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Factors Affecting on Methane Production from Glucose-Containing Wastewater Using an Anaerobic Batch Reactor.

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ABSTRACT

In this work, the process performance of an anaerobic batch reactor for the methane production from glucose-containing wastewater was studied. The anaerobic sludge taken from UASB reactor was operated on flax retting wastewater and activated seeding sludge from Zenin wastewater treatment plant. The anaerobic batch reactor was operated at ambient temperature, a hydraulic reaction time (HRT) of 24 h, and different chemical oxygen demand (COD) loading rates from 10 to 50 kgm^{-3} d^{-1} without and with pH control at 7. The results showed that at the optimum COD loading rate of 40 kgm^{-3} d^{-1} and pH 7. At these optimum conditions, the highest methane yield was 0.95 l methane/g glucose consumed. Under the optimum COD-to-nitrogen (COD: N) ratio of 100:2.4, the system with pH controlled at 7 gave the highest specific methane production rate of 30 l methane l^{-1} d^{-1}.

Keywords: batch reactor; glucose containing wastewater; methane production.

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INTRODUCTION

Over the last century, continued population growth and industrialization have resulted in the degradation of various ecosystems on which human life relies on. In the case of ocean and river quality, such pollution is primarily caused by the discharge of inadequately treated industrial and municipal wastewater. On initial discharge, these wastewaters can contain high levels of inorganic pollutants which can be easily biodegradable, but whose impact load on the ecosystems, either in Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD_5), or Chemical Oxygen Demand (COD), may be in the tens of thousands mg/L (Ng W.J., 2006). To combat this increasing burden on our aquatic environment, increasingly strict regulation on pollution discharge is being implemented by various governmental bodies, with focus primarily on waste reduction. The treatment systems developed by industry are frequently regarded as a regulatory obligation, increasing capital and running costs and yielding negative economic returns. Compliance to environmental legislations should not necessary lead to the creation of additional costs, but can instead provide a secondary source of income. One possible source of increased revenue available to industries is through taking advantage of the incentives awarded by the Clean Development Mechanism (CDM) under the Kyoto Protocol 1997.

In the treatment of wastewater, biological treatment appears to be a promising technology to attain revenue from Certified Emission Reduction (CER) credits, more commonly known as carbon credits from the CDM as methane gas is generated from anaerobic digestion and can be utilized as renewable energy. With appropriate analysis and environmental control, almost all wastewaters containing biodegradable constituents with a BOD/COD ratio of 0.5 or greater can be treated easily by biological means (Metcalf and Eddy, 2003). In comparison to other methods of wastewater treatment, it also has the advantages of lower treatment costs with no secondary pollution (Sponza and UlukÖy, 2005). Both aerobic and anaerobic processes can be used; the former involves the use of free or dissolved oxygen by microorganisms (aerobes) in the conversion of organic wastes to biomass and CO_2 while in the latter complex organic wastes are degraded into methane, CO_2 and H_2O through three basic steps (hydrolysis, acidogenesis including acetogenesis and methanogenesis) in the absence of oxygen. Aerobic biological processes are commonly used in the treatment of organic wastewaters for achieving high degree of treatment efficiency, while in anaerobic treatment, considerable progress has been achieved in anaerobic biotechnology for waste treatment based on the concept of resource recovery and utilization while still achieving the objective of pollution control (Yeoh, 1995; Seghezzo L., 1998). The various merits of both treatments are highlighted in Table 1, and both systems are capable of achieving high organic removals efficiency. In general, aerobic systems are suitable for the treatment of low strength wastewaters (biodegradable COD concentrations less than 1000 mg/L) while anaerobic systems are suitable for the treatment of high strength wastewaters (biodegradable COD concentrations over 4000 mg/L). According to Cakir and Stenstrom (2005), there exist cross over points, ranging from 300 to 700 mg/L influent wastewater ultimate BOD (BOD_u), which are crucial for effective functioning of aerobic treatment systems. The advantages of anaerobic treatment outweigh the advantages of aerobic treatment when treating influents in higher concentrations than the cross over values, and generally anaerobic treatment requires less energy with potential bioenergy and nutrient recovery. However, compared to anaerobic systems, aerobic systems achieve higher removal of soluble biodegradable organic matter material and the produced biomass is generally well flocculated, resulting in lower effluent suspended solids concentration (Leslie Grady C.P. et al., 1999). As a result, the effluent quality from an aerobic system is generally higher than the anaerobic system.

Table 1: Comparison of aerobic and anaerobic treatment*

Feature	Aerobic	Anaerobic
Organic removal efficiency	High	High
Effluent quality	Excellent	Moderate to poor
Organic loading rate	Moderate	High
Sludge production	High	Low
Nutrient requirement	High	Low
Alkalinity requirement	Low	High for certain industrial waste
Energy requirement	High	Low to moderate
Temperature sensitivity	Low	High
Start up time	2–4 weeks	2–4 months
Odor	Less opportunity for odors	Potential odor problems
Bioenergy and nutrient recovery	No	Yes
Mode of treatment	Total (depending on feedstock characteristics)	Essentially pretreatment

* Yeoh B.G. (1995); Leslie Grady C.P. et al.,(1999)

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Highly polluting industrial wastewaters are preferably treated in an anaerobic reactor due to the high level of COD, potential for energy generation and low surplus sludge production. However in practical applications, anaerobic treatment suffers from the low growth rate of the microorganisms, a low settling rate, process instabilities and the need for post treatment of the noxious anaerobic effluent which often contains ammonium ion (NH^{4+}) and hydrogen sulfide (HS^-) (Randa M.Osman et al., 2014); (Heijnen J.J. et al.,1991). In most applications, despite the efficiency of the anaerobic process is high, complete stabilization of the organic matter is impossible anaerobically due to the high organic strength of the wastewater. The final effluent produced by the anaerobic treatment contains solubilized organic matter. This is suitable for aerobic treatment, indicating the potential of using anaerobic–aerobic systems (Gray N.F., 2005) and subsequent post treatment using aerobic treatment is required to meet the effluent discharge standard.

Basics of anaerobic digestion

This section deals with anaerobic waste treatment methods only, as the most advanced and sustainable organic waste treatment method. Anaerobic digestion (WRAP 2010) is *"a process of controlled decomposition of biodegradable materials under managed conditions where free oxygen is absent, at temperatures suitable for naturally occurring mesophilic or thermophilic anaerobic and facultative bacteria and archaea species, that convert the inputs to biogas and whole digestate"*. It is widely used to treat separately collected biodegradable organic wastes and wastewater sludge, because it reduces volume and mass of the input material with biogas (mostly a mixture of methane and CO_2 with trace gases such as H_2S, NH_3 and H_2) as by-product. Thus, anaerobic digestion is a renewable energy source in an integrated waste management system. Also, the nutrient-rich solids left after digestion can be used as a fertilizer.

Biochemical reactions in anaerobic digestion

There are four key biological and chemical stages of anaerobic digestion as shown in Fig. 1:

- Hydrolysis
- Acidogenesis
- Acetogenesis
- Methanogenesis.

