Indian Journal of Experimental Biology
Vol. 55, July 2017, pp. 485-492

Spray drying as effective encapsulation method for phenol degrading bacteria

Nair M Lakshmi[1], K Umashankar[2], A Chandralekha[2], KSMS Raghavarao[2], Nayana M Jayan[1], Chandrasekharan Salini[1], Ashok Pandey[3] & Parameswaran Binod[1]*

[1]Microbial Processes and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram-695 019, Kerala, India

[2]Department of Food Engineering, CSIR-Central Food Technological Research Institute (CFTRI), Mysore-570 020, Karnataka, India

[3]Center of Innovative & Applied Bioprocessing, Sector 81, Mohali-160 071, Punjab, India

Received 01 February 2017; *revised 22 March 2017*

Encapsulated microorganisms are potential candidates for treatment of recalcitrant and persistent toxic compounds in wastewater and agriculture. Though extensive investigations have been done on bacteria with ability to degrade toxic phenolics, there is no product available for efficient removal of phenolic compounds from waste effluents. The present study aims at preparation of encapsulated microorganisms for the treatment of waste water containing phenol. *Acinetobacter seifertii* and *Bacillus pumilus* were the organisms used for effective degradation of phenol. Various encapsulation methods have been tried. Among the different carrier materials used, corn starch was found to be the best material with long shelf life. Spray drying and freeze drying were the two methods adopted for encapsulation. Based on cell viability, spray drying was found to be an effective method compared to freeze drying. *A. seifertii* could degrade phenol completely with in 12 h while *B. pumilus* degraded phenol completely within 24 h.

Keywords: *Acinetobacter seifertii*, *Bacillus pumilus*, Biodegradation, Bioremediation, Waste effluents

Phenol is an aromatic compound having a hydroxyl group attached to a benzene ring. Phenol is both naturally as well as synthetically produced organic compound. It is a white crystalline structure and is soluble in alcohol, glycerol and water to a lesser extent[1]. Phenol is a major component in the manufacturing of agriculture chemicals, pesticides, etc. It is found in dead organic matters like rotting vegetables and coals[2]. Effluents released out from various industries, such as petrochemical plants, leather manufacturing companies, paper industries, etc. contain a high concentration of a phenolic compound, which represent a major ecological issue due to their toxic effect throughout the environment[3,4]. Excessive exposure to this chemical may cause various health effect on eye, kidney, digestive system, skin, etc. It may also lead to genetic damage[5]. As such contaminated effluents are often released into water bodies, it also affects the marine genera of our ecosystem, and in case of fishes, it is lethal at a concentration of 5-25 mg/L[6-8]. As little as 0.005 mg/L of phenol can impart obnoxious taste and odour to the drinking water. Phenol is a potential human carcinogen as well and is of considerable health concern, even at a low concentration[9]. Phenol is listed among priority organic pollutants by the US Environmental protection agency[10]. Due to the adverse health effects of phenols, the World Health Organization (WHO) has set a guideline of 1 μg/L to regulate the phenol concentration in drinking waters[11-13]. Since phenol is causing serious health and environmental hazard, it is of prime importance to degrade such compounds before they are released into the environment.

One of the major biological approaches includes the use of microbes for phenol degradation. Another mechanism which is exercised includes adsorption[14]. Here, we deal with biological degradation of phenol. Many microbes such as bacteria and fungi are capable of degrading phenolic compounds and use it as a source of carbon and energy. The capacity of biodegradation is related to adaptation of microorganisms to the contaminated soil/aquatic habitat. Adaptation is mainly related to the synthesis of new enzymes capable of transforming even harmful xenobiotics as a source of energy. Therefore, much improvement is in progress for decontamination

*Correspondence:
Phone: +91 471 2515361 Fax: +91 471 2491712
E-mail: binodkannur@niist.res.in

of phenol and phenolic compounds by microbial degradation in recent past. Microbial degradation of phenol includes conversion of phenol to catechol. Further, this catechol gets converted by subsequent steps and enters the Krebs cycle[15].

Encapsulation of bacteria has opened up an intriguing way to immobilize bacteria. Survival of inoculated cells is a major fact responsible for degradation of phenol. Encapsulating the cells using carrier material can help in promoting shelf-life and survivability of bacteria. Availability of encapsulated microorganisms for degradation of phenolic wastes will decreases the pollution load in water and soil. Variety of encapsulated microorganisms and their applications are available in literature. Probiotic is a major area wherein successful encapsulation has been reported[16]. In the present study, we isolated and identified the microbial strain that is capable of degrading phenol. The strains which are isolated were encapsulated using spray drying technique. These encapsulated cells were evaluated for its phenol degradation efficacy.

