

STUDY ON DIFFERENT FACTORS AFFECTING COD REMOVAL CAPACITY OF *HALANAEROBIUM LACRUISEI* IN SALINE WASTEWATER

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Abstract. This study evaluated a number of factors (including salinity, pH and temperature) affecting the growth of *Halanaerobium lacruisei* bacteria in order to reduce organic compounds (COD concentration) in saline wastewater of a fish sauce processing plant. The results of the study showed that halophillic bacteria had good capacity of reducing COD concentration under specific conditions. Laboratory experiments with different bacterial culture conditions showed that 6 - 7 % salinity, pH 7.0 and 30 °C is the optimal culturing condition for *Halanaerobium lacruisei* treating COD in wastewater. In the laboratory condition, COD treatment capacity in saline wastewater of this microorganism could be up to nearly 88 % after 48 hours, ensuring that the effluent meets the environmental requirements. In pilot condition of culturing the bacteria (in plastic tank, stimulating the real condition), after 25 days, the COD removal decreased continuously, 80 % effectively compared to the inlet wastewater. The effluent after an effective treatment by this experimental method satisfied the requirements of type B wastewater standard specified in QCVN 40:2011/BTNMT. This study revealed that *Halanaerobium lacruisei* can be used effectively to treat the COD parameter in saline wastewater which can be applied in industrial wastewater treatment area as well.

Keywords: *Halanaerobium lacruisei*, saline wastewater treatment, COD removal.

Classification numbers: 3.3.3, 3.4.2, 3.4.4.

1. INTRODUCTION

The wastewater of aquatic product processing is characterized by a high level of pollution in both organic compounds and the concentration of nitrogen and phosphorus. Pollution and waste flows change in a wide range due to the fact that in a processing plant, there may be simultaneous or successive processing of various products such as fish, shrimp, crab, squid,

crustacean, with many processing capacity differs depending on material sources, and due to different technology and management levels. Obviously, saline wastewater is difficultly treated, especially in terms of organic compounds or nitrogen in order to satisfy the requirement of Vietnam National Standard [1].

Environmental culturing medium with NaCl concentration approaching saturation are often populated by dense microbial communities [2]. Although mostly isolated from the salted food, their natural habitats are hypersaline waters containing intermediate levels of salt concentration and hypersaline soils [3]. According to Kapdan *et al.* [4] and other authors [2], several groups of microorganisms and their requirement of particular salt concentration for the best growth are considered: (1) nonhalophiles (less than 0.2 M NaCl); (2) halotolerant (nonhalophiles tolerating high-salt concentrations); (3) slight halophiles (0.2 - 0.5 M NaCl); (4) moderate halophiles (0.5 - 2.5 M NaCl); (5) extreme halophiles (2.5 - 5.5 M NaCl) [2, 3, 4]. Many microorganisms are nonhalophiles or nonhalotolerant that can not inhabit in high-salt environments. Halophilic microorganisms can be isolated from different saline environments, and different species even belonging to the same genus are shown with various application [4].

Saline wastewater was defined as those with higher salt concentrations than seawater [3]. Various industrial activities such as tannery, agricultural production, chemical manufacturing, and petroleum production generate saline wastewaters [4, 5, 6]. Saline effluents are conventionally treated through physico-chemical methods because the biological treatment is strongly inhibited by salts, mainly NaCl [7, 8, 9]. However, since the costs of physico-chemical treatments are particularly high and the efficiency of biological treatments is relatively low, microorganisms for the treatment of saline wastewater are expected for better results.

