GREEN ENERGY PRODUCTION WITH SPECIAL REFERENCE TOWARD THE USE OF COW DUNG

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ABSTRACT

Green energy comes from natural sources such as sunlight, wind, rain, tides, plants, algae and geothermal heat. These energy resources are renewable, meaning they are naturally replenished. In contrast, fossil fuels are a finite resource that take millions of years to develop and will continue to be replenished with use. Renewable energy sources also have a much smaller impact on the environment than fossil fuels, which produce pollutants such as greenhouse gases as by-product, contributing to climate change. Gaining access to fossil fuels typically requires either mining or drilling deep into earth, often in ecologically sensitive location. Green energy, however, utilizes energy sources that are readily available all over the world, including in rural and remote areas that don't otherwise have access to electricity. Advances in renewable energy technologies have lowered the cost of solar panels, wind tribunes and other sources of green energy, placing the ability to produce electricity in the hands of the people rather than those of oils, coal utility companies. Green energy can replace fossil fuels in all major areas of use including electricity, water and space heating and fuel for vehicle. The ability of biomass and biofuels to contribute to a reduction in CO2 emissions is limited because both biomass and biofuels emit large amount of CO2 when burned. Furthermore, biomass and biofuels consume large amount of water. Other renewable sources such as wind, power, photovoltaic, and hydroelectricity have the advantage of being able to conserve water and reduce CO2 emissions.

INTRODUCTION

1.1 General Introduction

Anaerobic treatment is high rate reactor is increasingly recognized as the core method of an advanced technology for the environmental technology protection and resources preservation and it represent, combined with the other proper method a sustainable and appropriate waste water treatment system for developing countries (Lettinga et al.,1987;) 1993; Van Buuren, 1996; Lettinga, 1996; 1996b; Lettinga et al.,1997; Verstraete and Vandevivere,1999; Hammes et al., 2000;Mahmoud et al., 2004; Zeeman and Lettinga, 1999; Gijzen, 2001, Abbasi,1998; Grandoa et al., 2017; Jorge Ricardo Cunha et al., 2018; Huete et al .,2018).

Waste water purification is the clearest paradigm of environmentally friendly technologies Some negative aspect of development and urbanization can be diminished, or even eliminated though a comprehensive treatment of the domestic and industrial waste water directly and immediately enhancing the quality of the environment (Lucas seghezzo, 2004) The term 'sewage' refers to the waste water produced by a community, which may originate from three different sources a) domestic waste water, generated from bathroom and toilets and activities such as cooking, washing etc. b) Industrial waste water from industries using the same sewage system for their effluent (treated or not) and c) rain water, particularly in the case of sewer system constructed for both waste water and storm water (combined system) (Van Haandal and Lettinga, 1994; Cruz-salomon et al .,2017; Jiang Wu et al ., 2018; Shegnan Xu et al .,2018).

Domestic wastewater can be divided into divergent streams according to their origin. Generally two streams are distinguished: concentrated – black water from toilets (faeces, urine and Flushing water) and diluted – grey water from bath, wash and kitchen (Henze and Ledin, 2001). Most of the organic material, nutrients and pathogens in domestic wastewater are in black water (51% of COD, 91% of nitrogen, 78% of phosphorus; Terpstra, 1999), making its treatment of the greatest importance (Luostarinen et al., 2007).

One of the most notable developments in anaerobic treatment process technology is the upflow anaerobic sludge blanket (UASB) reactor. (Najafpour, 2006).

Domestic sewage treatment consists of an item that deserves ample documentation due to the environmental impact caused by such wastewater if directly discharged into receiving waters. In addition, due to an increase in the scarcity of clean water (Aiyuk et al., submitted for publication) there is need for appropriate management of available water resources. Some of the goals of environmental protection and resource conservation concepts are the re-use of treated wastewater, residues emanating there from, and other treatment byproducts (Lettinga et al., 2001; Yi, 2001). Consequently, by implementing these concepts, a wastewater like domestic sewage, apart from being sanitized, can become an important source of re-usable water, fertilizer, soil conditioner and energy.
Sunny Aiyuk et al., 2006; Boris Tartakovsky et al .,2015; Heng Li et al .,2018).

1.2 Introduction to Anaerobic Digestion

Anaerobic digestion is a process by which environmentally hazardous organic wastes from municipal, agricultural and industrial sources may be stabilized. The treatment has many side benefits, most notably the production of methane-rich biogas which can be used to generate electricity and heat. Anaerobic digestion is performed by a consortium of microorganisms. In the absence of oxygen the anaerobic bacteria break down organic matter producing methane and carbon dioxide. Several other methods of dealing with organic wastes exist, including aerobic digestion, direct application to land and combustion. These methods either utilize the available biomass as a fertilizer or a fuel, but not both as is the case with anaerobic digestion. Unfortunately the use of anaerobic digestion is not as widespread as the other options.

1.3 Advantages and disadvantages of anaerobic digestion

1.3.1 Advantages (*Seghezzo* et al.1998)

High efficiency. Good removal efficiency can be achieved in the system, even at high loading rates and low temperatures

Simplicity. The construction and operation of these reactors is relatively simple.

Flexibility. Anaerobic treatment can easily be applied on either a very large or a very small scale.

Low space requirements. When high loading rates are accommodated, the area needed for the reactor is small.

Low energy consumption. As far as no heating of the influent is needed to reach the working temperature and all plant operations can be done by gravity, the energy consumption of the reactor is almost negligible. Moreover, energy is produced during the process in the form of methane.

Low sludge production. The sludge production is low, when compared to aerobic methods, due to the slow growth rates of anaerobic bacteria. The sludge is well stabilized for final disposal and has good dewatering characteristics. It can be preserved for long periods of time without a significant reduction of activity, allowing its use as inoculum for the start-up of new reactors.

Low nutrients and chemicals requirement. Especially in the case of sewage, an adequate and stable pH can be maintained without the addition of chemicals.

Macronutrients (nitrogen and phosphorus) and micronutrients are also available in sewage, while toxic compounds are absent.

1.3.2 Disadvantages

Long start-up. Due to the low growth rate of methanogenic organisms, the start-up takes longer as compared to aerobic processes, when no good inoculum is available.

Low pathogen and nutrient removal. Pathogens are only partially removed, except helminthes eggs, which are effectively captured in the sludge bed. Nutrients removal is not complete and therefore a post treatment is required.

Possible bad odors. Hydrogen sulphide is produced during the anaerobic process, especially when there are high concentrations of sulphate in the influent. A proper handling of the biogas is required to avoid bad smell.

Necessity of post-treatment. Post-treatment of the anaerobic effluent is generally required to reach the discharge standards for organic matter, nutrients and pathogens.

1.4 Reduction of Startup period using Batch Reactors-Objective of the present study

Anaerobic treatment has been considered as one of the most effective and energy economical methods in treating domestic and industrial wastewater. However, the major drawback of anaerobic treatment is its extremely long start-up period; generally it requires 3-8 months for the micro-organisms to granulate. There are many different hypotheses trying to explain the mechanisms of anaerobic granulation process and none of them has been accepted unanimously. Observation is that the process of granulation had been enhanced successfully by pre-culturing the fresh sludge in batch reactor (Uyanik et al., 2000; Show et al., 2004). It has been expected that pre-culturing of sludge enhances the granulation process. Long start-up phase before steady state operation, if activated sludge is not sufficiently available. The table 1.1 is given below (Uyanik et al., 2000; Show et al., 2004).

