Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **4(9),** 60-67, September **(2015)** *Res.J.Recent Sci.*

Hydrocarbon Degradation and Biogas Production Efficiency of Bacteria isolated from Petrol Polluted Soil

Gopinath L.R.[1], Divya D.[1*], Gehitha T.R.[1], Bhuvaneswari R.[2], Archaya S.[1] and Merlin Christy P.[1]
[1]Department of Biotechnology, Vivekanandha College of Arts and Sciences for Women, Tiruchengode- 637 205, Namakkal, INDIA
[2]Department of Zoology, Namakkal Kavingnar Ramalingam Government Arts College for Women, Namakkal, INDIA

Available online at: www.isca.in, www.isca.me
Received 8th March 2014, revised 23rd June 2014, accepted 19th November 2014

Abstract

The present study framed to evaluate hydrocarbon degradation efficiency of bacteria isolated from petrol contaminated soil and its relevance in biogas production. Hydrocarbon degrading bacteria was isolated and selected by using Bushnell-Hass agar medium and identified as Pseudomonas stutzeri through biochemical analysis and 16S rRNA sequencing, and consequently obtained the accession number from the Genbank. The influence of various parameters like pH, temperature, incubation period and hydrocarbon concentration for the optimum degradation of petrol, diesel and crude oil with Pseudomonas stutzeri was examined during the study. The optimum pH and temperature for degradation of 1% hydrocarbons was found in the range of 8.0-8.5 and 30-55^{0}C respectively, whereas utmost degradation of 63.5% at pH 8.0 and 65.1% at temperature 30^{0}C was obtained on diesel. The present study was able to prove an incubation period of 7 days was fine for the degradation of all the three hydrocarbon used, of which greatest degradation of 74.4% was observed on crude oil. Since the growth of Pseudomonas stutzeri of different hydrocarbon concentration showed high growth occurs at 6% petrol, 4% diesel and 3% crude oil. Biogas production from cow dung with the incorporation of Pseudomonas stutzeri was able to increase the peak of methane production by 82.02% at 30th day from the peak of methane production of 57.29% at 45th day in control. Overall, around 24% increased methane production was observed in the treated tank in contrast to the control.

Keywords: Hydrocarbons, pollution, biodegradation, *Pseudomonas stutzeri*, bogas.

Introduction

The most important energy source used for industrial and domestic activity since last few decades is petroleum and its byproducts. A key problem associated with its production and transportation is it will release toxic compounds to the environment which may harmful to humans and other living organisms. Hence attention has been focused on the environmental problems due to the accidental release of hydrocarbons from the petroleum industry[1]. It is necessary to degrade these toxic substances into non-toxic substances in order to reduce environmental pollution. Mechanical and chemical methods were applied for degrading hydrocarbons, while the most accepted environmentally friendly method is the microbial degradation because the former two methods release less toxic compound to the environment besides hydrocarbon degradation[2,3]. Moreover, biodegradation offers environmental protection by means of degradation of pollutants like hydrocarbons and at the same time it is relevant for the degradation of various organic matters in the production of economically valuable alternative energy source biogas.

Biodegradation is the process in which organic materials having complex structures being converted to simpler substances in the presence of microorganisms. During biodegradation of hydrocarbon, the microorganisms convert hazardous substances into non-hazardous substances like nutrients, energy, etc., and hence this process is also referred to as bioremediation[4]. Microorganisms present in the soil, marine and freshwater habitat has the ability to degrade hydrocarbon promisingly[5]. Diverse microorganisms such as bacteria, fungi and protozoa are capable of hydrocarbon degradation, whereas the earlier study shows that compare to other organisms, bacteria have the potential to degrade hydrocarbons effortlessly[6]. Nevertheless various strategies could develop to ensure sustainability while that are restricted in some extent[7].

Environmental pollution by oil spills are the significant problem allied with most of the developing countries. Generally, hydrocarbon degrading microbes are associated with petroleum or oil spills contaminated sites[8]. Different types of bacteria involved in hydrocarbon degradation include *Pseudomonas sp*, *Proteus sp* and *Bacillus sp* and fungi include *Aspergillus sp.*[9,3]. In a recent study, petroleum degrading bacteria were isolated from cow dung and its activity at different pH, temperature and petroleum concentration were screened to find out potential hydrocarbon degrader[10]. However, it is crucial to identify efficient microbial strains from appropriate sources for bioaugmentation at the current scenario. In the light of the above aspects, the present study focused on the isolation and

identification of bacteria from petrol polluted soil, and to assess its ability to degrade various petroleum products such as petrol, crude oil and diesel.

