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EFFECT OF MIXING REGIMES AND IMPELLER GEOMETRY ON BIOGAS YIELD IN AN ANAEROBIC DIGESTER

PHD THESIS

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Abstract

Agitation is the most prominent factor, undeviating determines the performance of an anaerobic digester operated at higher solid content. The homogeneity of substrate for solid liquid phase and the uniformity of microbial community hinge on adequate mixing of slurry in an anaerobic digester. In spite of having clash of views on mixing intensity and sort of mixer usage multifaceted studies have been directed rendering worthful results. An absolute analysis was brought on the outcome of fusing the biogas production in an anaerobic digester by targeting the impeller geometry and mixing modes. This study demonstrates the effect of various mixing intensities, mixing intervals and different impeller geometry on the biogas yield in an anaerobic digester. Intermittent mixing (mixing in intervals) was applied as it is strongly recommended terms of quality and quantity of biogas along with lower power consumption for stirring as compared to continuous mixing.

For the first time, mixing performance of helical ribbon impeller under different mixing speeds and mixing intervals is compared in lab-scale digesters to analyse the effect on biogas yield. Computational fluid dynamics models of the digester were then developed to identify the turbulence characteristics present. Three lab-scale digesters were run for a period of four months under identical operating conditions at different mixing speeds and key indicators of digester stability were recorded alongside gas production. For more precise results all the three digesters were operated with identical parameters at the same time. Samples were taken twice a week in order to analyse the analytical measurements such as total solids content, volatile solids, volatile fatty acids, pH, ammonia concentration, FAS/TOC values. It has been shown that increased mixing speed leads to higher levels of turbulence. Experimental work has shown that in these digesters, increasing the mixing speed to a particular limit increase the stability of the methane generation process and accordingly has a detrimental effect on the gas production. Similarly, the abundance of methanogenic communities, dominated by the acetoclastic Methanosaeta, was adversely affected by increased VFA concentrations brought about by increasing mixing speeds. However, the digesters at low mixing speeds resulted in formation of dead zones which resulted in lower biogas yield than the digesters mixed at a higher speed, due to the formation of pockets of different environments in the digester which leads to uncontrolled digestion. As such, in the case of these digesters, minimal mixing represents the ideal scenario. Significant differences in biogas yields were observed at different mixing regimes. Further reducing in mixing interval time also resulted in higher biogas production as compared to longer break time between mixing operation.

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III. Nomenclature

AD	Anaerobic digestion
GHG	Greenhouse gases
BPR	Biogas production rate
VFA	Volatile fatty acids
COD	Chemical oxygen demand
DHR	Double helical ribbon impeller
SM	Swine manure
WWS	Wastewater sludge
COD	Chemical oxygen demand
SCOD	Soluble chemical oxygen demand
CFD	Computational fluid dynamics
VM	Vigorously mixed
CSTR	Continuously stirred tank reactor
SR	Slurry recirculation
NM	Natural mixing
MHVM	Minimal horizontal and vertical mixing
CARPT	Computer Automated Radioactive Particle Tracing
PIV	Particle image velocity

$(SMP)_V$	Specific mixing power input with respect to volume of slurry [W/m^3]	*s*
t	Time	*s*
T_{HRT}	Hydraulic return time	
V	Total system volume	l^3
v	Inlet flow rate	l^3/T
μ	Apparent viscosity	*Pas*
A	Frequency factor	*Pas*
E_a	Activation energy	*J/mol*
G_r	The specific biogas recirculation rate	$m^3/d\ m$
P_2	The head space pressure equal to 101325 Pa (atmospheric)	
P_2	The pressure at injection point	*Pa*
λ	The polytrophic exponent	
H	The head of slurry	*m*
g	Acceleration due to gravity	m/s^2
R	Universal gas constant	*J/mol/K*
T_{abs}	Absolute temperature	*K*
ORP	Oxidation reduction potential	
TS	Total solid	*%*
γ	Shear rate	γ_a'
n	Flow index	
k	Consistency index	Pas^n
ω	Rotational velocity	$rads^{-1}$
r_i	External radius of circle made by rotating stirrer tip	*mm*
r_d	Internal radius of digester	
τ_o	Yield stress	τ
rpm	Revolutions per minute	
d	day	
TS/l/d	Total solid content per litre per day	
C_2	Inter impeller distance	*mm*
C_b	Impeller distance from bottom	*mm*
C_t	Inter impeller distance	*mm*
P_{shaft}	Electric power consumption	*W*
CH_4	Methane	
$E_{speci.}$	Specific stirring electric energy consumption	kWh_{el}
$E_{speci.V}$	$E_{speci.}$ per 100 m^3 active digester volume	$kWh_{el}/100m^3{}_{active\ digester.d}$
$E_{stirrer,d}$	Daily total stirring electric energy consumption	kWh_{el}/d
$V_{Active\ digester}$	Active digester volume	m^3
$E_{spec.FM}$	$E_{speci.}$ per tonne added feedstock	kWh_{el}/t_{FM}
$E_{stirrer,d}$	Daily total stirring electric energy consumption	kWh_{el}/d
$\dot{m}_{FM,d}$	Daily added feedstock	
μ	Apparent viscosity	Pas
A	Frequency factor	Pas
T_{abs}	Absolute temperature	*K*
r^d	Internal radius of digester	*mm*
γ	Shear rate	s^{-1}

r^i	External radius of circle made by rotating stirrer tip	*Mm*
K	Consistency index	Pas^n
G	Average velocity gradient	s^{-1}
T_m	Mixing time	*s*
s_{ij}	The stress tensor due to molecular viscosity	Pa
t_{ij}	The stress tensor	Pa
M_{TS}	Weight of TS in slurry	kg
K_T	Constant for stirrer with two blades	

1 Introduction

1.1 Background and motivation

Drastically increasing world population and changing lifestyle of people is resulting in intensification of energy demand around the globe. In the present era, the fossil fuels and the conventional energy resources have major share in power production which is leading to the menacing issue of global warming and climate change. In 2012 the world energy consumption was estimated as 557 EJ/yr with only 10% from the biofuels and waste [1]. Due to the alarming consequences of use of fossils, mankind is forced to opt renewable energy resources and reduce carbon emissions[2]. The target is underpinned by the European Energy Policy for reduction of GHG emissions that is aimed at using less, cleaner, and locally produced energy, including energy recovery from waste. Global warming and increasing energy demand are serious matters of concern in present world. Increasing greenhouse gases emissions from the conventional energy resources is leading to drastic change in climate change due to global warming. In the present era there is absolute demand to focus on the renewable energy resources and pushing mankind to explore new renewable and non-conventional energy resources. Biogas power production is one of high demanding sources of renewable energy in this modern world. Bioenergy is recognized as a serious renewable energy alternative to fossil fuels. In turn, AD provides a very effective method of turning waste products into useful energy whilst reducing the potential for GHG emissions to atmosphere.

Biomass/organic matter is a source of greenhouse gases like methane, carbon dioxide, hydrogen sulphide and other gases when undergoes anaerobic digestion[3]. Biogas production utilizes organic waste from renewable resources and can be used in both small and large energy generation plants and in decentralized energy generation. Therefore, if sustainably managed, biogas could make significant contribution to energy security and mitigation of the GHG emissions. The biogas is mainly used for electricity and heat generation and as substitute for natural gas after upgrading and purification to biomethane.

AD is a microbial process which is carried in absence of oxygen resulting in biogas. A biogas plant can convert animal manure, green plants, waste from Argo industry and slaughterhouses into combustible gas. Biogas produced through an anaerobic digestion process consist of 50-70 % methane, 25-40% carbon dioxide and 2-8% of water vapours and traces of O_2, N_2, NH_3, H_2S. Biogas can be used for various heating and power generation purposes. The digestate is a mixture of solid and liquid which can be directly used as an organic fertilizer in the agricultural land to retain the nutrients and fertility of soil. Sometimes biogas is upgraded to enhance the calorific value of gas. Assuming biogas with 60% methane, the energy content will be 6 kWh/Nm^3 whereas when it is upgraded to 97% methane energy content will be 9.67 kWh/Nm^3[4].

1.1.1 Reduction of GNG by biogas production

1.1.2 Current status

Biogas sector is well developed in Europe and is ready to scale-up. According to data from the European Biogas Association, the combined production of biogas and its upgraded from, biomethane, could cover today 4.6% of the whole EU gas demand. By 2050, about 30-40 % of EU gas needs can be met by biogas/biomethane. As one of the global leaders in biogas production, European producers are now seeking new ways for cooperation with third countries to support the deployment of biogas outside EU borders[5].

Biogas production has increased in the EU, encouraged by the renewable energy policies, in addition to economic, environmental and climate benefits, to reach 18 billion m^3 methane (654 PJ) in 2015, representing half of the global biogas production. The EU is the world leader in biogas electricity production, with more than 10 GW installed and a number of 17,400 biogas plants, in comparison to the global biogas capacity of 15 GW in 2015. In the EU, biogas delivered 127 TJ of heat and 61 TWh of electricity in 2015; about 50% of total biogas consumption in Europe was destined to heat generation. Europe is the world's leading producer of biomethane for the use as a vehicle fuel or for injection into the natural gas grid, with 459 plants in 2015 producing 1.2 billion m^3 and 340 plants feeding into the gas grid, with a capacity of 1.5 million m^3. About 697 biomethane filling stations ensured the use 160 million $m^3$of biomethane as a transport fuel in 2015 [6].

1.2 Research relevance

Efficiency of AD depends on many key factors like substrate type, C/N ratio, HRT, pH value, temperature, OLR, mixing and hydrodynamic factors of anaerobic digester. The hydrodynamics is a paramount element that contributes in the evolution, mass transfer, structure and metabolism of microbial community in an anaerobic digestion process [7]. Agitation of an anaerobic slurry is vital to accomplish, primarily, the supply of substrate to be distributed uniformly, secondly, to keep continuous contact between the microorganisms and sludge, tertiary, the concentration of end product and prohibited biological intermediates have to be maintained at minimum levels [8]. The optimum mixing can boost the homogeneous distribution of nutrients and micro-organisms and can evade formation of surface crust and sedimentation [9]. Gerardi [10] acknowledge that the adjacent association between acetogens and methanogens can lead to effective methanogenesis which can be achieved by smooth and an adequate mixing.

Nearly 44% of the biogas plant failures are due to flaws in mixing [11]. The detrimental impacts of inadequate mixing are observed as abortive methane yield, defective stabilization of raw slurry, loss of digester volume and an increase in operational expenses [12]. It can also lead to sedimentation at bottom of digester, scum formation, short circuiting, uneven distribution of temperature and substrate and dead zones. Most of the studies acknowledges that the excessive mixing in an anaerobic digester can result in deteriorating methane production and unnecessary utilization of power [13][14]. The adverse repercussion of excessive mixing has been recognized by actuality that the high shear forces disrupt the microbial flocs and syntrophic relationships between methanogens and bacteria[13].

The exploration on mixing in an anaerobic digester in literature rests on mixing approaches such as mixing speed, mixing intervals but assessment of mixer type and mixer geometry is scanty. This research focuses on reviewing the performance characteristics of various impellers used for an agitation in an anaerobic digester. Informative viewpoint discloses the outcomes from the evaluation of diversified impellers in terms of hydrodynamics, mixing, power input and mass transfer properties within an anaerobic digester. Meanwhile the effect of mixing speed, mixing intervals on rheology of slurry is discussed in detail. An analytical view pivots around the development of mixer which can allocate an adequate mixing within the prescribed limits of mixing intensity and mixing time to enhance the biogas production in an anaerobic digester because mixing is not only concerned about homogeneity but also about the sustainable environment for bacteria during different phases of process. This work allows future experimental work to identify appropriate mixing regimes and better understanding of link between mixing and biogas production.

1.3 Objectives of the research

Effective mixing relies on the appropriate level of shear rate being applied to the substrate for the time necessary to achieve a required level of homogeneity throughout the digester.

- To perceive the optimum mixing intensity and mixing time for slurry in anaerobic digester to biogas production.
- Optimize the impeller geometry for mixing in an anaerobic digester.
- To understand the practicability of results of mixing in lab scale digester by using different impellers and mixing regimes to scaleup pilot scale biogas plant.
- To analyse the effect of impeller geometry on the entire active volume of digester.
- To optimize the mixing in large scale anaerobic digesters to decrease the energy consumption for agitation and enhance overall efficiency.

1.4 Layout of thesis

Following on the introduction this section gives an overview of the contents of the thesis. Chapter 1 is the brief introduction and objectives of research; it also includes the deep insight of literature available and literature findings on mixing in an anaerobic digester. Chapter 2 includes description of anaerobic digestion process and factors effecting the outcomes of the AD process. This chapter also includes the importance of appropriate mixing and types of mixing operation used in anaerobic digester at both lab-scale and commercial scale. Chapter 3 gives detailed description about mixing in an anaerobic digester. Chapter 4 demonstrates the methodology adopted for both experimental and numerical simulations to evaluate the effect of mixing in lab scale digester. Detailed description of experimental equipment and substrate properties is demonstrated. Chapter 5 includes experimental setup and procedures. Chapter 6 included results and discussion. Chapter 7 demonstrates methods adopted for evaluation of mixing in an anaerobic digester. Chapter 8 includes conclusion and new research findings.

2 Anaerobic digestion process

2.1 The process

Anaerobic digestion is a biological process which converts the organic biomass to the mixture of combustible gases in absence of oxygen (Fig. 1). Raw material for biogas production can be biodegradable municipal solid, sewage sludge, animal waste, crop/animal feed residues or purposely grown crops such as maize with the energy value of each feedstock being different. A biogas plant can convert any kind of organic waste into biogas. Biogas produced from the waste consists of 50-70 % methane, 25-40% carbon dioxide and 2-8% of water vapours and traces of O_2, N_2, NH_3, H_2S. Biogas can be used for various heating and power generation purposes. As Biogas is composition of various gases so, it cannot be directly used for internal combustion engine unless it is upgraded. For this purpose, it needs to be upgraded which refers to removal of carbon dioxide and other impurities.

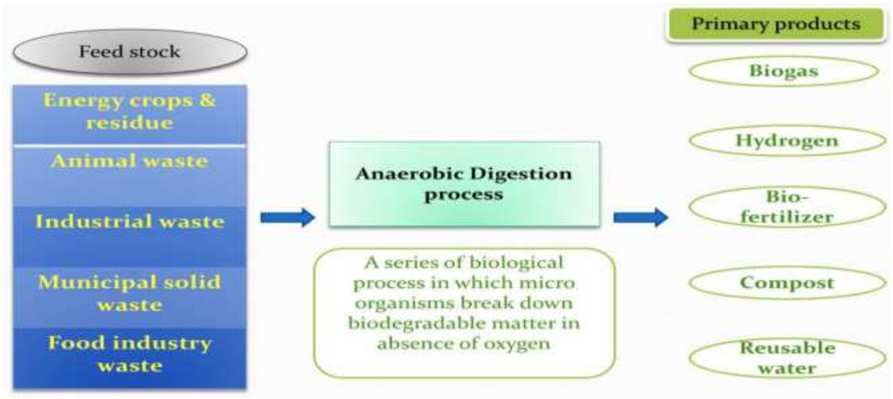


Figure 1. Biogas production process by anaerobic digestion.

The volumetric biogas potential of an biogas plant is a major economic metric as biogas generation determines the ratio of production of saleable energy and the capital invested in the volumetric capacity of the plant[15]. Therefore, AD system design should ideally support high biogas yields whilst maximizing OLR in the shortest HRT[16]. Overall efficiency of a biogas plant can be calculated in terms of biogas yields and the power consumption by plant itself. Biogas yield depends on various factors like substrate type, temperature, pH, HRT, OLR and mixing whereas the energy consumption by biogas plant is due to feeding systems, mixing of slurry and transportation of substrate within the plant. So, the design of biogas plant must be optimized to decrease the capital costs of installation as well as associated mixing energy and costs.

Before studying effect of mixing on biogas production it is very important to understand the anaerobic digestion process. The AD of organic material basically involves following steps; hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Fig. 2. The biological aspects of AD are dealt with in specialised literature[17]. AD is a complex process which requires strict anaerobic conditions ORP< 200 mV to proceed and depends on the coordinated activity of a complex microbial association to transform organic material into mostly CO_2 and methane (CH_4). Despite the successive steps, hydrolysis is generally considered as rate limiting[18]. The hydrolysis step degrades both insoluble organic material and high molecular weight compounds such as lipids, polysaccharides, proteins and nucleic acids, into soluble organic substances (e.g. amino acids and fatty acids). The components formed during hydrolysis are further split during acidogenesis, the second step. VFA are produced by acidogenic (or fermentative) bacteria along with ammonia (NH_3), CO_2, H_2S and other by-products. The third stage in AD is acetogenesis, where the higher organic acids and alcohols produced by acidogenesis are further digested by acetogens to produce mainly acetic acid as well as CO_2 and H_2. This conversion is controlled to a large extent by the partial pressure of H_2 in the mixture.

Hydrolysis

Hydrolysis is the initial step of decomposition. during which the complex organic matter (polymers) is decomposed into smaller units (mono- and oligomers). During hydrolysis, polymers like carbohydrates, lipids, nucleic acids and proteins are converted into glucose, glycerol, purines and pyridines. Hydrolytic microorganisms excrete hydrolytic enzymes, converting biopolymers into simpler and soluble compounds as it is shown below:

$$\begin{gathered}\text{Lipids} \longrightarrow \text{Fatty acids, glycerol}\\ \text{Polysaccharide} \longrightarrow \text{Monosaccharide}\\ \text{Protiens} \rightarrow \text{Amino acids}\end{gathered}$$

Acidogenesis

During acidogenesis, the products of hydrolysis are converted by acidogenic (fermentative) bacteria into methanogenic substrates. Simple sugars, amino acids and fatty acids are degraded into acetate, carbon dioxide and hydrogen (70%) as well as into volatile fatty acids (VFA) and alcohols (30%).

$$\text{Amino acids, fatty acids, sugars} \rightarrow \text{Long chain fatty acids}$$

Acetogenesis

Products from acidogenesis, which cannot be directly converted to methane by methanogenic bacteria, are converted into methanogenic substrates during acetogenesis. VFA and alcohols are oxidised into methanogenic substrates like acetate, hydrogen and carbon dioxide. VFA, with carbon chains longer than two units and alcohols, with carbon chains longer than one unit, are oxidized into acetate and hydrogen. The production of hydrogen increases the hydrogen partial pressure. This can be regarded as a „waste product “of acetogenesis and inhibits the metabolism of the acetogenic bacteria. During methanogenesis, hydrogen is converted into methane. Acetogenesis and methanogenesis usually run parallel, as symbiosis of two groups of organisms.

Methanogenesis

The final stage of methanogenesis produces methane by two groups of methanogenic bacteria: the first group splits acetate into methane and carbon dioxide and the second group uses hydrogen as electron donor and carbon dioxide as acceptor to produce methane.

Methanogenesis is a critical step in the entire anaerobic digestion process, as it is the slowest biochemical reaction of the process. Methanogenesis is severely influenced by operation conditions. Composition of feedstock, feeding rate, temperature, and pH are examples of factors influencing the methanogenesis process. Digester overloading, temperature changes or large entry of oxygen can result in termination of methane production.

$$\begin{gathered}Acetic\ acid \rightarrow Methane + Carbondioxide\\ Hydrogen + Carbondioxide \longrightarrow Methane + Water\end{gathered}$$

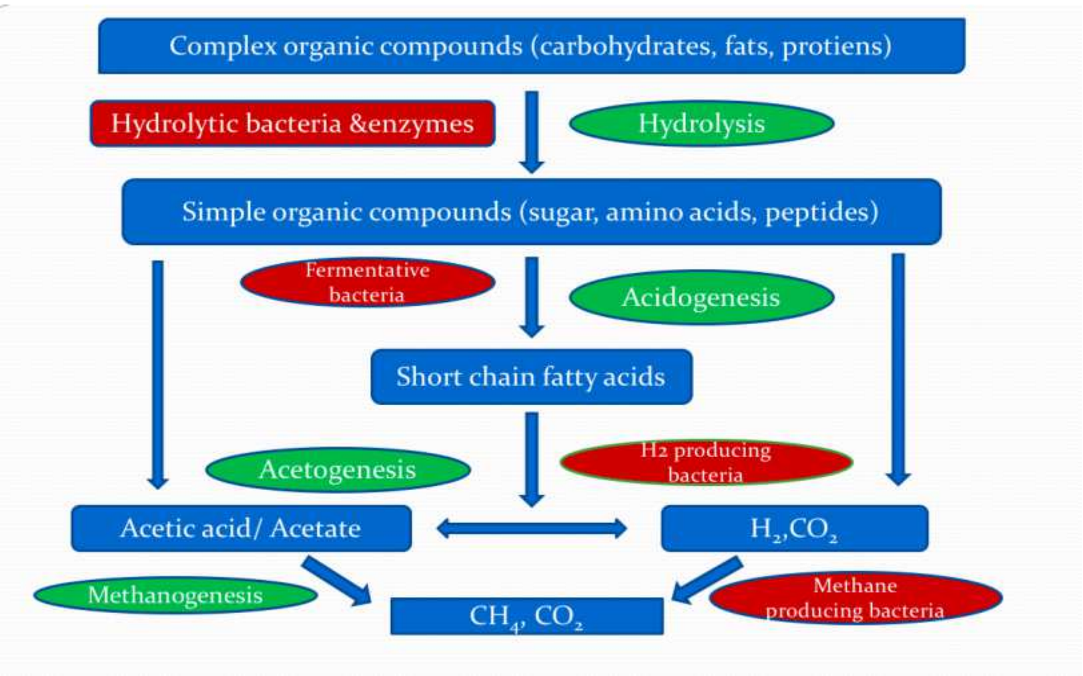


Figure 2. Subsequent steps in anaerobic digestion process.

The products

- Biogas
- Bio-fertilizer
- Wastewater
- Hydrogen

2.2 Factors affecting efficiency of anaerobic digestion

2.2.1 Temperature

Anaerobic digestion can take place in three temperature ranges: psychrophilic (below 20 °C), mesophilic (30-40 °C) and thermophilic (50-60 °C). At psychrophilic temperatures, the rate of digestion is slow. As such, the majority of sewage treatment plants operate in the mesophilic range. Whilst thermophilic digesters have higher organic loading rates and a higher pathogen destruction rate, the mesophilic range is preferable due to the reduced operational costs of heating, and the lower sensitivity of micro-organisms to toxic substances at lower temperatures[10]. The structure of the microbial communities that are active in each of the temperature ranges are different, and a change between mesophilic and thermophilic temperatures can cause a severe reduction in the biogas produced by the digester until the populations in the digester have stabilised and grown. Even changes in temperature of 2 °C have been shown to reduce the rate of biogas production. Temperature changes have different effects on different stages of the digestion process because of the communities of micro-organisms that are involved. The first stages of the digestion process (hydrolysis and acidogenesis) suffer very few ill effects from changes in temperature due to the mixed population involved in the process. This helps to ensure that at any temperature, there are some micro-organisms that are operating within their preferred temperature range. The later stages of the digestion process (acetogenesis and methanogenesis) require more specialised microorganisms and thus are more likely to be adversely affected by temperature changes[17,19]. As such, methane production is strongly temperature dependant. Moreover, fluctuations in temperature have a greater effect on the activity of methanogens than operating temperature itself. As the micro-organisms involved in anaerobic digestion all have different optimum operating temperatures, fluctuations in temperature can adversely affect some groups whilst being advantageous to others. Hence, fluctuations can cause changes in the activity of different microorganism groups, which in turn can lead to changes in the concentration of intermediary digestion products, such as organic alcohols and acids. This in turn will affect the overall performance of the digester. As such, it is important to maintain a stable operating temperature and process failure can occur if temperature changes are in excess of 1 °C/day. It is recommended that changes in temperature should be kept at less than 0.6 °C/day in order to avoid this situation arising [20].

2.2.2 Retention time

The retention time of a digester can be defined by the solids retention time (SRT), which indicates the average time that solids, and the micro-organisms that live on them, are in the anaerobic digester, or the hydraulic retention time (HRT), which is the average time that the liquid sludge is in the anaerobic digester. In a digester without a recycle, the HRT and SRT are equal. However, by recycling solids to the digester, the SRT and HRT can be decoupled and may vary considerably. As methanogens are slow-growing micro-organisms, the SRTs used in anaerobic digesters tend to be a minimum of 12 days, and often much longer. At lower SRTs, the wash-out of methanogens can have a detrimental effect on the long-term stability and performance of the digester. Furthermore, high SRTs provide the digester with a degree of buffering against the effects of shock or toxic loading. However, higher retention times require larger digestion vessels and as such there is a capital cost associated with an increased retention time. The degradation of volatile solids to methane and carbon dioxide in a digester are dependent on the HRT. In simple terms, the longer the HRT, the greater the volatile solids reduction will be, though it has been shown that increases in HRT greater than 12 days do not significantly increase the destruction of volatile solids. However, if the digested sludge is to be applied to land, the retention time must be sufficiently high to ensure that the volatile solids and indicator pathogen counts in the end product are low enough to comply with legislation.

2.2.3 Nutrients

The nutrients required for the micro-organisms involved in anaerobic digestion can be split into two groups, macronutrients and micronutrients. Macronutrients are those nutrients which are required in relatively large quantities by all micro-organisms, nitrogen and phosphorus. These are only available to micro-organisms in soluble form as ammonium nitrogen (NH_4 +-N) and orthophosphate phosphorus (HPO_4P). The exact nutrient requirements of the digester vary greatly depending on the organic loading rate. As a rule of thumb, a COD:N:P ratio of 1000:7:1 is used for high strength wastes and a ratio of 350:7:1 for low loadings. These ratios are based on the common empirical formula for cellular material, $C_5H_7O_2NP$ 0.1, and therefore assume that approximately 12 % of the dry weight of the bacterial cells in the sludge is nitrogen and 2 % is phosphorus. As such, the concentration of nitrogen and phosphorus in a digester can be estimated based on the COD of the influent and the COD removal in the digester. Alternatively, residual concentrations of 5 mg/L NH_4 +-N and 1-2 mg/L HPO_4 —P are recommended for digester effluent from a stable digester. By ensuring that there is residual nitrogen and phosphorus in the effluent, a check is made that bacterial growth is not limited by these elements in the digester.

Micronutrients are those nutrients that are required in relatively small quantities for microbiological growth and enzyme systems. For those micro-organisms involved in the conversion of acetate to methane, the critical inorganic micronutrients are cobalt, nickel, iron and sulphur. Methanogens also need traces of selenium, tungsten, molybdenum, barium, calcium, magnesium and sodium. These micronutrients are present in most municipal wastewater in sufficient concentrations for an anaerobic digester to run stably, though for industrial wastewater, it is often necessary to dose digesters with additional micronutrients in order to prevent digester upsets.

2.2.4 Alkalinity and pH

As with temperature, different micro-organisms have different optimum operating pH. Whilst most fermentative bacteria can function in a range between pH 4.0 and 8.5, the change in pH does have an effect on the products of fermentation. At low pH, the main products are acetic and butyric acid and at a pH of 8.0, the main products are acetic and propionic acid. Meanwhile, the microorganisms involved in methanogenesis are more sensitive to pH, with an optimum range of pH 6.8-7.2. For this reason, the digestion process is sometimes split into a two-stage process so that a more acidic pH can be maintained in the first stage thereby optimising hydrolysis and acidification, whilst the second stage is optimised for methanogenesis. The pH in an anaerobic digestion is normally maintained between pH 6.8 and 7.2, in order to prevent the predominance of acidogens which may cause the accumulation of volatile fatty acids (VFAs), causing a reduction in pH and eventually, process failure commonly known as souring. In a stable digester, the reduction of pH caused by VFA accumulation is countered by the activity of methanogens which produce alkalinity in the form of carbon dioxide, ammonia and bicarbonate.

Alkalinity can be considered as the buffering capacity of a digester to prevent rapid changes in pH. A stable digester will have a high alkalinity concentration in the form of carbon dioxide and bicarbonate ions. This makes it a useful indicator of stability and impending failure, as the accumulation of VFAs in a digester will result in a droping alkalinity before the pH of the digester starts to drop (Ward et al., 2008). A molar ratio of bicarbonate: VFA of at least 1.4:1 is recommended in order for a digester to remain stable.

2.3 Importance of appropriate system design

- An appropriate design of large-scale biogas plant is very necessary to optimize the initial capital investment and maintenance expenses. To be fit for purpose a digester must[16]:
- Maximize the degradation of volatile solids (VS) [within the design HRT].
- Provide a physical environment to optimize CH_4 production (including adequately mixing).
- Accommodate a high and sustainable OLR.
- Minimize HRT to reduce reactor volume.
- Reduce the overall power consumption by the system itself without effecting the biogas yield

3 Mixing in an anaerobic digester

3.1 Introduction

Efficiency of AD depends on many key factors like substrate type, C/N ratio, HRT, pH value, temperature, OLR, mixing and hydrodynamic factors of anaerobic digester. The hydrodynamics is a paramount element that contributes in the evolution, mass transfer, structure and metabolism of microbial community in an anaerobic digestion process [7]. Agitation of an anaerobic slurry is vital to accomplish, primarily, the supply of substrate to be distributed uniformly, secondly, to keep continuous contact between the microorganisms and sludge, tertiary, the concentration of end product and prohibited biological intermediates have to be maintained at minimum levels [8]. The mixing can boost the homogeneous distribution of nutrients and micro-organisms and can evade formation of surface crust and sedimentation [9]. Gerardi [10] acknowledge that the adjacent association between acetogens and methanogens can lead to effective methanogenesis which can be achieved by smooth and an adequate mixing.

