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ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Growth of *Rhodospirillum rubrum* on Synthesis Gas: Conversion of CO to H_2 and Poly-β-hydroxyalkanoate

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ABSTRACT: To examine the potential use of synthesis gas as a carbon and energy source in fermentation processes, *Rhodospirillum rubrum* was cultured on synthesis gas generated from discarded seed corn. The growth rates, growth and poly-β-hydroxyalkanoates (PHA) yields, and CO oxidation/H_2 evolution rates were evaluated in comparison to the rates observed with an artificial synthesis gas mixture. Depending on the gas conditioning system used, synthesis gas either stimulated or inhibited CO-oxidation rates compared to the observations with the artificial synthesis gas mixture. Inhibitory and stimulatory compounds in synthesis gas could be removed by the addition of activated charcoal, char-tar, or char-ash filters (char, tar, and ash are gasification residues). In batch fermentations, approximately 1.4 mol CO was oxidized per day per g cell protein with the production of 0.75 mol H_2 and 340 mg PHA per day per g cell protein. The PHA produced from *R. rubrum* grown on synthesis gas was composed of 86% β-hydroxybutyrate and 14% β-hydroxyvalerate. Mass transfer of CO into the liquid phase was determined as the rate-limiting step in the fermentation.

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KEYWORDS: carbon monoxide dehydrogenase; CO; hydrogenase; biohydrogen; *Rhodospirillum rubrum*; gasification; syngas; synthesis gas; producer gas; poly-β-hydroxyalkanoate

Introduction

Gasification is the high temperature (750–850°C) conversion of solid, carbonaceous fuels into flammable gases such as of CO and H_2 collectively known as producer gas, synthesis gas, or syngas (Guehenneux et al., 2005; McKendry, 2002b,c). If chemical equilibrium is attained during gasification processes, the products are essentially limited to the light gases CO, CO_2, H_2, and CH_4 (and nitrogen if air was used as a source of oxygen). However, gasifier temperatures and reaction times are rarely sufficient to attain chemical equilibrium and the raw synthesis gas also contains nitrogen, ammonia, carbonyl sulfide, hydrogen sulfide, various amounts of light hydrocarbons such as C_2H_2 and C_2H_4, as well as heavy hydrocarbons that condense to a black, viscous liquid known as "tar" (McKendry, 2002b; Worden et al., 1997). Other end products include mineral matter (ash) and unoxidized carbon (char) known collectively as gasification residue. The final gas composition is strongly dependent on the amount of oxygen and steam admitted to the reactor as well as the time and temperature of reaction. The sulfur and nitrogen compounds are costly to remove and can lead to the formation of greenhouse gases (Bredwell et al., 1999; Guehenneux et al., 2005; McKendry, 2002a,b,c).

In addition to its use as fuel, the CO and H_2 in synthesis gas are potential growth substrates for a number of diverse microorganisms (Bredwell et al., 1999; Brown, 2003; Klasson et al., 1991; Najafpour et al., 2003, 2004; Spima et al., 2003; Vega et al., 1989; Worden et al., 1991). H_2 has a high fuel value and is therefore a commercially valued end product (Najafpour et al., 2003, 2004). However, due to its toxicity and lower fuel value, the conversion of CO into hydrogen and/or biomass via fermentation would increase the commercial value of this process (Vega et al., 1989; Weaver and Maness, 1993; Wolfrum and Watt, 2002; Worden et al., 1997). Fortunately, CO can be utilized as a growth substrate by a variety of bacteria such as aerobic carboxydotrophs and anaerobic acetogens, sulfate-reducers, methanogens,

Abbreviations used: PHA, poly-β-hydroxyalkanoate

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and phototrophs (Ferry, 1995; Fox et al., 1996; Lorite et al., 2000; Meyers et al., 1990; Ragsdale and Kumar, 1996).

Rhodospirillum rubrum, a photosynthetic purple non-sulfur bacterium, appears particularly well suited for growth on synthesis gas. *R. rubrum* can utilize CO under anaerobic conditions as a sole carbon and energy source in the presence or absence of light (Kerby et al., 1992, 1995). When exposed to CO, both a carbon monoxide dehydrogenase and a CO-insensitive hydrogenase are induced (Bonam et al., 1989, 1984; Ensign and Ludden, 1991). The combined activities of these two enzymes result in the water-gas shift reaction: $CO + H_2O \rightarrow CO_2 + H_2$. Part of the CO_2 produced is assimilated into cell material and the remaining CO_2, along with the H_2, are released into the environment. The enrichment of H_2 during fermentation of synthesis gas has made *R. rubrum* a particularly attractive organism for the bioconversion of synthesis gas into value added products (Najafpour et al., 2003, 2004). Studies on the growth of *R. rubrum* utilizing artificial synthesis gas mixtures of CO, H_2, N_2, and CO suggest this bacterium should grow on synthesis gas (Najafpour et al., 2003, 2004). However, growth of *R. rubrum* on actual synthesis gas has not been reported nor has the problems associated with connecting a gasifier to a fermentor examined. This study examines both issues.

