

# Effects of biomass-generated producer gas constituents on cell growth, product distribution and hydrogenase activity of *Clostridium carboxidivorans* P7<sup>T</sup>

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## Abstract

In our previous work, we demonstrated that biomass-generated producer gas can be converted to ethanol and acetic acid using a microbial catalyst *Clostridium carboxidivorans* P7<sup>T</sup>. Results showed that the producer gas (1) induced cell dormancy, (2) inhibited H<sub>2</sub> consumption, and (3) affected the acetic acid/ethanol product distribution. Results of this work showed that tars were the likely cause of cell dormancy and product redistribution and that the addition of a 0.025 mm filter in the gas cleanup negated the effects of tars. *C. carboxidivorans* P7<sup>T</sup> can adapt to the tars (i.e. grow) only after prolonged exposure. Nitric oxide, present in the producer gas at 150 ppm, is an inhibitor of the hydrogenase enzyme involved in H<sub>2</sub> consumption. We conclude that significant conditioning of the producer gas will be required for the successful coupling of biomass-generated producer gas with fermentation to produce ethanol and acetic acid.

**Keywords:** Producer gas; Ethanol; Biomass; Tars; Nitric oxide

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## 1. Introduction

Due to the steadily increasing demand for ethanol, extensive research is being performed to develop an economically viable process for ethanol production. The gasification–fermentation process utilizing the fermentation of gasified lignocellulosic biomass to ethanol is being explored owing to the low cost and availability of biomass. The process involves the conversion of biomass to producer gas (a mixture of CO, CO<sub>2</sub>, H<sub>2</sub> and N<sub>2</sub>), following which the producer gas is converted to ethanol using microbial catalysts. The term “producer gas” denoted in this work refers to biomass-generated producer gas. It has been found that anaerobic bacteria such as *Clostridium ljungdahlii* and *C. autoethanogenum* can be used to convert CO, CO<sub>2</sub> and H<sub>2</sub> to ethanol and acetic acid [1,2]. The research described in this work utilized a novel *Clostridium* species, recently identified as *C. carboxidivorans* P7<sup>T</sup> [3], to

convert producer gas to ethanol and acetic acid [4].

In previous studies [5], certain effects of producer gas fermentation were observed. The process involved growing cells in a batch system under continuous flow of synthetic producer gas, following which the system was changed to a continuous liquid flow in which fresh media was added and products/cells were removed with no cell recycle. The term “synthetic producer gas” refers to a mixture of purchased compressed gases with a similar CO, CO<sub>2</sub>, and H<sub>2</sub> composition as the producer gas. After the cells reached a steady concentration, the synthetic producer gas was replaced with the producer gas that had been cleaned with two cyclones followed by two 10%-acetone scrubbers, all in series. Following the producer gas introduction, the cells stopped consuming H<sub>2</sub> almost immediately and the cells stopped growing after a delay of approximately 1.5 days. The cessation in cell growth led to cell washout from the reactor as a result of the continuous operation. In addition, an increase in ethanol production was also observed.

Producer gas via gasification typically contains tars, ash, and certain gaseous components [6,7]. It was hypothesized that one or more of these potential “contaminants” induced cell dormancy, stopped H<sub>2</sub> utilization, and affected product distribution. This work assessed whether tars, ash, ethylene, ethane, acetylene, and/or nitric oxide contributed to the above conditions. In addition to the producer gas cleaning described above, the inclusion of filters was also assessed to determine if any of the conditions could be eliminated.

## 2. Materials and methods

### 2.1. Biomass and producer gas

Producer gas was obtained by gasification of switchgrass. Switchgrass is a sustainable perennial herbaceous crop [8] which is advantageous owing to its high yields, low nutrient requirements, and geographically wide distribution [9]. The switchgrass was harvested, baled, chopped and then gasified in a fluidized-bed reactor as previously reported [5]. The exiting gas was passed through two cyclones in series to remove particulates (such as ash) and then through two scrubbers in series. Each 4-ft scrubber was packed with stainless steel pall rings containing a mixture of 90% water and 10% acetone at 20 IC that was continuously circulated through the scrubbers. The average residence time of the producer gas in each scrubber was 4 min. The producer gas was then compressed and stored at approximately 860 kPa in storage vessels. The producer gas analysis showed approximately 16.5% CO, 15.5% CO<sub>2</sub>, 5% H<sub>2</sub>, and 56% N<sub>2</sub> along with 4.5% CH<sub>4</sub>, 0.1% C<sub>2</sub>H<sub>2</sub>, 0.35% C<sub>2</sub>H<sub>6</sub>, 1.4% C<sub>2</sub>H<sub>4</sub> and 150 ppm nitric oxide (compositions based on measured species).

