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The kinetic of Biogas Production from Municipal solid waste & Sewage using two stages Fermentation

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Abstract: - Municipal solid waste (MSW) management is becoming a serious issue in all over the world. Anaerobic digestion (AD) is one of the technologies to convert that waste into useful form of energy. To fulfill the need, the present paper deals with the review of various operating parameters and their effects on AD. This paper also reviews different pre-treatment methods including mechanical, thermal, chemical and biological methods to improve the effectiveness of AD of MSW. In this research work the quality and content of methane in biogas generated from biogas plant is improved by co-digestion of MSW, cow dung along with the urine with better carbon to nitrogen (C/N) Ration. We took number of experiment using different ratio of MSW and additives to improve biogas. Rigorous experimentations concluded that the co-digestion of the MSW, cow dung and urine in the proportion of (50:40:10) with equal amount water in a portable bio digester for anaerobic digestion results into better methane production with maintaining C/N ratio and reducing time duration for flammable biogas production.

Key words: Bio-methanization, MSW, biogas, two stage anaerobic digestion.

Introduction: - Pune is one of the fast developing urban agglomerations in Asia and ranked eighth at national level. The present growth is due to various factors such as industrialization, educational institutes, information technology (IT) hubs and location of state and central government establishments. Pune, with a population approaching 3400000, is estimated to generate about 1400 metric tons of MSW daily (Mali et al. 2012). Methane and carbon dioxide are produced during the testing period due to the anaerobic degradation of organic contents of the substrate. The methane generated from the substrate is then measured and the methane potential of the substrate which is expressed as per mass of volatile solids added or chemical oxygen demand (COD) added can be calculated by subtracting the methane volume from a blank. As the organic material in the substrate is degraded through a series of complex microbiological processes, biogas is continually produced during incubation until there is no biodegradable material left. **(Feodorov,V. (2016).**

The process of anaerobic digestion is a biological process which makes use of anaerobic bacteria to break down organic waste, converting it into a stable solid and biogas, which is a mixture of carbon dioxide and methane. The anaerobic digestion process is very attractive because it yields biogas which can be used as renewable energy resources and also produce reduced stabilized material after treatment **(Wang et al., 2002)**. Thus, this study is designed to carry out a controlled high-rate biomethanization of Unsorted Municipal Solid Waste by the double-stage dry-wet digestion as pre-treatment option prior to landfill is given in Fig -1.

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(i) Hydrolysis,
(ii) Acidogenesis,
(iii) Acetogenesis, and
(iv) Methanogenesis.

The four steps of AD and their interrelationship are presented in Figure 2.

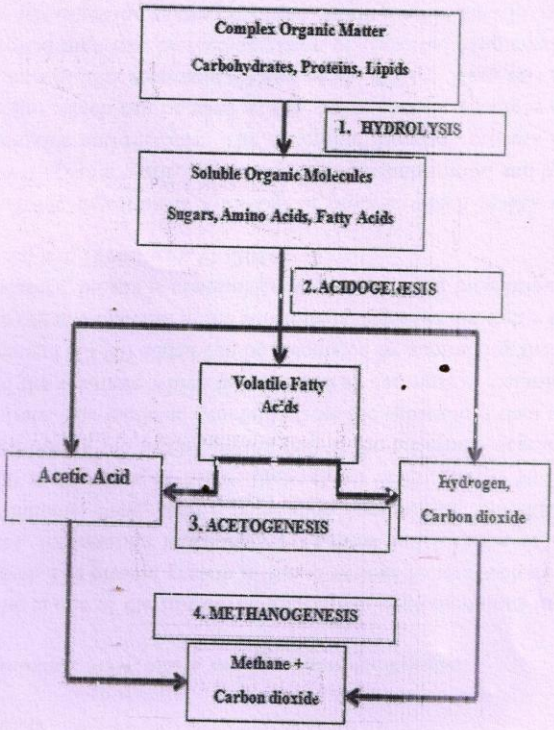

Fig 2. Anaerobic Digestion Process

Factors influencing anaerobic digestion:-

In an anaerobic digestion system, anaerobic microorganisms are highly susceptible to changes in environmental conditions. Some of the environmental conditions are temperature, pH, and toxicity. The two-stage system can use one, two, or all three water recirculation loops (R1, R2, and R3, shown in Fig. 3) in case of need. Using these recirculation loops brings many advantages, such as further controlling of pH (reduction of acidity due to using the high alkalinity effluent from the second reactor); mixing/ diluting of the high solid feedstock; and improving activities of bacteria.

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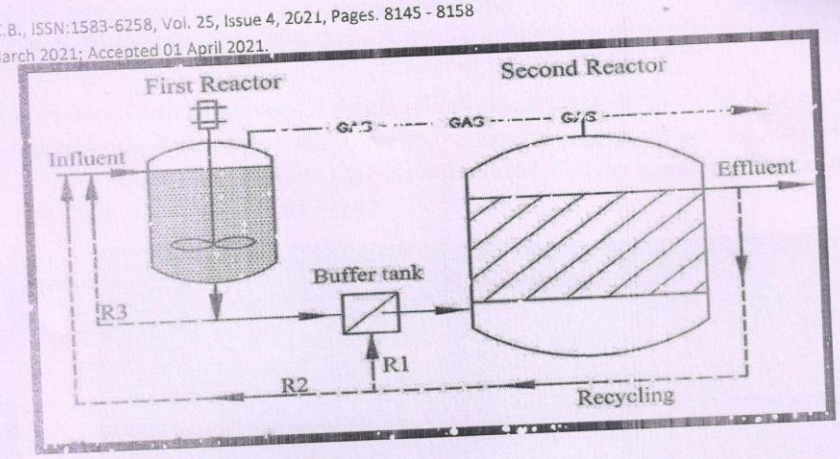

Figure 3:- Two-Stage Systems

Limitations to biogas production

But not everything that is used for anaerobic digestion can be converted to biogas, in other words there is no substrate that is digested to 100%. There are quite a few reasons for this; some of these are improper physical-chemical factors, usage of biomass to form new bacterial cells and presence of substances that are not easily biodegraded such as lignin. Carbon to Nitrogen ratio(C: N) is one of the most important physicochemical factor that effects the bio-conversion of biomass to biogas. 25-30 is the optimum C: N ratio that is ideal for biogas production. pH and temperature also effects the biogas production by interfering with microbial activity.

A pH which is around 7.0 to 7.2 is the best for maximum production but anywhere between 6.6 to7.6 is good. When pH drops to 5.0 it drastically effects the biogas production, because at this pH the growth and multiplication of cellulose degrading bacteria and amylolytic organisms is hampered. It was also found that microbial population was reduced by 2 to 4 times when pH drops below 5. In thermophilic anaerobic digestion a lot of biogas can be produced because of the faster reaction time, more methane content can be obtained and low hydrogen sulphide content in the biogas produced etc .Thermophilic anaerobic digestion can be hard to maintain at the high temperatures, can be more sensitive to fluctuations in temperature and heavy metals. Despite of the disadvantages thermophilic anaerobic digestion is often preferred over mesophilic digestion because of higher gas production yield and higher methane content (Cavinato C 2013).

Table 1 . Indicator for process imbalance in anaerobic digestion

Indicator	Principle
Gas production	Changes in specific gas production
Gas composition	Changes in the CH_4/CO_2 concentration ratio
pH	Drop in pH due to VFA accumulation
Alkalinity	Detects changes in buffer capacity

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Total volatile fatty acids (VFA)	Changes in total concentration of VFA
Individual VFA	Accumulation of individual VFA
COD or volatile solids reduction	Changes in degradation rate

Liquid displacement gas measurement The majority of laboratory volumetric gas meters are based on the liquid displacement method. These meters can be constructed with simple materials like glass/plastic jars or cylinders. Liquid displacement meters are simple, economic and they can work for a long period of time without maintenance. The preservation and collection of gases is the most important operation for any liquid displacement gasometer. Gasometers are the classical gas measuring unit which works with the principle of gas storing and does not provide the flowrates directly. The collection of the gas is usually done with the use of vessels containing a suitable liquid which is displaced as the gas gets collected. Wang J Y et al. 2002.

