THERMODYNAMICS OF ANAEROBIC DIGESTION: MECHANISM OF SUPPRESSION ON BIOGAS PRODUCTION DURING ACIDOGENESIS

TERMODINAMIKA PADA ANAEROBIK DIGESI: MEKANISME PROSES HAMBATAN PADA PRODUKSI BIOGAS SELAMA FASE ACIDOGENESIS

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ABSTRACT
Anaerobic digestion process consists of several stages in which different type of microorganisms are involved. The current study reviews some possible reactions occurred in anaerobic digestion particularly in acidogenesis stage. The paper also reviews the mechanisms of suppression and inhibition of anaerobic digestion in relation to some possible thermodynamic reactions during acidogenesis phase. Results of the study showed that ethanol and lactic acid are intermediate products that could potentially be produced during the anaerobic acid stage fermentation. Thus, those products should be carefully managed due to their acid effects in order to avoid the failure of anaerobic digestion.

ABSTRAK
Anaerobik digesi merupakan proses yang terdiri dari beberapa tahapan yang melibatkan berbagai jenis mikroorganisme pada setiap tahapannya. Studi ini memberikan penjelasan mengenai reaksi yang mungkin terjadi selama proses anaerobik digesi terutama reaksi pada fase acidogenesis. Paper ini juga menjelaskan mekanisme hambatan yang terjadi pada proses anaerobik digesi yang dihubungkan dengan beberapa reaksi termodinamika selama fase acidogenesis. Hasil studi menunjukkan bahwa etanol dan asam laktat merupakan produk menengah yang dapat dihasilkan selama tahapan fermentasi asam. Dengan demikian pembentukan produk tersebut harus dikelola dengan baik untuk menghindari kegagalan pada anaerobik digesi, karena produk tersebut dapat meningkatkan kandungan asam pada kultur anaerobik.

INTRODUCTION
Anaerobic digestion (AD) is a natural process involving a consortium of microorganisms to convert and decompose complex organic materials into simpler chemical molecules, and produces methane gas as the end-product *(Cheng, 2010; Van Lier, 2008)*. Some typical microorganisms involved in anaerobic digestion process include bacteria, yeast, fungi and protozoa *(Ali Shah et al., 2014; Suwannarat and Ritchie, 2015)*. Microorganisms involved in each stage of AD process (i.e acidogenesis and methanogenesis) are quite sensitive to some parameters including temperature, pH, hydraulic/solid retention time (HRT or SRT), solid content and organic loading rate (OLR), hydrogen partial pressure, volatile fatty acids concentration, alkalinity, and the concentration of free ammonia *(McCarty and Mosey, 1991; Appels et al, 2008; Darwin et al., 2016)*.

In anaerobic digestion, during the stage of acidogenesis all dissolved soluble organic compounds generated from hydrolysis process are firstly converted into intermediate products, such as volatile fatty acids (VFAs) and alcohols *(Henze et al., 2008)*, and then the products will be converted into acetate, CO_2 and H_2 in the stage of acetogenesis *(Appels et al., 2008; Cheng, 2010)*. The final stage of the anaerobic digestion is methanogenesis in which the acetate-utilizing methanogenic microorganisms would oxidize the acetate into methane. The methanogenesis would also be carried out by hydrogen-utilizing methanogens in which the microbes would utilize hydrogen as electron donor and carbon dioxide as acceptor to form methane as the end-product *(Appels et al., 2008; Henze et al., 2008).*

The crucial issue for operating anaerobic digestion process is to balance the equilibrium between the acidogenesis and methanogenesis. The rate of methane production would be significantly reduced when acids are accumulated in the digester. If this condition continuously happens, a potential risk in anaerobic digestion process is inevitable in which the process of anaerobic digestion could stop completely. This occurs as low pH caused by an accumulation of acids could generate acidic condition in the digester *(Yu and Fang, 2003)*.

Not many studies assessed the thermodynamics' reactions during anaerobic digestion process. In this present study, reactions involved during anaerobic digestion were evaluated through thermodynamics point of view in which free Gibbs energy was measured in some possible reactions in order to evaluate the mechanism of suppression in anaerobic digestion and some possible reactions occurred in anaerobic acid stage process.

