Section II. Hydrogen Production and Delivery

II.A Biological Processes

II.A.1 Biological Water Gas Shift Development

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Objectives

- Study the overall biological CO shift pathway at the physiological, biochemical, and genetic levels.
- Improve the rates and durability of H_2 production from CO by determining how CO regulates the overall water-gas shift (WGS) pathway by serving as an inducer, a substrate, and an inhibitor.
- Elucidate the genetic system of the CO shift reaction to (1) identify the various components involved and (2) allow manipulation of gene expression.
- Develop bioreactors that will promote an economic biological WGS reaction.
- Design, construct, and operate a bioreactor capable of operating at elevated system pressures.
- Measure the specific CO shift kinetics of the photosynthetic bacterium *Rubrivivax (Rx.) gelatinosus* CBS.

Approach

- Characterize the physiological and biochemical mechanisms in the CO shift reaction by which *Rx. gelatinosus* CBS produces H_2 from CO.
- Clone and characterize genes involved in the *Rx. gelatinosus* CBS CO shift pathway.
- Build on the understanding of the CO shift pathway, optimize the WGS process for maximum H_2 production.
- Develop reactor models and economic analyses to guide the research and development efforts of the biological WGS process.
- Scale up the WGS bioreactor design based on laboratory data, reactor modeling results, and economic analyses.

Accomplishments

- Completed physiological/biochemical study of the CO shift reaction and determined that CO acts as an inducer of the CO shift reaction at the gene transcription level and that at least 10% (v/v) CO is required to maximally induce the H_2 production activity.
- Identified several aspects of the CBS genome that will aid in the cloning of genes involved in H_2 production.
- Successfully operated trickle-bed bioreactors with volumes of 1L and 5L, and showed that the performance of the larger reactor could be predicted from the performance of the smaller one.

- Operated the 1-L bioreactor on a synthesis gas stream derived from biomass gasification, and investigated a number of materials for use as reactor supports.
- Determined the specific CO shift rate of *Rx. gelatinosus* CBS.
- Operated a 100-mL pressurized bioshift reactor at system pressure of 10 atm.
- Completed a "Work for Others" project with a refinery services company that demonstrated the ability of NREL-isolated microorganisms to effectively treat certain "real-world" refinery gas streams, and documented the attractive economics of the overall process. This work culminated in a National Renewable Energy Laboratory (NREL) Record of Invention (ROI).

Future Directions

- Determine if the CO shift reaction can serve as an energy-yielding step in darkness. If verified, this energy can be used to sustain cell growth and to induce new CO shift enzymes to support H_2 production for a longer duration without the need for light input.
- Investigate the enzymes and proteins involved in the initial CO oxidation step - namely the CO dehydrogenase (CODH) enzyme, its electron acceptor and electron mediators - to further understand the biochemistry of the CO shift pathway.
- Construct a large-insert genomic library in a broad host range plasmid. This will facilitate the cloning of CO-shift related genes as well as be useful in complementation experiments when a CO-shift mutant is obtained.
- Continue efforts to overcome the technical hurdles involved in operating the pressurized 1-L volume WGS bioreactor. Key issues to be resolved include system pressure control, in-place reactor sterilization, media addition and removal, and pH/ORP monitoring at elevated pressures.
- Once the bench scale system is optimized, design and construct a pilot-scale pressurized bioreactor at significantly increased scale.
- Continue to build on the specific rate measurement work completed, continuing experiments on the effect of trace levels of organic compounds on the specific CO shift rate of *Rx. gelatinosus* CBS.
- Determine how coproducts might affect the overall economics of biological hydrogen production, specifically on the co-production of single-cell protein (SCP).
- Continue work with industrial partners to investigate near-term applications for the biological water gas shift process.

Introduction

Microbial production of H_2 has gained substantial interest in recent years due to the wide variety of substrates microbes can use to generate H_2. These substrates can either be waste biomass or commercial waste streams containing organic compounds. Microbial H_2 production therefore has the dual benefits of generating a clean fuel and reducing waste. However, due to its heterogenous nature, biomass utilization by microbes is extremely slow. Instead, biomass can be gasified via a thermochemical process to generate a homogeneous synthesis gas, which contains hydrogen, carbon monoxide and carbon dioxide. The water-gas shift reaction, in which carbon monoxide is oxidized to carbon dioxide, and simultaneously water is reduced to hydrogen, is used to produce hydrogen from synthesis gas. The photosynthetic bacterium Rubrivivax gelatinosus CBS is one of a small number of organisms that can perform this reaction at ambient temperatures. This biologically-mediated shift reaction may be a cost-effective technology for the production of hydrogen from syngas.

Approach

Biological Hydrogen from Fuel Gases

To fully utilize the CO shift reaction as a renewable energy resource, it is necessary to understand the mechanism of how *Rx. gelatinosus*

CBS produces H_2 from CO. Carbon monoxide plays multiple roles in the microbial CO oxidation system, serving as a substrate, an inducer, and an inhibitor. In order to obtain optimal activity, we must understand how CO regulates the overall pathway, i.e., the effect and concentrations of CO on the maximal induction, synthesis, and maintenance of enzymatic activities in the CO pathway.

Another approach to gain insight into the CO shift is to clone and characterize genes involved in the pathway. For example, gene discovery will help in the understanding of the rate-limiting step during H_2 production, as well as how CO shift enzymes are produced and degraded.

Bioreactor Development for Biological Hydrogen Production

The overall goal of this part of the project is the scale-up of the low-temperature biological WGS reaction to provide a cost-effective technology for the conditioning of biomass-derived synthesis gas. Laboratory-scale data, reactor modeling results, and technoeconomic analyses are used to guide the development of the bioreactor and the biological WGS process. To date, these efforts indicate that favorable process economics are achievable if the WGS bioreactor can be operated at elevated pressures with improved volumetric mass transfer rates. The current approach is focused on examining the effect of reactor parameters on volumetric mass transfer rates, and designing and testing laboratory-scale bioreactors with higher system operating pressures. Once positive results are achieved in small-scale tests, a larger high-pressure reactor can be constructed to collect more detailed reactor data.

Results

Induction of CO Shift Activity by CO

A series of tests were conducted to determine if CO induces the CO-shift activity in *Rx. gelatinosus* CBS. Within two hours after adding CO to the gas phase of CBS cultures previously incubated in the absence of CO, CO consumption and H_2 production were detected (Figure 1). The addition of chloramphenicol (which inhibits new protein synthesis while exerting no effect on existing hydrogenase activity) along with the CO

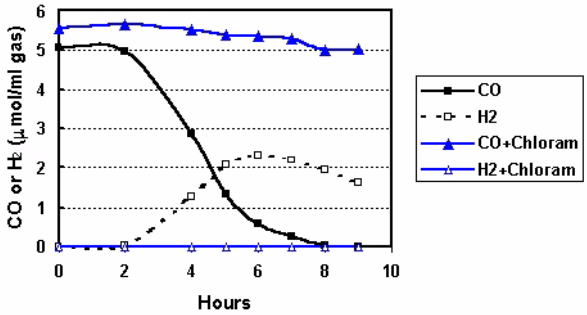

Figure 1. Effects of CO and a Protein Synthesis Inhibitor Chloramphenicol (Choloram) on Inducing CO Shift Activity

immediately stopped the appearance of the CO-shift activity for up to a seven-hour period (triangle), suggesting that CO acts as an inducer for the *de novo* synthesis of both carbon monoxide dehydrogenase (CODH) and hydrogenase of the CO-shift pathway. Consequently, in order to maintain CO shift activity, CO must be present to ensure that new enzymes are constantly being synthesized.

To determine if an optimal or minimal level of CO is required for maximal induction of the CO shift activity, *Rx. gelatinosus* CBS was cultured in various amounts of CO, ranging from 0%-50% (v/v). Data from Figure 2 suggests that at least 10% CO has to present at all times to ensure the maximal induction of the CO shift pathway.

Because CO facilitates the production of new CO shift enzymes, its constant presence is likely required to maintain steady-state levels of CO shift proteins. To verify this, two identical CBS cultures were

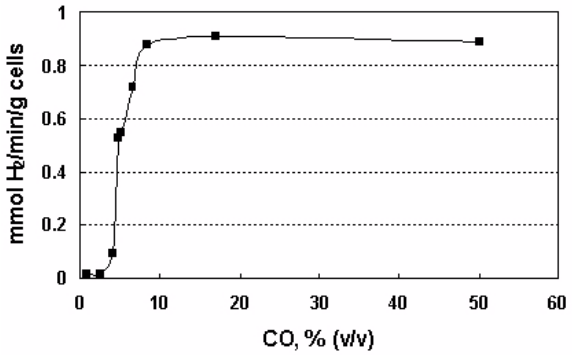

Figure 2. Effect of CO Concentrations on the Induction of Various Levels of CO Shift Activity

prepared and fed 20% CO during inoculation. To the control culture, no CO was fed once it was consumed within 10 hours, and to a parallel culture CO (20%) was fed daily. Results from Figure 3 indicate that, over a period of five days, the culture receiving CO daily was able to maintain a more constant CO shift rate, between day two and day five, 56% higher than that of the control without daily CO feeding.

Cloning the CO Shift Genes

To identify the genes involved in the CO shift pathway in *Rx. gelatinosus* CBS, a library of transposon mutants was created and then screened for CO shift activity. Mutants unable to either consume CO or produce H_2 would suggest that the gene(s) responsible for that function has been interrupted with the transposon insertion. After screening approximately 2000 mutants, two lines that were unable to consume CO or produce H_2 were identified. Biochemical analysis indicates that mutant GV1214 lacks the CODH activity, yet it still retains partial hydrogenase activity; mutant GV1762 retains 32% of the CODH activity, yet its hydrogenase activity is abolished completely. Sequence analysis suggests that the mutations occurred in a CODH gene and hydrogenase large subunit gene, respectively.

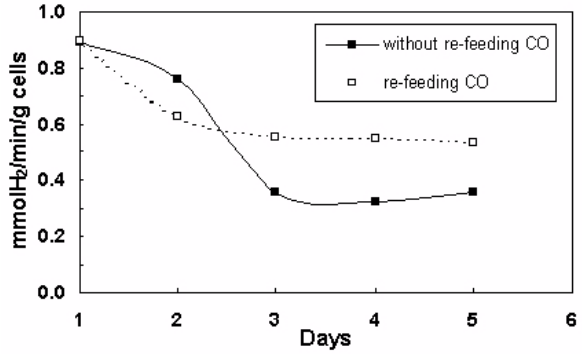

Figure 3. Effect of CO Feedings on Rates and Longevity of the CO Shift Reaction

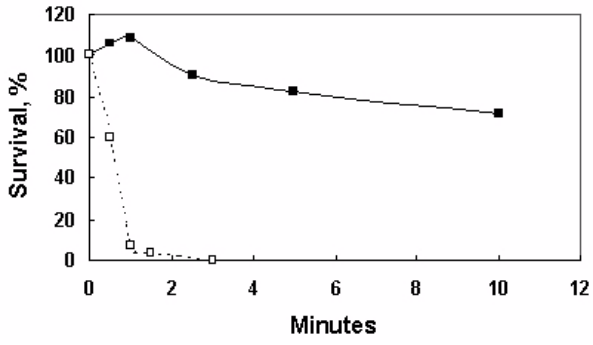

Figure 4. Photo-Oxidative Killing of Rx. gelatinosus

During the mutagenesis process, several mutants unable to produce carotenoid, the photoprotective pigment responsible for scavenging oxygen radicals, were identified. Without the carotenoid pigment, these colonies will die when exposed to both high light and O_2 simultaneously. Data from Figure 4 show that when a G1 culture was bubbled with air, the bulk of the cells were killed completely within one minute of illumination (open square). Little impact was observed with a parallel control containing CBS wild-type cells (closed square). Based on the susceptibility of the pigmented mutant to photo-oxidative killing, CO shift mutant can be selected from a population of cells cultured in CO as the sole carbon substrate.

Effect of Bioreactor Pressure and Inlet CO Concentration

Experiments were performed to investigate the effects of reactor pressure and carbon monoxide concentration on the volumetric productivity of trickle bed bioreactors. Figure 5 shows the apparent rate constant, k_{app} (a measure of reactor productivity), as a function of bioreactor operating pressure. As the system pressure is increased, the

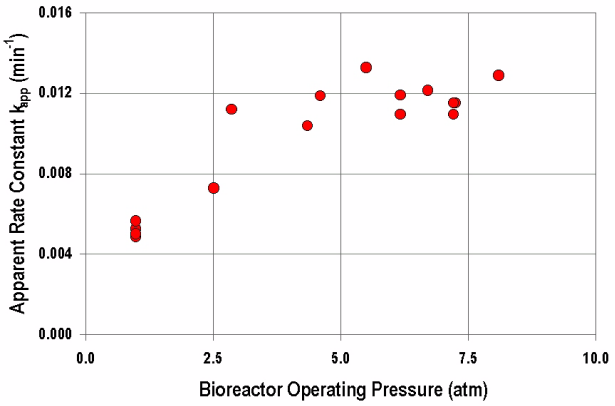

Figure 5. The effect of elevated operating pressure on the apparent bioshift rate constant k_{app}. At modest pressures, a significant increase in rate with pressure is seen. This effect appears to diminish at approximately 80 psia.

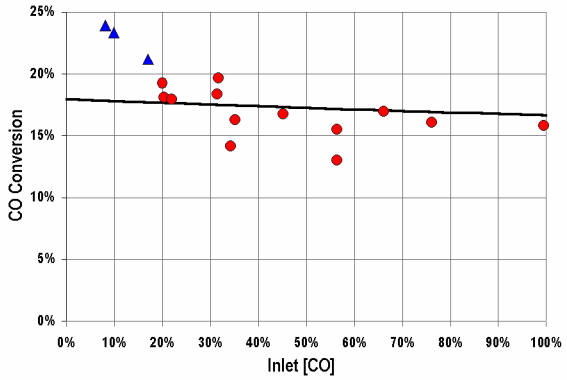

Figure 6. Effect of inlet CO concentration on CO conversion in a 1-L bioreactor. Curve is model prediction (Wolfrum 2002).

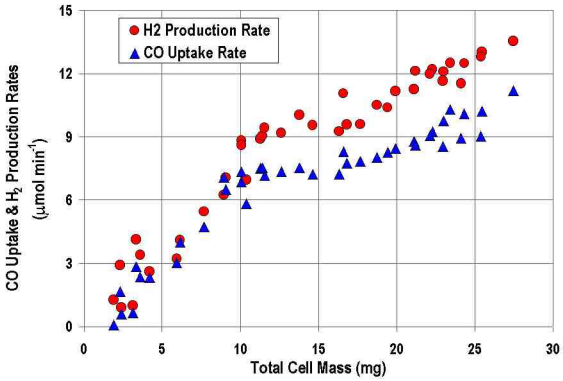

Figure 7. Hydrogen Production and Carbon Monoxide Uptake Rates vs. Total Cell Mass for Photosynthetic Bacterium *Rhodocyclus gelatinosus* CBS

apparent rate constant increases up to approximately 80 pounds per square inch absolute (psia), after which no further increase in reactor productivity occurs.

The effect of CO concentration on reactor performance was evaluated in a series of experiments in a 1-L bioreactor. The data in Figure 6 cover the range of CO inlet concentrations of 8% to 99%. The curve is a model fit using a previously developed reactor model (Wolfrum 2002). The data at low CO concentrations (triangle symbols) are believed to be unreliable because the measured concentration change is close to the repeatability of the analytical equipment (Agilent P200 gas chromatograph). The data in Figure 6 clearly indicate that the biologically-mediated CO-shift reaction is robust over a very wide range of inlet carbon monoxide concentrations; no poisoning of the microorganism occurred at high inlet CO concentrations.

Measurement of Specific H_2 Production Rate

The overall rate of CO uptake and H_2 production were measured at various cell densities.

Figure 7 shows a plot of hydrogen production and carbon monoxide uptake rates vs. the total cell mass for all experiments. When the total cell mass is less than approximately 0.012 g, there is a linear relationship between cell mass and H_2 production and CO uptake. At higher cell mass values, the CO uptake and H_2 production values do not appear to increase linearly with cell mass. This is an indication of bulk (gas-liquid) mass transfer limitations. The data in the linear region of the curves in Figure 7 were used to calculate the specific CO uptake rate and H_2 production rate.

The specific rate of H_2 production appears to be slightly higher than the specific CO uptake rate (0.80 vs. 0.73 mmol/min/g [millimole/minute/gram]), although theoretical stoichiometry predicts that they should be equal. However, at the 95% confidence level, the two parameters are equal. This is supported by the value of the H_2/CO ratio (1.07 ± 0.14), which cannot be distinguished from unity at the 95% confidence level.

Conclusions

- CO serves as an inducer for the CO shift reaction in *Rx. gelatinosus* CBS, and its constant presence is required to maintain this reaction both at a higher rate and for a longer duration.
- At least 10% CO has to be present at all times to ensure the maximal induction of the CO shift pathway in *Rx. gelatinosus* CBS.
- By identifying putative CO shift genes, researchers are beginning to understand, on the molecular level, how *Rx. gelatinosus* CBS responds to CO and produces H_2.
- A line of carotenoidless pigmented mutants has been isolated and could be incorporated

in a photo-oxidative killing scheme to enrich for CO-shift mutants.