The gas pressure inside the tube collected over the liquid solution is the sum of the biogas pressure and the vapor pressure. The pressure of biogas, (Pbio) can be obtained by subtracting the vapor pressure of liquid (Pw) at the temperature of measurement from the pressure of collected moist gas (P).

$$P_{Bio} = P - P_w$$

If the gas is collected over liquid, static pressure acts due to the difference of level (P_{level}),

$$P_{Bio} = P - P_w - P_{level} \text{ or } P_{Bio} = P - P_w + P_{level}$$

The produced biogas volume in normal condition can be converted to STP using Combine Gas law:

$$V_0 = V \times \frac{T_0}{T} \times \frac{P_{bio}}{P_0}$$

Here, V is the measured gas volume, V_0 is the volume of gas in standard temperature and pressure, P_0 is the standard pressure, T is gas temperature at the time of measurement, and T_0 is the standard temperature. Modified Arden Buck Equation can be suggested for the calculation of vapor pressure. Mohd. S. N. et al. 2015.

$$P_w = 6.1121 \exp\left(\left(18.678 - \frac{T_c}{234.5}\right) \times \frac{T_c}{257.14 + T_c}\right)$$

Tc is the temperature of gas in degrees Celsius. Pw is pressure in hP (1 hP = 0.1 kPa) Gasometers are usually height or weight types.Yu L et al.2013.

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Material and Methods:- A floating-drum biogas plant consists of a cylindrical digester and a movable, floating gasholder (drum). The digester is generally constructed underground (Fig 4) whereas the floating gasholder is above ground. Smaller household-scale systems may also be fully above ground (see Fig 4).

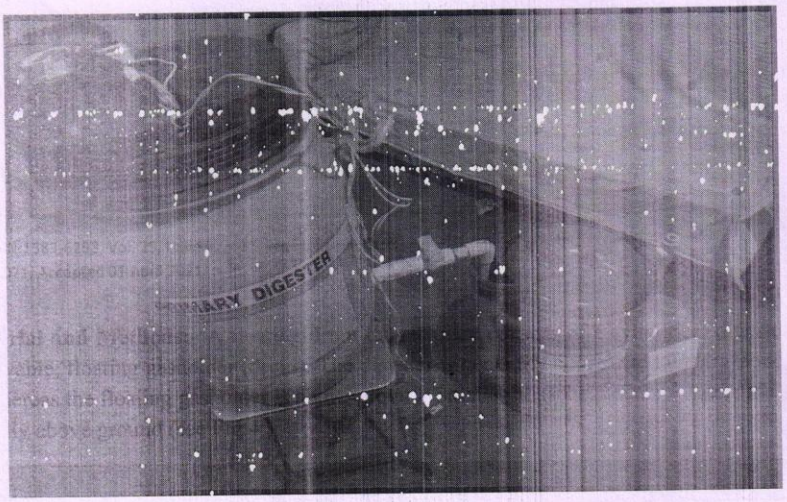

Fig:- 4 **Two stages bioreactor for experiment set**

Floating-drum plants consist of an underground digester (cylindrical or **dome-shaped)** and a moving gas-holder. The gas-holder **floats** either directly the fermentation slurry or in a water jacket of its own. The gas is collected in the gas drum, which rises or moves down, according to the amount of gas stored. Factors affecting floating-drum biogas plant is given in Table 2

Sl.No.	Factor	Floating drum
1	Gas storage	Internal Gas storage drum size (small)
2	Gas pressure	Up to 20 mbar
3	Skills of contractor	High: masonry, plumbing, welding
4	Availability of Material	yes
5	Durability	High; drum is weakness
6	Agitation	Manual steering
7	Sizing	Up to 20 m^3

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8	Methane emission	Medium

Table 2

The secondary digestor or fermenter was purchased from above ..... company. This fermenter works on floating dome principle. gasbag collected in this floating dome .The done rises upwards as the gas production begins & increases. The total gas production is to be measured with liquid displacement method.

Preparations of Feeding Constituents

MSW (Municipal Solid Waste) was collected from different part of city for mixing in biodigester. For additive purposes, we have selected animal urine and cow dung [9] as it maintains C/N ratio after anaerobic digestion and animal urine easily available. Thus, we collected cattle urine from cattle farm nearby to our experimentation location. The properties of the MSW, cow dung and urine are shown in table 3.

Table3. Properties of feeded mixture and types of the digesters

	MSW	COW DUNG	URINE
VFA (g/l)	8.7	0.3	155 µmole/l
VS (g/l)	170.2	13.8	-
COD (g/l)	12.8	155.3	-
TS (g/l)	229.6	24.2	-
PH (g/l)	6.6	9.2	5.7–5
C/N RATIO	30	8	19.56

*Traces of P, K, Ca and Mg found in PPM

Construction of biodigester For experimentation purpose we have prepared a lab scale portable biodigestor.Pawan P et al. 2000.

- **20L container.**
- **Connecting tube.**
- **Balloon for gas storage**
- **Flow control valve**
- **PVC pipe for inlet and outlet.**

Experimentations

The experiments were conducted in three different biodigesters as per tests. The biodigester compramises of the 20L container, Ballon for gas storage, PVC pipie for inlet and outlet,

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Connecting tube and flow control valves. The pressure is maintained on the top of biodigester by weight. The Biodigster is covered to restrict extrageneous factors like sunlight etc. The flow control valves are used to regulate the flows. The digester temperature kept at around the 35^{O}c.

Test 1: MSW with equal quantity of water.

Test 2: MSW mixed with sludge (3:1) and equal amount of water .Biogas collected in the storage tube, observations were recorded a regular intervals. For formation of biogas at small scale it needs at least 7-8 days. The reading were taken upto 25 to 30 days of feeding when biodigeter attains the stability .The tests were rigoursly repeated to confirm the results.

Test 3: MSW, Cow dung and urine (50:35:10) with equal amount of water.

Analytical Methods

The PH, TS, COD, VFA and TVS were determined by APHA 2005 standards. Gas chromatograph (Method-TCD) used to measure quality of the biogas.Model development for biogas production kinetic in batch mode. Biogas production kinetic in was studied by developing the equation closest to fundamental for biogas production in batch system. By assuming biogas production rate in batch condition is correspond to specific growth rate of methanogenic bacteria in the biodigester, biogas production rate predicted will obey modified Gompertz equation [A] as follows:

$$P = A \times \exp\left\{-\exp\left[\frac{Ue}{A}(\lambda - t) + 1\right]\right\}$$

In these equation, P is cumulative of specific biogas production, ml/gVS; A is biogas production potential, ml; U is maximum biogas production rate (ml/gVS.day); λ lag phase period (minimum time to produce biogas), day; and t cumulative time for biogas production, day. A, λ, and U constants can be determined using non linear regression. From the above equation, kinetic constant of biogas production rate will be expressed by U constant. The higher U exhibits the higher biogas production rate. Bodnar M et al. 2013

Sampling and Analysis

About 100 kg of fresh MSW samples are collected for sampling from different locations in Pune city. The samples are analyzed from January 2020 to November 2020 at intervals of one month. Two to three samples are collected for each month. Total twenty five samples are collected to get a representative characterization of MSW The collected samples are physically segregated into seven categories. Physico chemical characteristics of MSW from different part of city are given in Table. 4 & Table. 5

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Table 4. Physical analysis of MSW of from different Part of City(% on wet weight)

	MSW Composition						
	Plastic	Paper	Cloth	Metal	Stone	Glass	Organic
Minimum	2.4	1.2	0.4	0	1.4	0	46.0
Maximum	19.3	24.7	20.2	1.8	18.3	5.2	85.4
Average[1]	7.1	6.9	7.8	0.7	7.0	1.2	69.3
Stdev.	±4.1	4.5	5.4	0.6	5.2	1.4	9.6

[1]Average of 25 values.

