

ANAEROBIC DIGESTION OF PINEAPPLE WASTE USING A MICROBIAL
CONSORTIUM

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ABSTRACT

Anaerobic digestion of the agro-industrial pineapple waste has great potential to generate biogas, an alternative energy resource to compensate the deprivation of global fossil fuel over the years. The objectives of this study were to identify and characterize a microbial consortium (MC) from Palm Mill Oil Effluent (POME) as well as preliminarily evaluate its potential as a biogas producer through pineapple waste anaerobic digestion. The MC was subjected to gram and methylene blue staining techniques, Dinitrosalicylic Acid (DNS) method, 16S rRNA and Internal Transcribed Spacer (ITS) sequencing, and phylogenetic tree analysis. Next, gas production from the anaerobic digestion was analyzed by water displacement and Residual Gas Analyzer (RGA). Based on the phenotypic and genotypic identification, the MC was comprised of four bacteria, including *Bacillus cereus*, *Acinetobacter radioresistens*, *Klebsiella* sp., and *Stenotrophomonas maltophilia*, as well as a fungus *Aspergillus fumigatus*. The *A.radioresistens* strain in this study was found to be a novel facultative anaerobe strain with the potential of producing biogas. Higher overall cellulase activity of the MC was found in contrast to the amylase activity during the pineapple waste digestion process. The water displacement method showed potential gas production of 287 ± 135 cm³, comprising of 80.15% nitrogen, 16.94% oxygen, 2.63% water vapours, 0.19% carbon dioxide, 0.08% hydrogen sulphide and 0.01% hydrogen. In conclusion, this study had successfully identified and characterized the MC as well as determined its potential in biogas production, although the gas analysis showed an absence of the desired biogas, methane which could be acquired through further optimization of the biodigestion process.

ABSTRAK

Pencernaan anaerobik sisa nanas yang diperoleh dari industri pertanian dipercayai berpotensi menghasilkan biogas, satu sumber tenaga alternatif bagi mengimbangi kemerosotan bahan api fosil global sepanjang tahun. Objektif-objektif kajian ini adalah bagi mengenalpasti dan mencirikan konsortium mikrob (MC) dari efluen minyak kilang kelapa sawit (POME) serta membuat penilaian awal tentang potensinya sebagai penghasil biogas dari pencernaan anaerobik sisa nanas. MC tersebut disubjekkan kepada teknik-teknik pewarnaan gram dan methylene biru, metod Asid Dinitrosalisilik (DNS), penjujukan 16s rRNA dan *Internal Transcribed Spacer* (ITS) serta analisis pohon filogenetik. Kemudian, produksi gas dari pencernaan anaerobik itu dianalisis oleh metod sesaran air dan penganalisa sisa gas (RGA). Berdasarkan pengenalan fenotip dan genotip, MC itu terdiri daripada empat bakteria, termasuk *Bacillus cereus*, *Acinetobacter radioresistens*, *Klebsiella* sp., dan *Stenotrophomonas maltophilia*, serta *Aspergillus fumigatus* fungus. Strain *A.radioresistens* di dalam kajian ini ditemui sebagai strain anaerob fakultatif novel yang berpotensi menghasilkan biogas. Aktiviti selulase bagi MC secara keseluruhan adalah lebih tinggi berbanding aktiviti amilase semasa proses pencernaan sisa nanas. Metod sesaran air menunjukkan potensi penghasilan gas sebanyak $287 \pm 135 \text{ cm}^3$ yang terdiri daripada 80.15% nitrogen, 16.94% oksigen, 2.63% wap air, 0.19% karbon dioksida, 0.08% hidrogen sulfida dan 0.01% hidrogen. Kesimpulannya, kajian ini telah berjaya mengenalpasti dan mencirikan MC tersebut serta menentukan potensinya dalam menghasilkan biogas, walaupun analisis gas menunjukkan ketiadaan biogas yang dikehendaki, metana yang mungkin mampu diperoleh melalui pengoptimuman proses pencernaan tersebut yang lebih lanjut.