Materials and Methods

Sample collection

Soil samples were collected from various workshop sites (service stations) at Koothattukulam in Ernakulam district, Kerala, India, which has petroleum content in it. Samples were collected in a sterile autoclavable bags and was stored immediately in refrigerator.

Isolation of phenol- degrading bacteria

One gram of soil samples collected was taken and added to Bushnell Haas (BH) medium with a concentration of 500 mg/L phenol. Components of Bushnell Haas medium include magnesium sulphate (0.2 g/L), calcium chloride (0.02 g/L), ferric chloride (0.05 g/L), potassium dihydrogen phosphate (1.0 g/L), ammonium nitrate (1.0 g/L), potassium hydrogen phosphate (1.0 g/L), but it lacks carbon source which is an essential factor for the growth of microbes[17]. In this experiment, we added phenol as sole carbon source. It was then placed on a shaker for two days at 200 rpm. After two days samples from the flask was taken and serial dilution was performed till 10^6 dilutions, followed by spread plate in Bushnell Haas agar with a phenol concentration of 500 mg/L. Bacterial growth was checked, and two type of colony was observed in Bushnell Haas agar plates, which was then sub-cultured.

Characterization of the isolate

Gram staining was done and also morphological characteristics were observed. For molecular identification of bacteria, genomic DNA was isolated using genomic DNA purification kit. Briefly 2 mL of the overnight grown culture was pelleted and to the pellet added 180 µL of lysis solution T along with 20 µL RNase and 200 µL of proteinase k. It was then incubated at 55 °C for 30 min. 200 µL of lysis solution C was added, vortexed and again incubated at 55°C, followed by the addition of 200 µL of ethanol and mixed by vortexing for 5-10 s The content of the tube was transferred to a binding column and was centrifuged at 6500× g for 1 min. The elute was discarded and new 2 mL collection tube was placed. About 500 µL of wash solution added and centrifuged 3 min at 16000 ×g to dry the column. Again discarded the collection tube and placed a new 2 mL collection tube to which 8 µL elution buffer was added and centrifuged 6500 × g to elute the DNA. The DNA was further stored at 4°C.

Amplification of 16s rRNA sequence was then performed using universal primers [27F (5'AGAGTTTG ATCCTGGCTCAG3') and 1492R (5'GGTTACCT TGTTACGACTT-3')]. The PCR reaction was carried out in a 50 µL volume, containing 25 µL master mix, 2 µL forward and reverse primer, template DNA (1 µL) and nuclease free water 22 µL. The PCR amplification was performed as following conditions: Initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, and finally extension at 72°C for 10 minutes. After the PCR reaction, one µL of PCR products were also examined by electrophoresis. The resulting PCR products were sequenced. The nucleotide sequence obtained was then compared with database of sequence using BLAST.

Phenol degradation experiment with free cells

Bushnell Hass media was prepared with different concentrations of phenol (100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L) and was inoculated with 2% of the culture having OD 1.2. Samples were then collected at regular interval and centrifuged so as to get the supernatant. Finally, phenol assay were performed with the sample collected to detect the amount of phenol remaining in the media.

Phenol estimation assay

Phenol content in the medium was estimated spectrophotometrically by folins ciocalteu reagent.

Standard curve was plotted by using different concentration of phenol. Phenol concentration in the media was detected by adding 500 µL of the collected supernatant into dry tubes, followed by pipetting of 1 mL of 10% folins ciocalteu reagent, this was then mixed thoroughly and incubated 30 min in the dark at 30 °C. To this mixture four mL of 700 mM aqueous sodium carbonate was added and incubated in the dark for 2 h at room temperature. Finally, after 2 h, the absorbance of the solution was read at 735 nm against the blank sample. Folins ciocalteu's reagent contains phosphomolybdic and phosphotungstic acid, and during oxidation of phenol, it gets reduced to form mixtures of blue oxides of tungsten and molybdenum which was measured at 735 nm. The values obtained were compared with standard to determine the concentration of phenol[18].

