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Biodegradation of cellulose containing gum product utilized as salt binders and fuels in the slurry explosives by *Bacillus thuringiensis* (BAB-2592)

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Abstract

Cellulose based gums (guar gum, xanthan gum, tropica starch, acacia gum, maize starch etc.) are the basic ingredients used for binding and encapsulation of the air/N2 gas bubbles in the slurry explosives. The gumming materials are used in the two or more combinations considering the shelf-life of the finished products. Bacterial isolates from contaminated soil were grown in minimal medium and screened for gums (guar gum and xanthan gum) degradation. The isolates A1 exposed maximum growth selected for detailed studied. Morphological and biochemical analysis, as well as 16 rRNA gene sequence comparison, demonstrated that *B. thuringiensis* thereof are found prominent culture to decompose the carbon and for breaking of polymeric chain developed during production.

Key words : *Bacillus thuringiensis*, Biodegradation, Guar gum, Xanthan gum.

INTRODUCTION

Blasting by the use of commercial explosives is an important process during in mining for breaking of rocks [1]. The explosives used in Indian mining and excavation projects include (NG) based, ammonium nitrate fuel oil mixture (ANFO), Slurry, Emulsion and liquid oxygen (LOX) explosives [2]. Slurry explosives plays an important role in blasting operation in the opencast and underground mines.

Slurry explosive, as developed by Cook in 1960 contained trinitrotoluene (TNT), ammonium nitrate (AN), sodium Nitrate (SN), calcium Nitrate (CN), potassium pyro antimonate (PPA), water, guar gum, potassium dichromate, potassium antimony tartrate, and sodium nitrite [3-5]. However, recent slurry explosives contain oxidizing agents, fuel, water, thickening agent (viscofiers), crosslinking gents, gassing agents, and sensitizers. The oxidizing agents include nitrates of ammonia, sodium, potassium, calcium or combination of any two or three of these oxidizers. Fuels are generally water soluble reducing agents which include formamide, ethylene glycol, thiourea, urea, sugar and molasses [6] and thickening agent guar gum, tropica starch, acacia gum mazie etc. and organic polymer gum used to increase the viscosity of the solutionand used for binding and encapsulation of the air/N_2 gus bubbles in the slurry explosives [7, 1].

Huge quantity of slurry explosives are available in the magazines of manufacturers and users which has lost their explosive properties or seized explosive products which to be destructed by burning process only. The available method to destroy the deteriorated, expired and abandoned commercial explosives specified by the Petroleum and Explosive Safety Organization (PESO) in India is to burn the explosives in the presence of competent authority [8].The burning of commercial explosives is a very difficult process as they do not burn easily and burning generate huge amount of hazardous gases such as CO, CO_2, NO_x, SO_2 and other metal oxides, polluting the environment. The microbial method of decomposition has been used successfully for the Uni-molecular explosives such as TNT, RDX and HMX but this is not suitable for water gel slurry explosives. However, till date, No Biological methods has been ever developed and adopted for destruction of the slurry explosives.Development of suitable and alternative method of destruction of slurry explosive using microbial means is the need of the time and a lot of work is still to be done for the decomposition of slurry explosives using alternative method and its conversion into usable products [1].

This paper describes the method of biodegradation of guar gum and xanthan gum and to break the cross-linked gel of slurry explosives and, this paper also describes the results of investigation conducted for artificial destruction of explosive lattice using different microbes.

MATERIALS AND METHODS

Enrichment and isolation of Bacteria

In order to isolate slurry explosives degrading bacteria, enrichment cultures were set up using composite soil sample made of the fifteen samples. About 5g of soil equivalent dry weight was suspended in 50ml of mineral salt (MS) [9] medium containing Guar gum (GG) and Xanthan gum (XG) as the sole source concentration of 50 mg/l for liquid medium (MS-guar gum xanthan gum) and incubated at 30˚C on an orbital shaker at 150 revolution per minute (rpm). After five days incubation 5 ml aliquot was re-inoculated in the fresh MS medium and guar gum and xanthan gum was supplemented at a concentration of 100 mg/l and incubated under the same conditions for five days. After ten days incubation 5 ml aliquot was re-inoculated in the fresh MS medium and guar gum and xanthan gum was supplemented at a concentration 150 mg/l. At the last enrichment step the above mentioned process was repeated and Guar gum and xanthan gum concentration was increased from 150 mg/l to and 200 mg/l. Four successive enrichment cycles were carried out in the same way and the culture was serially diluted tenfold and 100 µl of the10^{-3} to

10^{-6} dilutions were streaked onto solid MS medium containing 200 mg/l of guar gum and xanthan gum and nutrient agar (NA) medium for isolation of a single colony. Each colony, considered as a different species, was repeatedly streaked on agar plates. Pure cultures were obtained by streaking for more than 10 times and were screened for Guar gum and xanthan gum degradation before using for subsequent study.

