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DISPOSAL METHOD OF MYCELIA WASTE IN ENVIRONMENTAL SOUND MANNER

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1. INTRODUCTION

What is Mycelium?

The vegetative mycelium of fungi and streptomycetes simultaneously performs a large number of different tasks. Besides producing and secreting enzymes for nutrient assimilation, mycelia transport nutrients and chemically differentiate to produce a plethora of secondary metabolites (Barka et al., 2016; Borkovich and Ebbole, 2010; Hopwood, 2007). Given that many of these metabolites are of great value to industry, much attention has traditionally been focused on the optimization of production performances in filamentous microbes. However, research in this direction has often been performed using “blind” screening procedures rather than strain optimization strategies based on a deep knowledge of the producing organism (Papagianni, 2004). What has for instance been largely ignored so far is where the production of all these compounds occurs within the mycelium, and how approaches to increase productivity correlate with changes in the localization of production.

The vegetative mycelium of several Streptomyces species is heterogeneous with respect to cellular morphology and physiology. More specifically, the vegetative growth of streptomycetes has been found to encompass two phases during which different cell types are formed (Manteca et al., 2005a, 2005b). The young mycelium that is established after spore germination is highly compartmentalized. The approximately 1-µm-wide compartments are thought to be separated by membrane structures and/or thin peptidoglycan-containing septa (Yagüe et al., 2013; Yagüe et al., 2016). This first compartmentalized mycelium, called MI mycelium, undergoes an ordered process of dismantling, which is followed by a second growth phase during which a multinucleated.

Usages of Mycelium in Antibiotic by fermentation process :-

Antibiotic is produced industrially through a fermentation process using the mycelium. This process involves growing the fungus in a nutrient-rich medium under controlled conditions, where it produces penicillin as a secondary metabolite. The resulting broth is then processed to extract and purify the antibiotic.

Process of Fermentation:-

- Inoculum Preparation:-
- The process begins with the selection of a suitable strain of mycelium often one that has been genetically improved for higher yield,

- The mycelium is cultured in a laboratory setting to create a concentrated inoculum, which serves as the starting material for large-scale production, according to Slide Share.

Fermentation:

- The inoculum is introduced into a large-scale fermenter containing a nutrient medium rich in sugars, nitrogen sources, and other essential minerals.
- The fermentation process is carefully controlled, maintaining optimal conditions for fungal growth and penicillin production. This includes
- Aeration: Ensuring sufficient oxygen supply for aerobic metabolism.
- Temperature: Maintaining a temperature range, typically around 25-26°C,
- pH: Controlling the acidity of the medium, often around a pH of 6.5
- Agitation: Ensuring proper mixing to distribute nutrients and oxygen evenly. During fermentation, mycelium secretes as a secondary metabolite.

Downstream Processing:

Filtration:

The fermentation broth is filtered to remove the fungal biomass (mycelia), according to Microbe Notes.

Extraction:
The rifampicin is extracted from the broth using organic solvents like butyl acetate or amyl acetate.
Purification:

The extracted penicillin is further purified through techniques like back extraction with a buffer, acidification, and re-extraction into an organic solvent

Crystallization:

The purified penicillin is then crystallized and dried to produce the final crystalline penicillin salt

- Product: - The final product is a crystalline Rifampicin salt, ready for use in pharmaceutical applications.
- Waste: - Mycelium waste, a by-product of fermentation processes, is increasingly recognized as a valuable resource rather than just waste.

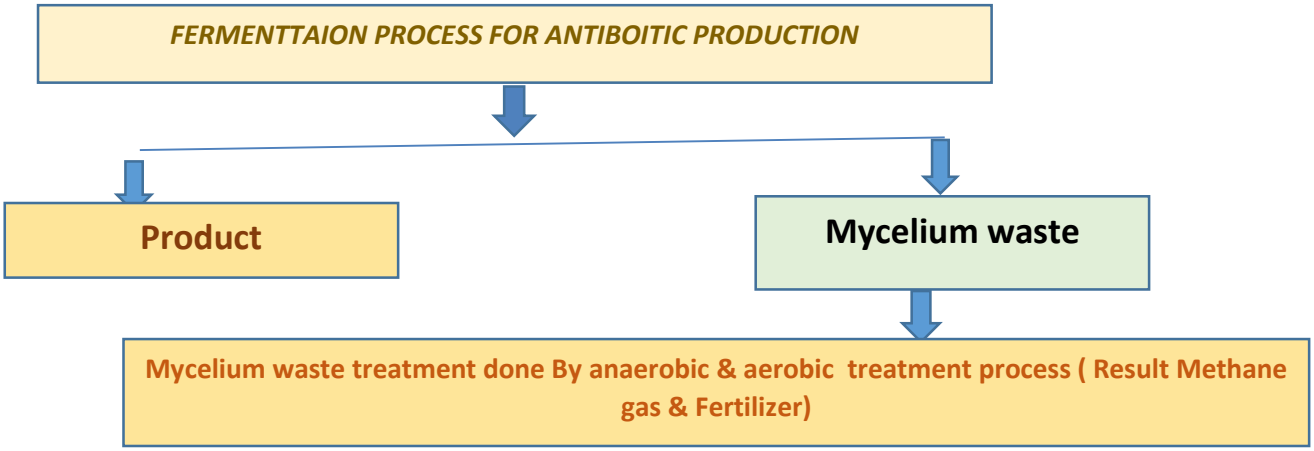
What is Mycelium Waste?

Rifamycin mycelial dreg (RMD) is a biological waste, and its residual rifamycin (RIF) is potentially harmful to both the environment and human health. In this work, thermally activated persulfate (PDS) oxidative degradation of RIF in RMD was developed for the first time. The effects of reaction temperature, initial PDS concentration, and pH on RIF degradation in RMD were investigated, and the treatment conditions were optimized using response surface methodology (RSM). The results showed that 90 °C, 50 mg/g PDS, and pH = 5.3 were the optimal pretreatment conditions, and 100% degradation efficiency of RIF (734 mg/kg) was achieved. SEM and FTIR analyses confirmed that the RIF was destroyed and decomposed after the oxidation reaction. The possible degradation pathways of RIF in the thermally activated PDS system were discussed through HPLC/MS and ESR analyses. The intermediate product was identified, and the toxicity of the final product was predicted to be low or nontoxic. In this work, a degradation pathway of RMD was proposed by

activating persulfate, which facilitates subsequent resource utilization and provides meaningful guidance for the practical treatment of antibiotic mycelium residue (AMR).

Mycelium waste contains harmful compounds such as residual antibiotics and metabolic intermediates and is an AMR with a moisture content of approximately 90.69%. The presence of RMD with high moisture content increases the difficulty of the treatment process. If Mycelium waste is not handled properly, it can cause serious environmental problems such as water pollution and odors, especially the development of resistance genes. It is important to explore efficient and simple disposal technology to meet more stringent standards. At present, the direct treatment methods of mycelium usually include incineration and pyrolysis. Which have the disadvantages of high cost and serious secondary environmental pollution? For the direct incineration or pyrolysis treatment of mycelium, some obstacles include high moisture content, which may release harmful gases during the direct high-temperature treatment process. Mycelium contains residual medium in addition to a small amount of antibiotics and high moisture content, and an unreasonable treatment method easily causes environmental pollution and ecological hazards. Importantly, there are multiple nutrients present in the mycelium waste, and unreasonable treatment results in the waste of recyclable resources. Therefore, considering its own particularity, an efficient, reliable and sustainable mycelium pre-treatment process is very important in reducing the concentration of antibiotics in mycelium; the removal of refractory organics by advanced oxidation processes has received more attention due to its superior oxidation capacity, broad environmental adaptability and fast reaction efficiency.

Treatment Methodology for Mycelium waste: - Mycelium waste is having high organic content. So we developed mycelium waste treatment technology.



2. Review of Literature

GUIDELINES FOR MANAGEMENT AND HANDLING OF SPENT MYCELIUM FROM BULK DRUG INDUSTRY

MAHARASHTRA POLLUTION CONTROL BOARD

Kalpataru Point, Sion (E), Mumbai – 400 022.

November, 2005

MAHARASHTRA POLLUTION CONTROL BOARD

GUIDELINES FOR

MANAGEMENT AND HANDLING OF SPENT MYCELIUM FROM BULK DRUG INDUSTRY

PREAMBLE:-

The spent (waste) mycelium from the bulk drug industry can exhibit hazard characteristics if not handled properly. For disposal of spent mycelium, it is subjected to bio composting followed by its use as agricultural manure. This guideline follows and implements the Maharashtra Pollution Control Boards' Pollution Control Policy in this regard. It aims to protect the health and safety of the community and the environment when handling mycelium and its further treatment.

1. GENERAL

1.1 Objective

The objective of this guideline is to provide general guidance and set the framework for the management of potential risks to human health and / or the environment resulting from activities involving handling of mycelium.

1.2 Area of validity

This guideline is binding for the Pharmaceutical and Bulk Drug Industries handling mycelium and holding Authorisation under Hazardous Wastes Rules, Consent to Operate under Water Act and Air Act from Maharashtra Pollution Control Board.

BASIC PRINCIPLES

Risk Management

When handling mycelium, risk management is based on an initial identification of the potential hazards followed by an evaluation of the associated risks and institution of the appropriate technical and organisational control measures.

Classification

Mycelium is classified into the appropriate risk group either according to international or national lists of organisms or based on a risk assessment verified by the State or Central Government Authority.

Hazard Identification

Prior to the commencement of activities with mycelium previously not handled at the site and / or novel types of handling, hazards must be identified and risks evaluated. This includes the classification of the mycelium into risk groups, considerations on the type and scale of handling of the mycelium, and the suitability of installations and equipment.

A *systematic* hazard analysis (e.g., Zurich Hazard Analysis) must be conducted prior to the handling of mycelium in World Health Organization risk group 3 and 4.

Safety Measures

Containment measures

The containment measures required for laboratories, production and other facilities must correspond to the risk group of the mycelium used and the way they are handled (e.g. scale, potential for aerosol formation). Facilities are can be designated as BL 1 (biosafety level 1, basic biosafety level) through BL 4 (biosafety level 4, maximum containment).

Facilities, in which mycelium of different risk groups are handled, must be designed to the level appropriate for the safe handling of the organisms in the highest risk group.

Labeling of Biohazard Areas and Material during Storage and Transportation Biological hazard zones i.e. BL2 and higher must be clearly labeled at the
entrance e.g., using the international biohazard warning sign (Schedule III and Schedule IV under Rule 6 of The Bio-Medical Waste (Management and Handling) Rules, 1998) and indicating the type of containment (BL2, BL3 or BL4) and the potential special hazards. Emergency contact persons responsible for biosafety in the work area should also be signposted.
Work practices PPE

Work practices and personal protective equipment (PPE) must be in line with the mycelium (risk group, exposure routes and means of transmission) and the processes used.

Operating Instructions

If hazardous mycelium (risk group 2 and above) are handled, operating instructions must be elaborated and made available in the work areas.

These operating instructions must include information on the potential effect of the mycelium and safety precautions in an emergency.

The operating instructions must be clear and written in a language understood by the workers. The instructions should be, wherever appropriate, complemented by oral instructions.

Maintenance / Cleaning

Safety equipment must be maintained in proper working order by carrying out routine maintenance according to an established program.

Maintenance and cleaning work in areas of category BL2 or higher may be carried out only after it has been ensured that the maintenance or cleaning staff are not exposed to biohazardous material.

The work may be carried out only under the supervision of a qualified person or by individuals who have undergone suitable safety training.

Inactivation / Waste disposal

Mycelium material which might pose a risk to the environment must be inactivated by disinfection, sterilization or incineration prior to final discharge into the environment.

The filtered Mycelium cake must be thoroughly washed with water or suitable reagent to remove any traces of active ingredient. The ability to differentiate between material before and after inactivation must be assured.

Disinfection

Methods used for disinfection must be periodically validated (in-house or contractor) to confirm their efficiency.

Emergencies

Written emergency procedures in case of spills or accidental release of mycelium must be established (first aid, prevention of spreading, decontamination and safe disposal of potentially contaminated material). Provisions for proper documentation and internal and external communication strategies must be included as part of the contingency plan.

1.3 Organisational Measures

The Industry generating mycelium shall obtain appropriate registration / approval to ensure evaluation biological risks in the work area.

The Principal Investigator (PI) (leader of project, head of laboratory, pilot plant, production plant or other person who directs biological activities) who

- is responsible for the safe handling of mycelium under his / her supervision
- establishes inventories of mycelium
- identifies those workers exposed to biohazardous material
- evaluates biohazards in the work area
- is the contact person to the local authorities in all biosafety matters
- initiates and supervises training programs
- performs routine performance checks

Glossary

Biohazardous agents See: Hazardous mycelium.

Mycelium Natural or genetically modified viable cells, cell clusters, spores, viruses or genomic elements capable of replication (pathogens but also nonpathogens).

BL Biosafety level (BL1 to BL4) according to known or expected risk category of organism or experiment. The levels are designated in ascending order by degree of protection provided to personnel, the environment, and the community.

Containment facilities Containment facilities are rooms and areas in which potentially hazardous mycelium are used. Disinfection Selective verified treatment aiming at removing the infectious activity of specific pathogens. Pathogens are thus rendered non-virulent.

Handling of Mycelium

Their production or use, i.e. application, storage, processing and treatment, packaging, repackaging, mixing, destruction and transport on-site and off-site.

Hazardous Mycelium

Inactivation Destruction of the biological activity of organisms, viruses and cell components, such as infectious materials.

Pathogens Disease-causing organisms

Principal investigator (PI)

Leader of project, head of laboratory, pilot plant, production plant or other person who directs biological activities.

Recombinant organism See: Transgenic organism

Transgenic Organism (virus, microorganism, animal, plant etc.) whose genetic material has been altered by any method that overrules existing natural barriers of sexual recombination and/or propagation, and still has potential to transfer and/or to multiply this new genetic material.

