BIOREACTOR DESIGN STUDIES FOR A NOVEL HYDROGEN-PRODUCING BACTERIUM

Edward J. Wolfrum, Andrew S. Watt
National Renewable Energy Laboratory
1617 Cole Boulevard, Golden, CO 80401

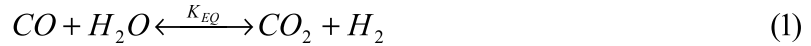
Abstract

Carbon monoxide (CO) can be metabolized by a number of naturally occurring microorganisms along with water to produce hydrogen (H_2) and carbon dioxide (CO_2). NREL researchers have isolated a number of bacteria that perform this so-called "water-gas shift" reaction at ambient temperatures. We have performed experiments to measure the rate of biological CO conversion and H_2 production in a trickle bed reactor (TBR). The reactor support material has a significant effect on the mass transfer coefficient, which in turn controls the overall reactor performance for this mass transfer-limited reaction. A simple reactor model taken from the literature is used to quantitatively compare the relative performance of the different support materials, including one support material tested at two different TBR sizes (1-L and 5-L). A TBR bioreactor was used to condition water-scrubbed synthesis gas from a biomass gasifier, and was unaffected by the presence of low concentrations of aromatic compounds over the course of the weeklong test.

Introduction

The biologically-mediated water-gas shift reaction may be a cost-effective technology for the conditioning of synthesis gas for storage or direct use within a hydrogen fuel cell, where the presence of even low concentrations of carbon monoxide are deleterious. NREL researchers have isolated a number of photosynthetic bacteria that can perform the water-gas shift reaction, in

which carbon monoxide is oxidized to carbon dioxide while simultaneously water is reduced to hydrogen (Weaver *et al.* 1980). The overall stoichiometry of this reversible reaction is:



$$CO + H_2O \xleftrightarrow{K_{EQ}} CO_2 + H_2 \tag{1}$$

One significant advantage to using bacteria to perform the water-gas shift reaction is their ability to operate at ambient temperature. Because the reaction occurs at ambient temperature, the reaction is not equilibrium-limited (at 25°C, K_{EQ}~5x10^{4}). The advantages of low operating temperature and lack of equilibrium limitation make the biological shift reaction a promising alternative to conventional shift technologies. Preliminary data already collected at NREL suggest that this reaction is far more rapid than the rate at which CO can be supplied to the bacterial culture (Markov *et al.* 1997). This is consistent with many other gas/liquid biological reaction systems, including most aerobic fermentations, where metabolic rates are commonly limited by the transfer rate of a gaseous substrate to the liquid media.

A number of researchers have investigated the biological conversion of gaseous substrates to produce fuels and chemicals. Vega and others (Vega *et al.* 1989a, et al. Vega 1989b) used batch (anaerobe bottles) and continuous (chemostat) experiments to investigate CO conversion to acetate using the bacterium *Peptostreptococcus productus*. The authors developed kinetic expressions for cell growth and CO utilization under mass transfer- and kinetics-limited operation in each reactor, but the resulting rate expressions predicted different levels of CO inhibition at higher substrate concentrations. Klasson *et al*. (1990) extended this work to investigate acetate production from CO by *P. productus* in a chemostat, a packed bubble column, and a trickle bed reactor. They developed a simple reactor model and used it to calculate mass transfer coefficients for each reactor tested. Kimmel *et al.* (1991) used a triculture of *Rhodospirillum rubrum*, *Methanosarcina barkeri*, *and Methanobacterium formicicum* to produce methane from synthesis gas using two different size trickle bed reactors. *R. rubrum* performed the water gas shift reaction to produce H_2 and CO_2, and the two methanogens subsequently converted these gases to CH_4. They compared the performance of the two reactors, but got considerably lower conversion rates in the larger column, even though they operated the larger column at slightly higher liquid velocities. The authors pointed to poor liquid distribution in the larger column as a likely cause of the differences between the two reactors.

In this work we use a monoculture of a unique photosynthetic bacterium, *Rubrivivax gelatinosus CBS2*, to carry out the water gas shift reaction (Eq. 1) and use a simple reactor model taken from the literature to analyze the mass transfer characteristics of a trickle bed bioreactor. We examine the influence of reactor support material on carbon monoxide conversion in a TBR, and then examine the relative performance of two different reactor reactors of identical geometry but different size. Finally, we present preliminary experimental data documenting the ability *of Rx. gelatinosus CBS2* to condition biomass-derived synthesis gas streams.

