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# Performance of Trickle-Bed Bioreactors for Converting Synthesis Gas to Methane

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## ABSTRACT

Carbon monoxide, $H_2$, and $CO_2$ in synthesis gas can be converted to $CH_4$ by employing a triculture of *Rhodospirillum rubrum, Methanosarcina barkeri,* and *Methanobacterium formicicum.* Trickle-bed reactors have been found to be effective for this conversion because of their high mass-transfer coefficients. This paper compares results obtained for the conversion of synthesis gas to $CH_4$ in 5-cm- and 16.5-cm-diameter trickle-bed reactors. Mass-transfer and scale-up parameters are defined, and light requirements for *R. rubrum* are considered in bioreactor design.

**Index Entries:** Synthesis gas; methane; trickle-bed reactor; triculture.

## NOMENCLATURE

| | | |
|---|---|---|
| $G$ | gas flow rate | mL/h |
| $\epsilon_L$ | liquid porosity | mL/mL |
| $h$ | column height | cm |
| $H$ | Henry's law constant | L·atm CO/mol CO |
| $K_La$ | mass-transfer coefficient | $h^{-1}$ |
| $P$ | partial pressure or tension | atm |
| $q$ | specific uptake rate | mmol/gcell·h |
| $R$ | ideal gas law constant | L·atm/mol·K |
| $S$ | cross-sectional area | $cm^2$ |
| T | temperature | K |
| $X$ | cell concentration | g/L |
| $Y_{CO}$ | mole fraction ratio between CO and inert | mol/mol |

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**Superscripts**
*i* inlet conditions
*o* outlet conditions
* equilibrium

**Subscripts**
G gas phase
L liquid phase

## INTRODUCTION

Increasing interest has occurred over the past few years in the biological utilization of gas-phase substrates. In addition to oxygen, substrates for microbial conversion include contaminants in gas streams (such as $H_2S$ and COS) and synthesis gas components (such as CO, $H_2$, and $CH_4$). A number of biological processes for the removal of $H_2S$ from gas streams have been described in the literature (*1,2*). The removal of other noxious gases, such as $SO_2$ and $NO_x$, has also been addressed (*3*). Methane has been utilized in the production of single-cell protein through methanol as an intermediate (*4*). Finally, CO and $H_2$ have been utilized in the production of organic acids (*5,6*), methane (*7*), and ethanol (*8*), using various pure and mixed-bacterial cultures.

The fermentation of synthesis-gas components (CO, $H_2$, $CO_2$) presents a unique set of problems not normally encountered in traditional aerobic fermentations. First, the limiting substrate (and quite often the carbon and energy source) is present in the gas phase, making efficient substrate utilization economically important. Secondly, the bacterial systems employed in synthesis-gas conversion are strict anaerobes. Thirdly, and probably most importantly, most of the gas-phase substrates are only slightly soluble in the liquid phase, thus making the transfer of substrate from the gas phase to the bacteria in the liquid phase very important.

Many contacting schemes may be employed for these mass-transfer-limited systems, including mechanically agitated reactors, bubble columns, packed columns, plate columns, spray columns and gas-lift reactors. A packed column or trickle-bed reactor is particularly effective because of the large mass-transfer coefficients obtained in this contacting device without expensive agitation. Although packed beds are normally operated countercurrently, cocurrent operation is possible with irreversible biological reactors, since the mean concentration driving force is the same for both modes of operation. Also, the capacity of cocurrent columns is not limited by flooding, and at any given gas and liquid flow rates, the pressure drop in the cocurrent column is less than in countercurrent columns (*9*).

