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Development and evaluation of a functional bioreactor for CO fermentation into ethanol

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Abstract

Background: In a conventional syngas fermentation process, gas was released into the fermentation broth through a single orifice or multiple orifices, except the hollow fiber membrane reactor. Consequently, a simplified bioreactor has been developed employing an innovative gas supply and effluent extraction systems.

Results: A continuous stirred tank bioreactor (CSTBR) has been developed by incorporating an innovative gas supply and effluent extraction system to ferment syngas into ethanol. The working volume of the bioreactor was controlled to 2 L. The CO gas was fermented in the developed bioreactor by using a microorganism (*Clostridium ljungdahlii*) with different gas (5–15 mL/min), media, and effluent flow rates (0.25–0.75 mL/min) and stirrer speed (300–500 rpm). Gas was diffused into the fermenting broth through an aqueous aeration tube commonly used in the small household aquarium, placed at the bottom layer throughout the periphery. The effluent was extracted from the top layer of the broth by using a membrane separator. Ethanol and acetic acid concentrations were varied from 0.17–1.17 and 8.50–23.68 g/L-effluent, respectively.

Conclusions: It seems that the performance of CSTBR can be enhanced with an innovative gas supply system, which may reduce the gas bubble size and result in higher lateral velocity at the releasing point, especially, throughout the periphery instead of the center of the reactor through a single or multiple orifice.

Keywords: Bioreactor, Continuous stirred tank, Microorganism, CO, Fermentation, Ethanol

Background

Liquid biofuels are identified to be the alternatives to fossil gasoline. Production and utilization of lignocellulosic ethanol (hereafter referred to ethanol) have been emphasized because it does not compete with food crops. Ethanol has been produced from lignocellulosic biomass (hereafter referred to biomass) by both biochemical and thermochemical conversion technologies. Although each process has its advantages and disadvantages in ethanol production from biomass, biochemical dominates over the thermochemical process (Subramani and Gangwal 2008). The biochemical conversion process requires higher pretreatment and enzyme costs, has low fermentability of the mixed sugar stream (C_5), and generates inhibitory soluble compounds (Munasinghe and Khanal 2010). In addition, non-carbohydrate materials in biomass cannot be converted into ethanol in the biochemical conversion process (Henstra et al. 2007) and require special care for downstream waste management. Conversely, several authors noted that thermochemical (gasification) conversion process converts all the components of biomass into syngas with nearly equal efficiency and effectiveness (Pereira et al. 2012; Weber et al. 2010), eliminates complex pretreatment and costly enzyme requirement (Munasinghe and Khanal 2010), and eases the downstream waste management processes. Moreover, industrial off-gas/natural gas/syngas extracted from biomass can be a cheaper feedstock for producing ethanol through syngas fermentation (Jiang et al. 2015).

Ethanol can be produced from syngas through a catalytic or bio-synthesis thermochemical conversion. The

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catalytic process requires expensive catalyst (metal-based or modified methanol catalysts), higher operating temperature, and pressure (Subramani and Gangwal 2008), which therefore increases the capital and operating costs (Datta et al. 2011). In contrast, some of the biological catalysts are able to convert syngas into ethanol more effectively, even at atmospheric pressure (Munasinghe and Khanal 2010; Henstra et al. 2007; Elshahed 2010; Heiskanen et al. 2007), while reducing the energy requirements, as well as the capital and operating costs (Daniell et al. 2012). Higher selectivity of biological catalysts reduces undesired byproducts and improves ethanol yield and downstream processes (Griffin and Schultz 2012; Abubackar et al. 2011). However, lower gas–liquid mass transfer and production of inhibitory compounds during fermentation were noted to be the main constraints to syngas fermentation into ethanol (Munasinghe and Khanal 2010; Huhnke 2013; Lee P 2010).

