DOE/MC/27226-3469
(DE94000008)

Development of Biological Coal Gasification (MicGAS Process)

Topical Report
July 1991 - February 1993

Kailash C. Srivastava

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Work Performed Under Contract No.: DE-AC21-90MC27226

For
U.S. Department of Energy
Office of Fossil Energy
Morgantown Energy Technology Center
Morgantown, West Virginia

By
ARCTECH, Inc.
Chantilly, Virginia

DISCLAIMER

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Distribution Category UC-109

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14100 Park Meadow Drive
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EXECUTIVE SUMMARY

This report contains a summary of the research work performed by ARCTECH during the period of July 1991 through February 25, 1993 in support of DOE/METC Contract #DE-AC21-90MC27226, Development of Biological Coal Gasification (MicGAS Process). The overall objective of this project is to develop a process for the direct biological conversion of low rank coals to methane with an ultimate aim to develop environmentally compatible fuel for electric power generation systems.

Laboratory and bench scale reactor research carried out during the report period confirms the feasibility of biomethanation of Texas lignite (TxL) and some other low-rank coals to methane by specifically developed unique anaerobic microbial consortia. The data obtained demonstrates specificity of a particular microbial consortium to a given lignite. For example, while Mic-1 consortium produced higher methane from TxL, the Mic-4 consortium was more effective with the German Brown coal or Neyveli lignite.

Development of a suitable microbial consortium is the key to the success of the process. The Mic-1 consortium was developed to tolerate higher coal loadings of 1 and 5% TxL (TxL) in comparison to initial loadings of 0.01% and 0.1% TxL. Moreover, the reaction period was reduced from 60 days to 14 to 21 days.

A significant advancement was made in reducing the cost of the culture medium for bioconversion by studying the effect of different growth factors on the biomethanation capability of Mic-1 consortium. By replacing the original yeast extract/Tryp soy broth mixture with Sheftone-T™, a 25 fold reduction in the cost was obtained. A further reduction in the cost of the culture medium will be due to the fact that with the Sheftone-T™ amendment, the addition of B-vitamins mixture was not required. Attempts to replace Sheftone-T™ with higher concentrations of less expensive NH_4Cl inhibited CH_4 production.

While surfactants did not enhance the biogasification of TxL, addition of 10 mM citrate or 1 mM ammonium oxalate did. Nevertheless, these effects have yet to be evaluated in a bench scale bioreactor.

Biomethanation of any complex substrate is a multi-step biochemical reaction performed by different groups of bacteria (organisms) that constitute the consortium. Typically, primary degraders carry out initial breakdown of the substrate. The product of this degradation is converted to smaller carbon chain compounds by the group of bacteria called fermenters. These small chain carbon compounds are converted to volatile fatty acids such as acetic acid (acetate) by acedogens. The acetate is the precursor of

methane whereby methanogens carry out the hydrogenation of acetate into methane and carbon dioxide. The chemical composition of the TxL indicates that this coal is low in hydrogen content. Therefore, the effect of hydrogen donors on biomethanation was studied by using methanol as a source of hydrogen. Mass balance of the substrates and products based on theoretical calculations and those with experimental data showed that methanol indeed enhanced the net biomethanation of TxL by the Mic-1 consortium. The laboratory scale data was confirmed in the bench scale reactor studies.

Four different bench scale bioreactor configurations, namely Rotating Biological Contactor (RBC), Upflow Fluidized Bed Reactor (UFBR), Trickle Bed Reactor (TBR), and Continuously Stirred Tank Reactor (CSTR) were evaluated for scale up studies. Preliminary results indicated highest biomethanation of TxL by the Mic-1 consortium in the CSTR, and lowest in the trickle bed reactor. However, highest methane production and process efficiency were obtained in the RBC.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF FIGURES

LIST OF FIGURES (cont.)

LIST OF FIGURES (cont.)

I. INTRODUCTION

One of the most abundant fossil fuels in the USA are the low rank coals. Yet at its best this vast reservoir is utilized in coal gasifiers only to eventually fuel the electric power plants. Not only is this method of coal utilization inefficient in terms of coal carbon converted to methane gas, it is also the major culprit for making the coal environmentally unfriendly and giving the public perception that coal is a "dirty fuel".

In recent years advance coal conversion technologies have led to better coal carbon utilization and less emissions of air toxics. This has been achieved through the modification of coal gasifiers. Nevertheless, the capital and operating costs together with the environmental implications associated with this method of power generation still make it relatively cost prohibitive.

Latest studies on coal chemistry have further confirmed the heterogenous nature of coal and identified a macromolecular higher molecular weight, and a lower molecular weight fractions comprising the lignites. The postulated empirical formula[1] for the macromolecular fraction is $C_{270}H_{240}N_3S_1O_{90}$. More specifically, the macromolecular fraction is comprised of 2, and 3- ring fused aromatics of a variety of chemicals interlinked through carboxyl, etheral and different aliphatic linkages. Further, the nitrogen and sulfur heterocyclics are the ones that make the coal most environmentally unfriendly in coal gasifiers as these compounds are oxidized to SO_x and NO_x[2].

A careful examination of the elemental composition of lignites indicates relative high oxygen content which makes them more amenable to microbiological degradation. At the same time, biological processes, albeit perceived to be slow, are much more environmentally friendly because of the mild reaction conditions of temperature and pressure which ultimately would lead to lesser to none emission of air toxics. The current awareness for a cleaner environment, stringent environment regulations and quest to obtain value added fuels necessitate seeking potential cost effective environmentally friendly technologies. Bioconversion of coals would provide such a technology for cleaner burning fuels. At the same time, the process by-products
would result into non-fuel value added specialty chemicals.

Studies conducted at ARCTECH have established the technical feasibility of biomethanation of low-rank coals by a variety of unique anaerobic microbial consortia (eg. Mic-1, Mic-2, Mic-3, Mic-4)[3]. This direct bioconversion has been confirmed by other scientists [4,5]. The work at ARCTECH has also demonstrated the specificity of certain anaerobic microbial consortium to a given lignite[5]. At the same time, ARCTECH has developed a conceptual process design and performed the preliminary economic study of the process based on the laboratory data demonstrating the efficient biogasification of TxL by the ARCTECH microbial consortium Mic-1[6].

This Topical Report summarizes recent experimental results obtained from laboratory and bench scale bioreactors to further enhance the biomethanation of TxL by the Mic-1 consortium. The report also describes the kinetics of methane production as a function of physical, chemical and biological parameters together with an overview of planned future research towards developing this (MicGAS) Process to the pilot scale.

II. MATERIALS AND METHODS

A. Microorganisms

The anaerobic consortia were derived from hind gut of wood-eating termites fed on coal[7]. Mic-1 was derived from *Zootermopsis* sp., while Mic-4 from *Nasutitermes* sp. Mic-2 was derived from a rotating biological contactor and Mic-3 was developed at ARCTECH by mixing consortia from different sources.

B. General Experimental Procedures

All the manipulations were carried out under anaerobic conditions according to the techniques described by Hungate[8] as modified by Bryant[9]. The anaerobic condition during media preparation, and in reaction as well as stock reservoir vessels was maintained by bubbling oxygen-free mixture of N_2 + CO_2 (80:20). All solutions, reagents and culture media were prepared in boiling water that was constantly purged with a mixture of deoxygenated N_2 + CO_2. For culture medium preparation each of the solid components (Table 1) were added sequentially and only after the one added before was completely dissolved. After preparation, the culture medium was dispensed into either pressure tubes, serum vials or Wheaton bottles. Serum vials and tubes were stoppered with butyl rubber stoppers and aluminum crimp sealed. The Wheaton bottles were stoppered with butyl stoppers and screw topped.

For cultivation of pure cultures of methanogens, the medium was prepared in the same manner as above, but the head space of the serum vials or Wheaton bottles was exchanged with a mixture of H_2 + CO_2 (80:20). All media contained resazurin indicator to ensure that the medium stayed anaerobic during inoculation and/or other additions. Anaerobic conditions were further ensured by adding to the complete medium 0.2 mL of a 2.5% solution of Na_2S.

Yeast extract + Tryp soy broth (YE/TSB) solution or alternate nitrogen amendments, chelators, or substrates other than coal were always added as concentrated solutions under aseptic and anaerobic conditions. Culture medium volume for any given experiment was always constant (usually 40 mL medium in a 60-mL serum vial). The medium was always prepared in such a manner that the medium components will not be diluted by additions where effects of chelators, surfactants, nitrogen supplementations, vitamins or substrates other than coal were examined. In experimental bottles containing TxL, appropriate TxL concentrations (0.01% to 20%) were added prior to dispensing the culture medium into the bottles. Thus appropriate amounts of coal (Texas or other

lignites) always had to be weighed for each individual bottle in any given experiment. All experiments were conducted with appropriate controls. Unless otherwise stated, two controls, one without the nitrogen amendment (eg. YE/TSB) and other without the TxL but containing nitrogen amendment were a part of each and every experiment. All studies were conducted with -325 mesh (44 μm) lignite (Texas or other) based on the results of previous studies at ARCTECH[10].

Anaerobic Glove Box (Coy Corporation, Ann Arbor, MI) was used to transfer all microbial cultures from Wheaton bottles to centrifuge bottles, washing the cells, or for isolation of individual cell types from the Mic-1 and Mic-4 consortia. In the glove box, anaerobic conditions were maintained with O_2-free mixture of N_2 + H_2 (60:40).

The evaluation of bioconversion of lignites was based on the production of total biogas, concentration (mole%) of each of the gases (CH_4, CO_2, N_2, and H_2S) of interest, volatile fatty acids (VFA), biomass growth, bacterial morphology, total soluble carbon, pH of the culture medium, and at the end of each experimental set the proximate composition (ash, carbon, hydrogen, moisture, oxygen and sulfur content) of the residual TxL. These parameters were monitored on at least weekly basis but depending on the experiment even at shorter intervals. The data collected were analyzed to obtain kinetics for the production of methane, VFA concentration, soluble carbon, and biomass. These data were also used to calculate mass and electron balance according to Datta and Andrews[11].

The total biogas production was measured by either syringe (for tubes, serum vials, and Wheaton bottles), or water displacement method (bench scale bioreactors). The head space gas composition was determined by gas chromatography using thermal conductivity detector (TCD). Concentration of H_2S was also monitored by gas chromatography but flame photometric detector (FPD) was used. Samples for headspace gas analyses were obtained through directly introducing the needle of a pressure tight gas syringe into the butyl rubber stoppers. The syringe was flushed with the headspace gas by moving the plunger a few times outward and inward. The sample was injected into the gas chromatograph by directly inserting the needle into the appropriate port. Prior to sampling, the pressure tight syringe was made anaerobic by flushing it with oxygen free CO_2 + N_2 mixture.

Gas chromatograph with a flame ionization detector (FID) was used to measure VFAs. The principal VFAs measured were: acetate, butyrate, caproate, isobutyrate, isocaproate, isovalerate, and valerate. Biomass growth was monitored by measuring total cell counts, dry cell weight, wet cell weight, absorbance at 660 nm (OD_{660}) and total cellular protein. Because of the interference with TxL particles, cellular protein was found to be the most appropriate and was the method of choice. The cellular morphology was observed by phase contrast and confirmed by epifluorescence[12] microscopy. Total soluble carbon was colorimetrically measured as chemical oxygen demand (COD) by a method available commercially from Hach Company[13]. The pH was potentiometrically measured using an

internal electrode standard. The proximate analysis of residual TxL was performed by a commercial analytical laboratory.

C. Culture Media

The composition of New Termite Medium (NTM) used for the cultivation of anaerobic microbial consortia is presented in Table 1. This was also the medium utilized for biogasification of TxL. The composition of the culture medium used for the maintenance of pure cultures of methanogens is presented in Table 2. Unless otherwise indicated, the complete medium was always autoclaved for 20' at 121 °C and 15 psi.

D. Culture Maintenance

The stock cultures were maintained frozen in 5% glycerol vials at -70 °C. The working stock cultures were prepared by thawing the frozen cultures at room temperature and subsequently transferred under aseptic and anaerobic conditions into 60-mL serum vials containing 40 mL NTM + 0.1% or 1% TxL. Unless noted otherwise, all cultures were incubated at 37 °C in a stationary incubator. The stock cultures were incubated for 14-21 days. The incubation time for all other cultures varied according to the experiment.

E. Inoculum Preparation

Inocula for all experiments were prepared by inoculating 500-mL Wheaton bottles containing 300-400 mL NTM + 0.1% TxL with the microbial consortia to be tested. Alternatively, in order to check the stability of consortia and avoid erroneous results due to residual TxL from inoculum(a), inoculum was prepared in bottles containing NTM without TxL. After appropriate incubation time (10 to 21 days), the cultures were aseptically harvested under anaerobic conditions, washed twice with the NTM (without YE/TSB), and resuspended in a quantity of NTM (without YE/TSB) that would give enough inoculum for the experiment to be performed. This method of inoculum preparation also provided a means of keeping the initial bacterial inoculum size constant for different treatments with in a given experiment. The bacterial numbers were quantified by measuring the absorbance of the resuspended bacterial suspension at 660 nm ($O.D._{660}$).

F. Kinetics of Methane Production

These experiments were performed to evaluate initial biomethanation of TxL by the Mic-1, consortium. The experiments were performed in 60-mL serum vials or Wheaton bottles containing 0.01% TxL suspended in NTM. Methane production as a function of time was evaluated.

G. Enhancement of Methane Production

These experiments were performed to determine parameters affecting the biomethanation of lignites by different Mic consortia. While most studies were done with TxL and Mic-1, other Mic consortia (2, 3 and 4) and lignites (Beulah, German brown, TxL and Neyveli) were also used in order to determine the potential of biogasification process for commercial application.

Table 1. Composition of New Termite Culture Medium (NTM)

Complete Medium *		Composition of Solutions					
		Pfenning's Trace Metals		Pfenning's Minerals			
Component	Quantity g/100 mL	Component	Quantity g/L	Component	Quantity g/L	Component	Quantity g/L
B-Vitamins[a]	0.50	NH_4Cl	8.00	H_3BO_3	0.300	Biotin	0.02
KCl	0.16	$CaCl_2 \cdot 2H_2O$	1.00	$CaCl_2 \cdot 2H_2O$	0.010	Cyanocobalamin	0.10
Pfenning's Minerals[a]	5.00	$MgCl_2 \cdot 6H_2O$	6.60	$CoCl_2 \cdot 6H_2O$	0.200	Niacin	0.20
Pfenning's Trace Metals[a]	0.10	KH_2PO_4	10.00	$FeCl_2 \cdot 4H_2O$	1.500	Pantothenic acid	0.10
Resazurin[a]	0.10	NaCl	8.00	$MnCl_2 \cdot 4H_2O$	0.030	p-Amino benzoic acid	0.10
$NaHCO_3$[b]	0.35			$Na_2MoO_4 \cdot 2H_2O$	0.025	Pyridoxine	0.50
Na_2SeO_3[a,b]	0.10			$NiCl_2 \cdot 6H_2O$	0.020	Riboflavin	0.10
Tryp Soy Broth (TSB)	0.10			$ZnSO_4 \cdot 7H_2O$	0.100	Thiamine	0.20
Yeast Extract (YE)	0.10						

[a] in mL,

[b] add after cooling, add each component with constant stirring, heat gently with constant stirring, cool and dispense into Wheaton bottles, autoclave and store. NTM should be made in water that is being simultaneously boiled and purged with oxygen free $N_2 + CO_2$ mixture (80:20). Cool, add reagents and dispense into appropriate containers.

Preparation of 2.5% Na_2S Transfer a pH meter and a Stir Hot Plate to a hood. In 2-L flask, boil 1 L of double distilled H_2O. Remove 100 mL. Bubble N_2 through 900 mL H_2O. Let cool down. Weigh 25 g $Na_2S \cdot 9H_2O$ on a plastic tray, transfer to hood rapidly. Wash Na_2S with distilled water. The yellow color is normal. Transfer crystals into boiled H_2O, stir to dissolve, make up to 1 L, add 7-8 mL conc. HCl, adjust pH to 9, dispense in 25 mL aliquots in 50-mL serum vials. Bubble N_2 through the vials, stopper, crimp seal and autoclave the vials.

* Bring in to the final medium just before inoculation, aseptically, and under anaerobic conditions, add 0.2 mL (for each 40 mL of culture medium) of 2.5% Na_2S prepared separately under anaerobic conditions and stocked in 25 mL quantities in 50 mL capacity serum vials.

Table 2. Composition of MS Medium (MSH) for the Cultivation of Methanogens

Complete Medium		Composition of Mineral Solutions and B - Vitamins					
		Minerals I, and II		Minerals III		B - Vitamins	
Component	Quantity g/100 mL	Component	Quantity g/100 mL	Component	Quantity g/100 mL	Component	Quantity (mg/L)
NH_4Cl	0.27	Minerals I		H_3BO_3	0.001	Biotin	2.0
B-Vitamins[a]	0.50	$CaCl_2 \cdot 2H_2O$	0.80	$CaCl_2 \cdot 2H_2O$	0.002	Calcium panthothenate	5.0
Mercaptoethanol sulfuric acid (MESA)	0.05	$MgCl_2 \cdot 6H_2O$	2.00	$CoCl_2 \cdot 6H_2O$	0.015	Cyanocobalamin	5.0
Minerals I[a]	0.50	KH_2PO_4	0.40	$CuCl_2 \cdot 2H_2O$	0.002	Folic acid	2.0
Minerals II[a]	0.50	Minerals II		$FeSO_4 \cdot 7H_2O$	0.010	Niacin	5.0
Minerals III [a]	1.00	K_2HPO_4	0.40	$MnCl_2 \cdot 4H_2O$	0.010	p-Amino benzoic acid	5.0
Resazurin[a]	0.10						
$NaHCO_3$[b]	0.50			$NiCl_2 \cdot 6H_2O$	0.002	Pyridoxine	10.0
$Na_2SeO_{3a,b}$	0.10			$Na_2EDTA \cdot 2H_2O$	0.050	Riboflavin	5.00
Tryp Soy Broth	0.20			$Na_2MoO_4 \cdot 2H_2O$	0.001	Thiamine	5.0
Yeast Extract	0.20			$Na_2WO_4 \cdot 2H_2O$	0.003	Vitamin B-12	0.1
				$ZnSO_4 \cdot 7H_2O$	0.010		

[a] in mL, [b] add after cooling, add each component with constant stirring, heat gently with constant stirring, cool and dispense in to Wheaton bottles, autoclave and store. NTM should be made in water that is being simultaneously boiled and purged with oxygen free $N_2 + CO_2$ mixture (80:20). Cool, add reagents and dispense into appropriate containers.