Nearly 44% of the biogas plant failures are due to flaws in mixing [11]. The detrimental impacts of inadequate mixing are observed as abortive methane yield, defective stabilization of raw slurry, loss of digester volume and an increase in operational expenses [12]. It can also lead to sedimentation at bottom of digester, scum formation, short circuiting, uneven distribution of temperature and substrate and dead zones. Most of the studies acknowledges that the excessive mixing in an anaerobic digester can result in deteriorating methane production and unnecessary utilization of power [13][14]. The adverse repercussion of excessive mixing has been recognized by actuality that the high shear forces disrupt the microbial flocs and syntrophic relationships between methanogens and bacteria[13].

Optimized mixing refers to attain homogeneity at lowest energy inputs. The slurry can be typically mixed by various modes such as mechanical mixing [21][22][23][24] referring to use of mechanical impellers and draft tubes, slurry recirculation [25][26][27][28] and biogas recirculation through digester [29][30]. Literature confirms that the mechanical mixing is considered as the most effective mixing mode in terms of power consumption [29]. Many different types of mixers and agitators were studied by the researchers to find the optimum design for mixing in anaerobic digester [31][32]. Variation in geometry that included changing of blade shape, size and angle, bottom and inter impeller clearances, position of impeller within the digester has been applied in previous researches. There is variation in results on effectiveness and efficiencies of different mixers due to different methods and setups, substrates and their concentration. Choosing appropriate impeller is very important as choice of impeller depends on various factors like liquid viscosity, the need for turbulent shear flows and design of digester etc. and equipment, maintenance and operation costs. Interesting fact that is being unfurled in this article will be to know the feasibility of published results on impeller geometry in lab scale to the pilot scale digesters.

The exploration on mixing in an anaerobic digester in literature rests on mixing approaches such as mixing speed, mixing intervals but assessment of mixer type and mixer geometry is scanty. This paper focuses on reviewing the performance characteristics of various impellers used for an agitation in an anaerobic digester. Informative viewpoint discloses the outcomes from the evaluation of diversified impellers in terms of hydrodynamics, mixing, power input and mass transfer properties within an anaerobic digester. Meanwhile the effect of mixing speed, mixing intervals on rheology of slurry is discussed in detail. An analytical view pivots around the development of mixer which can allocate an adequate mixing within the prescribed limits of mixing intensity and mixing time to enhance the biogas production in an anaerobic digester because mixing is not only concerned about homogeneity but also about the sustainable environment for bacteria during different phases of process. This work allows future experimental work to identify appropriate mixing regimes and better understanding of link between mixing and biogas production.

Mixing is one of most important operations involved in production of biogas. The key objective of mixing is to maximise the degree of homogeneity of a property such as concentration, viscosity, colour and temperature. Nearly 44% of the biogas plant failures are due to flaws in mixing [11]. The detrimental impacts of inadequate mixing are observed as abortive methane yield, defective stabilization of raw slurry, loss of digester volume and an increase in operational expenses [12]. It can also lead to sedimentation at bottom of digester, scum formation, short circuiting, uneven distribution of temperature and substrate and dead zones. Most of the studies acknowledges that the excessive mixing in an anaerobic digester can result in deteriorating methane production and unnecessary utilization of power [13][14]. The adverse repercussion of excessive mixing has been recognized by actuality that the high shear forces disrupt the microbial flocs and syntrophic relationships between methanogens and bacteria[13].

Mixing involves three physical processes classified as:

- Distribution (macro mixing)
- Dispersion (micro mixing)
- Diffusion (classified as macro-mixing or micro-mixing depending on the scale of the fluid motion)

The following factors are fundamental to understanding the effects of mixing on the AD process and so included in the research:

- Type of feedstock and the calorific value (CV) of the embedded VS.
- Substrate rheology of the primary/major feedstock.
- OLR.
- Process temperature.
- Effectiveness of mixing technique.
- HRT.
- Biogas yield
- CH_4 content
- Parasitic energy demand.

External Factors

To isolate the effects that mixing has on the AD process and ensure that results are not influenced by advanced techniques and practices, the following subject areas are excluded from the research:

- Digester design.
- Geographical influence of digester location.
- Digester operating practices beyond mixing and feeding.
- Implications and benefits of co-digestion.
- Using feedstock external to the farm, such as food waste.
- Environmental regulation.
- Product application post-digestion.

3.2 Necessity of mixing

- Individual microorganisms should be given ample opportunity to metabolize fresh feedstock.
- Products of metabolism need to be distributed without disrupting microbial symbiosis.
- Biogas must be removed.
- Temperature gradients within the substrate must be minimized.
- Floating/sinking layers must be avoided.
- Energy consumption should be minimized.
- Short-circuiting should be prevented.

3.3 Mixing techniques

Mixing in an anaerobic digester can be attained by three main methods. Comparative analysis of different mixing modes on various influential parameters in an anaerobic digestion process is demonstrated in Table 1.

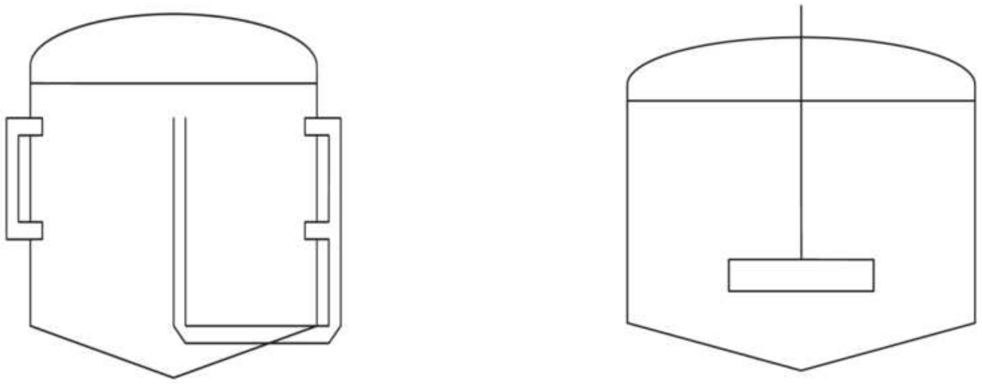
a. Slurry recirculation
b. Mechanical mixing
c. Internal gas mixing

3.3.1 Slurry recirculation:

In slurry recirculation method of mixing a large amount of digesting slurry is withdrawn from the center of digester and is pumped through external heat exchangers where the digested sludge is blended with the raw sludge and heated. It is then pumped back in the digestion tank through nozzles at the base of the digester or at the top to break the scum[18]. The flow rate in the recirculation should, however, be very large for ensuring a complete mixing of the tank which limits the sole use of this method of mixing. The minimum power required is 0.005–0.008 kW/m^3 of digester volume and may be higher, if friction losses are excessive. Other disadvantages of external pumped recirculation are plugging of the pumps by rags, impeller wear from grit and bearing failures[33]. Figure. 3(a) illustrates the slurry recirculation mixing in an anaerobic digester.

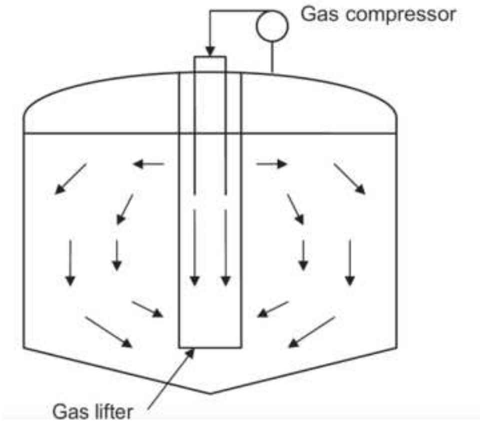
3.3.2 Mechanical mixing

Mechanical stirring systems generally use low-speed flat-blade turbines. In both systems, the sludge is transported by the rotating impeller(s), thereby mixing the content of the digestion tank. The mechanical pumping action is provided by centrifugal pumps, generally set up in an internal or external shaft tube to support vertical mixing. Literature confirms that the mechanical mixing is considered as the most effective mixing mode in terms of power consumption [29] Mixing is supported by the circulation of the sludge. Different types of paddle impellers are used at lower rpm to attain mixing in large scale biogas plants. Figure 3(b) represents the impeller mixing in digester.



(a) Slurry recirculation

(b) Mechanical mixing



c) Internal gas recirculation

Figure 3. Mixing modes in an anaerobic digester.

3.3.3 Internal gas mixing

In the gas mixing system Figure 3 (c), the biogas produced by the digester is collected and compressed. Then it is diffused through the diffusers in the digester volume. The mixing effect is attained as bubbles rush towards top pushing the slurry upward to the surface. Gas mixing is very effective against the scum formation in the digester, but it can lead to solid deposits at the bottom. The unit gas flow requirement for unconfined systems is 0.0045–0.005 m^3 /min[33].

Table 1. Comparative analysis of different mixing modes on various influential parameters in an anaerobic digestion process.

Parameters	**Mechanical mixing**	**Hydraulic mixing**	**Gas recirculation**
Viscous fluids	+ +	+	-
High TS content	+ +	+	-
Larger particle size	+	+	-
Lower power consumption	-	+	+ +
Low shear level	-	+	+ +
Minimum mixing time	+ + +	+	-
Better mass transfer	+ + +	+	-
Foaming	+ +	-	+
Low capital investment	-	+	+

a. From highly favorable (+ + +) to inefficient (-)

3.4 Mixing in large scale biogas plants

In a survey by Gemmeke et al. [34]in Germany it was observed that fast rotating submersible mixer (47%) is most commonly used in large scale biogas plants as compared to paddle agitators (7.4%), inclined shaft agitator (12.9 %) and reel agitators (6.8%). Similar results were demonstrated by Matthias [35] in Figure 4. and Figure 5. In study by Hopfner Sixt et al.[36] in Austria the number of agitators with low velocity and extended mixer blades for continuous operation was observed. In most of the BGP's paddle (36.6%) and submersible motor mixers (34.7%) are used.

Large scale biogas plants which are using 100% crop residue generally combines the low speed paddle impellers and high-speed submersible-motor propeller mixers. Operational characteristics of various of mixers used in large scale biogas plant are detailed in Table 2. Selection of impeller depends on type of substrate used for biogas production and are operated at different speeds as shown.

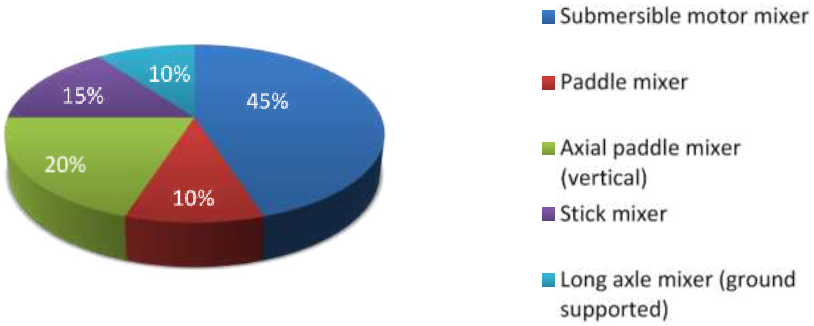


Figure 4. Distribution of impellers used for mechanical mixing in pilot scale biogas plants.

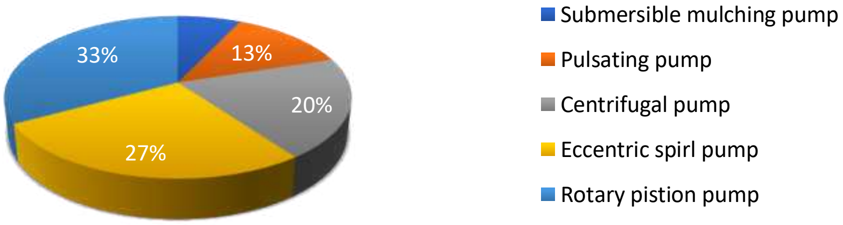


Figure 5. Distribution of various pumps for slurry recirculation in large scale biogas plants.

Table 2. The table represents use of various mechanical mixers according to the TS content in the slurry fed to the digester. It is very important to use appropriate geometry of impeller to enhance mixing efficiency and overall performance of anaerobic digester.

	Type of mixer	TS content	Approx. operation speed (rpm)	Installed power (kW)
1.	Submersible motor mixer	8 %	300-1500	0.25-35
2.	Central mixer	12 %	12-18	15
3.	Paddle mixer	14 %	10	-
4.	Shaft mixer	18 %	40-50	11

Electric power consumption by stirring at large scale digester

Substantial amount of energy is consumed by the mixing equipment used for mixing the slurry in digester. Power consumption can vary depending on the mode and time of mixing, viscosity of slurry and geometry of impeller. It is a major challenge to get homogenous substrate slurry by using least energy as energy consumption for agitation often demand large fraction of total energy consumption by biogas power plant. Mixing intensity is defined as power used per unit volume (P/V). Power consumption by an impeller in anaerobic digester dynamically is affected system characteristics such as impeller design, geometry and size of digester, impeller location, impeller speed and rheology of substrate being used in anaerobic digester. Accurate estimation of power is crucial for selection of power unit to achieve optimum mixing. Inadequate mixing unit will lead to unnecessary investment on machinery and higher power consumption rates leading to decrease of the efficiency of biogas plant[37]. According to United States EPA 5.8 W/m^3 of digester volume is recommended as power input for mixing in anaerobic digester but it is still conflicting subject[21].

Ecological and economic optimization is very crucial aspect to check the overall efficiency of a pilot scale biogas plant. Electric power is consumed by large number of equipments during the operation of pilot scale biogas plant which includes feeding systems, stirring system, desulphurization, conveyors and heating applications. 29-54 % of overall power need of biogas plant is utilized by the mixing equipment [38][39]. The percentage of power consumption by mixer varies with the size of digester. Biogas plants under 100 kWh uses only 2.7% of daily power production whereas, BGP with 250-500 kWh uses 5.5% for agitation and BGP with above 500 kWh production uses as high as 20.1% for mixing purposes [11]. Reports disclose that around 1 billion kWh/a electricity is consumed by mixing equipment in Germany costing around 200 million €/a for agitation [39].

Moreover, the electric power consumption for mixing also depends on HRT, TS content and feeding time. However, the direct interaction of specific electricity consumption to active digester volume and dry matter is not identified due to variation in geometry of impellers and physical properties of different substrates. Mixing energy demand can be optimized by changing the mixing time and design. According to Frey et al. [40] the energy consumption for mixing was reduced to 50% by adjustment of position of agitators in digester without any loss in mixing efficiency. Kress et al [41] noted 85% reducing in electric power consumption by minimizing the mixing time at pilot scale digester.

Specific stirring electric power consumption can be evaluated by two methods [42]:

i. Comparing power consumption to the active digester volume and mixing time

$$E_{speci.V} = \frac{\sum E_{stirrer,d}}{V_{active\ digester}} \tag{1}$$

The data represented in Figure 6 demonstrates the specific electric energy consumption by stirring in 13 large scale biogas plants along with average TS content during the operation [35][40][41]. The average $E_{speci.}$ is noted as 5.22 kWh$_{el}$/100m$^3{}_{active\ digester.d}$ but the individual values of most biogas plants hugely divert from mean value. The reason for higher energy consumption for higher TS content can be easily understood from the literature but it is hard to deduce significant ground of higher $E_{speci.}$ in some cases, at very low TS content of substrate. The cause of variation in energy consumption can be type of substrate design of digester and impeller. With increase in usage of energy crops for biogas production the energy demand for mixing has also increased. Moreover, the number of mixers also vary according to the size of digester. Usually, the BGP having capacity less than 250 kWh have one mixer and the number can increase to two or three depending on active volume[11].

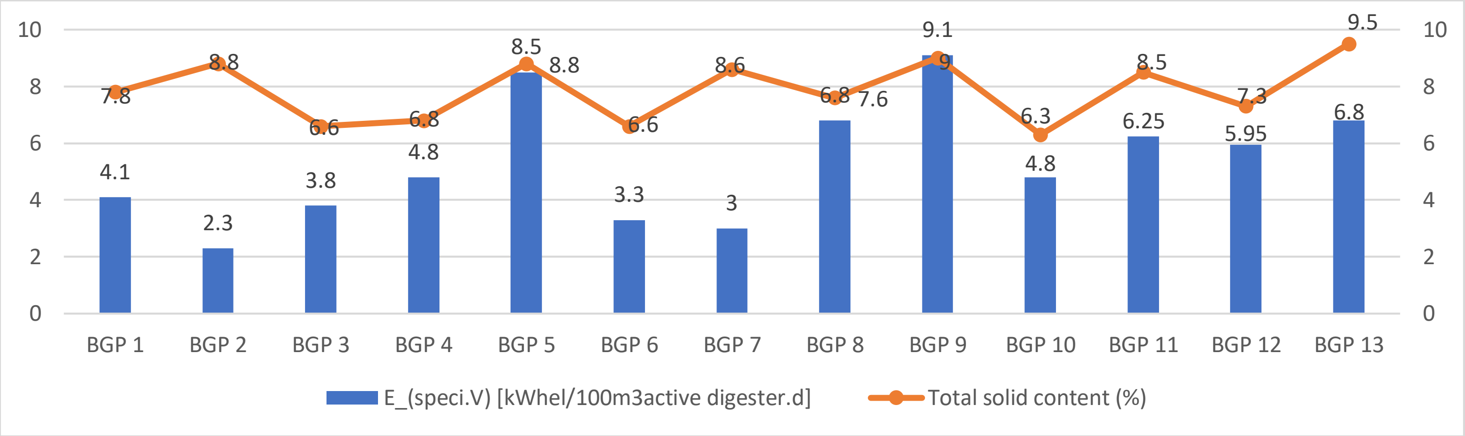


Figure 6.. Specific stirring electric energy consumption in comparison to the mean total solid content in large scale biogas plants.

ii. Comparing power consumption to daily added feedstock

$$E_{spec.FM} = \frac{\sum E_{stirrer,d}}{\dot{m}_{FM,d}} \qquad (2)$$

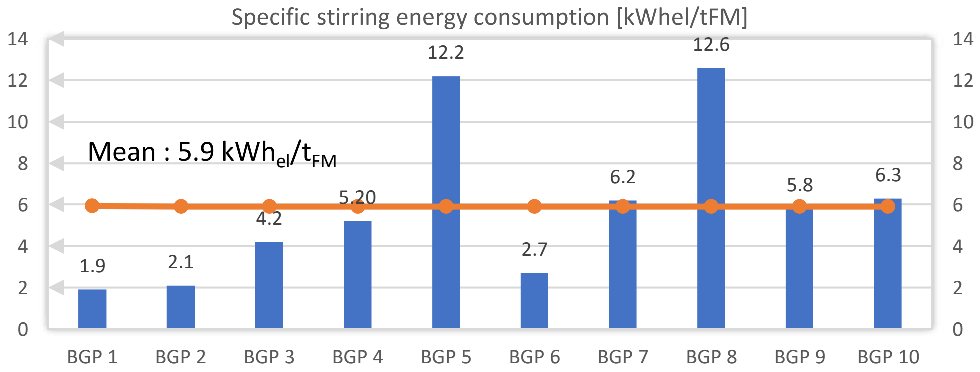


Figure 7. Specific stirring electric energy consumption in comparison to fed substrate mass in 10 BGPs in Germany.

Figure 7. represents the $E_{speci.}$ in comparison with amount of fresh feedstock fed to digester. The mean energy consumption is calculated as 5.6 kWh_{el}/t_{FM}. Significant variation in electric power consumption can be observed between the BGP's. This reason for this can be density of slurry, mixing intensity and mixing times. It has been seen that each of the BGP have their own mixing schemes and regimes. In actual conditions the BGP operators analyze the mixing in digesters by visual monitoring by which only surface can be monitored and information of mixing in other parts of digester is missed. This result in insecurity of failure of digester therefore, operator tend to increase the intensity and duration of mixing of slurry apart from recommended instructions.

H.J Naegele [39] studied the amount of electrical power consumption by full scale research biogas plant of university of Hohenheim located in a village of Eningen. The total electricity produced by biogas plant was 4063 kWh/day and from this amount 177 kWh/day (51%) was used by the agitators for mixing and it represented the most demanding group. The power consumption by inclined agitators was lower

than submersible motor mixing systems as while both agitators were operated for three minutes every 30 minutes. Higher stirring activity was demanded during larger feeding quantities to digester.
Literature lacks in sufficient information regarding specific mixing intensity, mixing time and geometry of impellers being used for large scale BGP's. But, it can be clearly stated from the literature that major share of electricity consumption by biogas plants itself stands with mixing of slurry in digester. It can be deduced that various reasons for higher power consumption by agitators are lack of compatibility between propeller and engine along with lack of knowledge of mixing processes and required mixing times because there is no common scenario of specific electricity consumption in different biogas plants. It is inferred that the use of slow rotating and larger agitating wings can cut the power consumption in BGP. Further, the variation in trend of specific energy consumption in biogas plants is observed in the large-scale biogas plants in comparison with total solid content and fed substrate. It is concluded that there is strong need to study mixing at pilot scales from economic point of view as significant amount of electric energy can be saved and future studies should be focused on geometry and positioning of impellers in actual conditions.

3.5 Mechanical mixing

3.5.1 Mechanical mixing

Mechanical mixing is considered as most effective mixing in terms of power consumed apart from gas mixing and pumped circulation. Many studies [43][44][45] have been published dealing with impact of mixing on biogas production in last year's using different designs, positions and configurations of impellers along with shape of digesters. Various factors which directly effects the mixing time and biogas production rates in a digester are impeller design, impeller bottom clearance and inter impeller clearance, impeller eccentricity, baffles and position of draft tube. Different setups and geometries were studied but there is variation in results on effectiveness and efficiencies of different mixers due to different methods used for evaluation along with different substrates and their concentration (Fig. 8). Choosing appropriate impeller is very important as choice of impeller depends on various factors like liquid viscosity, the need for turbulent shear flows and design of digester etc. The main objective of any impeller is to avoid stratification, dead zones and solid settling or even floating of substrate in a digester. For the small scale digesters coaxial impellers are used whereas in large equipment eccentric or inclined agitators can be used[46]. Usually, pumping effect is produced by rotary motion of impeller making slurry to flow in axial, radial and intermediate directions. However, in-vessel velocities are of course not necessarily an indicator of the degree of mixing. The sludge may be moving at a particular speed, but if all sludge in the immediate vicinity is moving at the same speed and in the same direction, then mixing is not occurring, rather the sludge is simply being moved within the vessel [22]. It was observed that ideal behavior of tank mixing may deviate due to variety of reasons associated with placement of inlets, outlets, stratification, and tank geometry. Moreover, presence of even a slight amount of density difference between the mixing fluids can strongly influence the progression of mixing[24].

3.5.2 Comparison of different impeller geometries

Lebranchu et al. [47] compared DHR and RT for mixing of cattle manure at different mixing intensities continuously in a 2 L lab scale digester. The digester equipped with helical mixer produced average 123 to 175 ml/h of biogas flow rates whereas digester with RT produced around 82 ml/h. Moreover, the digester equipped the RT showed large unmixed zones at almost all agitation speeds. The zones surrounding the impeller blades experienced higher shear rates which resulted in decline in broth viscosity to .22 Pa s and liquid velocity of 0.2 m/s whereas the volume average velocity was 0.0041 m/s. In case of helical ribbon, the maximal and volume average velocities were 0.034 m/s and 0.02 m/s respectively. K. Karim et al. compared different mixing modes i.e. biogas circulation, impeller mixing and slurry recirculation. It was observed that impeller mixing produced 22% more biogas than unmixed digester [48].

In the study[49] a MI with three blades, an AI, a RT with 45° inclined blades and PI were tested to know the mixing effect on this high viscosity mixture of OP and OMW. The comparison revealed that the marine impeller possesses good homogenization in the digester due to both axial and radial moments given to fluid. 6-blade RT with blade inclination of 45° performed much better than traditional RT resulting in increase in biogas production containing methane content of 82 V/V% (volume per volume percentage). The process efficiency of almost 17% was attained due to the effect to changing impeller motion from radial to axial and hence boosted the mixing efficiency. Best performance was noted by mixing with AI with biogas production of 22.6 Nl/l, and methane content of 84.4 V/V% [49]. A stronger tangential flow is generated by AI as compared to other impellers which makes it suitable for mixing viscous fluids [50].

M.S Vesvikar et al. [30] compared mixing obtained by sparging gas at different flow rates along with effect of draft tube clearance, shape of tank and tube size. Air was sparged at flow rates of 28.32 l/hr, 56.64 l/hr, 84.96 l/hr. Results indicated that flow pattern was not affected by change in flow rates. In case of draft tube diameter, the three different diameters were used. It was noted that there was no effect on flow pattern, but the active mixing volume increased with increase in diameter of draft tube along with reduction of dead zones. It was observed that by changing diameter to length ratio from 0.21 to 0.71 dead volume decreased by 60 %. Further no effect of draft tube clearance was observed on flow pattern and dead zones. It was concluded that a conical bottom and large draft tube diameter can be used to enhance the digester mixing and overall performance.

Jie Ding et al [51] compared normal impeller design of blade angle of 45° and diameter of 100 mm to an optimized impeller of same blade angle with external diameter of 120 mm along with four baffles each of width 20 mm around the inner tank in a 17 l continuously mixed tank reactor for Biohydrogen ($BioH_2$) production. It was observed that normal impeller generated more powerful vortex near bottom resulting in higher suspension of sedimentary activated sludge as compared to optimized impeller. Moreover, even at higher mixing speeds of optimized impeller there was very less influence on turbulence kinetic energy. It was concluded that optimized impeller can generate higher hydrogen yields even at slower speeds with less startup times.

Z. Trad et al. [52] demonstrated that the flow pattern of slurry was highly effected by off bottom and inter impeller clearance, the size and type of lower impeller. Different combinations of dual-impellers were studied in cylindrical, spherical bottom and unbaffled 5 l working volume reactor. Total of four Impellers were used where an elephant ear turbine was kept on the top in all experiments and the lower impellers were changed which were a four blade RT, a six blade RT and a MI. To study the effect of different off bottom C_b/H and inter impeller distances C_t/H total nine combinations were assessed by restricting power input below 10 W/m^3. It was observed that when the off-bottom clearance was decreased it restricted the circulation below the lower impeller and make it difficult to get suspension. With the usage of the 6RT70 (6 blade RT) and 3MP77 (3 blades marine impeller) impellers can reach faster homogeneous distribution and the adequate off bottom clearance was $C_b/H = 0.25$.

Fei Shen et al. [53] studied the mixing performance of various impellers in digester containing rice straw as substrate by using CFD simulations and experiments in a digester of working volume 8 l. Three different blades including the HEB, PB, disc mounted flat blade (DFB) were investigated at stirring rate between 20 rpm to 160 rpm. It was noted that at stirring rate of 80 rpm complete mixing of rice straw in vertical column was achieved by PB and HEB blades where flow velocity varied in range of 0-0.36 ms^{-1} whereas at same rpm in the triple impeller combination the flow velocity vectors varied from 0-0.44 ms^{-1}. The highest cumulative biogas production of 192.3 l was obtained at mixing speed of 80 rpm. In further experiments number of impellers were increased which resulted in generation of strong axial recirculation loop along with change in flow pattern which improved mixing performance. Impeller was rotated at lower speed in case of multi-impeller system resulting in lower shear rates and overall minimum cell destruction by dissipating the uniform overall power which is favourable for AD process. Use of triple impeller is recommended by Fei. Shen from the results obtained.

In a study by Pagilla et al.[29] gas mixing and mechanical mixing was compared at same operational conditions of feed (2.2 kg TS/m^3day), TSC (3.4%) temperature and OLR. It was observed that gas mixed digester formed more foam as compared to mechanical mixed as gas mixing provides favorable conditions for foam formation because of presence of bulk phase which stimulates the attachment of hydrophobic and surface-active compounds in slurry onto bubbles. Liquid film around the bubble is formed by surface active and hydrophobic compounds at surface of liquid in vessel which avoids bubbles from bursting and results in formation of foam potential.

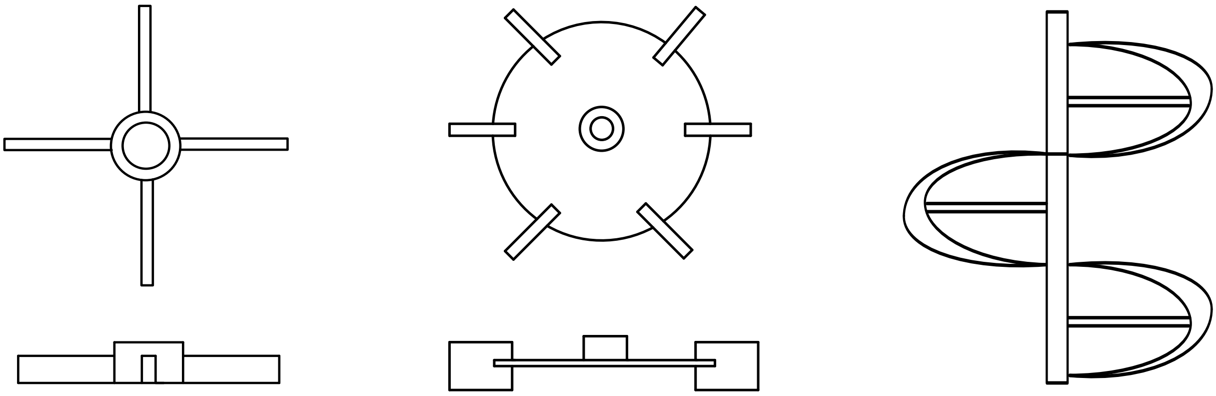
Binxin Wu [54] developed the computational fluid dynamic model of mixing by mechanical draft tube in egg shaped anaerobic digester. The direction of rotation and position of propeller were observed to identify the optimum position and primary pumping mode of propeller fixed in the tube. Two mixing methods i.e. mechanical draft tube mixing, and external pumped circulation were compared. In case of mechanical draft tube both upward and downward pumping modes were implied using an axial pump at rotating speed of 580 rpm. In up mixing mode two symmetrical vortexes were observed and two strong flow streams spread from top splash disc to side wall and on other hand in down pumping opposite flow paths were observed. It was concluded that up pumping in draft tube is more effective as compared to down pumping and also superior to external pump circulation in terms of power consumption. Optimum position of impeller for non-Newtonian fluid was determined as 0.914 m below the liquid surface.