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LIST OF ABBREVIATIONS

MC	-	Microbial Consortium
POME	-	Palm Oil Mill Effluent
DNS	-	Dinitrosalicylic Acid
PCR	-	Polymerase Chain Reaction
NJ	-	Neighbour Joining
RGA	-	Residual Gas Analyzer
ITS	-	Internal Transcribed Spacer
CO ₂	-	Carbon Dioxide
GC	-	Gas Chromatography
ETP	-	Economic Transformation Program
COD	-	Chemical Oxygen Demand
C/N	-	Carbon/Nitrogen
BOD	-	Biological Oxygen Demand
CH ₄	-	Methane
H ₂	-	Hydrogen
C/N	-	Carbon/Nitrogen
MJ	-	Mega Joules
GWh	-	Gigawatt Hours

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Anaerobic digestion is a biochemical process which drives the degradation of a wide variety of biomass through the microbial action (Adekunle & Okolie, 2015; Divya, Gopinath, & Christy, 2015). Four biochemical reaction stages are involved in the anaerobic digestion process, including hydrolysis, acidogenesis, acetogenesis and methanogenesis process (Divya *et al.*, 2015; Wang, 2014). Not only known to be a critical degrading component in solid waste and waste water treatment, the anaerobic digestion system is currently played a crucial role in generating an environmental-friendly renewable energy resource, biogas, an alternative to the finite fossil fuel (Divya *et al.*, 2015). Biogas is defined as gas produced as a result from organic matters degradation due to microorganisms' actions in which it includes methane, hydrogen, nitrogen, hydrogen sulphide and carbon dioxide (Minde, Magdum, & Kalyanraman, 2013; Divya *et al.*, 2015). Apart from the benefit of less greenhouse gaseous emission, the production of biogas can also lead to a decrease in energy production cost as this alternative energy resource can be harvested from biomass or industrial and agricultural wastes (Divya *et al.*, 2015)..

In general, the microbial communities which participate in the anaerobic digestion process to produce biogas are derived from animal manure such as cow dung and poultry manure (Eze & Agbo, 2010; Ukpabi *et al.*, 2017). Recent study has found in which the utilization of specific known novel microbial consortium is more effective to generate biogas in contrast to the unidentified microbial consortium from animal manure (Kumar *et al.*, 2014; Mirdamadian *et al.*, 2011). As the identity of each species in the microbial community is known, this made easier for the anaerobic digestions' regulation within an anaerobic digester as specific microbes can be dedicated to specific phase of anaerobic digestion process. Thus, the identification and creation of a novel anaerobic microbial consortium is essential to obtain satisfactory biogas production.

Other than animal manure, Palm Oil Mill Effluent (POME), a byproduct from the oil palm industry, has also been known to be a host of various anaerobic microbes which participate in the biogas generation via anaerobic digestion process (Meesap *et al.*, 2012; Neoh *et al.*, 2013; Wakil, Adelabu, & Fasiku, 2013; Wong, Teng, & Ong, 2014). A potential effective anaerobic microbial consortium (MC) can be developed from the POME-derived microbial inoculum in order to gain better quality and quantity of biogas production (Wakil, Adelabu, & Fasiku, 2013; Wong, Teng, & Ong, 2014). Therefore, the isolation of microbial colonies from POME is seen as a great platform to discover new MC which can be integrated into the anaerobic digestion process to effectively produce biogas.

Besides microbial consortium introduction, the utilization of biomass as feedstock in the anaerobic system is also a driving factor for biogas production (Divya *et al.*, 2015). Biomass can be comprised of organic materials such as animal manure, municipal solid waste and agro-industrial waste (Divya *et al.*, 2015). As the Malaysias' pineapple plantation intensifies in parallel with the 11th Malaysia Plan, it is forecasted that there will be an increase in pineapple waste generation (Ruekeith, 2015). Pineapple waste can be comprised of leaves, stalks, and crown which are discarded during the canned fruit making process and it accounts about 40% to 80% of the pineapple (Mohd Zain *et al.*, 2012). Therefore, the resulted increasing amount

of accumulated pineapple waste is seen as a potential feedstock for anaerobic digestion system to generate biogas. In addition to renewable energy generation, the anaerobic digestion on pineapple wastes also helps in mitigating the negative environmental repercussion of the particular agricultural wastes' inefficient management.

