Pertanika J. Sci. & Technol. 25 (S): 39 - 48 (2017)

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Bioconversion of Leachate to Acetic and Butyric Acid by *Clostridium butyricum* NCIMB 7423 in Membrane Fermentor

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ABSTRACT

Landfill leachate imposes a huge problem to the environment and human beings. This work focused on bioconversion of leachate to acetic and butyric acids by *Clostridium butyricum* NCIMB 7423. A continuous stirred tank reactor (CSTR) was applied and connected to fabricate membrane module. The leachate was collected from Pulau Burung Landfill Site (PBLS), Nibong Tebal, Penang. Prior to fermentation, leachate was treated to remove volatile fatty acid and adjusted to meet the minimum requirement of nutrients for anaerobic fermentation. Synthetic medium fermentation acts as a benchmark to the leachate fermentation. The outcomes indicated that the yield of acetic acid and butyric acid in synthetic medium fermentation was 0.70 g/L and 0.71 g/L, respectively. Meanwhile, leachate fermentation showed that the yield of acetic and butyric acid was 0.93 g/L and 1.86 g/L, respectively. High production of acetic and butyric acid showed that leachate fermentation is a green alternative to produce a cleaner product.

Keywords: Anaerobic, bioreactor, *C. butyricum*, fermentation, waste to wealth

ARTICLE INFO

Article history:
Received: 19 February 2017
Accepted: 17 July 2017

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INTRODUCTION

Equally, one of the aggressively developing countries in Asia, Malaysia faces the problem of solid waste disposal. A survey by the Malaysian government in 2013 shows that each day, a person produces 800 g of solid waste. On the other hand, those who dwell in the urban area produce 1.25 kg of waste per person daily. The government has estimated

ISSN: 0128-7680

that in 2013, Malaysian would be producing 30,000 to 33,000 tonnes of solid wastes a day. These amounts were alarming because, in the current situation, these had already exceeded the government's projection of 30,000 tonnes of solid waste daily for 2020 (Ismail, 2014). Waste disposal via landfill is the main method of disposal of municipal solid waste (MSW) in Malaysia (Abas & Wee, 2014).

Leachate is the main issue that links to the open landfill disposal. It is a percolated liquid from sanitary landfill (S. Q. Aziz, H. A. Aziz, Yusoff, Bashir, & Umar, 2010). It is reported that the leachate contains various contaminants such as organic materials, ammonia, heavy metals, phenol, nitrogen, and phosphorus. Various researchers constantly report that leachate contains these contaminant and imposes a great impact on an environment (Christensen et al., 2001; Kjeldsen et al., 2002; Ozturk, Altinbas, Koyuncu, Arikan, & Gomec-Yangin, 2003; Aziz, Adlan, Zahari, & Alias, 2004; Salem, Hamouri, Djemaa, & Allia, 2008; S. Q. Aziz, Yusoff, H. A. Aziz, Umar, & Bashir, 2009, Umar, Aziz, & Yusoff, 2010; Wang, Huang, Feng, Xie, & Liu, 2010; Kamaruddin, Yusoff, Aziz, & Basri, 2013; Raghab, Abd El Meguid, & Hegazi, 2013; S. Q. Aziz, H. A. Aziz, Bashir, & Mojiri, 2015). The issues on leachate treatment have existed for quite some time, but a universal solution has yet to be found (Wiszniowski, Robert, Surmacz-Gorska, Miksch, & Weber, 2006). Leachate treatment can be divided into aerobic treatment and anaerobic treatment. Aerobic treatment is a treatment of leachate in the presence of oxygen. In this process, the microorganisms will generate energy through enzyme-mediated electron transport using molecular oxygen as anelectron acceptor (Environmental Protection Agency, 2000). There are many types of aeration treatments, such as activated sludge reactor, rotating biological contactor (RBC) and aerated lagoons.

Anaerobic process is slightly different from the aerobic process where no oxygen is required. Electron acceptors in this process are inorganic compounds such as nitrate, sulphate and carbon dioxide (Environmental Protection Agency, 2000). The Environmental Protection Agency (2000) states that anaerobic process provides several benefits such as low sludge production, low energy demand (no oxygen required) and recovery of methane may provide energy. Anaerobic process is an effective process, but the remaining of BOD and COD are still high and at the end, leachate still needs to be treated with means of aerobic process to meet the effluent standard (Stegmann, Heyer, & Cossu, 2005).

The cost to treat leachate is a great barrier in the current situation. Leachate is not a profitable commodity, but the governments around the world need to spend a lot of annual expenditure to treat leachate. To date, lack of report has been published to turn the table around by making a profit from leachate, whilst at the same time attempting to treat this contaminant. The objective of this study is to implement membrane bioreactor in leachate treatment and at the same time produce a valuable product. This novelty treatment could solve a problem in leachate treatment and reduce the cost burden by the government.

