Preliminary study on oil extraction and biogas production from *Cymbopogon nardus* (Serai Wangi)

E Suali1, N S I Juasin, F A A Hamit, S M Anisuzzaman and M A Asidin
1Faculty of Engineering, Jalan UMS, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia
E-mail: 1emma.suali@gmail.com

Abstract. An essential oil from *Cymbopogon nardus* has many benefits, specifically in pharmaceutical, cosmetics and perfumery fields. However, extraction of *Cymbopogon nardus* produces abundant waste which usually left to decay naturally. The present study evaluated biogas potential from *Cymbopogon nardus* waste through anaerobic digestion method with rumen fluid as inoculum. The presence of methane which is the main gas that makes up the composition of biogas is verified by using GC-FID and Biogas Analyser. The waste was collected from the oil extraction of various part of *Cymbopogon nardus* plant. The experimental study on the extraction is important to identify the oil yield from various part of *Cymbopogon nardus* plant. The extraction was done through steam distillation with temperature varied from 40 °C to 100 °C. The comparison of oil yield from various part of the plant was done with an aid of GC-MS analysis. It was found that the leaf of *Cymbopogon nardus* contains most essential oil, which gives the highest oil yield about 1.5 % at 100 °C followed by the stem (0.3 %). The oil yield found in the plant flower was less than 0.3 % and none found in the root. Extraction at 100 °C resulted in higher oil yield compared to 40 °C, 60 °C and 80 °C. The citronellal content was the highest compound presents in the oil followed by geraniol. The average generation of biogas on the other hand were range from 0.4 ml/day to 12.5 ml/day. The F/I ratio which produced the highest volume of biogas is 10 (w/w).

Keywords: Citronella; *Cymbopogon nardus*; Essential oil; Biogas; Extraction; Steam distillation

1. Introduction
*Cymbopogon nardus* or known as *Serai Wangi* in local dialect of Malaysia and Indonesia is non-edible grass-like plant but has many benefits and uses [1]. In Malaysia, *Cymbopogon nardus* was mostly used as natural insect repellent, natural insecticides and household products such as detergent, soap and so on [2]. In recent studies, essential oil of *Cymbopogon nardus* were found to contain antibiotics for aquaculture [3-4]. Since antibiotic was banned for aquaculture, the oil from *Cymbopogon nardus* could be used as an alternative to commercial antibiotic.

Citronella oil contains many elements such as citronellal (16.9 %), citronellol (10.4 %), elemol (9.1 %) and geraniol (3 %) [5], whereas citronellal gives the distinctive lemon scents while geraniol give the rose-like odor and citrus smell. Citronellal which is the main content of oil yield is widely used for fragrance and perfumeries [1]. It was also reported useful for medicinal purposes. The benefits of the *Cymbopogon nardus* can be optimized through extraction of its essential oil. Extraction of *Cymbopogon nardus* is the process to segregate substances from its sources in order to obtain the
essential elements. The extraction process can be performed either in small or large scale under controlled temperature and pressure [6].

There are many methods to extract essential oil. This include supercritical fluid extraction (up to 2.8%) [9] steam or hydro distillation (less than 1%) [7,8] and solvent-less extraction (up to 1.3%) [10]. The oil yield from the plant is affected by the extraction method and the parameters used such as temperature, pressure, duration for extraction, and types of solvent. Hence, in order to achieve a high yield of oil, suitable method must be chosen and the parameter must be set in optimum conditions.

