Anaerobic Digestion of Slaughterhouse Wastewater: CO_2 Capture of Biogas Using *Chlorella vulgaris*

Nur Indradewi Oktavitri*, Wahyu Budi Pratiwi, Indah Purnamasari, Mufrihatul Hayati, Mega Rosita Fitrianingtyas, and Semma Hadinnata

Department of Biology, Faculty Science and Technology, Airlangga University, Kampus C Mulyorejo, Surabaya 60115, Indonesia

* ***Corresponding author:***

email: nur-i-o@fst.unair.ac.id

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Abstract: *Biogas quality from anaerobic digester influenced the combustion of biogas. A high percentage of CO_2 in biogas indicates the low quality of biogas. Abatement of CO_2 using microalgae, such as* Chlorella vulgaris *could enhance the quality of biogas. The aim of this research was to observe the ability of* C. vulgaris *on CO_2 removal from slaughterhouse wastewater biogas. In this research, two anaerobic digesters were provided with the different condition of biogas collector bag. The first digester was combined with only biogas collector bag, while another digester was combined with* C. vulgaris. *Slaughterhouse wastewater volume in each digester was 3.5 L. Observation time was 15 days and the samples were collected for every 5 days. The result showed that anaerobic digester was able to remove 63% of COD. Biogas composition of slaughterhouse wastewater after incubation for 15 days was 52.70% of air, 46.85% of $CH_4$and 0.45% of CO_2.* C. vulgaris *enhanced CO_2 removal from biogas up to 7%. The density of* C. vulgaris *decreased to 51 cell/mL. The biogas composition was probably influenced by the density of* C. vulgaris.

Keywords: *anaerobic digestion; biogas;* Chlorella vulgaris*; CO_2; density of* Chlorella vulgaris

INTRODUCTION

Nowadays, every country has a similar problem with energy. The increase in energy demand prompts us to find renewable energy [1]. One of the renewable energy is biogas. Biogas can be produced by fermenting organic materials in absence of air. Biogas is produced from organic materials, in particular by the cultivation of biomass [2]. Successful anaerobic digestion should be maintained to enhance biogas [3].

Microalgae have been intensively studied as a source of biomass for replacing conventional fossil fuels in the last decades [4]. The ability of *Chlorella* sp. as renewable energy is widely observed by researchers [4-6]. *Chlorella* sp. has the ability to grow and adapt in environment [6]. *Chlorella* sp. was utilized in various research of bioenergy, for example, for generating biogas [7] and purifying biogas from CO_2 [8]. Recent experiments focus on CO_2 capture from the anaerobic process of microalgae and photosynthetic bacteria. Different raw materials on anaerobic digestion could imply an unequal characteristic of biogas composition.

In this study, the source of biogas was obtained from slaughterhouse wastewater since the slaughterhouse wastewater contains methanogenic bacteria. Methane (CH_4) has a higher heat capacity than carbon dioxide (CO_2) [9]. CO_2 could influence biogas quality which triggers incomplete combustion [10]. Therefore, biogas needs an effective purification technology to remove the CO_2 gas. Analysis of biomass, density, the content of biogas, temperature, pH, and Chemical Oxygen Demand (COD) had been observed in this study.

EXPERIMENTAL SECTION

Materials

The *C. vulgaris* was collected from Balai Perikanan Budidaya Air Payau Situbondo. The specific medium used for cultivating *C. vulgaris* is Walne which contain

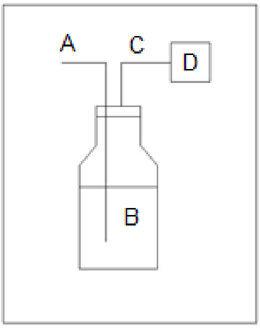

Fig 1. Anaerobic batch reactor control

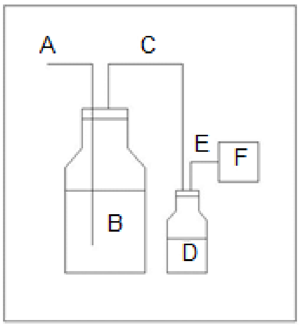

Fig 2. Anaerobic batch reactor treatment

20 g $NaH_2PO_4{\cdot}2H_2O$, 100 g $NaNO_3$, 5 g Na_2EDTA, 40 g Na_2SiO_3, 0.36 g $MnCl_2{\cdot}6H_2O$, 1.3 g $FeCl_3$, 10 H_3BO_3, 1000 mL distilled water [11]. The slaughterhouse wastewater samples were collected from Pegirian, Surabaya.

