

# ANAEROBIC ACIDIFICATION OF COCONUT WATER WASTE BY *LACTOBACILLUS ACIDOPHILUS* CULTURE FOR BIOTECHNOLOGICAL PRODUCTION OF LACTIC ACID

Darwin<sup>1</sup>, Ulfa Triovanta<sup>1</sup>, Ridho Rinaldi<sup>1</sup>, Atmadian Pratama<sup>1</sup>

<sup>1</sup> Department of Agricultural Engineering, Syiah Kuala University, Banda Aceh 23111, Indonesia

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## Abstract

The biotechnological production of lactic acid could be carried out via anaerobic acidification process. In order to achieve an optimal production of lactic acid, the role of inoculum would be essential. The current study aimed to investigate as well as evaluate the effect of inoculum concentration on the anaerobic acidification of coconut water waste for the production of lactic acid. Results showed that the addition of 20% inoculums to the reactor fermenting coconut water waste was sufficient for the optimal production of lactic acid. In the batch process anaerobic acidification of coconut water waste inoculated with 20% inoculums of *Lactobacillus acidophilus* culture had the yield of lactic acid production, which was about 1.62 mmol lactic acid/mmol glucose while under the continuous operation the yield of lactic acid production obtained, was about 1.15 mmol lactic acid/mmol glucose. During the acidification process in both batch and continuous modes pH dropped significantly from 5.1 to 3.7.

Keywords: acidification, lactic acid, coconut water waste

## INTRODUCTION

Coconut water is a slightly cloudy liquid obtained from the endosperm of coconuts (*Cocos nucifera* L.) when harvested for the production of coconut oil (Smith and Bull, 1976; Prado *et al.*, 2015). Coconut water is considered as an energy drink since it could provide an isotonic electrolyte balance for someone consuming it (Elumalai *et al.*, 2014). Due to rich in sugar content, coconut water could be used to replace the need of dextrose/glucose during the medical emergencies (Elumalai *et al.*, 2014; Obidoa *et al.*, 2010). Besides, it also could be used as an antidote for poisons (Elumalai *et al.*, 2014), and nutrient for healing wounds and illnesses (Radenahmad *et al.*, 2006; Roopan, 2016). Coconut

water is also known as a nutritive food source as it contains sugar, minerals, antioxidants, protein and fiber (Obidoa *et al.*, 2010).

In some tropical countries such as Indonesia and Malaysia coconut water from the mature coconut is largely wasted. This occurs as some people want to use coconut milk extracted from the grated pulp of mature coconuts for making some traditional foods (i.e. beef curry, cakes) (Raghavendra and Raghavarao, 2010; Marina and NurulAzizah, 2014; Surono, 2015), and normally coconut water is not used and would be disposed. The disposal of coconut water waste into water channel would generate serious problems. This is due to the fact that the biochemical oxygen demand of waste coconut water is somewhat high which is about

more than 40 kg/l (Smith and Bull, 1976). The contamination of this organic waste into water body could cut dissolved oxygen, and cause oxygen depletion in the water (Vos and Roos, 2005; Akpor *et al.*, 2014). Hence, the practice of disposing the organic waste would not only lower the quality of water but also affect the ecosystem in the water body and/or agricultural land (Su, 2014; Wen *et al.*, 2017).

The use of coconut water waste as substrate for biotechnological production of bio-products would be potential. This is due the fact that coconut water waste contains soluble carbohydrates including sucrose, glucose and fructose (Smith and Bull, 1976), and thereby could be used as carbon and/or energy sources for microbial growth and activities required for producing the end-products (Lee *et al.*, 2013). The production of bio-products utilizing the coconut water waste could be carried out through the biological processes, such as anaerobic digestion, anaerobic acidification and/or fermentation process. Those biological processes would involve microbiological processing to convert the soluble organic materials into some useful end-products (i.e organic acids and alcohols) (Puerari *et al.*, 2012; Mas *et al.*, 2014). Hence, evaluation of process parameters (i.e. pH, hydraulic retention time) during the conversion process is essential in order to achieve the optimal production of the expected end-product.

Study on the biotechnological production of lactic acid using coconut water waste as substrate via microbiological processing would be significant. This is because it could lower the risk of pollution caused by the disposal of untreated coconut water waste onto land and/or water bodies. One of the established technologies that could be applied to treat coconut water waste is anaerobic digestion (AD). AD is an effective microbiological processing that could be used to decompose and convert the organic materials into biogas containing methane as the main component (Molino *et al.*, 2013).

