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Newly isolated formaldehyde degrading *Bacillus cereus* from effluent of SIPCOT industrial area: identification and process parameter studies

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ABSTRACT

The aim of this research work was to explore formaldehyde (HCHO) degrading bacterium from industrial effluent. Formaldehyde biodegradation characteristics were evaluated in a batch process, under aerobic conditions. The experiments were performed with *Bacillus cereus* to reveal its formaldehyde degrading activity. Process parameters were studied with different experimental conditions of pH and inoculum concentration with initial HCHO concentration of 100, 200, and 300 mg/L to determine the effect of parameters on percentage degradation of formaldehyde. The process was carried out in a temperature controlled orbital shaker at 37°C for 2 d at 200 rpm speed. The experimental results showed that *B. cereus* exhibited the highest removal efficiency of formaldehyde by using surface immobilization beads, which reaches a maximum of 98% reduction at pH 7 and 15% inoculum size with initial formaldehyde concentration of 300 mg/L. The surface immobilization beads were analyzed by using the scanning electron microscopy before and after the biodegradation of formaldehyde to reveal the condition of beads and its contact with the substrate. A pure bacterial strain of *B. cereus* CIT (Accession number MN658860) was newly isolated and used for biodegradation of formaldehyde under different experimental conditions. From this study, microorganism has been identified by using 16s rRNA cell sequencing and it has confirmed that the isolated species was *B. cereus*. The growth of *B. cereus* was analyzed by growth curve and its formaldehyde degradation effect was observed by microbial inhibitory concentration. The isolated bacterial species has high potential for biodegradation of formaldehyde in wastewater.

Keywords: Formaldehyde degradation; *Bacillus cereus*; Surface immobilization; Scanning electron microscope analysis; Process parameters

1. Introduction

Volatile organic compounds (VOCs) are toxic pollutants, that are released into the environment is either by man-made or natural [1–3]. The toxic compounds that are released into the environment have been increased in the present scenario due to industrial development and organic fuels utilization [4]. These compounds degrade slowly and accumulate in the ecosystem and cause serious damage to the environment and also to the exposed human beings [5]. Increasing industrialization has provided many advantages to mankind but it also impacted the environment harmfully [6]. In the past decades industries released a large amount of these pollutants into the ecological systems [7]. Among these, formaldehyde is considered as one of the major VOCs emitted from industries because of its harmful effects on human health and high emission level in atmosphere [4]. Therefore, it is most important that the treatment of wastewater, before it gets discharged into the environment. Formaldehyde is the most dangerous and toxic organic compound present in industrial wastewater. The report on carcinogens reveals that, formaldehyde is the 25th most

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produced chemical (5 million ton/y) in the United States of America. Formaldehyde has been reported as toxic organic compound and has been enlisted among 45 organic substances that have detrimental effects on environment [8]. A variety of techniques have been reported for formaldehyde (HCHO) treatment. Various types of physical, chemical, and biological processes, involving photolysis, hydrolysis, biological decomposition, oxidation, adsorption, and simple evolution, are utilized to reduce the concentration of HCHO which are found in the environment [9]. The oxidation methods have good performance in removing degradable toxic contaminants such as HCHO, but the production of by-products and their release into the environment is one of the serious limitations of this method. Therefore, the use of complementary methods such as biological methods to eliminate such contaminants is mandatory [10]. Bioremediation was considered to be the most appropriate methods as it is eco-friendly and cheaper method compared to conventional adsorption and chemical oxidation processes [11].

Diverse metabolic capabilities of microorganisms have been worked by man in different ways in the biodegradation of waste materials [12]. The principle of bioremediation is based on consuming VOCs by microorganism as an energy resource in cellular respiration phase and as carbon resource in growth phase [13]. Microbial treatments for bioremediation of toxic and hazardous environmental pollutants are being preceded along vigorously [14]. Microorganisms play a major role in transformation of harmful organic pollutants to lesser or no harmful compounds due to the powerful catalytic activity of enzymes that are capable of changing toxicity and/or structure of contaminants [15]. The effectiveness depends upon type of pollutants and microorganisms that are chosen for the remediation studies.

Microorganisms such as Flavobacterium, Candida, Alcaligenes, Streptomyces, Cellulosimicrobium, Trichoderma, Penicillium, Methanospirillum, Sphingobium, Rhodococcus, *Aspergillus*, *Rhodotorula*, etc., have been isolated and characterized, which have shown notable biodegradation potential for different pollutants from environment. Microorganisms utilize the contaminants potentially as carbon and/or nitrogen sources for growth and metabolic activities [16]. Various microbes which are resistant to formaldehyde have the ability to degrade formaldehyde. *Pseudomonas putida* degrades formaldehyde with formaldehyde dismutase enzyme [17]. The study with *Pseudomonas putida* A2 is found to be capable enough to degrade about 400 mg/L of formaldehyde in 100 h as the sole carbon source. It has also been reported that gram-positive *Bacillus* and gram-negative coccus were found to be the most dominant species in the biological degradation of formaldehyde wastewater [18]. Though variety of microbial strains acts as an engine in the biodegradation process, in case of high formaldehyde concentrations significant inhibition phenomenon is observed as a major element and hence screening for high tolerance formaldehyde strain is utmost required [19]. *Bacillus* species has capability to tolerate harsh environmental conditions by forming antibiotics, quorum quenching enzymes and spores; thereby they can survive at various adverse living conditions [3]. *Bacillus cereus* is a gram-positive, rod-shaped bacteria which is isolated from the soil and waste effluent water naturally. Using immobilization beads of *B. cereus* with surface growth is the more effective for treatment of wastewater comparing to use of free cells. Sodium alginate was used for preparing immobilized beads to entrap bacterial cells and act as a supporting material which possess high porosity. Biodegradation studies in environmental processes will focus on both growth characteristics and degradation of toxic contaminants in the wastewater. Cell immobilization is an alternative method for enzyme immobilization in recent days. Selection of the support material plays a vital role in the process of immobilization. Different support materials, such as calcium alginate, alumina and nanomaterial's like nanofibers are used for immobilization of free cells by entrapment or physical adsorption methods [20]. The immobilized cells were more effective in treatment of the wastewater due to conversion rate of toxic substances into nutrient, production of biomass and carbon dioxide. Biodegradation rate using immobilized cells will be better when mass transfer resistance is absent [21].

