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Hydrogen-rich syngas fermentation for bioethanol production using *Sacharomyces cerevisiea*

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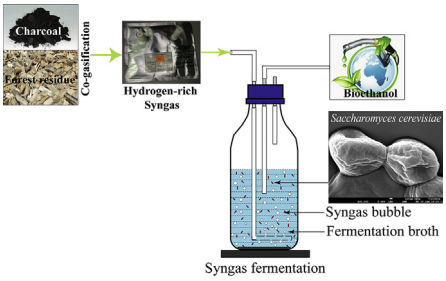
HIGHLIGHTS

- Hydrogen-rich syngas was converted into bioethanol using a tar free fermenter (TFF)
- *Saccharomyces cerevisiae* was suited microorganism for syngas fermentation
- Produced bioethanol was detected using (^{1}H) NMR and GC-MS analysis

GRAPHICAL ABSTRACT



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ABSTRACT

Bioethanol is an eco-friendly biofuel due to its merit that makes it a top-tier fuel. The present study emphasized on bioethanol production from hydrogen-rich syngas through fermentation using *Sacharomyces cerevisiea*. Syngas fermentation was performed in a tar free fermenter using a syngas mixture of 13.05% H_2, 22.92% CO, 7.9% CO_2, and 1.13% CH_4, by volume. In the fermentation process, effects of various parameters including syngas impurity, temperature, pH, colony forming unit, total organic carbon and syngas composition were investigated. The yield of bioethanol was identified by Gas chromatography-Mass spectrometry analysis and further, it was confirmed by Nuclear magnetic resonance (^{1}H) analysis. From GC-MS results, it is revealed that the concentration of bioethanol using *Saccharomyces cerevisiae* was 30.56 mmol from 1 L of syngas. Thus, hydrogen-rich syngas is suited for bioethanol production through syngas fermentation using *Saccharomyces cer-*

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TFF
Sacharomyces cerevisiea

evisiae. This research may contribute to affordable and environment-friendly bioethanol-based energy to decrease the dependency on fossil fuels.

Introduction

Worldwide energy demand is increasing due to rapid industrialization and urbanization [1–4]. Moreover, augmented consumptions of liquid fuels make the global energy demand critical and it will increase from 200 quadrillion Btu in 2020 to 229 quadrillion Btu in 2040 [5]. Biomass-based energy is an alternative energy source for the production of bioethanol owing to future fuel insecurity and environmental issues [4,6,7]. Gaurav, Sivasankari [8] reported that biomass is the fourth largest available renewable energy resource that can mitigate global warming. It contains a minor amount of sulfur (S) and emits the least amount of GHGs to the environment [2,9]. Woody biomass has higher lignin than non-woody biomass [10]. This lignin is difficult to degrade completely through the conventional fermentation process [11,12]. As a result, the conversion of hydrogen-rich syngas into other fuels like bioethanol is receiving increasing attention. Moreover, hydrogen-rich syngas is produced from biomass through some thermochemical conversion processes like pyrolysis, combustion, and gasification through various type of gasifiers [7,13]. The previous study revealed that the concentration of hydrogen and carbon monoxide increased by increasing charcoal ratio with forest residue, empty fruit bunch of palm oil and coconut shell [2,14–16]. The by-product (charcoal) and co-product (tar) are usually produced during gasification [2]. In the literature, it is also found that biochar provided nutrients to microorganisms for the enhancement of ethanol production during syngas fermentation [17]. Recently, Liakakou, Vreugdenhil [18] studied on syngas fermentation for second-generation bioethanol production from lignin-rich residues. Most of the organisms are grown better on CO than H_2 [18,19]. They also reported that syngas impurities (particles and tar compounds) can reduce the fermentability of syngas (hydrocarbons, HCl, HCN, COS, NH_3, and organic S-compounds). Furthermore, due to the presence of tar compounds in syngas, it is difficult to use directly as a power generation or transportation fuel purposes [20]. In addition, while biomass-based syngas is used as alternative fuels in internal combustion engines, co-product tar compounds are still great concerns because of the requirement to protect the environment as well as concern about safety and health hazards [21].

Syngas fermentation is a microbial process where syngas is used as carbon and energy sources, and finally, it is converted into valuable biofuels [22–26]. This syngas is usually produced from biomass gasification [12,25]. The biocatalyst of yeast and bacteria is used for the production of bioethanol [27,28]. The model microorganisms which are commonly used for bioethanol production include *Clostridium* sp., *Escherichia coli*, *Bacillus* sp., *Saccharomyces cerevisiae* and *Trichoderma reesei*, *Fusarium oxysporum* [29]. *Saccharomyces cerevisiae* is one of the most important biocatalysts because of its cost-effectiveness in comparison with other fermenting agents that are produced zero chemical wastes [30]. Moreover, bioethanol production by *Saccharomyces cerevisiae* has been playing a key role in fermentation industry [31]. This is an ideal biocatalyst for bioethanol production in a sugar-containing nutrient medium. However, biomass-based syngas is needed for purification because of impurities in syngas.

Various types of fermenters are used for syngas fermentation. The most common fermenters are Continuous Stirred Tank Reactor (CSTR), Bubble Column Reactor (BCR), Monolithic Biofilm Reactor (MBR), Trickle Bed Reactor (TBR), Microbubble Dispersion Stirred-tank Reactor (MDSR) and Membrane-based System Reactor (MSR) [25,27]. The ethanol production can be increased by the proper fermenter designs, which allow proper mass transfer rates, choice of biocatalysts with optimizing yields and efficient recovery methods [32]. Shen, Brown [33] stated that horizontal rotating packed bed (h-RPB) reactor is also more effective compared to CSTR for bioethanol production. Besides ethanol, there are some other products that are also produced during the fermentation process like methanol, acetic acid, higher alcohols (butanol) and acids [25].

