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# Microbial Degradation of Nitrate Salts Utilized as Oxidizing Agent in Slurry Explosives

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***Abstract: -*** Nitrate salts of ammonia, calcium, sodium, potassium are the oxygen suppliers for slurry explosives. These oxidizing agents are used in two or more combinations for providing the oxygen balance and to enhance the energy output required for breaking the rocks. Expired slurry explosives become unusable, hence requires destruction. The users and manufactures find these unusable slurry explosives very difficult to destroy or decompose. A suitable procedure is designed using microbial means to decompose the slurry explosives. Microbial action on Ammonium Nitrate is considered to break the gel lattices of the explosive more over. Cross-linking of gel will be broken to release other ingredients of the explosive like water and other salts including fuels, gumming materials etc. The oxygen balance for the formulation of slurry explosive will be disturbed due to decomposition of the Ammonium Nitrate and fuels responsible for explosive properties. The growth of bacterial namely *Pseudomonas* and *Bacillus* which are found suitable to decompose the Ammonium Nitrate and to disturb the Oxygen balance.

***Keywords: -*** *Ammonium Nitrate, Calcium Nitrate, Sodium Nitrate, Microbial Degradation, Slurry Explosive.*

## I. INTRODUCTION

Mineral deposits are the key parameters to identify the economic status of any country for its progress and development. Coal is the prime and cheapest source of energy and produced by opencast and underground coal mines [1]. Drilling and blasting are predominant methods to extract coal from coal mines and blasting operation is conducted using commercial explosives and blasting accessories such as slurry, emulsion and powder explosives in cartridge form or through direct charging in the blast holes and using initiating accessories such as cast boosters, detonating fuse, electric instantaneous and delay detonators [2]. India is consuming 10, 00,000 tonnes approximately slurry and emulsion explosives every year for blasting practices for mining coal and other minerals. The annual growth of consumption of commercial explosives is about 14-20 % and may rise in future because there is huge difference in coal production and requirement of energy in India.

Commercial explosives used in the coal mines are classified into two groups. 1. Permitted explosives 2. Non-Permitted explosives. Permitted explosives are specially designed explosives for underground coal mines and classified on the basis of gassiness of the mines while non-permitted explosives are used in opencast mines and classified as cap sensitive and non-cap sensitive explosives. Cap sensitive explosives act as primer and non-cap sensitive act as column explosive [3], [4]. The ratio of primer and column explosive is 1:3 generally but may be changed as per the geo-mining condition [5].

Slurry explosive plays an important role in conducting blasting practices in the opencast as well as underground mines [3].Sometime it is observed that the gel condition of the slurry explosives breaks down and water, along with chemical ingredients, oozed out from the slurry cartridges and their physical and chemical properties get deteriorated. It may be due to long storage of explosives in the explosive magazines or due to defect during its production at the production units [6], [7]. A significant point in this content is that these explosives lose their detonating cap sensitivity within 4-5 months and showed very low detonating power [8].

The deterioration of explosive properties may lead to poor blasting performance such as formation of boulders, toe problem and generation of ground vibration or complete failure of the blasting operations [9].

Oxygen balance is the key factor in the designing of explosive formulation and plays a very important role in generating explosion pressure on the rock body [7]. Nitrate salt of ammonium, sodium, potassium and calcium are the oxygen suppliers and having 60-75 % of total mass of the explosive cartridge and fuel –sugar, carbon, sulphur, urea, MEG, DEG, guar gum etc. are oxygen receiver [5]**.**

Those explosives whose properties have already got deteriorated or having very low intensity may be decomposed using microbes and may be converted into the usable by- products**.** Only a few aerobes able to use Trinitrotoluene (TNT) as a nitrogen or carbon source have been reported, and mineralization of this compound has been described even less frequently. The *Pseudomonas* strain isolated by [10], from soil around an explosive factory was found being able to grow with TNT as the sole nitrogen source. This strain of *Pseudomonas* eliminate TNT via the production of a Meisenheimer complex [11], [12].

An attempt has been made to deteriorate the nitrate salts of ammonium, sodium, potassium and calcium using different microbes in different experimental conditions [13]. This paper describes the method of deterioration of Ammonium Nitrate (AN), and to break the gel of slurry explosives and, this paper also describes the results of investigation conducted for artificial destruction of explosive lattice using different microbes.

## II. MATERIAL AND METHODS

### A. Isolation and selection of the microbes:

**S**oil samples were collected from the contaminated explosive industrial area and used for isolation and selection of microorganisms. The soil samples were then subjected to serial dilution method and plated on nutrient agar medium (NAM) and incubated in 28±2 $^{o}$C for 48 hours. Thereafter out of hundred colonies only eleven different colonies were randomly identified and picked up for further experiments. The selection of microbes for further study was done on the basis of Disk method by producing zone into minimal salt medium (MSM) containing Ammonium Nitrate (AN). Only two distinct types of isolates namely A1 and A5 were selected by showing zone in the said medium. Both the selected isolates were maintained and stored as a stock in nutrient slants at 4˚C for further study in use of decomposition of AN.

