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SHORT COMMUNICATION

A simple method for the determination of bioethanol from lignocellulosic materials using gas chromatography-flame ionisation detector (GC-FID)

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ABSTRACT

Aims: The utilisation of lignocellulosic biomass for bioethanol production reduces the dependency on fossil fuels as a source of energy and emission of greenhouse gas (GHG). However, studies in this emerging field are hampered by the cost of ethanol quantification methods. Due to the volatile nature of ethanol, the method for the quantification of bioethanol production should be reproducible and rapid to avoid any evaporation loss to the surroundings. Therefore, this study aimed to develop a simple, rapid and precise bioethanol quantification method using a gas chromatography-flame ionisation detector (GC-FID) without having to go through distillation process for ethanol purification.
Methodology and results: The bioethanol was produced via consolidated bioprocessing (CBP) using *Trichoderma asperellum* B1581 and paddy straw. The peak corresponding to ethanol was obtained at 2.347 min with a peak area of 189.66, equating to 0.159% (v/v) or 1.25 g/L ethanol. A comparison between the quantity of ethanol detected by GC-FID and spectrophotometric analysis (340 nm) showed no significant difference (p>0.05) in the amount of ethanol detected by GC analysis, thus validating the accuracy of the GC method.
Conclusion, significance and impact of study: This work presents a simple, precise and reliable method to determine the amount of bioethanol in the sample using a GC-FID. Currently, there are many GC-FID methods available for the determination of ethanol/alcohol in a human blood samples or in beverages but not in bioethanol samples. Thus, this method was developed to facilitate the determination of bioethanol in the samples produced from lignocellulosic materials.

Keywords: Bioethanol, GC-FID, quantification, spectrophotometric analysis

INTRODUCTION

Bioethanol is commonly known as ethyl alcohol (C_2H_5OH) and generated from the fermentation of fermentable sugars such as sucrose and glucose from the plant resources using microorganisms (Chin and H'ng, 2013). This kind of alcohol is considered as one of the most favorable substitute for fossil fuels as it can be integrated efficiently into conventional fuel systems without requiring any modifications to the engines (Zentou *et al.*, 2019). However, studies in this emerging field are hampered by the cost of ethanol quantification methods (Gerchman *et al.*, 2012). The determination of the ethanol content in samples can be achieved via spectrophotometric analysis, volumetric methods as well as gas chromatography (Pulungan *et al.*, 2018). Commonly, enzymatic assays are applied for the quantification of ethanol in biological samples, but the reproducibility of the enzyme-based methods is inadequate due to enzyme instability (Pinu and Villas-Boas, 2017). By contrast, gas chromatography (GC) is a robust instrument used for the quantification, separation, and identification of alcohols in diverse samples (Lu *et al.*, 2008). GC can be equipped with several types of detectors such as flame ionisation detector (FID), mass spectrometer (MS), flame photometric detector (FPD), thermal conductivity detector (TCD) and electron capture detector (ECD). Among them, the FID detector can maintain high accuracy with a wide dynamic range from ppm (parts per million concentrations) up to 100% and it is suitable for the analysis of carbon-containing organics (Liu *et al.*, 2018). Similar to FID, GC-MS projects a chromatogram with an additional spectral profile corresponding to each peak, hence allowing identification in association with databases of identified elements (Ottensmann *et al.*, 2018). Several studies have shown that gas

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chromatography-flame ionisation detector (GC-FID) is more reliable for quantitative analysis as the detector has higher sensitivity and lower noise compared to GC-MS, while GC-MS only offers specific biomolecule identification and qualitative information (Misra *et al.*, 2019).

The application of GC-FID instrument is more beneficial as the instrumentation is low cost which the FID cost was estimated around USD 17,000 compared to MS cost about USD 40,000 depending on the model, simple to maintain and can use hydrogen as a carrier gas instead of helium (Hložek *et al.*, 2014). However, the development and validation of the GC-FID method are important to confirm whether the application of this analytical procedure is suitable for its purposes (Godswill *et al.*, 2014). Several chromatographic conditions, such as split ratios and injection volume, need to be accurately identified to increase the sample outcome and the sensitivity (Sirhan *et al.*, 2019). Currently, there are many GC-FID methods available to determine ethanol or alcohol in human blood and beverages but lacks in quantification of bioethanol produced from lignocellulosic materials such as paddy straw. Hence, this study aimed to develop a simple, rapid and precise chromatographic method for the determination of bioethanol using FID and validate the outcome by comparison with spectrophotometric analysis.

MATERIALS AND METHODS

Bioethanol sample

The bioethanol sample was prepared using 2.72% (w/v) pre-treated paddy straw via consolidated bioprocessing (CBP) under optimised conditions using *Trichoderma asperellum* B1581 at a concentration of 1×10^6 spore/mL (Mohamed Ghazali, 2019). The ethanol sample was immediately harvested, filtered using a surfactant-free cellulose acetate (SFCA) syringe filter (pore size 0.2 µm) and quantified immediately using Megazyme® ethanol assay kit to minimise possible evaporation loss. The assays were read at 340 nm using micro plate reader.

