Growth & Adaptation Of Halophilic Bacteria In Saline Wastewater-New Way To Waste Treatment

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Abstract - ***Environmental pollution is a very bad issue today. Industrial polluted water or other types of wastewater which causes water pollution will increase day by day. So wastewater treatment is essential to make our environment & aquatic life free from pollution. Many industrial sectors generate highly saline wastewater like pickle, sausage, agro-food, petroleum, leather industries etc. The discharged wastewater containing high salinity and other wastes causes harmful effect to aquatic life, water portability, and agriculture. Thus, treatment of saline wastewater or decomposition of saline wastes is very much necessary. Saline effluents are mainly treated with chemical treatments because normal or moderate types of bacteria can not adapt to high saline environment so biological treatment is generally inhibited at high salt concentration (mainly the concentration of NaCl), Again the costs of chemical treatments is high, so alternative ways for the treatment of saline wastewater is now the interesting topic of research. In this case, halophilic bacteria is a good option as these bacteria can bear with an extreme saline environment. This study relates to the detection of Halobacillus sp and Halococcus sp of bacteria with an increasing salinity from 1% to 3.5% of waste water. This study may give a light to ship waste treatment.***

Keywords- ***halophilic, salinity, saline wastewater, mangrove, plate count, anaerobic reactor***

I.INTRODUCTION

Microorganisms can live any places like air, water, land area etc. Generally, the microorganisms which are better known they are a moderate type of bacteria that means grow in proper temperature, pH and other related parameters but there are also such types of microorganisms which can grow at high temperature, high salinity, acidic or basic ph etc extreme type of environment - they are the extremophiles. Halophilic bacteria or sea water bacteria are such types of bacteria. They are also salt-loving that means can grow and adapted at highly saline environment. This study is done with the growth and adaptation of two general types of halophilic bacteria like *halobacillus* sp. and halococcus sp. isolated from salty water of sundarban delta area. The wastewater sample collected from sundarban had the salinity of 1%-1.5%, in the lab these two species were isolated using different inoculums media and water salinity was increased from 1% to 3.5%. Decomposition capacity was seen by anerobic treatment. The species of bacteria which are cultured here are commonly found in sea water, salty lake and mangrove area. So the main aims of this project are-

- To detect the halophilic bacteria which can grow medium salinity ie from 1-5%
- To make the adaptation of that bacteria in saline wastewater with salinity 1-3.5% as this is the general range of salinity of industrial wastewater.
- To study the growth of these bacteria by plate count with an increasing salinity.
- To make an anaerobic digestion of salty wastewater and detect methane production.

II.PROPOSED METHODOLOGY

The total project work was done by the following method-

#Sample collection –

Saline wastewater was collected from a small village of Sundarban .

#Estimation of salinity-

Salinity was checked by salinity meter. 10 ml of sample was taken in a beaker and a probe of saline measuring kit was dipped into it then salinity was displayed in digital display section. sample was

#Increase of salinity-

The salinity of the samples was increased by adding of NaCl into the sample. The salinity of each sample was increased from 1% to 2% then 3.5%.

#Isolation of bacteria from wastewater sample – Two types of the bacteria halobacter sp and halococcus sp. were isolated from wastewater sea water in different agar media according to their species. They are halophilic agar and

halococcus agar.

Fig 1-Salinity Measuring Kit [Ref: JADAVPUR UNIVERSITY Pollution Control Lab]

#Making of inoculums - The two species of Halophilic bacteria were cultured in proper growth medium mixed with saline wastewater with three different salinities- 1%, 2% and 3.5%. and serial dilution was made with these for two species. The composition of the two media is given below-

For *halobacillus sp* –(gm/liter)

NaCl – 220 , KCl- 5, KNO_3- 1 , $MgSO_4$,$7H_2O$ -10, $MgCl_2$,$6H_2O$-20 , $CaCl_2$,$6H_2O$- 0.2, Yeast extract-5, Peptone – 5

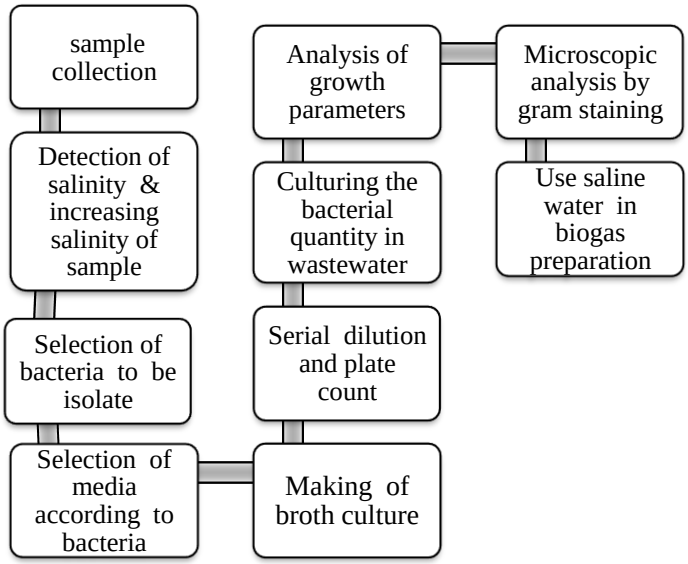
For *halococcus sp* – (gm/ liter)

Tryptone- 10, Yeast extract – 10, Peptone- 5, Trisodium citrate- 3, Potassium chloride- 2, Magnesium sulphate -25, Sodium chloride – 250

#Bacterial count in saline wastewater - Bacterial colonies were detected by plate count. Here two types of the bacteria were detected by the same selective agar media that are ha Halophilic agar and Halococcus agar.