The empty husk of the extracted *Cymbopogon nardus* usually left to decay naturally as well as the others part which not included in extraction process. It was also used as natural fertilizer for its own cultivation. This produces abundant waste to obtain a minute amount of essential oil from its leave. For every metric ton of citronella leaves distilled, only 8 kg of essential oil is produced leaving 992 kg of biomass residue discarded as a waste [11]. Thus, it is useful if ones can find a way to reuse the waste. In this study, a waste from *Cymbopogon nardus* extraction was tested for it potential as biogas producer. Biogas is a form of renewable energy that can be harvested from organic substrates which has acquired a lot of attention in the research field for decades [12]. Biogas generation through anaerobic digestion reduces emission of greenhouse gases compared to the burning of fossil fuels [13]. The process of anaerobic digestion is one of the oldest method to convert organic substance into biogas. Hence, anaerobic digestion aids a process of waste treatment and stabilization [14]. Biogas produced from organic substrate depends on the content, chemical composition and degree of biodegradability of the substrate used [15]. The performance of anaerobic digestion depends on few conditions namely hydraulic retention time, temperature, carbon to nitrogen (C/N) ratio, organic loading rate, partial pressure, pH, nature of the organic substrate, inoculum ratio and presence of oxygen in anaerobic environment [16].

The potential of various biomass as inoculum has been studied and considered for biogas production [17]. Different inoculum behaves differently in terms of the ability of converting organic substrate into biogas. It was reported that optimal feed to inoculum (F/I) ratio is within 1 to 10 [18]. Thus, in this study, a residue of *Cymbopogon nardus* extraction which include leaves, stem, root and flowers are tested for methane production. The residue include leaves, stem, root and flower of *Cymbopogon nardus*. It was claimed by many that no oil in the flower of *Cymbopogon nardus*. However, no scientific report to support the claim. Thus, it is interesting to conduct an oil extraction on the *Cymbopogon nardus* flower, stem and root besides leaves. In this study, oil yield of various parts (leave, stem, root and flower) of *Cymbopogon nardus* was compared.

2. Materials and Methods

2.1 Preparation of *Cymbopogon nardus*

*Cymbopogon nardus* was taken from Ladang Serai Tuaran at Kampong Selupoh, Tuaran, Sabah. The roots, stems, flowers and leaves of fresh *Cymbopogon nardus* plant was cut into a smaller pieces and weighed before drying 48 hr at 60 ℃ in the oven. The sample is finely grounded and stored in a sealed plastic bag and left in a cabinet at a room temperature without the presence of sunlight to prevent any possible oxidation reaction. The extracted husk was prepared according to the method reported in Alfa et al. [19].

2.2 Preparation of rumen fluid

Rumen fluid was used as inoculum. It was obtained from the Sabah Meat Technology Centre, Kinarut, Sabah. The rumen was used to provide sufficient bacteria for the digestion process. The method to prepare the inoculum was adapted from Ahmed and Kazda [20]. Inoculum that was freshly taken shortly after the process of slaughtering was sieved in order to remove the large particles and kept at 37 ℃ under anaerobic conditions for several days for degassing purpose.

2.3 Extraction of *Cymbopogon nardus*

The extraction of *Cymbopogon nardus* was conducted to determine the oil yield and oil content.
The extraction was performed by using steam distillation method as mentioned in [7-8]. The experiment was conducted in a Clevenger type distillation apparatus using water and hexane as solvents. The solvent-solvent extraction was performed using separatory funnel and the removal of hexane from the mixture of oil-hexane was performed using rotary evaporator. About 1.5 L of distilled water was added into the 2 L pear-shaped glass contained 150 g of sample. The straight glass condenser was connected to the pear-shaped glass. The temperature was set from 40 °C to 100 °C for the extraction of stems, flower, leaves and roots.

2.4 Anaerobic digestion
Potential of *Cymbopogon nardus* waste as biogas feedstock was studied and the experiment was performed in a batch operation. One hundred grams of waste feedstock was fed into respective biodigester and mixed with 100 ml water on a 1:1 w/v basis. The inoculum was fed into the digester according to the respective feedstock to inoculum ratio (F/I). The sample was kept in the bottle which acts as a mini bioreactor and left at a room temperature. The bioreactor A1 and A2, B1 and B2, C1 and C2, D1 and D2, and E1 and E2 were prepared with F/I ratio of 2, 2.5, 3.3, 5 and 10, respectively. The performance of the digestion was measured by the cumulative production of biogas. The study was conducted in duplicate in order to minimize the error and to avoid the risk of not getting results due to leakage considering samples in gaseous form is hard to contain and control. The solid retention time (SRT) of the anaerobic digestion was 40 days followed the method conducted by Owamah et al. [21] and poultry droppings. Measurement was taken at an interval of 2 days for each digester at 4 pm for a duration of 40 days.