Materials and Methods

Gasification of Biomass

Ground seed corn was gasified in a 25-cm diameter fluidized-bed operated at approximately 705°C (Fig. 1). During gasification, the reactor processed 10–20 kg/h of biomass. The reactor was designed to maintain a 30-cm deep bed at 1 atmosphere pressure. Additional details on reactor construction and operation are found in Emsick (2004). Tar, char, and ash were removed by cyclone(s) and heated particulate filter(s). The gas was then cooled and cleaned in an impinger train immersed in an ice bath prior to injection into the fermentor. Additional impingers(s) filled with activated carbon charcoal, char-ash mixture, or tar-char mixture were added, as subsequently described, when growth and/or CO oxidation rates were inhibited following the switch from the bottled gas mixture to producer gas.

Organism and Culture Conditions

Starter cultures of *R. rubrum* were grown under anaerobic, photosynthetic conditions on SMN plus malate media at ambient temperature (Kerby et al., 1992). Approximately 8 mL of starter culture was used to inoculate 8 L of RRNCO media (Kerby et al., 1995) in a 14 L Bioflo 110 bench-top fermentor (New Brunswick, Edison, NJ) with pH and dissolved O_2 probes and a standard Rushton impeller. Unless otherwise stated the concentrations of MOPS, $NaC_2H_3O_2$, and NH_4Cl in RRNCO were 10, 10, and 18.7 mM, respectively. Fermentation conditions were as follows: temperature: 30°C; synthesis gas or artificial synthesis gas flow rate: 0.7 L/min; agitation speed: 400, 500, 600, 700, or 750 rpm. In most cultures, the biotin and casein enzyme hydrolysate in SMN and RRNCO media were supplemented with yeast extract. In some cultures, 50 or 100% of the 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer, 50% NH_4Cl, and/or acetate were omitted.

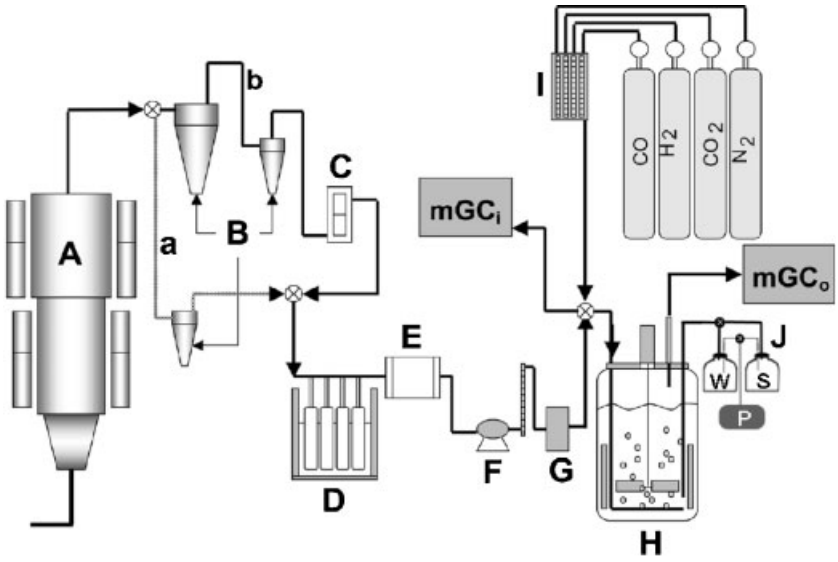

Figure 1. Schematic diagram of gasifier-stirred tank reactor. **A**: Fluidized-bed gasifier, (**B**) cyclones, (**C**) particulate filter, (**D**) impingers in ice bath, (**E**) additional filters (activated carbon charcoal, tar-char plug, or char-ash mix), (**F**) diaphragm pump, (**G**) flow meters, (**H**) stirred tank reactor, (**I**) flow regulator, (**J**) liquid sample port with vacuum pump (P), waste vial (W), and sample vial (S); mGC_i, gas chromatograph–monitoring gas composition entering fermentor; mGC_o: gas chromatograph monitoring gas composition in fermentor exhaust; a, fluidized-bed gasifier to fermentor line 1; and b, fluidized-bed gasifier to fermentor line 2.

