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# Palladium-Mediated Hydrogenation of Unsaturated Hydrocarbons with Hydrogen Gas Released during Anaerobic Cellulose Degradation

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**Among five hydrogenation catalysts, palladium on charcoal was the most reactive one when suspended in anaerobic culture medium, and Lindlar catalyst (Pd on $CaCO_3$) was the most reactive one when suspended in the gas phase of culture tubes. Palladium on charcoal in the culture medium (40 to 200 mg 10 $ml^{-1}$) completely inhibited growth of *Neocallimastix frontalis* and partly inhibited *Ruminococcus albus*. Lindlar catalyst (40 to 200 mg per tube) suspended in a glass pouch above the culture medium did not affect the rate of cellulose degradation or the ratio of fermentation products by these organisms. Acetylene added to tubes containing Lindlar catalyst in pouches, and either of the two organisms in monoculture or coculture with *Methanospirillum hungatei*, was reduced to ethylene and then ethane, followed by hydrogen production. Similar results were obtained with 1-pentene. Neither acetylene nor 1-pentene affected cellulose degradation but both inhibited methanogenesis. In the presence of Lindlar catalyst and propylene or 1-butene, fermenter-methanogen cocultures continued to produce methane at the same rate as controls and no olefin reduction occurred. Upon addition of bromoethanesulfonic acid, methanogenesis stopped and olefin reduction took place followed by hydrogen evolution. In a gas mixture consisting of propylene, 1-butene, and 1-pentene, the olefins were reduced at rates which decreased with increasing molecular size. These results demonstrate the technical feasibility of combining in one reactor the volatile fatty acid production by anaerobic digestion with chemical catalyst-mediated reductions, using the valuable by-product hydrogen.**

While anaerobic digestion has recently attracted renewed interest as a waste treatment process, it is still considered uneconomical if its sole purpose is biogas production on a large scale in a technologically advanced economy. One way of rendering the process economically feasible would be to direct the fermentation towards more valuable products than methane. Advances in the knowledge of metabolically distinct groups of organisms involved in anaerobic digestion have led to suggestions for alternative products and combinations of biological and chemical processes (11). One such alternative to biogas is the production of volatile fatty acids (18, 19). Hydrogen gas is a valuable by-product of this process and may contain up to half of the substrate energy recovered in volatile fatty acids (13). Thus, to contribute to the economic feasibility, hydrogen should be incorporated into the overall process.

This paper demonstrates the use of fermentation hydrogen in a chemical reaction. Cellulose was anaerobically degraded to acids and ethanol, and the hydrogen produced during this fermentation was simultaneously used for the palladium-catalyzed reduction of olefins in the gas phase of the fermentation vessel.

## MATERIALS AND METHODS

**Organisms.** *Neocallimastix frontalis* strain PN-1 was obtained from the culture collection of the Department of Scientific and Industrial Research, Palmerston North, New Zealand. *Ruminococcus albus* strain D-7 and *Methanospirillum hungatei* strain JF-1 were kindly supplied by M. P. Bryant, University of Illinois, Urbana/Champaign.

**Chemicals.** Lindlar catalyst (1 to 2 % Pd on $CaCO_3$) and Pd on charcoal (5%; moisture content, 45%) were obtained from British Drug Houses, Poole, England. Palladium on $BaSO_4$ (10%) and Pd on $Al_2O_3$ (5%) were obtained from Merck-Schuchardt, Munich, Federal Republic of Germany. Palladium on polyethyleneimine beads (1 to 2%; 40 mesh) was obtained from Pierce Chemical Co., Rockford, Ill. The gases were of at least commercial purity (New Zealand Industrial Gases, Wellington). All other chemicals were of reagent grade and were purchased from commercial sources.

**Culture media.** Anaerobic techniques for the preparation and use of media were based on those of Hungate (10), as modified by Bryant (4) and Balch and Wolfe (1). Two different types of media were used: medium A for the growth of all monocultures and also for the *N. frontalis-M. hungatei* coculture, and medium B for the *R. albus-M. hungatei* coculture.

Salt solutions (9) were used in the preparation of medium A. Solution I contained the following components (wt/vol): $KH_2PO_4$, 0.3%; NaCl, 0.6%; $(NH_4)_2SO_4$, 0.3%; $CaCl_2$, 0.03%; and $MgSO_4$, 0.03%. Solution II contained 0.3% (wt/vol) $K_2HPO_4$. The medium was based on that of Bauchop and Mountfort (2) and contained 16.5% (vol/vol) solution I, 16.5% (vol/vol) solution II, 17% (vol/vol) cell-free ovine rumen fluid, 0.1% (wt/vol) yeast extract (BBL Microbiology Systems, Cockeysville, Md.), 0.1% (wt/vol) Bacto-Peptone (Difco Laboratories, Detroit, Mich.), 0.5% (wt/vol) $NaHCO_3$, 0.02% (wt/vol) cysteine hydrochloride, 0.01% (wt/vol) $Na_2S \cdot 9H_2O$, and resazurin ($10^{-4}$ %). The gas phase was 70% $N_2$–30% $CO_2$.