Processing and treating coconut water waste would potentially generate some problems. This occurs since coconut water waste was somewhat too acidic in which its pH was between 4.4 and 5.6 (Chauhan *et al.*, 2014). In order to have an effective conversion, the process of AD should be carried out and maintained under the neutral pH (pH 6.8–7.2) (Ehimen *et al.*, 2013; Darwin *et al.*, 2017). Thus, in order to stabilize the process of AD, the low pH culture should be added alkaline and/or basic solution (i.e. NaOH, NaHCO<sub>3</sub>, CaCO<sub>3</sub>) (Siles *et al.*, 2010; Jasko *et al.*, 2011; Kuttner *et al.*, 2015). However, continuously adding buffer and/or alkaline solutions to the fermentation culture would be costly and become impractical process. Thus, applying the technique for operating the anaerobic reactor under the acidic condition would be a significant method.

The process of anaerobic acidification would be more favorable to treat coconut water waste that has low pH. This is highly significant as the process could cut the cost of using buffer solutions. In this current study, the anaerobic acidification was carried out without maintaining the pH culture. This is because the end-product that is expected from the acidification process of coconut water waste is lactic acid, and the production of lactic acid would be optimal under the acidic condition (pH  $\leq$  5.0) (Darwin *et al.*, 2018). The aim of the current study also included the investigation as well as evaluation on microbial processing of the lactic acid production using different concentrations of inoculum.

## MATERIALS AND METHODS

### Coconut Water Waste Collection and Culture Preparation

Coconut water waste (CWW) used for this experiment was obtained from several traditional markets located in Banda Aceh, Indonesia. The CWW was stored in the refrigerator at the temperature of  $4.5 \pm 0.5$  °C prior to using it for the experiment. As the experiment was carried out under the pure culture fermentation, the inoculums used for the experiment contained *Lactobacillus acidophilus* IIA-2B4. To investigate the effects of different concentration of inoculums added on the performance of fermentation process and the yield of end-product produced, for both batch and continuous experiments were carried out at 5, 10 and 20% of inoculums added.

### Experimental Design and Procedures

Anaerobic acidification was carried out at the steady state conditions in which the operational temperature was maintained under the mesophilic condition at  $35 \pm 1$  °C by using automatic thermostatic water bath for both batch and continuous reactors. To ensure the fermentation process was under the homogeneous condition, the culture was continuously stirred at about 85 rpm.

For the batch tests, three identical reactors with the working volume of 1 litre were utilized for the process of anaerobic acidification. To evaluate the performance of batch process, samples were withdrawn hourly for the analysis. For the continuous operation, the hydraulic retention time (HRT) applied was 10 days. As the working volume of the reactor was 3 litres, the loading rate applied was 300 ml/day. To assess the performance of the acidification process, pH culture was measured regularly during the feeding and discharging period according to the Standard Method (APHA, 2012). The samples of influent as well as effluent were analyzed on a daily basis. The anaerobic acidification process operated under the continuous mode, were carried out for 10 days in order to reach a steady state condition.

