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Effect of Environmental Factors on Bacterial Quorum Sensing

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Abstract

Biodegradation of complex hydrocarbons usually requires the cooperation of more than a single species of microorganisms including bacteria. This is particularly true in pollutants that are made up of many different compounds such as crude oil or petroleum, and where complete mineralization to CO_2 and H_2O is desired. An effort has been made to form the consortium of bacterial isolates (Qs1, Qs2 and Qs5) which are isolated from oil contaminated soil, and the effects of different environmental factors on these consortium has been studied . The growth of the consortium was studied at 6.5 pH and 35^{0}C like the individual bacterial isolates on the different hydrocarbons (xylene, toluene, hexane, diesel, benzene and petrol). These consortium of bacterial isolates, shared more efficient utilization of hydrocarbon as carbon source. This consortium shows confluent growth- at pH 6.0, 5.5, and 5.0 but survival rate decreases at pH above 6.5. Extremes in pH were shown to have a negative influence on the ability of microbial populations to degrade hydrocarbons. They also show the higher growth rate at the higher temperature range (up to 40^{o}C) but their growth rate decreases at lower temperature range (below 25^{0}C). It is suggested that the use of above bacterial consortium (at 35^{0}C temperature and 6.5pH) will be an effective and eco-friendly technology for the remediation of hydrocarbons.

Key words: Bacteria, Biodegradation, Consortium, Hydrocarbons, Quorum sensing.

Introduction

Bacteria are unable to insulate themselves from the environment and they react to any fluctuation by changing their own physiological functions. The membrane is the site of the primary contact of the cell with environment (1). Many external factors such as temperature, pH, nutrients, enzyme action and hydrocarbons affect the physio-chemical proteins of membrane and consequently their functioning. These changes include balance between bilayer and non-bilayer lipids, stability and fluidity of membrane as well as lipid protein interaction. Individual microorganisms can metabolize only a limited range of hydrocarbon substrates, so assemblages of mixed populations with overall broad enzymatic capacities are required to bring the rate and extent of petroleum biodegradation further (2). Microbial consortium containing a number of microorganisms which synthesize the degradative enzymes for different parts of the decomposition pathway is considered to be well suited to the degradation of aromatic hydrocarbons microbial consortium containing a number of microorganisms which synthesize the degradative enzymes for different parts of the decomposition pathway is considered to be well suited to the degradation of aromatic hydrocarbons (3).This research was carried out to explore the possibility of the use of selected bacterial cultures and a mixed bacterial consortium to degrade a crude oil at various pH, temperatures, and concentrations of hydrocarbons.

Materials and methods

Bacterial Isolation

Soil samples were collected in pre-sterilized glass bottle from an oil refinery in Guwahati, India. Enrichment and isolation of oil-degrading bacterial cultures were done using mineral salts medium contains KH_2PO_4 (1.3gl^{-1}), (K_2HPO_4 (1.8 gl^{-1}); NH_4Cl (4 gl^{-1}); $MgSO_4.7H_2O$ (0.2 gl^{-1}); carbon source (1% benzene, diesel, hexane, petrol, toluene, and xylene); and distilled water (1L) with pH 6.5 and then was incubated at 37 ^{0}C for 5-10 days. After the incubation period, serial dilution-agar plating technique on mineral salt medium was carried out (4). Hydrocarbons and other fine chemicals employed in the study were of the highest purity grade, produced by Merck (Germany). The isolated bacterial cultures were characterized by their morphological and biochemical characteristics (5).

Screening of Isolates

Bacterial cultures (24 hours old) were inoculated in mineral salts medium with the range (0 .006%-0.05 %) of different hydrocarbons (benzene, diesel, petrol, hexane, xylene, and toluene individually) as a carbon source. They were kept in a shaker at 200 rpm at 35^{0}C for a period of 96 hours (6). The growth was monitored through culture densities, measuring the absorption at 595 nm, spectrophotometrically (7).The isolate showed the maximum utilization (in terms of O.D. by spectrophotometer) at the concentration of 0.01% of selected hydrocarbons. The isolates with highest rate of hydrocarbon utilization were selected for the further study.