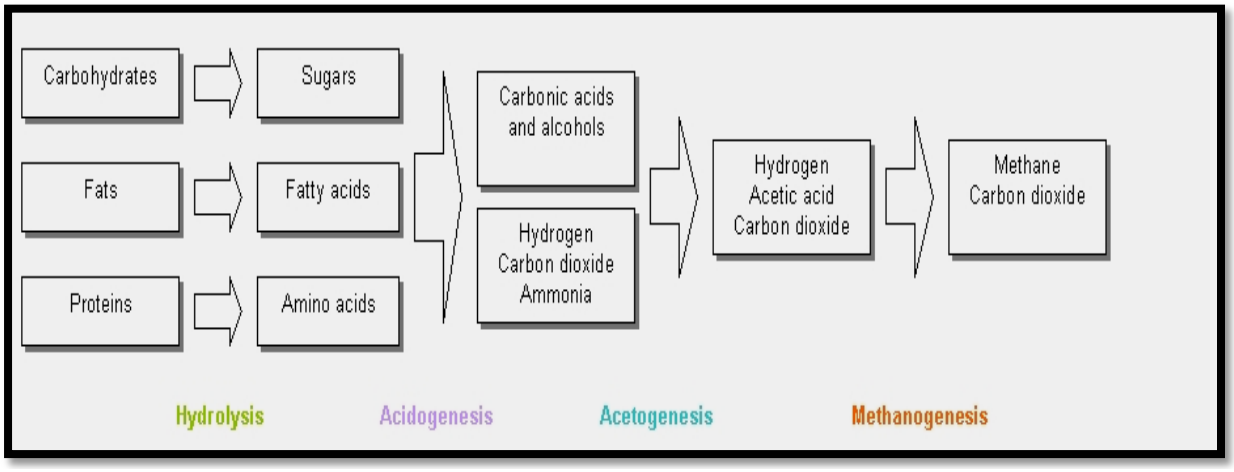

Fig. 1 the Four Distinct Processes of Anaerobic Digestion

In most cases biomass is made up of large organic compounds. In order for the microorganisms in anaerobic digesters to access the chemical energy potential of the organic material, the organic matter macromolecular chains must first be broken down into their smaller constituent parts. These constituent parts or monomers such as sugars are readily available to microorganisms for further processing. The process of breaking these chains and dissolving the smaller molecules into solution is called hydrolysis. Therefore hydrolysis of high molecular weight molecules is the necessary first step in anaerobic digestion. It may be enhanced by mechanical, thermal or chemical pretreatment of the waste. Hydrolysis step can be merely

biological (using hydrolytic microorganisms) or combined: bio-chemical (using extracellular enzymes), chemical (using catalytic reactions) as well as physical (using thermal energy and pressure) in nature.

Acetates and hydrogen produced in the first stages can be used directly by methanogens. Other molecules such as volatile fatty acids (VFA's) with a chain length that is greater than acetate must first be catabolised into compounds that can be directly utilised by methanogens. The biological process of acidogenesis is where there is further breakdown of the remaining components by acidogenic (fermentative) bacteria. Here VFA's are generated along with ammonia, carbon dioxide and hydrogen sulphide as well as other by-products.

The third stage anaerobic digestion is acetogenesis. Here simple molecules created through the acidogenesis phase are further digested by acetogens to produce largely acetic acid (or its salts) as well as carbon dioxide and hydrogen.

The final stage of anaerobic digestion is the biological process of methanogenesis. Here methanogenic archaea utilize the intermediate products of the preceding stages and convert them into methane, carbon dioxide and water. It is these components that makes up the majority of the biogas released from the system. Methanogenesis is – beside other factors - sensitive to both high and low pH values and performs well between pH 6.5 and pH 8. The remaining, non-digestible organic and mineral material, which the microbes cannot feed upon, along with any dead bacterial residues constitutes the solid digestate.

Factors that affect anaerobic digestion

As with all biological processes the optimum environmental conditions are essential for successful operation of anaerobic digestion (Table 2). The microbial metabolism processes depend on many parameters; therefore these parameters must be considered and carefully controlled in practice. Furthermore, the environmental requirements of acidogenic bacteria differ from requirements of methanogenic archaea. Provided that all steps of the degradation process have to take place in one single reactor (one-stage process) usually methanogenic archaea requirements must be considered with priority. Namely, these organisms have much longer regeneration time, much slower growth and are more sensitive to environmental conditions then other bacteria present in the mixed culture (Table 3).

However, there are some exceptions to the case:

- With cellulose containing substrates (which are slowly degradable) the hydrolysis stage is the limiting one and needs prior attention.
- With protein rich substrates the pH optimum is equal in all anaerobic process stages therefore a single digester is sufficient for good performance.
- With fat rich substrates, the hydrolysis rate is increasing with better emulsification, so that acetogenesis is limiting. Therefore a thermophilic process is advised.

Table 2: Environmental requirements (Deublein and Steinhauser 2008)

Parameter	Hydrolysis/ Acidogenesis	Methanogenesis
Temperature	25-35°C	Mesophilic: 30-40°C Thermophilic: 50-60°C
pH Value	5.2-6.3	6.7-7.5
C:N ratio	10-45	20-30
Redox potential	+400 to -300 mV	Less than -250 mV
C:N:P:S ratio	500:15:5:3	600:15:5:3
Trace elements	No special requirements	Essential: Ni, Co, Mo, Se

Table 3: Regeneration time of microorganisms

Microorganisms	Time of regeneration
Acidogenic bacteria	Less than 36 hours
Acetogenic bacteria	80-90 hours
Methanogenic archaea	5-16 days
Aerobic microorganisms	1-5 hours

Temperature

Anaerobic digestion can operate in a wide range of temperature, between 5°C and 65°C. Generally there are three widely known and established temperature ranges of operation: psychrophilic (15-20°C), mesophilic (30-40°C) and thermophilic (50-60°C). With increasing temperature the reaction rate of anaerobic digestion strongly increases. For instance, with ideal substrate thermophilic digestion can be approx. 4 times faster than mesophilic. However using real waste substrates, there are other inhibitory factors that influence digestion, that make thermophilic digestion only approx. 2 times faster than mesophilic.

The important thing is, when selecting the temperature range, it should be kept constant as much as possible. In thermophilic range (50-60°C) fluctuations as low as ±2°C can result in 30% less biogas production (Zupančič and Jemec 2010). Therefore it is advised that temperature fluctuations in thermophilic range should be no more than ±1°C. In mesophilic range the microorganisms are less sensitive; therefore fluctuations of ±3°C can be tolerated.

For each range of digestion temperature there are certain groups of microorganisms present that can flourish in these temperature ranges. In the temperature ranges between the three established temperature ranges the conditions for each of the microorganisms group are less favorable. In these ranges anaerobic digestion can operate, however much less efficient. For example, mesophilic microorganisms can operate up to 47°C, thermophilic microorganisms can already operate as low as 45°C. However the rate of reaction is low and it may happen that the two groups of microorganisms may exclude each other and compete in the overlapping range. These results in poor efficiency of the process, therefore these temperatures are rarely applied.

Redox potential

In the anaerobic digester, low redox potential is necessary. Methanogenic archaea need redox potential between -300 and -330 mV for the optimum performance. Redox potential can increase up to 0 mV in the digester; however it should be kept in the optimum range. To achieve that, no oxidizing agents should be added to the digester, such as oxygen, nitrate, nitrite or sulphate.

C:N ratio and ammonium inhibition

In microorganism biomass the mass ratio of C: N: P: S is approx. 100:10:1:1. The ideal substrate C:N ratio is then 20-30:1 and C:P ratio 150-200:1. The C:N ratio higher than 30 causes slower microorganisms multiplication due to low protein formation and thus low energy and structural material metabolism of microorganisms. Consequently lower substrate degradation efficiency is observed. On the other hand, the C:N ratio as low as 3:1 can result in successful digestion. However, when such low C:N ratios and nitrogen rich substrates are applied (that is often the case using animal farm waste) a possible ammonium inhibition must be considered. Ammonium although it represents an ideal form of nitrogen for microorganism's cells growth, is toxic to mesophilic methanogenic microorganisms at concentrations over 3000 mgL^{-1} and pH over 7.4. With increasing pH the toxicity of ammonium increases (Randa M. Osman, 2014).

Thermophilic methanogenic microorganisms are generally more sensitive to ammonium concentration. Inhibition can occur already at 2200 mgL-1 of ammonium nitrogen. However the ammonium inhibition can very much depend on the substrate type. A study of ammonium inhibition in thermophilic digestion shows an inhibiting concentration to be over 4900 mgL-1 when using non-fat waste milk as substrate (Sung and Liu 2003).

Ammonium inhibition can likely occur when digester leachate (or water from dewatering the digested substrate) is re-circulated to dilute the solid substrate for anaerobic digestion. Such re-circulation must be handled with care and examined for potential traps such as ammonium or other inhibitory ions build up.To resolve ammonia inhibition when using farm waste in anaerobic digestion several methods can be used:

- First possibility is carefully combining different substrates to create a mixture with lower nitrogen content. Usually some plant biomass (such as silage) is added to liquid farm waste in such case.