The purpose of this paper is to compare the performance of a triculture of *Rhodospirillum rubrum, Methanosarcina barkeri,* and *Methanobacterium*

*formicicum* in converting CO, $CO_2$, and $H_2$ in synthesis gas to $CH_4$ in two different-sized trickle-bed reactors. The packed columns were not used as fixed film reactors, but rather as a way of creating a large gas/liquid interfacial area. The triculture was chosen for this comparison from among a variety of pure- and mixed-culture systems because of its capability of producing $CH_4$ through the preferred $CO_2/H_2$ intermediates in a single reaction vessel (*10*). Comparisons of CO and $H_2$ conversions as a function of gas loading rate, $CH_4$ yields and productivities, and mass-transfer coefficients are made for the two reactors utilizing the triculture.

## THE BIOLOGICAL FORMATION OF METHANE FROM SYNTHESIS-GAS COMPONENTS

The conversion of CO, $CO_2$, and $H_2$ in synthesis gas proceeds by one of two mechanisms: $CH_4$ production through acetate as an intermediate or through $CO_2/H_2$ as intermediates. In utilizing the acetate pathway, synthesis-gas components are first converted to acetate by the equations

$$4\,CO + 2\,H_2O \rightarrow CH_3COOH + 2\,CO_2 \quad (1)$$

and

$$2\,CO_2 + 4\,H_2 \rightarrow CH_3COOH + 2\,H_2O \quad (2)$$

Such bacterial strains as *Peptostreptococcus productus* (*11*), *Acetobacterium woodii* (*12*), and *Eubacterium limosum* (*13*) have been utilized for this conversion. Acetate is then converted to $CH_4$ by the equation

$$CH_3COOH \rightarrow CO_2 + CH_4 \quad (3)$$

using *Methanosarcina barkeri* or *Methanothrix soehngenii* (*14*).

In utilizing the $H_2/CO_2$ pathway, CO is converted to $CO_2$ by the equation

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (4)$$

*Rhodospirillum rubrum*, a photosynthetic bacteria that grows in the presence of tungsten light on a variety of carbon sources other than CO, carries out the reaction of Eq. 5 (*15*). The $H_2$ and $CO_2$ produced by Eq. 4 and initially present in synthesis gas are converted to $CH_4$ according to Eq. 5, utilizing methane bacteria:

$$4\,H_2 + CO_2 \rightarrow CH_4 + H_2O \quad (5)$$

The conversion of $H_2$ and $CO_2$ to $CH_4$ by *M. barkeri* and *M. formicicum* has been demonstrated by Kluyver and Schnellen (*16*).

The $H_2/CO_2$ pathway has been shown to be more efficient than the acetate pathway in carrying out the conversion of synthesis gas to $CH_4$. Acetate tends to accumulate in the liquid medium in consequence of the slow growth of methanogens on acetate. Acetate accumulation is inhibi-

tory to both methanogens and acetogens (*17*). A triculture of *R. rubrum*, *M. barkeri*, and *M. formicicum* was thus formed in order to produce $CH_4$ from synthesis gas efficiently, in a single reaction vessel. Both methanogens were included in the triculture, since *M. formicicum* has been shown to be faster in utilizing CO and $H_2$ to produce $CH_4$, but *M. barkeri* is more tolerant of CO and sulfur gas contaminants than is *M. formicicum* (*18*).

## GASEOUS SUBSTRATE CONSIDERATIONS

The transport of a sparingly water-soluble substrate from the gas phase to the liquid phase may be described by the equation

$$\text{moles transported} = K_L a/H(P_G - P_L^*) \quad (6)$$

where $P_G$ is the partial pressure of the substrate in the gas phase, $P_L^*$ refers to the partial pressure of the substrate in equilibrium with the concentration of the substrate in the bulk liquid phase, $K_L a$ is the overall mass-transfer coefficient, and $H$ is the Henry's law constant. In the liquid phase, the substrate is consumed at a maximum rate by the cells, according to the equation

$$\text{mole consumed} = q\,X \quad (7)$$

where $q$ is the specific uptake rate and $X$ is the cell concentration. For a system not operating under mass-transfer limitation, the limiting step for substrate consumption is the microbial kinetics described by Eq. 7. On the other hand, under mass-transfer limitation, the uptake rate is generated by the mass-transfer limitation of the reactor and Eq. 6. It is important to realize that in both cases the moles of substrate transported from the gas phase to the liquid (Eq. 6) must be equal to the consumption rate by the cells (Eq. 7) at steady state.

