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Rapid determination of lactic acid in anaerobic biological treatment process using a portable sensitive lactate biosensor

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Abstract

The rapid and practical determination of lactic acid concentration during anaerobic digestion and in acidification samples is extremely important to ensure the digester is running properly and to avoid build-up of lactic acid. Thus, developing a simple and fast method for analyzing lactic acid in anaerobic digestion samples is important. The results of the present study revealed that lactic acid is an intermediate product of anaerobic digestion, which could be quickly measured using a portable lactate biosensor. We obtained linear standard curves from the relationship between various lactic acid concentrations of the standard solution and the lactate biosensor reading, which were tested at different temperatures (30, 45 and 55°C). A high value of coefficient determination, ~0.98–0.99, was obtained from all standard curves. This suggests that the evaluated system was accurate, reliable, and reproducible.

Key words: lactate, portable biosensor, anaerobic digestion, anaerobic acidification

Introduction

Anaerobic digestion is a series of biological processes that converts organic waste materials into certain useful end products such as bioenergetics(i.e., methane, hydrogen gas) and bioproducts (i.e., lactic acid, acetic acid) (Darwin et al., 2018a). Acidogenesis is the first stage of anaerobic digestion, which converts soluble organic compounds to organic acids and alcohols. Unlike volatile fatty acids (VFA), such as acetate, propionate, and butyrate, which are regularly measured to monitor the performance of anaerobic digestion, lactic acid is rarely measured because of limitations such as the use of complicated equipment, high cost, and a complicated analytical procedure. Lactic acid is an intermediate product of anaerobic digestion, which is generated during the acidogenesis phase (Darwin et al., 2018a). Regular monitoring of this organic acid is important to avoid acid accumulation in the anaerobic digester, which may lead to an upset digester because of a drop in the pH value.

Digester acidification via a reactor overload is one of the most common reasons for an upset digester (Franke-Whittle et al., 2014). Certain studies reported that a build-up of VFA is the primary reason for process deterioration in anaerobic digesters (Franke-Whittle et al., 2014; Ahring, 1995; Akuzawa et al., 2011). However, very few studies have revealed that lactic acid accumulation during the acidogenesis phase is the primary reason for an upset digester. Recent studies have revealed that anaerobic acidogenesis of starch wastes could potentially produce lactic acid as the primary end-product (Darwin et al., 2018a; Darwin et al., 2018b), and thereby it could interfere with the process of anaerobic digestion. As shown previously, lactic acid build-up caused the pH to drop significantly compared to VFA build-up (Darwin et al., 2018b). This occurs because lactic acid has a lower pKa (3.85) compared to VFA (pKa 4.76–4.87) (O'Hanlon et al., 2011). Thus, lactic acid build-up would generate higher proton concentration (H^+) compared to the VFA accumulation in the digester.

To ensure the proper process of anaerobic digestion, the concentration of organic acids should be regularly monitored. Currently, the organic acid analysis of anaerobic digestion samples can be readily achieved using gas chromatography (GC), which easily measures vo-

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latile organic acids; however, since lactic acid is a non-volatile acid, it could not be measured by GC(Darwin et al., 2018c). Thus, developing a simple and fast method for analyzing lactic acid obtained from anaerobic digestion samples would be very useful.

Several analytical methods for determining lactate concentration in anaerobic digestion samples are currently available such as titration (Vu et al., 2005) or colorimetric method (Madrid et al., 1999). However, the previously mentioned methods require complicated procedures in which certain chemicals and/or reagents such as NaOH, HCl, anthrone, sulfuric acid, and ethanol are required to change the color of the sample so that they can be easily detected through a spectrophotometer. High-pressure liquid chromatography (HPLC) could be used for measuring lactate concentration in fermentation samples as a reliable method (Vodnar and Socaciu, 2008). However, lactate analysis using HPLC would be a costly, tediousand complex procedure because the method for determining lactate using HPLC would require the column used for chromatography remains stable. As lactic acid is a polar molecule, it could generate no retention on reversed-phase column. Furthermore, as lactic acid contains carboxylic and hydroxyl groups, they could react with each other to produce dimers, trimers, and higher oligomers. Thus, the stability of the column is required to rectify the chain structure (Kishore et al., 2013). Another method that could be used for determining lactate concentration in anaerobic digestion samples is gas chromatography (GC) (Darwin et al., 2018c). However, the determination of lactate by GC would also be impractical because the method requires 60 min of pre-heating and use of periodic acid (Darwin et al., 2018c). Furthermore, lactate is a non-volatile organic acid; thus, the determination of lactic acid in the samples of anaerobic digestion would not be directly accomplished. Thus, using GC for lactate analysis would not be practical since the device could not be used in the field. Moreover, lactate analysis using GC requires a lot of procedure and chemical reagents; therefore, the determination of lactate concentration in the samples of anaerobic digestion could not be rapidly conducted.