- Second possibility is diluting the substrate to such extent, that concentration in the anaerobic digester does not exceed the toxicity concentration. This method must be handled with care. Only in some cases dilution may be a solution. If the substrate requires too much dilution, a microorganisms washout may occur, which results in process failure. Usually there is only a narrow margin of operation, original substrate causes ammonium inhibition, when diluted to the extent necessary to stop ammonia inhibition, and already a washout due to dilution occurs.

- It is also possible to remove ammonium from the digester liquid. This method is usually most cost effective but rarely used. One of such processes is stripping ammonia from the liquid. It is also commercially available (GNS 2009).

pH

In anaerobic digestion the pH is most affecting the methanogenic stage of the process. pH optimum for the methanogenic microorganisms is between 6.5 and 7.5. If the pH decreases below 6.5, more acids are produced and that leads to imminent process failure. In real digester systems with suspended biomass and substrate containing suspended solids, normal pH of operation is between 7.3 and 7.5. When pH decreases to 6.9 already serious actions to stop process failure must be taken. When using UASB flow through systems (or other systems with granule like microorganisms), which utilize liquid substrates with low suspended solids concentration normal pH of operation is 6.9 to 7.1. In such cases pH limit of successful operation is 6.7.

In normally operated digesters there are two buffering systems which ensure that pH persists in the desirable range:

- Carbon dioxide - hydrogen carbonate - carbonate buffering system. During digestion CO_2 is continuously produced and release into gaseous phase. When pH value decreases, CO_2 is dissolved in the reactor solution as uncharged molecules. With increasing pH value dissolved CO_2 form carbonic acid which ionizes and releases hydrogen ions. At pH=4 all CO_2 is in form of molecules, at pH=13 all CO_2 is dissolved as carbonate. The centre point around which pH value swings with this system is at pH=6.5. With concentrations between 2500 and 5000 mgL-1 hydrogen carbonate gives strong buffering.
- Ammonia - ammonium buffering system. With decreasing pH value, ammonium ions are formed with releasing of hydroxyl ions. With increasing pH value more free ammonia molecules are formed. The centre point around which pH value swings with this system is at pH=10.
- Both buffering systems can be overloaded by the feed of rapidly acidifying (quickly degradable) organic matter, by toxic substances, by decrease of temperature or by a too high loading rate to the reactor. In such case a pH decrease is observable, combined with CO_2 increase in the biogas. Measures to correct the excessive acidification and prevent the process failure are following:
- Stop the reactor substrate supply for the time to methanogenic archaea can process the acids. When the pH decreases to the limit of successful operation no substrate supply should be added until pH is in the normal range of operation or preferably in the upper portion of normal range of operation. In suspended biomass reactors this pH value is 7.4 in granule microorganisms systems this pH value is 7.0.
- If procedure from the point above has to be repeated many times, the system is obviously overloaded and the substrate supply has to be diminished by increasing the residence time of the substrate.
- Increase the buffering potential of the substrate. Addition of certain substrates which some contain alkaline substances to the substrate the buffering capacity of the system can be increased.
- Addition of the neutralizing substances. Typical are slaked lime ($Ca(OH)_2$), sodium carbonate (Na_2CO_3) or sodium hydrogen carbonate ($NaHCO_3$), and in some cases sodium hydroxide (NaOH). However, with sodium substances most precaution must be practiced, because sodium inhibition can occur with excessive use.

Inhibitory substances

In anaerobic digestion systems a characteristic phenomenon can be observed. Some substances which are necessary for microbial growth in small concentrations inhibit the digestion at higher concentrations

(Randa M.Osman, 2014). Similar effect can have high concentration of total volatile fatty acids (tVFA's). Although, they represent the very substrate that methanogenic archaea feed upon the concentrations over 10,000 mgL^{-1} may have an inhibitory effect on digestion (Mrafkova et al., 2003; Ye et al., 2008; Hema Krishna R. and Gilbert W.B., 2014).

Inorganic salts can significantly affect anaerobic digestion. Table 4 shows the optimal and inhibitory concentrations of metal ions from inorganic salts.

Table 4: Optimal and Inhibitory concentrations of ions from inorganic salts

	Optimal concentration [mgL^{-1}]	Moderate inhibition [mgL^{-1}]	Inhibition [mgL^{-1}]
Sodium	100-200	3500-5500	16000
Potassium	200-400	2500-4500	12000
Calcium	100-200	2500-4500	8000
Magnesium	75-150	1000-1500	3000

In real operating systems it is unlikely that inhibitory concentrations of inorganic salts metals would occur, mostly because in such high concentrations insoluble salts would precipitate in alkaline conditions, especially if H_2S is present. The most real threat in this case is sodium inhibition of anaerobic digestion. This can occur in cases where substrates are wastes with extremely high salt contents (some food wastes, tannery wastes…) or when excessive use of sodium substances were used in neutralization of the substrate or the digester liquid. Study done by Feijoo et al. (1995) shows that concentration of 3000 mgL^{-1} may already cause sodium inhibition. However, anaerobic digestion can operate up to concentrations as high as 16,000 mgL^{-1} of sodium, which is close to saline concentration of seawater. Measures to correct the sodium inhibition are simple. The high salt substrates must be pre-treated to remove the salts (mostly washing). The use of sodium substances as neutralizing agents can be substituted with other alkaline substances (such as lime).

Heavy metals also do have stimulating effects on anaerobic digestion in low concentrations, however higher concentrations can be toxic. In particular lead, cadmium, copper, zinc, nickel and chromium can cause disturbances in anaerobic digestion process.

In farm wastes,e.g. in pig slurry, especially zinc is present,originating from pig fodder which contains zinc additive as an antibiotic. Inhibitory and toxic concentrations are shown in Table 5.

Other organic substances, such as disinfectants, herbicides, pesticides, surfactants, and antibiotics can often flow with the substrate and also cause nonspecific inhibition. All of these substances have a specific chemical formula and it is hard to determine what the behavior of inhibition will be. Therefore, when such substances do occur in the treated substrate, specific research is strongly advised to determine the concentration of inhibition and possible ways of microorganisms adaptation.

Table 5: Inhibitory and toxic concentrations of heavy metals

Metal	Inhibition start[1] [mgL^{-1}]	Toxicity to adopted microorganisms[3] [mgL^{-1}]
Cr^{3+}	130	260
Cr^{6+}	110	420
Cu	40	170
Ni	10	30
Cd	70	600
Pb	340	430
Zn	400	600

[1]As inhibitory concentration it is considered the first value that shows diminished biogas production and as toxic concentration it is considered the concentration where biogas production is diminished by 70 %.

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MATERIALS AND METHODS

Biological biogas production using fermentative anaerobic bacteria is a promising means of supplying energy for the future because it is a non-polluting and non-energy-intensive process. This is because it can be operated at ambient temperature and at atmospheric pressure. It is even much more attractive if biomass residues and wastewaters are used as the raw material in the biological hydrogen production.

The biological biogas or methane production process is greatly influenced by many operational factors, including pH, temperature, oxidation-reduction potential and nutritional requirements. However, some factors, such as chemical oxygen demand-to-nitrogen (COD: N) ratio, or nutrient supplementation, have not been studied systematically to optimize the process. In this work, the effects of operating parameters (i.e. COD loading rate, pH, and COD: N ratio) on the efficiency of biological methane production from glucose-containing wastewater using serum bottles(500-1000ml) with mixed culture(anaerobic inoculum as seeding bacteria) were extensively investigated. The operating conditions were optimized in order to obtain the highest biogas production rate and yield.