These bacterial isolates were identified on the basis of morphological and biochemical tests like gram staining catalase, oxidose test and 16S rRNA sequence analysis .bacterial genomic DNA samples were extracted using a Instagene^{tm} matrix (BIO-RAD). The primers 27 F 5' (AGA GTT TGA TCB TGG CTC AG) 3' and 1492 R 5'(TAC GGY TAC CTT GTT ACG ACT T)3' were used for the PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 reaction mixture by using a EF-Taq (SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 2minutes, 35 cycles of 95 °C for 1minutes, 55°C, and 72 °C for 1minutes each were performed, finishing with a 10-minute step at 72 °C.

Based on the growth in the broth medium containing Guar gum, two species of bacteria i.e. *B. Thuringiensis* and *P.alcaligene* were selected for further investigation. Among the selected bacterial isolates, *B. Thuringiensis* degraded maximum 94.34 % guar gum and 79.90 % xanthan gum at 20 mg/L, followed by *P.alcaligene* (Guar gum 56.18% and Xanthan gum 42.86%) *B. Thuringiensis* was chosen for further study.

Growth kinetics of microorganisms

The bacterial isolate was grown in broth minimal medium supplemented with three concentrations of guar gum and xanthan gum .The flasks were then incubated at 30 °C on an orbital shaker (150 rpm), as well as in biochemical oxygen demand (BOD) incubator under static condition for 15 days. Sampling occurred at intervals of 5, 10 and 15 days. Bacterial growth was studied by measuring the optical density of the culture media using UV/VIS Spectrophotometer (VARIAN, Inc.) at 600 nm wavelength.

Biodegradation of Guar Gum and Xanthan Gum by B. Thuringiensis in agitating conditions

To assess the effect of agitation, on biodegradation of guar gum and xanthan gum by the microorganism, 250mL Erlenmeyer flasks holding 100mL broth minimal medium autoclaved at 121 °C for 20min were used. Broth pH was adjusted to 7.0, spiked with GG and XG as a sole source of carbon, at three concentrations i.e. (20, 50 and 100mg/L) and inoculated with *B. thuringiensis*. These flasks were incubated at 30 °C on an orbital shaker (150 rpm) for 15 days. Control flasks containing minimal medium and the gum powder, but without bacterial inoculation, were also maintained for compensation of abiotic degradation, if any.

Biodegradation of by Guar Gum and Xanthan Gum by B. Thuringiensis in static conditions

Another experiment was performed to study guar gum and xanthan gum degradation in static conditions. For this, the flasks were incubated at 30 °C in a BOD incubator for 15days. To minimize error, the study was carried out in triplicate and the results are means of the three. The samples were withdrawn aseptically after 5, 10 and 15days for estimation of residual Slurry explosives in the medium. The pH of culture broth was measured with a pH metre (Elico, India (P) Ltd) by calibrating with the standard pH buffer 4.0, 7.0 and 9.2.

Statistical Analysis of data

The experimental data was processed for calculating standard error of the means and multi-factorial analysis of variance as available in the SPSS statistical package (Stat Graphics Plus V. 11), and expressed at 0.05 probability level.

RESULT

The bacterial isolate used in the biodegradation study was Gram-negative, rod-shaped, fluorescent pigment producing in (triptic soy agar), catalase and oxidase positive. A partial 16S rRNA sequence, used to establish the identity of the isolate, revealed 97% sequence similarity with that of *B. thuringiensis*. The BLAST programme, used for gene homology search with the standard programme default, identified the microorganism as *B. thuringiensis.*

