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Evaluating Bacterial Cell Immobilization of *Brevibacillus formosus* BISR-1 and *Paenibacillus* sp. BISR-047 with Different Matrices

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***Abstract*:- Present scenarios of the world's biotechnological industries are, enhancement in enzyme productivity and development of new techniques for increasing their shelf life. Immobilization methodologies provide a base to fulfil all the requirements. Several natural and synthetic supports have been assessed for their efficiency for enzyme immobilization. In the present study several adsorption matrices have been used to study whole cell immobilization. *Brevibacillus formosus* BISR-1 and *Paenibacillus* sp. BISR-047, both the strains shows higher chitinase activity (425 IU/ml; 580 IU/ml, respectively) at 2 % entrapment material concentration in all approaches; whereas cells immobilized using agar beads performed better in entrapment outcomes as compared to agarose and alginate beads. In case of reusability, Agar-agar beads retained more than 95 % residual activity even up to 5th cycle and more than 60 % up to 10th cycle of re-use.**

Key words: Chitinase; immobilization; Paenibacillus sp.; Brevibacillus formosus; shelf life.

1.INTRODUCTION

Chitin and chitinase have received a growing interest over the last decades due to their versatile biotechnological applications and were produced from many microorganisms [1, 2]. Biotechnological methodologies based on whole cells immobilization have been developed rapidly over the last decades. Immobilized whole cells have been widely used at large scale in the production of pharmaceutical important compounds (antibiotics and drugs), and industrially important chemicals (chitosan, actone, butanol etc.). Generally, immobilization of cells could be carried out by either entrapment of the microorganisms in porous polymers or microcapsules or binding to an organic or inorganic support matrix [3]. Adsorption in addition to its simplicity has the possible advantages of reducing or eliminating the mass transfer problems associated with polymer entrapped cells, more particularly those using viable, metabolically active microorganisms. For a long time, basic studies of the physiological behaviour of immobilized viable cells have remained in the shadow of the applications. Natural immobilized cell structures are being increasingly investigated at the cellular level owing to their importance for human health and various areas of industrial and environmental relevance [4]. The chitinase purified from ***Paenibacillus*** sp. BISR-47 and ***Brevibacillus formosus*** BISR-1, characterized in our laboratory and their novel properties have been reported previously [5,6,7]. Both the previously reported microorganism have been found hyper-chitinase producing strains and shows strong insecticidal and fungicidal activities. In present study, efforts have been made to find out sustainability and production capability of both the microorganisms under immobilized and free state conditions.

2. MATERIALS AND METHODS

2.1 Enzyme immobilization through entrapment

Whole cell immobilization for extracellular chitinase production was determined in 250 ml Erlenmeyer flasks containing 50 ml nutrient broth (pH 7.0) for 48 h and the flasks were incubated at 37 ºC and 45 ºC (as per culture requirement), respectively at 180 rpm in an orbital shaker. After completion of incubation period, culture broth was harvested and the cell OD was adjusted to 0.5 (at 600 nm) and was used for preparing beads of three different types of entrapment material.

2.2 Whole cell entrapment through agar-agar and agarose

For cell entrapment a definite quantity of agar was added in distilled water and sterilized. After sterilization 10 ml of above cell suspension was mixed with molten agar to get a homogeneous suspension with a final concentration of agar 2 % and 4 %, respectively. 15 ml of this mixture was poured in a sterile 90 mm sterile Petri dish and allowed to solidify at room temperature. The solidified agar block was then cut into equal sizes with a cork borer of 6 mm diameter. The beads thus obtained were washed first with sterile distilled water and then with 50 mM sodium acetate buffer (pH 5.0) and stored at 4 ºC in screw cap glass bottles [8].Ten beads were used to inoculate 50 ml CC medium after washing again with sterile distilled water. Enzyme production was performed at respective temperatures (37 ºC and 45 ºC) by culturing the entrapped cells in 250 ml flask containing 50 ml CC medium (pH 7.0) at 180 rpm for 15 d and the chitinase activity in media was monitored at every 24 h. Agarose was taken instead of agar for whole cell entrapment through agarose and further for inoculation same procedure was adopted as above.