### 2.2. Microbial catalyst and culture medium

*C. carboxidivorans* P7<sup>T</sup> was provided by Dr. Ralph Tanner, University of Oklahoma. This bacterium is capable of fermenting producer gas, as well as sugars, to produce alcohols and acids. The bacterium was grown under strictly anaerobic conditions in a medium containing (per liter) 30 ml mineral stock solution, 10 ml trace metal stock solution, 10 ml vitamin stock solution, 0.5 g yeast extract, 5 g morpholinoethanesulfonic acid (MES), and 10 ml of 4% cysteine-sulfide solution. Resazurin solution (0.1%) was added as a redox indicator. The compositions of the minerals, vitamins, and trace metals stock solutions were previously described [5].

### 2.3. Chemostat studies

A BioFlo 110 Benchtop Fermentor (New Brunswick Scientific, Brunswick, NJ, USA) with a 3-l working volume was used for the fermentation studies involving continuous liquid feed and product removal (i.e. chemostat mode). The reactor consisted of an agitator, sparger, pH probe,

dissolved oxygen probe, ports for liquid inlet and outlet, jacket for temperature control and pumps for feed, product removal and pH control. The experimental setup is shown in Fig. 1. As shown, a four-way valve was used to introduce gas feed by switching between producer gas and synthetic producer gas. The gas was introduced through a sparger. Two liquid feed tanks were used to introduce sterile media into the bioreactor during chemostat operation. The liquid feed tanks were continuously purged with nitrogen to maintain anoxic conditions.

Although the pH of the reactor was controlled using a pH controller, MES was added as a buffer to prevent excessive fluctuations in pH during the course of the experiment. Prior to inoculation, the bioreactor was filled with 3 l of liquid media (without the vitamins) at pH 5.85 and autoclaved at 121 IC for 20 min. After cooling, the media was purged with nitrogen to provide an anaerobic environment and filter-sterilized vitamin stock solution (10 ml l<sup>-1</sup>) was added to the media to avoid the inactivation of vitamins during steam sterilization. Cysteine-sulfide (30 ml) was added to scavenge any remaining dissolved oxygen.

Once the anaerobic environment was obtained, the gas feed was changed from N<sub>2</sub> to synthetic producer gas (approximately 17% CO, 15% CO<sub>2</sub>, 5% H<sub>2</sub>, and balance N<sub>2</sub>) flowing at 160 cm<sup>3</sup> min<sup>-1</sup> at 25 IC and 137 kPa. A mass flow controller was used to mix bottled gases in the same composition as the CO, CO<sub>2</sub>, and H<sub>2</sub> in the producer gas to obtain the synthetic producer gas. The impellor agitation was 400 rpm and the temperature was 37 IC.

The bioreactor operation was divided into four stages. In all stages, the gas flow was continuous. In the first stage, the liquid was maintained in batch mode and the synthetic producer gas was fed to the bioreactor. The bioreactor was inoculated and the cells were allowed to grow until the cell

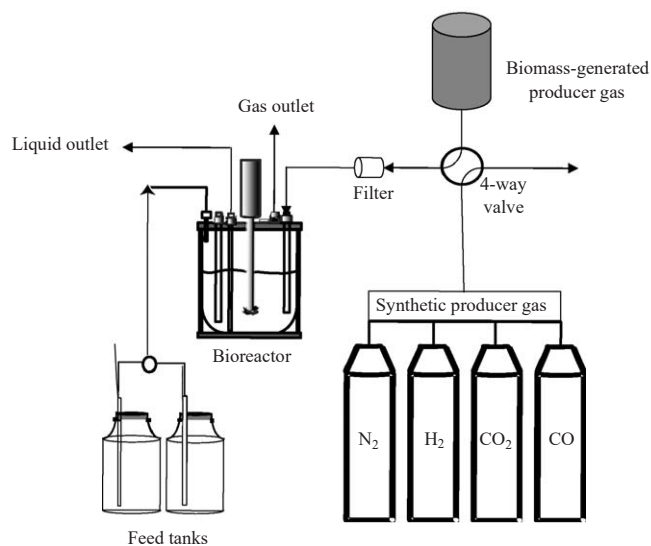


Fig. 1. Schematic of 3-l chemostat experiment. A four-way valve was used to switch the gas supply between synthetic producer gas and producer gas obtained from gasified switchgrass.

