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ESTIMATION OF BIOFILMS FROM SEWAGE TREATMENT PLANTS (STPS) OF MEWAR UNIVERSITY

Mukhtar Aminu Garba*
Abhishek Kumar Verma**
Abubakar Musab Umar***
Abba Umar Yakub****
Umar Isah Adam*****
Shehu Hadi Abubakar******
Ankita Mathur*******

ABSTRACT

Wastewater consists of myriad of micro flora that has a beneficial effect on the treatment process. Wastewater treatment process is generally classified into three categories i.e. primary, secondary and tertiary. The role of bacteria in wastewater has been marked for many years. Biofilms, are usually defined as the communities of microbes residing together in a matrix of EPS. These biofilms act as an invader to the external environment and prevent the residing bacteria from damages, desiccation and attack from environment damages. The microbial cells residing within biofilm are capable of act like division of labour, i.e. each microbial cell contributes towards development of biofilm and thereby protects the cells. Stress conditions such as variation in salinity, pH and temperature are quite common in wastewater treatment plant. The phenomenon effect of biofilms can safeguard it and could be a possible mechanism for future use in biodegradation.

Keywords: *Bacillus subtilis*, Bacteria, Biofilm, *E. coli*, organic matter, salinity, stress, Wastewater.

***Mukhtar Aminu Garba, ***Abubakar Musab Umar, ****Abba Umar Yakub, *****Umar Isah Adam, ******Shehu Hadi Abubakar** are Post-Graduate students at the Department of Microbiology (Life Sciences),Mewar University, Gangrar, Rajasthan-312901.

****Abhishek Kumar Verma** is an Assistant Professor at the Department of Biochemistry (Life Sciences),Mewar University,Gangrar,Rajasthan-312901

*********Dr. Ankita Mathur** is Assistant Professor at the Department of Microbiology (Life Sciences),Mewar University,Gangrar, Rajasthan-312901. She was awarded PhD in Nanobiotechnology(2017) on the topic "Effect of Nanoparticles (TiO2 NPs) on aerobic and anaerobic waste water bacterial isolate" from Vellore Institute of Technology(VIT,Vellore). Her research interest includes Nanotoxicology, nanoremediation and biofilm formation, drug interaction.

Corresponding Author E mail:-dr.ankita@mewaruniversity.co.in. # All authors carry equal contribution

INTRODUCTION

Wastewater consists of myriads of bacteria, protozoa and virus. The main role of these bacteria involves degradation of organic matter (proteins, carbohydrates and fats). There are generally two types of processes that are associated with bacterial waste water degradation-aerobic and anaerobic (Bitton *et al*., 2005).

Wastewater treatment is divided into three categories i.e. primary, secondary and tertiary. These treatments are independent of each other, the treatment processes starts with preliminary where the coarse particles are removed. The basic processes included in this are the operations of grit screening and removal of suspended larger particles. Processes include removal of organic and inorganic components. Further, the elimination of organic compounds (biodegradable) by utilizing treatment processes comes under secondary treatment process. During this process aerobic micro-organisms comes into action and metabolize the organic matter by using oxygen as a key ingredient. The secondary treatment controls the processes with three dimensions- activated sludge, trickling filter and rotating biological contractor. During the secondary treatment the role of microbes are more dominant as compared to the other processes. Tertiary treatment process is also known as advanced treatment process that involves removal of nitrogen, phosphorus and suspended solids (Droste *et al*., 2018).

Biofilms, are often defined as microbial communities residing in a self-encapsulated structure or a matrix that has been adhered to a surface. In biofilms, the micro-organisms reside together in a scaffold of extrapolymeric matrices that is made up of carbohydrate, protein and polymers. This matrix protects the residing bacteria from external environment damages and stress.

There have been many reports on the treatment of wastewater with biofilms. The process includes biodegradation, biosorption and bioaccumulation (Welander *et al*., 2004). The biofilms follows a process like- initiation, formation and development of biofilms. This process is intrinsic and involves changes in genotype, physiology and signalling. The residing bacteria within the biofilm form a consortium and follow a division of labour physiology. The bacterium assists one another and forms a protection to other bacteria in the shield (Mathur *et al.,* 2015). These may provide a suitable microenvironment and all together fight against the toxins and external factors (Gottenbos *et al*., 1999).

EPS or Extrapolymeric substances are those that are involved in matrix formation in biofilms. The major components present in the EPS are polymers, polysaccharides and extracellular DNA. These help in the dessication and protection of antibiotics and also from metals (heavy) and other toxic components (Kavitha *et al*.,2013).