MATERIALS AND METHODS

Characteristics of anaerobic acidogenesis

In anaerobic acidogenesis phase, all simple and/or soluble organic materials generated from the hydrolysis process, are then fermented into volatile fatty acids, lactic acid and alcohols *(Yu and Fang, 2002; Darwin et al, 2018a)*. In this phase some gases including hydrogen and carbon dioxide were produced *(Borja et al, 2005; Solera et al, 2002)*. As acidogenesis is the second step of the anaerobic digestion process, managing the appropriate operating conditions is important to prevent the failure of anaerobic digestion caused by organic acid build-up *(Darwin et al, 2018b)*.

Some studies revealed that operating conditions such as temperature, pH and HRT could significantly affect the rate of acidification process to produce fermentation end-products, such as VFA and alcohols *(Cha and Noike, 1997; Borja et al, 2005; Zhang et al, 2005)*. Bacterial population and substrate degradation in acidogenesis process are highly dependent on the changes of temperature and HRT especially at low temperature and short HRT. During the process of anaerobic acidogenesis, metabolic pathway could change drastically when pH culture changes. The shift of fermentation pattern may significantly affect the relative numbers of microorganisms present in the reactor *(Cha and Noike, 1997)*.

Some studies showed that the changes of fermentation pathway in acidogenesis are highly influenced by pH *(Thauer et al, 1977; Hwang et al, 2004, Cheong and Hansen, 2006)*. Microbial competition could occur at pH between 5.0 and 6.0, and the competition could involve VFA (e.g. propionate and butyrate) and ethanol producers *(Hwang et al, 2004)*. In anaerobic acidogenesis process, unstable condition may occur when all three types of acidogenic fermentation including butyrate, propionate and alcohol exist in the reactor, and this condition may occur at pH 5.0 *(Ren et al, 2007)*.The operational stability and the overall metabolic rate of the methanogenesis highly depend on the products generated from the acidogenic reactor *(Ren et al, 1997)*.

- *Fermentation end-products from acidogenesis phase*

Although acetate is known as the primary VFA species in anaerobic digestion, it does not always appear as the major VFA in the acidogenesis phase *(Angelidaki et al, 1999)*. The authors mentioned that the distribution of VFA composition produced in acidogenesis is influenced by operating conditions such as pH, temperature and hydrogen partial pressure. Butyrate and propionate were the main fermentation end-products of VFA produced from the anaerobic acidogenesis operated at pH of 5.0 *(Zhang et al, 2005)*. Some other intermediate products that could be directly used by methanogens include H_2/CO_2, formate, methylamine and methanol *(Bhatia et al, 1985; Ren et al, 1997)*.

Study conducted on anaerobic acidogenesis of dairy wastewater at several levels of pH, revealed that when pH increased hydrogen partial pressure decreased with an increase of methane production *(Yu and Fang, 2002)*. At pH 6.5, biogas produced from anaerobic digestion consisted of carbon dioxide and methane, and no hydrogen was detected. Some studies had revealed that propionate was the main fermentation end-product produced from anaerobic acdiogenesis operated at pH lower than 5.0 while acetate and butyrate were the main fermentation end-products produced from the acid stage fermentation operated at pH higher than 5.5 *(Harper and Pohland, 1986; Yu and Fang, 2002; Ren, 1996)*.

$$C_6H_{12}O_6 + 2\ H_20 \rightarrow 2\ C_2H_5OH + 2\ HCO_3- + 2\ H^+ \quad (1)$$

$$\Delta Go = -225.36\ kJ$$

During the process of anaerobic acidogenesis lactic acid could also be produced as the main fermentation end-products when pH of fermentation culture was too acidic (Darwin et al., 2018b and c). Further, under low pH (< 5.0) lactic acid could be the dominant fermentation end-product of starch fermentation *(Darwin et al., 2018c)*. Lactic acid tended to be formed when anaerobic digester received shock loading of glucose *(Zoetemeyer et al,1982)* as shown in Equation (2-3). At this condition, an accumulation of lactate in the digester may occur. As lactate is considered an intermediate product produced during the acidogenesis phase of anaerobic digestion, it could be used as the substrate for the second stage reactor or methanogenic reactor in order to enhance biogas production *(Pipyn and Verstraete, 1981)*.