S. No	*SOURCE*		*REFERENCE*
		DURATION	
1	Goodwin et al., 1990.Biological wastes, 32 (125-144).	300 Days	Tanaka and Matsuo (1986)
2	Lucas seghezo et al., 1998.	6 months 4 months 2.5 months > 2 months > 2 months > 6 months 4 months 5 months 2 months	Louwe Kooijmans and Van Velsen, 1986; Lettinga et al., 1987. Barbosa and san' anna, 1989. Draaijer et al., 1992. Gnanadipathy and polparsert, 1993. Vieira and Garcia, 1992. Schellinkhout and Osorio, 1994. Tang et al., 1995. Haskoning, 1996; Tare et al, 1997. Chernicharo and Borges, 1997.
3	Punal et al., (2000). Water Res. Vol 34 (2614-2619).	4-8 months, even more than one year	Jayantha and Ramanujam, 1995.
4	DeliaTeresa ponza, (2002).Process biochemistry 37 (1091-1101)	4 or 5 months	Wu et al., 1993
5	Yu Liu et al., 2003.water Res. 37 (661-673).	2-8 months	Yu Liu et al., (2003).
6	Weili zhou et al., 2006. Process biochemistry 41 (36-43).	3-8 months	Hulshoff pol et al., 1983; Hickey et al., 1991
7	Anushyaa Ramakrishnan and Gupta, 2006. Jornal of Hazardous materials (Article in press)	2-3 months to 1 year (or even more)	Bardiya et al., 1995. Maat et al., 1990. Shin et al., 1992

In the present study, an effect of pre-culturing on the granulation process was studied as follows:

1. Culture of granules in batch reactors with fresh seed slurry.

2. The developed granules were then used for studies on granular characteristics.

3. Studies on the performance of Batch reactors seeded with fresh goat dung slurry.

REVIEW OF LITERATURE

2.1 Granular Sludge Formation.

Anaerobic granulation is a complex process, in which microbiological, other biotic and abiotic factors combine (O'Flaherty and Lens 2003; Huete et al .,2018; Xinyu Zhu et al .,2018). In the past two decades, tremendous research progress has been made in our understanding of the microbiological characteristics of UASB granules and the interaction among different species in the granules. Granulation may be initiated by bacterial adsorption and adhesion to inert matter, to inorganic precipitates (Hulshoff Pol et al. 1987), and/or to each other through physicochemical interactions and syntrophic associations (Yu et al. 2001a). These substances serve as initial precursors (carriers or nuclei) for further bacterial growth. Filamentous bacteria (e.g. *Methanosaeta*) may also play a role in forming matrices which further embed other cells. These loosely adhered bacterial aggregates are then strengthened by extracellular polymers secreted by bacteria (Schmidt and Ahring 1996). The phenomenon of granule formation has been the focus of extensive scientific investigation and many mechanisms and models have been proposed **(Table 1.2)**.

Table 1.2 Proposed models and theories for granule formation (adapted from O'Flaherty & Lens 2003; Liu et al. 2003).

	Model	Reference
Physico-chemical theories	Inert nuclei model	Lettinga et al. (1980)
	Selection pressure model	Hulshoff Pol et al. (1983)
	Growth of colonised suspended solids	Pereboom (1994)
Structural/ecological theories	Bridging of microflocs by *Methanosaeta*	Dubourguier et al. (1987)
	Capetown model	Sam-Soon et al. (1987)
	Spaghetti model	Wiegant (1987)
	Multi-layered model	MacLeod (1990), Guiot et al. (1992) and Ahn (2000)
	Syntrophic microcolony model	Hirsch (1984)
Thermodynamic theories	Surface tension model	Thaveesri et al. (1995)
	Four-step model	Schmidt & Ahring (1996)
	Proton translocation–dehydration theory	Tay et al. (2000)
Other	Cellular automaton model	Wimpenny & Colasanti (1997)
	Cell to cell communication model	Davies et al. (1998)
	Cluster model	Gonzalez-Gil et al. (2001)
	General four-step model	Liu et al. (2003)

2.2 Structure of Granules.

Anaerobic granules are particulate biofilms, formed spontaneously by autoimmobilization of anaerobic bacteria in the absence of a support material (Lettinga 1995; Xinyu Zhu et al ., 2018). These dense particles, consisting of an intertwined mixture of the symbiotic anaerobic micro-organisms that work together in methane fermentation, are the secret to the successful operation of the modern, high-rate anaerobic digester (McCarty 2001). Each granule is a functional unit comprising of all the different micro-organisms necessary for the methanogenic degradation of organic matter (Sekiguchi et al. 1998). A typical granule may include millions of organisms per gram of biomass. However, none of the individual species in these micro-ecosystems are capable of completely degrading influent wastes (Liu & Tay 2002). Competitive and cooperative associations between the component micro-organisms of the granule are necessary, and so the consortium forms a unique microbial ecosystem within several millimeters of an aggregate (Grotenhuis et al. 1991; Harmsen 1996; Sekiguchi et al. 1998; McHugh et al. 2003; Huete et al .,2018). In order to

address syntrophic and competitive interactions within methanogenic granules and to establish links between microbial structure and function, the combined involvement of various techniques, such as immunological (Schmidt & Ahring 1999), microscopic (Sekiguchi et al. 1998; Moharram et al., 2017), histochemical, traditional enumeration methods, molecular methods (rRNA based methods; McHugh et al. in press) and bacterial activity (specific methanogenic activity (SMA) tests; Colleran & Pistilli 1994) and/ or competition studies, is required.

A specific spatial orientation of microbial consortia within the anaerobic granule is essential because the distance between micro-organisms, such as acetogens and methanogens, must be sufficiently small to ensure and maintain a low hydrogen partial pressure (MacLeod et al. 1990; Stams 1994; Harmsen et al. 1996; Chavalit Ratanatamskul and Thassana Siritiewsri., 2015). A layered structure of the granule has also been proposed, in which a central core of acetoclastic methanogens is surrounded by a layer of hydrogen- or formate- producing acetogens and hydrogen- or formate- consuming methanogens, and by an outside.

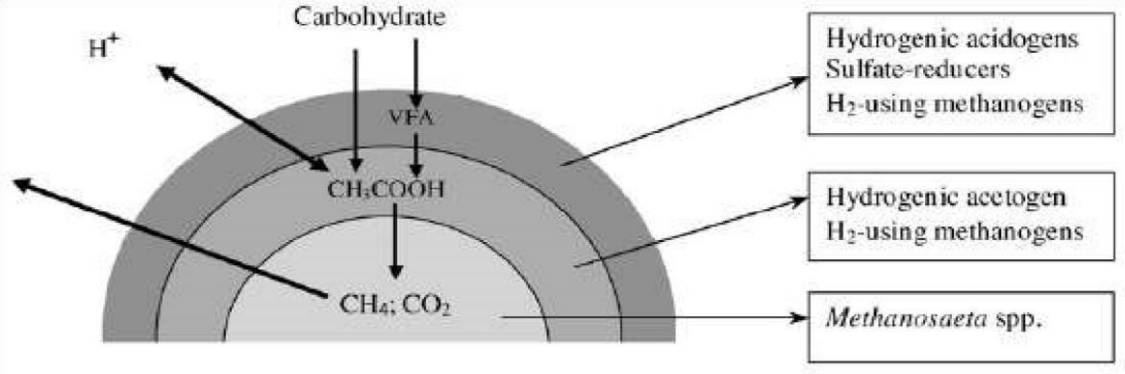


Figure 2.1 Schematic representation of the multi-layer model (Guiot and Pauss, 1992) **2.3**

Factors affecting granulation process in anaerobic digestion

Factors governing granulation are pH, temperature, composition and concentration

of organic compounds in wastewater, hydrodynamics, presence of multivalent cations, microbial ecology and production of exo-cellular polymeric substances by anaerobic bacteria (Yu et al., 2001a, 2000; El-Mamouni et al., 1998; Kalyuzhnyi et al., 1996; El-Mamouni et al., 1995; Florencio et al., 1995; Lens et al., 1995; Lettinga et al., 1991; Kosaric et al., 1990; Morgan et al., 1990; Haiyuan Ma et al.,2017).

2.3.1 Temperature

Operational temperature apparently affects the methanogens more than the acidogens (Chou et al. 2004; Huete et al .,2018). The optimum temperature range for digestion in mesophilic reactors is 30–40 °C (Henze and Harremoes 1983). For thermophilic methanogens, the optimum growth temperatures are Methanosarcina sp. 55–58 °C, Methanosaeta sp. 70 °C, Methanobacterium sp. 65–70 °C, and acetate utilizing mixed culture 60–65 °C (Zinder 1990; Zinder et al. 1984). Mesophilic granules, on the other hand, are sensitive to sudden temperature changes resulting in granule disintegration which may, in some cases, lead to reactor failure (Van Lier et al., 1990).The exact mechanism for this microbial adaptation process from mesophilic granules to thermophilic granules is still unclear.