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2.3 Whole cell entrapment through alginate

For cell entrapment a definite quantity of sodium alginate was dissolved in distilled water and a homogeneous suspension was prepared with a final concentration of sodium alginate 2 % and 4 % as above. This suspension was taken in a sterile syringe and allowed to pour drop by drop in pre-cooled 2% $CaCl_2$ solution used as a cross linking material under mild stirring conditions. The prepared beads (~2 mm diameter) were incubated up to 2 h in $CaCl_2$ solution for maturation. The beads thus obtained were washed first with sterile distilled water and then with 50 mM sodium acetate buffer (pH 5.0) and stored at 4 ºC in screw cap glass bottles [8]. Further for inoculation same procedure was followed as above.

The agar beads produced higher chitinase production than agarose and alginate beads. Therefore, only 2 % agar beads were used to study reusability of immobilized whole cells by varying number of cycles.

3. RESULTS

3.1 Whole cell entrapment through agar-agar

Obtained chitinase activity by agar-agar (2% and 4%) immobilized cells of isolate BISR-1 and BISR-047 has been shown in Fig. 1. The enzyme activity by isolates BISR-1 and BISR-047 was detected within first 24 h of incubation which increased gradually, reached its maximum value of 425 IU/ml (9 d) and 580 IU/ml (12 d) and then decreased in both the isolates, respectively. Entrapment through 2 % agar-agar showed higher activities as compared to 4 % in both the isolates.

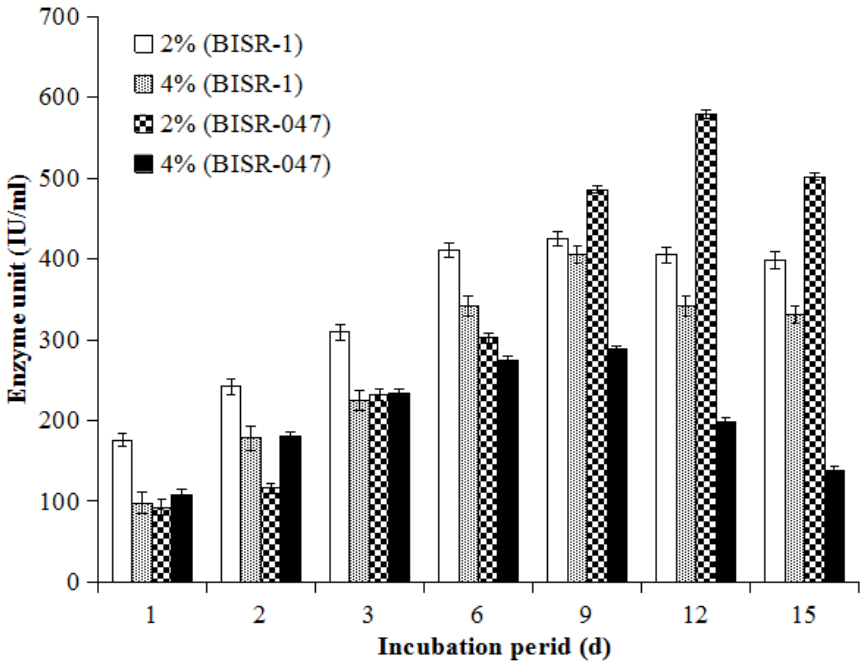

Fig.1: Cells immobilized on agar gel for chitinase production by B. formosus BISR-1 and Paenibacillus sp. BISR-047. Each point represents the mean of three independent experiments and error bar indicate SD.

3.2 Immobilization of whole cells in agarose

Chitinase activity obtained with agarose (2% and 4%) immobilized cells of isolate BISR-1 and BISR-047 has been shown in Fig. 2. The enzyme activity by isolates BISR-1 and BISR-047 was detected within first 24 h of incubation and then increased gradually, reached its maximum value of 399 IU/ml (9 d) and 256 IU/ml (6 d) and then decreased in both the isolates, respectively. Entrapment through 2 % agarose showed higher activities as compared to 4 % in both the isolates.