Medium B contained 5% (vol/vol) rumen fluid, 5% (vol/vol) Pfennig mineral solution, 0.1% (vol/vol) Pfennig metal solution, 0.5% (vol/vol) vitamin B solution, 2% (vol/vol) reducing solution (1.25% $Na_2S \cdot 9H_2O$–1.25% cysteine HCl), and a 7% (vol/vol) solution of 5% $NaHCO_3$. The

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gas phase was 80% $N_2$–20% $CO_2$ and the final pH was 7.2 (16).

For the preparation of both media, $NaHCO_3$ solution was added to medium cooled after boiling, which was then dispensed in 10-ml amounts into culture tubes (18 by 150 mm) modified for use with black rubber septum stoppers and aluminum serum cap closures (1). Prior to addition of medium, sisal (fiber from leaves of *Agave sisalona* L. commercially available as twine) or Whatman no. 1 filter paper strips were added to culture tubes in 100-mg amounts. When media were used for the maintenance of cultures, sterile sodium sulfide (medium A) or cysteine-sodium sulfide (medium B) reducing agent was added to individual tubes after autoclaving and usually 1 day to several hours before use. Experimental culture tubes received no added sodium sulfide.

**Culture techniques.** The techniques described previously by Hungate (10) as modified by Balch and Wolfe (1) were adapted for the maintenance and subculturing of cultures.

*M. hungatei* was maintained in agar slants of basal medium (16) modified to contain 0.2% (wt/vol) Trypticase (BBL), 30% clarified bovine rumen fluid, and 25 mM sodium formate. Before combining with cultures of *R. albus* or *N. frontalis* the methanogen was grown in liquid cultures by shaking in tubes containing basal medium with a 14% (vol/vol) solution of 5% $NaHCO_3$ and an 80% $H_2$–20% $CO_2$ gas phase initially at 202.6 kPa. To establish coculture with *R. albus* or *N. frontalis*, 1 ml of methanogen culture at full growth (optical density at 600 nm, 0.45) was inoculated into media immediately after inoculation with the cellulose degrader.

*N. frontalis* in monoculture or in coculture with *M. hungatei* was maintained in sisal medium, while *R. albus* was maintained in medium with filter paper strips. Every 6 days, 1/20 the volume of cultures was transferred to initiate further cultures.

Inoculation of experimental media containing filter paper strips was by transfer of 1/20 the volume of *N. frontalis* or *R. albus* cultures at full growth. Inoculation to give cocultures was by transfer of the same volume of stable *N. frontalis-M. hungatei* coculture and, for *R. albus-M. hungatei*, simultaneous inoculation of the two organisms which had been pregrown separately. The inoculum of cellulose degrader was equivalent to ca. $2.5 \times 10^3$ CFU of *N. frontalis* $ml^{-1}$ and, for *R. albus*, $5 \times 10^7$ cells $ml^{-1}$. The amount of methanogen in inocula ranged between $0.7 \times 10^7$ and $1.4 \times 10^7$ CFU $ml^{-1}$. Cell counts and CFU were determined by previously described procedures (2).

All incubations were at 37°C. With the exception of liquid cultures of *M. hungatei* which were incubated with shaking on a gyratory shaker (17), tubes for the maintenance of cultures were held in the vertical position without shaking. Experimental cultures were incubated either without shaking or, in the case of catalyst addition to medium, with shaking (17).

**Introduction of the catalytic system into experimental culture tubes.** Tubes which had been autoclaved were transferred to an anaerobic glovebox (Coy Laboratory Products, Ann Arbor, Mich.). Butyl septum stoppers were removed and replaced by the same type of stopper to which was attached a glass pouch containing 50 mg of palladium catalyst. The pouches were conveniently made by modification of Pasteur pipettes and were sterilized by autoclaving. Catalyst was preheated at 160°C for 2 h before introduction into pouches. After inclusion of the catalytic system, the gas phase of the culture tubes was readjusted to 70% argon–30% $CO_2$, and care was taken to ensure that the pouches were suspended clear of the culture medium.

In some experiments catalyst (40 to 200 mg) was added directly to growth medium instead of pouches.