The study deals with isolation, screening, identification of more efficient microorganism for degradation of HCHO from wastewater. The confirmation of the strain was performed by 16s rDNA studies and its efficacy was evaluated in a batch process. The isolated strain was immobilized and allowed for surface growth and used for formaldehyde degrading experiments with different parameters.

2. Materials and methods

The isolation of bacterial species was performed by serial dilution method and the cell sequencing was done by 16s rDNA sequence determination. The biodegradation study was performed in batch process using isolated species.

2.1. Chemicals and reagents

Analytical grade chemicals with purity greater than 99% were procured from HiMedia Laboratories Private Limited, India were used in this study. The chemicals used for the isolation and experimental study were Luria–Bertani (LB) agar, Luria–Bertani (LB) broth, yeast extract, beef extract, starch, sodium alginate, calcium chloride, sodium chloride, sodium hydroxide, hydrochloric acid, formaldehyde. The reagents used for analysis of formaldehyde concentration in samples before and after treatments by Hantzsch method were ammonium acetate, acetyl acetone and acetic acid. The Hantzsch reagents were prepared for reagent with pentane 2,4-dione and reagent without pentane 2,4-dione.

2.2. Sample preparation and isolation of microorganism

The industrial effluent containing formaldehyde was collected from State Industries Promotion Corporation of Tamil Nadu Ltd., (SIPCOT), Hosur, India. The initial formaldehyde concentration of effluent was analyzed by using Hantzsch reagents which was 100–120 mg/L. 10 mL effluent was diluted to 90 mL using sterile water in a 250 mL flask containing LB medium. Then medium with effluent was incubated at 37°C for 24 h in a rotary shaker incubator (Orbitek, Scigenics Biotech Private Limited, Chennai, India) at 200 rpm [22,23]. The sample culture was checked for its decrease in the turbidity and was plated on to LB agar with

serial dilution to isolate the colonies [24]. Colonies with distinct morphological characteristics such as colour, shape and size, were selected and purified with standard procedure [22]. Starch indicator was used for confirmation of *Bacillus* species. The broth medium was kept in the incubator for 24 h for the bacterial growth. Sub-culture was prepared from mother culture under optimal growth condition with standard procedure to carry out characterization of microorganism and experimental studies [22].

2.3. Characterization of microorganism

The isolated pure cultures were streaked in a separate petri plate for the growth. Visual observation was done using microscope to assess the growth and colony morphology. Gram staining was performed for identification using the microorganism from the fully developed colony [25].

2.3.1. DNA extraction, PCR, sequencing and phylogenetic analysis

2.3.1.1. Cell sequencing by 16s rDNA sequence determination

The isolates which have shown growth on formaldehyde concentration were considered as potential degraders. Among the isolated bacteria, *B. cereus* was found to be potent formaldehyde degraders. Hence, for cumulative identification, these isolates were sent to Yaazh Xenomics, Coimbatore, India, for the cell sequencing and DNA extraction was performed as per the standard protocol [26]. Amplification of 16s rDNA was done by colony polymerized chain reaction (PCR) method. PCR was performed based on the standard procedure with thermal cycling conditions. The reaction mixture about 25 μL containing 5 μL of DNA extract as the template, 1.5 μL of forward primer 8F (5′AGAGTTTGATCCTGGCTCAG3′) and reverse primer 1541R (5′AAGGAGGTGATCCAGCCGCA3′), 5 μL of deionized water, and 12 μL of Taq Master Mix [8]. 16s rRNA universal primers were used to perform single pass sequencing. Samples were prepared using standard protocol and subjected to electrophoresis (ABI 3730xl sequencer, Applied Biosystems). Obtained sequence was matched with National Centre for Biotechnology Information (NCBI) database using BLASTN [9]. MUSCLE 3.7 was employed to study multiple alignments of sequences [27]. Program PhyML3.0aLRT was used for phylogenecy analysis and HKY85 as substitution model. The program Tree Dyn 198.3 was used for tree rendering [28]. Phylogenetic tree shown in the supplementary Fig. S1 illustrates the evolutionary relationship among groups of organisms or among a family of related nucleic acid [28].

2.4. Growth analysis for resistant microorganism

The growth of bacterium in industrial effluents was determined in batch mode. A volume of 50 mL LB broth were prepared and inoculated with 1 mL of respective bacterial culture into the media. The culture along with positive control and negative control were incubated in shaking incubator at optimal condition with standard procedure [22]. The cell growth was measured by measuring optical density of the culture for every 1 h by using UV-Vis Spectrophotometer (Elico Ltd., India, SL 164 double beam) at 410 nm for the duration of 24 h [29].

2.5. Minimum inhibitory concentration (MIC)

To determine the ability of formaldehyde resistant microorganism of isolated microorganisms, 5 mL of the isolated microorganism from mother culture was taken and inoculated into freshly prepared 50 mL LB broth media, containing varying concentrations of 100, 200, and 300 mg/L, pH of 6, 6.5, 7 and inoculum volume 5%, 10%, 15% HCHO. Then the broth was incubated at specific conditions to observe HCHO resistant species [22].

2.6. Biodegradation of formaldehyde using isolated B. cereus

2.6.1. Preparation of aqueous solution

The aqueous solution used for experiment was prepared by using formaldehyde solution with 10% methanol as stabilizer which prevents evaporation of HCHO due to volatile in nature. The stock solution was prepared by taking 10 mL of formaldehyde in a 1,000 mL standard measuring flask and then distilled water is added to make the solution to 1,000 mL and stirred well. From the above stock solution, the working standard was prepared (100, 200 and 300 mg/L) by taking 10, 20, and 30 mL of stock solution in a 1,000 mL standard measuring flask, respectively and making the solution to 1,000 mL [30]. Totally 27 samples were analysed with different parameters.

2.6.2. Inoculum preparation

Newly isolated species *B. cereus* from the stock culture was sub-cultured and inoculated on nutrient broth medium containing beef extract, yeast extract, peptic digest of animal tissue and sodium chloride for bacterial growth. The optimum growth temperature was maintained for *B. cereus* at 37°C. The increase of turbidity reveals the growth of bacteria in the medium. The culture was acclimatized with 100 mg/L formaldehyde for its sustainability in the media [24]. Then the cells were utilized for immobilization beads preparation and for surface growth.