Annexure 1

World Health Organization Risk Group Definitions

Pathogens should be handled and contained depending upon their biological characteristics. These characteristics or factors provide for an indication of the associated risk of exposure and the risk of disease of the host. The factors that are associated with the risk of exposure are the host's work activity, proficiency, age, sex, immune status and medications being used. The factors associated with the risk of disease to the pathogen include virulence, infectious dose, route of infection, toxigenicity, agent's host range, and the availability of effective preventive measures and treatment. Other factors that determine how susceptible one is to an infectious agent are the host's natural defence mechanisms and the chance for opportunistic infection.

The World Health Organization (WHO) has classified infective microorganisms by risk group. These are numbered 1-4 as follows:

- **Risk Group 1** no or low individual community risk

A microorganism that is unlikely to cause human or animal disease, such as *E. coli* K-12 and *Saccharomyces cerevisiae*.

- **Risk Group 2 moderate individual risk, low community risk**

A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. Examples include Herpes viruses, HIV (clinical work), Varicella-Zoster virus, and polioviruses.

- **Risk Group 3 high individual risk, low community risk**

A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. Examples include HIV (non-clinical work), Mycobacterium tuberculosis, Coxiella burnetti, Brucella, and Hanta virus.

- **Risk Group 4 high individual and community risk**

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. Examples include: Ebola virus and Haemorrhagic fever.

Annexure 2

Biosafety Level Definitions Biosafety Level 1 (BL1)

Biosafety Level 1 is suitable for work involving agents of minimal potential hazard to personnel and the environment. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Personnel have specific training in the procedures and are supervised by a scientist with general training in microbiology or a related science. Contaminated materials that are to be decontaminated at a site away from the work place are placed in a durable leak-proof container that is closed before being removed from the work place. Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Biosafety Level 2 (BL2)

Biosafety Level 2 is similar to BL1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: personnel have specific training in handling pathogenic agents and are directed by competent scientists; access to the workplace is limited when work is being conducted; and certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment. Contaminated materials that are to be decontaminated at a site away from the work place are placed in a durable leak-proof container that is closed before being removed from the work place.

Biosafety Level 3 (BL3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Work Place personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. Contaminated materials that

are to be decontaminated at a site away from the work place are placed in a durable leak-proof container that is closed before being removed from the work place.

Biosafety Level 4 (BL4)

Requires maximum containment procedures.

Annexure 3

Case Study On Implementation Of Guidelines For Composting Of Mycelium

The prolonged use of chemical fertilizers, insecticides, herbicides for increasing crop productivity in our country has resulted in changes in microbiological population, nutrient imbalance, deterioration of physical property of the soil, drop in soil fertility.

Several methods have been developed by which organic residue or waste biomass can be converted safely into bio fertilisers.

Mycelium is generated during the fermentation process of the manufacture of Rifamycin B. Rifamycin B is further chemically converted to Rifampicin, a bulk drug used in the treatment of tuberculosis and leprosy.

At one Bulk Drug unit in Maharashtra, the fermentation process is a submerged fermentation and the fermenters are harvested for 265 hrs. The broth is deactivated with Formaldehyde at the end of harvesting and filtered through a Rotary Vacuum Drying Filter using Perlite as a Filter Aid. The Filtrate containing the product is taken for chemical synthesis and the mycelium on the Filter medium is removed from the Vacuum Dryer as a mycelium cake.

The deactivated mycelium is completely biodegradable and is used as a raw material for making bio fertiliser by the method of composting. With addition of different ingredients and other components, this composted mycelium has been proved as one of the best soil enricher, soil conditioner and agro manure.

Composting is a biochemical process in which diverse and mixed group of micro- organisms break down organic material into a black coloured humus substance.

In the early stage of composting mesophilic consume part of soluble carbohydrates resulting in the formation of more biomass and release of heat.

Due to this phenomenon, heap temperature is raised and this favours growth of thermophilic organisms.

Addition of water helps in maintaining moisture content in the range of 50-60% which helps in cooling the heap. A small addition of cow dung slurry helps as a seed for composting.

Breakdown of proteinaceous matter leads to liberation of ammonia and rise in pH. Turning of heap at regular intervals helps in aeration and enhance the composting rate.

Composting is complete in about 35 to 45 days time.

NADEP method has been adopted for composting of our mycelium.

In this method heaps of 1m height by 1m breadth and suitable length are made. In this heap, alternate layers of grass and fresh mycelia are spread.

Cowdung slurry is added for starting the composting process.

Heaps are covered by jute bags and the moisture content is maintained between 50- 60%.

Holes provided in the heap as well as turning of the heap help in aeration.

Chapter -2 Review of Literature 2

Anaerobic digestion

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• **Abstract**

Worldwide waste generation has become a topic of interest since the accumulation of this waste has prompted environmental hazards. Among which, anaerobic digestion provides green and efficient alternate solution for removal of toxic waste and energy production. Therefore, this review emphasizes on the recent data published in 2018 on topics related to anaerobic process, enhancement of biogas production, and fer- mentation efficiency. Furthermore, more focus was made on the factors influencing anaerobic digestion and the effect of trace elements as ionic salts as well as nanoparti- cles on overall biogas production, respectively. © 2019 Water Environment Federation

• **Practitioner points**

- Anaerobic digestion provides green and efficient alternate solution to deal with.
- This review focused on the conditions related to anaerobic process to improve biogas production and fermentation efficiency.
- The trace elements were focused on how to influence biogas production during anaerobic digestion.

• **Key words**

anaerobic digestion; biogas; nanoparticles; trace elements

SOLID Organic wastes such as agriculture, poultry, food, and sludge are ubiquitous resources that can be utilized for conversion to bioenergy. Among them, the main types of waste are rice straw (RS), corn stover, and wheat straw (WS) which account for approximately 47%, 28%, and 25% of total crop residue, respectively (Qin et al., 2018; Zhao, 2018). Hence, the removal of this waste has triggered severe environ- mental pollution problems. Anaerobic digestion (AD) is currently an attractive and environmentally friendly biological process for the conversion and treatment of var- ious complex biomass and toxic wastes. The process involves microbial break down of complex organic matter in the absence of oxygen into energy-rich product called “biogas.” Biogas is mainly a mixture of methane (CH_4) and carbon dioxide (CO_2) with less quantity of H_2S ammonia (NH_3) and moisture contents.

Overall, AD is a sequential process that is carried out by different groups of bac- teria and methanogenic archaea. The conversion of substrate into different products occurs in four stages namely hydrolysis, acidogenesis, acetogenesis, and methanogen- esis, respectively (Figure 1). During the first step, hydrolytic bacteria convert mac- romolecules of organic matter into simple monomers and oligomers. Hydrolysis is most important step of AD; however, it is still considered a rate limiting steps in AD for the degradation of numerous complex substrates reported by most researchers, due to liberation of toxic by-products (complex heterocyclic compounds) or non- desirable volatile fatty acids (VFAs) during this step (Batstone et al., 2018; Buffière, Dooms, Hattou, & Benbelkacem, 2018; Gonzalez, Hendriks, Lier, & van de Kreuk, 2018; Wang, Shen et al., 2018; Wang, Wang et al., 2018; Wang, Hawkins et al., 2018). Consequently, monomers from hydrolysis step are further converted into short chain

ANNUAL LITERATURE REVIEW :-

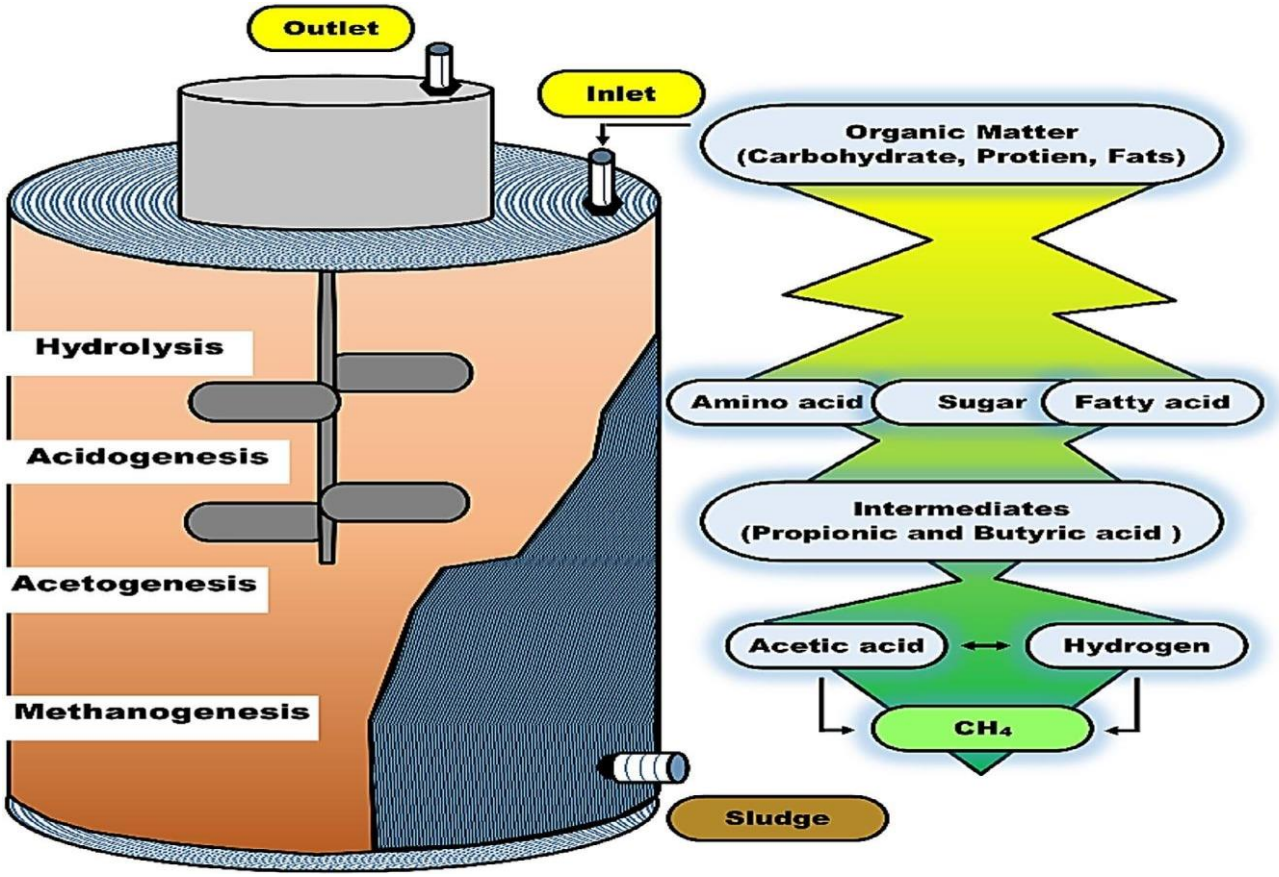

Figure 1. Schematic diagram of processes occurring within anaerobic digestion

fatty acid and volatile fatty acid including lactic acid, pyruvic acid, acetic acid, and formic acid during acidogenesis step, while acetogenesis involves the decomposition of organic acids formed during acidogenesis into acetic acid and hydrogen. Finally, during methanogenesis, acetic acid and hydrogen are converted into methane, and some other gases as final product of the process (Wang, Shen et al., 2018; Wang, Wang et al., 2018; Wang, Hawkins et al., 2018).

Factors such as temperature, pH, retention time (RT), C/N ratio, organic loading rate (ORL), substrate composition, and trace elements significantly affect the overall stability and per- formance of AD. Moreover, any fluctuation could cause drastic effects on the biogas yield and methane content of the reac- tor. Amidst all, trace elements (TEs) play an essential part in microbial metabolism and are extensively utilized as additives in anaerobic digestion to enhance the accumulation of VFAs. Generally, Fe, Ni, and Co are the most important trace elements in this field and have previously been studied considerably (Cai, Wang et al., 2018; Zhao, Mu, Zhao, Wang, & Zuo, 2018; Zhao, Westerholm et al., 2018; Zhao, Ji et al., 2018). In addition, many researchers have reported that TE augmentation could change the archaeal community structure (Cai, Wang et al., 2018; Lu et al., 2018; Zhao, Mu, et al., 2018;Zhao, Westerholm et al., 2018; Zhao, Ji et al.,

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biogas production. We analyzed research articles using key- words such as (effect of TEs on biogas) using "Science-direct (https://www.sciencedirect.com)" and "Springer (https://www. springer.com)" search engines. Subsequently, the Science- direct advance search yielded "465" entries, while Springer yielded "232" documents, respectively. However, the aforemen- tioned search directories, that is, Science-direct and Springer yielded "64" and "37" entries when the keywords were changed to (effect of TEs as nanoparticles on anaerobic digestion). After a sequential analysis, only "15" articles were assigned to TEs, while "06" were selected as TEs supplemented as nanoparticles for the enhancement of biogas during anaerobic digestion.

STAGES OF ANAEROBIC DIGESTION

Hydrolysis

Anaerobic hydrolytic bacteria are mostly found in different natural ecosystems such as soils, sewage, rumen of animals, compost, and AD sludge. Hydrolytic bacteria are mainly involved in the digestion of complex polymers (carbohy- drates, protein, and fat) into simpler soluble monomers (sugar, amino acid, and long-chain fatty acid) by the action of their various hydrolytic enzymes (Castellano-Hinojosa, Armato, Pozo, González-Martínez, & González-López, 2018). The deg- radation mechanism starts with formation of "cellulosome" a

multi-enzyme complex form by hydrolytic bacteria for deg- radation of organic substrates, in which different hydrolytic enzymes are released such as glucanases, hemicellulases, chi- tinases, and liganases. In cellulosome, complex various bonds are formed between bacteria, enzyme, and substrates and the covalent

bonds of substrates polymers are split in chemical reaction in the presence of H_2O. Normally, O_2 dissolved in water are utilized by facultative anaerobes, causing a low redox potential necessary for obligatorily anaerobic hydrolytic bac- teria (Fan, Wnag et al., 2018; Fan, Bi et al., 2018). The degra- dability of different polymers relies on the type, composition, and complexity of the polymers; for example, hydrolysis of carbohydrates takes place within a few hours, while hydroly- sis of proteins and lipids may take few days. Similarly, deg- radation of lignocellulose and lignin is slow and incomplete (Mulat & Horn, 2018; Schroyen, Vervaeren, Raes, & Van, 2018; Takizawa, Baba, Tada, Fukuda, & Nakai, 2018). It has been also confirmed that hydrolytic bacteria cannot produce enzymes without cellulosome.