Table 5. Chemical analysis of MSW of from different Part of City.

	pH	Moisture content %	Organic matter* %	Volatile solids*, %	C* %	N* %
Minimum	7.3	38.91	18.51	48.03	4.05	0.18
Maximum	8.9	58.91	48.86	74.47	19.28	0.97
Average[1]	7.85	48.08	32.83	62.61	11.62	0.59
Stdev.	±0.37	5.29	8.85	7.92	4.77	0.24

[1]Average of 25 values on dry wet basis

Results and Discussion:-

The Test 2 and Test 3 explored that the biogas was collected in the tank at faster rate as compared to Test 1 of mono-digestion. The time required for formation of biogas (test 2 and test 3) was reduced as compared to standard time required for biogas formation without additives (test 1). The collected biogas was feed into gas chromatography (Method TCD) machine and the outputs were recorded. Biogas consists of methane, nitrogen, carbon dioxide, hydrogen sulphite.

The Experimentations were done with different Co-digestion and mono-digestion the Bio gas generation was evaluated.

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- **Test run 1 (MSW)**
- **Test run 2 (MSW+ COWDUNG)**
- **Test run 3 (MSW+COWDUNG+URINE)**

a) Effect of co-digestion on the production of the Biogas

Above tests gave peak biogas production and burnable biogas at 26 days, 24 days and 22 days respectively. The Methane yield and Quality is found out to be best in test run 3. Urine contains the nitrogen which is favourable for the bacteria growth. The co-digestion with cowdung and urine enhances the concentration of soluble organic constituents which in turns reduces the time required for hydrolysis stage of Methanogenesis process and results into better biogas production.

b) Effect of co-digestion on Contents of Methane in produced biogas

The Methane contents were experimentally tested on weekly basis by Gas Chromatographs (Method TCD). The Experimentations were done to evaluate methane contents in biogas generated from different compositions. The Test run 1 (MSW), Test run 2 (MSW+ COWDUNG) and Test run 3 (MSW+COWDUNG+URINE) gave directly proportional biogas production along bio-digestion time in weeks. The Methane yield and Quality is found out to be best in test run 3.The co-digestion with Cowdung and urine breed the methanogenic bacteria and developed the better culture of bacteria which results into improved methane contents in generated biogas.

c) Effect of co-digestion on the pH

The pH is most drubbing factor for the growth and survival of microbes during AD methanogenesis. The drastic pH reduction inhibits the methane formation. The pH is a function of the Volatile Fatty Acid and carbon dioxide generations. Stable maximum pH obtained during co-digestion of the cow dung and MSW is 6.7 to 7.2. In all the tests with different additives the digester reaches to the stable pH that means the digestion is neutral at the end of the experimentations .The co-digestion helps to stabilize the PH which is essential for bacterial growth and hence the biogas production.

Physico chemical characteristics of MSW of landfill are given in Table 6 and Table 7.

Table 6. Physical analysis of MSW of Pune Nagar Nigan (% on wet weight

	MSW Composition						
	Plastic	Paper	Cloth	Metal	Stone	Glass	Organic
Minimum	2.4	1.2	0.4	0	1.4	0	46.0
Maximum	19.3	24.7	20.2	1.8	18.3	5.2	85.4
Average [1]	7.1	6.9	7.8	0.7	7.0	1.2	69.3

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Stdev.	±4.1	4.5	5.4	0.6	5.2	1.4	9.6

[1]Average of 25 values.

Table 7 Chemical analysis of MSW of Pune Nagar Nigan

	pH	Moisture content %	Organic matter* %	Volatile solids*, %	C* %	N* %
Minimum	7.3	38.91	18.51	48.03	4.05	0.18
Maximum	8.9	58.91	48.86	74.47	19.28	0.97
Average[1]	7.85	48.08	32.83	62.61	11.62	0.59
Stdev.	±0.37	5.29	8.85	7.92	4.77	0.24

[1]Average of 25 values on dry wet basis

The COD trend is in conformity with the other pollutants like OA, TS, VOS. Table 4.3 represents the effect of different operating temperature in terms of pollutant cumulative load production expressed as g/kgOTS. The parameters presented include COD and OA. Degradation and OA/COD ratio as percentages are also presented. It is important to note that the first-stage mechanism (dilution and liquid recirculation) regarded as optimum condition was maintained for the test conditions.

Table 8 Summary of essential parameters measured at the studied conditions.

Parameter	Ambient (26-28^{0}C)	30^{0}C	35^{0}C
COD/OTS(g/kg)	453±7.7	487±149	611±24
OA/OTS (g/kg)	113±10.8	136±51	169±4.4
Degradation (%)	35±0.9	34±2.0	36±5.0
OA/COD (%)	24±1.8	28±0.8	28±0.9

NB: Values expressed as mean ± standard deviation.

Anaerobic activities in the hydrolytic reactor. The gas phase study of the hydrolytic reactor was carried out by measuring the daily gas composition as presented in figure 4.7.

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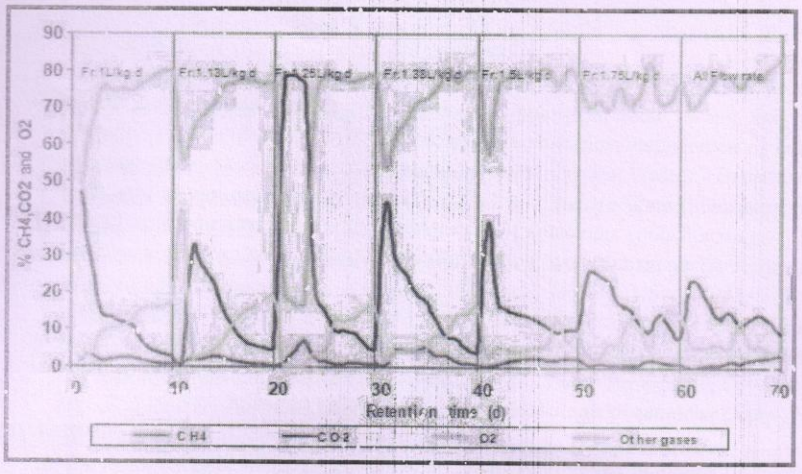

Figure 4. Gas production during hydrolysis as a function of operational time of each test.

Methane production in the hydrolytic reactor was generally not significant with an average value of 1.8±1.3% ranging from a minimum of 1% to a maximum of 7.2%. This behaviour of gas production was expected, due to the low methanogenic biomass concentration in the hydrolytic reactor as a result of the low pH and the effect of micro oxygen (average oxygen composition 8.3±5.1%) in the reactor. The methanogens are sensitive to oxygen, therefore the micro-oxygen application based on the reactor design was aimed at suppressing any appreciable methanogenic activity during hydrolysis.

These were obtained by solving the non-linear first–order equation by exponential regression analysis and the main parameters shown in Table 4.9.

Table 9. Statistical analysis of COD values

Flow rate (L/kg.d)	Correlation coefficient	K_H– Values	Standard deviation of error
		day^{-1}	
1	0.98	0.052	0.022
1.13	0.99	0.083	0.018
1.25	0.98	0.241	0.028
1.38	0.99	0.141	0.019
1.5	0.99	0.128	0.013
1.75	0.99	0.112	0.015
Stepwise decrease (all flow rate)	0.98	0.268	0.029

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Table 10. Hydraulic retention time, determined first-order hydrolysis rate constant and starting concentration of biodegradable waste

HRT (d)	K_H (d-1)	Starting COD concentration(g/l)
0.61	0.052	3190
0.54	0.083	2680
0.49	0.241	1800
0.44	0.141	2030
0.41	120	1627
0.35	0.112	1630
All HRTs	0.268	1630

The values of the kinetic constants obtained in this research are comparable with values of other studies on double stage fermentation of MSW. Two-stage anaerobic treatment system was operated for 30 days.