Clostridium is a strictly anaerobic and gram-positive bacteria. Their morphology is cylindrical-shape (Szymanowska-Powalowska, Orczyk, & Leja, 2014). *Clostridium* is a spore-forming bacteria and strains can be isolated from soil, wastewater, animal digestive systems, and contaminated dairy products (Zigov & Sturdik, 2000). *Clostridium butyricum* can utilize a variety of carbohydrate from mono to disaccharides and complex polysaccharides which

include glucose, lactose from whey, sucrose from molasses, starch, potato wastes, wheat flour, cellulose or dextrose (Bahl & Dürre, 2001; Kong, He, Chen, & Ruan, 2006; Tracy, Jones, Fast, Indurthi, & Papoutsakis, 2012; Azan, Lovitt, Nur, & Azwa, 2013).

The novelty of this research lies in the fact, to date no research has been documented on the treatment of leachate by using *C. butyricum* and at the same time producing a valuable product. The result of this research enables the understanding in leachate treatment as well as opening more possibilities for green and sustainable practices.

MATERIALS AND METHOD

C. butyricum National Collection of Industrial, Food and Marine Bacteria (NCIMB) 7423 which was used in this study was purchased from NCIMB Ltd. (Aberdeen, Scotland, United Kingdom). The medium formulation for *C. butyricum* NCIMB 7423 culture consisted of 10 g/L of glucose, 10 g/L of yeast extract, 5 g/L of di-potassium hydrogen phosphate, 10 g/L of ammonium phosphate, and 1 ml/L of 0.05% resazurin (Azan, Lovitt, Nur, & Azwa, 2013). All chemicals used were of analytical grade. The medium was prepared and adjusted to pH 6.5 (pH 2700, Eutech Instruments, USA) before being transferred into 50 ml or 100 ml serum bottles. The serum bottle was prepared using Hungate Method (Wolfe, 2011).

Leachate was collected from Pulau Burung Landfill Site (PBLS), situated within Byram Forest Reserve at 5º 24' N Latitude, 100º 24' E Longitude in Nibong Tebal, Penang (Aghamohammadi et al., 2007). A total of 125 L leachate was collected from the site. Approximately, 200 ml of raw leachate was taken for analysis to check for Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total volatile acid, and total carbohydrate. Leachate was pre-treated in a perspex column (diameter: 142 mm, height: 1200 mm) with 5 kg of limestone to remove volatile fatty acid as *C. butyricum* growth is inhibited by volatile fatty acid. Then, leachate was adjusted by adding glucose (4.18 g/L) to meet the minimal requirement of C:N for anaerobic fermentation (250:5) (Ammary, 2004) prior to fermentation.

A 2.5 L bioreactor (Minifors, Infors HT, Switzerland) with working volume up to 1.7 L was used in this study. The reactor was connected to the ceramic membrane assembly. The ceramic membrane used in this study was supplied by National University of Singapore (NUS). The flat sheet membrane with a size of 30.34 cm × 11.00 cm, had a pore size of 0.22 µm and was encapsulated in a fabricated stainless steel container. The inoculum was 10 L and prepared in a 12 L vessel using the synthetic medium for both synthetic and leachate medium fermentation. The inoculum was filtered using a membrane and the concentrated cell was used at the beginning of fermentation by adding fresh medium. For leachate fermentation, the medium used was only treated leachate with added glucose.

Sampling was carried out on an hourly basis. Sampling for both fermentations was the same. A total of 4 ml culture was removed from the sampling line and discarded, then 4 ml of culture was taken from the vessel. The sample was stored in the freezer at -20°C prior to analysis. Acetic and butyric acid content were measured using gas chromatography (GC). The method was carried out according to Standard Methods for the Examination of Water and Wastewater (American Public Health Association et al., 2005).

RESULTS AND DISCUSSION

The profile for leachate taken from Pulau Burung Landfill Site (PBLS) is tabulated in Table 1.

Table 1
Profile for raw and pretreated leachate of Pulau Burung Landfill Site (PBLS)

Analysis	Unit	Raw Leachate	Pretreated Leachate*
BOD	mg/L	N.D.	477.25
COD	mg/L	N.D.	794.00
Total volatile acid	mg/L	70.00	62.00
Total carbohydrate	g/L	0.16	0.16

N.D.: Not determined
*Treated by exposure to limestone for 30 minutes

From Table 1, the BOD of PBLS leachate after limestone pretreatment was 477.25 mg/L, exceeded the discharge limit. The leachate must undergo intensive treatment in order to surpass the stated discharge limit. The range of BOD measured by Aziz et al., (2009) for PBLS leachate was in the range of 67-93 mg/L, while a range of 252-730 mg/L was recorded earlier by Aghamohammadi et al. (2007).

