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Research Article

DIVERSITY OF MICRO-FLORA IN DIFFERENT COMBINATIONS OF PRESS-MUD FOR BIOGAS PRODUCTION

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Abstract- A bench scale model of Lab trials with 10 different combinations of Press-mud and other materials (FYM, sewage, spent wash, Leafy trash and green tops) were taken to find out pattern of distribution of different microorganisms responsible for Biogas production. Bacterial colonies observed in slurry at initial, 7th and 14th day were *Rhizobium spp., Azatobactor* spp., *Bacillus subtilis, Pseudomonas aurigunosa, Klebsilla pneumoniae, E. coli, Vibro cholera, Yarsinia enterocolitica, Micrococcus, Flovobacterium megningosepticum, Clostridium perfringens and Streptococcus lactis*. The bacterial strain obtained after 14th day were *Methanothrix soehngenii, Clostridium perfringens, Methanobacterium* sp., *Methanosaricina* spp., *Methanobrevibacter* spp., *and Bacillus licheniformis* but fungal colony were absent after 14th day of trail. The fungal colonies observed till 14th day were *Rhizopus, Penicillium chrysogenum, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatous, Microsporum* spp., *Rhizocktonia solani, Saccharomyces* spp., *Fusarium* spp. Thus, over all analysis indicated that there were only bacterial strains which play an important role in biogas production in utilizing the industrial waste *i.e.* press mud with different combination for production of biogas. The best results were seen in 80:20 combination *i.e.*, T1 which was composed of 80% press mud and 20% FYM and observed by maximum filling of biogas in balloon made it bigger in size than the other combinations.

Keywords- *Biogas, Press mud, Bacteria and Fungi*

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Introduction

Sugar industry plays an important role in the Indian economy since it is the largest producer of sugar in the world. During the process of the manufacturing of sugar, a sugar factory produces several wastes or byproducts such as molasses, spent wash, bagasse, and press mud *etc*., which have become valuable by products of the sugar industry. As far as press mud is concerned, Indian sugar factory produces about 5.0-5.5 millions tonnes of press mud annually. For sustainability of sugar industry, efforts are being made to generate more revenues from byproducts or wastes.

As we all know there is a growing need to reduce the use of fossil fuels for energy. A twofold reason exists for this: firstly, these resources are finite; secondly the utilization of these resources releases greenhouse gases which are known to contribute towards climate change. The rise in global population and energy use per person is adding to the unsustainable use of fossil fuels. Anaerobic digestion (AD) with concomitant biogas production is an environmentally attractive technology for the treatment of organic waste. Biogas has many environmental benefits like reduction of pollution, waste management, emission of CO_2-neutral renewable energy and the improvement of agricultural practices by application of left-over slurry as bio-compost [1].

AD is applied to a range of industrial waste streams, especially in the agro industry, which is a source of high concentrations of readily degradable organic material composed mainly of complex molecules. The biogas produces as a result of anaerobic reaction of various degradable substrates including filter cake from sugar industry serve as clean energy. This can reduce greenhouse effect and bioenergy being utilized in commercial activities. The end product of this anaerobic digestion named biofertilizer which used for the improvement of soil fertility and crop production. Despite the industrial-economic importance of the underlying microbiological events, little is known about the roles and activities of the microorganisms which inhibit the anaerobic niches [1].

Understanding the details of biogas production from press mud with combination of other organic wastes includes learning about the microorganisms involved in the process and knowledge about their roles allows the development of more efficient and stable systems. Metagenomics –analysis of the total genetic material of the microbial community-is a novel opportunity to expand our knowledge of microbiology of such complex communities. The digestion process takes place through various reactions and interactions among the methanogens and substrates fed into the digester as input.

Materials and Methods

Collection of Samples

The samples collected from different places were used for experimental set up. For the collection of raw materials (original samples) jute bags, collection bottles, polybags were used. Following raw samples were collected:

- Press mud
- Spent wash
- Sewage water
- Farm yard manure

Experimental Setup

A bench scale model was made for study to conduct experiment using combination of press mud with FYM, sewage, spent wash, Leafy trash and green tops, to determine the micro flora present in it and volume of biogas generation. An anaerobic conventional digester had been taken for the generation of biogas. The test was carried out by digesting 700 ml of slurry prepared in different experimental combination in 1400 ml of water (ratio 1:2) in a 2 lit. bottle. The flask was made air tight. A plastic pipe was attached to one balloon.