According to study by Wu et al. [31] digester shape have significant influence on the mixing of slurry. In this research the flow pattern of Egg-shaped digester was tested by Computation Fluid Dynamics. It was observed that mixing in Egg Shaped digester is more uniform which leads to reduction in power consumption, removal of dead zones, operational needs and energy demand to maintain the homogeneity of digester and amount of foam formation was reduced.

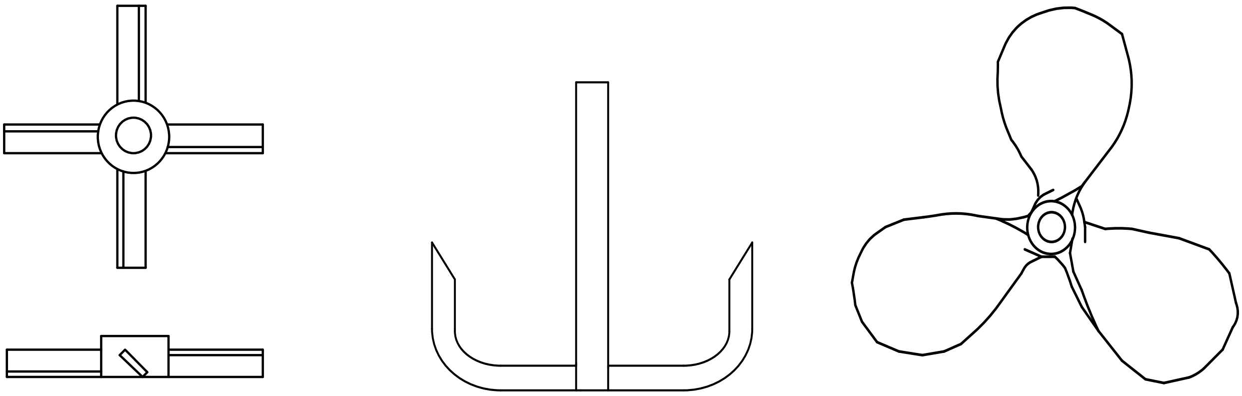
In above section, basic type of impellers has been discussed which were used in lab scale digesters. From the literature listed it is concluded that in slurry agitation the geometry characteristics of impeller determines their performance in mixing and biogas production (Table 2). Mostly the turbine impellers have been experimented to study the mixing effect of slurry rather. An idea of using paddle impellers can be encouraged because of better uniform viscosity distribution at lower shear rates and mixing speeds. Slow moving impellers with longer agitating wings can perform better in pilot scale digesters. It is observed that the impeller properties like pitch ratio, power number and axial flow number are closely related in attaining homogeneity in digester. These impellers should be modified to have uniform shear distribution so that the microorganisms remain unaffected and aim to reduce power consumption and improve flow pattern of slurry in digester. Subsequently, the impeller to be used for mixing slurry in an anaerobic digester should have nearly constant pitch as it will provide uniform velocity distribution at low shear rates. Consequentially, the scaleup of pilot scale mixing processes is key aspect for optimization of existing mixing and flow processes by keeping all dimensions in a fixed ratio, known as scale-up factor.

3.5.3 Effect of Mixing speed and mixing intensity

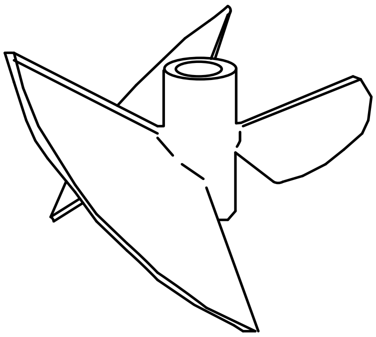
Two important parameters of mixing in anaerobic digester which can be examined are: intensity of mixing and mixing duration [48][55]. Lou et al.[56] Demonstrated that the mixing intensities have significant effect on hydrogen liquid gas mass transfer and biogas production. The levels of mixing intensity and duration affects the digester performance at different levels. Higher mixing intensities are favorable for reactor startup[57] and lower during methanogenesis. According to the previous research excessive mixing can enhance rate of hydrolysis and fermentation but on other hand syntrophic bacterial and methanogens association won’t be able to convert these fermentation products at the rate which they are formed due to inhibitory effect of the fermentation products which degrades the digestion performance[13]. Mixing time is time required to attain homogeneity in the digester and it depends on the impeller design, impeller speed, the number and placements of baffles, fluid properties and design of digester.



(a) 4-blade Rushton impeller (b) 6-blade Rushton impeller (c)Double helical ribbon impeller



(d) Pitched blade impeller (e)Anchor impeller (f) Marine impeller



(g) Elephant ear impeller

Figure 8. Different types of impellers used in lab scale experiments so far.

Hoffman et al. [58] compared the different mixing intensities at 1500, 500, 250 and 50 rpm to determine the effect of mixing intensity on methanogenic population, performance and juxtaposition of syntrophic microbes in an anaerobic digester in four laboratory scale 4.5 l unbaffled digesters. It was observed that different mixing speeds has no effect on overall biogas production rates. However, during startup, negative effect on biogas production rate was noted as microbial flocs were destroyed along with higher concentration of volatile fatty acids (4000 mg/ l) at intensive mixing of 1500 rpm. The work supports the fact that higher mixing intensities is just waste of energy. Fei Shen et al.[53] demonstrated the flow velocities at different rpm i.e 60, 80, 100. Flow velocities increased above 0.5 ms^{-1} at 100 rpm which resulted in lower digestion efficiency because of destruction of sludge structure and organisms as stated by Zhang et al[59].

D.A Stafford [23] at university of microbiology in Wales studied the effect of mixing rates on the biogas production at 140 rpm and 1000 rpm in primary sewage sludge in a 3 l digester mechanically mixed by magnetic stirrer. It was observed that low mixing speed nearly 150 rpm was appropriate for biogas production whereas at higher speed i.e above 700 rpm the gas production was reduced. Almost in all case the stirring tends to increase biogas production in initial stage from 10 seconds to 45 s.

Ratanatamskul et al. [28] studied the effect of slurry recirculation mixing and mixing time on performance of 10 m^3 anaerobic digester at mesophilic temperature range (35$\pm$2^{o}C). The digester was operated at HRT of 40 days and OLR of 5.83 kg COD/m^3/day. The sludge circulation rates were varied from 50 to 100% at with mixing time of 30, 60 and 90 min twice a day after each loading. Higher circulation rate of 100% improved the pH stability of digester due to higher alkalinity return but the biogas production rate was higher for 50% slurry recirculation rate with 24 m^3/day as compared to 22.5 m^3/day in 100% recirculation rate along with methane content of 54.1% and 60% respectively. Mixing time of 60 minutes was found optimum as highest COD removal efficiency of 90% was achieved along with highest biogas production of 0.71 m3/kg COD with CH_4 61.6%.

Jie Ding et al [51] analyzied role of impeller design by computational fluid dynamics (CFD) over a range of speeds to optimize biohydrogen production in reactors. A CSTR of working volume 17 l was operated in continuous flow mode by maintaining chemical oxygen demand of 3000 mg/ l at mesophilic temperature of 35^{o}C. An impeller having blade angle of 45^o and diameter of 100 mm was operated at different speeds of 50, 70, 90, 110 and 130 rpm. The flow fields, turbulence parameters, residence time distribution, $BioH_2$ and biogas yields were analyzed. It was noted that the average biogas yield increased from 11.8 l/day to 26.1 l/day when mixing speed of impeller changed from 50 to 70 rpm and spontaneously decreased at 130 rpm. Although the increasing speed of impeller improved the velocity distribution, but hydrogen yield was not increased. From the results it was observed that impeller speed of 70 rpm was optimum and produced highest $BioH_2$ as better velocity distribution was generated at lower speed.

J. Rivard et al[60] conducted laboratory scale experiment under high solid contents of 36% in a 20 liters digester to determine effect of mixing by varying mixing speeds from 1 to 25 rpm at 35^{o}C fed with MSW at OLR of 9.5 g VS/l d. A negligible difference in biogas production and methane content was observed. Mixing at 25 rpm was proved uneconomical in terms of power consumption and biogas production. Similar results were drawn by Z. Tian et al [61]for agitated and non-agitated digesters. Two digesters of volume 5 liters were fed with sugar beet at TSC 10.9% and operated at 55^{o}C. Digester 1 was under non-agitated conditions whereas Digester 2 was agitated continuously at 180 rpm by magnetic stirrer. It was observed that the peak production for digester 1 was 0.70 m^3/d (kg VS)$^{-1}$ on day 5 whereas for digester 2 it was 0.34 m^3/d (kg VS)$^{-1}$ on day 11.

R. Sindall et al.[62] derived a CFD model of a 6 liters laboratory scale digester with 4-B impeller to analyze the effect of varying mixing (unmixed, 50 rpm, 100 rpm, 200 rpm) and velocity gradient of particles at temperature of 35°C. At 50 rpm the velocity gradient was 7.2 s^{-1} and enhancing biogas production by 20% whereas at 100 and 200 rpm a sharp decrease of 18% and 56% was noted which supports the fact that there is a threshold above which intensive mixing becomes counter-productive. Accordingly, the threshold proposed by Sindall et al is between 7.2-9.7 s^{-1} and it was stated that above this velocity gradient value microbiological environment will be damaged. Hughes [63] proposed that Intermittent mixing at 140 rpm with resting time of 12 hours was optimum producing highest methane. But it was noted that here high mixing intensity had better result as compared to lower mixing intensities of 61.6 rpm and 36.96 rpm.

After continuous pre-run of three digesters from 0-19 days different mixing modes were analyzed which resulted that minimal mixing (mixing for 10 minutes prior to extraction/feeding) yielded highest methane as compared to intermittent (withholding mixing for 2 hours prior to extraction/feeding) and continuous mixing in digester but higher levels of Volatile fatty acids were noted in intermittent mixing. The methane production was improved in intermittent mixing by 12.5% and 14.6 % in lab scale and pilot scale digesters respectively as compared to continuous mixing[13]. It was noted that methane yield of maize Stover at low mixing intensity (20 rpm) was higher as compared to intensive mixing (70 rpm). Intensive mixing blurred the boundaries of upper and lower phases resulting in VFA's accumulation and loss of methanogens [64]. Similarly, Stroot PG et al. [65] supported the fact that low mixing can result in stable performance of anaerobic digester and further help to generate good contact between the substrate and microorganisms resulting in increasing the specific gas production[66].

Sulaiman et al[66] compared four different mixing regimes i.e natural mixing (NM), minimal horizontal mixing (MHM), minimum horizontal and vertical mixing (MHVM) and vigorous mixing (VM) in 500 m^3 digester using palm oil mill effluent as substrate. It was noted that highest methane productivity was produced during MHM at 1.4 m^3 m^{-1} /d whereas NM and MHVM produced 1.0 and 1.1 m^3 /d. This stated that minimal mixing is sufficient to create good contact between organisms and substrate and to release the entrapped biogas at the bottom in a digester.

It is observed that minimal intermittent mixing is favourable for effective anaerobic digestion process (Fig. 9). Further in intermittent mixing longer resting times can result in higher biogas production and in most of cases increasing mixing time intervals haven't shown any effect on biogas production but similar results can be obtained at lower power consumption. The direct influence of shear rate and mixing intensity is still controversial subject.

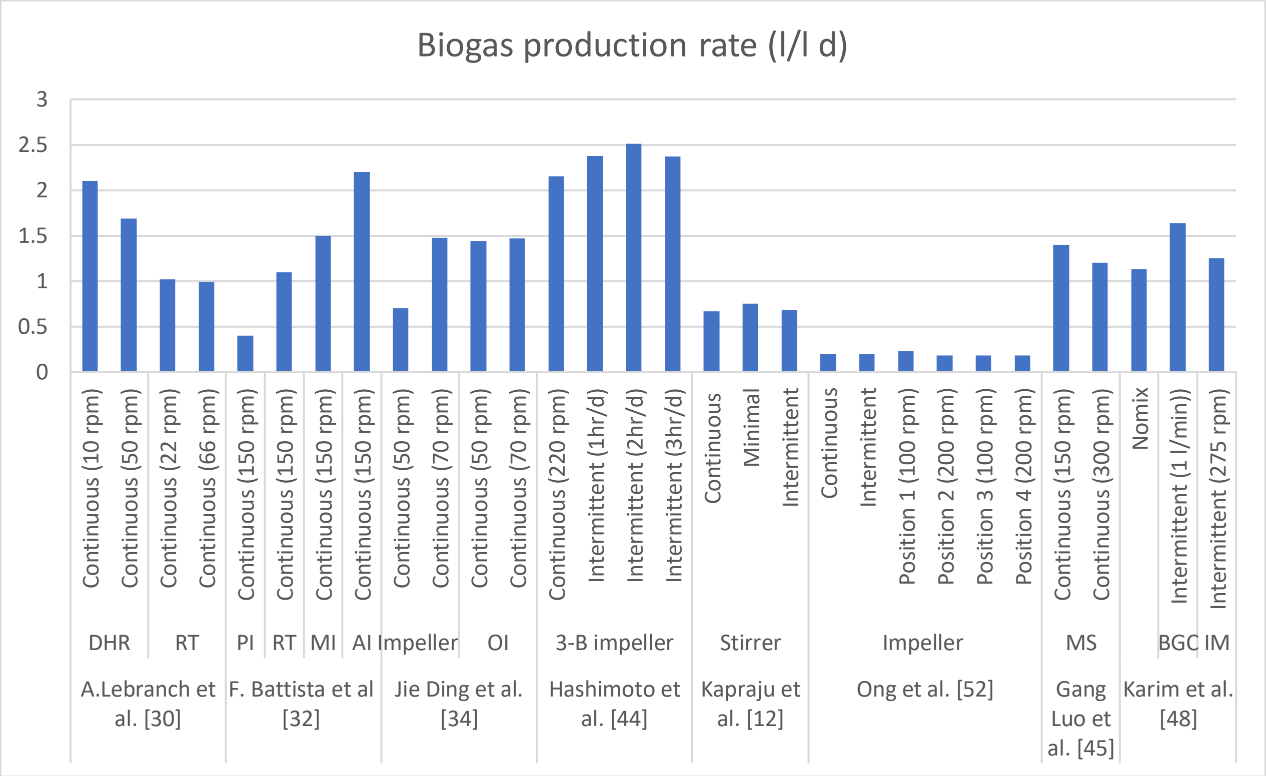


Figure 9. Efficiency comparisons of various impellers at lab-scale digester in terms of biogas yield. The figure represents the biogas yield of various experiments using different types of impellers and mixing regimes referring to mixing speed and mixing time. The data in the figure should be read in conjunction with Table 1. see describing various other factors considered during the anaerobic digestion process

Table 3. Comparison of various impellers and mixing modes in terms of biogas production rate in Anaerobic digestion. Detailed description of experiments is also demonstrated along with the parameters which effect the rate of anaerobic digestion.

Reference	Working Volume(l)	Feed stock	TS	HRT	Mixer type	Mixing type	Mixing speed (rpm)	Biogas production rate	Methane content (%)
A.Lebranchu. et al. [47]	2l	CM	8.8%	-	Double helical ribbon	Continuous	10 50 90	175±7 mlh^{-1} 141±1 mlh^{-1} 123±1 mlh^{-1}	64±1 58±1 57±1
				-	Ruston turbine	continuous	22 66 110	85±6 mlh^{-1} 83±8 mlh^{-1} 82±9 mlh^{-1}	64±0 64±1 59±2
F. Battista et al.[49]	2l	OP	221.4g/L	-	Pelton Impeller	Continuous	150	0.40±.03Nl/l	0.00±0.00
				15	Rushton Impeller	Continuous	150	11.6±0.5 Nl/l	82.07±3.57
				15	A Marine Impeller	Continuous	150	15.3±0.4 Nl/l	84.12±1.21
				16	Anchor impeller	Continuous	150	22.6±1.2 Nl/l	84.38±0.60
F. Shen et al. [53]	8l	RS	65 g/L	-	Triple Pitched Blade impeller	Intermittent (5 min after every 2 hours)	40 80 120 160	299 ml/g VS 370 ml/g VS 332 ml/g VS 327 ml/g VS	
Ratabatamskul and Saleart [28]	1000	FW	8%	40	Sludge recirculation	Intermittent	0.033 l/min l (30 min twice a day)	14.52 m^3/d	58.4
	1000	FW	8%	40	Sludge recirculation	Intermittent	0.016 l/min (60 min twice a day)	16.20 m^3/d	61.6
	1000	FW	8%	40	Sludge recirculation	Intermittent	0.0011 l/min 1 (90 min twice a day)	10.90 m^3/d	55.9
Sindall et al.[62]	6 l	MSW	2.9	-		Non-mixed	-	-	-
				-	4 blade rushton turbine	Continuous	50	Increased 20% of non-mixed	-
				-	4 blade rushton turbine	Continuous	100	Decreased 18% of non-mixed	-
				-	4 blade rushton turbine	Continuous	200	Decreased 56% of non-mixed	-
Rivard et al.[60]	20	MSW	36%	-	Impeller	Continuous	1	-	55.6
			36%	-	Impeller	Continuous	5	-	54.4
			36%	-	Impeller	Continuous	10	-	55.9
			36%	-	Impeller	Continuous	25	-	56.4
Jie Ding et al. [51]	17l	Molasses	13.5 g/L	8 hr	Normal impeller (diameter 100 mm)	Continuous	50 70	12 l/d 25.2 l/d	- -
					Optimized impeller (diameter 120 mm) with 4 baffles	Continuous	50 70	24.5 l/d 25 l/d	- -

Hashimoto et al.[67]	3l	BCW	14%	6	Three blade impeller	Continuous	220	2.15±0.1 l/Ld	49.4
				6		Intermittent	1 hr/d	2.38±0.1 l/Ld	49.7
							2hr/d	2.51±0.2 l/Ld	49.5
							3 hr/d	2.37±0.1 l/Ld	49.2
				4	Three blade impeller	Continuous	24 hr/d	3.96±0.6 l/Ld	56.4
						Intermittent	2 hr/d	3.57±0.2 l/Ld	55.6
Gang Luo et al.[56]	0.6l	CM	-	15	Magnet stirrer	Continuous	150	1.4± 0.1 (l/ld)	53±3
						Continuous	300	1.2±0.1(l/(ld)	68±2.5
Clark et al.[68]	1l	Nonfat powdered milk	-	-	Magnetic stir bar	Non-mixed		253.4 ml $(gVS)^{-1}$	-
						Continuous	500	327.7 ml $(gVS)^{-1}$	-
Z. Tian et al.[61]	5l	SB	10.9	-	Magnetic stirrer	Non-mixed		-	0.70 $m^3d^{-1}(kg\ VS)^{-1}$
						Continuous	180	-	0.34 $m^3d^{-1}(kg\ VS)^{-1}$
D.A Stafford[23]	3l	SS	-	-	Magnetic stirrer	Intermittent	90	80 mlh^{-1}	-
						Intermittent	150	88 mlh^{-1}	-
						Intermittent	250	89 mlh^{-1}	-
						Intermittent	400	82 mlh^{-1}	-
						Intermittent	600	80 mlh^{-1}	-
						Intermittent	800	79 mlh^{-1}	-
						Intermittent	1000	77 mlh^{-1}	-
Karim et al.[69]	3.73l	CM	51 g/l		Un mixed			0.84±0.07	64±3%
					Biogas mixed	Intermittent	1 l/min	0.94±0.07	56±3%
					Axial flow Impeller mixed	Intermittent	275 rpm	0.88±0.09	61±3%
					Slurry	Intermittent	0.82 l/min	0.85±0.09 (l/l d)	67±3%
			100 g/l		Un mixed			0.92±0.1	66±3%
					Biogas mixed	Intermittent	1 l/min	1.07±0.08	65±4%
					Axial flow Impeller mixed	Intermittent	275 rpm	1.14±0.13	65±3%
					Slurry	Intermittent	0.82 l/min	1.20±0.14 (l/l d)	66±4%
			150 g/l		Un mixed			1.13±0.14	64%
					Biogas mixed	Intermittent	1 l/min	1.64±0.32	66%
					Axial flow Impeller mixed	Intermittent	275 rpm	1.25±0.12 (l/l d)	63%
L. Tian et al.[70]	8l	CS	1.44	20	Impeller mixed	Continuous		427±63	-
			1.78			Continuous		487±56	-
			2.11 g/l d			Continuous		423±45 ml gTS^{-1}	-
	8l		1.44	20	Impeller mixed	Intermittent	80 rpm (after every 2 hr for 5 min)	459±58	-
			1.78		Impeller mixed	Intermittent		508±49	-
			2.11 g/l d		Impeller mixed	Intermittent		433±37 ml gTS^{-1}	-
	8l		1.44	20	Impeller mixed	Intermittent	80 rpm (after every 4 hr for 5 min)	430±61	-
			1.78		Impeller mixed	Intermittent		505±76	-
			2.11 g/l d		Impeller mixed	Intermittent		429±39 ml gTS^{-1}	-
	8l		1.44	20	Impeller mixed	Intermittent	80 rpm (after every 8 hr for 5 m	420±60	-
			1.78		Impeller mixed	Intermittent		454±69	-
			2.11g/l d		Impeller mixed	Intermittent		378±51 m	-

Rico et al.[45]	-	CM	6.1 %	20 20 20	Slurry recirculation Slurry recirculation Slurry recirculation	Continuous Intermittent Intermittent	1000 l/h 30 min 10 times/day 2.5 h/d	0.71 l/l d 0.70 l/l d 0.71 l/l d	67.2% 67.2% 67.2%
Kaparaju et al. [13]	3.6 l 500 l	CM	8.1% 7.5 %	15 20	Stirrer Impeller	Continuous Minimal Intermittent Continuous Intermittent	10 min prior to extraction /feed No mixing 2 hr before feed/extraction 5 min on/off No mixing 2 hr before feed/extraction	0.67 l/l d 0.75 l/l d 0.68 l/l d 1.198 l/l d 1.206 l/l d	64.1% 64.1% 63.0% 69.4% 67.1%
Sulaiman et al. [66]	5000000 l	Palm oil mill effluent	-	10	-	Natural Intermittent (HM) Intermittent (HVM) Intermittent (VM)	30 min every 6 hr 30 min every 6hr 30 min every 2 hr	$1.0\ m^3m^{-3}\ d^{-1}$ $1.4\ m^3m^{-3}\ d^{-1}$ $1.1\ m^3m^{-3}\ d^{-1}$ -	- - - -
Hoffman et al. [58]	4.5 l	CM	5%	83-15	Axial impeller	Continuous Continuous Continuous Continuous	1500 500 250 50	- - - -	67.4±5.0 67.4±5.0 67.4±5.0 67.4±5.0
Gomez et al.[58]	3 l	MSW	6%	47-37	Electrical Stirrer	Continuous Continuous Intermittent	80 200 200 rpm before and after feeding	0.3-0.5 l/l d 0.2-0.3 l/l d 0.3-0.6 l/l d	- - -
Ong et al.[71]	10 L	cm	80 g/L	10	Two six blade disc type turbine impellers	Continuous Intermittent Continuous Continuous Continuous Continuous	100 rpm 4*30 min/day 160 rpm (POI 1) 100 (POI 1) 200 (POI 2) 100 (POI 2) 200	0.2 l/l d 0.2 l/l d 0.23 l/l d 0.18 l/l d 0.18 l/l d 0.18 l/l d	45.2% 46.0% 45.7% 45.7% 46.0% 45.2%
Rojas et al.[72]	0.5 l	CM, Kitchen waste	7-13%	-	Magnetic stirrer	Un mixed Continuous	- 60 rpm	$318\ m^3/t$ oTS $699\ m^3/t$ oTS	51.0% 64.0%
Lin and Pearce et al.[57]	7 l	Potato processing wate	-	-	Four blade impeller	Unmixed Intermittent Intermittent	20 rpm (45min/h) 50 rpm (45 min/h)	0.40 l/l d 0.44 l/l d 0.45 l/l d	73.5% 76.5% 76.4%

		r				Intermittent	100 rpm (45 min/h)	0.47 l/l d	75.5%
						Unmixed	50 rpm (15 min/h)	0.45 l/l d	76.4%
						Intermittent	100 rpm	0.44 l/l d	75.5%

3.5.4 Effect of mixing on floating layer, crust and foam formation

Floating layer and crust formation are general problems faced during the digestion process in both lab scale and pilot scale biogas digesters. Lignocellulosic substrate present in the slurry exhibits properties like heterogeneity, high water holding capacity and low density. Due to these properties it floats on the surface of slurry and form floating layer which lead to imperfect association of micro-organisms and substrate, improper heat transfer and hence lower biogas production [70]. Whereas foam occurs due to formation of gas bubble on the upper layer of slurry as surface active and hydrophobic compounds form a liquid film and preventing bubbles to burst. Intensive mixing leads to formation of excessive bubbles which result in increase in attachment of surface active agents and hydrophobic compounds on bubbles and hence leads to higher foam formation[73].

According to Pagilla et al. [74] gas mixing causes more foam as compared to mechanical mixing. It was observed that the height of foam layer in gas mixed and mechanically mixed were 2.4 m and 1.3 m respectively. By agitation the network structure of floating layer can be disrupted by gas bubbles due to shear exerted by mixing [75][76]. However, in no mixing conditions gas bubbles remained trapped in slurry which are difficult to discharge and result in formation of floating layer [77]. Libin tian et al. [70] investigated the optimum mixing intervals to avoid floating layers formation by comparing agitated and non-agitated digesters a 10 l anaerobic digester using corn stover as substrate. Floating layer developed in non-agitated digester and volume of floating layer increased rapidly during first four days and then remained uniform whereas no layer was observed in digesters which were agitated. Moreover, the volume of floating layer depends on the TS content in the slurry. It was observed that volume increased by 48.72% when Total Solid content was increased from 4.30 % to 5.45 % and further for 7.36 %TS content it increased by 80.77%. In terms of biogas production, the yield decreased significantly in the digester with no agitation by 81.87%, 85.95% and 87.90% for floating test 1. Test 2, and test 3 respectively. Moreover, the gas released by the intermittently mixed digester was 70% more as compared to un mixed. This fact is supported by Stroot et al. [65] that floating layers of solids form due to insufficient mixing so increased mixing level is preferred.

Kowalczyk et al. [78] reported swelling and foam during long resting periods in initial days of operation in case of intermittent mixing but there was significant effect on biogas production. Whereas continuous mixing didn't show any foam and swelling. Kress et al. [41] found that short mixing time of 2 min and 30 min break didn't produced foam or swelling therefore increased OLR can avoid foam. According to V.S. Kshirsagar et al. [79] by increasing the surface velocity the scum on the surface of digester can be deformed. To overcome this problem the digester design was optimized by creating concrete flaps at the baseline of digester. It was observed that by increasing surface velocity the scum formation was reduced whereas domain velocity improved gas production rates.

Considerable difference in biogas production was missing between mixed and unmixed digester but mixing can be long term solution to avoid crust formation [80]. Optimized intermittent mixing and low mixing intensities can help to prevent scum, foam and floating layers resulting in improvement of overall AD process. Mechanical mixing is favourable to avoid the formation of foam in the digester.

3.6 Various effects of mixing on anaerobic digestion process

3.6.1 Effect of mixing on microbial community

The anaerobic digestion process involves different steps, such as hydrolysis, acidogenesis, acetogenesis and methanogenesis, for the production of biogas. Each step inhibits specific species of bacteria responsible for converting molecules from one form to another through biochemical reactions. Many researchers have observed that micro-organisms at all the stages exhibit dissimilar behavior at different shear rates [81][58]. Additionally, excessive mixing and high shear rates have a negative effect on biogas production rates[13][58]. This results in a lower rate of methanogenesis due to the reduced presence of methanogens and the dissipation of methanogenic centers in the vessel [81].

Recently, many studies have been published which have evaluated the effect of mixing at hydrolysis, the acidification and the methanogenic phases. In a recent work by Si-jia et al (2018) [82], the effect of mixing was analyzed through the physical separation of different phases. During hydrolysis and the acidification phase, slurry was mixed at different intensities of 30,60, 90 and 120 rpm, whereas during the methanogenic phase continuous mixing at 120 rpm was applied. The results showed that mixing at 90 and 120 rpm was favorable for hydrolysis and the acidification phase as an abundance of *Proteobacteria, Chloroflexi, Firmicutes, Actinobacteria* and *Bacteroidetes* was found.

Ghanimeh et al.[83] demonstrated microbial analysis at various mixing speeds between 80 to 160 rpm. It was observed that the agitated digester was dominated by *Thermotogae phylum* (89% at 80 rpm, 87% at 50 rpm and 85% at 160 rpm), which was gradually replaced by *Synergistetes*. In contrast, under non-mixing conditions *Synergistetes* dominated by 72% and *Thermotogae phylum* was reduced to as low as 5%. Further, it was observed that *Petrotoga genus* (phylum of *Thermotogae*) proliferated under mixing conditions and was absent in non-mixed digesters.