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOO^- + 2H^+ \quad (2)$$

$$\Delta Go = -198.2 \text{ kJ}$$

$$C_6H_{12}O_6 + H_2O \rightarrow CH_3\text{-}CHOH\text{-}COO^- + CH_3\text{-}CH_2OH + HCO_3^- + 2H^+ \quad (3)$$

$$\Delta Go = -211.91 \text{ kJ}$$

- *The mechanism of suppression of anaerobic digestion by low pH*

The process of anaerobic digestion is highly affected by pH. An optimum pH in anaerobic digestion for methane production ranges between 6.8 and 7.2 *(Ward et al, 2008; Gontupil et al, 2012)*. The growth of methanogenic bacteria is inhibited at pH lower than 6.6 *(Mosey and Fernandes, 1989; Chen et al, 2008)*. Some studies revealed that the optimum pH for hydrolysis as well as acidogenesis are between 5.5 and 6.5 *(Kim et al, 2004; Yu and Fang, 2002; Ward et al, 2008)*. A lowering in pH may significantly affect the dissociation of VFA and other organic acids by pushing the equilibrium towards the formation of free un-dissociated acids (Equation 4-6).

$$CH_3COO^- + H^+ \rightarrow CH_3COOH \quad (4)$$

$$CH_3\text{-}CH_2\text{-}COO^- + H^+ \rightarrow CH_3\text{-}CH_2\text{-}COOH \quad (5)$$

$$CH_3\text{-}(CH_2)_2\text{-}COO^- + H^+ \rightarrow CH_3\text{-}(CH_2)_2\text{-}COOH \quad (6)$$

RESULTS

Results of the study showed that a lowering in pH also could alter the free energy change of VFA producing and VFA consuming reactions. Oxidation of propionate and butyrate are not only limited by the lack of free energy change but their degradation is also repressed by a drop of pH (Fig. 1). Inhibitory effects generated from propionate and butyrate degradation to acetate may lower pH, and thereby could suppress the activity of methanogens (*Amani et al. 2011*).

When anaerobic digestion is operated under mesophilic condition at 35°C and 10^{-4} atm of hydrogen partial pressure, the conversion of ethanol to acetate is thermodynamically more favorable in comparison to the conversion of propionate and butyrate (Fig. 1). This is due to the fact that the reaction of ethanol degradation is exergonic at low pH. This indicated that the oxidation of ethanol to acetate is also not affected by acidic condition.

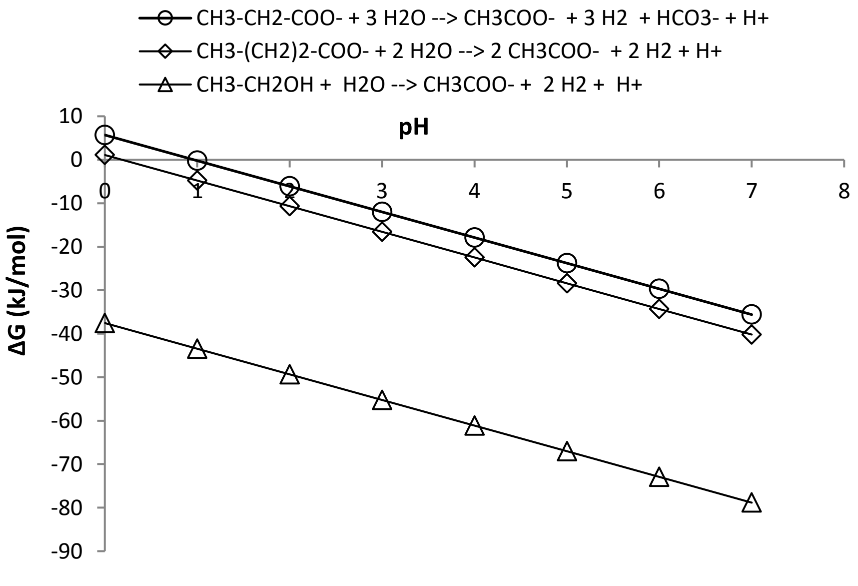

Fig.1 - Free energy change of VFA degradation as a function of pH

- *Problems of propionate accumulation in anaerobic digestion*

Study revealed that propionate was the major substrate affecting the degradation of VFA *(Wang et al, 2009)*. An increase of propionate concentration during anaerobic digestion could lower biogas production as well as methane yield. During anaerobic acidogenesis phase propionate formation should be monitored and prevented otherwise it could stimulate an accumulation of acid in the digester, and thereby could inhibit the growth of methanogens *(Amani et al, 2011)*. Propionate conversion to acetate tends to be more sensitive with hydrogen partial pressure in comparison to other intermediate products such as butyrate, ethanol and lactate in which these products are not too sensitive with hydrogen partial pressure in the reactor*(Ren et al,1997).*