It is the time that the depletion of fossil fuels occurs more rapidly when compared to its production, which point towards the exploration of sustainable energy resources in most of the countries[11]. Conversely, management of solid wastes and reduction of green house gases are necessary at this juncture[12,13]. In order to compensate these problems, an alternative energy source is needed. Biogas production technology ensures energy conservation in addition to environmental protection by means of degradation of organic materials[14]. Since, biogas is a renewable energy formed by the anaerobic digestion of organic matter by the sequential action of various microorganisms[15]. There are several strains of bacteria has been used in earlier for biogas production along with its other degradative applications, which includes *Bacillus, Staphylococcus, Proteus, Micrococcus, Klebsiella*, *Alcaligenes, Pseudomonas* and *Flavobacterium*[16,17,10]. Although, degradation potential of each organism depending upon the ability to utilize various substrates by producing suitable degradative enzymes. In this context, the current study also aimed to detect the biogas production efficiency of bacteria isolated from petrol polluted soil besides to detect its hydrocarbon degradation potential.

Methodology

The petrol contaminated soil sample was collected from near petrol bunks in Namakkal District, Tamilnadu and was aseptically transferred to the laboratory for the isolation of hydrocarbon utilizing bacteria. One gram of sample was primarily diluted in 100 ml sterile distilled water. Consequently, 1 ml diluent was serially diluted up to 10^{-6} and it was spread plated on Bushnell-Hass agar plate followed by incubated at 37^0C for 5-7 days[10]. Preliminary identification of the selected hydrocarbon degrading bacteria was done on the basis of morphological (Gram's staining) and physiological (IMViC, catalase and oxidase tests) characterization as per Bergey's manual.

Molecular identification was performed based on 16S rRNA sequencing. For sequencing, the genomic DNA from the bacteria was isolated as per Sambrook *et al*., 1989[18], and amplified with the universal primers such as 16SF "AGAGTTTGATCMTGGCTCAG" and 16SR "AAGGA GGTGATCCANCCRCA". The amplicon was run on agarose gel along with a marker of 1000 bps, and was then sequenced. This is followed by sequence similarity search was made by using the tool BLAST from the server NCBI[9]. Multiple sequence alignment of the query sequence along with sequences of four closely related strains was performed using the program Clustal W[19]. Phylogenetic analysis of aligned sequences was also conducted by means of Neighbor- Joining method through the software MEGA-4[20,21].

The efficacy of various hydrocarbon utilization of the isolate at four varied environmental conditions such as pH, temperature, incubation period and hydrocarbon concentrations was assessed[10]. For pH optimization, the isolate was individually inoculated into separate flasks containing 1% petroleum, 1% crude oil and 1% diesel incorporated Bushnell-Hass media of pH ranges 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5 respectively, and was incubated at 37^0C for 7 days. The temperature was optimized by the same manner, whereas the isolate inoculated flasks were incubated at different temperature ranges 15^0C, 20^0C, 25^0C, 30^0C, 35^0C, 40^0C, 45^0C, 50^0C, 55^0C, 60^0C, 65^0C and 70^0C, correspondingly.

Hydrocarbon degradation activity of the isolated strain for different hydrocarbons was detected through Bushnell-Hass media. For that, the isolate was individually inoculated on Bushnell-Hass media incorporated with 1% of three different hydrocarbons such as petrol, diesel and crude oil and was kept for 7 days at 37^0C for incubation. During the incubation period, the degradation rate was measured during each day time interval till 7^{th} day. This is followed by growth rate of the strain under a choice of petrol, diesel and crude oil concentration ranges from 1%-7% was studied after 7 days incubation period at 37^0C. Oil gravimetric method was used to find out the efficacy of hydrocarbon utilization by the isolate after incubation during optimization of all the four parameters[22].

Biogas production potential of isolated strain was studied by incorporating it into cow dung slurry in biogas production unit. For this, 20 liters of cow dung slurry were prepared by mixing cow dung and distilled water in the ratio 1:1 and then filled in a biogas unit. Followed by inoculum was mixed with cow dung slurry and then the unit was air tightened. Gas produced during five different time intervals (10^{th}, 20^{th}, 30^{th}, 40^{th} and 45^{th} day) until the 45^{th} day was collected and analysis of the obtained gas was performed through GC-MS analysis by using Poropak Q column[23].

Results and Discussion

For successful bioaugmentation and environmental protection, it is essential to introduce a suitable microorganism that has high capacity to degrade various hydrocarbons. Earlier studies showed that few microorganisms isolated from hydrocarbon polluted sites could survive in high hydrocarbon concentration and hence they can be used to reduce pollution with hydrocarbons[24,25]. However, in the present study, a prominent hydrocarbon degrading bacteria was successfully isolated and selected based on the highest zone of clearance accomplished on Bushnell-Hass agar medium.

Morphological studies evidenced that the isolate is Gram negative rod shaped motile bacteria and biochemical analysis showed positive outcomes for citrate, catalase and oxidase tests whilst negative results in the case of indole, methyl-red and Voges-proskaeur tests. Since the results of morphological and

biochemical studies, it is considered that the isolated strain belongs to the genera *Pseudomonas*. Stainer *et al* reported that bacteria belong to *Pseudomonas sp* have various interesting properties and are proficient for degrading various natural and synthetic organic compounds[26]. Different strains of *Pseudomonas sp* isolated from hydrocarbon contaminated environment implies like active degraders of hydrocarbon was genuinely accounted earlier[27,28]. However, these physiological studies as well revealed that the isolate can be used for biogas production because comparable pathways were also reported earlier in the case of biogas production[29,30].