Stroot et al. [65] revealed that methanogenic archaea and propionate oxidizing bacteria live in close vicinity in granules with hydrogen and formate as an electron carrier. For a thermodynamically stable reaction, the concentration of the electron carrier should be low, and therefore the high rate of propionate conversion observed can only be explained by the short diffusion distance possible in obligate syntrophic consortia. Excessive mixing distorts the granule structure and results in a declining rate of the oxidation of fatty acids, which can lead to digester instability. According to Vivilian et al. [81], higher mixing intensities inhibit hydrolysis, acidogenesis and methanogenesis due to the fact that higher VFA concentrations result in instability in the digestion process. Supporting this fact, R. Sindall et al.[62] demonstrated that due to increased turbulence (100 and 200 rpm) in an anaerobic digester, localized pockets of acetate are disturbed, which results in a decline in the ratio of acetoclastic methanogens to hydrogenotrophic methanogens. This, in turn, leads to a decline in biogas production rates.

In the two-stage process, acidogenic bacteria is grown in an acidogenic reactor with pH naturally low and a residence time between 1 and 4 days, whereas methanogenic bacteria is grown in a methanogenic reactor, which has a naturally much higher pH and a residence time of 15–20 days [19]. Mixing by MI gave excellent results for two-stage configurations, as efficiency jumped from 22.64% (mono-stage) to 30.24% (two-stage configuration) with a 1.34-fold improvement of the process. But AI mixing reduced the efficiency of the AD: the efficiency dropped from over 33% to 17.5%. Thus, the system should be configured so that it permits sufficient mixing without the mechanical stresses that can destroy methanogenic bacteria [49].

Z.Tian et al. [84] observed the presence of a different microbial community structure for agitated and non-agitated digesters. A higher diversity of species of methanogens was exhibited by non-agitated digesters, whereas a high proportion of *Petrotoga*-related (an anaerobic, thermophilic, xylanolytic, motile rod-shaped bacterium) species with *Methanosaeta*-related methanogens was observed in agitated digesters. *Petrotoga* enhances H_2 through the fermentation of sugar [85]. Continuing their research in 2014, Z. Tian et al [61] described the microbial community structure and digestion performance for non-agitated and agitated digesters (180 rpm). It was observed that *Methanosarcina (acetoclastic* methanogen) was more abundant than *Methanoculleus* (hydrogenotrophic methanogen) in non-agitated as compared to agitated digesters, which was the reason for higher methane production in non-agitated digesters. A relatively higher amount of *Acetanareobacterium, Ruminococcus* and *Ruminococcaceae* was found in agitated digester. These species produce hydrogen from cellulose, and further hydrogenotrophic methanogens are required to convert hydrogen to methane, but they were not found in enough quantity in agitated digesters, so a retardation in methane production was noted. Similar results were obtained by Ghanimeh et al.[83]: *Petrotoga* genus (phylum of Thermotogae) proliferated under mixing conditions and was absent in non-mixed digesters. It was also stated that the genus *Petrotoga* played an important role in the degradation of organic matter.

Kaparaju et al. [13] analyzed the microbial community in continuously mixed and intermittently mixed digesters. Results showed an abundance of small rod-shaped bacteria in continuously stirred digesters, whereas the presence of *Methanosarcina* and *Methanosaeta* was noted in intermittently mixed digesters. An appropriate balance of acetotrophic methanogenesis and hydrogenotrophic methanogenesis was observed in intermittently mixed digesters, which might be the reason for their higher biogas production. Considerable differences in levels of *Methanosaeta concilii* and *Methanosarcina* were noted by Hoffman et al. [58]. For *Methanosaeta concilii* the relative level of the small subunit rRNA was 2%, and this increased to 4% on the 6th day; furthermore, the level approached zero for mixing at 1500 rpm. For the digester mixing at 500 rpm, the levels of *Methanosaeta concilii* were between 3.2% to 4.8% for first 75 days and then decreased to 1% for the remaining period of operation. In addition, it was observed that the level of concilii was higher for 250 and 50 rpm compared to 1500 and 500 rpm from day 117 to the end. This is because *Methanosaeta concilii* cells have long filaments, and hence higher mixing intensities can affect the formation of filaments. Low levels of *Methanosarcina* were observed at low mixing levels of 50 rpm, whereas it increased for higher mixing intensities as *Methanosarcina* was between 2%- 4.5% at 1500 rpm and between 1%-5% at 500 rpm for the last 60 days. Moreover, with intensive mixing, a greater increase in the level of the hydrogenotrophic methanogenic family *Methanobacteriaceae* were observed; however, they remained constant at 250 and 50 rpm, and levels of acetoclastic methanogens were also high with intensive mixing (1500 rpm). They concluded that intensive mixing and continuous mixing are counter-productive in terms of biogas production. Mohammadrezaei et al. [86] observed that during the first 4 days of hydrolysis the biogas production was highest with mixing at 120 rpm compared to 0, 40 and 80 rpm. Whereas when the process approached methanogenesis, the higher mixing intensity reduced the biogas yield compared to mixing at slower rpm.

According to the literature survey, mixing intensity should be adjusted according to the specific stage of anaerobic digestion. As the digestion process approaches the final stage of methanogenesis, the shear rate should be decreased in correspondence with the lower mixing speeds of mixers. However, during the startup higher mixing speeds are favorable. An effective distribution of H_2 is a very important aspect which can be enhanced by optimized mixing.

3.6.2 Effect of mixing on CH_4 content

In general, the methane content of biogas depends on both the substrate composition and the operational parameters, which include HRT, OLR, TS and mixing schemes. This section will focus on the effect of mixing on methane content and its correlation with mixing intensity and mixing operation time.
In a study of lab-scale experiments, Lin and Pearce [57] observed that methane production was higher during intermittent mixing when compared to unmixed digester. Moreover, the methane production rate decreased when the duration of mixing was reduced from 45 min/h to 15 min/h. Lebranch [47] observed that at agitation rates of 50 and 90 rpm there was a decrease in CH_4 content from 64% to 57% compared to a rate of 10 rpm when using a helical ribbon impeller. On the other hand, it decreased from 64% to 59 % at a rate of 110 rpm compared to 22 and 66 rpm when mixing with a Rushton turbine. In research by D.A. Stafford at Cardiff University in Wales, the effect of mixing rates on biogas production was analyzed using low speed (140 rpm) and high speed (1000 rpm) in primary sewage sludge. It was observed that a low mixing speed of nearly 150 rpm maximized biogas production, whereas at a higher speed, i.e. above 700 rpm, gas production was reduced [23]. Similarly, S. Ghanimeh et al. [87] compared the different mixing schemes in two separate digesters. First, digester “A” was mixed at 100 rpm continuously, while digester “B” was mixed before and after feeding for a few minutes. Digester “A“ produced higher methane content compared to digester “B”. The methane yield in digesters “A” and “B” was 0.60 CH_4 l/g VS and 0.45 CH_4 l/g VS respectively. At an organic loading rate of 1.9 g VS/l/d, the peak methane content was 5.29 l/d and 5.10 l/d for digesters “A” and “B” respectively. In further studies, Ghanimeh et al [83] compared mixing intensities from 50 to 160 rpm. It was observed that a higher methane yield was obtained at 50 rpm, which was 26%- 41% higher than an 80-rpm mixing intensity.

The results support the fact that lower mixing intensities can enhance methane content. In a study by B. Wang et al.[88], different mixing intensities were applied along with a combination of unidirectional and bi-directional circulation of slurry. It was observed that the methane production increased significantly when the mixing speed was increased. Methane production increased by 77% and 220% with 10 rpm unidirectional mixing and 160 rpm bidirectional mixing respectively. This was because mixing boosted sludge liquefaction, which was helpful in the transport of substrate and nutrients, and also in mass transfer in the reactor. According to Z. Tian et al. [84], a non-agitated digester showed uniform CH_4 yields, CH_4 production rates and SCOD profiles, whereas a continuously mixing digester showed lower methane production rates from Run 4,5 and 6. For the proceeding runs 4,5 and 6 the unconverted SCOD was used for inoculation, which resulted in an excessive accumulation of SCOD, hence decreasing methane content in the final run, but overall methane production was similar for both agitated and non-agitated digesters. Hashimoto et al. [67] and M. Kim et al.[14] obtained almost similar results for both continuous and intermittent mixing in terms of methane content. According to C. Rojas et al [72], a significant stirring effect on the anaerobic digestion was noted only when seed sludge from a biogas plant was used as a starter.

Fig. 10 presents the variation in methane content due to different mixing regimes. Finally, it can be deduced that non-mixing and continuous vigorous mixing have negative impacts on methane, and it is just waste of energy to continuously agitate the anaerobic digester. It should be noted that the variation in methane content between different experiments can be due to properties of substrate and other operational parameters. Moreover, the mixing also depends on the type of impeller and the shape of the digester. Finally, lower mixing intensities should be preferred, but the uniform distribution of velocity and viscosity is a very important aspect which can lead to a higher mixing time.

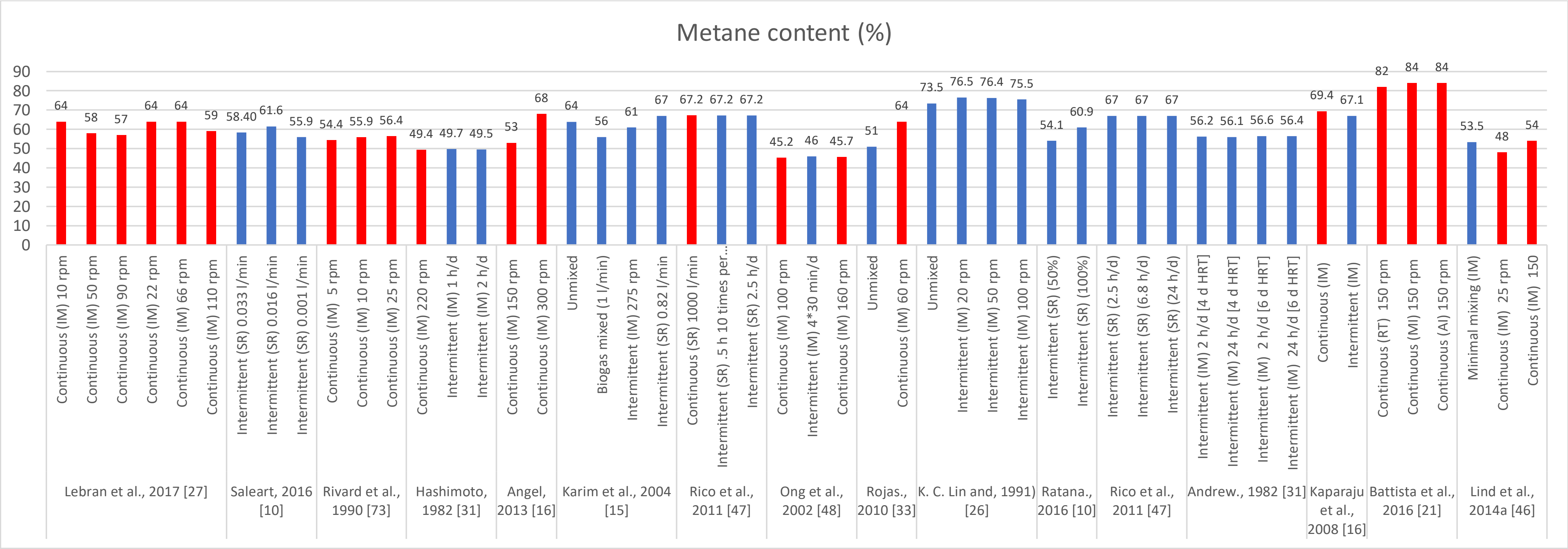


Figure 10. presents the variation in methane content (%) due to changes in the mixing intensity, mixing time and interval in the lab-scale digesters in literature. The variation among the results can be due to differences in substrate and digester geometry.

3.6.3 Effect of mixing on VFA concentration and TVS reduction

During the anaerobic digestion process, the major intermediate products are acetic acid, propionic acid and butyric acid. For an ideal anaerobic digestion, the pH range is between 6.8-7.2. Below pH 6.6 the growth rate of methanogens is greatly reduced [89] and an excessive decrease in pH can lead to microbial granule disintegration and can result in failure of the process [90][91]. Accordingly the optimum pH range for hydrolysis and acidogenesis is between 5.5-6.5 [92]. According to Drosg [93], the optimum values of the VFAs during methanogenesis should be less than 1 g/L. To determine the effect of mixing, Ratanatamskul et al [28] analyzed the production of various VFA and TVS reduction in a single-stage anaerobic digester at different mixing times of 30, 60 and 90 min/day by slurry recirculation. VFA concentration was noted as 3.5 g/l and 2.5 g/l, which were higher than the recommended values. It was observed that at a mixing time of 60 min/day, propionic acid and butyric acid were effectively converted into acetic acid, which led to the effective conversion of acetic acid into methane; this was observed as being the optimum mixing time. The significant rise in acetic acid and propionic acid was noted in the digester with intensive mixing, and this resulted in a fast diffusion from top to bottom. The feedstock, mixing intensity and the structure and volume of the reactor affect biogas production efficiency [64]. Supporting this fact, Stroot et al. [65] determined that the level of VFAs increased sharply under continuous mixing conditions because of an increase of acetate concentrations, which was due to an imbalance in the digestion process at higher mixing intensities. Increased hydrolysis and a lower growth rate of methanogens leads to higher VFA concentrations. The same results were obtained by Kim et al [94], as an intermittently mixed digester was observed to be more stable under mesophilic and thermophilic conditions, while a continuously mixed digester yielded an elevated propionate concentration and hence created an imbalance in the digestion process.

Ghanimeh et al.[83] noted that the digestion process was most stable at mixing speeds of 80 and 50 rpm as the VFA concentration was below 2 g/l. On the other hand, vigorous mixing at 120 to 160 rpm displayed an instability in the process and reduced removal efficiencies as the VFA concentrations were 3.3 and 1.8 times higher than the slower mixing. The increase in VFA levels at 120 rpm and 160 rpm was due to fact that with vigorous mixing there was damage to syntrophic microbial flocs, as noted by Suwannoppadol et al. [95]. Similar observations were noted by Latha et al. [96]. During impeller mixing at 50 and 200 rpm, the VFA concentrations were 1.2 g/l and 9.28 g/l respectively. The average VFA/ALK for 50, 100, 150 and 200 was found to be 0.15, 0.20, 0.19 and 0.28 respectively.

J. Jiang et al.[97] observed that at a certain level the higher shear rate increases the convection transfer of glucose in the direction of granules via boundary layer around the granule, creating disequilibrium in yield and utilization rates of volatile fatty acids, which give rise to accumulations within the granules. This leads to an inhibition of acetogenesis and methanogenesis because carbon dioxide and hydrogen are not fully consumed, which results in a reduction of methane content in biogas. By increasing the mixing intensity, the concentration of volatile fatty acids is increased. This work supports the idea of using a minimum mixing intensity. Under mixing conditions, lower values of pH were observed during the startup period because of an imbalance of hydrolysis, acidogenesis and methanogenesis.

In a study by Ismail et al. [98], the concentration of acetic acid and proponic acid was boosted with an increase in the Re_o from 100, 300 and 500. Minimum mixing can intensify the digestion process by ameliorating the concentration of volatile acids in the impregnable range, while at a high mixing intensity the pH drops and the digestion is interrupted, resulting in a decrease in biogas production. Rebecca A. et al. [58] analyzed four different mixing intensities, i.e., 1500, 500, 250, 50 rpm. It was observed that at 1500 rpm the concentration of volatile fatty acids was higher compared to the lower mixing intensities, and there was a negative effect on biogas production rates. This fact is supported by Sulaiman et al. [66], who analyzed the VFAs in a vigorously mixed (VM) anaerobic digester. It was observed that during VM the concentration of VFAs exceeded 3500 mg/l at the end of 13 days, which displayed the negative effect of VM on VFA utilization by methanogens. This agrees with the results of Stroot et al. [65], whose work supports the importance of minimal mixing for stability of digestion process. Z. Tian et al. [84] observed that in a continuously agitated digester at 100 rpm, the propionic acid accumulations were much higher than in the non-agitated digesters because the latter hindered the digestion process and inhibited methanogenesis. This indicated that continuous mixing declines the efficiency of anaerobic digestion. According to Lindmark et al.[99], biogas production decreased and the process was destabilized during high intensity mixing, but the results showed that VFA accumulation is not the only reason for a decline in biogas yield.

At high agitation rates, i.e., 50 and 90 rpm with a helical ribbon impeller and 110 rpm with a Rushton turbine, a high pH increase was noted during peak production [47]. For Re of 100 and 300, the pH was observed to be stable (between 6.8-7.5), but at an Re of 500 the pH started to decrease gradually from 6.8 to 4.7-5.3 in 4 days. It is concluded that mixing effects the pH values in an anaerobic digestion process, so it is necessary to control the mixing intensities during the agitation. Mixing is to be optimized so that a homogeneity of the mixture is maintained within the prescribed limits of the mixing intensity in order to control the pH levels in the digester [98]. Kaparaju et al. [13] observed low and delayed methane production for vigorous and continuous mixing in batch experiments because the methanogenesis was inhibited due to a homogenous distribution of volatile fatty acids in the digester. Propionate was consistently produced during vigorous mixing but was not consumed at the same rate. So, when the mixing scheme was shifted from vigorous to gentle mixing conditions, propionate was quickly consumed, and the digester attained a stable condition. According to Andrew G. et al. [67], there was a significant difference in biogas yields during continuous and intermittent mixing for HRT of 4 days. However, at HRT of 6 days the performance of both mixing regimes was the same. Moreover, mixing has a significant effect on digesters with a shorter HRT because, by mixing, the fresh substrate is introduced to microorganisms at a faster rate and reaction time decreases. Mixing does not affect the performance of digesters with a longer HRT[45][71].

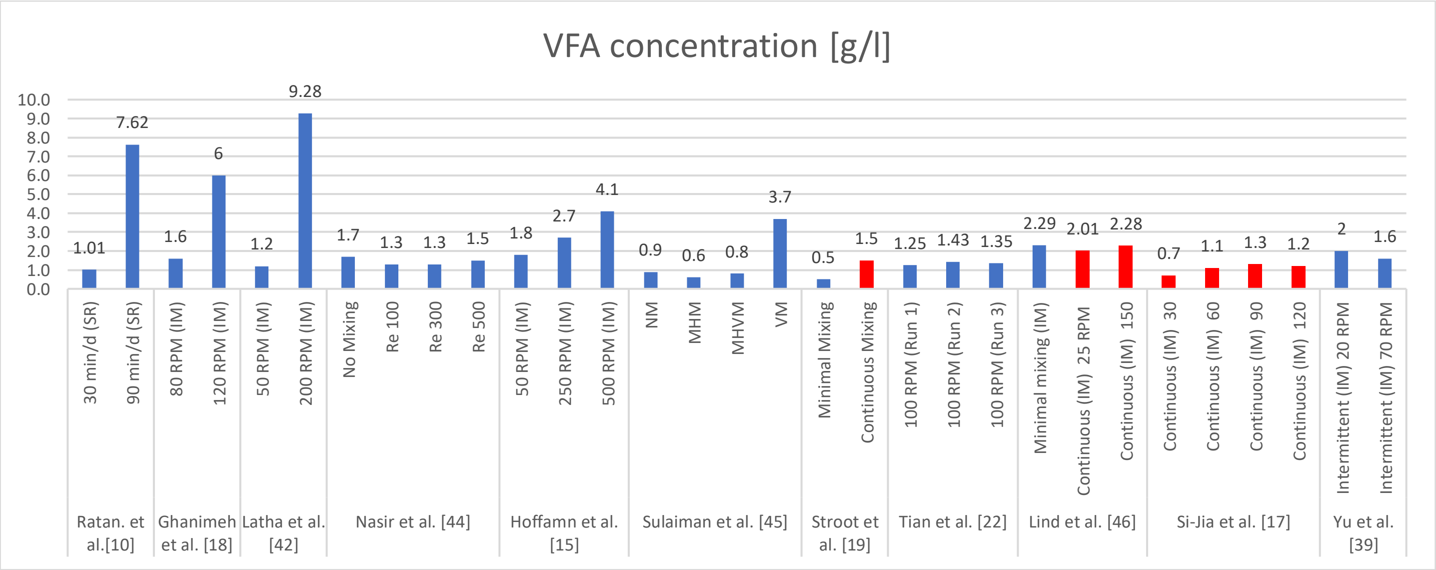


Figure 11. presents the effect of different mixing strategies on accumulation of TVFAs in the anaerobic digestion process. It can be clearly noted that at higher mixing intensities the concentration of VFAs increases rapidly.

3.7 Factors affecting mixing in an anaerobic digester

3.7.1 Effect of viscosity, shear stress and TS content

Rheological behaviour and bioreactor hydrodynamics are the key parameters that determine the efficiency of any mixing equipment in an anaerobic digester[47]. Rheological properties of slurry at various temperature, TS content, type of substrate have been studied extensively by many researchers [27,32,75,100–106]. According to studies, cattle manure and waste water sludges are non-Newtonian fluids because there is no linear relation between their shear rate and shear stresses [107]. Among all the factors which influence slurry rheology, TS content is closely associated with apparent viscosity. In comparison, high TS content results in a high viscosity of liquid, which requires greater stirring effort to achieve the same level of mixing. The impact of mixing seems also to depend on the type of waste fed into the system, as different substrate composition can lead to different microbial setups with varied tolerance, and differences in the abundance of toxins and inhibitors. It has been observed that the impact of mixing on biogas generation is perceptible only at higher TS concentrations (<10%) [69]. Figure 12 presents the behaviour of different fluids when shear stress is applied. Achkari-Begdouri [108] demonstrated the rheological properties of cattle manure at a TS content in the range of 2.5-12% TS and a temperature range of 20°-60°. It was observed that lowering TS content and a higher temperature make cattle manure slurries behave more like Newtonian fluid. This fact is supported by S. Baroutian et al.[109], who demonstrated that for waste water sludges temperature and solid concentration are critical parameters that influence mixed sludge rheology. It was noted that shear stress increases non-linearly with shear rate and decreases with an increase in temperature because at higher temperatures cohesive forces between molecules are reduced.

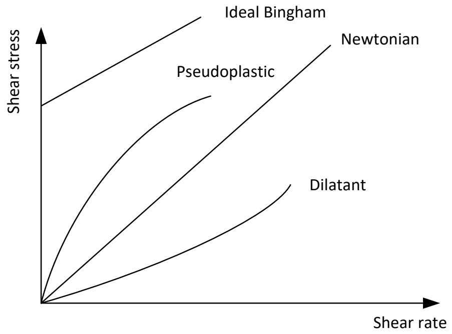


Figure 12. Different types of fluids and their behaviour with respect to shear rates.

Furthermore, apparent viscosity decreases with an increase in temperature. The effect of temperature on apparent viscosity can be evaluated with the Arrhenius model [110].

$$\mu = A\, exp^{(\frac{Ea}{RT_{abs}})} \tag{3}$$

Grinding plays a vital role in determining the viscosity of slurry. It has been noted that the fine particles dramatically reduce slurry's apparent viscosity. Accordingly, grinding solid manure before feeding it into the digester will decrease the mixing cost and enhance mixing and digestion efficiency [104]. Another method to decrease viscosity is filtration, which results in an improvement of the mixing efficiency of a Rushton turbine. These methods are beneficial from a rheological point of view, but on the other hand it also leads to an increase in the overall operational costs of methane production [49].

A study by J. Jiang et al.[97] revealed a close relationship between mean biogas production rates and mean methane content on the one hand, and the hydrodynamic shear rate in a digester on the other. The experiment was conducted in 450 ml CSTR mixed by helical ribbon at rotational speeds of 12, 18, 24, 36 and 60 rpm. Initially, the mean biogas production rate and mean methane content increased to their highest rate with an increase in shear rate but later decreased continuously. The maximum methane production was noted when a shear rate of 6.8 s^{-1} was applied. L.F.R Montgomery et al. [111] studied the rheological behavior of slurry consisting of agricultural residue. Various rheological models were developed, such as the Casson model, the power law and the Bingham plastic model. A non-Newtonian fluid character of slurry does not possess a constant viscosity but has rather an apparent viscosity (η_a), which can be calculated using Equation 5.

$$\eta_a = \frac{\tau}{\gamma} \tag{4}$$

Studies have revealed that apparent viscosity increases at very low shear rates, resulting in more power consumption. At a shear rate of 18 s^{-1} the apparent viscosity was 12.1 Pas and decreased to 6 Pas in the following 5 hours. At a shear rate of 36 s^{-1} the apparent viscosity dropped from 8.0 Pas to 4.7 Pas, which proved that apparent viscosity and non-Newtonian behavior is strongly time dependent.

Y. R. Chen [101] observed that at TS 2.84% slurry behaved like Newtonian fluid, whereas for TS>2.8% the behavior was non-Newtonian. It was concluded that the values of limiting viscosity (η_o) and the consistency index (k) increased as TS increased, and, on other hand, decreased as temperature increased. Similarly, Kumar et al. [112] demonstrated the fluid properties of animal waste slurries. The Power law was used to predict the k at a constant shear rate of 30 s^{-1} with respect to TS.

B. Wang et al. [88] demonstrated the properties of anaerobic inoculum and dewatered sludge which was collected from a sewage plant in Sweden. The viscosity and the flow curves of substrate showed shear thinning, yield stress, and non-Newtonian behavior. The minimum viscosity was observed at the top mixing speed (160) rpm, which was bidirectional.

Various numerical models have been developed by researchers to evaluate different aspects of the characteristics of slurry. The Power law model [100][113][112] was developed to determine the rheological characteristics of the medium based upon the local shear, dry matter content, and mean fiber length.

$$\tau = k \cdot \gamma^n \tag{5}$$

In a study by B. Wang et al., the Herschel Bulkley model (Eq. 6) was used to change rheogram data to the rheological behavior of fluid [88].

$$\tau = \tau_o + k \cdot \gamma^n \tag{6}$$

According to Doran et al., due to the non-Newtonian behavior of slurry, the turbulence during mixing is reduced and stagnant zones inside the digester are formed. It is necessary for the flow to be turbulent to ensure effective mixing and interchange of material between different locations, but on other hand, turbulent mixing can break the bacterial/archea morphology.

Power consumption for agitation depends directly on the fluid properties. Power consumption by an impeller can be related to the viscosity of slurry as proposed by Y. R. Chen et al[114]. According to the study, the higher the viscosity, the higher the power dissipation to attain homogeneous mixing. According to Keanoi et al. [115], higher biogas production is observed with mixing at higher levels of TS. There was a reduction in velocities in the vessel with increasing solid content due to increased viscosity and additional energy is required. Doubling the solid content increased the dead volume ninefold. At lower levels of total solid content, there is no impact of mixing on biogas yield. In one study, the mixing speeds were altered from unmixed to 100 rpm at TDS of 2.5, which resulted in no variation in biogas production, but at a TS of 5.4%, dead volume increased dramatically [22]. Higher mixing results in a waste of energy when the digester is fed with lower TS content.

Clarke & Greenwood [116] developed a formula to calculate the rpm of an impeller to generate specific shear rates. Accordingly, if the optimum shear rate values are known, the mixing speed of an impeller can be adjusted, which will depend on the geometry of the digester and the impeller. First, the rotational velocity has to be determined as per shear rate using Equation 7.

$$\omega = \frac{\gamma(r^d - r^i)}{r^i} \tag{7}$$

Furthermore, the rpm can be calculated as per Eq. 8

$$n = \frac{\omega \cdot 60}{2\pi} \tag{8}$$

The ability of movement of particles is reduced when solid particles increase and hence particles mix within the flow field [117]. According to Karim et al. [69], mixing is valuable only when the total solid content is greater than 10%. Results demonstrated that the mixed digesters produced 10-30% more biogas than unmixed digesters at higher TS content values.

Average velocity gradient helps to better understand mixing operations in the slurries. C. Ratanatamskul et al. [28] introduced a new parameter called the mixing intensity number. This parameter characterizes the velocity gradient and mixing time using the following equation (9).

$$G \cdot T_m = mixing\ intensity\ number \tag{9}$$

According to R. Sindall et al.[62], the threshold for an average velocity gradient lies between 7.2 to 14.3 s^{-1} to produce the maximum biogas yield[48]. Rivard et al. [60] observed that there was no significant difference in biogas production between mixing intensities of 1 and 25 rpm for a high organic loading of up to 9.5 g VS/d and TS content of 5% to 36% in a 20 l digester. A higher mixing intensity was just a waste of energy. At a concentration of 10% TS w/w, the fluid possessed a viscosity of 0.14-0.18 Pas, which resulted in inadequate mixing by RT, although the results were better at a TS concentration of 6%, in which viscosity was 0.06-0.08 Pas [49]. In research by Binxin Wu [54], six different values of TS content were examined (TS=0, 2.5%, 5.4%, 7.5%, 9.7% and 12.1%) for various mixing speeds (N= 400, 450, 500, 550, 600, 650, 700, 750 rpm). It was observed that with an increase of propeller speed, mixing intensity increases, but on other hand, poor mixing was observed at higher TS values. Furthermore, at a TS of 12.1%, dead volume was measured to be 87%, which demonstrates poor mixing at higher viscosity. The average velocity of fluid in a tank increases linearly, while the mixing energy level increases exponentially with an increase of rotation speed at a constant level of TSC. The effect of increasing solid content is significant on mixing characteristics and can be demonstrated in terms of velocity magnitude and volume of stagnant zones. Before optimization of mixing strategies, the value of solid content should be considered as it will effect the design of both the impeller and the digester.

The literature shows that high ORL can lead to the destabilization of a digester, which results in an increase of VFA and decrease of biogas yield. The failure of a digester due to high OLR can be avoided by optimized mixing. Intermittent mixing is enough to increase the efficiency of anaerobic digestion, whereas natural mixing involves evaluating the gas during digestion, and it can be controlled by feeding. When OLR is constant, natural mixing occurs at 6.4 $kgm^{-3}d^{-1}$ [118].