Growth kinetics of B. thuringiensis in broth culture

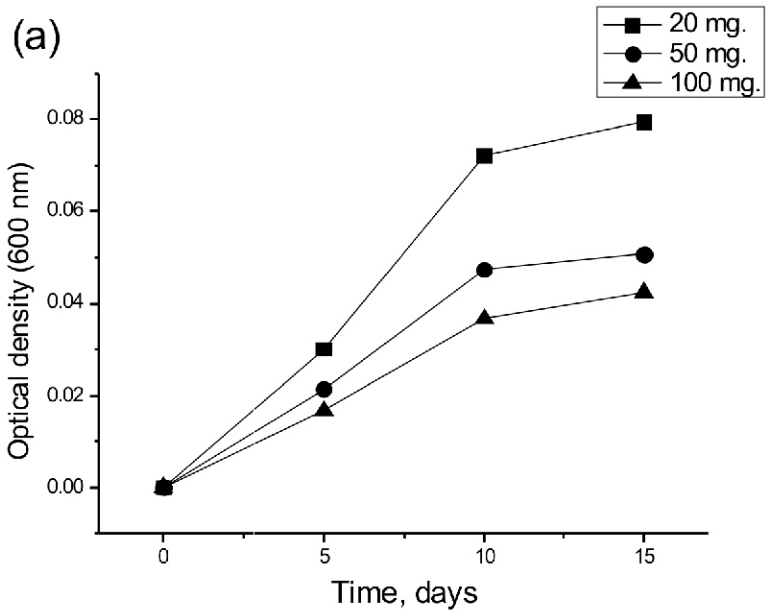
B. thuringiensis exhibited slow growth during the first five days incubation under both the conditions. Later on, it adapted to the medium, used GG and XG as a carbon source and grew exponentially up to the 10th day in GG and XG containing medium under agitating condition. Thereafter, the growth was almost stagnant up to the 15th day [Figure 1 (a) and (b)]. The growth of the bacterial isolate was slower ($p < 0.05$) under the static condition than the agitating condition [Figure 1(c) and (d)].

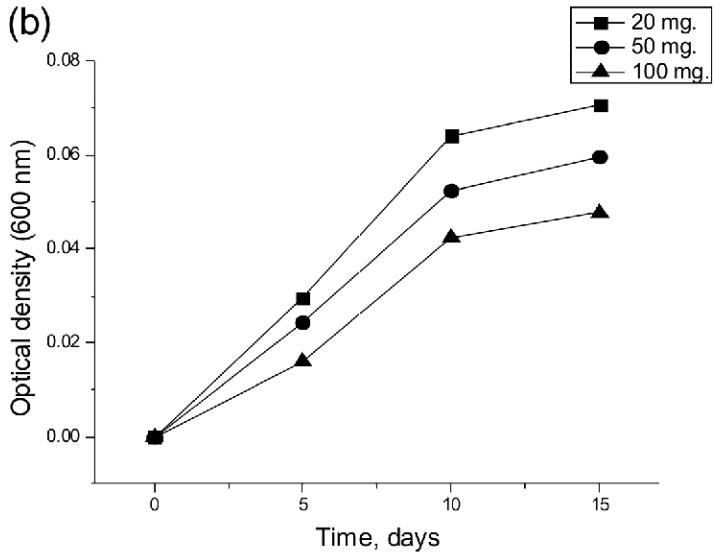
Biodegradation of guar gum and xanthan gum by B. thuringiensis in broth cultur

Biodegradation of guar gum and xanthan gum by *B. Thuringiensis* was determined by monitoring their disappearance and the appearance of metabolites during incubation over 15 days. The rate of GG and XG degradation in broth differed substantially under agitating and static conditions. During the first 5 days, biodegradation of GG and XG was slow and recorded 25.34 and 23.15 %, respectively, at 20 mg. in the agitating condition [Figure 2(a) and (b)], 20.41 and 18.41% in the static condition [Figure 3 (a) and (b)]. The degradation of both guar gum and xanthan gum at 50 and 100 mg. declined ($p < 0.05$), however. Later, the degradation at 20 mg. was enhanced in both agitating and static conditions (Figure 2 and 3). At the end of the experiment, biodegradation of GG in the agitating condition was 94.34% at 20 mg. followed by 83.12 and 74.12% at 50 and 100 mg. Degradation of XG at 20, 50 and 100 mg. was 89.35, 75.45 and 70.82%, respectively [Figure 2(a)and (b)]. In the static condition, degradation of GG at 20 mg/L was 79.9% followed by 61.02 and 51.12% at 50 and 100 mg., whilst the corresponding values for XG were 66.44, 40.89 and 35.12% [Figure3 (a) and (b)], after excluding the abiotic degradation. Degradation of XG was lower than that of GG in all the treatments (Figure 2 and 3). Biodegradation of gumming materials by *B. thuringiensis* was accompanied by decreased pH of the broth. In the agitating condition, pH varied from 7.0 to 4.53 and 7.0 to 5.02 in case of guar gum and xanthan gum, respectively. In the static condition, the pH variation was found to be 7.05.18 and 7.05.27 for GG and XG. There is a significant difference ($p < 0.05$) in the final pH of the broth in the agitating condition compared to the static condition (Table 1).