2.3.2 pH and alkalinity

It is generally agreed that a high partial pressure of hydrogen and a stable pH value close to neutrality are required to obtain good-quality granulated sludge (Gonzalez et al. 1998). The pH values inside a granule evidenced from the microprofile are lower than the bulk liquid (Lens et al. 1995).The proton translocation activity on the bacterial cell surface presumably pumps out protons into the immediate vicinity of the cell surface. This process may also create a proton conductance across the bacterial surface causing dehydration and consequently facilitate adherence to other bacterium or surface (Hulshoff Pol et al.2004). An acidogenic population is significantly less sensitive to pH fluctuations compared to methanogens, which has an optimum pH in the range of 6.3–7.8. Thus, in the extreme pH conditions, acid formation prevails over methanogenesis, resulting in accumulation of volatile fatty acid (VFA) in the reactor (Haandel and Lettinga 1994;

Carlos Rico et al., 2017).

The buffering capacity in a UASB reactor is mostly provided by the alkalinity (Florencio et al. 1995). Alkalinity also helps in neutralizing fluctuations in the volatile acid concentration often arising from variation in organic loading (Isik and Sponza2005). Thus, the ratio of alkalinity to chemical oxygen demand (COD) is one of the key parameters for granulation (Gonzalez et al. 1998). Optimum alkalinity in the influent reportedly ranges between 250 and 950 mg/l (Singh et al. 1999; Braga et al.,2017).

2.3.3 Organic loading rate

The OLR variation can arise from either variation in influent COD or variation in flow rate with constant COD. An increase in OLR beyond its optimum range leads to decrease in pH due to increase in the concentrations of the VFAs (Dohanyos et al.1985). Once the microbial biomass recovers and stabilizes, the extra VFAs are normally metabolized and the pH stabilizes (Myburg and Britz 1993). OLR of up to 104 kg COD m−3 day−1 has been reported for anaerobic digestion of sugar substrate (Wiegant and Lettinga 1985). Low OLR often causes acute mass transfer limitation leading to disintegration of the larger granules (Ahn et al. 2002). The granules loose their strength and stability because the decay starts at the center due to substrate limitation (Kosaric et al.1990). In contrast to these studies, Teer et al. (2000) and Tiwari et al., (2005) have not experienced any granule disintegration while operating a UASB reactor under low OLR (<1.5 kg COD m−3 day−1). **2.3.4 Shear due to upflow and gas production**

Granule formation and characteristics are strongly influenced by the upflow velocity of influent (Kosaric et al.1990) and the superficial velocity of biogas (Haandel and Lettinga 1994). The upflow velocity applied in most of the laboratory and industrial scale reactors is about 1 m/h, although values up to 6 m/h in laboratory scale reactors have been also reported (Haandel and Lettinga 1994).At high upflow velocities (above 1 m/h), the granules may disintegrate due to shearing, and the resulting fragments may wash out of the reactor (Kosaric et al.1990). Vigorous gas evolution at high organic loading may similarly result in shear-off of bacteria cells from granule surface leading to granule erosion (Syutsubo et al. 1997; Paolo Dessi et al.,2016)

2.3.5 Substrate characteristics

Substrate characteristics, both composition and strength, dictate the microstructure in the form of spatial distribution of different microorganisms in a granule (Batstone and Keller2001). For simple substrates such as acetate, only methanogens are needed to complete the degradation process and, hence, granules primarily consist of methanogens (Grotenhuis et al.1991). Layered structures have been reported mostly for complex but easily hydrolysable noninhibitory substrates of higher concentration, i.e., glucose, proteins, sucrose, and brewery wastes (Fang et al. 1994; Guiot et al. 1992; MacLeod et al. 1990) Fats, oil, and grease are known to have low biodegradability and report on applicability of UASB reactor to treat such waste is divided. Typically reported problems include foaming, scum formation, and sludge washout in the presence of lipids (Lettinga and Hulshoff Pol 1991; Ozturk et al. 1993).

2.3.6 Nutrients

The requirements for nutrients such as nitrogen, phosphorus, and sulfur of various syntrophic groups in a heterogeneous culture are rather complex. During the formation of granules, an excess of nitrogen and phosphorus in the substrate is helpful and can be eliminated after the start of the granulation process without any deleterious effect on the granules' development (Gonzalez et al. 1998). Singh et al. (1999) have reported that cell growth reduces drastically at a nitrogen concentration of less than 300 mg/l. In contrast, there are reports of inhibition of the process of granulation at higher concentrations of these nutrients (Jarrell and Kalmokoff 1988). Nitrogen, phosphorus, and potassium were indicated to retard the effect of shock loading and prevent the flotation of granule (Alphenaar etal., 1993; Blaszczyk et al.1994; Wu et al ., 2018).

2.3.7 Multivalent cations and heavy metals

Granulation is initiated by bacterial adsorption and adhesion to inert matters, to organic precipitates, and/or to each other through physico–chemical interactions and syntrophic associations (Dolfing 1986; Schmidt and Ahring 1996). The cations may accelerate this process through bridging between negatively charged groups on cell surfaces and linking exo-cellular polymers (Hulshoff Pol et al.2004; Morgan et al. 1991; Schmidt and Ahring

1996). In addition, multivalent cations condense the diffused double layers and facilitate flocculation due to Van der Waals forces (Liu et al. 2003; Schmidt and Ahring 1996).The predominant binding groups for metals on the surface of bacteria are carboxyl and amino groups in proteins (Artola et al. 1997). Prolonged exposure to low pH also affects the metal retention dynamics within the granular sludge (Singh et al. 1999). Heavy metals compete with other ions in the solution for these binding sites on the cell surface. The relative toxicities of some metals depend on pH, VFA concentration, HRT, type and form of metal ions, and strength and affinity of the binding groups present on the surfaces of prevalent microorganisms (Gould and Genetelli 1984; Lin and Chen 1999). The presence of inert solids in the granules offers some abiotic surfaces to interact with the metal ions and, in turn, increases the toxicity resistances of biogranules (Oleszkiewicz and Sharma 1990).

2.3.8 Trace elements and heavy metals

The trace element requirement of anaerobic microorganisms is specific because of many cobalt-, nickel-, and ironcontaining enzymes involved in the biochemistry of fermentation and methane production (Shen et al. 1993). Therefore, the lack of some key trace metal can severely limit the overall anaerobic conversion process and granulation. The toxicity of heavy metal towards anaerobic digestion is independent of total metal concentration in the digester but depends on the concentration of free metal species in the sludge (Lawrence and McCarty 1965; Mueller and Steiner1993. Active, inactive, and dead biomass is capable of binding and accumulating high quantities of heavy metals (Kuyucak and Volesky 1988). Some trophic group(s) or organisms within the anaerobic consortia in the digesters may be more severely inhibited by a pulse addition of toxic heavy metals than the methanogenic populations (Hickey et al.1989; Shegnan Xu et al .,2018).

A significant decrease in gas production and simultaneous accumulation of VFA in the UASB reactor was reported in the presence of heavy metals (Lawrence and McCarty 1965), and methane production decreased as the metal concentrations increased (Lin and Chen1999). Although acidogens are generally considered to be less sensitive to toxins compared to methanogens (Lin and Yang 1991), they are reportedly more sensitive to

chromium, nickel, zinc, and copper (Hickey et al.1989 Lin1993). Mixed acid seed sludge was more sensitive to metal ions (except Pb) than acetic acid seed sludge (Lin 1992).In addition, many metals precipitate as hydroxide at neutral pH. Metal precipitates are generally pH sensitive and their solubility increases with lower pH values.(Manoj et al 2006)

2.3.9 Microbial ecology of seed sludge

Acetogenic bacteria and Methanosaeta sp. have been reported to be key populations which significantly accelerate granule development (El-Mamouni et al.1997). The study by ElMamouni et al. (1997) ongranulation rates reported that granulation was rapid on nuclei enriched with Methanosaeta and syntrophic organisms, slightly poorer on nuclei enriched with Methanosarcina and very slow on acidogenic nuclei. Methanosarcina apparently plays no part in initial biofilm formation as it does not attach to either hydrophobic or hydrophilic synthetic support structure (Verrier et al. 1988).

