Journal of Basic and Applied Engineering Research
Print ISSN: 2350-0077; Online ISSN: 2350-0255; Volume 1, Number 9; October, 2014 pp. 17-21
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http://www.krishisanskriti.org/jbaer.html

Biodegradation of Carbon Containing Gumming Materials Utilized as Salt Binders and Fuels in the Mining Explosives

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Abstract: **Carbon based gums such as guar gum, xanthan gum, tropica starch, acacia gum, maize starch etc. are the basic ingredients used for binding and encapsulation of the air/N_2 gas bubbles in the mining 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 like as guar gum and xanthan gum degradation. The isolates A5 showed maximum growth selected for detailed studied. Morphological and biochemical analysis, as well as 16 rRNA gene sequence comparison, demonstrated that pseudomonas sp. thereof are found prominent culture to decompose the carbon and for breaking of polymeric chain developed during production. The study showed that pseudomonas sp. could be used effectively for biodegradation of guar gum and xanthan gum in the mining explosives.**

Keywords: **biodegradation, Guar gum, Xanthan gum, pseudomonas sp.**

1. INTRODUCTION

An explosive is a substance which produces, upon release of its potential energy, a sudden outburst of gas, thereby exerting high pressure on its surroundings (1). It is a mixture of oxidizing and reducing agents with sensitizers and stabilizers. Oxidizing agents have tendency to release oxygen and reducing agents have affinity to accept oxygen in explosives reaction. Stabilizers are used to protect the oxidizing and reducing agents, and the explosive compositions from deterioration. As the reaction front moves through detonation, the explosive releases large volume of gaseous products and energy on the surrounding rock, causing fragmentation, shattering or hearing (2).

There are two type of explosives: military and civil explosives. The composition of military explosive is classified so it is not public. Civil or Commercial explosives are chemical compounds, or mixture of compounds which undergo very rapid, self-propagating exothermic decomposition, when properly initiated. The commercial explosives are divided into two groups:

i) Detonating or high explosives, characterized by very high rate of reaction and high pressure;

ii) Deflagrating or low explosives, which burn slowly and develop much lower pressure (1).

Detonation indicates that the reaction is moving through the explosive faster than the local speed of sound in the un reacted part of the explosive, whereas, deflagration indicates that the reaction takes place at a rate below the speed of sound (3).

Slurry explosive, as developed by Cook in 1960 contained TNT, AN, sodium nitrate, calcium nitrate (CN), potassium pyro antimonate (PPA), water, guar gum, potassium dichromate, potassium antimony tartrate, and sodium nitrite (4,5). 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. These constitute approximately 60-70 % of the explosive. For high-density slurry explosives generally 40 - 50 % Ammonium Nitrate (AN) and 10-20 % sodium nitrate or calcium nitrate is preferred. Fuels are generally water soluble reducing agents which include formamide, ethylene glycol, thiourea, urea, sugar and molasses. Their proportions in the explosive mixture vary between 10-20 % depending upon the oxygen balance of the slurry composition, while water quantity varies between 10-20 %. Thickening agent –Guar gum, Xanthan gum and

organic polymer gum used to increase the viscosity of the solution, constitute about 1 to 2 % of the composition. Earlier slurry composition had low viscosity which caused segregation of constituents and loss of air bubbles, but a significant change in viscosity of the solution and stability of the slurry could be achieved by adding cross linking agents such as potassium dichromate, borax, boron compounds and antimony compounds which constitute 0.05 to 1.0 % of the explosive composition. Gassing agents (e.g. sodium nitrite, thiourea etc.) varying between 0.01-0.02 % are used to produce air pockets in slurry lattice and play an active role in detonation reaction. Aluminum powder and Monomethylaminenitrate (MMAN), the common sensitizers, vary between 4 and 6 % of the composition (6).

Slurry explosives, more popular in India both as cap and non-cap sensitive explosives in small and large diameter cartridge forms, are available in 25 to 200 mm diameters with the mass varying from 0.14 kg to 12.5 kg. Their VOD varies from 3000 to 4500 m/s and density varies from 1100-1250 kg/m^3. The bulk strength of slurry explosives is lower than NG based explosives. Compared to the NG based explosives slurry explosives are however, more water resistant and have less toxic fumes such as No_x and CO.

2. MATERIAL AND METHODS

2.1 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) 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 stating, 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$\mu\ell$ 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. *P. alcaligene* and *P. aeruginosa* were selected for further investigation. Among the selected bacterial isolates, P. *alcaligene* degraded maximum 89.80% guar gum and 58.35% xanthan gum at 20 mg/L, followed by *P. aeruginosa* (Guar gum 66.28% and Xanthan gum 42.56%) *P. alcaligene* was chosen for further study.

