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Effects of Variations in Sodium Chloride Concentrations on the Biodegradation of Heptane by *Alcaligenes* species

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Abstract
Conventional mineral salts medium (MSM) had been used for many years. A modification of the concentration of sodium chloride was attempted in this work to observe the effect or otherwise on microbial activities in reaction tubes. This study was undertaken to assess the biodegradation potentials of Alcaligenes species on heptane. Results showed the hydrocarbon degrading ability of Alcaligenes sp. under varying concentrations of sodium chloride salt for a period of sixteen (16) days at two to four days interval. Due to its rapid rate of multiplication, a steady increase in bacterial growth was observed during the experiment. Alcaligenes sp. showed appreciable growth on heptane with a reading of $6.5x10^8$cfu/ml on Day 16. Also, the regeneration rate of Alcaligenes sp. was found to be rapid on heptane in the presence of 4M concentration of NaCl with a reading of $1.18x10^9$cfu/ml on Day 16. This study shows that increased sodium chloride concentration aids the utilization of heptane by Alcaligenes sp. Hence, Alcaligenes sp. is a promising isolate that can be used for the bioremediation of hydrocarbon contaminated sites in saline environment.
Keywords: *Alcaligenes sp. Biodegradation, Heptane, Sodium Chloride, Saline.*

INTRODUCTION

Numerous anthropogenic activities have led to growing demands and use of hydrocarbon-based products (Meng *et al*., 2016). The prevalence of petroleum hydrocarbon spills in pristine environment has resulted in negative changes in the ecosystem thus, increasing universal environmental concerns (Uzukwu, 2017; Adam *et al*., 2019). When oil is spilled, it spreads out and moves on the surfaces of the contaminated sites. For instance, soils can become polluted with petroleum products and the run offs from these soils can pollute municipal water supplies thereby creating a public health menace (Masih and Taneja, 2006) hence, continuous pollution of the ecosystem endangers the public as well as erodes the aesthetic value of the environment.

Also, plant productivity is disturbed by the direct toxicity and recalcitrant nature of various components of hydrocarbons which inhibit the penetration of light, nutrients and water from entering the soil matrix (Ellis, 2011). This has necessitated the need to eliminate pollution from petroleum hydrocarbons and also devise an alternative *modus operandi* for the remediation of the environment which will be harmless and cost effective (Das and Chandran, 2011). Biodegradation of petroleum and other hydrocarbons is a complex procedure and its qualitative and quantitative aspects depend on the nature and number of hydrocarbons present, seasonal environmental conditions and the composition of microbial community (van Hamme *et al*., 2003; Gadd, 2007). Because hydrocarbons are a complex mixture of organic chemicals, oxygen, sulphur and nitrogen (Das and Chandran, 2011), a variety of indigenous microbial genera such as *Bacillus*, *Alcaligenes*, *Pseudomonas*, *Micrococcus*, *Sarcina*, *Aspergillus, Cladosporium*, *Fusarium*, etc. (Adekunle and Adebambo, 2007) are responsible for the breakdown of these molecules thus, several methodologies are utilized to decontaminate hydrocarbon-polluted environments (Adetitun, 2020; Chaillan *et al.,* 2006).

The presence of the appropriate microorganisms, energy and carbon sources that can be used by the organisms for cell maintenance and growth will determine how efficient biodegradation will be carried out (Varjani and Gnansounou, 2017).

Alcaligenes species are motile with one or more peritrichous flagella. They are halophiles and have the ability to survive the denaturing effects of salts as well as to maintain equilibrium between high environmental osmotic pressure and low water activity level outside their cells. Additionally, some strains of

Alcaligenes are capable of anaerobic respiration but they must be in the presence of nitrate or nitrite (Le-Borgne *et al*., 2008).

This study is aimed at assessing the ability of *Alcaligenes* sp. to utilize heptane as the only carbon source in mineral salts medium with varied concentrations of sodium chloride.

MATERIALS AND METHODS

Media and Collection of *Alcaligenes* sp. from Stock Culture

Media preparation was done according to the manufacturer's instructions. All microbiological processes were carried out aseptically (Fawole and Oso, 2007). Pre-isolated and purified cultures of *Alcaligenes* sp. was collected from the Microbiology Laboratory, Department of Microbiology, University of Ilorin, Kwara State, Nigeria. Molecular identification involving PCR and sequencing of the bacterium was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. BLAST was done using the online NCBI database.

Preparation of Mineral Salts Medium (MSM)

Two hundred and fifty mls of distilled water was used in the preparation of Mineral Salts Medium (MSM) having the composition of: 0.125g/L of KH_2PO_4, 0.35g/L of Na_2HPO_4, 0.05g/L of $MgSO_4.7H_2O$, 0.075g/L of KNO_3, 0.25g/L of $(NH_4)_2PO$ and the mixture homogenized. 20ml of MSM was measured into 20 reaction bottles and sterilized by autoclaving at 121^{o}C for 15 minutes (Adetitun *et al*., 2016).