Statistical optimization

The phenol degradation abilities of the culture *Bacillus pumilus* was further analyzed by a statistical optimization design. The levels of the significant parameters and the interaction effects between various medium constituents which influence the growth and phenol degradation were analyzed and optimized by Box-Behnken methodology[19]. In this study, the experimental plan consisted of 15 trials and the independent variables were studied at three different levels, low (-1), medium (0) and high (+1). The experimental design used for the study is shown in Table 1. The second order polynomial coefficients were calculated and analyzed using the 'Minitab' software (Version 15, Minitab Inc., USA) statistical package. The general form of the second-degree polynomial equation (2) is:

$$Yi = \beta o + \Sigma \beta iXi + \Sigma \beta iiXi^2 + \Sigma \beta ijXiXj \quad \ldots (2)$$

Table 1 — Box-Behnken experimental design matrix

Run Order	NH_4Cl	Glucose	KH_2PO_4	Phenol concentration at 48 h	% phenol degradation
-	0.3	0.25	0.25	48.9	2.2
2	0.3	0.5	0	9.7	80.6
3	0.5	0.25	0.5	47.4	5.2
4	0.3	0.25	0.25	28	44
5	0.5	0.25	0	42.5	15
6	0.5	0.5	0.25	48.7	2.6
7	0.1	0.25	0	50	0
8	0.5	0	0.25	0	100
9	0.3	0.5	0.5	50	0
10	0.3	0	0	0	100
11	0.1	0	0.25	0	100
12	0.3	0.25	0.25	50	0
13	0.1	0.25	0.5	9.5	81
14	0.3	0.25	0.5	50	0
15	0.1	0.5	0.25	50	0

Encapsulation of microorganisms

Feed preparation

Feed was prepared by adding predetermined (10% w/v) quantity of carrier materials along with 5% (w/v) microbial cells in 1000 mL of distilled water. The contents were mixed using a table top magnetic stirrer to get uniform slurry.

Spray drying

The microbial cells in the slurry was encapsulated using pilot scale spray drier (BE 1216, Bowen, UK; Diameter-0.76 m, height-0.72, cone height-0.74 m, 2-fluid nozzle type atomizer and water evaporation capacity of 5 kg/h). The operating conditions were standardised with reference to dry cell powder yield and cell survival of the preliminary experiments, which are: Inlet air temperature 120±2ºC, outlet air temperature 80 ± 2ºC, air pressure 2.5 psi, and feed flow rate 40±1 mL/min. The slurry was continuously stirred, while being fed to drier to avoid settling and maintain uniform concentration of feed. The spray dried powder samples were collected and evaluated for its efficacy.

Freeze drying

The feed containing microorganisms with carrier materials was subjected to freeze drying (Lyodryer LT58, ISI Lyophilization Systems Inc., USA) for comparison with respect to cell survival and powder yield. The processing conditions during the drying are same as of Chandralekha *et al*.,[16,21]. The powder samples obtained after drying were collected and kept for storage.

Cell survival and yield

Cell survival of dried microbial cells after drying was estimated using serial dilution method and spread plate method. The experimental procedure followed for estimation of cell survival was same as of Chandralekha *et al*.[16,21].

Cell survival was calculated using the following formula:

$$\textit{Cell Surivival}\ (\%) = \frac{N_r}{N_f} \times 100 \quad \ldots (2)$$

where Nr is log cfu/mL of rehydrated sample and Nf is log cfu/mL of feed solution

The yield of dried powder was calculated as the ratio of the weight of powder collected after drying to the total solid content in the feed.

The powder yield (%, w/w) was calculated by the following equation.

$$\textit{Dry cell powder (\%, w/w)} = \frac{W_p}{T_s} \times 100 \quad \ldots (3)$$

where 'W_p' is weight of the cell powder collected after spray drying and 'T_S' is the total soluble solid content in the feed.

Moisture analysis

Moisture content of dried cell powder after drying methods was measured using IR moisture meter (HMB100, Wenser, Chennai, India). The experimental procedure followed for measuring moisture in dried cells is same as of Chandralekha *et al.*[16]. The moisture content of all the samples was expressed in terms of % (w/w) dry basis. The reported results are mean of triplicate measurements.

Scanning electron microscopy (SEM)

Surface morphology of dried powder was observed using SEM. The sample preparation was carried out as described in Chandralekha *et al.*[16]. The images showing surface morphology microstructures of dried powder are reported.

Results and Discussion

Characterization of the isolate

By morphological analysis, one culture was found to be a cream slimy colony and the other was a cream colony with fluorescence. Gram staining showed that one culture was gram positive rod and the other gram negative cocco bacilli. Species identification was done by 16s rRNA sequencing. The sequence homology was compared with the database of sequence and showed 99% similarity to *Bacillus pumilus* and *Acinetobacter seifertii*. Hence, the species was confirmed to be *Bacillus pumilus* and *Acinetobacter seifertii*.