concentration started to level off. The pH was allowed to drop from an initial value of 5.85 to the lower pH setpoint of 5.25 in increments of 0.2 during the first stage. A deadband of 0.2 was used to avoid too much addition of acid or base by the pH controller. During the second stage, continuous liquid feed and removal was initiated at  $0.36 \text{ ml min}^{-1}$ . Once the cell concentration stabilized with continuous liquid feed, the third stage was initiated by switching the gas feed from synthetic producer gas to producer gas. The producer gas was additionally cleaned with a  $0.025 \text{ mm}$  filter (Millipore). In the fourth stage, the gas filter was replaced by a  $0.2 \text{ mm}$  filter with the producer gas continuing as the feed. In all stages, the cell concentration, pH, product concentration, and inlet/outlet gas compositions were analyzed.

#### 2.4. Batch studies

Batch experiments were conducted in 250 ml serum bottles with 100 ml of liquid media to assess the effects of residual tar in the producer gas on cell growth and product formation. The media composition was the same as described above except 10 g of MES was added per liter rather than  $5 \text{ g l}^{-1}$ . As there was no external pH control in the batch studies, a higher amount of buffer was used. The media was boiled and purged with nitrogen for 5 min to remove oxygen and then sterilized in an autoclave (Primus Sterilizer Co. Inc.) at  $121 \text{ }^\circ\text{C}$  for 20 min. The bottles were allowed to cool and the headspace was again purged with  $\text{N}_2$  for approximately 1 min. Cysteine sulfide (1 ml) was added to scavenge any remaining dissolved oxygen and the reactors were pressurized with a mixture of 80%  $\text{CO}$  and 20%  $\text{CO}_2$  at 10 psig. The reactors were then inoculated and placed at  $37 \text{ }^\circ\text{C}$  in a shaker (Innova 2100, New Brunswick Scientific). All studies were performed in triplicate. For one study, the 10% acetone solution used to scrub the producer gas was added to the bottles (1 ml). A second study was performed similar to the first. For controls, one study had no additional components added and one study involved the addition of a 10% acetone solution (1 ml) that had not been exposed to producer gas. The cell concentration, pH, and product concentrations were measured at regular time intervals.

#### 2.5. Analytical methods

The optical density (OD), which is proportional to the cell concentration ( $\sim 0.43 \text{ g l}^{-1}$  per OD unit), was determined using a UV-Vis spectrophotometer. Cell samples were collected in 4 ml cuvettes from the bioreactor and the OD was measured at 660 nm. A standard calibration chart was used within a linear range of 0–0.4 OD units to estimate the cell concentration. Samples with an OD greater than 0.4 units were diluted so that the OD was within the linear range of calibration. Gas samples were taken from the outlet and inlet lines of the bioreactor in gas tight syringes. The gas compositions for the chemostat

were determined using a gas chromatograph (3800 series, Varian Co., CA, USA) with a Hayesep-DB column (Hayes Separations Inc, Bandera, TX, USA) connected to a thermal conductivity detector (TCD) with argon as the carrier gas. The TCD was run at  $40 \text{ }^\circ\text{C}$  for 6 min, after which the temperature was ramped up to  $140 \text{ }^\circ\text{C}$  at  $100 \text{ }^\circ\text{C min}^{-1}$  for 20 min.

The liquid samples were centrifuged at  $1300g$  for 30 min. The cell-free supernatant was collected and then frozen at  $-18 \text{ }^\circ\text{C}$  until further analysis. The liquid products were analyzed for ethanol and acetic acid using a 6890 Gas Chromatograph (Agilent Technologies, Wilmington, DE, USA), equipped with a flame ionization detector and an 8 ft Porapak QS 80/100 column (Alltech, Deerfield, IL, USA).

### 3. Results and discussion

#### 3.1. Chemostat studies—cell growth

The cell concentration profile for one chemostat study is shown in Fig. 2. In the first stage, the cells grew and the cell concentration began leveling off on Day 8. Following the initiation of continuous liquid flow on Day 8 (stage 2), the cell concentration remained essentially constant. There was not much change in the cell concentration during the third stage following the introduction of producer gas. The  $0.025 \text{ mm}$  filter negated the previously observed decline in cell concentration observed with producer gas introduction using a  $0.2 \text{ mm}$  filter [5]. However, upon switching to the  $0.2 \text{ mm}$  filter (stage 4), the cell concentration declined after approximately 1.5 days.