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Liquid samples were collected aseptically and under anaerobic conditions in 110 mL serum vials sealed with butyl rubber stoppers (Fig. 1). Before use, the closed serum vials were subjected to five vacuum-purge cycles with argon. Two 110 mL samples were collected every 24 h with the first vial serving as a liquid waste bottle to purge the sample line. The optical density at 680 nm ($OD_{680\ nm}$), protein concentration, dry weight, and dissolved CO were determined by the methods of Bonam et al. (1984); Koch (1994); and Lowry et al. (1951), respectively. Liquid samples for dissolved CO analysis were determined using the continuous flow-syringe sampling port described by Kapic (2005).

Bioreactor Design and Operation

A modified 14 L Bioflo 110 bench-top fermentor (New Brunswick) was used in this study (Fig. 1). Liquid mass transfer rates and fermentor design in the stirred-tank reactor used in this study has recently been described (Kapic and Heindel, 2006). Modifications involved addition of a pressure release valve, a gas tight sampling port for 100 mL samples, and a continuous-flow syringe sampling port. Fermentations were generally started using an artificial synthesis gas mixture composed of 56.0% N_2, 17.2% CO, 16.3% CO_2, and 8.8% H_2. When the cultures reached the desired density the gas feed was switched to actual synthesis gas (Fig. 1).

Gas Analysis

CO, CO_2, CH_4, H_2, N_2, and O_2 concentrations in both the inlet and exhaust lines of a bioreactor were monitored with two separate 3-channel Varian CP-4900 quad-gas chromatograph systems with thermal conductivity detectors (TCD) (Varian, Walnut Creek, CA) and equipped with Molesieve 5A, Pora plot Q (PPQ), and CP-Sil-5CB or Hayesep columns (Varian). Input and output gas compositions were determined and compared in real time during fermentations. Gas concentrations were calculated as a volume percentage in gas samples and converted to molar concentrations based on gas density (Weast et al., 1989) for a given gas species at 30°C and atmospheric pressure. H_2S and carbonyl sulfide (COS) concentrations in raw synthesis gas were analyzed by a Shimadzu GC-17A gas chromatograph system with sulfur chemiluminescence detector (Model 335 SCD) (Sievers Instruments, Inc., Boulder, CO) equipped with GS-GasPro capillary column (30 m × 0.32 mm) (J&W Scientific, Palo Alto, CA).

Identification of Organic Compounds From Synthesis Gas

Organic compounds were extracted and concentrated from synthesis gas by bubbling the gas for 140 min at a flow rate of 20 mL/min into 20 mL hexane/acetone mixture (1:1 volume), in a 30 mL serium vial sealed with a Teflon lined silicon septa for 30 min of gas flow. Organic compounds extracted from syngas were analyzed by Shimadzu GC-17A gas chromatography (GC) system with flame ionization detector (FID) equipped with PTE-5 capillary column (30 m × 0.25 mm ID, 0.25 μm) (Supelco Co., Bellefonte, PA).

Polyhydroxyalkanoate Extraction and Analysis

Poly-β-hydroxyalkanoate (PHA) polymers were extracted and derivatized as described by Brandl et al. (1988); Daniels et al. (1994) with the following modifications. PHA fractions were extracted from 200 mg of lyophilized cells into chloroform and dried. The concentration of PHA was determined gravimetrically and calculated as the percentage of cell dry weight. To determine the polymer composition of PHA, methyl ester of purified PHA was analyzed by GC with an Agilent 6890 GC-mass spectrometer system (Agilent Technologies, Inc., Palo Alto, CA) equipped with a DB-Wax capillary column (30 m × 0.25 mm) (J&W Scientific).

Preparation of Filtering Materials

Three different filtering materials, activated carbon, char-ash mixture, and tar-char mixture, were constructed and tested for conditioning synthesis gas. Activated carbon filters were constructed by placing two impingers in series, each with approximately 250 mL of 6–14 mesh activated carbon (Fisher Scientific, Pittsburgh, PA). Tar-char material was obtained from the impingers in the gasifier-fermentor connecting *line a* (Fig. 1). Two impingers were then filled with tar-char material and the impingers were placed in an ice-bath. Char-ash was collected from the gasifier primary cyclone catch while still warm and stored in an airtight container. Two impingers were then filled with 350–450 mL of the char-ash mixture and placed in an ice-bath. Regardless of the filtration material used, the filter bed expanded during operation to fill the volume of the impingers but did not fluidize.

