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# Syngas fermentation in a 100-L pilot scale fermentor: Design and process considerations

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**Fermentation of syngas offers several advantages compared to chemical catalysts such as higher specificity of biocatalysts, lower energy costs, and higher carbon efficiency. Scale-up of syngas fermentation from a bench scale to a pilot scale fermentor is a critical step leading to commercialization. The primary objective of this research was to install and commission a pilot scale fermentor, and subsequently scale-up the *Clostridium* strain P11 fermentation from a 7.5-L fermentor to a pilot scale 100-L fermentor. Initial preparation and fermentations were conducted in strictly anaerobic conditions. The fermentation system was maintained in a batch mode with continuous syngas supply. The effect of anaerobic fermentation in a pilot scale fermentor was evaluated. In addition, the impact of improving the syngas mass transfer coefficient on the utilization and product formation was studied. Results indicate a six fold improvement in ethanol concentration compared to serum bottle fermentation, and formation of other compounds such as isopropyl alcohol, acetic acid and butanol, which are of commercial importance.**



[**Key words:** Syngas; Ethanol; Anaerobic; *Clostridium*; Biomass]

Ethanol is typically produced either by direct fermentation of fermentable sugars in sugar cane or sweet sorghum, or by enzymatic or chemical hydrolysis of starch, cellulose, and hemicellulose to sugars that are then fermented by microbes to produce ethanol. One of the major disadvantages with the utilization of straw, wood and other cellulosic biomass is the presence of a large proportion of non degradable components such as lignin. Gasification of cellulosic biomass can be one approach of overcoming this hurdle in the utilization of biomass. Gasification produces synthesis gas (syngas) in which CO and $H_2$ are the essential components for subsequent ethanol production. Fermentation of syngas offers several advantages such as higher specificity of biocatalysts, lower energy costs, greater resistance to catalyst poisoning and the requirement for a fixed CO:$H_2$ ratio (1, 2).

Since biological fermentation of syngas is irreversible in nature, it ensures complete conversion and prevents the formation of thermodynamic equilibrium. Production of acetate by utilizing CO, $CO_2$ and $H_2$ can be summarized by following reactions (3).

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \ (\Delta G^\circ = -37.8 kJ/gmol CH_3COOH) \quad [1]$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \ (\Delta G^\circ = -18.6 kJ/gmol CH_3COOH) \quad [2]$$

Stoichiometry for ethanol production is not available since an autotrophic ethanol producing microorganism has not been identified. Hence based on the reactions [1] and [2] above, following stoichiometry for ethanol production has been proposed (4).

$$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2 \ (\Delta G^\circ = -59.9 kJ/gmol C_2H_5OH) \quad [3]$$

$$2CO_2 + 6H_2 \rightarrow C_2H_5OH + 3H_2O \ (\Delta G^\circ = -23.2 kJ/gmol C_2H_5OH) \quad [4]$$

Organisms able to reduce $CO_2$ to acetate via the acetyl-CoA or the Wood–Ljungdahl pathway are termed acetogens. This class of bacteria are anaerobic and produce acetate as their major fermentation end product (5). Acetogens play an important role in scavenging the carbon dioxide and converting it to acetate. Acetogens are strictly anaerobic bacteria utilizing $C_1$ compounds such as $H_2$–$CO_2$, CO or formate (6).

Production of ethanol by acetogens follows an analogous path similar to the Wood–Ljungdahl pathway for acetate production by acetogens such as *Clostridium acetobutylicum* (7). Oxidation of $H_2$ to 2[$H^+$] or of CO with $H_2O$ to $CO_2$ and 2[$H^+$] produces the reducing equivalents for the conversion of $CO_2$ and CO to acetate or alcohols (8). Ethanol production is a non-growth related product formed during anaerobic syngas fermentation. From an economic perspective, several acetogenic organisms have been shown to produce ethanol. Prominent among these are *Clostridium ljungdahlii* and *Clostridium carboxidivorans*, which produced up to 460 mM and 175 mM of ethanol, respectively (9, Gaddy, J.L., Arora, D.K., Ko, C.W.,

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Phillips, J.R., Basu, R., Wikstrom, C.V. and Clausen, E.C., US patent 7285402, 2007). Recently *Clostridium* strain P11 was reported to produce 200 mM of ethanol using CO, and is currently the organism of choice at our research facility for syngas to ethanol conversion (Saxena, J. and Tanner, R.S. Abstr 106th Annu Meet Am Soc Microbiol, USA p. 422, 2006).

Commercialization of fermentation involves the scale-up of laboratory fermentation to pilot scale and industrial scale fermentors. With an increase in the fermentor volume, a larger deviation in the ideal behavior can be observed. A large "non-ideal" fermentor differs from its "ideal" laboratory counterparts in terms of macroscopic homogeneity of the fermentation broth, and influx of disturbances (10). Larger fermentors are also characterized by severe and uncertain changes in the extracellular environment. This is important because a constant interaction exists between the cells and the environment in terms of inward transport of the nutrients and outward transport of the products.

With an increase in fermentor size, mass transfer becomes a critical issue. Mass transfer can be increased by considering several design strategies, such as increasing the gas flow-rate per liquid volume (11) or use of micro-spargers to produce micro-bubbles (12). Mean bubble diameter and gas hold-up affects both the interfacial area and mass transfer coefficient, which in turn are affected by the fluid dynamics existing within a fermentor (13).

