PRODUCTION OF METHANE FROM COAL THROUGH BACTERIAL CULTURE AND ITS ROLE FOR SUSTAINABLE ENERGY AND ENVIRONMENT PROTECTION

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Abstract- Methane generated in the bio treatments with a consortium of bacteria developed indigenously and analyzed after 30 days span of time. There were three types of bio treatments based on varying concentrations such as; 10%, 20% and 50% a standard solvent of Hexane. But methane generation was observed in the treatment of 50%. Treatments were diluted in hexane. After analyzing through gas chromatography it is clearly obtained that coal carbon was metabolized by bacterial consortium with the end product of methane generation. Analysis was monitored for an increase in methane as an indication that coal carbon was metabolized by bacterial species with methane as an end product.

Keywords- Coalbed methane, gas chromatography, microbial consortium.

I. INTRODUCTION

Coalbed methane (CBM) can be produced through the thermogenically and biogenically during the conversion of organic substances during the process of coalification. It is observed through recent studies that coal bed methane can be produced biogenically through microbes during the decomposition and utilization of rich carbon compounds [1]. On utilization various nutrient material in coal bed methane wells methane generation can be enhanced [2-3]. Coal bed methane formation is categorized into two methods; one is thermogenic CBM in which CBM produced under high pressure and temperature during the process of coal maturation (coalification). Second is biogenic CBM in which metabolic activities are microbes involved because microbes have efficiency to use coal as a carbon source and generate methane [4-10] The goals of the current study were to prove the activity of methanogens for generation of methane gas from coal.

II. MATERIAL AND METHODS

1.1. Preparation of coal sample

Coal sample was collected from Lakhra Coal Field. The Lakhra coal field covering an area of 680 sq. km. is located 250 km. North East of Karachi in Jamshoro District of Sindh Province. It lies between latitudes 25^0 32' and 25^0 45' and Longitutes 68^0 0' and 68^0 15' E. The Lakhra coal field area is underlain by a sedimentary sequence belonging to Ranikot Group of Paleocene age, comprised of Sohnari and Laki Limestone members of early Eocene and Mancher formation of Pliocene age. The thickness of minerable coal seam in the area varies between 1.5 to 3.35 meters. The coal sample was grinded into powder form by grinder in the lab of Lakhra Power Plant. Different types of tests were performed for the analysis of coal sample (Table 1).

1.2.Bacterial Culture Preparation

Microorganisms named as Bacillus sp. were used in the present study were isolated from the contaminated soil & developed at Chemical Engineering Department, MUET Jamshoro.

TABLE I: Analysis of Coal

Parameters	Coal Sample	Moisture	Ash	V. Matters	Fixed Carbon	Total Sulfur	Calorific Value
Purchase Order Values	Collection Date / Time	10 ~ 30 %	30 Max. %	---------- %	22.40 %	7 Max %	5820 ~ 6500 Btu/lb
Coal Sample 1 (M)	25.07.13 /11:45	17.46	28.31	31.08	23.15	5.68	6139
Coal Sample 2 (E)	27.07.13 /14:45	18.37	28.08	30.61	22.94	5.98	6096

Maintenance of Culture

The strain was maintained MSM. Analytical grade chemicals were used to prepare MSM. Distilled water was added to the chemicals with shaking. Total 50 mL water was added. HCl (1N) and NaOH (1N) were used to optimize pH of media up to 7-7.2.

Composition of Nutrient Agar

Agar nutrient is utilized for the growth of microbes in medium where culture is multiplied in different stages. Agar is useful because at high temperature its solid state remains constant. When microbes grow in agar medium they are clearly observable in the form of colonies at the surface. Nutrient agar normally contains (mass/volume). It includes 1.5% agar, 0.5% Peptone, 0.5% NaCl 0.3% beef extract/yeast extract. These media was sterilized

it at 120 ℃ for 15min in steam sterilized autoclave.