Results

Growth of *R. rubrum* on Artificial Synthesis Gas Mixture

The laboratory gasifier used in this study was not designed for continuous operation, so to examine growth of *R. rubrum* on synthesis gas, cultures were maintained on an artificial gas mixture composed of 56.0% N_2, 17.2% CO, 16.3% CO_2, and 8.8% H_2 during growth on CO or 56.0% N_2, 17.2% Ar, 16.3% CO_2, and 8.8% H_2 to examine growth in the absence of CO. The cultures were then switched to synthesis gas when the gasifier was operating. The percentage of each gas in the artificial mixture was roughly based on the gas composition of the actual synthesis gas

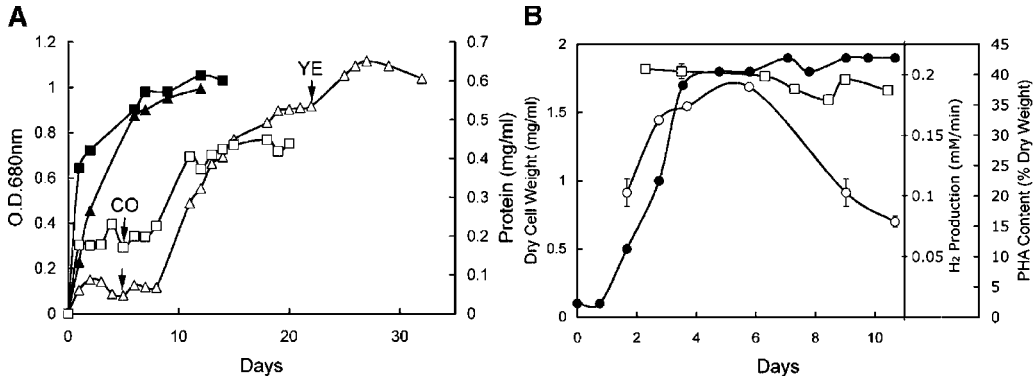

Figure 2. CO-dependent growth of *R. rubrum* in liquid medium. **A**: Cultures were initiated with artificial gas mixture (■, protein; ▲; OD_{680nm}) or initially purged with 72.2% N_2, 17% CO_2, and 9.5% H_2 gas mix (□, protein; △, OD_{680nm}) for the first 5 days followed by artificial gas mixture including CO. Additional yeast extract was added during the stationary stage of culture (YE). **B**: Cell dry weight (●) changes with H_2 (□) and PHA (○) production during the growth on artificial gas mixture.

(Emsick, 2004). *R. rubrum* showed similar doubling times of 11.1 ± 1.8 h on both bottled gas mixtures containing CO or on synthesis gas (Fig. 2). Growth was CO-dependent and the cell yield was limited by the supplemental vitamin mixture/yeast extract in RRNCO media (Fig. 2, Table I).

Dissolved CO concentration decreased from 350 μM to less than 88 nM within a few hours following inoculation and remained less than 0.88 nM in actively growing cultures (results not shown). CO oxidation and growth rates could be stimulated slightly with increased stirring rates, but even at highest stir speeds (900 rpm) the dissolved CO remained less than 0.88 nM (Fig. 3A). The consistency in H_2 evolution at different cell densities also suggests that dissolved CO or mass transfer of CO was the rate-limiting step in growth. As observed by Kerby et al. (1995); Svetlichny et al. (1991); and Uffen (1976), growth yields were limited by yeast extract which was used as a supplement for the vitamin mixture in RRNCO media (Figs. 2 and 3). However, the yeast extract may have also served as a supplemental carbon and/or energy source. Growth rates and yields were similar to those of *R. rubrum* under batch growth condition with a 100% initial CO headspace (Kerby et al., 1995).

Table I. The effect of supplemental carbon source, buffer, and nitrogen source on growth and PHA yields *R. rubrum*.

Medium modification	Cell yield (mg dry weight/L/day)	PHA (mg/day/L)
None	170	59.2
No acetate	130	11.5
5 mM MOPS	170	41.6
No MOPS	130	37.1
9.35 mM NH_4Cl	170	55.0

Concentrations of MOPS, $NaC_2H_3O_2$, and NH_4Cl in unmodified RRNCO were 10, 10, and 18.7 mM, respectively. Cultures were collected at late log or early stationary phase.