Conclusion:-

The Test run 1 (MSW), Test run 2 (MSW+ COWDUNG) and Test run 3 (MSW+COWDUNG+URINE) gave peak biogas production and burnable biogas at the 25 days, 23days and 21 days respectively. Methane yield is found out to be best in test run 3 due to the better C/N ratio maintained. Stable maximum pH obtained during co-digestion of the cow dung and MSW and mono digestion is 6.7 to 7.2.The Test 2 and Test 3 concluded that the biogas was collected in the storage tube at faster rate as compared to ideal rate of biogas formation Test 1 of mono-digestion. The retention time required for formation of burnable biogas (test 2 and test 3) was reduced as compared to standard time required for biogas formation without additives (test1). The maximum methane content in biogas obtained from the co-digestion of MSW, cow dung and urine was found out be around 69%.

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Biosurfactant: process optimization by classical one parameter at a time approach

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Abstract

In the present study, biosurfactant producing Pseudomonas sp. and Serratia sp. were isolated and screened from oil contaminated soil samples. The biosurfactant producing ability of these isolates was qualitatively and quantitatively analyzed by using haemolytic assay, oil displacement test and emulsification index. To improve the yield, biosurfactant production process was optimized by using classical one parameter at a time approach for pH, temperature, carbon source, nitrogen source and salt concentration. Bacterial growth and yield of the biosurfactant were found to be maximum at 27°C, pH 7, 4.5% NaCl conc. in presence of engine oil as a carbon source and $NaNO_3$ as nitrogen source in Bushnell Haas medium. The produced biosurfactant was extracted by solvent extraction method and characterized by FTIR spectrum range from 4000 to 400 cm^{-1}.

Keywords: Biosurfactant, haemolytic assay, oil displacement test, emulsification index, FTIR.

Introduction

Surfactants, the surface-active agents (SAA), are compounds capable of reducing surface and interfacial tension between liquids, solids and gases.[6] Biosurfactants are amphipathic compounds with hydrophilic and hydrophobic moieties, former is either a long chain fatty acid, hydroxy fatty acid or α-alkyl-β-hydroxy fatty acid and the later can be a carbohydrate, an amino acid, a cyclic peptide, a phosphate, a carboxylic acid or alcohol etc. These two groups confer the ability of surfactant to accumulate between fluid phases, thereby reducing surface and interfacial tension at the surface and interface regions respectively.

In recent few years, biosurfactant received renewed attention because they exhibited biodegradability, low toxicity, ecological acceptability and ability to be produced from renewable and cheaper substrates. Besides this role, biosurfactants can also help their producing strains to get access to nutrients.[4] Surfactants have been reported to have many industrial applications as adhesives, flocculating, wetting and foaming agents, de-emulsifiers and penetrants. An interfacial boundary exists between two immiscible phases. The hydrophobic portion concentrates at the surface while the hydrophilic portion is oriented towards the solution.

Biosurfactants are biologically produced by yeast or bacteria from various substrates including hydrocarbons, sugars, oils, alkanes and wastes. Chemical nature of biosurfactant includes peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides etc. Biosurfactants possess the characteristic property of reducing the surface and interfacial tension using the same mechanisms as that for chemical surfactants.

Biosurfactants are produced by a variety of microbes secreted either extracellularly or attached to parts of cells, predominantly during growth on water-immiscible substrates. The production of biosurfactants by microorganisms can be during exponential growth or it may be during the stationary phase of growth when the nutrient limiting conditions start prevailing in the growth medium. In case of growth associated biosurfactant production, there exists a parallel relationship between substrate utilization, growth and biosurfactant production.

Production of biosurfactants by some microorganisms might be attributable to the presence of certain genes that are turned on in the presence of particular hydrocarbons. These microorganisms are distributed among a wide variety of genera. The hydrocarbon utilizing and biosurfactant producing microbes are mainly from the genera *Bacillus, Nocardia, Pseudomonas, Acinetobacter, Flavobacterium, Arthrobacter, Rhodococcus, Mycobacterium, Corynebacterium* and *Candida.* The principle aim of the present study is to focus special emphasis upon the screening of over-producer microbial strains and optimization of cultural parameters.[14]

The genus *Pseudomonas* is capable of using different substrates such as glycerol, mannitol, fructose, glucose, n-paraffins and vegetable oils to produce rhamnolipid-type biosurfactants.[5,8] Several studies have been carried out to define the best ratio between carbon, nitrogen, phosphorus and iron needed to obtain high production yields. Optimization of physical and biochemical parameters that affect growth and biosurfactant producing ability of the organisms is the emerging area of research.[15]

Material and Methods

Sample Collection: Different soil samples were collected from oil contaminated sites of industrial area (18° 9' 0' N, 74°.35' 0'' E at 552 M elevation from above sea level) near Baramati (Pune, India) such as automobile workshops like Aditya auto care, Balaji oil mill, Edible oil mill and Dynamix Dairy Limited. The collected soil samples were used to analyze the physico-chemical parameters and to isolate the biosurfactant producing bacteria. All the samples were collected from the depth of 5 cm from the soil surface. They were collected in sterile polythene bags, tightly packed and carefully transferred to the laboratory for analysis and stored at 4°C aseptically until use.[12]

Enrichment and Isolation: Bacteria capable of degrading engine oil were enriched in the sterile Bushnell Hass (BH) broth medium (0.2gm magnesium sulfate, 0.02gm calcium chloride, 1.0gm potassium di- hydrogen phosphate, 1.0 gm di-potassium hydrogen phosphate, 1.0gm ammonium nitrate, 0.05 gm ferric chloride, 35gm sodium chloride and 20 gm agar in one liter distilled water) by inoculating 1 gm of oil contaminated soil sample into100ml of the medium in conical flask and allowed to incubate at room temperature (27°C) for one week on the rotary shaker. Absorbance was recorded at 540 nm after every 24 hrs.

After incubation all the samples were serially diluted and last three dilutions were spread on the sterile nutrient agar petri plates. The plates were then incubated at room temperature for 72 hrs. and observed for distinct bacterial colonies.[18]

Haemolysis test: The isolated bacterial strains were then streaked on sterile 5% blood agar plates. The plates were incubated for 48 hrs. at 27°C and observed for the presence of clear zone around the colonies which indicates the ability of isolate to produce biosurfactant. The diameter of zone of haemolysis relates with potential.[13,20] Total seven bacterial strains showing the presence of large, clear zones around the colonies on blood agar out of which two potent biosurfactant producer (selected by secondary screening) were selected for further study.

Identification of the isolate by morphological and biochemical methods: The screened two efficient bacterial strains were individually examined for colonial size, shape, color, margin, opacity, elevation, consistency, Gram character, capsule staining, spore staining, motility etc. Biochemical characterization of the isolates was also done by catalase, oxidase, indol, methyl red, Voges Proskauer, citrate utilization, nitrate reduction, H_2S production, amylase, gelatinase and lecithinase test. All the tests were performed by using standard protocols. On the basis of colonial, morphological and biochemical characteristics all the strains were identified up-to genus level.

Qualitative estimation of biosurfactant production

Inoculum preparation: One ml of 24 hrs. old bacterial culture was inoculated individually in 100 ml sterile BH broth medium having 3.5gm NaCl and 5% sterile engine oil as only carbon source. The inoculated medium was incubated at 27°C for one week on the rotary shaker at 130rpm.

Oil displacement test: Oil displacement is a method used to determine the appearance of the clear zone which occurs after adding cell free culture broth on oil water interphase. The diameter evaluation allows the surface tension reduction efficiency of a surfactant which is a reliable qualitative measures of biosurfactant.

The experiment was performed using 20 ml of distilled water in Petri plate followed by addition of 20µl of crude oil to the surface of the water. 10µl of cell free culture broth was then added to the oil surface. The diameters of the clear hollows visualized under visible light were measured after 60 sec.[10]

If biosurfactant is present in the cell free culture broth, the oil will be displaced with an oil free clearing zone and diameter of this zone indicates the surfactant activity, also called oil displacement activity. A negative control was maintained with distilled water (without surfactant) in which no oil displacement or clear zone was observed.