A portable lactate biosensor was initially utilized for the rapid determination of lactate concentration in someone's blood while conducting physical exercises in order to provide a useful source of training information such as optimal training intensity, which is measured on the basis of the individual anaerobic threshold (IAT) (Baldari et al., 2009; Baldari and Guidetti, 2000). A study conducted by Baldari et al. (2009) revealed that the portable lactate biosensor is an effective, simple, and affordable analyzer for detecting lactate concentration in the blood. Moreover, the sensor could demonstrate a high level of accuracy, linearity, and reliability.

Since the determination of lactate concentration in the blood sample using portable lactate biosensor is considered as an accurate method, it may also have the potential to be used in other biological samples such as fermentation, anaerobic digestion, and acidification samples. We aimed to evaluate the effectiveness of the portable lactate biosensor for the determination of lactate concentration on anaerobic digestion and anaerobic acidification samples. The evaluation of linearity, as well as reproducibility of the developed method, was also included.

Materials and methods

Instrument

An Accutrend Plus meter Cobas (Roche) lactate monitoring system working on an electrochemical assay method was used. The model of the lactate meter used was Accutrend and cobas. Moreover, the electrical equipment used was 8C79, RR0288468, with the dimensions of 154 × 81 × 30 mm having a weight of ~140 g. The allowed sample volume was 15–50 µl with a measurement time of 60 s, the calibration applied was plasma value-equivalent. The battery operation used for lactate biosensor was 4 × AAA 1.5 V alkaline manganese batteries (Power One alkaline, Micro LR03, No. 4103 AM4 MN2400 Varta Microbattery GmbH).

The lactate biosensor was used in corresponding test strips (BM-Lactate strips, Ref 03012654, Cobas, Roche). Reagents used per sample test analysis were 7.2 µg lactate oxidase (*Aerococcus viridians* rec.) 1.9 U; N,N-bis-(2-hydroxyethyl)-(4-hydroximino-cyclo hexa-2,5-dienylidene)-ammonium chloride 7.2 µg; and 2.18-phosphomolybdate 11.4 µg. Lactate concentration was measured by reflectance photometry at a wavelength of 657 nm for colorimetric lactate-oxidase (LOD) mediator reaction.

Standard solution and standard curve

A standard stock solution of sodium lactate (50 mM) was prepared from the analytical grade purified aqueous

Table 1. Lactate concentration from Accutrend readings at varioustemperature ranges

Standard solution [mM]	Mesophilic at 30°C lactate from Accutrend [mM]	Thermophilic at 45°C lactate from Accutrend [mM]	Thermophilic at 55°C lactate from Accutrend [mM]
0	0	0	0
5	2.9	3.4	2.8
10	6.1	7.3	7.5
15	11.6	13	11
20	14.4	15.3	13.2

solution of sodium lactate (60%) (VWR BDH Prolabo Chemicals). From this stock solution, desired standard solutions and/or different ranges of lactate concentrations were prepared. The standard curves for lactate were determined from the lactate biosensor reading responses to the standard solution, obtained as mentioned previously, with concentrations ranging from 0 to 20 mM. Standard curves were produced by testing various concentrations of lactate (5, 10, 15, 20 mmol/l) and different temperatures of the analyte such as 55°C, 45°C, and room temperature (30°C). These temperatures were selected as the process of anaerobic digestion and acidification are normally conducted under mesophilic (30°C) and thermophilic conditions (45–55°C).