The Chemical Oxygen Demand (COD) value for leachate after the pretreatment was 794 mg/L. The COD for PBLS leachate was reported to be in the range of 600-1300 mg/L by Aziz et al., (2010); the discharge limit is 100 mg/L (Ministry of Science and Environment, 1979). Again, the values reaffirmed that this leachate needed treatment before it could be discharged into the river. The BOD/COD ratio of the current leachate was 0.6. The values were in the range of 0.043-0.67 as reported by various researchers (Aziz et al., 2010). It was also stated that low BOD/COD value indicates that the leachate is stable and hard to treat biologically.

The volatile fatty acid (VFA) content in the current leachate was 70 mg/L. After subjected to limestone pretreatment, the VFA content reduced to 62 mg/L which accounted for 11.4% of reduction. *C. butyricum* NCIMB 7423 growth was inhibited by the VFA, including acetic and butyric acid, hence the VFA content needed to be lowered prior to fermentation. The figure may not be much but it was capable of sustaining the growth of *C. butyricum* NCIMB 7423 while preserving all nutrients and organic material that was crucial for bacterial growth. The details of the adsorption study were not considered as part of the current research since the main focus was only to obtain leachate that could sustain the growth of *C. butyricum* NCIMB 7423.

The introduction of membrane separation to the fermentation vessel can theoretically solve the problem during downstream processing which is costly and not energy efficient. To date, anaerobic membrane bioreactor is still in development and utilized particularly to treat low strength wastewater (Gao et al., 2010). The synthetic medium membrane fermentation was used as a benchmark to compare against leachate membrane fermentation. The membrane fermentation of *C. butyricum* NCIMB 7423 was achieved by connecting the membrane module to the stirred tank reactor. This set-up was reported by a researcher, but with different substrate (glycerol) (Szymanowska-Powałowska & Leja, 2014).

Figure 1(a) shows the growth curve of *C. butyricum* NCIMB 7423 on synthetic medium. From the figure, it can be observed that the fermentation duration was long (23 h) before *C. butyricum* NCIMB 7423 biomass started to decline. The membrane fermentation did not experience lag phase, and biomass was accumulated from the instantaneous separation of cells from the acetic and butyric acid produced, preventing inhibition by the products formed. The maximum biomass achieved by current fermentation was 0.29 g/L with 0.05 h^{-1} of specific growth rate. The biomass could be further increased, but the membrane had started to foul at biomass concentration of 0.29 g/L, resulting in membrane blockage.

Meanwhile, Figure 1(b) shows the membrane fermentation of altered-leachate. The fermentation condition was similar to that of the synthetic medium. The fermentation period was incredibly short compared to that of the synthetic medium fermentation. A specific growth rate of 0.33 h^{-1} was obtained, and the highest biomass achieved was 0.29 g/L, after which the membrane started to foul. The results suggest that *C. butyricum* NCIMB 7423 grew far more favorably in altered-leachate compared to synthetic medium. The short fermentation time may be due to high nutrient content in leachate, carbohydrate supply from the added glucose, and continuous withdrawal of acetic and butyric acids from the fermentation vessel. The possibilities of membrane altered-leachate fermentation were broad because high biomass can be attained in a short period of time.

Foulant layer which consists of extracellular polymeric substances (EPS) and microbial cells contributes to membrane fouling. Gao et al. (2010) claim that the accumulation of EPS reduces the effective pore size in the membrane and increases proportional resistance to permeate flux. In addition, they emphasize that the membrane fouling results in reducing membrane lifespan and membrane permeate flux. Consequently, the accumulation of acetic and butyric acid resulted in inhibiting the growth of *C. butyricum* NCIMB 7423.