Previous studies have shown that the type and amount of PHA produced by *R. rubrum* varied on media composition and carbon sources (Brandl et al., 1989; Ulmer et al., 1994; Weaver and Maness, 1993). On synthesis gas, the PHA content reached to 38% of the cellular dry weight at early stationary phase and was composed of approximately 86% β-hydroxybutyrate and 14% β-hydroxyvalerate (results not shown).

Conditioning of Synthesis Gas

Initial fermentations used black-steel tubing to connect the gasifier to the fermentor (Fig. 1, *line a*). The high molecular weight impurities in the synthesis gas were removed with a mini-cyclone and the gas cooled with a cooled-impinger system. Gas flow to the fermentor was regulated using a diaphragm pump and flow regulator. Using connecting *line a*, there was no negative effect on the growth and CO oxidation rates compared to that observed with the artificial synthesis gas mixture (Fig. 3A). The cause of the small increase in the growth and CO oxidations rates following the switch to synthesis gas was not determined, but was probably due to the higher CO concentration in the producer gas or to the presence of the reducing agent, H_2S (see below) (Table II, Fig. 3B). As in the case of artificial synthesis gas mixtures, dissolved CO was below detection (<0.88 nM) in growing cultures, suggesting gas-to-liquid mass transfer was the rate-limiting step in the rates of CO oxidation on synthesis gas (results not shown). The increased rate of CO oxidation and H_2 production shown in Figure 3A with increased stirring rate also suggested CO mass transfer was the rate-limiting step in these fermentations. Yield of *R. rubrum* grown on synthesis gas was 154 ± 22 mg dry weight/L/day.

Although the black metal pipe sample line (Fig. 1, *line a*) did not show any negative effects on growth of *R. rubrum*,

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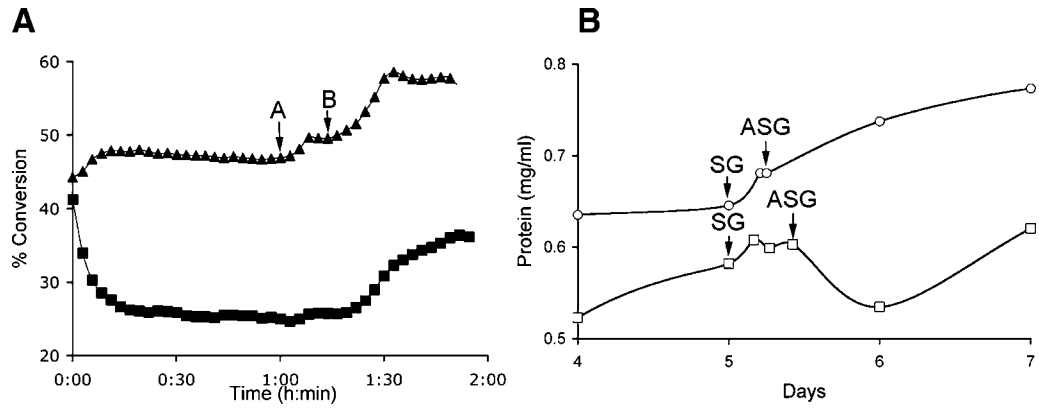

Figure 3. CO oxidation (■), and H_2 (▲) production rates, and protein concentration (○, □) of *R. rubrum* cultures growing on unconditioned synthesis gas from sample line a (Fig. 1). **A**: Agitation speed was changed from 400 to 500 (A) then to 650 (B) rpm during headspace gas analysis. **B**: Synthesis gas (SG) was provided to *R. rubrum* for 6 (○) and 9.6 (□) h, then replaced with artificial gas mixture (ASG).

this system proved problematic in prolonged operation. Char and tar accumulated in the tubing and impingers, which resulted in clogging and breaks in the sample line-impingers connecting points. The line breaks were followed by oxygen contamination and inactivation of the carbon monoxide dehydrogenase. An alternative line using stainless steel tubing with an additional cyclone and a particulate filter was constructed, which provided synthesis gas to bioreactor for more than 10 h without the significant char and tar accumulation (Fig. 1, *line b*). However, synthesis gas delivered through the stainless steel sample line inhibited CO oxidizing activity and resulted in cell death within 2 h of exposure.