In the literature, insufficient work has been attempted through syngas fermentation with *Saccharomyces cerevisiae* for biomass-based syngas containing a nutrient medium. Syngas fermentation has appeared as a promising fermentation technique for the conversion of biomass. However, bottleneck work has been done for the production of bioethanol using forest residue with charcoal co-gasification-based syngas along with tar purification system. Moreover, untreated syngas is difficult to use directly for power generation or transporting fuels purposes and it exists in a gaseous phase, and existing engines are needed for additional modification which is very expensive. Therefore, the aim of this study is to produce bioethanol through syngas fermentation using *Saccharomyces cerevisiae*.

Material and methods

Sample collection

Hydrogen-rich syngas was collected from the previous co-gasification process which was performed with the mixture of forest residue and charcoal at the blending ratio of 70:30. Collected syngas composition were hydrogen (13.05, % Mole), carbon monoxide (22.92, % Mole), carbon dioxide (7.9, % Mole) and methane (1.13, % Mole). The carbon, mineral and trace element containing by-product charcoal was also collected from a previous study [14]. *Saccharomyces cerevisiae* was obtained from the laboratory of Biochemical Engineering, International Islamic University Malaysia (IIUM), Malaysia and stored at 4 °C to prevent any type of contamination.

Cell culture and inoculum preparation

Saccharomyces cerevisiae was freshly cultured in 10 mL agar slants containing glucose (10 g/L), yeast extract (10 g/L), peptone (10 g/L) and agar (2%, w/v) as a growth medium to maintain stock culture [34]. Subsequently, the medium was sterilized by autoclaving at 121 °C for 20 min. It was sub-cultured in the petri plate and slant to grow up the new cells. The inoculum was prepared by dissolving 10 loops of *Saccharomyces cerevisiae* from sub-cultured cells in 10 mL of deionized (DI) water. The inoculum process was performed under the biosafety hood to protect contamination by indigenous microbial activities. After that, these were placed inside the incubator to maintain its temperature. The incubator temperature was set at 37 °C for new cells growth of *Saccharomyces cerevisiae* for 24 h and stored at 4 °C for further uses of syngas fermentation experiments.

Experimental setup and fermentation medium

The syngas fermentation was performed in a tar free fermenter (TFF) in the continuous recycling fed system (Fig. 1). Stored syngas was used as a carbon nutrient for *Saccharomyces cerevisiae*. By-product charcoal was also used as a nutrient of *Saccharomyces cerevisiae*. A peristaltic pump was used to control the flow of syngas to the TFF throughout the process. Prior to run the experiment, syngas was pass through (flow rate of 100 mL/min) acetone and methanol for the filtration of tar compounds. Fine particles were filtered using three series of the cotton filter. The effect of temperature and pH were observed by Eutech™ pH 700 m, colony forming unit (CFU) was observed by Stuart™ colony counter, total organic carbon (TOC) was observed by MERCK Spectroquant® Pharo 300 analyzer and syngas composition was investigated by Gas chromatography-mass spectrometer (GC-MS). Samples were collected every 24 h to observe the microbial cell growth profile.

In this study, the fermentation medium was prepared for performing syngas fermentation using *Saccharomyces cerevisiae*. A 500 mL impinger bottle was taken which was filled with 80% of fermentation broth and remaining 20% was considered as working volume. The medium was prepared and included: (1) yeast extract (0.2 gm) (2) peptone (0.8 gm), (3) KH_2PO_4 (0.4 gm), (4) $MnSO_4.7H_2O$ (0.2 gm), (5) $(NH_4)_2SO_4$ (0.8 gm), (6) by-product charcoal (0.7 gm) and (6) DI water (400 mL). Then, tar-free syngas was pass through the TFF, *Saccharomyces cerevisiae* was inoculated and connected with the biomass-based syngas bag. The pH was controlled within the ranges of 4.0–6.5. After that, the experiment was run inside the shaking incubator. The temperature and rotational speed were controlled at 37 °C and 200 rpm, respectively. The experiment was run for 16 days for the production of bioethanol. The syngas was recycled throughout the process to get the maximum production efficiency.

Product extraction and analysis

The yield of bioethanol from syngas fermentation was extracted from fermentation broth at the end of the process. In this study, the organic compounds were separated from the fermentation broth for further bioethanol detection using GC-MS and NMR (^1H) analysis.

Liquid-liquid extraction (LLE) using solvent

The organic compounds were separated from the fermentation broth using liquid-liquid extraction. Two solvents namely n-hexane (chromatogram pure grade, Sigma Chemicals, USA) and deuterochloroform, $CDCl_3$ (Sigma-Aldrich) were used to extract bioethanol in an aqueous solution; 5 ml of n-hexane or $CDCl_3$ were mixed with 40 ml sample (fermentation broth), and then vortexed vigorously using a vortex mixer (Vortex-Genie 2, Scientific Industries Inc., USA) for 10 min. After phase separation, the solvent phase was transferred to a new tube for further analysis.

Bioethanol detection and analysis

In this product analysis, 1 μL of extracted samples were used for both GC-MS and NMR (^1H) analysis. The bioethanol concentration was analyzed by GC-MS analyzer (Brand: Agilent, Model: 7890A). The used carrier gas was helium (He) with a flow rate of 1.0 mL/min. The initial and final temperatures were set as 70 °C and 325 °C, respectively. The oven temperature was set as 325 °C and oven program was set as 100 °C for 2 min, then 5 °C/min to 120 °C for 0 min and 5 min (post run). The total run time 6 min and MS was operated in the scan mode mass ranges from 40 amu to 1000 amu. For the quantitative analysis, standard ethanol (99.99%) was also prepared