The mass transfer between substrate and microbes is dependent on the level of syngas mixing with the fermentation media which provide greater interfacial area (gas–liquid) and retention time in the fermentation media. Higher agitation speed tends to produce finer bubbles, which result in decreased rising velocity in fermentation media, improved microbe accessibility to syngas, and improved mass transfer rates (Munasinghe and Khanal 2010). Syngas fermentation has been extensively studied with various types of bioreactors (Huhnke 2013; Shen et al. 2014; Richter et al. 2013; Mohammadi et al. 2012). The continuous culture was noted to be advantageous, when compared to a batch culture in a fermentation system (Richter et al. 2013). The mass transfer reported to be improved with an increase in the agitation speed (impeller speed) or gas flow rate; however, the process was not economical because of higher energy consumption and stress to microorganisms (Ungerman and Heindel 2007; Bredwell and Worden 1998). The higher the gas flow rate, the greater the syngas loss into the exhaust line (Bredwell et al. 1999). The stress, either mechanical, from inhibitory compounds, or other experimental parameters (especially, the cell density) affects the productivity (Mohammadi et al. 2011; Alsaker et al. 2010). Till to date in a conventional syngas fermentation process gas was released into the fermentation broth through a single orifice or multiple orifices (Mohammadi et al. 2012; Cotter et al. 2009; Younesi et al. 2006) in both the batch and continuous process, except for a few examples, especially, the hollow fiber membrane reactor (Shen et al. 2014; Richter et al. 2013). Consequently, this study attempts to develop a simplified continuous stirred tank bioreactor (CSTBR) employing an innovative gas supply and effluent extraction systems to enhance gas–liquid mass transfer, reduce inhibitory stress, and improve ethanol productivity.

Methods

Reactor development

A laboratory scale bioreactor (3 L) has been designed and developed to expedite the conversion process considering the following parameters: innovative, flexible, easy monitoring, durability, safety, easy assemble and disassemble, easy cleaning, and inexpensive. The low-cost gas diffuser was placed in the bottom layer and on the periphery of the fermentation broth, and an in situ cell retention filter was placed on the upper layer of the broth, which were the innovative ideas in this reactor development. A vent was also incorporated for flexibility of operation, i.e., the reactor can be operated in either anaerobic or aerobic condition. The material safety data sheet was strictly followed in the low-cost materials selection process to ensure the safety and durability. The reactor vessel was with a digital pressure gage, and a meter with a pH and temperature probe for the purpose of easy monitoring. The reactor lid (contains an O-ring which seals the gap between the vessel and the lid) was also made from a transparent PVC sheet. All the components were incorporated into the reactor through the lid which can easily be assembled and disassembled, which facilitates easy cleaning.

The working volume of the reactor was 2 L. The innovative gas diffusion method system may improve gas retention time, thus a higher gas–liquid mass transfer and ethanol concentration. The reactor was equipped with a micropump (GF-F155001, Gilson Inc., USA), gas flow meter (PMR1-0106018, Cole Parmer, QC, Canada), temperature and pH meter (PHE-1411, Omega Environmental, Inc., Laval, QC, Canada), and pressure gage (PHH-222, Omega Environmental, Inc., Laval, QC, Canada) to monitor and control the working temperature, pH, and pressure, respectively. The tubing for the micropump was selected based on the desired flow rates (F117938). The temperature and pH meter were braced with a temperature (TP-07) and pH probe. The reactor also consists of a membrane support to place the membrane evenly in the upper layer of the fermentation broth. The reactor was manufactured and assembled at the University of Guelph, Ontario, Canada.

Reactor tank, and media and effluent jar

Transparent PVC pipe (PVC-9002-86-2) has been used for the tank (3 L). Plexiglass sheet (VH-100 Acrylic Resin) was used for the base and the lid of the reactor. The ratio of diameter and height of the reactor tank was selected to be 2/3. Readily available glass jars (2 L) have been used as the media and effluent containers. The materials and accessories used for the bioreactor development are reported in the supporting information (Additional file 1: SI-1-1).