When anaerobic digestion is operated at neutral pH (pH 7) under the temperature of mesophilic condition (35^oC), the conversion of propionate to acetate could be inhibited due to high hydrogen partial pressure (Fig. 2). When hydrogen partial pressure is at about 10^{-2} atm, the degradation of propionate is impossible as the reaction is endergonic. Current study revealed that an oxidation of propionate to acetate could generate 1% of hydrogen. However, the conversion of propionate to acetate could be inhibited when the hydrogen produced was at about 1.5 %. The conversion of butyrate acetate would be inhibited at high hydrogen partial pressure in which the butyrate conversion may occur at about 10% of hydrogen produced. The degradation of butyrate into acetate could be inhibited when the hydrogen is produced at about 30%. The current thermodynamical study also revealed that the conversion of ethanol and lactate to acetate would not be inhibited at high hydrogen partial pressure.

As lactate is considered as a precursor of propionate production, it may also be regarded as an undesirable product from the process of acid stage fermentation. Thermodynamically propionate could be produced from the reaction of 1 mole of lactate and 1 mole of hydrogen (Equation 7). Even though lactate is regarded as the precursor of propionate, it also can be considered as a substrate for the second stage process (e.g. acetogenesis and methanogenesis). This is due to the fact that lactate can be converted into acetate and hydrogen *(Van Lier et al, 2008)*, and the reaction involved is thermodynamically possible to occur as shown in Equation 8.

$$CH_3\text{-}CHOH\text{-}COO^- + H_2 \rightarrow CH_3\text{-}CH_2\text{-}COO^- + H_2O \quad (7)$$

$$\Delta Go = -80.448 \text{ kJ}$$

$$CH_3\text{-}CHOH\text{-}COO^- + 2\,H_2O \rightarrow CH_3COO^- + 2\,H_2 + HCO_3^- + H^+ \quad (8)$$

$$\Delta Go = -4.04 \text{ kJ}$$

Some studies mentioned that the production of propionate is more favourable at low pH in comparison to the production of acetate and butyrate *(Yu and Fang, 2003; Hsu and Yang, 1991)*. The production of propionate with hydrogen could occur at pH between 4.0 and 5.0*(Borja et al, 2005)*. Some studies had revealed that propionate considered as undesired end-product of the acidification stage for the subsequent methanogenesis. This is due to the fact that methanogenesis of propionate is slower than other volatile fatty acids such as acetate and butyrate *(Yu and Fang, 2003; Bo et al, 2014; Cohen et al, 1984; Bengtsson et al, 2008).*

This condition may cause accumulated acid in the reactor *(Ren et al, 1997; Bhatia et al, 1985).*Low acetogenic rate of propionate occurred as propionate cannot be utilized directly by methanogens This phenomenon is also in agreement with Equation 9 in which at standard state condition the reaction of propionate conversion to acetate is thermodynamically impossible. The conversion of propionate to acetate may occur when the anaerobic process is operated at pH 7 but the reaction could be inhibited at a higher hydrogen partial pressure (Fig. 1 and 2).

$$CH_3\text{-}CH_2\text{-}COO^- + 3\,H_2O \rightarrow CH_3COO^- + 3\,H_2 + HCO_3^- + H^+ \quad (9)$$

$$\Delta Go = +\,76.484 \text{ kJ}$$

Research conducted on assessing optimal fermentation type of domestic wastewater added with molasses for the production of hydrogen in continuously-flow acidogenic reactor revealed that propionic acid type fermentation tended to occur at pH 5.5 with high redox potential *(Fukuzaki et al, 1990; Ren et al, 2007).* The attack of propionate producing bacteria could be prevented by maintaining pH level at 4.5 *(Ren et al, 1997)*. *McCarty and Mosey (1991)*revealed that propionate producers were suppressed at pH between 4.5 and 5.0.

An excess of propionic acid in the acidogenic reactor could suppress the process of anaerobic digestion that enable to lower pH. If this condition continuously occurs, the level of non-dissociated acids in the acidogenic reactor may go up. This condition could suppress the growth of methanogens in the conventional single-stage digester, and subsequently could lower methane productivity *(Angelidaki, Ellegaard, and Ahring 1999)*. Research carried out by *Maspolim et al (2014)* revealed that propionate tended to accumulate in the acidogenic reactor when *smithellapropionica* are present in the reactor. The propionate producer is related to the syntrophic propionate oxidizser *S. Propionica*. *Liu et al. (1999)* mentioned that *S. Propionica* may not be able to grow in the acidogenic reactor when pH in the reactor is 6.3.