Molecular level identification of the organism is important for the reason that which provide information at genetic level, which make possible to use them as widely for various industrial purposes. In the current study, DNA isolated from the strain was amplified and the amplicon showed a length equating to 274 base pairs compared to the marker on agarose gel (figure-1). BLAST analysis of the sequence obtained after 16S rRNA sequencing confirmed that the exacting organism is *Pseudomonas stutzeri.* An accession number JX442201.1 (*Pseudomonas stutzeri* strain ST-23) has been obtained after submission of the obtained sequence to NCBI-GENBANK. Phylogenetic analysis of *Pseudomonas stutzeri* ST-23 with five other closely related strains indicated that the targeted strain belongs to the minor clade and showed close relationship with the strain *Pseudomonas stutzeri* A1501. *Pseudomonas stutzeri* ST-23 has least divergence with the strains of the same species, including *Pseudomonas stutzeri* CCUG. Moreover, *Pseudomonas aeruginosa* PA38182, *Pseudomonas aeruginosa* PA01 and *Pseudoomonas mendocina* NK-01 were also arranged in the respective phylogenetic branch whilst showed considerable divergence from the targeted strain of *Pseudomonas stutzeri* (figure-2). Lalucat reported that *Pseudomonas stutzeri* showed close relationship with the strains of the same species that appeared as a single cluster in the phylogenetic trees analyzed, and all *Pseudomonas stutzeri* strains were positioned in the same phylogenetic branch while they were estranged from the other correlated species chosen such as *Pseudomonas balearica* and *Pseudomonas mendocina*[31].

An important environmental factor which strongly affects the growth of microorganism is pH. Hence it is an essential factor which needs to be maintained for proper hydrocarbon degradation[32]. Degradation efficiency of *Pseudomonas stutzeri* on different hydrocarbons at different pH levels shows that there is a gradual increase in degradation occurs from the pH 7.0 to 8.5 while the finest pH range of degradation was established as 8.0-8.5. Highest degradation of 63.5% for diesel followed by 57.2% for crude oil was observed at a pH close to 8.0 and 54.6% highest degradation was observed in the case of petrol at a pH of 8.5 (figure-3). Hence the study recognized that the growth of *Pseudomonas sp* decreased in low pH, while increased subsequently from 7.0 and finally peaked at 8.5. This is because of low pH of the environment leads to the production of acidic metabolites such as organic acids, which severely affects the growth of this organism and hence *Pseudomonas sp* tends to be growing at high pH of 8.00 for their proper growth[33]. However, it was clear that *Pseudomonas stutzeri* is vulnerable for acidogenesis while there has a possibility to use it for other stages of biogas production.