2.3.10 Exo-cellular polymer

The exo-cellular polymers produced by the bacteria are believed to play a critical role in maintaining structural integrity of granules (Schmidt and Ahring 1996; Shen et al. 1993). Several studies (Jia et al. 1996; Ross 1984) indicate that the ECP influences the formation of granules in UASB reactors. As ECPs are biopolymers accumulated on the surfaces of microbes, presumably some of the charges or the functional groups on the surface are associated to the ECP. The functional groups associated with the ECP of one microbial cell may increase ionic interactions between oppositely charged functional groups in the ECP of other microbial cells, leading to formation of a bond between the two cells.

In addition, ions in the media help in bridging between two like-charged functional groups of the cell ECPs (Hulshoff Pol et al. 1983; Schmidt and Ahring 1994). However, a high amount of ECP seems unnecessary for active granules and may cause deterioration in floc formation (Schmidt and Ahring 1996). ECP isolated from cells cultivated separately and added externally at the startup appears to have no effect on granulation in the UASB

reactors (Morgan et al. 1990). The addition of excess external ECP has been reported to show inhibitory effects on the granulation (Morgan et al. 1990)

2.3.11. Oxygen

The anaerobic treatment of dilute wastewaters can face a serious problem due to the possible presence of dissolved oxygen. Oxygen is considered as a suspect toxic compound since several investigations reported a detrimental effect, especially for the methanogens which are usually regarded as strict anaerobes (Hungate 1969 ;Whiteman et al ,1992).oxygen is a powerful reagent which generates potentially toxic radicals to all living cells, especially hydrogen peroxide and superoxide (Gottschalk and peinemann 1992 ; Morris 1979 ;Pfenning, N. 1978). The exclusion of molecular oxygen together with an environment of very low redox potential are postulated as being essential for the anaerobic microorganisms, especially the methanogens(Gottschalk and peinemamm 1992 ; Hungate 1969 ; Hungate 1984). The mechanism of oxygen – mediated inhibition was assumed to be due to the formed super oxide radicals. (Onderdonk et al, 1976). Ecosystems such as sludge digesters can be periodically subjected to stress by accidental entrance of air. This fact has been used as an argument in favour of the development of oxygen tolerant methanogens isolated in pure or enriched cultures from such ecosystems (Huser et al., 1982; Kiener and Leisinger, 1983). Methanosarcina that showed higher oxygen tolerance in cell aggregates compared with dispersed cells. The arrangement in cell aggregates is postulated to provide protection of the cells against oxygen, since individual cells showed higher sensitivity to oxygen (Kiener and Leisinger, 1983). Anaerobic microenvironments would be better protected and maintained in granular sludge because oxygen would hardly penetrate deep into the biofilm, contrary to dispersed sludge.

2.4 Role of Coagulant on Sludge Granulation

Initial stages of granulation depend upon the surface properties of bacteria and inert solids (Schmidt and Ahring, 1996). Surfaces of anaerobic bacteria, especially methanogens are typically negatively charged (Haandel and Lettinga, 1994).). Many natural and synthetic

polymers were used to enhance granulation but the reports once again, are mostly limited to high-strength wastewater (El-Mamouni et al., 1998; Guiot et al., 1991; Hughes et al., 1990).

2.5 Mechanism of anaerobic digestion

As the name anaerobic refers, the anaerobic digestion is carried out by microorganisms that can only live in an oxygen free environment. The decomposition of biowaste occurs in four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 1). Woody waste, in spite of being biodegradable, has a high lignin content which slows down the hydrolysis phase, and for that reason woody waste shall not be used as an input material in anaerobic treatment plants (United Tech, 2003), that type of waste should be preferable recycled as raw material for particle board production or thermally treated.

2.5.1 Hydrolysis:

During hydrolysis, the first stage, bacteria transform the particulate organic substrate into liquefied monomers and polymers i.e. proteins, carbohydrates and fats are transformed to amino acids, monosaccharides and fatty acids respectively. Equation 1 shows an example of a hydrolysis reaction where organic waste is broken down into a simple sugar, in this case, glucose (Ostrem, 2004).

Equation 1: $C_6H_{10}O_5 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2$

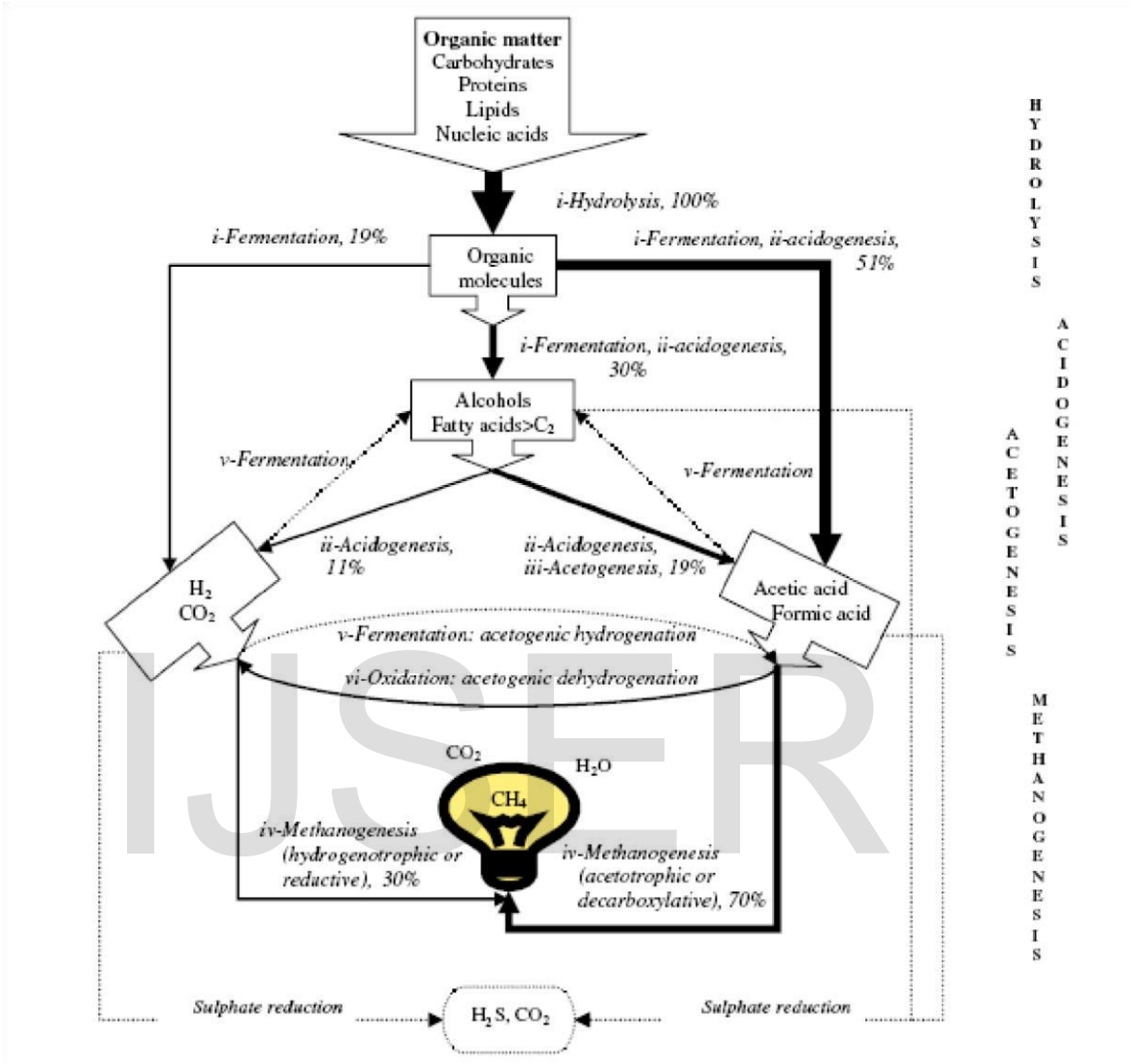


Fig.2.2: Schematic of the different metabolic steps and microbe groups involved in the complete degradation of organic matter to methane and carbon dioxide (arrow thicknesses show relative importance of degradation pathways with associated percentages; demarcation into trophic groups remains ill-defined (compiled from Van Haandel and Lettinga, 1994; McInerney, 1999; Poulsen, 2003).