Gas supply system

Syngas fermentation into ethanol was noted to be dependent on the mass transfer between gas and liquid (Huhnke 2013; Lee 2010). The mass transfer can be improved by increasing the residence time of gas in the aqueous media (Lee 2010). The bubbling technology helps in improving the gas retention time in the aqueous media. Although the size of bubbles affects the retention time, an aeration tube (that is commonly used in small household aquariums) has been selected to create a circular gas supply system (diameter is 4 cm smaller than the reactor's inside diameter) and placed at the center of the bioreactor with the help of a holder keeping a clearance of about 1 cm from the bottom (Additional file 1: SI-1-2). The purpose of the holder is so that gas can be diffused at the peripheral area of the fermentation broth, which can easily be replaced with other bubbling systems.

Effluent extraction system (membrane separator)

The membrane separation technology has been adopted to facilitate the continuous extraction of effluent from the fermentation broth excluding the microorganism; thus, the microorganism can be reused. An effluent extraction system has been developed and evenly placed in the top layer of the fermentation broth (Additional file 1: SI-1-3). The required length of the membrane fiber (PVDF, GE: 0.02 μm) has been determined based on the flux of the selected membrane (10 L/m^2/h) and the required effluent extraction rate. A membrane support has also been used to hold the membrane fiber at the same level (Additional file 1: SI-1-4) in the upper layer of the fermentation broth.

Experimental setup

The complete experimental setup was placed in an incubator (Heratherm IGS60, Thermo Electron LED GmbH, Germany) at 37 °C (Fig. 1). A bypass tube was used to connect the reactor and the media-jar. Another tube from the media-jar was connected to the exhaust tube to release the excess gas if any. The media and effluent (ethanol mixture) were supplied and extracted from the reactor with a micropump, respectively.

Microorganism and media

American-Type Culture Collection (ATCC#55380; *Clostridium ljungdahlii*) has been purchased from Cedarlane, Burlington, Ontario, Canada and used in this study.