It is concluded that the study of rheology is a very important aspect in designing an anaerobic digester. Slurry with higher viscosity faces problem of uneven velocity distribution within the digester. Higher mixing intensities and the resultant shear stress have a negative effect on flock formation and can reduce gas production. In a mechanically mixed digester, the shear rate near the impeller blades is very high but at distances away from blades the shear rate is relatively low, resulting in high apparent liquid viscosity and poor mixing. The optimum designing of a digester and impeller according to the rheological properties of slurry can help in a more uniform distribution of shear stress and viscosity, requiring less mixing time and minimum power consumption by agitating equipment. Hence, it can lead to lower capital investment and operational costs. Here, it should also be noted that the rheological data given in the literature can vary due to animal diets, manure treatment and handling, and measurement inaccuracies. Slurry possesses shear thinning behaviour because viscous forces are very sensitive to shear rate distribution during mixing. Moreover, to predict the rheology and behaviour of slurry, the composition of the slurry must be well understood.

4 Methodology

4.1 Inoculum feeding and substrates

The substrate consisted of a mixture of pig slurry (25% w/v) and chopped sweet sorghum (75% w/v). Fresh sweet sorghum was collected from plants and was chopped to particle length of less than 5 and stored frozen at -20°C. Sewage sludge for the lab-scale experiment was collected from a commercial biogas plant in Szeged to initiate the fermentation. The experiment was pre-run for at least 2 weeks to have the stable digestion process and constant biogas production.

Ultrapure nitrogen gas is used to spurge the system in the beginning of the experiment. The substrate was stored at ambient temperature and mechanically pre-treated by shredder pump. The digester was continuously fed with 5 gVS l^{-1} of cellulose every day. The digester is operated at the mesophilic temperature range (50°C) and HRT of 15 days.

The sludge is composition of 70% primer secondary sewage sludge 30% organic sweepings. Various characteristics of sewage sludge are demonstrated in Table 4. The TS content of slurry was maintained at 12.5%.

Table 4. Characteristics of sewage sludge from wastewater treatment plant in Miskolc.

Parameter	Value range
TS (%)	4.28
SS (g l^{-1})	57.8$\pm$10.0
Total carbon (%)	46.2
TVS (g l^{-1})	87.6$\pm$3.4
COD (g l^{-1})	141$\pm$6.4
VFA (g l^{-1})	4.15$\pm$1.38
pH	8.6
ρ (kg m^{-3})	1068
HRT (d)	15

Table 5. Detailed composition of substrate.

Type of feed stock	**Organic content**	**C:N ratio**	**DM %**	**VS % of DM**	**Biogas yield m^3kg^{-1} VS**	**Unwanted physical matter**	**Other unwanted matter**
Pig slurry	Carbohydrates, proteins, lipids	3-10	3-8	70-80	0.25-0.50	Wood shavings, bristles, water, sand, cords, straw	Antibiotics, disinfectants

4.2 Analytical methods

4.2.1 Gas analysis

Gas volume was measured continuously by means of direct mass flow controllers (DMFC, Brooks Instruments) attached to each gas exit port. Data collected from the digesters was stored in computer system by special software developed by Merat Ltd. Budapest, Hungary. Biogas composition was analyzed gas chromatograph (6890N Net-work GC system, Agilent Technologies). 250 µL sample was collected from the head space and injected into gas chromatograph equipped with 5Åmolecular sieve column (length 30 m. I.D. 0.53 megabore, film 23 µm) and thermal conductivity detector.

4.2.2 Volatile fatty acids

Volatile acids were determined by HPLC (Hitachi Elite, equipped with an ICSep ICE-COREGEL 64H column and a refractive index detector L2490), under the following conditions: solvent 0.1 N H_2SO_4, flow rate 0.8 mL min−1, column temperature 50∘C, detector temperature 41°C.

4.2.3 TS content and volatile content measurement

The quality of the dry matter was analysed by drying the substrate at 105°C for 24 hours and measuring the residues. Further heating of this residue at 550°C in the oven until its weight did not alter the overall organic solid material.

4.2.4 Statistical analysis

Statistical analysis was performed in Microsoft Excel using Student's unpaired t-Test, with a two-tailed distribution and in PASS using a permutational multivariate analysis of variance (PERMANOVA). The T test was performed in Microsoft excel for obtaining t values. Tests were performed between the same digesters at different rpm i.e at F1R10, F1R30, F1R67. Further, the tests were also undertaken between all three digesters at one particular rpm i.e F1R30, F2R30, F3R30 and so on.

4.3 Mixing operation

4.4 Rheology

Rheological study of slurry for anaerobic digestion process is very important aspect to design the digester, mixing and transport equipment. From the literature data it is confirmed that if TS>2.5% then the slurry possesses non-Newtonian shear thinning behavior and thixotropic characteristics in the laminar regime (approximately < 10-100).

For this instance, the power law model can be proposed to calculate the apparent viscosity and shear rate.

$$\mu_a = K \cdot \gamma_a'^{(n-1)} \qquad (10)$$

For a non-Newtonian shear thinning the value of n is always less than 1. For this instance, the rheological data for the waste waste sludge is taken from the literature presented in Table 6 (Figure 13) [119].

Table 6. Rheological properties of substrate.

Temperature (°C)	K (Pa s^n)	n	y (s^{-1})	η (Pa s)	ρ
37	0.19	0.56	0.237	0.01-0.03	1000.78

The average shear rate inside the vessel can be calculated as per the equation

$$\gamma_a' = k_s \cdot N \qquad (11)$$

Here k_s is Otto-Metzer constant which is directly associated with the impeller geometry. From the experimental measurements by Zhang et al. [120] value of k_s for helical ribbon impeller was k_s=34.8.

$$\tau = K \cdot {\gamma_a}'^{(n)} \tag{12}$$

Reynolds number represents the ratio of inertial forces to the viscous forces which determines whether flow is laminar or turbulent.

$$R_e = \frac{\rho \cdot N \cdot D^2}{\mu_a} \tag{13}$$

4.5 Power consumption

The relationship between speed of impeller, rheological characteristics and Reynolds number can be expressed by the following equation.

$$N_P = \frac{P}{\rho \cdot N^3 \cdot D^5} \tag{14}$$

Location of impellers in the vessels have a significant effect on the power consumption. Power consumption in multi impeller vessel is inversely proportional to the inter impeller spacing and impeller-bottom clearance. According to Gogate et al[121] if the inter impeller spacing is within range of $0.5<C/d<1.5$ the flow patterns generated by each of the impeller effects each other resulting in decrease in overall power consumption. On other hand if the inter impeller spacing is more than twice the diameter of impeller the power consumption also double as compared to single impeller.

4.6 Rheology of slurry

If shear stress is accepted as having the potential to disrupt the microbial communities that produce CH_4, the rheology (Fig. 14) of a substrate may have an impact on CH_4 production because:

- The levels of shear stress experienced by dairy farm slurry when pumped/mixed are primarily influenced by the solids content and temperature of the slurry, as well as the rate and length of time that the slurry is pumped/mixed. If recovery from thixotropy is permitted, rest periods associated with irregular mixing have an effect on the shear stress caused when mixing resumes.
- The only variable that has a contradictory impact on shear stress and apparent viscosity is increasing shear rate, which may be especially important when deciding between substrate homogeneity/heat distribution and minimizing microbial shear stress.
- Higher process temperatures reduce the shear stress to which microbial communities are exposed, especially when the percent TS is higher, the reduction of shear stress that occurs in slurry as temperature rises can have a positive impact on CH_4 development. As a result, thermophilic bacteria can experience up to 30% less shear stress than mesophilic bacteria.
- Optimal mesophilic and thermophilic temperatures are at or above 37°C, below which the rate of change of shear stress per degree of temperature change is much greater. As a result, microbial communities in lower temperature environments can experience more stable levels of shear stress during minor temperature fluctuations. However, these advantages may be minor as opposed to the possible benefits of running machines in a less viscous atmosphere when operating at a higher temperature.
- Conditioning's thixotropic effects can have a significant impact on the amount of shear stress that microbial species are exposed to. Shear stress levels post-shear are roughly 24 percent lower than those encountered during pre-shear conditions and 34-53 percent lower than Initial values

at mesophilic temperatures, for example (higher percentage reduction achieved at lower shear rate). Of necessity, the latter must be encountered before the former can be realized, but once accomplished, constant mixing can be encouraged to maximize the benefits of reduced shear stress. In contrast, due to changes in the viscosity of the fluid after resting, irregular mixing could occasionally expose microbial communities and mixing components to relatively high levels of shear stress. This could boost CH_4 productivity because microbial communities would be less stressed if the process was mixed constantly rather than intermittently. This may be especially important if an intermittent mixing regime requires lengthy periods of dormancy to enable the fluid to regain its viscosity.

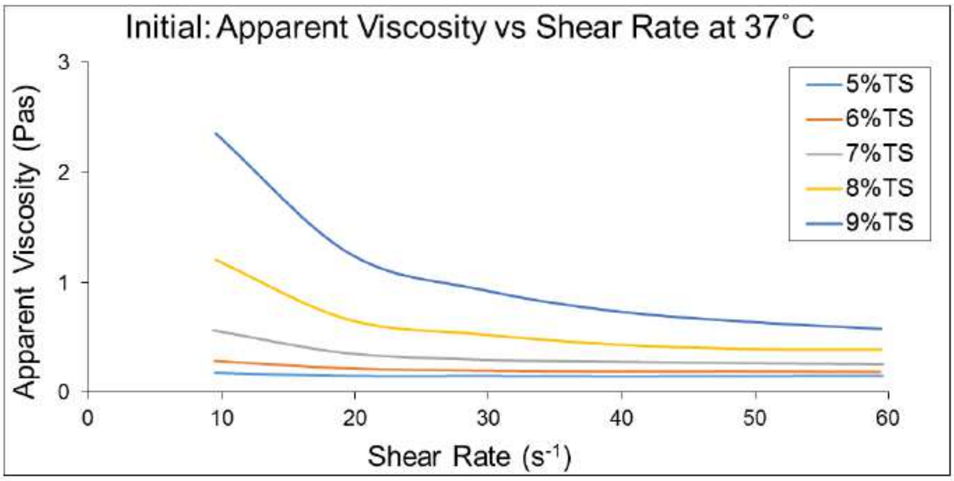


Figure 13. Effect of shear rate on apparent viscosity of slurry.

4.7 Experimental setup and procedures

The experiments were carried out in 3 parallel single stage continuously fed 5l lab-scale digester with head space of 1L custom-made from stainless steel by Biospin Ltd. Szeged, Hungary. These digesters were run under similar operating conditions of temperature and mixing speeds for one set of experiments. The schematic 2-D diagram of the experimental setup is shown in Fig 14. The reactors are equipped with helical ribbon impellers on single vertical shaft driven by variable speed motor to attain mixing. The key parameters (temperature, mixing speed and pH) were automatically controlled by computer software. The digesters were named as B1F1, B1F2 and B1F3 for the reference. All the impellers are operated by same electric motor in order to maintain identical mixing conditions. Fig. 15 illustrates the geometry and location of the impellers. The digester is equipped with 12 DC motor with all the controls to adjust the rpm of agitator and power consumption. The temperature in the reactor is maintained by the circulation of hot water through stainless steel pipes inside the vessel from an electrically heated thermostatic water bath with an accuracy of ±0.5 °C. Three sets of experiment were conducted parallelly to recognize the effect of varying shear rates on biogas production rates and methane content. The experiment lasted for 45 days including two weeks of pre-run phase. The agitation rate for R1, R2 and R3 was 10, 30 and 67 respectively. Intermittent mixing was applied for these experiments as supported by the literature. For this purpose, impellers were turned on for the period of 5 min every 1 hour.

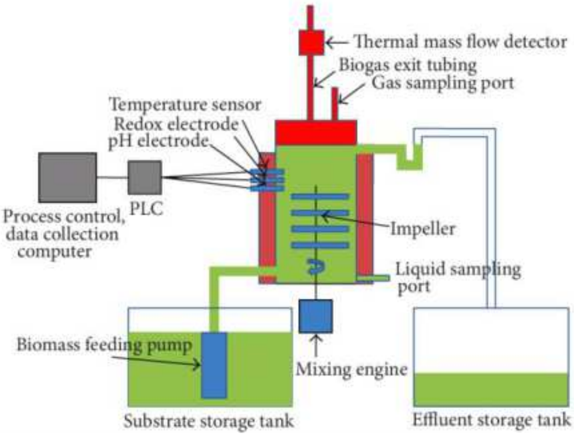


Figure 14. The schematic 2-D diagram of the experimental setup.

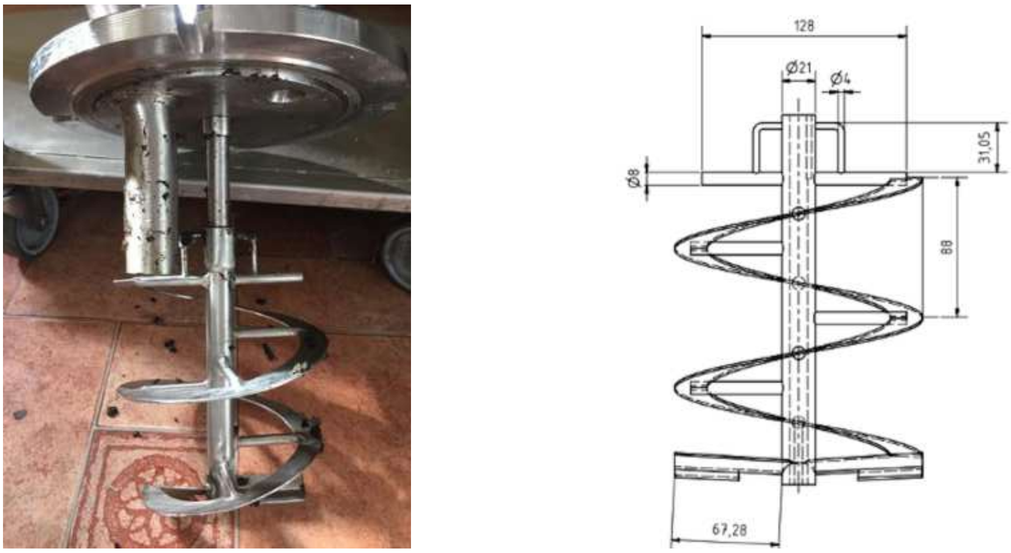


Figure 15. Representation of the geometry of the mixers inside the digesters.

Table 7. Geometrical specifications of experimental setup.

Parameter	Dimensions [mm]
Diameter of tank (D)	260
Height of liquid (H)	232
Diameter of impeller (*d*)	150
Height of blade (*h*)	15
Length of blade (*l*)	20
Off bottom clearance (C_1)	50
Inter impeller spacing (C_2)	88
C_1/ d	0.9
C_2/d	1.2

5 Results and discussions

5.1 Effect of mixing intensity on anaerobic digestion process

5.1.1 Effect of mixing intensity on biogas yield

All the three digesters were seeded with 5 l of incubated manure substrate solution. For first 14 days the digesters were fed with 5 g of cellulose until the digestion process became stable. The cumulative biogas production by all three digesters is given in Fig 16. The agitation rate values selected for each phase were chosen a priori to obtain comparable mean and maximum shear stress values for each configuration, enabling a more rigorous comparison of the three systems. Each of them has its own distinctive character. Table 8 represents the analytical data measured during the experiment. Fig 17. clearly states the difference in biogas production under various stirring rates. All the digesters exhibited comparable biogas production rates as slow agitation improved system stability through 1) reduced VFA's accumulation from 7.872 g HAc/l as compared to 4.634 g HAc/l, 2) lower propionate content of 0.456 g/l, and 3) enhanced VFA to alkalinity ratio (α) to 0.3. As a result, start-up of the digestion process was quite smooth and stable. During the first week there as negligible difference between the biogas production at all intermittent mixing intensities. It is therefore postulated that slow mixing helps to improve the stability and loading capacity of thermophilic digesters that treat substrates in the absence of an acclimatized seed. Similarly, Lin and Pearce [57] demonstrated that methane production was higher during intermittent mixing when compared to unmixed digester and on other hand study by Z. Tian et al.[84] proved that the continuous mixing resulted in declined biogas production rates[61,84]. From day 15 to 31 during the minimum mixing speed of 10 rpm lower biogas production was observed due to higher VFA's concentration and instabilities in AD process. The mean biogas production per day during these two weeks was recorded as 2.622 ml d^{-1} and overall cumulative volume of biogas produced during this period was 43.5 l. Further from day 32 to 48 the rotational speed of the mixer as increased to 30 rpm. Under these operating conditions the mean BPR was recorded as 2.85 l d^{-1} and the total biogas production under these conditions was 45.2 l. At both loading rates and shock rates the biogas production was higher at 67 rpm as a raise in rotational speed up to certain level is beneficial for decreases the mixing time and enhances heat, mass, nutrient homogeneity [122], efficient dispersion of metabolic waste, reduction on particle size due to shear forces and improvement in hydrolysis process. Mean BPR and total volume at higher mixing was noted as 3.2 l d^{-1} and 52.5 l respectively. The results demonstrated that there was 15-18 % higher biogas production at 67 rpm as compared to the slower mixing speeds. Fig 18. Represents the mean biogas production per day by all three digesters at various rotational speeds.

In fig 18. it can be clearly seen that all the three digesters (F1R67, F2R67, F3R67) at 67 RPM produced higher amount of biogas as compared to 10 rpm and 30 rpm. This study counters the results demonstrated by Hoffmann et al. [58] where it was proved that different mixing intensities (1500, 500, 250, 50) have no effect on the efficiency AD process. On other hand at higher mixing intensities the *Methanosarcina app*. and *M. concilii* were found abundant which also supports the fact that mixing intensities provides favourable environment for acetolactic methanogens. Moreover, intermittent mixing didn't destroy microbial flocs which apparently gave positive results in long term performance of a digester. Shear rate was noted as 5.6, 17.4, 38 s^{-1} at 10, 30 and 67 rpm according to equation 2. The results show close proximity with the study by Jiankai et al. where proposed optimal values for shear rate were between 28 to 48 s^{-1}. But on other hand same author reported in another study that under continuous mixing regime the optimal shear rate should be 6.8 s^{-1} for maximum biogas production. We obtained quite similar results statically as compared to study by Lebranch et al.[47].

According to our study the hydrodynamic shear (γ'_a) threshold is 39 s^{-1} which resulted in highest biogas production without disruption of microbial flocs. Additionally, the small scale of the digester in a lab is insufficient to answer all the questions related to mass transfer and mixing efficiency that can may rise at large scale biogas plant. For instance, for a large scale digester, the rotational speed of an impeller can be different to achieve homogenisation in terms of nutrient, temperature and dispersion of fresh substrate [123]. Finally, it can be concluded that geometry of the impeller as well as the digester will decide the optimal rotational speed of mixer along with consideration of rheological behaviour of the slurry.

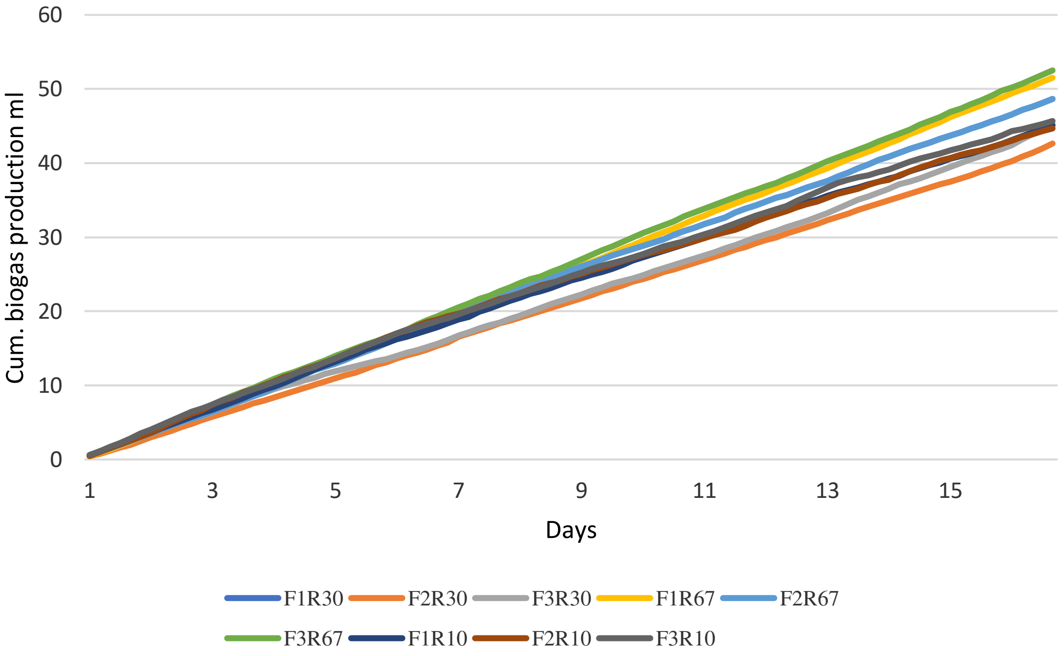


Figure 16. Cumulative biogas production rates in all three digesters.

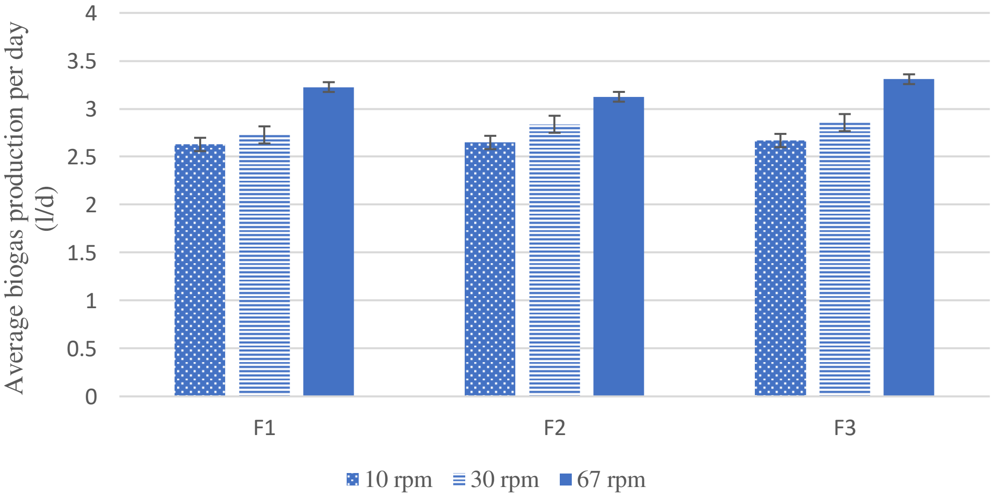


Figure 17. Average biogas production per day by all the digesters at 10, 30 and 67 rpm.

.Table 8. Represents the analytical data measured during the experiment.

	Batch 1 (10 rpm) (Period 15-30 days)			Batch 2 (30 rpm) (Period 31-48 days)			Batch 3 (67 rpm) (Period 48-64 days)		
Fermenters →	F1	F2	F3	F1	F2	F3	F1	F2	F3
Total Biogas production	45.1	44.2	43.5	48.6	42.6	45.2	51.5	48.6	52.5
VFA's (g/l)	7.3	6.1	5.8	3.1	3.8	4.9	1.1	1.9	2.2
pH	7.4	7.1	7.9	8.2	8.1	7.9	8.3	8.1	8.0
NH_4^+-N (g/l)	0.95	0.93	1.15	0.78	0.81	0.75	0.62	0.71	0.59
FAS/TOC Ratio	0.35	0.69	0.40	0.25	0.54	0.59	0.19	0.34	0.41

5.1.2 Statistical data analysis

Statistical analysis revealed that the methane production was consistently significant with $P < 0.05$ by Student's t-test. First of all, the t test was performed during the experiments on data from the all three digesters at identical rotational speed. The results proved that the biogas production rates from all the digesters at identical speed was similar as the p-values were above 0.05. The p values at identical impeller speed lies between 0.08 to 0.66 whereas values at different mixing speeds were noted lot below $p<0.05$. Table 9. represents the statistical analysis of biogas production at different mixing speeds. It can be clearly seen that the biogas production rates have quite significant difference between various mixing speeds.

Table 9. Represents the statistical analysis of biogas production at different mixing speeds.

	Data set			p values
10	F1	F2	F3	0.66454
30	F2	F3	F3	0.561287
67	F1	F3	F3	0.084101
F1	10	30		0.0032712
	30	67		1.0968E-12
	10	67		6.65976E-26
F2	10	30		0.00138614
	30	67		7.69224E-10
	10	67		0.001386142
F3	10	30		0.01789452
	30	67		2.47333E-12
	10	67		2.49809E-18

5.1.3 Effect of mixing intensity on VFAs

During the anaerobic digestion process the major intermediate products are acetic acid, propionic acid and butyric acid (Fig. 19). For an ideal anaerobic digestion, the pH range is between 6.8-7.2. Below pH 6.6 the growth rate of methanogens is greatly reduced [89] and excessive decrease of pH can lead to microbial granule disintegration and can result in failure of process [90][91]. So, accordingly the optimum pH range for hydrolysis and acidogenesis is between 5.5-6.5 [92]. According to Drosg [93] the optimum values of the VFAs during the methanogenesis should be less than 1 g/l. To determine the effect of mixing Ratanatamskul et al [28] analyzed the production of various VFA and TVS reduction in a single stage anaerobic digester at different mixing times of 30, 60 and 90 min/day by slurry recirculation. VFA concentration was noted as 3.5 g/l and 2.5 g/l which was higher than recommended value. It was observed that at mixing time of 60 min/day propionic acid and butyric acid were effectively converted to acetic acid which led to effective conversion of acetic acid to methane and which was observed as optimum mixing time. The significant rise in acetic acid and propionic acid was noted in the digester with intensive mixing which resulted in fast diffusion from top to bottom. The feedstock, mixing intensity and the structure and volume of reactor effects the biogas production efficiency [64]. Supporting the fact Stroot et al. [65] determined that level of VFA's sharply increased during continuous mixing conditions because in increase of acetate concentrations which was due to imbalance in digestion process at higher mixing intensity. Increased hydrolysis and lower growth rate of methanogens leads to higher VFA concentrations. Same results were obtained by Kim et al [94] as intermittently mixed digester was observed to be more stable under mesophilic and thermophilic conditions whereas continuously mixed digester gained elevation of propionate concentration and hence created imbalance in digestion process.

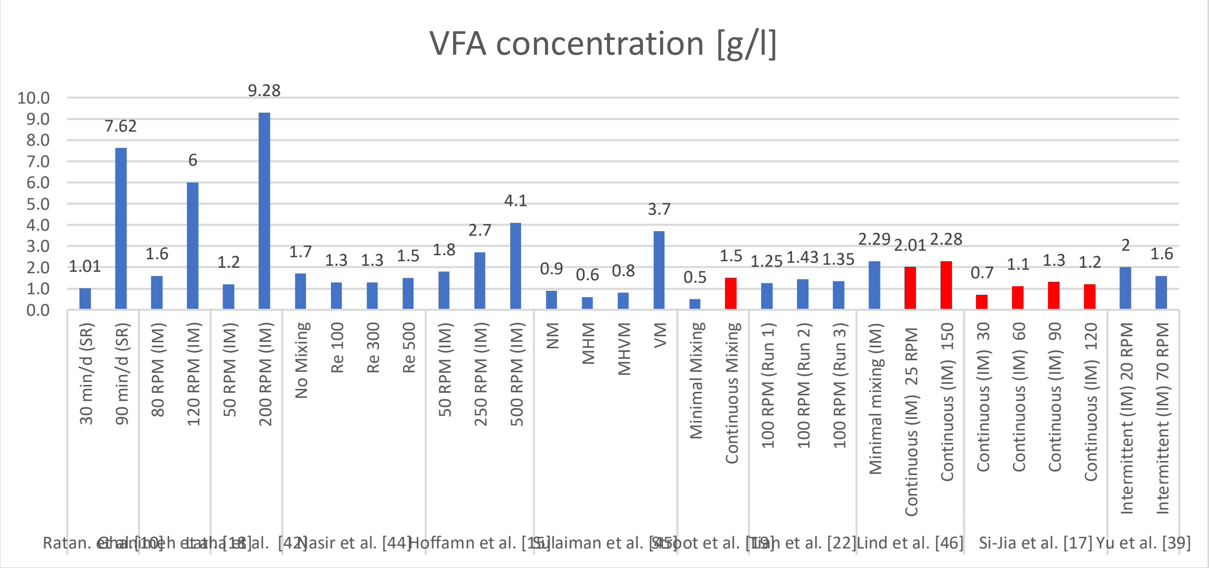


Figure 18. presents the effect of different mixing strategies on accumulation of TVFAs in the anaerobic digestion process. It can be clearly noted that at higher mixing intensities the concentration of VFAs increases rapidly.

VFA concentrations were measured continuously during the digestion process and serves as indicator in terms of stability/instabilities of digester in many studies [83][58]. But this study does not support the idea that at higher mixing destabilisation of AD process occurs due to accumulation of VFA's. The main intermediate products are acetic acid, propionic acid and butyric acid during the AD process and the pH range for optimal anaerobic digestion is between 6.8 and 7.2 [17]. Apparently, the growth rate of methanogens is significantly reduced below pH 6.6 and an extreme decrease in pH will contribute to the disintegration of microbial granules and the breakdown of the mechanism. For a stable AD process the FOS/TAC should be in range of 0.3-0.4 [124]. During the pre-run period the pH of F1, F2 and F3 was noted as 8.6, 8.1 and 8.1 respectively. FOS, TAC and ratio FOS/TAC was measured as 1.1, 0.2 and 0.15 respectively. Initially VFA's concentration recorded was 6.8-7.2 g HAc/*l* during the startup. Whereas, VFA concentration was stabilised at 1.5 -2.8 g HAc/*l* after one week of operation. At the minimum mixing of 10 rpm the was observed between 0.3 to 0.6 with average VFA levels of 7.4 g l^{-1}. Similar trend was observed by Ghanimeh et al. [83] where slower mixing resulted in enhance acidic levels to

15.6 g l^{-1} at minimum mixing. Furthermore, after increasing impeller rotational speed from 10 to 30 rpm significant change in VFA's level was noted down within range of 3.1 to 4.9 g l^{-1}.