Tar, char, and ash are mixtures of partially reduced carbon compounds, a variety of trace inorganic compounds, and activated carbon (Belgiorno et al., 2003; Fjellerup et al., 2005; Smisek and Cerny, 1970). The stainless steal tubing, a hot particulate filter, and the additional cyclone removed tar, char, and ash from the synthesis gas stream and solved the clogging problem (Fig. 1, *line b*). However, the composition of synthesis gas from *line b* differed from that observed in *line a*. The gas composition from *line b* showed a variety of volatile heavy organics such as phenol that were absent in *line a* (results not shown). Apparently the char, ash, and tar that accumulated in *line a* served as a filtering system for a variety of heavy organics. In an attempt to simulate the filtration effect of *line a*, char/tar-ash was captured from the gasifier primary cyclone and utilized as a filtering material. Figures 4 and 5 illustrate CO oxidation and H_2 production rates with synthesis gas filtered through

Table II. Gas compositions (% volume) of artificial synthesis gas mixture and synthesis gas before the bioreactor (input) and gases passed through the bioreactor (output).

	Artificial synthesis gas		Synthesis gas	
Gas species	Input	Output	Input	Output
H_2	9.53 ± 0.59	13.48 ± 1.07	9.71 ± 0.88	13.56 ± 1.08
N_2	55.19 ± 1.44	52.73 ± 1.48	44.74 ± 1.49	43.49 ± 1.12
O_2	bd[c]	bd	bd	Bd
CO	17.14 ± 0.60	11.43 ± 1.02	20.80 ± 1.78	15.11 ± 1.75
CO_2	16.32 ± 1.40	20.40 ± 1.72	14.71 ± 0.94	19.34 ± 1.46
CH_4	—	—	5.60 ± 0.40	5.10 ± 0.27
C_2H_2	—	—	0.21 ± 0.14	0.20 ± 0.11
C_2H_4	—	—	2.06 ± 1.24	1.92 ± 1.20
C_3H_8	—	—	0.05 ± 0.05	0.04 ± 0.02
H_2S[a]	—	—	0.044 ± 0.004	—
H_2S[b]	—	—	0.027 ± 0.008	—
COS[a]	—	—	0.011 ± 0.003	—
COS[b]	—	—	0.015 ± 0.001	—

[a]Gas samples from sample line a.
[b]Gas samples from sample line b.
[c]Below detection.

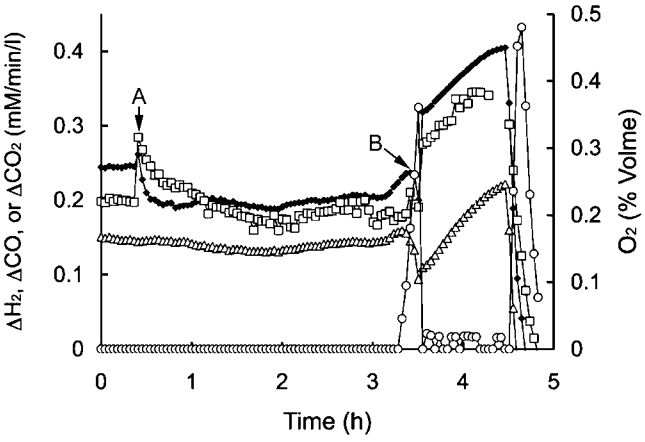

Figure 4. CO oxidation (◆), CO_2 production (□), and H_2 production (▲) rates of *R. rubrum* growing on synthesis gas conditioned by activated carbon charcoal (**A**), and tar-char plug (**B**) replaced activated carbon charcoal. Oxygen concentration (○) in synthesis gas was also monitored in real time.

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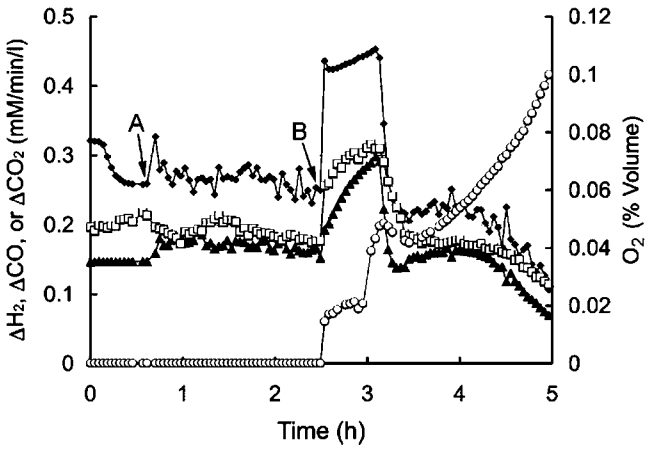