Corn Steep Liquor (CSL) offers a cheaper nutrient alternative compared to traditionally used yeast extract, thereby, improving the overall process economics. However, one of the main disadvantages of using CSL is its variable composition primarily due to the type of corn and the conditions employed for starch processing (14). Previously, CSL has been successfully incorporated in the industrial production of penicillin. CSL contains a group of active biological compounds favoring the metabolism of bacteria, yeast and mold (15). In a fermentation medium with *Zymomonas mobilis*, CSL provided a complete source of nutrients and other growth factors such as trace elements and vitamins (16). In a previous study it was observed that clarified CSL was 1.33 times more effective compared to whole CSL on a volume basis (16).

Objectives of the present study were to validate the fermentation of *Clostridium* strain P11 in a pilot scale fermentor, validate anaerobic fermentation procedure in a pilot scale fermentor and to assess the feasibility of fermentor run and the system utility designed over an extended fermentation period.

## MATERIALS AND METHODS

**Fermentor description** A pilot scale fermentor, Biostat D-75 (Sartorius BBI, USA) conforming to the ASME Section VIII, Division I code for an Unfired Pressure Vessel, was installed and commissioned to study syngas fermentation. The fermentor was installed in line with the existing fluidized bed and down draft gasifiers existing in our research facility (Fig. 1). Biostat D-75 is a stirred tank reactor (STR) which was operated in a semi continuous mode, i.e., syngas was purged continuously through the fermentation medium while the fermentation medium was not replaced. Vessel surface in contact with the liquid media is constructed of 316-L stainless steel (SS). The jacketed vessel consists of a closed loop circulation for steam and water for a precise temperature control via a PID (proportional-integral-derivative) control loop. The vessel is equipped with a pH probe, temperature probe, dissolved oxygen (DO) probe, foam sensor, two septum ports with 19-mm adapters for external media addition and needle inoculation and a single orifice sparger (SOS). The fermentor is equipped with four removable standard baffles, and a top driven agitator shaft mounted with three standard six blade Rushton impellers. The instrumentation and control of the fermentor includes a digital microprocessor controlled system, with an operator interface terminal (OIT) that ensures a direct digital control of the process parameters: temperature, speed, gas flow rate, pH, foam control and control loops for external media component addition. Measurement and control firmware includes Multi Fermentor Control System (MFCS) Data Acquisition (DA) system for real time data logging. Table 1 shows the detailed dimensions for the Biostat D-75.

**Utility sizing** The Biostat D-75 fermentor requires several utility requirements for optimum, and trouble-free operation (Fig. 2). The utility supply for the fermentor should also meet minimum quality requirements to ensure high quality processing and maintain a sterile fermentation environment. The different utilities required for the Biostat D-75 include clean and dry compressed air, utility steam, clean steam, softened water, and de-ionized water.

The utility requirements were sized to accommodate two additional 100-L fermentors with an additional 15% contingency. The utility system is flexible and can also handle the requirements of a much larger fermentor, for instance, a 1000-L fermentor. Table 2 shows the minimum flow capacity (pressure and flowrate) of the utilities installed on the fermentor required to ensure trouble-free operation of the fermentor. Plant steam and cooling water is required for maintaining the fermentor jacket temperature, which in turn maintains the desired fermentor temperature during sterilization and fermentation incubation. Clean steam is required for maintaining the differential pressure between the agitator seal housing and the fermentor vessel. Compressed process air is required for the operation of pneumatic valves on the fermentor.

A vertical high pressure tubeless natural gas fired boiler (Model HE-15 Lattner, Federal Corporation, USA) was installed to meet the steam requirement for the fermentor operation. The boiler package conforms to the American Society of Mechanical Engineers controls and cafety devices (ASME CSD-1) requirements for the state of Oklahoma code for pressure vessels, delivering 147 kW at 1135 kPa with an input of 200 kW. The main steam header was branched to a clean steam filtering station mounted with two sintered 316-L SS filters (Spirax Sarco, USA) with a steam filter efficiency of 2 μm or less. The unit conformed to the 3-A accepted practice number 609-