Instrumentation

Biomass analysis of *C. vulgaris* was conducted via gravimetric methods. Centrifuge with a speed of 40 rpm was used to homogeny the sample. Cells were collected through filtration and rinsed several times with distilled water. The filter paper which contained cells were pondered before drying. The temperature used for drying was 105 °C [12]. The density of *C. vulgaris* was counted using a Sedgewick–Rafter counting chamber with 10–100 times magnification by microscope [13]. The analysis of the content of biogas was observed by gas chromatography [14]. The specification of gas chromatography is GC9700. Meanwhile, COD analysis was conducted with the titrimetric method [15]. All samples were taken and analyzed twice (n=2) except gas composition. Statistical analysis was calculated by ANOVA method.

Anaerobic batch reactors

Two anaerobic digesters were used in this study. Two reactors were illustrated in Fig. 1 and 2. The first digester was used as a control, while the second digester called treatment digester.

Procedure

Seeding *C. vulgaris* was performed by growing *C. vulgaris* in media Walne. The growth of *C. vulgaris* was observed for every hour until OD_{480} up to 2.0 ± 0.02.

All digesters were illuminated at 4800 lux for 8 and 16 h without lightning [16]. The volume of each slaughterhouse wastewater samples in each anaerobic digester was 3.5 L. Each reactor had been connected to the biogas collector bag to capture methane as a result of the biogas process. In the second digester, biogas collector bag was connected with anaerobic digester of *C. vulgaris*, in order to capture CO_2. Analysis of CO_2 and CH_4 were conducted using gas chromatography. The examined parameters, such as COD, VSS, biomass, and density of *C. vulgaris*, were monitored for every 5 days in 15 days.

■ RESULTS AND DISCUSSION

Biogas Composition from Anaerobic Process

Anaerobic digestion was processed to decompose organic substance with a limited amount of oxygen. The presence of biogas indicated organic decomposition from 4 step processes i.e. hydrolysis; acidogenesis; acetogenesis; methanogenesis [17]. Anaerobic digestion involved various of a microorganism to support acidogenesis, acetogenesis, and methanogenesis process. Biogas was the result of methanogenesis process. The organic complex substance was hydrolyzed to simple organic monomer by hydrogenous and acidogenic bacteria. This process also produced volatile fatty acid, H_2, and acetic acid. The acetogenic bacteria converted volatile fatty acid to H_2, CO_2 and acetic acid. The last step was converting H_2, CO_2, and acetate to CH_4 and CO_2 [18]. Decreasing of COD level and production of biogas was the indication of the success of the anaerobic process. Fig. 3 shows that COD level decreases during anaerobic digestion of slaughterhouse wastewater.

Fig. 3(a) shows a comparison of COD level between control and treatment digesters, while Fig. 3(b)

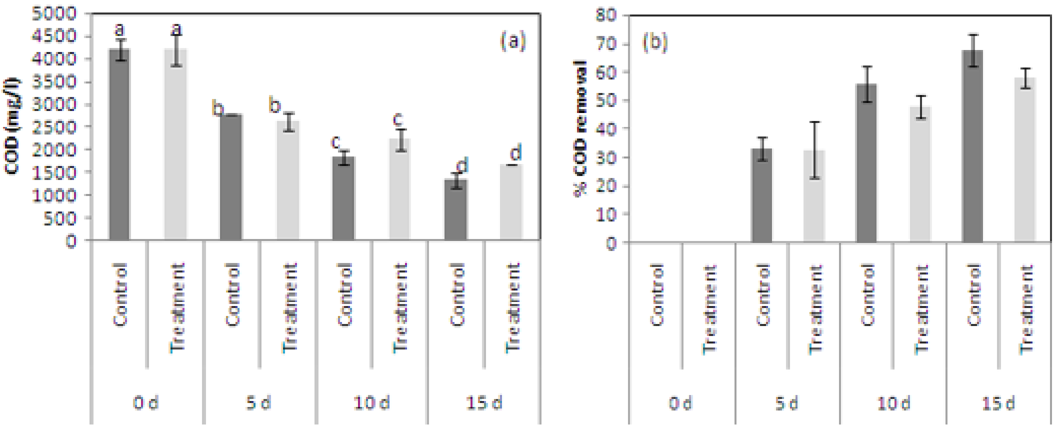

Fig 3. COD level (a) and removal percentage of COD (b) on the digesters as a function of time