DISCUSSION

B. Thuringiensis has been isolated from contaminated soil and cultured successively in broth medium containing guar gum and xanthan gum as the source of carbon. This isolate showed maximum growth as depicted by the optical density and hence


(a)
20 mg.
50 mg.
100 mg.
Optical density (600 nm)
0.00
0.02
0.04
0.06
0.08
0
5
10
15
Time, days


(b)
20 mg.
50 mg.
100 mg.
Optical density (600 nm)
0.00
0.02
0.04
0.06
0.08
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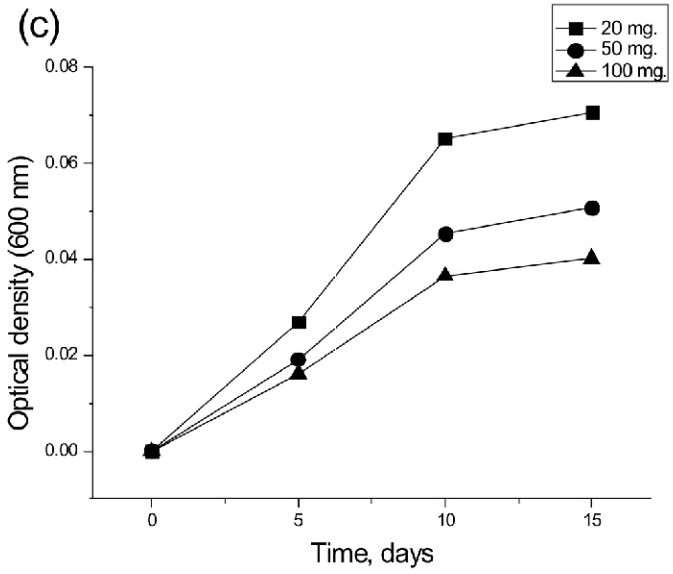
5
10
15
Time, days


(c)
20 mg.
50 mg.
100 mg.
Optical density (600 nm)
0.00
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0.04
0.06
0.08
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15
Time, days

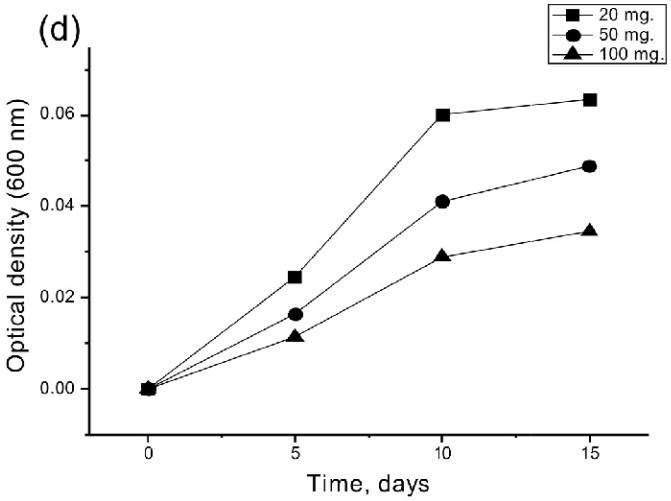

Figure 1. Growth kinetics of *B. thuringiensis* in broth culture: (a) Guar gum (b) Xanthan gum in agitating condition, (c) Guar Gum and (d) Xanthan in static condition.

