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Biochemic Studies of Methane Formation and Distillery Effluents

Ambedkar Kumar Verma[1, 2]
[1]*Department of Physics, National Institute of Technology, Patna, Bihar-800005, India*
[2]*Department of Chemistry, Swami Vivekanand University, Sagar, Madhya Pradesh-470228, India*
Corresponding Author:Ambedkar Kumar Verma

Abstract
The present study has to evaluate the distillery effluents.Practically, there is no use of the waste liquor remains after the recovery of alcohol and hence it is generally discarded. However, this liquor waste has a very high organic content and contributes to a very high SOD values. Distillery was spent wash is considered to be a highly polluting substance. The number of effluents can beassessing the extent of chemical pollution. Discussion of methods of treatment of effluents. Bio-methanation.

Keywords:*Biochemic Analysis, Bio-methanation, Distillery effluents, Molasses, Methods of treatment, Sugar industries*

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I. INTRODUCTION

In India, among agro-based industries, the second largest industry is the sugar industry after textiles. About 20 million tons per year of sugar is produced by sugar factories. Sugar industries also release above 5 million tons molasses as a byproduct per year. More than 250 alcohol industries are largely utilizing the molasses for the production of alcohol in distilleries. The production rate of molasses also increases with the increase of sugar production. About 1500 million liters of alcohol are annually yield by distilleries.

During the last decades, these distillery effluents have considerably increased with the extension of distillery industry causing immense pollution. Distillery effluent is not only a highly polluting waste but its amount is also significantly, very high. Because of the strict attitude taken by different state pollution Control Boards, from time to time many methods of treatment of this effluent have been suggested but most of them suffered from one major drawback or the other. During the past few years bio-methanation has drawn the attention of distilleries since this process not only treats the spent wash to a large extent but at the same time generates an important product in the form of biogas which can be utilized in the boilers and thus a huge amount of money can be saved which in turn results in saving coal, biogases or furnace oil.

In the present study the isolation of an active biogas producing bacteria has been isolated and various physical parameters like effect of temperature, PH, added carbon substrates, nitrogen sources, phosphate sources etc., were estimated. Since the growth of all bacteria is basically dependent on age and size or inoculums, these studies were also conducted on the isolated strains. Once the conditions were fully optimized, the isolated culture can be taken onto a full-scale treatment plant and its activity on this plant can be controlled.

The Bio-methanation being a microbiological process is highly dependent on the requirement of microbes for trace elements. Since most of these trace elements are heavy metals and all these are toxic to any microbial cell, hence optimum concentrations of a few selected heavy metal ions on the production of biogas have also been studied in the present investigations.

Application of distillery effluent on degraded soils is one of the most economical resources for the soil fertility amelioration through improvement in soil water-holding capacity, texture, structure, nutrients retention, roots penetration, and reduction in soil acidity (O'Brien et. al. 2002; Aravena et. al. 2007; Rato Nunes et. al. 2008).

Now a day in our country due to the increasing number of sugar mills and distillery units, application of distillery effluent on soil nearly become mandatory. However, its application in soil also results in environ mental problems (Cruz et. al. 1991) because apart from organic content and nutrients, sludge also includes heavy metals, colored compounds, dissolved inorganic salts, chlorinated lignin, and phenolic derivatives (Chandra et. al. 2004). These compounds may change soil physico chemical properties and soil enzyme activities. Soil enzymes activities play an essential role in catalyzing reactions which are necessary for the decomposition of organic matter and nutrient cycling in ecosystems, involving a range of plants, microorganisms, animals and their debris (Johansson et. al., 2000). Therefore, changes in enzymes activity

could alter the availability of nutrients for plant uptake and these changes are potentially sensitive indicators of soil quality (Ajwa et. al., 1999; Albiach et. al., 2000).