- Inlet CO concentration has little impact on the bioreactor conversion efficiency over the range 8%-99% CO (v/v%).
- Increasing the system pressure increased the bioreactor productivity up to approximately 80 psia, after which no increase in productivity was seen.
- The specific CO uptake rate was activity 0.73 ± 0.10 mmol/min/g, while the specific H_2 production rate was 0.80 ± 0.13 mmol/min/g. These two rates cannot be distinguished from each other at the 95% confidence level.

FY 2002 Publications/Presentations

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4. Wolfrum, Edward J. and Watt Andrew S. "Bioreactor Design Studies for a Hydrogen-Producing Bacterium." Applied Biotechnology and Bioengineering, 98-100, pp. 611-625 (2002).

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II.A.2 Maximizing Photosynthetic Efficiencies and H_2 Production in Microalgal Cultures

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Objectives

- Develop genetically engineered microalgae with enhanced photosynthetic solar conversion efficiencies and biomass/hydrogen production capabilities under mass culture conditions.
- Minimize absorption and wasteful dissipation of sunlight by the algae, a solution that requires reduction in the number of the chlorophyll (Chl) molecules in photosynthesis.
- Clone genes that determine the size of the Chl antenna in the model green alga *Chlamydomonas reinhardtii*. These genes could then be over-expressed or down-regulated to improve green alga solar conversion efficiency and biomass/hydrogen production.
- Create a library of genes conferring a 'truncated chlorophyll antenna size' in green algae.

Approach

- Apply DNA insertional mutagenesis and screening in the model green alga *Chlamydomonas reinhardtii* for the isolation of 'truncated Chl antenna' transformants.
- Apply biochemical, genetic and molecular analyses of the transformant cells, followed by DNA sequencing to identify genes that confer a 'truncated Chl antenna size'.

Accomplishments

- DNA analysis of the 'truncated Chl antenna size' *tla1* insertional transformant: Completed the DNA sequencing of the ARG7 insertion site in the *tla1* strain. Obtained full-length genomic DNA, cDNA and protein amino acid sequences for the *TLA1* gene.
- Initiate *tla1* mutant complementation studies: Successfully complemented the *tla1* strain with plasmid carrying the ble selectable marker and the isolated *TLA1* gene. Biochemical analyses are now under way to characterize the complemented strain. Initial results showed that the complemented strain has nearly wild type levels of Chl/cell and a low Chl *a* / Chl *b* ratio in its thylakoid membranes. The *Tla1* protein sequence analysis and the recovery of wild type phenotype provide a strong indication that the *TLA1* gene is a "Chl antenna size" regulatory gene. This is a first-time isolation and characterization of such a regulatory gene.
- Investigate mechanism of *CAO* (chlorophyll *a* oxygenase) gene expression: The *CAO* gene expression affects the biosynthesis and distribution of chlorophyll among the photosystems in green algae. The research proved that it is an important target for a truncated Chl antenna size in photosystem II (PSII). Beyond *CAO*, the work investigated the signal transduction pathway for the regulation of the Chl antenna size by irradiance. A novel molecular mechanism was discovered and analyzed.
- DNA insertional mutagenesis library: An additional 5,000 DNA insertional transformants were generated and screened by the Chl *a*/ Chl *b* ratio measurement. Among those, two putative "truncated

Chl antenna size" strains were identified. A biochemical and molecular analysis of these new transformants is now being undertaken.

- Solar conversion efficiency and H_2 production tests in wild type and *tla1* transformant: Scale-up tests were initiated under field conditions (in the University of California Berkeley [UCB] greenhouse) where photosynthetic productivity of wild type and *tla1* strains is conducted under mass culture conditions. Preliminary measurements of biomass accumulation and gas production (so far oxygen) support the notion of a better performance for the *tla1* versus the wild type strain.

Future Directions

- Functional analysis of the *TLA1* gene: Initiate a study of expression patterns for the *TLA1* gene under different irradiance conditions.
- Bring to a completion the *tla1* mutant complementation studies: Complete the biochemical analyses of the complemented strain by providing a full molecular and biochemical characterization of its properties.
- DNA insertional mutagenesis library: Proceed with the genetic, molecular and biochemical analysis of the two additional (recently isolated) putative "truncated Chl antenna size" strains that have been isolated.
- Solar conversion efficiency and H_2 production measurements in wild type and *tla1* mutant: Continue with the scale-up measurements under field conditions (in the UCB greenhouse) to fully assess photosynthetic productivity and H_2 production in wild type and *tla1* strains.

Introduction

Green algae growing under full sunlight, when photosynthetic productivity ought to be at a maximum, have disappointingly low solar conversion efficiencies. The reason for this inefficiency is that green algae have a genetic tendency to assemble large arrays of light absorbing chlorophyll antenna molecules in their photosystems [1]. At high solar intensities, the rate of photon absorption by the Chl antennae of the first few layers of cells in the mass culture far exceeds the rate at which photosynthesis can utilize them, resulting in dissipation and loss of the excess photons as fluorescence or heat. Up to 95% of absorbed photons could thus be wasted, reducing solar conversion efficiencies and cellular productivity to unacceptably low levels. In addition to the wasteful dissipation of excitation, and due to the high rate of photon absorption by the photosynthetic apparatus, cells at the surface of the mass culture are subject to severe photoinhibition of photosynthesis [2], a phenomenon that compounds losses in productivity. Moreover, cells deeper in the culture are deprived of much needed sunlight, as this is strongly attenuated due to filtering [1, 3, 4]. A genetic tendency of the algae to assemble large arrays of light absorbing chlorophyll antenna molecules in their photosystems is a survival strategy and a competitive advantage in the wild, where light is often limiting. Obviously, this property of the algae is detrimental to the yield and productivity in a mass culture.

The work aims to minimize the Chl antenna size of green algae and, thus, to maximize solar conversion efficiencies and the yield of biomass/ hydrogen production in mass culture.

Approach

A smaller, or truncated, chlorophyll antenna size in microalgae could alleviate the optical shortcomings associated with a fully pigmented Chl antenna, because it will minimize the over-absorption of bright incident sunlight by the photochemical apparatus of the algae. A truncated Chl antenna will diminish the over-absorption and wasteful dissipation of excitation energy by the cells, and it will also diminish photoinhibition of photosynthesis at the surface while permitting greater transmittance of light deeper into the culture. Such altered optical properties of the cells would result in much greater photosynthetic productivity and better solar conversion efficiency in the mass culture. Indeed, actual experiments [1, 5] showed that a smaller Chl antenna size results in a relatively higher

light intensity for the saturation of photosynthesis in individual cells but, concomitantly, in a 3-fold greater productivity of the mass culture. Thus, approaches by which to genetically truncate the Chl antenna size of photosynthesis in green algae merit serious consideration.

The Chl antenna size of the photosystems is not constant. In general, low light intensity during growth promotes a large Chl antenna size. Growth under high light intensities elicits the assembly of a smaller Chl antenna size [6-11]. Such adjustments of the Chl antenna size in response to irradiance are a compensation reaction of the chloroplast, as they are inversely related to the incident intensity. This regulatory mechanism functions in all organisms of oxygenic and anoxygenic photosynthesis [7, 12, 13]. It is possible to genetically interfere with this regulatory mechanism through DNA insertional mutagenesis and transformation of the algae and to direct the chloroplast biosynthetic/assembly activities toward a permanently truncated Chl antenna size.

Results

The foregoing suggests that, for purposes of biomass or H_2-production under ambient sunlight conditions, it is important to identify genes that confer a truncated Chl antenna size in the model green alga *Chlamydomonas reinhardtii*. Once a library of such genes is at hand, over-expression or down-regulation of expression of these genes, as needed, can be applied to other green algae that might be suitable for commercial exploitation and H_2-production.

The chlorophyll a oxygenase (*CAO*) gene encodes a chloroplast enzyme that catalyzes the last step in the Chl biosynthetic pathway, namely the conversion of Chl *a* into Chl *b*. A mutant with inactivated *CAO* was unable to synthesize Chl *b*, thereby lacking this auxiliary light-harvesting pigment. The assembly, organization and function of the photosynthetic apparatus was comparatively investigated in wild type and a Chl *b*-less mutant of *Chlamydomonas reinhardtii*, generated by DNA insertional mutagenesis [14]. It was shown that lack of Chl *b* diminished the photosystem II (PSII) functional Chl antenna size from 230 Chl (*a* and *b*) to about 95 Chl *a* molecules [15]. However, the functional Chl antenna size of photosystem I (PSI) remained fairly constant at about 290 Chl molecules, independent of the presence of Chl *b* (Table 1). This work provided evidence to show that transformation of green algae can be used as a tool by which to interfere with the biosynthesis of specific pigments and, thus, to generate mutants exhibiting a permanently truncated Chl antenna size. In support of the role of *CAO* in the Chl antenna size of photosynthesis, further work [11] showed that *CAO* gene expression is highly regulated in vivo according to the Chl antenna size needs of the organism. Thus, the *CAO* gene may be a target for a truncated Chl antenna size in PSII.

Chlamydomonas reinhardtii double mutant *npq2/lor1* lacks the β,ε-carotenoids lutein and loroxanthin as well as all β,β-epoxycarotenoids derived from zeaxanthin (e.g. violaxanthin and neoxanthin). Thus, the only carotenoids present in the thylakoid membranes of the *npq2/lor1* cells are β-carotene and zeaxanthin. The effect of these mutations and the lack of specific xanthophylls on the Chl antenna size of the photosystems was investigated [16]. In cells of the mutant strain, the Chl antenna size of PSII was substantially smaller than that of the wild type (Table 1). In contrast, the Chl antenna size of PSI was not truncated in the mutant. This analysis showed that absence of lutein, violaxanthin and neoxanthin specifically caused a smaller functional Chl antenna size for PSII but not for that of PSI. Thus, xanthophyll-biosynthesis genes, such as *lycopene* ε-*cyclase* and *zeaxanthin epoxidase* may be targets for a truncated Chl antenna size in PSII.

More recent DNA insertional mutagenesis and screening resulted in the isolation of a regulatory mutant, the phenotype of which was Chl deficiency and elevated Chl *a* /Chl *b* ratio [17]. This truncated light-harvesting Chl antenna (*tla1*) mutant apparently has a defect in the Chl antenna regulatory mechanism, the result of which is inability to produce a large Chl antenna size under any growth conditions. Table 1 shows that both the Chl antenna size of PSII and PSI were smaller in the *tla1* strain. This work provided further evidence to show that transformation of green algae can also be used as a tool by which to genetically interfere with the

molecular mechanism for the regulation of the Chl antenna size in green algae (Kanakagiri, Polle and Melis, unpublished).

The ultimate goal of our combined approaches is to develop customized strains of green algae which assemble only the minimum Chl antenna size of the PSII-core complex (37 Chl) and of the PSI-core complex (95 Chl molecules) (Table 1).

Table 1. Photosystem Chl antenna size in wild type and three *Chlamydomonas reinhardtii* mutant strains. The *cbs3* strain lacks Chl *b* and was isolated upon DNA insertional mutagenesis [15]. The *npq2/lor1* strain lacks all β,ε-carotenoids as well as the β,β-epoxycarotenoids. It contains zeaxanthin but lacks lutein, violaxanthin and neoxanthin from its thylakoid membranes [16]. The *tla1* strain was isolated upon DNA insertional mutagenesis [17]. Note that the *tla1* transformant has the smallest combined Chl antenna size of the three mutants described.

Table 1.

	Wild type	*cbs3* (Chl *b*-less)	*npq2/lor1* (Lut, Vio & Neo-less)	*tla1*	Goal (minimum Chl antenna size)
Chl-PSII	230	90	125	115[1]	37
Chl-PSI	290	289	294	160[1]	95

Notes:

Numbers show the Chl antenna size, i.e., the Chl (*a* and *b*) molecules specifically associated with each photosystem.
[1]Polle, Kanakagiri and Melis, unpublished.

Conclusions

Identification of three genes that confer a truncated Chl antenna size in the photosynthetic apparatus is a significant development in the direction toward cost-effective commercialization of green algae for biomass and H_2 production. Most promising in this respect is the cloning of the *TLA1* regulatory gene. A complete genomic and cDNA sequence of *TLA1* as well as the amino acid sequence of the *Tla1* protein are currently at hand (Kanakagiri and Melis, manuscript in preparation). It should be noted that this is a first-time isolation and characterization of a "Chl antenna size" regulatory gene. The *TLA1* gene may serve as a molecular tool in the elucidation of the function of the mechanism that regulates the Chl antenna size in photosynthesis. It may thus contribute to the identification of other genes that are important in this regulatory process. Further, *TLA1* may serve in the truncation of the Chl antenna size in a variety of green algae and, potentially, in non-oxygenic photosynthetic bacteria.

In conclusion, green algae with a truncated Chl antenna size are indispensable in efforts to substantially increase solar conversion efficiencies and the yield of biomass and H_2 production in photobioreactors under mass culture conditions.

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II.A.3 Efficient Hydrogen Production Using Enzymes of the Pentose Phosphate Pathway

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Objectives

- Cloning and expression of thermophilic pentose phosphate pathway enzymes
- Immobilization and stabilization of enzymes and cofactors for bioreactor development
- High stoichiometric yields of hydrogen from sugars derived from renewable resources using the optimized pentose phosphate pathway enzymes

Approach

- Isolate genes for thermophilic enzymes by polymerase chain reaction (PCR) and subclone into expression vectors for production in mesophilic host
- Employ and analyze immobilization methods for optimal performance
- Determine enzyme kinetics separately and in combination to optimize hydrogen production

Accomplishments

- Demonstration of the production of hydrogen by combination of enzymes from different thermophiles
- Isolation and subcloning of two key enzymes, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, from the thermophile Thermotoga maritima
- Production of hydrogen by enzymes encapsulated in lipsomes

Future Directions

- Isolate, clone, and express the remaining pentose phosphate pathway enzymes
- Demonstrate hydrogen production with thermophilic pentose phosphate pathway enzymes and compare with mesophilic counterparts
- Improve liposome encapsulation and investigate additional immobilization methods

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Introduction

The sugar glucose is the monomeric unit of which the major biomass components starch and cellulose are made. The abundant disaccharide sucrose, composed of glucose and fructose, is another common source of glucose. Methods to hydrolyze these bioproducts to glucose have been studied intensively by the food and bioethanol industries. But direct microbial production of hydrogen from glucose is generally not very efficient and often produces undesirable side products such as

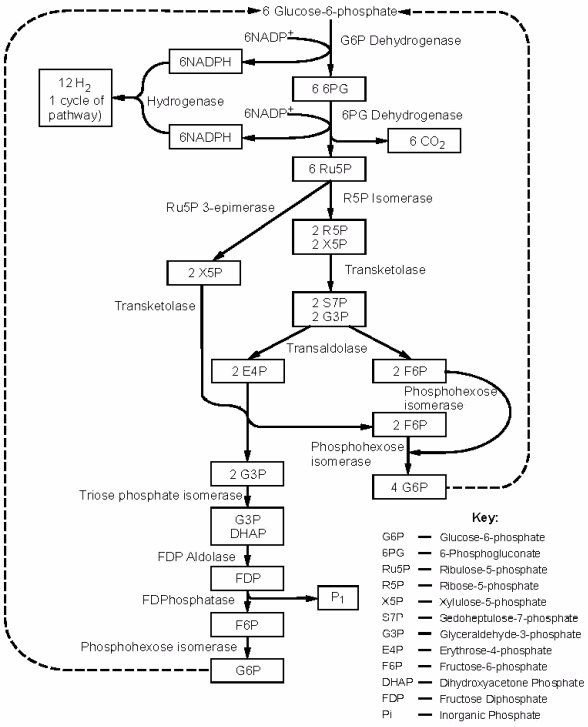

Figure 1. Production of Hydrogen from Glucose Using Pentose Phosphate Pathway Enzymes

hydrogen sulfide. Utilization of isolated enzymes has been shown to increase the efficiency of hydrogen production. Two enzymes, glucose dehydrogenase and hydrogenase, can be coupled through the cofactor nicotinamide adenine dinucleotide phosphate (NADP) to produce 1 mol H_2 per mol glucose (1, 2). By addition of the enzymes of the pentose phosphate pathway (Figure 1), a metabolic pathway found in all organisms, the yield can be increased to close to the theoretical value of 12 mol H_2/mol glucose. In this pathway, the cofactor NADP is reduced by the two enzymes glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, then used by hydrogenase for production of hydrogen gas (3). Utilization of the pentose phosphate pathway enzymes is currently limited by their expense and lack of long-term stability, as well as the instability and expense of the cofactor NADP. Development of cost-effective enzyme catalysts, cofactor stabilization, and bioreactor design is essential for practical application of this method for hydrogen generation from biomass components.

A popular method for obtaining stable biocatalysts is the utilization of enzymes originating from thermophiles, microorganisms that grow at high temperatures. Many of these enzymes can be conveniently produced from recombinant DNA constructs in mesophilic hosts such as Escherchia coli laboratory strains. The recombinant versions offer the advantages of simpler culture conditions, high yields, and simplified purification compared to production from the original thermophiles. However, some enzymes, such as hydrogenases, require certain special metal groups or other features and cannot be produced in common laboratory host bacteria.