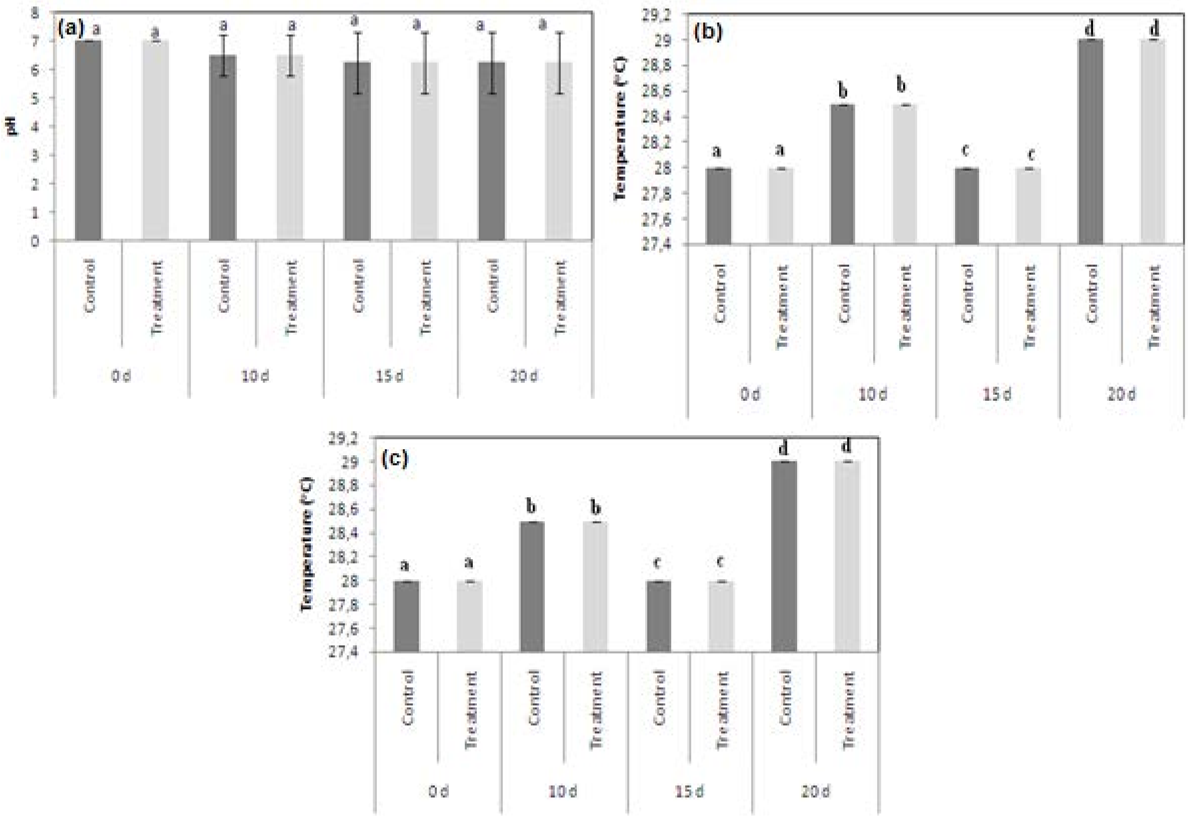

Fig 4. The value of pH (a) and temperature (b) at control and treatment reactor as a function of time

shows a comparison of removal percentage of COD between control and treatment digesters. The low case above bars in Fig. 3(a) shows that there is no significant difference between control and treatment digesters, for each treatment duration ($P < 0.05$, a > b > c > d). This condition was caused by the addition of *C. vulgaris* outside the main reactor, so it is relatively the same. Significant differences in COD level were influenced by additional contact time.