Experimental set-up

The anaerobic sludge and the anaerobic serum bottle reactor

An anaerobic biomass taken from a municipal sludge digester (co-digestion plant with food waste) contained sufficient metals content so that a further addition of metals did not significantly improve methane production (V. Facchin et al., 2013). The anaerobic sludge used for inoculation was obtained from a pilot-scale UASB reactor that treated the Flax Retting wastewater, Tanta CO., (Azza I. Hafez et al., 2012) and the sludge was acclimated with molasses in a laboratory scale UASB (effective volume 12 L), which was controlled at 35±2 ◦C by thermostat. The anaerobic sludge was first fed with 10 ml/L molasses as the carbon source, which enhanced the biological activity of the anaerobic microorganisms. This period lasted 15 days and the COD removal rate was over 85% before the addition of anaerobic sludge in the feeding water to the serum bottles. In the following 60 days, the molasses concentration was gradually increased in the feeding water which was from 10 to 15 mg/L. The molasses concentration of the influent was not increased until the removal rate of molasses was over 85%. The pH in the influent was adjusted to 6.8–7.1 using CaO or H_3PO_4.

Experimental design

Duplicate batch assays were carried out in 500-1000mL Schott bottles (Mainz, Germany) with a working volume of 80% of the total volume including 20% inoculum. Glucose was supplemented as a substrate to assays. Fig. 2 presents the reactor body as anaerobic Digester.

Inoculums

Anaerobic digester sludge from UASB Reactor mentioned previously was used as inoculum with raw sludge was taken from Zenin wastewater treatment plant Cairo, Egypt. Table 6. shows the characteristics of the anaerobic inoculums.

Table (6) Characteristics of the anaerobic inoculums

Soluble COD	(g/L)	2.31
Total solids	(g/L)	47.5
Volatile solids	(g/L)	20.8
pH		7.61

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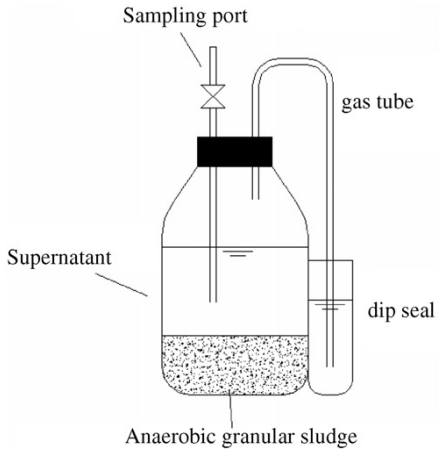

Fig 2: Schematic diagram of anaerobic serum bottle reactor

Wastewater Characteristics

Glucose anhydrous (AJAX Finechem) was used as a fermentation substrate because its small molecule can be easily assessed for the process performance of biogas production. Supplement nutrients for bacterial growth having compositions of 5.24 g NH_4HCO_3, 0.125 g K_2HPO_4, 0.015 g $MgCl_2{\cdot}6H_2O$, 0.025 g $FeSO_4{\cdot}7H_2O$, 0.005 g $NaHCO_3$, 0.125mg $CoCl_2{\cdot}5H_2O$, and 6.72 g $CuSO_4{\cdot}5H_2O$ (AJAX Finechem) (per liter of water) were added to the solutions containing different glucose concentrations. The feed solution was freshly prepared and was not used longer than 2 days in order to minimize the fluctuation in the feed composition due to self biodegradation with time.

Experimental procedure

In order to investigate the individual effects of three operational parameters (pH, COD loading rate, and COD: N ratio) on the biogas production performance, the following steps were systematically performed. Firstly, the COD loading rate was increased stepwise from 10 to 50 kgm^{-3} d^{-1} with 10 kgm^{-3} d^{-1} increments while keeping the temperature and COD: N ratio constant at ambient temperature, and 100:2.4, respectively. Secondly, the effect of pH was comparatively investigated by not controlling the system pH and by keeping the pH at 6.8-7, at which the highest biogas production activity was obtained, at various COD loading rates from 10 to 50 kgm^{-3} d^{-1}. Lastly, to study the effect of COD: N ratio, the COD: N ratio was varied at 100:1.4, 100:2.4, and 100:3.3 (mg/mg) by varying the NH_4HCO_3 amount. Each run was operated both without and with pH control over 2 weeks to ensure that the system reached steady state. The steady state condition was justified when the effluent COD and the gas production rate were nearly invariant with time. After that, samples of the influent, effluent and gas produced were analyzed and measured. Tables [7-8] summarize all the operating conditions used for the anaerobic serum bottles system in this work. When the anaerobic serum bottles system reached the steady state under a set of operating conditions, the next set was successively applied for the operation without replacing a new seed sludge.

Table 7: Operating condition used in this study under ambient temperature

COD loading rate (kgm^{-3} d^{-1})	Feed COD concentration ($mg\ l^{-1}$)	Feed glucose concentration ($mg\ l^{-1}$)	COD:N ratio	pH
10	10,000	9,375	100:2.4	
20	20,000	18,750	100:2.4	Without pH control
30	30,000	28,125	100:2.4	pH=7
40	40,000	37,500	100:2.4	
50	50,000	46,875	100:2.4	

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Table 8: Operating condition at optimum loading rate under ambient temperature

COD loading rate ($kgm^{-3} d^{-1}$)	Feed COD concentration ($mg\ l^{-1}$)	Feed glucose concentration ($mg\ l^{-1}$)	COD:N ratio	pH
			100:1.4	Without pH control
40	40,000	37,500	100:2.4	pH=7
			100:3.3	

Analytical measurements

Sludge and synthetic wastewater characterization

The mixed liquor volatile suspended solids (MLVSS) concentration of the granular anaerobic sludge was determined according to Standard Methods (APHA/AWWA/WEF, 2005). The COD in the feed or effluent sample was determined by the dichromate method using a COD analyzer (DR/2000, HACH). The amount of glucose in the feed or effluent sample was determined by a glucose assay kit (SIGMA) using a UV–vis spectrophotometer (2550, Shimadzu) according to Standard Methods (APHA/AWWA/WEF, 2005). The total nitrogen in the feed solutions was determined by the TNT persulfate digestion method.

Monitoring of methane production

Daily methane production was monitored using an inverted 1000 mL glass bottle filled with a 2% NaOH solution and connected to the digester by a capillary tube (Fig. 3). The volume of alkaline solution displaced from the 1000-mL bottle, which was collected and measured using a graduated cylinder, was assumed to be equivalent to the volume of the daily methane production. The CO_2 contained in the biogas did not affect the volumetric methane measurements as the CO_2 was dissolved in the alkaline solution.

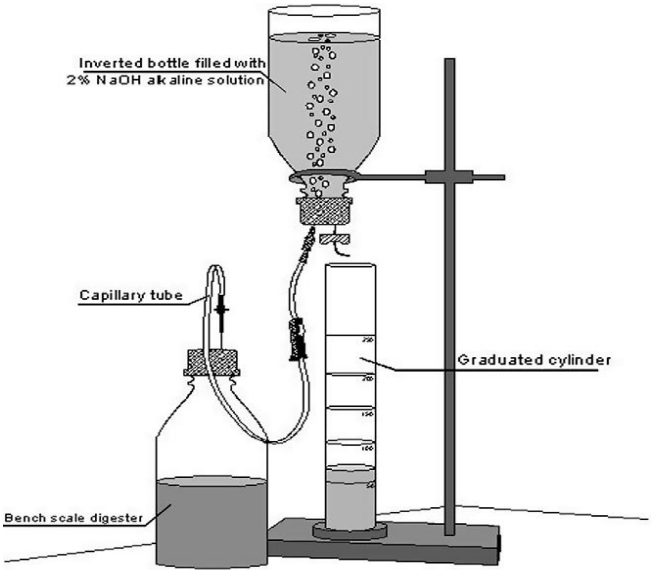

Fig 3: Experimental equipment used to measure the daily methane production.

pH and temperature monitoring

Temperature and pH of all mixtures investigated were monitored for at least once a day with a TFK 325 thermometer (WTW, Germany) and a pH meter (Professional Bench-top pH Meter BP3001, Singapore), respectively.