In the column reactors used in this study, the outlet concentration of, for example, CO may be related to the gas flow rate and column parameters by the following equation (*19*)

$$\ln(Y_{CO}^i/Y_{CO}^o) = [K_L a/H]\,\epsilon_L\,[ShRT/G] \quad (8)$$

where $Y_{CO}^i$ and $Y_{CO}^o$ are the molar fraction ratios of CO to an inert gas component (Ar) in the inlet and outlet gases, respectively. Thus, by varying the gas flow rate, $G$, and measuring the composition in the inlet and outlet gases, the mass-transfer properties of the column reactors may be estimated. The main assumptions made in the derivation of Eq. 8 are that mass-transfer-limiting conditions apply ($P_G >> P_L^*$) and that the gas flow rate does not change significantly along the length of the column.

In the case of the triculture used in the experiments, CO is the substrate for *R. rubrum*, which must be transferred from the gas to the liquid as described above. However, the situation for methanogens is somewhat

different, since the substrate, $H_2$, may be generated in the liquid phase through Eq. 4 or may be transported from the gas phase across the gas–liquid interface.

In addition to the mass-transfer concept discussed above, the inhibition of methanogens by CO (*10*) is an important factor to consider. Elevated levels of CO in the liquid severely inhibit the formation of $CH_4$, according to Eq. 5. It is therefore desirable to operate the triculture under conditions with $P_L^*$ close to zero, which is the case for CO mass-transfer-limiting conditions.

## MATERIALS AND METHODS

### Organisms and Medium

*Rhodospirillum rubrum,* ATCC 25903, and *Methanobacterium formicicum,* ATCC 33274, were obtained from the American Type Culture Collection, Rockville, MD. *Methanosarcina barkeri* was kindly supplied by M. P. Bryant, Department of Dairy Science, University of Illinois, Urbana, IL.

The medium used in all experiments contained the following components per 100 mL of medium: 5 mL Pfennig's mineral solution (*20*), 0.1 mL Pfennig's metal solution (*20*) with the addition of 10 mg/L $Na_2SeO_3$ (*21*), 0.5 mL B-vitamins solution (*21*), 0.1 g yeast extract (Difco, Detroit, MI), 0.58 g $NaCH_3COO{\cdot}3H_2O$ and 0.45 g $NaHCO_3$. Typically, 7 L of medium were prepared in a 13.5-L Pyrex™ carboy (New Brunswick Scientific, New Brunswick, NJ) and autoclaved at 2 atm for 45 min. Cooling was accomplished by bubbling a sterile mixture of He and $CO_2$ (75%/25%) through the medium. The cooled medium was reduced with 10 mL of $Na_2S$ (2.5%).

### Equipment

Two trickle-bed reactors were constructed from acrylic plastic for use in this study. As illustrated in Fig. 1, each reaction system was equipped with a packed absorption tower, a gas–liquid separator, a liquid-recycle loop, and a tungsten light supply for the growth of *R. rubrum,* located in the recycle loop. Physical characteristics of the two reactors are shown in Table 1. Each column was packed with either Intalox saddles or Pall rings.