The chemostat material balance for cells, neglecting death, is

$$\frac{dX}{dt} = \mu X - DX, \quad (1)$$

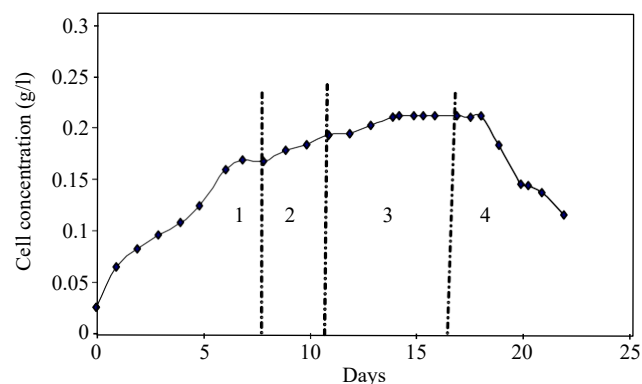


Fig. 2. Cell concentration profile in chemostat. In stage 1, the liquid phase was batch and cells were grown on synthetic producer gas. In stage 2, the liquid phase was changed to continuous. In stage 3, the gas supply was changed to filtered ( $0.025 \text{ mm}$ ) producer gas. In stage 4, the producer gas was cleaned with a  $0.2 \text{ mm}$  filter. The gas phase was continuous in all stages.

where  $X$  is the cell concentration,  $D$  is the dilution rate defined as the ratio of the liquid feed rate ( $F$ ) to the liquid volume ( $V$ ), and  $\mu$  is the cell growth rate. The cell balance is important for understanding the experimental observations. During batch growth (stage 1),  $D \ll \mu$  and integration of Eq. (1) yields a slope of  $\mu$  when  $\ln(X/X_0)$  is plotted versus time.  $X_0$  is the initial cell concentration. For this study,  $\mu$  was  $0.0074 \text{ h}^{-1}$  during stage 1. For stage 2, when the cell concentration was nearly constant ( $dX/dt \approx 0$ ),  $\mu \approx D$ . Thus, a liquid flow rate of  $F \approx 0.36 \text{ ml min}^{-1}$  was chosen to maintain  $D$  just below  $0.0074 \text{ h}^{-1}$  ( $D \approx 0.0069 \text{ h}^{-1}$ ).

On Day 11, the gas was switched from the synthetic producer gas to producer gas that had passed through a  $0.025 \text{ mm}$  filter. The cell concentration remained independent of time, thus the cells were still growing in the presence of  $0.025 \text{ mm}$ -filtered producer gas since  $\mu \approx D$ . This result was confirmed in two additional chemostat experiments. Upon replacing the  $0.025 \text{ mm}$  filter with a  $0.2 \text{ mm}$  filter, the cell concentration began declining after approximately 1.5 days. Assuming  $\mu \approx 0$  (i.e. no cell growth) in Eq. (1) when the cell concentration begins to decline, integration yields:

$$\ln \delta X = X_0 \delta - D t \quad (2)$$

In Eq. (2),  $X_0$  represents the cell concentration just as the decline begins. Using the cell concentration data in Stage 4 (the declining portion), a plot of  $\ln(X/X_0)$  versus time with Eq. (2) yielded  $D \approx 0.0068 \text{ h}^{-1}$ , which is in agreement with the value of  $D$  used in the experiment. This agreement demonstrated that cells were not dying, but rather were remaining in a non-growth state and were washing out of the reactor. This was also observed in previous studies with the use of a  $0.2 \text{ mm}$  filter [5]. As noted below in the product formation section, acetic acid was still being produced in stage 4 providing evidence that the cells were not dead.

With regards to cell dormancy, preliminary studies with similar concentrations of ethylene, acetylene, or ethane added to batch cultures grown under "clean"  $\text{CO}/\text{CO}_2$  gases showed no difference in growth characteristics. During the chemostat run, the filter inlet and outlet compositions of ethylene, acetylene and ethane in the producer gas were measured and it was found that there was no detectable change in the compositions—again suggesting that these gases do not contribute to cell dormancy.