2.7. Immobilized beads with surface growth

The beads were formed by extruding 5% sodium alginate solution with species into calcium chloride solution with a needle dropper. Dropped beads were kept in the calcium chloride solution for 1 h to harden the beads and washed three times with sterile distilled water. These beads were taken in inoculated broth medium for surface growth and incubated in temperature controlled orbital shaker.

2.8. Experimental procedure for biodegradation in batch process

The experimental studies were performed in the 250 mL batch reactor (conical flask). It consists of 150 mL formaldehyde solution with varying concentration and surface immobilized beads in each of the conical flask. The

experimental studies were performed with different experimental conditions of pH value (6.5, 7.0 and 7.5) and inoculum concentration (5%, 10% and 15%) to determine the effect of parameters on the degradation process [31]. The pH of the solution was adjusted with 2 N NaOH or 2 N HCl [32]. The incubations were done on the temperature controlled orbital shaker at 37°C at 200 rpm for 2 d [22,23,33,34]. Samples were withdrawn at regular time interval and the concentration of formaldehyde was determined by Hantzsch method by using UV-Visible spectrophotometer at 410 nm (Elico Ltd., India, SL 164 double beam). This experiment was repeated by varying the formaldehyde concentration to determine the effect of concentration on the degradation process for species *B. cereus* (MN658860).

Formaldehyde degradation efficiency was calculated by using the formula:

$$E_{(\mathrm{HCHO})}(\%) = \left[\frac{(C_0 - C_F)}{C_0}\right] \times 100$$

where C_0 – initial formaldehyde concentration (mg/L) and C_F – final formaldehyde concentration (mg/L).

2.9. Scanning electron microscopic analysis

Calcium alginate beads with surface growth and without surface growth immobilized cells were examined with VEGA3 TESCON scanning electron microscope (SEM; Czech Republic). It uses a focused beam of high-energy electrons for generation of variety of signals at surface of solid specimens. Signals derived from electron-sample interactions reveals information about sample. Standard protocols were followed for SEM analysis.

3. Results and discussion

3.1. B. cereus – MN658860

General physio chemical characterization was done for the isolated bacteria and staining was performed. The isolates which have shown growth on formaldehyde concentration were considered as potential degraders. Among the isolated bacteria, the potential degrader was subjected to the 16s rDNA sequence analysis and the sequence were deposited in NCBI and it was published with GenBank accession number MN658860 and named as *B. cereus* CIT (Accession Number MN658860).

3.1.1. 16s rDNA sequence determination

Though many bacterial isolates were obtained from industrial effluent, their efficiency in degrading formaldehyde was inferior to *B. cereus*. Hence the rest of the isolates were omitted and *B. cereus* alone included in the current investigation. The study reveals that the strain isolated was close to the members of genus *B. cereus*. The partially amplified 16s rDNA sequence was submitted to NCBI database search. The highest sequence similarity conformed that it is closely related to *B. cereus*. Details are given in the supplementary Fig. S2.

3.2. Growth curve analysis and minimum inhibitory concentration

For the growth curve analysis (Fig. 1) the initial concentration of formaldehyde taken was 300 mg/L which was the maximum concentration level used for biodegradation experiment. There is lag in the growth of cells from initial time to 2 h which shows adaptation period. During the time period of 2–5 h there is gradual and rapid increase in the growth of cells which shows the exponential growth period. Optical density increased as the time increases at the beginning, and reached the maximum value at 5 h. Inhibitory effect was observed as value of the optical density declined rapidly with further increase in time; no growth was found for the time higher than 7 h and maximum concentration of formaldehyde was about 300 mg/L at corresponding optical density. The biodegradation of formaldehyde decreased at concentrations higher than 300 mg/L [35]. After analysing the incubated samples dosed with HCHO, it was revealed that the microbial growth was inhibited due to the presence of formaldehyde. For higher HCHO concentrations (above 300 mg/L) degradation percentages decreased as the formaldehyde concentration increased. The experiments were carried out using positive and negative control. The absorbance value(s) were related to the values obtained by Jarusutthirak et al. [36] in their studies.

3.3. Biodegradation of formaldehyde using B. cereus (MN658860.01)

The biological treatments of wastewater systems are cost effective, but their efficiency is directly proportional to performance of the microbial community associated with the specific system [37]. A pure culture of *B. cereus* was isolated and used for the biodegradation of formaldehyde under different experimental conditions of pH, inoculum size and HCHO concentration. Identification and cell sequencing was made to confirm the species as *B. cereus*. The experimental results were shown that the removal efficiency of formaldehyde by using surface immobilization beads reaches a maximum of 98% reduction at pH 7, 15% inoculum size and in initial formaldehyde concentration of 300 mg/L.

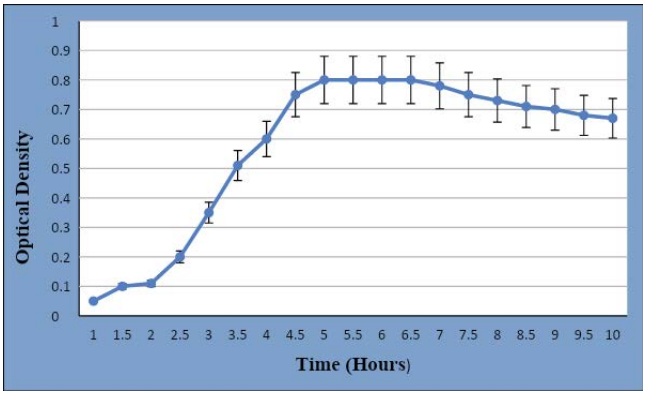

Fig. 1. Growth curve analysis with the initial concentration of formaldehyde 300 mg/L (standard deviation is shown by error bars).

3.4. Process parameters

3.4.1. Effect of pH on percentage degradation

The speed of reactions relies on the pH of the environment, and pH directly or indirectly affects the oxidation of chemical materials [9]. Jarusutthirak et al. [36], in their study reported that the pH range of 5–7 was optimum pH for formaldehyde removal. Under acidic condition the formaldehyde degradation efficiency was low and it was increased to maximum level at neutral pH. Then at basic condition with pH 7.5 and above again the degradation efficiency was reduced to appropriate level. At pH 7.0, the maximum degradation efficiency was achieved for 300 mg/L of initial HCHO concentration with inoculum size 15%.