Moreover, hydrolytic bacteria present in AD are classified in five different phyla: Firmicutes, *Bacteroidetes*, *Fibrobacter*, *Spirochaetes*, and *Thermotogae* (Batstone et al., 2018). However, *Firmicutes* and *Bacteroidetes* are considered the most abundant taxa of hydrolytic bacteria in AD. The relative abundance of hydrolytic bacteria mostly depends on type of inoculum, oper- ating temperature, cell retention time (CRT), and substrate (Wang, Hawkins et al., 2018; Wang, Wang et al., 2018; Wang, Shen, et al. 2018). Other parameters such as high accumulation of VFAs, LCFAs, increased H_2 partial pressure, and humic acid inhibit the activity of hydrolytic bacteria (Di Marcoberardino, Foresti, Binotti, & Manzolini, 2018; Kofoed et al., 2018; Li, Hao, van Loosdrecht, & Luo, 2018). Such inhibition causes either permanent deformation of enzymes complexes (due to changes in enzymes chemical structures) or temporary reduction of hydrolysis due to bindings of inhibitors to enzyme active sites or substrate–enzyme complexes (Angelidaki et al., 2018; Wang, Shen et al., 2018; Wang, Wang et al., 2018; Wang, Hawkins et al., 2018). Different studies have been reported previously to study the effect of VFAs, LCFAs, high hydrogen partial pres- sure, and humic acid on hydrolysis; however, very few studies have been found in recent literature. Batstone et al. (2018) stud- ied the inhibitory effect of humic acid on hydrolysis (carbo- hydrates and proteins) and acetotrophic methanogenesis. The study revealed that cellulose hydrolytic activity was inhibited at HA concentration of 5 g/HAL or above, while protein hydroly- sis and acetotrophic methanogenesis were least affected at this concentration.

Acidogenesis

During this step, the products of hydrolysis, such as sugar, amino acid, and long-chain fatty acid, acts as substrate for acidogenic bacteria. These bacteria further degrade such sub- strates into short chain organic acid which mostly contain C1-C5 molecules of carbon. Organic acids formed during this stage are butyric acid, propionic acid, acetate, and acetic acid with the release of some other compounds such as alcohols, hydrogen, and carbon dioxide. Normally, the concentration of the intermediately formed hydrogen ions affects the kind of the products of fermentation; that is, the fewer reduced compounds (acetate) are formed with the increase in hydrogen partial pres- sure and vice versa (Oh, Kang, & Azizi, 2018; Westerholm, Müller, Singh, Karlsson Lindsjö, & Schnürer, 2018; Zhou, Yan, Wong, & Zhang, 2018). Generally, two types of acidogenic bacterial communities are present in AD, that is, facultative anaerobic acidogens and obligatory anaerobic acidogens. The first stage of the process is carried out by facultative anaero- bic acidogens, while obligatory anaerobic acidogens are active in later stage of anaerobic digestion. Importantly, acidogenic bacteria are mostly the members of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* phyla, whereas most of the bacterial species found in accordance to their abundance during high fermentation period in AD confirming their role in this phase are *Clostridium* (*Firmicutes*), *Peptococcus* (*Firmicutes*), *Bifidobacterium* (*Actinobacteria*), *Desulfovibrio* (*Proteobacteria*), *Corynebacterium* (Actinobacteria), *Bacillus* (*Firmicutes*), *Pseudomonas* (*Proteobacteria*), and *Desulfobacter* (*Proteobacteria*), respectively (De Vrieze, Pinto, Sloan, & Ijaz, 2018). Different operational parameters and factors includ- ing digester design, temperature, cell retention time, and type of substrate may alter the abundance and population of aci- dogenic bacteria in AD. Among all other factors, substrate composition and concentration are considered to be the most influential and are reported by many researchers; for exam- ple, a mesophilic syntrophic acetate oxidizing bacterium (*Syntrophaceticus schinkii spp*.) was significantly enriched by acetate substrates during AD (Cai, Wang et al., 2018; Zhao, Mu et al, 2018; Zhao, Westerholm et al., 2018; Zhao, Ji et al., 2018). Similarly, *Clostridium spp.*, a cellulosic waste degrading bac- terium, was found to be dominant in high cellulose substrate (Liu, 2018; Silva Rabelo, Soares, Sakamoto, & Varesche, 2018; Zou, Ye, & Zhang, 2018).

Acetogenesis

The third phase, Acetogenesis, involves the metabolism and transformation of organic acids (propionic, butyric, penta- tonic acid) and alcohols into acetate. Acetate formation occurs by different mechanism performed by two different group of acetogenic bacteria (Wang, Shen et al., 2018; Wang, Wang et al., 2018; Wang, Hawkins et al., 2018). The first group of bacteria normally called homoacetogenic bacteria which constantly reduced H_2 and CO_2 into acetate. For instance, during ethanol fermentation, carbon dioxide and hydrogen are used to produce acetate; if the partial pressure of H_2 gets increased because of accumulation of hydrogen, then the activity of acetate forming bacteria will be ceased which results in loss of acetate production (Fu, Conrad, & Blaser, 2018; Fu, Luo et al., 2018). Normally, such process is outcompeted by hydrogeno- trophic methanogens because this pathway is less favorable in the AD process. In contrast, hydrogenotrophic methanogens utilize hydrogen in the production of methane which in turn decreased the hydrogen pressure. However, the activity of hydrogenotrophic methanogens is extremely critical to maintain low hydrogen partial pressure in AD (Di Marcoberardino et al., 2018; Kofoed et al., 2018). Many acetogenic bacteria

belong to the genus *Syntrophomonas* (e.g., *Syntrophobacter wolinii* and *Syntrophomonas wolfei*) could also produce acetate from various organic acids and are called "syntrophic fatty- acid oxidizing" microorganisms. Volatile fatty acids (VFAs) conversion into acetate is also dependent on hydrogen partial pressure which must be very low for efficient conversion of volatile fatty acids (VFAs) into acetate. Any variation in such interaction would result in VFA accumulation in the system and will affect overall AD performance. Some bacteria called syntrophic acetate oxidizing (SAO) bacteria are rarely present in acetogenic step; such bacteria normally stabilize the AD pro- cess particularly when the process goes through environmental fluctuations. For example, under elevated ammonia concentra- tion, VFAs, heavy metals, and sulfide, SAO dominantly con- vert acetate to H_2 and CO_2, which are frequently consumed by hydrogenotrophic methanogens for methane production. The decrease in the acetate concentration in turn supports the activity of acetoclastic methanogens which contribute more than 70% of methane production because of increase in the pH of the digester (Algapani et al., 2018; Kofoed et al., 2018). Both mesophilic and thermophilic SAO in AD has been reported which includes *Pseudothermotoga lettingae*, *Thermacetogenium phaeum*, *Syntrophaceticus schinkii*, and some in the phylum of *Spirochaetes* (Jiang, Banks, Zhang, Heaven, & Longhurst, 2018; Jiang, Ru et al., 2018; Westerholm et al., 2018).

Methanogenesis

Methanogenesis is the final step of AD which is carried out by methanogenic archaea. Among others, H_2 oxidation, CO_2 reduction, and utilization are the unique characteristics of methanogens. Moreover, methanogenic bacteria use H_2 and CO_2 along with formate, methanol, and acetate as substrates in the absence of O_2 and produce methane as final product (Wagner, Watanabe, & Shima, 2018; Lyu, Shao, Akinyemi, & Biology, 2018). Methanogens are slow-growing microorgan- isms having strictly anaerobic nature (highly sensitivity towards O_2) and can only degrade limited organic compounds as car- bon and energy source (Lyu et al., 2018). They are generally well known for biogas production, and so far, 65 different spe- cies of methanogens have been found by researchers, which are grouped in five orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales* (Wang, Shen et al., 2018; Wang, Wang et al., 2018; Wang, Hawkins et al., 2018). In addition, they are further categorized into three different groups on the basis of substrate utilization that are as follows: (a) methylotrophic methanogens—metha- nogens that utilize methyl and other single carbon compounds,

(b) hydrogenotrophic methanogens—these organisms con- sume CO_2 and H_2 as carbon and energy source and converts into methane, and (c) acetoclastic methanogens—these metha- nogens convert acetate into methane. Amidst others, aceto- clastic methanogens are accountable for majority of methane production and are relatively higher in their relative abundance such as the genus *Methanosaeta* (Gomez Camacho & Ruggeri, 2018; Peng, Chang et al., 2018; Peng, Zhang et al., 2018). In contrast, another genus *Methanosarcina* of acetoclastic meth- anogens has versatile metabolic pathways and is relatively tolerant to distresses (e.g., low pH and high VFAs) (Centler et al., 2018; Peces, Astals, Jensen, & Clarke, 2018; Peng, Chang et al., 2018; Peng, Zhang et al., 2018; Tian et al., 2018). On the contrary, hydrogenotrophic methanogens have relatively lower abundance in AD as compared to acetoclastic methanogens. In addition,

hydrogenotrophic methanogens are more tolerant in harsh conditions than acetoclastic methanogens. Normally, a drastic population shift in methanogenic community from ace- toclastic to hydrogenotrophic was observed under perturbed conditions (Guermazi-Toumi, Chouari, & Sghir, 2018; Kadier et al., 2018; Ziels et al., 2018).

FACTORS INFLUENCING ANAEROBIC PROCESS

The performance and long-term stability of AD are influ- enced by different biotic and abiotic factors. The production of main product of the process, that is, biogas, depends on both operational parameters (such as temperature, pH, retention time, C/N ratio, organic loading rate, substrate composition, optimum amount of essential nutrient, trace elements) and the diversities and activity of different microbial communities involved in different stages of AD process. Some of these basic factors are discussed as follows.

Temperature

Temperature is the most important factor that influences the AD process because various methanogens are sensitive to fluc- tuation in temperature. Even a slight variation in operating temperature of AD can alter the biological activities including the inhibition of some anaerobic bacteria, especially meth- ane-forming bacteria (Liu, Wachemo et al., 2018; Liu, Singh et al., 2018; Liu, Sun et al., 2018; Liu, Si et al., 2018; Wang, Wang, et al., 2018). AD process has been operated by differ- ent temperature ranges, although an optimum temperature is necessary for stable and successful fermentation. Overall, three different types of AD process have been developed based on different temperature ranges that are psychrophilic AD (10–20°C), mesophilic AD (30–40°C), and thermophilic AD (50–60°C), respectively (Hupfauf et al., 2018; Liu, Wachemo et al., 2018; Liu, Singh et al., 2018; Liu, Sun et al., 2018; Liu, Si et al., 2018). Mesophilic AD and thermophilic AD are com- mon processes used for the production of biogas; the selection of each process depends on numerous factors. Because of high operating temperature, thermophilic AD compromises several advantages such as high metabolic rates, high biogas yields, and deactivation of pathogens. However, higher temperature between 40 and 50°C inhibits the activity of methane-forming bacteria. In contrast, mesophilic AD can maintain high organic loading rates but has lower metabolic rate. Normally, most of the methanogenic microorganisms are mesophilic, while only a few are thermophilic; this is because thermophilic methano- gens are more sensitive to sudden thermal changes then meso- philic methanogen (Wang, Hawkins, et al., 2018; Wang, Wang, et al., 2018; Wang, Shen, et al., 2018). Relative abundance and community diversity of AD are also affected by temperature fluctuations. Higher temperatures exert selective pressure on the community resulting in the enrichment of tolerant strains and decrease in diversity (Wang, Hawkins, et al., 2018; Wang, Wang, et al., 2018; Wang, Shen, et al., 2018). Thus, the meso- philic AD has more diverse archaeal community than the ther- mophilic AD process (Kim, Lee, Han, & Hwang, 2018; Liu, Borghei et al., 2018; Liu, Singh et al., 2018; Liu, Sun et al., 2018; Liu, Si et al., 2018; Zhang, Jia et al., 2018; Zhang, Liu, Yang, He, & Wang, 2018; Zhang, Zhang, & Chung, 2018).

Retention time

HRT is an important parameter and should be detained for an appropriate period to remove dead zones and achieve an efficient syntrophy for the generation of active microorgan- isms. Generally, the metabolic activity and growth of anaero- bic microorganisms are slow, so it is recommended for stable operation of AD to keep retention time twofold greater than the generation time of slow growing methanogens. Optimal value of HRT varies from one process to another depending on quality and composition of feedstock, process temperature, process technology, and type of digester (Kadier et al., 2018; Schmidt, McCabe, & Harris, 2018; Schmidt, McCabe, Harris, & Lee, 2018; Guermazi-Toumi et al., 2018; Xu, Sun et al., 2018; Xu, Yang et al., 2018; Xu, Zhang et al., 2018; Ziels et al., 2018). However, it is necessary to give 10–15 days HRT for retaining and returning their biomass in order to avoid washing out from the reactor. In contrast the regeneration time of hydrolytic and acid-forming bacteria is shorter having lower risk of washing out from digester. Mostly, longer startup phase of biogas plant, that is up to 3 months, is also due to slow growth rate of metha- nogens. This is also one of the reasons that immediately after feeding, the amount of required inoculating sludge is neces- sary to start the plant at its full capacity, which is mostly not available. On the contrary, to overcome the cost of biogas plant because of reduction in volume of the digester, lower retention time is considerably helpful despite compromising on quan- tity and quality of biogas products (Tan, Saritpongteeraka, Kungsanant,

Charnnok, & Chaiprapat, 2018; Tan, Nishimurs et al., 2018; Tang, Wu, Esquivel-Elizondo, Sørensen, & Rittmann, 2018). Higher HRT in anaerobic digestion appar- ently results in higher reduction of volatile solid and hence gives higher biogas yield. However, an optimum duration of HRT is still necessary for stability of AD. For instance, increase in retention time >12 days does not contribute significantly in digestion of volatile solids, as biogas generation remains high in initial stages but when the process proceeds, yield gradually decreases (Tabatabaei, Sulaiman, Aghbashlo, Chisti, & Valijanian, 2018). It has also been noted that higher HRT during AD increases volatile mass removal capacity, required digester volume, and provide buffering capacity for protec- tion against the effects of shock loadings and toxic compounds in wastewater and sludge of the reactor (Buffière et al., 2018; Haryanto, Triyono, & Wicaksono, 2018; Solé-Bundó, Salvadó, Passos, Garfí, & Ferrer, 2018).

pH

Anaerobic digestion performance is also significantly affected by pH of the process; and therefore, it is a basic parameter for the growth of different microorganism in various stages (Wang, Hawkins, et al., 2018; Wang, Wang, et al., 2018; Wang, Shen, et al., 2018). In a two-stage AD, it is more significant to adjust higher pH in the second stage than that in the first stage. *Methanosarcina* is the only genera of methanogens that can tolerate lower pH 6.5 and below, while at pH < 6.7, the metabolism of all other methanogenic bacteria remains ceased. In AD, actually natural mechanisms have been evolved to sus- tain the pH with in neutral range which is normally ensured by natural buffering procedure in digester. CO_2 is released con- tinuously and escapes into air but when the pH falls the CO_2 gets dissolved in substrate and remains in the form of neutral molecules. However, at rising pH, the dissolved carbon is con- verted to carbonic acid and vice versa (Sträuber, Bühligen, Kleinsteuber, & Dittrich-Zechendorf, 2018).