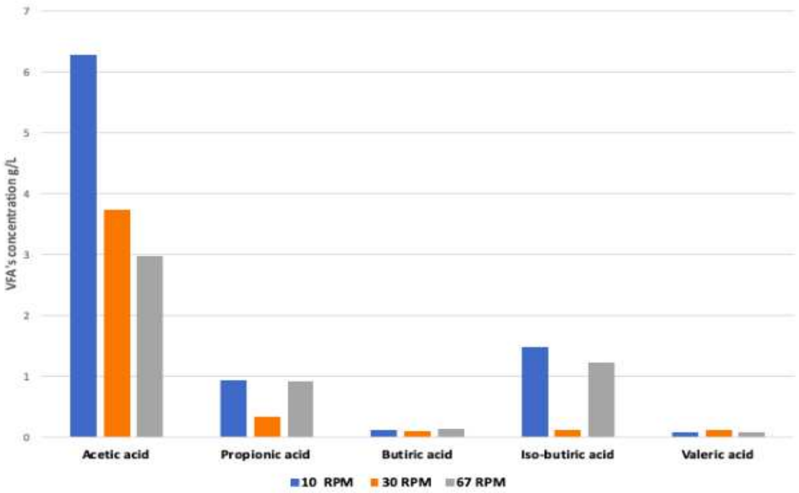


Figure 19. VFA's concentration at different mixing intensities.

The results indicated that VFA's degraded at higher rate at higher mixing intensities (67 rpm). Raised ph values were noted during higher VFA content during start-up up to 8.5 and later stabilised to 7.8. Results also demonstrate that either the VFA's degradation is more rapidly at 67 rpm or the production is slower after the overload and feeding. Methanogenic activity can be reduced by dispersion of VFA's at high mixing intensities as it can effect establishment of methanogenic zones [81]. 67 rpm mixing intensity lead to reduced production of VFA which contributed to the high biogas production of the 10 and 30 rpm mixing speed. Raise in VFA's concentration lead to damage of microbial flocs along with reduction of removal efficiencies. The pH value stayed in the range 7.8–8.2 throughout but fell gradually over the course of the experiment. Higher shear enhances the convective transport of glucose to granules via the boundary layer around the granule, while the mass transfer within the granule for further acetogenesis and methanogenesis is still regulated by low molecular diffusion. As a result, imbalances in the output and consumption rates of VFAs arise and contribute to aggregation within granules. The VFA's and pH values are summarised in Fig. 19 & 20. Ammonia is produced by the biological degradation of the nitrogenous matter, mostly in the form of proteins and urea. Several pathways for inhibition of ammonia have been suggested, for example a change in intracellular pH, rise in energy demand for maintenance and the inhibition of a particular enzyme reaction. The average ammonia concentration was recorded between range of 0.71-0.93 g/l during the whole experiment. The results insinuate that mixing is compulsory when the VFA levels increase to disperse the localised inhibiting environments.

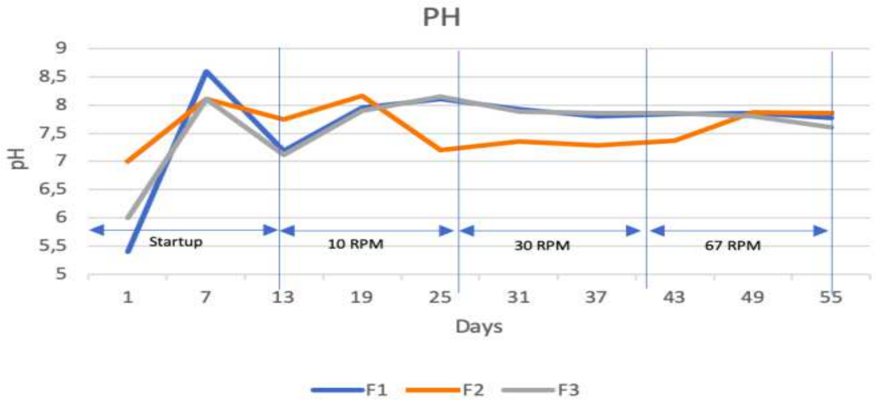


Figure 20. pH during the experiment under various mixing intensities.

5.1.4 Conclusion

The mixing intensity (shear rate) and the length of time that shear rate is applied by an effective mixing system defines the degree of mixing achieved. The uniform shear rate can be considered as a tool to achieve stability of digestion biodegradation process. Higher mixing intensity of 67 rpm for 5 min h^{-1} produced 15-18 % higher biogas production as compared to 10 rpm and 30 rpm without creating any instability in terms of VFAs accumulation and dead zones. Furthermore, higher mixing speed can lead to reduction in dead zones to less than 2%. After analysing the results from the current study and literature it is concluded that mixing is a very important aspect, which significantly affects the biogas production rates but the impeller design is the principal factor. Large diameter impeller at medium mixing speed is best combination in direction of optimization of mixing in an anaerobic reactor.

5.2 Effect of mixing interval on anaerobic digestion process

5.2.1 Methodology

The experiments were carried out in 3 parallel single stage continuously fed 5l lab-scale digester with head space of 1L custom-made from stainless steel by Biospin Ltd. Szeged, Hungary. These digesters were run under similar operating conditions of temperature and mixing speeds for one set of experiments. The schematic 2-D diagram of the experimental setup is shown in Fig 13. The reactors are equipped with helical ribbon impellers on single vertical shaft driven by variable speed motor to attain mixing. The key parameters (temperature, mixing speed and pH) were automatically controlled by computer software. The digesters were named as B1F1, B1F2 and B1F3 for the reference. All the impellers are operated by same electric motor in order to maintain identical mixing conditions. The temperature in the reactor is maintained by the circulation of hot water through stainless steel pipes inside the vessel from an electrically heated thermostatic water bath with an accuracy of $\underline{+}$0.5 °C. Three sets of experiment were conducted parallelly to recognize the effect of varying shear rates on biogas production rates and methane content. The experiment lasted for 45 days including two weeks of pre-run phase. The agitation rate for R1, R2 and R3 was 67 rpm. Intermittent mixing was applied for these experiments as supported by the literature. For this purpose, impellers were turned on for the period of 5 min every 1,2,3 and 4 hours respectively.

5.2.2 Experimental analysis

5.2.2.1 *Start-up phase*

All the digesters were filled with sewage sludge collected from a wastewater treatment plant in Szeged. The digesters were pre-run until a stable biogas production rate was obtained. The OLRs were set at 5 g for the entire experiment and was fed once a day. The mash was forced into the digester through a funnel/plug arrangement. It was feasible to reduce air intrusion into the digester while feeding using this method. During the start-up phase process instabilities were observed that might be caused by increased hydrogen concentrations, which result in a better breakdown to propionic acid rather than acetic acid, carbon dioxide, and hydrogen[125]. It was observed that, until the end of first two weeks of pre-run, the operation of digesters became stable with 0.23–0.26 mL day^{-1} gas production. After the pre-run period of 15 days, the biogas production was constant and the VFA and alkalinity (FOS/TAC) ratio was recorded as 0.39, which is considered normal as it indicates that the digestion process was stable. Different mixing intervals were started at day 15.

5.2.2.2 *Effect of mixing intervals on biogas yield*

According to the results of previous study [126] the mixing speed of 67 rpm was selected for further investigation of interval time between the mixing operations on biogas production rate. All the three digesters were run with identical parameters such as TS content, temperature and mixing regimes. Mixing interval time of 1 hour was selected in initial stage (after start-up phase) of experiment from day 1 to day 20 and further increased to 2, 3 & 4 hours accordingly. The use of three parallel digesters is preferred to obtain the more precise data on effect of varying parameters during the whole experiment. Accordingly, the similar trends in all the digesters were observed at particular defined mixing regime.

Results from this study found that the BY is closely related to the mixing interval time of the slurry. In the fig.21, *F* represents the digester, H represents the resting time (hours) between mixing operations. In Fig 22(d) the green bars in graph represents the daily biogas production in fermenter 1,2 & 3 at one hour resting time. According, to the obtained results (Fig .21 *(a)(b)(c)*) the daily maximum biogas yield during resting time of 1, 2, 3 and 4 hours was noted as 3.84 *l*/d, 3.36 *l*/d, 3.12 *l*/d and 2.94 *l*/d respectively whereas the minimum of the same mixing operations were observed as 2.43 L/d, 2.76 L/d, 2.34 L/d and 2.22 *l*/d respectively. The average daily biogas yield during all the mixing regimes was noted as 3.3 L/d, 2.9 *l*/d, 2.8 *l*/d, 2.5 *l*/d as depicted in Table 3. Similar results were demonstrated by K. Latha et al.[96] where mixing regimes were continuous. 15 min/hr and 30 min/hr. Maximum biogas yield was observed at mixing rate of 15 min/hr between 50 rpm-200 rpm. The observed higher biogas yields at minimum resting time is attributed to have favoured better interaction among methanogenic and acetogenic granules and further enhanced bacterial contact between substrate and microbes. The maximum, minimum and total BY are represented in Fig 22.

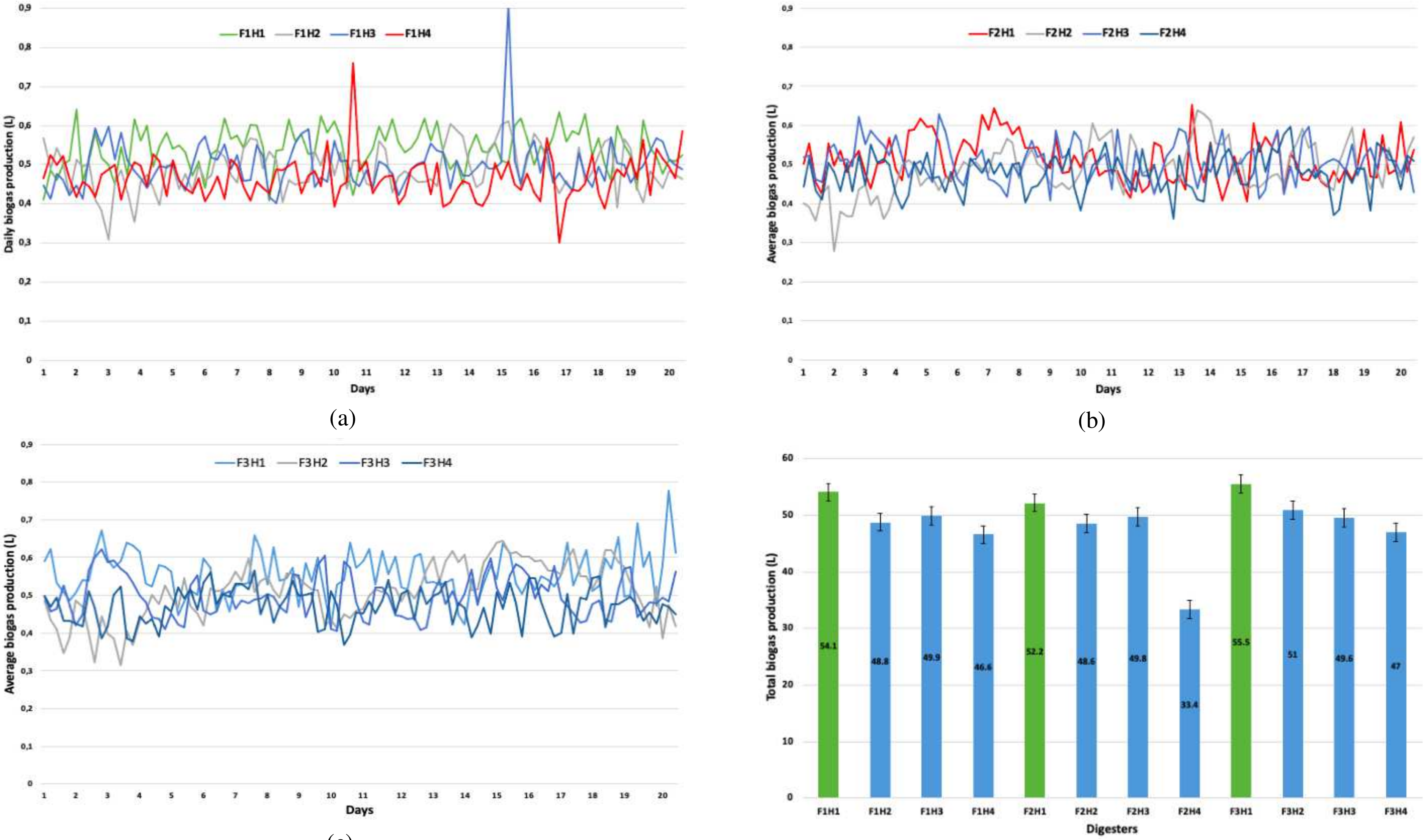
In terms of biogas production almost similar trends can be clearly noted in all the three digesters. Fig 21 demonstrates the biogas daily and cumulative biogas production rates in digesters. The overall biogas production in fermenter 1 at resting time of 1, 2, 3 and 4 hours was 54.1 *l*, 48.8 *l*, 49.9 *l* and 46.6 *l* respectively. The higher yield of biogas at breaktime of 1 hour is consequences of better chemical equilibrium along with better buffer action gained during the waiting time. The effect of increasing mixing interval was observed from the day 35 when the mixing time was reduced from 1 hour to 2 hours. The daily biogas production was dropped from 0.59 *l* to 0.41 *l* in F1, from 0.52 *l* to 0.41 *l* in F2 and from 0.61 *l* to 0.55 *l* in F3. This variation at different mixing intervals might be attributed to the more frequent mixing, which allowed for more interaction between the substrate and the microorganisms.

The mixing interval of 4 hrs represented the adverse effect of BY as compared to other intervals. The BY was recorded as 46.6 *l*, 33.4 *l* & 47.3 *l* in all the three digesters respectively at this particular mixing interval. The main reason of lower BY was the settling of solid particles at the bottom of digester due to longer break times. BY as low as 14-30% lower was recorded in all the digesters in comparison with reduced mixing interval time. Formation of floating layers in digester is also one of the main driving factors responsible for deviation in BY at various mixing intervals [70]. Lowering the mixing interval time led to prevented floating layer formation which was responsible for smooth discharge of biogas from the slurry. Floating layer is also directly associated with the OLR and TS content [13]. In this case the OLR was 1 $gVSL^{-1}d^{-1}$ which is between the optimum range where the reduction in gap between the mixing operation can led to reduction in formation of floating layer.

Figure 22 demonstrates the reduction of VS content in all the digesters at different mixing intervals. Similar trends as in BY can be observed. The highest VS reduction rate (64.2 % - 68.5 %) was observed at lower mixing intervals. On the other hand, at mixing interval of 4 hrs between the mixing operation the VS reduction was recorded as 58.3 ± 1.4 %, 53.6 ± 2.8 % and 56.7 ± 2.5 % for F1, F2 & F3 respectively. The average VS reduction at various mixing interval of 1, 2, 3 & 4 hours was recorded as 66.1%, 59.1%, 60.3% & 56.2% respectively (Table 11). According to recent study by H. Caillet et al [127] the variation in both TS and reduction in VS was also found when the different samples were taken from both top and bottom of a lab scale digester under various mixing speeds of 30, 40, and 50 rpm. The local study of TS and VS contents showed the effect of mixing on the displacement of solid matters. As a result, biogas production can be enhanced by appropriate mixer design, mixing speed and mixing interval times. Furthermore, it is suggested that intermittent mixing is adequate for the anaerobic digestion process. Based on these findings, it can be infered that biogas output increased with reactor design and that the operating parameters (intermittent mixing mode at lower mixing intervals and OLR) which can be favourable to the substrate and microorganisms.

Table 10. Comparison of biogas production form all three digesters under similar working conditions.

	Batch 1 (1 hour resting time) (Period 15-35 days)			Batch 2 (2 hours resting time) (Period 35-55 days)			Batch 3 (3 hours resting time) (Period 55-75 days)			Batch 4 (4 hours resting time) (Period 75-95)		
Fermenters →	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
Total Biogas production (*l*/d)	54.1	52.24	55.5	48.9	48.6	51.2	49.9	49.8	49.6	46.6	33.4	47.3
Maximum BP (daily) (*l*/d)	3.84	3.24	3.71	3.36	3.20	2.91	2.98	3.12	2.34	2.94	2.82	2.35
Minimum BP (daily) (*l*/d)	2.50	2.44	2.43	2.36	2.39	2.42	2.34	2.53	2.77	2.31	2.22	2.43
Average (*l*/d)	2.70	2.61	2.77	2.44	2.43	2.55	2.49	2.82	2.48	2.33	2.51	2.36



(a) (b)

(c) (d)

Figure 21. (a)(b)(c) represents the daily biogas production for continuous 20 days at different mixing intervals for digester 1, 2 & 3 respectively. (d) represents the overall biogas production from all three digesters.

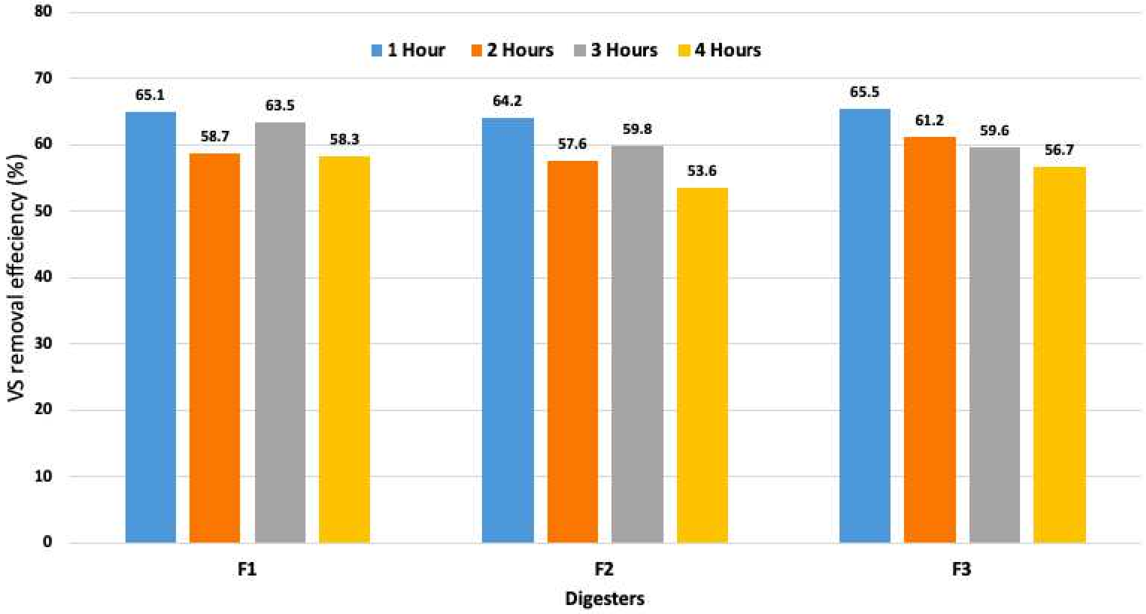


Figure 22. Performance of the all three anaerobic digesters for total volatile solids removal with different mixing intervals at 67 rpm.

Table 11. Represents the total VS reduction (%) in all the digesters at various mixing interval.

Digesters	Mixing intervals (hours)	VS removal efficiency (%)	Total biogas production (L)
F1	1	65.1 ± 3.4	54.1 ± .24
	2	58.7 ± 2.1	48.9 ± .31
	3	63.2 ± 3.6	49.7 ± .25
	4	58.3 ± 1.4	46.6 ± .14
F2	1	64.2 ± 2.5	52.2 ± .32
	2	57.6 ± 3.2	48.6 ± .13
	3	58.8 ± 5.2	49.8 ± .26
	4	53.6 ± 2.8	33.4 ± .27
F3	1	68.5 ± 2.7	55.5 ± .17
	2	61.2 ± 3.4	51.2 ± .30
	3	59.6 ± 3.2	49.6 ± .23
	4	56.7 ± 2.5	47.3 ± .21

5.2.2.3 Impact of mixing intervals on VFA concentration, alkalinity, pH and ammonia

The mixing intensity, mixing mode and frequency directly influences the AD bioprocess equilibrium and have major impact on overall biogas production yields. VFA such as acetic acid, propionic acid, butyric acid, iso-butyric acid and valeric acid are produced during acidogenesis reaction. The rise in VFA concentration has an effect on the efficiency with which substrates are converted to biogas. In this section the effect of mixing operation on VFA, pH, FAS/TOC ratio and free NH_3 is analyzed (Table 12). All the parameters were measured twice a week after completion of mixing cycle to obtain the homogeneous sample. Rheology of substrate is one of major parameters which have significant effect on performance during mixing operation. So, the digesters were operated at 4.2% TS content throughout the experiment so that the rheological parameters of slurry can remain constant and more precise results can be obtained.

Table 12. Performance of the digesters at different mixing intervals. Average values

	Batch 1 (1 hour resting time) (Period 1-20 days)			Batch 2 (2 hours resting time) (Period 20-40 days)			Batch 3 (3 hours resting time) (Period 40-60 days)			Batch 4 (4 hours resting time) (Period 60-80)		
VFA's (g/l)	7.3	6.1	5.8	3.1	3.8	4.9	1.1	1.9	2.2	2.4	4.3	3.1
pH	7.2	7.0	7.7	7.1	8.0	7.7	8.5	7.9	8.1	7.6	8.0	7.9
NH_4^+-N (g/l)	0.94	0.89	9.15	0.88	0.82	0.85	0.66	0.75	0.58	0.78	0.82	0.99
FAS/TOC Ratio	0.34	0.49	0.45	0.35	0.44	0.60	0.29	0.24	0.42	0.24	0.35	0.44

The average pH of substrate throughout the experiment remain between the optimal limits of 6.8-7.5. For one hour resting time the average pH recorded in all the digesters is 7.2, 7.0 and 7.7 in digesters 1, 2 and 3 respectively whereas during the maximum resting time of 4 hours the pH values recorded for the digesters refers to 7.6, 8.0 and 7.9 in digesters 1, 2, and 3 respectively (Fig. 23). Higher pH values at higher resting time is due to lower accumulation of VFAs during that period. The average VFA levels were noted as 5.876 *g*/*l*, 4.417 *g*/*l*, 5.338 *g*/*l*, 7.799 *g*/*l* for the resting time of 1, 2, 3 and 4 hours respectively followed by increase in biogas production respectively (Fig 24). On other hand according to Caillet et al. [127] the increase in VFA content had no detrimental impact on biogas generation. In terms of biogas production and changes in ammonium and VFA concentrations, no substantial fluctuation in these two concentrations was found to explain differences in biogas output. Higher VFA concentrations and lower ammonium concentrations resulted in greater biogas output. Whereas in our study the ammonia concentration was found to be in equilibrium during the whole experiment (Fig. 26). Similarly as per the findings of Franke et al.[128] the higher VFA levels (8-10 gl^{-1}) and pH values didn't destabilize the anaerobic process.

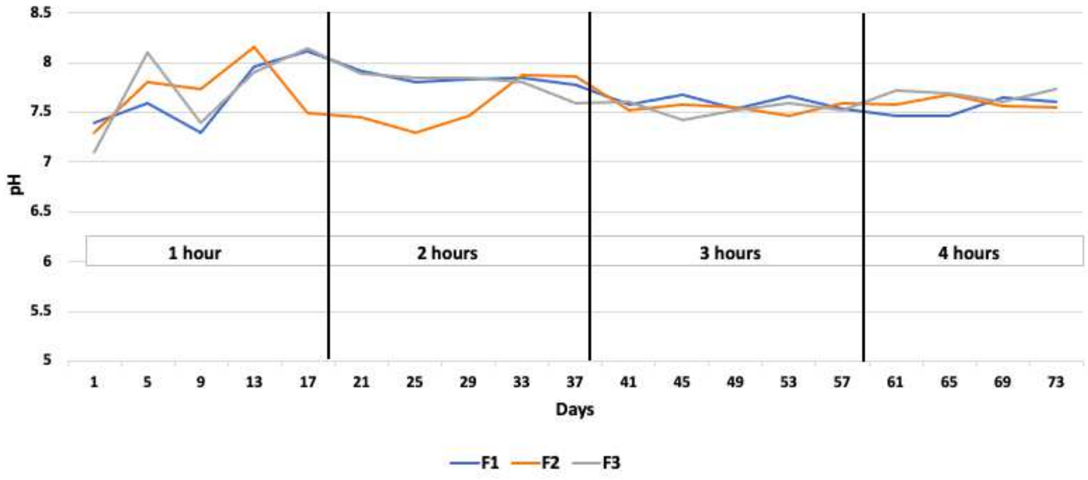


Figure 23. Ph levels during the different mixing intervals throughout the experiment.

The concentrations of VFA and alkalinity, as well as the corresponding ratios of VFA-to-alkalinity (FAS/TOC) were used to assess the system's stability (Table 11). The average FAS/TOC was recorded as .39 which was reported below the threshold value of .5 for a stable process to avoid failure of thermophilic digesters during transient conditions [129]. As a result, the startup with stable digesters was deemed successful prior to commencing varied mixing intervals to prevent the impact of shock loading. Greater alkalinity resulted in increased biogas generation. This outcome was predicted since the digestive environment's alkalinity was more conducive to the AD process. Furthermore, a rise in VFA concentration resulted in an increase in pH. The average pH for VFA concentrations of 5.8 gl^{-1} was 7.6, whereas the average pH for VFA concentrations of 7.79 gl^{-1} was 7.9.

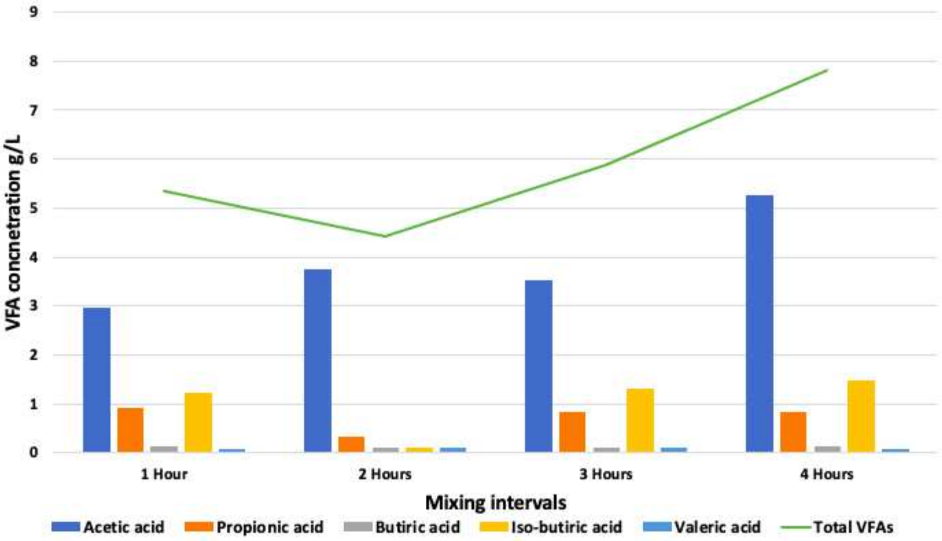


Figure 24. VFA concentration during different mixing intervals.

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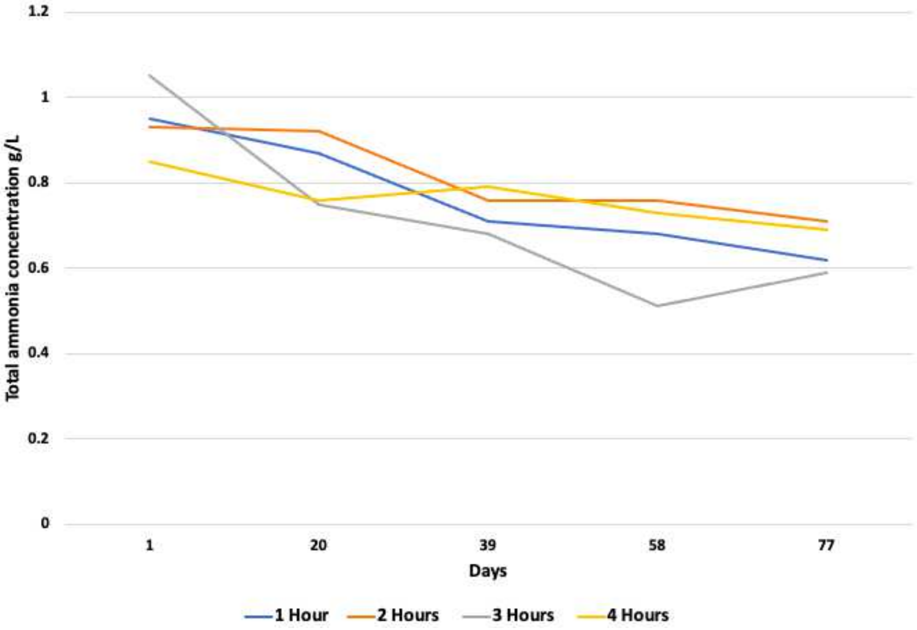


Figure 25. Total ammonia concentrations during the entire experiment.