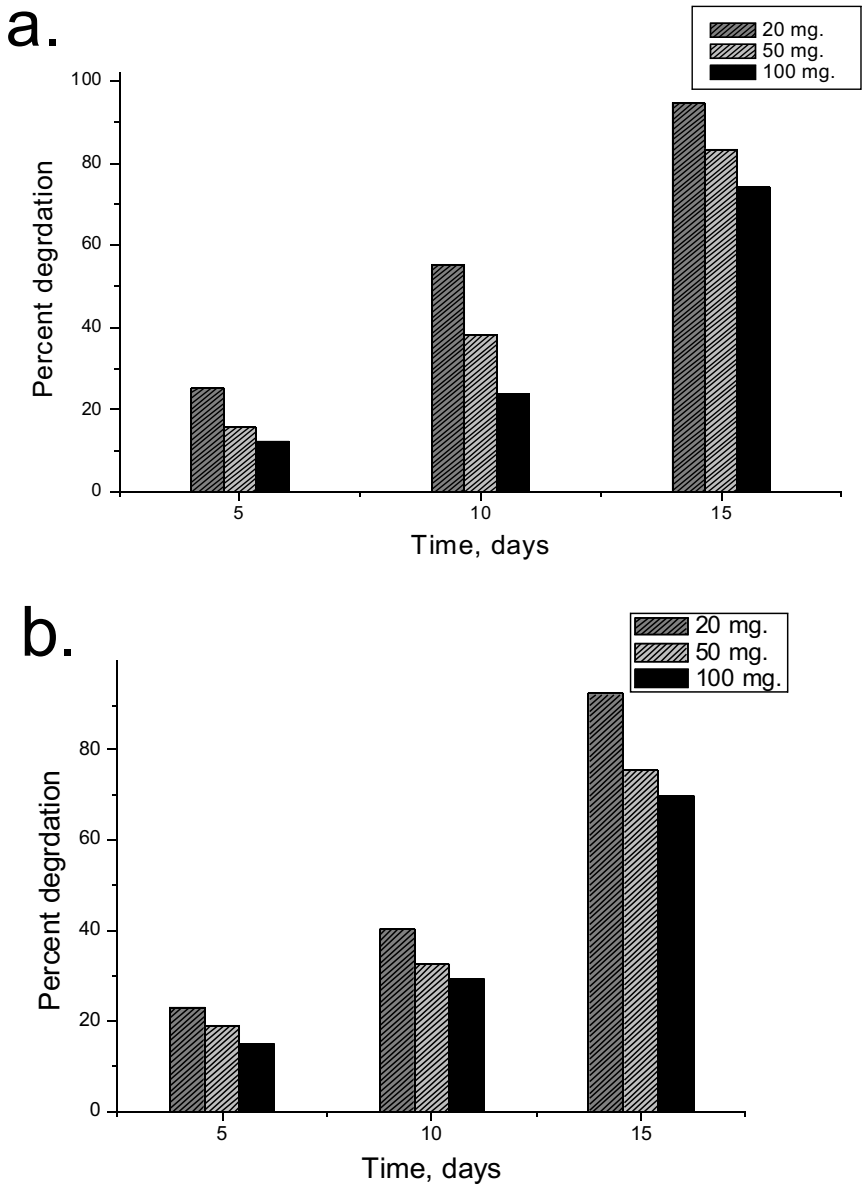

Figure 2. Microbial degradation by *B. thuringiensis* in broth culture: (a) GG (b) XG in agitating condition