The present study deals with the effect of different type of stress and its impact on bacteria isolated from wastewater and also its biofilm. The effect of biofilm has a protective role on the bacteria towards its stress. Thereby, this model can act as biodegradation model for the future where biofilms can be used as a defense against the toxic metals and stress.

MATERIALS AND METHOD

Characterization of wastewater

Wastewater was collected from Mewar University Sewage Treatment Plant (STP) in the sterile bottles. Collected water was analyzed for physical- chemical characterization. pH was estimated to be 7.0, conductance around 168±0.8 mS and dissolved oxygen to be 5.9 mg/L.

Isolation of bacteria

The wastewater collected from Mewar University STP was utilized for the bacteria isolation. Collected wastewater was initially filtered and used for isolation. Standard isolation procedure was followed and subsequently serial dilution and primary isolation was performed (Yazdi *et al.,* 2001)

Estimation of Biomass

An aqueous solution of the bacterial cells was used for this method. For this the cells were grown in nutrient broth maintaining a temperature of 37ºC. Bacterial cells were grown for a period of 24 hours and after that the cells were harvested by centrifugation at 4000 g for 10 minutes. Further, the sediment was washed twice with distilled water. Subsequently, the sample growth was checked periodically by taking the aliquot and analysing optical density with UV Visible Spectrophotometer. For the purpose of biomass measurements, dry weight and wet weight were calculated accordingly (Orduna *et al*., 2010)

Static Biofilm formation

Bacterial cells (isolated) were grown on slides that were inserted in nutrient broth media. The cells were made to grow till mid-logarithm phase. Consecutively, the slides were removed and the biofilm grown were washed with sterile distilled water and kept for drying. Further, the dried slides were washed with 1% crystal violet and the extra dyes removed with 95% ethanol solution. The retained biofilm biomass was analyzed and absorbance was taken at 590 nm (Meritt *et al*., 2006)

Effect of Salinity, pH on Biofilms

For analysing, the effects of salinity, a range of 0.5-5 µg/mL of NaCl were prepared. These concentrations of NaCl were utilized. The extracted biofilms were treated with varying concentration of NaCl and absorbance was taken at 595 nm. A plot was prepared for the same (Sun *et al*., 2010). To analyze the effect of pH, three different pH viz. 3, 7 and 9 were utilized for the experiment.

Statistical Significance

The results were done in duplicates and presented in ±SE form. A two way ANOVA test was performed utilizing Bonferroni Post tests

RESULTS AND DISCUSSION

Isolation of Bacteria

The isolated bacteria were characterized as pale, white colonies and further after microscopic, biochemical characterization were recognized as *Bacillus subtilis* and *E. coli* species. Although these species were further, characterized on the basis of Gram staining, *Bacillus subtilis* possess Gram positive nature whereas *E. coli* possess Gram negative nature. The microscopic images are displayed in Figure 1.