1. Culture Development. The original cultures of different consortia obtained from the hind gut of different termite species demonstrated little methane production with very long retention times. Consequently, these cultures were enriched to enhance the methane production and reduce the retention time. Mic-1 consortium was the one of choice for further enhancement as it demonstrated the highest biomethanation of TxL. Therefore, Mic-1 consortium was repeatedly transferred and allowed to grow in to stepwise increasing concentration (0.01% to 1%) of -325 mesh (44 μ) TxL suspended in the NTM.

2. Coal Microbial Interactions. The methods described under this section were used to study the parameters that supposedly influence the biomethanation of TxL. The parameters considered were TxL chemical composition, addition of chemicals other than the NTM components and biological factors.

a. Effect of Solids Loadings. In order to enhance the economic efficiency of MicGAS Process solids loadings of 20% or greater should be used. Therefore, biomethanation of TxL was studied on laboratory scale in serum vials and in two different bench scale bioreactor configurations. Seven sets of serum vials containing NTM, NTM without YE/ TSB (Table 1) and NTM + 0.1%, 1.%, 5%, 10% and 20% TxL respectively were prepared. The control vials were not, but test vials were inoculated with Mic-1 consortium.

b. Effect of Trace Elements and Coal Mineral Components. During solids loading experiments methane production by Mic-1 and Mic-2 consortia was inhibited at higher solids loadings of TxL. This could possibly be due to soluble inhibitors found in coal[3]. Therefore, it was deemed necessary to identify which component(s) of coal causes these inhibitory effects. Several metals, including iron, manganese, magnesium and aluminum have been found to occur in Beulah lignite at concentrations which could affect the growth of microorganisms[14]. An experiment to evaluate the effects of trace and major elements of Beulah lignite on biogasification by Mic-3 was carried out in 22-mL pressure tubes containing 10 mL NTM. The NTM wss supplemented with Beulah lignite (0.1%, w/ v) and one of the following test elements: oxides of iron (6.2 μmoles/ mL), manganese (14.1 μmoles/ mL), magnesium (24.8 μmoles/ mL), aluminum (9.8 μmoles/ mL) and ash from Beulah lignite (1.0 mg/ mL). Controls were prepared in which no metals or ash were added. In addition, tubes were prepared with test elements but without the lignite. The tubes were stoppered with butyl rubber stoppers, aluminum crimp sealed, and autoclaved. After cooling, the experimental tubes were inoculated with the Mic-3 consortium. All (inoculated and uninoculated) tubes were incubated. The tubes were sampled to determine methane, cellular protein and VFA concentrations.

The experiment was repeated with Mic-1 and TxL. The metal oxides were replaced with metal so as to provide better solubility and control of metal salts concentration in the experimental system. The metal salts were added as concentrated solutions to give a final concentration of 10 mM.

c. Effect of Agitation. Earlier studies using Mic-1 and Mic-2 consortia indicated that methane production was inhibited by solids loadings greater than 1%[3]. This could be due possibly to less interaction between the coal particles and bacteria constituting the microbial consortia. It was hypothesized that at higher solids loadings, agitation may be required to allow optimal contact between coal particles and bacteria and/ or enzymes. To further examine the effects of solids loadings and agitation on biogasification, Mic-1 consortia were grown in vials that contained NTM + 0.1%, 1% and 5% TxL. These cultures, as well as control (no TxL) were cultivated with and without agitation (gentle mixing at 100 rpm). In addition, samples were examined microscopically to monitor bacterial cell populations attached to the coal particles.

d. Effect of Autoclaving on Biomethanation of Texas Lignite by Mic-1 Consortium. Five groups (each composed of four sets in triplicate) of 60-mL serum vials were prepared. The first group of four sets contained only NTM. The second group of 4 contained NTM without YE/ TSB. The third group of 4 sets contained NTM without YE/ TSB + 1% TxL, and the fourth group contained NTM + 1% TxL. The final group of 4 sets contained NTM + 10% TxL. Two sets from each group were autoclaved while the other two sets were not. One set of the two from each group was inoculated with Mic-1 consortium and other was used as abiotic control. The parameters described in section II B were monitored.

e. Effect of Chelators and Surfactants. The bases for this study was to enhance biomethanation of TxL by either pretreating the TxL with these chemicals or incorporate them in the culture medium. The surfactants used were sodium dodecyl sulphate (SDS), Triton X-100 (TX 100), and Igepal CO-720 (IGP720). The chelators or sequestering agents used were ethylene diamine tetraacetic acid (EDTA), ammonium oxalate (AMMOX), and sodium citrate (citrate). TxL (0.1%, 1%, and 10%) suspended in NTM was supplemented with the surfactants at 0.05%, 0.1% and 0.2% (w/ v). The EDTA, AMMOX, and citrate were used at 1 and 10 mM final concentrations. All treatments were prepared by addition of concentrated solutions of test substances to complete NTM. Controls consisted of NTM with and without test substances, TxL and Mic-1 consortium respectively.

f. Development of Efficient and Economic Culture Medium. In order to develop an economically viable process and obtain efficient biomethanation of TxL it was imperative to reduce the cost of the culture medium. As YE/ TSB accounted for most of the cost, alternatives to this amendment were sought. Preliminary studies[15] indicated that in general replacement of YE/ TSB with Sheffield products resulted in not only economical but more effective biomethanation of TxL by the Mic-1 consortium. Therefore, sets of serum vials containing TxL suspended in NTM were prepared in which the YE/ TSB was supplemented with these products (Table 3). Again, the controls were NTM without 0.2% YE/ TSB, NTM, and NTM without YE/ TSB + 0.2% of each of the test products. Based on the results of this experiment, the NTM without YE/ TSB was always amended with different concentrations of selected Sheffield

Table 3A. Cost Effectiveness of Different Replacements for YE/TSB During Biomethanation of Texas Lignite[a] by Mic-1 Consortium

Product	Product Manufacturer	Cost ($/lb.)
YE/TSB	Difco	35.45
BHI Solids	Marcor Development Corp.	21.77
Meat Peptone	Marcor Development Corp.	8.85
Testone 900	Universal Foods	4.35
Sheftone-M™	Sheffield Products	1.72
Sheftone-T™	Sheffield Products	1.42

Table 3B. Comparative Cost Effectiveness of YE/TSB and Different Concentrations of Sheftone-T™ During Biomethanation of Texas Lignite[a] by Mic-1 Consortium

Product	Product Manufacturer	% in the NTM[b]	Cost ($/lb.)	CH_4[c]	Total Cost[d]
YE/TSB	Difco	0.20	35.45	24.2	178.82
Sheftone-T™	Sheffield Products	0.20	1.42	87.9	2.01
Sheftone-T™	Sheffield Products	0.10	1.42	36.4	2.43
Sheftone-T™	Sheffield Products	0.05	1.42	7.0	6.32

[a] TxL was used at 0.1% (w/v)
[b] Quantity (w/v) of product used in NTM
[c] Cumulative methane produced (cc/g TxL) at 14 day of incubation
[d] Cost (in U.S. $) of the nutrient amendment required for the production of 1 ft^3 of methane from TxL.

Product (Sheftone-T™) and was henceforth called %SNTM. The number before the % sign would indicate the amount (w/v) of Sheftone-T™ used).

In another experiment, the effect of B Vitamins solution (Table 1) on the performance of most suitable Sheffield product was studied. In this case, serum vials containing 0.2% SNTM + TxL were control. The test was 0.2% SNTM without B-vitamin solution + TxL.

In order to further economize the process cost, chemostat cultures containing 0.2% SNTM supplemented with 4 times concentration of NH_4Cl (32 as opposed to 8 g L^{-1}) present in original NTM (Table 1) and inoculated with Mic-1 consortium were incubated at 37 °C. The control was 0.2% SNTM. The chemostats were simulated from 1 L capacity aspirator bottles.

g. Effect of Pretreatment on Biogasification of Texas Lignite. Mic-1 was grown on TxL. After 14 days, the TxL was harvested, washed with tap water, and dried. This residual TxL was resuspended in fresh SNTM, inoculated with Mic-1 consortium and evaluated for products. In another set of experiments, TxL was extracted with tetrahydrofuran (THF) in a soxhlet. The THF soluble fraction (mobile phase, MP) and residual TxL were separated, dried and stored. The biomethanation of THF residual, THF mobile and Mic-1 treated residual TxL was evaluated in different sets of experiments, inoculated with the Mic-1 consortium. Abiotic controls were not inoculated with Mic-1, whereas biotic control consisted of 0.2% SNTM + treated TxL + Mic-1.

h. Isolation of Individual Bacterial Components of Mic-1 Consortium. Experiments to isolate bacteria from biomethanation consortia (Mic-1 and Mic-4) were conducted in NTM supplemented with agar (2%, w/v) and either -325 mesh TxL (0.5% w/v) or biosolubilized TxL (1.25%, w/v). This agar "overlay" was melted and poured onto Petri plates. Control plates were prepared using overlays containing no TxL supplements. The samples (diluted 10^{-4} or non diluted Mic consortium) were centrifuged at 12,000 rpm to allow the bacterial cells to settle down. The supernatant, which contained free floating bacteria was removed. The settled TxL was resuspended in sterile medium and inoculated directly into isolation medium at 45°C, a temperature at which agar remained molten but did not adversely affect bacteria in the short time they are exposed to this temperature. By eliminating the free floating bacteria, it was hoped that little or no dilution will be required, allowing bacteria attached to the TxL particles (putative primary TxL degraders) to be isolated. All manipulations were performed in an anaerobic glovebox.

H. Bioreactor Studies

The purpose of bioreactor studies was to generate information that could be used for preliminary economic and commercial feasibility studies on scaling-up the laboratory experiments. In all the bench scale bioreactor studies, the control was NTM containing

0.2% of either YE/ TSB, selected Sheffield Product, or NTM without B-vitamins solution + selected Sheffield Product. The treatments contained either 1, 5, 10, or 20% TxL. After charging the culture medium (in both control and treatment reactors), all reactors were purged with oxygen free N_2 + CO_2 (80:20) mixture. Four different configurations of bioreactors were used. Performance of these bioreactors was evaluated on the basis of the production of total biogas, the headspace concentration (mole%) of each of the gases of interest (CH_4, CO_2, N_2, and H_2S), the concentration of VFAs, biomass growth, bacterial morphology, total soluble carbon, pH of the culture medium, and the proximate composition of residual TxL.

1. **Rotating Biological Contactor (RBC).** A rotating biological contactor (RBC, total volume 7.6 L) was constructed and used for several experiments (Figure 1). This reactor contained 6.2 L of NTM and varying (1-10%) concentrations (w/ v) of TxL (1:1, -325 mesh:micronized). Inoculum consisted of 500 mL of fresh anaerobic sewage sludge and 200 mL of Mic-1. In later experiments the inoculum was only Mic-1 or mixture of Mic-1 and Mic-4.

2. **Upflow Fluidized Bed Reactor (UFBR).** Another type of bench scale reactor, upflow fluidized bed reactor (UFBR) was basically a cylindrical column. The reactor was provided with a conical bottom so as to provide effective fluidization through pumping either the liquid (culture medium), or a portion of head space gas. The advantage of this reactor is that it provides better interaction between the microorganisms and the substrate (lignite). In earlier studies at ARCTECH, UFBR constructed of plexiglass were used (Figure 2). The total volume of the reactor was 0.68 L. The working volume was 0.6 L. Because of the large number of seams between various parts, these reactors were prone to gas leaks. Leaks of this nature make it extremely difficult to accurately determine total gas production. In addition, such leaks may result in the introduction of oxygen into the anaerobic system, which inhibits methanogenesis. In order to alleviate these problems, new UFBR constructed of glass (Figure 3) were obtained. These reactors measure 2 inches in diameter by 24 inches in height. The advantages of using these reactors include less of a likelihood of leakage, and easy conversion from batch operation to continuous mode. The total volume of these reactors were 1.25 L with a working volume of 0.8 L.

Three plexiglass UFBRs, each with working volume of 550-560 mL and headspace volumes of 140-150 mL were used. Fluidization was provided by circulating the headspace gas with peristaltic pumps. Texas lignite was added to two of the reactors, while the third reactor was run as control with NTM only. All the three reactors were inoculated with anaerobic sewage sludge + Mic-1 + Mic-4 consortia (200 + 100 + 100 mL). Throughout the fermentation, TxL and YE/ TSB were added to the two experimental reactors, and YE/ TSB to the control reactor. Thus, after 34 days of reactor run, the reactors with TxL contained 0.24% YE/ TSB + 1% TxL and 0.25% YE/ TSB + 0.5% TxL, respectively. The control reactor contained 0.25% YE/ TSB. All reactors were monitored for TxL biomethanation products.

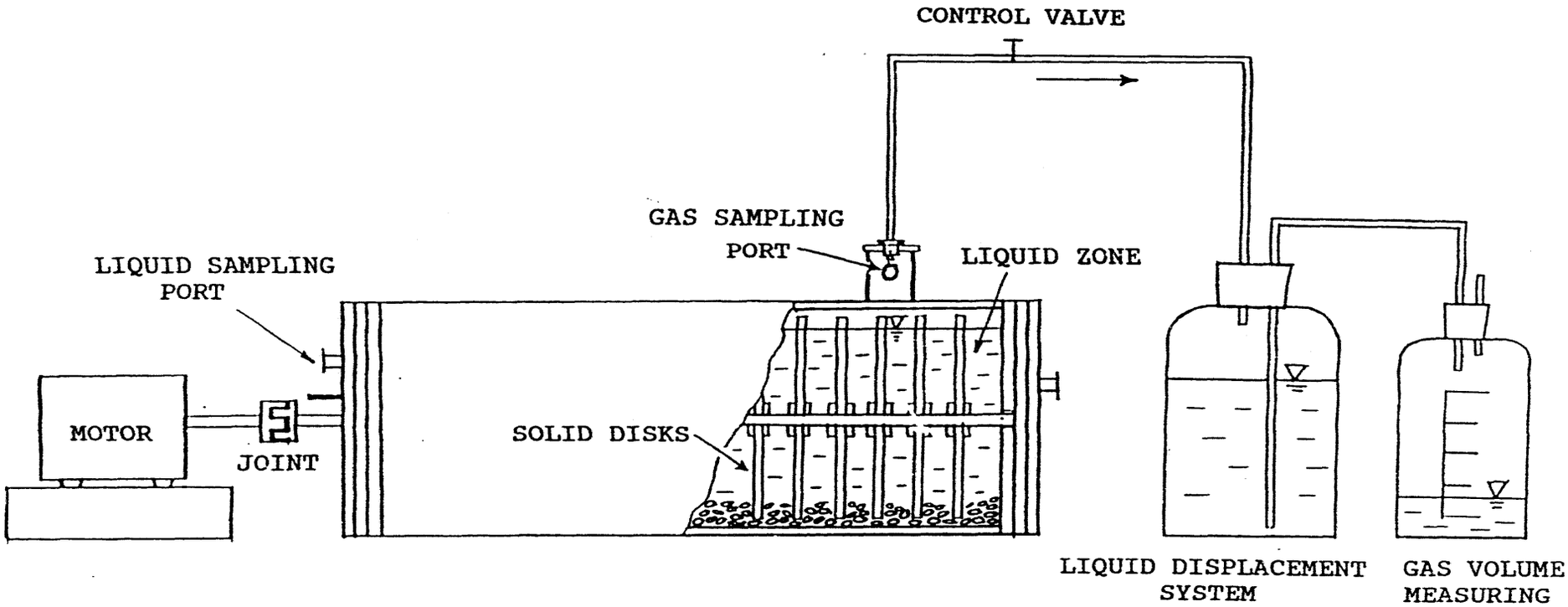

Figure 1. Experimental Set-up for the Biomethanation of Texas Lignite by Mic-1 Consortium in a Rotating Biological Contactor (RBC).