COD significantly decreased from 4000 to 1500 mg/L in both reactors (Fig. 3) after 1-2 weeks of operation of the anaerobic process. Removal percentage of COD level on both digesters approximately reached 60% after 15 days. Ahmad et al. concluded that

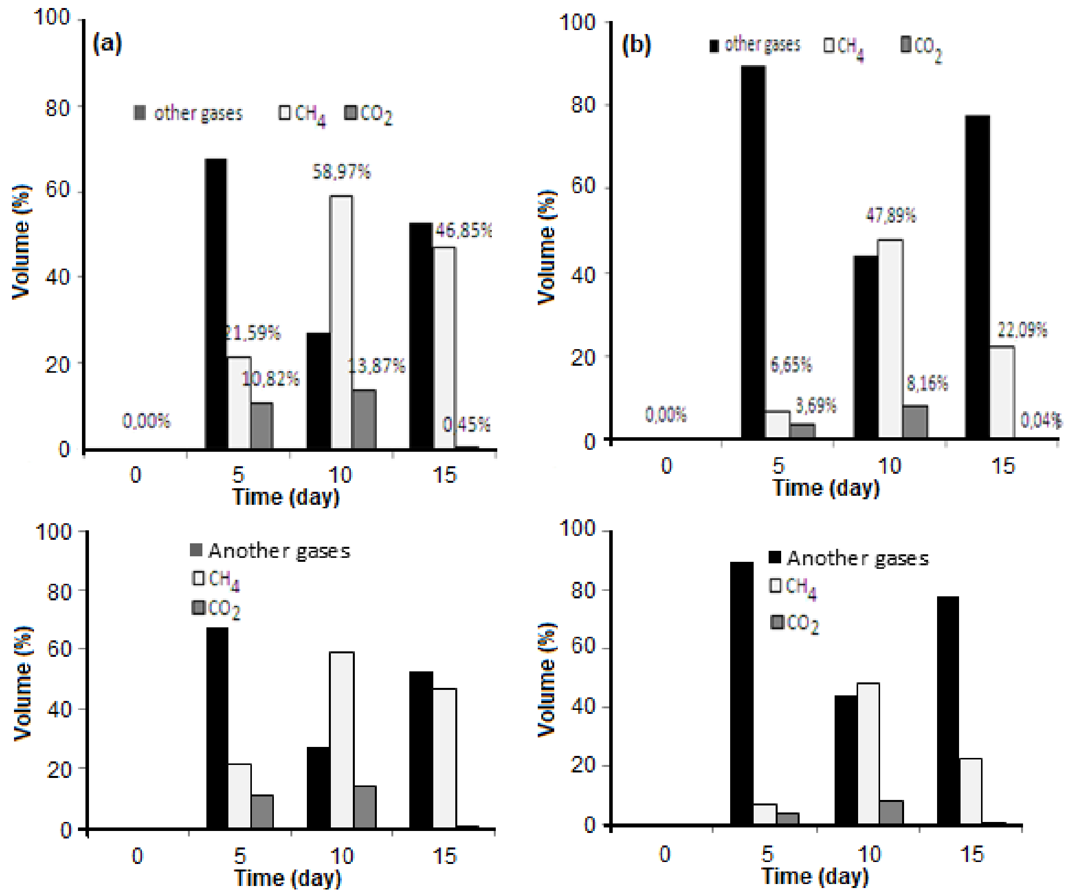

Fig 5. Biogas composition of control (a) and treatment of reactor (b) (I choose above graph)

Table 1. The composition of biogas from anaerobic digestion after incubation 5, 10 and 15 days

Biogas Composition (%)	Time (Days)					
	Control			Treatment		
	5	10	15	5	10	15
Other gases	67.58	27.15	52.70	89.66	43.95	77.87
CH_4	21.59	58.97	46.85	6.65	47.89	22.09
CO_2	10.82	13.87	0.45	3.69	8.16	0.04

anaerobic digestion depends on the condition inside the digester such as pH and temperature [19]. Gerardi [18] recommended that the pH and temperature for the anaerobic process was 5.5–7 and 27–29 °C, respectively [18]. The pH value and temperature in this research are within that range (Fig. 4).