2.5.2 Acidogenesis

In the second stage, acidogenic bacteria transform the products of the first reaction into short chain volatile acids, ketones, alcohols, hydrogen and carbon dioxide. The principal

acidogenesis stage products are propionic acid (CH_3CH_2COOH), butyric acid ($CH_3CH_2CH_2COOH$), acetic acid (CH_3COOH), formic acid ($HCOOH$), lactic acid ($C_3H_6O_3$), ethanol (C_2H_5OH) and methanol (CH_3OH), among other. From these products, the hydrogen, carbon dioxide and acetic acid will skip the third stage, acetogenesis, and be utilized directly by the methanogenic bacteria in the final stage (Figure 2). Equations 2, 3 (Ostrem, 2004) and 4 (Bilitewski et al., 1997) represent three typical acidogenesis reactions where glucose is converted to ethanol, propionate and acetic acid, respectively.

Equation 2: $C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2$

Equation 3: $C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O$

Equation 4: $C_6H_{12}O_6 \rightarrow 3CH_3COOH$

2.5.3 Acetogenesis

In the third stage, known as acetogenesis, the rest of the acidogenesis products, i.e. the propionic acid, butyric acid and alcohols are transformed by acetogenic bacteria into hydrogen, carbon dioxide and acetic acid (Figure 2). Hydrogen plays an important intermediary role in this process, as the reaction will only occur if the hydrogen partial pressure is low enough to thermodynamically allow the conversion of all the acids. Such lowering of the partial pressure is carried out by hydrogen scavenging bacteria, thus the hydrogen concentration of a digester is an indicator of its health (Mata-Alvarez, 2003). Equation 5 represents the conversion of propionate to acetate, only achievable at low hydrogen pressure. Glucose (Equation 6) and ethanol (Equation 7) among others are also converted to acetate during the third stage of anaerobic fermentation (Ostrem, 2004).

Equation 5: $CH_3CH_2COO^- + 3H_2O \leftrightarrow CH_3COO^- + H^+ + HCO^{-3} + 3H_2$

Equation 6: $C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2$

Equation 7: $CH_3CH_2OH + 2H_2O \leftrightarrow CH_3COO^- + 2H_2 + H^+$

2.5.4 Methanogenesis

The fourth and final stage is called methanogenesis. During this stage, microorganisms convert the hydrogen and acetic acid formed by the acid formers to methane gas and carbon dioxide (Equations 2.20, 2.21 and 2.22) (Verma, 2002). The bacteria responsible for this conversion are called methanogens and are strict anaerobes. Waste stabilization is accomplished when methane gas and carbon dioxide are produced.

Equation 8: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

Equation 9: $2C_2H_5OH + CO_2 \rightarrow CH_4 + 2CH_3COOH$

Equation 10: $CH_3COOH \rightarrow CH_4 + CO_2$

2.6 Anaerobic Digestion Systems

There are several possibilities to design anaerobic digestion systems. A system can be as simple and cheap as a single static cylindrical digestor or as complex and expensive as a multi digestor system with moving parts and intelligent sensors that support the operation of the plant. The efficiency of the plant is directly affectedby the type of system installed and the way it is managed. Simple plants are easy to design but require constant monitoring and are less efficient, while complex plants are designed to detect errors and warn operators, thus making them more efficient.

Although the microbial processes are the same for all anaerobic digestion processes, each plant is unique and should be designed according to its own input parameters and economic possibility. At the moment of designing a new plant, or making an old facility more efficient, several factors must be taken into consideration. Some of these factors are the capacity of the plant, the types of waste to be treated, the area available, the climate of the region, the demographics and the location of the plant.

Two factors have a dominant influence on the biogas production: the content of digestible matter of the waste treated and the transfer rate of this waste fraction into the digester.

Depending on the water content in the digester and the way of feeding the digester, the anaerobic digestion process can be classified into wet and dry fermentation and continuous and discontinuous fermentation.

Wet fermentation refers to total solid content in the Digestor with less than 12 percent of dry matter, while dry fermentation refers to digester feed with 30 percent of dry matter or more.

Continuous fermentation processes are realized in Wet Anaerobic Digestion Plants and Plug Flow Anaerobic Digestion Plants. Discontinuous fermentation processes are realized in Batch Dry Fermentation.

2.7 Background of the study with special reference to camel dung

The dungs of the Ruminant animals (cattle, buffalo, sheep, and goat) have been studied and used for the anaerobic digestion. These ruminant animals produce significant amounts of methane as part of their normal digestive process. In the rumen (large fore-stomach) of these animals, microbial fermentation converts their feed into products that can be digested and utilized by the animal. Methane is also produced in smaller quantities by the digestive processes of other animals, including humans, but emissions from these sources are in significant (USEPA, 2011a; Huete et al .,2018).

Whereas the methane that is exhaled by the ruminant animals is impossible to capture, a large proportion of methane produced by the manure of these animals can be captured. Livestock manure keeps releasing methane due to the anaerobic decomposition of organic material contained in the manure by bacteria exited along with the manure from the animal (Chhabra et al., 2009; Alvarino et al., 2017).

Special importance has been given to study and experiment the camel dung and its production and generation of methane. The reasons for the selection of camel dung are due to the availability of camel in the region of Rajasthan and so far the study and the experiment has not been performed by anyone. The camel dung has been collected from

various places of Rajasthan and the experiment has been performed. The performed experiment is also compared with the dungs of sheep, cow, buffalo and goat. This is the new and the present innovation conducted by the process of anaerobic digestion.

2.8 Aim of study with reference to camel dung

The study of the camel dung involves the sludge characteristics like pH, TS, SV, SVI sieve size of granules and the methane generation potential from it. The table **1.3** gives the evidence of biogas generation from livestock manure (Metcalf and Eddy, 2004; Batzias et al., 2005; Deublein and Steinhauser, 2008) is documented as follows.

Animal	*Total manure (kg/head/day)*	*Total solid (TS) (kg/head Day)*	*Biogas yield factor (m3/kg of dry matter)*	*C/N*	*VS (% of fresh manure)*
COW	20	4.0	0.20-0.50	18-25	13
BUFFALO	25	4.5	0.15-0.32	18-25	
SHEEP/ GOATS	1-5	0.6	0.56-0.65	13	12
PIG	1.8	0.6	0.37-0.61	29	
POULTRY	0.1	0.03	0.31-0.54		17
HORSES	24	7.1	0.20-0.30	24-25	
RABBITS	0.2	0.1	0.36		

3.1 Batch reactor

3.1.1 Substrate

The substrate used in the study was synthetic wastewater containing sucrose. Raw synthetic wastewater had a COD of 1000 mg/l (per gram of sucrose substrate), it was diluted accordingly to get the COD of 3000 mg/l.

3.1.2 Inoculum

For the start-up of the batch reactors, Fresh Camel Dung Slurry is used as seed.

3.1.2.1 Fresh Camel Dung Slurry(FCDS)

FGDS was made by soaking the goat dung overnight that are collected from village near by NIMS University campus. The characteristics of FCD Sludge are shown in Table 3.1.

The sludge was sieved with 0.2mm mesh and then inoculated in the reactors. To this, 1ml of trace elements solution/ liter of the wastewater were added. The trace element solution was prepared as per Singh et al, 1996 (**Table 1.4**).

Table 1.4 Characteristics of Seed Sludge

S.No.	*Parameters*	*FCD sludge*
1.	pH	7.5-7.9
2.	TS g/l	85.6800
3.	Particle size, mm	0.212
4.	Settling velocity, m/h	10.00
5.	Sludge Volume Index, mg/l	40.00

3.2 Batch Reactor Set-up

Three reactor set up (Fig 3.1) were used as batch reactors. The working volume of each reactor was 2 L. The reactor was sealed air tight with a lid. Through the lid, a tube was connected, which was used as gas outlet. The gas evolved was collected in a collection bottle filled with 0.1 N

H_2SO_4 solutions. The gas bubbled through the solution and was collected at the top of the bottle. As the gas collected in the bottle, the increased pressure resulted in displacing H_2SO_4. The H_2SO_4 solution collected was taken to be the amount of biogas evolved (Figure 3.1). To ensure a minimum amount of dead space, all the tube lengths were kept as short as possible and the level of the liquid in the flask was as close to the lid as possible.