Phenol degradation experiment with free cells

Bacillus pumilus and *Acinetobater seifertii* was tested to determine the potential of the organisms to degrade phenol. Bushnell Haas medium was supplemented with different concentration of phenol ranging from 100-400 mg/L. Media was then inoculated with *Bacillus pumilus* and *Acinetbacter seifertii*, sampling was done at regular intervals and phenol degradation was checked (Fig. 1 A and B)

Statistical optimization

From the study it was found that by 48 h of incubation, 100% phenol was degraded in run number 8, 10 and 11. These three experimental media were devoid of glucose. The study showed that in the absence of glucose and in the presence of phenol as the sole carbon source, the *Bacillus* strain used in the present study could completely utilize phenol. If glucose is present in the presence of phenol, organisms utilize the glucose and hence, phenol degradation does not happen. In order to find the best condition, the experimental result was analysed using the software Minitab and various surface plots were prepared. Fig. 2A shows the surface plot of interaction between glucose and KH_2PO_4. It was evident from the plot that as the concentration of glucose was increasing, the percentage phenol degradation was decreasing, and maximum percentage phenol degradation was observed in the absence of glucose and KH_2PO_4. The interaction between glucose and ammonium chloride is shown in Fig. 2B. This analysis also shows that maximum phenol degradation occurred in the absence of glucose and lowest concentration of ammonium chloride. The interaction between ammonium chloride and KH_2PO_4

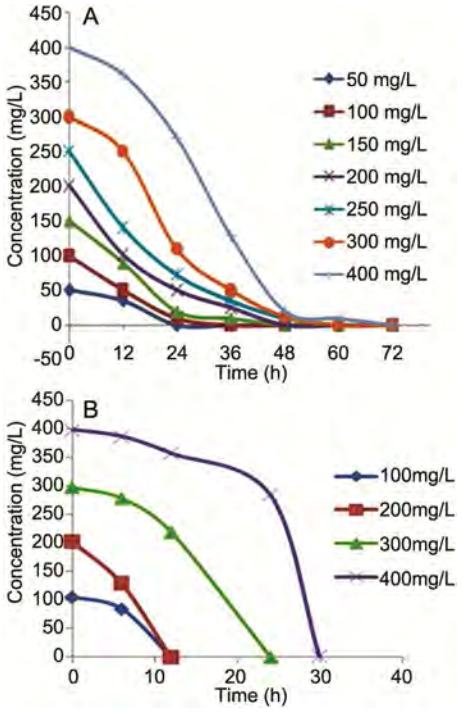

Fig. 1 — Phenol degradation of *Bacillus pumilus*

shows the highest concentration of ammonium chloride with low KH_2PO_4 or highest concentration of KH_2PO_4 with low concentration of ammonium chloride. The surface for interaction between KH_2PO_4 and ammonium chloride is shown in Fig. 2C.