Fig. 4 shows that pH shows no significant difference between control and treatment reactor. On the other hand, the temperature fluctuates inside the reactor. Methane production is directly correlated with organic decomposition [20]. In which it was obtained by COD value for both of reactor. Angelidaki et al. [21] reported that the relationship biogas will result from every 0.5 L/g of COD removal [21]. Therefore, it was predicted that the volume of biogas production also had an equal value from both digesters, control digester, and treatment digester which were combined with *C. vulgaris* in biogas collector bag.

The European Commission of waste treatment [22] described that the content of biogas from anaerobic digestion does not only consist of CO_2 and CH_4 but also other gases such as O_2, N_2, H_2S, among others [22]. Table

1 and Fig. 5 show the components of biogas are CO_2, CH_4, and other gases.

Biogas production was detected after 5 days, composed of methane, carbon dioxide, and other gases. Several days were required to produce biogas by the anaerobic process. The anaerobic process was conducted by various bacteria to convert complex organic compound to biogas. Moreover, methanogenic bacteria were slow to grow and sensitive to an anaerobic condition [23].

At the early 5 days of the experiment, air dominated the biogas composition in both digesters. It consisted of O_2, $H_2$and H_2S. After the 5 days, the percentage of air decreased by around 20%, approximately. The autotrophic microorganism utilized the oxygen for their metabolism (such as hydrolysis bacteria, microalgae, etc.) [17-18]. Hence, air content in the anaerobic digester of *C. vulgaris* decreased by half of the initial volume in 10 days. Moreover, after 15 days of incubation, the percentage of air increased again above 50%. Anaerobic digester of *C. vulgaris* had the highest percentage of air after 15 days of anaerobic process. It was probably because carbon dioxide from biogas was used by microalgae for photosynthetic which produces oxygen [17-18].

The highest value of methane detected after 10 days of incubation indicated that methanogenic bacteria mostly degraded the carbon to methane (CH_4). The microorganism needed particular time to degrade the organic substance and it depends on the growth rate of the microorganism [24]. The CH_4 will be highly produced if the organic material reduced significantly [21]. Due to the experiment, CO_2 was obtained below 20%, approximately. The ratio between CH_4 and CO_2 depends on the oxidation state of the carbon. For a compound $C_nH_aO_b$, the anaerobic digestion process can be described as equation 1 [21]:

$$\mathrm{CnHaOb}+\left(n-\frac{a}{4}-\frac{b}{2}\right)H_2O\rightarrow\left(\frac{n}{2}+\frac{a}{8}-\frac{b}{4}\right)CO_2+\left(\frac{n}{2}-\frac{a}{8}+\frac{b}{4}\right)CH_4 \quad (1)$$

CO_2 Adsorption by Microalgae

The treatment reactor consisted of microalgae in biogas collector bag (illustrated in Fig. 6. The effect of *C. vulgaris* existence seen from the decreasing of CO_2 in biogas bag. The efficiency of CO_2 adsorption by *C. vulgaris* was reflected by equation 2: Reduction of CO_2 composition in the reactor.

$$\begin{aligned}&\text{Reduction of } CO_2 \text{ composition in reactor}\\&=\left(\%CO_2 \text{ in control reactor}\right)-\left(\%CO_2 \text{ in treatment reactor}\right)\end{aligned} \quad (2)$$

Fig. 6 Indicates that CO_2 was used in *Chlorella* photosynthesis. Reduction of CO_2 composition in reactor reached up to 7%. Ouyang et al. [5] described that CO_2 removal reached above 50% using illumination of [25] also reported several research treated waste used *Chlorella* sp. for efficiencies. The research of Ramaraj et al. [26] resulted that *Chlorella sp.* removed CO_2 to 17% from the biogas composition of the biomass of microalgae.