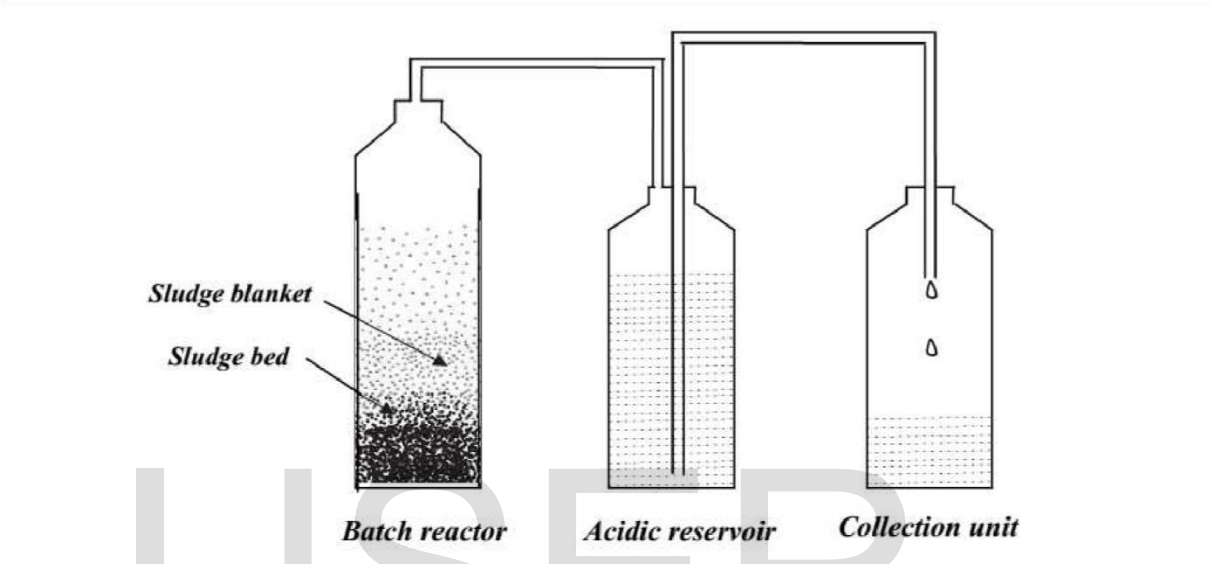
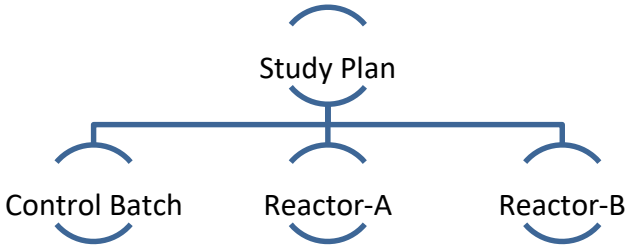


Figure 2.3 Batch reactor set up

Experimental and reactor design:



Control Batch → Synthetic waste water

Reactor- A & B→ Sieved sludge 0.212 mm of 15 g VSS

3.4 Analytical method

3.4.1 Total Solids

For total solids 25 ml of sample were taken in crucible and dried at 103ºC to 105ºC for 1 hour. Cool dish in desiccators to balance temperature, and weight (APHA, 1998).

Calculation:

$$\text{mg total solids/L} = \frac{(A-B) \times 1000}{\text{Sample volume, ml}}$$

A = Weight of dried residue + dish mg, and B= Weight of dish, mg.

3.4.2 Chemical oxygen demand (COD)

The chemical oxygen demand is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.

Take 20 ml sample in the flask of reflux unit and add 10 ml of potassium dichromate, a pinch of each silver sulphate and mercuric sulphate and 30 ml of sulphuric acid. Attach condenser to the mouth of flask and heat for atleast 2 hours to reflux the contents. Cool the flask, detach from unit and dilute its contents to about 140 ml by adding distilled water. Add 2-3 drops of ferroin indicator solution and titrate against ferrous ammonium sulphate solution. At the end point blue green colour of contents changes to reddish brown. Run simultaneously distilled water blank in similar manner.

Calculation:

$$\text{COD (mg/l)} = \frac{(A-B) \times N\ FAS \times 8000}{}$$

20 ml (sample)

A = ml FAS used for blank, B = ml

FAS used for sample, and

N = Normality of FAS.

3.4.3 Settling Velocity

Settling Velocity of the granules was examined by sampling the granular sludge from the middle and lower parts of the reactor. The granules were quickly transferred into the top of a glass column (2.5mm diameter X 1metre height) filled with tap water. The first granules to be sediment on the bottom of the column were observed following by other granules at I, 2, 3 minute intervals were noted. The settling velocities of all the samples were calculated from the time required for sedimentation from top to the bottom of the column by correcting the volume of granule samples withdrawn Laguna *et al*., 1999.

3.4.4 Sieving

For estimating the size distribution of the sludge particles taken from the bottom sampling port, solid samples were classified into 5 fractions using laboratory sieves with various openings (0.2, 1.0, 2.0, 3.0 & 4.0mm). The sludge particles were first placed in the sieve with the biggest opening (4.0mm). The particles were gently submerged in water and shaken to let the smaller particles pass through this sieve. The particles passing through were collected in a container. The smaller particles collected in the above sieve were then placed in the next sieve (3.0mm) and the above procedure were repeated until all of the four sieves were used (Yu *et al* ., 2001a; Ghangrekar *et al*., 2005).

3.4.5 Biogas analysis

For batch experiments, Biogas produced by the reactors was collected over water to which sulfuric acid (final concentration 0.1 N H_2SO_4) had been added to reduce the solubility of CO_2.

3.4.6 pH

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment, e.g., acid base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control is pH dependent. At a given temperature the intensity of the acidic or basic character of a solution is indicated by pH. pH is defined by Sorenson ,(1909) is –log (H+). pH was determined by pH sensitive glass electrode connected to a digital pH meter. Since pH depends greatly on temperature, temperature compensation was done during the calibration of the pH meter. The apparatus was calibrated using Buffer 7.

3.5 Batch reactor Start-up and Steady state performance:

Duplicate reactors were started with FGD sludge of 15g VSS. The effluent was decanted and replaced with synthetic wastewater of same strength of 3000 mg/l COD in an interval of every 7 days. All the reactors were operated for complete degradation of organic matter present in the feed.

RESULTS AND DISCUSSION

The results of the present study are discussed as the Performance of the reactors in terms of biogas yield and granular characters.

4.1 Performance of the reactors

Granulation is initiated by bacterial adsorption and adhesion to inert matters, to organic precipitates and/ or to each other through physico-chemical interactions and syntrophic associations (Dolfing 1986; Schmidt and Ahring 1996). It is hypothesized that the spontaneous immobilization of bacterial cells through electrostatic charge attraction, results in agglomeration of dense and active biogranules enhancing the reactor performance (Show., *et al* 2004).Hence in this study pre-culturing of granules and their biogas production potential has been evaluated.

4.1.1 COD removal and Biogas yield

The performance of the reactors were evaluated in terms of COD removal efficiency and biogas yield as summarized in fig. 4.1 and 4.2. The biogas produced as CH_4/g of COD removal achieved more than 90% when steady state was achieved for all reactors was respectively consistent with values of 0.30 to 0.32 CH_4 g^{-1} COD day^{-1}.These values are close to the stoichiometric quantity of 0.35 m^3 kg^{-1} COD removed reported by Cronin and Lo (1988) and Oleszkiewick and Barry (1986).