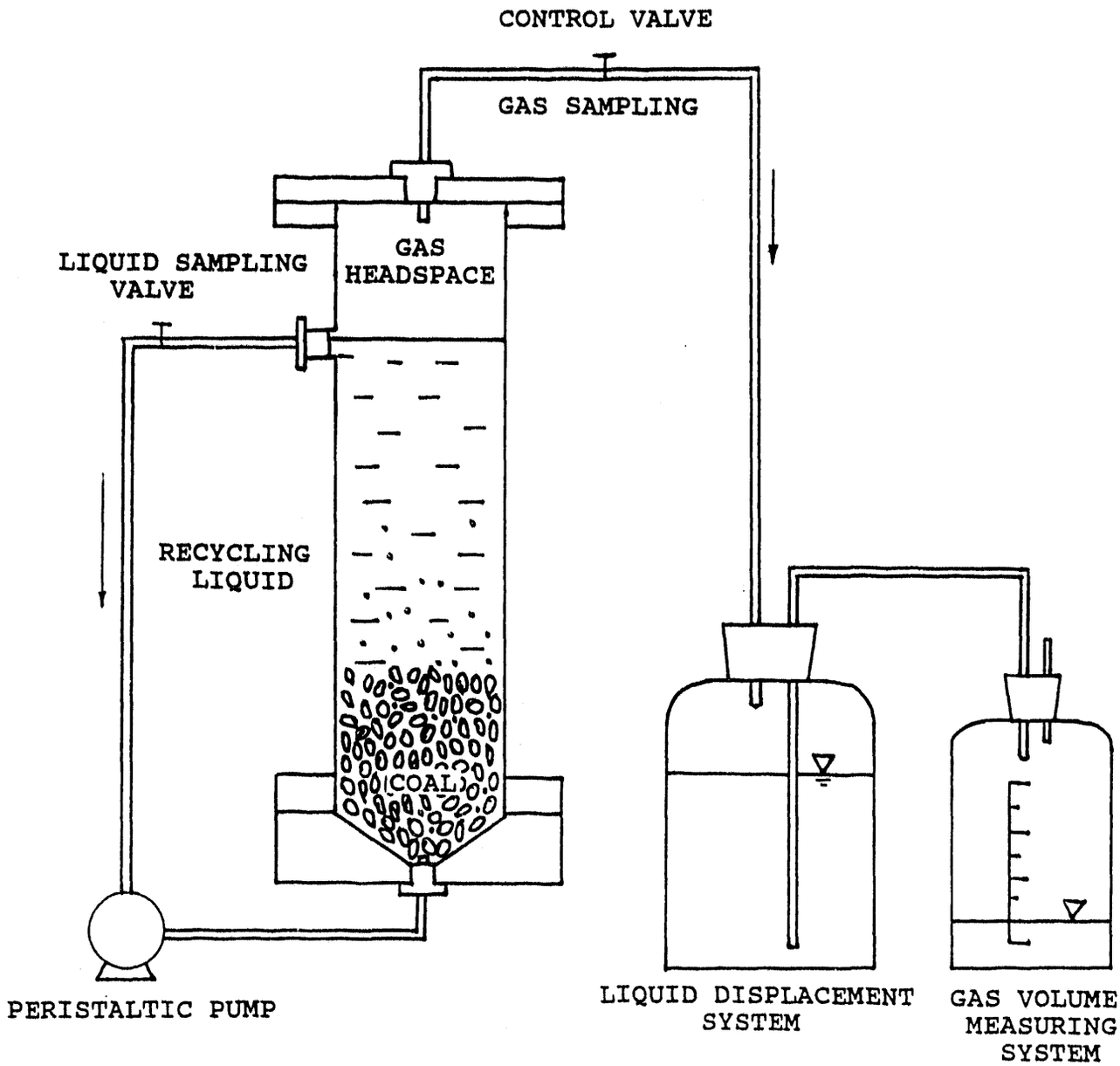

Figure 2. **Experimental Set-up for Biomethanation of Texas Lignite by Mic-1 Consortium in an Upflow Fluidized Bed Reactor (UFBR) Made of Plexiglass.**

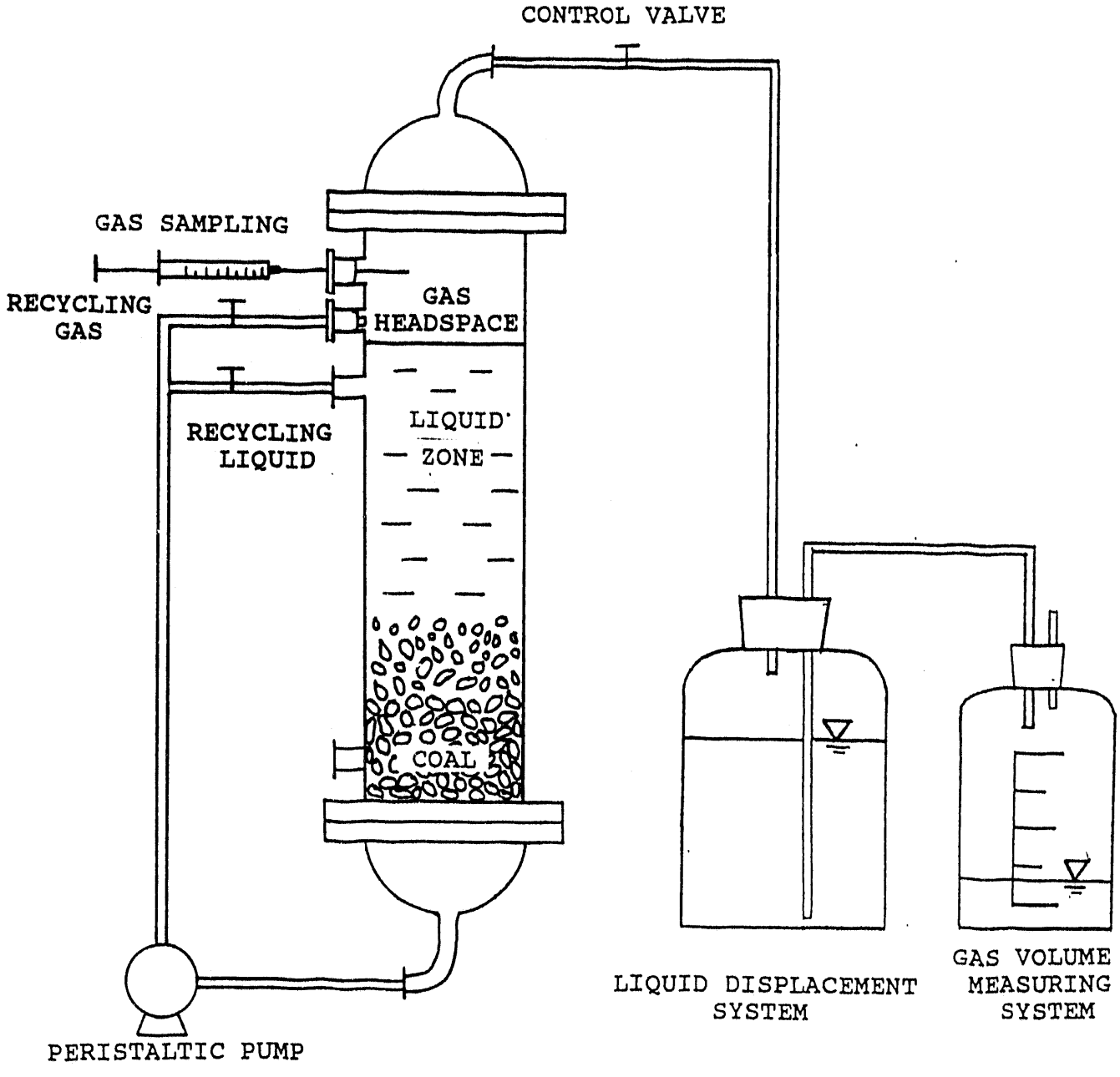

Figure 3. Experimental Set-up for Biomethanation of Texas Lignite by Mic-1 Consortium in a Modified Upflow Fluidized Bed Reactor (UFBR) Made of Glass.

Once the glass UFBR were obtained, two UFBR were set up with 700 mL 0.2% SNTM and 100 g of TxL. A third reactor was prepared without TxL to serve as control. Each of the reactors were inoculated with 100 mL sewage sludge culture, 60 mL Mic-4 and 40 mL Mic-1 (total inoculum was 20% v/v). The final volume was 1 L, with 10% TxL solids loadings. After 5 days, a portion of the headspace gas was recirculated through the bottom of the reactor to facilitate mixing of TxL with the liquid phase. Total gas, CH_4, and CO_2 production were monitored over time. In addition, VFA concentrations, soluble carbon, and cellular protein concentrations in the liquid phase were also monitored. After a month, the operation of these reactors had to be discontinued because of the clogging of the bottom inlet port by TxL particles.

To avoid the problems of clogging and poor circulation associated with pulverized TxL, the three upflow reactors were restarted with 10 mesh (>1.0 mm) TxL. The reactors contained 0, 5 or 10% TxL. Each reactor was inoculated with 4% (v/v) Mic-1, 4% (v/v) Mic-4 and 2% (v/v) sewage sludge in 0.2% SNTM. The headspace gas was recirculated through the liquid phase to facilitate mixing. The reactor operation was carried for about 6 weeks and then stopped.

These reactors were recommissioned with 800 mL 0.2% SNTM + 4X NH_4Cl to study the effect of methanol as a hydrogen donor. Again Reactor 1 was used as control and contained 0.2% SNTM. Reactor 2 contained 0.2% SNTM + 5% TxL + 0.5% (v/v) methanol (4 mL methanol was added), and reactor 3 was charged with 0.2% SNTM + 5% TxL.

3. Trickle-Bed Reactor. A trickle bed reactor, TBR was constructed (Figure 4). This reactor had a total volume of 2 L and working volume of 1 L. It could be run with or without recycle. It was charged with TxL (300 g of -9 mesh, 4 g each of -325 mesh and micronized) and 700 mL NTM. The reactor was inoculated with 200 mL of sewage sludge and 100 mL of Mic-1.

4. Tank Reactors. Three types of tank reactors, continuously stirred tank, simulated anaerobic chemostat and simulated tank reactors were used.

a. Continuously Stirred Tank Reactor (CSTR). A Bioflo (New Brunswick Scientific Co.) fermenter with 2 L capacity vessel and stainless steel flange top (Figure 5) was used as a CSTR. Fermentation was carried out at agitation of 25 rpm and 37 °C. One percent TxL + NTM were initially charged into the vessel and biomethanation monitored for 34 days. Periodically TxL and YE/TSB were added to the vessel. Thus, at the end of 34 days, the total TxL was 5%, and YE/TSB 0.2%.

b. Simulated Anaerobic Chemostats (SAC). These were made out of 1-L aspirator bottles that had a glass aspirator tube at the bottom (Figure 6). The bottles were stoppered with a two holed rubber stopper. One additional hole (port A) ran through only half of the stopper thickness. Port A was used to sample the headspace gas with

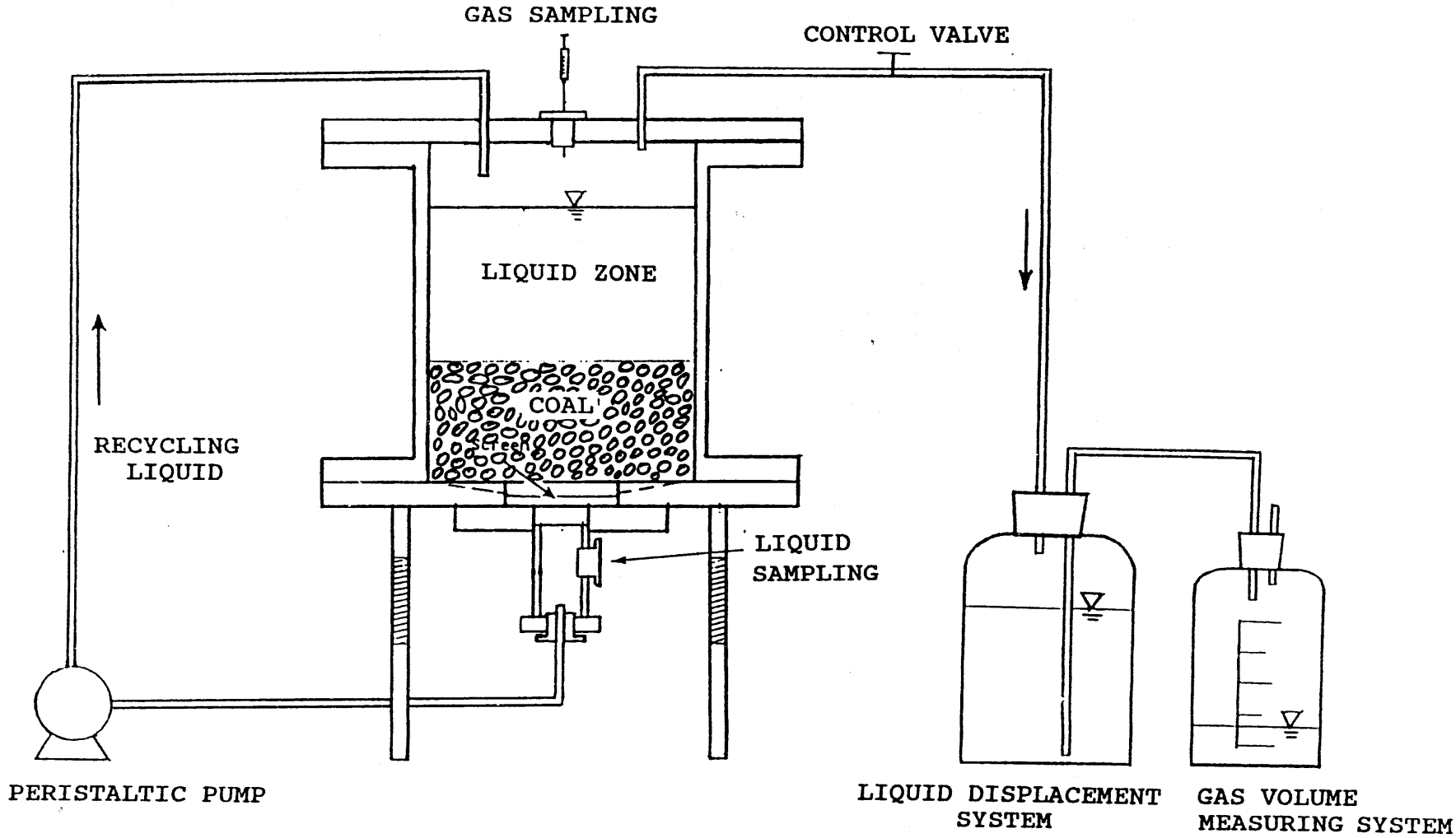

Figure 4. Experimental Set-up for Biomethanation of Texas Lignite by Mic-1 Consortium in a Trickling Bed Reactor (TBR).

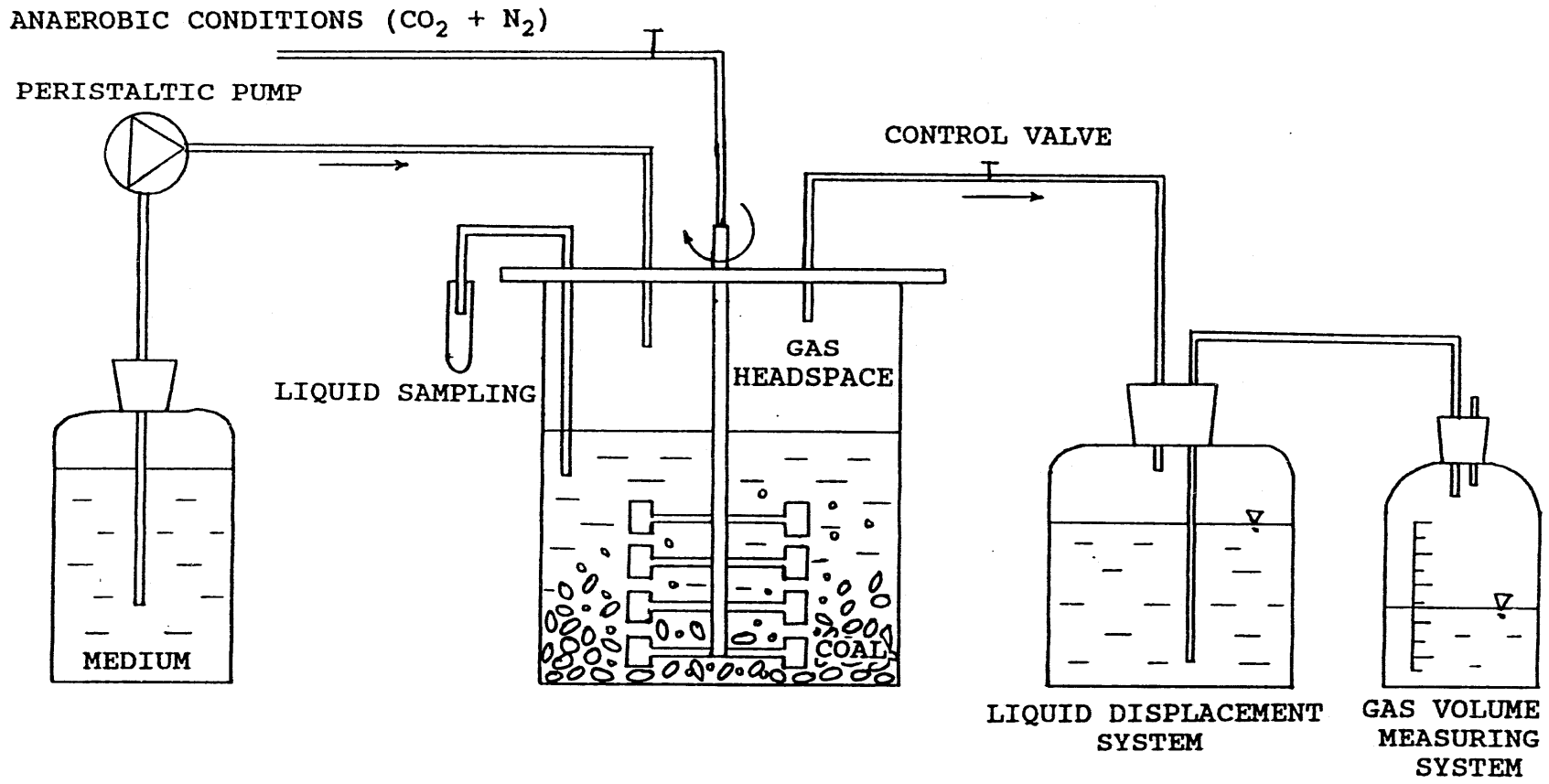

Figure 5. Experimental Set-up for Biomethanation of Texas Lignite by Mic-1 Consortium in a Continuously Stirred Tank Reactor (CSTR).