The reactor installation in Fig. 1 was assembled to operate in a cocurrent mode, which has been found to present fewer problems with cell clogging and channeling. The liquid make-up stream was combined with the recirculated liquid and entered the top of the reactor. Gas and liquid flowed in a downward mode through the packing and exited in a combined stream at the bottom of the reactor. The separation of the two streams occurred in a separator outside the reactor. Constant temperature was maintained by locating the equipment in a constant-temperature environment. External cooling surrounded the light source to assure constant

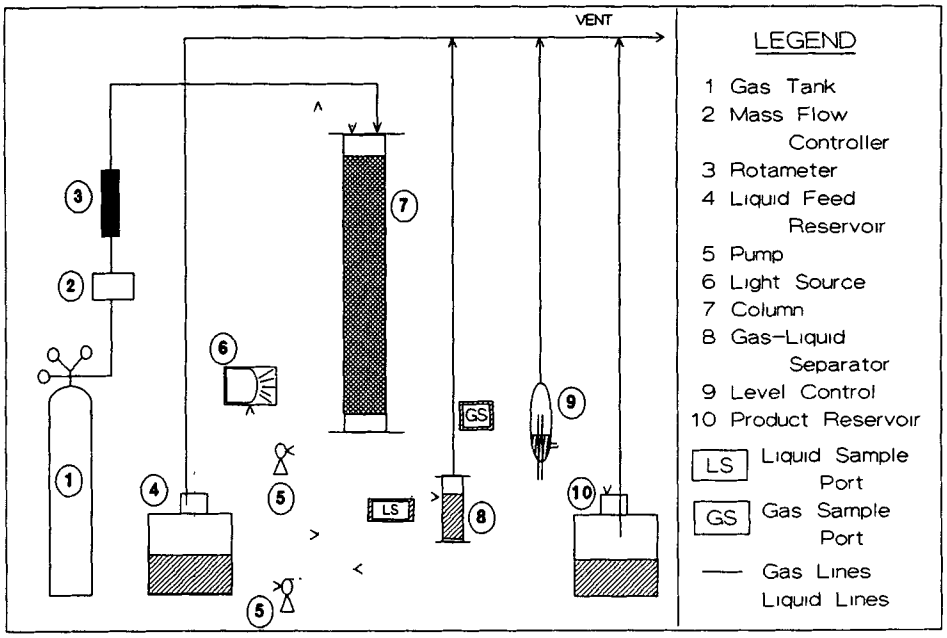


Fig. 1. Schematic diagram of the equipment setup for the trickle-bed bioreactors.

Table 1
Trickle-Bed Reactor Characteristics

| | Small Reactor | Larger Reactor |
|---|---|---|
| Column height, cm | 51.5 | 121.9 |
| Column inner diameter, cm | 5.1 | 16.5 |
| Packing | | |
| a. Intalox saddles | | |
| nominal diameter, mm | 6.35 | 13 |
| column porosity | 0.60 | 0.78 |
| b. Pall rings | | |
| nominal diameter, mm | -- | 16 |
| column porosity | -- | 0.87 |
| Operating temperature, °C | 37 | 37 |
| Operating pressure, atm | 1 | 1 |
| Liquid recycle rate, mL/min | 125,229,278 | 3465,3544 |
| Light supply, % of maximum | 50,50,70 | 75,75 |
| Inlet gas composition, in mole percent | | |
| Ar | 14.82 | 14.74 |
| CO | 55.62 | 54.42 |
| $CO_2$ | 9.67 | 9.72 |
| $H_2$ | 19.68 | 21.11 |

temperature. Sterilization of the equipment was accomplished with carboxide (Union Carbide Corporation, Danbury, CT), a mixture of 10% ethylene oxide in $CO_2$.

## ANALYTICAL METHODS

### Gas Analysis

A Perkin-Elmer (Norwalk, CT) gas chromatograph, Model Sigma 300, and Perkin-Elmer integrator, Model LCI-100, were used for the analysis of the gas-stream compositions. The column used was a 1.8-m×3-mm stainless steel column packed with Carbosphere®, 60/80 mesh (Alltech, Deerfield, IL). Helium was used as the carrier gas at a flow rate of 40 mL/min. The oven temperature was held constant at 135°C and the injector and thermal-conductivity-detector temperatures were 175°C.