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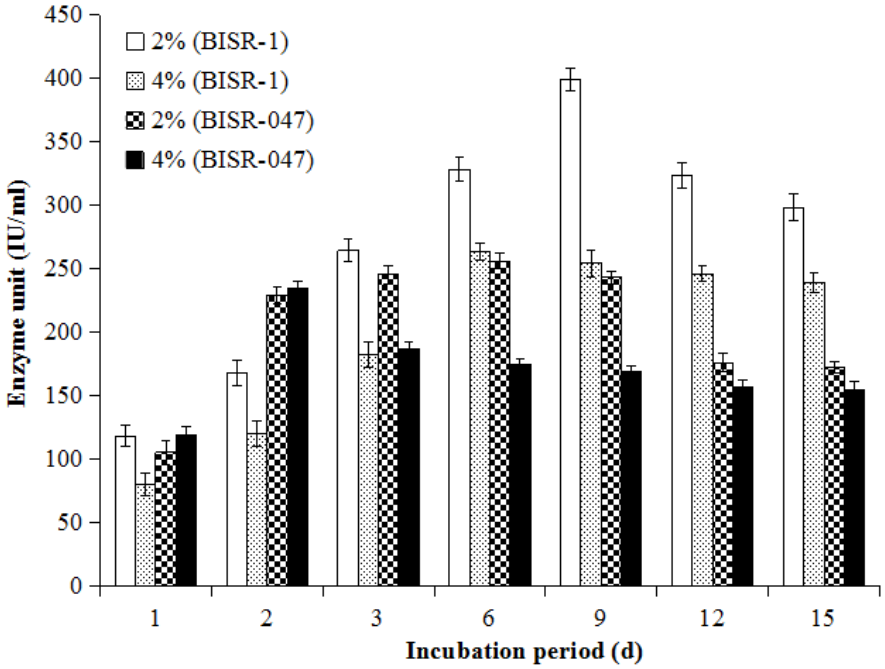

Fig. 2: Cells immobilized on agarose gel for chitinase production by B. formosus BISR-1 and Paenibacillus sp. BISR-047. Each point represents the mean of three independent experiments and error bar indicate SD.

3.3 Immobilization of whole cells in alginate

Chitinase activity obtained by alginate (2% and 4%) immobilized cells of isolate BISR-1 and BISR-047 has been shown in Fig. 3. The enzyme activity by isolates BISR-1 and BISR-047 was detected within first 24 h of incubation and then increased gradually, reached its maximum value of 342 IU/ml (6 d) and 394 IU/ml (9 d) and then decreased in both the isolates, respectively. In this case also, the entrapment through 2 % alginate showed higher activity as compared to 4 % in both the isolates.

Overall, both the strains showed higher chitinase activity at 2 % entrapment material concentration in all the three approaches, whereas cells immobilized using agar beads performed better in entrapment experiments as compared to agarose and alginate beads