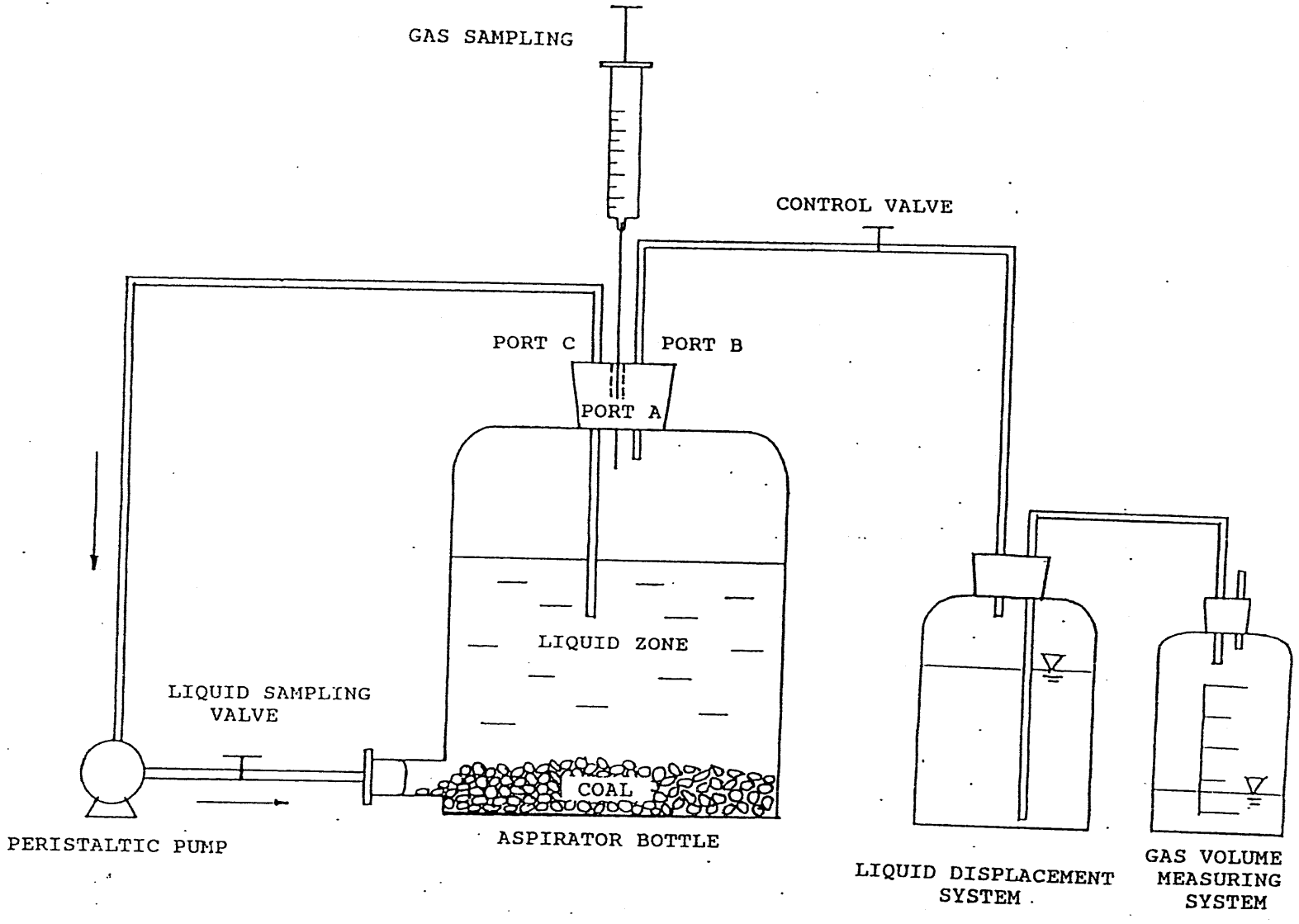

Figure 6. **Experimental Set-up for Biomethanation of Texas Lignite by Mic-1 Consortium in a Simulated Anaerobic Chemostat Bioreactor (SACB).**

a pressure tight syringe. Through one of the holes (Port B) of the stopper passed a small glass tube running into the bottle above the liquid level. This port was used for measuring total gas production by the water displacement method. Through the second hole (port C) was passed another glass tubing that ran just below the liquid level inside the aspirator bottle.

c. Simulated Tank Reactors (STR). One liter aspirator bottles (Figure 7) simulating tank reactors were set up to monitor methane production from sewage sludge, 0.2 % SNTM + sewage sludge and 0.2% SNTM + sewage sludge + TxL by the indigenous sewage sludge microorganisms. The STR were incubated under static and agitated conditions.

III. RESULTS AND DISCUSSION

A. Project Planning

A detailed project plan covering all the experimental protocols was submitted and approved by the METC Program Manager at the time.

B. Kinetics of Methane Production

In order to enhance and optimize the bioconversion of TxL to methane, it was essential to have an understanding of the kinetics of the biogasification reactions. Previous studies at ARCTECH examined the growth characteristics of Mic-3, and the kinetics of conversion of acetate to methane by this consortium. During the period of this report growth characteristics of another biogasifying consortium, Mic-1, were completed.

1. Preliminary Studies with Mic-1 Consortium. Initial data on the biomethanation of TxL by Mic-1 consortium is presented in Table 4. In general, natural microbial populations have a basic threshold for tolerating certain concentrations of a given substrate to make certain products for their metabolism. This hypothesis clearly explains and is supported by the results presented in Table 4.

However, it is also known that in general the microbiota from any given habitat have also the capability to utilize other substrates albeit not at the same rate at which these can utilize the substrates in their natural habitat. Because of this capability, certain microorganisms can be adapted or "tricked" to utilize a substrate similar to the one in their natural habitat. This was the rationale used for getting the termite hind gut natural microflora from the TxL fed termites. However, as is evidenced by the scientific literature on the role of termite hind gut microflora[16], the microorganisms present in the hind gut of soil or wood eating termites predominantly utilize the carbohydrate moieties and only a limited amount of lignin or humus like material (which are structurally similar to TxL) are utilized.

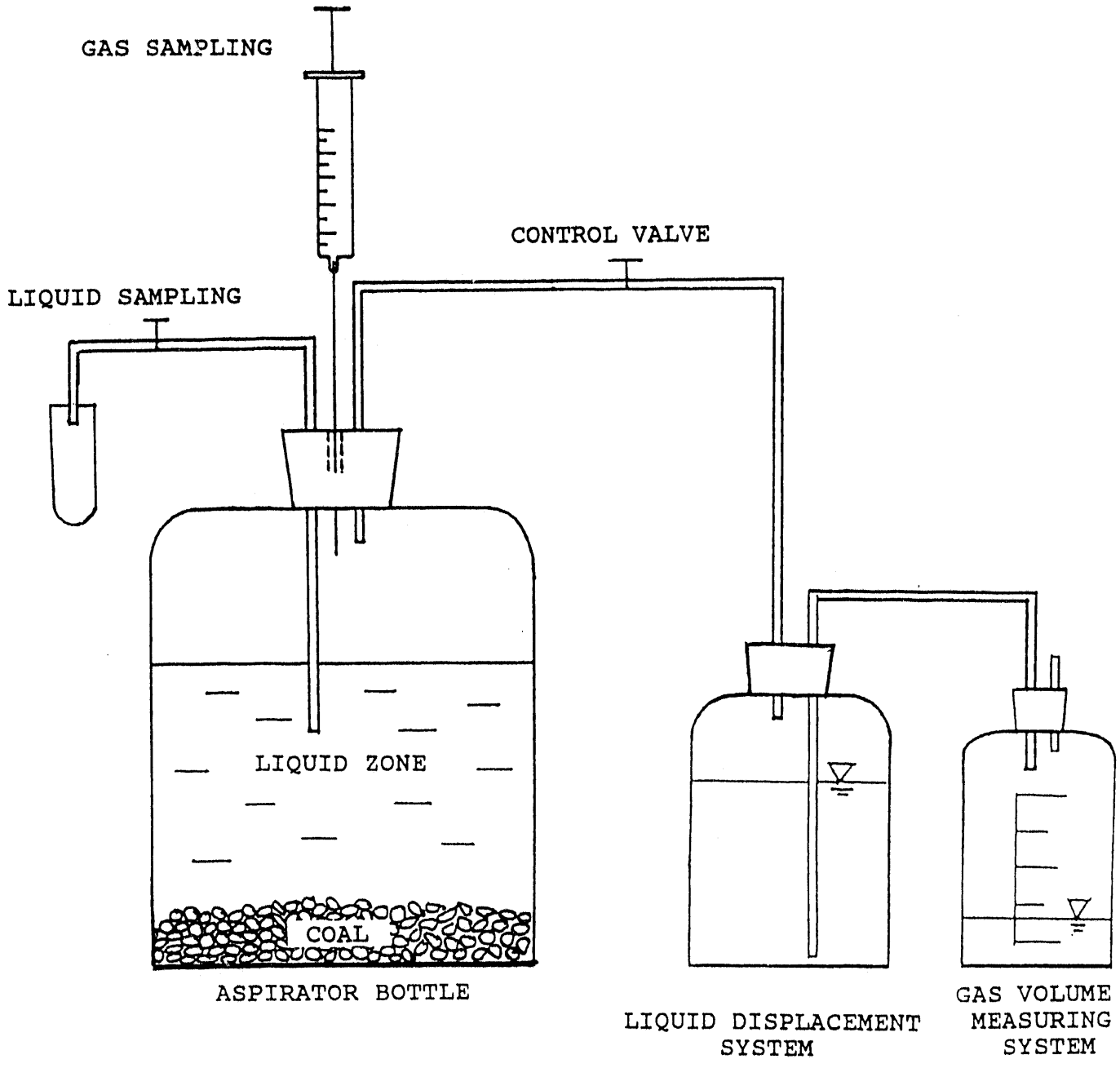

Figure 7. Experimental Set-up for Biomethanation of Texas Lignite by Mic-1 Consortium in a Simulated Tank Reactor (STR).

Table 4. Methane Concentrations in Mic-1 Reaction Mixtures Before and After Adaptation		
Parameter	Methane Production	
	Before Adaptation	After Adaptation
Coal solids (%)		0.010.1 - 1.0
Mole %	10.00	40 - 50
cc/g of coal	40.00	96 - 193
Time (days)	60.00	21 - 28

2. Culture Development. Adaptation is a technique that enables microbiologists to manipulate microorganisms so that they can produce higher concentrations of desirable products from a given substrate. During the process, substrate consumption and production of product(s) are monitored and quantified as a function of time. At the same time morphological changes in a given population are also monitored so as to get an understanding of the microbial species constituting the consortium that gives the desired product(s). Thus, during the initial studies on biomethanation of TxL by the Mic-1 consortium, the potential of this consortium to tolerate higher TxL concentrations, bacterial morphology, and products produced were evaluated.

a. Enrichment of Mic-1 for Coal Utilization. Mic-1 was obtained as a result of enrichment of microbiota from the hind gut of termites belonging to *Zootermopsis* sp. Enrichment is a technique to manipulate growth and specific substrate conversion by a given group of microorganisms. Through this technique an increase in methane production from 10 to 50 mole% reaching up to 193 cc/g TxL was obtained (Table 4). Thus a five fold increase was achieved as a function of adaptation. Furthermore, the methane production by the Mic-1 consortium from 1% TxL started at day 3 and reached a maximum within 21-28 days compared to approximately 2 months time in initial experiments with 0.01% TxL. The maximum rate of methane production was observed to occur between 11-14 days. These results demonstrated that the Mic-1 consortium has the ability to adapt to utilize higher TxL carbon concentrations. Moreover, the consortium has significantly improved during the adaptation period. This also led us to believe that the Mic-1 consortium has the potential to tolerate even higher TxL solids loadings, a positive indication for further improvement in the process research.

b. Bacterial Morphology. Commonly observed morphologies constituting the Mic-1 consortium (Table 5) were single cells, chains, or clumps of cocci, rods (predominantly short, $< 3\ \mu m$) of various lengths and thickness, curved and straight rods, both motile and non-motile rods, spore forming rods and coccobacilli. Four

morphologies of methanogens were evident: *Methanococcus* sp., *Methanothrix* sp., *Methanosarcina* sp., and short rods. The short rods appeared after two weeks of incubation. As expected, the overall numbers of methanogens increased with time. Notable changes occurring in the consortium as a function of time were an increase followed by a decrease in the number of short rods, appearance of coccobacilli (possibly *Methanosarcina* sp.), and the appearance of short curved rods.

Table 5. Bacterial Morphology of Mic-1 Consortium at the Beginning and After Two Weeks of Cultivation

Predominant Morphologies	Methanogens	Major Cultural Changes
cocci short rods (< 3μm), long rods, and long filaments	*Methanococcus* sp., *Methanosarcina* sp., *Methanothrix* sp., short rods	Increase in "coccobacilli". At 2 weeks: appearance of short curved rods, increase followed by decrease in the number of short rods, increase in number of methanogens

c. Products of Biogasification. The carbon in TxL was converted to CH_4 (Figure 8). Also, the total soluble carbon concentration as measured by the chemical oxygen demand (COD) of the aqueous phase (broth) of the culture medium was lowest at 1% TxL solids loadings (Figure 8B). Moreover, higher COD were observed in the treatments without TxL. This indicates less biological activity as compared to those containing TxL. Finally, in TxL containing treatments the decrease of COD as a function of time was indicative that the TxL carbon was being bioutilized with very little biomass production (bacterial growth). The cellular protein (indicator of biomass) of the consortia grown in presence of 1% TxL was the highest (Figure 9). The exponential growth phase occurred between 3-14 days with a specific growth rate of 0.043. Among the VFAs, the highest concentration was that of acetic acid (Table 6) which declined with the methane production. This indicates that the Mic-1 consortium is composed of bacterial species that are different both morphologically and physiologically. Furthermore, the metabolism of one type bacteria is dependent on the other and each metabolic group complements the activity of the other.

C. Enhancement of Methane Production by Mic-1 Consortium

In order to enhance the biomethanation of TxL by the Mic-1 consortium it was imperative to have basic understanding of the metabolism of this consortium and study the process parameters. TxL is a complex substrate and preliminary studies showed that it is degraded into acetic acid by the Mic-1 consortium. It was decided to compare the

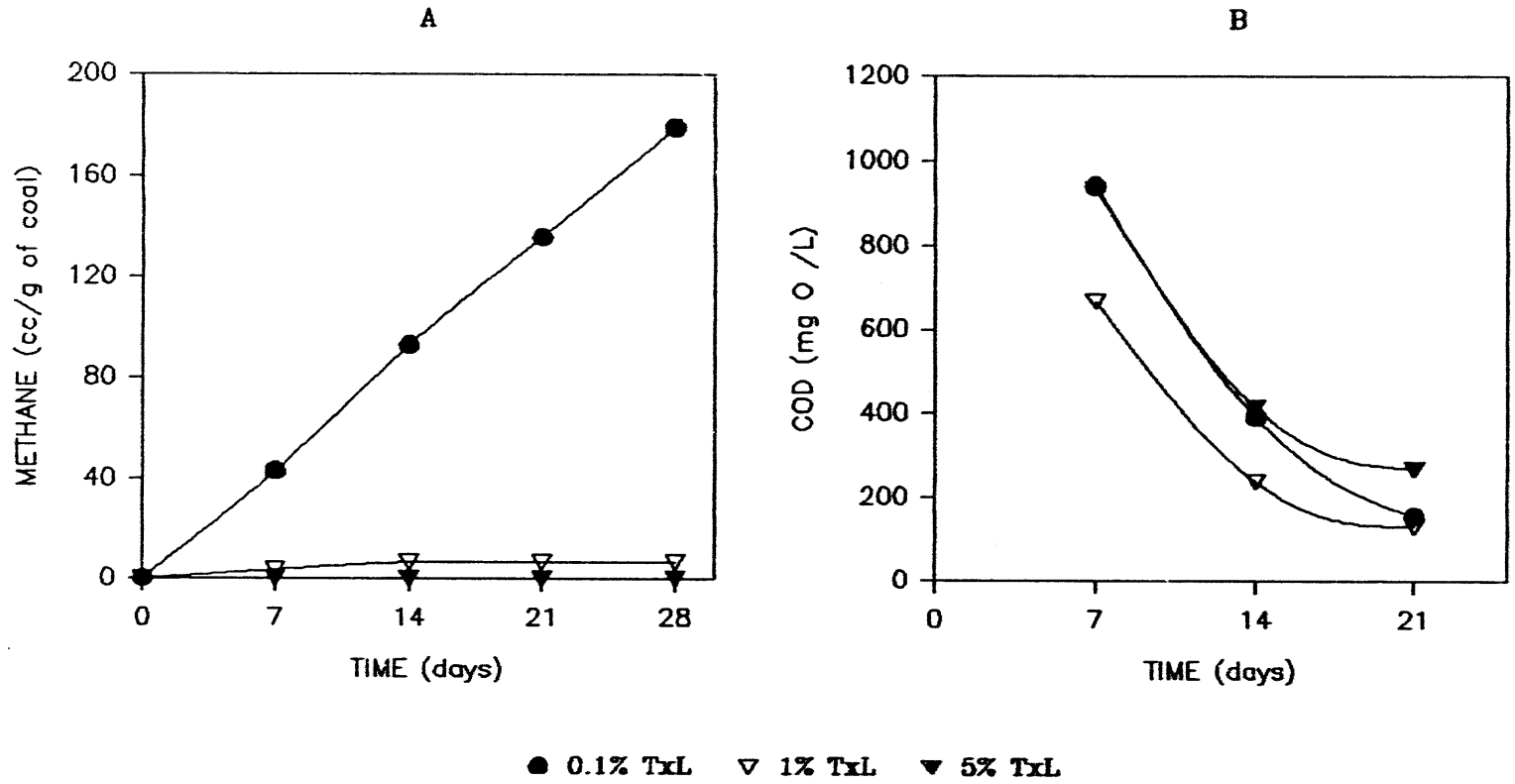

Figure 8. Cumulative Methane (A) and Total Soluble Carbon (B) Production During Biomethanation of Texas Lignite by Mic-1 Consortium.

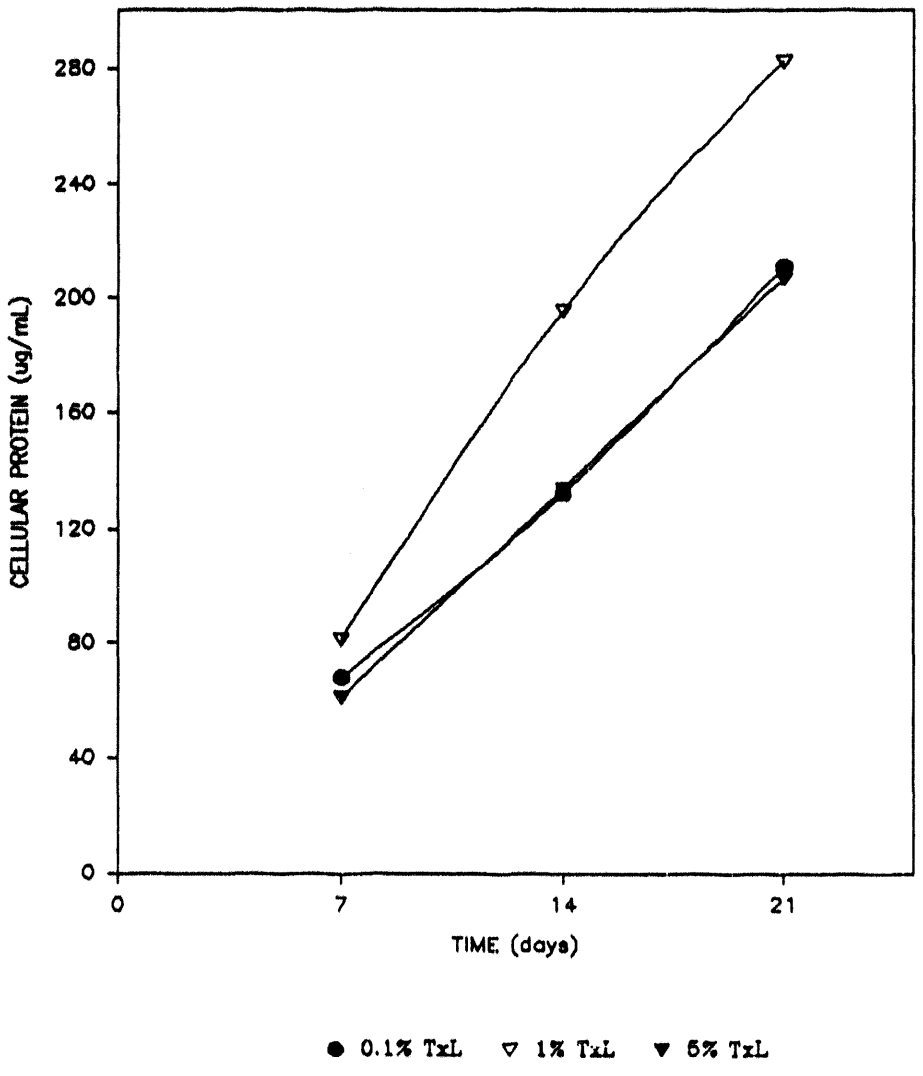

FIGURE 9. Biomass Production During Biomethanation of Texas Lignite at Different Concentrations by Mic-1 Consortium.