Utilizing specific DNA primers that flank the desired gene sequences, copying and amplification of target genes for cloning can be rapidly carried out using PCR. The cloning of thermophilic enzymes is simplified by the availability of the complete genomic sequences of thermophiles such as Methanococcus jannaschii (4) and Thermotoga maritima (5) as a result of the DOE Microbial Genome Program, as well as the availability of bacterial strains from the American Type Culture Collection.

Approach

Enzyme and whole cell activities of the thermophile Thermotoga maritima were examined for comparison with and complementation of the recombinant enzymes and the Pyrococcus hydrogenase.

The target genes were determined based on the successful production of hydrogen using commercially available pentose phosphate pathway enzymes originating from mesophilic organisms such as yeast and rabbits. Based on the published genomic sequences of thermophiles, DNA primers are designed and synthesized for amplification of the target genes by PCR. The amplified genes are then cloned into an expression vector so they can be produced in laboratory host strains of Escherichia coli. These recombinant thermophilic enzymes will be tested for activity, then combined with the hydrogenase produced by the thermophile Pyrococcus furiosus (M. M. Adams, University of

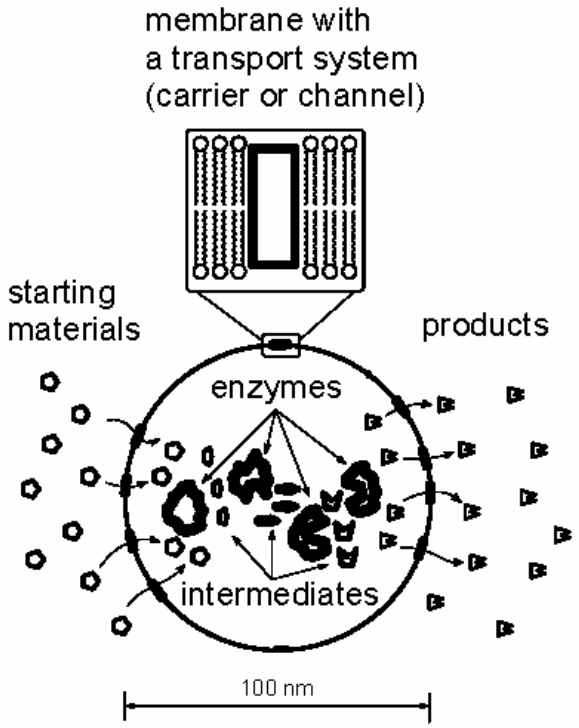

Figure 2. Liposomes as Nanobioreactors

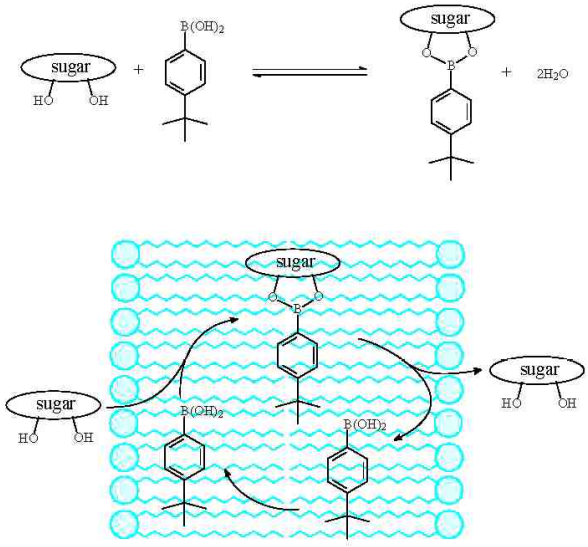

Figure 3. Glucose Transporter Mechanism

Georgia) and the cofactor NADP for optimization of the production of hydrogen from glucose.Parallel to the cloning endeavor, methods of immobilization and stabilization, as well as cofactor recycling, are being

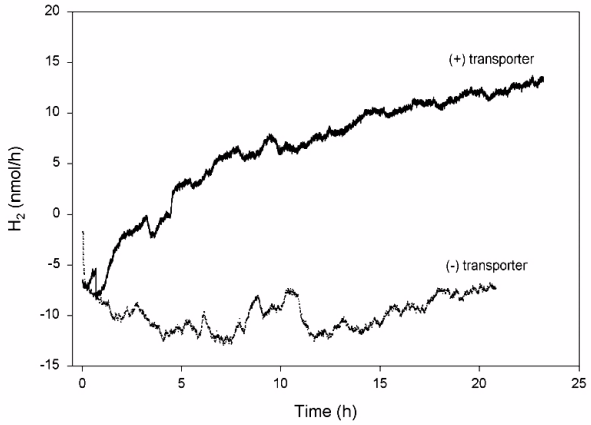

Figure 4. Production of Hydrogen from Glucose by Encapsulated Glucose Dehydrogenase, Hydrogenase, and NADP in Presence (+) or Absence of Glucose Transporter

investigated for suitability in a bioreactor for the production of hydrogen. Methods that are being examined include sol gel immobilization, NADP analogs, and liposome encapsulation. The formation of liposomes, small vesicles composed of lipid bilayers that enclose aqueous solutions, does not require covalent linking of enzymes and can easily entrap multiple enzymes and cofactors in one step.

Results

The intrinsic production of hydrogen by Thermotoga whole cells and cell extracts was found to be low. The cell extracts produced hydrogen at a rate of 0.1 µmol/h. The key enzyme glucose 6-phosphate dehydrogenase was detected in the cell extracts. Addition of Pyrococcus hydrogenase increased the rate of hydrogen production by the cell extract to 1.9 µmol/h.

Primers were designed and used to clone the genes for the pentose phosphate pathway enzymes glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase from genomic DNA prepared from T. maritima. Activity of the cloned enzymes was detected when the recombinant genes were expressed in E.coli.

Liposomes were prepared containing glucose dehydrogenase, hydrogenase, and the cofactor NADP+ encapsulated in lipid bilayers. The bilayers

are permeable to hydrogen gas but keep proteins and other water-soluble molecules inside the liposome, similar to natural cell membranes (Figure 2). The compound tertbutylphenyl boronic acid was incorporated into the liposomes to transport glucose across the lipid bilayer (Figure 3). Hydrogen production was demonstrated when this transporter was included in the liposomes with the two enzymes at an encapsulation efficiency of 1% (Figure 4). A modified procedure using dried, reconstituted lipid vesicles to encapsulate the enzymes increased the efficiency to 30-50%

Conclusions

The Thermotoga enzymes were demonstrated to be compatible with the Pyrococcus hydrogenase, indicating that the utilization of recombinant enzymes coupled with this hydrogenase will be successful. Without hydrogenase supplementation, Thermotoga whole cells and extracts produced hydrogen at a low rate. Cloning and expression of two of the pentose phosphate pathway enzymes from Thermotoga was accomplished. Liposome encapsulation was shown to be a promising method for immobilization of enzymes for the production of hydrogen. The enzymes and cofactor are kept inside the liposomes and are not diluted or lost, while hydrogen passes through the liposome membrane and is removed from the solution by the carrier gas.

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II.A.4 Biohydrogen Production from Renewable Organic Wastes

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Objectives

- Develop different strategies for selective growth of hydrogen producing bacteria (e.g., heat selection and pH control) in a mixed culture environment.
- Apply nucleic acid based technique to identify and quantify the hydrogen-producing bacterial population in a complex microbial community background.
- Optimize process to achieve sustainable hydrogen production in continuous flow bioreactors.

Approach

- Evaluate technical and practical feasibility of sustainable hydrogen production in continuous flow bioreactors.
- Investigate the effect of heat treatment of sludge on hydrogen production rate through batch and continuous experiments.
- Apply nucleic acid based techniques for microbial identification and quantification.
- Find the correlation between hydrogen yield and the *Clostridium* sp. in the continuous bioreactor.

Accomplishments

- Evaluated the effect of environmental factors on hydrogen production using mixed culture.
- Successfully enriched naturally available mixed seed to culture hydrogen producing bacteria.
- Enhanced the hydrogen production through preheat treatment of seed inoculum at 70-90^{o}C for 15 - 20 minutes followed by repeated heat treatment of settled returned sludge.
- Found positive correlation of hydrogen production with *Clostridium* sp. in the continuous bioreactor.

Future Directions

- Develop pilot-scale hydrogen production demonstration project using real wastes.
- Evaluate the full-scale plant design.
- Prepare design manual with detail guidelines for biohydrogen production from organic wastes.
- "Identify and quantify the hydrogen-producing bacterial population in a complex microbial community background using nucleic acid based techniques.

Introduction

As a sustainable energy supply with minimal or zero use of hydrocarbons, hydrogen is a promising alternative to fossil fuels. It is a clean and environmentally friendly fuel, which produces water instead of greenhouse gases when combusted. The waste streams from corn, soybean, and meat processing plants pose a major burden on the environment. Aerobic wastewater treatment of these wastes requires energy input to provide aeration, whereas anaerobic digestion processes can achieve the dual benefits of energy production in the form of hydrogen or methane, and waste stabilization. By using hardware similar to that used in industrial methane fermentation, the economics of hydrogen fermentation could be favorable due to its faster reaction rate.

For global environmental considerations, production of hydrogen by biological reactions from renewable organic waste sources represents an important area of bioenergy production. However, the viability of biohydrogen production using mixed culture in a continuous mode has not yet been evaluated. This study evaluated the potential application of mixed culture to produce hydrogen and also examined two important operating strategies, heat treatment, and pH, control to achieve sustainable hydrogen production.

Approach

The first phase of this study involved the design and development of a completely mixed bioreactor for continuous hydrogen production using mixed culture. The experimental setup is shown in Figure 1. The bioreactor was equipped with mixer, pH control unit, external settling tank and heating chamber. Upon successful start-up, operation of the bioreactor was optimized to maximize hydrogen production. The process optimization included determination of optimum operating pH, temperature, and duration of heating of settled biomass, and COD loading rate.

Detail microbial study is crucial to correlate hydrogen production to the microbial group involved. For this purpose, a nucleic acid based technique - terminal restriction fragment length polymorphism (T-RFLP) - was used to identify the abundant populations in the bioreactors in a complex microbial community background.

Figure 1. Experimental Setup of Continuous Bioreactor

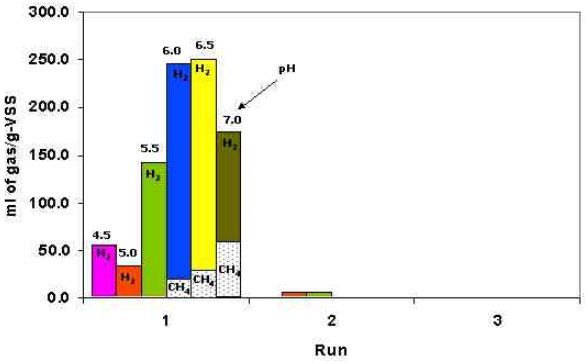

Figure 2. Effect of pH on Hydrogen Production in Batch Studies

Results

According to the batch test result in Figure 2, the pH of 5.5 was found optimum for hydrogen production without any detection of methane in the gas phase. This pH was therefore selected for all continuous phase research. Batch tests showed that heat treatment of seed inocula at 70-90^{o}C for 15-20

minutes enhanced the hydrogen production by more than five times with respect to the control (without heat treatment), as shown in Figure 3. Such an increase was likely due to more favorable conditions for spore-forming hydrogen producers by reducing nonspore-forming hydrogen consumers. An interesting observation was the occurrence of a lag phase during hydrogen production. Heat treatment could potentially damage the cells. Thus, the HRT should be longer than the lag time in the continuous operation so that hydrogen producers can adjust themselves to a new environment.

In the continuous operation, a substrate concentration of 20 g/L was chosen to improve hydrogen production because high substrate concentrations were favorable to hydrogen producers. The seed sludge was preheat-treated at 100°C for 15 minutes, whereas repeated heat treatment was applied only to returned sludge from reactor R1. After 10 days of operation (in steady-state), hydrogen production of reactor R1 was similar to that of reactor R2 but higher hydrogen content was maintained in reactor R1 (Figure 4). Methane was not detected in these two reactors. The average VFA concentrations were measured at 5,220 and 5,100 mg/L as acetic acid for reactors R1 and R2, respectively. Approximately 92% and 93% of sucrose were converted into H_2 and VFA in reactors R1 and R2, respectively. H_2 yield (0.113 m^3 H_2/kg COD) of reactor R1 was higher than that (0.075 m3 H_2/kg COD) of R2 based on maximum values. Repeated heat treatment was, therefore, effective in selecting or even activating spore-forming hydrogen-producers.

Microbial studies conducted at University of Illinois Urbana Champaign showed that *Clostridium butyricum, C. beijerinckii, C. botulinum, C. putrificum and C. sporogenes* were predominant in both reactors. Clostridia are obligate anaerobic acidogenic bacteria that can form spores (endospores) to protect themselves against unfavorable environmental conditions, such as high temperature, desiccation, radiation, or toxic chemicals; however, when favorable conditions return, they can germinate and become vegetative cells. Some of *Clostridia's* spores require heat treatment to increase the germination of spores.

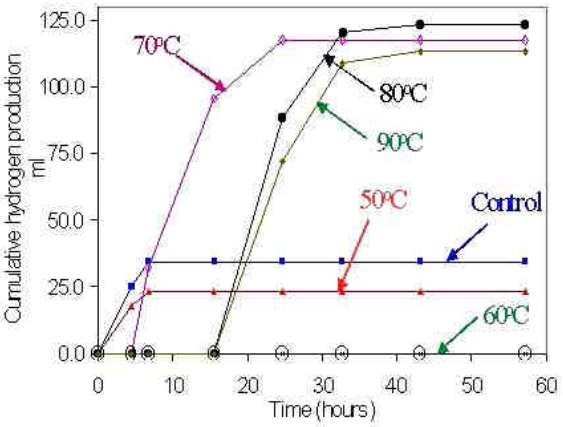

Figure 3. Effect of Heat Treatment on Hydrogen Production in Batch Studies

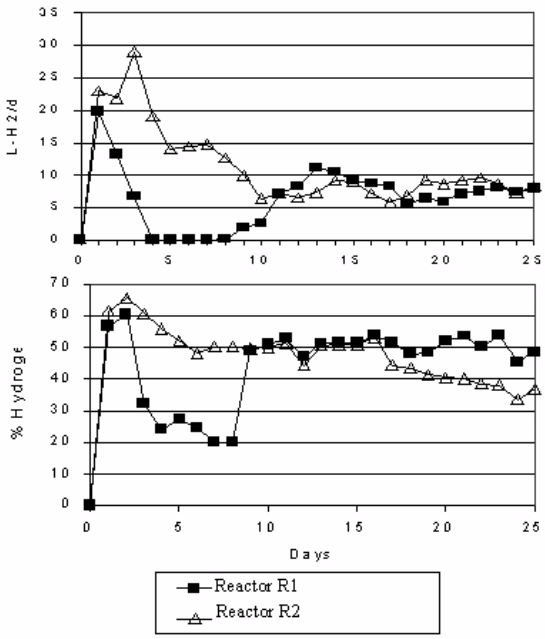

Figure 4. Hydrogen Production Rate and Percentage of Hydrogen in Biogas for Continuous Anaerobic Reactor with (Reactor R1) and Without (Reactor R2) Repeated Heat

Conclusions

- Both initial heat treatment of the inoculum and repeated heat treatments of the biomass during operation promoted hydrogen production by eliminating non-spore forming hydrogen consuming microorganisms and by selecting for hydrogen producing, spore forming bacteria.
- An operational pH of 5.5 was shown to be optimal for hydrogen production.
- Sustainable hydrogen production was possible with pH control and repeated heat treatment of settled sludge at 70-90^{o}C for 15-20 min.
- Terminal restriction fragment length polymorphism (T-RFLP) analysis showed that *Clostridium* and *Bacillus* species were the dominant populations in the bioreactors.
- A positive correlation was observed between the total abundance of *Clostridium* species and hydrogen production during part of an operational run.

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II.A.5 Photobiological Algal H_2 Production

Task 1: Molecular Engineering of Algal H_2 Production

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Task 2: Cyclic Photobiological Algal H_2 Production

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Objectives

Molecular Engineering of Algal H_2 Production

- Study two cloned *Chlamydomonas reinhardtii* hydrogenases at the molecular biology, biochemical and structural level.
- Use the information from these studies to design mutated genes with potentially higher tolerance to O_2.

Cyclic Photobiological Algal H_2 Production

- Build a new photobioreactor system with monitoring and feeedback control capability for key biophysical and biochemical parameters and determine the effect of chosen parameters (pH, light intensity, temperature) on inactivation of O_2 evolution and/or rates of H_2 photoproduction by sulfur-depleted *Chlamydomonas reinhardtii*.