Table-1 *Macroscopic characters and microscopic characters of bacterial strain on petri plates. of original samples*

SN	Sample	Macroscopic Characters				Microscopic Characters	
		Colour	Margin	Elevation	Texture	Shape	Gram stain
1	Press mud	White	Regular	Raised	Smooth	Rod	-ve
		Off white	Irregular	Flat	Slimy	Rod	-ve
		White	Irregular	opaque	Smooth and slimy	Rod	+ve
		White	Irregular	Flat	Sticky	Rod	-ve
		White	Regular	Raised	Slimy	Rod	-ve
2	Sewage	White	Regular	Convex raised only in center	Slimy	Rod	-ve
		Translucent	Regular	Slightly raised at center	Moist	Comma	-ve
		Gray white	Regular	Raised	Slimy	Rods and Coccus	-ve
		White	Irregular	Flat	Sticky	Rod	-ve
		Yellow white	Regular	Pin headed	Smooth	Coccus	+ve
3	Leafy trash and green tops	White	Irregular	Flat	Sticky	Rod	-ve
		White	Irregular	Opaque	Smooth and slimy	Rod	+ve
		Yellow white	Regular	Pin headed	Smooth	Coccus	+ve
		Translucent	Regular	Convex	Smooth and shiny	Rod	-ve
		Translucent	Regular	Slightly raised	Smooth	Rod	+ve
4	Spent wash	White	Irregular	Opaque	Smooth and slimy	Rod	+ve
		White	Irregular	Flat	Sticky	Rod	-ve
5	FYM	White	Irregular	Opaque	Smooth and slimy	Rod	+ve
		White	Irregular	Flat	Sticky	Rod	-ve
		Translucent	Regular	Slightly raised	Smooth	Rod	+ve

Table-2 *Common Visual (Macro and Microscopic characteristic) of Different treatments*

Days of Observations	Macroscopic & Microscopic Characteristics
Initial Day	It mostly consists of white color and translucent colony with irregular and regular margin and have smooth and slimy texture. Under microscope it was observed that mostly rod shape bacterial strain were seen with gram staining of –ve and +ve appearance.
7th Day	Presence of white color and translucent colony with regular margin and have smooth and slimy texture. Under microscope it was observed that mostly rod shape bacterial strain were seen with gram staining of –ve and +ve appearance.
14th day	Presence of yellowish white color colony with filamentous structure and have smooth texture. Under microscope it was observed that rod shape bacterial strain with gram staining of –ve appearance. And the remaining colonies were same as day 7th and 14th respectively.
21st day	Macroscopic characters changed drastically with the presence of yellowish white color colony, greenish white, translucent with the presence of regular margin and filamentous structure and have smooth texture. Under microscope it was observed that rod and Coccus shape bacterial strain with gram staining of –ve and +ve appearance and mostly +ve strain were present in large amount.
28th day	Macroscopic characters were similar with table 21st day with the presence of yellowish white color colony, greenish white, translucent and bluish off-white colony with the presence of regular, irregular margin and filamentous structure and have smooth texture. Under microscope it was observed that rod and Coccus and clubbed shape bacterial strain with gram staining of –ve and +ve appearance and mostly +ve strain were present in large amount.

For this experiment different treatments/ combinations were taken and biogas production was determined per week. The sets were performed on the basis of their characters like methanogenic, non-methanogenic, aerobic and facultative anaerobic. As a control each experiment set was remain undisturbed for a month. Setup of a small biogas plant *i.e.*, anaerobic digester for investigating micro flora and gas (methane) produced during the experiment were analyzed. Different combinations were tried for to get the best result and press mud was common in all the treatments. Ten setups were conducted with different combination and ratios as shown below:

List of Different Combinations and Ratios:

Treatments	Samples	Ratios
T1	Press mud : FYM	80:20
T2	Press mud : FYM	60:40
T3	Press mud : FYM	30:70
T4	Press mud : Sewage	50;50
T5	Press mud : FYM : Sewage	50:25:25
T6	Press mud : Spent wash : FYM	50:25:25
T7	Press mud : Spent wash : FYM : Sewage	50:17:17:16
T8	Press mud : FYM : Leafy trash and green tops	50:40:10
T 9	Press mud : Spent wash : FYM : Leafy trash and green tops	50:25:15:10
T 10	Press mud : Spent wash : FYM : Leafy trash and green tops	50:25:15:10

Isolation of Bacterial Strain

Initially Original samples or raw samples were taken for isolation of microbial strains (Press mud, Spent wash, FYM, Sewage). A small amount of original sample (press mud, FYM, sewage, spent wash) was taken in a test tube containing 9 ml of sterile distilled water. By serial dilution, the sample was diluted to 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5}.....10^{n} times. 0.1 ml of 10^{-9} and 10^{-10} for press mud and sewage; 10^{-3} and 10^{-4} for spent wash; 10^{-8} and 10^{-9} for FYM, times diluted samples were taken and plated in petriplate containing Nutrient Agar Media (NAM) by pour plate technique. The inoculated plates were incubated at 37˚C for 24-48 hours

Similarly, Initial day slurry samples of different experiments were taken.

1 ml of slurry was taken in a test tube containing 9 ml of sterile distilled water. By serial dilution, the sample was diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4},10^{-5}.....10^{n} times. 0.1 ml of 10^{-6},10^{-7} and 10^{-8} times diluted samples were taken and plated in petri plate containing Nutrient Agar Media (NAM) by pour plate technique. The inoculated plates were incubated at 37˚C for 24-48 hours. The same was repeated on 7th, 14th, 21st and 28th of experiment set up.

Physiological and Biochemical Tests

The methods used was based on physiological and biochemical tests given by Somasegaran and Hoben (1985) [2] and Josey *et al*., (1979) [3] respectively, and well described by Cappuccino and Sherman (1999) [4] to identify the bacteria strains.

Isolation of Fungi

1 ml of Initial day slurry was taken in a test tube containing 9ml of sterile distilled water. By serial dilution, the sample was diluted to 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5}.....10^{n} times. Spread plate technique is been performed. Potato Dextrose Agar Media (PDA) was poured in a petriplate and left for solidification. 0.1 ml of 10^{-8},10^{-9} and 10^{-10} timed diluted samples were taken with the help of micro pipette and plated in petriplate containing Potato Dextrose Agar Media (PDA). With the help of spreader, the diluted sample was spreaded in whole plate. The inoculated plates were incubated at 28°C for 24-48 hours. The plates were incubated at 25 + 2°C for five days. Fungi appearing on the medium were mounted over a clean slide and staining was done with lacto phenol cotton blue and was observed under the microscope. The same was repeated on 7th, 14th, 21st, and 28th of experiment set up. The fungi were identified by using standard manuals such as Manual of soil fungi Gillman, (1957) [5].

Table-3 *Characters of fungal strain in original samples*

SN	Sample	Macroscopic Characters				Microscopic Characters	Fungus
		Colour	Margin	Elevation	Texture		
1	Press mud	Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
		Blue green	Regular	Flat	Velvet Like	-Hyphae are Septate and haline. -Conidiophores are simple or branched. -Phaldes are bunched in brush like clusters. -Conidea are single, round, smooth walls in chain.	*Penicillium chrysogenum*
		Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
		Persian blue	Regular	Flat	Rough	-Columnar, -Uniseriate conidial heads. -Conidiophores are short, smooth-walled and have conical shaped terminal vesicles, which support a single row of phialides on the upper two thirds of the vesicle.	*Aspergillus fumigatous*
2	Sewage	Black	Irregular	Flat	Rough	-Conidiophores end with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
		White with orange center	Regular	Raised	Cottony, feather like appearance	-Forms both macroconidia and microconidia on short conidiophores. -Macroconidia are hyaline, multiseptate, variable in form, fusiform, spindle-shaped to obovate	*Microsporum spp.*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
3	Leafy trash and green tops	Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
		Greenish white	Regular	Flat	Rough	-Hyphae often branch at a 90° angles -The mycelium consists of hyphae partitioned into individual cells by a septum containing a dough-nut shaped pore.	*Rhizocktonia solani*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
		Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
4	Spent wash	Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
		Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
		White	Regular	Raised	Smooth	-Blastoconidia (cell buds) are observed. -They are unicellular, globose, and ellipsoid to elongate in shape. -Hyphae are absent.	*Saccharomyces spp.*
5	FYM	Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
		Blue green	Regular	Flat	Velvet Like	-Hyphae are Septate and haline. -Conidiophores are simple or branched. -Phaldes are bunched in brush like clusters. -Conidea are single, round, smooth walls in chain.	*Penicillium chrysogenum*
		White with orange center	Regular	Raised	Cottony, feathery appearance	-Forms both macroconidia and microconidia on short conidiophores. -Macroconidia are hyaline, multi septate, variable in form, fusiform, spindle-shaped to obovate	*Microsporum spp.*