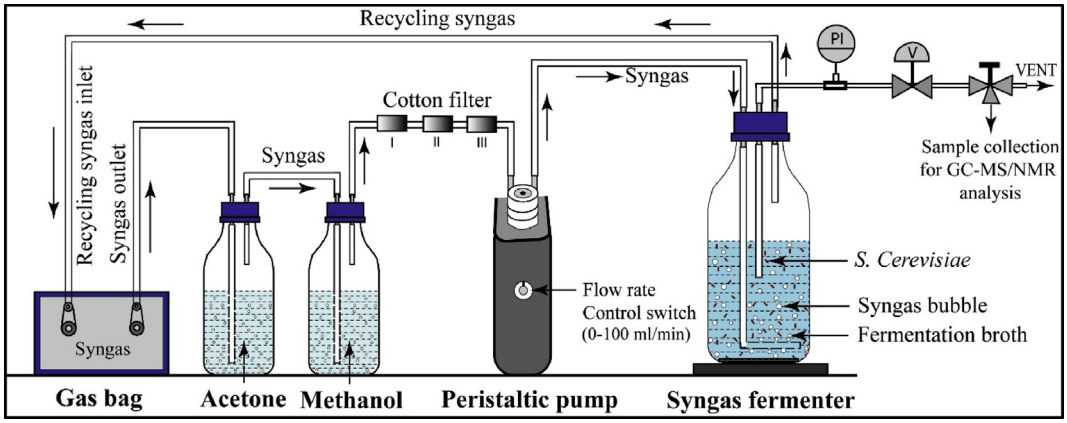

Fig. 1 – Experimental setup for bioethanol production through syngas fermentation using *Saccharomyces cerevisiae*.

to 1%, 2% and 3% (10 μL, 20 μL and 30 μL in 1 mL n-hexane solution). Correspondingly, the MS fraction of extracted samples were matched with the standard ethanol (99.99%) and ultimate bioethanol concentration was calculated. The concentration of bioethanol using 50 mL, 100 mL, 250 mL, 500 mL and 1000 mL syngas capacity bag was evaluated. Moreover, for final confirmation of bioethanol, NMR (^{1}H) analysis was also performed. In this analysis, ^{1}H NMR (500 MHz) spectra were recorded using BRUKER-500 spectrometer (Model: Bruker Ultra Shield Plus 500 MHz) and chemical shifts were stated relative to $CDCl_3$ (TMS, 0.00 ppm).

Results and discussions

Cell growth and characterization

The freshly cultured *Saccharomyces cerevisiae* was prepared for syngas fermentation is shown in Fig. 2. In this figure, it is observed that the new microbial cell was cultured in slant (Fig. 2a) and petri plate (Fig. 2b). This type of microorganism has the ability to maintain some limiting conditions for the production of bioethanol. The obtained *Saccharomyces cerevisiae* was used in the previous recent works for bioethanol production [35,36] and it was found that high yield of second-generation bioethanol generated by using this strain of *Saccharomyces cerevisiae*. The freshly cultured *Saccharomyces cerevisiae* was also characterized by FESEM analysis for its confirmation (Fig. 2c and d). From this morphological analysis, it is shown that the surface of the cell body is smooth, and the shape of the body was spherical. The FESEM images of *Saccharomyces cerevisiae* colony is consistent with the literature reported by Zhao, Lin [37]. Therefore, this freshly cultured *Saccharomyces cerevisiae* was capable for syngas fermentation and suited of the production of bioethanol.

Effect of syngas fermentation

Effect of syngas impurity on microbial cell growth

In this study, the effect of syngas impurity on the growth of *Saccharomyces cerevisiae* was observed in comparison with treated and untreated syngas (Fig. 3). Both fermentation processes were run until the 16th day, and the effect of impurity was observed through microbial colony counting. From this study, it is shown that *Saccharomyces cerevisiae* cells entered the stationary growth phase (2nd day) after the inoculation in fermentation broth. After the 2nd day, it was observed that the trend of cell growth decreased rapidly when untreated syngas was used. Subsequently, growth of the cell was gradually decreased, and microbial cell concentration remained constant until the 11th day. After that, there were no microbial cells were observed. On the contrary, due to the used of treated syngas significant lag phase was observed (Fig. 3) which is responsible for bioethanol production [38]. The maximum growth of *Saccharomyces cerevisiae* was found on the 2nd day (Fig. 3). However, the cell growth rate was abruptly increased at 1st and 2nd day during syngas fermentation. From this study, it was found that the maximum microbial cell mass concentration on fermentation broth was 500 times higher when treated syngas was used instead of untreated