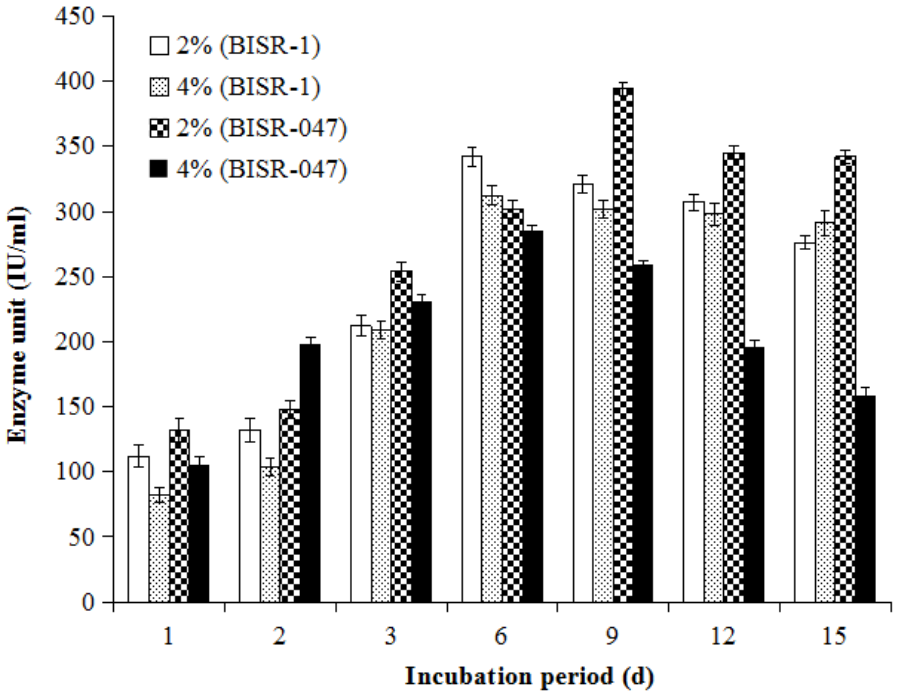

Fig. 3: Cells immobilized on alginate gel for chitinase production by B. formosus BISR-1 and Paenibacillus sp. BISR-047. Each point represents the mean of three independent experiments and error bar indicate SD.

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(Fig. 1, 2 and 3). Therefore, agar-agar material (2 %) was chosen for further experiments to evaluate re-usability potential of the immobilized cells of isolates BISR-1 and BISR-047.

In order to evaluate the re-usability of immobilized whole cells, the chitinase production was studied at optimal conditions for 10 cycle of 2 d each using 2 % agar-agar beads. After each cycle, the beads were washed thoroughly with sterile distilled water under aseptic conditions and re-used to inoculate the next batch. After each cycle, enzyme activity was measured, residual activity was calculated and the data have been presented in Fig. 4. Data clearly indicates that agar-agar beads retained more than 95 % residual activity even up to 5th cycle and more than 60 % up to 10th cycle of re-use.