Table 6. Predominant Volatile Fatty Acids (VFA) Produced During Biomethanation of Texas Lignite by Mic-1 Consortium			
VFA	Concentration (ppm)	VFA	Concentration (ppm)
Butyric acid	22	Isovaleric acid	61
Valeric acid	26	Propionic acid	132
Isocaproic acid	41	Acetic acid	200
Isobutyric acid	48		

physiology and metabolism of Mic-1 consortium on the simpler substrate. Therefore following aspects were studied.

1. **Physiology and Metabolism of Mic-1 Consortium.** The Mic-1 was grown in NTM supplemented with either sodium acetate (0.5% w/v), solubilized TxL or particulate TxL. The particulate, and solubilized TxL were added on an equal carbon basis (0.057% carbon/bottle, as determined by ultimate analysis). A set of bottles containing NTM without TxL carbon or acetate were also prepared. The bottles were periodically sampled to measure the parameters described in section IIB.

a. Growth Characteristics of Mic-1 Consortium. Growth curves of Mic-1 grown on various substrates were obtained by plotting the cellular protein concentration as function of time (Figures 10 and 11). The bottles containing acetate exhibited a small increase in biomass during the first 4 days of incubation, followed by a decrease until day 8 (Figure 10). A period of exponential growth occurred from day 8 to day 18. This exponential growth phase corresponded to the period of maximum methane production, indicating that methanogens are actively growing at this time. The 8 day lag phase and simultaneous growth and methane production are similar to the results obtained in an experiment performed earlier with Mic-3. The first growth phase (days 2-4) corresponds to an increase in acetate concentrations in the culture, indicating that an acetogenic population was growing at that time. Because a similar increase in acetate concentrations occurred in all of the cultures, including the control, the acetogens were probably utilizing medium components such as yeast extract and TSB.

Growth characteristics of Mic-1 grown on TxL and solubilized TxL were similar (Figure 10). Both cultures demonstrated a short lag phase (3 days), and an exponential growth phase lasting until day 14. There was a sharp decrease in biomass immediately following inoculation in the culture containing TxL. A similar decrease was observed in the control culture. This is probably due to the fact that carbon in solid TxL is not readily utilized by the bacteria, and is therefore not able to

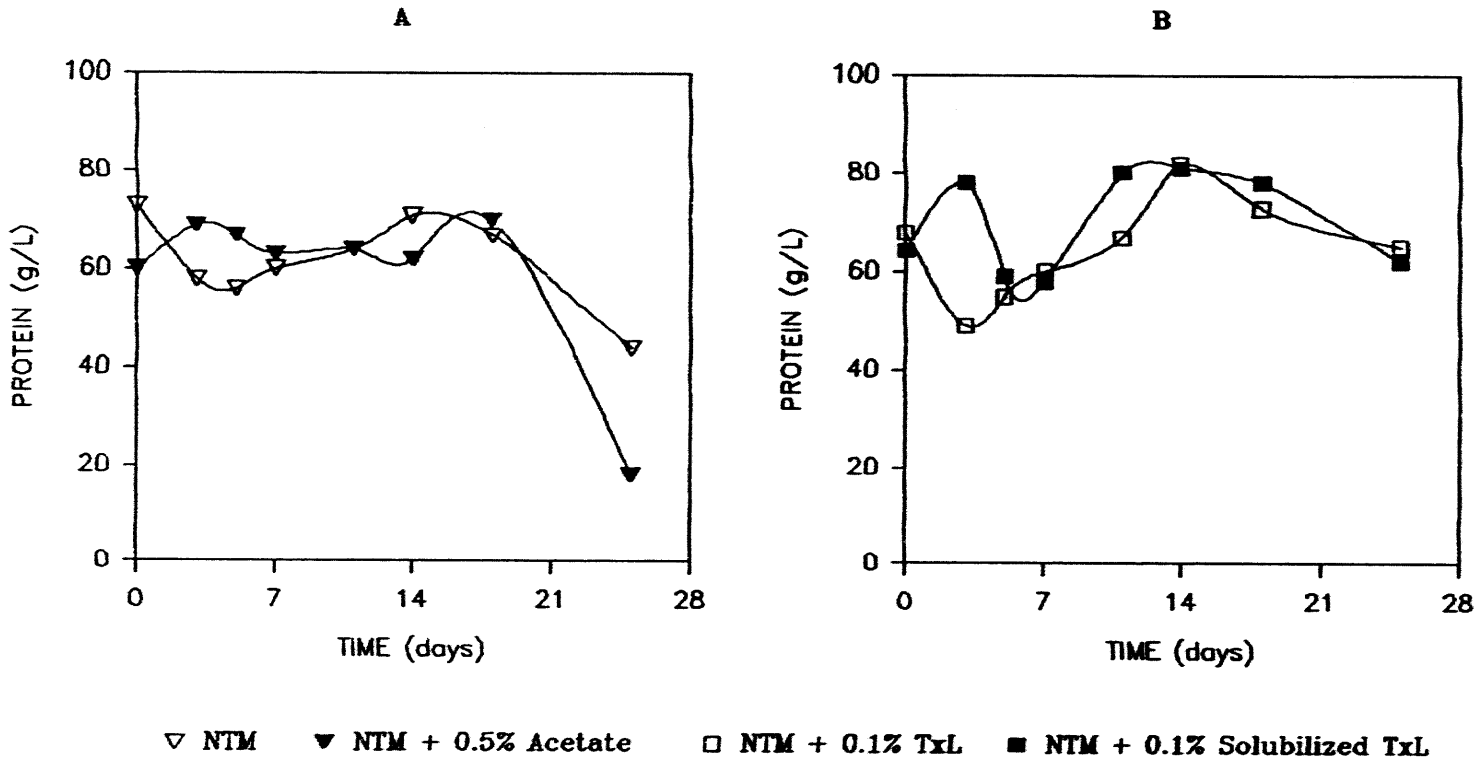

Figure 10. Cellular Protein Concentrations of Mic-1 Consortium Grown in NTM with 0.5% Acetate (A) and 0.1% Texas Lignite (B).

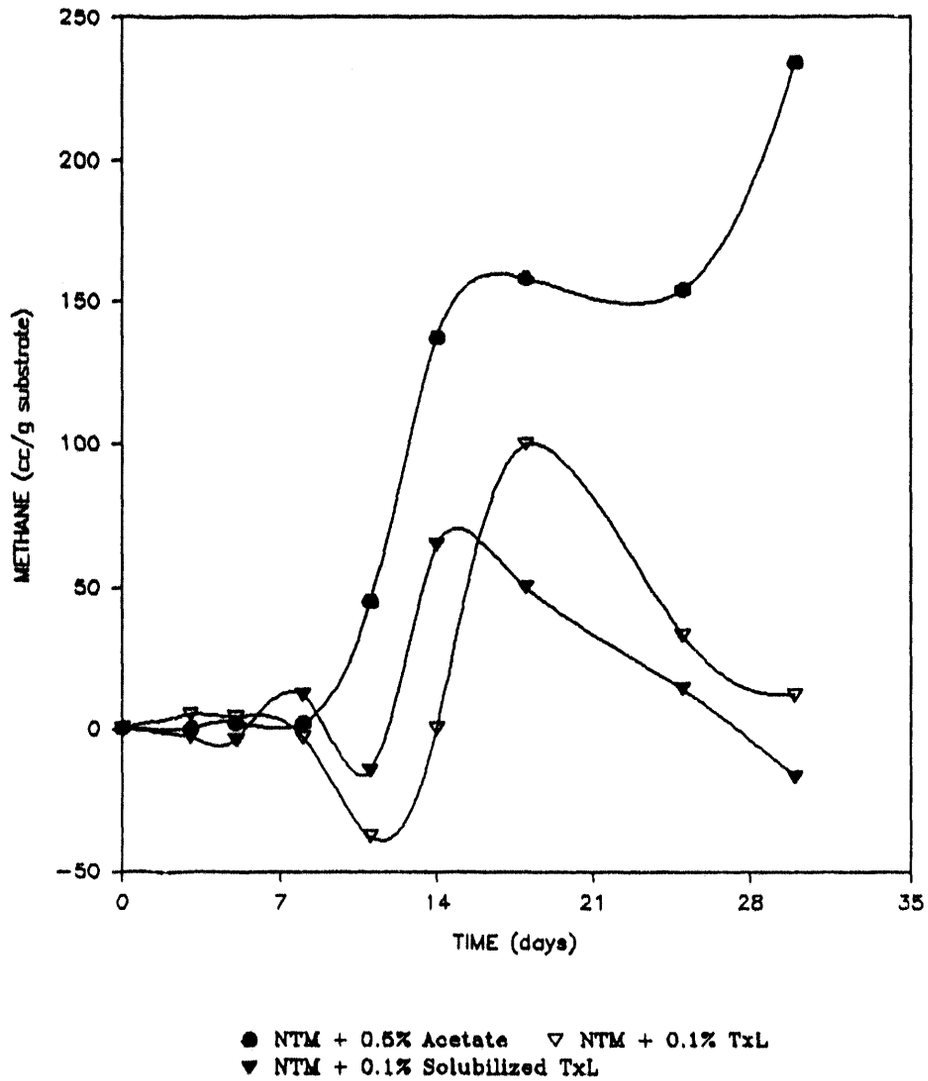

Figure 11. Methane Production from Different Growth Substrates by Mic-1 Consortium. Methane Production in Control (NTM) Deducted.

support growth of certain microbial populations immediately. Biomass increased after 3 days, indicating that the TxL was utilized after that time. The initial decrease in biomass was not observed in the culture containing acetate or solubilized TxL. Solubilized TxL is more readily utilized by microorganisms, and is therefore able to support more growth earlier than solid TxL.

The cultures containing readily metabolizable carbon sources (i.e. acetate and solubilized TxL) would be expected to have greater biomass than the control culture. However, there was little difference in the amount of biomass of Mic-1 grown on various substrates. It is possible that cells were lysing during the experiments, thus preventing biomass from accumulating. Using the growth curves obtained from the protein data, it was possible to determine the specific growth rates and biomass doubling time of Mic-1 grown on different substrates. These results are shown in Table 7.

Table 7. Specific Growth Rates (μ) and Doubling Times (T_d) of Mic-1 Grown on Various Substrates

Substrate	Exponential Phase (days)	μ (days^{-1})	T_d (days)
Texas lignite (TxL)	3 - 14	0.043	16.2
Solubilized TxL	3 - 14	0.042	16.5
Acetate	8 - 18	0.061	11.4
Medium Control	3 - 11	0.066	10.5

b. Methane Production by Mic-1 Consortium Grown on Different Substrates. Methane production in cultures containing each of the substrates is illustrated in Figure 11 and acetate concentrations in these cultures are listed in Table 8. The earliest (between days 8 and 11) methane production was observed in the culture containing acetate. Methane production in cultures containing soluble TxL and the ones containing TxL began between days 11 and 14. The acetate containing culture produced the highest amount of methane (225 cc/ gram of acetate) at day 30. This was expected, because acetate is a readily metabolizable substrate for methanogens. The cultures containing soluble TxL and TxL produced less methane.

Methane production in the cultures containing TxL or TxL product as a substrate fell off after approximately 18 days of incubation. This indicates that the Mic-1 cultures experienced a loss of a particular bacterial population at that time. Because methane production in the acetate-grown cultures continued until day 30, it appears that methanogens are not the population being lost. The decreased methane production occurred in cultures containing both solubilized and solid TxL, and corresponded to a decrease in acetate concentration in both cultures. This indicates that in these

Table 8. Acetate Concentration in the Culture Broth of Mic-1 Consortium Growing on Different Substrates

Days	Acetate Concentration (ppm) in the Culture Broth of Mic-1 Grown on			
	Control	Acetate	Solubilized TxL[a]	Solid TxL[a]
0	25.0	1,823.0	88.0	35.0
1	287.0	1,982.0	291.0	279.0
2	368.0	2,311.0	333.0	340.0
3	352.0	2,009.0	340.0	354.0
4	356.0	1,933.0	391.0	328.0
8	110.0	1,495.0	160.0	142.0
11	0.0	869.0	55.0	20.0
14	0.0	116.0	0.0	0.0
18	0.0	0.0	0.0	0.0
22	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0

[a] TxL - Texas lignite

cultures the acedogenic population was less active than the methanogenic population. Loss of primary TxL degrading bacteria could also affect methane production in the culture containing solid TxL. However, results from this experiment did not provide sufficient evidence for the latter assumption.

The conversion of acetate to methane by Mic-1 growing in a medium containing acetate as the primary carbon source is presented in Figure 12. An increase in acetate concentration occurred during the first two days of incubation. This was perhaps due to metabolism of medium components (yeast extract and TSB) by acedogens.

Methane production and acetate utilization occurred concomitantly, beginning between days 4 and 8. By day 18, 100% of the acetate had been utilized and methane production ceased at this time. Maximum cell growth occurred during this time, indicating that cell growth and methanogenesis occurred concomitantly. Overall, approximately 70% of the carbon in acetate was converted to methane and carbon

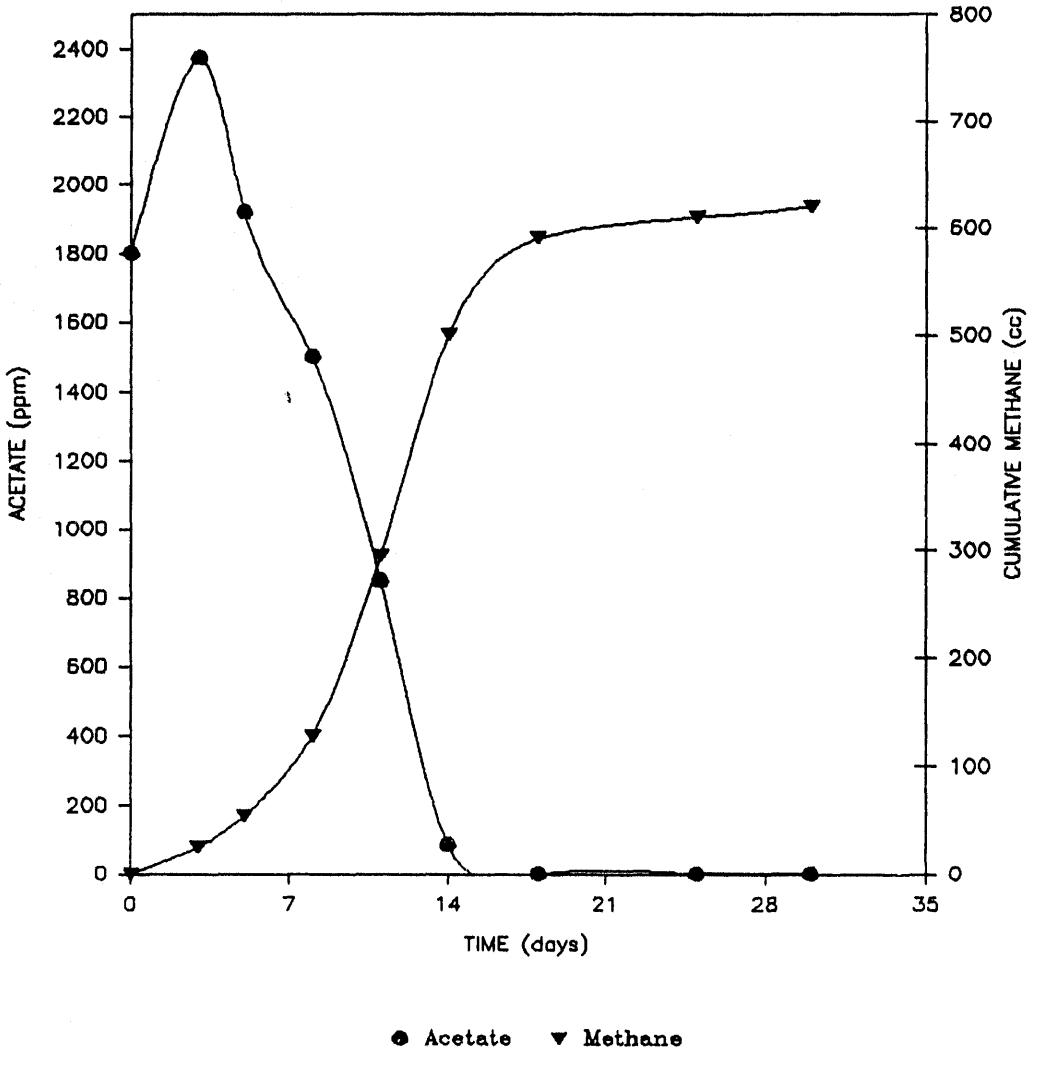

Figure 12. Acetate Utilization and Methane Production by the Enriched Mic-1 Consortium Grown on Acetate.

dioxide, leaving ~ 30% of the acetate carbon unaccounted for. This is very similar to the metabolism of Mic-3. A small amount of the 30% of the carbon not accounted for was converted to VFA and biomass. It is believed that the remainder may have been converted to undetectable intermediates. Another possibility is that only the methyl portion of the acetate molecule was metabolized by methanogens, resulting in accumulation of carbonate and/ or bicarbonate ions in the liquid phase. However, if this was the case the pH of the culture medium should go up rather than going down.