Experimental

Materials

The reactor design used in this work was a trickle bed bioreactor (TBR), shown schematically in Figure 1. Both a 1-L and a 5-L TBR assembly were used. The 1-L TBR assembly consisted of a 2” ID (nominal) glass pipe 24” long. Rubber stoppers (#11 size) were inserted at each end of the glass pipe and acted as end caps. The reactor support rested on a stainless steel mesh approximately 3” above the bottom of the reactor. This space provided a sump area where the recirculating liquid collected and could be conveniently sampled for cell density and pH. The gas inlet and outlet fittings which passed through the rubber stopper end caps were 1/8” OD stainless steel tubing, and the liquid inlet and outlet fittings were ¼” OD stainless steel tubing. The inlet fittings (gas and liquid) were located in the center of each end cap, with the corresponding outlet fittings offset slightly. The liquid drained into the reactor sump by gravity and was recirculated using a peristaltic pump and ¼” ID flexible tubing (MasterFlex #24 Norprene tubing) back to the top of the TBR. The 5-L TBR assembly was conceptually similar to the 1-L assembly except that the reactor was composed of 3” diameter glass tubing, the rubber stoppers were larger (#14 size), and gas and liquid inlet and outlet fittings were ¼” stainless steel.

Four different reactor supports were tested in the 1-L TBR assembly. Two nonporous glass beads (3mm and 6mm diameter) were tested. Two porous materials were also tested: a cellulosic sponge material, cut into approximately 1-cm cubes; and mixture of northeastern mixed hardwoods milled to approximately 1.5-cm pieces. The 6mm-diameter glass bead support was also tested in the 5-L TBR assembly.

The microorganism used in this work was *Rubrivivax gelatinosus CBS2*, isolated from the natural environment by the Weaver group at NREL (Maness and Weaver 1994). The minimal culture media (M-1 basal) had the following composition (amounts are for 1 L of final media preparation): basal salts (120 mg $MgSO_4 \cdot 7H_2O$, 75 mg $CaCl_2 \cdot 2H_2O$, 11.8 mg $FeSO_4 \cdot 7H_2O$, 20 mg EDTA); trace elements (2.8 mg H_3BO_3, 1.6 mg $MnSO_4 \cdot H_2O$, 0.75 mg $Na_2MoO_4 \cdot 2H_2O$, 0.24 mg $ZnSO_4 \cdot 7H_2O$, 0.04 mg $Cu(NO_3)_2 \cdot 3H_2O$, 0.8 mg $CoCl_2 \cdot 6H_2O$, 0.8 mg $NiCl_2 \cdot 6H_2O$); phosphates (1.2 g KH_2PO_4, 1.8 g K_2HPO_4); vitamins (1.0 mg thiamine HCl, 15 ug biotin, 1.0 mg nicotinic acid, 10 ug B-12, 0.1 mg p-aminobenzoic acid); and ammonia (1.5 g NH_4Cl). In addition, malate (5 g/L) and yeast extract (0.5 g/L) were added as carbon sources. The media was prepared using deionized water and stock solutions of basal salts, trace elements, vitamins, buffers, ammonia, and D,L-malic acid. Yeast extract was added as a powder immediately prior to sterilization. The stock solutions were in turn prepared using stock chemicals from various manufacturers, which were used as received.

Methods

Pure cultures of *Rx. gelatinosus CBS2* were grown and periodically subcultured under sterile conditions using 20-mL screw-top test tubes and 200-mL serum flasks. These vessels were kept under incandescent illumination until used to inoculate the TBR assemblies. The microorganisms were not exposed to carbon monoxide during the growth/subculture process.