Many studies on the biological saline wastewater treatment are based on the use of aerobic and anaerobic halophilic microorganisms, degradative capabilities, and degradation pathways. Anaerobic organisms such as *Halophillic sp.* [5] isolated from saline sediment and seawater such as *Halanaerobium lacusrosei*, *Haloanaerobacter chitinovorans*, *Haloanaerobium congolense*, *Haloanaerobium praevalens*, and *Haloanaerobium alcaliphilum* have been reported effective in removing the organic pollutants [4, 5, 10]. In Viet Nam, scientists inside and outside the military have done a number of researches on this topic and isolated and selected strains of microorganisms capable of decomposing organic matter in saline conditions, testing proteinase activity, and study the effect of external conditions such as temperature, initial pH, salt concentration, substrate concentration, etc. on the activity of these bacteria [10, 11, 12].

Nowadays, wastewater treatment with microorganisms is considered the most optimal technology for treating the saline wastewater [13, 14]. This is considered to be biological treatment technology and it is always environmentally friendly. Wastewater treatment with microorganisms is highly effective by providing and supplementing the microorganism strains capable of handling organic substances, participating in the domestic conversion processes. However, studies on aerobic granulation and sludge application in wastewater treatment in our country are still limited. Prior to that fact, we conducted research on the topic of COD treatment in saline wastewater at food processing plants. The objective of this study was to determine the best conditions for microbial culture, conduct experiments on COD treatment under laboratory conditions and treat real wastewater in a hypothetical environment.

2. MATERIALS AND METHODS

2.1. *Halanaerobium lacruisei* bacteria

Currently, there has been many strains of anaerobic bacteria that have been isolated such as *Haloanaerobacter chitinovorans*, *Haloanaerobium congolense*, *Haloanaerobium lacusrosei*, *Haloanaerobium praevalens*, etc. These bacteria strains were investigated with high ability to remove organic matter. Anaerobic halophilic *H. lacusrosei* microbial culture was obtained from the laboratory of Biotechnology, Institute of Science and Technology, Ministry of Defense as pure culture. The culture was cultivated under aseptic conditions in the laboratory of School of Environmental Science and Technology- Hanoi University of Science and Technology (HUST). The bacteria was seen with micro-rod-shaped, non-spore-forming, gram-negative, living in arbitrary respiration and capable of growing at 25 - 35 °C, pH = 4.0 - 8.0. The study also tested on a laboratory scale for wastewater processing fish sauce in Vinh Tuy Ward, Hoang Mai, Ha Noi. The saline wastewater has an average COD of 1420 mg/L and salinity of 110 g/L (11 %).

Table 1. Analysis of the wastewater from fish sauce production plant, Ha Noi (5/2020) (Analysed at INEST-HUST).

	Parameter	Unit	Value	QCVN 24:2009, column B
1	pH	-	5.2	5.5-9
2	COD	mg/L	1420	50
3	BOD	mg/L	1120	100
4	SS	mg/L	102	100
5	N _{total}	mg/L	491	30
6	P _{total}	mg/L	2,35	6
7	Oil and grease	mg/L	205	20
8	Turbidity	NTU	67	-
9	Color	Pt-Co	231	70
10	Salinity	%	11	-



Figure 1. Image of *Haloanaerobium lacruisei* culturing petri dish.

2.2. Microorganism culture media preparation and biomass harvesting

2.2.1. Culture media preparation

The microorganism culture medium was proceeded: Weigh the amount of chemicals as the medium to grow microorganisms, then proceed optimally with the addition of beef extract

powder bacto™. The amount of peptone used in the original experiment was 1.25 g, the amount of salt used was 50.0 g/L, equivalent to 5 %. The experiment was carried out as: the titration with distilled water, plug the cotton plugs into the flasks and wrap the foil, pasteurize the environment, place the flasks in the rack of the pasteurizer and then pasteurize at 121 °C for 15 minutes.

2.2.2. Culturing the bacteria and obtaining the biomass

Culturing media after pasteurization was put into a sterile incubator, turned on the UV light in the incubator for 10 minutes and then turned off. The microorganism was taken and transplanted into the medium near the alcohol lamp to ensure they were not contaminated. The flasks were shaken after inoculation with microorganisms for 50 minutes, at a rate of 100 RPM. The microorganisms were kept in the incubator ensured the temperature when rearing 28 - 32 °C, then ABS optical absorption was measured to determine the growth of microorganisms every 2 hours at 600 nm to determine the growth calibration curve. Total cell count could be figured out through the standard method TCVN 11039-1:2015- Food additive - Microbiological analyses - Part 1: Determination of total aerobic count by plate count technique.