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RESULTS AND DISCUSSION

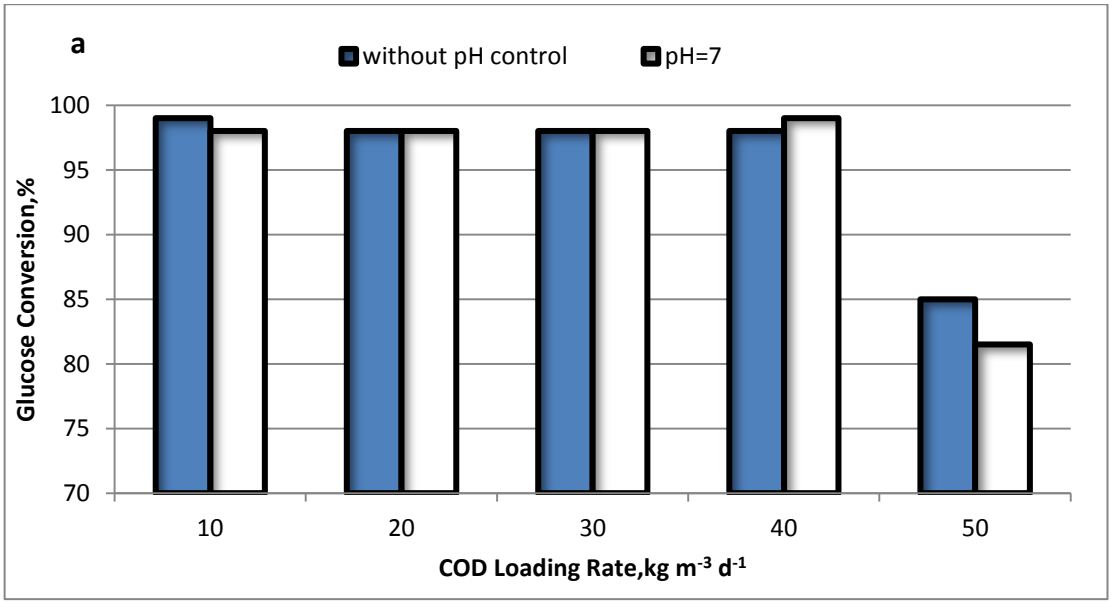
Effect of COD loading rate on organic removal

Fig. 4 shows the effect of COD loading rate on glucose conversion and COD removal of the two systems without and with pH control. At COD loading rates in the range of 10–40 kgm^{-3} d^{-1} for both systems (without and with pH control), glucose was converted by more than 98% (Fig. 4a). The high percentage of glucose conversion is due to its small molecules, the smallest of the carbohydrates, so it can easily be consumed by methane-producing bacteria. However, glucose conversion at the highest COD loading rate (50 kgm^{-3} d^{-1}) decreased to 85 and 81.5% for the systems without and with pH control, respectively, suggesting that the system was operated under an overloading condition.

The percentage of COD removal for the system with pH control at 7 increased with increasing COD loading rate and reached the maximum value of 80.2% at a COD loading rate of 40 kgm^{-3} d^{-1} (Fig. 4b), consistent with the increases in biogas production, as clearly shown in the following sections. With increasing COD loading rate beyond 40 kgm^{-3} d^{-1}, the COD removal rapidly decreased to 42.1% at a COD loading rate of 50 kgm^{-3} d^{-1}, consistent with the decrease in glucose conversion. In the case without pH control, the system exhibited a similar trend of COD removal efficiency. In a comparison between the systems without and with pH control, both maximum COD removal and optimum COD loading rate increased with pH control.

Effect of COD loading rate on gas production

Fig. 5 depicts the specific gas production rate and yield of gas production as a function of COD loading rate of the two systems with and without pH control. For the system with pH control, the specific gas production rate dramatically increased with increasing COD loading rate from 5 $l\ l^{-1}d^{-1}$ at 10 kgm^{-3} d^{-1} to 30 $l\ l^{-1}d^{-1}$ at 40 kg m^{-3} d^{-1} and decreased rapidly to 15 $l\ l^{-1}d^{-1}$ at 50 kgm^{-3} d^{-1} (Fig. 5a). The decrease in gas production rate at high COD loading rates was observed for both the systems with and without pH control. This is due to the toxicity effect of VFA accumulation in the bioreactor, which was discussed in literature before. The comparative results between the systems without and with pH control at 7 show that, at any given COD loading rate in the range of 10–40 kgm^{-3} d^{-1}, the specific gas production rate increased when the system pH was controlled, especially at a COD loading rate of 40 kgm^{-3} d^{-1}, which is considered to be the optimum COD loading rate for the highest gas production rate (30 $l\ l^{-1}d^{-1}$). At a COD loading rate of 50 kgm^{-3} d^{-1}, the gas production rate from both the systems without and with pH control decreased substantially because of the overloading effect, as well as the toxicity derived from the excessive amounts of NaOH or H_3PO_4 used for pH adjustment for the case of pH control.



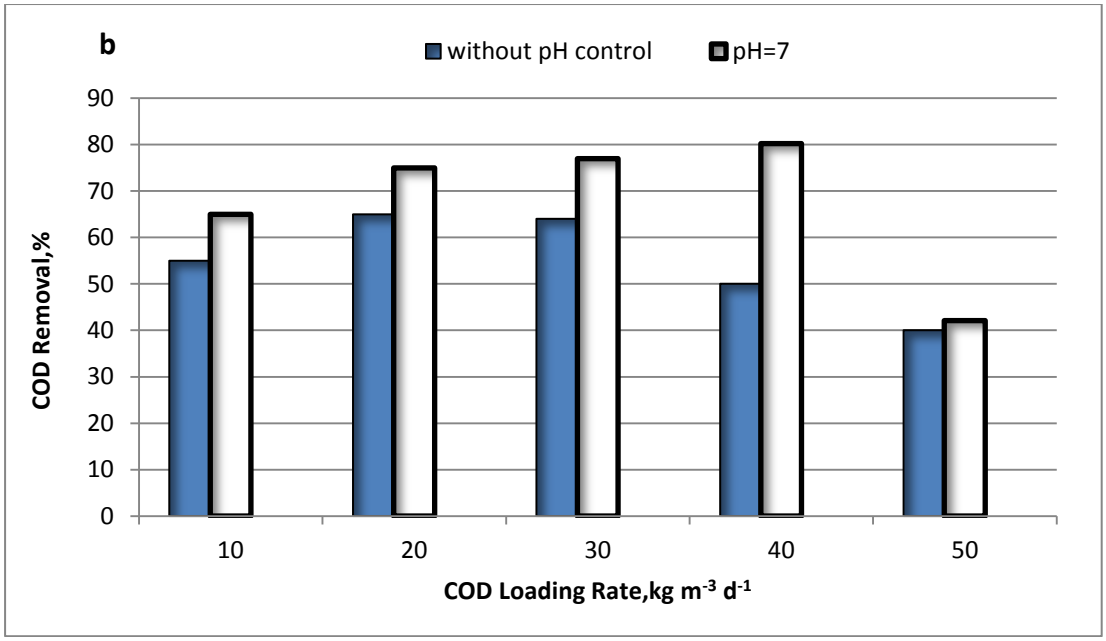


Fig. 4. Effects of COD loading rate and pH on (a) glucose conversion and (b) COD removal at ambient temperature and 24 h HRT.

The yield of biogas production is defined as the ratio of produced biogas in liter to COD consumed in gram (Fig.5b) and the ratio of produced biogas in liter to glucose consumed in gram (Fig.5c). For the system with pH control, the yield of biogas increased with increasing COD loading rate from 0.77 l of biogas / g COD consumed at 10 kgm^{-3} d^{-1} to reach a maximum of 1.04 l of biogas/g COD consumed at the COD loading rate of 30 kgm^{-3} d^{-1} (Fig. 5b). In the case of the system without pH control, the gas yield had a similar trend except that the optimum COD loading rate shifted to 40 kgm^{-3} d^{-1}, corresponding to a maximum biogas yield of 1.2 l biogas / g COD consumed.

The yield of biogas production in liter to glucose consumed in gram, For the system with pH control, the yield of biogas increased with increasing COD loading rate from 0.54 l of biogas / g glucose consumed at 10 kgm^{-3} d^{-1} to reach a maximum of 0.95 l of biogas/g glucose consumed at the optimum COD loading rate of 40 kgm^{-3} d^{-1} (Fig. 5c). When the COD loading rate further increased to 50 kgm^{-3} d^{-1}, the biogas yield decreased drastically. In the case of the system without pH control, the gas yield had a similar trend except that the optimum COD loading rate shifted to 30 kgm^{-3} d^{-1}, corresponding to a maximum biogas yield of 0.8 l biogas / g glucose consumed.