5.2.3 System mixing intensity for semi-continuous mixing mode

The importance of efficient sludge mixing in anaerobic digesters has been recognized as a key design criterion for full-scale anaerobic digesters. For application in the design and operation of systems incorporating mechanical mixing devices, Camp and Stein[130] coined the term velocity gradient:

$$G = [\frac{P}{\mu V}]^{1/2} \tag{15}$$

Where G is the average velocity gradient, P the power dissipation, V the reactor volume, and μ the liquid viscosity. For this particular design and construction of setup the value of G is 10 S^{-1} as a slow mixing value was applied to the system by adjusting the mixing power to achieve this velocity gradient.

Due to biochemical process of anaerobic digestion which includes various microbes and formation of flocs the velocity gradient is not the only parameter which is determine the overall biogas production rates but also the mixing time and the interval between the mixing regimes. So, the parameter of velocity gradient mixing time integral in the case of semi-continuous mixing mode is calculated by the following equation:

$$mixing\ intensity\ no. = G \times T_m \tag{16}$$

Where T_m is the mixing time in seconds. The mixing intensity number can be accurately calculated and can be used to determine the appropriate mixing time of the impeller (Fig 26). In this case the mixing intensity number of 72000 is the found to be the optimum mixing intensity number which means mixing the slurry every hour for 5 min at 67 rpm can result in highest biogas production as compared to other mixing regimes (Table 13).

Table 13. Comparison between total biogas production and the mixing intensity number.

Resting time (hours)	Mixing time (seconds per day)	Mixing intensity no.	Total biogas production (L)
1	120	72000	54.1
2	60	36000	51.2
3	40	24000	49.9
4	30	18000	46.6

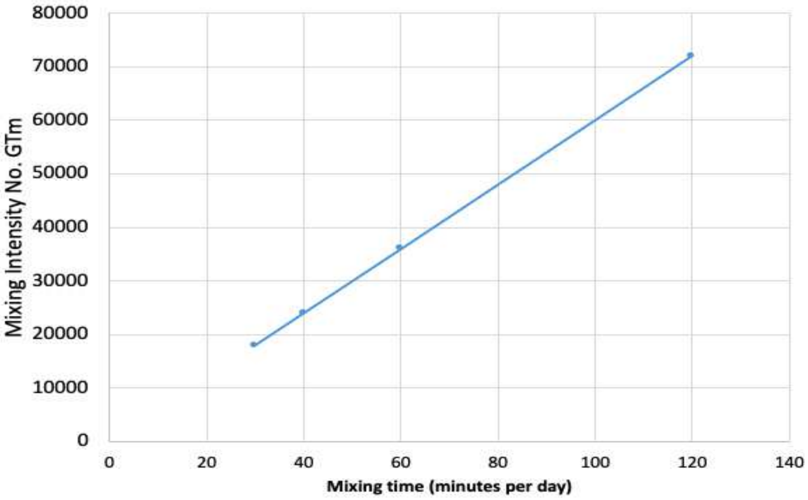


Figure 26. Relationship between mixing intensity number and mixing time.

5.2.4 Conclusion

Three digesters were operated under identical inculcation and operating parameters with various mixing intervals. It is concluded that the efficiency of the mesophilic digester is directly associated with the mixing time interval. The mixing regime also has effect on the physicochemical properties of the substrate. The digester performance was better under the minimum resting time of 1 hour at 67 rpm impeller speed. During this mixing regime the biogas yield was 5-12% higher as compared to longer resting times. The FAS/TOC Ratio was below 0.5 and the VS reductions was noted as 66.1 %. Drop in biogas yield can be due to VFA accumulations to some extent along with formation of floating layers and sedimentation at longer break time between the mixing operations. The appropriate agitation interval might not only accomplish high biogas generation, but also boost anaerobic digestion's energy efficiency. The findings can be used to run an anaerobic digester in an efficient and cost-effective manner.

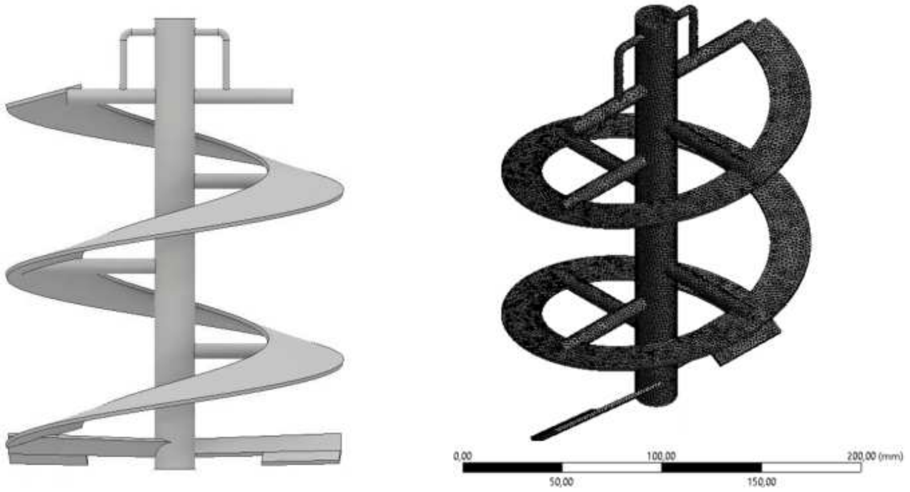
5.3 CFD Analysis

Computational fluid dynamics (CFD) is the application of computer models to simulate flow patterns utilizing basic equations, boundary conditions, and flow rates in order to predict the outcomes of an experimental system. The CFD simulation in this study is performed using ANSYS 2021. For simulation, transient simulation is used to determine the velocity distribution in the fermenter. The isometric view of grid generated is shown in Figure 27. The turbulence model k-ω is used for the simulations. The time step was constant, the value was 10^{-5} s. FLUENT was set up to iterate until the convergence parameters were satisfied, to reach the convergence, in all steps having a maximum 50 inner iteration steps per time step based on the 2,945,850 cells.

In this study, a single-phase model is used to reduce the simulation time. In this model, the solid particle containing liquid was considered as a homogenous phase with the density and viscosity values of the liquid–solid mixture. It should be noted that single-phase models are reliable when the percentages of the solid and fluid volumes coexisting in the container are approximately equal. Additionally, as the solid particles be finer and the difference in the densities of the two phases be less, application of a single-phase model would be more logical. The reason is that the mixture will be more homogenous, and its behavior will approach that of mono-phase systems, in this state. In the simulated systems, densities of the solid and liquid phases are 998 kg m^{-3} and 1000 kg m^{-3}, respectively, and their volumetric percentages are 50%. In the CFD simulation, the mixture of slurries (substrate) was assumed to be incompressible and pseudo-plastic fluid. The power law model was used to describe the slurry rheological properties as mentioned in the previous section. The velocity profile was viewed, and the flow patterns were compared at various mixing speeds. The hydrodynamics of each agitation condition used experimentally were numerically simulated.

The volume-averaged velocity magnitudes were obtained as (Equation (17)):

$$<\| u \|> = \frac{1}{V_L} \iiint_{V_L}^{1} \| u \| \, (V) dV \tag{17}$$



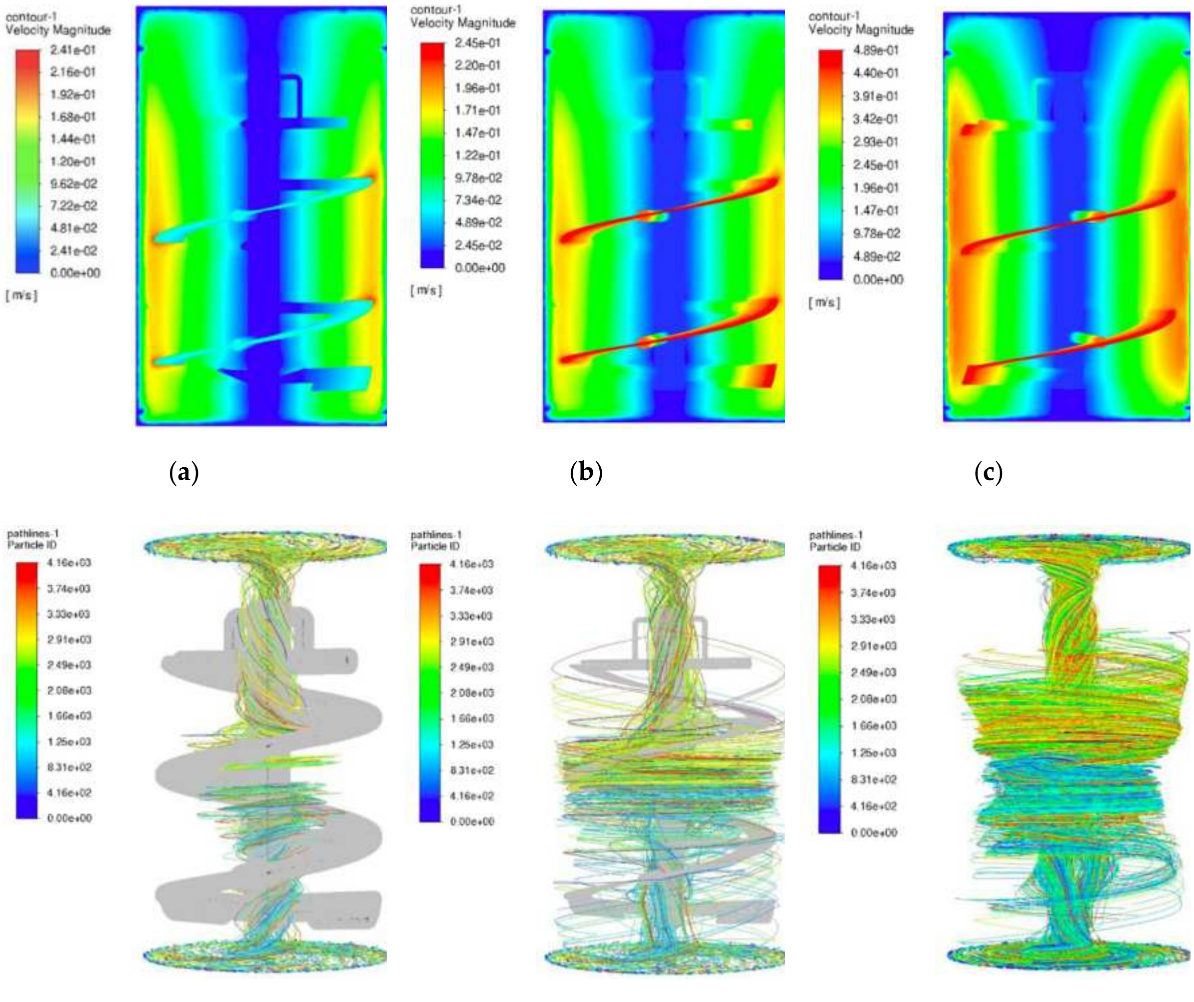
(**a**) (**b**)

Figure 27. 2D geometry (a) of impeller and Isometric view (b) of the tetrahedron elements of the helical ribbon impeller.

5.3.1 Numerical Simulation of Digester Hydrodynamics

Simulations revealed the presence of higher unmixed zones at lower mixing speeds characterized by reaching near zero velocities (Figure 28 (a)(d). The colour intensity of contours and streamlines indicates the magnitude of velocity in each region. The liquid flows theoretically downwards between the blades and the tank wall, inwards along the bottom of the tank, upwards near the shaft, and radially outwards at the surface of the digester. The impeller drives the fluid towards the walls of digester where the shear rate is maximum. On the other hand, a little movement is observed in axial direction near to the shaft. The red colour near the walls of the digester (Figure 28 (b)(c)) indicates the higher velocities between the interference of the impeller blades and the walls of the digester. Furthermore, the larger magnitude of velocities is readily seen as the mixing speed increases.

It can be observed that increasing the impeller's rotating speed causes a reduction of dead zones. A higher rotational speed, on the other hand, necessitates more energy consumption, which directly results in increase of operating and maintenance expenses. The flow field outlines show that increasing the rotating speed from 10 to 30 rpm has no discernible effect on the elimination of stagnant areas, but the energy consumption skyrockets. Furthermore, exceeding a specific rotating speed might damage the microbial growth and seedling habitat. Despite the impeller's interference, the overall flow pattern is consistent with what has been described in the literature. The radial and axial flow, along with a dominating annular flow, is enough to suspend and shear the sludge granules in the reactor.



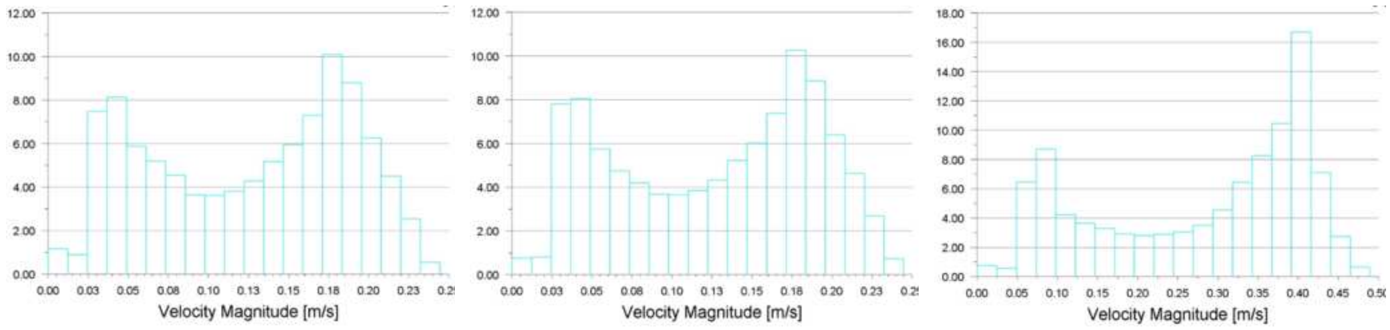
(**a**) (**b**) (**c**)

(**d**) (**e**) (**f**)

Figure 28. (a) 10 rpm; (b) 30 rpm; (c) 67 rpm; (d) 10 rpm; (e) 30 rpm; (f) 67 rpm.

According to this study, slurry homogeneity was attained at a speed of 67 rpm. In this situation, increasing the rotating speed of the mixer will have no effect on mixing performance. Previous experimental findings also show that raising the impeller speed to a particular optimal level might improve the mixing system's performance. Beyond that point, the power consumption skyrockets, with just a minor beneficial impact to mixing performance and reduction in biogas production rates.

Figure 30 represents the volume percentage in the function of velocity magnitude at 10, 30, and 67 rpm. It is observed that, in Figure 29 a,b, there is negligible difference in the velocity magnitudes and the volume percentage under the velocities is less than 0.05 ms^{-1}. The maximum velocity at 67 rpm was recorded as 0.5 ms^{-1} which is almost twice the velocities recorded at 10 and 30 rpm which are recorded as 0.25 and 0.24 ms^{-1} respectively (Table 14). The mixing intensities can be easily evaluated in terms of dead zone volume. The parts of the reactor with no flow or very low velocities are known as dead zones or stagnant zones. Dead zones are undesirable because that volume of the reactor remains isolated from the rest of the reactor volume and get no mixing, resulting in a reduction in the effective reactor volume. The dead zone volume under lower mixing speeds was observed to be comparatively very high. Under minimal mixing speed of 10 and 30 the dead volume was recorded as 18% and 17%, respectively; whereas under higher mixing intensity it was reduced to just 2%. Inside a dead zone volume, the pH and temperature gradient occur, which results in decrease of the digester's effectiveness and apparently decline in biogas production and sometimes even digester failure.



(a) (b) (c)

Figure 29. Volume percentage in the function of velocity magnitude at 10, 30, and 67 rpm. (a) 10 rpm; (b) 30 rpm; (c) 67 rpm.

It can be inferred that raising the impeller's rotating speed is not always beneficial in improving the mixing pattern. Vortices can develop in some places as the rotating speed increases, which can lead to disruption of biomass activity, phase interaction, and heat and mass transport. As a result, based on its rheological properties, the ideal impeller speed and optimum mixing pattern for each non-Newtonian fluid should be investigated independently which directly depends on the total solid content and temperature.

Table 14. Comparison of maximal and average velocities under the different mixing conditions.

Rpm	**Torque (Nm)**	**Maximum Velocity (m s^{-1})**	**Average Velocity (m s^{-1})**	**Dead Volume**
10	3.9×10^{-6}	0.5	0.08	18%
30	3.9×10^{-6}	0.24	0.10	17%
67	1.33×10^{-5}	0.25	0.12	2%

5.3.2 Effect of geometrical characteristics on flow patterns and mixing efficiency

Mixing is a physical operation which is highly dependent on the design and geometry of the vessel and the impeller. In the current study, impeller speed, geometry and slurry rheological are considered as the principal factors that determine the efficiency of mixing system in an anaerobic digester. According to Amiraftabi et al. [131] the helical ribbon impeller provides stronger radial flow movement as compared to axial flow under different mixing speeds. Due to the optimum geometrical design used in this experiment, the greater amount of the slurry is pushed towards the walls where the hydrodynamic shear is low and very little is drawn towards the shaft of the impeller. Due to larger diameter the maximum mixing happens near to the clearance between the walls of the digester and the ribbons of impeller. Moreover, due to low bottom clearance the mixing effect can observed in the entire active volume of the digester and efficiency of the impeller is significant[121]. Furthermore, the non-Newtonian characteristic of slurry results in decrease of viscosity near the high shear zones close to the blades which creates a low viscosity film between the blades and walls that is significantly influenced impeller geometry. The weakening of core network of shear-thinning fluid increases both the molecular and the mass diffusions leading to an effective method of mixing. The results in this study also indicate that the increase in impeller rotational speed reduces the mixing time and enhances the uniformity of nutrients, heat and mass.

5.3.3 Practical implication of this study

It is inferred from this study along with literature that the geometry characteristics of the impeller decide their efficiency in mixing and biogas output in slurry agitation. In most studies the turbine impellers were analysed to study the mixing effect of the slurry [53][62][62][71][57]. The concept of using paddle impellers can be encouraged by greater consistent distribution of viscosity at lower shear rates and mixing speed [57][132]. Slow moving propellers with longer agitating wings can do better in pilot scale digesters. It is reported that the impeller characteristics, such as pitch ratio, power number and axial flow number, are closely related to achieving homogeneity in the digester. These impellers can be adjusted in order to provide a consistent shear distribution such that the microorganisms remain unharmed and seek to reduce the energy consumption and increase the flow pattern of slurry in the digester [51]. Eventually, the impeller in an anaerobic digester can have almost constant pitch as it guarantees a consistent distribution of velocity at low shear speeds. As a consequence, the scaling-up of pilot scale mixing processes is a crucial feature for maximizing current mixing and flow processes by holding all measurements within a set ratio, known as a scale-up factor [133]. Minimum periodic mixing is observed to be favourable for a successful anaerobic digestion operation [63,67,134]. Intermittent mixing with longer resting periods may result in higher biogas output and, in most situations, increased mixing time cycles have not seen much impact on biogas production, but comparable results can be produced at lower power consumption [69]. The direct effect of the shear rate and the mixing speed is discussed in this study.

6 Methods Adopted for Evaluation of Mixing Efficiency in an Anaerobic Digester

Effect of slurry mixing in an anaerobic digester on biogas production is intensively studied in last few years. This subject is still debatable due to fact that this process involves three phase solid-gas-liquid along with involvement of microbes responsible during biochemical reactions which are highly vulnerable to changes in hydrodynamic shear stresses and mixing conditions. Moreover, complexity in direction of optimization of mixing magnifies due to implication of both fluid mechanics and biochemical engineering to study effect of mixing in anaerobic digestion (AD). The effect of mixing on AD is explored using recent literature and theoretical analysis, concentrating on the multi-phase and multi-scale aspects of AD. The tools and methods available to experimentally quantify the function of mixing on both the global and local scales are summarized in this study.

The major challenge for mixing in anaerobic digester is to minimize dead zones, uniform distribution of viscosity and shear at low mixing intensities without disrupting the microbial flocs and syntrophic relations between the bacteria during the AD process. This chapter posses critical analysis of various techniques and approaches adopted by researchers to evaluate the effectiveness of mixing regimes and mixing equipment. Most of the studies describe biogas production performance and hydrodynamic characteristics of the digesters separately but evaluation of mixing requires interdisciplinary experts which include mechanical engineers, microbiologist and hydrodynamic experts. Through this section the readers will be guided to intensive literature regarding agitation, best possible way to scrutinize the agitation problems and can approach to answer to the question “why is optimization of mixing in anaerobic digester still debatable subject?”

The present chapter has been considered with the following motives:

- To determine the best combination of techniques and approaches towards evaluation of mixing efficiency in anaerobic digesters.
- To understand the importance of interdisciplinary approach in optimization of mixing in anaerobic digester.
- To discover the local and global parameters involved in analysis of mixing in bioreactors.
- To draw the directions for future research and scope in field of optimization of mixing in digesters in terms of power consumption and biogas production rates.

6.1 Correlation between lab-scale and large-scale mixing in anaerobic digesters

It have been observed that most of the studies on evaluation of effect of mixing regimes on biogas production are limited to lab-scale digesters[47][135][65][68][52] and only few represents the results regarding the full scale biogas plants[66][136][137]. The major challenge in mixer setup design is to scaleup from a laboratory or pilot scale to full scale unit. Effective design and scale-up from laboratory to large scale bioreactor includes optimization of design and operating parameters, including thorough knowledge of biokinetics and hydrodynamics.

For some specific cases generalized power correlation between N_p versus Reynolds number for various impellers are available for scale up as shown in Fig 30. Another possible solution for scaleup is based on geometrical similarities between the laboratory and full-scale plant equipment. However, it is not always possible to have lab scale and large-scale digesters geometrically similar. Furthermore, in some cases it can be possible to obtain geometrical similarities, but it is very hard to have dynamic and kinematic similarities which will lead to divergence from the predicted results.

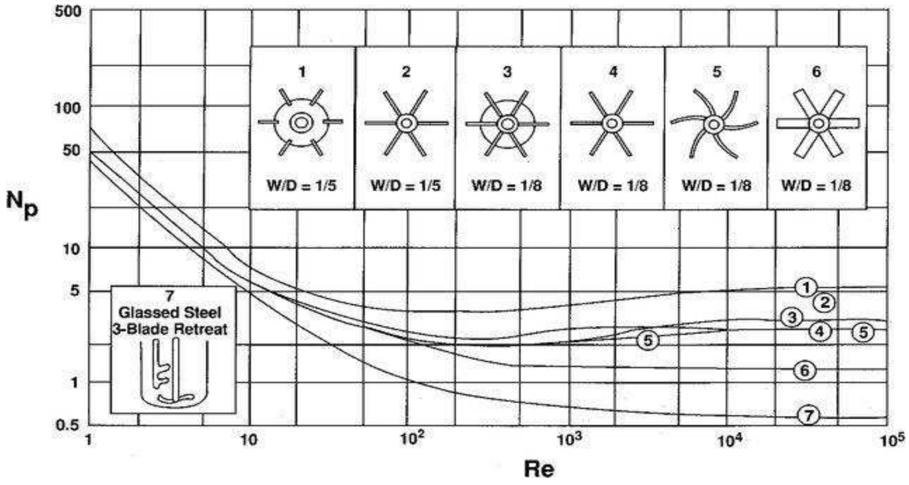


Figure 30. Power number N_p versus Reynolds number R_e for turbines and high-efficiency impellers[138].

Basically, the similarities between the different size of vessels can be classified as:

- Geometrical similarity.
- Kinematic similarity.
- Dynamic similarity.

Geometrical similarity refers to similarity between ratio of all corresponding dimensions in digesters of different sizes which includes the diameter, off bottom and inter impeller clearance, tank shape etc. The optimum ratio of vessel diameter to the impeller diameter for a given power input is a crucial factor in scaleup. This ratio is strongly influenced by the nature of agitation problem. For a constant power input the impeller speed will be higher if the impeller diameter is smaller. Correspondingly, for lower speed of impeller the diameter of the impeller should be increased in the anaerobic digester because lower mixing intensity is preferred for maintaining favorable environment for bacteria.

When constant power per unit volume and geometrical similarities are maintained in scaling up, the impeller speed changes with $D_a^{-2/3}$. The power per unit volume is

$$\frac{P}{V} = \frac{N_P n^3 D_a^5 \rho}{(\pi/4) D_t^2 H} = \left[\frac{4 N_P \rho}{\pi} \left(\frac{D_a}{D_t}\right)^2 \left(\frac{D_a}{H}\right)\right] n^3 D_a^2 \qquad (18)$$

The terms inside the bracket are constant so, $n^3 D_a^2$ must be constant.

Hence,

$$\frac{n_2}{n_1} = \left(\frac{D_{a1}}{D_{a2}}\right)^{2/3} \qquad (19)$$

During scale up of anaerobic digester, in order to maintain P/V constant, reduction in impeller speed can lead to longer mixing time hence leading to higher power consumption. Dynamic similarity is attained when the ratio of all corresponding forces is same whereas kinematic similarity refers to similarity in ratio of the velocities at corresponding points. Additionally, these similarities have utmost importance specially during scaleup of an anaerobic digester and are presented together because they are interrelated in a fluid system. Equality of the groups in the following equation ensures the dynamic and kinematic similarities.

$$f\left(\frac{D^2N\rho}{\mu},\frac{DN^2}{g},\frac{Pg_c}{D^5N^3\rho}\right)=0 \tag{20}$$

$$f(R_e,F_r,N_P)=0 \tag{21}$$

Power consumption in large scale biogas plants can be accurately predicted from the curves of N_p versus R_e number (Figure 31.). Usually, the power consumed by the impeller per unit volume of slurry has been used as a measure of mixing effectiveness[21]. Power use of the anaerobic digester impeller dynamically influences the characteristics of the device, such as the configuration, the geometry and the scale of the digester, the position of the impeller, the speed of the impeller and the rheological behaviour of the substrate in the anaerobic digester. Precise measurement of the strength is important for the power unit selection for optimal mixing. Poor mixing units would contribute to excessive expenditure in equipment and higher energy use rates, which will reduce the productivity of the biogas project[37].

6.2 Parameters for evaluation of mixing in anaerobic digester

Numerous parameters are evaluated to study the mixing efficiency in an anaerobic digester. From the intensive literature review it have been observed that the approach to study mixing efficiency depends on the scale of the biogas digester. For instance, in the lab-scale experiments general focus is on the determination of amount of biogas production rate [28][139], methane content [57][87], behavior of microorganisms [83][62][56]and dead zones [47][52] by varying the rotational speed of the impeller. Each factor has its own significance and importance. Here it will not be erroneous to say that determination of biogas production rate should not be the only parameter to evaluate the efficiency of an impeller at various mixing speeds.

Another parameter to 63nalyse the effect of mixing intensity on biogas production is determination of velocity gradient. Various studies can be found dealing with the simulations and experimental calculation of average velocity[58][140], velocity gradient (G)[141][28] and mixing energy level (G_L)[142] and their effect on biogas production rates and microbial flocs. These parameters directly depend on the impeller geometry, impeller speed, position and diameter along with physical and rheological properties of slurry such as density and viscosity. According to U.S EPA recommendations MEL of 5-8 W/m^3 and G of 50-80 s^{-1} are favourable. Rivard et al. [142] and Wu [141] calculated values of MEL above 8 W/m^3 for slurries at higher solid concentration which resulted in higher power consumption per unit volume. The value of G and G_L is valuable parameter to predict the dead zones along with positive and negative effects of mixing intensity on microorganisms.

Table. 14 demonstrates the comparative analysis of different mixing modes on various influential parameters in an anaerobic digestion process. From the mentioned mixing modes, mechanical mixing can be considered as most favourable mixing methods due to its positive response to all the influential parameters. Whereas, under the high TS content in slurry the pneumatics mixing is ineffective to some extent due to increase in viscosity of the fluid[143]. Furthermore, in case of hydraulic mixing chances of dead zones and unmixed regions are relatively higher under any conditions. Consequently, the rheology of the slurry is key parameter that should be underlined during the analysis of mixing and designing of mixing equipment for an anaerobic digester[144].

Geometry of the digester tank and the mechanical mixer is only considered at the lab-scale experiments which makes it easier to determine the hydrodynamics characteristics of digesters. Whereas, at the full-scale biogas plant the mixing is evaluated in terms of power consumed per unit volume and per unit fresh feedstock added to the digester [145][39][41]. It is noted that geometrical aspect is missing while studying effect of mixing at large scale biogas plant. Accordingly, the power consumption should not be the only parameter to study effect of mixing because mixing is physical process that is directly associated with the dimensions of the setup. Without knowing the exact geometrical configuration, it is impossible to determine the mixing time for slurry by the impeller and range of hydrodynamic stresses produced by the impeller blades.

6.3 Importance of geometrical constraints and rheological study of slurry

Mixing is a physical process which produce physical motion of the fluid between different parts of whole volume. The mixing can be classified into various mechanisms such as: bulk flow in laminar and turbulent regimes and both eddy and molecular diffusion. These classifications are determined by the physical and rheological properties of fluid and the geometry of the impeller and the vessel tank. The design of mixing equipment in an anaerobic digester involves the selection of type, size and operating conditions that can perform a desired service corresponding to the slurry rheology. The method of predicting the process performance, characteristics of mixing equipment generally depends on the empirical methods involving correlation of dimensionless groups and model relationships. Moreover, in case of mixing in an anaerobic digester it is even more complex to draw a correlation between microorganisms, mixing intensity and biogas production rates. In many studies dealing with the evaluation of mixing in digester at lab-scale or large scale, the geometry of the mixers and the digesters is missing[146][87][83]. It is very hard to determine the flow patterns, shear stresses and dead zones when geometrical characteristics are absent. Whereas, some studies combine the two approaches of biogas production performance and hydrodynamics of digester[82][62][81].

For a non-Newtonian shear, the value of n is less than 1. For this case, the rheological data for wastewater sludge are taken from the literature [50].