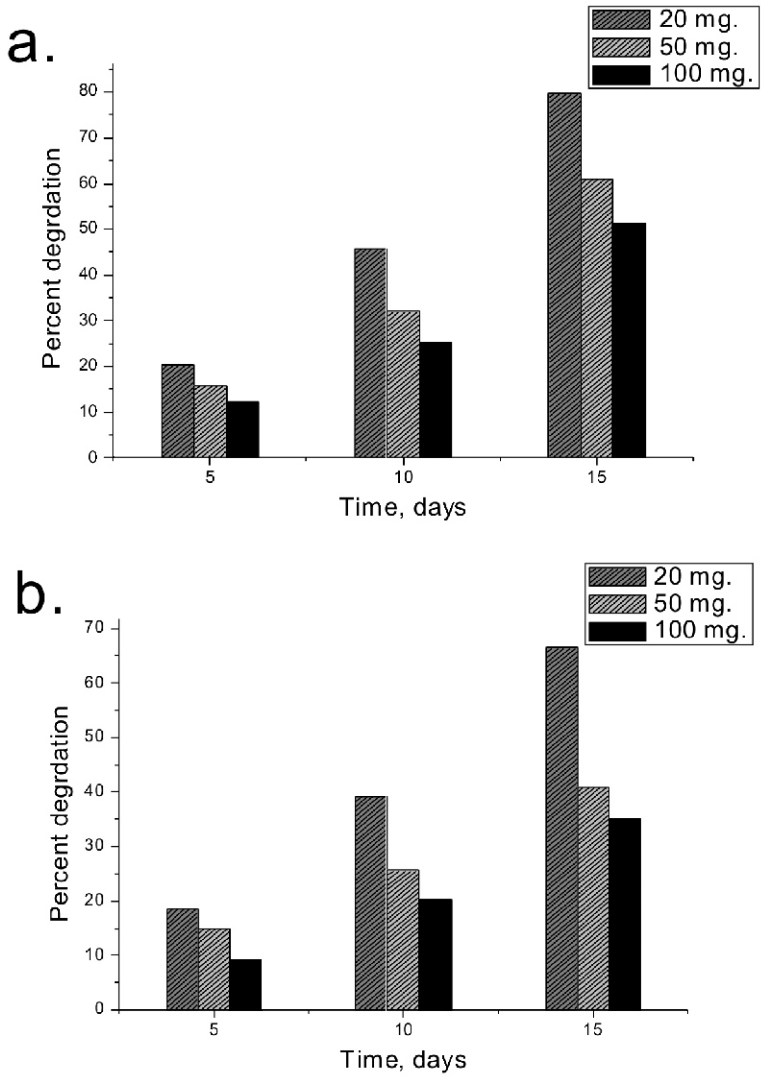

Figure 3. Microbial degradation by *B. thuringiensis* in broth culture: (a) GG (b) XG in static condition

Table 1: pHchange of broth culture as a result of guar gum and xanthan gum degradation by *B. thuringiensis.*

	Conc. (mg.)	Incubation period (Days)					
		Agitating condition			Static condition		
		5	10	15	5	10	15
Guar gum	20	6.11±0.04	8.19±0.11	8.53±0.07	6.16±0.05	6.69±0.04	7.23±0.03
	50	6.31±0.01	6.65±0.15	6.56±0.05	6.04±0.07	6.62±0.07	6.94±0.05
	100	5.75±0.07	6.69±0.12	6.28±0.01	6.28±0.04	6.48±0.02	6.51±0.14
Xanthan gum	20	6.21±0.03	7.19±0.11	8.83±0.06	6.10±0.17	6.92±0.08	7.89±0.10
	50	5.78±0.05	6.31±0.13	7.18±0.15	5.89±0.04	6.41±0.03	6.96±0.06
	100	5.97±0.06	6.18±0.10	6.83±0.09	5.72±0.09	6.18±0.16	6.61±0.05

Note: Values are average of three repeats, after excluding control value, ±SE

was selected for further study. In broth culture, an increase in optical density with disappearance of demonstrated the use of guar gum and xanthan gum as a carbon and energy source.

B. Thuringiensis used both the isomers of guar gum and xanthan gum either as carbon or sulphur source. This might be due to the prolific growth of the bacterial isolate during the incubation period and induction of a suitable enzyme system responsible for the degradation. During the first 5days, degradation of guar gum and xanthan gum was slow, which might

represent a lag phase. Afterward, guar gum and xanthan gum degradation was enhanced as the incubation proceeded, most likely due to exponential growth of the bacterium as shown by optical densities at different concentrations and the induction of enzymes responsible for degradation. Maximum degradation of guar gum ad xanthan gum by B. Thuringiensis was recorded at 20mg/L after15days incubation. The degradation of guar gum and xanthan gum was lower at 50 and 100mg/L in both conditions. The biodegradation in the agitating condition was more pronounced in the static condition. This demonstrates that the agitating condition might be more conducive for bacterial growth, or agitation might have resulted in more bioavailability of substrate to the bacterial isolate and more oxygen in the medium for its activity. There was a parallel decrease in pH of the culture medium as the biodegradation proceeded. This reduction in pH of the broth might be due to dehalogenation of and formation of acidic substances.

CONCLUSION

The success of bioremediation may depend on the augmentation of the best suited microbial species coupled with a search for optimum environmental condition(s) for their activity at the desired pace. From the present study, we can conclude that tremendous potential for use in biodegradation of guar gum and xanthan gum contaminated habitats especially with lower concentrations. *B. thuringiensis* thereof are found prominent culture to decompose the carbon and for breaking of polymeric chain developed during production. The study conclude that *B. thuringiensis* could be used effectively for biodegradation of guar gum and xanthan gum in the mining explosives.

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