If anaerobic digestion is operated in the two-stage process, *S. propionica* could be detected in the methanogenic reactor which could consume propionic acid to less than 0.1 mM *(Maspolim et al, 2014).*

- *Speed degradation of intermediate products for methane production*

An increase of methane yield occurs when the concentration of acetate, butyrate and ethanol in the digester increases *(Angelidaki et al, 1999; Ren et al, 2003; Uellendahl and Ahring, 2010; Wang et al, 2009).* However, the growth of acidogenic bacteria may be inhibited when there was an increase of propionate concentration in the digester, and thereby could slower the conversion of VFAs (e.g. butyrate and propionate) and ethanol into acetate. This condition may significantly limit the growth of methanogenic bacteria.

Ren et al. (1997) revealed that several characteristics that should be considered for optimizing methanogenesis include: (1) intermediate products that should be directly used by methanogens, such as acetate; (2) products that should be readily converted into methanogenic substrates by hydrogen-producing acetogens; (3) fermentation products that should contain less propionate. Due to the high rate of hydrogen-producing acetogenesis, the intermediate products including acetate, butyrate and ethanol are regarded as the optimal fermentation products for methanogenesis *(Ren et al, 1997; Hwang et al, 2004)*.

Methane also can be produced from hydrogen and carbon dioxide or bicarbonate as shown in Equation 11. Homoacetogenesis may occur when the formation of acetate as the only end product is derived from the reaction of hydrogen and carbon dioxide or bicarbonate (Equation 12).

Although methane can be produced from hydrogen and carbon dioxide, almost 65-70% of methane formation is derived from acetate *(Thauer et al, 1977)*. Methane formation derived from hydrogen and carbon dioxide has significant impact in the process of anaerobic digestion. This occurs as methanogens consume hydrogen that enables to lower the hydrogen partial pressure in the digester. This condition could support the anaerobic oxidation of VFA (e.g. butyrate and propionate) to acetate and hydrogen, and thereby could prevent acid accumulation in the digester *(Thauer et al, 1977)*.

$$CH3COO- + H2O \rightarrow CH_4 + HCO_3- \quad (10)$$

$$\Delta Go = -31.05\ kJ$$

$$4\ H_2 + HCO_3- + H+ \rightarrow CH_4 + 3\ H_2O \quad (11)$$

$$\Delta Go = -135.604\ kJ$$

$$4\ H_2 + 2\ HCO_3- + H+ \rightarrow CH_3COO- + 4\ H_2O \quad (12)$$

$$\Delta Go = -104.552\ kJ$$

The reactions mentioned in Equation 9 and 10 show that both reactions are thermodynamically not possible to occur as the free energy is positive. The reactions are considered as synthrophic acetogenesis, which involve the oxidation of propionate and butyrate to form acetate and hydrogen. Anaerobic oxidation of both propionate and butyrate tends be inhibited even at a slightly higher hydrogen partial pressure (Fig. 2). The oxidation of propionate and butyrate to acetate can easily proceed when hydrogen is consumed by methanogens to form methane, or it may occur when homoacetogens take hydrogen to form acetate *(Thauer et al, 1977)*. *Amani et al. (2011)* revealed that the anaerobic conversion of propionate is thermodynamically more unfavorable in comparison to butyrate (Fig. 1 and 2). This is due to the fact that propionate formation tends to generate acid accumulation in the anaerobic digester.