Chemicals

All chemicals used in this study were reagent-grade from commercial sources. The standard solution used was absolute ethanol (99.8% v/v) (VWR Chemicals, USA) and the calibration curve was determined for reference in the ethanol quantification.

Calibration curve for the reference

FID analysis is considered an ideal tool to determine the concentration of a specific compound with minimal sample preparation. The identification of ethanol was performed by applying the calibration curve using the absolute ethanol (99.8% v/v). The strategy to obtain a linear calibration curve was based on the literature by estimating the amount of ethanol in the sample (Li *et al.*, 2009; Brus, 2015). In this study, the calibration curve was constructed via GC-FID using five ethanol concentrations, 0.03% (v/v), 0.05% (v/v), 0.1% (v/v), 0.3% (v/v) and 0.5% (v/v).

Ethanol quantification

All GC experiments were performed using an Agilent Technologies 6890N Network GC System (USA) coupled with an FID. The capillary column used was DB-5 with a capacity reaching a maximum temperature of 325 °C, 30 m nominal length, 320 µm nominal diameter, 0.25 µm nominal film thickness and 1.2 mL/min nominal initial flow with an average velocity of 25 cm/sec. The sample preparation and measurement procedures were as follows: 5-50 µL of sample solution was placed into a 2 mL Agilent screw top vial and placed in the sample tray for GC measurements (Li *et al.*, 2009). The sample injection volume was 1 µL and each sample was injected at least three times. The GC operating conditions were set as follows: (i) oven (initial temperature 110 °C, initial time 6.00 min, equilibration time 3.00 min, post time 0.00 min, run time 6.00 min); (ii) split front inlet (initial temperature 200 °C, pressure 54.8 kPa, split ratio 30:1, split flow 36 mL/min, total flow 40 mL/min, using helium gas) and (iii) FID back detector (temperature 200 °C, airflow 450 mL/min, hydrogen flow 40 mL/min, makeup gas using helium).

RESULTS AND DISCUSSION

A GC-FID method was developed and optimised for various important parameters such as injection conditions, oven temperature and split ratios (Keyfi and Varasteh, 2016). The parameters for the validation of the GC-FID method included method selectivity, linearity, accuracy, repeatability, ruggedness, the limit of detection (LOD) and limit of quantitation (LOQ) (Zuas *et al.*, 2016). The method linearity was found to be high with a good correlation coefficient value of 0.99998 for the target compound, ethanol (Figure 1). The R^2 value of >0.998 is considered as an indication of adequate fit of the data to the regression line and the LOQ must not be lower than LOD, but it may be equivalent or higher (Armbruster and Pry, 2008). LOD and LOQ were calculated from the calibration curve constructed based on dilute standard solutions, 0.03% to 0.5% (v/v), as 0.007% and 0.021%, respectively.

Optimum qualitative and quantitative GC analyses comprise several features such as good resolution with sharp and symmetric peaks, good reproducibility of retention times and fine precision in quantitation based on peak area measurements (Al-Bukhaiti *et al.*, 2017). The analysis time can be reduced by either manipulating the operational parameters (temperature programme rate, the linear velocity of carrier gas, etc.) or changing column parameters to a smaller inside diameter (ID) with a thinner film (Maštovská *et al.*, 2001). The selection of a non-polar DB-5 column (30 m × 320 µm × 0.25 µm) helps to generate the best peak efficiencies with the optimum

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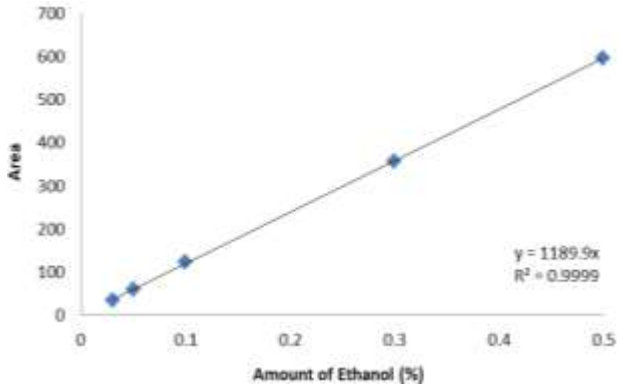

Figure 1: The calibration curve using ethanol standard for quantitative measurement in GC-FID with gradient 1189.9.

signal-to-noise ratio as the thinner film (<0.3 mm) can minimise column bleed (Watson, 2019). In addition, the application of wide diameter columns will cause coelution and collection of impure fractions leading to poor resolution and severe loss in efficiency (Sciarrone *et al.*, 2015). Regarding the oven temperature, the initial temperature was set at 110 °C. Setting the correct temperature programme is crucial as too low a temperature will result in a longer analysis time, broader peaks and reducing the sensitivity of the signal height (de Zeeuw, 2015). Another typical issue for peak broadening is the volume of sample injections, if the volume is too large, it can cause column overloading (Woodman, 2010). In this study, the sample injection volume was 1 µL and did not cause peak broadening. One of the critical parameters for developing a GC-FID method is the split ratio. The amount of analyte reaching the column is controlled by the split ratio and with very narrow GC columns (<100 µm ID) it can be as high as 1:1000+, with the split ratio eventually influencing the sensitivity and the peak width (LCGC Europe, 2016). Since the ID of the DB-5 column is 320 µm, this method used a split ratio of 30:1 with a split flow of 36 mL/min.