Growth Study of Consortium

A loop of overnight grown culture was used to inoculate 100 ml sterile mineral salt medium. The flasks were kept in a shaker at 200 rpm for 24 hours at 35^{0}C. Equal volumes of culture broth from the selected isolates (Gh6, Gh7, Gh8, Gh9, and Gh10) were used to prepare the bacterial consortium. This bacterial consortium (1 ml) was inoculated in 50 ml mineral salt me-

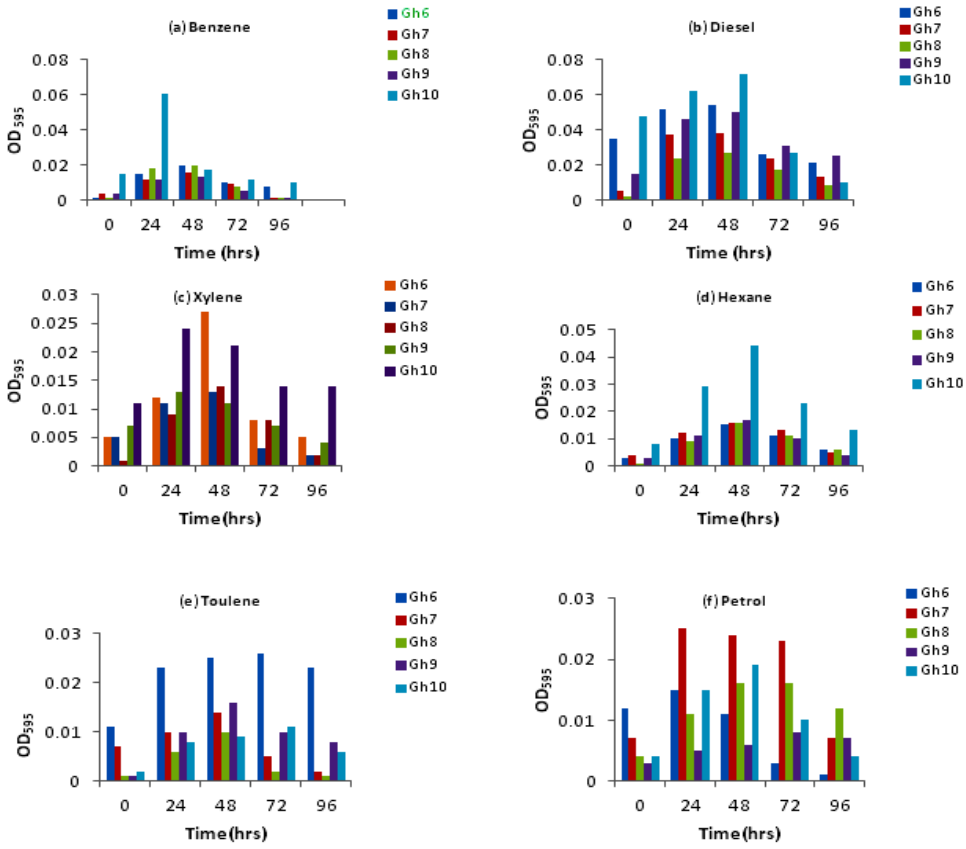

Figure1. Growth of individual isolates (control) on different hydrocarbons.

Table 1. Morphological characterization of selected bacterial isolates.

S. No.	Bacterial isolate	Colony shape	Colony colour	Opacity	Elevation	Texture
1	Gh6	Round	Cream	Translucent	Convex	Smooth
2	Gh7	Round	Cream	Translucent	Convex	Smooth
3	Gh8	Round	Cream	Translucent	Raised	Smooth
4	Gh9	Round	Cream	Translucent	Convex	Smooth
5	Gh10	Round	Cream	Translucent	Pulvinate	Smooth

dium with 0.01% of different hydrocarbon. The growth was monitored through culture densities, measuring the absorption at 595 nm, spectrophotometrically at regular interval of 24 hours (8). Quantitative estimation of protein was also performed (9).