2.3. Experimental methods

Wastewater composition: Synthetic wastewater used in experimental studies was diluted with various salt content (NaCl) and the COD concentration in wastewater were from 800 to 1200 mg/L.

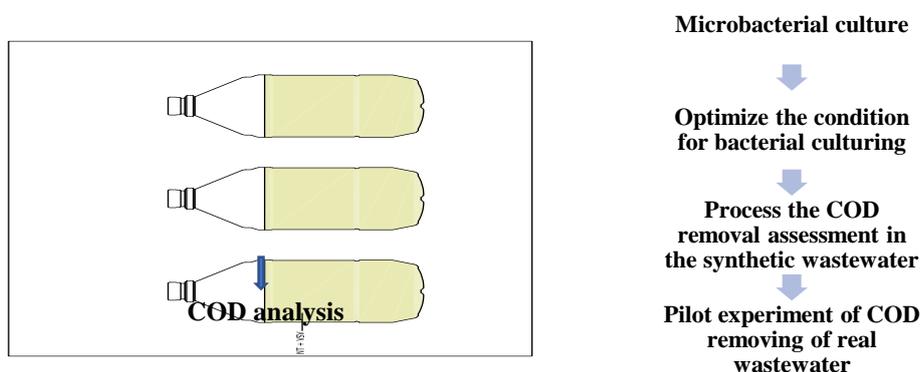


Figure 2. Experimental set-up and procedure of optimizing the COD removal condition in saline wastewater.

2.3.1. Experimental set-up

The anaerobic reactor was inoculated with *H. lacusrosei* at the beginning of the experiment and operated with effluent recycle at 20 g/L salt and 700 mg/L COD concentration for 30 days to allow immobilization with the microbial medium. The liquid phase of the system was refreshed with new synthetic media every week to provide nutrients. The system was loaded with fresh medium at the beginning of each batch experimental condition and it was operated for at least 15 days until the system reached equilibrium conditions, which was defined as obtaining almost the same effluent COD concentration (standard deviation less than 5 %).

2.3.2. Study the effect of environmental factors on bacteria's ability to remove COD

Several environmental factors that influence COD's removal ability were investigated in this study: NaCl concentration, pH and temperature. In this experiment, 2 concentrations of COD were investigated to find the COD removal capacity, 600 and 800 mg/L respectively. COD concentration was measured by the oxidizing agent method of potassium dichromate ($K_2Cr_2O_7$) because it is relatively cheap, easy to purify and has the ability to almost completely oxidize all organic substances. pH effect was performed by taking a specific concentration and varied the pH values from 1 - 8 using dilute NaOH/HCl solutions. The samples were agitated for specific time, filtered and then analyzed. Other parameters were measured using standard laboratory methods [15].

2.3.3. Study the effect of environmental factors on bacteria's ability to treat COD in real wastewater (pilot experiment)

COD value was fixed at 700 mg/L and fix the concentration of NaCl salt, pH and culturing temperature during treatment period. COD removal from real wastewater was investigated in order to determine the capacity. The experiment was carried out in 2.0 L glass bottles containing 1.5 L of mixed waste water and 150 mL of solution containing biomass of cultured microorganisms.

2.3.4. Statistical data processing

The classical statistical analyses were processed using IBM SPSS software version 20. The probability level $P < 0.05$ was considered to be significant.

3. RESULTS AND DISCUSSIONS

3.1. Culturing the *Halanaerobium lacruisei* bacteria and establishing the standard curve of bacteria density

Figure 3 showed the calibration curve for determining the density of microorganisms determined by the counting method on the counting chamber and the correlation between the corresponding microorganism density absorbed by UV light at a wavelength of 600 nm. The graph shows a very high correlation between the two factors above, showing that the method of determining microbial density by absorbing UV wavelength is relatively accurate, giving high reliability ($R^2 > 0.9$).