**Addition of unsaturated hydrocarbons to culture systems.** Unsaturated hydrocarbons (chain length, $C_2$ to $C_5$) were added by syringe in amounts ranging from 80 to 420 μmol usually after growth had established, which was approximately 48 and 72 h after inoculation of *R. albus* or *N. frontalis*, respectively.

**Growth measurement.** In the absence of unsaturated hydrocarbons growth of monocultures of *R. albus* or *N. frontalis* was determined by measurement of $H_2$ production. Under the same conditions growth of cocultures was determined by measurement of methane production.

**Measurement of gases.** Hydrogen was analyzed on a Fisher-Hamilton gas partitioner equipped with a thermal conductivity detector (2). Hydrocarbons, ($C_1$ and $C_2$) were determined by gas chromatography, using a steel column packed with Porapak Q at 70°C in a Carle model 5900 gas chromatograph equipped with a flame ionization detector. Longer-chained hydrocarbons were separated isothermally at 65°C, using a glass column (2 m by 4 mm) packed with Durapak C-8 in a Tracor model 560 gas chromatograph equipped with a flame ionization detector. The amounts of hydrocarbons were determined by comparison of peak areas of known standards. Under conditions where hydrocarbons were found to dissolve in water, the total amount of gas was corrected for that dissolved, using the formula $X_t = X_g + X_g \cdot \alpha V_L (273 + \nu)/273 V_g$, in which $X_t$ is the total amount of gas, $X_g$ is that remaining in the gas phase, and $V_L$ and $V_g$ are the volumes of liquid and gas space, respectively. The symbols $\nu$ and $\alpha$ refer to the temperature (degrees Celsius) and the Bunsen absorption coefficient, respectively.

**Measurement of cellulose and fermentation end products in culture media.** When no further growth of cultures occurred, as evident from no further $H_2$ or $CH_4$ production, or reduction of unsaturated hydrocarbon, growth media were harvested by centrifugation at 8,000 × *g* and 4°C for 15 min. The supernatant was removed and stored at −18°C until required for determination of glucose or fermentation end products. The pellet was suspended in 5 ml of distilled water, and residual cellulose was determined by the method of Updegraff (25).

Quantitative determination of glucose in the culture supernatant was achieved by using glucose oxidase in a procedure based on that of Huggett and Nixon (8). For measurement of acetate and ethanol, 2-ml portions of culture supernatant were acidified with 0.1 ml of 6 N HCl and analyzed by gas chromatography as previously described (2). The compounds were quantitated by comparison of retention times and peak area with those of known standards.

## RESULTS

**Selection of catalyst.** Lindlar palladium (50 mg), palladium on charcoal (50 and 100 mg), palladium on $Al_2O_3$ (50 mg), palladium on polyethyleneimine beads (50 mg), and palladium on $BaSO_4$ (25 mg) were placed in the pouches of culture tubes containing medium reduced with cysteine. Hydrogen and either propylene or acetylene were added (130 μmol), and the uninoculated tubes were incubated at 37°C. The rate of propylene hydrogenation was fastest with Lindlar catalyst (300 μmol $h^{-1}$) followed by palladium on $Al_2O_3$ (240 μmol $h^{-1}$) and then by palladium on $BaSO_4$ and palladium-polyethyleneimine beads (210 μmol $h^{-1}$). Palladium on char-

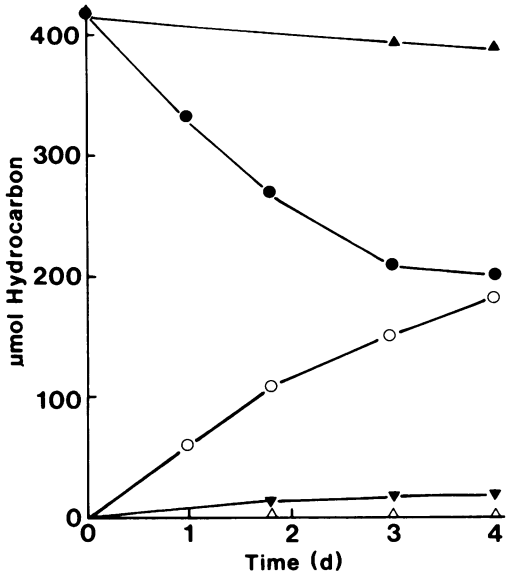


FIG. 1. Propylene reduction in medium containing *R. albus* and palladium on charcoal. Medium A (10 ml) amended with 100 mg of Whatman no. 1 filter paper strips and palladium on charcoal (40 to 200 mg) was inoculated with 0.5 ml of a 4-day-old culture of *R. albus* and incubated on the shaker at 37°C. Propylene (430 µmol) was added immediately after inoculation. Symbols: propylene—(●) 100 mg of catalyst, (▲) 200 mg of catalyst; propane—(▼) 40 mg of catalyst, (○) 100 mg of catalyst, (△) 200 mg of catalyst.