### 3.2. Chemostat studies—pH changes

The pH of the medium was initially adjusted to 5.85 before inoculation. Once the cells started growing, the pH started to drop due to the production of acids. The pH was allowed to drop during stage 1, but the pH setpoint was adjusted from 5.85 to 5.25 over the first 3 days to minimize a rapid drop in pH. Once the pH was 5.25, the setpoint was maintained at 5.25 during stage 2 with a dead band of 0.2. After the introduction of producer gas (stage 3), the pH

began to rise and was controlled at a value of 5.35. After about 4 days, the pH was allowed to rise. The pH reached a value of 5.75 and then started to decrease as the cells washed out of the reactor.

### 3.3. Chemostat studies—substrate utilization

In the presence of synthetic producer gas, the cells consumed  $\text{CO}$  and  $\text{H}_2$  and produced  $\text{CO}_2$ . The inlet gas contained  $0.165 \text{ mole min}^{-1}$  of  $\text{CO}$  and  $0.15 \text{ mole min}^{-1}$  of  $\text{CO}_2$  while the outlet gas contained  $0.14 \text{ mole min}^{-1}$  of  $\text{CO}$  and  $0.16 \text{ mole min}^{-1}$  of  $\text{CO}_2$ . An overall carbon balance showed that more than 97% of the utilized carbon was accounted for in the production of  $\text{CO}_2$ , ethanol, acetic acid, and cell mass. Upon switching from synthetic producer gas to producer gas, the  $\text{H}_2$  consumption immediately ceased, irrespective of the filter size.

Previous studies using a  $0.2 \text{ mm}$  filter also demonstrated an immediate cessation of  $\text{H}_2$  uptake [5]. Thus, the producer gas component(s) inhibiting the hydrogenase enzyme responsible for  $\text{H}_2$  uptake is not affected by the filter size. Gases like nitric oxide and acetylene, which have been identified in the producer gas, are known to be inhibitors of hydrogenase [10–12] and would not be filtered out using the present setup. Preliminary studies with nitric oxide have shown that this gas reversibly inhibits the hydrogenase enzyme(s) of *C. carboxidivorans* P7<sup>T</sup>. Hydrogenase inhibition occurred at 60 ppm (10% inhibition) and 100% inhibition occurred at concentrations greater than 130 ppm—consistent with the 150 ppm nitric oxide in the producer gas that inhibited  $\text{H}_2$  utilization. A model has been developed to quantitatively predict the hydrogenase activity in the presence of nitric oxide but the results are beyond the scope of this article.

### 3.4. Chemostat studies—product formation

Fig. 3 shows the change in the ratio of the product concentration ( $P$ ) to the cell concentration ( $X$ ) with time

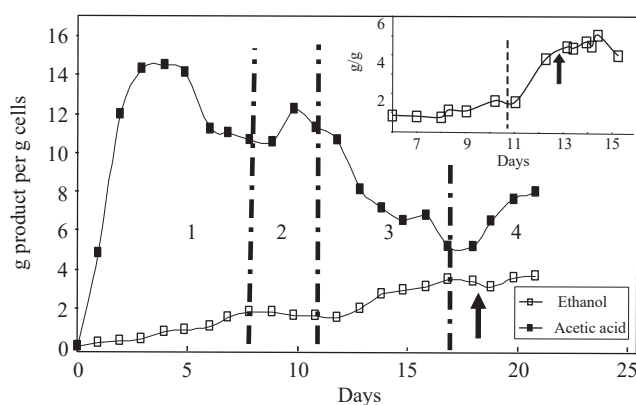


Fig. 3. Ethanol and acetic acid profile in chemostat. The stages are the same as described in Fig. 2. The inset is from a previously published experiment [5].

for both ethanol and acetic acid. The transient mass balance for  $P$  ( $\text{g l}^{-1}$ ) in a well-mixed chemostat is

$$\frac{dP}{dt} = q_p X - DP, \quad (3)$$

where  $q_p$  is the product formation rate per cell mass [ $\text{g (g cells)}^{-1} \text{time}^{-1}$ ] and  $D$  is the dilution rate [ $\text{time}^{-1}$ ]. Since  $(P/X)$  is shown in Fig. 3, time differentiation of  $(P/X)$  shows

$$\frac{d(P/X)}{dt} = \frac{X \frac{dP}{dt} - P \frac{dX}{dt}}{X^2}. \quad (4)$$

Substitution of Eq. (3) into Eq. (4), with some rearrangement, gives Eq. (5) that is useful for understanding the results shown in Fig. 3

$$\frac{d(P/X)}{dt} = \frac{q_p}{X} - \frac{P}{X^2} \frac{dX}{dt} - \frac{P}{X} D.$$

During stage 2, both  $X$  (Fig. 2) and  $(P/X)$  (Fig. 3 for both ethanol and acetic acid) were relatively constant such that the derivatives in Eq. (5) are small compared to the last term. Thus,  $q_p$  for ethanol and  $q_p$  for acetic acid is approximately six times that of ethanol during the fermentation of synthetic producer gas. It is noted that  $(P/X)$  for acetic acid may be slightly increasing during stage 2 such that  $q_p$  would be slightly greater than  $D(P/X)$ . Nevertheless,  $q_p$  for acetic acid is still much greater than that for ethanol.