2. Coal Microbial Interactions. Effect of solids, coal chemical components, extraneous chemicals other than coal components, pretreatment of TxL, culture medium components and constituents of Mic-1 either individually or in combination with other Mic-1 isolates was evaluated.

a. Coal Solids Loadings. Based on the laboratory scale studies on biomethanation of Texas and Beulah lignites an economic study of the MicGAS Process was performed by Fluor Daniels. Their study indicated that for the MicGAS Process to be economically viable, a minimum of 20% TxL solids loading will be required. Data on TxL biomethanation by Mic-1 consortium at solids loadings of 1% to 10% shows an inverse relationship between the TxL solids loadings and methane production (Figure 13). Although not encouraging, these data support previous studies at ARCTECH with Mic-2 consortium. Postulated explanation for this inhibition could be any or all of the following: 1. as TxL carbon is utilized more inorganic materials indigenous to TxL become leached and dissolved into the culture medium and might accumulate in concentrations high enough to inhibit microbial growth and metabolism, 2. At higher solids loadings the thickness of slurry may not permit closer association between microbes and the TxL particles, 3. Exhaustion of available H_2 to complete the biomethanation, and 4. Changes in the pH of the culture medium perhaps due to release of excessive CO_2. Recent literature provides proof that some of the TxL components such as Fe, SiO_2[17] are inhibitory to anaerobic microbial acidogenesis[18-20]. Nevertheless, these postulations need to be experimentally verified.

b. Trace and Major Elements of Texas Lignite. Studies performed at ARCTECH have indicated that biogasification by Mic-1 and Mic-2 is inhibited at TxL solids loadings greater than 1%. Increasing TxL solids loadings to 20% or more is a primary goal for ARCTECH's MicGAS Process. Therefore, it was imperative to identify which component(s) of TxL or lignites in general cause these inhibitory effects. Several metals, including iron, manganese, magnesium and aluminum, have been found to occur in Beulah lignite at concentrations which could affect the growth of microorganisms[14].

Since Mic-3 was found to be the most suitable consortium for biomethanation of Beulah lignite[21], this system was used to test the hypothesis that metallic components in lignite ash may be inhibitory to microorganisms. Results from the experiment evaluating methane production by Mic-3 with the various test elements are presented

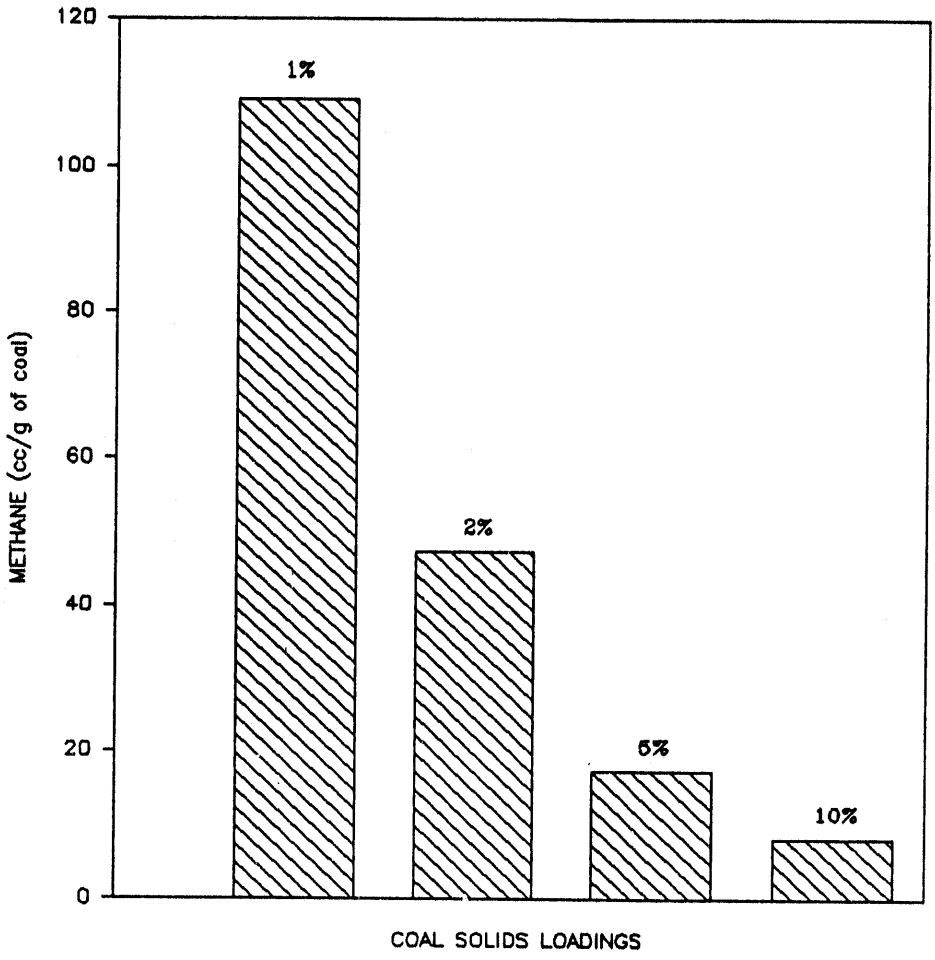

Figure 13. Effect of Coal Solids Loadings on Biomethanation of Texas Lignite by the Enriched Mic-1 Consortium.

in Figure 14. Methane production was significantly lower in the cultures containing iron and magnesium oxides as compared to the control cultures. The cultures containing manganese and aluminum oxides and Beulah ash exhibited slightly less methane production than the control cultures. However, this difference was not significant. There was no significant difference in the cellular protein concentrations in the cultures with the various test elements.

Significantly higher levels of acetate (120-190 ppm), butyrate (20-40 ppm) and isobutyrate (20-30 ppm) were observed in cultures containing magnesium. All other cultures did not contain detectable levels of these acids. This indicates that the acetate-utilizing methanogens present in the Mic-3 consortium are inhibited by magnesium. These results reaffirm the fact that anaerobic microbial acidogenesis, specially of n-butyric acid[19] a precursor of acetic acid which is the basic substrate for methanogenesis is inhibited by some metallic components in lignites. There were no significant differences in the VFA concentrations in the cultures containing iron, manganese, aluminum, ash, or the control cultures. In a recent publication[22] aluminum was shown to enhance methanogenesis by providing additional hydrogenesis which is vital for methanogenesis, especially in lignites as evidenced by the recently proposed empirical formula for the German Brown coal.

The experiment was repeated with TxL and Mic-1. The metals were used as salts rather than metal oxides because most metal oxides are not readily soluble in water, and therefore it is difficult to control the concentration of the metal. To avoid this problem, metal salts ($FeCl_3$, $MnCl_2$, $MgCl_2$ and $AlK(SO_4)_2$) were prepared as sterile stock solutions and added to the medium to give a final concentration of 10 mM. During a preliminary trial, it was found that most of the metal salts reacted with sodium sulfide used as a reducing agent, to form insoluble precipitates. This experiment will be rerun with cysteine HCl substituted for Na_2S normally used to reduce the culture medium.

c. Effect of Agitation. Among the three TxL solids loadings (0.1%, 1%, and 5%) tested, methane production was highest in both static and agitated cultures containing 0.1% TxL (Figure 15) and lowest at 5% solids loadings. Both the agitated and the static cultures produced ~ 80-85 cc CH_4/ g TxL. However, the maximum CH_4 was produced by day 21 in the static cultures as opposed to day 28 in the agitated cultures (Figure 15A). Methane production was significantly lower in the cultures containing 1% TxL (Figure 15B) maximum being 3 to 5 cc/ g TxL. Similar to the 0.1% loadings, the static cultures achieved maximum CH_4 production sooner than the ones that were agitated. There was no significant difference between the fluidized and static cultures in a reactor loaded with 5% TxL (Figure 16).

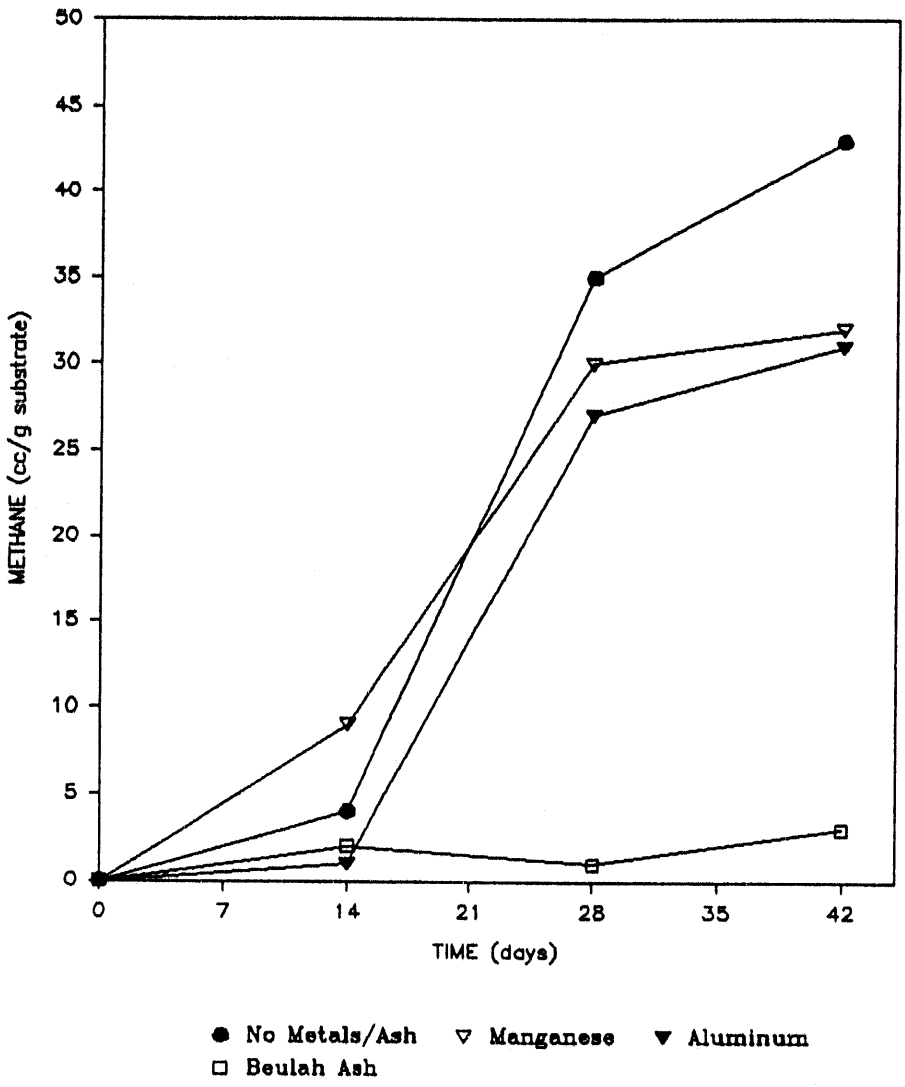

Figure 14. Influence of Coal Components and Metals on Biomethanation of Beulah Lignite by Mic-3 Consortium. Methane Produced in Control Deducted.

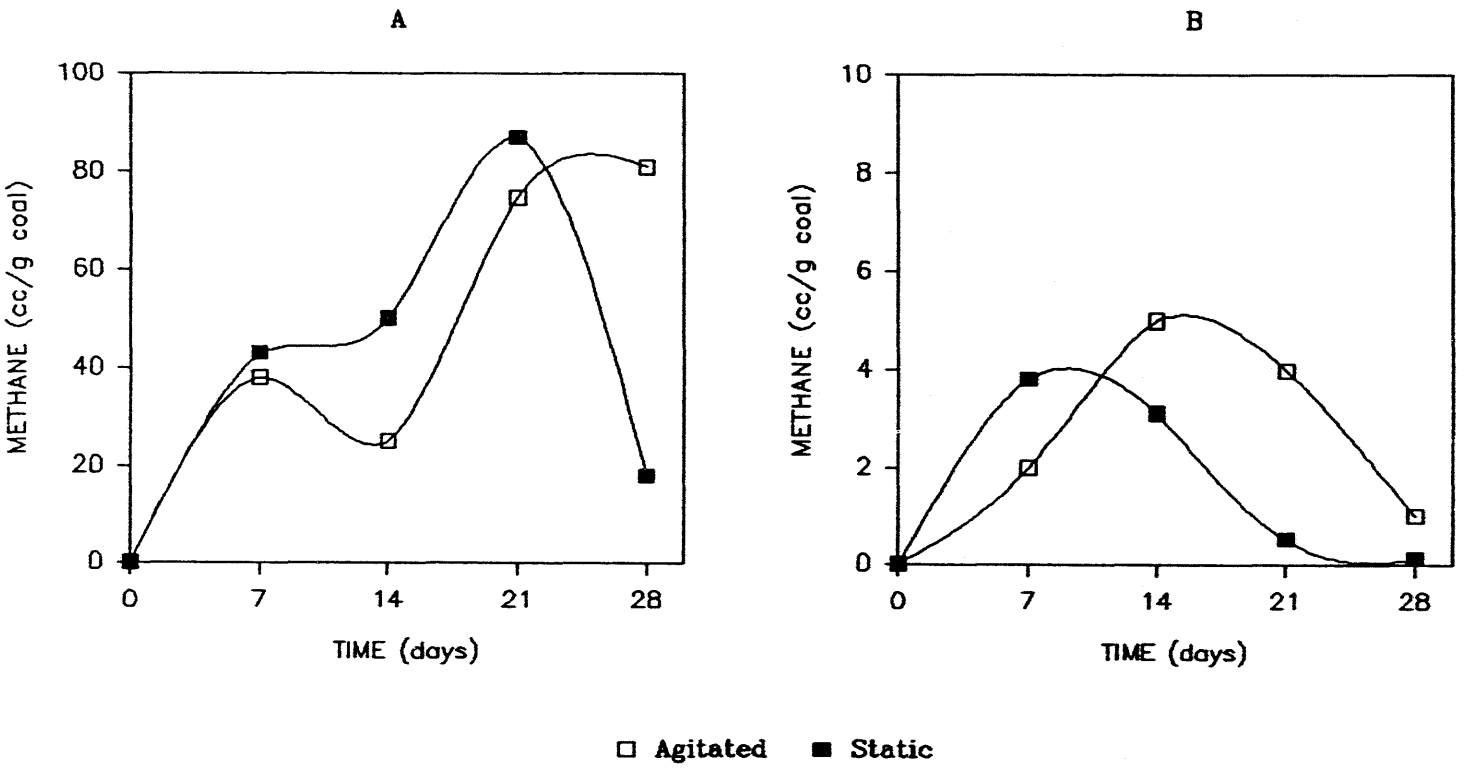

Figure 15. Methane Production by Mic-1 Grown on 1% (A) and 10% (B) Texas Lignite Under Agitated and Static Conditions.

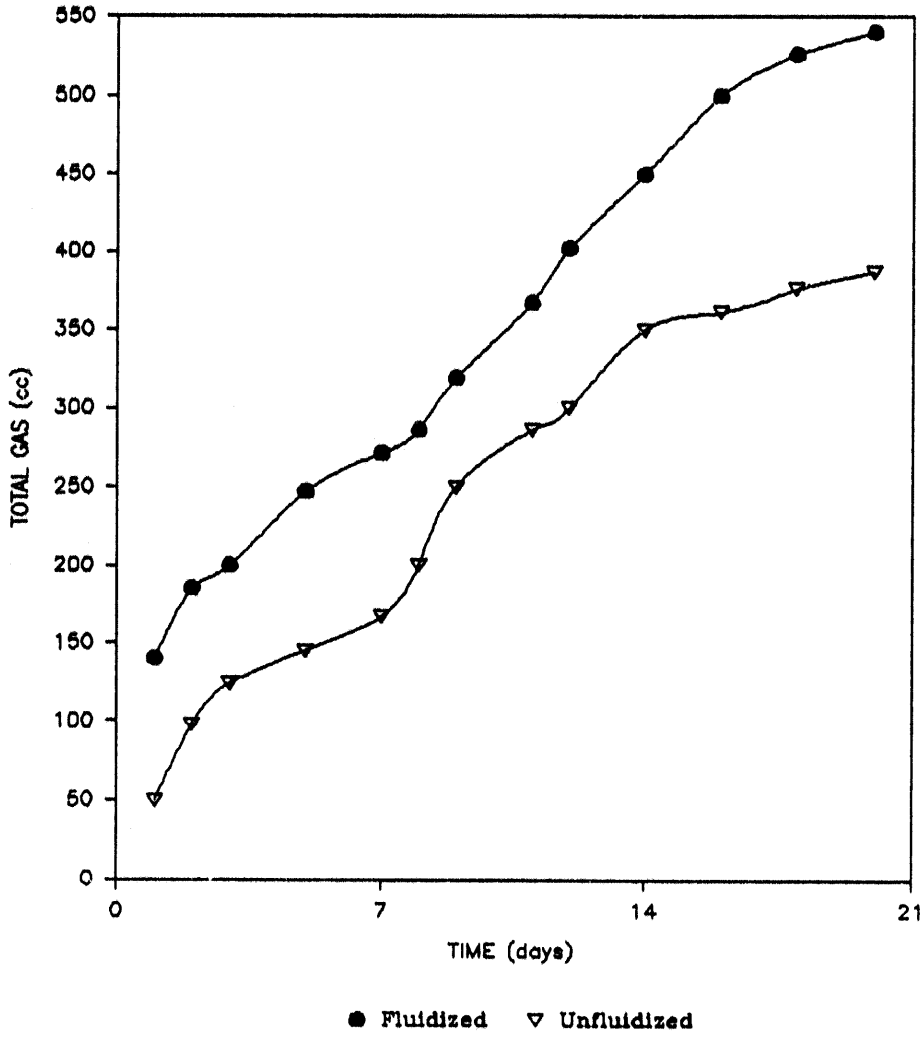

Figure 16. Methane Production by Mic-1 Grown on 5% Texas Lignite Under Fluidized and Unfluidized Conditions in an Upflow Fluidized Bed Reactor.