Substrate composition

The yield of biogas in AD is directly influenced by the composi- tion and degradability of feedstock used as substrate. Almost all substrates that contain carbohydrates, proteins, and fats as key component are used for biogas production. Most widely used feedstocks for biogas production are cow and pig manure, municipal sewage sludge, food waste, fruit/vegetable waste, paper, and pulp, respectively. Feedstock containing more car- bohydrates, fats, and proteins produce more volume of biogas; for example, food and vegetable wastes produce more methane then cattle manure and sludge (Maragkaki et al., 2018; Okonkwo, Onokpite, & Onokwai, 2018; Valenti et al., 2018). It is obvious that carbohydrates, proteins, and cellulose are the main components in majority of substrates, which mostly accounts for biogas production. Amount of biogas obtained from digestion of different component of substrates varies, upon complete conversion of its components, therefore Zhao, Mu et al. (2018); Zhao, Westerholm et al. (2018); Zhao, Ji et al. (2018) and Cai, Wang et al. (2018) studied the influence and kinetics of substrates composition on methane production from food and vegetable wastes (FVW) in mesophilic AD, while using acclimated anaerobic granular sludge (AGS) and waste activated sludge (WAS) as microbial and nutritional reg- ulators. Different substrate organic composition, that is, carbo- hydrate/protein/cellulose in ratio 75:15:10, 65:30:5, 50:45:5,
40:55:5, 25:70:5, and 15:83:2 on methane generation were applied during the study, the result proclaimed that highest methane yield 411 ml/g-VS_{added}, which was obtained at optimal carbohydrate/protein/cellulose mass ratio 50:45:5,respectively, while methane production from the other ratio such as 40:55:5, 25:70:5, 65:30:5, 15:83:2, and 75:15:10 was 382 ml/g-VS, 338 ml/
gVS, 326 ml/g-VS, 288 ml/g-VS, and 234 ml/g-VS, respectively. Furthermore, overall carbohydrate degradation was 92.1%– 93.8% except in substrate ratio 15:83:2 which showed a less degradation about 82.5%. The kinetics of substrate conversion and methane yield was also evaluated in this study. A modified Gompertz model was used for this purpose, and correlation coefficient (R^2) of 0.995–0.999 was achieved. The model was better fitted with increasing carbohydrate concentration in substrate than protein, and therefore, methane yield and pro- duction were first enhanced and then reduced correspond- ingly, when the concentration of carbohydrates was

and that of proteins increased, respectively. Similarly, Zhao, Mu et al (2018); Zhao, Westerholm et al. (2018); Zhao, Ji et al. (2018) and Cai, Wang et al. (2018) evaluated the impact of sub- strate concentration and temperature on conversion of acetate, propionate, and hydrogen. During this experiment, two differ- ent bioreactors were operated at two different temperature ranges, that is, mesophilic 37°C and thermophilic 55°C, respec- tively. Initially, HRT was kept on 23 days and was later reduced to 7 days. Degradation of different concentration of acetate (20, 40, and 60 g-COD/L) and coffee (20 g-COD/L) was studied; the result showed that the degradation rate of propionate was higher in thermophilic, while acetate degradation rate was higher at mesophilic conditions. Both in mesophilic and in thermophilic batch reactor fed with 20 g-COD/L coffee powder for 24 hrs, acetate concentration was decreased from 1030 to 310, 166, and 103 mg/L within 1.5, 3.5, and 5.4 hr for meso- philic reactor; however, in thermophilic reactor, concentration of acetate was slightly increased from 350 to 386 mg/L within

3.5 hr. Likewise, the overall acetate concentration after 24 hr was lower in mesophilic reactor than thermophilic. In contrast, propionate concentration in mesophilic reactor decreased from 34 to 15 mg/L within 5.5 hr, while in thermophilic, it remained 8 mg/L at the beginning of the experiment and then increased to 11 mg/L after 24. However, Rmax (ml/g VSS^{-d}) value in Gompertz model for propionate degradation was higher than mesophilic, thus confirming higher conversion rate of propionate in thermophilic reactor. Consequently, the overall methane production in the process was 300 ± 31 ml/g- COD_{in} at HRT 7 days in mesophilic reactor fed with coffee powder 20 g-COD/L, while it was 267 ± 37 ml/g-COD_{in} for thermophilic reactor. Effect of different acetate concentration (20, 40, and 60 g-COD/L) and temperature were also investi- gated in this study, higher gaseous hydrogen consumption rates (G-H_2CR) observed were 0.26, 0.54, and 0.79 ml/hr, respectively, for thermophilic acetate reactor and 0.15, 0.14, and 0.21 ml/hr, respectively, for mesophilic acetate reactor. The author predicted that G-H_2CR was observed in both tem- perature ranges, which indicates that the bacterial and archaeal communities have developed mechanisms at higher feeding. In addition, relative abundance of hydrogenotrophic methano- gens was also high, which showed that at high acetate concen- tration and temperature, methanogenesis was directed toward the syntrophic acetate oxidation pathway. Vivekanand, Mulat, Eijsink, and Horn (2018) determined the synergistic effect of different substrates' composition in anaerobic co-digestion. In this research, three different substrates, that is, manure, fish ensilage, and whey, were evaluated for biogas production using biomethane potential test (BMP). The result obtained from this study disclosed that accumulative methane yields for cellulose (control), whey, manure, and fish ensilage digested as mono- substrates were 363, 274, 180, and 740 ml/gVS, respectively, while the degree of biodegradation was highest for fish ensilage up to 99% and lowest for manure 28%. Synergistic effect of dif- ferent substrate combination was also studied in this research, corresponding to all mixing ratios (85:15; 75:15; 50:50; 25:75; 15:85) of substrate whey and manure, has generally shown no synergistic effect on methane yield. Furthermore, the

degradation was decreased with increasing ratio of manure. Similarly, co-digestion of whey and fish ensilage at 85:15 ratio increased the biogas yield by 13%. However, at higher fish ensi- lage concentration (15%–85%) in co-digestion with whey bio- degradation was decreased from 91% to 83%, and thus, only lower fish ensilage concentration resulted in synergisms due to co-digestion. On the contrary, 84% increase in biogas occurred synergistically at 85:15 substrate ratio of manure and fish ensi- lage, and also, biodegradation was ranged between 79% and 109%, during co-digestion of manure and fish ensilage. Moreover, it was demonstrated that in co-digestion, substrate composition and biodegradability in fact increased the meth- ane yield in few cases from individual feedstock treated in mono-digestion. Liu, Borghei et al. (2018); Liu, Singh et al. (2018); Liu, Sun et al. (2018) and Liu, Si et al. (2018) evaluated the substrate induced responses in mesophilic laboratory scale semi-continues anaerobic reactor, using grass–manure and milled feed wheat as substrates (MFW). In this experiment, methane potential from substrates was determined by biome- thane potential test (BMP); the results revealed that during mono-digestion, average methane values were 291 ± 46, 300 ± 38, and 326 ± 42 for cellulose, MFW, and grass–manure, respectively. However, the final methane potential of all sub- strates marked a mean value 357 ± 45 ml CH4/g VS, while showing no significant difference between different substrates or inoculum as confirmed from the pairwise *t* test, $p > .05$. In contrast, during co-digestion of grass–manure mixture with MFW in CSTR, the total methane production was increased 29% with co-digestion compared to mono-digestion of grass– manure. The initial methane content in the process was increased from 3818 ± 158 to 5317 ± 304 ml CH_4/day between days 0–42 and 56–112,

respectively, which was further increased to 6669 ± 439 ml CH_4/day on Day 140 and finally a stable methane production 5362 ± 205 ml CH_4/day was obtained for 182–231 days. Moreover, it was suggested that addition of MFW to all four CSTR operated for grass–manure significantly increased volumetric methane production; how- ever, a parallel decrease was observed in specific methane pro- duction and substrate degradation efficiency.

Organic loading rate

Organic loading rate is the amount of volatile solid (VS) of organic matter fed into digester per day. Volatile solid (VS) normally contribute to the degradable portion of solid organic matter, while the nondegradable portion including some non- digestible volatile solids is termed as "fixed" solid. It is obvi- ous that higher OLR results in higher biogas yield; however, a higher retention time is required for complete conversion and digestion of organic matter by microorganisms. Also, feeding too much volatile solid into digester will result in higher pro- duction and accumulation of volatile acid, which influences pH and alkalinity of the digester. The actual loading rate is related to the design of reactor, biomass degradability, and microbial activity. Furthermore, mass transfer between incoming waste and biomass, temperature, pH, toxicity level, and staging and configuration of phased digesters are the most influential fac- tors for obtaining maximum OLR (Azzahrani, Davanti, Millati, & Cahyanto, 2018; Musa, Idrus, Hasfalina, & Daud, 2018). Mostly, for the operation of AD reactor for any feedstock, a total solid content of 8.0%–10.0% is generally desirable. It has been noted that in case of dairy manure, a total solid concentration of 15.2% gives highest biogas yield; moreover, for fresh dairy manure, having total solid concentration between 13.0% and 15.0% seems most ideal. Different studies have been reported regarding effect of OLR on AD process. Tan, Saritpongteeraka et al. (2018); Tan, Nishimurs et al. (2018), Tang et al. (2018) evaluated the effect of OLR on thermophilic anaerobic diges- tion of stillage produced from ethanol fermentation of waste paper and kitchen wastes. During the study, three batches of stillage with similar VS and VTS 1.5% (w/w) and 10% (w/w), respectively, were used for thermophilic AD. It was cleared from the result that biogas production was enhanced from 504.1 ± 45.5 ml/g to 605.4 ± 52.7 ml/g VTS with increase in OLR from 2 g VTS/(L^{-1} day^{-1}) to 8 g VTS/(L^{-1} day^{-1}); however, fur- ther increase in OLR to 14 VTS/(L^{-1} day^{-1}) resulted in decrease biogas production up to 518.3 ± 29.6 ml/g VTS. Additionally, the methane content in the biogas was 70% at OLRs of 2 g VTS/ (L^{-1} day^{-1}) and 4 g VTS/ (L^{-1} day^{-1}), which gradually decreased to approximately 60% and 66% at OLRs of 6 and 14 g VTS/ (L^{-1} day^{-1}). However, the highest methane production was 389.6 ± 44.0 ml/g VTS at OLR of 4 g VTS/ (L^{-1} day^{-1}) and 388.1 ± 33.8 ml/g VTS at OLR of 8 g VTS/ (L^{-1} day^{-1}), respec- tively. Similarly, Morken, Gjetmundsen, and Fjørtoft (2018) studied the performance and kinetics during anaerobic co- digestion of dairy cow slurry (DCS) and municipal food waste (MFW) at increased OLR. Four different reactors were oper- ated with constant DCS and changing MFW concentration, that is, 0%, 14.0%, 24.5%, and 32.2% (w/w), respectively, for co- digestion. The specific methane produced in this experiment was 110% higher in reactor operated at highest OLR compared to the one with lowest OLR. Furthermore, 477% more meth- ane production per unit volume of the reactor was obtained subsequently, when OLR was increased from 1.83 to 5.04 g VS L^{-1} day^{-1}. Meanwhile, the HRT was also reduced from 25.3 to 17.2 days. In summary, the relationship between the kinetic constant and the OLR was found to be linear; therefore, it was predicted that efficiency of the process increases corresponding to increasing OLR. In another experiment, Hassan and Dahlan (2018) studied the performance, biogas and methane produc- tion in modified anaerobic hybrid baffled (MAHB) bioreactor at different OLR, that is, 1, 2, 3, and 4 g COD/L day^{-1}, respec- tively, while treating recycled paper mill effluent (RPME). The overall biogas production was increased from 7.21 L/day to the highest 12.51 L/day at OLR 2 g COD/L day^{-1} and then gradu- ally decreased to 6.8 L/day upon further increment in OLRs 4 L/day. In addition, correlation between biogas, methane, and OLR showed that both biogas and methane were increased from 10.99 L/day to 12.51 L/day and 6.45 L/day to 7.92 L/day, respectively, when the OLR was increased from 1 g COD/L/ day to 2 g COD/L/day. However, further increasing OLR to 3 g COD/L/day, both biogas and methane productions were started to decrease and even a constant value of biogas methane production was noted at OLR 4 g COD/L day^{-1}. The author predicted that the reasons might be the presence of toxic material in RPME such as high OLR, which normally affects that activity of anaerobic

microorganism. Also, at higher, that is 3 or 4 g COD/L day^{-1}, the conversion of organic matter to VFAs and then VAFs to methane can also be reduced. The overall COD removal efficiency and VS degradation were also evaluated in this study; both COD removal efficiency and VS degradation were increased from 86.5% to 97.9% by increas- ing the OLR from 1 g COD/L day^{-1}; however, a slight decrease was noted in the values when the OLR was further increased to 4 g COD/L day^{-1}. Likewise, during the mesophilic anaero- bic treatment of malting wastewater in submerged anaerobic membrane bioreactor (SAnMBR), the biogas production was decreased from 0.345 ± 0.007 to 0.308 ± 0.025 L/g $COD_{removed}$ when the OLR increased from 1.36 to 3.18 kg COD/m^3/day, while the methane content in the biogas was 70.9% ± 2.0%. Similarly, the COD removal efficiency also followed the same pattern and was decreased from 94.1% ± 2.5% to 90.2% ± 1.4% (Maleki, Catalan, & Liao, 2018). C/N Ratio

