

**TREATMENT OF PETROLEUM  
WASTEWATER USING  
HALOALKALOTHERMOPHILIC  
BACTERIAL CONORTIUM  
UNDER EXTREME CONDITION**

## Introduction:

Bacteria are the most important microbial community that can do novel things, and vary in their species, and they have a significant impact on degradation of Polycyclic Aromatic Hydrocarbons (PAHs) in wastewater under extreme conditions.

## PROBLEM:

Petroleum wastewater treatment with the existing technology is one of the longest process that requires time, effort and cost. Especially the treatment of petroleum wastewater that contains PAHs, an organic waste that accumulates constantly and does not dissolve in water (hydrophobic), in addition to being toxic and carcinogenic.

## RESEARCH QUESTION:

How can we treat the petroleum wastewater and decrease the PAHs in the wastewater under extreme condition?

## Hypothesis:

If haloalkalothermophilic was developed in extreme conditions (halophilic, thermophilic and alkalophilic) then, PAHs will decrease in the wastewater.

## Scientific importance:

This process can be used instead of other ways that harm the environment since it is an eco-friendly process. Also, this process costs less than other techniques.

## Methodology:

### 1- Development of bacteria

Enrichment of bacterial consortium in mineral salt medium with phenanthrene as sole carbon source under extreme conditions (salinity-40 g/L, Temperature-50 °C and pH-10). Identical transfers of the culture to obtain potential amount of extremophilic consortium.

### 2- Measuring protein

To monitor the bacterial growth

### 3- Selection of PAHs

Phenanthrene (PHN), Fluorene (FLU) and Pyrene (PY)

### 4- Biodegradation Study

Two controls used in the study, PAHs+Medium as abiotic control and Medium+ Consortium as biotic control. Test flasks with PAHs+Medium+Consortium.

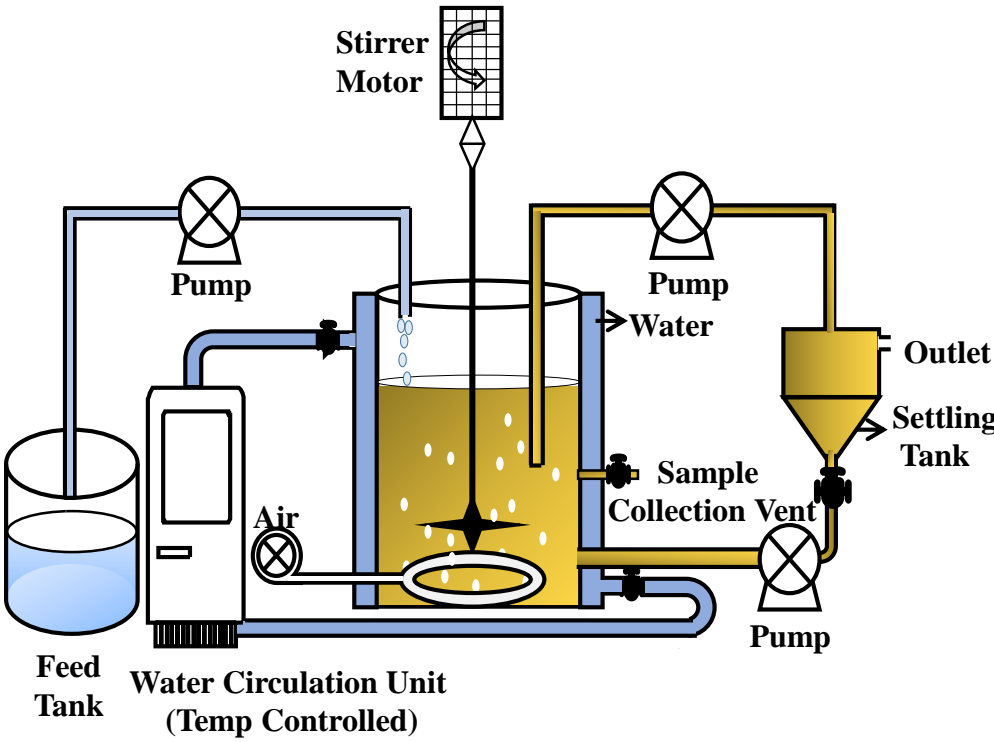
PAHs analyzed in HPLC equipped with C18 Column as stationary phase, Acetonitrile (1mL/min) as mobile phase and UV detector (254 nm).

### 5- Lab Scale bioreactor study

Continuous Stirred Tank Reactor (CSTR) was used to analyze the treatment efficacy of the extremophilic consortium under extreme condition. Reactor was operated different OLR (Organic Loading Rate) and HRT (Hydraulic Retention Time). Optimized OLR -0.17 kg COD/m<sup>3</sup>.d, 0.7 L/d flow rate and HRT (Hydraulic Retention Time) of 12 days. COD, BOD, MLSS and MLVSS parameters were analyzed as per APHA (2005) Standard methods.