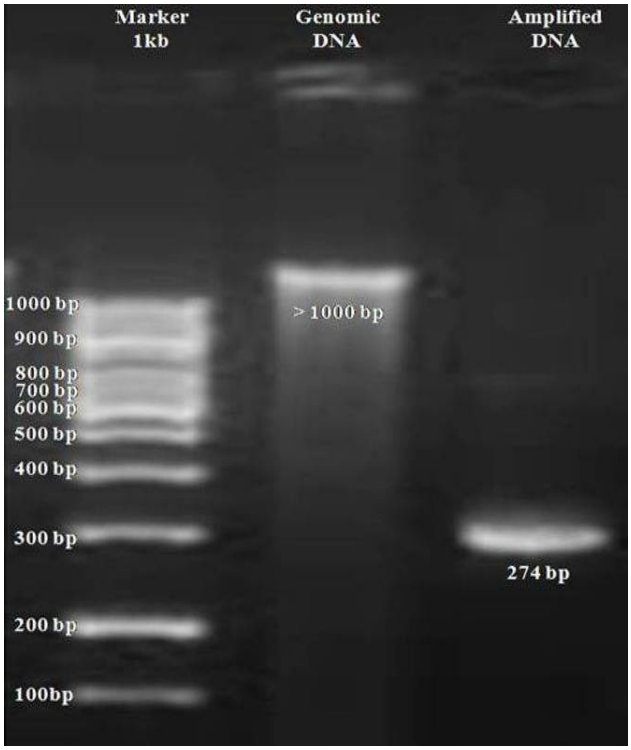

Figure-1
Isolated and amplified DNA

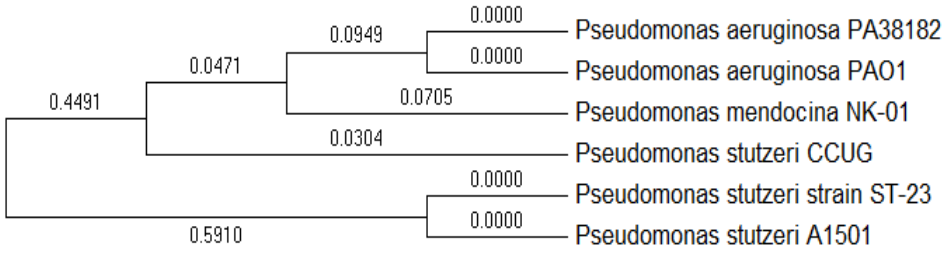

Figure-2
Phylogenetic analysis of *Pseudomonas stutzeri* strain ST-23

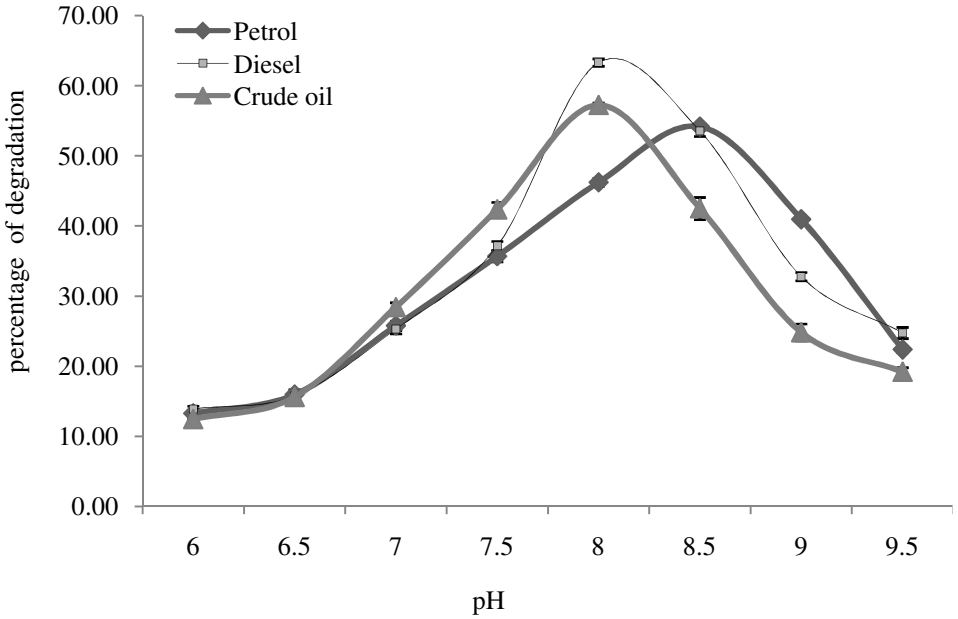

Figure-3
Hydrocarbon degradation by *Pseudomonas stutzeri* at different pH

Temperature optimization is essential because which cruelly affect the growth of microorganisms. The temperature at which the organism grow well is depending upon the habitat where they grown[34]. The competence of *Psudomonas stutzeri* for hydrocarbon degradation at different temperature was tested also provided good results, in which optimum temperature for degradation was observed in the range 30-50^{0}C. Specifically, the high range of 65.1% degradation was found for diesel at 30^{0}C, followed by 59.0% in the case of petrol at 40^{0}C and finally 57.4% for crude oil at 50^{0}C after incubation (figure-4). A similar study by Joshi and Pandey, indicated that the optimum temperature for degradation of petroleum hydrocarbon by *Pseudomonas sp* is 37^{0}C with an efficiency of 40.01%, which was relatively lower than the efficiency of *Pseudomonas stutzeri* obtained during the present study[10].

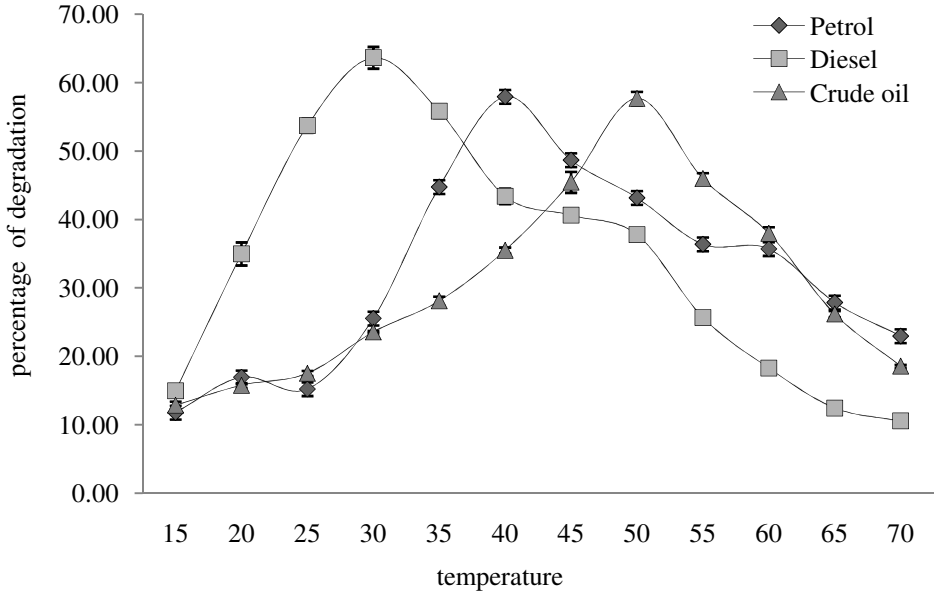

Figure-4
Hydrocarbon degradation by *Pseudomonas stutzeri* at different temperature