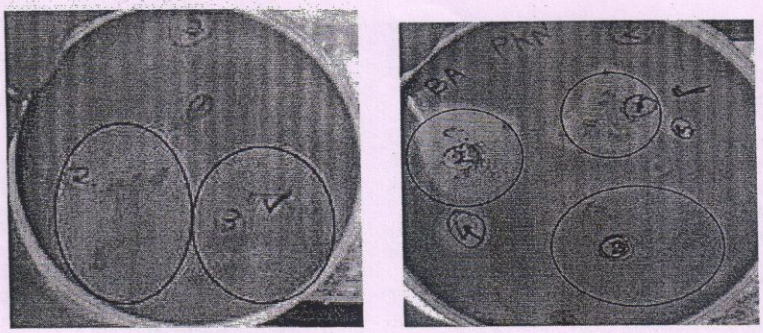
Emulsification index: The determination of emulsification capacity of the suspension towards hydrocarbon i.e. engine oil is performed by Cooper and Goldberg[3] method. Sterile BH agar medium was distributed in 20 ml amount in sterile test tubes. Tubes were allowed to stand for solidification. Then hydrocarbon source and suspension were inoculated. 2 ml hydrocarbon and 1 ml cell suspension of the sample cultures were prepared in sterile test tubes containing saline and homogenized by vortexing for 2 min. and inoculated in BH agar tubes. These tubes were kept for incubation at 37°C for 24 hrs. Results were recorded after incubation.[3,5,10]

The emulsification activity was investigated after 24 hrs. and the emulsification index (E_{24}) was calculated for selected both bacteria. Emulsification index (E_{24}) was calculated by dividing the height of emulsion by the total height of the liquid column and then multiplied by 100.

$$EI = (a / b) \times 100$$

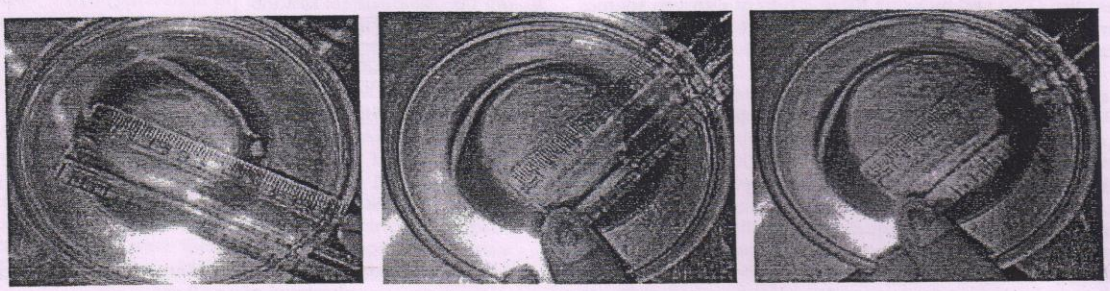
where a is height of the emulsified layer (cm) and b is height of the total liquid column (cm)

Optimization of the biosurfactant production: The growth and biosurfactant production process were optimized by using classical method. Various factors were selected for optimization aiming to obtain higher productivity of the biosurfactant. Effect of physical factors like pH, temp. and salt conc. on surfactant production was determined followed by evaluation of effect of carbon and nitrogen source. All the experiments were carried out in triplicates; standard errors were calculated and shown by error bars.



Isolate 5 Isolate 6

Figure 1: Plates showing zone of haemolysis.



Isolate 5 Isoalate 6 Consortium

Figure 2: Oil displacement by two screen isolates and consortium.

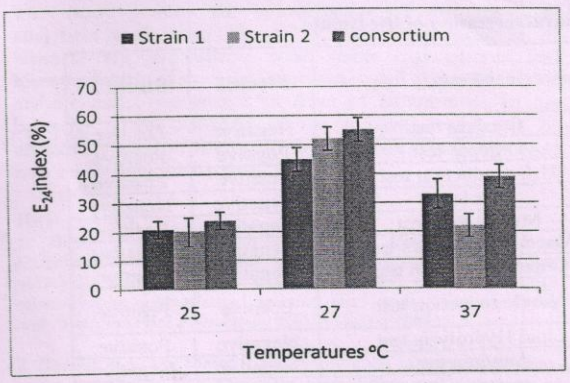
The quantitative analysis including emulsification index[7] and surface tension measurement test was found to be more reliable method for the quantification of the soluble biosurfactant producer. If it is reduced, the surface tension below 40 dynes/cm (Bodour and Miller-Maier, 1998) and or maintained at least 50% of the original emulsion volume in 24 hrs. after the formation of emulsification determines the productivity of bioemulsifiers.

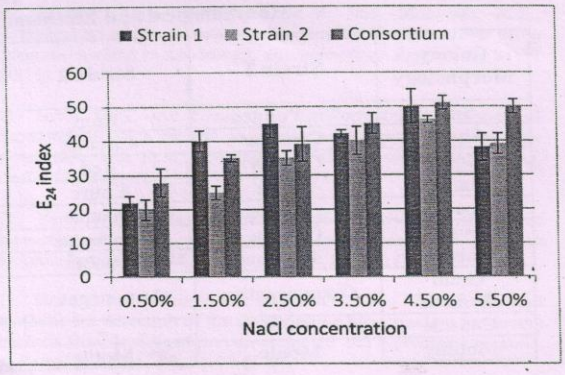
Identification of screened isolate: On the basis of the results of morphological and biochemical test by using Bergey's manual of determinative bacteriology, both the selected isolates were identified as *Pseudomonas* sp. and *Serratia* sp. respectively.

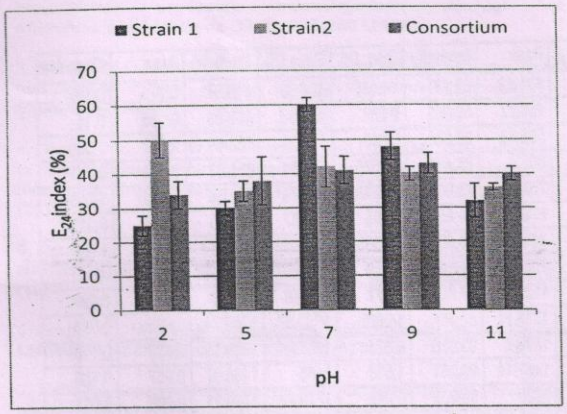
Optimization of biosurfactant production process: The cell growth and accumulation of metabolic products were strongly influenced by medium composition and other growth factors viz. temperature, pH, culture state etc. Thus, optimization process can give high yield of metabolites. The biosurfactant production was measured by emulsification index test. Temperature is one of the important physical factors influencing the growth and the biosurfactant production ability of the organism. The optimum temp. for biosurfactant production was found to be 37°C. A further increase or decrease in the incubation temperature leads to decrease in biosurfactant yield.

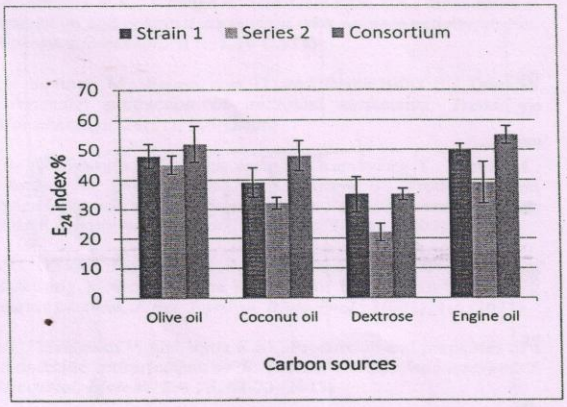
The pH (hydrogen ion conc.) also plays vital role in the growth of organism and production of secondary metabolites like biosurfactant. In the present study, maximum yield was obtained at pH 7 by isolate 1. The salt conc. in the production medium is critical factor for maintaining the osmotic balance and stimulating growth. The selected isolates produced maximum biosurfactant at 4.5% of NaCl, further increase or decrease of which showed negative effect on final yield. The effect of different carbon and nitrogen source supplemented in BH broth was determined. The observed yield indicates that presence of engine oil and $NaNO_3$ as a carbon and nitrogen source best supports the biosurfactant production.