Preparation of Agar Plates

Growth medium composed of agar and nutrients present at Petri dish are used as culture microorganisms. Microorganisms when grow separately they form colonies. For calculation of microorganism concentration in liquid culture plates are analyzed. Plates will show culture promoted in the form of genetically growth in different colonies. Streaking is a technique used for culture testing in which a loop of wire known as sterile is inoculator is applied. When microbes grows successfully in the form of colonies then these colonies stored for further modification when they are separately applied for any type of growth [11].

Preparation of Slants

Bacterial growth media equally distributed in 10 test tubes accurately covered with cotton-plugged and aluminum foil. The tubes were sterilized at 120 °C, 15 psi for 15 minute in autoclave. The autoclaved tubes were laid down in slanting position for some time for solidification of media. The slants were inoculated with S. cerevisiae by streaking, and then incubating at the temperature of 37 °C for 24 h.

Preparation of the Culture of Bacillus Sp:

The bacterial culture is prepared by applying following steps.

(a) Preparation of Bacillus Sp: growth medium

50mL distilled water and following chemicals were added and the pH was kept 7 to 7.2

(b) Chemical Composition of Inoculums Medium

The composition of a nutrient broth is as follows;

Beef extract: 0.3 % (minerals and carbohydrates), Peptone: 0.5 % (protein and nitrogen source), NaCl: 0.5 % (electrolyte). The pH value of the inoculums solution was adjusted with acid and base solution. Then MSM was inoculated with colony picked off an agar plate and culture obtained after 24 hour of growth was used to inoculated second pre culture at 2 %(v/v). After sterilizing cultural media contained in conical flask were incubated in incubator at 370C to grow the microorganism and was used for further experiment. The resulting broth was to inoculate the bioreactor.

Treatment series of sample and culture

In this method, bacteria Bacillus A 4 was selected. Different treatment series were organized with some variation. Three types of treatments (T 1, T 2, and T 3) were prepared by applying different concentration of nutrient media and culture which are as under (Table 2). All these treatment series were applied in conical flasks and flasks were covered by aluminium seals. After the settlement of treatments optical density (OD) of these treatment series was observed through spectrophotometer (spectro UV-VIS Double Labomed Inc. USA Serial # Double 001151) in the lab of Biochemical at Chemical Department of MUET on the first day of sample treatment preparation. 1 ml of each sample treatment was taken and diluted three times with distilled water (Table 3) and finally optical density (OD) was observed at 680 nm. Optical density was observed at the range of 0.8 nm where blank was adjusted at zero. After then the samples treatment series were kept at shaking incubator in Biochemical lab (Table 4).

On the second day of treatment two optical densities were taken. One of each sample treatment and second was of culture of bacterial species. In this test sample treatment and culture were diluted one time with distilled water and OD was observed at 680 nm of both samples whereas blank was set at zero. It is cleared from the optical density readings that OD was in increasing order. It shows microbes started their activity in a progressive way. After then these sample treatments were kept continuously in shaking incubator for one month.

TABLE II: The treatment series of sample and culture

Treatment Series	Quantity of Coal Sample	Quantity of Culture	Quantity of Media	Treatment Series
Treatment # 1	5 gm	2 ml of culture	20 ml of Medium	Treatment # 1
Treatment # 2	5 gm	3 ml of culture	30 ml of Medium	Treatment # 2
Treatment # 3	5 gm	3 ml of culture	50 ml of Medium	Treatment # 3

TABLE III: Dilution of samples

Amount of culture + Amount of Distilled water
1 ml of culture + 1 ml of distilled water
1 ml of Sample 1 + 1 ml of distilled water
1 ml of Sample 2 + 1 ml of distilled water
1 ml of Sample 3 + 1 ml of distilled water

TABLE IV: OD results

Samples	OD Range
Culture	0.68 nm
Sample 1	1.26 nm
Sample 2	0.984 nm
Sample 3	0.291 nm

Treatment series of sample and culture

Methane was monitored over time in treatments by removing 0.3 ml of the headspace using a gas tight syringe with a pressure lock and analyzing by gas chromatography (GC).