Dick and Tabatabai (1992) expressed those measurements of several enzymatic activities have been used to establish indices of soil biological activity. Cellulase and Urease are the two important enzymes which play a significant role in soil environment. Cellulase is a core enzyme which contains exo, endo and β-glucosidases. This enzyme synergistically acts on cellulose, the most abundant polysaccharide of plant cell walls and representing significant input to soils (Richards, 1987). Urease catalyzes the hydrolysis of urea and amides to carbon dioxide and ammonia. It acts on carbon-nitrogen (C-N) bonds other than peptide linkage (Bremner and Mulvaney, 1978; Karaca et. al., 1999). Urease is a constitutive intracellular enzyme with three subunits of α, ß and γ and two nickel ions. Furthermore, liberation of these enzymes by microbes during litter decomposition may be influenced by too many factors like temperature, pH and substrate concentration in the soil environment (Linkins et. al., 1984).

The physico chemical properties of amended and un-amended soil, including organic matter, cations exchange capacity etc. were estimated using standard methods (Kalra et. al. 1988, APHA 2005). Moisture content was determined by wet oxidation method. Soil pH was determined using an electrode and a 1:1 soil/water mixture (Thomas, 1996).

Electrical conductivity was estimated by the addition of 100 ml of water to 1 g of soil sample in the conductivity meter. The method described by Johnson and Ulrich (1960) was employed for estimating 70% water holding capacity. Organic C and Total Nitrogen content was measured by using the Walkely and Black method (Nelson and Sommers, 1996), and Microkjeldhal method (Jack son, 1973), respectively. The extractable heavy metal concentrations in soil samples were measured by atomic absorption spectrometry after extraction with aquaregia.

Enzymatic activity diminished with increasing available concentration of metals (Tyler, 1974; Kizilkaya et al., 2004). Increased levels of heavy metals will react of enzymes causing inhibition or inactivation of the enzymatic activity (Nannipieri, 1994). Metals also indirectly affect soil enzymatic activities by altering the microbial community which synthesizes enzymes (Kandeler et al., 1996).

The organic matter-heavy metal fractions which are readily available for plant uptake occur in organic matter and soil solutions. This would prevent the heavy metal from interacting directly with the active sites of enzyme, thus affecting the enzyme, activity (Doelman and Haanstra, 1984). The rapid decomposition of organic matter which occurs after the application of distillery effluent to soil increases the proportion of available metals as a result of mineralization of organically complexed metals (Dudley et. al., 1986).

Decreased activity of cellulase at higher concentrations of effluents may be due to the exposure of cell free enzyme to highly concentrated effluent. But, inhibitory effect of organic matter (Gianfreda and Bollag, 1994, 1996), high acidity (Ruggiero et. al., 1996) and short living enzymes in the soil environment (Ahn et. al., 2002) are also the reasons for the de creased activity. Similar observation was made by Sreenivasulu (2005) that, at high concentration of fungicide in soil, the cellulase activity was inhibited.

According to Joshi et al. (1993), enzyme activity was greatly increased in soils high amount of substrate and increased enzyme activity was positively correlated with fungal, bacterial number and moisture content of litter. None the less, high significant correlation between cellulase activity and soil respiration was observed by Splading (1979) and microbial biomass by Kanazawa and Miyashita (1987) and Donnelly et al. (1990). Additionally, by increasing the effluent concentration in the control sample, the cellulase activity was increased, maximum at 50%, there after decreased.

Effluent originating from distilleries known as spent wash leads to extensive water pollution. A study was conducted to know the quality of effluent generated from the distillery, for the purpose of proper treatment and dilution of effluent before discharge in water stream or on land. Physico-chemical characteristics of distillery effluent samples such as colour, odour, Total Solids, Total dissolved solids, Total Suspended Solids, pH, Electrical Conductivity, Total hardness, Calcium, Magnesium, Alkalinity, Chloride, Dissolved Oxygen, Biological Oxygen Demand, Chemical Oxygen Demand, Ammonical Nitrogen, Total Phosphorus, and Total Potassium were analysed and it was observed that the characteristics of spent wash and PTDE (primary treated distillery effluent) have high load of chemical and organic pollutants.

But when PTDE was diluted with 50% and 75% of water, all the values of physicochemical properties were decreased. The decrease in these values show that the toxicity of distillery effluent decreases with increasing dilution. Thus, the characteristics of spent wash and PTDE do not allow its discharge into a waterbody, hence it requires treatment and dilution before discharge.