Hydrocarbon degradation activity is restricted in some groups of microorganisms and that is mainly depending upon the type and concentration of hydrocarbons they can able to utilize. Furthermore, the time duration for the degradation is also coupled with the activity of microorganism used for the purpose. Several workers have tested microorganisms in order to determine its hydrocarbon degradation activity in earlier[6]. Among the hydrocarbon degraders, some strains of *Pseudomonas sp* were extensively studied previously because of its higher degradation activity[35,27]. Conversely, in the present study degradation activity of *Pseudomonas stutzeri* on 1% hydrocarbon up to 7 days showed that a high degradation rate of 73.4 % on crude oil, 68.2% on petrol and 67.8% on diesel was observed at 7th day (figure-5). This is higher and promisingly comparable to the results of Joshi and Pandey, 2011; in which 41.23% degradation was obtained for degradation of toluene after 7 days incubation[9].

Despite the fact that sometimes higher concentration of hydrocarbon in the environment would cause lethal effects on microorganisms which also reduce hydrocarbon degradation rate[36]. There were some reports on microbial degradation showed that better growth occurs at lower concentration ranging from 0.5-1.5%[32]. Although, the present study concurrent with those earlier publications and endeavor to determine the tolerance of *Pseudomonas stutzeri* at various concentrations of three different hydrocarbons such as petrol, crude oil and diesel. Growth of *Pseudomonas stutzeri* under different hydrocarbon concentration after seven days showed high growth of 0.940 occurred at 4% diesel, 0.870 at 6% petrol and 0.840 were at 3% crude oil (figure-6). It was also exposed from the study that the isolated *Pseudomonas stutzeri* capable of degrading petrol, crude oil and diesel effectively.

Today, the depletion of fossil fuels occurs more rapidly that mainly enforces the production of biogas as a renewable energy source from diverse organic compounds[37]. Various organisms are capable of degrading these compounds and are ubiquitous in nature. Few studies reported that some hydrocarbon degraders are gifted with a property of biogas production. One of the bacteria capable of degrading hydrocarbon exploited for biogas production is *Pseudomonas aeruginosa*, which showed moderately higher methane production at 30th day[38]. It has been seen in the present study that physiological characterization of *Pseudomonas stutzeri* proves they can able to carry out some metabolic pathways competence with the function of hydrolysers, and hence they might be used for digestion of organic matters. By considering the above concept, the present study also utilized an efficient hydrocarbon degrader *Pseudomonas stutzeri* strain ST-23 in order to evaluate its biogas production efficiency on the potential substrate cow dung. However, *Pseudomonas stutzeri* incorporated biogas production unit provided more biogas compared to that of control throughout the study period. The results indicated that consequently methane concentration increased with decreasing carbon dioxide concentration from the 10th day to 30th day. Highest methane concentration of 82.02±0.11% was observed at 30th day in *Pseudomonas stutzeri* treated biogas plant, whereas only 46.22±0.86% production rate was observed at 30th day in control. The concentration of methane was increased to 57.29±0.99% in control, at the same time it was decreased to 57.51±0.74 in the case of *P. stutzeri* treated plant from 30th day to 45th day (table-1). Hence there is a decrease of highest production peak to 30th day from 45th day in *P. stutzeri* treated plant and as a result about 24% increase in methane production was observed in it compared to control.

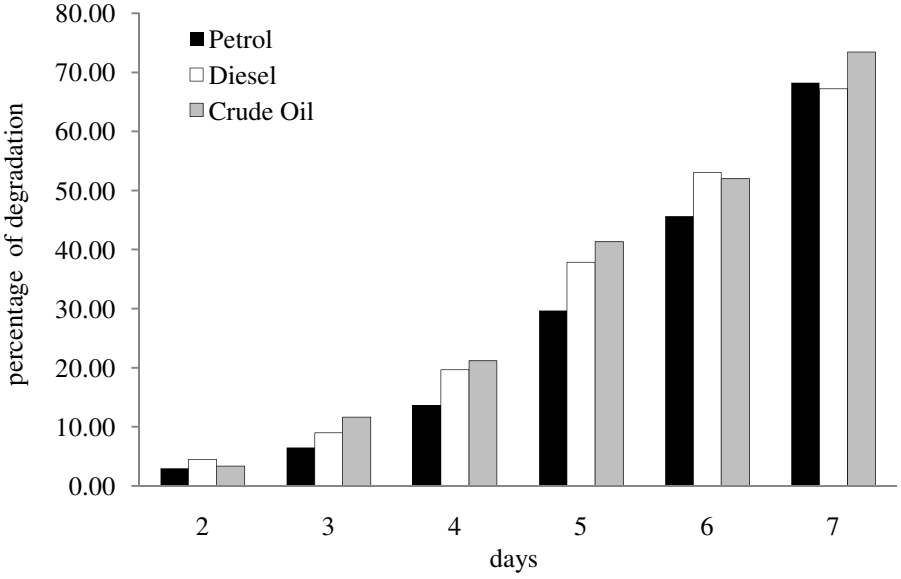

Figure-5
Degradation of 1% hydrocarbon by *Pseudomonas stutzeri* at different incubation period