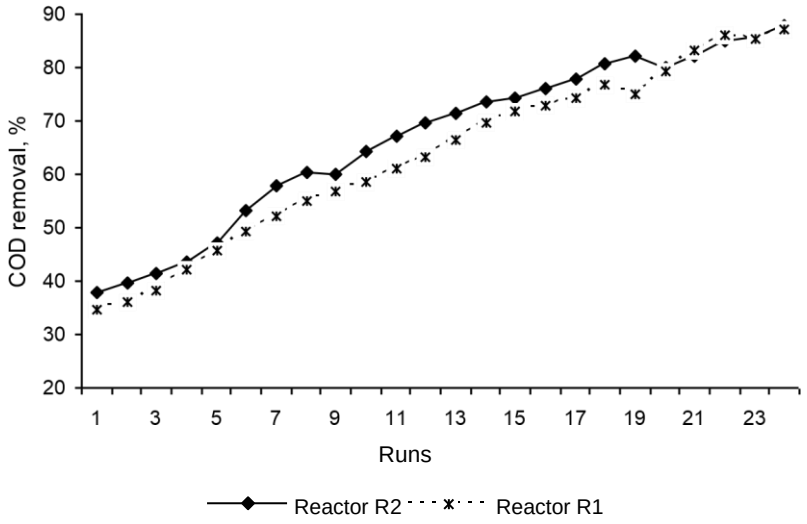


Figure 2.4 Performance in COD removal from batch reactor pre-cultured with Fresh Camel Dung (FCD) Slurry.

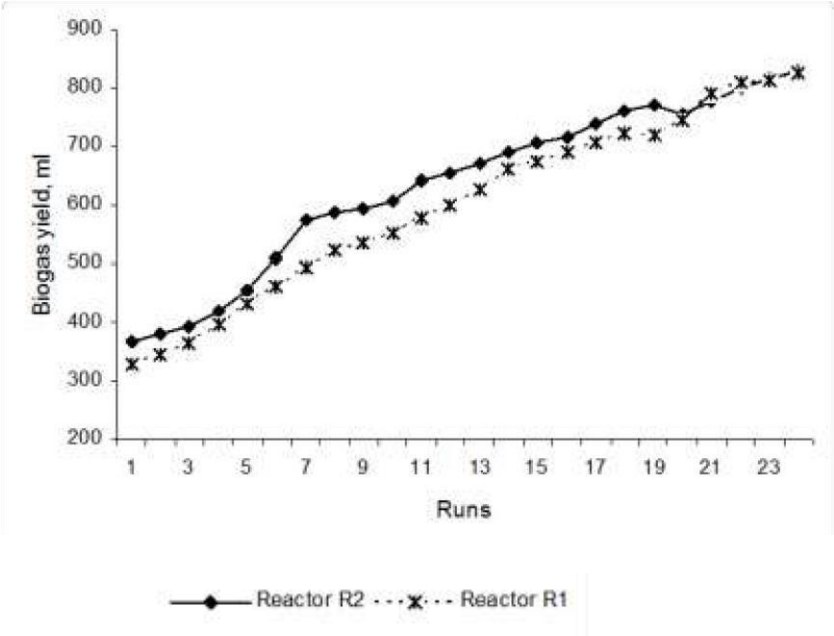


Figure 2.5: Performance in Biogas yield from batch reactor pre-cultured with Fresh Camel Dung (FCD) Slurry.

4.2 Granule characteristics

The purpose of this study is to enhance the granulation process by pre-culturing of granules in batch reactor. Hence granule characteristics was studied during each organic loading rate.

4.2.1 Settling Velocity (SV)

The development of granules with high settleabilities is a prerequisite for successful operation of the anaerobic process. Settleablity is dependent on density and size of the granules. Density of granules is greatly influenced by the presence of inorganic precipitates and the inclusion of gas (Holshoff pol *et al*., 1986). Settleability of granules could be indicated by measurement of settling velocity (SV). Granular sludge can be divided into three fractions based on the reported settling velocities :a poor settling fraction , a satisfactorily settling, and a good settling fraction, with settling velocities up to 20 m/h, from 20 to 50 m/h, and over 50 m/h, respectively (Schmidt and Ahring, 1996).

Settling velocity of granules ranging from 0 to 52 m/h was reported by Blaszezyk *et al*., (1994). The sludge from the control reactors have 5 m/h SV at 1.5 g COD/l/day and the pre-cultured granules from the batch reactors exhibit the granular character in good settling fraction (in the range 76-78 m/h (0.212-0.500 mm) and 86 – 84 m/h (0.500<) the same OLR. Good granular sludge has SV of 18-100 m/h (Dolfing and Mulder, 1985; Maaskant and Zeevalkink, 1983; Thaveersri et al., 1994; Park *et al.*, 1997; Lens *et al.*, 1998). However, the settling velocity of granules examined in the study is not in the range reported by Blaszezyk et al., (1994).

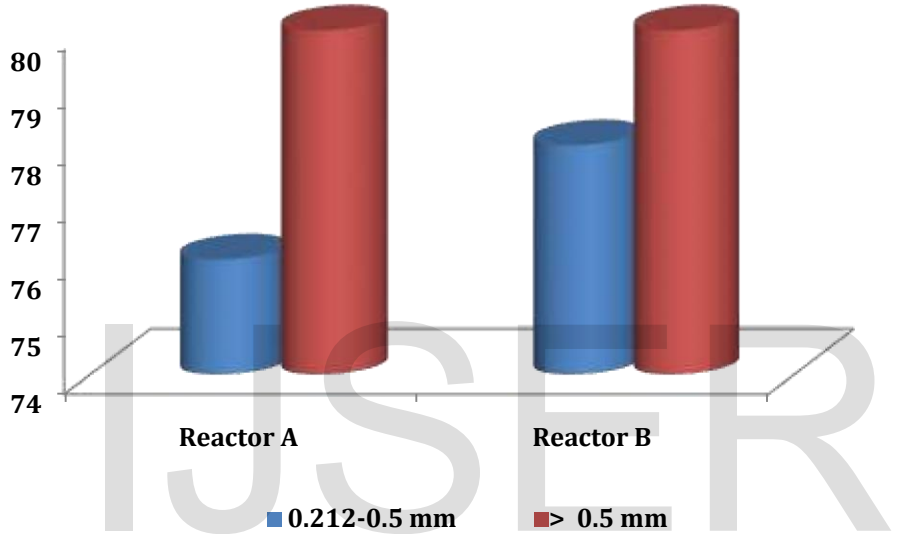


Figure 2.6: Settling Velocity of granules in the First month of Batch reactors.

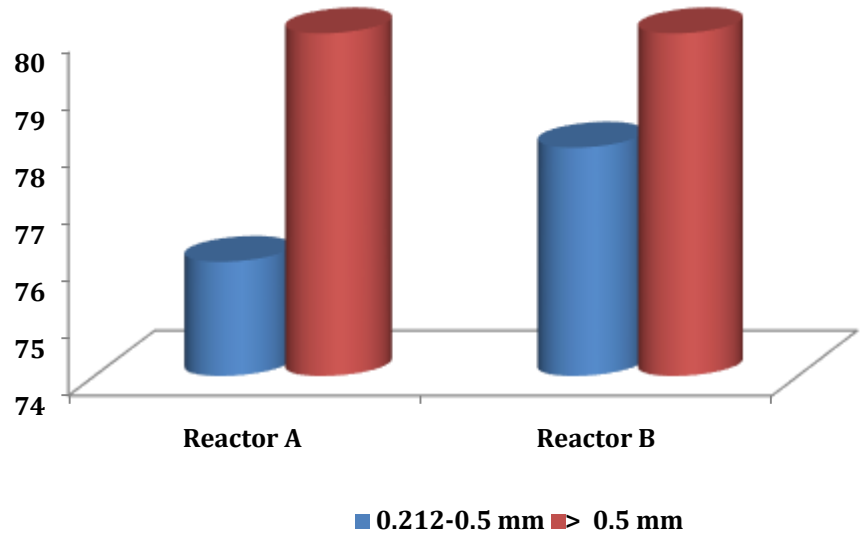


Figure 2.7: Settling Velocity of granules in the Second month of Batch reactors.

4.2.2 Sludge volume index (SVI)

Reduction in SVI, which is an important physical parameter, was generally considered as an indicator of improvement in granule settleability. Prakash and Gupta (2000) has reported that SVI of 25 ml/g SS for granules of size 0.25 to 3 mm diameter. At the end of the present study reactors (A & B) granules has SVI of 15 & 17 ml/g respectively. It is reported that for granular sludge, SVI vary from 10 to 20 ml/g (Maat and Habets, 1987; Yan and Tay, 1997). Reduction in SVI and enhanced granule settleablity due to the improved bacterial adhesion possibly caused by spontaneous immobilization of bacterial cells in to a complex network structure for enhanced bacterial aggregates (Wirtz et al., 1996; Yoda et al., 1989).