Perusal of literature indicates that efforts have been made on physical treatment of waste water using sand and soil mixture for the treatment (Huisman and Wood, 1974; Sarkar et. al.; 1994; Bhagat et. al., 1999; Setvik et. al., 1999; Weber-Shirk, 2002; Rooklidge and Ketchum 2002; Ausland et. al., 2002; Prasad et. al., 2006).

COD of raw sewage was found 10933.33 mg/L and was reduced by 78.96% in filtered effluent. Similarly 76-82% removal of COD from waste water using sand intermittent filtration bed has been reported by Van Buuren et al. (1986). Our findings are also similar to the findings reported by Rao et al. (2003).

Both MPN and SPC of raw effluent were found 350/100 ml and 61x105 / ml respectively. Significant decline in both MPN and SPC values in filtered effluent was found to be 95.14% and 67.12 % respectively. Even in absence of dissolved oxygen, bacterial population was much higher in raw effluent and surprisingly it declined in filtered effluent as evident by recorded data. The reduction in bacterial population in treated effluent may directly be related with consumption of organic components, their retention and death while passing through the filtration bed.

But in the present conditions, it could not be stated whether existing bacteria are aerobic / anaerobic/ facultative aerobic/ facultative anaerobic in nature.The present findings established a positive co relation between temperature, BOD, MPN and SPC. The declined trend in temperature is in accordance with decline in BOD, MPN and SPC. It may be because of the influence of temperature as governing factor in each step of physiological behaviour of bacterial species inhabited in the effluent. However, depletion of all these is also related with retention time. Christianae et al. (1998) also reported that water detention time is important factor in removing of organic matter from wastewater when it was passed through intermittent filter containing non-woven textile coupons. The spent wash has been considered as waste generated from the distillery processing units and hence it can be graded as a high potassium containing organic liquid fertilizer (Samuels 1980).

Effluents of sugar industries and other distilleries are spoiling of agricultural land and damaging the homeostasis of ecosystem. Therefore, the aim of the present study is to assess the extent of chemical pollution of the river water and soil as it is increasingly polluted by industrial effluents.

II. MATERIALS AND METHODS

All the glassware's were cleaned with warm chromic acid washed several times with tap water and finally rinsed with distilled water at least three times. All the culture tubes and flasks were of corning glass. Sterilized non adsorbent cotton was used for plugging the culture tubes and flasks. All the flasks, tubes and media were generally autoclaved at 15 psi for 15 minutes. In certain cases where sterilization by autoclaving was not possible, steaming for 1 hour was done on three consecutive days. Incubation temperature for the isolation and other studies was usually 37°C. The chemicals used in the study were of analytical grade.

Sodium and Potassium

Sodium is present in a number of minerals, the principal one being rock salt (sodium chloride). The increased pollution of substantial increase in the sodium content of drinking water in different regions of the world. Sewage, industrial effluents, sea water intrusion in coastal area, and the use of sodium compounds for corrosion control and water softening processes, all contribute to the sodium concentration in water because of the high solubility of sodium salts and minerals. In ground water sodium concentration may be less than 1 mg/l or exceed 300 mg/l depending upon the geographical conditions.

Interferences

Chlorine in the samples or nitrogen trichloride which normally coexist with NO_2 produces a false colour. This can be minimized by addition of the NED dihydrochioride reagent first and then the sulfanilic acid reagent; an orange colour still may result when a substantial Nd_3 concentration is present. Under such circumstances check for free chlorine and NCI_3 residuals. NO_2 determinations should be carried out in filtered, turbidity free sample

Apparatus:

1. Colorimeter or spectrophotometer that can be operated at 543 nm.
2. Nessler tubes or 100 ml capacity and volumetric flask.

Reagents:

1. Sulfanilamide reagent: Dissolve 5 g sulfanilamide in a mixture of 50 ml conc. HCI and about 300 ml water. Dilute to 500 ml. with water. The solution is stable for many months.

2. NED - dihydrochioride Solution: Dissolve 500 mg N- (1-napthyl) ethylenediamine dihydrochioride in 500 ml water.

The estimation of sodium and potassium is based on the emission spectroscopy, which deals with the excitation of electrons from ground state to higher energy states and coming back to its original state with the emission of light.