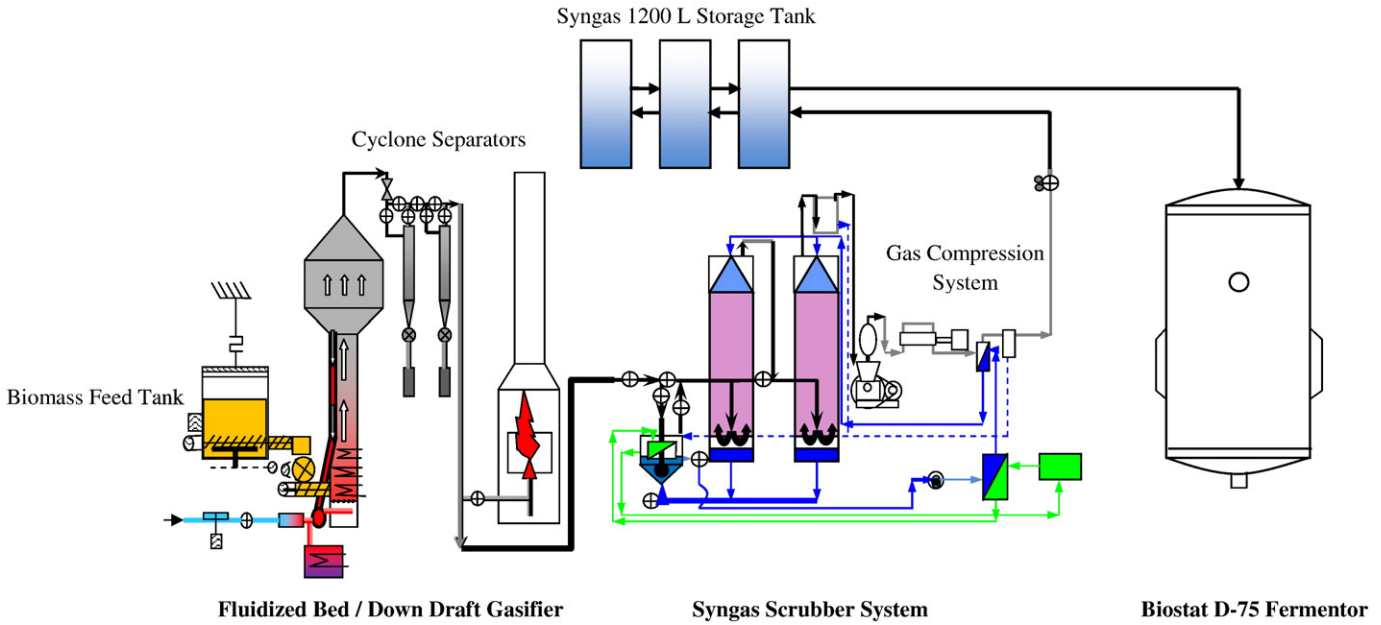


FIG. 1. Schematic representation of the Oklahoma State University Syngas-Fermentation pilot scale process.

**TABLE 1.** Dimensions of the Biostat D-75 fermentor used for syngas fermentation studies.

| Parameters | Reference symbol | Biostat D-75 |
|---|---|---|
| Total volume | $V_t$ | 0.1 $m^3$ |
| Working volume (maximum) | $V_w$ | 0.075 $m^3$ |
| Working volume (minimum) | $V_w$ | 0.03 $m^3$ |
| Vessel diameter | $D_t$ | 0.406 m |
| Vessel height | $H_t$ | 0.914 m |
| Impeller diameter | $D_I$ | 0.152 m |
| $D_I/D_t$ Ratio | | 0.38 |
| Agitator speed range (1119 $Js^{-1}$) | | 60–600 rpm |
| Tip speed | | 4.796 $ms^{-1}$ |
| Maximum working pressure (176.67 °C and full vacuum) | | 3.48 bar |

**TABLE 2.** Utility requirements for Biostat D-75.

| Utility | Capacity |
|---|---|
| Cooling Water (515 kPa) | $3.15 \times 10^{-4}$ $m^3$ $s^{-1}$ |
| Plant utility steam (412 kPa) | $8.44 \times 10^{-3}$ kg $s^{-1}$ |
| Clean steam (308 kPa) | $2.52 \times 10^{-3}$ kg $s^{-1}$ |
| Process and instrumentation air (653 kPa) | $2.08 \times 10^{-3}$ $m^3$ $s^{-1}$ |
| Electrical | 120–208/3/60 15 amps |

01 for the production of culinary steam meeting the clean steam requirement for the fermentor. A condensate receiver (Model FHC-112, Armstrong, USA) was installed to continuously return the steam condensate back to the boiler feed tank. A modular water treatment system (US Filter-Siemens, USA) consisting of pre-filters, activated carbon, and ion-exchange resin based twin water softener was installed for pre-treating water to the required quality standards for fermentor and boiler operation. An anion–cation based exchange bed unit was installed to deliver de-ionized quality water (18 MΩ resistivity), required for the media preparation and fermentor jacket circulation. The jacket circulation water was continuously fed through a chiller (Model #8301, Unitrol, USA) with a nominal rating of 35 kW.

A 0.00873 $m^3$ $s^{-1}$ capacity rotary screw compressor, Model Ingersoll Rand UP6-5-TAS (Ingersoll Rand, USA), was installed to deliver high quality (dust- and oil-free) compressed air for the operation of pneumatic valves on the fermentor. The compressor was pre-equipped with integrated dryer and prefilters. Two additional filters were installed in series to remove oil aerosols and water up to 0.01 μm, and dry particles removal down to 1 μm.

Several modifications were sought on the fermentor to improve the performance during syngas fermentation. Mixing plays an important role in the distribution of gas and the overall mass transfer coefficient. One approach considered in the present research was the installation of a 5.1-cm SS micro-sparger (Mott Corporation, USA) for increased gas mass transfer in the fermentation medium. The positive effect of a micro-sparger in improving the mass transfer coefficient by generating very fine gas bubbles for effective gas dispersion has been indicated earlier. The bubble size generated from a micro-sparger is a function of the pore size, gas exit velocity, liquid viscosity, and face velocity across the sparger pores. The bubble size was not evaluated either by us or by the manufacturer purely due to the lack of equipment to test the bubble size. However, visual examination indicated that the bubbles generated were much smaller compared to the SOS and uniform in size, larger in number, and uniformly distributed throughout the fermentation medium. Table 3 shows the dimension of the micro-sparger installed on the Biostat D-75 fermentor. The syngas feed inlet line was modified to install a new bypass line to prevent the dosing of plant steam into the fermentation medium during the fermentor sterilization.

A stainless steel exhaust gas condenser was fabricated in-house to minimize the evaporation of fermentation medium and to prevent the loss of fermentation gas products in the exhaust gas stream. The main fermentation products during ethanol fermentation are ethanol, 2-propanol and 1-butanol. Since these products are volatile at the fermentation temperature of 37 °C, it is necessary to capture volatilized products for accurate analysis of the fermentation products. Water from the 1900-L water tank was used as the cooling medium in a cross flow direction to maximize the fermentation product condensation in the condenser.