The formaldehyde degradation with surface immobilized beads of *B. cereus* at various HCHO concentrations at pH 6.5 ranging from 100 to 300 mg/L was shown in (Fig. 2a–c). The maximum degradation of formaldehyde (85.15%) was achieved at a concentration of 300 mg/L with inoculum size 10% at pH of 6.5 and 8 h (Fig. 2c). The surface immobilized beads of newly isolated strain *B. cereus* could degrade formaldehyde at initial concentrations at pH 7.0 varying from 100–300 mg/L was shown in (Fig. 3a–c). The maximum degradation of formaldehyde (98.05%) was achieved at a concentration of 300 mg/L with inoculum size 15% at a pH of 7 and 8 h (Fig. 3c). The surface immobilized beads of *B. cereus* degrade formaldehyde at initial concentrations at pH 7.5 varying from 100–300 mg/L was shown in (Fig. 4a–c). The maximum degradation of formaldehyde (93.40%) was achieved at a concentration of 300 mg/L with inoculum size 10% at a pH of 7.5 and 3 h (Fig. 4c).

The experimental studies reveal that the degradation was low at acidic and basic condition but maximum degradation achieves at neutral pH. Under alkaline conditions, the radical oxidation potential of hydroxide decreases, consequently the efficiency of the process also decreases [38]. The experimental results show that for surface immobilized beads the percentage degradation was 85.15% at pH 6.5 and it was increased to 98.05% at pH 7.0, further it was decreased to 93.40 at pH 7.5. The study revealed that the optimum pH for the degradation of formaldehyde was 7.0 with maximum degradation of 98.05%.

3.4.2. Effect of initial concentration of HCHO on percentage degradation

It is very clear that the concentration of pollutants is one of the most important parameter that varies in different industries. Hence, studying the effect of the initial concentration of pollutants on the degradation efficiency is required [39]. Based on the result from the experiments it is evident that at initial time the degradation was low due to adaptation period and as time increases the degradation also increases in most experimental conditions. The degradation efficiency of HCHO by *Bacillus* species was maximum at lower concentration in the range of 100–400 mg/L and decreases after 400 mg/L [40]. Similarly at initial HCHO concentration of 100 mg/L the degradation was achieved at low level and it was increased at 200 mg/L reasonably. But at initial concentration of 300 mg/L the degradation of formaldehyde was achieved as 98.05% for immobilized beads with surface growth. At low concentration of HCHO, the hydroxyl radicals can easily remove a large percentage of the contaminants present in the reactor but, by increasing the concentration of the contaminant, the amount of these radicals will be insufficient for its further degradation [39,41]. By increasing the initial concentration of formaldehyde, the amount of contact and exposure of the formaldehyde to hydroxyl radicals declines, which causes more utilization of hydroxyl radicals and decreases the removal efficiency [42].

3.5. SEM analysis of surface immobilized beads

The surface immobilization bacterial beads were analyzed by using the SEM before and after the biodegradation of formaldehyde to reveal the surface growth on the beads (Fig. 5a and b). The view field range from 415 μm–2.07 mm and width range are 10.29–9.82 mm.

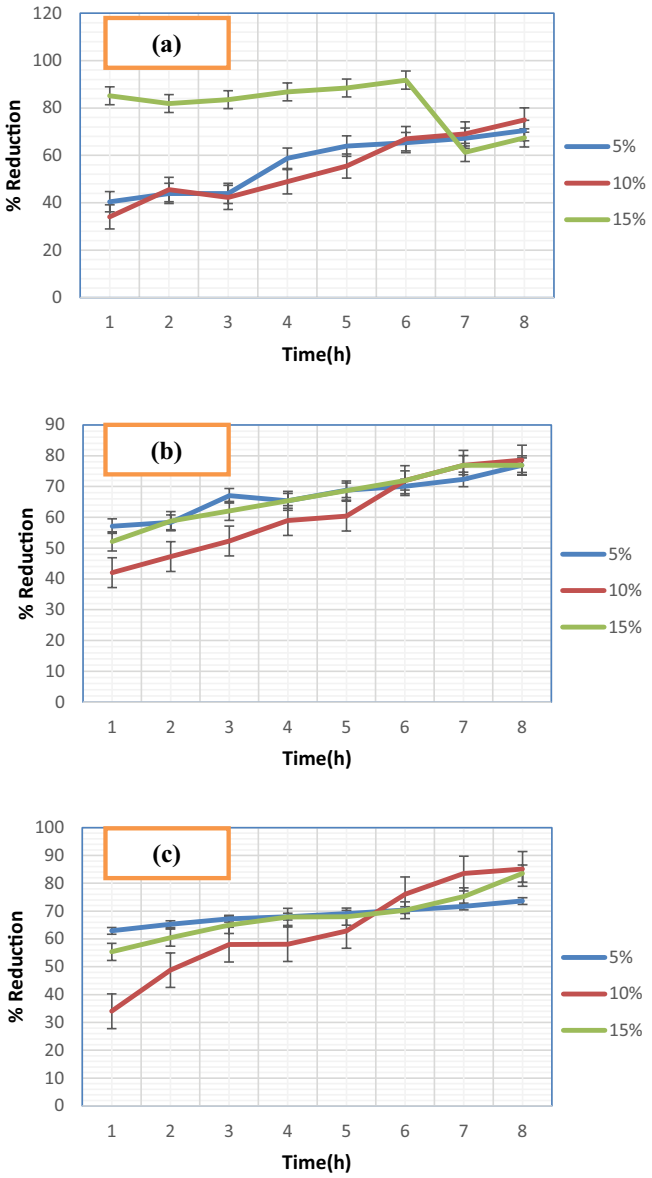

Fig. 2. Reduction of different concentrations of HCHO at pH 6.5 (a) 100 mg/L, (b) 200 mg/L, and (c) 300 mg/L.