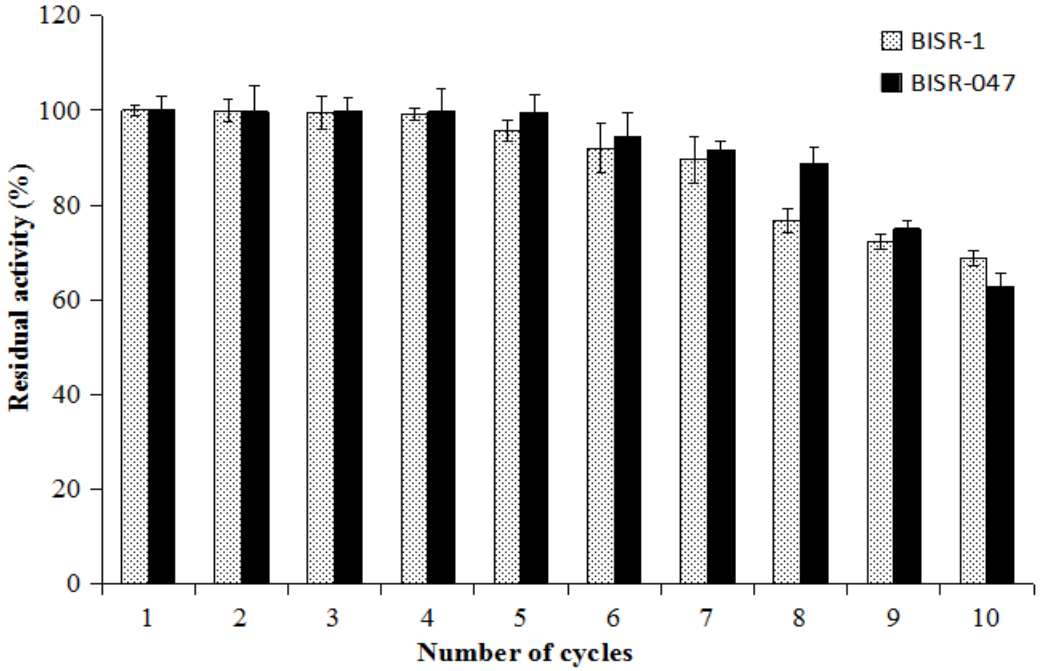

Fig. 4: Re-usability of immobilized cells with 2% agar gel for chitinase production by B. formosus BISR-1 and Paenibacillus sp. BISR-047. Each point represents the mean of three independent experiments and error bar indicate SD.

4. DISCUSSION

Immobilized cells have many important advantages in terms of industrial application since they retain their original biological functions with increased stability and cell productivity, and they can be reused for repeated or continuous processes without cell wash-out and with easy separation of the cells from the reaction system. Several methods of immobilization are previously reported [9]. A comparative analysis with previously studied different methods of whole cell immobilization by various microbes has been presented in table (Table 1). We adopted commonest methods used in immobilization of whole cells in the form of gel entrapment using two different concentrations (2 % and 4 %) of agar, agarose and alginate. These substrates are commonly used in many of the previous studies probably because of their mild effects on the cells. The entrapped cells were used for chitinase production and the enzyme activity was monitored regularly in isolates BISR-1 and BISR-047 (Fig. 1, 2 and 3). The data presented here clearly indicates that entrapment of cells with agar shows comparatively higher response than agarose or alginate by analyzing enzyme activity. Further, the entrapment of cells with 2 % agar showed higher response than 4 % in case of both the isolates. It showed an activity of 425 IU/ml at 9 d (Fig. 1) when entrapped in agar beads, and was comparatively higher (378 IU/ml) with free cells of isolate BISR-1 (Fig. 5), whereas an activity of 580 IU/ml at 12 d (Fig. 1) was observed with agar entrapped beads, and was comparatively higher (352 IU/ml) with free cells of isolate BISR-047 (Fig. 5). The enzyme activity obtained after immobilization was higher than free cells of both the isolates and could be due to the better attachment of cells with the support material. Similarly, El-sharif et al., [10] have previously reported that cells of *B. lichenifomis* produce higher chitinase activity (1.25 U/ml) by cells entrapped with 2 % agar, as compared to the free cells. *Bacillus* cells were immobilized by entrapment into different gel materials such as calcium alginate, k-carrageenan, polyacrylamid, cellulose and agarose and then used as biocatalysts for fermentative production of various enzymes [11]. The supports used for cell adsorption are wide and varied, which includes ceramics polyurethane foam [12] and luffa sponge [13] The re-usability of the 2 % agar entrapped cells of both the isolates (*B. formosus* BISR-1 and *Paenibacillus* sp. BISR-047) was also investigates in the present study up to 10 cycles (Fig. 4). In both the isolates, agar beads were found to retained more than 95% (up to 5 cycle) and more than 60 % (up to 10 cycles) residual activity. A 86.5% residual

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activity after 5 cycle of re-use has been reported previously with cells of *Bacillus* sp. R2 immobilized by agar gel [1, 14]. Our result of re-usability of immobilized cell was in accordance with previous findings [15,16]. Contrary to this, a 85% residual activity after one cycle of re-use has been reported by cells of *Gongronella* sp. JG immobilized with alginate [17]. Similarly, a 75 % loss in enzyme activity after 5th of cycle of reuse has been reported by cells of *B. amyloliquefaciens* immobilized with alginate [18].