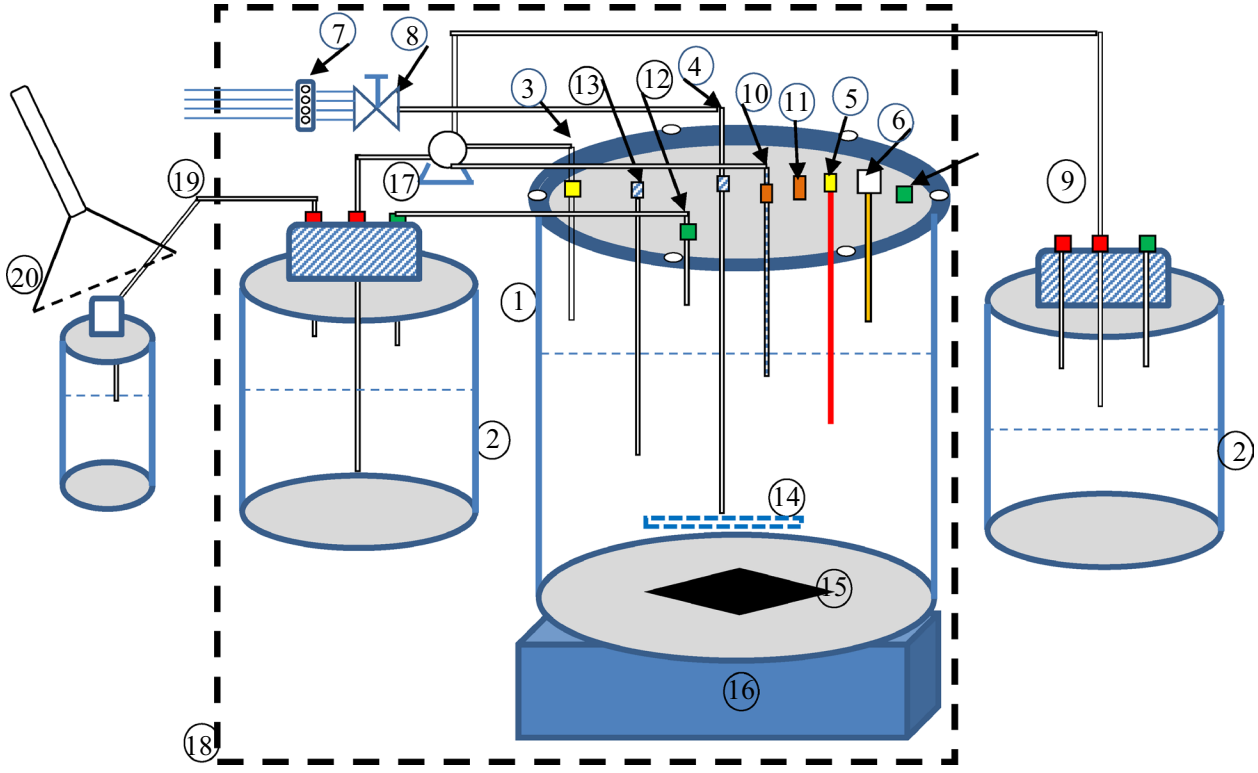

Fig. 1 Schematic diagram of the experimental setup. *1*. Reactor (3 L), *2*. Glass bottle (2 L), *3*. Medium inlet, *4*. Gas inlet, *5*. Temperature probe, *6*. Pressure meter, *7*. Gas controller, *8*. Control valve, *9*. Vent, *10*. Membrane sampler/extraction port, *11*. Gas sampling port (gas impermeable butyl rubber stopper), *12*. Bypass line, *13*. pH meter, *14*. Diffuser, *15*. Stirrer, *16*. Stirring plate, *17*. Pump, *18*. Incubator, *19*. Exhaust tube, and *20*. Fume hood

The recommended broth media have been prepared in the laboratory based on the preparation manual supplied by Cedarlane. The components of the broth media and the production procedure are reported in the supporting information (Additional file 1: SI-1-5). Freeze-dried (0.4 mL) microorganism was aseptically transferred into the broth media in test tubes, cultured and propagated anaerobically in the broth media at 37 °C in an incubator, and then used in the syngas fermentation process.

CO fermentation

The fermenting media (the components have been reported in Additional file 1: SI-1-5) and propagated microorganism were poured into the reactor with a working volume of 2 L and placed in an incubator for fermentation (Additional file 1: SI-1-6). An anaerobic chamber has been used to pour the microorganism and media into the reactor (Additional file 1: S-1-7), which was equipped with a vacuum pump and N_2 supply. The fermentation experiment was conducted at anaerobic condition and at the atmospheric pressure. The pH and stirrer speed in the fermentation broth were controlled to 4.0–5.0 (by adding 1 N NaOH if required, i.e., it was added manually only if pH dropped to less than four) and 300–500 rpm, respectively (Richter et al. 2013; Mohammadi et al. 2012; Younesi et al. 2006). The syngas, media, and effluent flow rates were controlled to 5–15 mL/min, 0.25–0.75 mL/min, and 0.25–0.75 mL/min, respectively. The reactor was operated continuously, after the initial 2 days of batch condition. The quality and composition of syngas from biomass was noted to be dependent on the type of feedstock and gasification parameters. All of the components of feedstock can be converted into syngas (usually, H_2, CO, CO_2, and CH_4) with a trace amount of other gases and a few residues (tar and ash) (He and Zhang 2011). Although a wide range of syngas composition has been used in the syngas fermentation process (Shen et al. 2014; Richter et al. 2013; Kundiyana et al. 2011), only the CO was fermented in this study (Chang et al. 1998) to prove the concept of the developed reactor. Fermentation media contains dextrose, acetate, etc., which might also be a trace carbon source in the fermentation process.

Analytical method

The GC–MS system (Agilent Technologies, USA) consists of a gas chromatograph (7890A) and a mass spectrometer (59756MS) that have been used to analyze the liquid effluent and quantify the presence of ethanol and acetic acid. The GC–MS system was equipped with a Bruker BR-SWAX column (30 m × 0.25 mm I.D. with a 0.25-μm phase thickness). The oven was programed to maintain the initial temperature at 44 °C for 3.5 min and allowed to increase at a rate of 5 °C/min to 200 °C. Immediately after this phase, 70 °C/min heating rate was maintained until it reached to 250 °C. The carrier gas (He) flow rate was 1 mL/min. The sample was injected manually in a splitless mode at 280 °C. Liquid samples (0.5 mL) were transferred into sealed vials (15 mL) and incubated for 5 min at 75 °C. The incubated sample was then equilibrated with a 75 μm carboxen–polydimethylsiloxane (CAR–PDMS) fiber immersed in the headspace for 20 min. The volatile compounds were then thermally desorbed in the injector port by manually injecting and exposing the fiber for 8 min. The mass spectrometer was scanned from *m/z* 10–150 at an interval of 1 s. The ionization was created by an electronic impact at 200 °C, while the transfer line was kept at 250 °C. The data were obtained in a positive ion mode. The compounds were also extracted manually with a SPME holder (Supelco, USA), a hotplate, and a metal support with clamps.