coal gave the slowest rates (<180 µmol $h^{-1}$). The relative rates of acetylene hydrogenation in the presence of the various catalysts were similar to those observed for propylene hydrogenation, the fastest rate being obtained with Lindlar catalyst (400 µmol $h^{-1}$) and the slowest being obtained with charcoal palladium (270 µmol $h^{-1}$). Lindlar catalyst was chosen for further experiments, using the pouch system.

To select the most suitable catalyst for use in liquid culture media, the same quantities of catalysts as in the previous experiment were added directly to culture tubes containing reduced medium. The tubes then received 130 µmol of $H_2$ and the same amount of acetylene, propylene, butene, or 1-pentene. The uninoculated tubes were incubated by shaking at 37°C. Palladium on charcoal catalyzed the hydrogenation of all four hydrocarbons, and the fastest hydrogenation rate was achieved with propylene (33 µmol $h^{-1}$), followed by acetylene and 1-butene (17.4 µmol $h^{-1}$) and 1-pentene (5.8 µmol $h^{-1}$). Propylene reduction was complete after about 5 h of incubation. The same rates were achieved with either medium A or B. In the presence of the other catalysts only acetylene was reduced, and the rates were equal to or slightly slower than that obtained with palladium on charcoal. Palladium on charcoal was selected for experiments with the catalyst in the culture medium.

**Effect of catalysts on metabolism, hydrogenation rate, and fermentation pattern.** When *N. frontalis* was incubated in medium A containing palladium on charcoal (40 to 200 mg) in the presence and absence of propylene (430 µmol), no cellulose was degraded and no olefin hydrogenation was observed. In control tubes containing propylene but no catalyst, cellulose degradation was normal as indicated by an $H_2$ production of about 120 µmol in 6 days. Thus, palladium on charcoal inhibited growth of the fungus.

When *R. albus* was incubated in the same medium containing palladium on charcoal, hydrogenation of propylene to propane occurred (Fig. 1), and the fastest hydrogenation rate (2.5 µmol of propane $h^{-1}$) was achieved with 100 mg of catalyst. After 96 h of incubation some $H_2$ accumulated, and slightly more than 50% of the initial propylene had been hydrogenated. Increasing the amount of catalyst beyond 100 mg led to a decline in hydrogenation and cellulose degradation, so that with 200 mg of catalyst cellulose utilization was completely inhibited. At low concentrations, the catalyst was ineffective as shown by $H_2$ accumulation (Table 1).

The previous experiment was repeated with Lindlar catalyst in pouches above the culture instead of Pd on charcoal suspended in the medium. With 50 and 100 mg of catalyst per tube, the propylene reduction rate was 5 µmol of propane $h^{-1}$ and the reaction was completed within 72 h, but with 20 mg of catalyst the rate was <1 µmol of propane $h^{-1}$. Cellulose degradation was not affected by the catalyst in the pouch (Table 1).

Neither Lindlar catalyst nor Pd on charcoal significantly affected the ratio of acetate to ethanol produced (Table 1), thus suggesting that these catalysts were not reactive enough to cause a shift in the fermentation pattern towards more oxidized products.

Since Pd on charcoal suspended in the culture medium

TABLE 1. Effect of catalytic hydrogenation systems on the fermentation of cellulose by *R. albus*[a]

| Catalyst | | Cellulose utilized (mg)[b] | $H_2$ produced (µmol)[b] | mol of $C_2$ product 100 mol of hexose units$^{-1}$[b] | | Acetate/ethanol |
|---|---|---|---|---|---|---|
| Type | Amt (mg) | | | Acetate | Ethanol | |
| Palladium/charcoal | 0 | 48 | 380 | 62.5 | 63 | 1.0 |
| | 40 | 73 | 511 | 58 | 55 | 1.05 |
| | 100 | 37 | 87 | 70 | 57 | 1.2 |
| | 200 | <1 | Nil[c] | | | |
| Lindlar palladium | 0 | 54 | 300 | 55 | 63 | 0.87 |
| | 20 | 48 | 203 | 66 | 83 | 0.80 |
| | 50 | 45 | 6.1 | 63 | 76 | 0.83 |
| | 100 | 47 | Nil[c] | 58 | 70 | 0.83 |

[a] Media (10 ml) containing 100 mg of Whatman no. 1 filter paper strips were inoculated with 0.5 ml of a 4-day-old culture of *R. albus*. Propylene (430 µmol) was added immediately after inoculation. Catalyst was present either in the culture fluid (palladium/charcoal) or suspended above the culture fluid in glass pouches (Lindlar palladium). Cultures were incubated at 37°C by shaking (palladium/charcoal) or were held in the vertical position without shaking (Lindlar palladium).