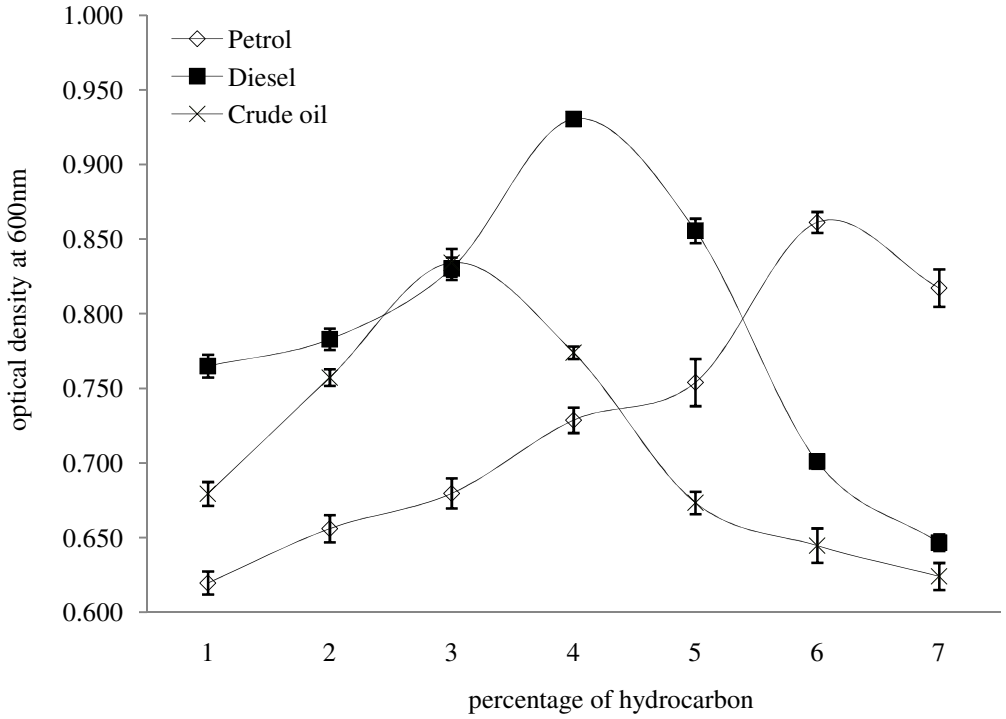

Figure-6
Growth of *Pseudomonas stutzeri* on different concentrations of hydrocarbon

Table-1
Biogas characteristics during the experiment

Gas produced	**Control**				
	10^{th} day	20^{th} day	30^{th} day	40^{th} day	45^{th} day
Methane	10.27 ± 0.16	33.70 ± 1.39	46.22 ± 0.86	51.94 ± 1.61	57.29 ± 0.99
Carbon dioxide	65.38 ± 0.04	45.99 ± 0.43	33.63 ± 0.71	25.30 ± 0.53	17.89 ± 0.87
Hydrogen sulfide	22.11 ± 0.89	15.78 ± 0.69	11.33 ± 0.16	11.51 ± 1.27	10.08 ± 0.49
Others	2.23 ± 1.51	4.53 ± 0.91	8.81 ± 0.23	11.25 ± 0.71	14.75 ± 0.62
Pseudomonas stutzeri					
Methane	12.17 ± 0.68	42.80 ± 0.34	82.02 ± 0.11	75.86 ± 0.48	57.51 ± 0.74
Carbon dioxide	65.17 ± 1.22	37.82 ± 0.21	9.71 ± 0.13	13.15 ± 0.30	13.05 ± 1.40
Hydrogen sulfide	20.30 ± 0.35	15.44 ± 1.11	5.52 ± 0.87	8.72 ± 1.13	21.85 ± 0.78
Others	2.36 ± 0.59	3.94 ± 0.74	2.76 ± 0.66	2.27 ± 0.92	7.58 ± 0.92

Conclusion

An efficient strain for the degradation of hydrocarbon was profitably isolated and identified as a new strain of *Pseudomonas stutzeri*, through morphological, biochemical and taxonomical studies. The degradation activity of *Pseudomonas stutzeri* under different environmental parameters such as pH, temperature, time intervals and various hydrocarbon concentrations has been studied, which shows better results and thus this would be the preferable choice for biodegradation of hydrocarbons or bioaugmentation of hydrocarbon polluted sites instead of others. Incorporation of *Pseudomonas stutzeri* into a biogas production unit containing cow dung as substrate offered an increase of 24% biogas production on 30^{th} day with a drop of highest production peak to 30^{th} day from 45^{th} day in contrast to control. Hence, this study concluded that *Pseudomonas stutzeri* would have immense application in both bioremediation of hydrocarbons as well as biogas production from various organic compounds through biodegradation and thereby it would be feasible to diminish global environmental problems and energy crisis as an eco-friendly manner.