**Fermentation** *Clostridium* strain P11 fermentation medium was maintained in the laboratory in serum bottles by a periodic subculturing regime. Protocol for the inoculum preparation for the fermentor involves repeated inoculum transfer also termed as "passaging." *Bottled Gas* refers to the mixed gas purchased commercially having a customized composition similar to gas produced by gasifying switch-grass at the OSU Biomass Gasification Facility. *Producer Gas* refers to syngas produced by gasifying switch-grass at our facility. The composition of the bottled gas was 5% $H_2$, 15% $CO_2$, 20% CO, and 60% $N_2$. Composition of the producer gas varied between the different gasification batches, feedstock, and process conditions. Differences in gas composition were observed in the quality of gas generated from the fluidized bed and downdraft gasifier. The gas composition from the fluidized bed was comparable to the bottled

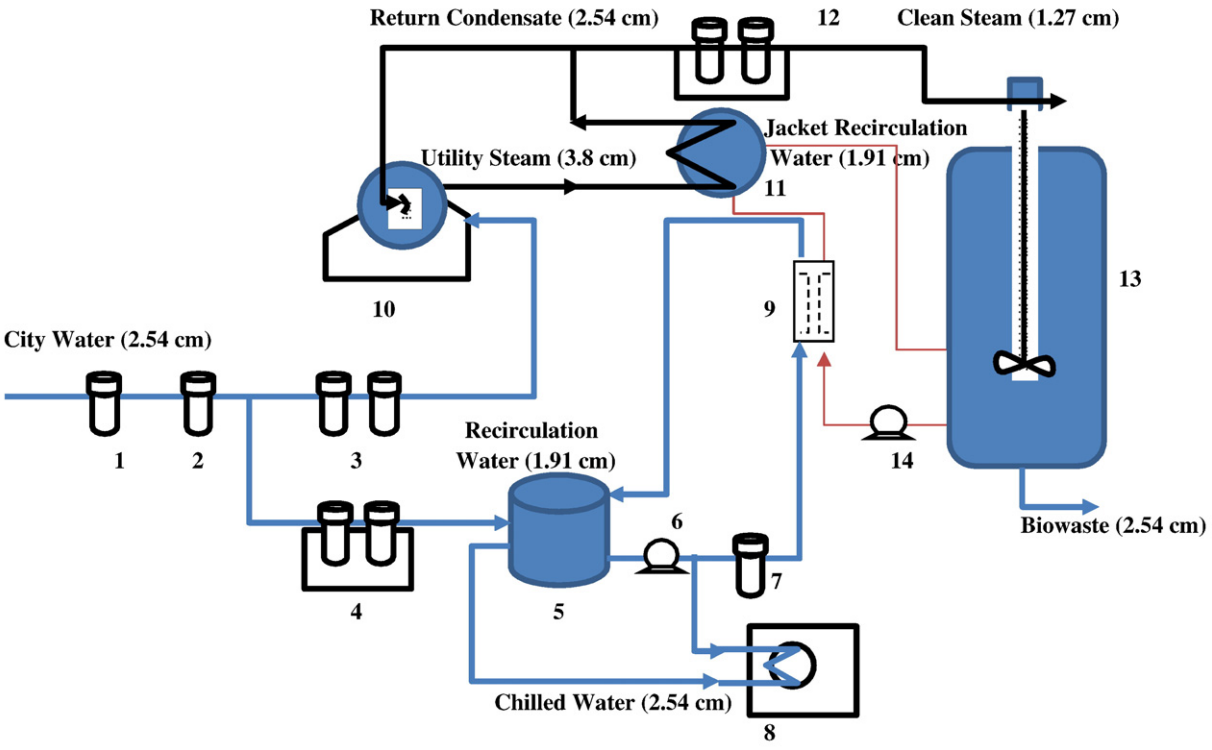


FIG. 2. Schematic representation of the utility design for the Biostat D-75 fermentor. Piping dimensions are shown in parentheses. The steam and water utilities used in the fermentor are shown. Temperature inside the fermentor is maintained by jacket circulation water. (1) Water prefilter; (2) activated carbon bed; (3) twin water softener bed; (4) anion–cation exchange bed; (5) 1900 L water storage tank; (6) circulation pump; (7) 0.2 μm stainless steel filter; (8) water chiller; (9) plate heat exchanger; (10) vertical boiler; (11) Tubular heat exchanger; (12) clean steam filtration station; (13) Biostat D-75 fermentor; (14) water circulation pump.

**TABLE 3.** Engineering design for micro-sparger installed at the end of SOS in the Biostat D-75 fermentor.

| Design parameters | Engineering data |
|---|---|
| Sparger outer diameter | 0.95 cm |
| Porous length | 5.08 cm |
| Mean pore size | 2 μm |
| Impingement length | 5.08 cm |
| Media grade | 2 |
| End fitting | 1.27 cm MNPT [a] to 0.95 cm FNPT[a] reducer bushing |
| Material | 316-L SS porous/316 SS hardware |

[a] MNPT—male national pipe thread (US standard), FNPT—female national pipe thread (US standard).

syngas ($H_2$—5%, CO—20%, $CO_2$—15%). On the other hand, the gas composition from the downdraft gasifier ranged from 7.0% to 12% $H_2$, 12% to 18% CO, and 10% to 17% $CO_2$. Sampling and instrument error of approximately 0.1% existed during the gas analysis. $O_2$ was also observed in the producer gas from both gasifiers ranging between 0.04% and 2.6%.