pH and Temperature Studies

The influence of pH on the growth and degradation of 0.01% of hydrocarbon (as described above) was studied at every 24 hour time interval up to 96 hours. To maintain the pH of media citrate–phosphate buffer (pH 5.5-6.5), phosphate buffer (pH 7 and pH 8), and Borax buffer (pH 8.5 and pH 9) were used (3). The flasks were inoculated with bacterial consortium incubated at 35^0C. The growth of consortium at different hydrocarbon at different time intervals was monitored. The effect of temperature (25–45^0C) on the growth and utilization of hydrocarbons was studied using mineral salts medium with 0.01% hydrocarbon at pH 6.5 with incubation time of 96 hours. The growth of consortium was determined.

Results and Discussion

Morphological identification (Table 1) and number of biochemical tests (Table 2) of selected isolates were

Table 2. Gram staining and cellular characterization in terms mode of arrangements.

S. No.	Bacterial Isolates	Gram Reaction	Shape	Mode of arrangement
1	Gh6	**+ve**	Bacilli	Dispersed
2	Gh7	**+ve**	Cocci	Discrete
3	Gh8	**+ve**	Bacilli	Diplobaccilli
4	Gh9	**+ve**	Bacilli	Dispersed
5	Gh10	**+ve**	Bacilli	Dispersed

carried out. Studies on the growth pattern (Figure:1) of bacterial isolates (Gh6, Gh7, Gh8, Gh9 and Gh10) and their consortium at pH 6.5 and 35 ± 1^0C have shown confluent growth. Therefore, further study of environmental factors was carried with the consortium.

Effect of pH on Growth

It is evident from Figure:2 (a, e ,and f) that the consortium showed the optimal growth at pH 6 when it

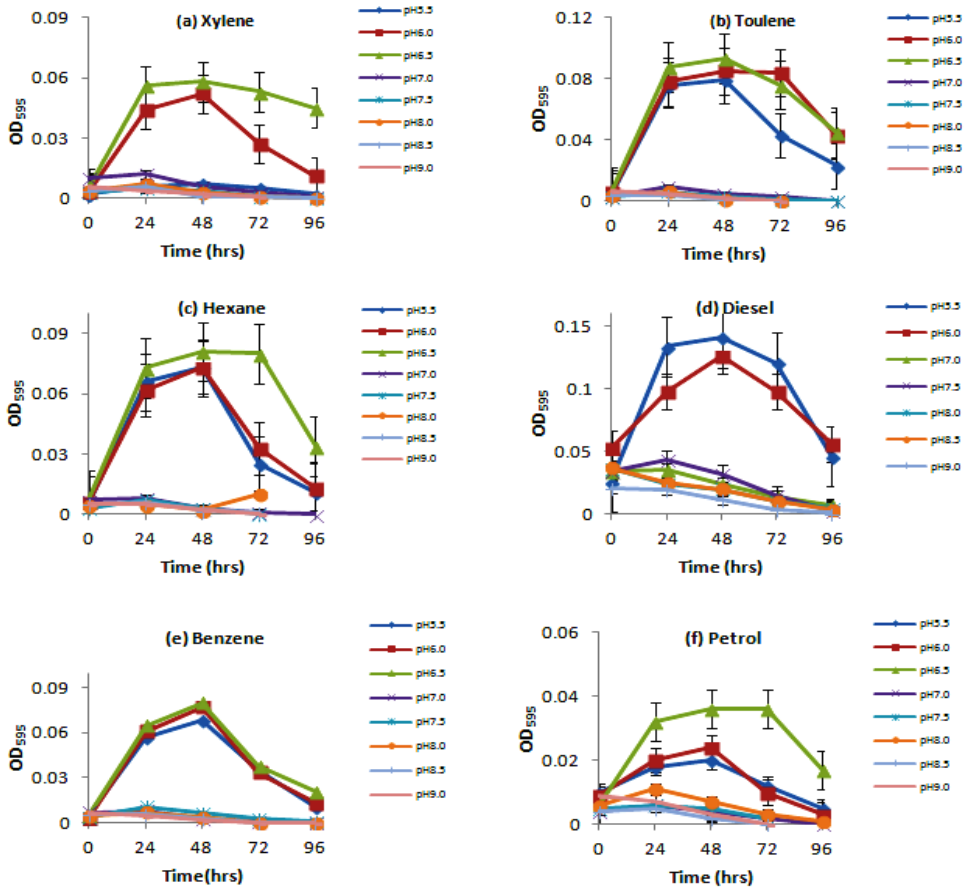

Figure2. Growth of consortium Qs1 at different pH.