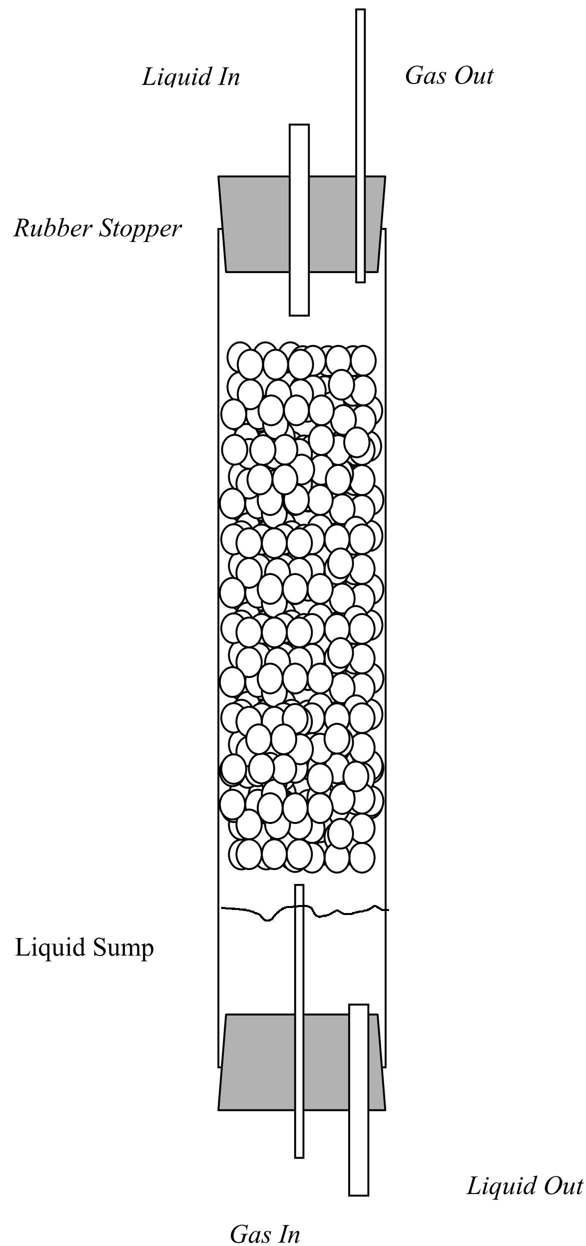


Figure 1. Schematic Diagram of countercurrent Trickle Bed Bioreactors (TBRs) used in the present work.

The 1-L TBR experiments proceeded as follows. The reactor, including the external liquid recirculation loop, was assembled, autoclave sterilized, and allowed to cool. The assembly was then installed in a canopy hood, and the gas inlet fitting connected to the source gas (20%CO, 0.5% He as tracer gas, balance N_2). The reactor was then rinsed with sterile M-1 media while gas flow was initiated. After several reactor volumes of gas were allowed to flow through the reactor, the reactor was drained of any remaining media and inoculated with one serum flask of

Rx. gelatinosus. Default gas and liquid flowrates were established (200mL/min liquid recirculation rate, 25 accm gas flowrate) and the reactor sump was illuminated with a 65W incandescent lamp for several days. CO uptake (and concomitant H_2 production) was induced within approximately 48 hours. Once H_2 production reached a steady state, the lamp was turned off and the reactor loosely covered with black cloth. The operating conditions of the reactor (gas and liquid flowrates) were periodically adjusted, and the outlet gas composition was monitored over time, using a portable gas chromatograph (Agilent Inc. P200). The reactor typically required 12 hours or more to reach steady state after a change in operating conditions. These steady state values were recorded, and then the operating conditions were changed. The total liquid volume in the reactors (including the reactor sump) was approximately 200mL. There was some liquid loss due to evaporation. Periodically, ~20mL aliquots of sterile M-1 media were added to the reactor to replenish the liquid. No effect on reactor productivity (CO shift rate) was seen as a result of these media additions.

The 5-L TBR experiments proceeded in a similar fashion, except the reactor was not autoclaved. Rather, it was washed with standard laboratory soap and rinsed thoroughly with deionized water passed through a sterile 0.2µm filter. Larger liquid innocula were used, typically 600 mL. The default gas and liquid flowrates for the 5-L TBR assembly were 65 accm and 500 mL/min, respectively.

As mentioned above, the inlet gas stream contained 0.5% He as an inert tracer to compensate for changes in the volumetric gas flowrate across the reactor. The water gas shift reaction causes an increase in the volumetric gas flow rate, since 2 moles of gas (H_2, CO_2) are produced for every mole of CO consumed (water is supplied by the media). This volume change would bias CO outlet concentrations low, since CO would not only be consumed by the microorganisms but also diluted by additional gas flowrate. Similarly, outlet hydrogen concentrations would be biased low due to dilution. Since helium is neither consumed nor produced in the reaction, its molar flowrate is constant. Thus, any change in helium concentration must correspond to a change in the overall gas flowrate. This correction factor was applied to all outlet concentration measurements.

Reactor Modeling

The performance of TBR reactor assembly can be modeled as simple plug-flow reactor, with the overall reaction rate controlled by a mass transfer coefficient. This model was developed by Gaddy et al. for a number of different reactor geometries, including CSTRs, packed bubble column reactors, and trickle bed reactors (Vega *et al.* 1989a,Vega *et al.* 1989b, Klasson *et al.* 1990). When the reaction rate is limited by the rate of mass transfer, the steady-state liquid concentration of reactant (in this case, carbon monoxide) can be assumed to be zero, and the controlling equation for an ideal trickle bed reactor is:

$$v_z \frac{dC}{dz} = -\frac{k_L a}{H} C \tag{2}$$

where v_z is the axial gas velocity, C is the gas-phase concentration of reactant, k_La is the overall mass transfer coefficient (based on empty bed reactor volume) and H is the Henry's Law coefficient of the reactant, a measure of its solubility in the liquid phase. Often the mass transfer coefficient is based on the liquid holdup volume, and the term k_La in the Eq. 2 is replaced by $k_La \cdot e_L$, *where* e_L is the liquid porosity (the ratio of the liquid holdup volume to the empty bed reactor volume). In this work we base the mass transfer coefficient on the overall reactor volume, so no such correction is necessary. Eq. 2 can be integrated to give:

$$C_o = C_i \exp(-\frac{k_L a}{H} t_{EBCT}) \quad (3)$$

where C_o and C_i are the outlet and inlet reactant concentrations, and t_{EBCT} is the empty bed contact time of the reactant in the reactor, calculated as the empty bed volume of the reactor divided by the volumetric gas flowrate. By using an overall mass transfer coefficient in Eqs. 2 and 3, we treat the reactor system as a "black box," a view reinforced by the use of t_{EBCT} as the independent variable. Thus, reactors with variable geometries, capacities, and support materials can be directly compared using this equation.