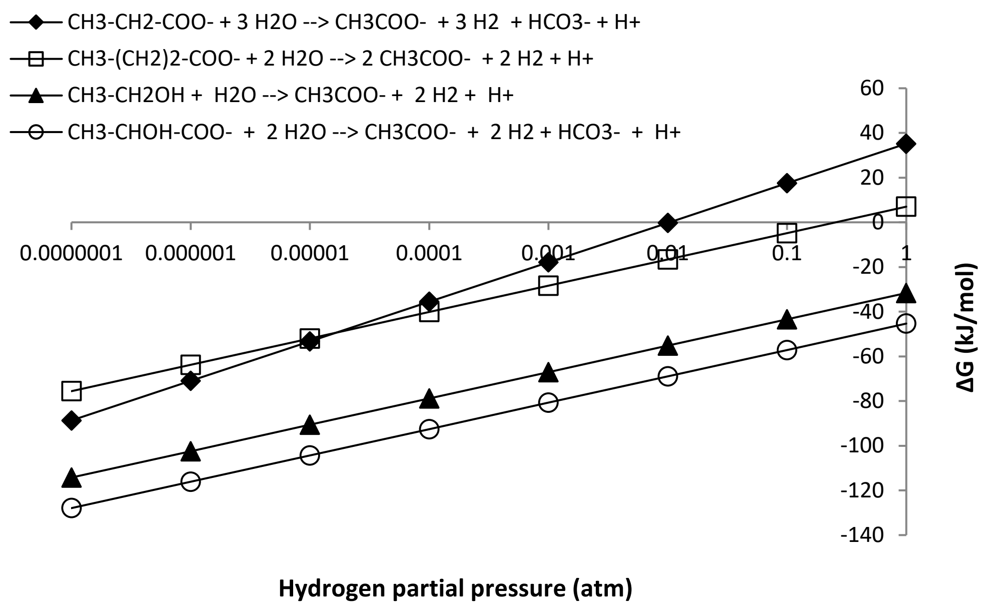

Fig. 2 - Free energy change of VFA degradation as a function of the hydrogen partial pressure

Study conducted by *Amani et al. (2011)* revealed that anaerobic oxidation of acetate to methane may stimulate propionate to be degraded thermodynamically. The study revealed that the maximum conversion of propionate could be accomplished when the concentration of butyrate and acetate in the digester were extremely low. Even if acetate oxidation may stimulate the degradation of propionate, the presence of acetate in the digester also can contribute to lower pH, and thereby could generate the inhibitory effect on the growth of methanogens as well as acetogens. However, the inhibitory effect generated from acetate is extremely lower in comparison to the inhibitory effect caused by propionate and butyrate *(Amani et al, 2011).*

Although propionate and butyrate to some extent may enhance the formation of methane, they may extremely inhibit the growth of anaerobic microorganisms when their concentration is high in the digester. This condition may lead to the decrease of biogas production. High concentration of propionate and butyrate in the digester may lower pH in the digester, and thereby could significantly suppress the growth of anaerobic microbes, particularly methanogens *(Ahring 1995; Thauer et al, 1977; Kim et al, 2004)*.

Study conducted on the effects of VFA concentrations on methane yield and methanogenic bacteria revealed that ethanol, acetic acid and butyric acid concentrations of 2400, 2400 and 1800 mg L^{-1}, respectively, led to no significant inhibition of the activity of methanogens. However, propionic acid concentration of 900-951 mg/l extremely inhibited the growth of methanogens *(Demirel and Yenigun, 2002; Wang et al, 2009)*.

The studies also revealed that propionic acid concentration of 900-951 mg/l may generate an accumulation of acids due to the decreasing rate of acid degradation.

Study of Gibbs free energy showed that at an extreme low pH the reaction to form acetate and propionate may be shifted by the reaction of ethanol and butyrate (depicted in Fig. 3). Further, during the low pH, the formation of 1 mol of propionate along with 1 mol of acetate as well as 1 mol of hydrogen is possible to occur. The reaction to form propionate is highly dependent on the hydrogen partial pressure in comparison to acetate, butyrate and ethanol (Fig. 3 and 4).

The conversion of propionate to acetate at neutral pH would be inhibited due to high hydrogen partial. Anaerobic conversion of propionate to acetate could generate 1% of hydrogen, and its conversion would be inhibited when the hydrogen produced was at about 1.5 %.