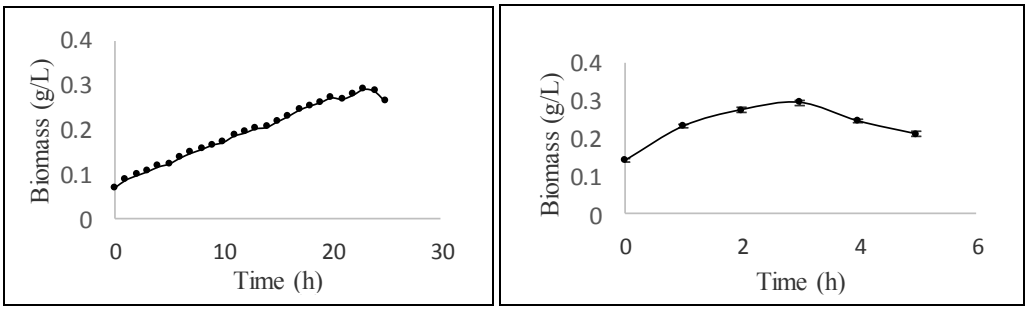
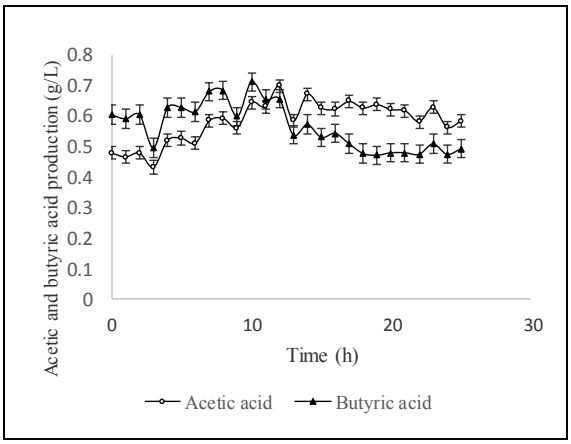
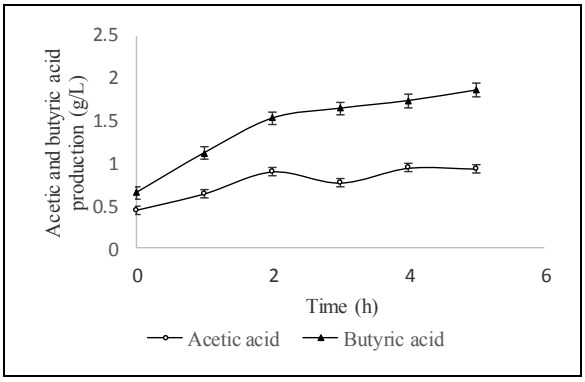


Figure 1. The growth curve of *C. butyricum* NCIMB 7423 in: (a) synthetic medium; and (b) altered-leachate. Data point are mean ± standard deviation (n=3)

For the acid production, Figure 2 shows the trends of acetic and butyric acid production throughout the 25 h fermentation using synthetic medium and altered-leachate medium.The concentrations of acetic and butyric acid were constant throughout fermentation. It should be noted that the fermentation sampling point was from the main vessel (level of acetic and butyric were maintained) and not from the permeate collection bottle (accumulation of acetic and butyric acid). The clear fluid could be observed at the permeate collection bottle which indicated that only product and liquid passed through.



(a)



(b)

Figure 2. Acetic and butyric acid production by *C. butyricum* NCIMB 7423 in: (a) synthetic medium; (b) and altered-leachate medium

Using synthetic medium, for the first 11 h, the production of butyric acid exceeded the production of acetic acid (Figure 2(a)). This condition was favorable because of the high commercial value of butyric acid compared to acetic acid. From 12 h onwards, the production of butyric acid seemed to have reduced while production of acetic acid increased. According to Saint-Amans, Girbal, Andrade, Ahrenss and Soucaille (2001), the product of *C. butyricumis* not regulated at the genetic level, but at the enzyme level which is directly affected by the substrate concentration thus explaining the sudden changes in acid production. The highest production of acetic and butyric acid recorded in the fermentation vessel was 0.70 g/L and 0.71 g/L, respectively. It verifies that both acids were removed continuously from the fermentation vessel.

The highest productions of acetic and butyric acid by *C. butyricum* NCIMB 7423 in altered-leachate membrane fermentation were 0.93 g/L and 1.86 g/L, respectively (Figure 2(b)). The current production was higher compared to that produced during synthetic medium fermentation. Throughout the fermentation (from 0 h to 5 h), butyric acid production was higher than acetic acid. Figure 2(b) shows that the concentration of acetic and butyric acid

increased steadily, suggesting that the membrane was slowly starting to foul because of the high biomass of *C. butyricum* NCIMB 7423 resulting in accumulation of acetic and butyric acid in the fermentation vessel. The current findings are favorable as the production of both acids exceeded that produced in the synthetic medium at a faster rate.

CONCLUSION

The current study successfully demonstrates the production of acetic and butyric acid in leachate using membrane reactor. The results show acetic (0.93 g/L) and butyric acid (1.86 g/L) were highly produced in altered-leachate compared to acetic (0.70 g/L) and butyric acid (0.71 g/L) in synthetic medium. These findings open a revolutionary way to biologically treat landfill leachate and at the same time, taking advantage of the treatment.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Government of Malaysia (203.PTEKIND.6711373) and UniversitiSains Malaysia (1001.PTEKIND.811262) for the research grants that financed this study. Besides that, great thanks to School of Industrial Technology, School of Civil Engineering and Solid Waste Management Cluster for the use of various facilities in their school.

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