Approach

Molecular Engineering of Algal H_2 Production

- Clone and sequence the [Fe]-hydrogenase genes (encoding for the enzymes that releaseH_2 gas), *hydA1* and *hydA2*, in *C. reinhardtii*.
- Raise antibodies specific against each of the enzymes to investigate differences in the physiological role of the two cloned algal hydrogenases and to decide which enzyme to target for mutagenesis.
- Use the antibodies in detailed protein expression studies.
- Characterize the differences between the purported amino acid sequences and structures of HydA1 and HydA2 to identify what specific regions of the protein to target for future mutagenesis in order to improve the O_2-tolerance of the system.

Cyclic Photobiological Algal H_2 Production

- Evaluate the effects of pH, light intensity, and temperature on the inactivation of photosynthetic O_2 evolution by sulfur-depleted algal cultures.

- Build a photobioreactor with feedback control to monitor biophysical and biochemical culture parameters.
- Determine the effects of adding controlled amounts of sulfate during H_2-production to maintain electron transport from H_2O to the hydrogenase at higher levels.

Accomplishments

Molecular Engineering of Algal H_2 Production

- Cloned and sequenced the *hydA1* and *hydA2* [Fe]-hydrogenase genes in *C. reinhardtii*.
- Developed a new high-throughput screening procedure based on a DNA plasmid, constructed to study the expression of *in vitro* mutated hydrogenases introduced into wild-type algae.
- Generated antibodies against oligopeptides specific to the HydA1 and HydA2 proteins to facilitate the expression studies. Heterologous expression of the two algal hydrogenases further confirmed the specificity of the antibodies.
- Completed biochemical studies of the physiological changes of *C. reinhardtii* upon anaerobic induction under photoheterotrophic and photoautotrophic conditions and correlated these results to the expression of the *hydA1* and *hydA2* transcripts.
- Completed preliminary structural modeling studies of the proteins encoded by *hydA1* and *hydA2*.

Cyclic Photobiological Algal H_2 Production

- Evaluated the effects of pH, high light and/or high temperature on the inactivation of photosynthetic O_2 evolution by sulfur-depleted algal cultures.
- Investigated the effects of adding controlled amounts of sulfate during H_2-production to maintain electron transport from H_2O to the hydrogenase at higher levels.
- Found that the residual PSII electron transport activity in sulfur-deprived cultures limits the rate of H_2 photoproduction by the system.
- Developed the use of a fluorescence method to externally monitor the health of the cultures in the bioreactors and to determine when they will start to produce H_2.

Future Directions

Molecular Engineering of Algal H_2 Production

- Continue to study mutant hydrogenase genes, differentiating between the roles of HydA1 and putative HydA2 in H_2 photoproduction under different physiological conditions. These studies will determine which of the enzymes should be the focus of future mutagenesis efforts to generate O_2-tolerant mutants.
- Study the regulation of HydA2 expression using the antibody developed this year. Correlate expression studies of HydA2 using activity assays, antibodies, and nucleic acid hybridization techniques.
- Improve the frequency of *C. reinhardtii* transformation by electroporation in NREL's laboratory.
- Generate mutant hydrogenase genes by either random or site-directed mutagenesis, based on information obtained from structural analyses.

Cyclic Photobiological Algal H_2 Production

- Complete a feasibility study on the operation of a new photobioreactor that produces H_2 for longer periods of time as an alternative to significantly increasing H_2-production rates.
- Design, construct, and test a photobioreactor with immobilized algal cells.

- Identify and test physical and chemical variables to optimize the H_2-production rates with the new photobioreactors.
- Eliminate the need for costly centrifugation as a means to change the growth medium from sulfur-replete to sulfur-depleted.

Introduction

Hydrogen metabolism, catalyzed by [Fe]-hydrogenases in green algae, was first observed 60 years ago in *Scenedesmus obliquus*. Since then, hydrogenase enzymes that either uptake or evolve H_2 have been found in many green algae. Hydrogen production by green algae has significant advantages over other photobiological systems: ATP is not required, high theoretical efficiencies are possible, and water is used directly as the source of reductant without the need to produce biomass or to store intermediary carbon metabolites. Research is underway in several areas to maximize the hydrogen production capabilities of green algae:

1. *Molecular Engineering of Algal H_2 Production.* Algal H_2 photoproduction is sensitive to O_2, a co-product of photosynthesis, and this sensitivity is one of the major factors currently limiting the use of algal systems for H_2 production. One research goal is to engineer the metabolic pathways in the green alga, *Chlamydomonas reinhardtii*, involved in H_2 photoproduction to decrease their O_2 sensitivity and eventually lead to development of a mutant hydrogenase gene that produces H_2 under aerobic conditions.

2. *Cyclic Photobiological Algal H_2 Production.* Previous research has shown that removing sulfate from the algal growth medium partially inactivates the photosynthetic H_2O-oxidizing activity of the cells and results in the establishment of anaerobic conditions in the culture. The resulting anaerobiosis leads to induction of the hydrogenase activity. This research effort is focused on further developing the sulfur-deprivation approach, elucidating the pathways involved in H_2-production under sulfur-deprivation, and developing approaches to improve the H_2-production yield of the process.

Approach

Molecular Engineering of Algal H_2 Production

Mutagenesis can be used to decrease the O_2 sensitivity of [Fe]-hydrogenases and thus lead eventually to a system that produces H_2 under aerobic conditions. The first step in the process is to clone and sequence the [Fe]-hydrogenase genes (encoding for the enzymes that releases H_2 gas), *hydA1* and *hydA2*, in *C. reinhardtii*. In order to investigate differences in the physiological role of the two cloned algal hydrogenases, antibodies specific against each of the two enzymes have been raised and will be used for detailed protein expression studies. This information will help us to determine which enzyme to target for mutagenesis in order to improve the O_2-tolerance of the system. Characterization of the differences between the purported amino acid sequences of HydA1 and HydA2, combined with structural modeling of the two enzymes, will be used to identify what specific regions of the protein to focus on in our mutagenesis studies, which will be done concomitantly with random error-prone PCR mutagenesis.

Cyclic Photobiological Algal H_2 Production

This work stems from past research that demonstrated the feasibility of using sulfur-deprived algal cultures to photoproduce H_2 continuously over a number of days. In this case, the approach is to control the environment in the photobioreactor to maximize the H_2-production activity of the system. This includes evaluating the effects of pH, light intensity, and temperature on the inactivation of photosynthetic O_2 evolution by sulfur-depleted algal cultures. A photobioreactor with feedback control was built to support this effort. *In situ* fluorometry is also being developed as a method to externally monitor the physiological state of the H_2-producing cultures. Finally, the effects of adding controlled amounts of sulfate during H_2-production to maintain electron transport from H_2O to the hydrogenase at higher levels are evaluated.

Results

Molecular Engineering of Algal H_2 Production

Two [Fe]-hydrogenase genes from *C. reinhardtii*, *hydA1* and *hydA2*, have been cloned and sequenced. The *hydA2* gene has a similar degree of homology to other [Fe]-hydrogenases as *hydA1*, and it contains all the motifs characteristic of other [Fe]-hydrogenases. Additional analysis shows that *hydA2* is a distinct nuclear gene that is located on a different chromosome from *hydA1* (see Figure 1A). Exposure of *C. reinhardtii* to anaerobic conditions is accompanied by the induction of hydrogenase activity. Figure 1B shows that the induced H_2 photoproduction reaches steady-state levels after about 90 minutes of anaerobic treatment. The *hydA1* and *hydA2* transcripts are induced almost equally under anaerobic conditions, when grown either photoheterotrophically (Fig. 1B) or photoautotrophically (not shown). Moreover, both transcripts are equally inactivated by exposure to O_2 (not shown).

Two oligopeptides containing sequences that are specifically found in either HydA1 or HydA2 were designed, coupled to a carrier protein and used as antigens to generate specific antibodies in rabbits. The specific antibody reaction to HydA1 and HydA2 was characterized using cell extracts from *E. coli*, and demonstrates that the anti-HydA1 antibody shows very little cross-reaction with the HydA2 protein and vice-versa. These antibodies are being tested against cell extracts from *C. reinhardtii* and will be used for detailed protein expression studies in the future.

The predicted structures of HydA1 and of the putative HydA2 hydrogenase were generated by homology modeling of the purported amino acid sequences against the known structure of the [Fe]-hydrogenase from the anaerobic bacterium, *Clostridium pasteurianum* (Cpl). The core regions of the HydA1 and HydA2 exhibit a very high degree of structural similarity with each other and with Cpl. Figure 2 shows a surface view of HydA1. Careful analyses of the model structures of the two algal hydrogenases will help to direct further efforts in the generation of O_2-tolerant mutants for sustained H_2 production under aerobic conditions.

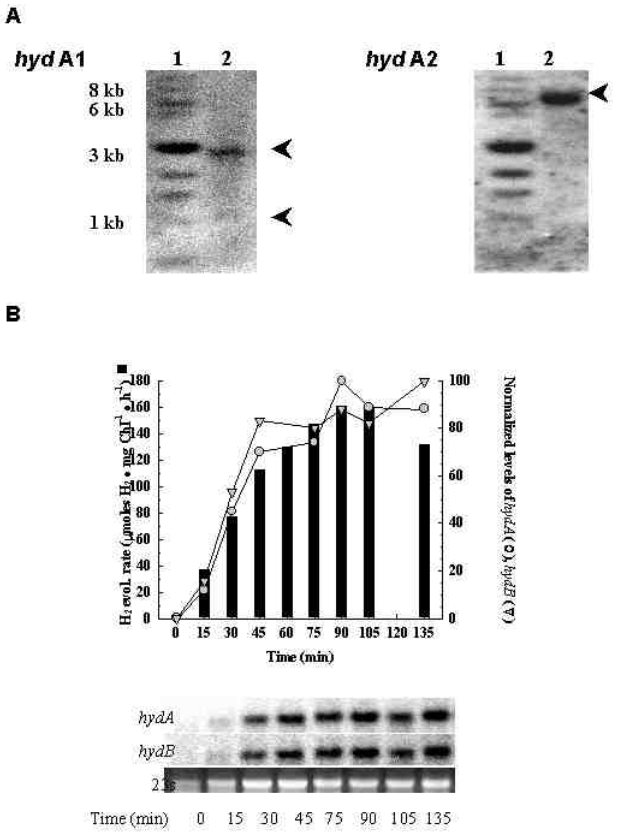

Figure 1. (A) Southern blot analyses of *PstI*-digested *C. reinhardtii* DNA probed with, respectively, *hydA1*- or *hydA2*-specific DNA probes. The arrows indicate the location of the restriction digest fragments. (B) Results from Northern blot analyses of RNA isolated from cultures grown photoheterotrophically and then anaerobically-induced for different periods of time and probed with *hydA1*- or *hydA2*-specific probes. The bars represent H_2-production activity measured concomitantly. Gels at the bottom show the original Northern blot data.

Cyclic Photobiological Algal H_2 Production

Sustained production of H_2 by green algae can be achieved by the reversible inactivation of photosynthetic H_2O-oxidizing activity, catalyzed by Photosystem II (PSII). Past research has shown that the removal of sulfate from the growth medium can accomplish this inactivation. Current research efforts are focused on the elucidation of the pathways involved in H_2-production under sulfur-deprivation

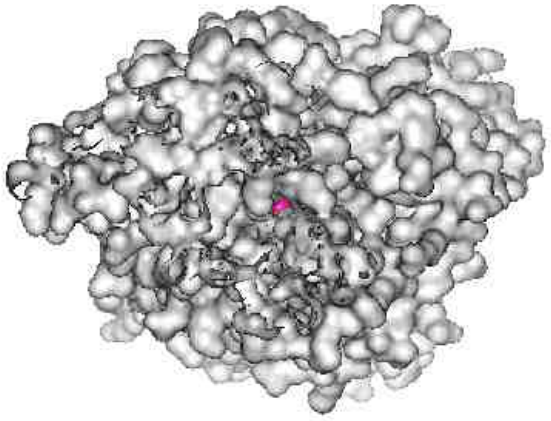

Figure 2. Surface model of HydA1. The red dot in the center of the structure represents the [2Fe2S] center of the catalytic H-cluster.

and on approaches to improve the H_2 production yield of the process.

First, a pH titration of various metabolic activities during sulfur-deprivation was used to identify those favoring H_2 production. The maximum yield and initial rates of H_2 photo-production by sulfur-deprived cultures were observed at pHs that favor the residual activity of PSII (Figure 3), strengthening previous observations that photosynthetic water oxidation is the main source of electrons for H_2 production. At the equivalent optimal initial pH of 7.7, hydrogenase activity is close to its maximum rate. In contrast, starch and protein degradation peak at acidic pHs, and generate fermentation products (acetate, formate, and ethanol). If fermentation products are desired, the algal system is robust enough to shift its metabolism to a fermentative mode upon lowering the pH inside the photobioreactor.

A second approach focused on the addition of limiting amounts of sulfate at different points during the process. When added at the start of anaerobiosis, a concentration of 5 µM sulfate will allow H_2 photoproduction to start at the expected time and to proceed at rates similar to those of samples to which no sulfate was re-added. However, addition of sulfate at concentrations of 1 µM or higher 14 h after the start of H_2 photoproduction will interrupt the process temporarily and result in lower final yields. These results show that it is possible to find a maximum concentration of sulfate that will not

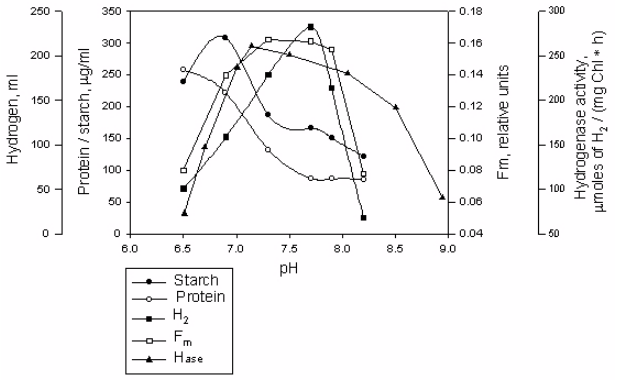

Figure 3. pH titrations of the amounts of starch and protein degraded, the amount of H_2 produced, the maximum Chl *a* fluorescence yield (a measure of PSII capacity) and the *in vitro* hydrogenase activity. As observed in the text, the pHs used in the titration of hydrogenase activity only were set to the actual values measured *in vitro*, and do not represent the initial pH at sulfur deprivation. In order to correlate them to the pH titrations of the other parameters, it is necessary to subtract 0.4 pH units from each value, which corresponds to the change in pH of the cultures from the start of sulfur deprivation to the early H_2 photoproduction phase.

inactivate H_2 photoproduction, and that this concentration is different depending on the time at which sulfate is added. These results open up the possibility of maintaining high PSII capacity during the H_2-production phase by continuously supplying the medium with specific amounts of sulfate.

To further understand factors that currently limit the rate of H_2 photoproduction, PAM fluorometry measurements were used to externally monitor the *in situ* PSII photochemical activity of the algal culture inside the photobioreactor vessels. These results show that during the early stages of sulfur deprivation (O_2-production and consumption phases), PSII photochemical activity gradually declines. However, at the time anaerobiosis is established, an additional, abrupt, and partially reversible down-regulation of PSII photochemical activity occurs, as shown in Figure 4. This drop begins at the exact time that the O_2 concentration in the culture suspension reached zero. These changes in PSII activity are characterized by complex

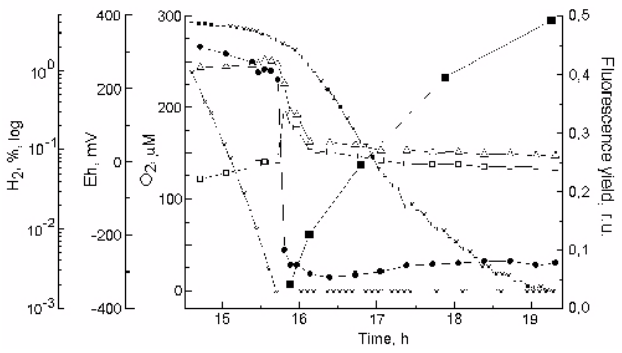

Figure 4. Time course of physiological parameters and H_2 production in *C. reinhardtii* cells during incubation under sulfur-deprived conditions. F_t (open squares), $D_\Delta F/F_m'$ (solid circles), F_m' (open triangles), dissolved oxygen (pO_2, crosses), redox potential (E_h, asterisks) and H_2 content (%) in the gas phase of the culture vessel (solid squares). Incubation in sulfur-deprived medium started at 0 h.

dynamics during the course of cell adaptation to the nutrient stress. The redox state of the PQ pool has been identified as the primary factor in regulating the activity of PSII water-splitting capacity during all stages of sulfur deprivation. Thus, the redox potential of the PQ pool depends on the relationship between the rates of photosynthesis, chlororespiration, respiration, and H_2 production.

Conclusions

Molecular Engineering of Algal H_2 Production

- Characterization of *hydA2* confirmed that it is expressed at the mRNA level and is located on a different gene than *hydA1*. In addition, its expression is regulated by anaerobiosis. The *hydA2* gene has a similar degree of homology to other [Fe]-hydrogenases as *hydA1*, and it contains all the motifs characteristic of these hydrogenases in algae.
- The *hydA1* and *hydA2* transcripts are induced almost equally under anaerobic conditions, and equally inactivated by exposure to O_2. The level of both transcripts is further modulated by the growth condition of the culture prior to the anaerobic treatment.
- Modeled structures of HydA1 and HydA2 are very similar to each other and to the core structures of the [Fe]-hydrogenase from the anaerobic bacterium, *Clostridium pasteurianum*, for which there is a solved X-ray crystal structure. A gas channel from the surface to the catalytic center of the enzyme was identified and will direct further mutagenesis work.