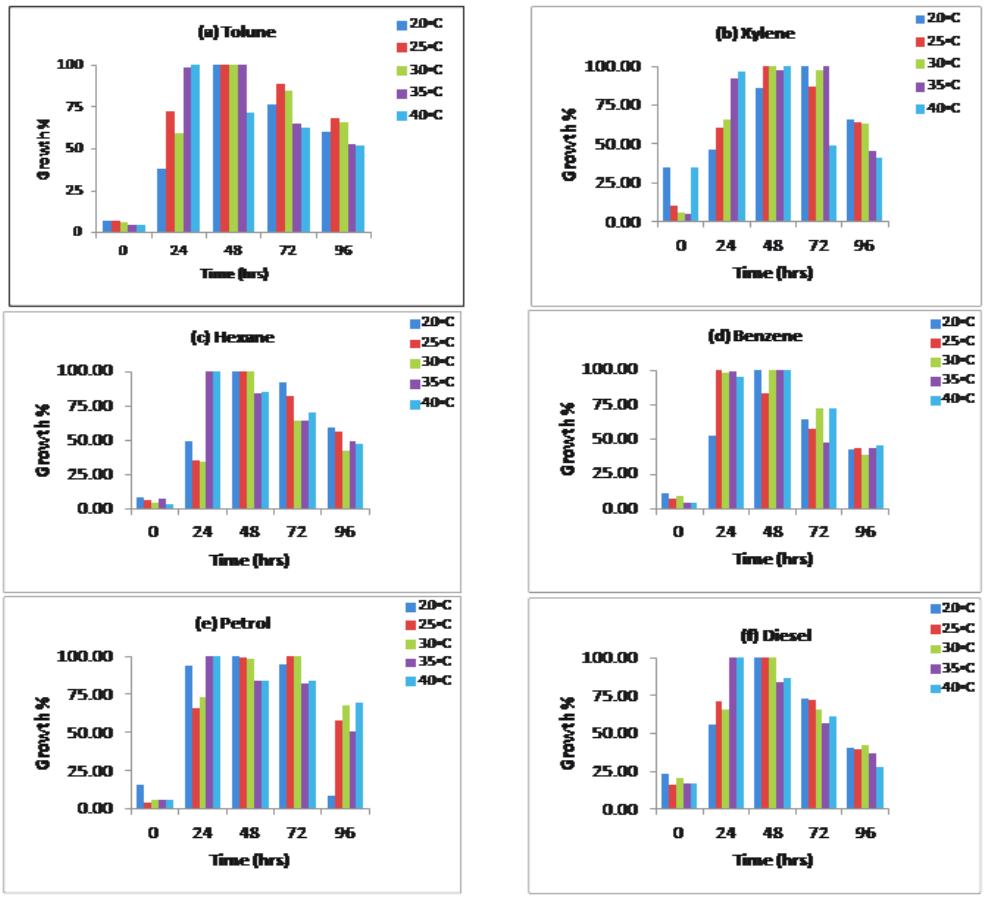

Figure3. Growth of consortium Qs1 at different temperature.

used petrol, xylene, and diesel as a carbon source .When the concentration of OH^- increased in the medium, accumulation of different lipophillic compounds occur at varying depths in the bilayer which results in the membrane losing its integrity (10) .It was also noted that the consortium showed the maximum growth at pH 6.5 (recorded in terms of O.D. by spectrophotometer) because at this pH cell membrane decreases the cell fluidity by

increasing the quantity of branched and saturated fatty acids to tolerate benzene and toluene (11).