### B. Identification of bacterial strains:

Identification of strains selected from screening were characterized by morphological, biochemical tests according to the methods described by Bergey's Manual of Determinative Bacteriology [14], [15].)The microscopic identification was carried out by gram's staining method using oil immersion microscope [16].

### C. Microbial Degradation of Nitrate Salts Utilized as Oxidizing Agent in the Slurry Explosives

Mineral salt medium was used to study the for decomposition of AN. Microorganism required environment conditions like as pH and temperature as well as food material like carbon substrate etc. Here microbial degradation of AN was done at different pH, temperature and carbon substrate. The optical density was then measured by using spectrophotometer. Optical density shows the bacterial concentration in a suspension. Decomposition of AN was confirmed by Disk method. Zone of decomposition was observed after following this method.

#### *1). Effect of pH on the Decomposition of AN:*

For the study of effect of pH on decomposition of AN, 20 ml MSM containing 2 % w/v AN was prepared in different flasks and pH of all the flasks was maintained from 6-9. Equal amount of inoculate of isolates was added for decomposition. The flasks were incubated for 48 hrs. at 37°C and shaking 150 rpm. After 48 hrs. the growth was determined by spectrophotometer [17]. The broth was centrifuged and activity of the supernatant was determined by Disk method.

#### *2). Effect of Temperature on the Decomposition of AN:*

The effect of temperatures on decomposition of AN was studied and sample was prepared in different flasks (25 ml MSM containing 2 % w/v AN) and equal amount of inocula of microbes was added. The flasks were incubated for 48 hrs. at room temperature, 31°C, 37°C, and 50°C. After 48 hrs. the growth was determined by spectrophotometer [17].Further the broth was centrifuged and activity of the supernatant was determined by Disk method.

#### *3). Effect of Carbon Sources on the Decomposition of AN:*

Glucose, Fructose, Sucrose, Xylose, and Inositol were used as carbon substrates to study the effect of carbon source on decomposition of AN. 25 ml MSM containing 2 % w/v AN was prepared in different flasks and the carbon source (0.5g/100 ml) was added in all the flasks. An equal amount of inoculums of microbes was added in all the flasks. The flasks were incubated for 48 hrs at 37°C and 150 rpm. After 48 hrs the growth was determined by spectrophotometer. Further the broth was centrifuged and activity of the supernatant was determined by Disk method.

## III. RESULTS AND DISCUSSION

### A. Isolation and identification of ammonium nitrate degrade microbes:

In the present study, the collected soil samples were evaluated *in vitro* for AN degrade bacteria in nutrient ager medium (NAM) plates supplemented with 1.5% (w/v) agar. Initially, 11 isolates were isolated on the basis of clearance around their colonies on NAM plates. Out of 11 bacterial isolates, 2 isolates showed decomposition of AN were selected for the further studies. All of the bacterial isolates were rod shaped. These

isolates were characterized, by a series of biochemical reactions and identified as genus *Pseudomonas* and *Bacillus* (Table 1).

**Table 1. Morphological and Biochemical Characteristics of the Isolates**

| Characteristics | A1(Pseudomonas) | A5(Bacillus) |
|---|---|---|
| **Morphological characterization** | Abundant, thin, white growth | Dirty, , abundant, thick, white, growth |
| **Gram Strain** | -Rod | + Rod |
| **Glucose** | - | + |
| **Fructose** | - | + |
| **Sucrose** | - | + |
| **Xylose** | - | + |
| **Inositol** | - | + |
| **Catalase** | + | - |
| **$H_2S$ production** | - | + |
| **Litmus Test** | - | + |
| **Indole Test** | - | + |
| **Citrate utilization test** | + | + |
| **Methyl Red test** | - | + |
| **Voges Proskaure test** | - | + |

**B. Microbial Degradation of Nitrate Salts Utilized as Oxidizing Agent in the Slurry Explosives**

***1). Effect of pH***

Results shown in Graph 1 revealed that in case *A*1 *(Pseudomonas)* the optical density was increased upon increasing the pH at 8 and, thereafter decreased. In case of A5 *(Bacillus)*. It was found that optical density of the decomposed sample was maximum at pH 6 and as the pH increased the optical density was decreased.