2.5 Analytical procedures
2.5.1 Gas Chromatography Flame Ionization Detector (GC-FID)
The gas type analyses were performed using GC-FID, Agilent Technologies GC 7890B with HP 5MS column. Initial temperature of 105 °C and detection temperature of 150 °C. Gas samples were tested using manual injection by using Agilent manual syringe with the volume of 10 ml.

2.5.2 Biogas analyzer
The composition of the biogas was confirm using Geotech’s Biogas 5000 Portable Biogas Analyzer. The device is reliable in measuring gas levels of methane, carbon dioxide, oxygen and hydrogen sulfide.

2.5.3 Gas Chromatography-Mass Spectrometry (GC-MS)
The oil content analysis was performed to detect the presence of valuable component which are citronellal, citronellol and geraniol. Citronella oil obtained from the experiment was analyzed using Agilent 6890/5973 GC-MS, Model G1530A. The analysis was performed by analyzing the peaks obtained from the GC-MS which represent the composition of citronella oil. The composition include citronellal, citronellol and geraniol. The presence of the valuable components are detected by comparing its retention time with the commercial standards solution with a brand of Eau de Senteur. The GC-MS composed of the capillary column which has 0.25 internal diameter, 30 m long, 0.25 μm film thickness. The gas carrier is helium with the flow rate of 1.0 ml/min and the temperature is set up varied from 50 °C to 300 °C at 8 °C/min with the injector temperature of 280 °C. The oven temperature was at 80°C.

3. Results and discussions
3.1 Essential oil yield of *Cymbopogon nardus*
The yield of *Cymbopogon nardus* oil ranged from 0 % to 1.5 % whereas oil yield found in leaves was the highest about 1.5 % followed by the stem approximately 0.28 %. It was found that there are oil yield obtained from the flower about 0.24 % and none from the root. The oil in aromatic plant is stored
in a special glands called glandular gland. These special gland located in different parts of plant but mostly found in the leaves of the plants. Hence, more oil is obtained from the leaves compared to the other parts. Other than that, higher yield of oil observed from leaves may be due to less fibers in leaves which can store more oil compared to stem.

The ages of the plant affect the oil color which the younger plant produces lighter color of oil compared to the old aged plant. The old plants are richer in more resinous and have darker oils due to the continuing evaporation of the lighter fraction of the oil. Hence, more components are presence in the oil from leaves and stem compared to the flowers. However, the characteristics of the oil obtained from the flowers similar to the oil obtained from the leaves and stem. It only varied in term of color whereas the flowers produces light-yellow oil compared to oil obtained from the leaves and stem which have dark-yellow color.

3.2 Effect of temperature on extraction yield

The extraction temperature is one of the most important variable in the extraction process as its affect the amount of product yield. Thus, in this study, effect of temperature was conducted on the oil yield of the leaves. The leaves was selected than the others part of the *Cymbopogon nardus* because it has the highest oil yield. It was found that the highest extraction temperature was at 100 °C, which gives the highest percentage oil yield about 1.53 %, followed by the extraction temperature of 80 °C and 60 °C which gives the percentage oil yield of 0.6 % and 0.54 %, respectively. The lowest amount of oil yield was obtained at 40 °C, which only 0.03 %. Table 1 presents the comparison oil yield of the leaves at various temperature.