For efficient AD process, a proper composition of feedstock in term of carbon and nitrogen is necessary for balanced C/N ratio. Both carbon and nitrogen are essential for the growth of anaerobic bacteria. Carbon is utilized as energy source, while nitrogen is essential for protein synthesis and building cell structures. Microorganisms during AD consume carbon 25–30 times faster than nitrogen. Therefore, to meet this require- ment, microbes need a 20–30:1 ratio of C to N with the largest percentage of the carbon being readily degradable. An optimal C/N is important parameter in AD. At higher C/N ratio, the nitrogen will no longer react with left over feedstock carbon; as a result, gas production will be slow. Similarly, at lower C/N, excess nitrogen accumulates in the form of ammonium ion (NH^+), which results in increased level of total ammonia nitrogen (TAN) and pH of the digester. Higher concentration of TAN and elevated pH of digester have toxic effect on metha- nogen activities and would sometimes cause possible failure of the AD process (Braz, Fernandez-Gonzalez, Lema, & Carballa, 2018). In order to adjust the proper C/N ratio, normally differ- ent substrates having different carbon and nitrogen composi- tion are mixed for co-digestion. Optimal C/N ratio normally fluctuates by changing feedstock used for AD. Different studies have shown the effect of C/N ratio on the performance and pro- ductivity of AD process. For instance, Betenbaugh et al. (2018) studied synergistic co-digestion of algal biomass and cellulose to optimize C/N ratio for higher biogas and methane produc- tion. During the experiment, algal-bacteria biomass (low C/N ratio) was co-digested with cellulose (high C/N ratio); it has been noted that overall methane yield was improved when the C/N ratio of the algal biomass increased from 5.7 to 20–30. Likewise, C/N ratio 21 and 34 obtained by algae to cellulose substrates ratios 35%: 65% and 20% : 80%, respectively, were considered optimal C/N ratio with highest methane yield 385 and 403 ml g VS^{-1}. Zahan, Othman, and Muster (2018) opti- mized the C/N ratio during mono- and co-digestion of differ- ent substrates such as chicken litter (CL), yoghurt whey (YW), organic fraction of municipal solid waste (OFMSW), hay grass C/N Ratio

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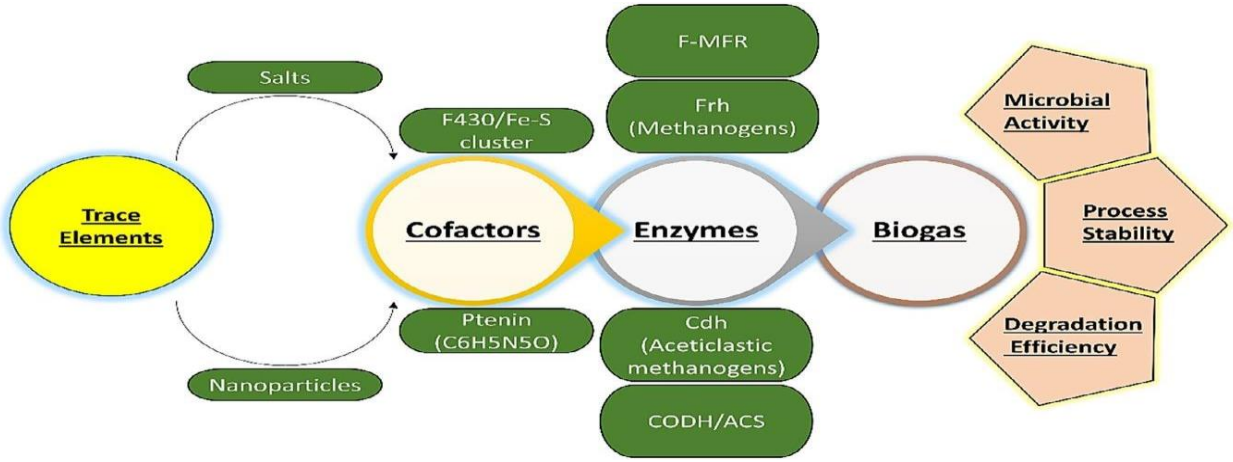
digestion of differ- ent substrates such as chicken litter (CL), yoghurt whey (YW), organic fraction of municipal solid waste (OFMSW), hay grass (HG), and WS. The results proclaimed that anaerobic co-digestion (ACoD) improved the ultimate methane yield because of two, three, or four different substrates mixing. The author concluded that it might be due to the increased degradability and optimized C/N obtained by different substrate combina- tion. Moreover, highest biogas produced from mono-digestion was 316.73 mlN/gVS_{added} from CL at 3% VS at C/N ratio 13.2. Similarly, for the co-digestion, CL was used as main substrate due to its highest biogas capability in mono-digestion, while the other substrates were tested as co-substrate to improve the C/N ratio. The optimum methane yield was achieved by mix- ing CL (30%–35%) and (65%–70%) mixture of YW, HG, and WS at a C/N ratio of (26–27.5). The aforementioned study was demonstrated by modified Gompertz model and surface (opti- mization) model. Consequently, Ammar, Korai, Shahbaz, Li, and Zou (2018) studied the influence of C/N ratio in anaerobic co-digestion of municipal solid waste (MSW) and food waste (FW) at different C/N ratio 20–40. The study depicted that increasing C/N ratio in response decreases the biogas produc- tion due to the nonavailability of sufficient organic nitrogen for microbial growth. However, biogas produced from co-diges- tion of all substrates mixture was relatively higher than mono- digestion control (MSW), which portrayed that the addition of FW as co-substrate balanced the C/N ratio. Moreover, a higher biogas and methane yields were observed to be 827 and 474.44 ml/gVS, at C/N ratio 20, respectively.

EFFECT OF TRACE ELEMENT IN ANAEROBIC DIGESTION

Unlike other parameters such as organic loading rate (OLR), alkalinity, concentration of toxic compounds etc, the availabil- ity of micro and macro-nutrient effectively alter maintenance and operation of anaerobic digesters. Given the importance, the macronutrients (P, N, K, Na, Mg, and Ca) are prevailing the biological processes of the digestion system, along with the attribute of the digestate as fertilizer. In contrast, micronutrients or trace elements (TE) (Mn, Zn, Fe, B, Co, Ni, Cu, Mo, Se, Al, V, and W) are vital for the growth and metabolism of anaer- obic microorganisms, and any deficit in the TEs results in lower methane production (Cai, Wang et al., 2018; Fahlbusch, Hey, Sauer, & Ruppert, 2018; Zhao, Mu et al., 2018; Zhao, Westerholm et al., 2018; Zhao, Ji et al., 2018). Generally, trace elements act as an imperative role in microbial metabolism and are extensively utilized as additives in anaerobic digestion to mitigate the accumulation of volatile fatty acids (VFAs). Up till now, Fe, Co, and Ni are the key trace elements and have already been studied considerably (Cai, Wang et al., 2018; Crest et al., 2018; Schmidt, McCabe, & Harris, 2018; Schmidt, McCabe, Harris et al., 2018; Zhao, Mu et al., 2018; Zhao, Westerholm et al., 2018; Zhao, Ji et al., 2018). Spatially, the addition of TEs has also been suggested as a mean to augment the methane pro- duction of organic substrates deprived of energy depletion and relatively low working cost (Joo, Delicio, Muniz, & Baek, 2018; Peng, Chang et al., 2018; Peng, Zhang et al., 2018; Xu, Sun et al., 2018; Xu, Yang et al., 2018; Xu, Zhang et al., 2018). Consequently, it is essential to supplement these components to the reaction mixture. Thus, this has usually been done by means of a co-sub- strate that contributes the shortfall of these elements in the waste to be treated or by supplementation with exogenous products. Rendering to the published literature, TEs such as cobalt (Co), iron (Fe), nickel (Ni), molybdenum (Mo), and sele- nium (Se) have been acquired to be fundamental for the activity of microbial enzymes, especially in methanogens (Chandolias, Wainaina, Niklasson, & Taherzadeh, 2018; Jiang, Banks et al., 2018; Jiang, Ru et al., 2018; Wintsche, Jehmlich, Popp, Harms, & Kleinsteuber, 2018). For instance, Fe is used for catalysis and electron transport as Fe–S (Fe_2S_2, Fe_3S_4, or Fe_4S_4 clusters). Similarly, another enzyme associated in methanogenesis is the Frh enzyme complex with an Fe–Ni active site and four Fe_4S_4 clusters which grows as bulky aggregates, elevating metal requirements by around eight times (Berghuis et al., 2018; Wagner et al., 2018; Wintsche et al., 2018). Likewise, Ni either binds to the center of a porphyrin unique to methanogens, known as cofactor F430 or Fe–S clusters, respectively (Boll, Estelmann, & Heider, 2018; Merrouch et al., 2018). Furthermore, Ni–Fe enzymes are comprised in all hydrogenases which oxi- dize H_2 and decrease coenzyme F420, ferredoxin, among other electron carriers (Zhang, Jia et al., 2018; Zhang, Liu et al., 2018; Zhang, Zhang et al., 2018). Nevertheless, Acetoclastic methano- gens utilize two metalloenzymes to alter the methyl group from acetate to (CH_3-H_4SPT). In particular, the amplest metal-rich acetoclastic enzyme is acetyl-CoA/CO dehydrogenase synthase (Cdh), which splits the methyl group from acetyl-CoA and moves it to CH3-H4SPT (Lu et al., 2018). The Cdh complex in general has one Fe_4S_4 cluster attached an Ni–Ni site, four Fe_4S_4 clusters, and a

$NiFe_4S_4$ cluster and relegates a 2 × $[Fe_4S_4]$ ferre- doxin. However, Co is involved in methyl group transfer and can be found in cobamide. CH_3-H_4M(S)PT-coenzyme M methyltransferase (Mtr), which is utilized by all methanogens to transfer the methyl group from CH_3-H_4M(S)PT to HS-CoM, has eight Fe atoms and two cobamide cofactors (each with one Co). Besides, another typical coenzyme in methanogenic path- ways is methyl coenzyme M reductase (Mcr), accountable for the production of CoM–CoB heterodisulfide with electrons sourced from HS-CoB and reduction of CH_3-S-CoM to CH_4. Mcr comprises two coenzyme F430 Ni tetrapyrroles (Phillips et al., 2018), while Mo is existing in a pterin ($C_6H_5N_5O$) cofac- tor to catalyze two-electron redox reactions. Thus, these TE are an indispensable part of cofactors and enzymes involved in methanogenesis and hence can directly affect microbial activ- ity, degradation efficiency, and process stability in an AD sys- tem (Montiel-Rozas et al., 2018; Wawra et al., 2018; Zwicker et al., 2018) Above all, the optimum supplementation of TE is perilous for metabolic activities and microbial reproduction in AD (Figure 2). Therefore, lack of TE might extremely affect the microbial activities. In contrast, excessive TE augmentation might also be lethal for anaerobic consortia and elevated con- centrations of TE can restrain the use of digestate in agricul- tural purposes and cause environmental pollution. Much has been published on supplementation of TE on influencing of AD from multifarious substrates. For instance, Table 1 illus- trates some of the data published on TEs augmenting biogas production. Slaughterhouse wastes are deemed as a precious



resource for the production of energy by means of anaerobic technologies (Arshad et al., 2018; Fardad et al., 2018; Lendormi et al., 2018; Loganath & Mazumder, 2018; Wang, Hawkins, et al., 2018; Wang, Wang, et al., 2018; Wang, Shen, et al., 2018). Likewise, Eftaxias, Diamantis, and Aivasidis (2018) studied the effect of TE limitation on the mesophilic anaerobic digestion of thermally pretreated emulsified slaughterhouse wastes (TESW). In this study, continuous stirred tank reactor (CSTR) with sludge recirculation was operated at elevating organic loading rate (OLR) from 1.5 to 10 g L/day. The authors reported 96% COD removal, biogas yield up to 0.53 L/g COD and biogas methane content 77% under optimized conditions. Due to high lipid content of the waste, the process efficiency declined because of VFA accumulation and extreme sludge flotation which was indicative of mass transfer limitations caused by lipid adsorption onto the anaerobic biomass. Similarly, Schmidt, McCabe, and Harris (2018) and Schmidt, McCabe, Harris et al. (2018) also studied the effect of TE for biogas qual- ity, quantity, and process stability using CSTR with slaughter- house wastewater. The results proclaimed that addition of TE (Fe, Ni, Co, Mn, and Mo) enhanced degradation efficiency and improved process stability and higher biogas production. In this research, the process stability was evident with the control reactors produced 84% less biogas per day compared to the reactors containing trace elements. The maximum biogas pro- duction was up to 932 and 425 $ml_N\ g^{-1}$. In addition, the authors predicted that this study can be used by biogas plant operators using meat processing wastewater to optimize biogas production. It can also help to overcome process instabilities as a result from overloading, high FOG concentration, and low temperature and improve start up after shutdown periods. Among other renewable bioenergies, H_2 has the high density of 143 MJ/kg and with a possible maximum electricity generation of 118.68 MJ/kg by a fuel cell. Biohydrogen production is most feasible and efficient energy production method

among various H_2 production technologies with the advantage of altering organic wastes. In analogy, Keskin et al. (2018) determined the effect of TEs (Fe, Ni, Zn, Co, Cu, Mn, Al, B, Se, Mo, and W) in biohydrogen production from fruit and vegetable wastes using