Determination of volume of MSM and heptane used

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

Since 1g=1L

For 20ml to be used,

$$20\text{ml} = \frac{20\text{g}}{1000}$$

$$= 0.02\text{g}$$

$$\text{Volume} = \frac{0.02\text{g}}{0.684\text{g/cm}^3}$$

$$= 0.0292$$

In µl, 0.0292 ×1000= 29µl

Volume of Heptane to be used is 29µl for 20ml of MSM.

Calculation of NaCl Molar concentrations

Rapid Labs Limited NaCl was used. Precisely, 58.5 g of the powdered salt was suitable for preparing 1L of solution.

58.5g = 1L

1Molar of NaCl

1000ml= 58.5g NaCl

$$20\text{ml} = \frac{58.5\text{g} \times 20\text{ml}}{1000\text{ml}}$$

1M =1.17g of NaCl in 20ml of reaction mixture

2Molar of NaCl

2 ×1M

=2 ×1.17g NaCl

2M=2.34g NaCl in 20ml of reaction mixture

4Molar of NaCl

4×1M

=4×1.17g NaCl

4M=4.68g NaCl in 20ml of reaction mixture

RESULTS

Confirmation of *Alcaligenes* sp.

The sequences below are those obtained for *Alcaligenes* sp.

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Confirmation of *Alcaligenes* sp.

The sequences below are those obtained for *Alcaligenes* sp.

ATCTTTTGGGTATACTGGGCGTAAGCGTG
CGCAGGCGGTTATTTAAGACAGATGTTGA
ATCCCCGAGCTTCACTTTGGACTGCCTTT
GAAATAGCGAATCTGGATGTGTGAAAAG
GAGGTGGATTCCCGTGTACAGGTGAAATT
CGTAGATTTCGGAGGAACACCAATGGCG
AAGGCGGTTTCCTGGTCCTTTACTGACGC
TGAGCACGAAAAGCGTGGGAGCAAAAGG
GATTAATACCCTGGTAGTCCACGCCTAAA
ACGATTAAAGTAACTCGTCGGGCCGTATG
GCCTCGGTGCCCCACTTAAGCGTGAAGTT
TACCGCCTGGGGAGTACGGTCGCAAGATT
AAAACTCAAAGGAATTGACGGGGACCCG
CACAAGCGGTGGATGATGTGGTTTAATTC
GAAGCAACGCGAAAAACCTTACCTACTCT
TGACATGTCCGGAATGGGCATTAGATTTG
GTAGTGCTTCGTAAGAGAACCGGAACAC
AGGTGCTGCATGGGTGTCGTCAGCTCGTC
GTGTGAGATGTTGTGGTTAAATCCCCCAA
CAAGCGCAACCCTTGTCATTATTTGCTAC
CAACATGTTACTTGGCACTTCTGAGCGGG
GTGGCCGACCGAACGAAGGTGGGGATGA
CGTCAATTCCTCCTGCCCCTTATGGCTTA
GGGGTTCGCGCGCCACACAATGGCTAGG
ACGGAGGAGAGCCTGCCCACCAGGACAG
AGGATATCCAACTAACCTGATCGTACTCT
CTATCGCAGTATTGCACTCTGCTGCTCTG
AGTGCATGAATCTCTAATTATCTAGTATC
AGCATATCAGCGTGAGCGAAACCCCCCC
C A A A A A A A A A A A A A A A A A

A graphical representation of the growth rate of *Alcaligenes* sp. on heptane is depicted in Figure 1. It was observed that the growth of the organism began with a lag phase which lasted from Day 0 to Day 5. The result also showed that the log growth phase of the organism began at Day 5 and lasted to Day 16. The lowest bacterial count was observed to be $1.41x10^6$cfu/ml on Day 0 while highest bacterial count was observed to be $6.5x10^8$cfu/ml on Day16. The steady line of growth increase showed that the organism was able to degrade the heptane excellently.