Conditioning Biomass-Derived Syngas

The longer-term goal of this project is to use the biological water gas shift reaction to condition biomass-derived synthesis gas. One concern with this process is the presence of organic compounds in the synthesis gas that may be toxic to microorganisms. These organic compounds are generally referred to as "tars," and have a broad range of molecular weights and chemical structures. To test this, we operated a bioshift reactor on a slipstream of biomass-derived synthesis produced at NREL's Thermochemical User Facility (TCUF).

Biomass-derived synthesis gas was produced by feeding an 8-inch diameter fluid bed reactor at the TCUF with a mixture of hard and soft woods at 12 kg/h, with superheated steam providing the fluidization at 25 kg/h. The bed temperature was approximately 580°C, sufficient to generate both pyrolysis gas and vapors. Thermal (steam) cracking of the vapors in the secondary thermal cracker (780-800°C) converted the vapors to syngas. Cyclones and wet scrubbing cleaned the syngas of char/ash and high molecular weight tars. A slipstream of this gas was taken directly downstream of the wet scrubber.

A 1-L reactor containing *R.gelatinosis CBS2* immobilized on knife milled (sieved to 10-12 mesh) northeastern mixed hardwoods was fed synthesis gas for a total of 29 hours over four days. Since the TCUF operates only during the day, at night the bioreactor was fed a synthetic syngas mixture. The water scrubbing removed most of the high molecular weight and the most polar "tars." However, GC data indicated the presence of both acetylene and ethylene, and mass spectroscopy indicated the presence of both benzene and toluene in the feed to the bioreactor at approximately 5000 and 3000 ppmv, respectively.

Results and Discussion

The results of a typical experiment (a 1-L TBR reactor with cellulose support) are shown in Figure 2. This figure shows the variation in conversion, mass balance and ratio of hydrogen

produced to carbon monoxide consumed ($H_2/(CO_{in}–CO_{out})$) vary with time. The mass balance data were very stable at 98.0% +/- 0.8% over the entire course of the experiment, which lasted about 25 days. The H_2/CO ratio is also quite stable at 101.4 +/- 3.6%, and is in agreement with theoretical stoichiometry. The conversion goes from zero to approximately 60% over the first four days of the experiment. This represents the induction period of a non-induced culture; the time necessary for the microorganisms to activate the necessary enzyme pathways to metabolize CO. Once this steady-state value is reached, the reactor conditions (gas and liquid flowrates) are systematically varied, causing dramatic changes in CO conversion. During these rapid fluctuations in CO conversion, the mass balance and H_2/CO ratio values remain essentially constant, giving us confidence that our analytical system is functioning well and that the bioreactor system as a whole is performing properly. The data in Figure 2 are reduced to yield approximately 12 discrete conversion versus gas flowrate data points.

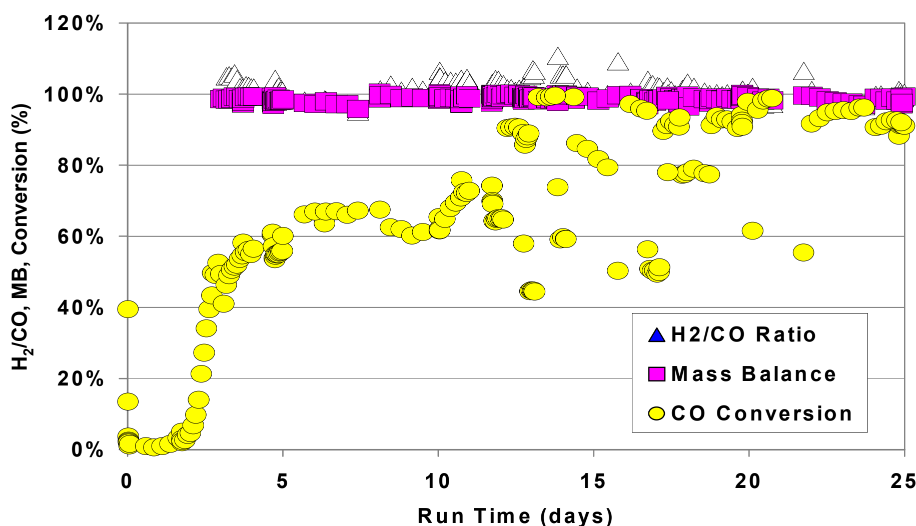


Figure 2. Typical performance of the 1-L TBR assembly. The liquid recirculation rate was 200 mL/min and the gas flowrate was 25 accm.