*Clostridium* strain P11$^T$ originally was obtained from Dr. Ralph Tanner, University of Oklahoma. All subculture fermentations were conducted in 250 mL serum bottles each with a 100 mL final working volume. *Clostridium* strain P11 subculture currently maintained in the laboratory was used as the starting inoculum at the rate of 10% of the working volume. The bacterium was maintained under strictly anaerobic conditions in a semi-defined medium containing following components (per L): 30 mL mineral stock solution, 10 mL trace metal solution, 10 mL vitamin solution, 10 g corn steep liquor (CSL), 10 g morpholinoethanesulfonic acid (MES), 10 mL of 4% cysteine sulfide solution, and 0.1 mL 1% resazurin indicator (17). Detailed composition of the stock solutions has been described previously (Huhnke, R.L., Lewis, R.S., and Tanner, R.S., US patent 005755408, 2008). CSL was previously centrifuged at 13000 revolutions per minute (rpm) for 10 min in a micro-centrifuge to remove the solids. Removal of solids was found to be necessary to minimize the variability in subculture preparation because of the varying solids concentration in CSL. Removing the solids also helps in cell optical density measurement in a UV spectrophotometer. Bottled syngas was used for all inoculum preparation. The serum bottles were purged with bottled gas every 24 h. All fermentation serum bottles were incubated at 37 °C with a constant agitation of 150 rpm on an orbital shaker.

Passage 1 was prepared by inoculating the previously maintained *Clostridium* strain P11 inoculum and fermenting the serum bottles for 36 h. In a similar procedure, Passage 2 and Passage 3 were prepared which were then incubated for 72 and 60 h, respectively. Passage 3 was prepared in five serum bottles giving a total inoculum volume of 500 mL required for the preparation of the final inoculum. The final inoculum was prepared in a 7.5 L Bioflo 110 (New Brunswick Scientific Corporation, USA) STR operated in a semi-continuous fermentation mode.

Preparation of the final inoculum in a 7.5-L STR (NBSC, USA) is an elaborate process. The total working volume in the reactor was 5 L. The only difference between the media composition in serum bottles and the 5-L fermentor was the reduced addition of MES (at 0.5% $vv^{-1}$), addition of CSL with solids, and the addition of cysteine sulfide after sterilization in the fermentor. These media components were prepared in Pyrex bottles and added externally into the fermentation medium. All media components were weighed and added to the reactor, and the volume was made to 4.5 L by adding deionized water (DI). Dissolved oxygen (DO) and pH probe calibration procedures are explained in the section below. Experience has shown that it is a good practice to check the condition of gaskets and O-rings on the head-plate, and for any leaks from the fermentor by purging compressed nitrogen gas before sterilization. Immediately after sterilization, $N_2$ was continuously purged through the fermentor. The medium components were autoclaved and cooled to ambient temperature before adding 1% ($vv^{-1}$) cysteine sulfide. $N_2$ is continuously purged for another 3 h and later switched to syngas for 3 h. Passage 3 inoculum is then added at the rate of 10% ($vv^{-1}$) under sterile anaerobic conditions.

**Biostat D-75 fermentor operation** The fermentor operation requires a thorough manual cleaning followed by sterilization-in-place (SIP) before the start of a fermentation batch. Experience working with pilot scale fermentors has shown that it is a good practice to test the SIP procedure prior to attempting fermentation. This procedure ensures that the fermentor is performing normally, and any leaks or other maintenance requests are rectified before the start of the actual fermentation run. During this preliminary SIP, the modified bypass line was opened to the fermentor rather than to the drain.

For accurate process measurement, it is necessary that the attached probes are properly maintained and calibrated according to the manufacturer's recommendation. Easyferm Plus VP 120 pH probe (Hamilton, USA) was calibrated with respect to standard solutions before SIP. From a good manufacturing practice (GMP) perspective, it is necessary to ascertain the condition of the DO probe membrane, and replace the electrolyte solution. Calibration of the DO probe is usually done during or after SIP, and is calibrated against two reference points "zero" and "span." During sterilization, the DO probe was zero calibrated, but the 100% span was not calibrated due to the limitation existing in the calibrating procedure of a DO probe in a larger anaerobic fermentor. For the 100% span calibration of the DO probe that is performed after SIP, high purity compressed air has to be purged through the fermentation medium. This step poses significant process limitation under anaerobic fermentation conditions because purging of compressed air needs to be followed by purging with nitrogen gas to make the medium anoxic. This step proves uneconomical and impractical under pilot scale and commercial production environments. Our research group is in the process of finding a solution for the optimal functionality of the DO probe.

A total fermentation working volume of 70 L was prepared in a manner similar to the media preparation in a 7.5-L fermentor. Following the pre-SIP, fermentation media components were weighed and added to the fermentor. Detailed *Clostridium* strain P11 media composition has been described earlier. The fermentor with fermentation medium, was sterilized with the bypass line opened to the drain.