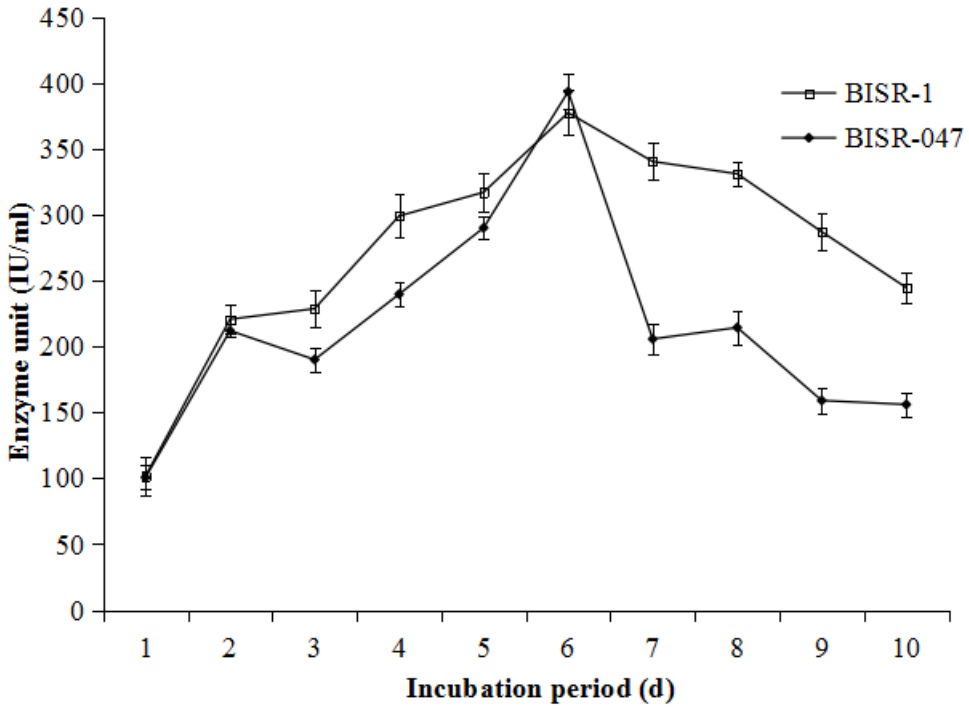

Fig. 5. Time course of extracellular chitinase production from isolates B. formosus BISR-1 and Paenibacillus sp. BISR-047 on colloidal chitin medium. Each point represents the mean of three independent experiments and error bar indicate SD.

Table 1: Types of immobilization matrix used to immobilize various microorganisms and their applications in previous studies.

Microorganisms	Immobilization matrix	Applications	References
Asprgillus awamori	Polyurethane foam	Glucoamylase production Reticulate	[19]
Aspergillus niger	Polyurethane Sponge cubes	Olive mill waste water treatment	[20]
Rhodobacter sphaeroides	Agar	Hydrogen production from tofu waste water	[21]
Pseudomonas putida	Alginate	Biodegradation of phenolic industrial waste water	[22]
Chlorella vulgaris, Azospirillum brasilense	Alginate	Removal of ammonium and phosphorous ions from synthetic waste water	[23]
Paecilomyces sp., *Microbrevisluteum*	Gravel solid support	Removal of colour and detoxification of pulp and paper mill effluent	[24]
Rhizopus oryzae	Reticulated Polyurethane foam	Biodiesel production	[25]
Trichoderma Viride	Alginate, Agar	Biosorption of Cr VI	[26]
Klebsiella oxytoca	Alginate and cellulose triacetate	Treatment of cyanide waste water	[27]
Bacillus subtilis	Chitosan	Biosorption of copper II	[28]
*Brevibacillus formosus*BISR-1	Agar, alginate and agarose	Hyper-chitinase activity	Present study
Paenibacillus sp.BISR-047	Agar, aginate, agarose	Hyper-chitinase activity	Present study

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CONCLUSION

In the present study a comparative analysis of both the strains (BISR-1 and BISR-047) have been studied to find out their shelf life and production capabilities in Free State and immobilized state. Results of this study shows that immobilized cells are more stable and shows higher chitinase activity than free state. The advantages of Immobilizing of whole cell rather than a purified enzyme are numerous: the expense of separation, isolation and purification of the enzyme is obviated; a wide scope of reactions is possible including multistep reactions utilizing several enzymes; maintain ace of the enzyme in its native state enhances its stability; and the presence of cofactors and continued biosynthesis within the cell contribute to the longevity of enzyme activity.