| Temperature (°C) | Mass of oil (g) | Oil yield (%) |
|------------------|----------------|---------------|
| 40               | 0.050          | 0.03 ± 1      |
|                  | 0.051          |               |
|                  | 0.049          |               |
| 60               | 0.543          | 0.543 ± 1     |
|                  | 0.541          |               |
|                  | 0.542          |               |
| 80               | 0.905          | 0.603 ± 1     |
|                  | 0.903          |               |
|                  | 0.902          |               |
| 100              | 2.295          | 1.530 ± 1     |
|                  | 2.294          |               |
|                  | 2.293          |               |

It is showed that the temperature affect the oil yield and higher extraction was obtained at higher temperature compared to the lower temperature. This is due to viscosity of the oil which get lowered at high temperature causes more oil released from the intact cell.

3.3 GC-MS analysis of essential oil components

The concentration for each constituent is different for each sample. There are also others compounds presents beside essential oil. Others GC-MS analysis from stem and flowers were not shown. The presence of others peak besides essential oil compounds showed that the oil extracted contains other impurities. However, the impurities were not identified for this study. It would be interesting to study in the future about others potential of *Cymbopogon nardus* extract to produce others compound besides citronellal, citronellol and geraniol. The summary of peak appearance represents essential oil components for this study is shown in Table 2. Table 2 shows concentration of compound presence in extracted citronella for each component extracted from various parts of the plant. The presence of citronellal, citronellol and geraniol compounds in the oil extracted were determined by comparing with
the retention time and the spectra fragmentation of the standards, which appeared around 13.8 min, 14.8 min and 15.6 min for citronellal, citronellol and geraniol, respectively. The concentration of the compounds were obtained by substituting the area under the curve (y) of the compound into the linear equation obtained from the calibration curve for the respective compound. From the study, all compounds have high concentration in leaves followed by stem and flowers. The empty husk from the extraction was further treated for biogas production.

| Table 2. Concentration of compounds in various part of the plant |
|---------------------------------------------------------------|
| Plant Components        | Citronellal | Citronellol | Geraniol |
| Leaves                  | 10.36       | 12.31       | 13.56    |
| Stems                   | 9.54        | 10.51       | 11.32    |
| Flowers                 | 6.26        | 5.78        | 7.68     |

3.4 Effect of F/I ratio on biogas yield
There are 10 bioreactors labelled as A1 to E1 and A2 to E2 were prepared to study the potential of waste husk *Cymbopogon nardus* to generate biogas. The daily gas generated from the bioreactors were summarized in Figure 1. The volume of biogas were quantified by measuring the volume of water displaced. In the duration of 40 days, approximately a total of 309 ml of biogas was produced from various reactors. Volume of biogas produced differs from each reactor. As can be seen, the reactors A1, A2, B1, B2, D2, and E2 shows no displacement of water. Despite no displacement of water, no conclusion can be made yet prior to analyzing the gas in the headspace of the reactor using GC-FID and Biogas Analyzer. The aforementioned reactors may produce biogas as well but the volume is not significant, hence no water displacement. Although, the fact that the reactors may have leakage can also be considered. The reactors which showed a significant water displacement were C1, C2, D1, and E1. The average production of biogas from each biogas-producing reactors are 0.6 ml/day, 0.4 ml/day, 1.95 ml/day and 12.5 ml/day from the reactor C1, C2, D1, and E1, respectively.

In general the production of biogas started around day 6 and showed gradual increase until day 26. From day 0 until day 6, there is no gas produced. The late start in gas production could be attributed to the nature of the substrate where presence of lignin is the limiting factor of the anaerobic digestion process. This situation similar as reported in Alfa et al. [19] where the substrate for lignin degradation prior to digestion increases decomposition rate of substrate. Substrate such as citronella grass containing lignin decomposes at a slower rate compared to animal intestinal wastes which have undergone prior digestion within the digestive system of animals. Reactor E1 with F/I of 10.0 showed the most significant volume of gas produced among all of the reactors. Reactor E1 generated the highest cumulative volume of gas with 250
As the F/I ratio increases, the biogas generated also increases. Low efficiency of gas production might be due to insufficient methanogens. Optimal F/I ratio must be reached in order to accelerate the methanogen activities and start-up of biogas activity. The F/I of 10.0 generated the most amount of biogas while the decreased in F/I also showed decreased in biogas production.