Plackett–Burman statistical design. The maximum biohydro- gen production in this experiment varied between 31 and 76 mlH_2/g VS. Among other TEs, Zn and Ni were found most effective by Plackett–Burman tool. In summary, the biohydro- gen production potentials were enhanced by two to three times with TE addition in comparison with control. The microbial profile of the reactors in this study was also evaluated by DGGE analysis, which revealed that the reactors were inhabited by spore-forming microorganisms, that is, *Clostridium* species, known to have high H_2 production abilities. Yearly, 1.3 billion tons of food produced for human consumption are wasted orlostglobally.In general, food waste is rich in oil (22%–31% of the food waste dry matter), which would be an ultimate and promising substrate. Zn is considered an essential nutrient and a cofactor of several methanogenic enzymes, while it is also commonly found in food waste (7.8–75 mg/L). Thus, the effect of Zn supplementation on biogas production and short-/long- chain fatty acids accumulation during anaerobic co-digestion of food waste and domestic wastewater was evaluated by Chan, Toledo, de Iu, and Shim (2018). This study comprised the Zn supplementation as ($ZnSO_4$ and $ZnCl_2$; 50, 70 and 100 mg/L Zn^{2+}) in an upflow anaerobic sludge blanket reactor operated under mesophilic condition at pH 7.6 and 10 days of hydraulic retention time. The results obtained from this study disclosed that with increasing Zn from 50 to 100 mg/L, methane yield and chemical oxygen demand removal efficiency increased by 30–65 and 10%, respectively. It was also notable that the SCFA accumulation was decreased together with acetate; however, SCFA other than ^{COD}acetate might have been converted to biogas (methane) through pathways other than hydrogenotrophic and acetotrophic methanogenesis. LCFA concentration within the reactor also decreased ($p < .05$), after the microelement supple- mentation could be related to the contribution of biological and physical (precipitation) removal. The maximum biogas yield recorded for ZnSO4 and ZnCl2 was 0.28 ± 0.02 LCH_4/g COD and 0.37 ± 0.01 LCH_4/g COD. Various other studies conducted on effect of TE on AD of food waste also reported the positive effect of TE on overall biogas production (Crest et al., 2018; Yazdanpanah et al., 2018). Consequently, Yazdanpanah)

(2018) reported that the TEs adversely impacted methanogenic activity by 20%–58% in the specific methane activity (SMA) tests. The maximum biogas production for Fe, Ni, Se, Mo, and Co from reactors with food waste including iron-rich inoculum was 100 ± 2.6, 103 ± 6.6, 46 ± 0.1, 91 ± 1.3, and 88 ± 4.9 mlCH_4 g^{-1} day^{-1}, respectively. Crest et al. (2018) also studied the effect TEs and granular activated carbon (GAC) on the anaerobic digestion performance of consecutive batch reactors treating food waste. For the comparison, two batch reactors were set up, one with TE supplementation, while the other was supplemented with GAC. In particular, Fe, Co, Mo, Ni, Se, Zn, Cu, and Mn were utilized as TEs during the whole experiment. The study portrayed a synergistic effect of both activated car- bon and TEs on enhancing methane emission, where activated carbon favored biomass acclimation and improving acetic acid. Activated carbon also preferred the growth of archaea and syn- trophic bacteria, whereas TE addition permitted a faster con- sumption of propionic acid, reducing significantly the batch duration and improving the average methane productivities. The maximum methane production from two batches fed with TE was 419 ± 27 and 584 ± 14 ml/day, while for GAC, the meth- ane production varied from 406 ± 3 and 600 ± 31 ml/day, respectively. The authors concluded that TEs and GAC might be a feasible approach to stabilize FW AD by favoring VFA consumption. As the Al and Zn, Al can bind to the cell mem- brane, thereby reducing microbial growth and affect methane production (Aslam, Yang, Lee, & Kim, 2018; Cai, Wang et al., 2018; Xing & Jin, 2018; Zhao, Mu et al., 2018; Zhao, Westerholm et al., 2018; Zhao, Ji et al., 2018), while Zn in greater concentra- tion causes total inhibition of the process (Cai, Wang et al., 2018; Stowhas et al., 2018; Zhao, Mu et al., 2018; Zhao, Westerholm et al., 2018; Zhao, Ji et al., 2018). However, among the other agricultural wastes, the poultry sector is one of the most important area generating large amounts of organic wastes (Keskin et al., 2018). Besides, chicken manure is quite an appropriate substrate for anaerobic digestion because of its high biodegradable organic matter (450 L biogas/kg VSS). Till date, chicken manure has been well adopted as a substrate for biogas production through anaerobic digestion. For example, Keskin et al. (2018) determined the effect of TE's on basal medium for biogas production from chicken manure. The opti- mum basal medium in this study was designed through Taguchi statistical analysis, along with the effect of TEs on AD. In total,

27 runs with two replicates were carried out over 140 days in which the biogas production between 161 and 628 ml CH_4/g dry matter was recorded from the BMP tests. Moreover, the sta- tistical model remained significant with R^2 = 0.9999 and the Fe, Ag, Co, Mn, Mo, Se, and W were submitted as the significant TEs, whereas the maximum methane production for DM (1%), DM (5%), and DM (10%) were 131, 3,726, and 3,090 CH_4 g^{-1} DM, respectively. It was noted that among all the trace element added, Fe and Co were found to be effective for enhancement of methane in all 27 runs. Methane production from nitrogen- rich chicken manure and the effect of TEs were also investi- gated. In this study, Molaey, Bayrakdar, Sürmeli, and Çalli (2018) studied the effect of mix doses of TE and different total ammonia nitrogen (TAN) concentration in batch experiments

As compared to control, batch digestion supplemented with TEs (Ni 1 mg/L, Co 1 mg/L, Mo 0.2 mg/L, Se 0.2 mg/L, W 0.2 mg/L, and Fe 5 mg/L) at TAN concentration of 3,000 mg/L and 4,000 mg/L, the collective CH_4 production rate and CH_4 production improved by 5%–6% and 7%–8%. The results also showed that with increasing concentration of TAN (6,000 mg/L), the influence of TE augmentation was signifi- cantly high and the cumulative CH_4 production and produc- tion rate were increased by 20% and 39.5%, respectively. Therefore, the authors attributed this enhancement to the stim- ulation by TE supplementation because of syntrophic acetate oxidation (SAO). In another experiment, Molaey et al. (2018) also studied the effect of TE supplementation in anaerobic digestion of chicken manure while linking it to the methano- genic population dynamics at TAN concentration up to 5,000 mg/L for 332 days. During this experiment, the addition of (Se) enhanced the methane yield to 50% and reached
0.27 ± 0.01 L/g VSS added. On the contrary, deficiency of TEs other than Se, the CH_4 yield decreased and dropped to 0.13 L/g VS added. The results of metagenomic analysis portrayed the Se addition improved the CH_4 production and digestion stabil- ity by enhancing the activity and number of hydrogenotrophic *Methanoculleus bourgensis*. However, the addition of TE mix (Co, Ni, Mo, W, and Se) altered the hydrogenotrophic com- munity; hence another hydrogenotrophic methanogen, *Methanobrevibacter*, became dominant within the digester. Finally, the CH_4 yield increased and reached steady state at
0.32 ± 0.01 L/g VS, along with the conversion of propionate to acetate and consumption of acetate via SAO. The SAO bacteria are known to be slow-growers, which might be affected by TE element deficiency, so the authors recommended to use TEs in mixtures rather individual, as the CH_4 yield increased to
0.32 ± 0.01 L/g VS during their experiment (Molaey et al., 2018). Biohythane production especially through two-stage process is gaining attention as a green energy process for both waste treatment and energy production (Liu, Borghei et al., 2018; Liu, Singh et al., 2018; Liu, Sun et al., 2018; Liu, Si et al., 2018). Theprocess encompasses several advantages compared to biogas or hydrogen production. This comprises increase in the net energy balance and allowable organic loading rates beside an increase in the methanogenic activity leading to high production rates and chemical oxygen demand (COD) reduc- tion proficiency. As a rule of thumb, all the substrates utilized in AD are converted to volatile fatty acid (VFA) (acidification) which undergo VFA metabolism (acetogenesis) to produce methane (methanogenesis). The actual hindrance with VFA metabolism is the preclusion of VFA accumulation; for exam- ple, propionic and butyric acids can halt CH_4 production, increase the duration of substrate methanization, and stimulate process failure. Thus, Ezebuiro, Techamanoon, and Körner (2018) evaluated the synergistic and antagonistic effects of TEs on VFA degradation and CH4 production during the methani- zation of a mixture of volatile fatty acids. The experiments were carried out in 1L batch mesophilic reactors with mixture of VFA (butyric, acetic, and propionic acids), supplemented with TEs (Ni, Co, Se, and Mo). The results exhibited that TE sup- plementation triggered variable effects on VFA degradation rate (−10% to + 139%) and methane production (−33% to 55%), respectively. Moreover, the synergistic effect of TEs on VFA degradation was recorded as VFA*Se, Ni*Mo, VFA*Mo, and Ni*Se and the antagonistic interaction was Co*Mo. Comparably, the significant synergistic effect for methane pro- duction included Ni*Co and the antagonistic interaction was VFA*Ni. The methane production for Ni, Co, Se, and Mo was 1.42, 1.48, 148, and 131 Nml/day,whilethe overallmethane pro- duction increased up to 31%–48% due to the addition of the selected TEs and 60%–123% increase in VFA degradation. The synergistic effect of Ni and Co on CH_4 is evident the metabo- lism of the -CH_3 group by the metallo-enzyme (ME). Carbon monoxide dehydrogenase/acetyl CoA synthase (CODH/ACS) is slow when not catalyzed by Ni and Co, whereas Co is required for propionate degradation in the methyl-malonyl-CoA path- way, which also affirms the qualitative findings of this study (Ezebuiro et

al., 2018). The worldwide annual mean coffee pro- duction during recent years (2013–2016) was about 9 million tons. The methane yield of coffee husk and pulp in AD has been described by many researchers, and the results suggest that these residues might have improve performance as compared to other agricultural residues (Aquino, de Gurgel, Adarme, Baêta, & dos Santos, 2018; Chala, Oechsner, Fritz, Latif, & Müller, 2018; Chala, Oechsner, Latif, & Müller, 2018; Juliastuti et al., 2018; San Martin Ruiz, Reiser, Hafner, & Kranert, 2018). Therefore, (Chala, Oechsner, Fritz, et al., 2018; Chala, Oechsner, Latif, et al., 2018) studied the anaerobic stability and perfor- mance of coffee husk and pulp with and without TE supple- ment using 20-L mesophilic continuous stirred tank reactors for 140 days of experiment. The TEs utilized in this study were a mixture of Fe, Ni, Zn, Co, Mn, Mo, Se, W, and B. During the experiment, the OLR was increased from 2.5 (HRT = 40 days) to 6.0 kg VS m^{-3} day^{-1} (HRT = 16.7 day). Notably, the effect of TEs on specific methane yield (SMY) was statistically signifi- cant (p < 001) at higher OLRs (5.0–6.0 kg VS m^{-3} day^{-1}). Moreover, the TEs improved the anaerobic stability through an optimum alkalinity ratio (VFA/TIC < 0.3) and repressed the accumulation of VFA. Overall, the highest CH_4 productivity from pulp with and without TE remained 1.272 and 0.965 m^3 m^{-3} day^{-1} at an OLR of 6.0 and 5.0 kg VS m^{-3} day^{-1}, while the husk produced 0.895 and 0.795 m^3 m^{-3} day^{-1}, respec- tive, at an OLR of 6.0 kg VS m^{-3} day^{-1}. The SMY yield of pulp at OLR 5.0 kg VS m^{-3} day^{-1} with and without TEs was 217 ± 4.7 and 193.1 ± 8.2 L/kg VS; however, husk yielded 149.2 ± 6.0 and 135 ± 4.9 kg/VS, respectively. During their study, the authors observed that mono-digestion of husk and pulp is prone to lack Zn, Mo, Fe, and Ni in long-term anaerobic fermentation. In conclusion, the author recommended further research on husk and pulp to investigate AD performance at higher OLR (>6 kg VS m^{-3} day^{-1}) and to optimize co-digestion with animal manure to meet the TE requirements along with the examina- tion of dry fermentation. Brewery's spent grains (BSG) is also a by-product of crushed husk of malted barley grains obtained after the extraction of fermentable polypeptide and starch (Bougrier, Dognin, Laroche, & Cacho Rivero, 2018; Bougrier, Dognin, Laroche, Gonzalez, et al., 2018; Ravindran, Jaiswal, Abu-Ghannam, & Jaiswal, 2018

worldwide yearly production of BSG is estimated to be
38.6×10^6 tons (Bougrier, Dognin, Laroche, et al., 2018; Bougrier, Dognin, Laroche, Gonzalez, et al., 2018; Ravindran et al., 2018). Hence, the opportunity of obtaining energy from this by-product could have an economic interest. Therefore, Bougrier, Dognin, Laroche, et al. (2018); Bougrier, Dognin, Laroche, Gonzalez et al. (2018) evaluated the effect of TEs (Fe, Cu, Ni, Mn, and Co) on anaerobic digestion of BSG. The result proclaimed that control reactors showed instability after 3 months, with a decrease in performance and even collapse, while supplemented reactors had COD removal rate of 60%– 65% and a methane production varied between 282 ± 14–374 ± 17 NL CH_4/kg VS added. Both low and high concentration of TEs revealed positive effects on methane from all the tested samples with 65%–70% biodegradability of the substrate. The authors suggested a supplementation solution based on remaining trace element concentration in the soluble phase of the digestate. In addition, based on 50% bioavailability of TEs in BSG for Fe, Mn, and Na and on non-bioavailability for K, Mg, Ni, and Co, no copper addition was necessary. The authors also suggested that these guidelines and further analy- ses on bioavailability and the study of interaction among TEs are required to well estimate real need for anaerobic digestion of such wastes. Agriculture wastes such as wheat straw, corn straw, and rice straw are the main sources for potential biomass energy production in china (Qin et al., 2018; Zhao, 2018). But

<5% of the total crop straw produced in China is utilized for biogas production (Fu, Conrad et al., 2018; Fu, Luo et al., 2018; He, Zhang, & Zeng, 2018; Wang, Hawkins, et al., 2018; Wang, Wang, et al., 2018; Wang, Shen, et al., 2018). However, due to the high C/N of crop straw, anaerobic systems frequently become uneven because of the accumulation of VFA, which can be improved via TEs addition or co-digestion with livestock manure (Cai, Wang et al., 2018; Zhao, Mu et al., 2018; Zhao, Westerholm et al., 2018; Zhao, Ji et al., 2018). In this regard, (Zhao, Mu et al., (2018); Zhao, Westerholm et al. (2018); Zhao, Ji et al. (2018) and Cai, Wang et al. (2018) deliberated the effect of rare TEs on anaerobic digestion of rice straw. During the experiment, the methane yield increased by 59.3%, 47.1%, and 48.9% in the first 10 days following the addition Mo (0.01 mg/L), Se (0.1 mg/L), and Mn (1.0 mg/L), respectively, whereas the maximum biogas production for each TE was Mn (19.7 ml g^{-1}Vs day^{-1}), Mo (18.1 ml g^{-1}Vs day^{-1}), and Se (17.2 ml g^{-1}Vs day^{-1}). On the contrary, toxic effects and the accumulation of VFAs were observed when the Se, Mn, and Mo and concentra- tion raised from 100, 1,000, and 100 mg/L. This could possibly be due tothe disruption of protein structure and

function through binding of functional groups to protein molecules or replacement of the original metals in protein prosthetic groups (Chen, Steele, & Stuckey, 2018; Chen, Zhou et al., 2018; Tang et al., 2018). Moreover, high level of Mn, Se, and Mo might dis- rupt the function of extracellular polymeric substances (EPS), which has an important role in sheltering the digestive system against potential inhibition by high concentration of metals (You et al., 2018; Zhang, Jia et al., 2018; Zhang, Liu et al., 2018; Zhang, Zhang et al., 2018). The authors also concluded that addition of TEs altered the bacterial community structure,

CONCLUSION

Anaerobic digestion is considered an efficient, sustainable, and environmental friendly approach for complex biomass and waste utilization. Anaerobic digestion yield biogas which has been the focus of many countries as an alternate energy source. However, many factors affect the overall performance and stability of the anaerobic digestion. Among all, TEs play an important role in enhanced degradation efficiency, higher biogas production, and an improved process stabil- ity. Moreover, it also alters the bacterial community structure involved in anaerobic digestion. On the contrary, it may also drastically affect the biogas content of the digestor. Thus, fur- ther research on bioavailability and interaction among TEs is required to estimate the effect of TEs supplementation for biogas enhancement.