The composition of the two media is given below -

For *halobacillus sp* –(gm/liter) NaCl – 200 , Yeast e extract-3, agar- 20



For *halococcus sp* – (gm/ liter) Tryptone- 10, Yeast extract – 10, Peptone- 5, Trisodium citrate-3, Potassium chloride- 2, Magnesium sulphate -25, Sodium chloride – 250, agar – 20

The plates were then kept in incubator 9 days for *halobacillus* sp. and 15 days for *halococcus* sp.

#Anaerobic treatment of wastewater sample - Wastewater sample with an increasing salinity from 1% to3.5% were treated in a small lab-scale anaerobic reactor and methane gas formation was detected by flame test.

Picture1– Lab scale bioreactor

[Ref- Jadavpur University pollution control lab]

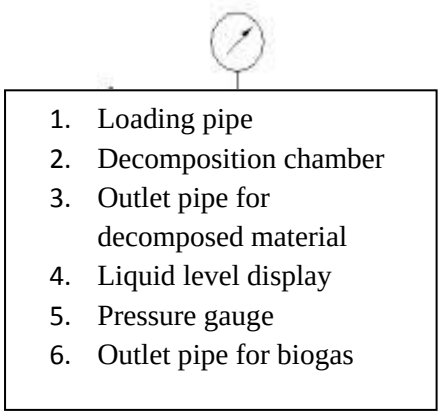


Diagram1 – Linear diagram of the lab scale reactor

III.RESULT AND DISCUSSION

This paper is related to the studies of growth and adaptation of two types halophilic bacteria present in wastewater coming from mangrove industrial area. Some parameters were fixed for the proper growth of these bacteria after some literature survey, these are given in table 1.

TABLE 1- Parameters for the growth of halophilic bacteria

Parameters	**Values**
Temperature for growth	35˚c (**Abram and Gibbons 1960)**
pH for Growth	6.8-7.2 (**Abram and Gibbons 1960)**
NaCl concentration for media	200-250gm/l **(HiMedia lab)**
Antibiotic concentration for media (Bacitracin HiMedia)	0.3mg/l **(Oran 2006)**

In this study salinity was the main parameter which was changed from 1% to 3.5%. within this range of salinity, the bacterial density of halophilic bacteria was detected by optical density value. growth curve of both bacteria obtained by spectrometric values shown in figure 1 for *halobacillus* sp and figure 2 for *halococcus* sp

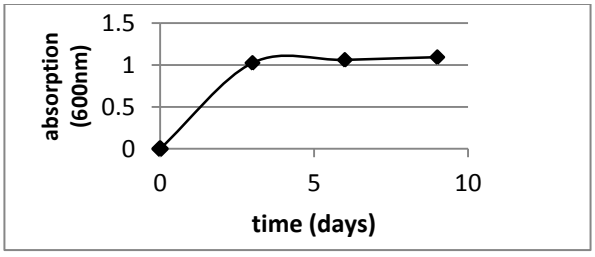


Figure 1

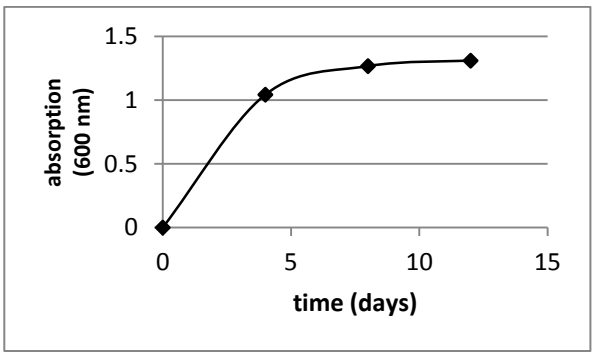


Figure 2

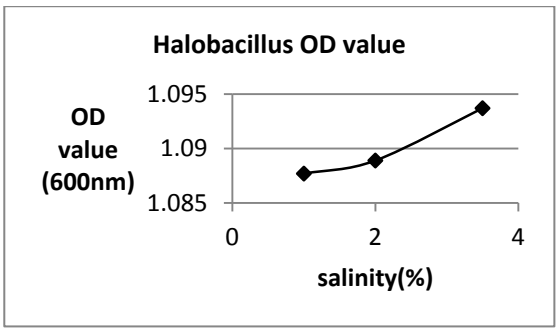


Figure- 3

In figure 1 and 2, it is shown that in the first stage bacteria were grow rapidly that means they are in growth or log phase, after that in the second condition, their growth rate will go to a static condition or in stationary phase. then the growth of these bacteria will be retarded as they go to lag or death phase. For halophiles, the death phage is quite difficult to understand as they can adapt themselves quickly & again start to grow in extreme conditions. With the changing of salinity, the optical density will also change, as a graphical representation of this changing at 600nm has shown below in figure 3& 4. Figure 3 is for halobacillus sp and figure 4 is for halococcus sp.