Following sterilization $N_2$ and syngas was purged continuously through the fermentor as explained in the previous section. Cysteine sulfide (1% $vv^{-1}$) was dosed anaerobically using a peristaltic pump, followed by the addition of *Clostridium* strain P11 inoculum (10% $vv^{-1}$). During the fermentation growth phase, bottled syngas was purged through the fermentation medium. After seven days, gas source was switched to switch-grass producer gas. This minimizes interference or inhibition of *Clostridium* strain P11 cell growth from tar and other compounds during the growth phase of the cells and reduces the variability due to producer gas composition. Temperature, pH, agitation rate, and gas flow rate were registered real time using Biostat D-75 MFCS software.

Samples were collected to determine the time course for bacterial cell biomass, substrate, and product concentrations (ethanol, acetic acid, 2-propanol and 1-butanol). The cell biomass was determined by measuring sample absorbance at a wavelength of 660 nm ($A_{660}$) in 1 cm pathlength cuvettes using a UV-visible spectrophotometer (Cary 50 Bio, Varian, USA). Product concentrations were separated by HPLC with an Aminex-HPX-87H ion exchange column (Biorad, Hercules, CA, USA). The eluent was 0.01N $H_2SO_4$ pumped at 0.6 mL $min^{-1}$ and column temperature was 60 °C (Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J. and Templeton, D., NREL, Laboratory Analytical Procedure, 2005). Separated components were detected by a refractive index detector (1100 Series, Agilent, Santa Clara, CA, USA). Peaks were identified by comparing with known standards (Sigma-Aldrich, St. Louis, MO, USA). Ethanol production was calculated by subtracting initial concentrations from concentrations at other time points. The inlet and outlet gas samples were analyzed using a gas chromatograph (6890 Agilent Co., USA) equipped with a Hayesep-DB column and a thermal conductivity detector with argon as the carrier gas. A ramped temperature profile included 40 °C for 6 min after which the temperature was increased to 140 °C for 20 °C at an increment of 100 °C $min^{-1}$.

## RESULTS AND DISCUSSION

A fermentor is central to the economical production of useful biological products like ethanol, acetic acid, 2-propanol and 1-butanol. An ideally designed fermentor will provide a controlled environment for optimal growth and product formation. Of the different fermentor designs currently available in the market today, STR either in a batch or in continuous mode is the most desired and widely used fermentor system for industrial applications.

**Fermentor design and operation** The present study did not evaluate the effect of increasing gas flow rate at constant impeller speed due to limited availability of the constant supply of producer gas. In this study, we have successfully incorporated the use of a micro-sparger to improve the mass transfer rates without considering changes in the impeller configuration or the gas flow rates. Micro-spargers have been shown to produce micro-bubbles that improved the overall mass transfer coefficient in fermentation systems (12).

The impeller blade and baffle design used in Biostat D-75 as well as other vessel geometries used in the present study corresponds to a standard reactor configuration (18). A dual six-blade impeller along with four symmetrically located baffles was found to reduce vortex formation, thereby improving the mass transfer performance.

The fermentor was run for a period of 59 days without any major downtime in the utility requirement. Unexpected failure was observed in the operation of the air compressor (49 days) leading to the unavailability of compressed air to the fermentor. This resulted in fermentor temperature to drop to 30.2 °C after a failure time of 6 h. On the resumption of compressed air supply, the bacterial cells resumed metabolism, as evident by the change in product profile and cell densities. No failure was observed in case of steam boiler, and as per the manufacturer's recommendation, it was necessary to blow-down and monitor the feed water quality on a daily basis. This practice also qualified per se GMP requirement. The performance of utility set-up

**TABLE 4.** Product concentration recorded during the P11 fermentation run in Biostat D-75.

| Product | Time period (day) | Concentration ($gL^{-1}$) |
|---|---|---|
| Ethanol | 59 | 25.26 |
| 2-propanol | 24 | 9.25 |
| Acetic acid | 24 | 4.82 |
| 1-butanol | 13 | 0.47 |
| Total cell biomass concentration | 59 | 1.13 |

on the fermentor validates the sizing and the functionality of the designed system to meet the utility requirement of the fermentor, in order to keep the fermentation medium viable and sterile.

The process parameters on the fermentor included syngas/producer gas flow-rate (0.9 standard liters per minute, SLPM), agitation (150 rpm), and temperature (37 °C). Syngas and producer gas was purged continuously through the fermentation medium, which was different from the subculture bottle fermentation where the gas exchange was done every 24 h. Agitation in the fermentor was also different compared to serum bottles and it is assumed that with Ruston impellers, the gas mass transfer rates will be improved when compared to mass transfer rates in serum bottles.

**Fermentor product profile** The main products analyzed from the Biostat D-75 fermentation were ethanol, acetic acid, 2-propanol, and 1-butanol (Table 4). The product profile can be divided into two main phases considering ethanol productivity as the criteria: Phase 1 extends from day 0 to day 24 (acetogenic phase) and Phase 2 from day 24 to day 59 (solventogenic phase) (Fig. 3). Results for 1-butanol are not discussed because of low productivity.

In Phase 1, ethanol concentration increased from 0.17 $gL^{-1}$ on day 1 to 4.46 $gL^{-1}$ with a productivity of 0.18 $gL^{-1}$ $day^{-1}$ at the end of 24 days. The productivity for acetic acid was 0.15 $gL^{-1}$ $day^{-1}$, with the concentration increasing from 1.24 $gL^{-1}$ on day 1 to 4.82 $gL^{-1}$ at the end of 24 days. Comparatively, 2-propanol showed a higher productivity of 0.39 $gL^{-1}$ $day^{-1}$ with concentration increasing from 0 $gL^{-1}$ on day 1 to 9.25 $gL^{-1}$ at the end of 24 days.