Figure 5. CO oxidation (◆), CO_2 production (□), and H_2 production (▲) rates of *R. rubrum* growing on synthesis gas conditioned by activated carbon charcoal (**A**), and char-ash mixture (**B**) replaced activated carbon charcoal. Oxygen concentration (○) in synthesis gas was also monitored in real time.

the char-tar, char-ash, and activated charcoal filters. The oxidation rates were observed with synthesis and bottled gas from *line b* if the gas was filtered through an activated charcoal filter were similar, which is less than one standard deviation of the individual ferementations. Surprisingly, synthesis gas filtered through tar-char or a char-ash filter resulted in an immediate increase in carbon monoxide dehydrogenase and hydrogenase activities with subsequent increased growth rates over filtration though an activated charcoal filter or with bottled gas mixtures (Figs. 4 and 5, Table III). The reason for this increase has not been determined. One possible explanation for the stimulation is the ineffective filtering of ammonia or other nutrients from the synthesis gas (Emsick, 2004) or to the removal of a trace toxins such as COS (Hyman et al., 1989). However, since the stimulation was immediate it is unlikely that additional nutrient(s) is the explanation for this effect.

A more plausible explanation is a change in redox potential change. Na_2S is a volatile reducing agent added to RRNCO media, however, much of the Na_2S is lost via oxidation or volatilization in these multi-day fermentations. H_2S is a normal component of synthesis gas that was not included in the bottled mixture (Table II). The carbon monoxide dehydrogenase from *R. rubrum* is redox sensitive and lowering redox potential has been shown to stimulate activity (Feng and Lindahl, 2004; Heo et al., 2001). Comparison of gases filtered though the different materials also suggest H_2S was responsible for the stimulation of carbon monoxide activity. In contrast to char-ash or tar-char filters, H_2S was removed by activated carbon filters, and no stimulation of carbon monoxide dehydrogenase activity was observed when producer gas was filtered through activated charcoal filters (Figs. 4 and 5, Table III).

In an attempt to identify the toxic compound(s) in synthesis gas the organic compounds were extracted and examined by GC-FID. Figure 6 showed chromatograms of polar organic compounds extracted from unfiltered synthesis gas (A) and synthesis gas filtered though activated carbon charcoal filter (B) syngas. With activated carbon charcoal filter, approximately 20 compounds were removed from unconditioned syngas. Of the compounds removed by the activated charcoal filter only phenol (9.3 min) and 2,6-dimethoxyphenol (14.6 min) were identified using authentic standards (Fig. 6).

Table III. Effect of carbon monoxide source and gas filters on CO oxidation and H_2 production rates by *R. rubrum*.

Gas	Filter	CO oxidized (mol/day/g protein)	H_2 produced (mol/day/g protein)
ASG[a]	None	1.07	0.74
SG[b]	Carbon	0.79	0.62
SG	Char + ash	1.03	0.67
ASG	None	0.91	0.57
SG	Carbon	0.76	0.53
SG	Char + ash	1.40	0.65

[a]Artificial synthesis gas mixture.
[b]Synthesis gas.

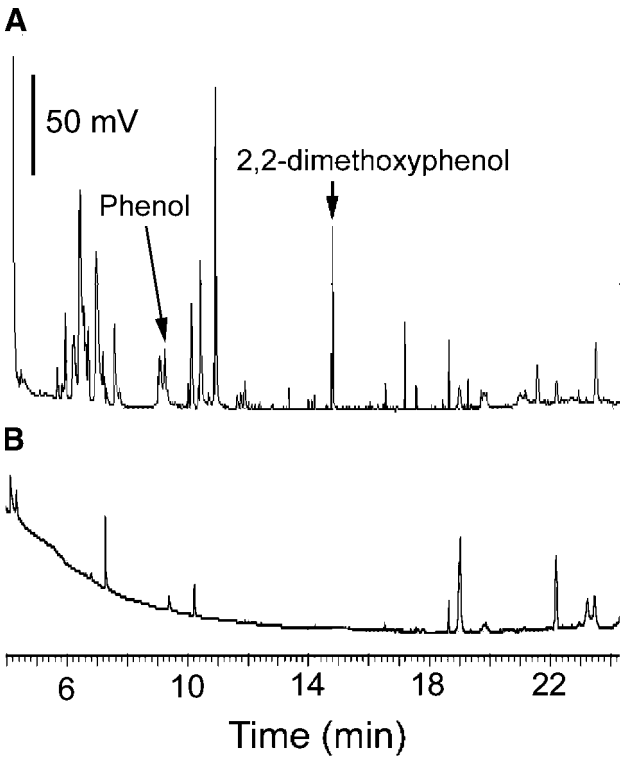

Figure 6. Chromatograms of polar organic compounds extracted from unfiltered synthesis gas (**A**) and synthesis gas filtered though activated carbon charcoal filter (**B**).