[b] Determined after 96 h for the palladium/charcoal system and 72 h for Lindlar palladium, during which time no glucose accumulated in the culture fluid. Values for the palladium/charcoal system are means of duplicate determinations.

[c] The detection limit for hydrogen was $5 \times 10^{-4}$ atm (50.5 Pa).

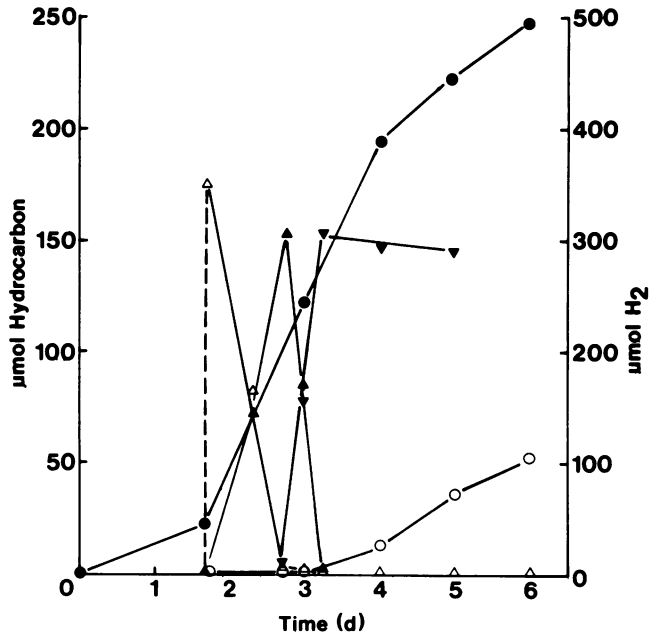


FIG. 2. Hydrogenation of acetylene to ethylene and ethane catalyzed by Lindlar palladium, utilizing $H_2$ from the fermentation of cellulose by *R. albus*. Medium (10 ml) containing 100 mg of Whatman no. 1 filter paper strips was inoculated with 0.5 ml of a 4-day-old culture of *R. albus* and incubated at 37°C. Catalyst (50 mg) was suspended in a glass pouch above the medium. Acetylene was added after 40 h. Symbols: (△) acetylene; (▲) ethylene; (▼) ethane; (○) hydrogen; (●) hydrogen in control cultures receiving no acetylene.

markedly reduced the cellulolytic activity of the organisms and catalyzed the reduction at a lower rate than Lindlar catalyst in the pouch, the latter was chosen for further experiments.

**Hydrogenation of acetylene, ethylene, and 1-pentene.** After cultures of *R. albus* had been incubated for 40 h in tubes containing medium A with filter paper and pouches with Lindlar catalyst, 176 μmol of acetylene was added to the tubes. Ethylene appeared during acetylene reduction, and after the disappearance of acetylene, ethylene was reduced and ethane was produced. After completion of this reaction, hydrogen accumulated to about 100 μmol, whereas in controls containing no acetylene hydrogen had accumulated to about 500 μmol (Fig. 2). Similar experiments showed that acetylene completely inhibited ethylene reduction so that the ratio between acetylene added and hydrogen produced determined the final product(s) (results not shown).

In the absence of Lindlar catalyst, addition of acetylene (88 μmol) to cocultures of *R. albus* and *M. hungatei* resulted in inhibition of methanogenesis and accumulation of $H_2$ (Fig. 3A). In the presence of catalyst, acetylene also inhibited methanogenesis, but no hydrogen appeared and acetylene was reduced to ethylene and then ethane (Fig. 3B). When the reduction was complete, $H_2$ rather than methane accumulated. As in experiments with *R. albus* monocultures, increasing the amount of acetylene added to the culture systems resulted in increased production of ethylene.

The results obtained with 1-pentene instead of acetylene were similar to those obtained with acetylene. 1-Pentene inhibited methanogenesis and was reduced to pentane, after which $H_2$ accumulated. Up to 340 μmol of 1-pentene per culture tube could be hydrogenated. During hydrogenation of 1-pentene a transitory intermediate appeared with a retention time slightly longer than that for pentene on the Durapak column. The intermediate was not characterized.