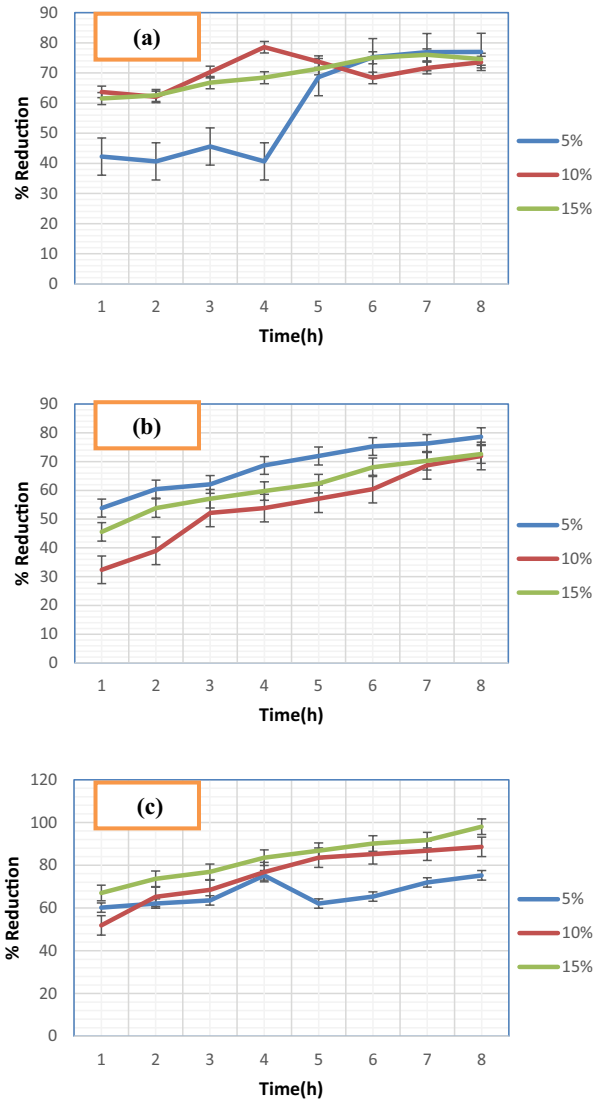

Fig. 3. Reduction of different concentrations of HCHO at pH 7.0 (a) 100 mg/L, (b) 200 mg/L, and (c) 300 mg/L.

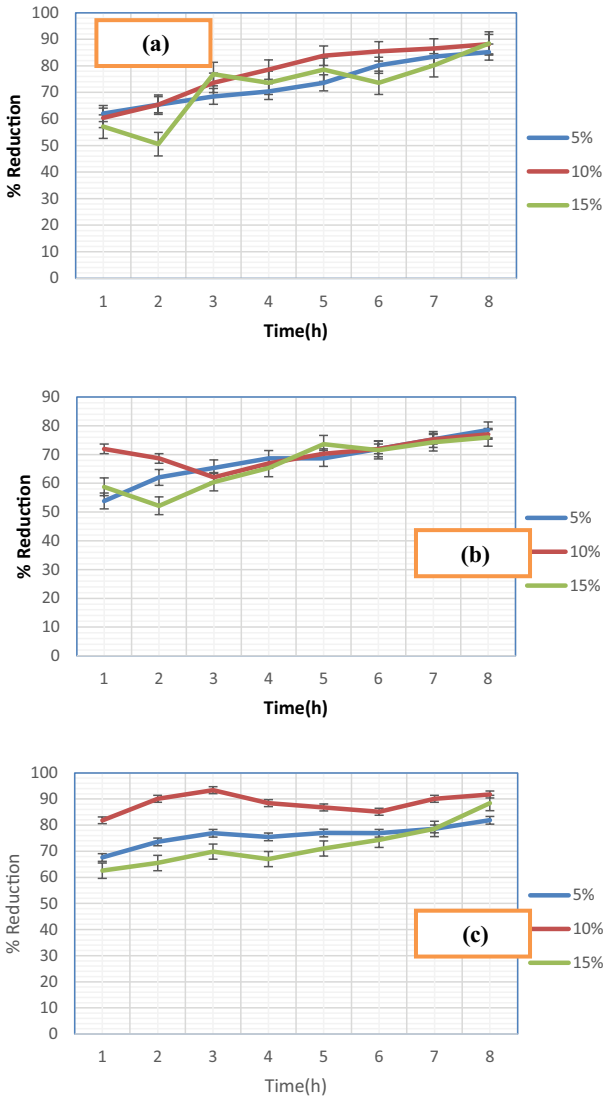

Fig. 4. Reduction of different concentrations of HCHO at pH 7.5 (a) 100 mg/L, (b) 200 mg/L, and (c) 300 mg/L.

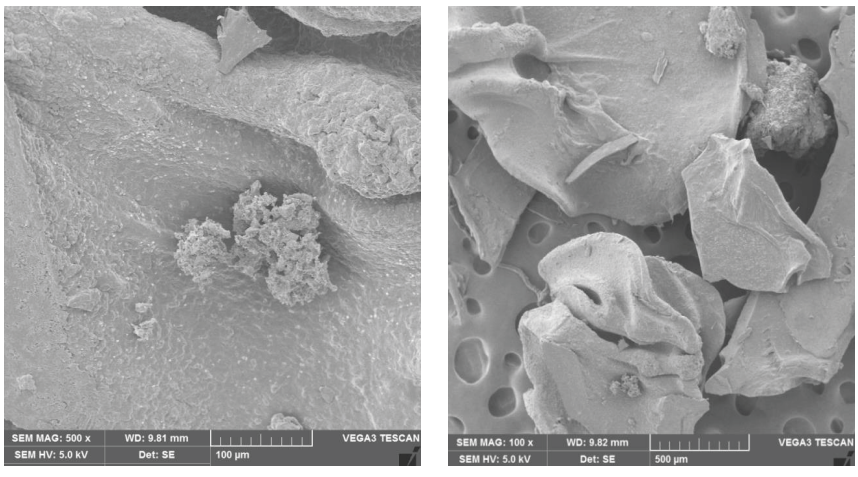

Fig. 5. Scanning electron microscopy analysis (a) before surface growth and (b) after surface growth.