Inoculum reacts differently with different substrate due to the differences in chemical compositions and/or physical structures. The findings in the current study however cannot be compared to any previous study related to inoculum ratio variation on *Cymbopogon nardus* for biogas production as there is a lack of data and no published data on the notion. From the current study, the F/I ratio which produced the highest volume of biogas is 10.0. Study varying the range up to a higher F/I ratio should be further investigated to find the optimal F/I ratio as F/I ratio below 10.0 showed insignificant amount of gas produced.

### 3.5 Validation of methane gas in biogas sample using GC-FID and biogas analyzer

The validation of methane gas was conducted for each reactor. There are two methods were applied to analyze the contents of gas present in each bioreactor, GC-FID and biogas analyzer. GC-FID is the most preferable method as it accurately shows the type of gas in sampling. However, GC-FID does not detect gases that present in non-standard gas (air). Thus, further testing was conducted using biogas analyzer. The first analysis using GC-FID shows that only reactor A1 and D1 contains methane. The analysis result under GC-FID is shown in Figure 2. Figure 2 shows the peak appearance of methane gas for both A1 and D1. There are no peak for the others sample which were taken from each bioreactor. This probably due to the gas present which was not of standard gas (air), thus cannot be detected by the FID detector. Based on GC-FID analysis, it can be confirmed that the reactors; A1 and D1 contained methane gas. As the highest peak can be found around 2.315 to 2.865 minutes which confirms the presence of methane according to the standard reference. The A1 samples showed a delayed in the highest peak appearance which was at around 2.865 minutes due to technical difficulties while conducting manual injection using the GC-FID. To support the gas analysis result from the GC-FID, further testing was conducted by using biogas analyzer. Biogas analyzer is used to measure the fractions of gases presents in the sample. The analysis results of biogas analyzer were summarized in Table 3 with focuses on methane, carbon dioxide, oxygen and hydrogen sulfide.
The reactor with the highest CH4 content is from the reactor D1 at 10.1% while the lowest is from C2 at 5.9%. The highest content of CO2 produced is from B1 with a percentage of 38.1% and the lowest from A1 with 11.0%. The reactor containing the highest oxygen is from reactor A1 with a percentage of 15.8% while the lowest is from D1 with 0.7%. Impurity gas which is H2S is also present in the gas where reactor E1 contained the highest with 52 ppm while the lowest one is 4 ppm from reactor A1. When the ratio of F/I is increased, it is predicted that the H2S content will also increase due to increasing sulfur content. However, the formation of hydrogen sulfide in the gas samples does not follow a specific increasing trend when in theory, as the F/I is increased, the H2S content should follow as well. It is important to determine the physicochemical properties of the substrates prior to the study in order to predict the H2S content in the gas sample.

### Table 3. Biogas composition in each bioreactor

| Reactor | CH4 (%) | CO2 (%) | O2 (%) | H2S (ppm) |
|---------|---------|---------|--------|-----------|
| A1      | 7.8     | 11.0    | 15.8   | 4         |
| A2      | 7.5     | 26.6    | 3.8    | 18        |
| B1      | 8.1     | 38.1    | 11.6   | 24        |
| B2      | 8.7     | 27.4    | 3.5    | 12        |
| C1      | 6.5     | 26.4    | 2.3    | 14        |
| C2      | 5.9     | 27.3    | 2.0    | 8         |
| D1      | 9.6     | 31.0    | 0.7    | 19        |
| D2      | 10.1    | 19.6    | 6.8    | 11        |
| E1      | 6.3     | 22.5    | 4.5    | 52        |
| E2      | 7.8     | 26.4    | 1.2    | 21        |