This observation that  $q_p$  for acetic acid is much greater than that for ethanol is contrary to what was previously observed [4] in which more ethanol was produced than acetic acid. *C. carboxidivorans* is an acetogen that produces primarily acetic acid during the growth phase. A switch to solventogenesis (ethanol production) occurs when the medium conditions are no longer favorable for growth. In many cases, the initiation of solventogenesis in

clostridium bacteria is associated with the onset of sporulation [13–15]. It is feasible that the initial inoculum in the previous experiments contained a mix of cells that were both acid and ethanol producing.

When producer gas was introduced through a 0.025 mm filter in stage 3,  $(P/X)$  for ethanol nearly doubled and approached a steady value (where  $q_p = D(P/X)$ ). Thus, in comparison with stage 2,  $q_p$  for ethanol approximately doubled. On the other hand,  $(P/X)$  for acetic acid decreased from 11.3 to 5.1  $\text{g g}^{-1}$  of cells over 6 days. Initially,  $d(P/X)/dt = -1 \text{ g g}^{-1} \text{ day}^{-1}$  but then began leveling off towards zero. Since  $X$  remained essentially constant (i.e.  $dX/dt$  is small) and  $D(P/X)$  initially began at 1.85 but leveled off at 0.85  $\text{g g}^{-1} \text{ day}^{-1}$  (with  $D = 0.007 \text{ h}^{-1}$ ), Eq. (5) shows that  $q_p$  for acetic acid decreased compared to stage 2 but still remained positive. Even if the net value of  $q_p$  is positive, acetic acid could be both produced and then consumed to make ethanol. Additional experiments need to be performed to determine if the increase in ethanol is due to the consumption of acetic acid rather than from direct conversion of CO to ethanol. However, this increase in ethanol production by the cells suggests that some constituent of the producer gas could be making the cells more solventogenic as compared to the synthetic producer gas.

In stage 4, the filter was changed to 0.2 mm and washout of the cells began after about 1.5 days. The arrow in Fig. 3 indicates the time at which washout of the cells began. Prior to washout,  $q_p = D(P/X)$  as previously explained in stage 3. However, at the onset of cell dormancy in which washout occurs,  $dX/dt = -X$  as described in the cell growth section. Thus, substitution into Eq. (5) shows that  $q_p = d(P/X)/dt$  during cell dormancy. Therefore, prior to washout,  $q_p$  for ethanol and acetic acid were positive. Throughout cell dormancy,  $q_p$  for ethanol was negligible