Influence of Reactor Support

Figure 3 shows the effect of the four reactor support materials on CO conversion in the 1-L TBR assembly. The abscissa in this figure is the space velocity, which is the ratio of the volumetric gas flowrate to the empty bed reactor volume, the inverse of the empty bed contact time. The nature of the reactor support clearly has a profound influence on the performance of the TBR. The same data are replotted according to Eq. 3 in Figure 4. The slopes of these lines are the quantity k_La/H (the solid lines in Figure 3 are the model fits of Eq. 3). Since the Henry's Law coefficient for carbon monoxide (at 25°C) is 57,800 atm (mole fraction)$^{-1}$ or 42.3 (unitless) (Foust 1980), the overall mass transfer coefficients for the four supports are: 0.85 min^{-1} (hardwood), 0.72 min^{-1} (cellulose), 0.38 min^{-1} (3mm glass beads) and 0.19 min^{-1} (6mm glass beads). Thus, by varying the reactor support material, the performance of the reactor can be altered by over a factor of 4.

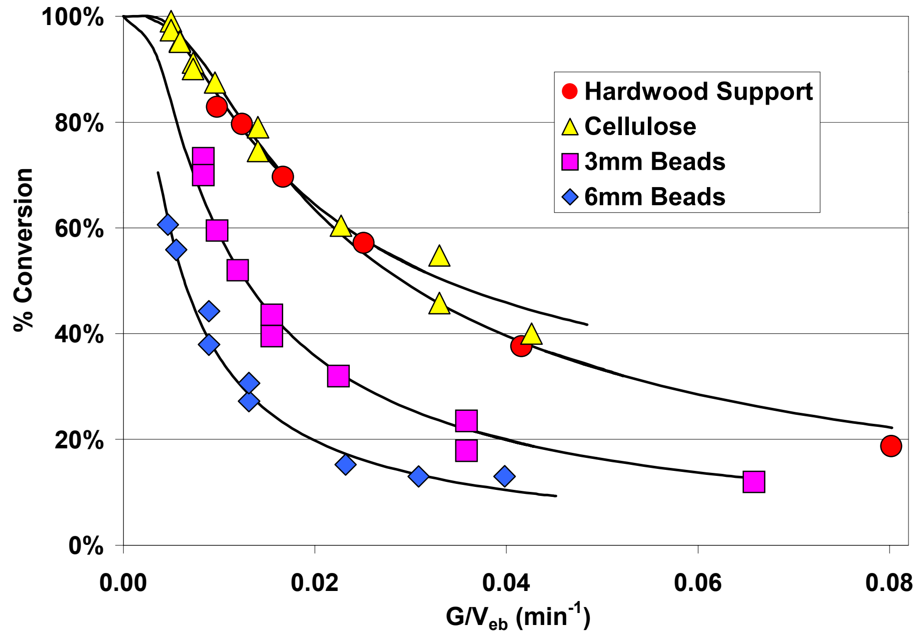


Figure 3. Comparison of CO conversion vs. space velocity in the 1-L TBR assembly using different support materials.