Acknowledgement

The second author grateful to the Department of Science and Technology, Government of India for providing INSPIRE fellowship to conduct a part of this research. The authors are thankful to Vivekanandha College of Arts and Sciences for Women for providing necessary facilities for present research work.

References

1. Peixoto R.S., Vermelho A.B. and Rosado A.S., Petroleum Degrading enzymes; bioremediation and new prospects, *Enz. Res*., **2011**, 1-7 **(2011)**

2. Das N. and Chandran P., Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview, *Biotechnol. Res. Internat*., **2011**, 1-13 **(2011)**

3. Oboh B.O., Ilori M.O., Akinyemi J.O. and Adebusoye S.A., Hydrocarbon Degrading Potentials of Bacteria Isolated from a Nigerian Bitumen (Transand) Deposit, *Nat. and Sci.*, **4(3)**, 51-57 **(2006)**

4. Bhattacharya S., Das A., Palaniswamy M. and Angayarkanni J., Molecular Characterization of *Pleurotus ostreatus* PO-3 Involved in Mycoremediation of Benzo (a) Pyrene, *Int. Res. J. of Biol. Sci*., **2(8)**, 48-53 **(2013)**

5. Atlas R.M. and Bartha R., Abundance, Distribution and Oil Degradation Potential of Microorganisms in Raritan Bay, *Environ. Pollut*., **4**, 291-300 **(1973)**

6. Ojo O.A., Petroleum-Hydrocarbon Utilization by Native Bacterial Population from a Waste Water Canal Southwest Nigeria, *Afr. J. Biotechnol*., **5(4)**, 333-337 **(2006)**

7. Mishra S.R., Mohanty M.K. and Pattanaik A.K., Preparation of Biodiesel from Crude Oil of Simarouba glauca Using CaO as a Solid Base Catalyst, *Res. J. of Rec. Sci*., **1(9)**, 49-53 **(2012)**

8. Rahman K.S., Rahman T., Perumalsamy L. and Banat I.M., Occurrence of Crude Oil Degrading Bacteria in Gasoline and Diesel Station Soils, *J. of Bas. Microbiol.*, **42**, 284-291 **(2002)**

9. Shukor M.Y., Hassan N.A.A., Jusoh A.Z., Perumal N., Shamaan N.A., MacCormack W.P. and Syed M.A., Isolation and Characterization of a *Pseudomonas* Diesel-Degrading Strain from Antartica, *J. Environ. Biol*., **30(1)**, 1-6 **(2009)**

10. Joshi P.A. and Pandey G.B., Screening of Petroleum Degrading Bacteria from Cow Dung, *Res. J. of Agricul. Sci.*, **2(1)**, 69-71 **(2011)**

11. Akhwanzada S.A., Mat T. and Razman B., An Analysis of Malaysian Renewable Energy Target Using Simulation Modelling Approach, *Res. J. of Rec. Sci*., **3(1)**, 38-44 **(2014)**

12. Maw T.T. and Raskar S.S., Management of Agriculture Waste from Market yard Through Vermicomposting, *Res. J. of Rec. Sci*., **1(ISC-2011)**, 289-296 **(2012)**

13. Ravuru N.R. and Suvikram Y.V.N.S., Carbon Credits- A step to sustainable future of the world, *Res. J. of Rec. Sci*., **1(ISC-2011)**, 388-397 **(2012)**

14. Divya D., Gopinath L.R. and Merlin Christy P., A review on Trends, Issues and Prospects for biogas production in Developing Countries, *Internat. Res. J. of Environ. Sci.*, **3(1)**, 62-69 **(2014)**

15. Omer A.M., Biomass and Biogas for Energy Generation: Recent Development and Perspectives, *Res. in Biotechnol*., **2(2)**, 36-49 **(2011)**

16. Adegunloye D.V., Adetuyi F.C., Akinyosoye F.A. and Doyeni M.O., Microbial Analysis of Compost Using Cowdung as Booster, *Pak. J. of Nutr.*, **6(5)**, 506-510 **(2007)**

17. Sharifi-Yazdi M.K., Azimi C. and Khalili M.B., Isolation and Identification of Bacteria Present in the Activated Sludge Unit, in the Treatment of Industrial Waste Water, *Iran. J. Publ. Heal.*, **30(3-4)**, 91-94 **(2001)**

18. Sambrook J., Fritsch E.F. and Maniatis T., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press **(1989)**

19. Thompson J.D., Higgins D.G. and Gibson T.J., CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Weighing, Position-Specific Gap Penalties and Weight Matrix Choice, *Nucl. Acid. Res*., **22**, 4673-4680 **(1994)**