### Other Measurements

Rates of gas flow through the columns were measured by a precalibrated rotameter mounted before the gas inlets of the columns. The liquid flow rates and liquid recycle rates were determined during the experiment by timing medium-broth displacement in a pipet. Liquid holdup in the columns was measured by abruptly shutting off the liquid-recycle pump and collecting the liquid in a calibrated vessel as it drained. This procedure was repeated for several recycle rates; thus, calibration curves correlating liquid recycle rate and liquid holdup were obtained for the various setups used. The temperatures inside the columns were monitored by the use of thermocouples mounted through the walls of the columns.

## RESULTS AND DISCUSSION

The triculture was successfully incubated in the two trickle-bed reactors, with the components of synthesis gas converted to $CH_4$ through $H_2$ and $CO_2$ as intermediates in a single reactor unit. After an initial start-up period of approx 10–14 d, both reactors stabilized at a constant conversion rate. Both reactors showed $CH_4$ production from CO, $H_2$, and $CO_2$ at all of the experimental conditions used. The overall performance of the reactors is illustrated in Fig. 2, where $CH_4$ production is plotted as a function of the estimated $H_2$ consumption at various light intensities (percentages of maximum light intensity of a 40-W bulb) and liquid recycle rates. The estimated $H_2$ consumption was calculated from the measured disappearance of $H_2$ and CO from the gas stream, since the rate of CO uptake by *R. rubrum* equals the rate of $H_2$ production, as described in Eq. 4. From the data of Fig. 2, it is seen that the average $CH_4$ yield on $H_2$ was found to be

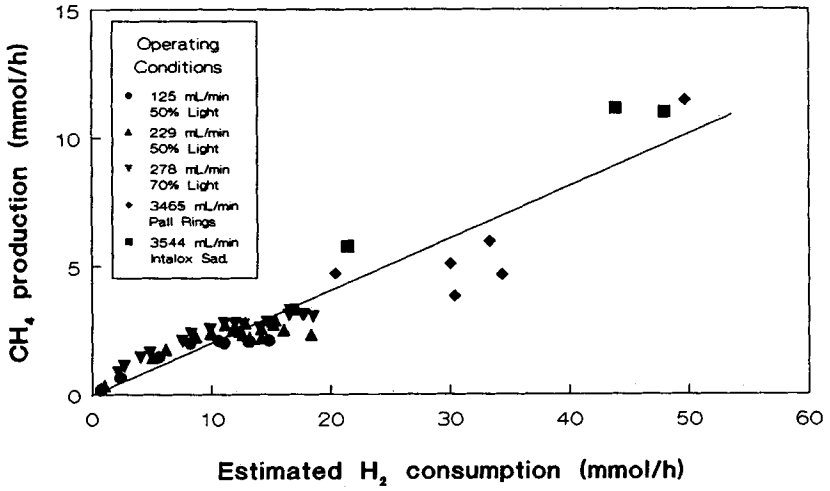


Fig. 2. Determination of the average $CH_4$ yield on $H_2$ (◆and ■ represent data for large column).

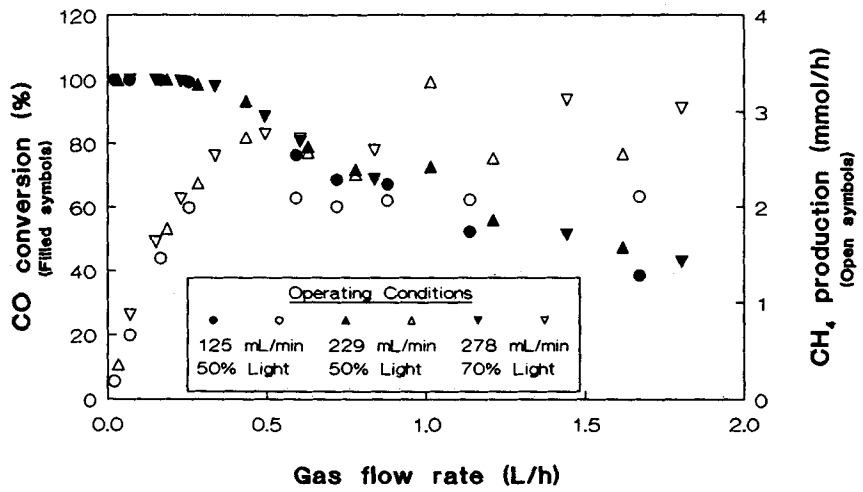


Fig. 3. CO conversion and $CH_4$ production as a function of gas flow rate for the experiments conducted in the small column.