Sample preparation and analysis procedure

Samples were obtained from two different types of anaerobic microbial cultures, including anaerobic digestion and anaerobic acidification process operated under mesophilic conditions (30°C). First, samples of 2 ml were collected from the digester of anaerobic acidification of *Lactobacillus plantarum* cultures fed with taro starch asa substrate. Taro (*Colocasia esculenta*) is a tropical tuber crop produced primarily for its underground corms that contain 70–80% starch (Kaushal et al., 2015). Since taro contains highlevels of carbohydrates, it would be feasible to use it as a substrate for anaerobic acidification. Samples of 2 ml anaerobic digestion cultures were withdrawn from the reactor of anaerobic digestion of the leachate, which a water-based solution of compounds generated from the waste that normally contains both dissolved and suspended materials (Christensen et al., 2001). In this experiment, the leachate used for anaerobic digestion was the landfill leachate collected from the landfill area located in the Javanese Village, Banda Aceh, Indonesia. Each anaerobic reactor had 3 l of working volume. The process of anaerobic acidification was operated with a hydraulic retention time (HRT) of 5 days, while anaerobic digestion was operated with a HRT of 25 days. Since the measuring range of lactate concentration was 0.7 to 26 mmol/l, each sample was diluted 10 timesto obtain an accurate measurement. The samples were analyzed under the ambient or room temperature for determining lactate concentration. Moreover, to have reproducible results, the experiments were repeated three times.

Results and discussion

The effectiveness of the lactate determination method using a portable lactate biosensor, its linearity as well as reproducibility in lactate analysis was evaluated in this study. To determine the relationship between the lactate biosensor readings and the concentrations of lactate solution, a set of lactate standard solutions were prepared from 0 to 20 mmol/l. Rathee et al. (2016) mentioned that to obtain an extensive linear range that has a significant merit, the performance of sensor should be evaluated. Thus, it could be explained as the range of analyte concentration in which the biosensor could run and respond to the changes linearly according to the concentrations used (Table 1).

The results of the conducted experiments showed that the lactate biosensor readings of the standard solution containing lactate produced a linear standard curve (Fig. 1). The coefficient of determination (R^2) of the standard curve obtained from the lactate measured at the temperature of 30°C with various ranges of concentrations was ~0.986. This indicated that the determination of lactate concentration in the solution containing

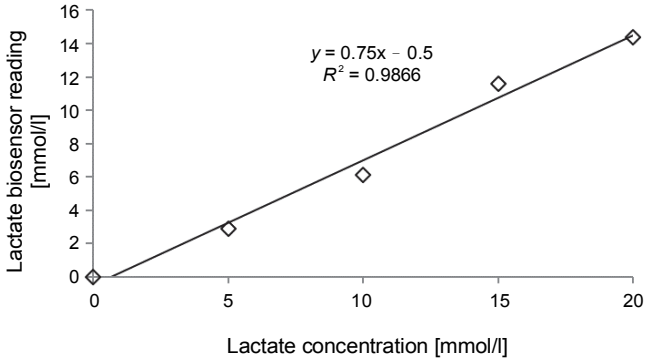

Fig. 1. The linearity of the lactate standard solution measured with the lactate biosensor at a temperature of 30°C

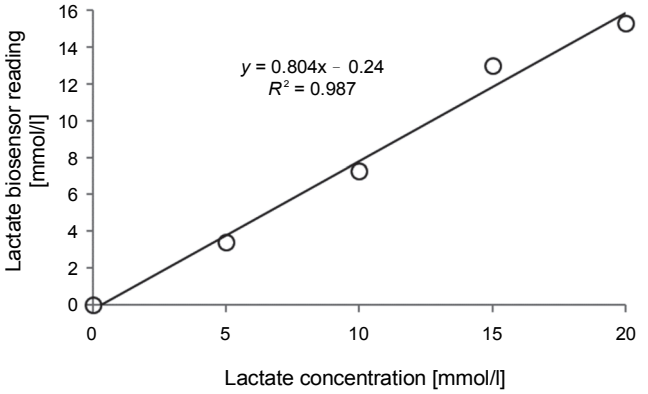

Fig. 2. The linearity of the lactate standard solution measured with the lactate biosensor at a temperature of 45°C