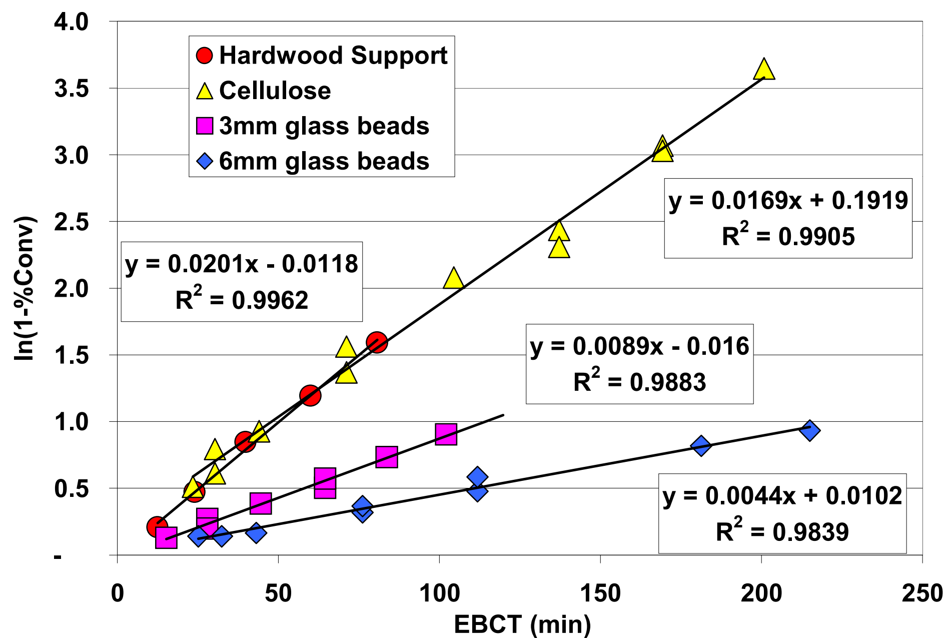


Figure 4. ln(C_o/C_i) vs. EBCT for the 1-L TBBR experiments. The liquid recirculation rate was 200 mL/min.

Reactor Scaling

The ability of this simple model to predict reactor performance at different scales is tested in Figures 5 and 6, where the influence of the same reactor support (6mm-diameter glass beads) in the 1-L and 5-L TBR assemblies are compared. The performance of the two reactors is essentially identical. Note that the superficial liquid phase velocities were slightly different: 1.06 cm s^{-1} and 1.18 cm s^{-1} in the 1-L and 5-L TBR assemblies, respectively. Since the liquid velocity in the larger reactor was slightly higher, we would expect slightly better performance in this reactor. Nonetheless, these results give us considerable confidence in our understanding of the reactor dynamics, and in our ability to accurately predict the performance of larger reactors from the performance of smaller ones.

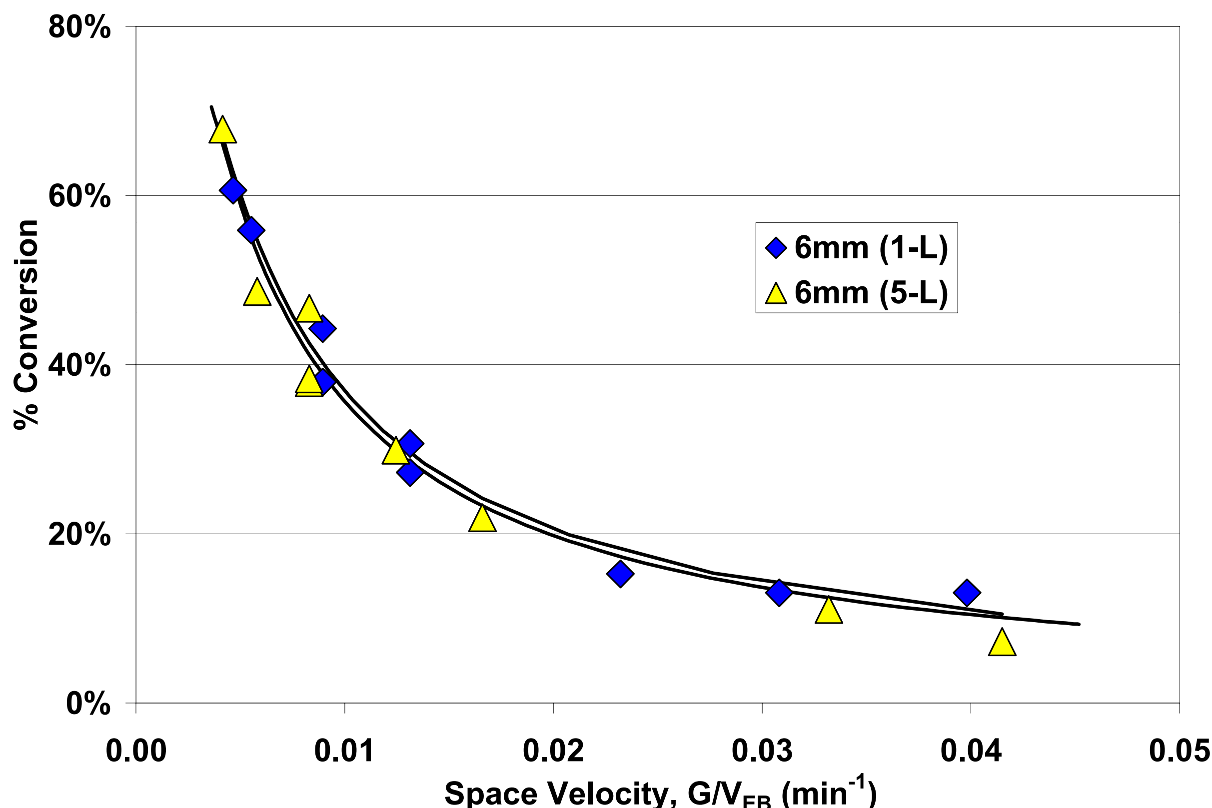


Figure 5. Comparison of CO conversion vs. space velocity using a 6mm-diameter glass bead support in the 1-L and 5-L TBR assemblies. The liquid recirculation rate was 200 mL/min for the 1-L TBR and 500 mL/min in the 5-L TBR, giving similar superficial liquid velocities.