Growth kinetics of isolates

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.

2.2 Biodegradation of Carbon Containing Gumming Materials by isolates in agitating condition

To assess the effect of agitation, on biodegradation of Guar gum and Xanthan gum by the bacterial isolate, 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 *P. aeruginosa*. These flasks were incubated at 30 °C on an orbital shaker (150 rpm) for 15 days. Control flasks containing minimal medium and the pesticide, but without bacterial inoculation, were also maintained for compensation of abiotic degradation, if any.

2.3 Biodegradation of Carbon Containing Gumming Materials by isolates in static condition

Another experiment was performed to study slurry explosives 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.

2.4 Statistical Analysis of data

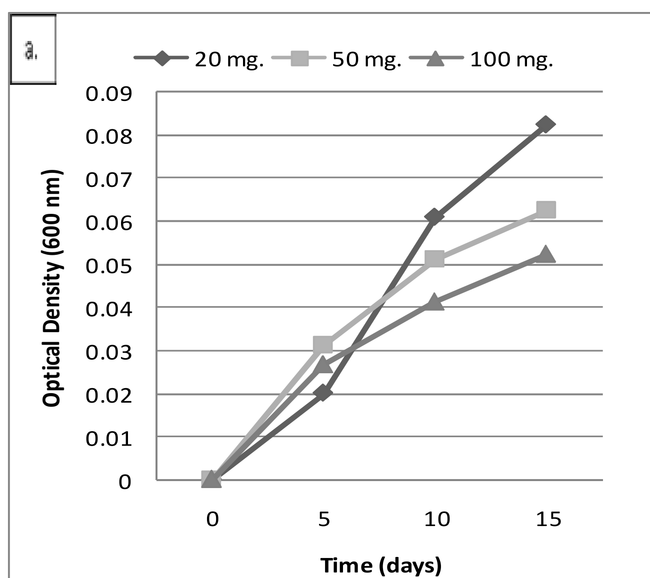
The experimental data was processed for calculating standard error of the means and multi-factorial analysis of variance as available in the SPSS statistical package (Stat Graphics Plus V. 11), and expressed at 0.05 probability level.

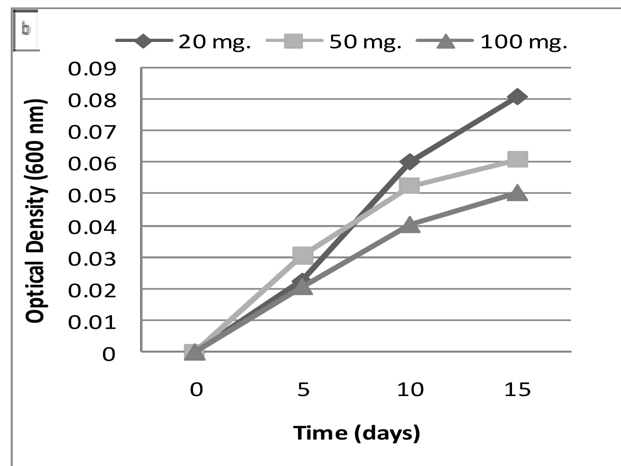
3. RESULT AND DISCUSSION

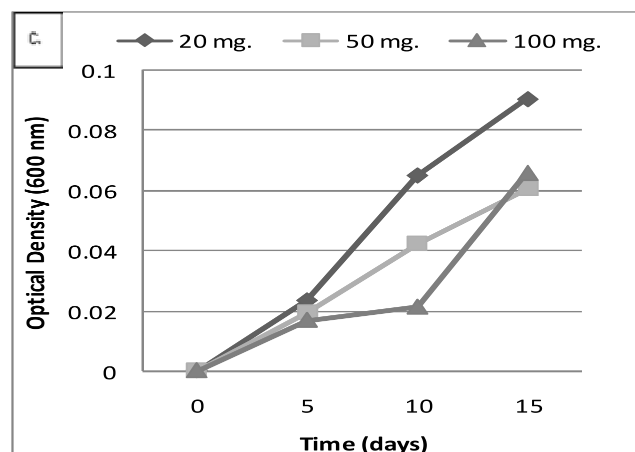
The bacterial isolate used in the biodegradation study was Gram-negative, rod-shaped, fluorescent pigment producing in (triptic soy agar), catalase and oxidase positive. A partial 16S rRNA sequence, used to establish the identity of the isolate, revealed 97% sequence similarity with that of *P. aeruginosa*.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