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Discussion

R. rubrum was used as a biocatalyst in an attempt to upgrade the quality of synthesis gas by decreasing the concentration of CO, increasing the concentration of H_2, and producing PHA as a model product. *R. rubrum* has a number of properties which make it a good test system in our attempts to improve the commercial value of synthesis gas. Metabolically, *R. rubrum* is capable of growth under aerobic or anaerobic conditions in the presence or absence of light on a variety of growth substrates (Truper and Pfenning, 1978). With respect to synthesis gas, *R. rubrum* can utilize CO as a sole energy and electron source reducing H^+ to H_2 as a terminal electron acceptor (Ensign and Ludden, 1991). The bacterium can also utilize H_2S as a sole energy or electron source, with the extracellular deposition of S^0 (Brune, 1995; Truper and Pfenning, 1978). The removal of this toxic and corrosive gas would improve the quality of the post-fermentation gas. Volatile sulfur and nitrogen compounds are costly to remove and can lead to the formation of greenhouse gases (Bredwell et al., 1999; Guehenneux et al., 2005; McKendry, 2002a,b,c). This bacterium also has a well developed genetic system and the structural genes for carbon monoxide dehydrogenase, the CO-induced hydrogenase, and biosynthetic genes for PHA production and degradation in this bacterium have been characterized (Clemente et al., 2000; Donohue and Kaplan, 1991; Ensign et al., 1989; Ensign and Ludden, 1991; Kerby et al., 1992, 1995; Reiser et al., 2000; Williams and Taguchi, 1995). In addition, the culture conditions for induction of PHA synthesis on a variety of organic compounds have been determined in *R. rubrum* (Fuller, 1995). Lastly, to make this process more cost effective, the media composition of *R. rubrum* was modified in this report to minimize cost. MOPS, acetate, and $NiCl_2$ are the most expensive media components in RRNCO media. The MOPS and acetate in the media could be removed without affecting growth rates or H_2 production. However, removal of acetate resulted in a 50% decrease in PHA production. Ni is a critical component of the carbon monoxide dehydrogenase and could not be reduced without affecting growth on CO. The results presented here demonstrate that *R. rubrum* can be cultured on synthesis gas although several technical problems in gas conditioning need to be addressed before the process can be commercialized.

The major issue to be addressed is the mass transfer of CO into the liquid phase. A commercial stirred tank reactor was used in this project to examine the potential problems in coupling a gasifier to fermentors and as observed in earlier studies using artificial gas mixtures (Bredwell et al., 1999; Cowger et al., 1992; Kapic, 2005; Kerby et al., 1992, 1995), mass transfer of CO was the rate-limiting step in growth. However, the results presented in this study demonstrate the potential of *R. rubrum* to lower the CO concentration in synthesis gas below toxicity levels, that is, <0.9 nM. Thus, if the mass transfer rate of CO into the liquid phase is increased via altered fermentor design or sequential fermentor systems, this bacterium has the potential to convert essentially all of the CO in synthesis gas to biomass, CO_2, and H_2.

A second potential problem identified in this study, which may eventually prove beneficial, was the accumulation of tar, char, and ash in the connecting lines and impingers. Tar, char, and ash appear to remove high molecular weight end products of incomplete gasification, which can prove toxic to *R. rubrum*. Activated carbon was shown in this study to remove toxic compounds from the synthesis gas but the use of activated carbon would increase fermentation costs. Tar-char and char-ash mixtures also removed toxic compounds from synthesis gas and appeared to stimulate carbon monoxide dehydrogenase activity. However, the tar containing mixtures were difficult to manipulate and would frequently clog the impingers trains resulting in cracking and oxygen leaking. Although technical improvements are required to prevent clogging, the results presented here indicate a waste product of the gasification process could be used as filtering material for conditioning synthesis gas.

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