Cyclic Photobiological Algal H_2 Production

- H_2 production can be optimized by controlling the pH of the medium at the start of sulfur deprivation.
- Different maximum amounts of sulfate can be re-added to the system at different points of the sulfur-deprivation process without decreasing H_2 production.
- Overall, the rates of H_2-production in the system are limited by the rates of electron transport from the H_2O-oxidizing complex to hydrogenase under anaerobic conditions. H_2-production during sulfur-deprivation provides the means to reactivate residual photosynthetic electron transport capacity, which is rapidly down-regulated by the establishment of anaerobiosis.
- The technique of PAM fluorescence is a practical and inexpensive means to externally monitor the start of H_2 photoproduction by the cultures and could be further developed for use with future applied systems.

FY 2002 Publications/Presentations

Molecular Engineering of Algal H_2 Production

1. Seibert, M., Flynn, T. and Ghirardi, M.L. (2001) "Strategies for Improving Oxygen Tolerance of Algal Hydrogen Production". In *BioHydrogen II* (J. Miyake, T. Matsunaga, and A. San Pietro, Eds), Pergamon, Amsterdam, pp. 65-76 (2000, CD ROM 2000).

2. Forestier, M., Zhang, L., King, P., Plummer, S., Ahmann, D., Seibert, M., and Ghirardi, M. "The Cloning of Two Hydrogenase Genes from the Green Alga, *C. reinhardtii*". Proceedings of the *12th International Congress on Photosynthesis, 18-23 August 2001, Brisbane, Australia*, CSIRO Publishing, Melbourne, Australia. http://www.publish.csiro.au/ps2001 (2001).

3. Zhang, L., King, P., Seibert, M. and Ghirardi, M.L. "Direct Evidence for Different Electron Transport

Pathways Linked to H_2 production in the green alga *Chlamydomonas reinhardtii*". Abstracts of the *11th Western Photosynthesis Conference, January 3-6, 2002, Asilomar Conference Center, Pacific Grove, CA*, p. 44.

4. Forestier, P., King, P., Posewitz, M., Schwarzer, S., Happe, T., Zhang, L., Ghirardi, M.L., and Seibert, M. "A Second Putative [Fe]-Hydrogenase Gene (*hydA2*) in *C. reinhardtii* is Also Expressed Under Anaerobic Conditions". Submitted (2002).

5. Flynn, T., Ghirardi, M.L., and Seibert, M. "Accumulation of Multiple O_2-Tolerant Phenotypes in H_2-Producing Strains of *C. reinhardtii* by Sequential Application of Chemical Mutagenesis and Selection". *Int. J. Hydrogen Energy 27, 1421-1430*.

6. Ghirardi, M. "The Cloning of Two Hydrogenase Genes from the Green Alga *Chlamydomonas reinhardtii*". Invited plenary lecture at the *12th International Congress on Photosynthesis, 18-23 August 2001, Brisbane, Australia.*

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1. Kosourov, S., Tsygankov, A., Ghirardi, M.L., and Seibert, M. "Sustained Hydrogen Photoproduction by *Chlamydomonas reinhardtii*—Effects of Sulfur Re-addition". Proceedings of the *12th International Congress on Photosynthesis, 18-23 August 2001, Brisbane, Australia*, CSIRO Publishing, Melbourne, Australia, (2001).

2. Kosourov, S., Ghirardi, M.L. and Seibert, S. "Influence of Extracellular pH on Hydrogen Photoproduction by Sulfur-Depleted *Chlamydomonas reinhardtii* Cultures". Abstracts of the *11th Western Photosynthesis Conference, January 3-6, 2002, Asilomar Conference Center, Pacific Grove, CA*, p. 33.

3. Antal , T. K., T. E. Krendeleva, T. V. Laurinavichene, V. V. Makarova, A. A. Tsygankov, M. Seibert, and A. B. Rubin (2001) "The Relationship between Photosystem 2 Activity and Hydrogen Production in Sulfur Deprived *Chlamydomonas reinhardtii* Cells'" *Doklady Akademii Nauk (Biochemistry and Biophysics)* 381, 371-375.

4. Kosourov, S., Tsygankov, A., Seibert, M, and Ghirardi, M.L. "Sustained Hydrogen Photoproduction by *Chlamydomonas reinhardtii*—Effects of Culture Parameters". *Biotechnol. Bioeng*. 78 p. 731-740 (2002).

5. Laurinavichene, T.V., Tolstygina, I.V., Galiulina, R.R., Ghirardi, M.L., Seibert, M., and Tsygankov, A. "Different Methods to Deprive *Chlamydomonas reinhardtii* Cultures of Sulfur for Subsequent Hydrogen Photoproduction". *Int. J. Hydrogen Energy 27, 1245-1249.*

6. Tsygankov, A., Kosourov, S., Seibert, M., and Ghirardi, M.L. "Hydrogen Photoproduction under Continuous Illumination by Sulfur-Deprived, Synchronous *Chlamydomonas reinhardtii* Cultures". *Int. J. Hydrogen Energy 27, 1239-1244*.

7. Seibert, M. "Hydrogen Gas Production in *Chlamydomonas*—Effects of Sulfate Re-addition to Sulfur-Deprived Cultures," Invited plenary lecture at the *Biohydrogen Joint Workshop of COST Action 841 and IEA Annex 15*, (September 2002).

Special Recognitions & Awards/Patents Issued

Molecular Engineering of Algal H_2 Production

1. Seibert, M., Flynn, T., and Benson, D. "Method for Rapid Biohydrogen Phenotypic Screening of Microorganisms Using a Chemochromic Sensor," U.S. Patent No. 6,277,589.

2. Seibert, M., Flynn, T. and Benson, D. "Apparatus for Rapid Biohydrogen Phenotypic Screening of Microorganisms Using a Chemochromic Sensor," U.S. Divisional Patent pending.

Cyclic Photobiological Algal H_2 Production

1. Seibert, M., Makarova, A., Tsygankov, A., and Rubin, A.B. "A Fluorescence Technique for On-line Monitoring of the Physiological State of Hydrogen-Producing Microorganisms," Patent application submitted.

II.B Biomass-Based

II.B.1 H_2 Production by Catalytic Reforming of Pyrolysis Vapors

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Task 1: Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquids, K. Magrini-Bair, S. Czernik, R. French, Y. Parent, M. Ritland, E. Chornet

Task 2: Production of Hydrogen from Post-Consumer Wastes, S. Czernik, R. French, C. Feik, E. Chornet

Task 3: Hydrogen from Biomass for Urban Transportation, Y.D. Yeboah, K.B. Bota, Z. Wang, M. Realff, D. Day, J. Howard, D. McGee

Task 4: Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam Reforming of Peanut Shells Pyrolysis Products, R.J. Evans, S. Czernik, E. Chornet, C.J. Feik, R. French, S.D. Phillips

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Objectives

Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquids

- Formulate a fluidizable particulate catalyst for the production of hydrogen (H_2) by reforming biomass-derived pyrolysis oils. The catalyst support must be able to withstand reforming conditions for extended operation.

Production of Hydrogen from Post-Consumer Wastes

- Develop a robust thermo-catalytic process for producing hydrogen from readily available non-recyclable post-consumer materials and residues, such as spent plastics, organic solid residues, and waste grease.
- Demonstrate the feasibility of the production of hydrogen from synthetic polymers and from waste grease with a yield of 80% of the stoichiometric potential.

Hydrogen from Biomass for Urban Transportation

- Demonstrate process to produce hydrogen from peanut shells and other agricultural residues for use in an urban bus/transportation demonstration.
- Integrate a pilot-scale version of the pyrolyzer that is currently used to produce activated carbon from peanut shells with the pilot-scale catalytic steam reformer designed and constructed in Phase I.

Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam Reforming of Peanut Shells Pyrolysis Products

- Partner with interested parties to demonstrate that the thermochemical conversion of biomass to hydrogen is commercially viable over the next three years.
- Scale up the National Renewable Energy Laboratory's (NREL's) thermochemical biomass-to-hydrogen production process.
- Determine gas composition over the catalyst life cycle and compare the purity requirements needed for the shift reactors and pressure swing adsorption unit.
- Participate in Phase 2 testing work that will occur in Georgia at Scientific Carbon's activated carbon production facility.

Approach

Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquids

- Identify, refine, and optimize robust, available catalyst support materials.
- Formulate and evaluate multicomponent reforming catalysts made with the best supports.

Production of Hydrogen from Post-Consumer Wastes

- Identify and evaluate appropriate waste streams.
- Develop and refine the thermochemical processes (i.e., pyrolysis or partial oxidation followed by catalytic steam reforming) used to produce hydrogen from wastes.

Hydrogen from Biomass for Urban Transportation

- Develop decision models for selecting a feedstock, a process, and alternatives, and characterize the coproducts of the process.
- Design, construct, test, and integrate the pilot-scale pyrolyzer and the pilot-scale steam reformer, and begin long-term catalyst activity testing.
- Design a separation and storage system for hydrogen, and develop analytical systems for monitoring transportation system performance.
- Develop partnerships and collaborations for future bus/transportation demonstrations, the use of other feedstocks, and the development of new processes and markets for the co-products.

Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam Reforming of Peanut Shells Pyrolysis Products

- In NREL's Thermochemical User's Facility (TCUF), scale up and shake down catalytic steam reforming process as derived from the bench-scale experimentation carried out at NREL to develop the process concept.
- Provide technical support for the shakedown of the pyrolyzer/reformer system in Georgia.
- Support the partnership development that will be necessary for the bus demonstration. Identify other biomass resources in the region that could be used to produce hydrogen.

Accomplishments

Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquids

- Tested 11 commercially-available spherical alumina catalyst supports to determine optimum particle size, surface area, and attrition resistance.

- Prepared and evaluated several viable catalysts from the two alumina supports that showed the best performance in simulated reforming operations.

Production of Hydrogen from Post-Consumer Wastes

- Demonstrated a fluidized bed catalytic steam reforming process for the production of hydrogen from "trap grease".
- Studied pyrolysis/reforming of several types of common plastics using a micro-reactor interfaced with a molecular-beam mass spectrometer (MBMS).
- Designed a bench-scale fluidized bed pyrolysis reactor.

Hydrogen from Biomass for Urban Transportation

- Collected bio-oil and determined solubility parameters and physical property estimation methods for the components of the bio-oil product of peanut shell pyrolysis.
- Completed the design and construction of a pilot-scale (1/8th-scale) pyrolyzer. Began integration of the pyrolyzer with the reformer.
- Prepared the catalyst and feed samples for the pyrolyzer-reformer integration shakedown and long-term catalyst testing at Scientific Carbons Inc.
- Acquired additional analytical instruments for determining and monitoring the composition of the pyrolyzer and reformer inlet and output streams.
- Developed partnerships with several institutions and organizations including the Federation of Southern Cooperatives (for feedstock/farmer training in future phases); Albany State University (summer student interns and community acceptability study); Dougherty County, City of Albany and Georgia's Water Gas & Light Commission (future bus/transportation demonstration).

Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam Reforming of Peanut Shells Pyrolysis Products

- Developed a new heater control system to protect the reactor from overheating in case of heater malfunction.
- Successfully operated the 30-cemtimeter (cm) catalytic steam reformer on methane and peanut shell pyrolysis products at NREL.
- Experiments with peanut shell pyrolysis vapor reforming duplicated the 5-cm bench-scale unit results with the aqueous fraction of wood pyrolysis oil. This is the first time whole pyrolysis vapors have been processed in the fluid bed reforming process.
- Developed a computer system to to ensure that the unit in Georgia can be monitored during operation and utilize the new control strategy to protect the reactor.

Future Directions

Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquids

- Produce attrition-resistant alumina supports and develop an appropriate "recipe" for adding promoters during support preparation.
- Develop a "recipe" for producing a broad range of robust promoted nickel (Ni) fluidization catalysts, focusing on adding stabilization agents (magnesium [Mg], manganese [Mn]) to Ni to improve reforming performance.
- Provide the best catalysts to the Post-Consumer Waste Hydrogen Production project.
- Produce a pilot-scale quantity of catalyst for use in the scale-up evaluation for the Steam Reforming of Peanut Shell Pyrolysis Vapors project.

Production of Hydrogen from Post-Consumer Wastes

- Demonstrate the process concept for producing hydrogen by pyrolysis and subsequent reforming of plastics and achieve 80% of the theoretically possible conversion of plastics to hydrogen.
- Construct and demonstrate a bench-scale two-step pyrolysis reforming system.
- Continue using the MBMS reactor system to quantitatively evaluate the process performance.
- Demonstrate the "trap grease"-to-hydrogen process in long-duration tests using the attrition-resistant catalysts. An alternative reactor option that uses a circulating bed to prepare 80 μm catalyst particles will also be considered.
- Analyze the catalyst used in "trap grease" reforming tests to determine if it was affected by possible contaminants in the feed.

Hydrogen from Biomass for Urban Transportation

- Perform liquid-liquid equilibrium experiments on representative bio-oil compounds and develop property estimation methods.
- Develop models and heuristic solution methods for the network of process steps to account for feedstock, location, process and the uncertainties of these factors.
- Complete integration and testing of the pyrolyzer, reformer, analytical instruments, and accessories, and begin long-term catalyst testing at Blakely, GA.
- Design pressure swing adsorption (PSA) separation and hydrogen storage systems for the produced hydrogen.
- Establish additional partnerships to prepare for the urban bus demonstration, identify alternative feedstocks, and develop new markets for the coproducts.

Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam Reforming of Peanut Shells Pyrolysis Products

- Complete detailed analysis of the product over the catalyst life cycle.
- Continue to provide technical support for the shakedown testing in Georgia. The goal is 100 hours of operation.
- Recommend a design and implementation plan for the shift and separation steps for the Phase 3 demonstration in Georgia in 2003.

Introduction

Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquid

Bio-oil reforming requires a multi-functional catalyst which can (1) steam reform the oil organic components into carbon dioxide (CO_2), carbon monoxide (CO) and H_2; (2) shift the produced CO with steam to make more CO_2 and H_2; (3) gasify carbonaceous residues formed on the catalyst surface mostly from non-volatile bio-oil components; and (4) resist attrition. CoorsTek Ceramics produces spherical aluminas, with a range of physical properties, which could meet these requirements. Nickel-based catalysts, the most widely used shift catalysts, with potassium and magnesium as additives to aid coke and shift gasification and stabilize nickel crystals, respectively, are being evaluated to optimize catalyst performance.

Production of Hydrogen from Post-Consumer Wastes

At present, hydrogen is mostly produced by catalytic steam reforming of hydrocarbons: natural gas and naphtha. The goal of this research is to develop a technology for producing hydrogen from alternative resources, specifically from two types of waste post-consumer materials: plastics and "trap grease". The concept proposed for plastics is a two-stage process: fast pyrolysis to convert polymers to a

gas/vapor stream of monomers and other low-molecular weight compounds, followed by catalytic steam reforming of this gas to yield hydrogen and carbon oxides. "Trap grease", a waste material recovered from traps on sewer lines, does not require a depolymerization step and can be directly steam reformed to produce hydrogen. The challenge is to efficiently convert plastic polymers and the constitutive molecules of "trap grease" (predominantly free fatty acids and glycerides) to hydrogen at a cost similar to that for the existing natural gas-based technologies.

Hydrogen from Biomass for Urban Transportation

Clark Atlanta University has teamed with the National Renewable Energy Laboratory (NREL) and other partners in Georgia to develop and demonstrate a biomass-to-hydrogen process that will use peanut shells as a biomass feedstock for hydrogen production, and then use this hydrogen as a transportation fuel. Specifically, a pilot-scale reactor on site at Scientific Carbons Inc., a company in Blakely, Georgia, that produces activated carbon by pyrolysis of densified peanut shells, is being used to test the concept. The vapor by-products from the pyrolysis, which are currently used as fuel for steam generation, will be converted to hydrogen using NREL's catalytic steam reforming process. In the transportation demonstration, the hydrogen produced from peanut shells will be combined with compressed natural gas to raise its energy density and create a more viable transportation fuel.

Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam Reforming of Peanut Shells Pyrolysis Products

The steam reforming of biomass pyrolysis oil, when integrated with the production of high-value products, is a promising near-term approach to the production of renewable hydrogen. Based on bench-scale work at NREL, a team from Georgia is utilizing the NREL TCUF and its staff to develop a 7 kg/hr reactor that will be operated at NREL this year before being run at the production site in Georgia on densified peanut shells. The application in Georgia is at a plant that makes activated carbon from peanut shells and has pyrolysis by-products available for conversion. The key technical goals for the shakedown at NREL are to ensure the safety of the reactor and obtain preliminary performance data on the catalyst, especially physical attrition and deactivation.