In both mono- and coculture experiments with *R. albus*, cellulose degradation was not significantly affected if acetylene or 1-pentene was added to cultures after they had established growth. In the presence of 88 to 360 μmol of acetylene, *R. albus-M. hungatei* cocultures degraded 50 to 60% of the initial cellulose in 7 days, and cultures not exposed to acetylene converted 55% of the substrate. However, if acetylene was added at the time of inoculation, growth was inhibited, and if pentene was added, the lag phase was prolonged.

**Hydrogenation of 1-butene and propylene.** Addition of

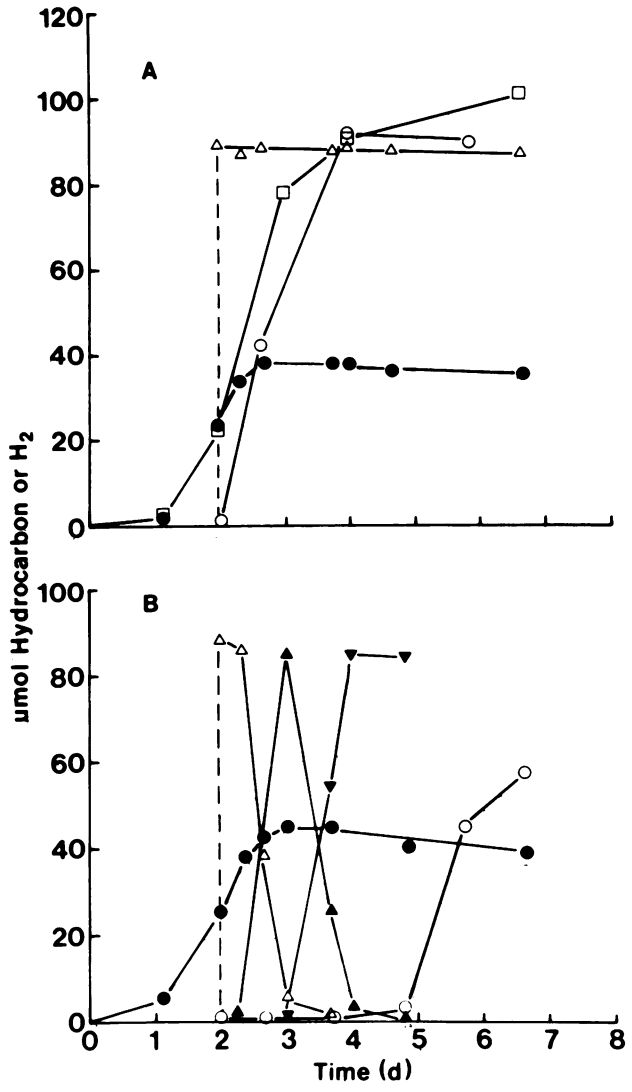


FIG. 3. Effect of Lindlar catalyst and acetylene on gas metabolism of an *R. albus-M. hungatei* coculture. Medium A (10 ml) containing 100 mg of Whatman no. 1 filter paper strips was inoculated with 0.5 ml of a 4-day-old *R. albus* culture and 1 ml of a 48-h-old *M. hungatei* culture and incubated at 37°C. Catalyst (50 mg) was suspended above the medium in a glass pouch. Acetylene was added to the cultures after 48 h. (A) In absence of catalyst; (B) in presence of catalyst. Symbols: (△) acetylene; (▲) ethylene; (▼) ethane; (●) methane; (○) hydrogen; (□) methane in controls receiving no acetylene.