4.2.4 Granule size

According to Dolfing (1987), three kinds of particles may be distinguished:

(a) Flocs, which are aggregates with a loose structure. After settling , they usually cannot be individualized;

(b) Pellets, which are aggregates with a more dense structure than flocs. After settling, they are still individualized entities;

(c) Granules, which are dense pellets with a granular shape. They are firm, withstand a certain amount of compression and are referred to as “well flocculated sludge (Lettinga et al., 1980).

At the end of the period with OLR of 3 g COD/g VSS, there was 52 & 54 % of 0.2 – 0.5 mm; and 28, 26 % of < 0.5 mm sized granules in studies reactors. Whereas, it was 90 %

sludge in < 0.2 mm in the control reactor. In general, development on and above 0.5 mm size has been considered as granules by many researchers (Wang et al., 2004; Show et al., 2004; Bellouti et al., 1997; Yan and Tay,1997). However, few have considered 0.16 mm or less as granules (Tiwari et al., 2005, Chou and Huang, 2005; Pever et al., 2006; Lalit et al., 1997).

Bio particles less than 0.3 mm, instead of calling them as granules, they could be regarded as pellets, which are aggregates with a more dense structure than flocs (Dolfing, 1997). Smaller the bioparticles the more their number, particulate matter such as inorganic precipitates, metabolites, bacterial secretions, etc, could have been involved/ embedded in the granulation process (Yan and Tay, 1996).

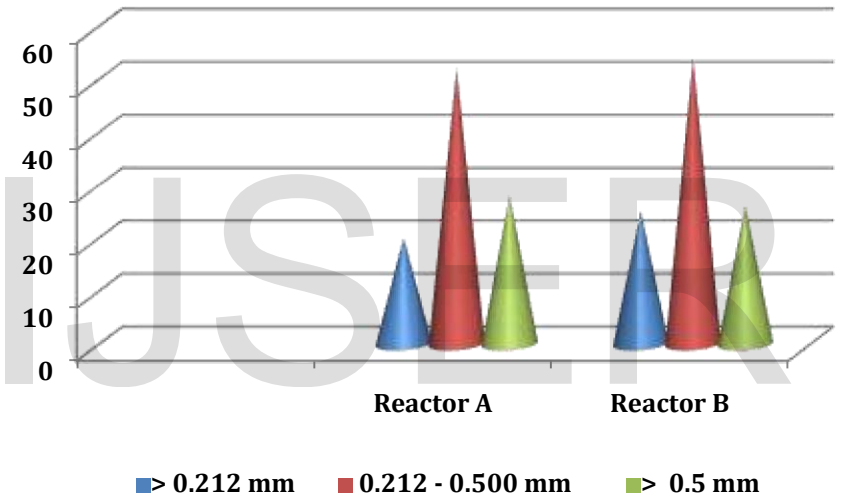
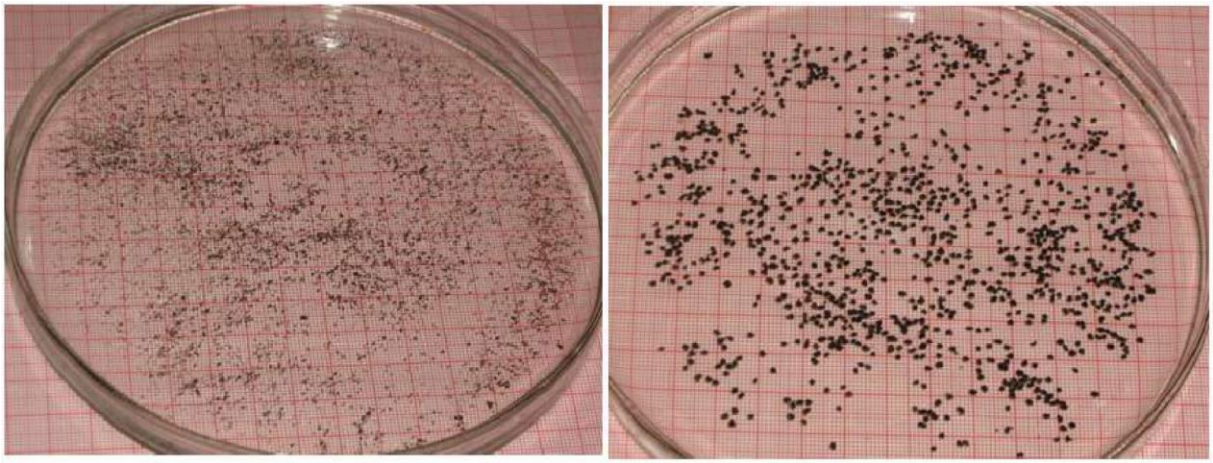


Figure 2.8: Size distributions of granules in the First month of batch reactors.



0.212 – 0.5 mm **0.5 mm**

Figure 2.9: Granules developed from Pre-culturing of FCD slurry.

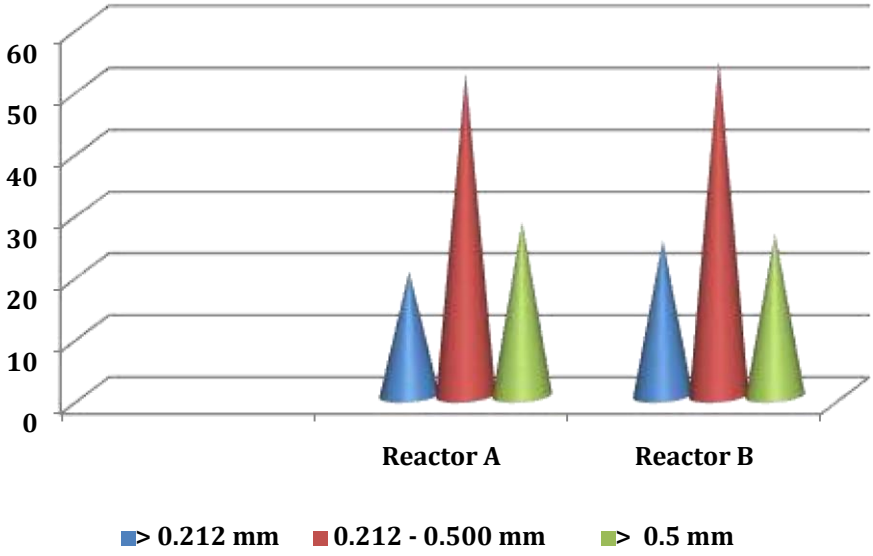


Figure 2.10: Size distributions of granules in the Second month of all reactors.

4.3 Sludge parameter results:

The data of the sludge parameters taken on weekly basis for complete 3 months are as follows:

The table 1.5 of SV Data:-

Sieve size	30th Day		60th Day	
	Reactor-A	Reactor-B	Reactor-A	Reactor-B
0.2-0.5 mm	60	64	76	78
0.5< mm	72	76	86	84

The table 1.6 of SVI Data:-

Days	Reactor-A	Reactor-B
30th	32	35
60th	14.9	16.8

Sieve size	30th Day		60th Day	
	Reactor-A	Reactor-B	Reactor-A	Reactor-B
0.2< mm	40	35	20	25
0.2-0.5 mm	45	43	52	54
0.5< mm	15	22	28	26

5.1 Conclusion

From the present study it cloud be concluded that for *first time* the (FCD) Fresh Camel Dung Slurry has been investigated as the suitable substrate for the anaerobic digestion of waste material. The Innovated process is highly efficient to yield the green energy in terms of methane gas. This could be used as an efficient substrate for the high rate reactors. The performance and the granular character of the studied reactor is summarized as follows:

1. The batch reactors performed well and achieved up to 98% COD removal and biogas yield of 0.35 CH_4 $^{g-1}$ COD removed/ day.

2. The granules were acclimatized with the feed and flocculent in nature.

3. Continual feeding of the FCD slurry, granules were developed in the batch reactors of size greater than 0.5 - 2 mm within a month.

4. The granules were having good settling velocity. Granules with higher settling velocity are desirable for the sludge in UASB to reduce washout of the active sludge from the reactor.

Hence from this study, it can be concluded that the pre-cultured granular sludge can be directly seeded to the UASB reactors. Their by the stat-up period could be reduced and better operation of the reactor may be achieved.

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