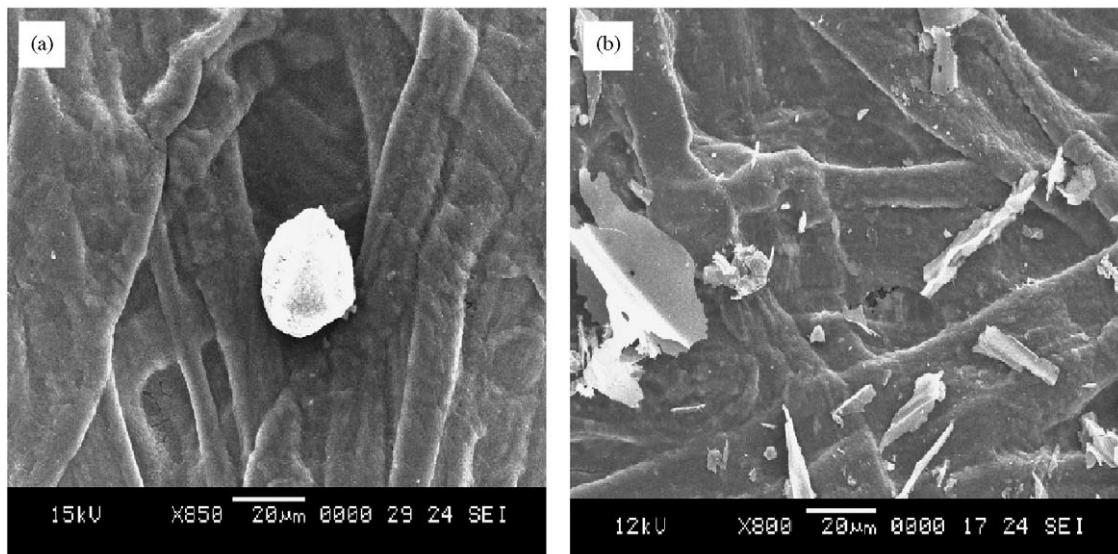


Fig. 4. Scanning electron microscope analysis of: (a) 0.025 mm filter from bioreactor run and (b) 0.025 mm filter with ash from gasifier.

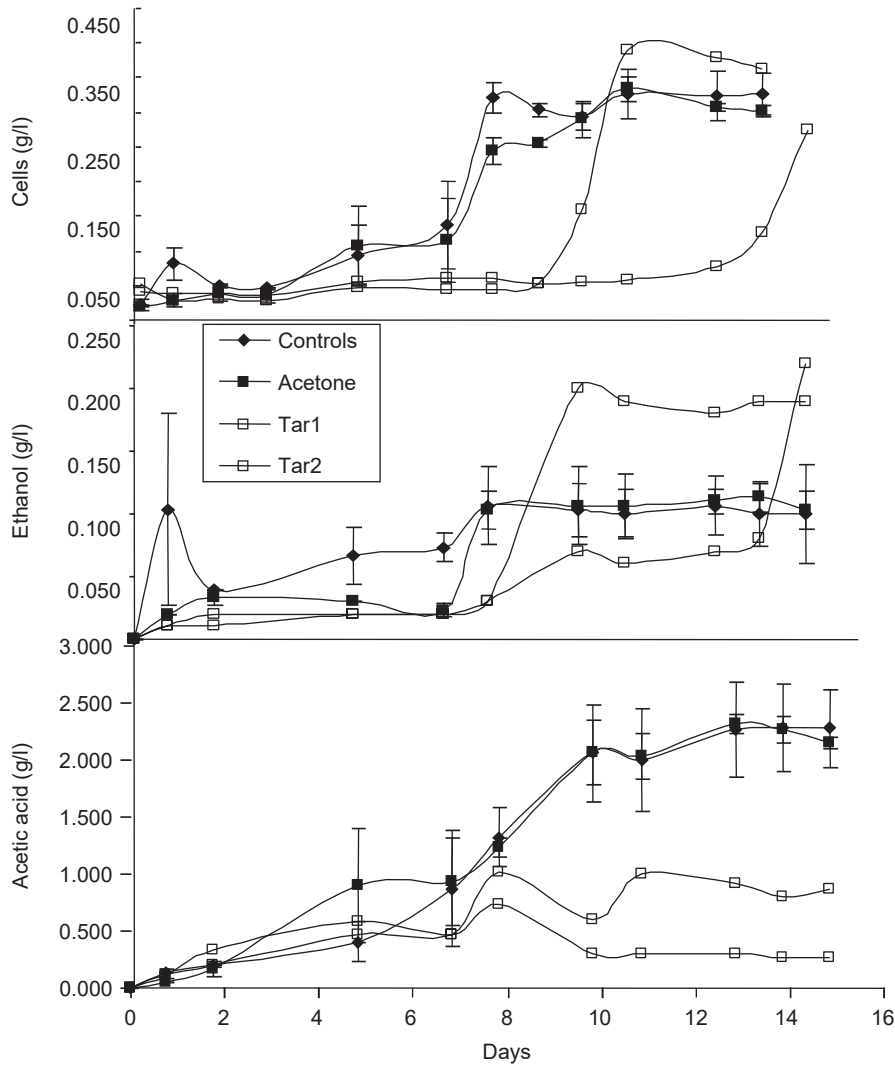


Fig. 5. Cell, ethanol, and acetic acid concentrations in 100-ml batch studies. The control study contained media. The acetone study contained media supplemented with 1-ml of acetone. The error bars represent the standard error ( $n = 3$ ). The two tar studies contained media supplemented with 1-ml of acetone scrubbing solution used to clean the producer gas. As the onset of growth varied upon exposure to the acetone scrubbing solution (tar experiments), the results are shown separately.

since ( $P=X$ ) remained essentially constant, although  $q_p$  for acetic acid increased.