3.1 Growth kinetics of P. aeruginosa in broth culture

P. aeruginosa 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)].







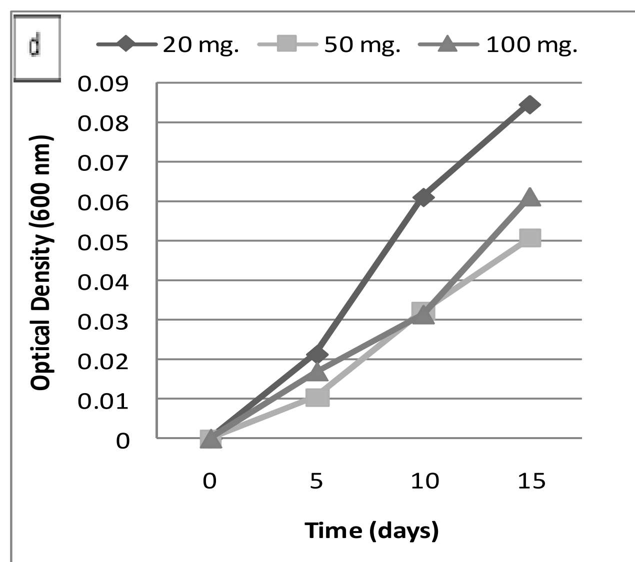
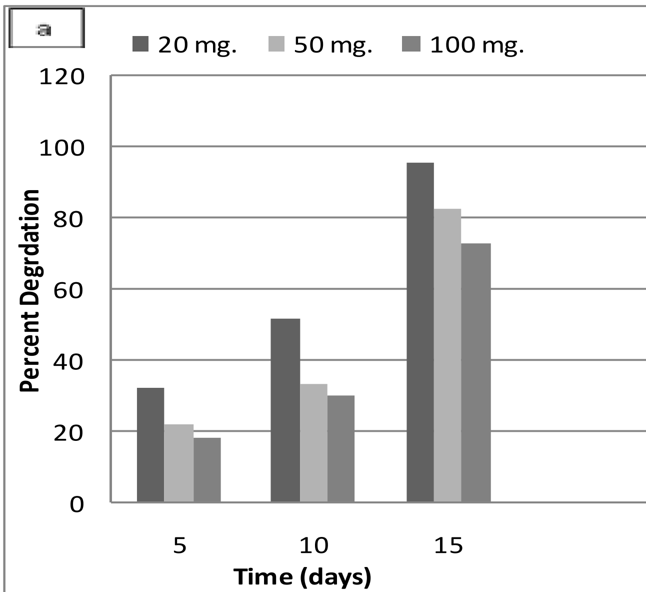


Fig. 1. Growth kinetics of *P. aeruginosa* in broth culture: (a) Guar gum (b) Xanthan gum in agitating condition, (c) Guar Gum and (d) Xanthan in static condition.

3.2 Carbon Containing Gumming Materials degradation by P. aeruginosa in broth culture

Biodegradation of Gumming Materials isomers by *P. aeruginosa* 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 31.85 and 28.85 %, respectively, at 20 mg. in the agitating condition [Figure 2(a)], 29.33 and 23.85% in the static condition [Figure 2(b)].

The degradation of both gumming materials 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(a) and (b)). At the end of the experiment, biodegradation of GG in the agitating condition was 95.80% at 20 mg. followed by 82.35 and 72.82% at 50 and 100 mg. Degradation of XG at 20, 50 and 100 mg. was 92.80, 86.35 and 79.82%, respectively [Figure 2 (a)]. In the static condition, degradation of GG at 20 mg/L was 81.8% followed by 69.35 and 49.82% at 50 and 100 mg., whilst the corresponding values for XG were 79.38, 44.22 and 39.85% (Figure 2(b)), after excluding the abiotic degradation. Degradation of XG was lower than that of GG in all the treatments (Figure 2(a) and (b)). Biodegradation of gumming materials by *P. aeruginosa* 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 7.0–5.18 and 7.0–5.27 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).