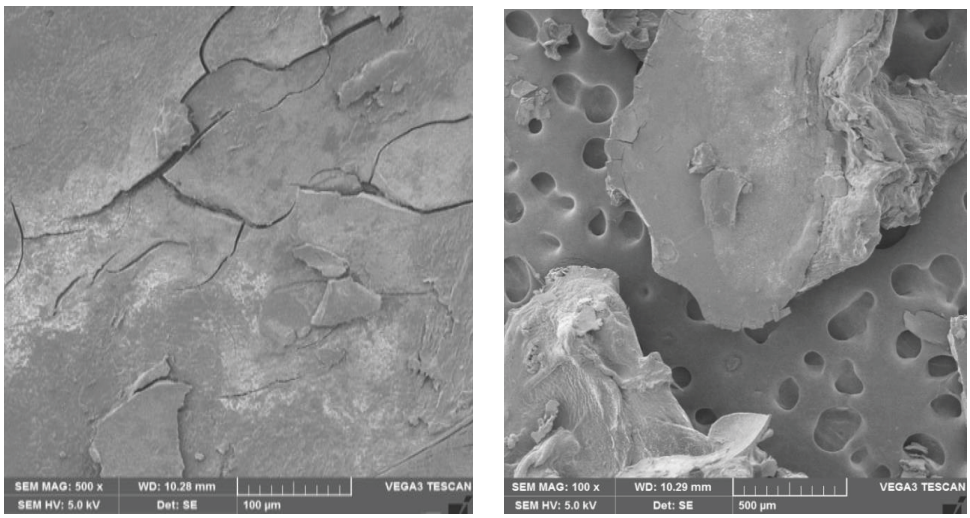

Fig. 6. Scanning electron microscopy analysis of surface immobilization beads (a) before treatment, view field 415 µm and (b) after treatment, view field 2.07 mm.

Further, the surface immobilization beads were analyzed by using the SEM before and after the biodegradation of formaldehyde to reveal the condition of beads and increasing contact with the substrate (Fig. 6a and b). The analysis shows that there are changes on surface of immobilized beads. Initially beads were having rough surfaces, after experiment the surfaces become flat due to repeated usage, as shown in Fig. 6b. The repeated usage of beads causes erosion of cells present on the surface of the matrix so that number of cells, which are in contact with substrate decreases that leads to decreased activity [30]. The pores present in the surface of the bead appeared to be coarse and uneven, which is helpful to increase specific surface area of the beads and increase diffusion of substrates [28]. The images observed by SEM indicated that the structure of the beads was beneficial for increasing activity of surface immobilized cells [13].

4. Conclusion

A pure culture of *B. cereus* (MN658860) was newly isolated and used for biodegradation of formaldehyde under different experimental conditions of pH, inoculums size and HCHO concentration. Identification and 16s rRNA cell sequencing were performed to confirm the isolated species was *B. cereus*. The growth of *B. cereus* was analyzed by growth curve and its formaldehyde degradation effect was observed by microbial inhibitory concentration. The isolated pure culture of *B. cereus* was used for biodegradation of formaldehyde and the experiment was conducted at various conditions of pH, inoculum size and HCHO concentration. The experimental results confirmed that removal efficiency of formaldehyde by using surface immobilization beads reaches a maximum of 98.05% reduction at pH 7, 15% inoculum size with initial formaldehyde concentration of 300 mg/L. The surface immobilization beads were analyzed by using SEM before and after the biodegradation of formaldehyde to reveal the condition of beads and increasing contact with the substrate.

5. Limitation and scope of the study

Hence the data obtained in this research work were laboratory scale process and through modeling, there is considerable scope to extend this in future. In this context, it is valuable to consider the following:

- The kinetics of microbial growth study.
- The degradation can be predicted by using mixed culture of microorganisms.
- Degradation of formaldehyde by using the combination of both microbial and photo catalytic methods in various concentration.

References

[1] S.K.S. Patel, M. Singh, V.C. Kalia, Hydrogen and polyhydroxybutyrate producing abilities of *Bacillus* spp. from glucose in two stage system, Indian J. Microbiol., 51 (2011) 418–423.
[2] A. Kansal, Sources and reactivity of NMHCs and VOCs in the atmosphere: a review, J. Hazard. Mater., 166 (2009) 17–26.
[3] A. Talapatra, A. Srivastava, Ambient air non-methane volatile organic compound (NMVOC) study initiatives in India – a review, J. Environ. Prot., 2 (2011) 21–36.
[4] O.J. Prado, M.C. Veiga, C. Kennes, Effect of key parameters on the removal of formaldehyde and methanol in gas-phase biotrickling filters, J. Hazard. Mater., 138 (2006) 543–548.
[5] P.A. Kumar, M. Ray, S. Chakraborty, Hexavalent chromium removal from wastewater using aniline formaldehyde condensate coated silica gel, J. Hazard. Mater., 143 (2007) 24–32.
[6] T. Shindhal, P. Rakholiya, S. Varjani, A. Pandey, H.H. Ngo, W. Guo, H.Y. Ng, M.J. Taherzadeh, A critical review on advances in the practices and perspectives for the treatment of dye industry wastewater, Bioengineered Mater., 12 (2020) 70–87.
[7] X. Chen, P.S. Stewart, Biofilm removal caused by chemical treatments, Water Res., 34 (2000) 4229–4233.
[8] D. Paliulis, Removal of formaldehyde from synthetic wastewater using natural and modified zeolites, Pol. J. Environ. Stud., 25 (2016) 251–257.
[9] S.A. Mokhtaria, M. Gholamib, A. Dargahic, M. Vosoughi, Removal of polycyclic aromatic hydrocarbons (PAHs) from contaminated sewage sludge using advanced oxidation process