Approach

Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquids

The mechanical strength of the fluidized catalyst is a significant process issue that must be solved. To address this operational problem, a two-step approach was taken to (1) identify and develop economical and attrition resistant support materials that could withstand high-temperature fluidization, and (2) prepare reforming catalysts from the best supports. The catalysts, containing nickel oxide (NiO), manganese oxide (MgO) and/or potassium oxide (K_2O), were then evaluated for attrition resistance and activity in a fluid bed system.

Production of Hydrogen from Post-Consumer Wastes

To prove this process concept, a two-stage micro-scale reactor interfaced with a molecular-beam mass spectrometer was used to qualitatively evaluate several types of plastic. Samples of plastics were pyrolyzed in the bottom part of the reactor; then the created gases and vapors were steam reformed in a fixed bed of a commercial Ni catalyst located in the upper part of the reactor. The product gas was analyzed by mass spectroscopy. "Trap grease" was catalytically steam reformed in a 2"-diameter fluidized bed reactor using the same commercial nickel catalyst. Observed catalyst losses, and the resulting hydrogen conversion efficiency losses, highlight the need for either further process evaluation using a fluidizable attrition-resistant catalyst, or reconfiguring the bed to allow the use of existing commercial catalysts.

Hydrogen from Biomass for Urban Transportation

In Phase 1 of this project, decision models for selecting a feedstock, a process, and alternatives were developed, and a 7 kg/hr fluidized-bed catalytic steam reformer system was designed and constructed. In Phase 2, the emphasis is on integrating and testing the pilot-scale pyrolyzer at

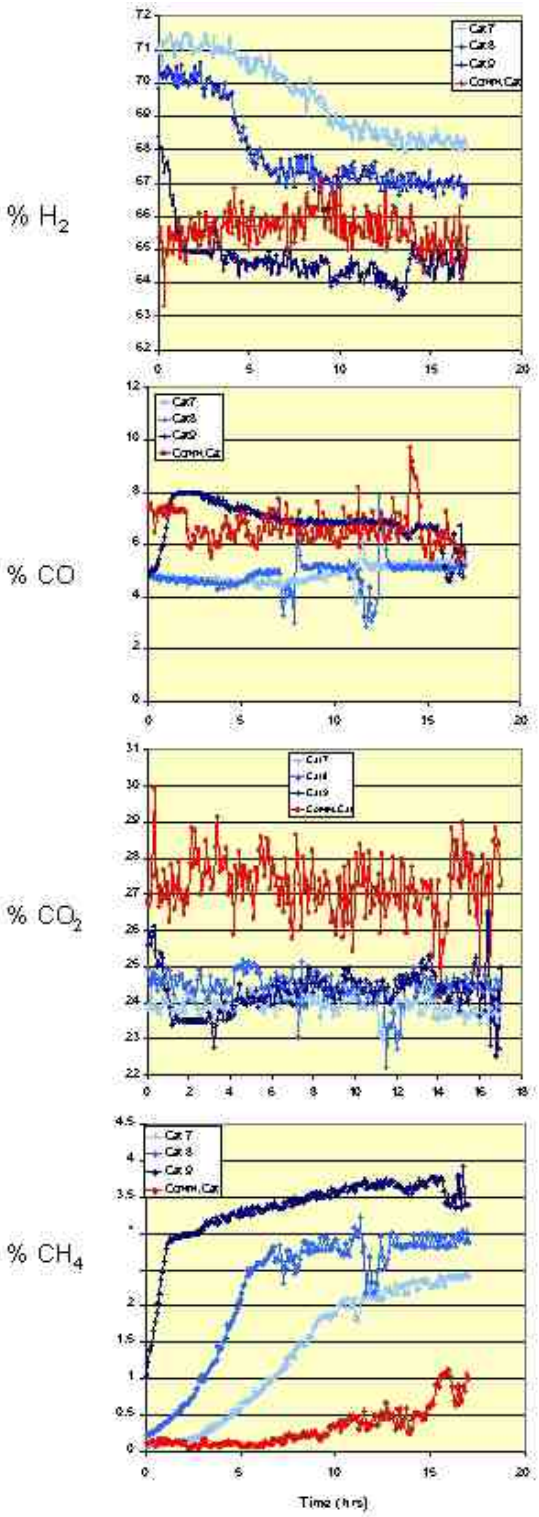

Figure 1. Reaction Gas Composition Data Versus Reaction Time for Catalysts 7-9 and CK-11 NK

Scientific Carbon's activated carbon production facility with the pilot-scale reformer constructed and tested in Phase 1. In addition, Phase 2 will involve engineering research and pilot-scale process development studies in the use of peanut shells as feedstock for the pyrolysis-steam reforming process to produce hydrogen. The hydrogen produced in Phase 3 will be blended with CNG and used to power a bus in nearby Albany, Georgia. Partnership-building and outreach play a key role in this collaborative project.

Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam Reforming of Peanut Shells Pyrolysis Products

The peanut shell-to-hydrogen concept is a modification of the biomass-to-hydrogen process that has been under development at NREL since 1993. This process concept was first characterized at the bench scale to determine the concept viability and to define key process parameters. Based on past results with the bench-scale fluid bed reactor, a pilot-scale unit was constructed and tested in NREL's Thermochemical User's Facility (TCUF). The 20-kg/hr fluid bed pyrolysis unit at TCUF was used to feed the steam-reforming unit for the shakedown tests. The TCUF is designed to test new thermochemical process concepts by interfacing key unit operations in the existing TCUF facility. This approach minimizes risk and disruption by taking advantage of process control systems and analytical capabilities, which would not be available for shakedown or proof of concept in an industrial environment. By performing the initial system tests at NREL, the experience of the NREL researchers with the process and the analytical equipment of TCUF can be used to monitor and optimize the system performance.

Results

Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquids

Eleven commercial catalysts were tested for attrition resistance under simulated reforming conditions. Two alumina supports supplied by CoorsTek Ceramics (90% and 99% alumina) showed the best combination of attrition resistance and surface area. In addition, they consist of alpha

alumina, the desired support for reforming because of surface acid sites, which contribute to hydrocarbon cracking, and are readily available in the kg amounts required for testing. These materials were used to prepare several simple Ni-based catalysts to evaluate reforming and gasification activity with pyrolysis oil in the 2" fluidization reactor.

The sample catalysts, prepared from CoorsTek supports, were evaluated by comparing their performance with that of the industrial catalyst, C-11 NK. Preliminary evaluations (Catalysts 1-6) led to the development of Catalyst 7, which showed a slightly better short-term performance and a 20% improvement in the shift reaction when compared to C-11 NK. Catalyst 7 contained 1.8% Ni, 0.15% Mg, and 0.1% potassium (K), on 99% alumina with 0.2-1.4 m^2/g surface area. Two additional catalysts (Catalysts 8 and 9), with varying amounts of Ni, Mg, and K, were prepared in an effort to establish the boundary compositions for the three-component, multifunctional catalyst. Figure 1 plots H_2, CO, CO_2, and CH_4 concentration versus reaction time for Catalysts 7-9 and C-11 NK.

Production of Hydrogen from Post-Consumer Wastes

Pyrolysis and reforming of several types of common plastics (polyethylene, polypropylene, polyvinyl chloride, polyethylene terephthalate, polyurethane, and polycarbonate) were studied qualitatively, using a micro-reactor interfaced with a MBMS. Each type of plastic pyrolyzed at 550-750ºC. This was followed by steam reforming of vapors in a fixed bed of C-11 NK catalyst at 750-800ºC. The composition of the product gas (mass spectrum) was observed for different values of the steam-to-carbon ratio and space velocity that changed depending on the size of plastic samples. Preliminary tests showed that at process conditions similar to those used for reforming natural gas, polymers were almost completely converted to hydrogen and carbon oxides.

"Trap grease" was also evaluated as a low-cost feedstock for hydrogen production, via catalytic steam reforming. The suitable process conditions are similar to those for natural gas reforming. At 850°C,

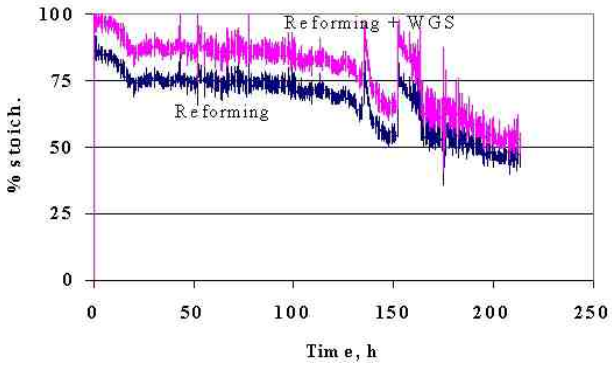

Figure 2. Yield of Hydrogen from Reforming "Trap Grease"; t=850°C, S/C=5, GC1 VHSV=970 h-1

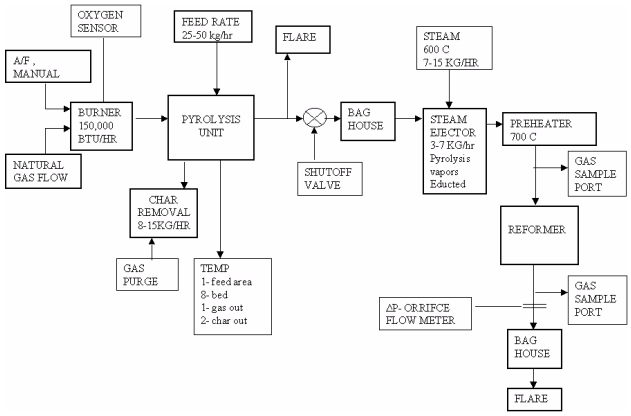

Figure 3. Schematic Flow Diagram of the Biomass Pyrolysis-Reformer Process

using steam-to-carbon ratio 5, and with a space velocity of 1000 h-1, hydrogen was produced with a yield of 25 grams (g) per 100 g of grease during 135 hours of continuous testing. This yield could be increased to over 28 g H_2/100 g grease (85% of the stoichiometric potential) if CO were further converted by water-gas shift. At the end of the testing, this yield decreased to 16.4 g/100 g grease (48% of the stoichiometric potential), as shown in Figure 2.

Hydrogen from Biomass for Urban Transportation

A schematic flow diagram of the biomass pyrolyzer-reformer designed and constructed for Phase 2 is shown in Figure 3. The pyrolyzer is designed to process up to 188 lbs (85 kg) per hour of pelletized biomass into char and pyrolytic off-gas.

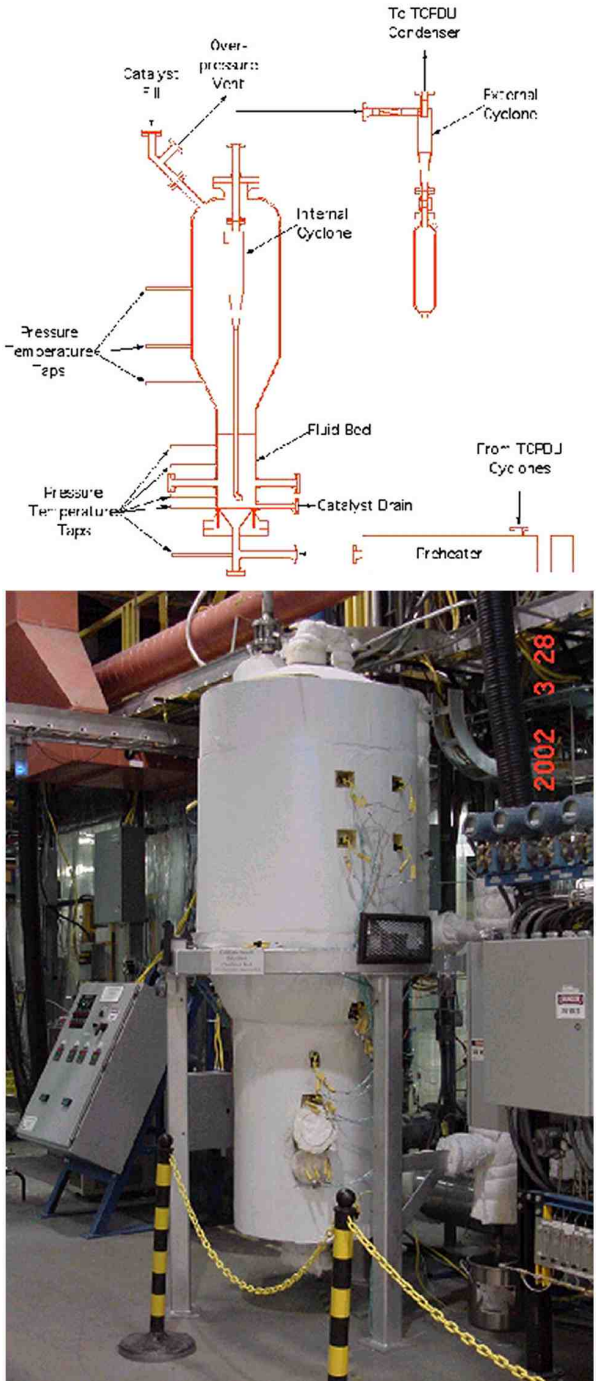

Figure 4. Catalytic Fluid Bed Steam Reforming Reactor

After carbon particulates are removed from the off-gas, it is heated to 700°C and then fed into the bottom of a steam reformer. Literature data and thermodynamic models were employed to evaluate a large number of organic solvents for the extraction of phenol from aqueous bio-oils. Propyl acetate and methyl isobutyl ketone (MIBK) were identified as good solvents as a result of this evaluation. These two solvents were used in extractions of bio-oil samples.

The hydrogen produced from peanut shells will be combined with compressed natural gas (CNG). This will require the development of a safe hydrogen-compressed natural gas storage system. Key design issues under evaluation include gas storage pressure, slow-fill versus fast-fill refueling stations, hydrogen/natural gas mixture, and materials of construction for storage cylinders. Given a target gas pressure of 3,600 psi and variable composition ranges from 75% to 85% CNG (i.e., 15% to 25% hydrogen), a tank volume of 1.78 cubic meters, diameter of 0.61 m, and length of 6.1 m is required. Due to the limitations placed on the system by the CNG distribution pipelines and the corrosive properties of hydrogen, the 80:20 mixture was selected.

Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam Reforming of Peanut Shells Pyrolysis Products

A schematic and photograph of the pilot-scale catalytic fluid bed reformer are shown in Figure 4. The 30-cm catalytic steam reforming reactor was successfully operated on peanut pyrolysis vapor at a feed rate of 7 kg/hour of vapors. The results are in agreement with those obtained from the 5-cm bench-scale reactor used for the reforming of the aqueous fraction of pyrolysis oil. Typical gas compositions at the outlet of the reformer are shown in Figure 5. These data show that the yield of hydrogen is approximately 90% of maximum.

The fluid bed pyrolysis unit was fed at a maximum feed rate of 20 kg/hour of peanut shell pellets. Because peanut shells are high in lignin (32%) and protein (8%), the char yield was approximately 35%, which is higher than the yield from typical biomass. Vapor contact time was explored by varying the weight hourly space velocity (WHSV) (weight of feed/hr/weight of catalyst). A plot of H_2 yield on an oil basis versus the inverse of WHSV is shown in Figure 6. A smaller WHSV represents more catalyst contact; therefore, plotting

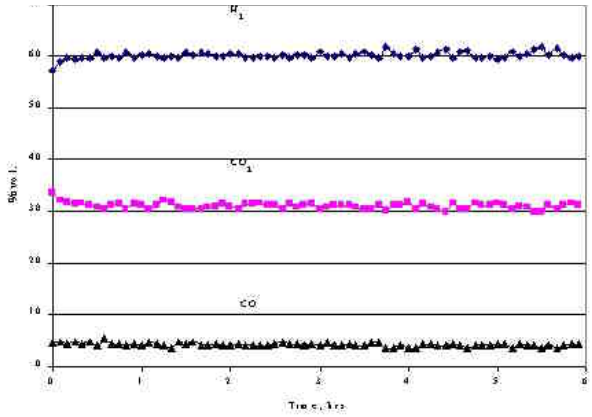

Figure 5. Gas Composition from Reforming Peanut-Shell Pyrolysis Vapors

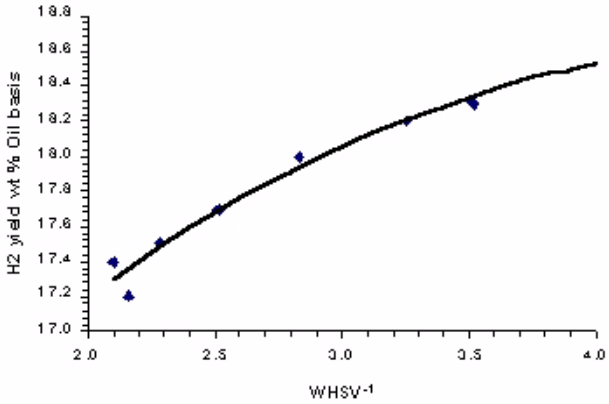

Figure 6. Variation in Estimated H_2 Yield based on Contact Time (inverse WHSV is proportional to contact time)

the inverse, which is proportional to contact time, presents a clearer picture of the effect of increasing catalyst contact. A 5% relative increase in H_2 yield was noted at higher contact time.