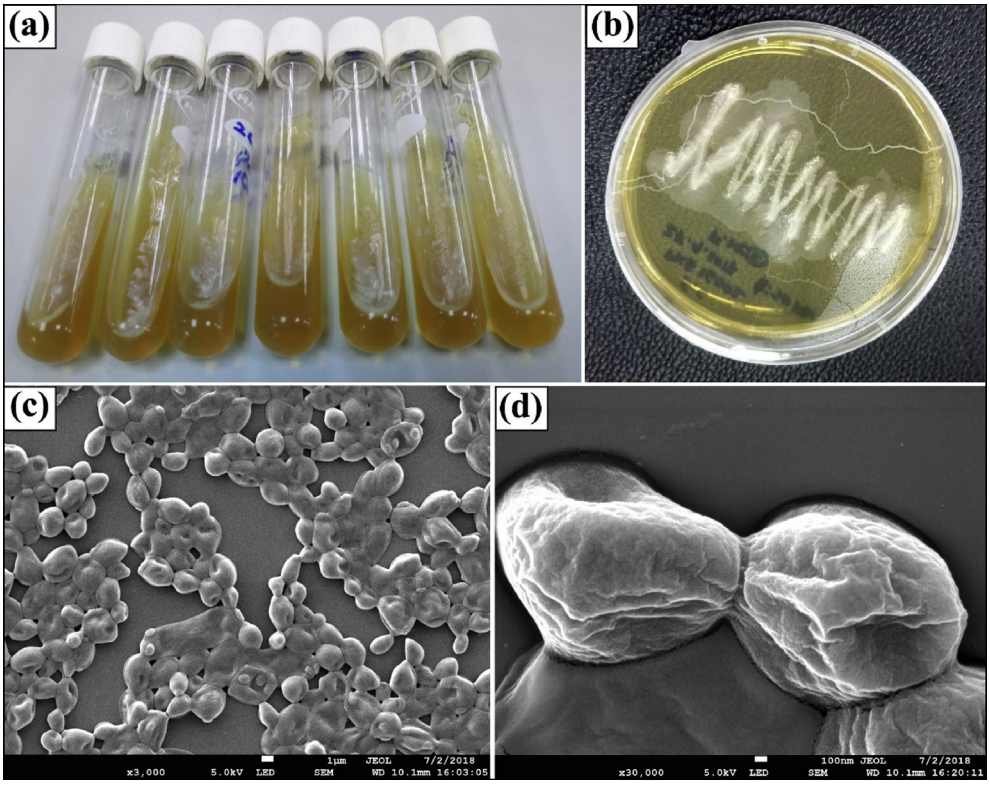

Fig. 2 – Cell Growth Culture of *Saccharomyces cerevisiae*: (a) in petri plate (b) in slant (c) FESEM image of *Saccharomyces cerevisiae* group colony (b) FESEM image of *Saccharomyces cerevisiae* single colony.

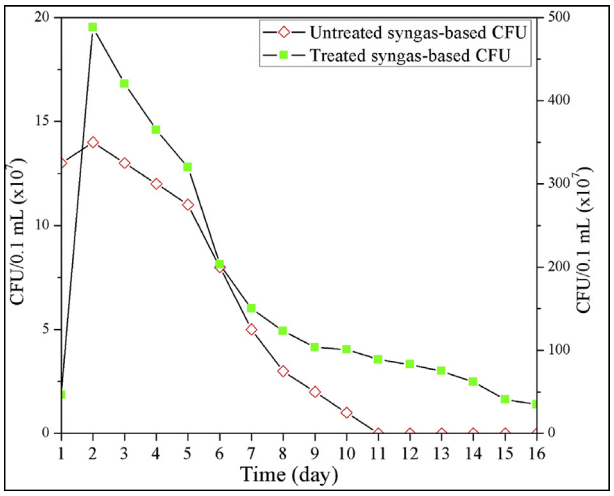

Fig. 3 – Effect of syngas impurity on syngas fermentation.

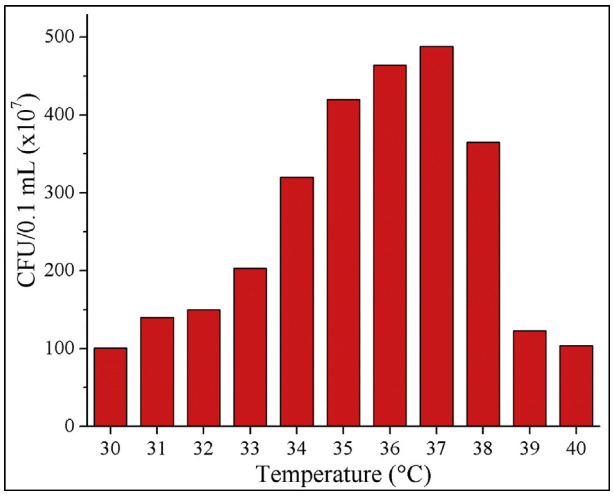

Fig. 4 – Temperature effect on syngas fermentation.

syngas. The outcome of this observation revealed the strong robustness that affected the yield of bioethanol production. A similar effect was found in the literature where potential inhibitors of tar and nitric oxide (NO) affected the product formation for microbial cell growth during syngas fermentation [39,40]. Therefore, high molecular weighted tar compounds and particles were purified from raw syngas before inoculated to the fermentation broth using TFF.

Effect of temperature on microbial cell growth

The temperature effect was investigated on the microbial cell growth during syngas fermentation as shown in Fig. 4. From this analysis, it was shown that maximum microbial growth was at a temperature of 37 °C. Therefore, based on the optimum temperature, the whole syngas fermentation process was performed at 37 °C for achieving the maximum yield of bioethanol.

Effect of pH on microbial cell growth

The effect of pH on the whole syngas fermentation process using *Saccharomyces cerevisiae* was observed. This parameter has a significant effect on the growth of *Saccharomyces cerevisiae*. In the case of bioethanol production, the pH ranges from 6.60 to 4.31 until the 16th day (Fig. 5). In this study, starting incubation pH of 6.60 was observed to negatively impact *Saccharomyces cerevisiae* metabolism. The pH level was 6.24 in the 5th day and 5.95 in the 15th day. The pH was changed quickly from 6.60 to 6.24 which followed 1st day to 3rd day. After the 10th day, the pH level was gradually decreased until the 16th day. As a result, the pH level reduced to 4.31 at the end of the process (16th day). Thus, the changes of pH from initial to final stage was occurred by stimulating the production of bioethanol which resulted in phase separation. According to the Wood-Ljungdahl pathway, there is a fast growth of microbial cells and the production of organic acids was followed in the initial phase. Therefore, the accumulation of organic acids, the pH drops, which causes phase transitions [41]. Richter, Martin [42] also used microorganisms for the conversion of syngas to ethanol through the

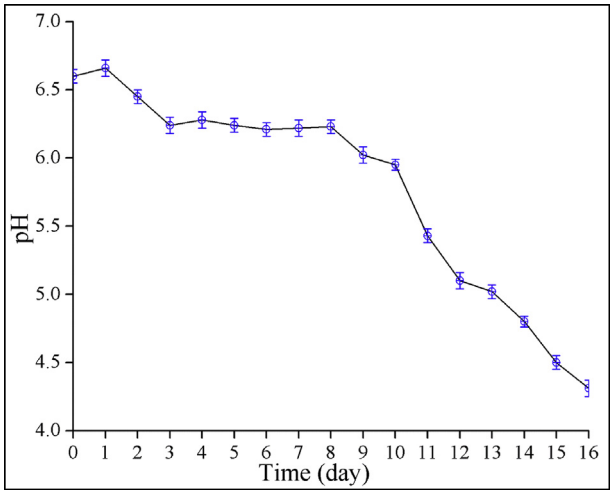

Fig. 5 – Effect of pH on syngas fermentation.