Results and discussion

pH profile during CO fermentation

Several studies revealed that CO can be converted into many value-added products (ethanol, acetic acid, propanoic acids, propanol, nonanoic acid, benzaldehyde etc.) in the presence of microorganisms (Younesi et al. 2006; Roy 2014). In the initial stages of this experiment, pH was decreased rapidly and then stabilized to about 4.5. The production of organic acids might result to this rapid change. The rapid change in pH was observed in the first 2 days, while the experiment ran on the batch process, and decreased from 5.8 to about 5.0. The pH varied from 5.0 to 4.5 in the continuous process. This variation was caused by the media, effluent and gas flow rates, and ethanol productivity. During the initial stage, acetic acid might also be the dominant product, which causes the rapid fall of pH in the fermentation broth (Fig. 2).

Effect of stirrer speed on products production

Ethanol and acetic acid production rate was observed to be 0.22, 0.30, and 1.17 g/L-effluent and 11.14, 15.20, and 23.68 g/L-effluent for a stirrer speed of 300, 400, and 500 rpm, respectively, while media and effluent rate was 0.25 mL/min (Fig. 3). The bars in the figures are the standard errors of the replicated experiments (i.e., the experiment replicated twice). Ethanol and acetic acid production was found to be increased with an increase in stirrer speed ranging from 300–500 rpm. The ratio of ethanol and acetic acid was found to be increased from 0.02 to 0.05. The acetic acid concentration was rapidly decreased at this stage, probably due to conversion of acetic acid into ethanol by the microorganism (Ukpong et al. 2012; Ramachandriya et al. 2011). The ethanol concentration was also reported to be significantly improved

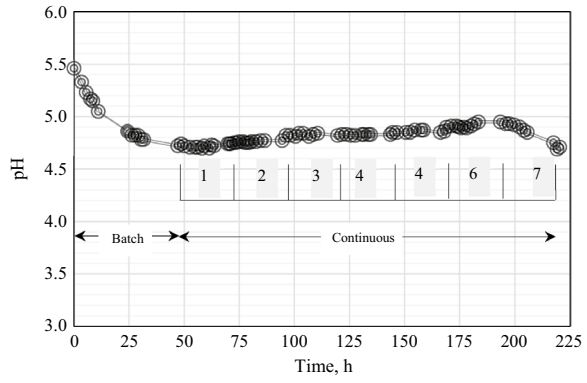

Fig. 2 Variation of pH during the experiment. 1*^; 2*^; 3*^; 4**. Gas: 10 mL/min, media and effluent: 0.25 mL/min; 5***. Gas: 15 mL/min, media and effluent: 0.25 mL/min; 6***. Gas: 10 mL/min, media and effluent: 0.5 mL/min; 7***. Gas: 10 mL/min, media and effluent: 0.75 mL/min; *300 rpm; **400 rpm; ***500 rpm; ^ Gas: 5 mL/min, media and effluent: 0.25 mL/min