Results and Discussion

Visual Observation in The Different Combinations

Samples after being serially diluted in sterile distilled water were plated on Nutrient Agar Plates and then incubated for 24-48 hrs, at 37°C. The results of investigation revealed that the original samples (FYM, sewage, spent wash, press mud, Leafy trash and green tops) show the presence of bacterial and fungal colony and there macro and microscopic characters are present in [Table-1]. The Initial slurry sample of 10 different Treatments *i.e.*, showed the presence of bacterial and fungal colony.

The slurry sample of 7th day also showed the presence of bacterial colony and only two fungal colony. The observation of slurry sample of 14th day revealed that no growth in PDA (Potato Dextrose Agar Media) plates, means that the fungal colony was absent and growth was seen in NAM (Nutrient Agar Media), indicating the presence of bacterial colony.

Table-4 *Characters of fungal strain in Slurry sample at initial day*

SN	Sample	Macroscopic Characters				Microscopic Characters	Fungus
		Colour	Margin	Elevation	Texture		
1	Press mud : FYM (80:20)	Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
		Grey, green brown	Regular	Flat	Wooly	-Septate brown hyphae. -Conidiophores are Septate, brown, simple or branched. -Conidia are Septate, brown, muriform, oval, chains or single.	*Alternaria*
		Blue green	Regular	Flat	Velvet Like	-Hyphae are Septate and haline. -Conidiophores are simple or branched. -Phaldes are bunched in brush like clusters. -Conidea are single, round, smooth walls in chain.	*Penicillium chrysogenum*
		Greenish white	Regular	Flat	Rough	-Hyphae often branch at a 90^{o} angles -The mycelium consists of hyphae partitioned into individual cells by a septum containing a dough-nut shaped pore.	*Rhizocktonia solani*
2	Press mud : FYM (60:40)	Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
		Grey, green brown	Regular	Flat	Wooly	-Septate brown hyphae. -Conidiophores are Septate, brown, simple or branched. -Conidia are Septate, brown, muriform, oval, chains or single.	*Alternaria*
		White, olive brown on surface	Regular	Slightly raised	Wooly	-Septate hyphae -Conidiophores are brown in color.	*Curvularia*
		Blue green	Regular	Flat	Velvet Like	-Hyphae are Septate and haline. -Conidiophores are simple or branched. -Phaldes are bunched in brush like clusters. -Conidea are single, round, smooth walls in chain.	*Penicillium chrysogenum*
		White with orange center	Regular	Raised	Cottony, feather like appearance	-Forms both macroconidia and microconidia on short conidiophores. -Macroconidia are hyaline, multiseptate, variable in form, fusiform, spindle-shaped to obovate	*Microsporum spp.*
3	Press mud : FYM (30:70)	Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
		Grey, green brown	Regular	Flat	Wooly	-Septate brown hyphae. -Conidiophores are Septate, brown, simple or branched. -Conidia are Septate, brown, muriform, oval, chains or single.	*Alternaria*
		Blue green	Regular	Flat	Velvet Like	-Hyphae are Septate and haline. -Conidiophores are simple or branched. -Phaldes are bunched in brush like clusters. -Conidea are single, round, smooth walls in chain.	*Penicillium chrysogenum*
		Persian blue	Regular	Flat	Rough	-Columnar, -Uniseriate conidial heads. -Conidiophores are short, smooth-walled and have conical shaped terminal vesicles, which support a single row of phialides on the upper two thirds of the vesicle.	*Aspergillus fumigatous*
4	Press mud : Sewage (50:50)	Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
5	Press mud : FYM : Sewage (50:25:25)	Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
		White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
6	Press mud : Spent wash : FYM (50:25:25)	Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
		White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*