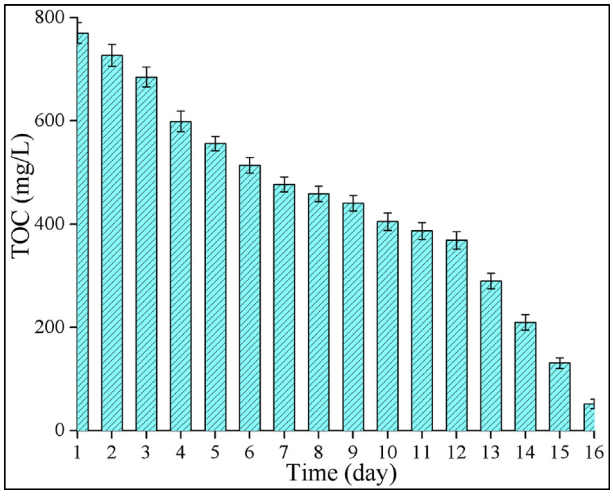

Fig. 6 – Effect of TOC on syngas fermentation.

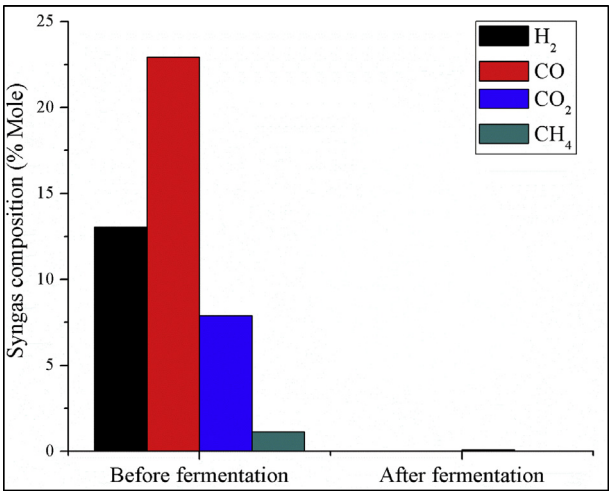

Fig. 7 – Effect of syngas composition before and after syngas fermentation.

fermentation system and they reported the effect of pH shift (4.31–5.60) on ethanol production. Therefore, bioethanol production was verified by the change of pH from the initial stage to the final stage. Similar observations were made in fermentation with *Saccharomyces cerevisiae* where the level of bioethanol needed to switch from acidogenesis to solventogenesis depended on the external pH of the fermentation broth during the syngas fermentation process. In the literature, it was reported that the ability of acetic acid generation was reduced when the pH value was around 4.5 and bioethanol concentration was not increased when pH decreased to 4.5 [43]. The findings of this study revealed the optimized pH level for bioethanol production by using *Saccharomyces cerevisiae*. In the fermentation media with pH level higher than 4.5, *Saccharomyces cerevisiae* cells were activated an adaptive response and resume cell growth after a long lag phase [43,44]. Thus, pH is one of the most important factors for bioethanol production.