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REFRENCES

[1] B.A. Cheba, T.I. Zaghloul, A.R. EL-Mahdy, M.H. EL-Massry. Enhanced Production of *Bacillus* sp. R2 Chitinase through Cell Immobilization. ACT Biotechnol. Research Communications., volume 1: pp. 8-13, 2011.

[2] F. Berini, C. Katz, N. Gurudev, M. Casartelli. Microbial and viral chitinases: Attractive biopesticides for integrated pest management. Biotechnology Advances. volume 36, pp. 818-838, 2018.

[3] H.A. Enshasy, M.A. Farid, A.I El- diwany. Oxytetracycline production by free and immobilized cells of *Streptomyces rimosus* in batch and repeated batch cultures. Progress in Biotechnology, volume11, pp. 437-443,1996.

[4] S. Datta, L.R. Christena, Y.R.S. Rajaram. Enzyme Immobilization: An Overview on Techniques and Support Materials. 3 Biotech, volume 3, pp. 1-9, 2013.

[5] S. Meena, R.K. Gothwal, J. Saxena, M. K. Mohan, P. Ghosh. Chitinase production by a newly isolated thermotolerant *Paenibacillus* sp. BISR-047. Ann. Microbiol., volume 64 pp. 787-797, 2013.

[6] S. Meena, R.K. Gothwal, M. K. Mohan, P. Ghosh. Production and purification of a hyperthermostable chitinase from *Brevibacillus formosus* BISR-1 isolated from the Great Indian Desert soils. Extremophiles, volume18 pp. 451-62, 2014.

[7] S. Meena, R.K. Gothwal, J. Saxena, M. K. Mohan, P. Ghosh. Effect of metal ions and chemical compounds on chitinase produced by a newly isolated thermotolerant *Paenibacillus* sp. BISR-047 and its shelf-life. Int.J.Curr.Microbiol.App.Sci., volume 4 pp. 872-881, 2015.

[8] K. Adinarayana, B. Jyothi, P. Ellaiah. Productions of alkaline protease with immobilize cells of *Bacillus subtillus* PE-11 in various matrices by entrapment technique. AAPS pham. Sci. tech., volume 6, pp. 391-397, 2005.

[9] F. Shiraishi, K. Kawakami, S. Kono, A. Tamura, S. Tsuruta, K. Kunsunoki. Characterisation of production of free gluconic acid by Gluconobacter suboxydans adsorbed on ceramic honeycob monolith. Biotechnol. Bioeng., volume 33, pp. 1413-1418, 1989.

[10] M.F. El-sharif, A.S. Youssef, M.A. Hassan, H.M.G. Hassan. Immobilization and soli state fermentation methods for chitinase production from *Bacillus licheniformis*. Life science journal, volume 10, pp. 3036-3043, 2013.

[11] N.S. Landau, N.S. Egorov, I.B. Gornova, S.B. Krasovaskaya, A.D. Virnik. Incorporation of Bacillus firmus cells in triacetate cellulose fibres and films and their use in proteinase biosynthesis. Prikl. Biokhim. Mikkrobiol.,volume 28, pp. 108-113, 1992.

[12] R. Haapala, E. Parkkkinen, P. Suominen, S. Linko. Production of extracellular enzymes by immobilized *Trichoderma reesei* in shake flask cultures. Appl. Microbiol. Biotcchnol., volume 43, pp. 815-821, 1995.

[13] J.C. Ogbonna Y.C. , Lin, Y.K. Lin, H. Tanaka. Loofa *(Luffa cylindrica*) sponge as a carrier for microbial cell immobilization. J. Ferment. Bioeng., volume 78, pp. 437-442, 1994.