Comparing the two systems, improvement of the process performance, in terms of COD removal and biogas production efficiency (specific biogas production rate, and biogas yield), can be achieved by maintaining the system pH at 7. The present results agree well with many works as in literature section, which showed an optimum pH at 7 for biogas production. An explanation of why the system with pH controlled at 7 gave the higher process performance will be given in the next section.

Effect of nitrogen content on organic removal

Nutrient supplementation has been used for improving the treatment of wastewater containing relatively resistant organic wastes, including highly concentrated lipid wastes (K. Nakano and M. Matsumura, 2001). Nitrogen is classified as a macronutrient, which is one of the necessary nutrients for bacterial growth (McCarty and Rittmann, 2001). The results of the glucose conversion and COD removal for both systems without and with pH control at various COD: N ratios under the optimum COD loading rate of 40 kgm^{-3} d^{-1} are shown in Fig. 6. For any given operating conditions, glucose was degraded by higher than 98%, and the COD: N ratio of 100:2.4 (stoichiometric ratio or optimum ratio for the organic decomposition activity and growth of anaerobic bacteria (S.J. Jahren etal., 2002)) gave the highest glucose conversion for both systems (Fig. 6a). The slight decrease in glucose conversion at the ratio of 100:1.4 for both the systems possibly results from

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insufficient nitrogen for bacterial metabolism. Interestingly, in the case of the system with pH control at the lowest COD: N ratio (100:3.3), or the highest nitrogen-to-COD ratio, the glucose conversion decreased as compared with that at the stoichiometric COD: N ratio (100:2.4).

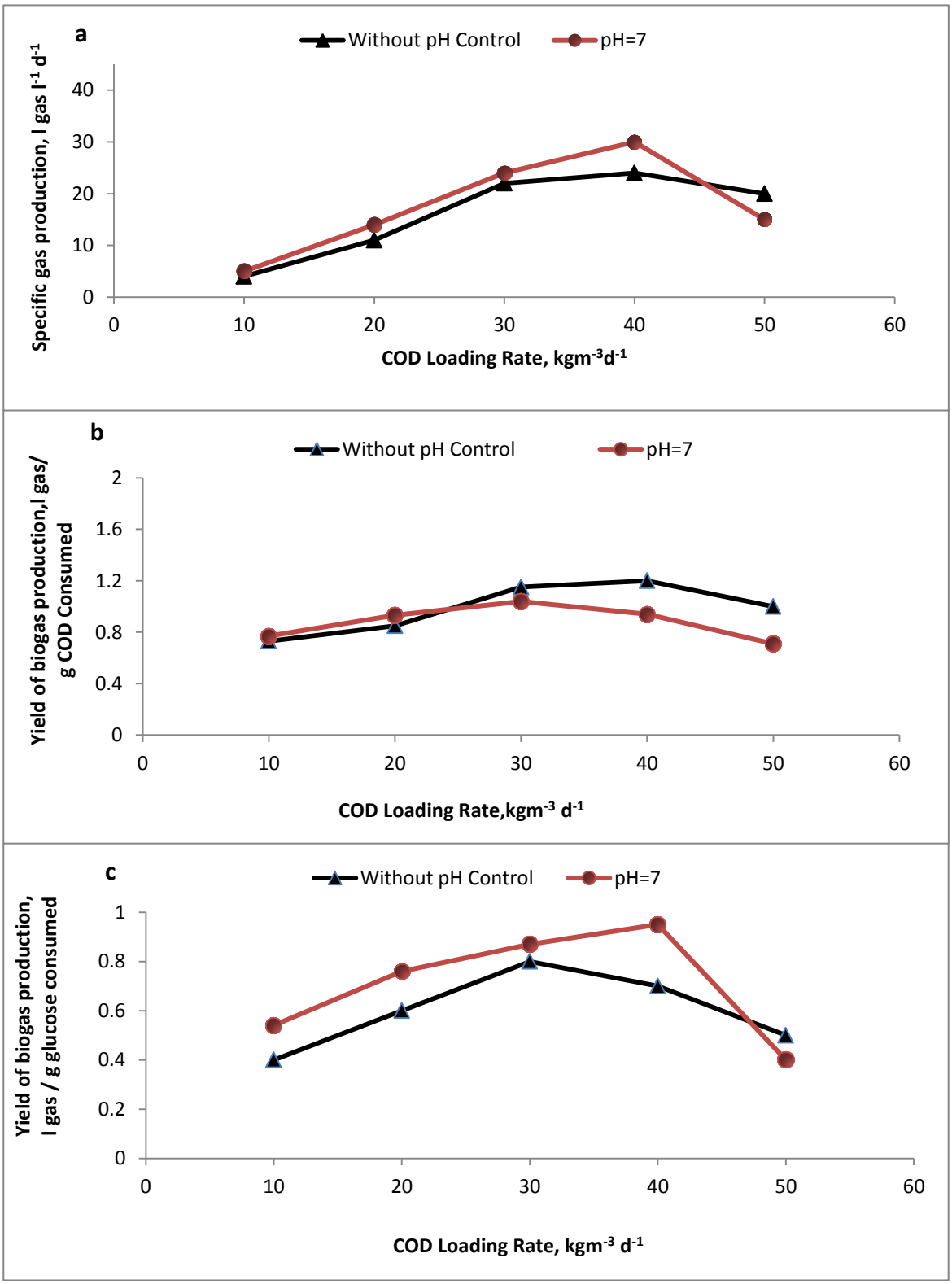

Fig. 5: Effects of COD loading rate and pH on (a) specific production rate, (b) yield of gas production based on COD consumed, and (c) yield of gas production based on glucose consumed at 37 ◦C and 24 h HRT.

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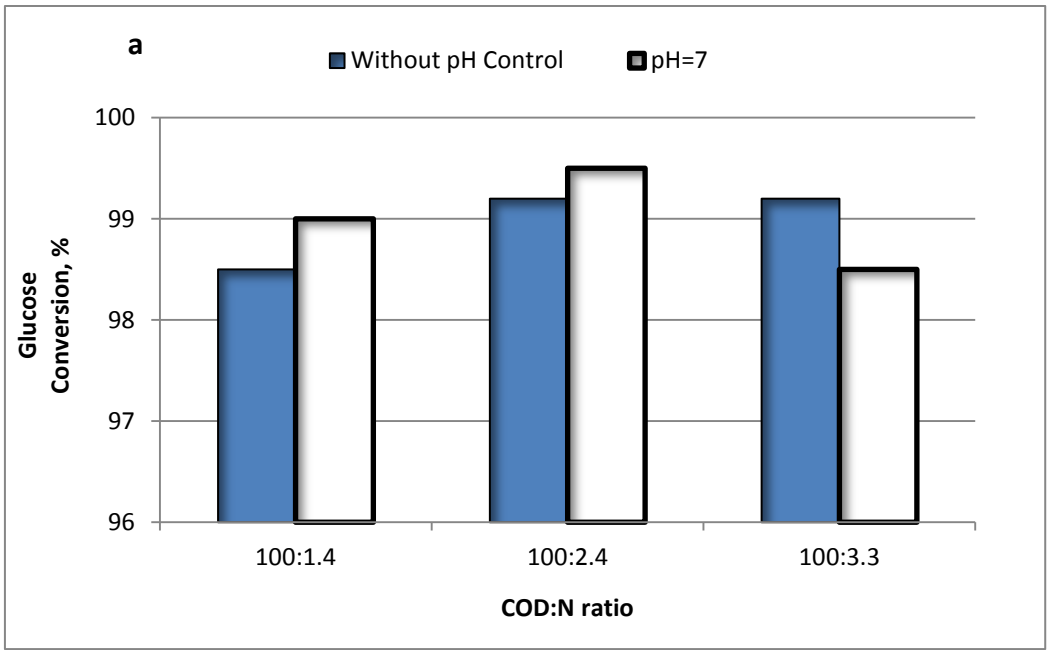
The percentage of COD removal for the system without pH control rapidly decreased from 48.6 to 31.3% when changing COD: N ratio from 100:2.4 to 100:1.4 (Fig. 6b). This trend was also observed for the system with pH control, in which the maximum COD removal (80.2%) was obtained at the stoichiometric COD: N ratio of 100:2.4. A possible explanation is the insufficiency of nitrogen for bacterial metabolism at a COD: N ratio of 100:1.4 (McCarty and Rittmann, 2001).