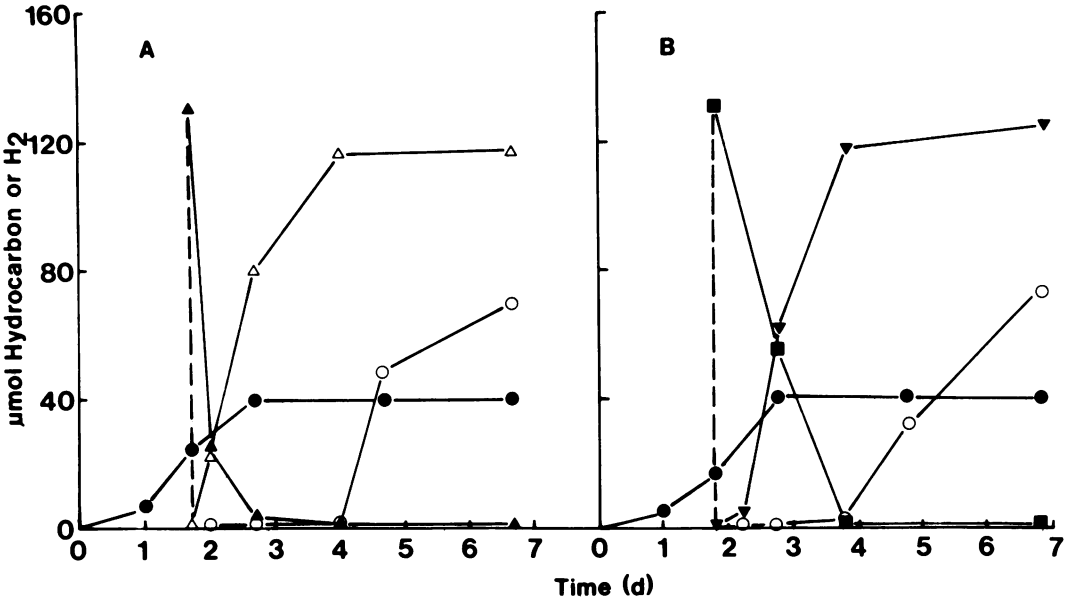


FIG. 4. Lindlar palladium-catalyzed hydrogenation of 1-butene (A) and propylene (B), utilizing $H_2$ produced by BES-treated cocultures of *R. albus* and *M. hungatei*. Inoculation and incubation were as in the legend to Fig. 3. BES was added at the same time as olefin to give a final concentration of 100 µM. Symbols: (▲) 1-butene; (△) butane; (■) propylene; (▼) propane; (○) hydrogen; (●) methane.

1-butene or propylene (130 µmol) to catalyst-containing culture tubes in which *R. albus* in coculture with *M. hungatei* had initiated growth on cellulose resulted in little or no olefin hydrogenation. No $H_2$ was released into the gas phase, and when cultures reached full growth (7 days) the total $CH_4$ was 100 µmol, which was the same as for control cultures receiving no olefin (see Fig. 3A for time course of $CH_4$ production in control cocultures). Addition of bromoethanesulfonic acid (BES) to the cultures (100 µM) at the time of olefin addition resulted in inhibition of methanogenesis and hydrogenation of butene and propylene (Fig. 4). When this reaction was complete, $H_2$ but not $CH_4$ accumulated. The results indicated that propylene and 1-butene did not inhibit methanogenesis and that hydrogen availability for olefin reduction required the addition of BES.

The addition of propylene or butene did not impair the activity of *R. albus*. After 7 days the amount of cellulose utilized was the same as that in control cultures receiving no olefin.

**Hydrogenation of olefin mixtures.** Simultaneous addition of 1-pentene, 1-butene, and propylene each at 65 µmol to tubes in which *R. albus* in coculture with *M. hungatei* had initiated growth on cellulose resulted in concomitant hydrogenation of all three olefins and in inhibition of methanogenesis (Fig. 5). The rates of hydrogenation decreased with increasing chain length.

***N. frontalis.*** The experiments described for *R. albus* were repeated with *N. frontalis*. The results were similar, but the *N. frontalis* coculture was slightly more sensitive to inhibition by the olefins. Despite the higher cellulolytic activity of *N. frontalis* the hydrogenation rates and yields in its presence were generally lower than with *R. albus*, suggesting that the fungus produced less hydrogen per unit of substrate fermented.

## DISCUSSION

The results demonstrate the technical feasibility of combining the acidogenic phase of anaerobic digestion with a hydrogen-consuming chemical reaction within the same reactor. The biological production of volatile fatty acids and simultaneous utilization of the valuable by-product hydrogen is possible, and such a combination may be economically more interesting than the traditional biogas production. The