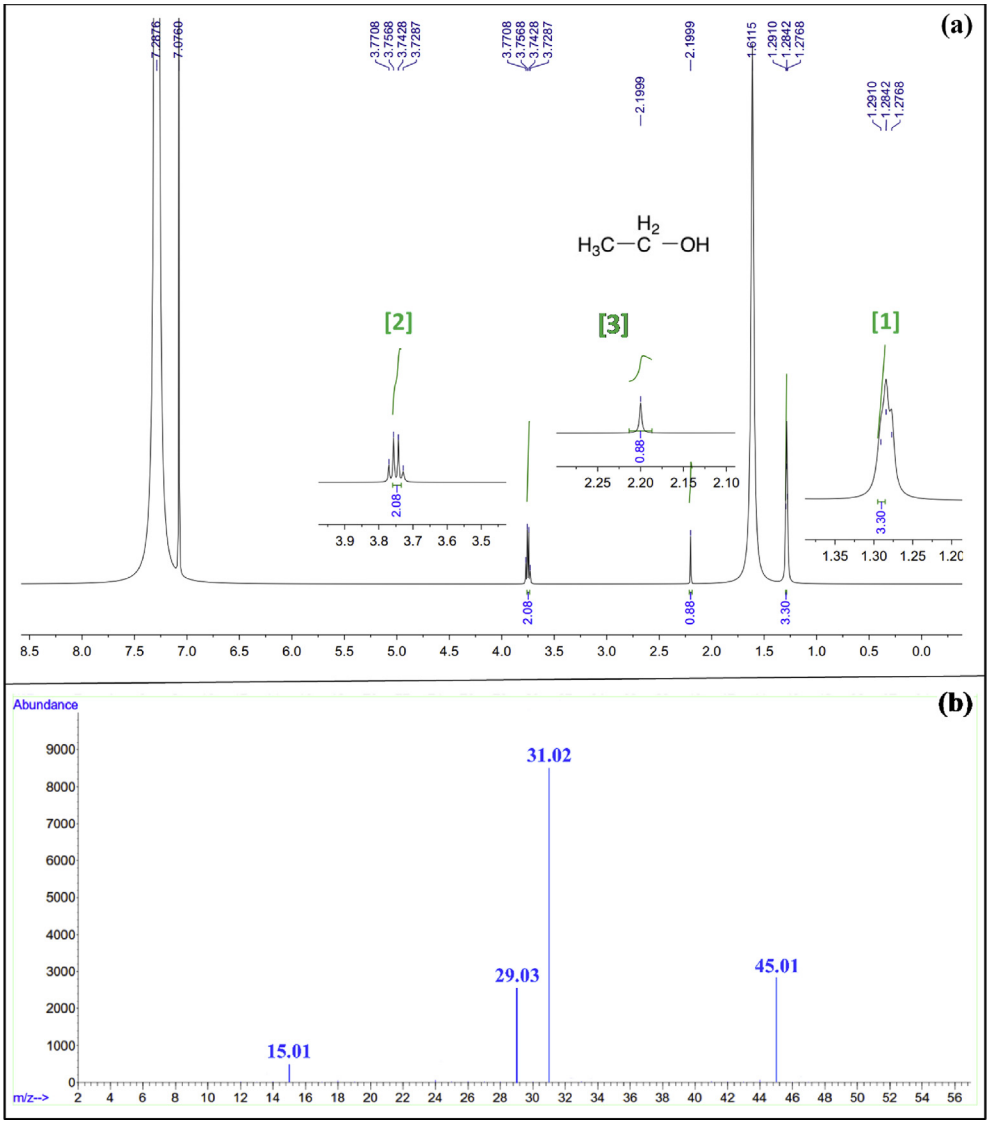

Fig. 8 – Syngas fermentation using *Saccharomyces Cerevisiae*: (a) ^{1}H NMR, 500 MHz ($CDCl_3$): $\delta = 3.75$ (q, $J = 7.00$ Hz, 2H [CH_2]), 2.19 (s, 1H [OH]), 1.28 (t, $J = 3.70$ Hz, 3H [CH_3]). (b) *Saccharomyces cerevisiae*-based bioethanol MS fraction (15.01:29.03:31.02:45.01).

Effect of time (day) on total organic carbon (TOC)

In this study, total organic carbon (TOC) in fermentation broth was also monitored throughout the whole process and results are shown in Fig. 6. Syngas and charcoal were the main gaseous carbon sources for *Saccharomyces cerevisiae*. These nutrients provided gaseous carbon of CO, CO_2, CH_4, etc. for the metabolism of *Saccharomyces cerevisiae*. From Fig. 6 it is found that organic carbon content was reduced from 770 gm/L to 52 gm/L from the initial stage to final stage, and *Saccharomyces cerevisiae* absorbed carbon slowly throughout the whole process. As a result, *Saccharomyces cerevisiae* was received carbon content from syngas and charcoal slowly and active until the 16th day. It is also observed that microorganism was slowly died due to the reduction of carbon sources. Therefore, during their lifetime bioethanol was produced which was detected by GC-MS and NMR (^{1}H) analysis (Fig. 8).

Effect of fermentation on syngas composition

Syngas composition was analyzed before and after syngas fermentation as shown in Fig. 7. Before fermentation, the composition of syngas were H_2-13.05%, CO-22.92%, CO_2-7.9%, CH_4 -1.13%, and after fermentation it was changed to 0.00%, 0.00%, 0.08% and 0.00%, respectively (Fig. 7 and S3). In this analysis, it is clearly shown that syngas composition was reduced significantly from the initial value, and it was found that except CO_2, the other two carbon-containing gases (CO and CH_4) were dissolved entirely with the fermentation broth. Therefore, it is evident that carbon-containing gases CO, CO_2, and CH_4 were mixed with fermentation broth, and microbes were taken gaseous carbon enormously. From this analysis, it can be deduced that *Saccharomyces cerevisiae* was taken carbon nutrient from syngas, and produced bioethanol which was detected by GC-MS and NMR (^{1}H) analysis (Fig. 8).

Bioethanol production and analysis

At the end of syngas fermentation, bioethanol was separated from fermentation broth and analyzed by NMR (^{1}H) and GC-MS study. The formation of bioethanol was confirmed by NMR (^{1}H) and yield was calculated by GC-MS analysis (Fig. 8a). The formation of bioethanol was detected by ^{1}H NMR spectrum when syngas was fermented using *Saccharomyces cerevisiae* (see Table 1). From this analysis, it is clearly shown that bioethanol gives a triplet signal at 1.28 ppm, which referred to the methyl group (-CH_3) along with the neighboring methylene group (–CH_2–) (Fig. 8a). A quartet signal indicated the presence of methylene group (–CH_2–) and the position of the peak of this signal at 3.75 ppm further confirmed the methylene group is connected with an oxygen atom. Additionally, a singlet peak appeared at 2.19 with one proton integral value which revealed that the presence of the hydroxyl group in the ethanol molecule. These results are consistent with the literature reported by Zuriarrain, Zuriarrain [45].

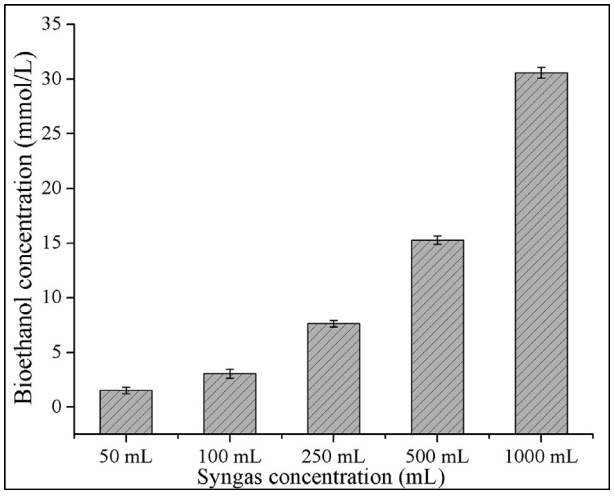

Fig. 9 – Bioethanol concentration from syngas fermentation using *Saccharomyces cerevisiae* considering 50 mL, 100 mL, 250 mL, 500 mL and 1000 mL containing syngas.

Table 1 – ^{1}H NMR data of bioethanol generated from *Saccharomyces cerevisiae*-based syngas fermentation.

Syngas fermentation	Peaks	^{1}H NMR (δ_H ppm)
S. cerevisiae based syngas fermentation	1	1.28 (t, CH_3)
	2	3.75 (*q*, OCH_2)
	3	2.19 (s, OH)

For the further confirmation of the formation of bioethanol molecule GC-MS analysis has been performed. From this analysis, it was observed that the MS fraction of bioethanol was 15.01:29.03:31.02:45.01 (Fig. 8b) which was similar as the standard MS fraction of 15:29:31:45 (S1).