GC studies were carried out on an Agilent model 6890 network GC system connected with flame ionization detector (FID) and split injector (Agilent Technologies, Sanata Clara, CA, USA), hydrogen generator (Parker Balston, Analytical Gas System, H2-90, Parker Hnnifin Havorhill, MA, USA) and pure nitrogen (British Oxygen Company Karachi);computer with Chemstation software controlled the gas chromatograph. Capillary column HP-5 (30m × 0.32 mm id) with film thickness 0.25 µm (J & W Scientific GC Column, USA) was used throughout the study.

Methane analysis was performed using gas chromatography (GC). GC studies were carried out on an Agilent model 6890 network GC system equipped with a Capillary column HP-5 (30m × 0.32 mm id) with film thickness 0.25 µm and FID detector. The flow rate of nitrogen was 1 ml/ minute. Pressure was applied at the range of 6.12 Psi. Initial oven temperature was 100°C at holding time of 2 minutes. Ram time was 20°C. Final oven temperature was 250°C and holding time was 2 minutes. Total run time was 11.50 minutes. Inlet temperature was 270°C and detector temperature was 280°C. Hydrogen pressure was 45 Psi and air pressure was 450 Psi. 0.1 µl of each treatment sample was diluted in 1 ml of hexane and finally applied for analysis at GC.

III. RESULTS AND DISCUSSION

The coal samples were collected from Lakhra sites and then analyzed in fuel testing laboratory (Table 1). Methane generated in the bio treatments with a consortium of bacteria developed indigenously and analyzed after 30 days span of time. There were three types of bio treatments based on varying concentrations such as; 10%, 20% and 50% a standard solvent of hexane (Merk,Germany).But methane generation was observed in the treatment of 50%.

Treatments were diluted in hexane. After analyzing through gas chromatography it is clearly obtained that coal carbon was metabolized by bacterial consortium with the end product of methane generation. Analysis was monitored for an increase in methane as an indication that coal carbon was metabolized by bacterial species with methane as an end product. Retention time of methane generation was 2.6min observed by gas chromatography as shown in figure 1. Methane generated from coal through bacteria at the time of completion of one month. Total methane generation process took place in two phases.

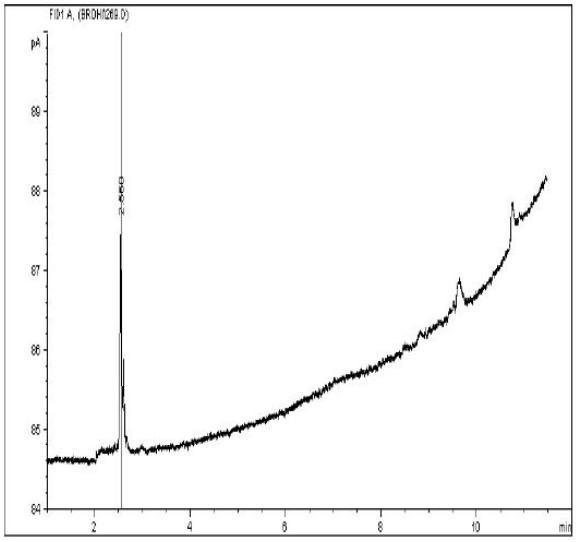


Fig. 1: Retention time of Methane

In phase one bacteria inoculums growth act upon complex organic compounds present in coal and convert them and generate methane gas through fermentation.

In phase two methanogenic substrates are totally converted into methane gas which is the result of metabolism of different organic substrates.

CONCLUSION

Through the process of bioassay organic matter present in the coal can be converted into methane under certain conditions at laboratory scale. Bioassay technique provides a lot of supporting methods involved in the methane generation from coal through biogenetically. Bioassay method is totally dependent of microbial activity in which microbial consortium is applied in different coal samples which is responsible for conversion of organic matters into methane form. For analysis of coal maturity in term of rank, depth, nitrogen content and caloric value, the bioassay method is supporting one.

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