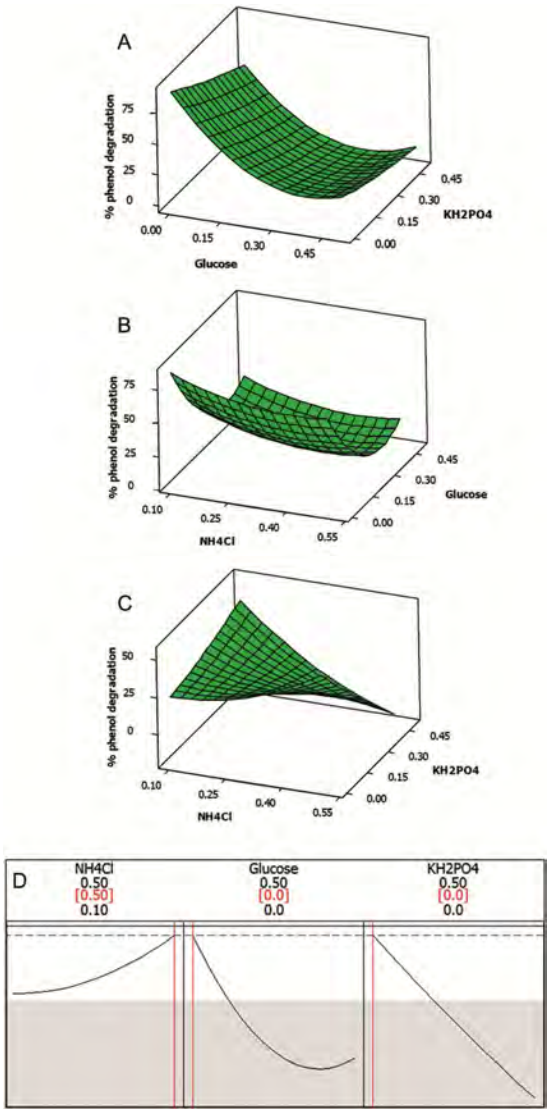

Fig. 2 — Phenol degradation of *Acinetobacter seifertii*

From the statistical analysis, it was found that presence of glucose was decreasing the phenol degradation. It was obvious that as the glucose was present in the culture medium, bacteria preferred the carbon source as the primary source of energy. However, when the glucose was getting depleted, the organism chose other carbon sources. The optimization plot as analysed by the software is shown in (Fig. 2D) shows that ammonium concentration of 5 g/L in BH medium was sufficient for phenol degradation by *Bacillus pumilus*. About 50 mg/L glucose could be degraded by *Bacillus pumilus* within 24 h.

Encapsulation of cultures

The microbial cultures which are characterised were cultured further for developing biomass using shake flask method. The biomass obtained was subjected to encapsulation by spray drying technique. Different carrier materials were employed in our previous studies[16,20]. Among the different carrier materials used, the corn starch which gave the best result (with respect to cell survival and powder yield) was chosen for encapsulation (Table 2).

About 5% (w/v) of cells were used in each trial, N_f value (log cfu/mL) of the feed was found to be constant (that is 60×10^{15} for *Bacillus pumilus* and 60×10^{18} for *Acinetobacter seifertii* during trials). Feed for spray drying was prepared by mixing 5% (w/v) of microbial cells with 10% (w/v) of carrier material. The feed formed was spray dried and dried culture powder was obtained. It was observed that after spray drying the cell survival for *Bacillus pumilus* was 95% and for *Acinetobacter seifertii* was 92% (10×10^{15} cfu/mL and 22×10^{17} cfu/mL). The powder yield with corn starch was 49% (w/w) for *Bacillus pumilus* and 50% (w/w) for *Acinetobacter seifertii*.

The results observed were similar to the results of Chandralekha *et al*.[20] where yeast was encapsulated using corn starch as a carrier material cell survival of 67% and powder yield of 59% (w/w) was observed which was reported as the best among different carrier material used.

Table 2 — Comparison of spray drying and freeze drying methods of encapsulation

Microbial culture	Carrier material	Spray drying				Freeze drying			
		Survival (%)	Yield (%)	Moisture (%)	Final cell count (cfu/ml)	Survival (%)	Yield (%)	Moisture (%)	Final cell count (cfu/ml)
Bacillus pumilis	CS	95	49	8.6	10*10^15	97	90	7.1	20*10^15
Acinetobacter seifertii.	CS	92	50	8.5	22*10^17	96	95	5.7	12*10^18

CS- Corn starch

Freeze drying of *Bacillus pumilus* and *Acinetobacter seifertii* was carried out as control. The cell survival was observed to be 97% for *Bacillus pumilus* (20×10^{15} cfu/mL) and 96% for *Acinetobacter seifertii* (12×10^{18} cfu/mL). More than 95% (w/w) of powder yield was obtained for both the cultures. Similar results were observed in our previous studies as well[16,201]**.** The sample obtained was in the form of dry cake/flakes. In Freeze drying, powder yield after drying was higher compared to spray drying technique.

The moisture content values of dried microbial cell powder are shown in Table 2. Dried samples which can be considered as stable at moisture content less than 10% are categorized as microbiologically stable[21]. It was observed that the moisture content of spray dried samples were 8.6% for *Bacillus pumilus* and 8.5% for *Acinetobacter seifertii* and for freeze dried samples it was around 7%.

Phenol degradation by spray dried cultures

Phenol degradation of spray dried cultures of *Bacillus pumilus* and *Acinetobacter seifertii* was checked after spray drying (Fig. 3A) and similar pattern of phenol degradation was observed as that of native organisms.