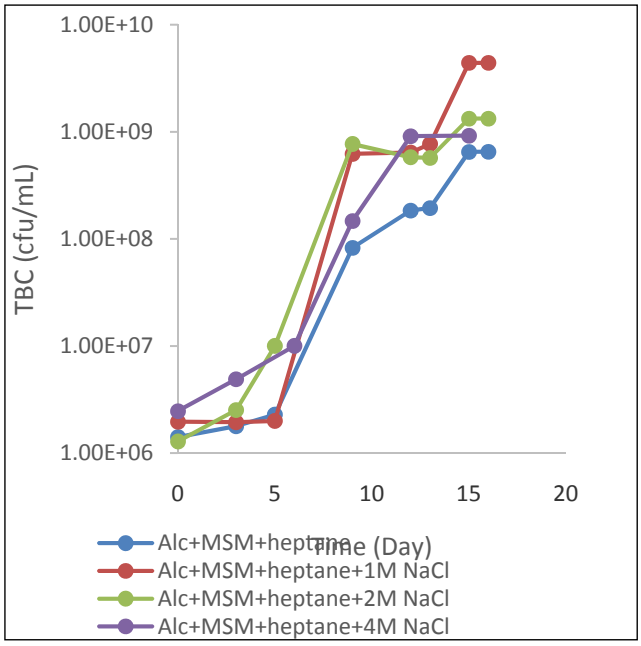

Figure 1: Growth of *Alcaligenes* sp. on mineral salts medium containing heptane with different NaCl concentration

The result of the effect of varying concentrations of sodium chloride showed significant growth changes with various concentrations. In the reaction mixture with 1M NaCl concentration, there was a rapid multiplication of bacterial cells when plated. The log phase of the organism began on Day 0 to Day 3 which had a reading of $1.94x10^6$cfu/ml. Although, a slight decline was noticed on Day 11, thereafter microbial growth increased till Day 15 which had a reading of $4.41x10^9$cfu/ml.

The regeneration of *Alcaligenes* sp. on heptane and 2M NaCl concentration is also indicated in Figure 1. The effect of this concentration of salt was similar to that of the 1M concentration. Similarly, the log phase began at Day 3 and lasted till Day 9. A decrease was observed from day 10 to day 13 and then an increase in growth was seen till Day 16. The lowest total bacteria count was recorded as $1.28x10^6$ cfu/ml on Day 0 while the highest reading was recorded on Day 15 as $1.33x10^9$ cfu/ml.

A higher concentration of NaCl salt (4M) was introduced into the reaction tubes. A rapid biodegradation of heptane was observed due to the addition of the salt. The log phase began right from Day 0 which had a bacterial count of $2.4x10^6$ cfu/ml and continued to increase up to Day 16.

DISCUSSION

In many ecosystems there is an adequate indigenous microbial community capable of extensive oil biodegradation, provided that environmental conditions are favorable for oil-degrading metabolic activities (Kim *et al.,* 2005).

The findings in this study indicated that *Alcaligenes* sp. was able to degrade heptane at any molar concentration of NaCl. *Alcaligenes* sp have been reported to thrive in high salt environment (Oren, 2002). The growth rate of *Alcaligenes* sp. is rapid and dynamic. The highest activity was observed in the reaction mixture with 4M, followed by the reaction mixture with 2M and then 1M.

This indicates that the rate of biodegradation was determined by how much NaCl was available to the organism in the medium. This is in agreement with the work done by Okoro and Amund (2015), who explained the high rate of biodegradability of heptane by *Alcaligenes* sp. when provided with favorable conditions such as a halophilic environment. The high increase exhibited by the organism in this halophilic environment compared to the neutral environment of the species in pure culture was due to a synergistic mechanism because of their ubiquity and diverse enzymatic activities under this extreme condition.

Okoro and Amund (2015), reported that *Alcaligenes* sp. is an excellent hydrocarbon degrader as the organism degraded hydrocarbons such as n-alkanes and polyaromatic hydrocarbons. Ajay and Vinay (2012) also confirmed a progressive and complete biodegradation by *Alcaligenes* sp. The research of Ijah and Antai (2003) also proved a progressive rate on degradation of Nigerian crude oil by *Alcaligenes* sp. In addition, the study done by Adetitun *et al.* (2019) validated the ability of *Alcaligenes* Strain 3k to make use of kerosene, hexadecane, cyclohexane among others as sole carbon sources and the isolate was able to degrade various lignocelluloses compounds.

Furthermore, as portrayed in the research of Igwo-Ezikpe *et al.* (2009), a strain of *Alcaligenes* (*Alcaligenes faecalis)* was shown to have emulsifying and hemolytic potentials on engine oil, kerosene, hexadecane, hexane among others and also capable of degrading

same. This is linked to their ability to stimulate the production of extracellular proteins, carbohydrates and biosurfactants that have been found beneficial for industrial purposes as well as for bioremediation. Apparently, indigenous microorganisms have made bioremediation of contaminated sites a generally accepted approach (Ali *et al*., 2020) and this suggests that each microbial genus has its role in hydrocarbon transformation processes (Ghazali *et al.,* 2004).

CONCLUSION

The findings from the growth patterns of *Alcaligenes* sp. in this experimental study revealed *Alcaligenes* sp. capability in efficiently utilizing heptane solely and in reaction with varying concentrations sodium chloride thereby enhancing biodegradation and bioremediation in an eco-friendly way.

CONFLICT OF INTEREST

The authors hereby declare no known conflict of interest associated with this work.

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