The low percentage of CO_2 removal is probably caused by the environment in *C. vulgaris* tank, which inhibited the growth of *C. vulgaris*. Biogas composition from slaughterhouse wastewater contained a high percentage of other CO_2 gases (Fig. 6), which probably contains H_2S. H_2S is toxic to *C. vulgaris* [6]. Therefore,

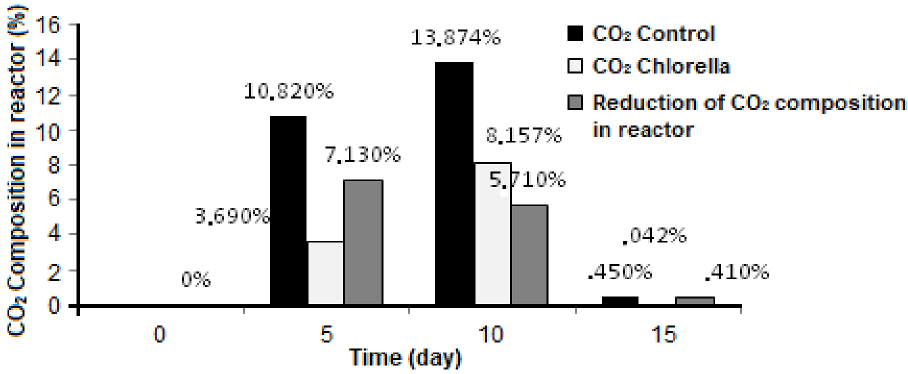

Fig 6. The efficiency removal of CO_2 in treatment reactor

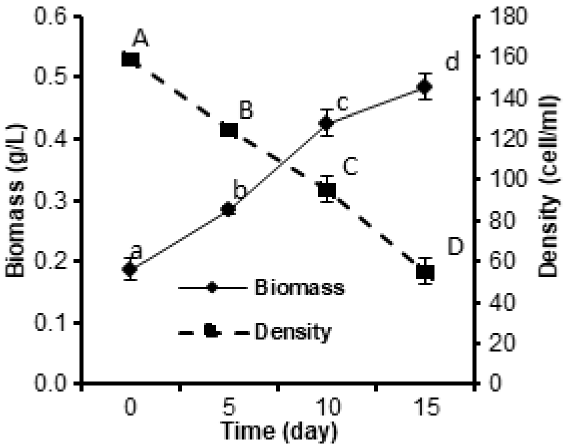

Fig 7. Value of biomass and density of *C. vulgaris*

the *C. vulgaris* population decreases. The cell of *C. vulgaris* can be represented by its biomass and density (Fig. 7). Previous studies [5,24] also reported that the growth of microalgae can be indicated by biomass.

Biomass and density of *C. vulgaris* were calculated under different incubation condition for 15 days. The upper case letters indicate the significant difference of the density ($p < 0.05$, A > B > C > D), while lower case letters indicate a significant difference of the biomass ($p < 0.05$, a < b < c < d).

Fig. 7 displays the trend of biomass and density of *C. vulgaris* as a function of time. It is shown that the biomass has an opposite trend compared to the density of microalgae. It indicates that gravimetric methods for biomass analysis did not describe the existence of microalgae correctly. The dead cell of microalgae becomes the part of suspended solid which then were collected beneath the reactor [27]. Therefore, biomass analysis was used to observe the suspended solid which located in the bottom part of the sample. The method used to be recognized as gravimetric methods. The dead cell of microalgae released H_2S and can be detected in this experiments. Therefore, on the last day of operation time, the density of microalgae decreased, while the percentage of air enhanced significantly.

This research observed the response of *C. vulgaris* in utilizing the light and CO_2 from biogas photosynthesis [8]. Photosynthetic rate exceeded the respiration rate of *C. vulgaris* cell by10–100 times [28]. Due to the reproduction of microalgae, one mature cell can produce into four new ones every 16–20 h [28] and the reproduction type of *C. vulgaris* is asexual and rapid [6]. Even, Ouyang et al. [5] recommended cultivating *Chlorella* sp. in low and high intensity of light. Meier et al. [24] reported that the growth of microalgae can be inhibited by the low percentage of CO_2. Percentage of CO_2 provides sufficient support to microalgae growth, if the concentration was above 15% [29]. Great CO_2 concentration was required for carbon balance that was used by microalgae [30]. It can be concluded that the density of microalgae (Fig. 7) decreases alongside the percentage of CO_2 in biogas (Fig. 6).

CONCLUSION

This study shows that *C. vulgaris* enhanced CO_2 removal from biogas up to 7%.The density of *C. vulgaris* decreased to 51 cell/mL and it was influenced by biogas composition.

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