Conclusions

Fluidizable Catalysts for Producing Hydrogen by Steam Reforming Biomass Pyrolysis Liquids

- Most of the alumina materials tested exhibited improved attrition resistance under steam reforming conditions compared to that of commercial fluidized reforming catalysts though surface areas are about an order of magnitude less.
- The best support materials were CoorsTek 90% and 99% alumina particles with surface areas of 0.2-1.4 m^2/g. Attrition losses for the materials were less than 0.5 wt% per day.
- Catalysts made from the CoorsTek supports and containing Ni, Mg, and K oxides exhibit increased shift/gasification activity and significantly improved reforming ability compared to the industrial material for the first 3 hours of reaction. From 20-23 hours, we observed a reduction in shift/gasification activity for the NREL and commercial catalysts.
- While this new support/catalyst system works about as well as the commercial catalyst (C-11 NK), further optimization of support and catalyst composition may yield even more active and selective catalysts.

Production of Hydrogen from Post-Consumer Wastes

- Qualitative tests for producing hydrogen by pyrolysis/reforming of plastics showed promising results.
- NREL's fluidized bed catalytic steam reforming process for the production of hydrogen from "trap grease" also showed promising results in a bench-scale system.
- The fluidized bed process performance significantly decreased after 135 hours of uninterrupted operation. This low conversion to hydrogen was most likely due to the catalyst losses by attrition and elutriation from the reactor, though some deactivation effects cannot be excluded at this time.

Hydrogen from Biomass for Urban Transportation

- The project is ready to begin the shakedown and testing phase of the entire pyrolyzer/ reforming system at the Scientific Carbon facility in Blakely, Georgia.
- The hydrogen storage system design is underway, and some of the system constraints have been identified.
- The partnership efforts are building interest in the project and helping to lay the groundwork for the future bus demonstration.
- Engineering Scale-Up of Renewable Hydrogen Production by Catalytic Steam

Reforming of Peanut Shells Pyrolysis Products

- The 30-cm catalytic steam reforming reactor was successfully operated on peanut shell pyrolysis vapor at a feed rate of 7 kg/hour of vapors.
- Experiments with peanut pyrolysis vapor reforming duplicated the results in a 5-cm bench-scale unit with the aqueous fraction of wood pyrolysis oil.
- The whole vapors from peanut shells, which have a unique composition, i.e., high levels of lignin and protein, can be successfully reformed. Over the time on stream tested to date, no significant deactivation of the catalyst was noted.

II.B.2 Supercritical Water Partial Oxidation

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Objectives

- Show feasibility of supercritical water partial oxidation (SWPO) in pilot-scale preliminary testing.
- Develop improvements to SWPO hardware.
- Optimize SWPO operating parameters and hydrogen yields.
- Demonstrate an integrated SWPO pilot-scale system including hydrogen separation.

Approach

- Test various feedstocks and reactor designs using existing General Atomics (GA) pilot plant.
- Identify suitable SWPO feedstocks.
- Identify improvements to reactor and system design.
- Implement and test improvements when possible.

Accomplishments

- Showed basic feasibility of SWPO at a pilot scale.
- Established suitable operating conditions, e.g. reactor and preheat temperatures.
- Confirmed prior laboratory-scale results showing high hydrogen yields.
- Identified suitable feedstocks.
- Attained reliable pumping of thick feed slurries.
- Identified improvements to reactor design.

Future Directions

- Update a Development Plan for commercializing SWPO (Phase I).
- Implement second-generation pilot-scale SWPO reactor (Phase II).
- Characterize SWPO operating parameters and hydrogen yields (Phase II).
- Demonstrate an integrated SWPO pilot-scale system including hydrogen separation (Phases III & IV).

Introduction

General Atomics is developing Supercritical Water Partial Oxidation (SWPO) for the efficient and environmentally advantageous gasification of and hydrogen production from low-grade fuels such as biomass, municipal/solid waste (MSW) and high-sulfur coal.

SWPO involves carrying out oxidative reactions in a supercritical water environment - akin to high-pressure steam - in the presence of limited quantities of oxidant, typically pure oxygen or air. The key potential advantage of the SWPO process is the use of partial oxidation in-situ to rapidly heat the gasification medium, resulting in less char formation and improved hydrogen yield. Another major advantage is that the high-pressure, high-density aqueous environment is ideal for reacting and gasifying organics. The high water content of the medium should encourage formation of hydrogen and hydrogen-rich products and is highly compatible with high water content feeds such as biomass materials. By the same token, the high water content of the medium is effective for gasification of hydrogen-poor materials such as coal. The pressurized nature of the SWPO process naturally lends itself to the liquefaction and sequestration of carbon dioxide (CO_2). The combination of high pressure and the cold sink available with a liquid oxygen oxidant enables ready liquefaction of CO_2. It may then be recycled, injected for oil recovery or otherwise handled to reduce greenhouse effects.

Approach

Figure 1 provides a representative process flow diagram for the gasification tests carried out. A number of different configurations were tested over the course of the project. The particular configuration shown is that used in conjunction with the "large" reactor vessel. This reactor has a volume of about 10 liters and at the flow rates tested provides a residence time of about 75 seconds at 650°C operating temperature and about 60 seconds at 800°C operating temperature. Figures 2 through 4 show photographs of three skids comprising the SWPO system.

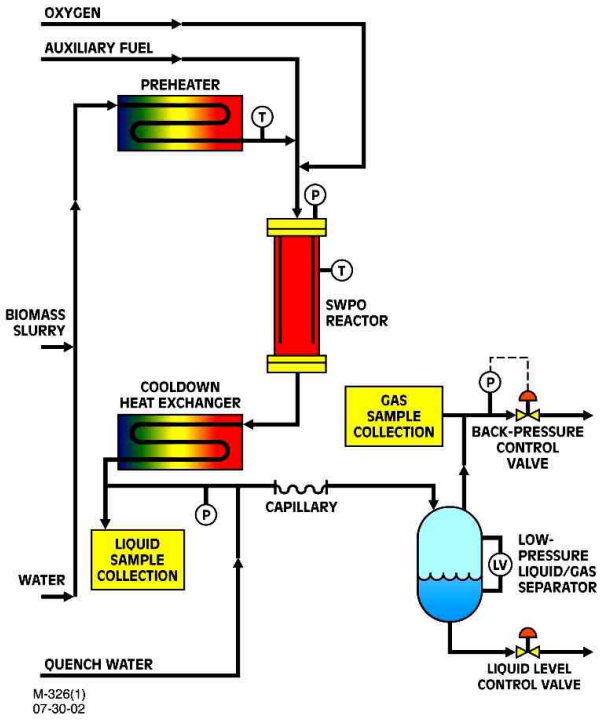

Figure 1. Process Flow Diagram for the SWPO System

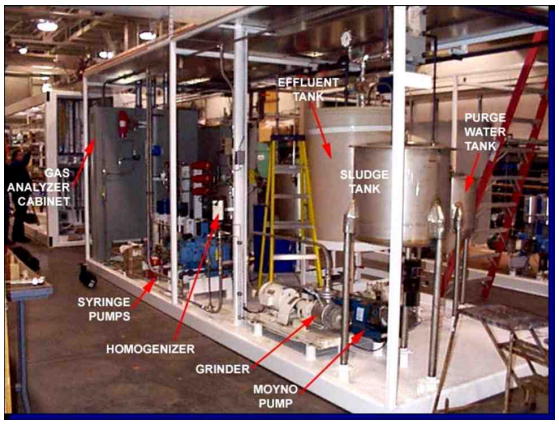

Figure 2. SWPO System Feed Skid

Pressurized slurry (or water during startup and shutdown) is fed to the preheater, where it is preheated to a temperature of 250°C, or other suitable temperature depending on the feed material. It was found during testing that slurry preheating had to be limited to avoid char formation and plugging of the preheater. In addition, the pumpable

concentration of biomass slurry was limited, for example to about 10-15 wt% for wood flour. Due to limited resources for advancing solutions to these limitations during the Phase I effort, it was decided to utilize a liquid fuel to help attain the desired reactor operating temperature. Thus, as shown in Figure 1, high-pressure auxiliary fuel (ethanol) and oxygen are combined with the preheated slurry at the reactor inlet. Oxidation of the organics results in a typical reactor temperature of 650°C. In the reactor, the feed is converted primarily to CO_2, H_2O, hydrogen gas (H_2), methane (CH_4) and CO. The liquid effluent is collected in a tank, while the gases are vented through the facility carbon filter and released to the atmosphere.

Table 1 provides a synopsis of the Phase I pilot-scale testing carried out. Several runs with composted municipal solid waste (MSW) were carried out with a pipe reactor in the absence of oxidant, i.e., they were indirectly heated and did not utilize partial oxidation. The pipe reactor had an ID of 0.815 inches and a length of about 90 feet.

Table 1. Summary of Gasification Runs

Feed	No. of Runs	Reactor Type	Run Conditions
40% corn starch	1	Small vessel	605°C
40% coal	2	Small vessel	530°C; 620°C
30-40% MSW compost	2	Small vessel	570°C
30-40% MSW compost	5	Pipe	620°C- 650°C
30% corn starch	1	Pipe	650°C, 300°C preheat
10% wood + 10% coal	2	Large vessel	800°C, 250°C preheat, EtOH fuel[2]
10% wood	3	Large vessel	650°C, 250°C preheat, EtOH fuel[2]
10% wood	3	Large vessel	800°C, 250°C preheat, EtOH fuel[2]
10% corn starch	4	Large vessel	650°C, EtOH fuel[2]
40% corn starch	2	Large vessel	650°C, EtOH fuel[2]
15% wood	2	Large vessel	650°C, 250°C preheat, EtOH fuel[2]
20% MSW compost	1	Large vessel	650°C, 250°C preheat, EtOH fuel[2]

Notes:
1. All tests are at 3400 psi.
2. Ethanol (EtOH) is oxidized with approxiately stoichiometric oxygen in the reactor to bring the preheated stream up to the target reactor temperature.

Results

The major results observed over the past year are as follows:

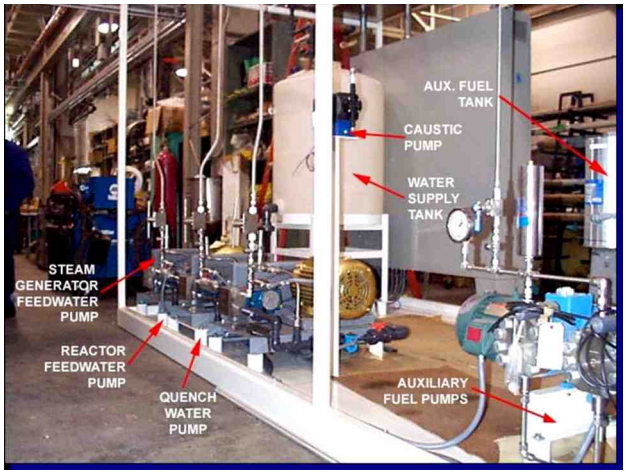

Figure 3. SWPO System Pump Skid

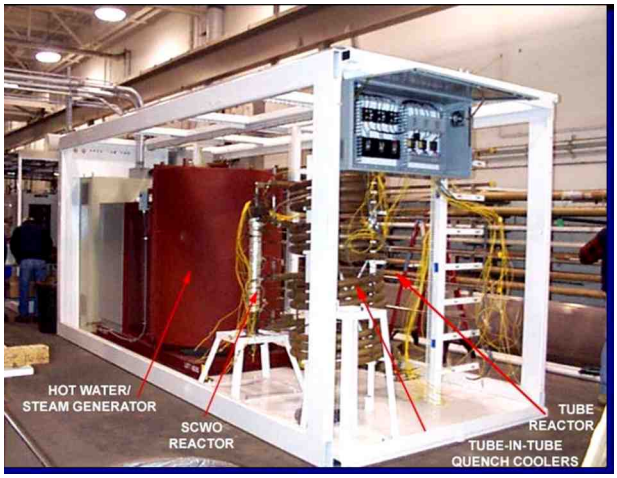

Figure 4. SWPO System Reactor Skid

1. Using direct mixing with supercritical water for heat-up of a biomass slurry entering the reactor is problematic because a relatively large amount of supercritical water is required. This dilutes the biomass slurry and requires oxidation of most of the biomass to attain the desired final temperature of about 650°C. Limited preheat and partial oxidation have been established as the preferred means of heat-up.
2. Significant effort was required to obtain reliable operation of a dual syringe pump for pumping thick slurries to high pressure. A number of improvements over the basic design were implemented, including magnetic sensing of the position of the driving pistons. The pump is now operating in highly reliable fashion.

3. The maximum pumpable concentration of wood flour slurries is in the range of 10-15 wt% dry basis. The maximum pumpable concentration of composted MSW is in the range of 30 wt% dry basis. A mixture of 10 wt% wood plus 10 wt% coal is readily pumpable.
4. Preheat temperatures in excess of about 250°C are conducive to charring with many biomass feeds. Preheating is being limited to 250°C.
5. A vessel reactor is preferable to a pipe reactor, allowing better temperature control and heat conservation and better ash and char handling.
6. Coal does not gasify well at 3400 psi and temperatures up to 800°C, but excellent oxidation has been achieved for coal at the same conditions.
7. Results for gasification of wood slurries have generally confirmed the high hydrogen yields reported in earlier laboratory testing at the University of Hawaii (UH). Table 2 compares the GA pilot scale results with UH's results. In addition to some temperature and pressure differences as shown in the table, the UH tests utilized an activated carbon catalyst with external heating, while the GA tests utilized no catalyst and partial oxidation with ethanol fuel. Hydrogen yields (Table 2, last column) are similar for both sets of tests, and both show a significant takeup of hydrogen from the water to make gaseous products.

Table 2. Comparison of GA Pilot-Scale Tests with UH Lab-Scale Tests

Organi-zation	Feed	T °C	P psi	% H_2	% CH_4	% C_2	% CO	% CO_2	g H/ 100g feed
GA	9% wood + CMC[1,2]	650	3400	44	33	0.3	7	16	9.9
GA	9% wood + CMC[1,2]	800	3400	38	30	0.2	4	28	10.8
UH Ref. 1	10.4% corn starch[3]	650	4061	45	14	0.0	2	35	9.1
UH Ref. 1	5% wood + 5.5% corn starch[3]	650	4061	34	23	0.2	3	45	8.2
UH Ref. 1	5% wood + 5.6% corn starch[3]	650	4061	50	10	0.0	4	39	11.4
UH Ref. 1	5% wood + 6/1% corn starch[3]	650	4061	43	14	0.2	3	37	9.3
UH Ref. 2	11% wood + 4.25% corn starch[3]	650	4061	57	6	NA	4	33	12.2

Notes:
1. CMC is carboxymethyl cellulose suspension agent.
2. Average of 3 runs, some ethanol fuel present.
3. Activated carbon catalyst used.

Conclusions

A versatile pilot plant for exploring gasification in supercritical water has been established at the General Atomics' facility in San Diego. Preliminary testing of the SWPO process has found hydrogen yields of about 10 grams per 100 grams of feed, comparable to those found in prior laboratory-scale work carried out at UH. As in that prior work, a significant amount of the hydrogen found in the gas phase products is derived from the water/steam matrix. Potential improvements to the SWPO process include higher energy feed injector nozzles for the reactor to achieve better dispersion of the incoming feed, second-generation reactor designs with higher solids residence times, and means for feeding more concentrated slurries or dry feed.

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2. Hong, G.T. and M.H. Spritzer, "Supercritical Water Partial Oxidation", 2002 Annual Report to USDOE Hydrogen Program, available at www.eren.doe.gov/hydrogen/docs/2002toc.html.

II.B.3 Biomass-Derived Hydrogen from a Thermally Ballasted Gasifier

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Main Subcontractor: Iowa State University, Ames, Iowa

Objectives

- Determine whether switchgrass is a suitable fuel for the ballasted gasifier.
- Obtain time-resolved concentrations of important fuel components evolved.
- Identify process conditions that maximize the production of hydrogen (H_2).
- Evaluate methods for removing contaminants from the producer gas.
- Evaluate methods for mediating the water-gas shift reaction in the product gas.
- Estimate the economics of H_2 production from switchgrass.

Approach

- Prepare switchgrass fuel and feeder.
- Prepare 5-ton per day bubbling fluidized bed gasifier and a latent heat ballasting system.
- Prepare slipstream for upgrading producer gas.
- Prepare gas sampling and analysis system.
- Perform gasification trials.
- Perform cost estimate of the gasification system.

Accomplishments

- The 5-ton per day biomass gasifier has been modified to operate as a ballasted gasifier.
- The gas sampling system and a slipstream of the gasifier effluent have been prepared.
- The gas conditioning system has been designed and constructed.
- Analytical methods for measurement of trace contaminants (hydrogen sulfide [H_2S], Ammonia [NH_3], and hydrochloric acid [HCl]) have been established.
- Shakedown trials of the gasifier have been performed.
- Producer gas composition has been characterized for gasification of switchgrass.
- The steam reformer has been demonstrated to reduce total tar by 95% and condensable (heavy) tar by greater than 99%.
- The catalytic water-gas shift reactor has been demonstrated to reduce carbon monoxide (CO) to less than 0.5 %-vol.