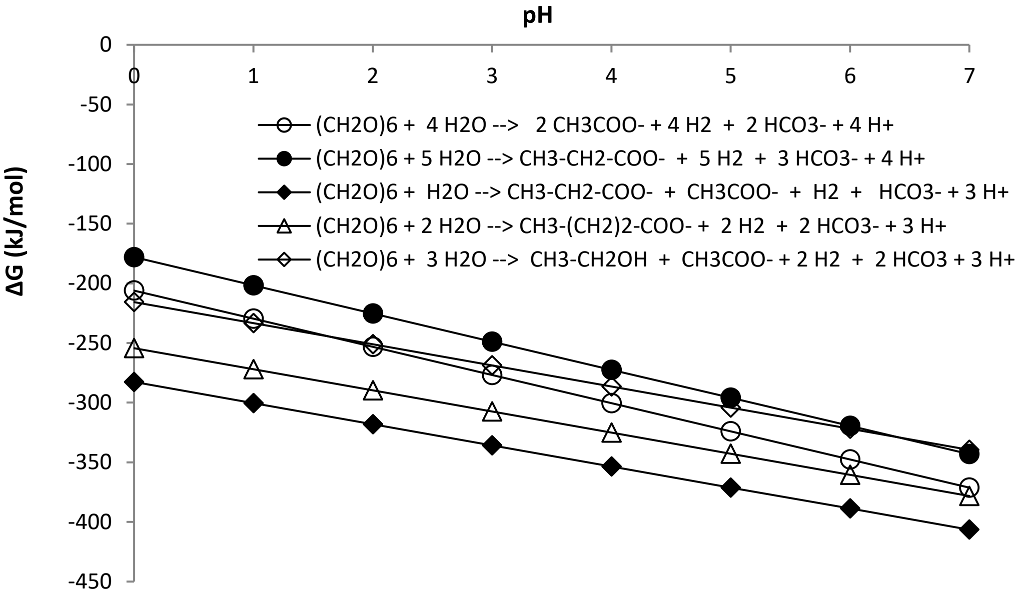

Fig. 3-Free energy change of VFA formation as a function of pH

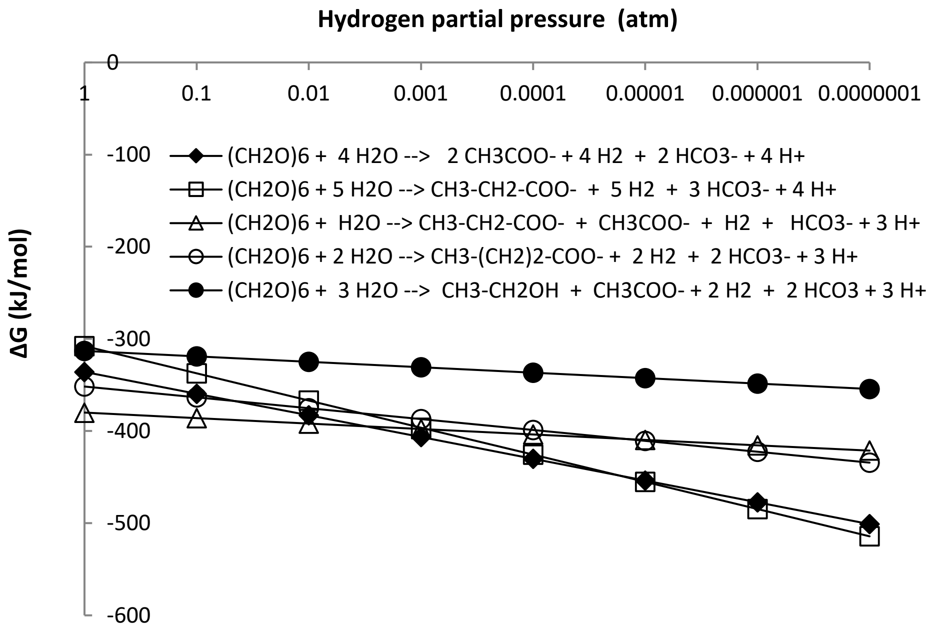

Fig. 4 - Free energy change of VFA formation as a function of the hydrogen partial pressure

CONCLUSIONS

The present study revealed that low pH in anaerobic digester could shift the free energy change in the reactions of both VFA production and VFA utilization. The current result showed that the degradation of VFAs (i.e. propionate and butyrate) during the process of anaerobic digestion is not only caused by the lack of free energy changes but it could also affected by low pH. The VFAs that could not be degraded and oxidized during the anaerobic digestion, would significantly suppress the methanogens' activity due to an increase of proton concentration in the digester caused by acid build-up.

The results of the current study showed that the formation of ethanol as an intermediate product during the stage of acidogenesis is more favorable in comparison to the formation of propionate, lactate and butyrate. This is due to the fact that at the low pH, the reaction of ethanol conversion is thermodynamically spontaneous. This suggested that the oxidation of ethanol to acetate is also not affected by the acidic condition, and thereby could balance and optimize methane production.

This study is significant for enhancing methane production during the process of anaerobic digestion, and also could be useful for developing further research on selectively producing useful fermentation end-products during anaerobic acidogenesis. Further, the study also provided an overview in regards to the intermediate products that potentially accumulated during the phase of acidogenesis.

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