Phase 2 corresponded with a linear increase in ethanol production while the acetic acid and 2-propanol concentration was constant. The ethanol concentration increased to 24.57 $gL^{-1}$ with a productivity of 0.87 $gL^{-1}$ $day^{-1}$ at the end of 59 days. The productivity for ethanol was comparable to productivity observed with *C. carboxidivorans* in a 7.5-L fermentor (19).

Production of acids in the first stage is necessary for the production of solvents in the second stage (20). From a bioenergetics point of view, production of acid is necessary for the generation of ATP and no consumption of electrons, whereas, alcohol production is associated with consumption of electrons and no production of ATP. 2-propanol concentration also decreased in Phase 2 reducing from 9.25 $gL^{-1}$ at the end of 24th day to 8.86 $gL^{-1}$ on 59th day with a corresponding productivity of −0.02 $gL^{-1}$ $day^{-1}$. Reason for decrease in 2-propanol concentration is unknown. 2-propanol production has not been previously reported during a syngas fermentation. In related work in our lab, both P11 and *C. carboxidivorans* were cultured in glucose media containing acetone (unpublished data). P11 reduced acetone to 2-propanol, but *C. carboxidivorans* did not. This indicates that P11 has the necessary enzyme or enzymes to reduce acetone to 2-propanol.

The ethanol concentration in the fermentation run is higher compared to previously reported concentrations during syngas fermentation with *C. carboxidivorans* and *Clostridium* strain P11 as the microbial agent (Saxena, J. and Tanner, R.S. Abstr 106th Annu Meet Am Soc Microbiol, USA p. 422, 2006). Formation of 2-propanol has not been shown in previous studies while the production of acetic acid was comparable to other studies (19, 21). Probably the higher productivity in the present run could be due to pilot scale fermentor, gas sparging strategy, process modifications done on the fermentor, and comparatively longer fermentor incubation time compared to previous studies.

Fig. 4 shows the cell concentration with corresponding pH profile observed during the Biostat D-75 fermentation. The cell concentration increased to 0.44 $gL^{-1}$ at the end of day 2 with a corresponding pH of 5.62. From day 3 to day 7, the cell concentration essentially remained constant, however the pH dropped exponentially to 4.95. The gas source was changed to biomass producer gas at the end of day 7, which caused further lag in the fermentation with no change in pH or cell concentration. This is probably because the bacterial cells were adapting to the producer gas environment. Producer gas components such as nitric oxide and acetylene has been shown to inhibit hydrogenase activity and result in a growth lag in acetogens (22). The total cell density in the fermentation broth peaked at the end of 14 days reaching a concentration of 0.84 $gL^{-1}$ before plateuing until the end of the fermentation. This may also correspond with the switch in *Clostridium* strain P11 metabolism from acetogenic to solventogenic phase, in accordance with the non-growth associated production of solvents (19). The total cell concentration at the end of 59th day was 0.87 $gL^{-1}$. The cell densities observed in the present study were much higher compared to earlier studies with *C. carboxidivorans* $P7^T$ (19, 21).

The pH continued to decrease to 4.74 observed at the end of 24 days before the fermentation switched to solvent production. Regulation of pH further alters the flow of electrons either to acetate or solvent production and it is necessary for the bacterial cells to

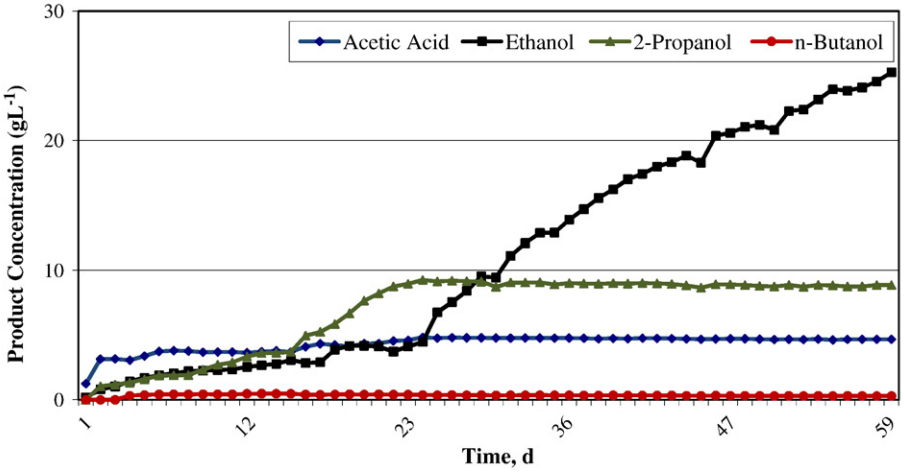


FIG. 3. Product profile for Biostat D-75 fermentor during the time course of fermentation.