Because the methane production reached a maximum at the COD: N ratio of 100:2.4 for the system with pH control (as shown next in Fig. 7), this led to the high COD removal. The increase in the percentage of COD removal when the pH was controlled can be explained by the aforementioned reason whereby the toxicity due to the organic acid accumulation is reduced by adding NaOH to decrease the free acid form, which is more toxic than the ionized form. A further increase in nitrogen at the COD: N ratio of 100:3.3 (greater than the stoichiometric ratio) showed a decrease in the organic removal for both glucose and COD. This can be explained in that, at a COD:N ratio of 100:3.3, the nitrogen content in the feed increased to 1,320mgl^{-1} in terms of ammonium-nitrogen, which is close to the reported toxicity level of ammonium-nitrogen of 1,500mgl^{-1} (K. Vijayaraghavan and T.K. Ramanujam,2000).

Effect of nitrogen content on gas production

Fig. 7 shows the results of specific biogas production rate, and biogas yield as a function of COD: N ratio. For the system without pH control, the specific gas production rate increased from 12.8 to 24 l l^{-1}d^{-1} when the COD: N ratio was changed from 100:1.4 to 100:2.4, and rapidly decreased to 11 l l^{-1}d^{-1} at a COD: N ratio of 100:3.3 (Fig. 7a). This trend was also observed for the system with pH control, in which the gas production rate increased from 18.6 to 30 l l^{-1} d^{-1} when the COD: N ratio was changed from 100:1.4 to 100:2.4, and greatly decreased to 13.3 l l^{-1}d^{-1} at a COD: N ratio of 100:3.3. The aforementioned explanation of the effect of nitrogen content on the organic removal can be used for that on the biogas production. The addition of nitrogen to the feed with insufficient nitrogen was also reported to enhance the biogas production by stimulating the microflora in the bioreactor and improving their degradation activity (Lee et al., 1993). In this present work, a comparison between the systems without and with pH control shows that the gas production rate increased when the system pH was controlled, at all operating conditions, especially at a COD: N ratio of 100:2.4, at which the gas production rate increased to 30 l l^{-1} d^{-1}. A possible reason might be the same as explained above for the effect of COD loading rate.



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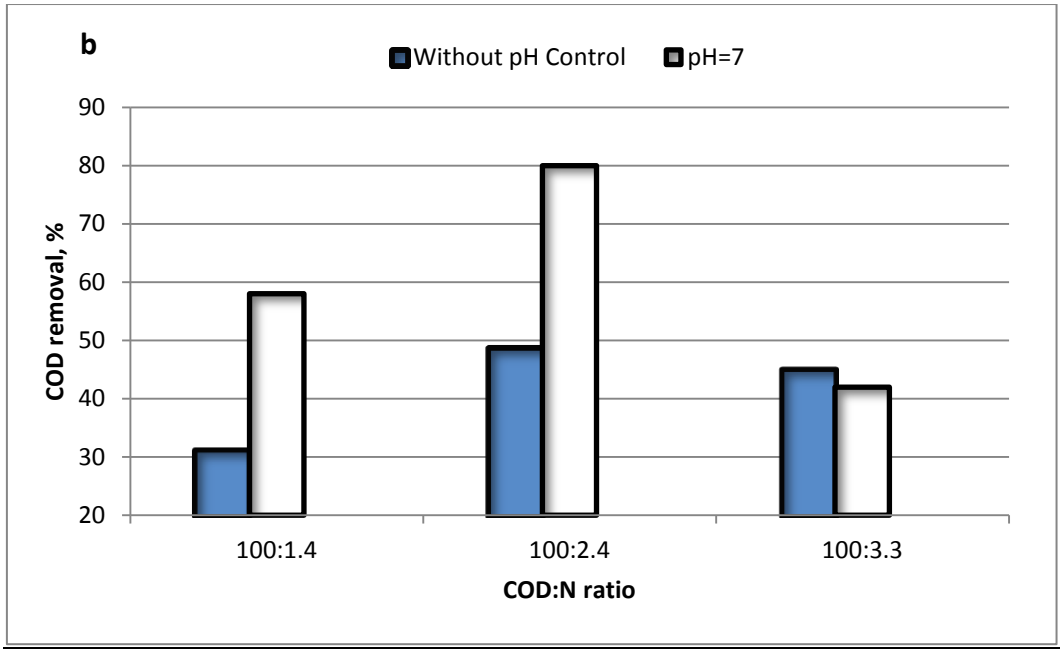
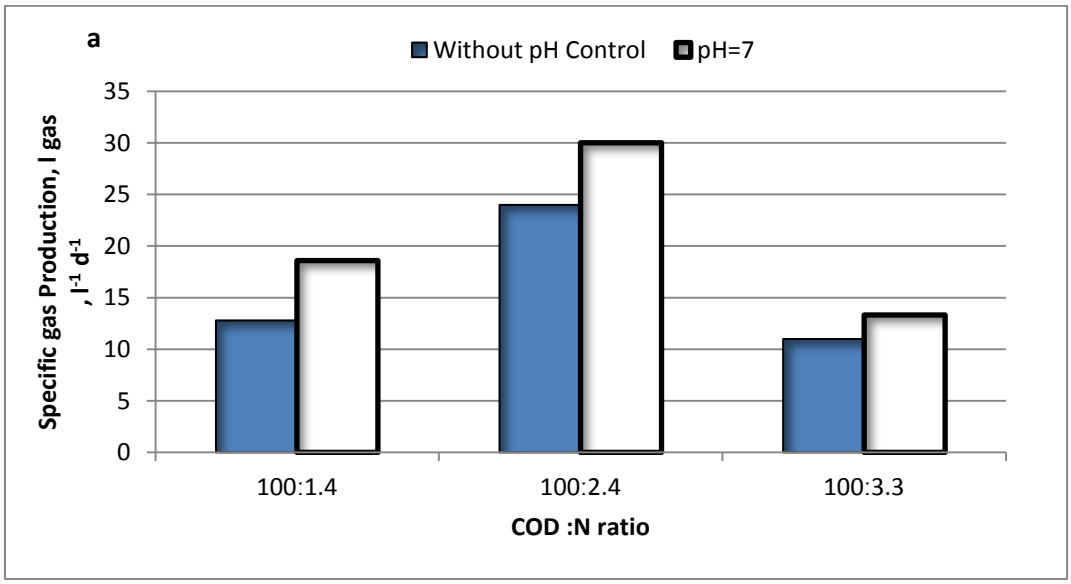


Fig. 6: Effects of COD:N ratio and pH on (a) glucose conversion and (b) COD removal at a COD loading rate of 40 kgm^{-3} d^{-1}, ambient temperature, and 24 h HRT.

For the system with pH control, the highest yield of biogas (0.94 l biogas/gm COD consumed as in Fig. 7b) and (1.46 l biogas/gm glucose consumed) was obtained at the stoichiometric COD: N ratio of 100:2.4 (Fig. 7c). The decrease in the yield of biogas was obtained at the COD: N ratio of 100:1.4 or 100:3.3. The decrease in the biogas yield under either deficient or excess N condition in the present work agrees well with the work of Argun et al. (2008), who reported that high nitrogen concentrations inhibited hydrogen formation by dark fermentation probably by shifting the metabolic pathway of microflora.