In all experiments, the effect of consortium of the *Pseudomonas* sp. and *Serratia* sp. on final yield of the surfactant was also checked. The result suggests the synergetic effect of the consortium on final yield.









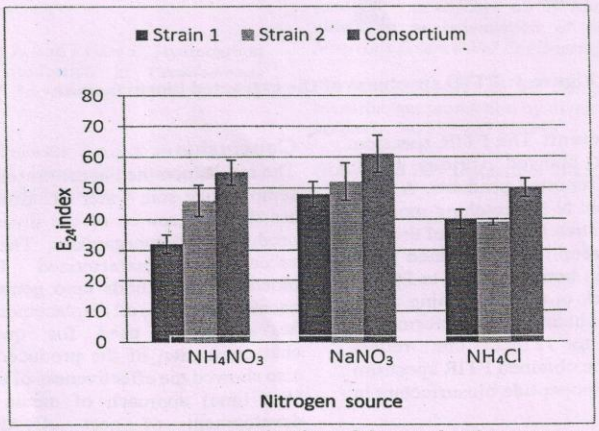
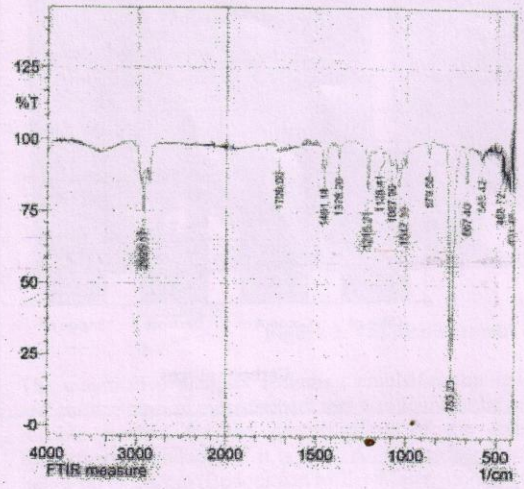


Figure 3: Classical optimization of the production process

Table 3
Morphological and Biochemical characterization of the isolate

Colony Morphology	Strain 1	Strain 2	Biochemical Tests	Strain1	Strain 2
Size	Pin Pointed	1mm	Catalase test	Negative	Positive
Shape	Circular	Circular	Oxidase test	Positive	Positive
Color	Creamy	Red	H_2S production test	Positive	Positive
Margin	Entire	Entire	Indol test	Negative	Negative
Opacity	Opaque	Opaque	Methyl Red test	Negative	Positive
Elevation	Convex	Umbonate	Vogues prauskaurs test	Negative	Negative
Consistency	Rough	Smooth	Citrate utilization test	Negative	Positive
Gram character	Gram negative	Gram negative	Nitrate reduction test	Positive	Positive
Capsule	-	+	Lipid Hydrolysis test	Negative	Positive
Motility	Motile	Motile	Amylase test	Negative	Negative



No.	Peak	Intensity	Corr. Int	Base (H)	Base (L)	Area	Corr. Are
1	421.46	82.481	14.437	424.36	418.57	0.273	0.188
2	468.72	89.389	9.686	472.58	465.83	0.186	0.16
3	585.42	93.856	0.656	586.39	583.49	0.073	0.005
4	667.4	85.457	12.675	679.94	655.83	0.759	0.561
5	753.23	23.968	73.248	793.74	714.66	14.657	13.696
6	879.58	94.963	5.085	898.87	864.15	0.313	0.319
7	1047.39	63.866	10.663	1064.75	1020.39	2.1	1.025
8	1087.9	91.647	0.242	1106.22	1086.93	0.523	-0
9	1128.41	95.269	2.277	1144.8	1117.8	0.369	0.097
10	1215.21	82.354	14.667	1232.57	1200.74	1.038	0.624
11	1378.2	93.886	4.324	1387.84	1368.55	0.327	0.176
12	1461.14	90.281	1.818	1465	1436.07	0.857	0.159
13	1709	95.806	0.249	1710.93	1706.11	0.086	0.003
14	2925.17	74.816	13.318	2944.46	2881.77	5.096	1.847

Figure 4: FTRI spectrum of the extracted biosurfactant.

FTIR Analysis of the biosurfactant: The FTIR spectrum of the extracted biosurfactant showed various bands characteristics of the previously reported products. A band at 2925.17 cm^{-1} was resulting from N-H stretching mode. A broad peak around 1709.00 cm^{-1} (stretching mode of the CO-N bond) may be due to amide group.[11] The obtained FTIR spectrum showed lack of bands in between 2882 to 2935.8 cm^{-1} which may be due to the lack of C-H stretching mode of CH_3 and CH_2 group in alkyl chains.[16] The deformation vibrations from 1461.14 cm^{-1} to 1215.21 cm^{-1} reflect aliphatic chains (-CH_3, -CH_2-). The obtained FTIR spectrum clearly indicates the presence of lipopeptide biosurfactant in the extract.[1]

Conclusion

The results obtained in the present study showed that natural samples like soil, water etc. from the oil contaminated soil are the rich source of the diverse group of biosurfactant producing microorganisms. The obtained isolates can be successfully characterized by morphological and biochemical methods upto genus level. The methods like haemolytic assay, oil displacement method and E_{24} index can be effectively used for qualitative and qualitative characterization of the produced biosurfactant. The study also showed the effectiveness of the classical (one parameter at a time) approach of media optimization and in the development of cost effective and high yielding fermentation medium.

The study proves the effectiveness of the advanced analytical techniques like FTIR for characterization of biosurfactant. Nowadays, solid waste management and bioremediation of the oil contaminated soil and water fields are the major challenges in front of community, so the bacterial strain obtained in the present study can be effectively used in future for this purpose which can open new horizon for industries.

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Study of antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from burn patients swab

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Abstract
The present study performed on patients admitted in burns unit to determine *Pseudomonas aeruginosa* of burn wound infection and to study their antibiogram. It is an opportunistic pathogen that develops life threatening infection in patients with immunological system defect like burn patient.
In this study during the period of last six month [1June to 31December2018] total samples of 135 burn patients swab was collected using sterile cotton swabs from hospitalized in the Kasturba Hospital, Mahalaxmi, Mumbai. *Pseudomonas aeruginosa* was identified by standard bacteriological method. The antibiotic resistance patterns using different antimicrobial agents like [Ceftazidime, Cefepime, Colistin, Gentamicin, Amikacin, Ciprofloxacin, Imipenem and Levofloxacin] were performed for all the isolates using Kirby Bauer's disc diffusion method.
Pseudomonas aeruginosa were isolated from 135 clinical samples and all these isolates multidrug resistance *Pseudomonas aeruginosa*. The resistance rate to various antibiotics were as Ceftazidime [26.66%] Cefepime [8.88%] Colistin [14.81%] Gentamicin [34.07%] Amikacin [13.33%] Ciprofloxacin [32.59%] Imipenem [16.80%] and Levofloxacin [15.83%]
This hospital-based study will be help to implement better infection control strategies and improve the knowledge of antibiotic resistance patterns among clinicians. The finding of study also helpful for identifying the common bacteria causing burn wound infection. To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies in our country.