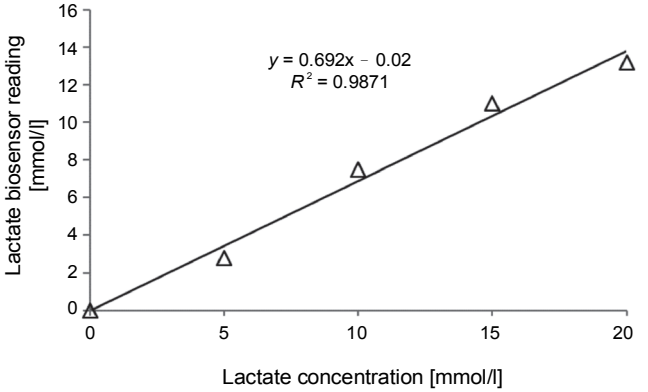

Fig. 3. The linearity of the lactate standard solution measured with the lactate biosensor at a temperature of 55°C

lactate was linear. Thus, the method of lactate determination using the portable biosensor could potentially be applied to measure lactate concentration in other biological samples such as anaerobic digestion and acidification samples.

The previous test shown in Figure 1 revealed that there wasalinearity of lactate concentrations used for preparation of a standard curve at the room temperature (30°C). To evaluate whether the linearity of lactate concentration determined by the portable biosensor would be applied at higher temperatures, other temperatures were set for the analysis. Results showed that linear standard curves were obtained from the analysis of lactate concentration at 45 and 55°C (Fig. 2 and Fig. 3). Both the standard curves obtained from the temperatures tested had a coefficient determination of 0.987, which indicated that the determination of lactate concentration using portable biosensor was linear. The results of these tests suggested that the determination of lactate concentration in the samples obtained from the process of anaerobic digestion operated at the mesophilic (30°C) and thermophilic (45–55°C) conditions could be conducted using a portable lactate biosensor. This is highly significant since the linearity of the lactate concentration measured by a lactate biosensor was reproducible in which the test was repeated with various concentrations of the standard solutions under different temperatures that are normally applied for anaerobic digestion and anaerobic acidification.

The above mentioned lactate measurement method was used for determining lactate concentration in relevant real-world applications in which the lactate to be analyzed was generated during the fermentation process. For such an analysis, samples were drawn from the digester of the anaerobic acidification process of taro starch (*Colocasia esculenta*) inoculated with *Lactobacillus plantarum* as a lactate-producing bacterium. Results showed that within 2 h of incubation, the lactate detected inthe sample of anaerobic acidification was of taro starch. The concentration of lactate increased significantly from 6.5 to 23 mmol/l. After 10 h of incubation, the concentration of lactate reached 25 mmol/l, which suggested that the lactate biosensor could effectively be used to monitor lactate concentration that was gradually formed during anaerobic acidification.

The previous test shown in Figure 4 has shown that the portable lactate biosensor could be used to measure lactate concentration from the fermentation of pure culture in which the broth was inoculated with the lactate-producing bacterium (*L. plantarum*). In such a case, the increase in lactate concentration was a result of the for-

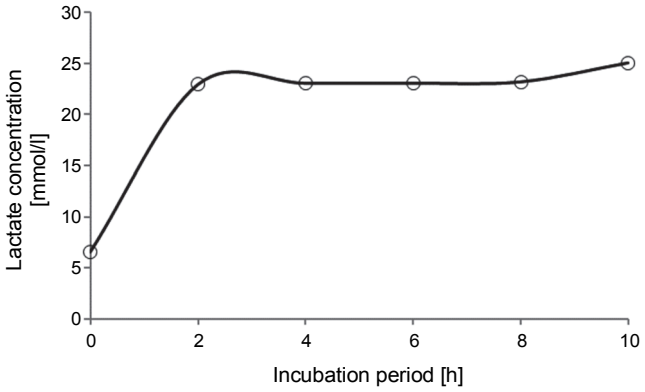

Fig. 4. Lactate concentration detected during the process of anaerobic acidification of taro starch