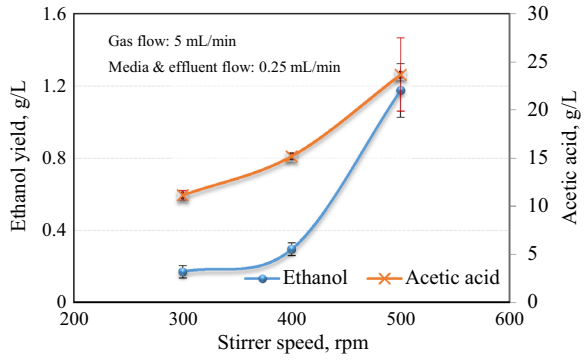

Fig. 3 Effect of stirrer speed on ethanol and acetic acid yield

with *C. ljungdahlii* when the pH dropped to 4.0–4.5 (Klasson et al. 1993), because the microorganism produces less acid, which prevents a further pH drop (Datar et al. 2004) and induced the higher ethanol concentration. Bakker et al. (1994) noted that an increase in agitation intensity leads to an increase in gas–liquid mass transfer. At a certain gas flow, an increase in impeller speed leads to depression of the gas radially. In this stage, the gas bubbles reached the vessel wall, but did not recirculate. The gas bubbles recirculate throughout the tank, if the impeller speed is increased further, known as complete dispersion, beyond that, flow patterns does not change; however, mass transfer rate increases. Stirrer speed helps raising the rotational movements of fermenting broth in the reactor; thus, the gas bubble released in the broth gets greater lateral speed (curvilinear motion because of the resultant speed induced by buoyancy and lateral speed), which allowed the gas bubble to travel a longer path before being diffused into the headspace of the reactor. Bubble movement toward the center of the vortex was caused by the convective flow and the pressure gradient. The tangential velocity of the vortex was noted to be proportional to the agitation speed. The higher the stirrer speed, the stronger the vortex (Sudiyo and Andersson 2007). Agitation also helps to break the bubble and enhance gas retention time and increase the gas–liquid interfacial area for mass transfer (van Kasteren JMN et al. 2005; Bakker et al. 1994). The gas conversion rate was noted to be increased with an increase in agitation speed (Younesi et al. 2006). The mass transfer also reported to be dependent on the reactor configuration (Huhnke 2013). It seems the combination of reactor configuration and higher stirrer speed not only helped to break the bubble, but also induced greater lateral speed for the bubble, consequently, increasing the gas–liquid interfacial area which might have improved the gas–liquid mass transfer. The combination of higher initial lateral speed of the gas bubble, longer retention time, and greater interfacial area led to the improvement of product concentration.

Effect of gas, media, and effluent flow rates on products production

The product concentration was dependent on the gas, media, and effluent flow rates. Ethanol and acetic acid concentrations varied from 0.27–1.17 and 8.59–23.68 g/L, respectively, depending on the gas flow rates (Fig. 4a). The product concentration was found to decrease with an increase in gas flow rate, which seems to be supported by another researcher (Shen et al. 2014). The authors reported that after a certain gas flow rate, ethanol production decreased with a further increase in gas flow rate; thus, for the optimum production, gas flow rate might need to be selected based on the working volume of the reactor. High gas flow rate adversely affects the gas conversion (Bredwell et al. 1999). Devarapalli et al. (Huhnke 2013) have also reported that gas–liquid mass transfer characteristic was affected by gas, media, and effluent flow rates. The culture may have also become inhibited by the increased supply of CO. The combination of increased flow rate and stirrer speed might have induced stress to the microorganisms (Ungerman and Heindel 2007; Bredwell and Worden 1998; Alsaker et al. 2010) and resulted in lower product concentration.

Product concentration was also decreased with the increase of media and effluent flow rates, which might be because of the higher dilution rate. Ethanol and acetic acid concentrations varied from 0.21–0.31 and 8.50–20.69 g/L, respectively (Fig. 4b). Shen et al. (2014) reported that in the case of CSTBR, dilution rate had an effect on the syngas fermentation process. Lower dilution