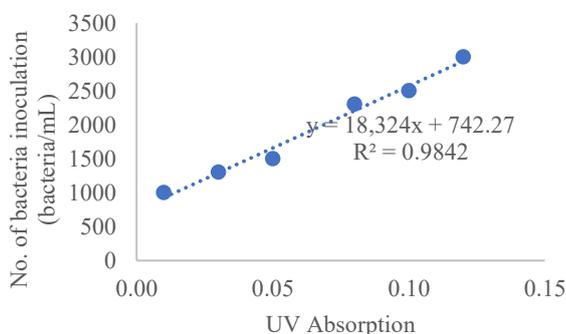


Figure 3. Calibration curve for determining the number of bacteria inoculation based on the optical density.

3.2. COD removal efficiency

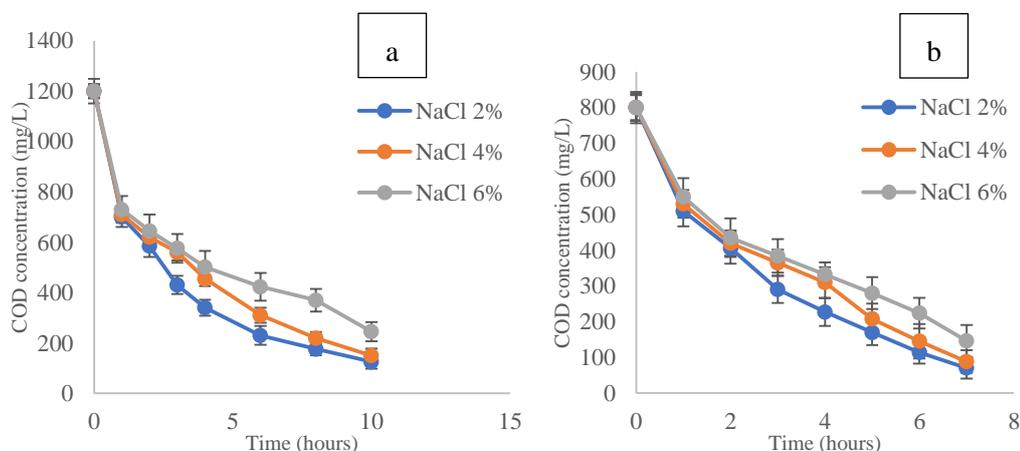


Figure 4. Experimental results of COD treatment with different concentrations of NaCl (a. COD_{initial} = 600 mg/L; b. COD_{initial} = 800 mg/L).

The experiment was performed with NaCl content ranging from 2.0 % to 6.0 %, equivalent to 20 - 60 g/L NaCl with the results shown in Figure 4. The results showed that the effect of pH on COD treatment ability of microorganisms is quite obvious, in which the higher the concentration of NaCl, the less ability to remove COD. At low salt concentration of 2.0 %, the treatment efficiency was up to 94.17 % after 7 days, when the NaCl salt concentration was 4.0 %, the efficiency drops to 88.33 % and if the salt concentration increased up to 6.0 %, COD removal capacity effectively reduced to 82.5 %. On the first day, COD decreased rapidly and gradually over the following days, indicating that in the first day, *Halanaerobium lacruisei* was able to adapt to the habitat (high adaptability). According to the experiment's result, NaCl concentration for the next experiments was chosen as an average of 4.0 %.

Experimental results showed that the initial concentration of COD at 600 mg/L gave treatment results higher than the concentration of 800 mg/L. Figure 5 and 6 together revealed that too high COD concentration affected the removal ability of microorganism. Moreover, NaCl concentration also played an important role in this study. The appropriate salt concentration for microorganisms to grow was at 4.0 % NaCl, the ability to treat COD could be better than NaCl 2.0 % and 6.0 %. These research results figured out the similarities with the previous results in Kapdan study [4].