Effect of Temperature on Growth

The bacterial consortium and individual bacterial cultures showed maximum growth in different hydrocarbon at 35^{0}C (Figure: 3). When there was decrease in temperature (25-20^{0}C) the growth of consortium also decreased(in terms of O.D. by spectrophotometer) and with increase in temperature the rate of hydrocarbon, utilization typically in the range of 35–40^{0}C increased (in terms of O.D. by spectrophotometer) for up to 24 hours because at the lower temperature water solubility of hydrocarbons decreases.

The optimum growth of the consortium at 35^{0}C was because as temperature rises, the lipid membranes are melted and trans-gauche rotations are able to propagate freely up and down the acyl chain which help the movement of benzene, toluene, and xylene (Figure: 3: a, b, and d) inside the bacterial cell (1) hence consortium can utilize diesel and petrol as a carbon source which is exhibited by the optimum growth at 25^{0}C. At this temperature, there was a change in branched and unsaturated fatty acid, which in turn helps in the lowering in the surface tension of the ell envelope (Figure: 3 :e, and f) (12). 30^{0}C is the optimum temperature for hexane utilization as a carbon source because at this temperature in active transport , proton motive force decreases and the amount of saturated fatty acid increases which help in the utilization of hexane (Figure: 3c).

The present findings are in agreement with AL-Saleh *et al* . (13) , Bordenave, *et al* .(14) Vasudevan and Rajaram (15) and Rahman *et al.* (16) that have illustrated the ability of bacterial consortia to utilize crude oil. Hamme *et al*.,(17) reported that mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil or diesel fuel. Such mixed cultures display metabolic versatility and superiority to pure cultures. The individual isolates and bacterial consortium showed optimal values of pH 6.5 and temperature (25- 35^{0}C) for maximum utilization of hydrocarbon. The researcher reported pH range from 6.0-7.0 as the optimal range for hydrocarbon degradation by bacterial consortium (16). Extremes in pH were shown to have a negative influence on the ability of microbial populations to degrade hydrocarbons (18). It seems that positive effects are related to variation in isoelectric state with respect to pH, which maintains the membrane integrity and fluidity. At low temperatures, the viscosity of the oil is increased, volatilization of alkanes is reduced, and the water solubility is decreased, delaying and decreasing the onset of biodegradation. Similarly, Rahman, *et al.* (16) reported 30 - 40^{0}C as optimum temperature for the degradation of crude oil by individual and mixed consortium of bacterial cultures. The present study revealed that the mixed bacterial consortium achieved maximum crude oil degradation at pH range 6.0-7.0 and 25-40^{0}C. Hence, it can be suggested that the use of above bacterial consortium under optimized conditions would be a better choice for further studies. This is because in their native state bacteria are highly under the control of environmental conditions, whereas, they are more stable and resilient when grouped together in consortia.

Other articles in this theme issue include references (19-34).

References

1. Mrozik,A., Piotrowska-Seget, Z. and Łabużek S., Cytoplasmatic Bacterial Membrane Responses to Environmental Perturbations . *Pol. J. Environ. Stud.* 2004, **13 (5)**: 487-494.

2. Sorkhoh, N.A., Ibrahim, A.S., Ghannoum, M.A., Radwan, S.S., High temperature hydrocarbon degradation by Bacillus stearothermophilus from oil-polluted Kuwait desert. *Appl. Microbiol. and Biotechnol*. 1993, **39**: 123–126.

3. Sathishkumar, M., Binupriya, A.R., Baik, S., Yun, S.E., Biodegradation of Crude Oil by Individual Bacterial Strains and a Mixed Bacterial Consortium Isolated from Hydrocarbon Contaminated Areas. *Clean.* 2008, **36 (1)**: 92–96.

4. Boboye, B., Olukunle, O. F., and Adetuyi, F. C., Degradative activity of bacteria isolated from hydrocarbon-polluted site in Ilaje, Ondo State, Nigeria. *Afr. J. Microbiol.* 2010, **4 (23)**: 2484-2491.