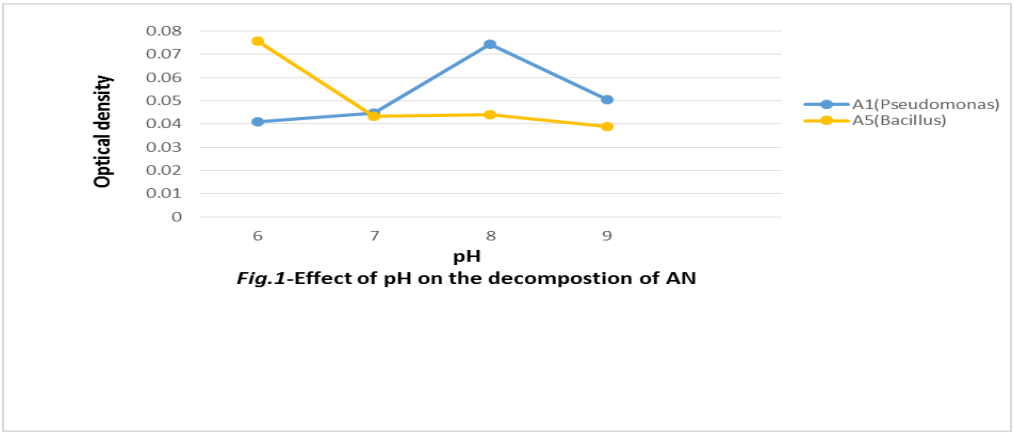


***Fig.1*-Effect of pH on the decompostion of AN**

**Values are mean of there replicates**

The decomposition of AN was significant in basic media and highest at pH 8 which was also confirmed by the Disk method Test. The maximum decomposition activity at pH 8 was 14 mm when A1 *(Pseudomonas)* was used for degradation of AN. These results showed that A5 *(Bacillus)*, is effective microbe in low pH.

**Table 2. Effect of decomposition of AN**

| Ph | A1*(Pseudomonas)* | A5*(Bacillus)* |
|---|---|---|
| **6** | 7±1.15 | 12±1.1 |
| **7** | 10.33±1.20 | 6±1.15 |
| **8** | 11.66±1.4 | 4.6±1.45 |
| **9** | 11±2 | 3.33±1.76 |

**Values are mean of there replicates; ± SE**

***2. Effect of Temperature***

Graph 2 showed the results of effect of temperature on decomposition of AN indicating that decomposition of AN increased upon increasing the temperature. A1 *(Pseudomonas)* and A5 *(Bacillus).* Microbes showed better growth and decomposition of AN.

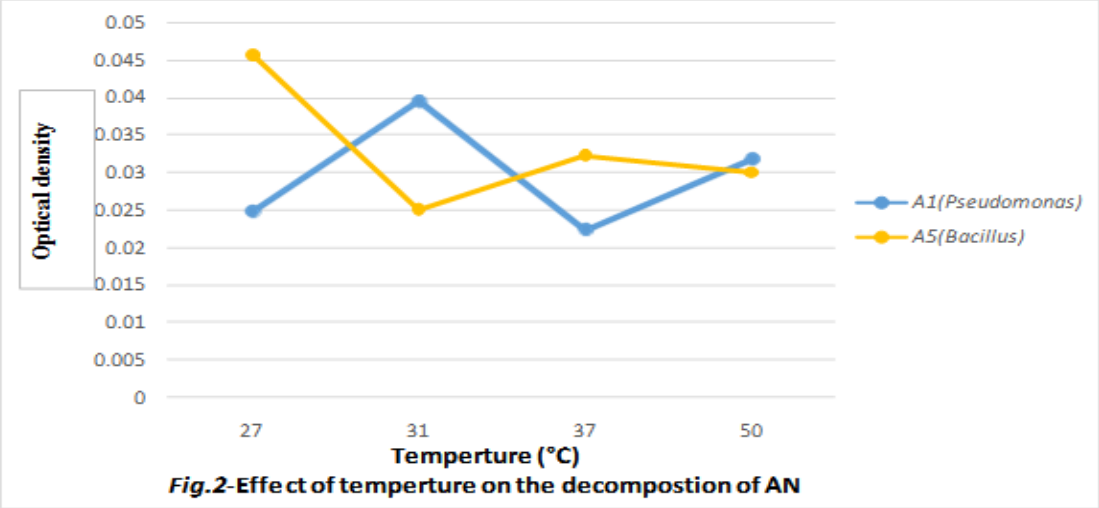


***Fig.2*-Effect of temperture on the decompostion of AN**

The decomposition of AN was significant in basic media and found highest decomposition at 50 **°C** (Disk method Test). The maximum decomposition activity at 50 °C was 13 mm. In case of A5 *(Bacillus)* the maximum decomposition activity was 12 mm at 37 °C.