Future Directions

- Design and construct multi-contaminant control system based on sorbent injection. This system will remove particulate, sulfur, and halogen contaminants.
- Improve trace contaminant instrumentation. These improvements will give on-line capability for sulfur, NH_3, and some tar species.
- Evaluate effectiveness of multi-contaminant control system.
- Evaluate additional catalysts for removal of tar and NH_3 and enriching H_2 content via water-gas shift reaction.
- Identify appropriate separation technology to purify H_2.
- Perform thermal system and cost estimate analyses.

Introduction

The goal of this project is to optimize performance of an indirectly heated gasification system that converts switchgrass into H_2-rich gas suitable for powering fuel cells. We have developed a thermally ballasted gasifier that uses a single reactor for both combustion and pyrolysis. Instead of spatially separating these processes, they are temporally isolated. The producer gas is diluted with neither nitrogen (N_2) nor the products of combustion. The heat released during combustion at 850°C is stored as latent heat in the form of molten salt sealed in tubes immersed in the fluidized bed. During the pyrolysis phase, which occurs at temperatures between 600 and 850°C, the reactor is fluidized with steam or recycled producer gas rather than air. Heat stored in the phase change material is released during this phase of the cycle to support the endothermic reactions of the pyrolysis stage.

Because air is not used during the gas-producing phase of the cycle, N_2 does not dilute the product gas, resulting in relatively high concentrations of H_2 and CO in the producer gas compared to conventional gasifiers. The CO, along with steam used to fluidize the reactor, can be shifted to additional H_2 by the water-gas shift reaction.

Approach

The approach to this project is to employ a pilot-scale (5-ton per day) gasifier to evaluate the thermally ballasted gasifier as a means for producing H_2 from switchgrass. Gasification at the pilot scale is important for obtaining realistic process data, especially for calculating energy flows through the system and assessing the practicality of feeding switchgrass into the gasifier. A series of gasification trials are being performed to evaluate the effect of biomass feed rate (fixed steam rate) and the effect of biomass/steam rate (fixed biomass feed rate) on H_2 production.

A slipstream from the gasifier will be used to evaluate gas cleaning and upgrading options during the first year of research. This slipstream will include a guard bed designed to remove H_2S and HCl and some tar, a steam reformer designed to crack the remaining tar and decompose NH_3, and high-temperature and low-temperature catalytic water-gas shift reactors to remove CO from the product gas and increase its H_2 content. A series of gasification trials will be performed to evaluate the effectiveness of these four reactors in removing tar and contaminants, and shifting producer gas towards increased H_2 and decreased CO.

Results

Most of the first year was consumed in constructing the experimental equipment required for the gasification system and the gas analysis system. The gasification system is illustrated schematically in Figure 1. The 5-ton per day bubbling fluidized bed gasifier was modified to operate as a ballasted gasifier. This entailed installation of the ballast system within the fluid bed reactor. The ballast system consists of 25.4 mm diameter stainless steel tubes 610 mm in length. Each of the ballast tubes was filled with 0.3 kg (0.66 lb) of Lithium Fluoride (LiF). An air pocket was left in each of the tubes to allow for expansion of the LiF. Forty-eight ballast tubes cover about 15% of the bed cross-sectional area. This represents a total latent heat storage capacity of 15,100 kJ.

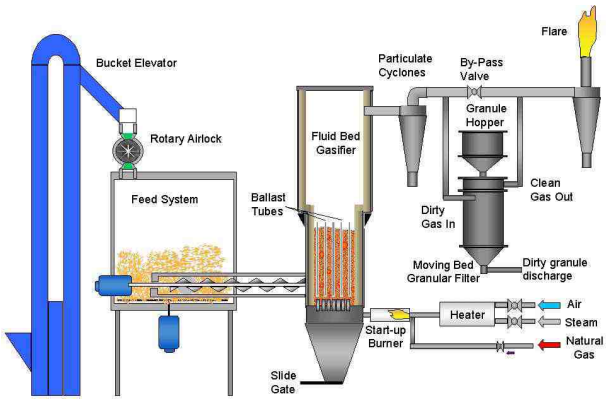

Figure 1. Schematic of the Gasifier System

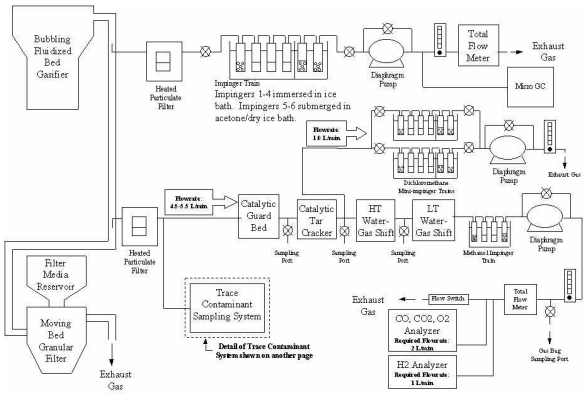

Figure 2. Overall Schematic of Gas Sampling System and Slipstream from Gasifier Effluent

Several other modifications have been made to the gasifier system. An additional fuel hopper was added to the system to enable the use of multiple fuels. Both fuel hoppers were outfitted with load cells to accurately measure fuel flow into the gasification reactor. The moving bed granular filter, which will be evaluated for hot gas cleanup of particulate material in the producer gas, has been improved by the addition of a new auger system to remove dirty filter media from the bottom of the filter. The exhaust system, which was in poor repair, was replaced and upgraded to minimize the impacts of temperature on the system. Additionally, an automated high-temperature valve was installed to enable division of the pyrolytic gases from the combustion gases.

Figure 2 is a flow diagram of the gas sampling system and the slipstream used on the producer gas effluent. Producer gas is extracted at two locations in the producer gas effluent. The first sampling point is positioned just downstream of the gasifier to provide raw gas characterization. This sampling is performed isokinetically to obtain a representative particulate loading. The isokinetically-sampled gas passes through a Mott Hyline porous metal filter operated at 600ºC to prevent condensation of tar. The gas then passes through a series of impingers containing varying combinations of dichloromethane (DCM) and glass beads. The impingers remove all condensable species, including tars and water. The Varian Micro-Gas Chromatograph draws a small portion of the sample stream in order to characterize the gas, specifically for H_2, CO, carbon dioxide (CO_2), N_2, methane (CH_4) and ethylene (C_2H_4). The remaining portion of the sample stream is passed through a total volumetric gas meter and exhausted to atmosphere.

The second extraction point is for the purpose of producing a slipstream of the gasifier effluent, which is used to evaluate an experimental gas conditioning system. A heated filter, similar to the one previously described, removes particulate matter, but gas is not sampled isokinetically, as is done in the first gas extraction location. After passing through the gas conditioning system, described in the next section, the producer gas passes through on-line gas analyzers that monitor CO, CO_2, Oxygen (O_2) and H_2. The slipstream also provides a particulate-free gas for the quantification of trace contaminants such as H_2S, HCl, hydrogen cyanide (HCN), and NH_3.

Based upon previous work on the catalytic conversion of tars, new reactors have been designed and constructed. Improvements include the addition of standard flanges for better leakage control, upgraded reactor materials for improved characteristics in high-temperature environments, and welded joints in all areas inside the heated enclosures to vastly reduce leakage potential. Two more reactors were added for high-temperature and low-temperature H_2 shift reaction purposes. Figure 3 shows the current design for the gas conditioning system. The reactor bodies are made of 1¼ inch pipes constructed with Haynes 230 and pre-manufactured flanges made of 304 stainless steel. The 3/8 inch inlet and outlet pipes are constructed

with Haynes 230 material and have 3/8 inch stainless steel tube adapters welded to them. Each reactor has a volume of approximately 16.5 cubic inches (270 cubic centimeters).

The trace contaminant sampling system draws a slipstream from the gas passing into the catalytic reactor system. This sample is drawn following the porous metal filter through which all of the gas passes. The slipstream progresses through a condensing coil to remove all tars. The flow is then split into two smaller streams. The first stream is passed through a nafion membrane to remove all moisture from the gas, and a sample is drawn with Draeger tubes to quantify H_2S content. The second stream passes through bubblers plumbed in parallel to each other to simultaneously quantify HCl, HCN and NH_3. Following sampling, these streams are exhausted to the atmosphere.

During the first year, most gasification trials were performed with the gasifier operated in conventional, air-blown mode. Experimental results included characterization of the gas obtained from gasification of switchgrass, preliminary evaluation of the performance of the steam reformer, and preliminary evaluation of the catalytic water-gas shift reactors.

Air-blown gasification of switchgrass produced tar loadings of 117 g/nM^3, which is within 10% of the tar loadings obtained from gasification of obsolete seed corn, the model fuel used in this test program. Particulate loadings from air-gasification of switchgrass were 19.3 g/nm^3. The steam reformer was able to reduce total tar by 95% and condensable (heavy) tar by greater than 99%. The catalytic water-gas shift reactor reduced CO to less than 0.5 %-vol.

Conclusions

Most of the first year was occupied in setting up the 5-ton per day pilot plant gasifier to operate in thermally ballasted mode and preparing the slip stream for gas conditioning studies.

Switchgrass was successfully gasified and shown to produce a gas with characteristics similar to producer gas from a model gasification fuel, namely obsolete seed corn.

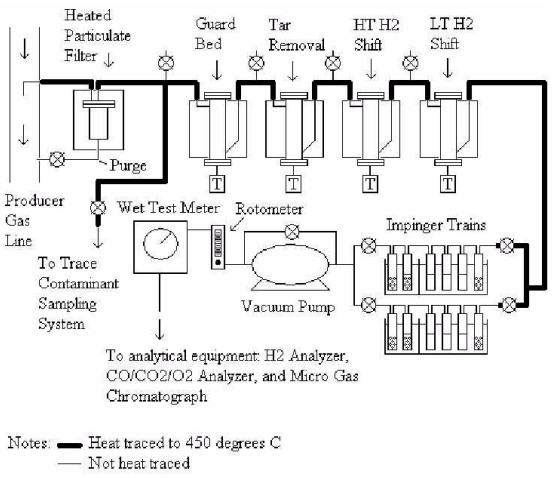

Figure 3. Gas Conditioning System

The steam reformer was able to eliminate up to 95% of heavy tars.

The water-gas shift reactors reduced CO in the producer gas to 0.5 %-vol.

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1. Cummer, K. and Brown, R. C., "Ancillary equipment for biomass gasification," accepted for publication in Biomass and Bioenergy, Fall 2001.

2. Pletka, R., Brown, R. C., and Smeenk, J., "Indirectly heated biomass gasification using a latent heat ballast. Part 1: Experimental evaluations," Biomass and Bioenergy 20, 297-305, 2001.

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4. Zhang, R., Brown, R. C., Suby, A., Cummer, K., "Catalytic cleaning of hot producer gas," Fifth International Biomass of the Americas Conference, Orlando, FL, September 17-21, 2001 (conference cancelled because of national emergency).

5. Nunez, J. S., Cummer, K., Brown, R. C., Smeenk, J., "Use of a moving bed filter for cleanup of hot producer gas," Fifth International Biomass of the

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8. Nunez, J., Cummer, K., Suby, A., Dvorak, B., Smeenk, J., Brown, R. C., "Improving prospects for gasification of wastes through gas conditioning: Dust removal," Renewable Energy and Organics Recycling Conference, Des Moines, IA, October 29-31, 2001.

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II.B.4 Techno-Economic Analysis of Hydrogen Production by Gasification of Biomass

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Objectives

- To determine the economics of hydrogen production by gasification of three biomass candidates: bagasse, switch grass, and nutshells.
- To optimize hydrogen production for transportation use.
- To identify the economic and technical barriers associated with biomass gasification.

Approach

- Develop a simulation to quantify hydrogen production.
- Determine the optimization of hydrogen by testing various cases through simulation.
- Analyze research and simulation results to determine technical and economic feasibility.

Accomplishments

- Developed a model using a Hysis Simulation package.
- Explored the resource base for nutshells and bagasse to determine their availability.
- Gathered literature for an ultimate dry basis analysis of the feedstocks.

Future Directions

- Develop a more detailed process flow schematic with a complete gas cleanup system.
- Perform laboratory testing on biomass feedstocks and analyze hydrogen production.

Introduction

The future application of hydrogen as a non-polluting fuel is dependent on the convergence of cost effective technologies for its manufacture, delivery, and end-use. DOE is actively pursuing research in all these areas to enable the private sector to demonstrate the technical viability of hydrogen technologies. Once viable from a technical viewpoint, commercial acceptance requires that these technologies demonstrate cost effectiveness in the marketplace. Key markets for hydrogen technologies are the transportation, stationary industrial, residential, and commercial energy markets. The prime mover targeted for hydrogen is fuel cell systems that are capable of very efficient and clean conversion of hydrogen to electricity, either with or without byproduct heat recovery.

Approach

The object of this project is to assess the cost of hydrogen production from three candidate biomass feedstocks and identify the barriers for commercialization of this technology. This is to be accomplished by first assessing the resource base. A process flow scheme will be developed for each feedstock that includes the following sections: feed preparation, followed by gasification or pyrolysis, a reforming section to reduce heavy hydrocarbons in the gas, a shift conversion process to maximize hydrogen production, and a gas purification process to provide gas meeting end-use specifications. The process design will then be used to perform an economic analysis to determine the cost of producing hydrogen. Throughout this effort, possible barriers to implementation of the technology will be identified, and a cost sensitivity analysis examining the major cost elements of the process will be performed. The resultant package will identify areas where targeted research will have the greatest benefits. The project will also identify the current influence of government incentive programs for biomass production and recommend changes that will further stimulate integration of biomass as an energy feedstock.

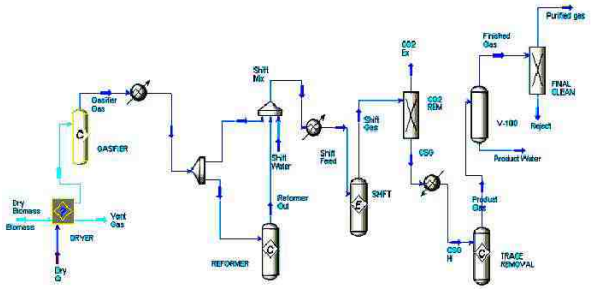

Figure 1. Hysis Simulation Flow Sheet

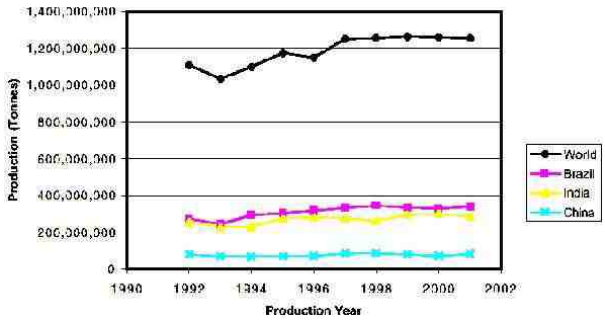

Figure 3. Sugar Cane Production for the World, Brazil, India, and China (1992 - 2001)

Results

This project is still in the analysis phase to determine the economics of hydrogen production from biomass. A process flow sheet has been developed (see Figure 1) and a resource base for nutshells and bagasse has been analyzed. Nut production varies, as with any agricultural crop, due to changing climates and weather conditions. Generally, the production of nuts has increased over the past 10 years. Heating values for nutshells range from 7500 - 9000 BTU/lb on a dry basis.

Bagasse is leftover material from the production of sugar cane. The majority of sugar cane production comes from Brazil, India, and China (the United States ranks tenth in world production of sugar cane). Data on world and major sugar cane producing countries can be seen in Figure 2. The majority (85%) of bagasse is used to meet the thermal demands of the processing factory. The remaining 15% can be used for power generation needs. During the crushing season in Brazil, there would be roughly 165 ton/day of bagasse fiber if a given factory operated at capacity. Based on this estimate, four plants have the ability to produce around 660 tons of bagasse per day for around 200 days per year, which would satisfy the needs of a small hydrogen production facility. The heating value of bagasse is around 7200 - 8000 BTU/lb on a dry basis.

Switchgrass is an energy crop being studied for its potential as a fuel source. Switchgrass is beneficial in that it has yet to be cultivated and therefore can potentially be grown in locations close to the gasifier and ultimately reduce hydrogen production costs by reducing transportation costs. Typical heating values for switchgrass range from 7700 - 8200 BTU/lb on a dry basis.

Conclusions

- Gasification technologies, in combination with advancements in hot gas cleanup technologies, allow for hydrogen production with little environmental impact.

- Biomass can be used as a valuable and environmentally clean product, instead of a waste product, if used to produce hydrogen.
- Hydrogen production costs for transportation use will be significantly higher than current fossil fuel prices, but hydrogen from biomass has potential to become a competitive fuel with various government incentives and advances in technology.
- Hydrogen from biomass yielded production values ranging from 5.7 - 9.1 scf H_2 / lb biomass during initial case studies (this is assuming that all the CO is shifted to H_2).
- A typical hydrogen from biomass plant will be able to operate economically at around 600 tons/day for bagasse. A plant producing hydrogen from nutshells will be significantly lower. Switchgrass has yet to be determined.

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