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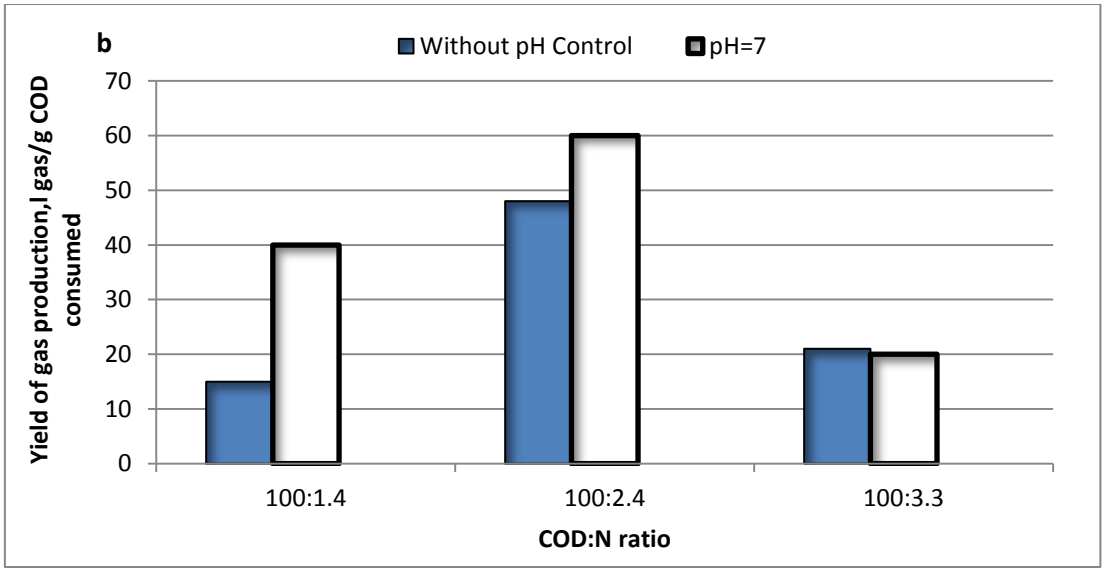

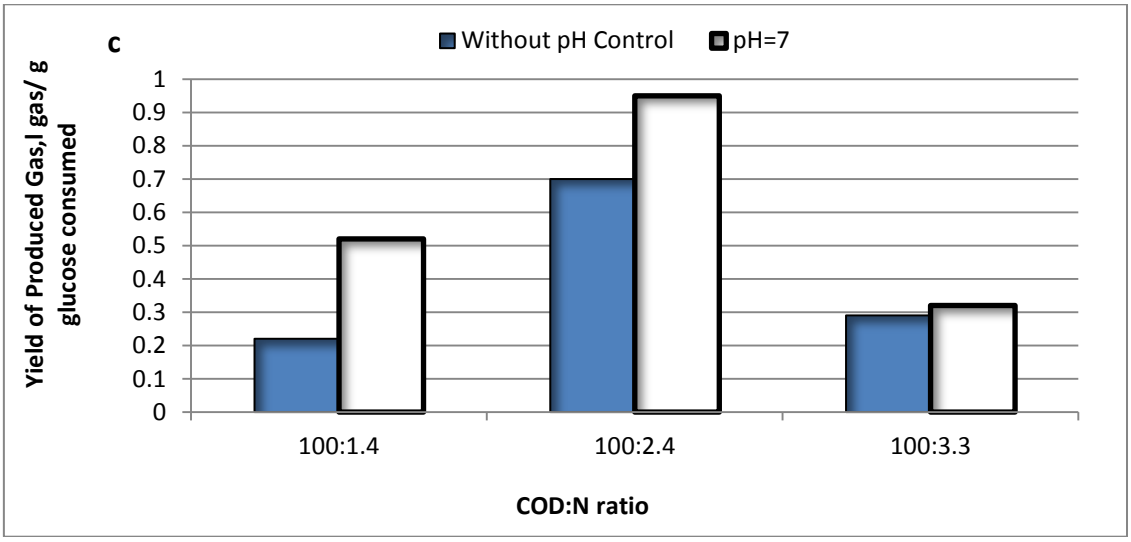

Fig.7: Effects of COD: N ratio and pH on (a) specific gas production, (b) yield of gas production based on COD consumed, and (c) yield of gas production based on glucose consumed at a COD loading rate of 40 kgm^{-3} d^{-1}, ambient temperature, and 24 h HRT.

Effect of nitrogen content on microbial concentration

The concentration of biogas-producing bacteria can also be quantified by using MLVSS. The experimental data of the MLVSS at various COD: N ratios are shown in Fig. 8. For the system without pH control, the microbial concentration, in terms of MLVSS, only slightly changed with varying COD: N ratio. However, for the system with pH control, the MLVSS significantly increased with adjusting COD: N ratio from 100:1.4 to 100:2.4, and then decreases with further adjusting to 100:3.3. The highest microbial concentration was found to correspond to the stoichiometric COD:N ratio of 100:2.4. The present results also confirm that a pH of 7 is suitable for the growth of methane-producing bacteria, as mentioned above.

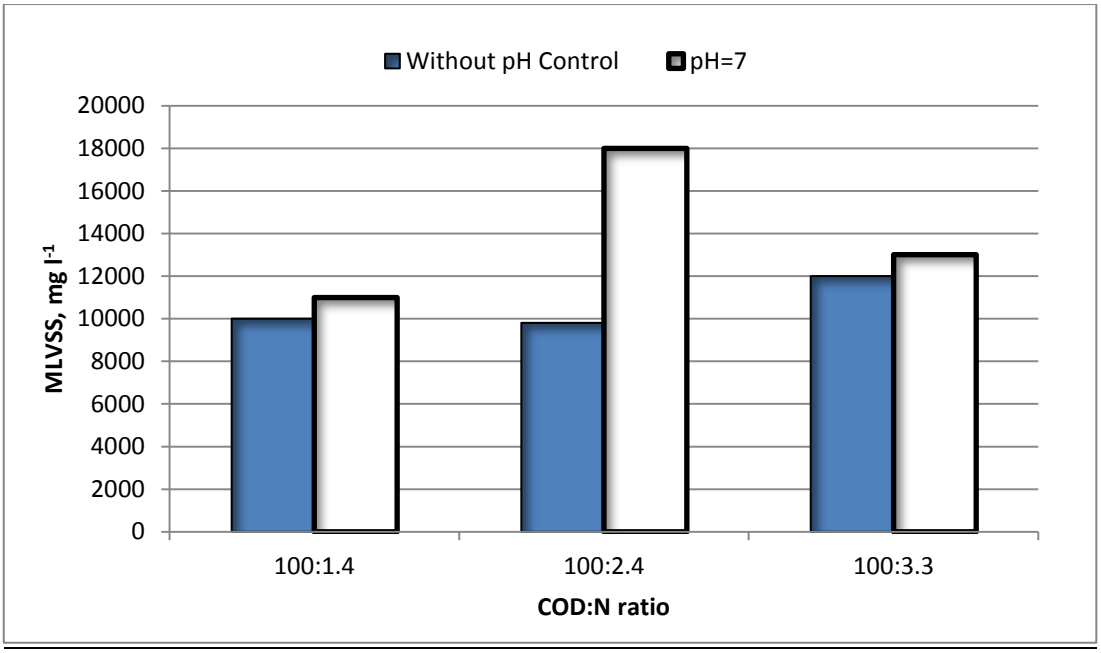

Fig. 8: Effects of COD: N ratio and pH on MLVSS at a COD loading rate of 40 kgm^{-3} d^{-1}, ambient temperature, and 24 h HRT.

CONCLUSION

Biogas production from glucose containing wastewater by liquid fermentation using anaerobic serum bottles system was found to be dependent on several factors, including COD loading rate, pH, and COD: N ratio. For the anaerobic system with pH control at 7, the results showed that pH control could enhance the biogas production by reducing the toxicity from the accumulation of VFA produced from the acidogenesis process. The maximum biogas production was achieved at a COD loading rate of 40 kgm^{-3} d^{-1} under pH control at 7, ambient temperature, and 24 h HRT.

Additionally, an insufficient amount of nitrogen in the feed could cause a decrease in both organic removal and biogas production efficiency because nitrogen is necessary for bacterial growth and metabolism. A stoichiometric COD: N ratio of 100:2.4 was found to be optimum for the biogas production.

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