### 6- Bacterial Community Analysis

Next Generation Sequencing Techniques was used to identify the bacterial strains present in the consortium under extreme condition.



Previous studies:

The previous work focused on enriching bacterial strains for biodegradation of PAHs present in the petroleum wastewater under normal conditions.

Saudi Arabia with sea water salinity 40 g/L and temperature nearly 50°C was unable to treat the petroleum wastewater effectively.

Limited research work was performed around the world on combination of extreme condition.

The present study is focused on use of extremophilic bacterial strains in the treatment process which will overcome the environmental factors such as temperature, pH and salinity.

The consortium was subjected to phylogenetic analysis at various stages of the experiments (beginning, during and at the end of experiment) in order to confirm the stability of the bacterial strains present in the consortium.

Variables:

constant :consortium

Independent: halophilic (40 g/L), thermophilic (50 °C) and alkalophilic (pH10)

Dependent: COD,BOD

# Graphs tables:

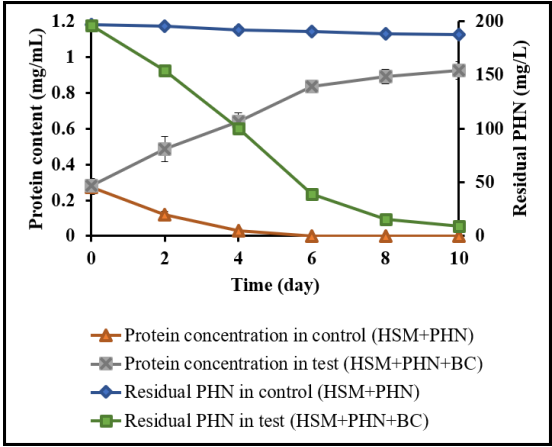


Fig.1. Biodegradation of Phenanthrene (PHN) (200 PPM) by extremophiles bacterial consortium.

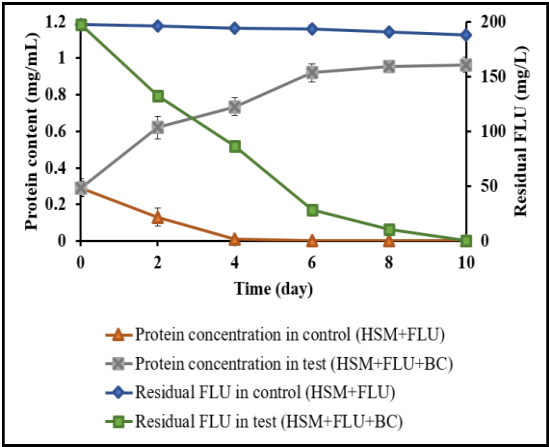


Fig2. Biodegradation of Fluorene (FLU) (200 PPM) by extremophiles bacterial consortium.

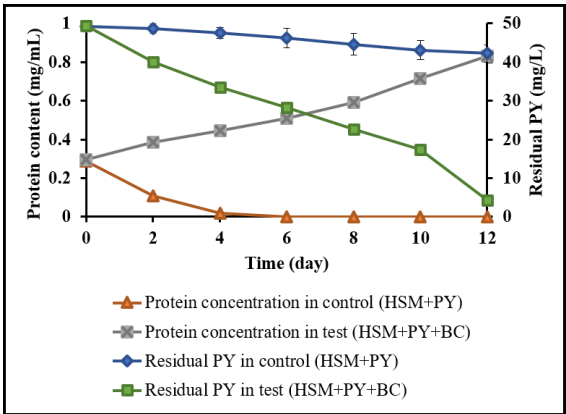


Fig.3. Biodegradation of Pyrene (PY) (50 PPM) by extremophiles bacterial consortium.

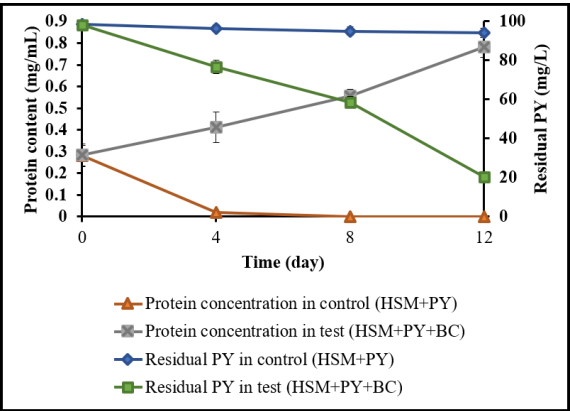


Fig.4. Biodegradation of Pyrene (PY) (100 PPM) by extremophiles bacterial consortium.