ACKNOWLEDGMENTS

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PROCESS FOR TREATMENT OF MYCELIA WASTE ;-

Mycelia waste sample collected & sent to external agency for feasibility study of anaerobic decomposition of mycelium waste because of as per literature found it may be chances of anaerobic decomposition .

Jan. 26th, 2024

Sample Description: Dried Mycelium Powder

Sample collected by: User

Sample preparation: 40% concentration of Mycelium

#	Parameter	UOM	Raw Effluent Value	After feasibility study of 5 days HRT
1	pH		9.6	7.78
2	Chemical oxygen demand.	mg/l	72930	26692
3	Biochemical oxygen demand	mg/l	43810	8760
4	Total suspended solids.	mg/l	46194	18120
5	Total dissolved solids	mg/l	290230	107448
6	Total solids.	mg/l	336424	28568
7	Oil and Grease	mg/l	BDL	BDL

#	Parameter	UOM	Value	%
1	Chemical oxygen demand destroyed.	mg/l	46238	63.40
2	Biochemical oxygen demand destroyed.	mg/l	35050	80.00
3	Biogas Coefficient, (biogas produced per kg of COD degraded)	m3/kg	0.35	

#	Parameter	UOM	%
1	Methane	%	49.7
2	Carbon Dioxide	%	47.2
3	Hydrogen Sulphide	%	3.1

#	Parameter	UOM	Value
1	Mycelium generation (60kl/batch in 48 hours)	Kl/d	30
2	TCOD of Mycelium per day @ 40% concentration)	Kg	2187
3	TCOD destroyed	kg	1387.14
4	Biogas Generation @ 0.35kg/kg of COD destroyed	m3/d	485.49

Conclusions

- The COD: BOD ratio is suitable for Anaerobic Digestion.
- The overall COD destruction is 63.40 %, on plant scale it shall be ± 5%
- The overall BOD destruction is 80.00 %, on plant scale it shall be ± 5%
- Biogas generation is 485 m3/d, Energy equivalent to 240 kg/d of HSD shall be generated based on this treatability of the sample.
- The overflow from Anaerobic digester shall provide continuous Microbial culture to Aerobic system.
- The qty. of sludge for disposal would be substantially reduced resulting into financial saving.
- A pilot plant CSTR anaerobic study for two months may be done at your place. Pilot plant on rental basis is available with us.
- The purity of Biogas shall improve with reduction in CO_2 and H_2S. This has been the hands-on experience of operating. CSTR based plants at various chemical sites.

As per feasibility report found 49%Metahne gas generation & 47.2% Carbon dioxide gas generation along with COD reduction found about 65% TDS reduction 50% . So Feasibility report given positive result . we have started pilot trail .

Installed 200 ltr SS tank with agitator , Feed pipe line deep , Circulation pump installed for continuous circulation . one pipe line from collection of Gas in HDPE drum .

Pilot trail started after setup of equipment .

- **First charged 50 Ltr Cow dung slurry & maintain PH 10.5 by addition of lime .**
- **Maintain four days agitation & maintain PH also**
- **Sixth day charged 50 Ltr Mycelia slurry & Maintain PH about 10 by using of lime slurry**
- **Continuous agitation PH maintaining done**
- **After 5 days found Gas generation started**
- **Addition of 10 ltr Mycelia waste alternative days .**
- **After 10 th day we found about 30 Kg gas accumulated in tank .**
- **We have taken sampling found methane is available**
- **After purification we can conduct burning test . in burning test found positive .**

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This certificate is digitally & electronically signed.

LAB SERVICE DIVISION PG 1 OF 1

ANALYSIS & BIODEGRADABILITY STUDY REPORT

CLIENT'S NAME & ADDRESS	REPORT NO	04/ENV/ANA/11-23
Mr.Vivekanand pandit	DATED	09/12/2023
	LAB REFERENCE NO	ENV/SW/12-23
	SAMPLE RECD. ON	24/11/23
	ANALYSIS CARRIED ON	24/11/23
	ANALYSIS COMPLETED ON	09/12/23

SAMPLE OF	SAMPLE COLLECTED BY/METHOD
SOLID WASTE	CLIENT / GRAB

RESULTS OF ANALYSIS (RAW)

SR.NO.	PARAMETER	UNIT	SOLID WASTE
1	pH (40 % SOLUTION)	--	.60
2	CHEMICAL OXYGEN DEMAND	Mg/l	72930
3	CHEMICAL OXYGEN DEMAND(Filtered)	Mg/l	N.A.
4	BIOCHEMICAL OXYGEN DEMAND (Filtered)	Mg/l	43810
5	TOTAL SUSPENDED SOILDS	Mg/l	46194
6	TOTAL DISSOLVED SOLIDS	Mg/l	290230
7	TOTAL SOLIDS	Mg/l	336424
8	OIL & GREASE	Mg/l	BDL

RESULTS OF ANALYSIS (AFTER ANAEROBIC TREATMENT)

SR.NO.	PARAMETER	UNIT	AFTER ANAEROBIC TREATMENT
1	pH	--	.78
2	CHEMICAL OXYGEN DEMAND	Mg/l	26692
3	BIOCHEMICAL OXYGEN DEMAND	Mg/l	8760
4	TOTAL SUSPENDED SOILDS	Mg/l	18120
5	TOTAL DISSOLVED SOLIDS	Mg/l	107448
6	TOTAL SOLIDS	Mg/l	28568
7	OIL & GREASE	Mg/l	00

ANAEROBIC TREATABILITY STUDY REPORT

SR.NO	PARAMETER	UNIT	RESULT
1	COD BASED BIODEGRADABILITY	%	3.4
2	BIOGAS COEFF.	M³/Kg COD removed	.35

BIOGAS ANALYSIS

SR.NO.	PARAMETER	UNIT	RESULT
1	METHANE	%	9.7
2	CARBONDIOXIDE	%	7.2
3	HYDROGEN SULPHIDE	%	3.1

- The samples analysed as per Manual by CPCB, IS – 3025 and APHA, 1981 "Standard Methods for Examination of Water and Wastewater".

FOR ENVIRO - SAFE
SATYAJIT ANAND BAJIKAR
(LAB INCHARGE)

FOR ENVIRO - SAFE
(ANALYST)

Note : This analysis report is extended as Technical Assistance only & ENVIRO – SAFE will not be involved in any legal dispute arising out of this report.
Above Results pertain only to the sample tested.

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Lead Auditor For ISO 9001, ISO 14001, ISO 45001, ISO 22000, ISO 50001, ISO 28001.
SHOP ACT NO. : 183100031003117O (old)/ 18310003126520G5 (New) w.e.f. 01/01/2019
UDYAM REGISTRATION NUMBER (MSME): UDYAM-MH-26-0052530
GSTN NO.: 27ABAPB1654N1ZR PAN NO.:ABAPB1654N
OFFICE & LABORATORY :Rudra Co.Op.H.S.G., 'A' Wing, Block No.2, Niranjan Park Lane, Manik Baug, Sinhagad Road, Pune – 411051
Tele – Fax : 020 24352539 Mobile : 9822301753 Email: enviro_consult@hotmail.com

After successful pilot trial we have design one large scale plant for capacity of 600 KLD . In 500 KLD plant 420 Kld Low COD effluent & 80 Kld High COD(Mycelia waste) effluent .Treatment scheme is mention below .

LIST OF EQUIPMENT UTILISE FOR TREAMENT OF LOW COD EFFLUENT & MYCELIA WASTE.

Sr. no.	ETP unit / Item	Input		Output		
		1	2	1	2	3
1	LCOD Equalization tank A	LCOD Effluent from Production		Feed to Static Mixer		
2	LCOD Equalization tank B					
3	LCOD Equalization tank C					
4	Static Mixer1	From LCOD Eq. Tank	Chemicals from Central Dosing system	to Pipe Flocullator		
5	Pipe Flocculator	From Static Mixer		Lamella Clarifier / DAF		
6	Lamella Clarifier / DAF	From Pipe Flocullator		to CSTR 1 Feed	Sludge to Sludge thickhner	
7	Anaerobic Digester I	From Feed tank & Settling tank of CSTR2	Recycled Biomass	to Degaser Pond	Gas to Gasholder	
8	Degaser Pond	From CSTR I		to Settling tank	Biomass Recycling CSTR1	
9	Settling tank	From Degaser Pond		to Aeration tank 1A/B/ 2A/2B	Biomass to CSTR1	Sludge wasting to Sludge thickhner
10	Gas Holder	From CSTR I / 2		to Flare	Boiler	
11	Aeration tank 1A/B	From Settling tank		to SC1		
12	Secondary clarifier1	From Aeration tank 1A/B		Sludge recycling to AT1	Sludge to CSTR1	Sludge wasting to Sludge thickhner
13	Aeration tank 2A/B	From Settling tank		to SC2		
14	Secondary clarifier2	From Aeration tank 1A/B		Sludge recycling to AT1	Sludge to CSTR1	Sludge wasting to Sludge thickhner
15	RC Feed sump	From SC1/2	Chemicals from Central D	Reactivator clarifier		
16	Reactivator clarifier	From Feed Sump		to Glass Media Filter via feed tank		
17	RO Feed tank	From Glass Media		to RO1		
18	RO1 Plant	Feed from RO feed tank		Permeate to Permeate tank	Rejects as RO 2 feed	
19	RO1 Permeate tank	RO1		for reuse		
20	RO1 rejects/RO2 Feed tank	to RO2				
21	RO2 plant	from RO1 Rejects tank		Permeate to Permeate tank	Rejects to Evaporatin system	
22	RO2 Permeate tank	From RO2 plant		for reuse		
23	RO2Rejects tank	From RO2 plant		Rejects to evaporation system		
24	HCOD Equalization tank D	HCOD effluent from plant		To CSTR		
25	HCOD Equalization tank E					
26	Anaerobic Digester 2	from HCOD Eq. tanks		Degaser Pond		
27	Degaser Pond			to Settling tank	Biomass Recycling CSTR2	
28	Settling tank	from Degaser pond		to CSTR1	Biomass Recycling CSTR2	Sludge wasting to Sludge thickhner
29	Sludge Thickhner	from LC/Settling tanks/ Sec. clarifiers/ Rectivator clarifier/ Back wash from glass media filter		to MDSP	Supernant to Eq. tank	
30	MDSP	From Sludge thickhner				
31	Central Dosing system			to Lemella calrifier	to Recativator clarifier	To MDSP

Description & Working of Equipment :-

Equalisation tank :- An equalization tank is a reservoir used in wastewater treatment plants to buffer and stabilize the flow and composition of incoming wastewater, acting as a buffer to ensure a steady flow to downstream processes. It collects raw sewage during peak hours, then releases it at a constant, average rate, which helps prevent damage to equipment and improves treatment efficiency. These tanks are also designed to mix the incoming wastewater, creating a more uniform mixture and reducing shock loadings.