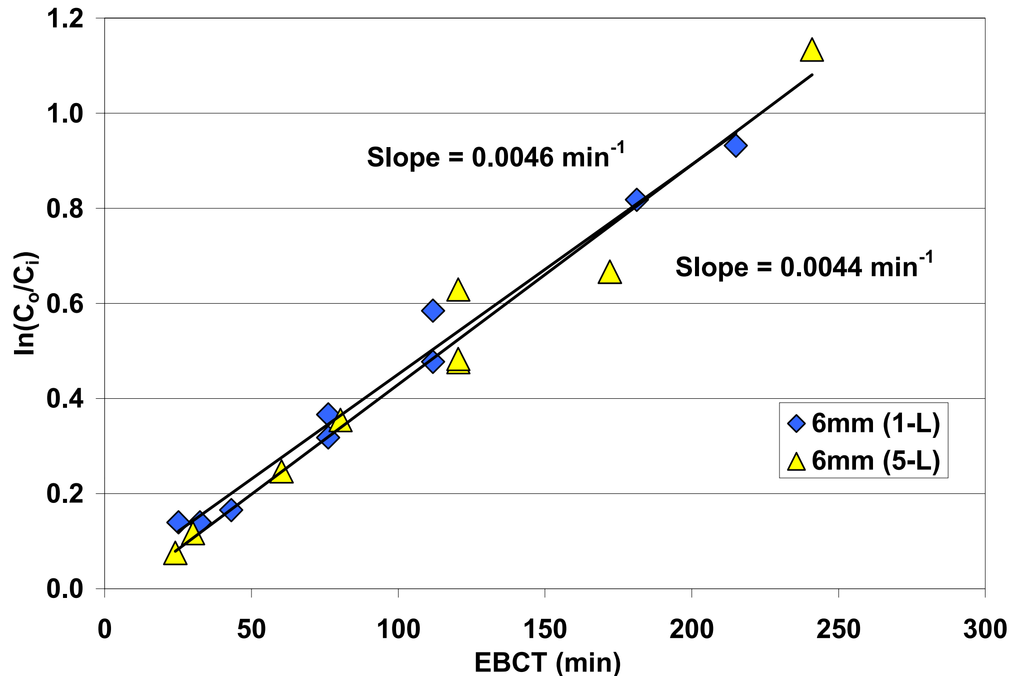


Figure 6. ln(C_o/C_i) vs. EBCT for the 6mm diameter glass bead support in the 1-L and 5-L TBR assemblies. Superficial liquid velocities were 1.06 cm s^{-1} and 1.18 cm s^{-1} in the 1-L and 5-L TBR assemblies, respectively.

Conditioning Biomass-Derived Syngas

Figure 7 shows the results of the biomass-derived syngas conditioning experiment performed at the NREL TCUF. A 1-L bioreactor conditioned the water-scrubbed synthesis gas during the day for four consecutive days, running with bottled synthesis gas overnight. Over the course of this experiment, the conversion efficiency was essentially constant at 25%, and the carbon mass balance was substantially closed. The variation in the outlet concentrations of both carbon monoxide and hydrogen at the beginning of days 2-4 is an artifact of the switch between bottled gas and process gas. This was the first test of the bioreactor using biomass-derived syngas, rather than synthetic carbon monoxide mixtures, and the overall performance was encouraging. Concerns that tar components of syngas (and permanent gases including ethylene and acetylene) may be toxic or inhibitory to *R. gelatinosus* were unsubstantiated by this experiment. We are presently performing rigorous toxicity experiments in the laboratory to further investigate this important issue.

Conclusions

A trickle bed reactor was used to examine the influence of reactor support material on the conversion of carbon monoxide to hydrogen by a novel photosynthetic bacterium in a trickle bed bioreactor. A simple reactor model taken from the literature was used to quantitatively compare the performance of the different supports, and to compare the performance of a single support in two different reactor sizes. The nature of the support affects the mass transfer coefficient, which

in turn controls the overall reactor performance. The two reactor sizes performed approximately the same, giving us confidence in the scale-up of this reaction.