Similar results were observed in previous studies in which producer gas was cleaned with a 0.2 mm filter [5]. When producer gas was introduced, an increase in the ethanol concentration was observed for about 1.5 days while the cell concentration remained relatively stable. Thus,  $q_p$  for ethanol increased. After cell dormancy (washout) began, ( $P=X$ ) for ethanol (plotted using the reported data of  $P$  and  $X$  and shown as the inset in Fig. 3) was essentially constant. Since  $q_p \diamond \frac{dP=X}{dt}$  during washout,  $q_p$  for ethanol was negligible.

### 3.5. Filter analysis

As it was conclusively shown that the filter size affected cell dormancy and  $q_p$  for ethanol and acetic acid, an analysis of the filter was performed. Particulates trapped by

the filter could include ash or tar generated during the gasification process. The 0.025 mm filter was evaluated using a scanning electron microscope (JEOL JSM 6360). Fig. 4a shows the filter used to clean the producer gas. The particle shown was one of the larger agglomerates seen on the filter. There were also several smaller particles of similar appearance spread over the filter. The trapped particulates differ from ash shown in Fig. 4b. Thus, ash was not the culprit leading to cell dormancy, but tar particulates were the likely candidates.

### 3.6. Batch studies

To assess the effects of tars on cell growth and product distribution, batch experiments were performed. Fig. 5 shows the cell growth and product formation in the presence of media, media supplemented with ‘‘clean’’ 10% acetone, and media supplemented with the 10%

acetone solution from the scrubbers. The stored producer gas was bubbled through a 10% acetone solution to identify potential tar species in the gas. A GCMS analysis of the acetone solution identified benzene (327 mg ml<sup>-1</sup>), toluene (117 mg ml<sup>-1</sup>), ethylbenzene (131 mg ml<sup>-1</sup>) and *p*-xylene (92 mg ml<sup>-1</sup>), in addition to less abundant species like *o*-xylene and naphthalene. The “clean” acetone studies showed no difference with the studies containing media alone. However, the presence of tars in the scrubbed acetone solution showed a significant delay in cell growth. The delay is much longer than the washout period shown in Fig. 3. For the studies with tars, the results are shown separately for two of the three studies since the onset of growth varied. The third study is not shown since growth was not observed. A likely scenario is that the tars initially inhibit growth (but do not cause death), which is consistent with the washout of cells in the chemostat. After a long period of time, the cells can adapt to the tars and begin to grow (as observed in batch studies). The adapted growth would not be observable in the chemostat since the washout of cells would occur before the cells had time to adapt. This hypothesis must still be assessed. In addition, studies must be performed to assess whether there is an acceptable limit of the tar constituents denoted above in which cell growth is not affected.

The cells in the presence of tar produced much lower quantities of acetic acid and much higher amounts of ethanol than the controls. Thus, there was a change in the product distribution, although these results appear contrary to the results of Fig. 3 in which the 0.2 mm filter resulted in a decrease in ethanol production and an increase in acetic acid production. However, the cells in the batch studies had a chance to adapt to the tars, unlike the cells in the chemostat studies in which the cells washed out. This may explain the discrepancy, although further studies need to be performed. Nevertheless, the key point is that the tars appear to affect the cell dormancy and product distribution.

#### 4. Conclusions

When fermenting biomass-generated producer gas with *C. carboxidivorans* P7<sup>T</sup>, results showed that tars promoted cell dormancy and a redistribution of ethanol and acetic acid production. However, cells could adapt and grow in the presence of tars following prolonged exposure. Preliminary studies showed that nitric oxide inhibited the hydrogenase enzyme. The additional cleaning of producer gas using a 0.025 mm filter prevented growth inhibition although the filter cleaning did not eliminate the hydrogenase inhibition.

The extent of gas cleanup is a critical issue when applied to producer gas fermentation. Cells are sensitive to many chemical species and the potential for numerous species to be generated during biomass gasification is high. These studies showed that cleaning the producer gas with a cyclone, 10% acetone scrubbing bath, and a 0.025 mm filter

enabled the cells to remain viable and produce ethanol and acetic acid. Further studies need to be conducted to assess the reasons for the shifts in ethanol and acetic acid production. Similarly, scavenging of nitric oxide appears critical for maintaining hydrogenase activity. Gas clean-up issues will likely vary depending upon the biomass since some biomass contains species, such as sulfur compounds, that may affect cell function in fermentation. Nevertheless, this work identified some key gas clean-up issues (tar removal and potential nitric oxide removal) for the fermentation of producer gas to ethanol and acetic acid.

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