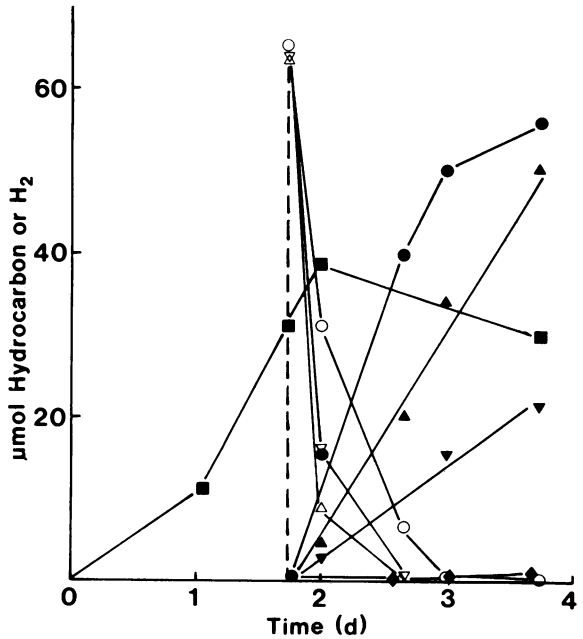


FIG. 5. Lindlar palladium-catalyzed hydrogenation of an olefin mixture, utilizing $H_2$ from cellulose fermentation by an *R. albus-M. hungatei* coculture. Inoculation and incubation were as in the legend to Fig. 3. 1-Pentene, 1-butene, and propylene at 65 µmol each were added to the culture after a 40-h preincubation. Symbols: (◆) hydrogen; (■) methane; (○) propylene; (●) propane; (△) 1-butene; (▲) butane; (▽) 1-pentene; (▼) pentane.

hydrogenation proceeded under mild conditions (atmospheric pressure, mesophilic temperature), thus requiring no special reaction vessel and no organisms adapted to extreme temperatures or pressures.

Palladium on charcoal suspended in the culture medium inhibited *N. frontalis* and *R. albus* to varying degrees, thus reducing the rate of product formation. In contrast, Lindlar catalyst in the pouch had no effect on the fermentation rate. Moreover, the hydrogenation rates in the gaseous phase were markedly higher than in the aqueous phase. While these results point out the advantage of keeping the hydrogenation catalyst in the gas phase, they also indicate the limits of such a system: hydrogen will only diffuse from the aqueous phase into the gaseous phase along a concentration gradient and the hydrogen concentration in the liquid phase required for this gradient may be too high to allow anaerobic fatty acid oxidation, which is a major pathway of hydrogen and volatile fatty acid production in the anaerobic digestion of mixed biomass, e.g., sewage sludge (12, 13). However, if the substrate did not contain a significant amount of fatty acids (e.g., lignocellulose), complete conversion of the fermentable hydrolysate to volatile fatty acids and hydrogen should be possible since this fermentation hydrogen would originate from substrates with relatively low redox potentials (28).

While the unsaturated hydrocarbons had little effect on established cultures of the fermenters, their effect on the methanogens varied from none (e.g., butene) to complete and irreversible inhibition (e.g., acetylene). These results are consistent with those of other workers (20, 23) and add pentene to the list of unsaturated hydrocarbons which inhibit methanogenesis. In combinations of fermentations and hydrocarbon reductions where the hydrocarbon does not simultaneously inhibit methanogenesis (e.g., butene), addition of an inhibitor specific for methanogenesis such as BES is necessary.

In our experiments, catalytic reduction of the unsaturated hydrocarbons was their only significant reaction. However, hexadecene is degradable by methanogenic consortia (21), and acetylene can be metabolized anaerobically (6, 22) as well as reduced by nitrogen-fixing enzymes (7, 14). Thus, if a mixed acidogenic microbial population is exposed to an unsaturated hydrocarbon, its capacity to degrade this hydrocarbon might become sufficiently strong to compete with the catalytic reduction.

Acetylene was reduced in preference to ethylene (Fig. 2), and while propylene, butene, and pentene were reduced simultaneously, the reaction rate decreased with increasing molecule size (Fig. 5). These results indicate at least partial control of the reductions by their free energy. Such control mechanisms may be used to arrive at predetermined product compositions.

Propylene reduction did not affect the ratio between acetate and ethanol among the fermentation products (Table 1), but methanogens in coculture with fermenters are known to cause an increased production of oxidized fermentation products (2, 15, 27). In cocultures, the presence of catalyst/hydrocarbon combinations which did not inhibit methanogenesis could not cause a shift of the electron flow from methanogenesis to olefin reduction. Thus the methanogens had a higher apparent affinity to hydrogen than the catalyst/hydrocarbon systems. This higher apparent affinity may be due to the fact that no phase transfer is involved in the fermenter-methanogen system, while in our fermenter-catalyst-hydrocarbon system the fermentation hydrogen had to diffuse into the gas phase before it could interact with the catalyst and electron acceptor. The close physical proximity between $H_2$ producers and $H_2$ consumers has been demonstrated in various situations including endosymbiosis of methanogens in anaerobic protozoa (24, 26), and gas metabolism evidence has been given in support of the juxtaposition of $H_2$-producing and methanogenic bacteria (5). In the light of these reports it is not surprising that in our system methanogenesis was favored over catalytic olefin reduction in the gas phase. To outcompete methanogens as $H_2$ consumers, the catalytic olefin reduction would have to occur similarly to methanogenesis in close proximity to the $H_2$ source, i.e., in the culture medium outside the cell wall of $H_2$ producers (3). We shall report on such a system elsewhere.

## ACKNOWLEDGMENTS

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