20. Saitou N. and Nei M., The Neighbor-Joining Method: A

New Method of Reconstructing Phylogenetic Trees, *Mol. Biol. and Evol.*, **4**, 406-425 **(1987)**

21. Tamura K., Dudely J., Nei M. and Kumar S., MEGA4: Molecular Evolutionary Genetics Analysis Software Version 4.0, *Mole. Biol. and Evol.*, **24**, 1596-1599 **(2007)**

22. Chrzanowski L., Kaczorek E. and Olszanowski A., The Ability of Candida Maltose for Hydrocarbon and Emulsified Hydrocarbon Degradation, *Pol. J. of Environ. Stud*., **15**, 47-51 **(2006)**

23. Meher K.K. and Ranade D.R., Isolation of Propionate Degrading Bacterium in Co-culture with a Methanogen from a Cattle Dung Biogas Plant, *J. Biosci*., **18(2)**, 271-277 **(1993)**

24. Yousefi Kebria D., Khodadadi A., Ganjidoust H., Badkoubi A. and Amoozegar M.A., Isolation and Characterization of a Novel Native *Bacillus* Strain Capable of Degrading Diesel Fuel, *Inter. J. of Environ. Sci. and Technol*., **6**, 435-442 **(2009)**

25. Okerentugba P.O. and Ezeronye O.U., Petroleum Degradation Potentials of Single and Mixed Microbial Cultures Isolated from Rivers and Refinery Effluent in Nigeria, *Afr. J. of Biotechnol.*, **2**, 288-292 **(2003)**

26. Stanier A., Levi-Minzi and Riffaldi R., Maturity Evaluation of Organic Wastes, *Biocy.*, **29**, 54-56 **(1998)**

27. Mittal A. and Singh P., Isolation of Hydrocarbon Degrading Bacteria from Soils Contaminated with Crude Oil Spills, *Ind. J. of Experi. Biol.*, **47**, 760-765 **(2009)**

28. Adeline S.Y.T., Carol H.C.T. and Aw C.S., Hydrocarbon-Degradation by Isolate *Pseudomonas lundensis* UTAR FPE2, *Mal. J. Microbiol*., **5(2)**, 104-108 **(2009)**

29. Archer D.B., The Microbial Basis of Process Control in Methanogenic Fermentation of Soluble Wastes, *Enzy. Microb. Technol.*, **5**, 162-169 **(1983)**

30. Zinder S.H., Microbiology of Anaerobic Conversion of Organic Wastes to Methane: Recent Developments, *Amer. Soc. for Microbiol.*, **50**, 294-298 **(1984)**

31. Lalucat J., Bennasar, A., Bosch R., Garcia-Valdes E. and Palleroni N,J., Biology of *Pseudomonas stutzeri*, *Microbiol. Mol. Bio. Rev*., **70(2)**, 510-547 **(2006)**

32. Zhao D., Wu W., Zhang Y., Liu Q., Yang H. and Zhao C., Study on Isolation, Identification of A Petroleum Hydrocarbon Degrading Bacterium *Bacillus fuciformis sp.* and Influence of Environmental Factors on Degradation Efficiency, *China Petrol. Process. And Petrochem. Technol.*, **13(4)**, 74-82 **(2011)**

33. Naveenkumar S., Manoharan N., Ganesan S., Manivannan S.P. and Velusamy G., Isolation, Screening and Invitro Mutational Assessment of Indigenous Soil Bacteria for Enhanced Capability in Petroleum Degradation, **1(4)**, 498-513 **(2010)**

34. Ma Y., Wang L. and Shao Z., *Pseudomonas*, the Dominant Polycyclic Aromatic Hydrocarbon-Degrading Bacteria Isolated from Antartic Soils and the Role of Large Plasmids in Horizontal Gene Transfer, *Environ. Microbiol.*, **8**, 455-465 **(2006)**

35. Tang X., Zhu Y. and Meng Q., Enhanced Crude Oil Biodegradability of *Pseudomonas aeruginosa* ZJU After Preservation in Crude Oil Containing Medium, *Wor. J. Microbiol. Biotech*., **23 (1)**, 7-14 **(2007)**

36. Zhou C., Lin Q. and Su G., Isolation, Characterization and Study on Properties of a Strain for Degradation of Offshore Petroleum Hydrocarbons, *J. of Jimei Univer (Natural Science Selection).*, **14(1)**, 24-28 **(2009)**

37. Dhanalakshmi S. and Ramanujam R.A., Biogas Generation in a Vegetable Waste Anaerobic Digester: An Analytical Approach, *Res. J. of Rec. Sci*., **1(3)**, 41-47 **(2012)**

38. Potivichayanon S., Sungmon T., Chaikongmao W. and Kamvanin S., Enhancement of Biogas Production from Bakery Waste by *Pseudomonas aeruginosa*, *Wor. Acad. of Sci. Eng. and Technol*., **56**, 498-501 **(2011)**