[14] B.A. Cheba, T.I. Zaghloul, A.R. EL-Mahdy, M.H. EL-Massry. Effect of Metal Ions, Chemical Agents, and Organic Solvent on *Bacillus* Sp. R2 Chitinase Activity. Procedia Technology, volume 22 pp. 465 -470, 2016.

[15] M. Angelova, P. Sheremetska, M. Lekov. Enhanced polymethylgalacturonase production from *Aspergillus niger* 26 by calcium alginate immobilization. Process Biochem., volume 33pp. 299-305, 1998.

[16] C. Hemachander, N. Bose, R. Puvanakrishnan. Whole cell immobilization of Ralstonia pickettii for lipase production. Process Biochem., volume 36 pp. 629-633, 2001.

[17] P. Zhang, W. Zhou, P. Wang, L. Wang, M. Tang. Enhancement of chitosanase production by cell immobilization of *Gongronella* sp. JG. Braz J Microbiol., volume 44 pp. 189-195, 2013.

[18] S. Guleria, A. Walia, A. Chauhan, C.K. Shirkot. Molecular characterization of alkaline protease of *Bacillus amyloliquefaciens* SP1 involved in biocontrol of Fusarium oxysporum. Int. J. Food Microbiol., volume 232, pp. 134-143, 2016.

[19] E. Bon, C. Webb. Passive immobilization of *Aspergillus awamori* spores for subsequent glucoamylase production. *Enzyme Microb. Technol*., volume11, pp.495-499, 1989.

[20] N. Vassilev, M. Fenice, F. Federici, R. Azcon. Olive mill waste treatment by immobilized cells of *Aspergillusniger* and its enrichment with soluble phosphate. *Process Biochem.*, volume 32, pp. 617-620, 1997.

[21] H. Zhu, T. Suzuki, A. Anatoly. Tsygankov, Y. Asada, J. Miyake. Hydrogen production from tofu waste water by *Rhodobactersphaeroides* immobilized in agar gels. *International J. Hydrogen Energy.*, volume 24, pp. 305-310, 1999.

[22] G. Gonzalez, G. Herrera, M.T. Garcia, M. Pena. Biodegradation of phenolic industrial waste water in a fluidized bed bioreactor with immobilized cells of *pseudomonas putida*.Bioresource Technology, volume 80 pp. 137-142, 2001.

[23] D. Bashan, E. Luz, M. Moreno, J.P. Hernandez, Y. Bashan. Removal of ammonium and phosphorous ions from synthetic wastewater by the microalgae *chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillumbrasilense*. *Water Research.*, volume 36, pp. 2941-2948, 2001.

[24] P. Singh, I. Thakur. Removal of colour and detoxification of pulp & paper mill effluent by microorganisms in two step bioreactor., *Journal of Scientific and Industrial Research*, volume 63, pp. 944-948, 2004.

[25] S. Hama, H. Yamaji, T. Fukumizu, T. Numata, S. Tamalampudi, A. Kondo, H. Noda, H. Fukudo. Biodiesel fuel production in a packed-bed reator using lipase producing *Rhizopusoryzae* cells immobilized within biomasss support particles, *Biochemical Engineering Journal*. Volume 34, pp. 273-278, 2007.

[26] N.R. Bishoni, R. Kumar, K. Bishon. Biosorption of Cr (IV) with *trichodermaviride* immobilized fungal biomass and cell free CaAlginate beads. *Indian Journal of Experimental Biology*., volume 45, pp. 657-664, 2007.

[27] C.Y. Chen, C.M. Kao, S.C. Chen. Application of *Klebsiella oxytoca* immobilized cells on the treatment of cyanide wastewater. *chemosphere*., volume 71, pp. 133-139, 2008.

[28] Y.G. Liu, T. Liao, Z.B. He, T.T. Li, H. Wang, X.J. Hu, Y.M. Guo, H.E. Yuan. Biorsorption of Copper (II) from aqueous solution by *Bacillus substilis* cells immobilized into chitosan beads. *Trans. Nonferrous Met. Soc*., volume 23, pp. 1804-1814, 2013.