0.20 mol/mol for both the small and the large columns, regardless of the liquid recycle rate or light intensity. This yield is slightly lower than the theoretical yield of 0.25 mol/mol, found from Eq. 5.

The conversion of CO and production of $CH_4$ from the triculture in the two columns is plotted as a function of the gas flow rate in Figs. 3 and 4, respectively. As is noted in the figures, the CO conversion approached or reached 100% for both reactors at low gas flow rates, but dropped as the gas flow rate was increased. It is interesting to note that almost identical CO conversions were obtained for different operating conditions within

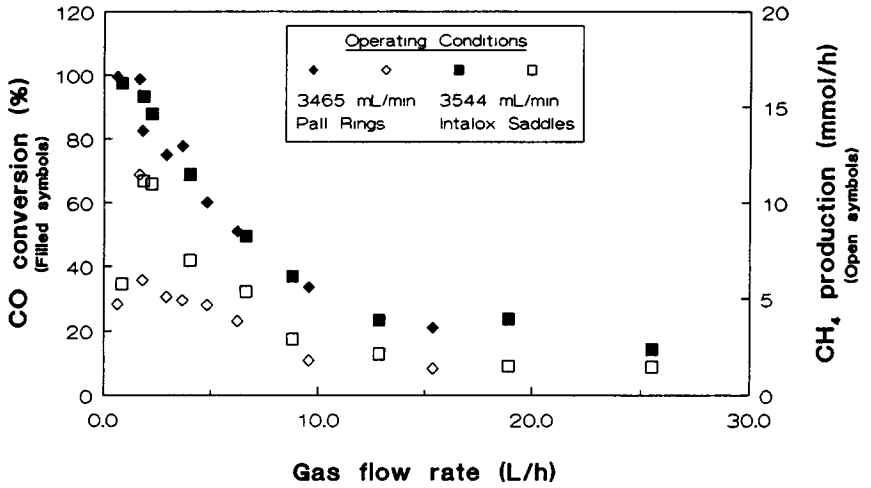


Fig. 4. CO conversion and $CH_4$ production as a function of gas flow rate for the experiments conducted in the large column.

columns of a single size. Methane production showed the same type of repetitive behavior within the columns. Methane production in the smaller column (Fig. 3) increased linearly with increasing gas flow rate before leveling off at a $CH_4$ production rate of 2–3 mmol/h. The data obtained from the large column showed a sharp peak in $CH_4$ production (Fig. 4) of 11.8 mmol/h, followed by a sudden decrease as the gas flow rate was further increased. It is assumed that this sudden drop in $CH_4$ production resulted from elevated levels of dissolved CO, which are inhibitory to the methanogens. It thus follows that mass-transfer-limiting conditions were not obtained at higher gas flow rates in the large column, probably because of limiting *R. rubrum* cell concentrations in the reactor.

The relation of Eq. 8 for finding the mass-transfer coefficient is illustrated in Fig. 5, where $\ln (Y^i_{CO}/Y^o_{CO})$ is plotted as a function of $ShRT/G$. As is seen in the figure, the data collected in the smaller reactor fell on a straight line through the origin with a slope of approx 0.044 mol/L·atm·h. Using values for $\epsilon_L$ of 0.0832, 0.106, and 0.119 for the three liquid recycle rates studied, values of $K_La$ in the range of 450–640/h (based on liquid holdup) may be obtained. Based on reactor volume, the value of the mass-transfer coefficient ($K_La\epsilon_L$) remained constant at 53/h. The same relationship is also plotted in Fig. 5 for the larger column. As is noted, the data from the two experiments conducted in the large column fell nearly on a straight line, but since mass-transfer-limiting conditions were not reached in the large column, the relationship presented in Eq. 8 is no longer valid, and a $K_La$ value may not be obtained from this data. Charpentier (*9*) reported that an average value of 0.008/s may be used for low gas and liquid flow rates and for packings with diameters >2 mm. This value is based on oxygen and reactor volume and, if corrections are made in order to