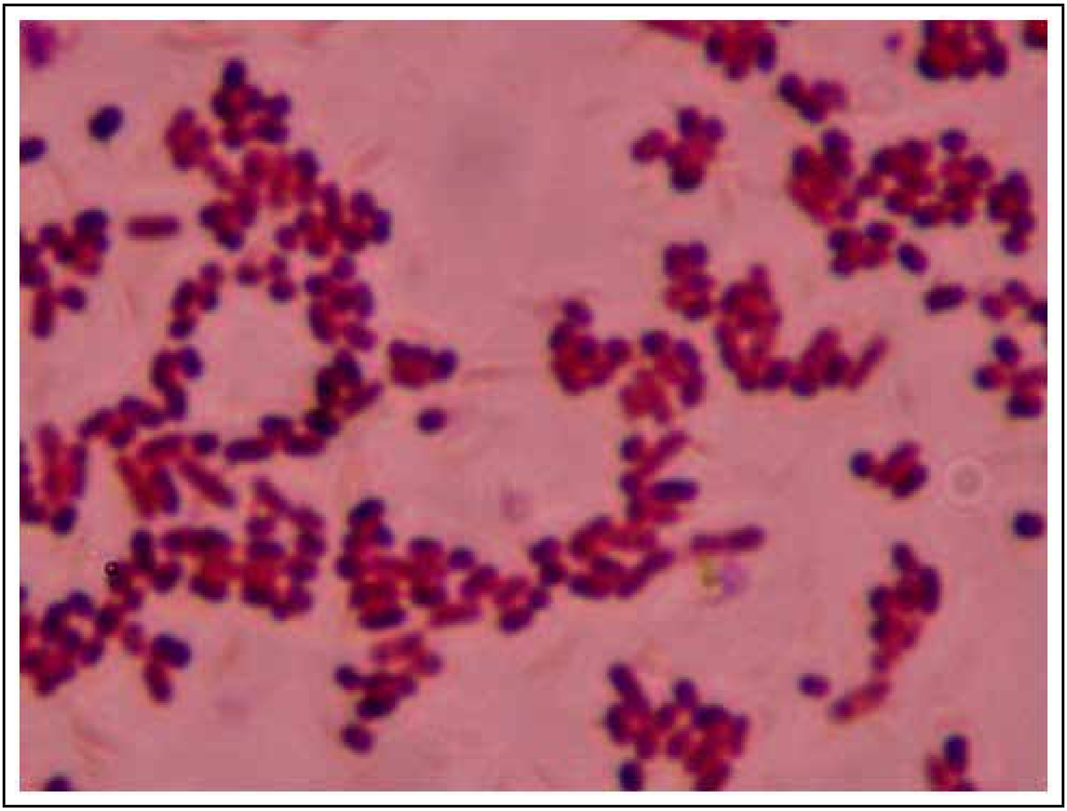

Figure1: Colonies of *Bacillus subtilis* and *E. coli*

Biomass Estimation

The biomass for the *Bacillus subtilis* was estimated to be around 50.1±0.01% whereas the estimated biomass for *E coli* was determined to be 48.0±0.01%. This biomass was calculated with respect to the bacterial cells. For biomass estimation there have been reports demarking the weight (dry) of the pellet of bacterial cell. An average percentage in bacteria has been recorded from 10 to 29% whereas the content of water (intracellular) has been ranged from 33-92%. This depends on the type of bacteria, intracellular content (Orduna *et al.,* 2010).

Effect of Salinity and pH on the biofilms

The estimation of biofilms for the bacterial strain *Bacillus subtilis* and *E. coli* possess an increase in the biofilm formation with increase in the concentration of NaCl. At the initial concentration, of 0.5µg/mL of NaCl the biofilm formation was 0.80±0.01 and 0.75±0.01 nm in *Bacillus subtilis* and *E. coli* respectively. At the highest concentration of NaCl i.e. 5µg/mL the biofilm formation in *Bacillus subtilis* is 2.31± 0.01 and in *E. coli* is 0.84 ± 0.01 nm (Figure 2).

Biofilms may exhibit an exaggerated response towards NaCl. According to Xu *et al.*(2010), aggregation of the biofilm cells elevate with a concentration of 10% NaCl. In case of *S. aureus,* biofilms have increased with the concentration of NaCl (Kennedy and O Gara *et al*., 2004). In wastewater biofilms, there is a common spectacular feature of association towards metabolic pathway in terms of carbohydrate, amino acids (Wang *et al*., 2009). The coverage rate of biofilm decreases with the increase in NaCl and simultaneously higher NaCl concentration has attributed towards more compact biofilm morphologies.

There has been a remarkable and noticeable change in the biofilm formation with respect to the concentration. The difference in biofilm formation between both the isolates was found to be significant with respect to the control ($p<0.0001$).

With respect to the other parameter, i.e. pH, the biofilms grown were significantly higher, i.e. at an acidic pH (3) the biofilms grown were higher in *Bacillus subtilis* .A similar kind of trend was observed with increase in pH, i.e. *Bacillus subtilis* showed more biofilm formation as compared to the other isolate. An alteration in pH affects the growth of biofilm, the mechanistic approach involves the excretion of extrapolymeric substances i.e. changes in polymeric content especially carbohydrate and protein content (Sehar *et al*., 2016).