Table-4 *Characters of fungal strain in Slurry sample at initial day [Conti…]*

SN	Sample	Macroscopic Characters				Microscopic Characters	Fungus
		Colour	Margin	Elevation	Texture		
7	Press mud : Spent wash : FYM : Sewage (50:17:17:16)	Black	Irregular	Flat	Rough	-Conidiophores ends with a sac like structure. -Conidia are attached to phialides in chains.	*Aspergillus niger*
		White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
8	Press mud : FYM : Leafy trash and green tops (50:40:10)	Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
		Blue green	Regular	Flat	Velvet like	-Hyphae are Septate and haline. -Conidiophores are simple or branched. -Phaldes are bunched in brush like clusters. -Conidea are single, round, smooth walls in chain.	*Penicillium chrysogenum*
9	Press mud : Spent wash : FYM : Leafy trash and green tops (Fine particles) (50:25:15:10)	Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*
10	Press mud : Spent wash : FYM : Leafy trash and green tops (Chopped) (50:25:15:10)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Green in color with white color margin	Regular	Flat	Wrinkled growth	-Dense mats of mycelium with conidia. -Conidial heads are radiate. -Conidia are globose to subglobouse. -Pale green in color.	*Aspergillus flavus*

Similarly, observation on 21st day showed no growth in PDA but bacterial colony was seen on NAM. The same trend was seen o 28th day of Experiment *i.e.*, the presence of bacterial colony and absence of fungal colony. This indicates that bacteria play a major role in production of biogas and fungus does not exist in biogas production plant after certain days which were confirmed from our results. On an average the macroscopic character of original samples and slurry samples were seen as off-white, smooth, slimy, shiny, regular and irregular in the case of bacteria. For fungus, growth was mostly seen in original samples not in slurry sample. The macroscopic character was rough, elevated, regular or irregular, feathery, valvate, raised and flat [Table-2].

Bacterial Flora in The Different Combinations

With the help of Biochemical test, the confirmation and identification of bacterial strain were carried out in different combinations. It was observed that the micro flora *Bacillus subtilis, Micrococcus, Pseudomonas aurigunosa, Clostridium perfringens, Yarsinia enterocolitica, Flovobacterium megningosepticum, Rhizobium* spp., *E. coli., Azatobactor* spp., *Klebsilla pneumoniae, Vibro cholera* out of these *Bacillus subtilis, Pseudomonas aurigunosa, Clostridium perfringens* which were present in most of the original samples. The micro flora observed at initial day in different combinations were *Bacillus subtilis, Micrococcus, Pseudomonas aurigunosa, Clostridium perfringens, Yarsinia enterocolitica, Flovobacterium megningosepticum, Rhizobium* spp., *E. coli., Azatobactor* spp., *Klebsilla pneumoniae, Vibro cholera* out of these *Bacillus subtilis, Pseudomonas aurigunosa, Clostridium perfringens* which were common as in original samples. The distribution of the species in the biogas-producing microbial community at the beginning of the experiments (week 0) was very similar to those revealed in earlier studies on the microbial community composition in the conventional AD process with animal manure and maize silage as substrates [6-9].

Test observations at 7th day showed the presence of *Bacillus subtilis, Clostridium perfringens, Klebsilla pneumoniae, Streptococcus lactis*. In this samples there is presence of *Streptococcus lactis* which was not observed in previous observations (Original Samples & initial sample) remaining colonies were same. At 14th day different biochemical observations were seen by the presence of new bacterial strains *Methanothrix soehngenii, Clostridium perfringens, Klebsilla pneumoniae, Bacillus licheniformis* out of this *Clostridium perfringens* is common in all experimental treatments.