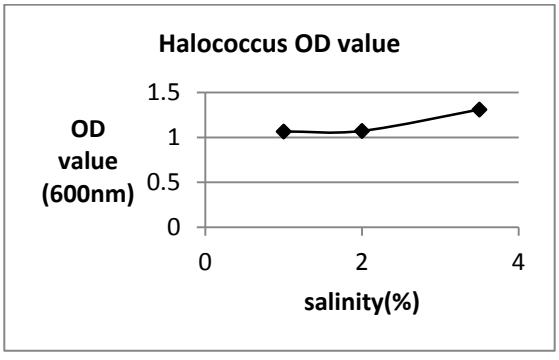


Figure 4

There is also the indirect method of detecting the growth of bacteria ,the bacterial count was detected by plate count with a 10 fold dilution and the graphical representation has shown in Figure 5 for *halobacillus* sp and *halococcus* sp.

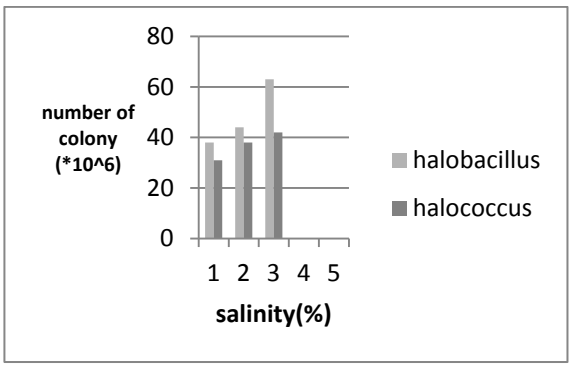


Figure 5

In the both cases, we can see that with increasing salinity bacterial count increases. Both of these bacteria will adapt to higher salinity for that there is a good growth in 2% salinity than 1% and highest growth at 3.5% salinity. This is the range of average salinity of wastewater coming from an industrial area like food, agriculture, leather industry etc.

This bacterial growth profile study reveals that the species of these bacteria can be adapted themselves to higher salinity, they can get their proper nutrition from the waste material of waste water, so they can grow in the saline wastewater. Again by anaerobic treatment of the saline wastewater with an increasing salinity and decomposition occurrence was detected by methane flame test.

The time of production of methane gas with increasing of salinity are shown by a chart in two different seasons of the year like summer & winter. Though these bacteria increase in number in higher salinity but they need more time for methane production as salty environment retard methane production but these salt-loving bacteria can able to be active in high salinity & decompose waste. Methane gas production is done in normal temperature that means in simple room temperature, the parameters which were maintained are shown in table 2 .

Table 2 Parameters for methane production (ref: Jadavpur University Pollution Control Lab)

	Parameters	**Values**
1	Temperature at the time of production (room temperature)	In summer: average 34˚c
		In winter: average 15˚c
2	Pressure	1 atm (˜ 15 psi)
3	pH	7.2
4	Salinity	1-3.5%

Figure 6 show how the methane production time changes with the season that means with the temperature and with an increasing salinity.

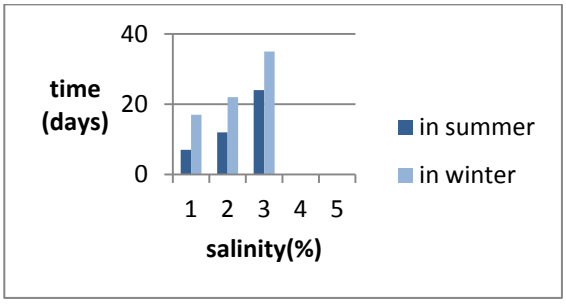


Figure -6

In low salinity high flame was produced as decomposition rate was high and the opposite incident occurred at high salinity.

(a) (b)

Picture 2- (a)high flame and (b) low flame of methane

IV. CONCLUSION

This project will give an opportunity to detect if these bacteria can simply survive and decompose wastes, there was a satisfactory result. They need some time to update themselves but they can do it. They do not need any extra effort to maintain pressure or temperature in the reactor. This is very good as the process is not going to be cost effective. So they are the best option for saline wastewater treatment and can make industrial environment pollution free.

V. FUTURE SCOPES

This project has a good microbiological and environmental values. The halophilic bacteria can also be used for ship waste treatment to make the aquatic life of sea pollution free Furthermore the gene sequences of these bacteria can be detected so that a proper and best mother culture can be produced for the treatment of salty wastewater found in agro, food or leather industries. These bacteria can also use for the bench scale biogas production from the manure or other waste found in mangrove village area and also commercially by setting up biogas plants there.

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