(hydrogen peroxide and sodium persulfate), Desal. Water Treat., 213 (2021) 311–318.
[10] A. Dargahi, R. Shokoohi, G. Asgari, A. Ansari, D. Nematollahic, M.R. Samarghandi, Moving-bed biofilm reactor combined with three dimensional electrochemical pretreatment (MBBR – 3DE) for 2,4-D herbicide treatment: application for real wastewater, improvement of biodegradability, RSC Adv., 11 (2021) 9608–9620.
[11] M. Wang, S. Xu, S. Li, R. Zhu, Isolation of formaldehyde-degrading bacteria and the evaluation of the degradation characteristics, J. Ind. Eng. Chem., 75 (2019) 224–229.
[12] G.C. Okpokwasili, C.O. Nweke, Microbial growth and substrate utilization kinetics, Afr. J. Biotechnol., 5 (2005) 305–317.
[13] Y. Hajizadeh, M. Rezaei, Biodegradation of formaldehyde from contaminated air using a laboratory scale static-bed bioreactor, Int. J. Environ. Health Eng., 2 (2013) 18–25.
[14] S. Varjani, A. Bajaj, H.J. Purohit, V.C. Kalia, Bioremediation and circular biotechnology, Indian J. Microbiol., 61 (2021) 235–236.
[15] K.S. Rajmohan, C. Ramya, S. Varjani, A review on occurrence of pesticides in environment and current technologies for their remediation and management, Indian J. Microbiol., 60 (2020) 125–138.
[16] S. Mishra, Z. Lin, S. Pang, W. Zhang, P. Bhatt, S. Chen, Recent advanced technologies for the characterization of xenobiotic-degrading microorganisms and microbial communities, Front Bioeng. Biotechnol., 9 (2021) 632059, doi: 10.3389/fbioe.2021.632059.
[17] M. Eiroa, A. Vilar, L. Amor, C. Kennes, M.C. Veiga, Biodegradation and effect of formaldehyde and phenol on the denitrification process, Water Res., 39 (2005) 449–455.
[18] N. Adroer, C. Casas, C.D. Mas, C. Sola, Mechanism of formaldehyde biodegradation by *Pseudomonas putida*, Appl. Microbiol. Biotechnol., 33 (1990) 217–220.
[19] P. Kaszycki, M. Tyszka, P. Malec, H. Kołoczek, Formaldehyde and methanol biodegradation with the methylotrophic yeast *Hansenula polymorpha*. An application to real wastewater treatment, Biodegradation, 12 (2001) 169–77.
[20] A. Rasheed, T. Ghous, S. Mumtaz, M.N. Zafar, K. Akhter, R. Shabir, Zain-ul-Abdin, S.S. Shafqat, Immobilization of *Pseudomonas aeruginosa* static biomass on eggshell powder for on-line preconcentration and determination of Cr(VI), Open Chem., 18 (2020) 303–313.
[21] S.C.S. Martins, C.M. Martins, L.M.G. Fiuza, S.T. Santaella, Immobilization of microbial cells: a promising tool for treatment of toxic pollutants in industrial wastewater, Afr. J. Biotechnol., 12 (2013) 4412–4418.
[22] C. Guo, S. Lili, S. Ge, Y. Diansi, D. Rui, Isolation and characterization of formaldehyde-degrading bacteria from furniture factory, Inf. Technol. Agric. Eng., 134 (2012) 261–267.
[23] S. Ray, V.C. Kalia, Polyhydroxyalkanoate production and degradation patterns in *Bacillus* species, Indian J. Microbiol., 57 (2017) 387–392.
[24] B. Bharathiraja, J. Jayamuthunagai, I.A.E. Selvakumari, A.A. Dino, B. Abirami, B.A. Nayagam, S. Varjani, Biodegradation of sulfanilic acid using *Bacillus cereus* AAA2018 from textile industry effluent contaminated soil, Indian J. Exp. Biol., 58 (2020) 869–874.
[25] S. Liu, Y. Zhang, P. Zeng, H. Wang, Y. Song, J. Li, Isolation and characterization of a bacterial strain capable of efficient berberine degradation, Int. J. Environ. Health Eng., 16 (2019) 1–12.
[26] N. Akhtar, N. Ilyas, H. Yasmin, R.Z. Sayyed, Z. Hasnain, A. Elsayed, A. Hesham, E.I. Enshasy, Role of *Bacillus cereus* in improving the growth and phytoextractability of *Brassica nigra* (L.) K. Koch in chromium contaminated soil, Molecules, 26 (2021) 1569, doi: 10.3390/molecules26061569.
[27] U. Edwards, T. Rogall, H. Blocker, M. Emde, E.C. Bottger, Isolation and direct complete nucleotide determination of entire genes: characterization of a gene coding for 16S ribosomal RNA, Nucleic Acids Res., 17 (1989) 7843–7853.
[28] A. Dereeper, V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, S. Dufayard, V. Guindon, M. Lefort, J. Lescot, M. Claverie, O. Gascuel, Phylogeny.fr: robust phylogenetic analysis for the non-specialist, Nucleic Acids Res., 36 (2008) 465–469.
[29] A. Qureshi, A. Kapley, H.J. Purohit, Degradation of 2,4,6-trinitrophenol (TNP) by *Arthrobacter* sp. HPC1223 isolated from effluent treatment plant, Indian J. Microbiol., 52 (2012) 642–647.
[30] K. Veenagayathri, N. Vasudevan, Biodegradation of formaldehyde under saline conditions by a moderately halophilic bacterial consortium, Curr. World Environ., 5 (2010) 31–38.
[31] D.S. Yu, G. Song, L.L. Song, W. Wang, C.H. Guo, Formaldehyde degradation by a newly isolated fungus *Aspergillus* sp. HUA, Int. J. Environ. Sci. Technol., 12 (2015) 247–254.
[32] S.K.S. Patel, J.K. Lee, V.C. Kalia, Integrative approach for producing hydrogen and polyhydroxyalkanoate from mixed wastes of biological origin, Indian J. Microbiol., 56 (2016) 293–300.
[33] H. Zhao, Y. Geng, J. Fan, K. Tao, T. Hou, Isolation and identification of *Paracoccus* sp. FD3 and evaluation of its formaldehyde degradation kinetics, Biotechnol. Bioprocess Eng., 18 (2013) 300–305.
[34] J.F. Zhan, S.T. Jiang, L.J. Pan, Immobilization of phospholipase A1 using a polyvinyl alcohol-alginate matrix and evaluation of the effects of immobilization, Braz. J. Chem. Eng., 30 (2013) 721–728.
[35] S. Varjani, V.N. Upasani, Comparing bioremediation approaches for agricultural soil affected with petroleum crude – a case study, Indian J. Microbiol., 59 (2019) 356–364.
[36] C. Jarusutthirak, K. Sangsawang, S. Mattaraj, R. Jiraratananon, Treatment of formaldehyde-containing wastewater using membrane bioreactor, J. Environ. Eng., 138 (2012) 265–271.
[37] H.R. Lotfya, I.G. Rashed, A method for treating wastewater containing formaldehyde, Water Res., 36 (2002) 633–637.
[38] K. Hasani, M. Moradi, S.A. Mokhtari, H. Sadeghi, A. Dargahi, M. Vosoughi, Degradation of basic violet 16 dye by electroactivated persulfate process from aqueous solutions and toxicity assessment using microorganisms: determination of by-products, reaction kinetic and optimization using Box–Behnken design, Int. J. Chem. React. Eng., 19 (2021) 261–275.
[39] A. Dargahi, M. Vosoughi, S.A. Mokhtari, Y. Vaziri, M. Alighadri, Electrochemical degradation of 2,4-dinitrotoluene (DNT) from aqueous solutions using three-dimensional electrocatalytic reactor (3DER): degradation pathway, evaluation of toxicity and optimization using RSM-CCD, Arabian J. Chem., 15 (2022) 103648, doi: 10.1016/j.arabjc.2021.103648.
[40] J.K. Lee, V.C. Kalia, Mapping microbial capacities for bioremediation: genes to genomics, Indian J. Microbiol., 60 (2020) 45–53.
[41] A. Dargahi, M. Moradi, R. Marafat, M. Vosoughi, S.A. Mokhtari, K. Hasani, S. Moghadami, Applications of advanced oxidation processes (electro-Fenton and sono-electro-Fenton) for degradation of diazinon insecticide from aqueous solutions: optimization and modeling using RSM-CCD, influencing factors, evaluation of toxicity, and degradation pathway, Biomass Convers. Biorefin., (2021), doi: 10.1007/s13399-021-01753-x.
[42] K. Hasani, M. Moradi, A. Dargahi, M. Vosoughi, Evaluation of cefixime toxicity treated with sono-electro-Fenton process by bioassay using microorganisms, Avicenna. J. Environ. Health Eng., 8 (2021) 22–27.