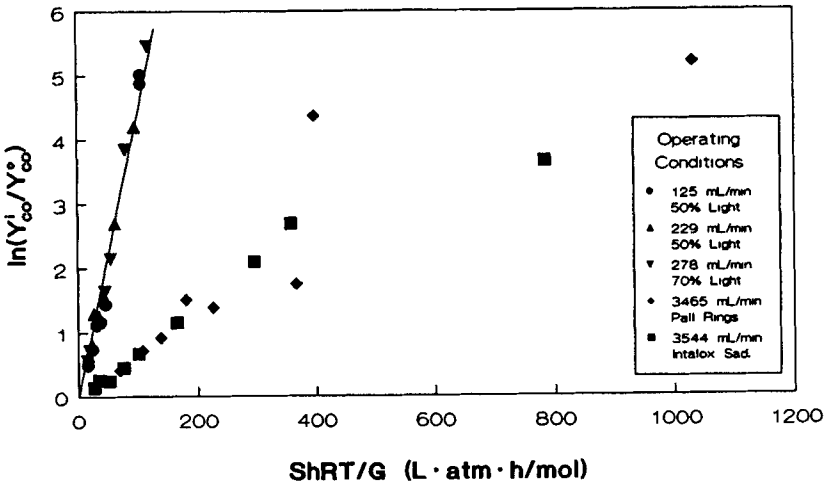


Fig. 5. Demonstration of the mass-transfer relationship (◆ and ■ represent data for the large column).

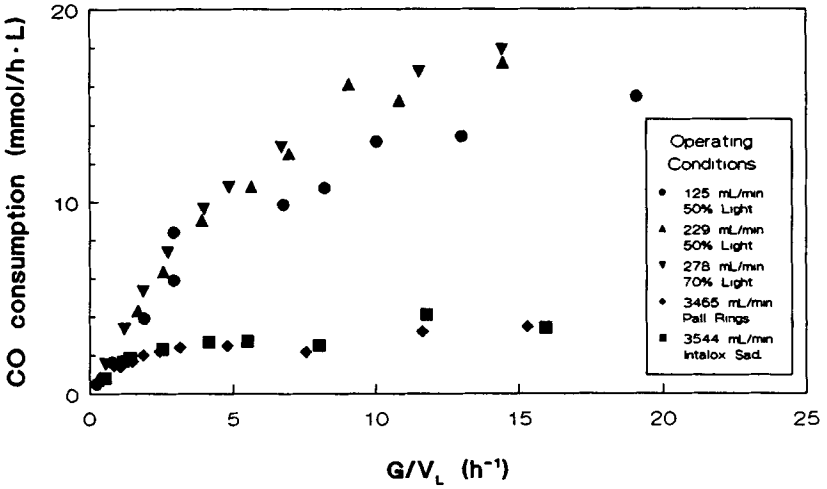


Fig. 6. CO consumption based on empty reactor volume as a function of gas loading (◆ and ■ represent data for the large column).

apply to the conditions in the smaller column, a $K_L a \epsilon_L$ value of 30.5/h may be obtained. This compares fairly well with the value of 53/h calculated from the data in the smaller column.