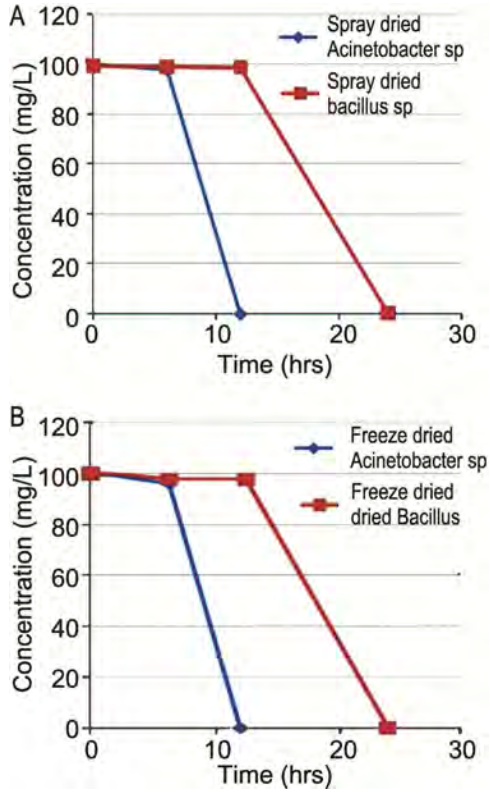

Fig. 3 — Surface plot for interaction between (A) glucose and KH_2PO_4; (B) glucose and ammonium chloride; (C) KH_2PO_4 and ammonium chloride concentration on % phenol degradation; and (D) Optimization plot of phenol degradation by *Bacillus pumilus*

Phenol degradation by freeze dried cultures

Similarly, phenol degradation of freeze dried cultures of *Bacillus pumilus* and *Acinetobacter seifertii* were checked after freeze drying (Fig. 3B). *A. seifertii* was able to degrade phenol completely within 12 h while *B. pumilus* could degrade phenol completely within 24 h.

SEM analysis of spray dried and freeze dried cultures

SEM images of spray dried microbial cells were captured at 1000X resolution to observe the morphological surface. The morphology of dried microbial cells was found to be almost spherical shaped in both spray dried and freeze dried samples. No surface dents and cracks were observed on the surface powder of both the cultures. This was due to proper expansion or "ballooning" of droplets that occurred at drying. SEM images of spray dried *Bacillus* and *Acinetobacter* encapsulated with cornstarch is shown in Fig. 4 A and B. Similarly, SEM images of freeze dried *Bacillus* and *Acinetobacter* encapsulated with cornstarch is shown in Fig. 4 C and D.

Different works have been reported on degradation of phenol using single or consortium of microorganisms[22-33].There are different factors that control the degradation of phenol. Some of the environmental factors include pH, temperature, nutrient, oxygen availability, soil availability, etc. Phenol was used as sole carbon source by the microorganisms by addition of phenol into BH medium. *Bacillus pumilus* and *Acinetobacter seifertii* was proved to be two potent strains that degrade phenol. Even though various organisms have been reported to degrade phenol, to our knowledge this is the first report using *Bacillus pumilus* and *Acinetobacter seifertii* for phenol degradation. From the optimization studies, it is clear that, in the absence of glucose organisms use phenol as the sole carbon source. However, if glucose is present, then the organisms prefer glucose as the primary source and phenol depletion is halted. Only when glucose gets depleted the organisms opt other carbon sources.

Encapsulation is one of the widely used techniques since last two decades for various applications, such as ethanol production, lactic acid production, etc. Encapsulation can be done for food ingredients, enzymes, cells or other materials in small capsule. Different carrier materials are used for encapsulation. The commonly used materials are maltodextrin,