The GC-MS results are shown in Fig. 8b. According to the literature, the MS for ethanol is 45. In this study, the obtained MS value from GC-MS analysis was 45.01. From the fragmentation data, it is shown that MS 31 is corresponding to $[CH_2$–$OH]^+$. The fragmentation of 31 is indicated that $[CH_2OH]^+$ that changed to the more stable cation of $[CH_3$=$O]$. The MS of 15 is corresponding to $[CH_3]^+$. In addition, the MS of 29 is corresponding to $[CH_3CH_2]^+$. Therefore, it is concluded that *Saccharomyces cerevisiae* was assisted to generate bioethanol from hydrogen-containing syngas. Moreover, by-product charcoal was contributed and assisted to the microbes for syngas fermentation by supplying carbon nutrient, trace elements and minerals which was detected by XRF analysis (S2). The concentration of bioethanol production using *Saccharomyces cerevisiae* using 50 mL, 100 mL, 250 mL, 500 mL and 1000 mL containing syngas was 1.53 mmol, 3.07 mmol, 7.64 mmol, 15.28 mmol and 30.56 mmol, respectively (Fig. 9). From the above analysis, it is confirmed that bioethanol was produced through syngas fermentation using *S. cerevisiae*. Therefore, biomass-based syngas was suited for bioethanol production.

Conclusions

In this study, hydrogen-rich syngas was taken from the co-gasification of forest residue and charcoal (70% and 30%) and further, it was fermented for the production of bioethanol using *Saccharomyces cerevisiae*. In the meantime, various effects were investigated throughout the syngas fermentation process. The results show that forest residue and charcoal-based hydrogen containing syngas are suitable for

bioethanol production. The concentration of bioethanol was relatively low, and therefore further research is required to determine how bioethanol production can be enhanced. The major findings of this study are as follows:

(1) The produced bioethanol was detected by NMR (^1H) spectra analysis and corresponding to methyle group (CH_3-), methylene group (–CH_2–) and hydroxyl group (OH).
(2) The bioethanol yield concentration was calculated using *Saccharomyces cerevisiae* and 50 mL, 100 mL, 250 mL, 500 mL, and 1000 mL of syngas were converted into 1.53 mmol, 3.07 mmol, 7.64 mmol, 15.28 mmol and 30.56 mmol of bioethanol, respectively.
(3) Therefore, hydrogen-containing syngas and by-product charcoal are the potential source of bioethanol for the fulfilment of future energy demand.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijhydene.2019.07.246.

REFERENCES

[1] Oumer AN, Hasan MM, Baheta AT, Mamat R, Abdullah AA. Bio-based liquid fuels as a source of renewable energy: a review. Renew Sustain Energy Rev 2018;88:82–98.
[2] Monir MU, Abd Aziz A, Kristanti RA, Yousuf A. Gasification of lignocellulosic biomass to produce syngas in a 50 kW downdraft reactor. Biomass Bioenergy 2018;119:335–45.
[3] Yousuf A, Sultana S, Monir MU, Karim A, Rahmaddulla SRB. Social business models for empowering the biogas technology. Energy Sources B Energy Econ Plan Policy 2017;12:99–109.
[4] Archer SA, Steinberger-Wilckens R. Systematic analysis of biomass derived fuels for fuel cells. Int J Hydrogen Energy 2018;43:23178–92.
[5] Capuano L. International energy outlook 2018 (IEO2018). Washington, DC, USA: US Energy Information Administration (EIA); 2018. p. 21. 2018.
[6] Wyman CE. Ethanol production from lignocellulosic biomass: overview. Handbook on Bioethanol. Routledge; 2018. p. 1–18.
[7] Basu P. Biomass gasification, pyrolysis and torrefaction: practical design and theory. Academic press; 2018.
[8] Gaurav N, Sivasankari S, Kiran GS, Ninawe A, Selvin J. Utilization of bioresources for sustainable biofuels: a Review. Renew Sustain Energy Rev 2017;73:205–14.
[9] Sahu SG, Chakraborty N, Sarkar P. Coal–biomass co-combustion: an overview. Renew Sustain Energy Rev 2014;39:575–86.
[10] Widjaya ER, Chen G, Bowtell L, Hills C. Gasification of non-woody biomass: a literature review. Renew Sustain Energy Rev 2018;89:184–93.
[11] Raud M, Tutt M, Olt J, Kikas T. Dependence of the hydrolysis efficiency on the lignin content in lignocellulosic material. Int J Hydrogen Energy 2016;41:16338–43.
[12] Sikarwar VS, Zhao M, Fennell PS, Shah N, Anthony EJ. Progress in biofuel production from gasification. Prog Energy Combust Sci 2017;61:189–248.
[13] Li Q, Song G, Xiao J, Sun T, Yang K. Exergy analysis of biomass staged-gasification for hydrogen-rich syngas. Int J Hydrogen Energy 2019;44:2569–79.
[14] Monir MU, Abd Aziz A, Kristanti RA, Yousuf A. Syngas production from Co-gasification of forest residue and charcoal in a pilot scale downdraft reactor. Waste and Biomass Valorization; 2018.
[15] Monir MU, Abd Aziz A, Kristanti RA, Yousuf A. Co-gasification of empty fruit bunch in a downdraft reactor: a pilot scale approach. Bioresour Technol Rep 2018;1:39–49.
[16] Monir MU, Yousuf A, Aziz AA, Atnaw SM. Enhancing Co-gasification of coconut shell by reusing char. Indian J Sci Technol 2017;10:1–4.
[17] Sun X, Atiyeh HK, Zhang H, Tanner RS, Huhnke RL. Enhanced ethanol production from syngas by Clostridium ragsdalei in continuous stirred tank reactor using medium with poultry litter biochar. Appl Energy 2019;236:1269–79.
[18] Liakakou E, Vreugdenhil B, Cerone N, Zimbardi F, Pinto F, André R, et al. Gasification of lignin-rich residues for the production of biofuels via syngas fermentation: comparison of gasification technologies. Fuel 2019;251:580–92.
[19] Daniell J, Köpke M, Simpson S. Commercial biomass syngas fermentation. Energies 2012;5:5372–417.
[20] Chaurasia A. Modeling of downdraft gasification process: studies on particle geometries in thermally thick regime. Energy 2018;142:991–1009.
[21] Munasinghe PC, Khanal SK. Chapter 4 - biomass-derived syngas fermentation into biofuels A2 - pandey, ashok. In: Larroche C, Ricke SC, Dussap C-G, Gnansounou E, editors. Biofuels. Amsterdam: Academic Press; 2011. p. 79–98.
[22] Sayed ET, Abdelkareem MA. Yeast as a biocatalyst in microbial fuel cell. Old yeasts-new questions. InTech; 2017.
[23] Foo JL, Susanto AV, Keasling JD, Leong SSJ, Chang MWJB. bioengineering. Whole-cell biocatalytic and de novo production of alkanes from free fatty acids in Saccharomyces cerevisiae, vol. 114; 2017. p. 232–7.
[24] Davis R, Molloy S, Quigley B, Nikodinovic-Runic J, Solano F, KEJAm O'Connor, et al. Biocatalytic versatility of engineered and wild-type tyrosinase from R. solanacearum for the synthesis of 4-halocatechols. 2018. p. 1–11.
[25] Asimakopoulos K, Gavala HN, Skiadas IV. Reactor systems for syngas fermentation processes: a review. Chem Eng J 2018;348:732–44.
[26] Rodionova MV, Poudyal RS, Tiwari I, Voloshin RA, Zharmukhamedov SK, Nam HG, et al. Biofuel production: challenges and opportunities. Int J Hydrogen Energy 2017;42:8450–61.
[27] Acharya B, Roy P, Dutta A. Review of syngas fermentation processes for bioethanol. Biofuels 2014;5:551–64.
[28] Yousuf A, Khan MR, Islam A, Monir MU, Ab Wahid Z, Pirozzi D. Application of electroporation technique in biofuel processing. MATEC web of conferences. EDP Sciences; 2017. p. 01085.
[29] Alfenore S, Molina-Jouve C. Current status and future prospects of conversion of lignocellulosic resources to