Functions of an equalization tank:

- **Flow regulation: It absorbs fluctuations in flow rates, releasing wastewater at a steady rate to prevent the downstream processes from being overwhelmed.**
- **Pollutant load stabilization: It smooths out variations in pollutant concentrations, ensuring a more consistent and effective treatment process**
- **Homogenization: Mixing is used to create a homogeneous mixture of the wastewater, which is crucial for efficient biological and chemical treatment processes**
- **Shock loading prevention: It prevents "shock loadings," which can occur when large volumes of wastewater with high concentrations of pollutants enter the treatment system at once**
- **Emergency storage: It provides an emergency storage capacity to hold excess inflow**
- **pH stabilization: Mixing can help in the process of stabilizing the pH levels, which is important for downstream biological processes.**
- **Solid suspension: Continuous mixing prevents solids from settling at the bottom of the tank**

Static Mixter :- Static mixers are motionless devices that continuously blend fluid streams into a homogenous mixture without moving parts, using stationary internal elements to force the fluid into complex flow patterns that promote mixing. Fluid movement is provided by external forces, such as a pump. Static mixers are ideal for non-viscous liquids and gases but are not suitable for mixing solids or highly viscous fluids, as these can cause build-up and blockages

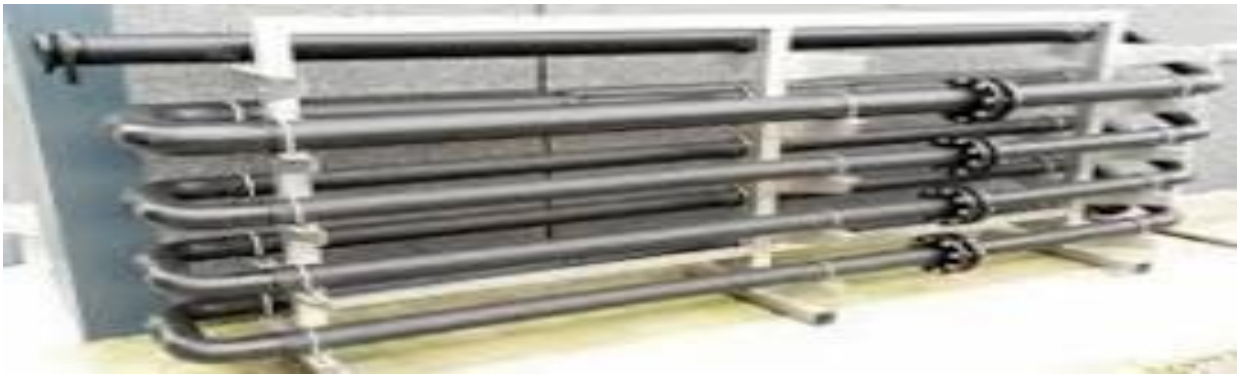
Functions of Static Mixter :-

Flow and Elements: The fluid is pumped through a tube or channel containing stationary mixing elements.

Flow Division and Redistribution: These elements divide the fluid streams and redirect them into a series of complex paths

Mixing Action: As the fluid passes through these elements, it moves in alternating clockwise and counterclockwise motions, colliding with each other and the mixer walls. This action ensures thorough mixing and dispersion, creating a homogeneous product from separate streams.

PIPE FLOCULATOR :- A pipe flocculator is a type of wastewater treatment equipment that uses a series of pipes to mix chemicals with wastewater to promote the formation of larger particles called "flocs". It is a plug flow reactor where a chemical coagulant is added to the water, and then a flocculant is dosed and mixed in to create a turbulent flow. This process groups fine particles together into larger, heavier flocs that can then be more easily separated from the water through sedimentation or filtration



- **Coagulation: A chemical coagulant, such as alum or ferric chloride, is added to the wastewater to neutralize the negative charges on suspended particles.**
- **Mixing: The water and coagulant flow through a series of pipes that have changes in diameter, bends, and other features to create turbulence. This turbulent mixing ensures the coagulant is evenly distributed throughout the water.**
- **Flocculation: A flocculant is then added and mixed with the water. The high turbulence from the pipe design causes the particles to collide and stick together, forming larger, heavier aggregates called flocs**
- **Retention: The pipe sections provide the necessary retention time for the flocs to grow to their full size**
- **Separation: The water with the now-larger flocs is then sent to a separator, such as a clarifier or a lamella separator, where the flocs can settle out or be filtered**

LAMELA CLARIFIER:- A lamella clarifier is a compact water treatment device that uses a series of inclined plates to remove suspended solids from liquids. Dirty water flows upward between the plates, where solid particles settle down onto the plate surfaces due to gravity, accumulating as sludge at the bottom. The clarified water, now with a lower solid content, moves up and is collected at the top for discharge.

Function of Equipment :-

- **Inlet:** Pretreated liquid containing suspended particles enters the clarifier, typically through a distribution duct.
- **Flow:** The liquid is directed into the open spaces between the angled plates
- **Sedimentation:** As the liquid flows upward, suspended particles settle downwards onto the inclined plates, where they lose momentum
- **Sludge collection:** The settled particles accumulate on the plates and eventually form a sludge layer at the bottom, which is then removed
- **Outlet:** The clarified water, with most of the solids removed, flows to the top of the clarifier for collection

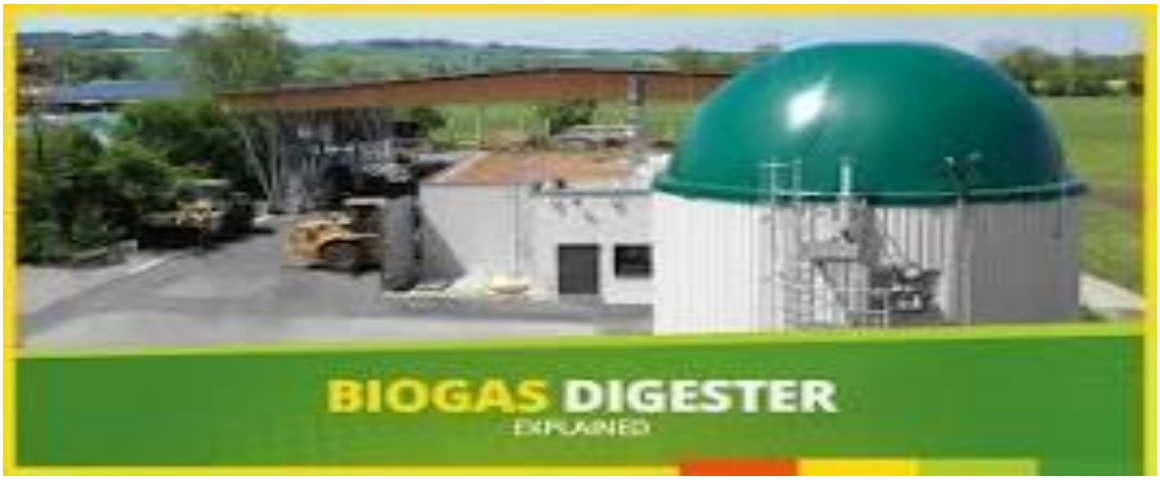
Lamela Clarifier:- A lamella clarifier in an ETP uses a series of inclined plates to separate suspended solids from wastewater, providing a much larger settling area than a conventional clarifier in a smaller footprint. It's ideal for industries needing a compact, efficient solution for clarifying water with suspended and colloidal particles, and it's often used in primary and secondary wastewater treatment stages.

Function of Lamella Clarifier:-

- **Inlet:** The liquid containing suspended particles enters the clarifier and is distributed into the spaces between the inclined plates.
- **Settling:** As the liquid flows upward through the angled plates, solids settle downward and accumulate on the plate surfaces. The inclined plates significantly increase the settling surface area within a small footprint, which enhances the settling process compared to a traditional tank.
- **Sludge removal:** The settled solids slide down the plates into a sludge collection zone at the bottom, where they are then removed.
- **Clean water outlet:** The clarified liquid flows over the top of the plates and is discharged as clean water.

ANEROBIC DIGESTOR: - *Anaerobic digestion is a biological process used in wastewater treatment to break down organic matter in the absence of oxygen. This process occurs in a sealed vessel called a bioreactor, where microorganisms convert waste into two main products: biogas (a mixture of methane and carbon dioxide) and nutrient-rich biosolids (digestate). The biogas can be captured and used as a renewable energy source, while the remaining solids are treated and be used for fertilizer.*

Function of Anaerobic Digester: - The breakdown of organic material happens in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.



- **Hydrolysis:** Complex organic polymers are broken down into simpler molecules like sugars and amino acids.
- **Acidogenesis:** Microorganisms convert the simple molecules into volatile organic acids, such as acetic acid.
- **Acetogenesis:** Other microbes further convert these acids into acetate
- **Methanogenesis:** Methane-producing archaea convert acetate into methane (CH4) and carbon dioxide (Co2)

Input materials: Mycelia waste use as a input material in anaerobic Bio digester along with lime & Cow dung.

Out Put material:-

- **Biogas:** The primary output is a renewable energy source that can power the treatment plant or be sold.
- **Biosolids/Digestate:** The leftover liquid and solid material is nutrient-rich and can be used as fertilizer in agriculture.

Degasser Pond: - Degassing' is the removal odorous, or even noxious vapor from a tank, vessel, or pipeline under pressure/vacuum and controlling that vapor through chemical reactions, typically Oxidation. Oxidation uses high temperatures to convert VOC's to water vapor and carbon dioxide.

Settling Tank: - A settling tank, or clarifier, in an ETP (Effluent Treatment Plant) uses gravity to separate suspended solids from wastewater, with solids settling to the bottom as sludge and clarified water flowing out. These tanks are crucial for both primary and secondary treatment stages, working by reducing turbulence to allow particles to settle, which a key step in purification is.

Function of Settling Tank :-

Influent: Wastewater enters the tank, often after pre-treatment steps like screening and grit removal

Settling zone: The flow slows down significantly within the tank, creating a calm environment. Gravity pulls suspended solids downward, where they accumulate as sludge.

Removal: The clarified liquid, which now has fewer suspended particles, is discharged from the top, while the sludge at the bottom is periodically removed.

Aeration Tank:- An aeration tank is a large basin in wastewater treatment plants that introduces air to wastewater to support aerobic microorganisms. These microorganisms break down organic pollutants, significantly reducing the biological oxygen demand (BOD) of the water. Aeration tanks are a crucial part of the activated sludge process, essential for making wastewater clean enough to meet environmental standards.

Function of Aeration Tank:-

- **Pollutant breakdown: Aeration tanks provide oxygen for aerobic microorganisms to consume organic matter, converting it into carbon dioxide, water, and new cells.**
- **Biological Oxygen Demand (BOD) reduction:** By breaking down organic pollutants, the tanks effectively reduce the BOD, a measure of water pollution
- **Activated sludge process:** The process encourages the growth of microorganisms that clump together to form "flocs," which can then be settled out of the water.
- **Air distribution:** They are designed to distribute oxygen uniformly throughout the wastewater, which is vital for efficient microbial activity.
- **Odor control:** Aeration helps control odors that can arise during the treatment process.
- **Sludge stabilization:** Aeration tanks are a key component for stabilizing sludge and ensuring it is ready for subsequent treatment or disposal.

Secondary Clarifier:- A secondary clarifier in an Effluent Treatment Plant (ETP) is a sedimentation tank that follows the biological treatment stage (like the activated sludge process) to separate microbial biomass from the treated wastewater. Its main purpose is to allow settled solids to sink to the bottom, producing clear effluent that can move on to further treatment or discharge, and returning the settled sludge (activated sludge) to the biological process.

Function of Secondary Clarifier :-

Process: Wastewater flows into the tank, where the flow is slowed to a calm state. Gravity causes the biological flocs (microorganisms that consume organic waste) and other suspended solids to settle to the bottom of the tank

- **Output:** The clear, treated water is drawn from the top of the tank.
- **Sludge return:** The settled solids, called activated sludge, are collected and returned to the aeration basin to help maintain the biological treatment process.
- **Sludge removal**: Excess or waste sludge is removed from the tank to prevent the system from becoming overloaded.

Gas Holder for Methane Gas (Bio Gas) :- Gas holders for storing biogas are available in several types, with fixed dome, floating drum, and flexible membrane being the most common. Fixed dome holders are rigid, built-in structures, while floating drum holders are movable and often made of steel, and flexible membrane holders use two membranes—one for gas and another for air to maintain pressure

Type of Biogas Holder :-

Fixed Dome: These are rigid holders, typically built with bricks, concrete, or other permanent materials. The gas collects in the upper part of the dome and pushes out digested slurry to make room.

Floating Drum: These have a gas-tight drum, often made of steel, that floats on the surface of the liquid in the digester or on a water-filled trench. The drum rises as it fills with gas and falls as the gas is used, maintaining an optimal pressure.

Volute Press: - A volute press is a type of sludge dewatering machine used in wastewater treatment that separates liquid from solid sludge. It works by using a screw that rotates within a cylinder made of fixed and moving rings, creating pressure to squeeze out liquid as the sludge moves towards the outlet. This process thickens the sludge and significantly reduces its volume and water content.

Function of Volute Press:-

- Thickening: After a polymer flocculant is added, the sludge is pre-thickened in a separate flocculation tank or within the initial "thickening zone" of the press
- Dewatering: The screw then pushes the sludge through a cylindrical filter made of alternating fixed and moving rings
- Pressure: The gap between the rings gets progressively smaller, and the screw pitch changes, increasing pressure on the sludge.
- Separation: This pressure forces the water (filtrate) out through the small gaps, while the thickened, dewatered sludge cake is discharged at the end

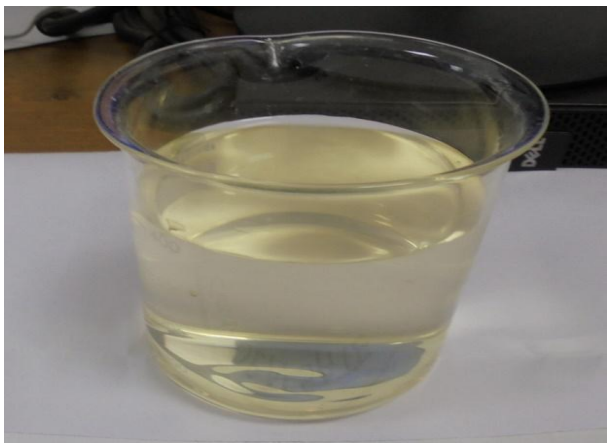
Paddle Dryer: - A paddle dryer works by using a rotating shaft with heated paddles to continuously mix, agitate, and heat a wet material, which causes moisture to evaporate. Heat is transferred indirectly from the heated paddles and dryer walls, while an exhaust system removes the resulting vapor to produce a dry product

Operating Method:-

- **Feeding:** Wet material is fed into the dryer.
- **Heating and agitation:** A heating medium, such as steam or thermal fluid, is circulated through the hollow shafts and paddles, transferring heat to the material indirectly.
- **Mixing:** The rotating shaft turns the paddles, which constantly mix and stir the material, ensuring it comes into contact with the heated surfaces. This process continuously renews the material's surface, promoting evaporation
- **Moisture removal:** As the material is heated, moisture evaporates from it. The evaporated moisture is then removed from the dryer. In a vacuum dryer, this process happens more quickly under low pressure
- **Discharge:** The dried product is conveyed to the discharge port. In some designs, the direction of the agitator can be reversed to help discharge the final product.

Images :-

FEED



TREATED WATER

DRY POWDER USE AS A FERTILIZER FOR AGGRICULTURE