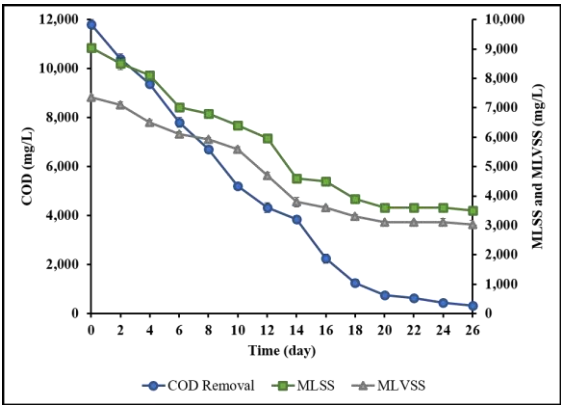


Fig5. Treatment of petroleum wastewater in (CSTR) by extremophiles bacterial consortium.

Graphs tables:

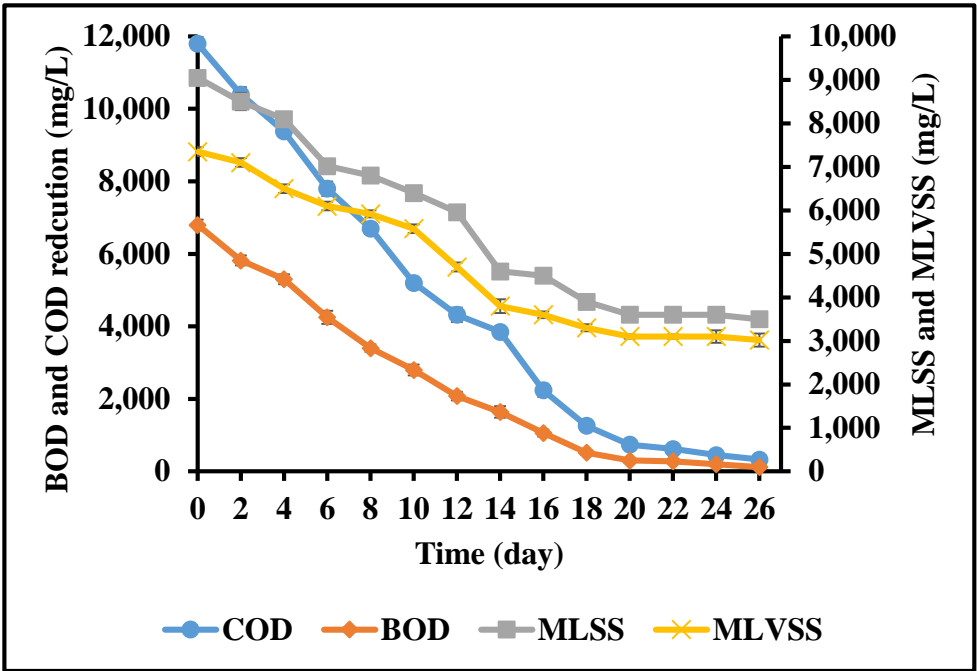


Fig.6. Treatment of petroleum wastewater in CSTR under extreme condition.