5. Cappuccino, J. G. and Sherman, N., In: Microbiology: a laboratory manual. Pearson Education, USA, 2004, pp.156-160.

6. Kim, I.S., Lee, H., Trevors, J.T., Effects of 2, 2', 5, 5'-tetra- chlorobiphenyl and biphenyl on cell membranes of Ralstonia eutropha H850. *FEMS Microbiol. Lett.* 2001, **200**: 17.

7. Kumar, G., Singla, R., and Kumar, R., Plasmid Associated Anthracene Degradation by *Pseudomonas* sp. Isolated from Filling Station Site. *Nature and Science*. 2010, **8 (4)**: 89.

8. Varjani, S.J and Upasani, V.N., Comparative Studies on Bacterial Consortia for Hydrocarbon Degradation. *Int. J. Innov. Res. Sci. Eng. Technol*. 2013, **2 (10)**: 5377-5383.

9. Lowry, O.H., Rosenbrough, N.H., Farr, A.L., Randall, R.J., Protein measurement with the folin-Phenol reagent. *J.Biol.Chem*. 1951, **193**: 265-275.

10. Sikkema, J., De Bont, J.A.M., Poolman, B., Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev*. 1995, **59**: 201.

11. Gutierrez, J.A., Nichols, P., Couperwhite, I., Changes in whole cell-derived fatty acids induced by benzne and occurrence of the unusual 16:1ω6c in *Rhodococcus sp.* 33. *FEMS Microbiol. Lett*. 1999, **176**: 213.

12. Härtig, C., Loffhagen, N., and Harms, H., Formation of *trans* Fatty Acids Is Not Involved in Growth-Linked Membrane Adaptation of *Pseudomonas putida. Appl.Environ.Microbiol*. 2005, **71 (4)**: 1915-1922.

13.AL-Saleh, E., Drobiova, H. and Obuekwe., C., Predominant culturable crude oil-degrading bacteria in the coast of Kuwait. *Int. Biodeter. Biodegr.* 2009, **63 (4)**: 400–406.

14. Bordenave, S, Goñi-Urriza, M. S., Caumette, P. and Duran, R., Effects of heavy fuel oil on the bacterial community structure of a pristine microbial mat. *Appl. Environ. Microbiol*. 2007, **73 (19)**: 6089–6097.

15. Vasudevan, N. and Rajaram, P. Bioremediation of Oil Sludge Contaminated Soil. *Environ. Int.* 2001, 26: 409–411.

16. Rahman, K.S.M., Vasudevan, N. Lakshmanaperumalsamy, P., Enhancement of Biosurfactant Production to Emulsify Different Hydrocarbons, *J. Environ. Pollut*. 1999, **6**: 87–93.

17. Hamme, J. D., Odumeru, J. A. and Ward, O. P., Community Dynamics of a Mixed-Bacterial Culture Growing on Petroleum Hydrocarbons in Batch Culture. *Can. J. Microbiol*. 2000, **46**: 441 – 450.

18. Rahman, K.S.M., Rahman, J. T ., Lakshmanaperumalsamy, P., Banat, I. M., Towards Efficient Crude Oil Degradation by a Mixed Bacterial Consortium. *Bioresour. Technol.* 2002, **85**: 257 – 261.

19. Singh, V. K., Singh, M. P. Bioremediation of vegetable and agro-wastes by *Pleurotus ostreatus*: a novel strategy to produce edible mushroom with enhanced yield and nutrition. *Cell. Mol. Biol.* 2014,