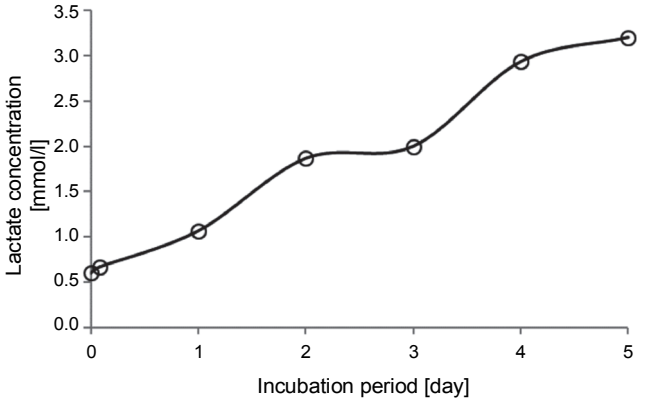

Fig. 5. Lactate production of anaerobic digestion of leachate

mation of endproduct produced from the acidification of taro starch inoculated with *L. plantarum*.

To ensure the lactate method could be used for analyzing the sample that contains a low concentration of lactate (0.5–0.7 mmol/l), the sample obtained from the process anaerobic digestion of leachate was used. This experiment was performed in a similar manner as that followed for the anaerobic digestion of landfill leachate where the formation of organic acids, particularly lactic acid, would be in the low concentration range. This happened because the landfill leachate contained high concentration of ammonia (Fang et al., 2012). Once the landfill leachate containing high concentration of ammonia is processed in anaerobic digestion, the hydroxideion (OH^-) would be generated and result in an increase of pH in the anaerobic culture (Strik et al., 2006). Thus, a high level of pH and ammonium concentration in the digesters would potentially inhibit the formation of organic acids such as lactic acid (Sauer et al., 2008; Yenigün and Demirel, 2013).

The acidification process was operated with a short HRT of 5 days, while the anaerobic digestion was operated at an HRT of 25 days. Thus, just because of incubation time, the amount of lactate formed in the anaerobic digester could be lower than the lactate produced from the digester during the acidification process (Darwin et al., 2018a; Darwin et al., 2018d). The experimental results in the process of anaerobic digestion showed that using the portable lactate biosensor, the concentration of lactate at 2 h of incubation was 0.67 mmol/l, and then the concentration of lactate reached 3.2 mmol/l after 5 days of incubation (Fig. 5). The results suggested that the portable lactate biosensor could effectively be used to regularly monitor the concentration of lactate produced during the continuous process of anaerobic digestion of organic wastes.

The results obtained support the use of portable lactate biosensor as an accurate and reliable instrument that can be easily used for the regular monitoring of lactate concentration in fermentation processes such as anaerobic digestion and acidification. The standard curves that have been produced for lactate and different ranges of temperature analysis have shown the reliability and accuracy of the determination of lactate using portable lactate biosensor. To obtain a consistent reading for the determination of lactate in the fermentation samples, the portable lactate biosensor that is used should not be interchangeable. Certain studies have revealed that the same portable lactate analyzer should be used throughout all the measurements in order to reach a consistent and accurate reading of the lactate concentration (Baldari and Guidetti, 2000; Buckley et al., 2003; Medbø et al., 2000).

The standard curves with the coefficient of determination of ~0.99 obtained in this study showed that the linearity and accuracy of lactate biosensor have been met. Baldari et al. (2009) also mentioned that the closer a measurement system coefficient reaches 1, the lesser error variance it reflects and the more the evaluated and developed method can be considered as reliable and accurate.

Conclusions

The determination of lactate concentration in fermentation samples such as the anaerobic acidification of taro starch and anaerobic digestion of leachate could successfully be reached using a portable lactate bio-

sensor. The linear standard curves from different levels of temperature testing showed a highly sensitive calibration between the lactate concentration and the portable lactate biosensor reading. The linear relationship was depicted using a high value of coefficient determination (R^2) of 0.99, which indicated that the evaluated system was accurate and reliable.

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