The more soluble a component is in the stationary phase, the higher the retention time (T_R). The T_R of the ethanol standard (2.342 min) was used as a reference for ethanol quantification (Figure 2). The amount of a substance in the sample can be measured quantitatively as the area under the peak is directly proportional to the concentration (Christian, 2004). The T_R and peak area of samples were compared with those of standard ethanol to confirm the presence of ethanol (Sudhaker and Jain, 2016). The ethanol in the sample was detected at 2.347 min with a peak area of 189.66 equating to 0.159% (v/v) or 1.25 ± 0.02 g/L ethanol (Figure 3). The T_R of the sample showed no significant difference (p>0.05) compared to the T_R of ethanol standard. If the T_R of an analyte is not within the established range, a counteractive action must be engaged to restore the system or develop a new calibration curve for that particular compound (U.S. EPA, 2003).

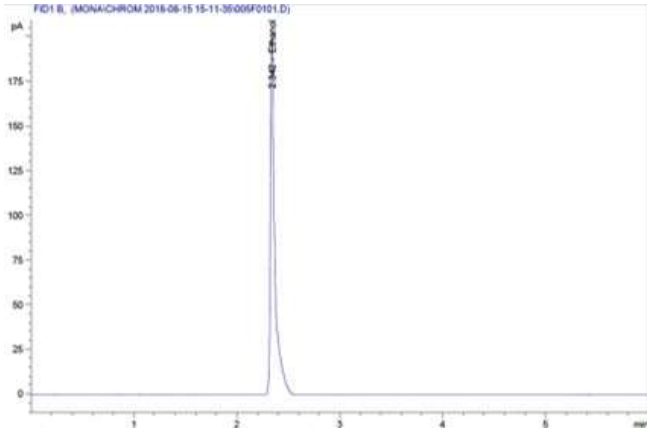

Figure 2: The peak of ethanol standard with retention time of 2.342 min.

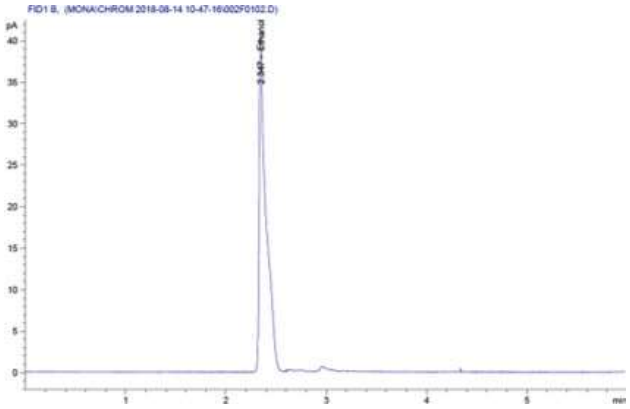

Figure 3: The peak of ethanol from sample at retention time of 2.347 min.

In this study, the sample prepared via CBP was collected and directly transferred into GC vials for immediate analysis without distillation. Normally, the major components left in the broth after alcoholic fermentation were ethanol and total water; hence, distillation process has been used as the main purification method for ethanol recovery (Zentou *et al.*, 2019). Although no distillation was involved, the result shows the sample was clean, only consisting of ethanol (single peak) with no impurities or other compounds detected, demonstrating the capacity of GC-FID method to quantified ethanol without distillation. Moreover, using appropriate settings or procedure for the GC-FID contributes to create a clear peak and the amount of ethanol could be analysed within the linear range (Brus, 2015). An appropriate GC procedure tends to minimise the possible sources of analytical errors during analysis, such as contamination, measurement errors and instrumental error (Miricioiu *et al.*, 2016). In addition, the developed method was rapid, with a 6 min run time and a 3 min equilibrium time per sample compared to a

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conventional GC-FID method which takes more than 20 min to complete (Lin *et al.*, 2014).

A comparison of the quantity of ethanol detected by GC-FID and spectrophotometric analysis using the Megazyme® ethanol assay kit was performed to validate the efficiency of the GC-FID method. The amount of ethanol detected using GC-FID and the microplate reader (spectrophotometry) was 1.25 ± 0.02 g/L and 1.11 ± 0.02 g/L, respectively, with no significant difference (t-test; $p>0.05$) in the amount detected by both methods, hence, validating the accuracy of the GC-FID method.

CONCLUSION

A simple and rapid GC-FID method was successfully developed for the quantification of bioethanol sample with the amount of ethanol detected using GC-FID was 1.25 ± 0.02 g/L, which was slightly higher than the amount of ethanol detected using spectrophotometric analysis, 1.11 ± 0.02 g/L. However, there was no significant difference between the means ($p>0.05$), hence, validating the method developed.

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