60 (5): 2-6. doi: 10.14715/cmb/2014.60.5.2
20. Vishnoi, N., Singh, D. P., Biotransformation of arsenic by bacterial strains mediated by oxido-reductase enzyme system. *Cell. Mol. Biol.* 2014, **60 (5)**: 7-14. doi: 10.14715/cmb/2014.60.5.3
21. Srivastava, A. K., Vishwakarma, S. K., Pandey, V. K., Singh, M. P., Direct red decolorization and ligninolytic enzymes production by improved strains of *Pleurotus* using basidiospore derived monokaryons. *Cell. Mol. Biol.* 2014, **60 (5)**: 15-21. doi: 10.14715/cmb/2014.60.5.4
22. Kumari, B., Rajput, S., Gaur, P., Singh S. N., Singh D. P., Biodegradation of pyrene and phenanthrene by bacterial consortium and evaluation of role of surfactant. *Cell. Mol. Biol.* 2014, **60 (5)**: 22-28. doi: 10.14715/cmb/2014.60.5.5
23. Pandey, V. K., Singh, M. P., Biodegradation of wheat straw by *Pleurotus ostreatus. Cell. Mol. Biol.* 2014, **60 (5)**: 29-34. doi: 10.14715/cmb/2014.60.5.6
24. Pathak, V. V., Singh, D. P., Kothari, R., Chopra, A. K., Phycoremediation of textile wastewater by unicellular microalga *Chlorella pyrenoidosa*. *Cell. Mol. Biol.* 2014, **60 (5)**: 35-40. doi: 10.14715/cmb/2014.60.5.7
25. Pandey, A. K., Vishwakarma, S. K., Srivastava, A. K., Pandey, V. K., Agrawal, S., Singh, M. P., Production of ligninolytic enzymes by white rot fungi on lignocellulosic wastes using novel pretreatments. *Cell. Mol. Biol.* 2014, **60 (5)**: 41-45. doi: 10.14715/cmb/2014.60.5.8
26. Singh, M. K., Rai, P. K., Rai, A., Singh, S., Alterations in lipid and fatty acid composition of the cyanobacterium *Scytonema geitleri* bharadwaja under water stress. *Cell. Mol. Biol.* 2014, **60 (5)**: 51-58. doi: 10.14715/cmb/2014.60.5.10
27. Singh, M. P., Pandey, A. K., Vishwakarma, S. K., Srivastava, A. K., Pandey, V. K., Singh, V. K., Production of cellulolytic enzymes by *Pleurotus* species on lignocellulosic wastes using novel pretreatments. *Cell. Mol. Biol.* 2014, **60 (5)**: 59-63. doi: 10.14715/cmb/2014.60.5.11
28. Chandra, P., Singh, D. P., Removal of Cr (VI) by a halotolerant bacterium *Halomonas* sp. CSB 5 isolated from sāmbhar salt lake Rajastha (India). *Cell. Mol. Biol.* 2014, **60 (5)**: 64-72. doi: 10.14715/cmb/2014.60.5.12
29. Tewari, S., Arora, N. K., Talc based exopolysaccharides formulation enhancing growth and production of *Hellianthus annuus* under saline conditions. *Cell. Mol. Biol.* 2014, **60 (5)**: 73-81. doi: 10.14715/cmb/2014.60.5.13
30. Kumar, M., Singh, P., Tripathi, J., Srivastava, A., Tripathi, M. K., Ravi, A. K., Asthana, R. K., Identification and structure elucidation of antimicrobial compounds from *Lyngbya aestuarii* and *Aphanothece bullosa*. *Cell. Mol. Biol.* 2014, **60 (5)**: 82-89. doi: 10.14715/cmb/2014.60.5.14
31. Arun, N., Vidyalaxmi, Singh, D. P., Chromium (VI) induced oxidative stress in halotolerant alga *Dunaliella salina* and *D. tertiolecta* isolated from sambhar salt lake of Rajasthan (India). *Cell. Mol. Biol.* 2014, **60 (5)**: 90-96. doi: 10.14715/cmb/2014.60.5.15
32. Prakash, S., Singh, R., Lodhi, N., Histone demethylases and control of gene expression in plants. *Cell. Mol. Biol.* 2014, **60 (5)**: 97-105. doi: 10.14715/cmb/2014.60.5.16
33. Singh, A. K., Singh, M. P., Importance of algae as a potential source of biofuel. *Cell. Mol. Biol.* 2014, **60 (5)**: 106-109. doi: 10.14715/cmb/2014.60.5.17
34. Dixit, S., Singh, D. P., Role of free living, immobilized and non-viable biomass of *Nostoc muscorum* in removal of heavy metals: An impact of physiological state of biosorbent. *Cell. Mol. Biol.* 2014, **60 (5)**: 110-118. doi: 10.14715/cmb/2014.60.5.18