At 21st day biochemical test results in drastic change with the presence of *Clostridium perfringens, Bacillus licheniformis, Methanothrix soehngenii, Methanosaricina* spp., *Methanobacterium* spp., *Methanobrevibacter* spp. These all the bacteria were responsible for methane production and biogas generation. On 28th day the biochemical test results were similar to 21st day with the presence of *Clostridium perfringens, Bacillus licheniformis*, *Methanothrix soehngenii, Methanosaricina* spp., *Methanobacterium* spp., *Methanobrevibacter* spp. It is important to mention that all the above bacterial flora was present in all the combinations but their action depends on quantity/counts present in slurry. All the bacteria were responsible for methane production and biogas generation. Several characteristic alterations were observed relative to the initial microbial composition (week 0) as a result of the adaptation process (week 5). The classes Clostridia, Bacilli and Gamma-proteobacteria constituted the majority of the Bacteria in the biogas digester was state by 12.

Fungal Flora in The Different Combinations

In Original samples macroscopic view in the Petri plate the morphology of fungal colony was observed by presence of different color colony with regular, irregular margin and with the presence of rough texture. Microscopic examination revealed the presence of Septate and *Asepted hyphae* with the presence of conidiophores with Conidea. The fungal colonies which were present were *Rhizopus, Microsporum* spp., *Aspergillus flavus, Penicillium chrysogenum, Saccharomyces* spp., *Rhizocktonia solani, Aspergillus niger, Aspergillus fumigatous*. The fungal colonies which were present at initial stage in different treatments were *Rhizopus*, *Microsporum* spp., *Aspergillus flavus*, *Penicillium chrysogenum*, *Saccharomyces* spp., *Rhizocktonia solani, Aspergillus niger, Aspergillus fumigatous*, *Fusarium* spp. On 7th day it was observed that the presence of white and pale white color colony with regular and irregular margin with the presence of rough texture.

The fungal colonies only present were *Rhizopus* and *Fusarium* spp. On 14th day the observation revealed that no growth in PDA (Potato Dextrose Agar Media) plates, means that the fungal colony was absent and no fungal colony was seen further.

Table-5 *Characters of fungal strain in Slurry sample at 7th day*

SN	Sample	Macroscopic Characters				Microscopic Characters	Fungus
		Colour	Margin	Elevation	Texture		
1	Press mud : FYM (9:1)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
2	Press mud : FYM (6:4)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
3	Press mud : FYM (3:7)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
4	Press mud : Sewage (5:5)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
5	Press mud : FYM : Sewage (5:2.5:2.5)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
6	Press mud : Spent wash : FYM (5:2.5:2.5)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
7	Press mud : Spent wash : FYM : Sewage (5:1.6:1.6:1.6)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
8	Press mud : FYM : Leafy trash and green tops (5:2.5:2.5)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
9	Press mud : Spent wash : FYM : Leafy trash and green tops (Fine particles) (5:1.6:1.6:1.6)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad. -Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*
10	Press mud : Spent wash : FYM : Leafy trash and green tops (Chopped) (5:1.6:1.6:1.6)	White	Regular	Umbonate	Smooth and slimy	- Septet hyphae -Chlymydospores present.	*Fusarium spp.*
		Pale white color	Irregular	Raised	Cottony	-Hyphae broad.-Asepted hyphae. -Rhizoids and stolons present. -Sprongiophore brown, oval or round in shape.	*Rhizopus*

Conclusion

Analysis indicated that there were only bacterial strains which play an important role in biogas production and utilizing the industrial waste *i.e.*, press mud for production of biogas. Though all the combination produced biogas but the best results were from 80:20 combination *i.e.*, Treatment no. 1 which is composed of 80% press mud and 20% FYM which was observed by maximum filling of biogas in balloon made it bigger in size than that of other combinations. Use of mixed culture of microbes isolated from slurry of best combination may be used for generating more energy and reducing the waste generated from industry can be used in other wastes combinations to increase the biogas productivity. A technology may be developed for genetic manipulation of microorganism to maximize their influence.

Application of research

This finding might be a feasible option as it provides benefits to the environment through energy and nutrient recycling, while also mitigating odors, pathogens and atmospheric CH_4. Promising future waste-to-profit activities may enhance the economic performance of the overall waste management system in sugar factory.

Research Category: Biogas Production

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