The total soluble carbon measured as chemical oxygen demand (COD) in the culture broth of Mic-1 consortium grown in static and agitated modes at different solids loadings of TxL are presented in Table 9. In all cases, the COD decreased throughout the course of the experiment. As would be expected, the control cultures demonstrated higher COD than the ones containing TxL. This suggested that even though TxL would provide more soluble carbon, the rate of soluble carbon utilization by the Mic-1 consortium was higher in the cultures containing TxL.

Table 9. Total Soluble Carbon[a] in the Liquid Phase of Mic-1 Cultured in Static or Agitated Mode at Different Solids Loadings of Texas Lignite

Solids Loadings (%)	Soluble Carbon (ppm)[b] in the Two Culture Modes					
	Day 7		Day 14		Day 21	
	Static	Agitated	Static	Agitated	Static	Agitated
0.0	1,156	1,100	603	733	441	499
0.1	941	866	392	454	155	216
1.0	667	717	238	281	130	138
5.0	935	993	415	369	268	259

[a] measured as Chemical Oxygen Demand
[b] data represents average of three replicates

A similar trend was observed for the cellular protein (Table 10) demonstrating that cellular growth was being supported by the TxL solubilized carbon. However, it was also evident that higher TxL solids loadings inhibit both growth (Table 10) and COD Table 9). The cellular protein data coupled with data on COD concentrations and observations on methane production by Mic-1 at different TxL loadings tested (Figure 13) strongly indicate that the methanogenic population was inhibited by one or more components at higher solids loadings.

Another important observation was that even before adding the Mic-1 inoculum to the culture vessels, the CO_2 concentration in the headspace gas of the cultures containing 5% TxL was significantly higher than those containing lesser TxL or in the controls. This could be attributed to the fact that all serum bottles were autoclaved (sterilized) and higher CO_2 would be released in the ones containing higher TxL loading. Therefore, immediately before inoculating, all culture vessels were aseptically reflushed with the oxygen free N_2 + CO_2 (80 : 20) mixture to ensure that the headspace gas was consistent in all the treatments. However, after 28 days of experimental period, the CO_2 concentration in the cultures grown on 5% TxL was 34 to 36 mole % as

Table 10. Biomass Production[a] in the Liquid Phase of Mic-1 Cultured in Static or Agitated Mode at Different Solids Loadings of Texas Lignite						
Solids Loadings (%)	Biomass[a,b] in the Two Culture Modes					
	Day 7		Day 14		Day 21	
	Static	Agitated	Static	Agitated	Static	Agitated
0.0	47.7	58.6	36.7	51.7	55.3	63.1
0.1	67.8	68.5	64.2	69.1	78.8	79.0
1.0	81.2	84.4	114.1	116.1	87.2	115.1
5.0	60.8	56.1	72.9	76.1	62.0	69.4

[a] measured as total cellular protein in $\mu g\ mL^{-1}$
[b] data represents average of three replicates

compared to 20-25 mole % in other cultures. The concentration of CO_2 in the headspace is vital to methanogenesis. However, in spite of the higher CO_2 at higher TxL loadings of 5% less CH_4 was produced. Two hypotheses, one that the system was deficient in H_2 another essential limiting factor for biomethanation, and the other lower culture medium pH could possibly account for this observation. These factors were further investigated in a series of experiments where abiotic, autoclaved, and unautoclaved conditions were used for the biomethanation of TxL by the Mic-1 consortium.

d. Effect of Autoclaving on Biomethanation of Texas Lignite by Mic-1 Consortium. In general, higher methane was produced in the presence of 1% TxL than in NTM without TxL (Figure 17). However, it was found that at higher Solids loadings (10%) the biomethanation of TxL was inhibited in the treatment that were autoclaved than the ones that were not (Figure 18). Furthermore, the pH of culture medium was much lower in the autoclaved than in the non autoclaved treatments.

The COD was much higher in the autoclaved treatments containing 10% TxL (Figure 19). These data clearly explain that the inhibition of biomethanation at higher solids loading is related to TxL components one of which could be CO_2. Another important indication is that the pH of culture medium needs to be buffered for efficient biomethanation of TxL.

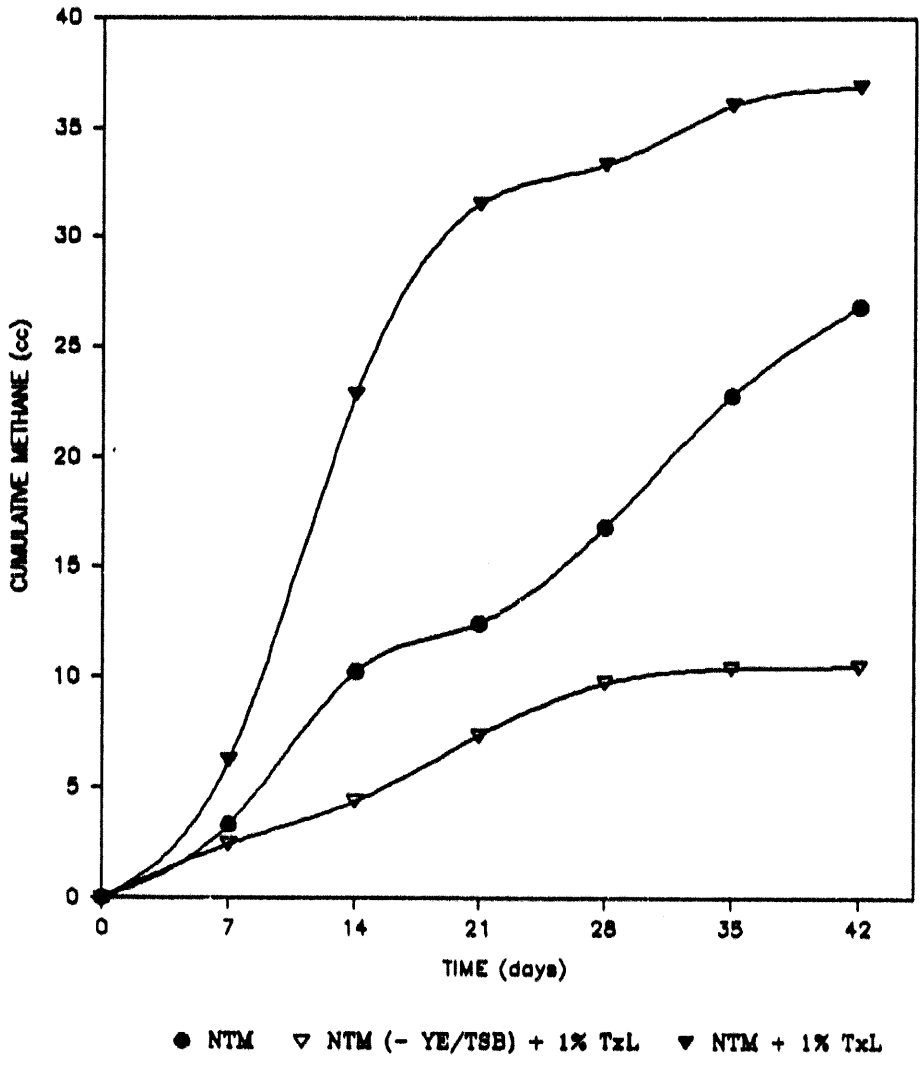

Figure 17. Influence of Texas Lignite and Yeast Extract + Tryp Soy Broth (YE/ TSB) on Biomethanation of Texas Lignite by Mic-1 Consortium.

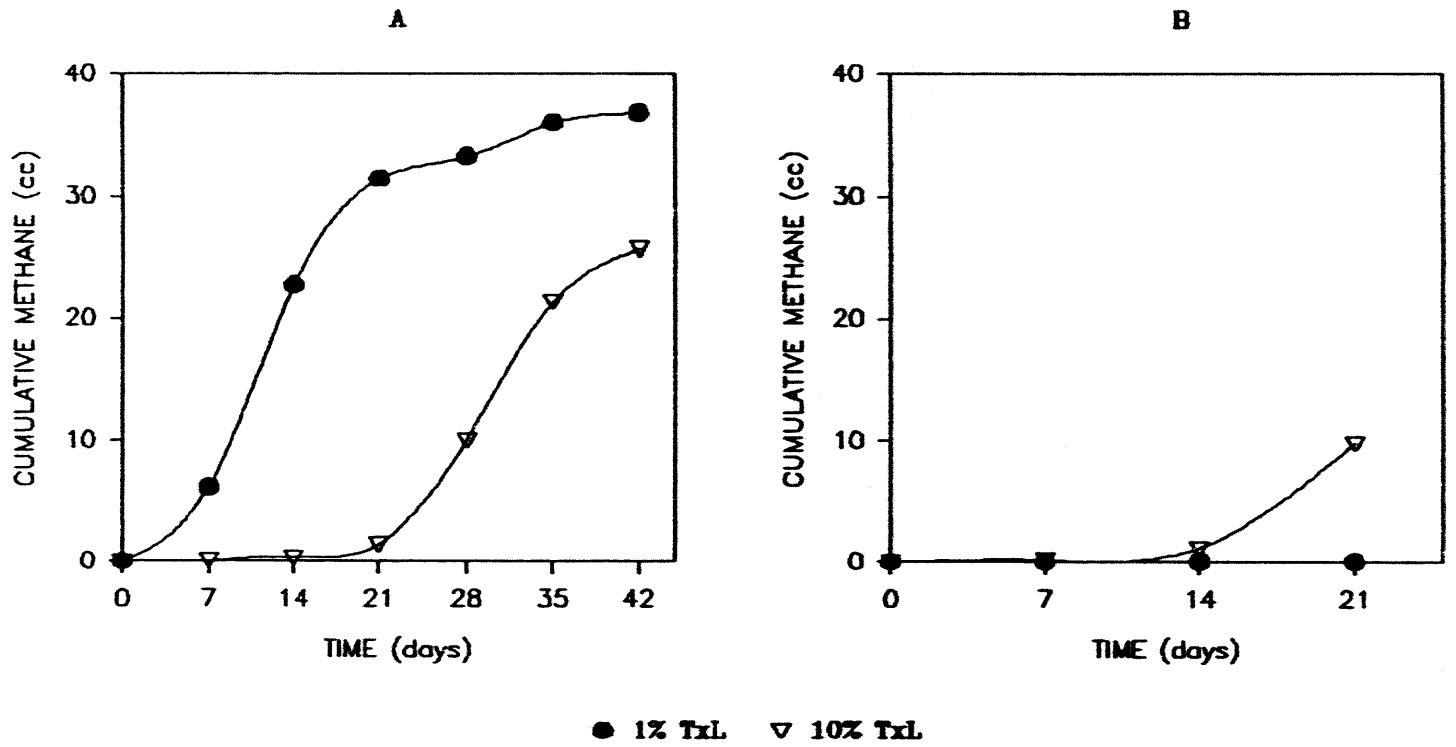

Figure 18. Cumulative Methane Production from Autoclaved (A) and Unautoclaved (B) Texas Lignite Using NTM.

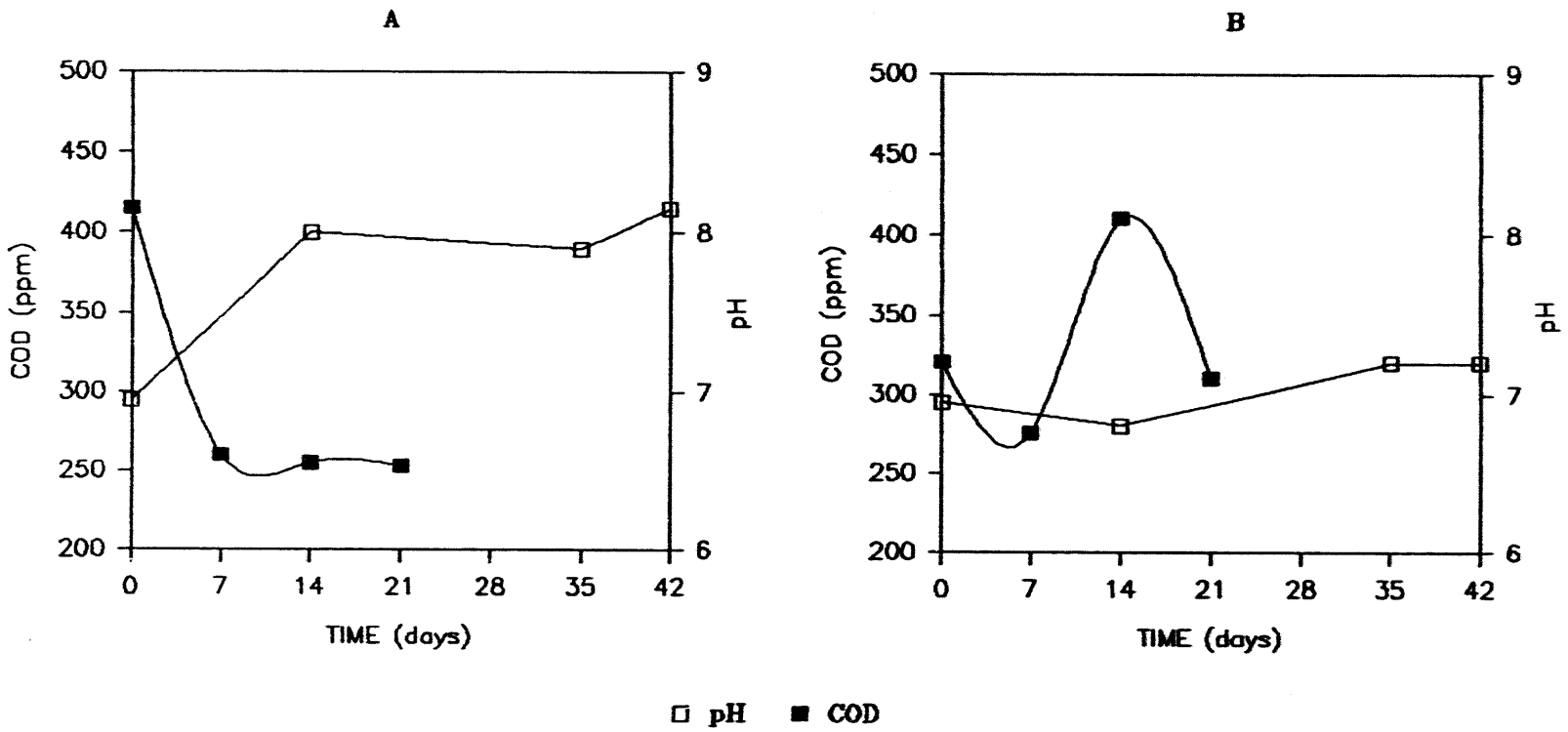

Figure 19. Time Course of Total Soluble Carbon (COD) and pH During Biomethanation of Texas Lignite by Mic-1 at 1% (A) and 10% (B) Solids Loadings.

e. Effect of Chelators and Surfactants. Coal and some of the coal by-products like biphenyl are considered xenobiotics. Literature reports indicate that modifying the surfaces of xenobiotics, such as TxL makes them more amenable to biodegradation[23]. At the same time chelators/ and or sequestering agents can sequester some of the trace metals indigenous to TxL. The chelating or sequestering affinity of a given agent is specific towards each metal, For example EDTA has the highest affinity for zinc but relatively little affinity for calcium. It so happens that zinc is one of the metal ion that does inhibit anaerobic acedogenesis[19]. The hypothesis to be tested in these experiments was that if the metals are chelated or sequestered by addition of these agents, there should be an increase in biomethanation of TxL by Mic-1. Similarly, enhanced biomethanation of TxL by Mic-1 in treatments containing surfactants would further prove the hypothesis.

Data from these experiments showed (Figures 20 to 23) that at low concentrations (0.1 mM and 1.0 mM) EDTA had little effect on biogasification (Figure 20). This effect could be due to stronger chelating properties of EDTA. For the first 14 days, AMMOX at 0.1 mM and 1.0 mM had little effect, while at 10.0 mM; it inhibited early methane production (Figure 21). However, by day 21, methane production was higher in cultures containing each of three AMMOX concentrations. In contrast, significantly higher methane production was observed in cultures supplemented with different concentrations of citrate (Figure 22). Thus, there is a direct correlation between the concentration of citrate and biomethanation (Figures 22). A comparison of effect of AMMOX and citrate showed citrate to be most effective sequestrant (Figure 23) for biomethanation of TxL. Nevertheless, at higher TxL solids loadings, even citrate was not effective (Figure 24). The data presented are net methane production from TxL after deducting the methane produced from controls (NTM + citrate, oxalate, or EDTA).