To more easily compare the performance of the small and large trickle-bed reactors, Figs. 6 and 7 were generated. The gas loading rate was chosen as the independent variable and was defined as the average gas flow rate divided by the liquid holdup in the column. The liquid holdup was correlated to the liquid recycle rate through calibration charts. Carbon monoxide consumption and $CH_4$ productivity for the two columns are plotted as a function of the gas loading rate in Figs. 6 and 7. Both the CO consumption and the $CH_4$ productivity were based on empty reaction volume in

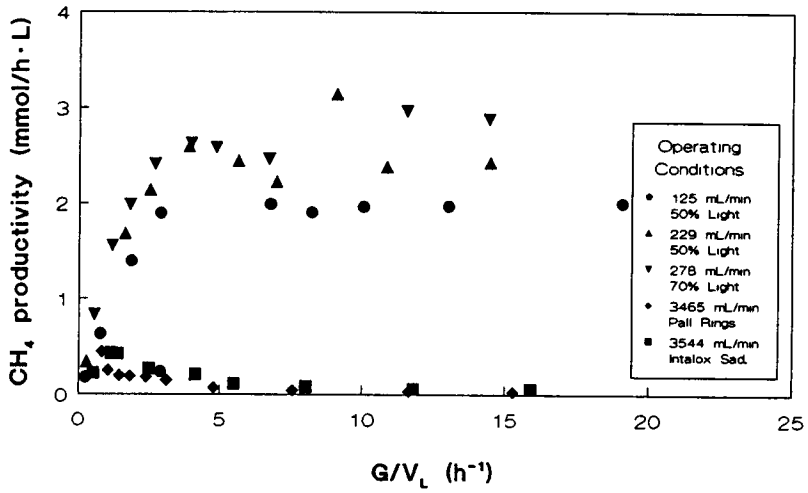


Fig. 7. $CH_4$ productivity based on empty reactor volume as a function of gas loading (◆and ■ represent data for the large column).

the packed section. As is noted, both reactors were studied within the same gas loading ranges. Both the CO consumption rate and the $CH_4$ productivity were 10-fold higher in the smaller column. Methane productivities of 2–3 mmol $CH_4$/h·L were obtained in the smaller column for gas loading rates of 4/h and above. The $CH_4$ productivity in the larger column reached only 0.4 mmol $CH_4$/h·L at a very low gas loading rate and was always lower than the values obtained in the smaller column for similar gas loading rates. The superficial liquid velocity in the larger column was up to 2.5 times greater than that in the small column for some experiments. Also, the liquid holdup per packing area was in the same range. This would indicate similar or "better" operating conditions in the larger reactor, promoting a higher mass-transfer coefficient. In speculating on the reasons for the opposite behavior, it should be realized that the smaller column had a smaller packing, which assured narrowed channels and thereby promoted radial distribution to a larger extent than in the larger column. Also, as mentioned, the cell concentration of *R. rubrum* may have been lower in the large column.

## CONCLUSIONS

The overall performance of the two columns must be considered successful. The yield of methane on hydrogen was estimated at 0.20 mol/mol, in which the $H_2$ consumption was estimated as the decrease in $H_2$ in the gas phase plus the addition $H_2$ formed by *R. rubrum* according to Eq. 4. The calculated yield of 0.20 is close to the theoretical yield of 0.25 mol/mol.

The poor performance of the large trickle-bed reactor is likely caused by low cell concentrations or by poor distribution of liquid throughout the column. With an initial porosity of 0.78–0.87 for the packing, large voids were available for gravity liquid flow. Poor distribution of liquid resulted in decreased radial distribution, so that part of the packing was unwetted. The resulting gas conversion was low, and performance was upset. The smaller trickle-bed reactor had an initial porosity of 0.60, which was considerably less than that of the larger column. This, together with a smaller packing in the small trickle-bed reactor, assured narrowed channels in the column and promoted radial distribution of the liquid. It may be desirable to reduce the packing size in the large column even though this is not standard procedure, since the ratio of packing "diameter" to column diameter should be kept at approx 1:9 for packed beds. Cell concentrations must be kept at a maximum at all times to assure mass-transfer limitations and high methane production.

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