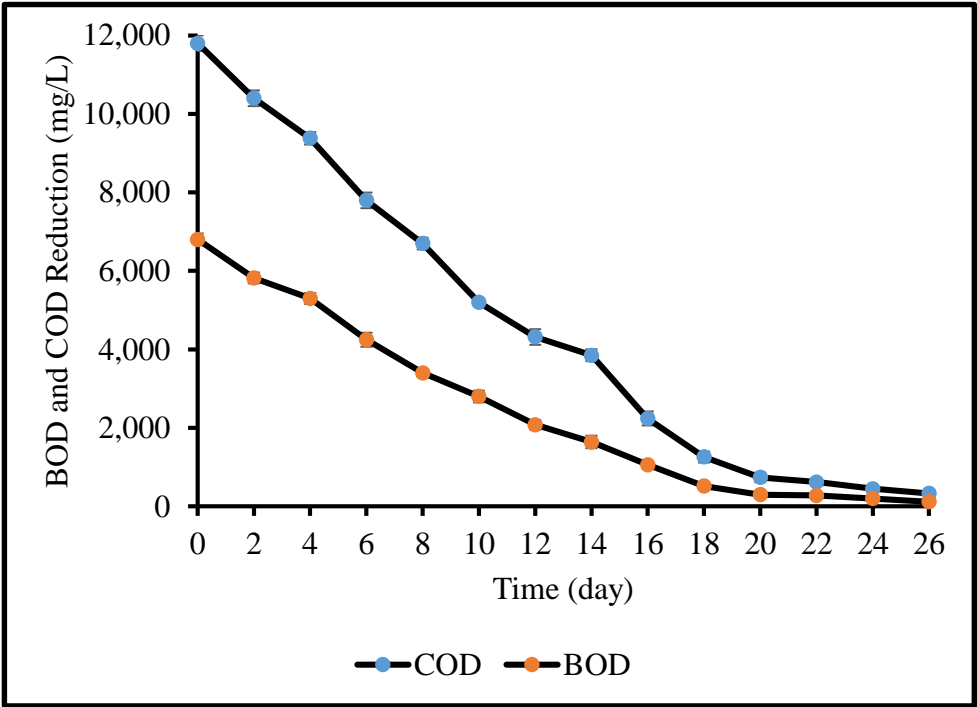


Fig.7. BOD and COD reduction during the treatment of petroleum wastewater under extreme condition

## RESULT AND DISCUSSION:

Extremophiles bacterial consortium was enriched from contaminated site and desalination plant samples. The extremophiles consortium utilized PHN as sole carbon source under halothermoalkalophilic condition.

Different petroleum hydrocarbons (PAHs) was analyzed at different concentrations (PHN 200 ppm, FLU 200-500 ppm, PY 50-100 ppm).

The results of present study showed the bacterial consortium was capable of degrading PHN (100 ppm)  $95 \pm 2.1\%$  in 10 days “Fig. 1”. FLU degradation was also studied by extremophiles bacterial consortium at different concentrations 200, 500 ppm under halothermoalkalophilic condition. Complete degradation of FLU (200 ppm) was recorded by the consortium in 10 days “Fig. 2”. Degradation of PY at 50, 100 ppm was  $91 \pm 2.4\%$ ,  $79 \pm 2.8\%$ , in 12 days, respectively “Fig. 3-4”.

Increase in PAHs concentration lead to decrease the ability of the bacterial consortium to degrade and increased the time required for degradation.

The COD (Chemical Oxygen Demand) removal efficiency of the extremophiles bacterial consortium was  $97 \pm 1.1\%$  in 26 days. MLSS (Mixed Liquor Suspended Solids) and MLVSS (Mixed Liquor Volatile Suspended Solids) was maintained at 3.5 g/L and 3 g/L in the reactor (Fig. 5).

The bacterial community analysis using next generation sequencing technique confirmed the presence of the following bacterial strains such as *Ochrobactrum* (62%), *Marinobacter* (24%) , *Pseudomonas* (8%), and *Bacillus* (4%), *Sphingomonas* (1%) and other strains (1%).

The COD removal  $97 \pm 1.5\%$  and BOD (Biochemical Oxygen Demand) removal  $98 \pm 1.7\%$ , MLSS (Mixed liquor suspended solids) and MLVSS (Mixed liquor volatile suspended solids) was maintained 3.5 g/L and 3 g/L in the reactor (Fig.6 and 7).



## Conclusion:

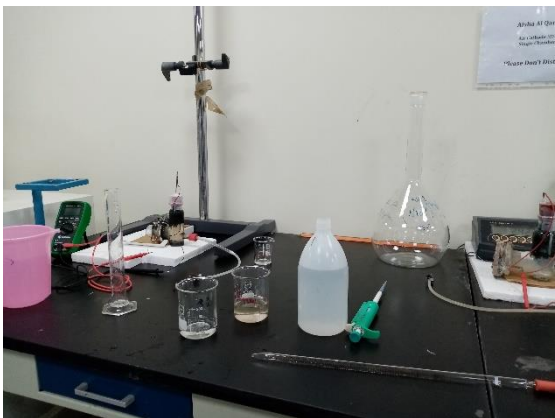
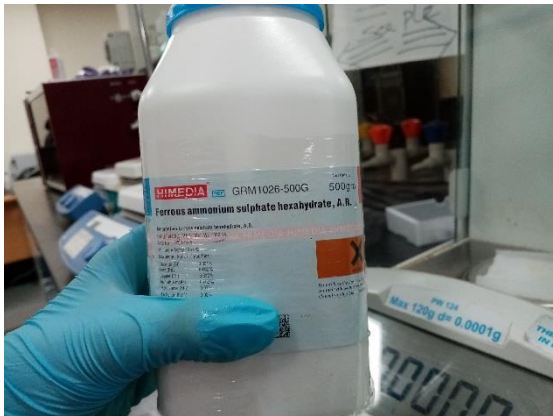
Development and employment of haloalkalothermophilic consortium revealed the potential treatment of petroleum wastewater under extreme condition.

## Future work:

In the near future, it could be applicable to produce energy (electricity) while haloalkalothermophilic consortium breaks petroleum hydrocarbons operated in microbial fuel cell reactor.

Extremophilic consortium can be used in the future in other types of wastewater treatment.

Documents:



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