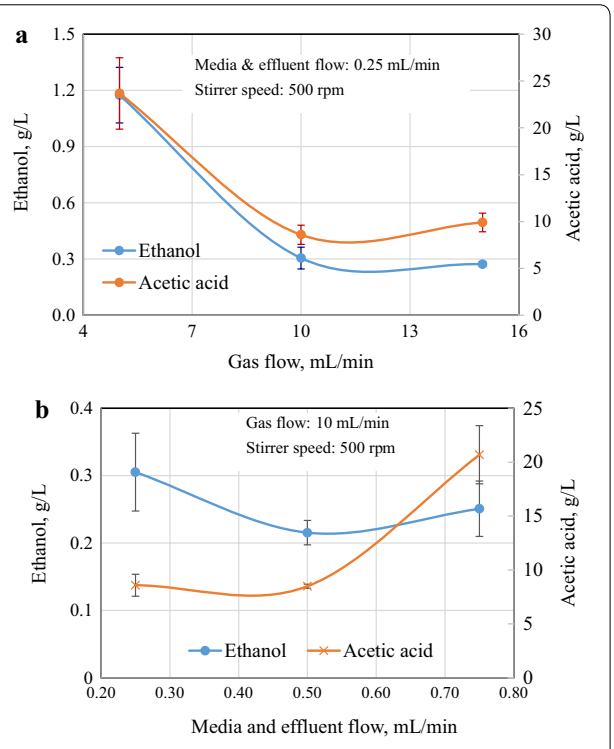

Fig. 4 Effect of gas, media, and effluent flow rates on ethanol and acetic acid yields

rate improved productivity (Richter et al. 2013; Mohammadi et al. 2012). The greater media flow increases the dilution rate, thus the ethanol concentration was decreased. *However, the acetic acid concentration observed to be improved*, might be because of greater gas flow rate which restricts ethanol productivity after certain flow rate (Shen et al. 2014) as well as media (content a bit of acetate) flow rate. Therefore, the highest ethanol concentration was observed at a flow rate of 0.25 mL/min in this study. It seems the productivity of acetic acid can be controlled by restricting the gas, media, and effluent flow rates (Fig. 4).

The combination of ethanol and acetic acid productivity varied from 10.83–24.06 g/L when only CO was fermented in this study. The ethanol concentration was reported to be 0.55 g/L (Younesi et al. 2005), where synthesis gas had been used. The combined (ethanol and acetic acid) production from synthesis gas (CO, H_2, CO_2, Ar) fermentation in a CSTBR was also reported to be 11 g/L (Younesi et al. 2006). The ethanol concentration was 20.7 g/L in the case of a two stage CSTBR equipped with a hollow fiber membrane module (Richter et al. 2013). In contrast, the maximum productions of ethanol and acetate were 6.50 g/L and 5.43 g/L, respectively (Ungerman and Heindel 2007), but the combined yield was 11.93 g/L. A wide range of ethanol concentration (0.02–0.65 g/L) has also been reported in the case of batch processes (Abubackar et al. 2011; Kundiyana et al. 2011; Younesi et al. 2005, 2006; Najafpour and Younesi 2006). The formation of ethanol inhibits the activity of *C. ljungdahlii* (Younesi et al. 2005); thus, continuous removal of ethanol from the fermentation broth improves ethanol production. The ethanol production in a bubble column reactor was 0.56 g/L (Rajagopalan et al. 2002). Conversely, higher ethanol concentration (23 g/L) was reported in the case of hollow fiber membrane reactor (Shen et al. 2014). Table 1 represents a brief summary of the biosyngas fermentation processes and ethanol concentration reported in the literature.

Ethanol concentration was noted to be dependent on the mass transfer efficiency (Munasinghe and Khanal 2010; Huhnke 2013; Lee et al. 2012), and the characteristics of the biofilm (Shen et al. 2014). The mass transfer was also dependent on the reactor configuration, agitation speed, syngas, and media flow rates (Huhnke 2013; van Kasteren JMN et al. 2005). Hollow fiber membrane reported to be effective in increasing the gas–liquid mass transfer, thus the ethanol production (Shen et al. 2014; Richter et al. 2013; Lee et al. 2012). Gas–liquid mass transfer was noted to be dependent on the retention time of gas in the fermentation broth and the size of the bubble. The ethanol production was observed to be dependent on the bioreactor design, especially gas–liquid mass transfer. The position of the syngas supply systems in the reactor may also have an influence on gas retention time (Additional file 1: SI-1-8), thus the gas–liquid mass transfer. Gas supply throughout the periphery in a CSTBR improves the gas retention (Additional file 1: SI-1-8) time compared to the gas supply at the center because of higher speed of the gas bubbles at the periphery (Additional file 2: SI-2). The broth circling near the outer rim moves faster and experiences greater forces (since the force is proportional to velocity) compared to the middle or the center. Therefore, the gas bubble released at the outer surface gets greater initial horizontal velocity and travels longer distances before ending to the surface layer of the broth, thus increased the retention time. In addition, the bubbles tend to move toward the center (lateral movement) due to the vortex resulted by the stirrer (Sudiyo and Andersson 2007), and help improving gas–liquid mixing, thus gas–liquid mass transfer. The effluent extraction method helps in situ cell retention and extraction of products and water mixture, which might have stipulated in the higher ethanol concentration compared to some of the other studies.