Microbiological methods are more suited because of low handling and maintenance costs. The antipollution drive can be classified under the headings aerobic and anaerobic digestion. Aerobic methods of treatment include aerated lagoons and activated sludge method. The aerobic methods generate energy in the form of biogas. A brief description of the two methods is given below:

1.1 Aerobic Processes

The light ends produced by cracking reaction are removed in the stripper column. The off -gas from the stripper is sent to the fuel gas, but flared if it is under high pressure [3].

1.1.1 Aerated lagoonss

Use of algae and bacterial cultures has been suggested for the treatment of distillery spent wash and a net reduction in BOD in the range of 77 to 80% have been reported. But the removal of algae is another problem and the organic matter concentration in ponds including algae often exceeds the concentration of influent.

1.1.2 Activated Sludge Method

In this process, the bacterial cells grown on spent wash are agglomerated in flocs. The three major requirements for this process are:

i. A mixed population of aerobic microorganisms must be able to degrade the obnoxious components of the spent wash
ii. Sufficient population of the cells must be able to grow in the aeration tank

1.2 Anaerobic Processes

The Anaerobic processes are cheap to install and financially viable to maintain and the handling costs are minimum. These can be broadly classified into three categories:

i. anaerobic lagooning process.
ii. anaerobic ammonification process
iii. anaerobic digestion followed by methane recovery which can be used as energy source in boilers during the process.

III. RESULT AND DISCUSSION

Biochemic Studies of Spent Wash or Distilleries Effluents

Alcohols collected from the fermented wort has distillery spent wash which is nearly 12 to 15 times volume of the produced alcohol. A normal distillery of 3.0 $x10^4$litre per day capacity approximately generated 450 m^3 of spent wash per day. The spent wash thus produced is highly acidic in nature and contains innumerable organic and inorganic substances coming from sugarcane juice and those formed during the processing of cane juice, molasses fermentation and distillation of fermented broth. The composition of the spent wash is shown below in the Table1.

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Table 1: Representative composition of the spent wash by mass.

Composition of the spent wash	**Specification**
Appearance	Dark Brown
Turbidity	Very high
Temperature when discharged	80°C - 90°C
pH	3.5-4.5
Brix	6°C - 9°C
Total solids	$7.1x10^4$ - $9.1x10^4$ mg/L
Suspended Solids	40-60 mg/L
Inorganic solids	$1.5x10^4$ - $3.1x10^4$ mg/L
Ash	2.5 - 3.8%
Potassium as K20	0.65%
Total nitrogen	0.085%
Phosphorus as P_2O_5	0.15%
Calcium as CaO	0.33%
Magnesium as MgO	0.21%
Sesqueoxide	0.06%
Sulfates	0.37%
Chlorides	0.37%
Carbonate	0.01%
Organic solids	$5.5x10^4$ - $6.1x10^4$ mg/L
Glycerol	0.24 - 0.35%
Lactic acid	0.25 - 0.65%
Succinic acid	0.5 - 0.6%
Free ammonia	30.4 mg/L
Albuminoid ammonia	22.1 mg/L
Fused oil	0.1 – 0.4%
Biochemical Oxygen Demand (B.O.D.) 5 days	$4.01x10^4$ - $6.02x10^4$ mg/L
Biochemical Oxygen Demand (B.O.D.) 20 days	$5.45x10^4$ - $8.45x\ 10^4$ mg/L
Chemical Oxygen Demand (C.O.D.)	$7.0x10^4$ - $1.2x10^5$ mg/L
Oxygen Absorbed (OA) (1 hr)	$2.0x10^4$ - $3.0x10^4$ mg/L

In addition, the detailed organic composition of spent wash (expressed in mg/L) includes carbohydrates (6.7 - 21.2%), proteins (15.1- 31.0%), free amino acids (2.1 - 4.3%), glycerol (4.5 - 7.5 %) and total titrable acid (4.9 - 18.9%). Because of high content of organic compounds, the BOD and COD values are very high as depicted in the table above. When allowed to decompose in open ponds, the spent wash produces foul smell, thus rendering surroundings completely unhygienic and highly polluted. It is still more hazardous when discharged into rivers, lakes nullahas as it results in the complete depletion of dissolved oxygen and destroys aquatic life.

We have studied the effect of distillery effluents both treated and untreated on the seedling growth of pisumsativum. Anaerobic digestion1 and reduction in C.O.D. values due to aging of inoculum was also reported.