Here k_s is Otto-Metzer constant which is directly associated with the impeller geometry. Table 15. represents the Value of Otto-Metzner constant (k_s) for different types of impellers with respect to the D_t/D_a ratio in the vessel. Equation 6 is used to calculate mean shear rate under specific conditions of mixing. Apparently, it is very crucial to extract the volumetric curve (shear stress v/s shear rate) for the slurry used in a digester experiment.

Table 15. Represents the value of Otto-Metzner constant (ks) for different types of impellers with respect to the D_t/D_a ratio in the vessel[144].

T/D_a Impeller	$\geq$1.5	1.184	1.111	1.072	1.047
Anchor impeller	19	39	51.5	71.5	84
6-B Rushton turbine	11.6	-	-	-	-
2-B Paddle impeller	10	-	-	-	-
3-B Marine impeller	10	-	-	-	-

Optimization of impeller geometry rests in achieving uniform distribution of velocity within minimum mixing time at low rpms. Impeller choice have high importance in case of bioreactor.

6.4 Approaches towards evaluation of mixing efficiency

6.4.1 Computational Fluid Dynamics (CFD) simulations

CFD is a powerful tool for modelling of mixing operations in fluid dynamics. The mixing in anaerobic digesters has been modelled using CFD[51][79][147]. CFD analysis in an anaerobic digester can be used to study the flow fields, velocity contours, movement of dissolved components, turbulence, particle trajectories and dead zones under different operating conditions. CFD provides the numerical simulation of viscosity distribution, flow pattern along with numerous output parameters such as turbulence levels, vorticity and particle velocity[148]. Modelling by CFD can help to reduce the initial cost of the experimental setup, optimization of full-scale anaerobic digesters. After modelling the Cfd can be validated against the experimental results. On other side the CFD focuses on the fluid dynamics but not on the kinematics i.e. anaerobic digestion.

The 3-D geometry is first constructed in computer aided design software. The mesh is generated by dividing the entire volume of the geometry. The mesh is relatively fine near the impeller blades and it can be coarse far away from the impeller and size functions are used to control the mesh growth. The properties of different phases such as liquid, solid and gas are defined. Depending on the nature of problem i.e. single phase or multiphase, different turbulence models and solvers are selected to observe the effect of geometry and boundary condition on mixing in an anaerobic digester. Table 16. represents the Summary of CFD simulation outcomes and methods used for solving governing equations and turbulence models.

Terashima et al [149] introduced a new parameter called uniformity index by numerically evaluating laminar flow. According to Mendoza et al.[150] for determination of dead zones and the flow inside the digester distribution of streamlines and velocities is very important. In another study by Manea et al.[151] the optimum geometry and rotational speed was obtained by three dimensional numerical simulation. Few studies have been reported dealing with simulations at large scale digesters. B. Wu et al. [54,97,117,141,152] predicted the flow pattern in large scale digesters using large eddy simulations, turbulence models, Eulerians multiphase models and sliding mesh methods along with mixing characteristics of impeller. However, the structural differences could preclude a clear application of operational information from laboratory-scale findings to full-scale designs.

Table 16. Represents the various fluid models developed by using CFD in context of evaluating mixing in anaerobic digesters on both lab-scale and large-scale biogas plants.

Study	**Turbulence model**	**Rheology of slurry**	**CFD software/ method**	**Results**
Wu & Chen et al. 2008 [117]	*k*–ε turbulence model single equivalent phase	Non-Newtonian	ANSYS Fluent/ finite volume	Flow pattern and dead zones
H. Caillet et al. 2018 [153]	LES- turbulence model	Newtonian and non-Newtonian	Open FOAM software/ Finite volume	Temperature and velocity profile
Binxin Wu et al. 2010 [54]	*k*–ε turbulence model	Non-Newtonian	ANSYS Fluent/ Multiple reference frame	Mixing energy levels and dead zones
J. Ding et al. 2010 [51]	*k*–ε turbulence model	Non-Newtonian	ANSYS CFX	Optimized impeller design/ velocity distribution
R. Meroney et al.2009 [24]	*k*–ε turbulence model	Newtonian	ANSYS Fluent/ Finite volume	Digester volume turnover time/ Mixture diffudion time
J. Bridgeman 2012 [22]	Standard *k*–ε (S *k*–ε), Realizable *k*–ε (R *k*–ε),	Non-Newtonian	FLUENT	Flow patterns combined with biogas yield

Vesvikar et al. 2005 [154]	k–ε turbulence model	Newtonian	CFX/ Finite difference	Gas distribution and flow patterns
Dama et al. 2000 [155]	-	Newtonian	FLUENT / Finite difference	Velocity profile and flow patterns
B. Wu et al. 2010 [54]	-	Newtonian and non-Newtonian	FLUENT	Mixing energy levels
M. Terashima et al. 2009 [149]	*Single phase/ laminar*	Non-Newtonian	ANSYS CFX/ Finite element	Sludge distribution
Mohammadrezaei et al. 2017 [156]	k–ε turbulence model	Non-Newtonian	FLUENT/ Multiple reference frame	Optimum mechanical stirrer and flow patterns
Li et al. 2004 [157]	k-ω & k–ε turbulence model	-	CFX	Flow and velocity prediction by impeller
Elena et al. 2012 [158]	*Navier stokes equation*	Newtonian	Mixsim	Nominal mixing speed and optimum geometry of impeller
Y. Zang et al. 2015 [159]	k–ε turbulence model	Non-Newtonian	FLUENT	Flow pattern
Fei Shen et al. 2013 [160]	Standard k–ε (S k–ε)	Non-Newtonian	Mixsim	Optimum mixing speed
Ahmed et al. 2009 [161]	k–ε turbulence model	Non-Newtonian	ANSYS CFX/ Eulerian approach	Power consumption and mixing time
C. Maier et al. 2010 [162]	Euler-euler multiphase	Non-Newtonian	FLUENT/ Finite volume	Scaleup process/ mixing behaviour
Huang et al. 2014 [163]	-	Non-Newtonian	FLUENT	
H.Azargoshasb et al. 2015 [164]	RNG k–ε	-	FLUENT	VFA's concentration profiles
Karaeva et al. 2015 [165]	k–ε	Newtonian	COMSOL Multiphysics	Geometrical parameters in hydraulic mixing
Torotwa et al. 2018 [166]	k–ε model	Newtonian	COMSOL Multiphysics Euler-Euler multiphase	Flow pattern in gas mixing
Leonzio et al. 2018 [167]	k–ε model	Non-Newtonian	COMSOL Multiphysics Euler-Euler multiphase	Shear rate, velocity gradient and flow pattern
Manea et al. 2012 [158]	*No turbulence*	Non-Newtonian	Multiple reference frame	Impeller geometry and flow pattern

6.4.2 Linking CFD to actual biogas production

It cannot be denied that the CFD modeling is one of ways to analysis the mixing efficiency of the mixing equipment but on other hand it is very important to link the CFD findings to the actual biogas production on all scales of anaerobic digestion. For example, studying effect of shear stresses, mixing time, dead zones and flow regime on the microbes and biogas production at different phases of anaerobic digestion process. Determining the effect of mixing in two stage anaerobic digester is also interesting fact to study. Effect of hydrodynamic shear rates on different categories of organisms can be better understood by physical separation of hydrolysis and methanogenesis.

6.4.3 Mixing time

Mixing period is one of the parameters used to describe the mixing of the liquid process in the stirred reactors. Mixing time is the time taken to reach a certain degree of homogeneity. Higher mixing time corresponds to higher power consumption by the mixing operation which will reduce the overall efficiency of the biogas plant.

Tracer technique is one of very popular techniques to calculate the mixing time[168][169][170] and determination of hydrodynamics of the digester by RTD. It is calculated on basis of time take by particle to enter and leave the digester. A known mass of chemical tracers such as lithium or fluoride is added through inlet and concentration is detected and monitored at outlet. RTD curves can be generated from the data obtained which is used to analyze the hydeodynamic characteristics of the digester in terms of dead zones, short circuiting and breakthrough time[168]. Significance of smooth exponential decay refers to perfectly mixed digester. Typically, the tracer concentration at a point within the tank varies with time, and the time taken forth variation to reduce below a certain level, say, within 5% of the fully-mixed concentration, is taken as the mixing time. The same method can be used in a numerical calculation by injecting a neutrally-buoyant, virtual tracer at a given location. The concentration is governed by the following equation.

$$\frac{\delta \rho \phi}{\delta t} + \nabla \cdot \left(\rho u \phi - \Gamma_{\phi} \nabla \phi \right) = S_{\phi} \tag{22}$$

Some researchers [171] suggested an equation for determination dead zones.

$$\frac{C_{so}(t)}{C_o} = \exp\left(-\frac{1-f}{ar(1-d)T_{HRT}} \left(t - L - \frac{p(1-f)rT_{HRT}}{1-f} + \beta ar(1-d)T_{HRT} \right) \right) \tag{23}$$

Where $= -\frac{\ln(1-f)}{1-f}$, and as f→0, β→0.

6.4.4 Optimize impeller design

Various variables that specifically influence the mixing period and the output rate of biogas in the digester are the design of the impeller, the bottom clearance of the impeller and the clearance of the impeller, the eccentricity of the impeller, the baffles and the location of the draft channel. Various shapes and geometries have been tested, but there is a difference in the efficacy and performance outcomes of the various mixers owing to the different approaches utilized for measurement for various substrates. Impeller choice is critical because its selection depends on different factors. The key goal of the impeller is to prevent dead areas, stratification and firm forming. Coaxial impellers are used for small-scale digesters, whereas eccentric agitators may be utilized in large installations [73]. Typically, the pumping action is created by the rotary movement, which allows the slurry to move in radial, axial and intermediate directions. However, the speed of slurry the in-vessel is not usually a measure of the degree of mixing. The sludge may travel at a specific pace, but if all the sludge in the immediate vicinity travels at the same speed and in the same direction, the sludge is not mixed, rather the sludge is simply pushed inside the vessel [56]. It has been found that the ideal behaviour of tank mixing can deviate due to a variety of reasons associated with the location of inlets, exits, stratification and tank geometry. In addition, the existence of even a small variation in density between the mixing fluids can have a direct impact on the success of the mixing [55].

Fig 9. represents the data from literature where various different types of geometries of impellers were compared and outcome in terms of flow patterns, particle velocity, dead zone volumes, shear stresses induced, and biogas production rates was analyzed. It is clearly observed that the impellers with larger diameters such as double helical ribbon impeller, anchor impeller performed better as compared to high shear impellers such as RT. Above literature rests on experimentation on the lab scale digesters, but optimization of mixing on a pilot scale and large scale is the prominent issue to be explored. The basic prerequisite for the scale-up of the pilot scale of the digester is similarity among the geometric, dynamic and kinetic conditions of the lab-scale digester. Previous studies lack the scaling up of mixing equipment using the distinct forms of lab-scale mixer mentioned above.

6.5 Biogas production rates and methane content

Mixing effect at various mixing intensities and different geometries is also analyzed on basis of biogas production rates and the methane content. In the literature it has been observed that researchers compared the production rates by changing the rheological properties, temperature, TS content and geometry of the digester. Biogas production is recorded continuous throughout the experiments. Because gasses have limited densities, it is generally not feasible to gather gas and determine its mass. In the case of gasses which are basically not soluble in water, it is necessary to extract the gas created by displacing the water from the bottle. This approach is quite straightforward in economic terms and operates over a longer period of time without maintenance. The process of displacement of water is one of the traditional methods of calculating regular gas output (Fig. 31). In this process, the amount of water transported by gas implies the volume of biogas generated by the digester. Some mistakes can occur due to variations in ambient temperatures, so it is really important to report changes.

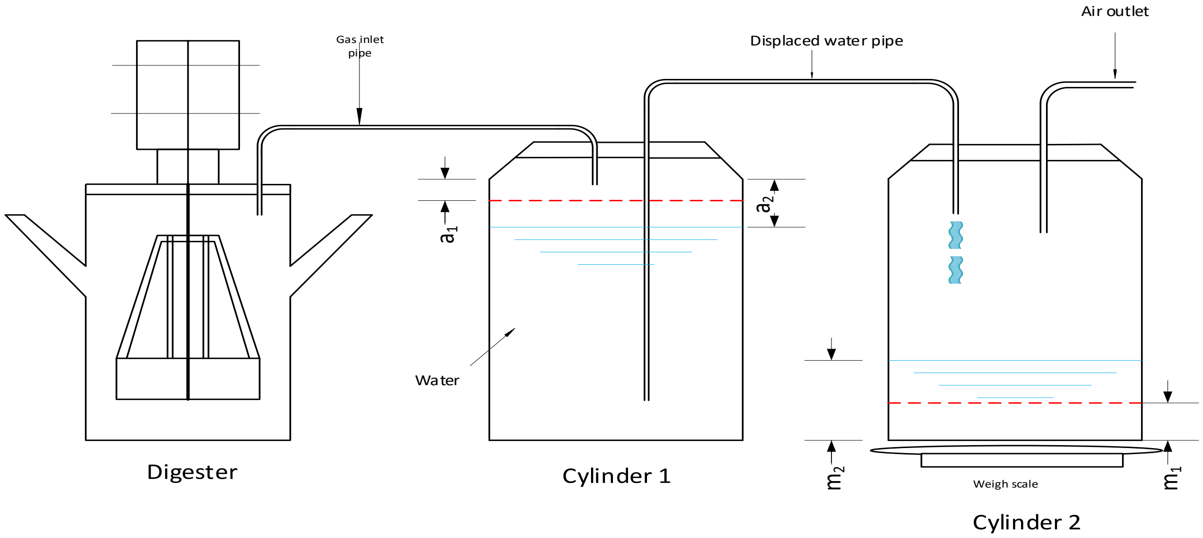


Figure 31. Apparatus for analysis of biogas volume.

6.6 Present scenario and future directions for analysis of mixing in an anaerobic digester.

To proceed research in any field, it is utmost important to consider the present scenario in that field. This section aims to categorize the literature on basis of evaluation of mixing techniques implemented in the experiments at both lab-scale and large-scale digesters. It has been observed that anaerobic digestion is a biological and chemical process whereas mixing of slurry is the physical operation which makes optimization of mixing in digester even harder challenge. For this purpose, it is very important to adopt an interdisciplinary approach to study the effect of mixing intensity, shear rate, shear stresses and flow patterns on microorganisms, bacteria and their syntrophic relations. Optimization of mixing at large scale will remain challenge until the understanding of actual description of effect of mixing on anaerobic digestion process. CFD provided ultimate solution for mixing issues but physical modeling still varies as far as literature is concerned which have resulted in hurdle to accept a general model in AD. Optimization isn't just about increasing the production of biogas. By reducing the mixing, there is also an ability to minimize power demand as well as maintenance and operational cost. It has been shown that intermittent mixing approach can produce the same amount of biogas/methane and even increase the output of gas combined with a continuously mixed system, whereas reducing the maintenance and energy demand of the process. The digester architecture and the common use of the continuously mixing are fair to question.

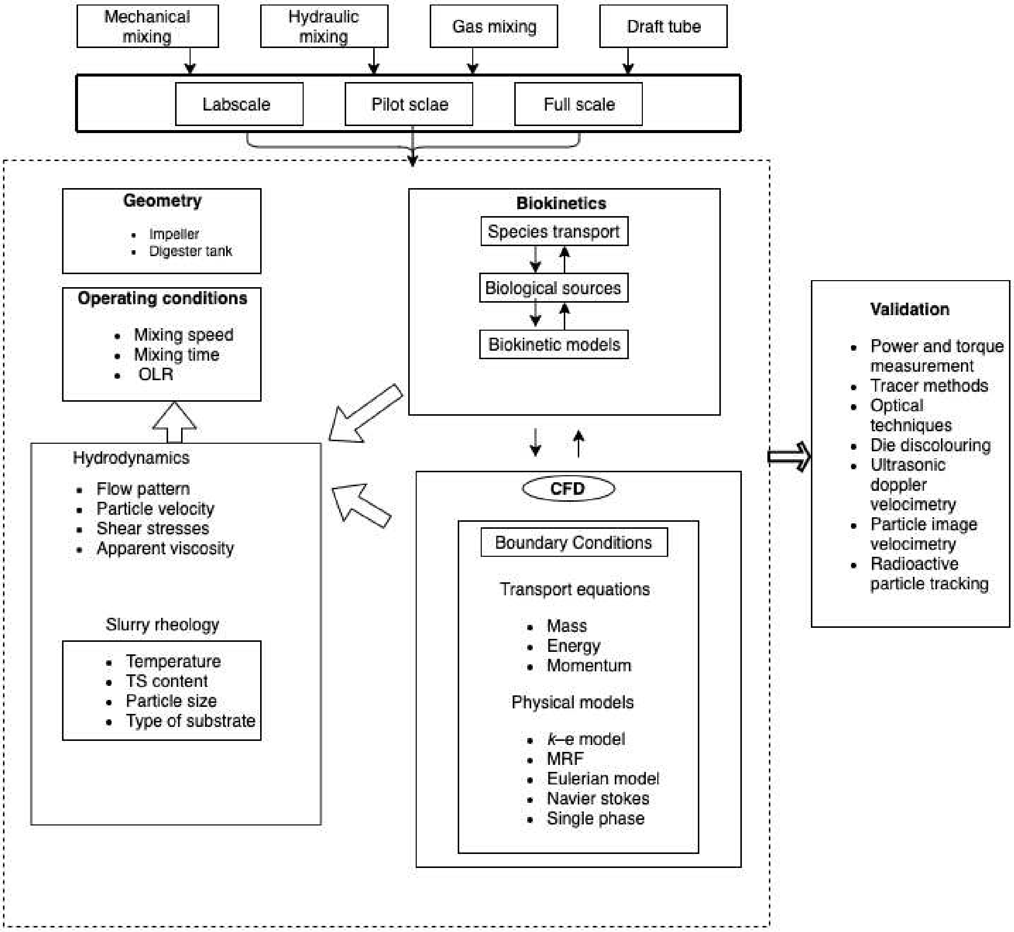


Figure 32. represents the correlations between biochemical processes, operating condition, mixing, and setup geometry, including experimental and modelling tools for analysis of mixing in an anaerobic digester.

Several investigations were undergone to analyze various forms of mixer geometry and digester designs and other forms of bioreactors used in the AD process. This involves tracer method research, numerous lab experiments as well as modelling. Recently Leonzio [167] studied the mixing properties in digester using different geometric configurations. Results proposed an innovational mixing system consisting of external recirculating pump. Accordingly, the dispersion of fluid tangent to the lateral surface resulted in lower dead zones and greater homogeneity of velocity distribution. Table 17. summarizes the large number of studies in terms of multiscale analysis of mixing and their short comings. It can be clearly derived from the table that lack of appropriate resources and analysis methods for evaluation of mixing can lead to unnecessary confusion in this field. Recent experimental and simulation results can help to better understand and improve the mixing properties in a bioreactor. Fig. 33 represents the correlations between mixing, biochemical processes, operating condition and setup geometry, including experimental and modelling tools for analysis of mixing in an anaerobic digester.

Table 17. Representation of data referring to the various approaches adopted by researchers to evaluate the effect of mixing in an anaerobic digester.

Reference	Digester scale	V	Numerical Appr.	Empirical Approach	Digester Geometry	Mixer Geometry	Microbial analysis	Biogas yield analysis	CH_4 yield analysis
Robert et al.[172]	LS	10000 m^3	CFD	o	♦	♦	o	o	o
Subramanian et al.[146]	LS	2911 m^3	o	♦	o	o	♦	♦	o
Ghanimeh et al. [83]	Lab-S	9 L	o	♦	o	o	♦	♦	♦
Ratanatasmskul et al. [28]	PS	12 m^3	o	♦	o	o	o	♦	♦
L. Yu et al. [139]	Lab-S PS	1 L 70 L	CFD CFD	♦ ♦	♦ ♦	♦ ♦	o o	♦ ♦	o o
Kshirsagar et al. [79]	PS	n.a.	CFD	o	♦	n.a.	o	o	o
Mohammadrezaei et al. [86]	Lab-S	1.2 m^3	CFD	♦	♦	♦	o	♦	o
R. Sindall et al. [62]	Lab-S	6 L	CFD	♦	♦	♦	♦	♦	o
Gang Luo et al. [56]	Lab-S	1 L	o	♦	o	o	♦	♦	♦
Rico et al. [45]	PS	1.5 m^3	o	♦	o	o	o	♦	♦
Z. Tian at al. [61]	Lab-S	5 L	o	♦	o	♦	♦	o	♦
R. Bello et al.[173]	n.a.	n.a.	MATLAB	♦	o	o	o	o	o
N. Stalin et al.[174]	PS	168 L	o	♦	♦	n.a.	o	♦	o
Andrew G. et al. [67]	Lab-S	4 L	o	♦	o	♦	o	♦	♦
H. K Ong et al. [71]	Lab-S	10 L	o	♦	o	o	o	♦	♦
K. C. Lin et al.[57]	Lab-S	7 L	o	♦	♦	♦	o	♦	♦
X. Zhai et al. [135]	PS	1.6 m^3	CFD	♦	♦	♦	o	o	♦

A. Noorpoor et al. [175]	LS	30 m^3	CFD	o	◆	◆	o	o	o
V. A. Vanlin et al. [81]	Lab-S	1 L	CFD	◆	o	o	◆	o	◆
A. Sulaiman et al. [66]	PS	500 m^3	o	◆	o	o	◆	◆	◆
Mohammad et at. [156]	LS	1200 L	CFD	o	◆	◆	o	o	o
H. Caillet et al.[153]	LS	n.a.	CFD	◆	◆	◆	o	◆	◆
F. Battista et al. [49]	Lab-S	2 L	o	◆	o	o	o	◆	◆
Terashima et al. [149]	LS	n.a.	CFD	o	◆	◆	o	o	o
Lebranch et al. [47]	Lab-S	2 L	CFD	◆	◆	◆	o	◆	◆
Hughes [63]	Lab-S	1 L	o	◆	o	o	o	◆	◆
Bridgn [22]	Lab-S	6 L	◆	◆	◆	◆	o	◆	o
James et al. [176]	Lab-S	9 L	o	◆	o	◆	o	◆	o
K. Latha et al. [96]	Lab-S	4.5 L	o	◆	◆	◆	◆	◆	o
J. Jiang et al. [97]	Lab-S	4.1 L	o	◆	◆	◆	o	◆	◆
Ismail et al. [98]	Lab-S	4.5 L	o	◆	◆	◆	o	◆	◆
Hoffman et al. [58]	Lab-S	4.5 L	◆	◆	◆	◆	◆	◆	◆
Suliaman et al. [66]	PS	500 m^3	o	◆	o	o	◆	◆	◆
Stroot et al. [65]	Lab-S	2 L	o	◆	o	o	o	◆	◆
M. Kim et al. [14]	Lab-S	4 L	o	◆	o	o	o	◆	◆
B. Wang et al. [88]	Lab-S	0.5 L	◆	◆	o	o	o	o	◆
Lindmark et al. [99]	Lab-S	1 L	o	◆	o	o	o	◆	◆
Semen et al. [177]	LS	2659 m^3	o	◆	o	◆	o	◆	◆
Peng Wei et al. [178]	Lab-S	7.2 L	CFD	o	◆	◆	o	o	o
Fei Shen et al.[160]	Lab-S	8.0 L	CFD	◆	◆	◆	o	◆	o
C. Maier et al.[162]	LS	n.a	CFD	◆	◆	◆	o	o	o

LS: Large scale; Lab-S: Laboratory scale; PS: Pilot scale; ◆: data available; o: missing elements; n.a.: data not available

6.7 Implementations

After careful analysis of literature on optimization of mixing it is concluded that due to involvement of microbiology, chemical aspects and hydrodynamics, mixing efficiency is considered as one of complex subject to optimize and unfurl various facts related to it. Intensive literature has been reviewed which provides evidence of both positive and negative effects of mixing on biogas production under the various operating conditions. Each study describes the outcome of results in different manner due to reason that the mixing evaluation technique and the setup of the experiments is totally different from each other. CFD provided ultimate solution for mixing issues but physical modelling still varies as far as literature is concerned which have resulted in hurdle to accept a general model in AD. The optimized multi-scale modelling approach that applies prototypes to various scales tends to provide the best balance in terms of efficiency, robustness and precision for technology and scale-up applications. Further research should be focused on the analysing the effect of mixing by physical separation of different steps of AD. Moreover, the design of the impeller and the digester tank can be changed according to mixing requirements in the slurry. Here some following conclusions can be drawn:

- Multidisciplinary approach is very important to evaluate the effect of impeller and digester geometry on biogas production rates.
- Evaluation of mixing in digester without disclosing the geometry of digester and impeller is not valuable enough unless the amount of shear stresses produced and time to mix is known.
- Scale up of lab scale digester and scale down of full-scale digester should be focused to optimize the mixing intensity and time of operation.
- Results can vary due to lot of factors intertwined in the AD process so, it is very important to focus on the parameters on actual working of full-scale biogas plant.
- The impact of agitation on microbial populations is only marginally discussed in AD and we believe that this study will prompt future work in this area.

7 New scientific results

1. This research demonstrated that the higher mixing intensity of 67 rpm for 5 min h^{-1} produced 15-18 % higher biogas production as compared to 10 rpm and 30 rpm without creating any instability in terms of VFAs accumulation and dead zones. Furthermore, higher mixing speed lead to reduction in dead zones to less than 2%. Large diameter impeller at medium mixing speed is best combination in direction of optimization of mixing in an anaerobic reactor.
2. It is concluded that the efficiency of the mesophilic digester is directly associated with the mixing time interval. The mixing regime also has effect on the physicochemical properties of the substrate. The digester performance was better under the minimum resting time of 1 hour at 67 rpm impeller speed as compared to resting time of 2, 4 and 6 hours. During this mixing regime the biogas yield was 5-12% higher as compared to longer resting times. The FAS/TOC Ratio was below 0.5 and the VS reductions was noted as 66.1 %. Drop in biogas yield can be due to VFA accumulations to some extent along with formation of floating layers and sedimentation at longer break time between the mixing operations.
3. CFD results demonstrated that under minimal mixing speed of 10 and 30 the dead volume was recorded as 18% and 17%, respectively; whereas under higher mixing intensity (67 rpm) it was reduced to just 2%. Inside a dead zone volume, the pH and temperature gradient occur, which results in decrease of the digester's effectiveness and apparently decline in biogas production and sometimes even digester failure
4. Multidisciplinary approach including the hydrodynamics and biochemical process is very important to evaluate the effect of impeller and digester geometry on biogas production rates. Evaluation of mixing in digester without disclosing the geometry of digester and impeller is not valuable enough unless the amount of shear stresses produced and time to mix is known. Scale up of lab scale digester and scale down of full-scale digester should be focused to optimize the mixing intensity and time of operation.
5. The intermittent mixing is strictly recommended as compared to continuous and un-mixing in terms of biogas yield and energy point of view. Geometrical similarity of lab-scale experiments and pilot scale biogas plants along with the rheological characteristics of slurry should be considered keeping the ratio of dimensions constant.
6. Digesters operated at lower TS content and longer HRT were unaffected by mixing but at higher TS levels mixing have significant effect on biogas yields due to increase in viscosity of slurry. Reduction of dead zones is the primary motive during the mixing to obtain homogenous mixture. Design of impeller, speed of mixing should be optimized according to design of digester and rheological properties of slurry

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3. Buta, Singh; Zoltán, Siménfalvi; Zoltán, Szamosi, M Rosas-Casals Decentralized biomass for biogas production. Evaluation and potential assessment in Punjab (India) November 2020 Energy Reports 6:1702-1714 DOI: 10.1016/j.egyr.2020.06.009
4. Buta, Singh; Zoltán, Siménfalvi; Zoltán, Szamosi, Critical Analysis of Methods Adopted for Evaluation of Mixing Efficiency in an Anaerobic Digester *Sustainability* 2021, *13*(12), 6668; https://doi.org/10.3390/su13126668
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6. Buta, Singh; Zoltán, Siménfalvi; Zoltán, Szamosi Techniques for evaluation of mixing efficiency in an anaerobic digester Solutions for Sustainable Development 2019-09-19 | book-chapter DOI: 10.1201/9780367824037-19 Part of ISBN: 9780367824037
7. Buta, Singh; Siménfalvi, Zoltán; Szamosi, Zoltán Comparison of mixing efficiency of different impellers for agitation of slurry in anaerobic digester November 2020 DOI: 10.1063/5.0024243
8. Buta, Singh; Siménfalvi, Zoltán; Szamosi, Zoltán Hydrodynamic factors in an Anaerobic Digester, Multiscience XXXII. MicroCAD International Multidisciplinary Scientific Conference Miskolc-Egyetemváros, Hungary: Miskolci Egyetem, (2018) pp. 1-9., 9 p. DOI: 10.26649/musci.2018.009 Publication:30418826
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Conferences

1. MultiScience - XXXII. micro CAD International Multidisciplinary Scientific Conference University of Miskolc, 5-6 September 2018
2. 6th International scientific conference on advances in mechanical engineering (ISCAME 2018) 11-12 October 2018 Debrecen, Hungary
3. The 14th Miklós Iványi International PhD and DLA Symposium 29-30 Oct 2018 University of Pécs, Hungary
4. RING - Fenntartható Nyersanyag-gazdálkodás Tudományos Konferencia tájékoztató, Pecs, 8-9 November 2018
5. EIT Climate-KIC Summer Programme (Paris-Zurich-Leoben) 1st July-4th August 2018
6. Buta Singh: Evaluating specific power consumption for mixing in digester of a large-scale biogas plant: A case study, 5th International Congress on Water, Waste and Energy Management (WWEM-19), Paris, 22-24 July 2019.
7. Buta Singh: Comparison of mixing efficiency of different impellers for agitation of slurry in anaerobic digester, International Conference on Mechanical, Materials and Renewable Energy (ICMMRE 2019), 6-7 December 2019, Sikkim, India.

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