Conclusions

This study revealed that the developed bioreactor can be used for syngas fermentation into ethanol. The ethanol

Table 1 Syngas fermentation parameters and ethanol yields

Experimental conditions						Concentration	Reference
Reactor	Microbe	Cell density	Syngas	Temp. °C	pH		
CSTR (3 L)	*C. ljungdahlii*, ATCC#55380	–	100 % CO	37	4.0–5.0	Ethanol: 0.17–1.17 g/L; acetic acid: 8.50–23.68 g/L	This study
Two stage: 1 L CSTR and 4 L bubble column	*C. Ljungdahlii*, ATCC#55383	10 g/L	60 % CO, 35 % H_2, and 5 % CO_2	37	5.5 (stage 1) 4.4–4.8 (stage 2)	Ethanol: 20.7 g/L	Richter et al. (2013)
CSTR (2 L)	*C. ljungdahlii*, ATCC#55383	2.34 g/L	55 % CO, 20 % H_2, 10 % CO_2 and 15 % argon	37	4.0	Ethanol: 6.50 g/L Acetate: 5.43 g/L	Mohammadi etal. (2012)
CSTR	*C. ljungdahlii, ATCC# 55383*		55 % CO, 5 % CO_2, 20 % H_2, and 15 % Ar			Ethanol: 0.55 g/L, Acetate: 1.3 g/L	Younesi et al. (2005)
CSTR (20 L)	*C. ljungdahlii*, ATCC#55383	2.0	55 % CO, 20 % H_2, 10 % CO_2, and 15 % Ar	37	4.5	Ethanol and Acetate: 11.0 g/L	Younesi et al. (2006)
HFM-BR (8 L working volume)	*C. carboxidivorans P7*	–	50 % CO, 30 % H_2,20 % CO_2	37	4.5–5.5	Ethanol: 23.93 g/L	Shen et al. (2014)
HBM-BR (3.3 L working volume)	*A. bacchi strain CP15 (56 %) and Clostridium propionicum (34 %)*		20–39 % CO, 24–25 % CO_2, 10–43 % H_2, 10–12 % N_2	37	–	Ethanol: 8 g/L	Liu et al. (2014)
HFM-R (125 mL)	*C. ljungdahlii*, ATCC#55383	–	50 % CO, 30 % H_2, and 20 % CO_2	35	–	Ethanol: 6 g/L; Acetate: 2.3 g/L	Lee (2010)
Gas lift reactor	*Eubacterium limosum*	0.75 g/L	100 % CO	37	6.8	Butyrate, acetate, Ethanol: 1.75 g/L	Chang et al. (1998)

CSTR Continuous stirred tank reactor; *HFM-BR* Hollow fiber membrane biofilm reactor

yield was observed to be varied from 0.17–1.17 g/L-effluent, while only CO was fermented. The yield was found to be dependent on the experimental parameters. It seems that an innovative gas supply system may reduce the gas bubble size and provide higher lateral velocity at the releasing point, especially, throughout the periphery instead of the center of the reactor through a single or multiple orifice.

Additional files

Additional file 1. Accessories of the developed bioreactor.

Additional file 2. Bubble movement.

Authors' contributions

PR has conducted the experiments and analysis for this work. He was guided by Dr. AD and Dr. SC. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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