It is, therefore, essential that this spent wash which not only has high BOD value but contains many other injurious substances, should be subjected to proper treatment so as to bring down the pollution loads to sufficiently low levels. From time to time many physical, chemical and microbiological methods have been suggested for the treatment of spent wash. Physical and chemical methods suggested are based on adsorption, ion-exchange, membrane processing and chemical oxidation.

Combined ion exchange and solvent extraction technique (C.I.E.S.E.) has been employed for the separation of different metal ions using synthetic organic ion exchange papers and inorganic exchanger.Concentration of spent wash in multiple effect evaporators for the recovery of potash has been suggested by Reich, Chakrovorty and Bhaskaran. Many internal companies supply equipment for the recovery of potash including Dorr Oliversfluidizeci bed unit. (GA Technologies San Diego California), Particulate Solid Combustion System of John Zinc Co (Tulsa), Dumeg System of Thermal Transfer Process (Monroville, Pa) etc. A few Indian companies also supply know-how on the incineration of distillery spent wash including M/s. Kinetic Technology, New Delhi and MIS. Thermax Pvt. Ltd. Pune etc. Chatterjee et al have shown that a full plant in operation at Walchandnangar Industries, Walchandnagar is giving profit at the rate of Rs. 2,28,620/- per year. Alternatively, the concentrated spent wash can be mixed with press mud and can be sold as potash rich fertilizer. However, the process is highly capital intensive and large amount of energy is needed to concentrate the spent wash from 70 to 80°Bx.

Pretreated vegetables when used with dung samples produce appreciable methane though the dung samples are not equally effective for biomethanation.The aerobic methods generate energy in the form of biogas. Considerable biogas generations with different biomass mixed with dung samples as inoculums had been found to be dependent on pH, extent of pulverization and other physical conditions.

Biochemic Studies of Methane Formation

The four well known genera Methanosarcina, Methanobacterium, Methanobacillus and Methanococcus which involve in methane formation called **methane bacteria.** They convert the organic acids into methane molecule from molecular hydrogen and carbon dioxide as electron acceptor followed by oxidation.

$$4H_2 + CO_2 \xrightarrow{Bacteriase} CH_4 + 2H_2O$$

This oxidation reaction provides only energy and but no carbon for molecular growth. However, organic material oxidizes to produce carbon skeleton and carbon dioxide. It has been reported that organic acids like formic acid, acetic acid, propionic acid, butyric acid, valeric acid, isovaleric acid and caproic acids are good substrate for methane bacteria. Whenever Methanobacteriumsuboxydans growth on valeric acid substrate it degraded to produce acetic and propionic acids. Among the methane bacteria, fermentation of acetic acid is the most important reactions in the chain reactions.

$$CH_3 - COOH \xrightarrow{Bacteriase} CH_4 + CO_2$$

To enlighten the extra sources of methane in anaerobic digestions, Jerris and Mc Carty used radioactive tracer techniques. These inferences are summarized below in figure 1:

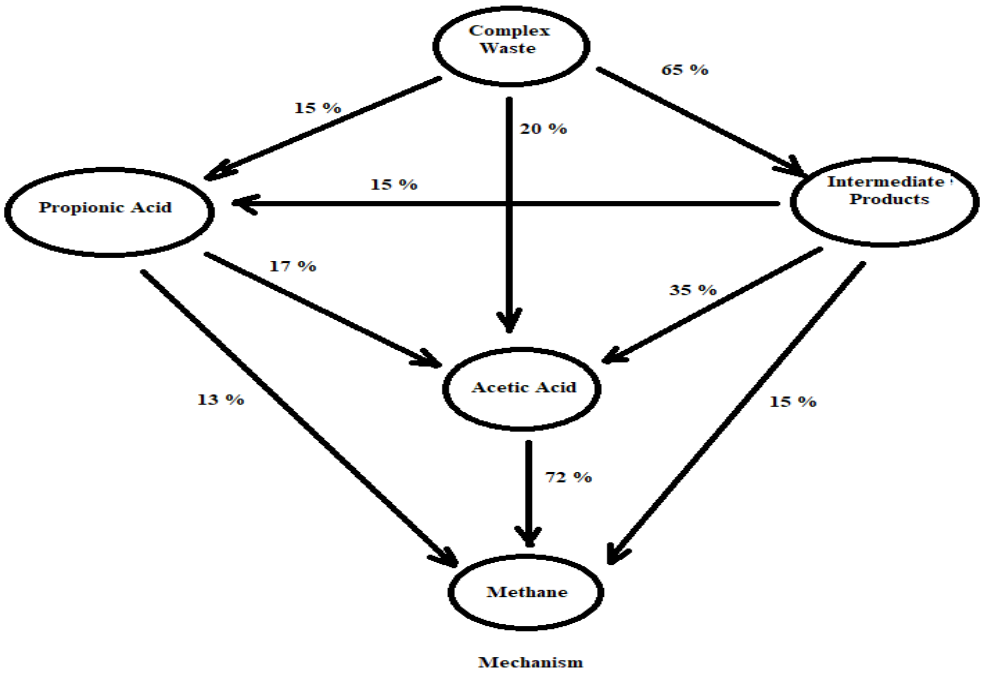

Figure 1: Schematic diagram of the methane formation.

Figure:1 shows that intermediate products including valeric acid are also a source of propionic as well as acetic and formic acid which are directly involved with the CO_2 during the formation of methane. Thus, whole process of biogas production (**bio-methanation**) involves three steps:

Step-1: Hydrolysis

$$Organic\ Nutrients \xrightarrow{Bacterial\ \ cells} Smaller\ complex\ organic\ compositions$$

Step-2: Fermentation

$$Smaller\ complex\ organic\ compositions \xrightarrow{Acid\ \ Producing\ \ Bacteria} Volatile\ Organic\ Acid\ + Alcohols$$

Step-3: Methane Formation

$$Volatile\ Organic\ Acid \xrightarrow{Methane\ \ producing\ \ Bacteria} Biogas\ (Methane) + Carbon\ dioxide$$

Assessment of Energy Formulation

Methane rich biogas as a byproduct is a very useful source of energy which can be obtained by employing anaerobic digestion system for treatment of distillery effluent. Large amount of coal-biogas/ furnace oil can be saved by subsequently use of biogas in boilers. Distillery produces producing 30,000 litres of alcohol per day, and also produces spent wash about 4,50,000 litres per day with. 80000 mg/l or more COD load. On an anaerobic digestive treatment, spent wash produces biogas to an extent of about 2800 m^3/day which is assumed that the COD is reduced to an amount of 70%. On account of 200 m^3 of biogas produces energy which is equivalent to that required to produce one ton of steam. Hence, total steam generated is equivalent to that of combustion of 14 to 15 tons of biogas. It is estimated at about 30,000 litres of alcohol/day released by the distillery and requires about 75 tons of steam per day. Thus, approx. 20% of fuel can saved by using biogas which is necessary for the alcohol production. Anaerobic treatment of effluents is used to save the expressive amount of energy while aerobic process requires. The advancement of the anaerobic treatment of effluents are lesser amount of organic solids production, lower nutrient necessity, about 75-100% extent of steam requirements for methane production, longer preservation of active anerobic sludge, etc. However, it has some limitations such as longer starting time to process, high BOD value range (5000-8000 ppm), requirements of post treatment and about 20-100% diluted water.

IV. CONCLUSION

It was observed that the Indiandistilleries by and large use molasses for the fermentative production of ethanol which is ultimately recovered through analyzer as well as rectifier column. The waste liquor after recovery of alcohol is practically of no use and hence discarded. It has, however, a very high organic content which contributes to a very high SOD values because of which the waste liquor known as vinasses, distillery was spent wash is considered to be a highly polluting substance.These effluents affect not only the lives of flora and fauna nearby the water bodies in the industrial area but also the agricultural lands and river beds.The COD values reduced to an amount of 70 percent. A 200 m^3 volume of biogas produces energy which is equivalent to that produced by one ton of steam. The amount of effluent generated is nearly 15 times the volume of alcohol produced by the Indian cane molasses distilleries.Anaerobic treatments have more advantages over the aerobic treatments.Distillery effluents should be release after the primary treatments. Due to the scarcity of water availability, it is justifiable to be encourage for irrigations.

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