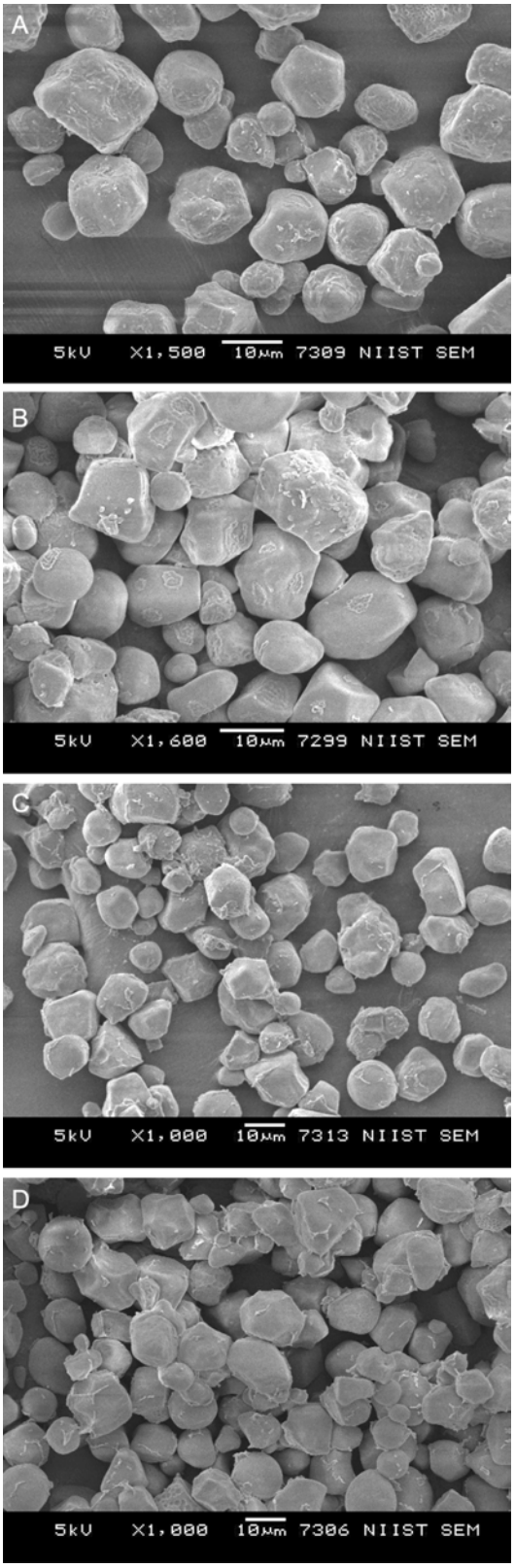

Fig. 4 — Phenol degradation by spray dried and freeze dried *Bacillus pumilus* and *Acinetobacter seifertii*

cornstarch, gellan gum, silica gel, sodium alginate, etc. In our study, encapsulation was carried out using cornstarch as the carrier material. Corn starch was found to be effective carrier material in protecting the cell as compared with other materials like malto dextrin, whey protein, sodium caseinate[33]. Among the two methods that we adopted, spray drying and freeze drying, comparing the cost and cell viability, the former was found more reliable as compared to the latter. SEM analysis shows that bacteria were encapsulated in cornstarch.

Summarily, we can say that out of the two organisms isolated *Acinetobacter seifertii* was found to degrade phenol at faster rate when compared to *Bacillus pumilus* and among the encapsulation technique used spray drying was found to be more effective than freeze drying, since its more cost effective and also cell viability is more for spray dried culture.

Acknowledgement

We gratefully acknowledge CSIR network project CSC0127 for funding this work.

References

1 Guido Buscaa, Silvia Berardinelli , Carlo Resini & Laura Arrighi, Technologies for the removal of phenol from fluid streams: A short review of recent developments. *J Hazard Mater*, 160 (2008) 265.

2 Khazi Mahammedilyas Basha, Aravindan Rajendran & Viruthagiri Thangavelu, Recent advances in the Biodegradation of Phenol: A review. *Asian J Exp Biol Sci*, 1 (2010) 219.

3 Siti Aqlima Ahmad , Nor Aripin Sh*amaan, Noorliza Mat Arif, G*an Bee Koon, Mohd Yunus Abdul Shukor & Mohd Arif Syed, Enhanced phenol degradation by immobilized Acinetobacter sp.strain AQ5NOL 1. *World J Microbiol Biotechnol*, 28 (2012) 347.

4 Gurusamy Annaduraia, Ruey-Shin Juangb & Duu-Jong Leea, Microbiological degradation of phenol using mixed liquors of *Pseudomonas putida* and activated sludge. *Waste Manage*, 22 (2002) 703.

5 Michałowicz J & Duda W, Phenols – Sources and *Toxicity*. *Polish J Environ Stud*, 16 (2007) 347.

6 *A*rinjay Kumar, Shashi Kumar & Surendra Kumar, Biodegradation kinetics of phenol and catechol using Pseudomonas putida MTCC 1194. *Biochem Eng J*, 22 (2005) 151.

7 Muftah H. El-Naas, Shaheen A Al-Muhtaseb & Souzan Makhlouf, Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. *J Hazard Mater*, 164 (2009) 720.

8 Manoj Kumar Ghosh, Uttam Kumar Ghosh & Samit Kumar, Minimization of Phenols and Phenolic Compounds in Pulp and Paper Industries: Biological Approaches. *J Chem Eng*, 5 (2011) 595.