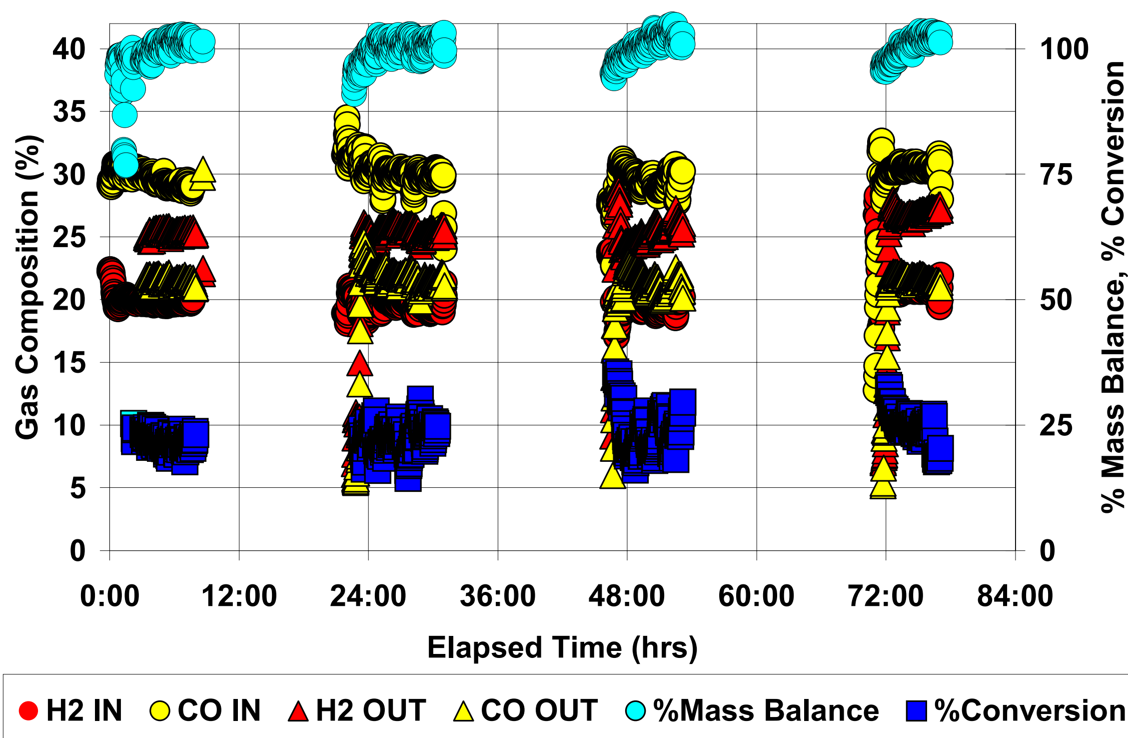


Figure 7. Results from four-day experiment using *Rx. gelatinosus CBS2* to condition biomass-derived synthesis gas.

Acknowledgments

The authors would like to acknowledge Pin-Ching Maness and Paul F. Weaver of the NREL Basic Sciences Center, who provided guidance and advice throughout the course of this work. Mr. Christopher Doelling performed a number of preliminary experiments.

References

A.R. Pedersen and E. Arven. "Removal of Toluene in Waste Gases Using a Biological Trickling Filter," 1995. *Biodegradations*. 6:109-118.

A.S. Foust; L.A. Wenzel; C.W. Clump; L. Maus, and L.B. Anderson. 1980. *Principles of Unit Operations, 2nd Edition*. New York: John Wiley & Sons.

Kimmel, D. E.; Klasson, K. T.; Clausen, E. C., and Gaddy, J. L. 1991. "Performance of trickle-bed bioreactors for converting synthesis gas to methane." *Applied Biochemistry and Biotechnology,* 28/29:457-469.

Klasson, K. T.; Elmore, B. B.; Vega, J. L.; Ackerson, M. D.; Clausen, E. C., and Gaddy, J. L. 1990. "Biological Production of Liquid and Gaseous Fuels from Synthesis Gas." *Applied Biochemistry and Biotechnology,* 24/25:857-873.

Maness, P.-C. and Weaver, P. F. 1994. "Production of poly-3-hydroxyalkanoates from CO and H2 by a novel photosynthetic bacterium." *Applied Biochemistry and Biotechnology,* 45/46:395-406.

Markov, S.; Weaver, P. F., and Seibert, M. 1997. "Spiral tubular bioreactors for hydrogen production by photosynthetic microorganisms: design and operation." *Applied Biochemistry and Biotechnology,* 63/65:577-584.

Vega, J. L.; Clausen, E. C., and Gaddy, J. L. 1989a. "Study of Gaseous Substrate Fermentations: Carbon Monoxide Conversion to Acetate. 1: Batch Culture." *Biotechnology and Bioengineering.* 34:774-784.

Vega, J. L.; Antorrena, G. M.; Clausen, E. C., and Gaddy, J. L. 1989b. "Study of Gaseous Substrate Fermentations: Carbon Monoxide Conversion to Acetate: 2. Continuous Culture." *Biotechnology and Bioengineering..* 34:785-793.

Weaver, P. F.; Lien, S., and Seibert, M.1980. "Photobiological production of hydrogen." *Solar Energy,* 24(1):3-45.