11	3455	543	84
12	3455	550	84

Table A.21 Raw data of continuous operation of packed bed reactor at COD_0=3372±57 mg/L, 4% salt concentration and θ_H=30 h

Time (day)	CODo (mg/l)	CODe (mg/L)	E_{COD} (%)
0	3400	3400	0
1	3356	982	71
2	3356	1004	70
4	3356	990	71
5	3445	987	71
8	3445	846	75
9	3445	992	71
10	3445	915	73
12	3315	1015	69

Table A.22 Raw data of continuous operation of packed bed reactor at COD_0=3300±33 mg/L, 5% salt concentration and θ_H=30 h

Time (day)	CODo (mg/L)	CODe (mg/L)	E_{COD} (%)
0	3300	3300	0
1	3350	3245	3
2	3350	2987	11
3	3350	2945	12
4	3276	2534	23
6	3276	2321	29
8	3276	1850	44
9	3290	1984	40
10	3290	1996	39
12	3310	2010	39
13	3310	1990	40
14	3245	1845	43
15	3245	1820	44
16	3287	1875	43
19	3287	1890	43

Table A.23 Effect of initial COD concentration on COD removal in continuously operated anaerobic packed bed reactor

θ_H (h)	CODo (mg/L)	CODe (mg/L)	Lo (g/Ld)	E_{COD} (%)	Rate (g/L d)
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	1910±100	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 A.24 Effect of hydraulic retention time on COD removal in continuously operated anaerobic packed bed reactor

θ_H (h)	CODo (mg/L)	CODe (mg/L)	Lo (g/Ld)	E_{COD} (%)	Rate (g/L d)
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)