### Analytical Methods

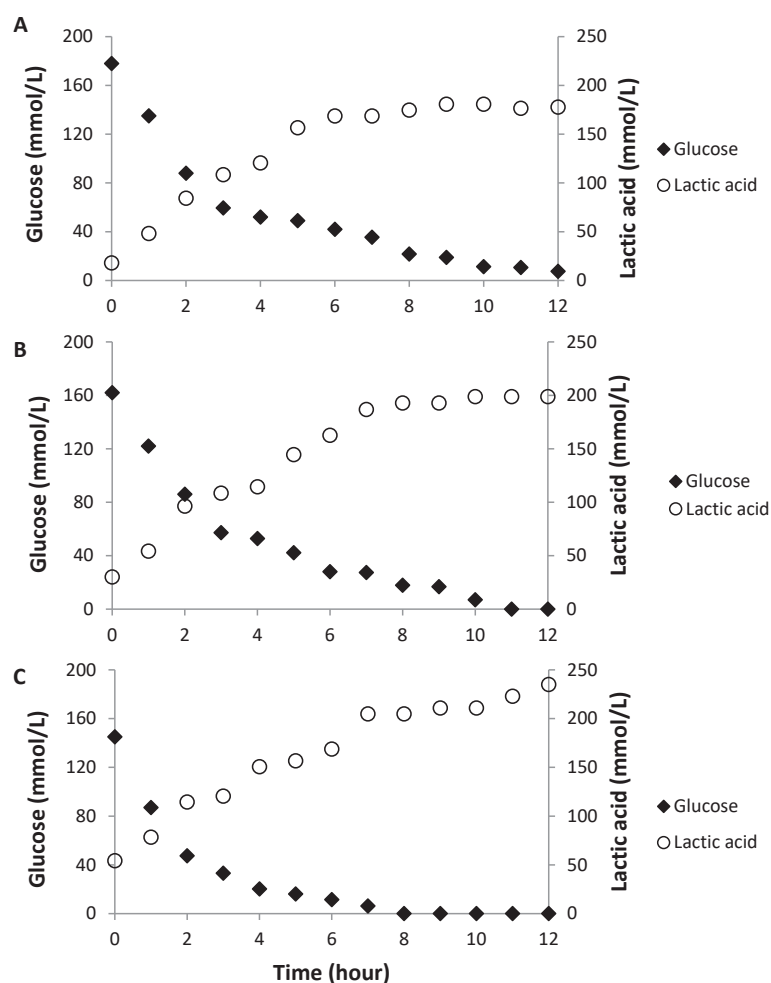
All samples taken from the influent and effluent were analyzed for pH, lactic acid and glucose concentration. To assess the substrate uptake during the fermentation process, 2 ml of samples were used for the determination of glucose content in the fermentation broth. The glucose analysis was conducted using glucose meter, BioSensor AGM-2100 with an assay method of electrochemical method (Gold electrode). The glucometer used was in corresponding to test strips (Gold Plated Test Strip, allmedicus). All the procedures for the glucose measurement were carried out according to the method developed by Darwin (2019a). For measuring glucose concentration, samples of 2 ml fermentation broth were taken from the reactor of anaerobic acidification of coconut water waste from the time of 0 to 12 h of incubation, and the samples were diluted 10 times prior to the analysis. The glucose analysis was conducted under the ambient temperature. Lactate analysis was carried out using an Accutrend Plus meter Cobas (Roche) lactate monitoring system, working on an

electrochemical assay method and using test strips (BM-Lactate strips, Ref 03012654, Cobas, Roche). All the procedures used for the determination of lactate concentration on the fermentation samples, followed the method developed by Darwin (2019b). Sample of 2 ml for lactate analysis was collected from the reactor of anaerobic acidification of coconut water waste. The sample taken was filtered prior to the measurement of lactate concentration using lactate biosensor. The response of lactate biosensor reading was correlated to the standard solution of lactate ranging from 0 to 20 mM.

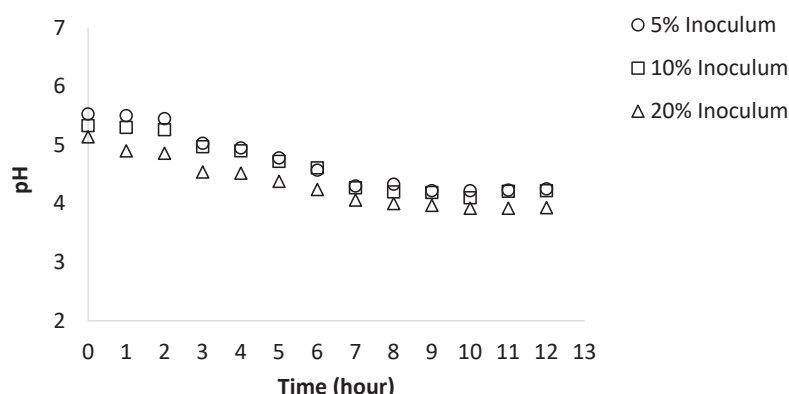
## RESULTS

### Batch Operation

In order to evaluate the effectiveness of lactic acid production from the fermentation of coconut water waste inoculated with *Lactobacillus acidophilus* culture, various concentrations of the inoculum were introduced into the batch digesters. Results from the batch experiments showed that anaerobic



1: Lactic acid production and glucose uptake of the batch digesters inoculated with *Lactobacillus acidophilus* culture, under the different concentration of inoculum: (A) 5%, (B) 10%, and (C) 20% of inoculum



2: pH profile of the batch digesters inoculated with different levels of inoculum concentration

acidification of coconut water waste inoculated with *Lactobacillus acidophilus* culture could easily produce lactic acid as the end-product. Results revealed that the addition of 20% inoculums into the batch digester containing coconut water waste could enhance the production of lactic acid in comparison to the digester inoculated with 5 and 10% inoculums (Fig. 1). The maximum yield of lactic acid production obtained from the batch digester inoculated with 20% inoculums was quite high at around 1.62 mmol lactic acid/mmol glucose, which was about 80% of the theoretical lactic acid production (2.0 mmol lactic acid/mol glucose). It was higher than the yield obtained from the digesters inoculated with 5 and 10% inoculums in which the yields reached were only 1.02 and 1.23 mmol lactic acid/mmol glucose, respectively.