9 Kumar A, Kumar S & Kumar S, Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194. *Biochem Eng J*, 22 (2005) 151.

10 Khleifat KM, Biodegradation of phenol by *Ewingella Americana* : Effect of carbon starvation and some growth conditions, *Process Biochem*, 41 (2006) 2010.
11 Yan J, Jianping W, Jing B, Daoquan W & Zongding H, Phenol biodegradation by the yeast *Candida tropicalis*in the presence of *m*-cresol. *Biochem Eng J*, 29 (2006) 227.
12 Kumar A, Yadav AK, Sreekrishnan TR, Satya S & Kaushik CP, Treatment of low strength industrial cluster wastewater by anaerobic hybrid reactor. *Bioresource Technol*, 99 (2008) 3123.
13 Hsieh FM, Huang C, Lin TF, Chen YM & Lin JC, Study of sodium tripolyphosphate-crosslinked chitosan beads entrapped with *Pseudomonas putida* for phenol degradation. *Process Biochem*, 43 (2008) 83.
14 Nadia Roostaei & Handan Tezel F, Removal of phenol from aqueous solutions by adsorption. *J Environ Manage*, 70 (2004) 157.
15 Paula van Schie M & Lily Young Y, Biodegradation of Phenol: Mechanisms and Applications. *Biorem J*, 4 (2007) 1.
16 Chandralekha A, Hrishikesh Tavanandi A, Amrutha N, Umesh Hebbar H, Raghavarao KSMS & Ramachandra Gadre, Encapsulation of yeast (*Saccharomyces cereviciae*) by spray drying for extension of shelf life. *Drying Technol*, 34 (2016) 1307.
17 Lin J, Reddy M, Moorthi V & Qoma BE, Bacterial removal of toxic phenols from an industrial Effluent. *Afr J Biotechnol*, 7 (2008) 2232.
18 Essam Abdul-Hafeez Y, Nazira Karamova S & Olga Ilinskaya N, Antioxidant activity and total phenolic compound content of certain medicinal plants. *Int J Biosci*, 5 (2014) 213.
19 Box GEP & Behnken DW, Some new three level designs for the study of quantitative variables. *Technometrics*, 2 (1960) 455.
20 Chandralekha A, Anupama Rani, Hrishikesh A Tavanandi, N Amrutha, UmeshHebbar & Raghavarao KSMS, Role of Carrier Material in Encapsulation of Yeast(*Saccharomyces cerevisiae*) by Spray Drying. *Drying Technol*, (2016) (in press DOI:10.1080/07373937.2016.1230626)
21 Hari, Reginold Jebittas & Sivaraman, Production and characterization of Sugar cane juice powder. *J Sugarcane Res*, 3 (2013) 20.
22 Ajaz M, Nabeela Noor, Sheikh Ajaz Rasool & Shakeel Khan A, Phenol Resistant Bacteria from Soil Identification-Characterization and Genetical Studies. *Pak J Bot*, 36 (2004) 415.
23 Gayathri KV & Vasudevan N, Enrichment of Phenol Degrading Moderately Halophilic Bacterial Consortium from Saline Environment. *J Bioremed Biodegrad*, 1 (2010) 104.
24 Prpich GP & Daugulis AJ, Enhanced biodegradation of phenol by a microbial consortium in a solid-liquid two phase partitioning bioreactor. *Biodecomposition*, 16 (2005) 329.
25 Jame SA, Rashidul Alam AKM, Fakhruddin ANM, & Alam MK, Degradation of Phenol by Mixed Culture of Locally Isolated Pseudomonas Species. *J Bioremed Biodegrad*, 1 (2010) 102.
26 Chakraborty S, Bhattacharya T, Patel TN & Tiwari KK, Biodegradation of phenol by native microorganisms isolated from coke processing wastewater. *J Environ Biol*, 31 (2010) 293.
27 Jiang H, Fang Y, Fu Y & Guo QX, Studies on the extraction of phenol in wastewater. *J Hazard Mater*, 101 (2003) 179.
28 Nair CI, Jayachandran K & Shashidhar S, Biodegradation of Phenol. *Afr J Biotechnol*, 7 (2008) 4951.
29 Agarry SE & Solomon BO, Kinetics of batch microbial degradation of phenols by indigenous *Pseudomonas fluorescence*. *Int J Environ Sci Technol*, 5 (2008) 223.
30 Marrot B, Barrios-Martinez A, Moulin P & Roche N, Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor. *Biochem Eng J*, 30 (2006) 174.
31 Boyd SA, Shelton DR, Berry D & Tiedje JM, Anaerobic biodegradation of phenolic compounds in digested sludge. *Appl Environ Microbiol*, 46 (1983) 50.
32 van Schie PM & Young LY, Isolation and characterization of phenol-degrading denitrifying bacteria. *Appl Environ Microbiol*, 64 (1998) 2432.
33 Adav SS, Chen MY, Lee DJ & Ren NQ, Degradation of phenol by aerobic granules and isolated yeast *Candida tropicalis*. *Biotechnol Bioeng*, 96 (2007) 844.