The experiment investigated the effect of the initial pH of the wastewater environment to be varied from 4.0 to 10.0 at 30 °C. Results showed that environmental pH also greatly affects the ability of growth and removal of COD in wastewater. Based on the graph in Figure 6, we could see that microorganisms could be grown in the wide pH range of the wastewater environment. However, the optimal pH value for COD treatment of this microorganism was neutral pH (~ 7.0). When pH = 4.0, 10.0, after the first day COD treatment decreased from 800 mg/L to about 700 mg/L and the following hours decreased more slowly, while pH = 7.0 after the first day COD halved from 1200 mg/L to 602 mg/L and decrease gradually the following days.

The effect of pH and temperature on the COD removal capacity was mentioned in this study agreed well with many other authors [4, 6, 10]. The previous authors showed that increasing in the sewage temperature from 15 - 35 °C could increase the COD removal [15]. In

addition, different levels of input COD had a great influence on the ability of bacteria, possibly because the input C source is too high to inhibit the activity of this bacterium.

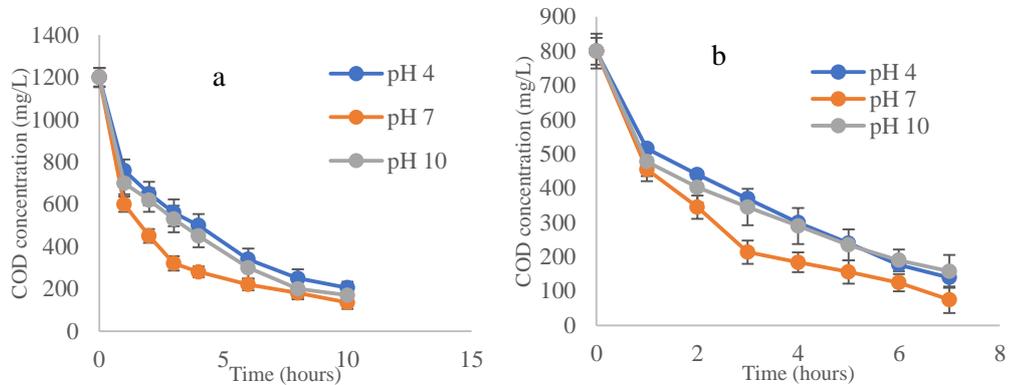


Figure 5. Experimental results of COD treatment with different pH.

(a. COD_{initial} = 600 mg/L; b. COD_{initial} = 800 mg/L).

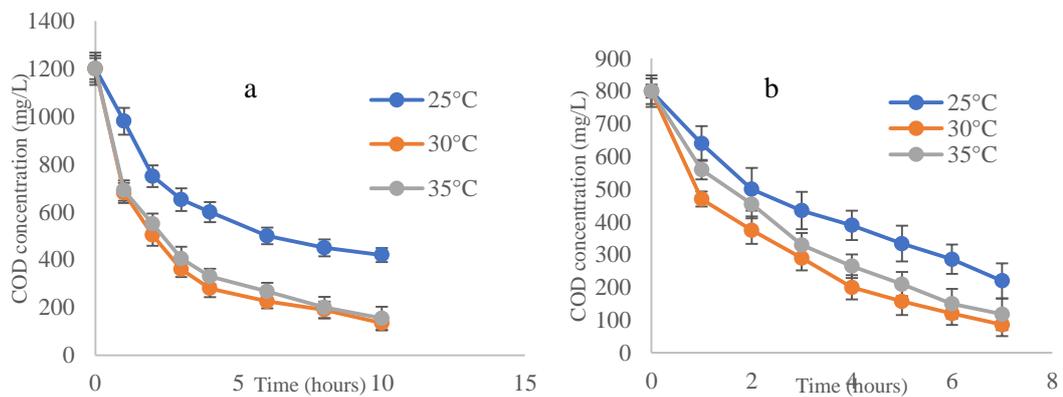


Figure 6. Experimental results of COD treatment with different temperatures

(a. COD_{initial} = 600 mg/L; b. COD_{initial} = 800 mg/L).