The results of the experiments showed that 10 and 20% inoculum applied into the batch digesters could convert a hundred percent of substrate into lactic acid (Fig. 1). However, the digester inoculated with 20% inoculums could convert substrate faster than the digester added with 10% inoculums in which the 20% inoculums could complete the conversion of substrate within 8 days of incubation while 10% inoculums required 10 days of incubation to complete the conversion process. Further, the digester inoculated with 20% inoculum had the highest rate of substrate conversion during the fermentation process, which was about 21 mM glucose per hour in comparison to the digesters inoculated with 5 and 10% inoculums, the substrate conversion rate accomplished were only 14 and 16 mM glucose/hour, respectively.

### Continuous Operation

Results from the batch experiments revealed that anaerobic acidification of coconut water waste inoculated with 20% inoculums of *Lactobacillus acidophilus* could enhance lactic acid production. In order to evaluate to what extent the fermentation of coconut water waste inoculated with *Lactobacillus acidophilus* supports the development of a stable

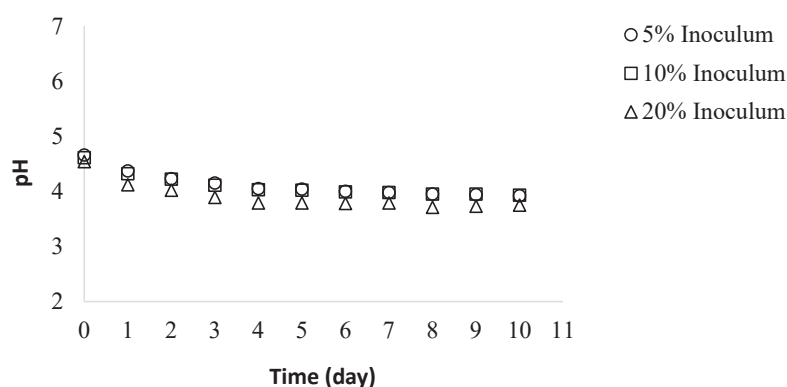
culture in a continuous process, the fermentation of coconut water waste were tested in a continuous system with various concentration of inoculums.

The results revealed that 20% inoculum was fairly sufficient for the process of acidification of coconut water waste to produce lactic acid. The digester inoculated with 20% inoculum performed quite well in which lactic acid production rate reached was higher than of the reactor inoculated with 5 and 10% inoculums. In terms of lactic acid production rate obtained, within 5 days of the incubation process, the reactor inoculated with 20% inoculums could reach 154 mmol/L of lactic acid production, which was significantly higher than the reactor added with 5 and 10% inoculums, which only had 127 and 133 mmol/L of lactic acid production, respectively (Fig. 4).

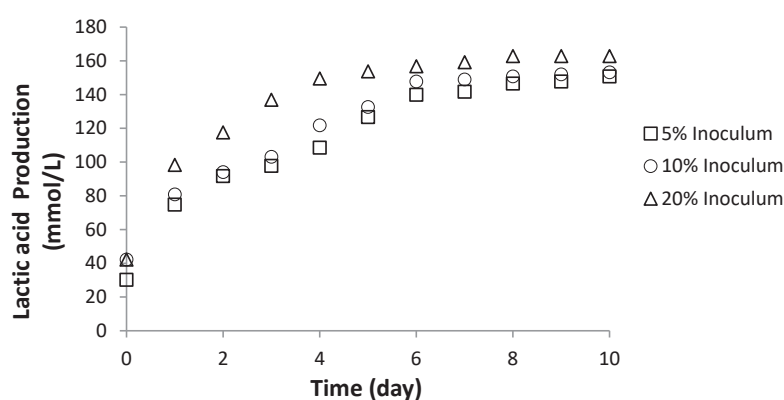
Results showed that lactic acid productivity of the reactors inoculated with 20% inoculums was higher (146 mmol/L.day) than the productivity of the digesters added with 5 and 10% inoculums, which were about 122.6 and 128.5 mmol/L.day, respectively. The results of the experiment revealed that anaerobic acidification of coconut water waste inoculated with *Lactobacillus acidophilus* produced lactic acid at the concentration of more than fifty percent (150–160 mmol/L) of the total lactic acid expected (280 mmol/L). The continuous reactor added with 20% inoculums reached the maximum yield of 1.15 mmol lactic acid/mmol glucose while the yields of lactic acid obtained from the reactors inoculated with both 5 and 10% inoculums were 0.94 and 0.92 mmol lactic acid/mmol glucose, respectively.