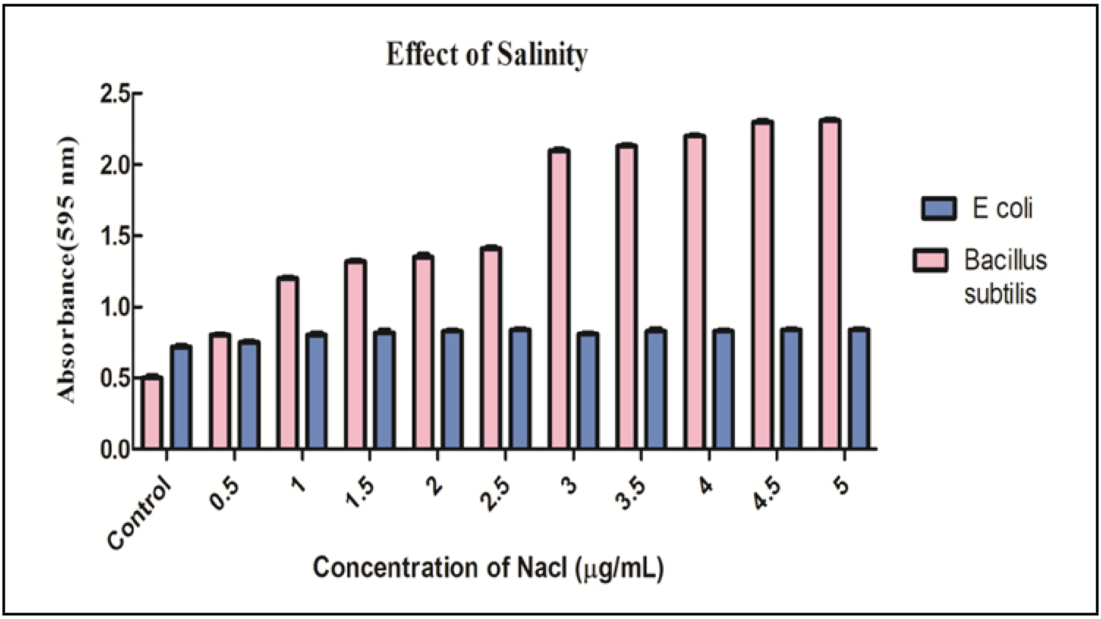

Figure 2: Effect of salinity on *E. coli* and *Bacillus subtilis* biofilms

Table 1: Biofilm formation of *Bacillus subtilis* and *E. coli* at varying pH

Organism	**pH 3**	**pH 7**	**pH 9**
E. coli	0.23±0.01	0.50±0.02	0.80±0.001
Bacillus subtilis	0.31±0.01	0.56±0.01	0.93±0.002

CONCLUSION

Wastewater bacteria are capable of tolerating a particular amount of stress in the form of concentration of NaCl and variations in pH. These might be the result of the biofilm developed over time. These biofilms serves as a barrier and are useful for degradation of organic matter present in the waste water. These could serve as defender and would be an ideal model in future to combat degradation of pollution by materials (nano), heavy metal toxicity etc.

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AUTHOR(S) CONTRIBUTION

Designing: AM

Experimentation: MG, UY, IA,SA

Analysis of Data:AKV,AM

REFERENCES

1. Bitton, G. (2005). *Wastewater Microbiology*. John Wiley & Sons.
2. Droste, R. L. & Gehr, R. L. (2018). *Theory and Practice of Water and Wastewater Treatment*. John Wiley & Sons.
3. Wilderer, P. A. & McSwain, B. S. (2004). "The SBR and its biofilm application potentials". *Water Science and Technology*, **50**(10):1-10.
4. Mathur, A., Kumari, J., Parashar, A., Lavanya, T., Chandrasekaran, N. & Mukherjee, A. (2015). "Decreased phototoxic effects of TiO_2 nanoparticles in consortium of bacterial isolates from domestic waste water". *PLoS One*, 10(10), e0141301
5. Gottenbos, B., Van der Mei, H. C. & Busscher, H. J. (1999). "Models for studying initial adhesion and surface growth in biofilm formation on surfaces". *Methods in Enzymology*, **310**, 523-534.
6. Kavita, K., Mishra, A. & Jha, B. (2013). "Extracellular polymeric substances from two biofilm forming *Vibrio* species: Characterization and applications". *Carbohydrate Polymers*, **94**(2): 882-888.
7. Sharifi-Yazdi, M. K., Azimi, C. & Khalili, M. B. (2001). "Isolation and identification of bacteria present in the activated sludge unit, in the treatment of industrial waste water". *Iranian Journal of Public Health*, 91-94.
8. Li, E. & Mira de Orduña, R. (2010). "A rapid method for the determination of microbial biomass by dry weight using a moisture analyser with an infrared heating source and an analytical balance". *Letters in Applied Microbiology*, **50**(3): 283-288.
9. Merritt, J. H., Kadouri, D. E. & O'Toole, G. A. (2006). Growing and analyzing static biofilms. *Current Protocols in Microbiology*, (1), 1B-1.

10. Sun, C., Leiknes, T., Weitzenböck, J. & Thorstensen, B. (2010). "Salinity effect on a biofilm-MBR process for shipboard wastewater treatment". *Separation and Purification Technology*, **72**(3):380-387.
11. Xu, H., Zou, Y., Lee, H. Y. & Ahn, J. (2010). "Effect of NaCl on the biofilm formation by foodborne pathogens". *Journal of Food Science*, **75**(9):M580-M585.
12. Kennedy, C. A. & O'Gara, J. P. (2004). "Contribution of culture media and chemical properties of polystyrene tissue culture plates to biofilm development by *Staphylococcus aureus*". *Journal of Medical Microbiology*, **53**(11): 1171-1173.
13. Sehar, S. & Naz, I. (2016). "Role of the biofilms in wastewater treatment". *Microbial biofilms-Importance and Applications*, 121-144.