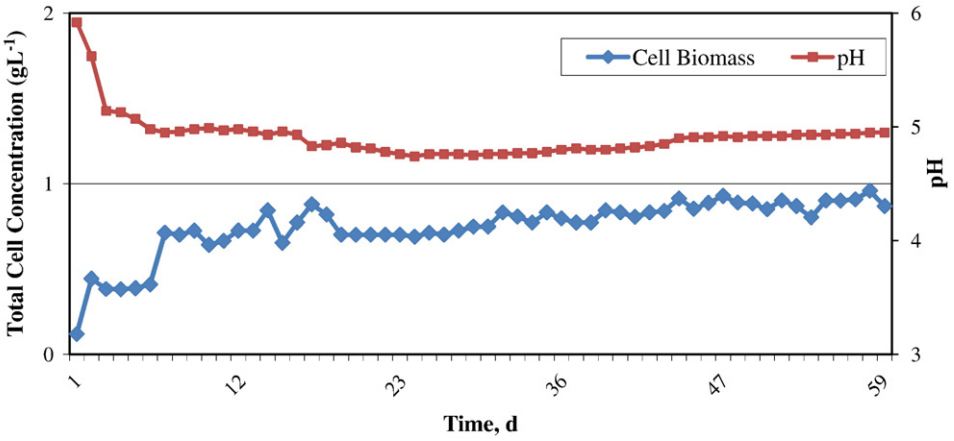


FIG. 4. *Clostridium* strain P11 cell concentration and pH profile observed during Biostat D-75 fermentation run.

produce acid to ensure subsequent solvent production (20). It is also expected that the drop in pH observed during the time course of fermentation, may also increase the mass transfer performance due to the presence of carbonic acid ($H_2CO_3$), produced by simultaneous chemical reaction and $CO_2$ mass transfer during syngas fermentation (23). The authors observed that presence of carbonic acid resulted in the decrease of medium pH which had an instantaneous effect on the solubility of CO and a greater sensitivity to its mass transfer (23).

Large scale production of inoculum is one of the critical factors for maintaining a viable inoculum volume in large scale fermentations and for multi stage inoculum development. Once the process operating conditions have been optimized, the reaction rate can be improved by increasing the microbial cell density. A high *Clostridium* strain P11 cell concentration was observed in the fermentation medium, which was beneficial in efficiently converting the syngas components, and may have resulted in high productivity observed in the present run. A high cell concentration was shown to impact substrate utilization (21). In ethanol fermentation using yeast cells it was found that high cell densities was necessary to achieve high fermentation rates (24). It can also be deliberated that a high cell concentration, the inoculums will have a greater probability of survivability and adaptation in presence of inhibitors.

The ethanol productivity was much lower than that observed for fermentations of glucose and other monosaccharides to ethanol. Low cell densities and low mass transfer rates are the main reasons for low ethanol productivity during syngas fermentations. Recently, several patent applications have been filed detailing the use of hollow fiber membrane reactors for syngas fermentation (Tsai, S-P., Datta, R., Basu, R., Yoon, S-H., and Tobey, R., US patent 2009-0029434, 2009; Tsai, S-P., Datta, R., Basu, R., and Yoon, S-H., US patent 2009-0215163, 2009; Tsai, S-P., Datta, R., Basu, R., and Yoon, S-H., US patent 2009-0215153, 2009). In these reactors a biofilm forms on the side of the membrane in contact with the liquid media with syngas diffusing through the membrane and into the biofilm. Mass transfer rates are much greater in these reactors and the cells are immobilized in the reactor by the biofilm, thus, increasing cell density and preventing cell washout if continuous operation is used. Other novel strategies such as altering media composition, nutrient feed rate, operating conditions such as temperature, agitation and pH, and syngas composition and feed-rate, have also been mentioned in order to maintain a steady state condition associated with ethanol productivities greater than 10 $gL^{-1}$ and a stable culture growth (Gaddy, J.L., Arora, D,K., Ko, C-W., Phillips, J.R., Basu, R., Wikstrom, C.V., and Clausen, E.C., US patent 7285402, 2007).

**Effect of contaminants on the fermentor product profile** The effect of biomass producer gas components, specifically nitric oxide and acetylene, has been shown to inhibit the hydrogenase activity and subsequent uptake of hydrogen. A study with nitric oxide and *C. carboxidivorans* showed that at a nitric oxide concentration of 150 ppm, hydrogenase activity was consistently inhibited (19). In addition, the authors also observed that tar in biomass producer gas was responsible for cell dormancy rather than ash. This may be the reason for the lag observed in cell growth when the feed gas was switched from bottled gas to producer gas.

Acetogens are strict or obligate anaerobes. However, in the present study the producer gas generated from the gasifier was constantly contaminated with oxygen, reasons for which are presently being investigated. Oxygen concentration as measured by headspace gas analyzed on the gas chromatography ranged between 400 and 26000 ppm (data not shown). In spite of the $O_2$ presence in the gas feed, *Clostridium* strain P11 inoculum demonstrated growth and product formation. Ability of *Clostridium* strain P11 to grow and metabolize under micro-aerophilic conditions (5% $O_2$ concentration) is surprising and encouraging from a process scale-up perspective. Acetogens isolated from anoxic soils and termite guts were speculated to contain certain mechanisms to cope with $O_2$ in an in-situ environment. Acetogens such as *Moorella thermoacetica* and *Clostridium magnum* displayed peroxidase and NADH oxidase activity which enables it to grow in media supplemented with 21% $O_2$ ($vv^{-1}$) (25). It can be speculated that under prolonged exposure to $O_2$ in the producer gas, it is possible that some of these oxygen consuming enzymes were over expressed enabling *Clostridium* strain P11 to grow and metabolize the syngas components to ethanol, 1-butanol.

**Conclusion** In this study, we have shown the successful installation and operation of a pilot scale fermentor with improved productivity compared to serum bottle fermentation and bench scale STR's. The study also elaborates the sizing and design of utilities required for a trouble free operation of a fermentor. We were able to operate the fermentor over an extended period of time. We have also shown a staggered procedure for preparing final inoculum for the pilot scale fermentor. A direction for future research to assess the effect of different nutrients using a statistically designed procedure has been indicated.

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