### DISCUSSION

As shown in Fig. 2, pH in the digester inoculated with 20% inoculums is lower than the digester added with 5 and 10% inoculums in which the pH dropped from 5.14 to 3.93. Results also showed that low pH in the digester inoculated with 20% inoculums indicated that the production of lactic acid is somewhat optimal. This occurs since the



3: pH profile of the continuous digesters inoculated with different levels of inoculum concentration



4: Lactic acid production of the continuous digesters inoculated with *Lactobacillus acidophilus* culture under the different concentration of inoculum

oxidation of glucose into lactic acid could result in an increase of proton concentration in the fermentation culture. Thus, lactic acid accumulated in the digester, could significantly lower the pH culture. A drop of pH occurs due to the fact that lactic acid possesses low  $pK_a$ , which was about 3.86 (Eyal and Canari, 1995).

The result is in an agreement with the study by Gonzalez *et al.* (2008) revealed that an extremely acidic pH (3.0) would be more desired to have a maximum production of lactic acid. The study added that at pH values around 5.0, lactic acid is found in the dissociated form while at pH values around 3.0, lactic acid is found in the undissociated form. This suggested that the amount of dissociated lactic acid (lactate anions) and undissociated lactic acid found in the fermentation culture would be based on the equilibrium of lactic acid at such operating conditions (Russo and Kim, 1996; Dey and Pal, 2012).

The result of the current study is in agreement with study conducted by Shirai *et al.* (2001) revealing that the presence of inoculums and initial substrate concentration were essential factor in the fermentation process to reach a balanced process and an optimal production of the expected end-product. The study found that high initial

inoculums and substrate (i.e. glucose) concentration could significantly lower the time and enhance the production of lactic acid from the fermentation of shrimp waste (Shirai *et al.*, 2001). This suggested that sufficient amount of inoculum added, could significantly enhance substrate uptake by microbes, and thereby could increase the formation of the expected end-product (Boulanger *et al.*, 2012; Hobbs *et al.*, 2018).

High production of lactic acid in the reactor inoculated with 20% inoculum was also followed by an increase of proton concentration in the culture, as a consequence pH in the reactor dropped from 4.5 to 3.7 that was quite lower than pH in the reactor inoculated with 5 and 10% in which the pH dropped from 4.6 to 3.9 (Fig. 3). Low pH in the continuous process suggested that the formation of acidic metabolite such as lactic acid could significantly contribute for lowering the pH in the fermentation broth (Huang *et al.*, 2005; Mousavi *et al.*, 2011).

Based on the results, anaerobic acidification of coconut water waste inoculated with different concentrations of inoculums (5–20%), produced lactic acid at about 46–58% of the total expected lactic acid production. This is due to the fact that the oxidation of one mole of glucose theoretically



could generate 2 moles of lactic acid as the end-product (John *et al.*, 2006). The results suggested that high concentration of inoculums applied could potentially enhance the conversion rate, lower the period of lag or stationary phase, and accelerate

the log/exponential phase during the fermentation process (Robinson *et al.*, 2001; Rolfe *et al.*, 2012). Thus, the operating time could be reduced significantly, and the large amount of coconut water wastes could be treated and converted rapidly.

## CONCLUSION

The current study showed that the addition of 20% inoculum of *Lactobacillus acidophilus* to the digesters fermenting coconut water waste would be feasible to optimize the production of lactic acid. During the process of anaerobic acidification of coconut water waste the gradual formation of lactic acid could lead to the decrease of pH culture. The rapid oxidation and/or conversion of substrate into the end-product (lactic acid) could be accomplished by adding suitable amount of inoculum. Under the batch operation 20% inoculums added to the digesters fermenting coconut water waste could optimize the production of lactic acid at about 80% of the theoretical lactic acid production.

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