**Table 3. Effect of decomposition of AN**

| Temperature (°C) | A1*(Pseudomonas)* | A5*(Bacillus)* |
|---|---|---|
| **27** | 6.33±1.5 | 10±0.57 |
| **31** | 3.66±1.5 | 6.33±0.88 |
| **37** | 6.66±1.5 | 12±1.45 |
| **50** | 13±1.5 | 10±1.15 |

***3). Effect of Carbon Substrate***

Results of investigation shown in Graph 3 revealed that in case of A1 *(Pseudomonas)* the decomposition of AN increased upon addition of sucrose, xylose and inositol and decreased when glucose and fructose was added as carbon substrate. The AN decomposition was significant when sucrose fructose and inositol were used as carbon substrate and decomposition of AN was not significant in case of glucose, and xylose.

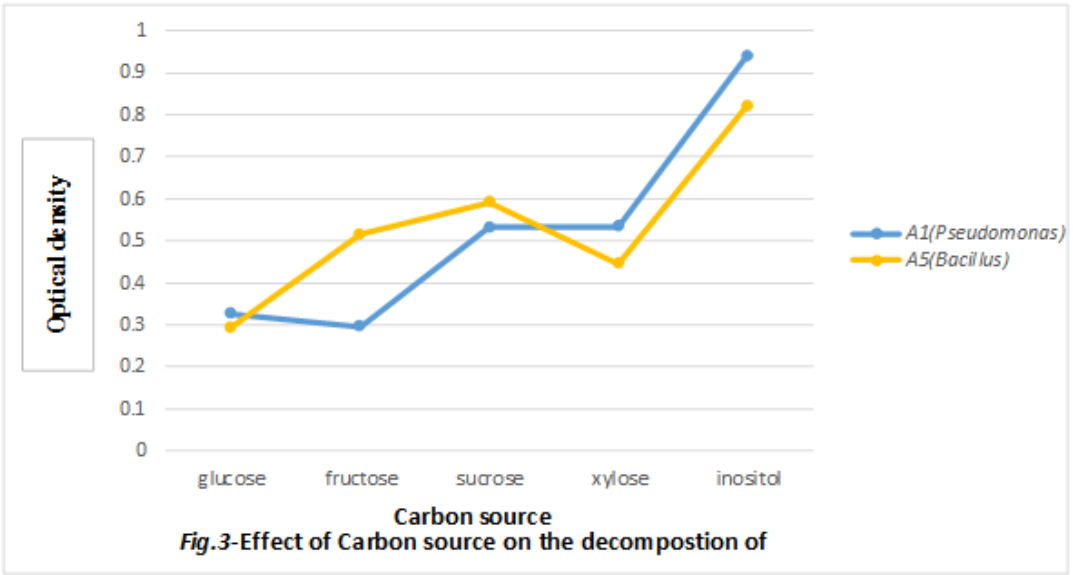


***Fig.3*-Effect of Carbon source on the decompostion of**

Inositol substrate showed maximum decomposition of AN in basic media. The maximum decomposition activity for A1 *(Pseudomonas)* upon addition of inositol and sucrose as substrate was 16 and 18.66 mm and decomposition activity for A5 *(Bacillus)* was 13.6 and 16 mm respectively. Degradation of was not significant in case of A5 *(Bacillus)* and decomposition activity showed that A5 *(Bacillus)* may not be suitable microbes for AN.

**Table 4. Effect of decomposition of AN**

| carbon source | A1*(Pseudomonas)* | A5*(Bacillus)* |
|---|---|---|
| **Glucose** | 5.33±0.88 | 4.33±0.88 |
| **Fructose** | 5±1.1 | 4.66±0.88 |
| **Sucrose** | 16±1.1 | 13.6±1.45 |
| **Xylose** | 13±1.1 | 8.66±0.88 |
| **Inositol** | 18.66±1.2 | 16±1.52 |

## IV. CONCLUSIONS

The following conclusions are drawn on the basis of experimental results:

- **i.** The pH plays an important role in the decomposition of AN and, low pH is suitable for the growth of A5 *(Bacillus)* and higher decomposition activity at pH 8 is found to be suitable for A1 *(Pseudomonas).*
- **ii.** Temperature is very crucial in the decomposition of AN at 50 **$^{o}$C** and may decompose the cross-linked gel which increase the efficacy of the microbes. High summer in India may be help microbial degradation slurry explosives.
- **iii.** Carbon substrate is a key factor for decomposing the AN and disturb the oxygen balance of the slurry explosives and accelerate the decomposition of slurry explosives.
- **iv.** A1 *(Pseudomonas)* may be effective microbe to decompose the AN may lead to change the oxygen balance of the slurry explosive formulation and lost its explosive properties.

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