Multiple scientific studies have shown evidence of the pineapple wastes' capacity in serving as feedstock in biogas production (Chulalaksananukul, Sinbuathong, & Chulalaksananukul, 2012; Ugwoke & Ekpe, 2011). In this present study, four anaerobic bacteria and a fungus isolated from POME were determined in terms of their phenotype and genotype criteria. The microbes were then mixed together in order to create an effective MC for biogas generation. Next, the efficiency and capacity of this microbial inoculum to produce biogas using pineapple waste was examined and the gas released from the particular anaerobic digestion process was analyzed.

1.2 Problem Statement

In the execution of anaerobic digestion, anaerobic microbes are known to own the potential to facilitate the process via utilization of biomass or agricultural waste (Wang, 2014). Although a number of scientific studies centralized in the anaerobic degradation for biogas production have been performed over the years, the search for efficient anaerobic microbes must continue due to the huge diversity of the microbial world. The microbial identification study and databases help to widen the knowledge spectrum regarding the potential biogas producer-anaerobic microbes.

Besides the use of an efficient MC, the carbon sources or organic materials are the key factors affecting the anaerobic digestion process (Divya *et al.*, 2015). Pineapple waste has a high potential to serve as a substrate for anaerobic digestion to generate biogas (Reungsang & Sreela-or, 2013). As the production and plantation of pineapple is predicted to increase in parallel with the 11th Malaysia Plan, the need to find a measure to address the issue of increasing pineapple waste generation is extremely crucial (Lun, Wai, & Ling, 2014; Ruekeith, 2015). This is because the increasing accumulation of pineapple fruit wastes will potentially cause unfavorable environmental liabilities. Hence, the utilization of pineapple waste as an optional feedstock in anaerobic digestion for biogas production is seen as an excellent measure to mitigate the environmental concern, along with creating a new clean renewable energy resource (Chulalaksananukul *et al.*, 2012).

Next, the generation of biogas from anaerobic digestion system is an advantage to the world population due to its capacity to mitigate global high rate of fossil fuel consumption to support increasing demand and population (Divya *et al.*, 2015). Besides that, the increment of greenhouse gas emission to the atmosphere, particularly in Malaysia, can also be addressed by the production of biogas which is environmentally friendly (Abdeshahian *et al.*, 2016). Thus, through the use of biogas, despite fossil fuel such as crude oil and coal, a clean energy can be used to generate electricity and heat to the global population (He, 2010; Onojo *et al.*, 2013).

1.3 Objectives

The objectives of this study were:

1. To identify the microbial consortium used for anaerobic digestion of pineapple waste.
2. To evaluate the performance of biogas production by microbial consortium.

1.4 Scope of Study

The following scopes are performed to acquire the objectives:

1. Isolation of POME-derived microbial species.
2. Phenotype profiling of microbial consortium: Identification and characterization of microbes according to gram staining, methylene blue staining as well as Dinitrosalicylic Acid (DNS) assays for cellulase and amylase activities.
3. Genotype profiling of microbial consortium: Identification of the microbial consortium through 16s rRNA and Internal Transcribed Spacer (ITS) sequencing techniques prior to phylogenetic analysis using the Neighbour-Joining (NJ) method.

4. Investigation of the microbial consortium's capacity to generate biogas through pineapple waste anaerobic digestion process using water displacement method within 4 days of retention time.
5. Identification and analysis of gas composition using Residual Gas Analyzer (RGA).

1.5 Significance of Study

The current established database on anaerobic microbes which are involved in biogas production needs to be updated regularly. Hence, more research studies on new potential microbial inoculum should be performed. Next, POME has also been seen as an alternative source, other than animal manures to acquire efficient microbial consortium to be integrated into the anaerobic digestion process for biogas production. Furthermore, both the evolutionary study of anaerobic microbial consortium and its hydrolytic enzymes activity can help to gain in depth understanding of specific function of each microbial species in the consortium. As the pineapple cultivation for exportation expands annually, this would definitely result in massive amount of waste generation which can lead to a serious environmental problem. Therefore, in this study, the utilization of pineapple waste as a substrate in anaerobic digestion to potentially generate the desired biogas could account for economic and environmental benefits to global population.

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