The typical constituent of biogas is methane (50% to 80%), carbon dioxide (25% to 50%), nitrogen (0% to 10%), hydrogen (0% to 1%), hydrogen sulphide (0% to 3%) and oxygen (0% to 2%). The result obtained using the analyzer does not correlate with the literature. There is no general trend as well in terms of the composition analyzed based on the variation of F/I. Therefore, the effect of F/I variation on the biogas composition is inconclusive. Errors in the experiment is probably attributed to the nature of the sample, where it is hard to detect contamination and leakage. Leakage of sample to the atmosphere may even contaminate and affect the accuracy of the result obtained.
4. Conclusions
It was proven that, the *Cymbopogon nardus* leaves, stem and flower contains oil. However, the highest oil yield was from the leaves. Citronellal was the major compound of the extracted oil. The waste of *Cymbopogon nardus* was also confirm to produce biogas especially methane.

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References
[1] Chen W and Viljoen A M 2010 *S. Afr. J. Bot.* 76 643
[2] Kpoviessi S, Bero J, Agbani P, Gbaguidi F, Kpadonou-Kpoviessi B, Sinsin B and Quetin Leclercq J 2014 *J. Ethnopharmacol.* 151 652
[3] De Toledo L G, Ramos M A, Spósito L, Castilho E M, Pavan FR, Lopes É, Zocolo G J, Silva F A, Soares T H, Dos Santos, A G, Bauab TM and De Almeida M T 2016 *Int. J. Mol. Sci.* 17 1252
[4] Wei LS and Wee W 2013 *Iran J. Microbiol.* 5 147
[5] Kamari FEL, Taroq A, Atki YE, Aoum I, Oumokhtar B, Lyoussi B, Abdellaoui A 2018 *Int. J. Pharm. Sci. Rev. Res.* 50 14
[6] Ruenroengklin N, Zhong J, Duan X, Yang B, Li J and Jiang Y 2008 *Int. J. Mol. Sci.* 9 1333
[7] Hawthorne SB, Grabanski CB, Martin E and Miller DJ 2000 *J. Chromatogr. A* 892 421
[8] Khajeh M, Yamini Y and Shariati S 2010 *Food Bioprod. Process.* 88 227
[9] Manaf M A, Mustapa AN and Mustapa K 2013 IEEE Business Engineering and Industrial Applications Colloquium (BEIAC), 7-9 April, Langkawi, pp. 73-78
[10] Spigno G, Tramelli L and De Faveri DM 2007 *J. Food Eng.* 81 200
[11] Manurung R, Melinda R, Abduh MY, Widiana A, Sugoro I and Suheryadi D 2015 *Pak. J. Nut.* 14 919
[12] Achinas S, Achinas V and Euverink GJW 2017 *Eng.* 3 299
[13] Scarlat N, Dallemand JF and Fahl F 2018 *Renew. Energ.* 129 457
[14] Aribaaatar J, Panico A, Esposito G, Pirozzi F and Lens PN 2014 *Appl. Energ.* 123 143
[15] Raposo F, De la Rubia MA, Fernández-Cegrí V and Borja R 2012 *Renew. Sust. Energ. Rev.* 16 861
[16] Mao C, Feng Y, Wang X and Ren G 2015 *Renew. Sust. Energ. Rev* 45 540
[17] Raposo F, Banks CJ, Siegert I, Heaven S and Borja R 2006 *Process Biochem.* 41 1444
[18] Tumutegyereize P, Muranga FI, Kawongolo J and Nabugoomu F 2011 *Afr. J. Biotechnol.* 10 18243
[19] Alfa IM, Okuofu CA, Adic DB, Dahunsi SO, Oranusi SU and Idowu SA 2012 *Int. J. Green Chemist. Bioprocess.* 2 34
[20] Ahmed S and Kazda M 2017 *Anaerobe.* 46 114
[21] Owamah HI, Alfa IM and Dahunsi SO 2014 *Renew. Energ.* 68 366