Keywords: antibiotic resistance pattern, antibiotics, burn wound infection, multidrug resistance (MDR), *Pseudomonas aeruginosa*

Introduction

Burn injury is a major significance problem in the world. It has been estimated that 75 % of all deaths following burns are related to infection. It concluded that the burns patients were most commonly infected with the *Pseudomonas aeruginosa* gram-negative bacteria. Burns are one of the most common and devastating forms of trauma and a major public health concern in all around the world. Globally an estimated 75% death occurs annually. And most importance to do not require hospitalized in longer time. The emergence worldwide of antimicrobial resistance among a wide variety of human bacterial and fungal burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections (Virendra S Kolhe1, Antibiotic Resistance Pattern in Aerobic Gram Negative Bacterial Infection in Burn Patient's at Tertiary Care Hospital in Maharashtra, May 2017). Antibiotics first introduced were considered as a miraculous drug. Unfortunately, most of the cheaper antibiotics lost their efficacy due to emergence of resistance among bacteria. Expensive and complicated antibiotics were introduced to tackle simple infections. *Pseudomonas aeruginosa* is an aerobic, nonfermenting, Gram-negative bacillus. It is commonly involved in opportunistic nosocomial infections. *Pseudomonas aeruginosa* develops resistance against maximum all antibiotics by several mechanisms like, multi-drug resistance like that aminoglycoside modifying enzymes and mutations in different chromosomal genes (Abdul Samad1, 2017) [1]. Pseudomonas aeruginosa is an aerobic, motile, gram negative rod. It belongs to the family of pseudomonadaceae. *Pseudomonas aeruginosa* being an opportunistic human pathogen, it is the fatly cause of nosocomial infections, generally patients are admitted to intensive care units (ICU). It can become resistant through mutations in the chromosomal genes which regulate the resistance genes (Mohanasoundaram, 2011 Jun) [7]. It has been found by many messengers that the distribution of various species of bacteria from burn wound surfaces. Burn patients are ideal hosts for opportunistic infections. The burn site remains relatively sterile during the first 24 hour; thereafter, colonization of the wound by gram negative bacteria (Latika Sharma, 08 January 2017) [5]. Now a day, the most widespread methods used for microbial monitoring of burn wounds are swab culture and biopsy culture. The swab culture is a non-invasive and less expensive method, (Mohammad Ali Bahar, August 2008.). Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality (AL-Aali, 2016) [2]. *Pseudomonas aeruginosa* is most challenging organisms involved in a multiplicity of infections. It is highly leading cause of nosocomial infections and is associated with a high mortality rate. Regional variations in antibiotic resistance patterns for different organisms including *Pseudomonas aeruginosa* also occur, which could be due to differences in antibiotic prescribing practices (Mubashir A. Khan, 2016) [8]. With advancements in burn care over the last 50 years, infection is now the leading cause of death after extensive burn injuries.

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Research Article

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Study of antibiotic resistance pattern of bacteria isolated from patients of urinary tract infections UT in Beed (M.S.), India.

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ABSTRACT

Urinary tract infection is the second most common infection present in community practice. This study is aims to study the antibiotic resistance pattern of bacteria isolated from patients of urinary tract infection in Beed (M.S.), India. This study also revealed a significant association between gender and UTIs; female is more common to have UTIs they have a significant higher risk of UTIs than male. The prevalence of UTI was higher among female than male patients in a ratio of (3.1:1). Six isolates were isolated from 58 patients of UTI. In which E. coli (n=31, 53.44%), E. faecalis (n=11, 18.96%), *Styphylococcus aureus* (n=08, 13.79%), Klebsilla pneumoniae (n=03, 5.17%), and *Stapylococcus saprophyticus* (n=03, 5.17%). *Pseudomonas aerogenosa* (n=2, 3.44%). *E.coli* was the most prevalent organism causing UTI among patient studied. The overall percentage of resistance of all isolates to antimicrobials agents were found Amphicillin 65.51%, Cefotaxime 60.34%, ciprofloxacin 29.31%, Amoxicillin 63.79%, Amikacin 6.89%, Nalidixic acid 51.2%, Imipenem 12.06%, Norofloxacin 22.41%, Gentamycin 12.06%, Ceftriaxone 41.37%, Chloranphenicol 79.31%. All six isolates show sensitivity to antibiotic Amikacin, Imipenem and Gentamycin.

Keywords: Urinary tract infection, Antibiotic resistance, Uropathogens, Antimicrobial agents.

INTRODUCTION

Urinary tract infection is the second most common infection present in community practice. Worldwide, about 150 million people are diagnosed with UTI each year (Schaeffer, 1999). Almost 95% cases of UTIs are caused by bacteria (Bishop *et al.*, 2007). Several studies show geographic variations in etiologic agents of UTIs and their resistant pattern to antibiotics (Gupta, 2003). A study carried by Theodore (2006)

in Nigeria found 141 out of181 (77.9%) urine samples gave significant growth and common isolates were *E. coli, K. pnenumonia, S. a ureus* (Thodore 2006, Ebie *et al.,* 2001).

Study of bacteriology and antibiotic resistance pattern of UTI bacteria from Iran shows most common isolates are *E.coli K. pneumoniae* and *S.aureus A. houmannii* and most isolates shows higher resistance to tested antibiotics (Youseti *et al.,* 2009; Al-Jebouri *et al.,* (2013). Al-Mijalli (2017) studied bacterial uro pathogens in urinary tract infections and antibiotic susceptibility pattern in Riyadh Hospital, Saudi Arabia. He concluded that old women were more affected by UTIs than men. *E.coli* is main causative agent. Most of the strains are *E.coli* and *Klebsilla* are resistant to antibiotics. Fluoroquinolones should only be used for the treatment of UTIs.

Gul *et al.* (2004) studied antibiotic resistance profile of indigenous bacterial isolates from UTIs patients. She isolated 65 bacterial isolates Gram Negative bacteria showed more resistance to those antibiotics as compare to Gram Positive organisms. Thus, it was concluded that UTIs pathogens should decreased susceptibility most of the antibiotics usually used for the treatments of UTIs. Study of antibiotic resistance pattern of *E. coli* isolated from urine specimens in eastern India and found that 45% isolated specimens were resistant to Ceftriaxone and 20% resistant to Piperacillin, 12.5% to Amikacin, 25% to gentamycin, 16% to Macrolide cases (Ray *et al.*, 2015).

They isolated Gram Positive bacteria, Gram Negative bacteria and fungi from UTIs patients' urine and also observed that majority of positive cases fall between ages 21-30 years (Priyadharsini *et al.*, 2014). However, it more appropriate to use an antibiotic with a narrow spectrum activity that affects only Gram Positive and Gram-Negative bacteria because of concerns about infection with resistant organisms. Moreover, the extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance which has become a major problem worldwide (Kumar *et al.*,2006).

MATERIAL AND METHODS

Isolation and identification of UTI isolates: 58 urine samples from UTI patients of known sex and age were collected from different pathological laboratories of Beed city. Mid-stream urine specimens were collected as aseptically as possible, in a sterile wide mouth container. All specimens were processed by the laboratory within two hours of collection or kept refrigerated at 4^{0}C until delivery to laboratory, and subsequently processed no more than 18 hours after initial collection.

For the isolation of UTI causing strain loop full of urine sample was streaked on to nutrient agar plate and incubated 37^{0}C for 24 hours. Next day individual colonies were selected and identified on the basis of morphological, cultural and biochemical characteristics (Cheesbrough, 2000).

For Identification of Gram negative bacteria:To check morphological characteristics, Gram staining, capsule staining (Manvals method) and motility test were performed. To check cultural characteristics and growth pattern, MacConkey's agar, Eosine Methylene blue agar, Endoagar (Hi –media) were used. For biochemical characteristics, sugar fermentation (lactose, glucose, mannitol, maltose, sucrose and xylose), TSI, IMViC and nitrate test were performed (Thomos, 1995).

For identification of Gram-Positive bacteria: To check morphological characteristics, Gram staining and capsule staining (Maneval's method) was performed. To check the growth pattern, different media including Nutrient agar, mannitol salt agar and blood agar were used. For biochemical characteristics, sugar fermentation, oxidase, catalae, coagulase test were performed (Thomas, 1995). Stock cultures were maintained in nutrient agar slants at 4^0 C.

Determination of antibiotic resistance profile by The Kirby Baur test: Identified UTI isolates were subjected to antibiotic resistance screening by Disk diffusion method. For this purpose, lawn of UTI isolates were made on Mueller-Hinton agar (Hi-media) with the help of wire loop. Then commercially available antibiotic disks were placed on lawn of culture and plates were incubated 37^0 C for 24 hours. Next day presence or absence of Zone of inhibition around the antibiotic disk was observed and measured. (Thomas, 1995). Antibiotics used for Antibiotic sensitivity test: Amhicillin, Cefotaxime, Ciproflaoxacin, Amoxicillin, Amikacin, Nalidixicacid, Imipenem, Norofloxacin, Ceftriaxone, Gentamicin, Chloramphenicol.