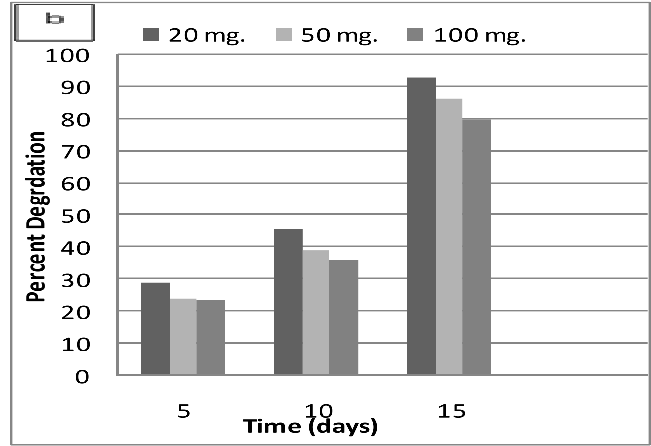
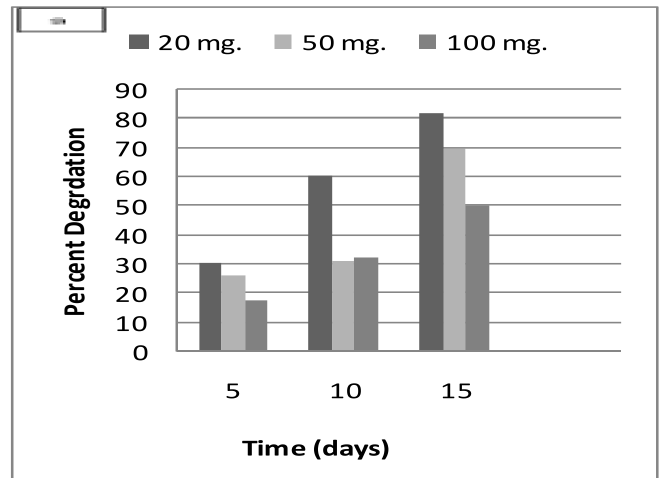


Fig. 2. Microbial degradation by *P. aeruginosa.* in broth culture: (a) GG (b) XG in agitating condition



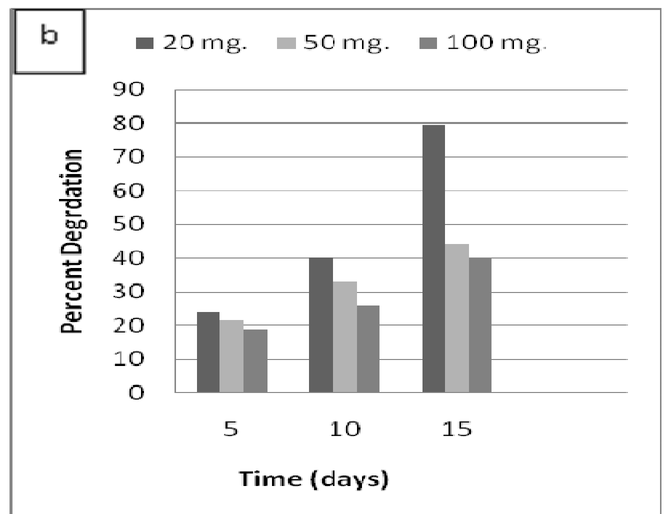


Fig. 3. Microbial degradation by *P. aeruginosa.* in broth culture: (a) GG (b) XG in static condition

Table. No.-1 pH change of broth culture as a result of gumming materials degradation by isolates

	Conc. (mg.)	Incubation period (Days)					
		Agitating condition			Static condition		
		5	10	15	5	10	15
Guar gum	20	6.21±0.06	8.15±0.11	8.49±0.07	6.10±0.05	6.66±0.04	7.17±0.03
	50	6.41±0.31	6.61±0.15	6.50±0.05	6.08±0.07	6.58±0.07	6.84±0.05
	100	5.95±0.07	6.65±0.12	6.23±0.01	6.24±0.04	6.42±0.02	6.46±0.14
Xanthan gum	20	6.19±0.03	7.15±0.11	8.78±0.06	6.09±0.17	6.88±0.08	7.85±0.10
	50	5.88±0.05	6.27±0.13	7.13±0.15	5.87±0.04	6.37±0.03	6.99±0.06
	100	5.94±0.06	6.12±0.10	6.79±0.09	5.67±0.09	6.14±0.16	6.56±0.05

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

4. CONCLUSIONS

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 concludes that has *P. aeruginosa* tremendous potential for use in biodegradation of gumming materials contaminated habitats especially with lower concentrations. *Pseudomonas aeruginosa* thereof are found prominent culture to decompose the carbon and for breaking of polymeric chain developed during production. The study conclude that pseudomonas sp. could be used effectively for biodegradation of guar gum and xanthan gum in the mining explosives.

ACKNOWLEDGEMENTS

The authors are highly grateful to Director, Indian School of Mines, Dhanbad. and Director, Central Institute of Mining and Fuel Research, Dhanbad and. for the encouragement and support.

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