Supplementary information

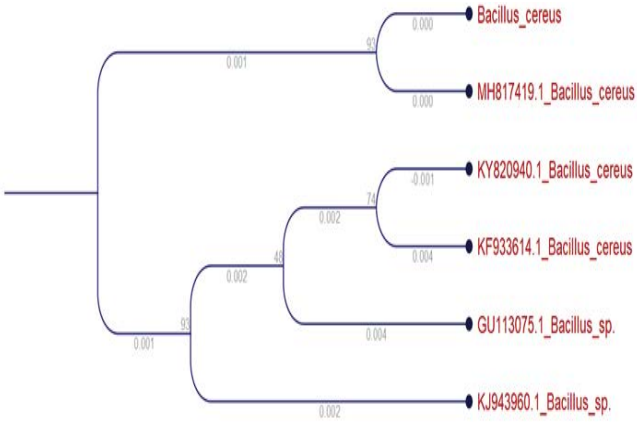

Fig. S1. Phylogenetic tree.

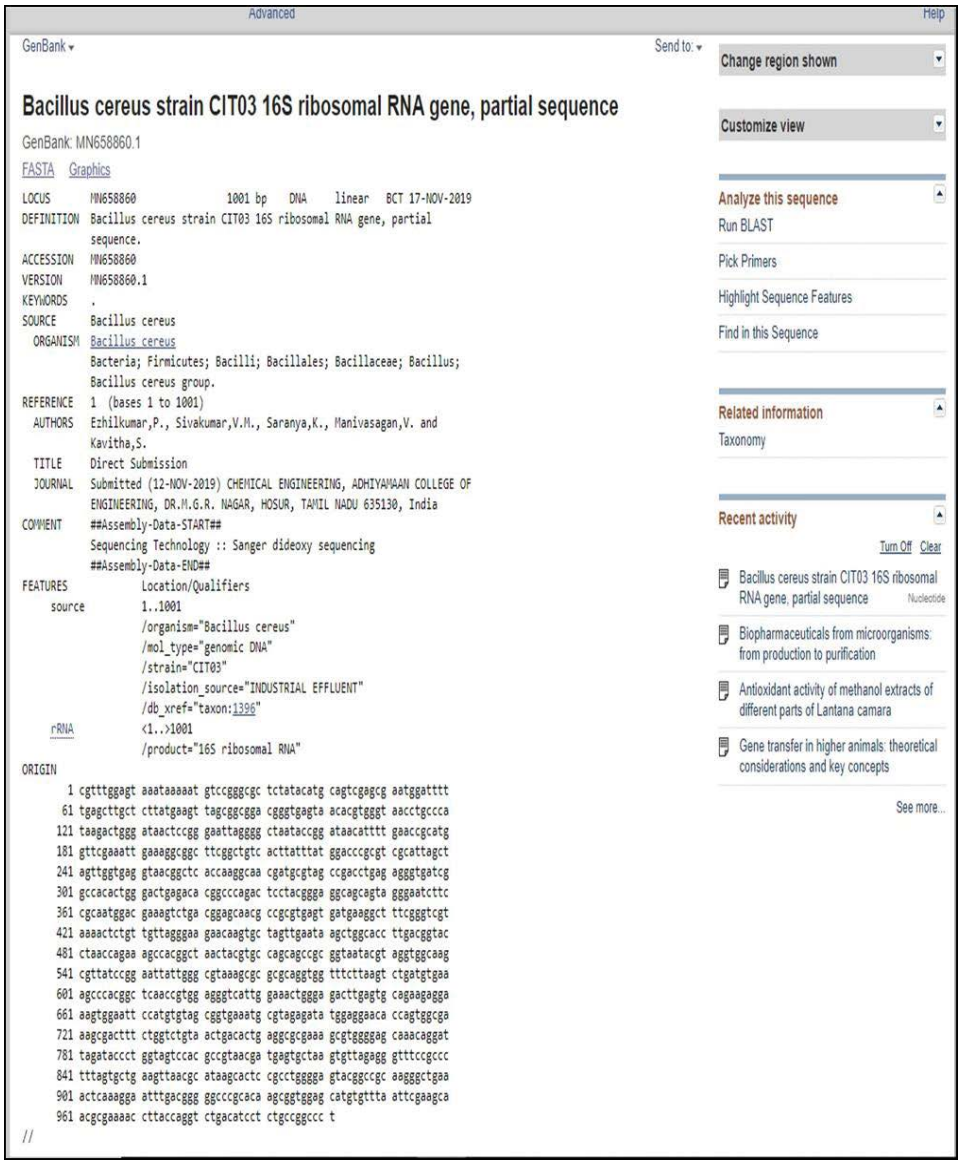

Fig. S2. 16S ribosomal RNA gene sequencing (*Bacillus cereus* CIT03).