RESULT AND DISCUSSION

Urinary tract infections (UTIs) are among the most commonly prevalent infections in clinical practice. The purpose of the present study is to describe the susceptibility and resistance profile of multidrug resistant isolates from urinary tract infections.58 isolates from different pathological laboratories of Beed district city (M.S.) India, were isolated and identified by conventional methods. Identification of the causative organism and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat the patient in early stage of UTI (Khan and Shan, 1981). Three Gram positive organisms *S.aureus, E. fecalis and S. saprophyticus* and three Gram negative isolates *E. coli, K. pneumoniae, P. aerogenosa* were isolated from 58 samples of urine.

In this study revealed a significant association between gender and UTIs female are more common to have UTIs they have a significant higher risk of UTIs than male. The prevalence of UTI was higher among female than male patients in a ratio of (3.1:1) and this was almost similar to that at AL –Nass *et al.* Woman are more prone to have UTI than men because in females, the urethra much shorter and closer to the anus than the males and they lack the bacteriostatic properties of prostatic secretions (Al-jebouri, 1999) (Table 1). Furthermore, these results shows a significant association between age group and UTIs Vulnerable age group is (21-30years) found.

All infections were mono cultures, no cases of multiple infections were observed. Following isolates were isolated E. coli (n=31, 53.44%) was the most commonly isolated microorganism, followed by E. faeclis (n=11, 18.96%), *Styphylococcus aureus* (n=08, 13.79%), *Klebsilla pneumoniae* (n=03, 5.17%), and *Stapylococcus sapropyticus* (n=03, 5.17%). *Pseudomonas aerogenosa* (n=02, 3.44%) (Table 2). However, *E. coli* was the most prevalent organism causing UTI among patient studied this conclusion was found elsewhere (Menikandan *et al*, 2011).

The overall percentage of resistance of all isolates to antimicrobials agents were Amphicillin 65.51%, Cefotaxime 60.34%, ciproflaoxacin 29.31%, Amoxicillin 63.79%, Amikacin 6.89%, Nalidixic acid 51.2%, Imipenem 12.06%, Norofloxacin 22.41%, Gentamycin 12.06%, Ceftriaxone 41.37%, Chloranphenicol 79.31% (Table 3 and 4). Isolated uropathogens from patient's urine revels sensitivity to Amikacin, Imipenem and gentamycin antibiotics (Figure1).

Table 1: Distribution of UTI patients in relation to their age groups

Sr. No	Age group	No. of Male	No. of Female
1	1-10	-	02
2	11-20	-	08
3	21-30	05	17
4	31-40	01	09
5	41-50	02	05
6	51-60	05	03
7	61-70	01	00
		14	44

Table 2: Percentage of Gram Positive and Gram negative bacteria isolated from UTI patients.

Sr. No.	Bacterial Isolates	Total organisms	Total percentage of UTI
1	*E.coli*	31	53.44
2	*K.pnemoniae*	03	5.17
3	*P. aerogenosa*	02	3.44
4	*S.aureus*	08	13.79
5	*E. faecalis*	11	18.96
6	*S.saprophyticus*	03	5.17

Table 3: Antimicrobial susceptibility of Gram negative isolates to tested antibiotics

Antibiotics	*E.coli* Number (%)		*K.pnemoniae* Number(%)		*P.aerogenosa* Number (%)	
	S	*R*	*S*	*R*	*S*	*R*
Amphicilin	02(6.45)	29(93.54)	00(0)	03(100)	00(0)	02(100)
Cefotaxim	10(32.25)	21(67.74)	00(0)	03(100)	01(50)	01(50)
Ciproflaoxacin	16(51.61)	15(48.38)	03(100)	00(0)	02(100)	00(0)
Amoxicilliin	04(12.90)	27(87.09)	00(0)	03(100)	00(0)	02(100)
Amikacin	30(96.77)	01(3.22)	02(66.6)	01(33.3)	02(100)	00(0)
Nalidixic acid	13(41.93)	18(58.06)	02(66.6)	01(33.3)	02(100)	00(0)
Imipenem	31(100)	00(00)	03(100)	00(0)	01(50)	01(50)
Norofloxacin	26(83.87)	05(16.12)	02(66.6)	01(33.3)	00(0)	02(100)
Gentamycin	28(90.32)	03(9.67)	03(100)	00(0)	02(100)	00(0)
Ceftriaxone	20(64.57)	11(35.48)	01(33.3)	02(66.6)	01(50)	01(50)
Chloramphenicol	01(3.22)	30(96.77)	00(0)	03(100)	00(0)	02(100)

S=sensitive, R-resistant

Table 4: Antimicrobial susceptibility of Gram positive isolates to tested antibiotics

Antibiotics	*S. aureus* Number (%)		*E. faecalis* Number (%)		*S.saprophyticus* Number (%)	
	S	*R*	*S*	*R*	*S*	*R*
Amphicilin	04(50)	04(50)	11(100)	0(0)	03(100)	0(0)
Cefotaxime	03(37.5)	05(62.5)	06(54.5)	05(45.4)	03(100)	0(0)
Ciproflaoxacin	06(75)	02(25)	11(100)	0(0)	03(100)	0(0)
Amoxicillin	05(62.5)	03(37.5)	09(81.8)	02(18.1)	03(100)	0(0)
Amikacin	08(100)	0(0)	09(81.8)	02(18.1)	03(100)	0(0)
Nalidixic acid	05(62.5))	03(37.5)	03(27.2)	08(72.7)	03(100)	0(0)
Imipenem	06(75)	02(25)	07(63.6)	04(36.3)	03(100)	0(0)
Norofloxacin	06(75)	02(25)	08(72.7)	03(27.2)	03(100)	0(0)
Gentamycin	07(87.5)	01(12.5)	08(72.7)	03(27.2)	03(100)	0(0)
Ceftriaxone	01(12.5)	07(87.5)	08(72.7)	03)27.2)	03(100)	0(0)
Chloramphenicol	03(37.5)	05(62.5)	05(45.4)	06(54.5)	03(100)	0(0)

S=sensitive, R-resistant

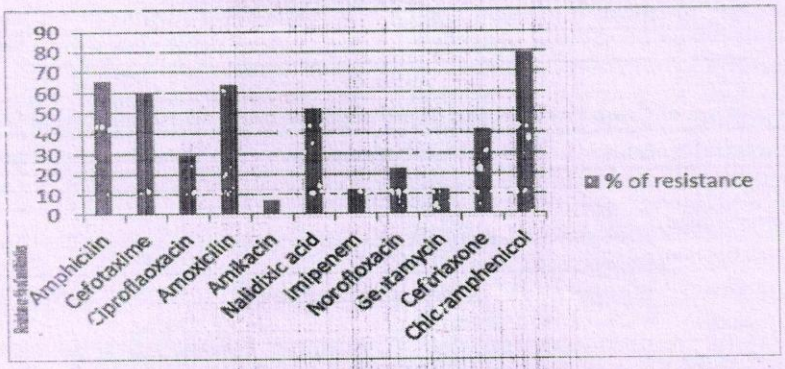

Figure:1 Percentage of resistance of bacterial isolates to antimicrobial agents.

CONCLUSIONS

This study indicates that *E.coli* is the most frequent uropathogen. Amhicillin, Cefotaxime, Ciproflaoxacin, Amoxicillin, Nalidixicacid, Norofloxacin, Ceftriaxone, Chloramphenicol did not have good in vitro coverage for many of uropathogens in this study. Isolated uropathogens revels sensitivity to Amikacin, Imipenem and gentamycin antibiotics. Updates on the knowledge of antibiotic resistance pattern of uropathogens is important for timely modifying the drug of choice for empirical therapy which is required for early and effective treatment of infection.

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Conflicts of Interest: The authors declare no conflict of interest.

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