biofuels using yeasts and bacteria. Process Biochem 2016;51:1747–56.
[30] Hossain N, Zaini JH, Mahlia T. A review of bioethanol production from plant-based waste biomass by yeast fermentation. Int J Technol 2017;8:5–18.
[31] Akhtar MN. Biofuel: a stepping stone towards sustainable development. Int J Technol BioSci 2016;5:1–12.
[32] Shen Y, Brown R, Wen Z. Syngas fermentation of Clostridium carboxidivoran P7 in a hollow fiber membrane biofilm reactor: evaluating the mass transfer coefficient and ethanol production performance. Biochem Eng J 2014;85:21–9.
[33] Shen Y, Brown RC, Wen Z. Syngas fermentation by Clostridium carboxidivorans P7 in a horizontal rotating packed bed biofilm reactor with enhanced ethanol production. Appl Energy 2017;187:585–94.
[34] Islam MA, Yousuf A, Karim A, Pirozzi D, Khan MR, Wahid ZA. Bioremediation of palm oil mill effluent and lipid production by Lipomyces starkeyi: a combined approach. J Clean Prod 2018;172:1779–87.
[35] Elgharbawy AA, Alam MZ, Moniruzzaman M, Kabbashi NA, Jamal P. Chemical and structural changes of pretreated empty fruit bunch (EFB) in ionic liquid-cellulase compatible system for fermentability to bioethanol. 3 Biotech 2018;8:236.
[36] Elgharbawy AA, Alam MZ, Moniruzzaman M, Ahmad Kabbashi N, Jamal P. High yield of second-generation ethanol in an ionic Liquid-Cellulase integrated system for single-step processing of empty fruit bunch. Biofuels 2019:1–12.
[37] Zhao J, Lin M, Chen G. Facile recycling of Escherichia coli and Saccharomyces cerevisiae cells from suspensions using magnetic modification method and mechanism analysis. Colloids Surfaces B Biointerfaces 2018;169:1–9.
[38] Sinharoy A, Baskaran D, Pakshirajan K. Sustainable biohydrogen production by dark fermentation using carbon monoxide as the sole carbon and energy source. Int J Hydrogen Energy 2019;44:13114–25.
[39] Ahmed A, Cateni BG, Huhnke RL, Lewis RS. Effects of biomass-generated producer gas constituents on cell growth, product distribution and hydrogenase activity of Clostridium carboxidivorans P7T. Biomass Bioenergy 2006;30:665–72.
[40] Xu D, Tree DR, Lewis RS. The effects of syngas impurities on syngas fermentation to liquid fuels. Biomass Bioenergy 2011;35:2690–6.
[41] Abubackar HN, Veiga MC, Kennes C. Biological conversion of carbon monoxide to ethanol: effect of pH, gas pressure, reducing agent and yeast extract. Bioresour Technol 2012;114:518–22.
[42] Richter H, Martin M, Angenent L. A two-stage continuous fermentation system for conversion of syngas into ethanol. Energies 2013;6:3987–4000.
[43] Liu C, Luo G, Wang W, He Y, Zhang R, Liu G. The effects of pH and temperature on the acetate production and microbial community compositions by syngas fermentation. Fuel 2018;224:537–44.
[44] De Klerk C, Fosso-Kankeu E, Du Plessis L, Marx S. Assessment of the viability of Saccharomyces cerevisiae in response to synergetic inhibition during bioethanol production. Curr Sci 2018;115:1124–32.
[45] Zuriarrain A, Zuriarrain J, Villar M, Berregi I. Quantitative determination of ethanol in cider by 1H NMR spectrometry. Food Control 2015;50:758–62.