3.3. Pilot experiment

The monitoring of the activated sludge process in the period more than 20 days led to conclusions that COD reduction from 743 to 176 mg/L. COD concentrations in the first 2 - 3 days were fluctuating because microorganisms needed time to adapt to the untreated wastewater environment. The high efficiency of the system was established by the process water quality parameters obtained for the treated water, which were also validated by the industry provided the wastewater sample [3, 4, 5]. As could be seen in the Figure 8, the pre-treatment wastewater was yellowish-orange in color, however the wastewater after treatment became transparent and the decreased turbidity. It revealed that *Halanaerobium lacruisei* could decompose organic matter in the wastewater (carbohydrate, cellulose, protein, etc.).

For real wastewater samples, the pollutant removal efficiency (80 %) was lower than that of synthetic wastewater samples (88 %) with similar content. This could be explained by the fact that in the real wastewater sample, the organic compounds were different from the synthetic organic compounds in the wastewater, so during the treatment, they continued to decompose through many reaction processes. There were many different products that cannot be processed by microorganisms (such as xenobiotics). Pilot experiments on real wastewater showed that COD treatment capacity of *Halanaerobium lacruisei* was quite good with a period of over 24 days. The results indicated that when applied this isolated bacteria system in real wastewater, up to > 80 % of COD was removed.

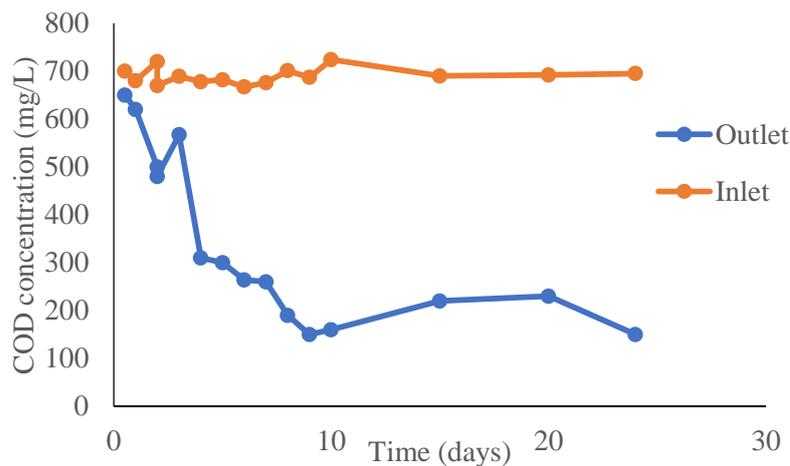


Figure 7. COD concentrations of the pilot experiment.



Figure 8. Comparison of wastewater color of pre-treatment (A) and post-treatment (B) wastewater.

4. 4. CONCLUSIONS

The research isolated a strain of *Halanaerobium lacruisei* microorganism and figured out the optimal condition (salinity, pH and culturing temperature) for culturing the bacteria.

Moreover, COD removal capacity of the bacteria in saline wastewater was also investigated. This study evaluated the main factors affecting the ability to treat COD concentration in saline wastewater: salinity, pH and temperature. The experimental results revealed that 6 - 7 % salinity, pH 7.0 and 30 °C was the optimal culturing condition for *Halanaerobium lacruisei* and also optimal condition for treating COD in wastewater. These results also indicated that operation of anaerobic biological wastewater treatment system at elevated temperatures can have adverse effects on removal capacity. COD removal ability bacteria is quite high in optimal conditions with efficiency up to more than 80 %. In conclusion, this study showed that this strain bacteria could be applied well for treating the organic compounds in the industrial saline wastewater.

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