Methane production was severely inhibited in all cultures supplemented with surfactants even at the lowest test concentration of 0.05%. No methane production was observed in cultures containing either Triton X-100 or SDS. In the cultures containing Igepal CO-720, the highest methane production was 1 mole%. It is quite possible that some or all the bacterial species constituting the Mic-1 consortium might have lysed at the used concentrations of these surfactants. At least two of these, SDS and Triton X-100 are used to break open the bacterial cells for the extraction of microbial nucleic acids[24]. These results clearly indicate that even at the lowest concentration, surfactant amendment will not enhance biomethanation of TxL by the Mic-1 consortium.

f. Development of Improved Growth Medium. The Fluor Daniels study also recommended that for the MicGAS Process to be economically attractive, the NTM must be improvised to replace YE/ TSB with low cost nutrient sources. Consequently, several alternatives to the YE/ TSB were evaluated[7]. Recent results indicate that at least five of these products supported higher methane production from TxL than that

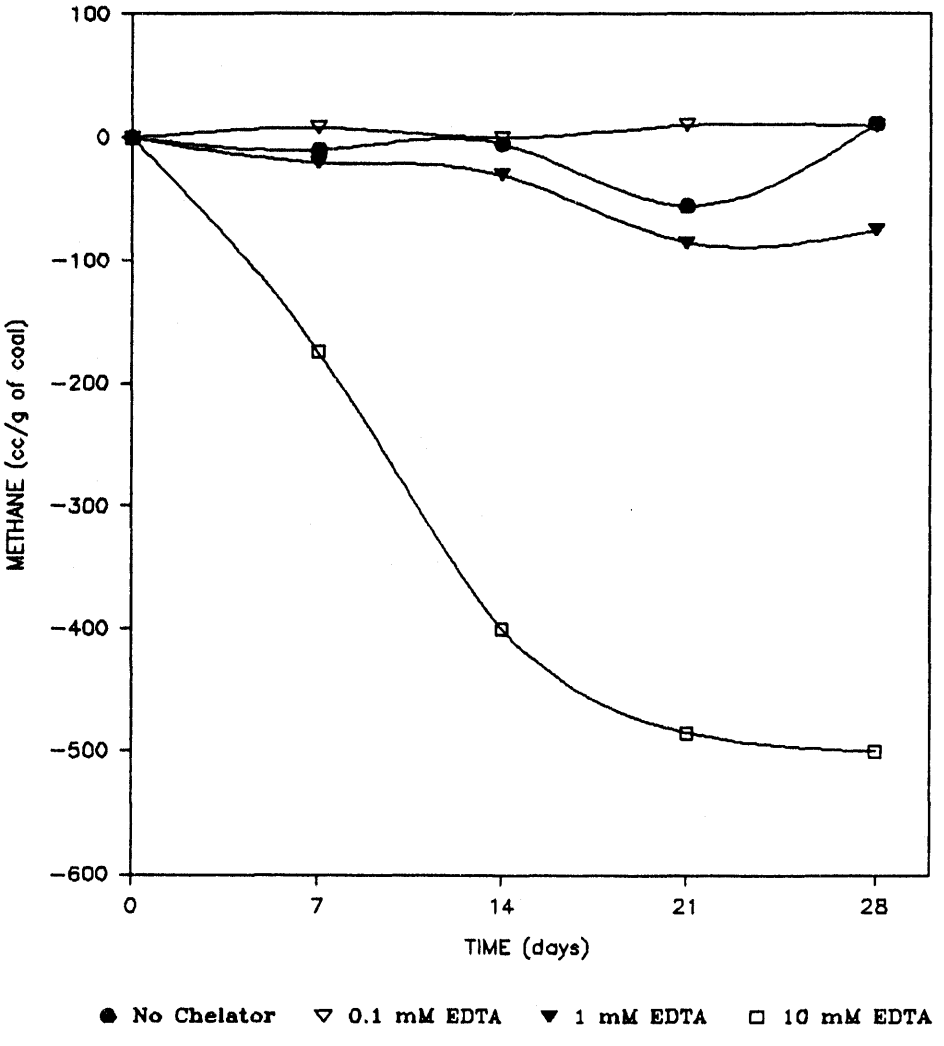

Figure 20. Biomethanation of Texas Lignite by Mic-1 Consortium at Different EDTA Concentrations. Methane Production in Control Deducted.

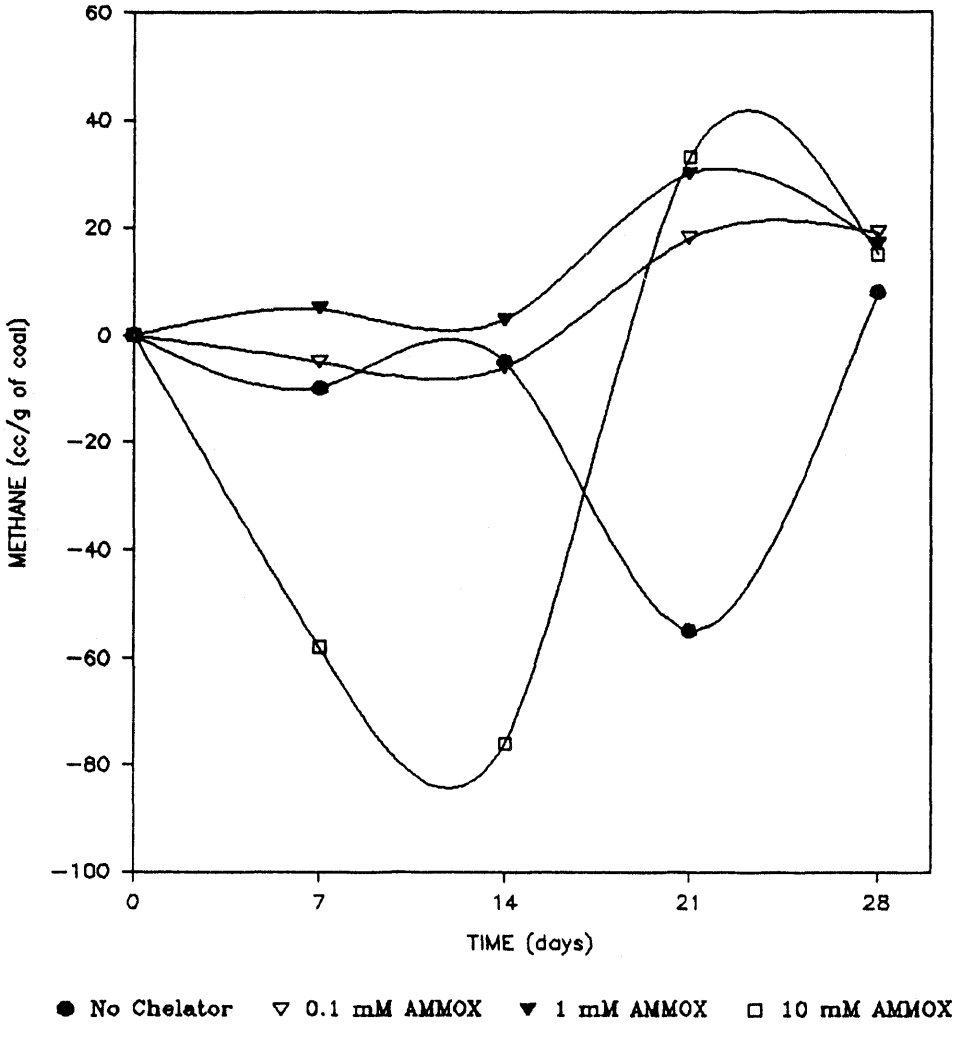

Figure 21. Biomethanation of Texas Lignite by Mic-1 Consortium at Different Ammonium Oxalate (AMMOX) Concentrations. Methane Produced in Control Deducted.

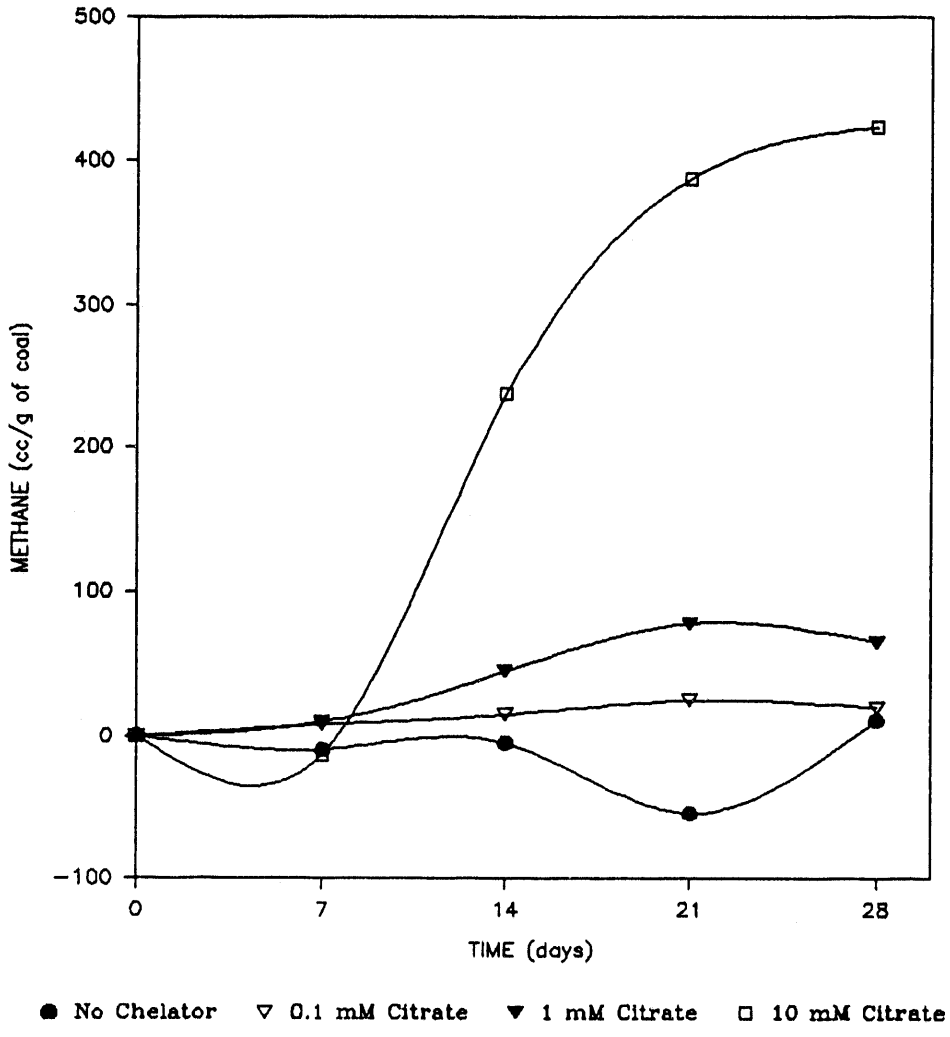

Figure 22. Biomethanation of Texas Lignite by Mic-1 Consortium at Different Sodium Citrate Concentrations. Methane Produced in Control Deducted.

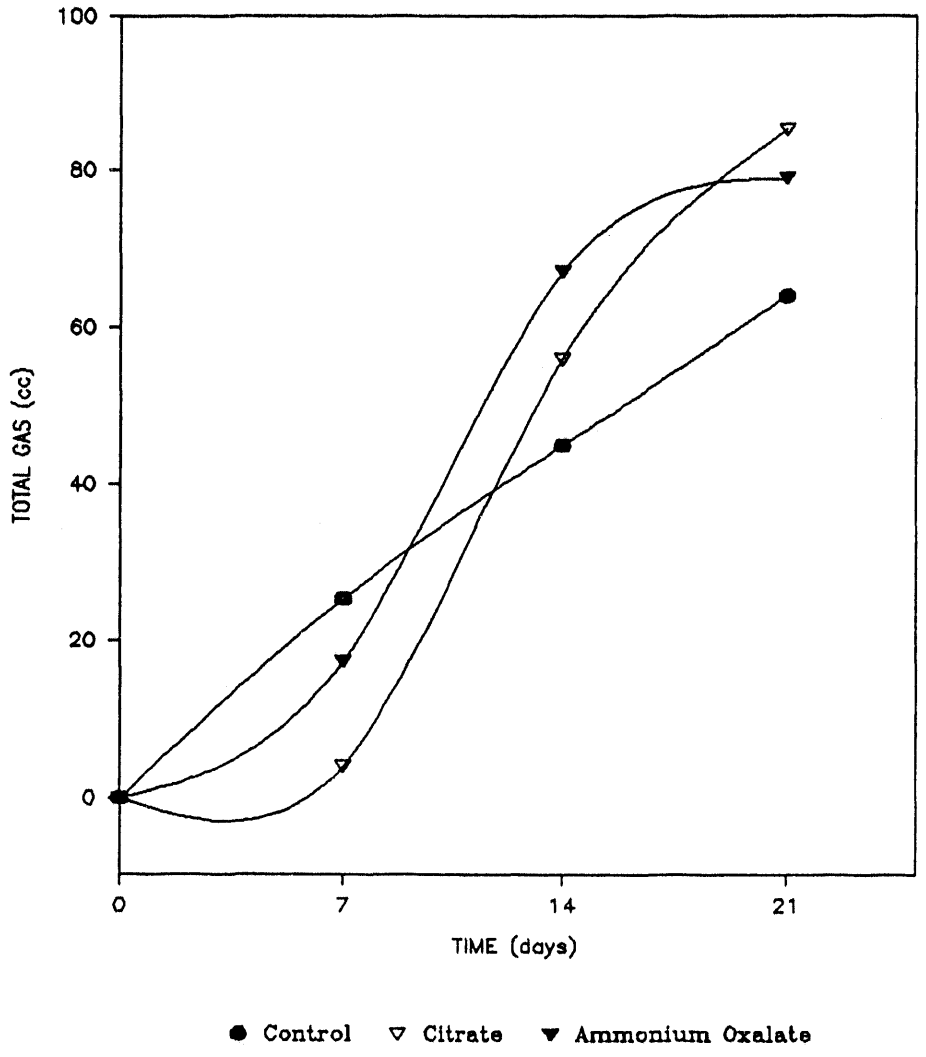

Figure 23. Biomethanation of Texas Lignite by Mic-1 Consortium. Comparative Effects of Ammonium Oxalate and Sodium Citrate.

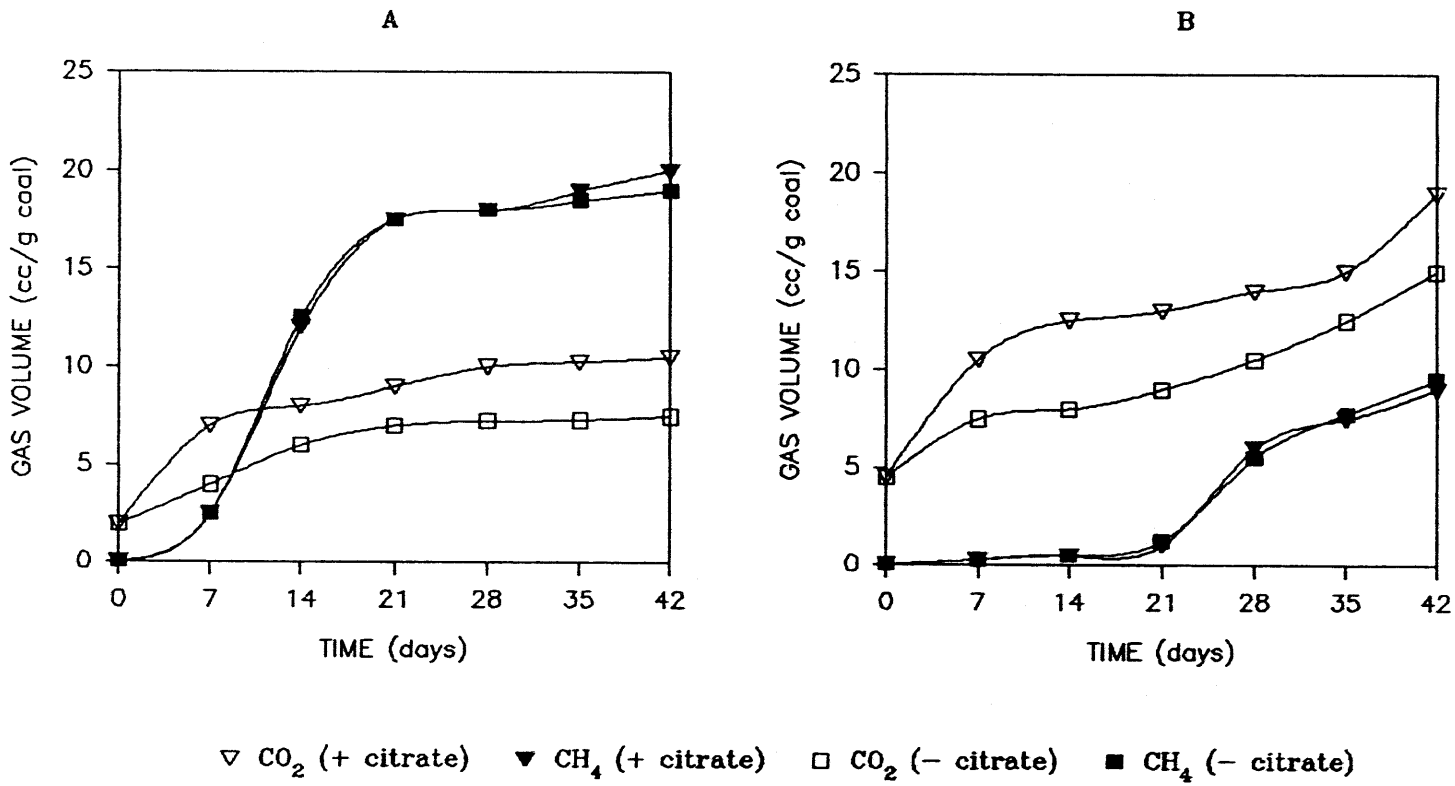

Figure 24. Influence of Sodium Citrate (10mM) on Biomethanation of 1% (A) and 10% (B) Texas Lignite by Mic-1 Consortium.

obtained with YE/ TSB. The highest methane production was in the presence of Sheftone-T™ (Table 3 and Figure 25).

In order to evaluate the cost effectiveness of a range of materials that could replace YE/ TSB for the efficient biogasification of TxL by Mic-1 the following equation was developed.

$$\frac{(P * N)}{(M * C)} * K = \$/ ft^3$$

where P is the price of the nutrient in dollars/ pound, N is the percent concentration of the nutrient in the medium, M is the methane produced per gram of TxL, and C is the percent concentration of TxL in the medium. The constant K is a conversion factor to calculate the cubic feet of methane produced. Using this equation, it is possible to determine the cost of nutrient required to produce one cubic foot of methane from TxL. With this information, the most cost effective concentration of nutrient for the MicGAS Process could be determined. Comparative costs for the selected products that could replace YE/ TSB are presented in Table 3B. Sheftone-T™ (Sheffield Products, Norwich, N.Y.) was found to be least expensive. Hence in all further studies this product replaced YE/ TSB and NTM was renamed % SNTM. The number before the % sign indicates the quantity (w/ v) of Sheftone-T™ used for a given experiment.

During screening for potential alternatives to YE/ TSB, the potential test materials were evaluated at 0.2% (w/ v). However, it is desirable to use these nutrients at a lower concentration, thereby further reducing medium costs. Results from an experiment to determine the minimum concentration of Sheftone-T™ required for efficient biomethanation of TxL by Mic-1 demonstrated that at concentrations of Sheftone-T™ <0.1%, the methane production was severely curtailed, and was less cost effective than when used as 0.2% SNTM (Table 3B).

B-vitamins. The above described biogasification medium still contained a solution of B-vitamins (Table 1). However, the manufacturer's description of the composition of Sheftone-T™ indicates that it may contain adequate vitamins for the MicGAS process. The cost of SNTM could be further reduced by omitting the vitamins solution from it.

As shown in Figure 26, methane production was not effected by the absence of B-vitamin solution. In fact, after 14 days of incubation, methane production was somewhat higher in cultures without additional vitamins (198 cc/ gram of TxL) than in culture which contained the additional vitamins (156 cc/ gram of TxL). These results indicate that B-vitamin solution need not be added to the biogasification medium when

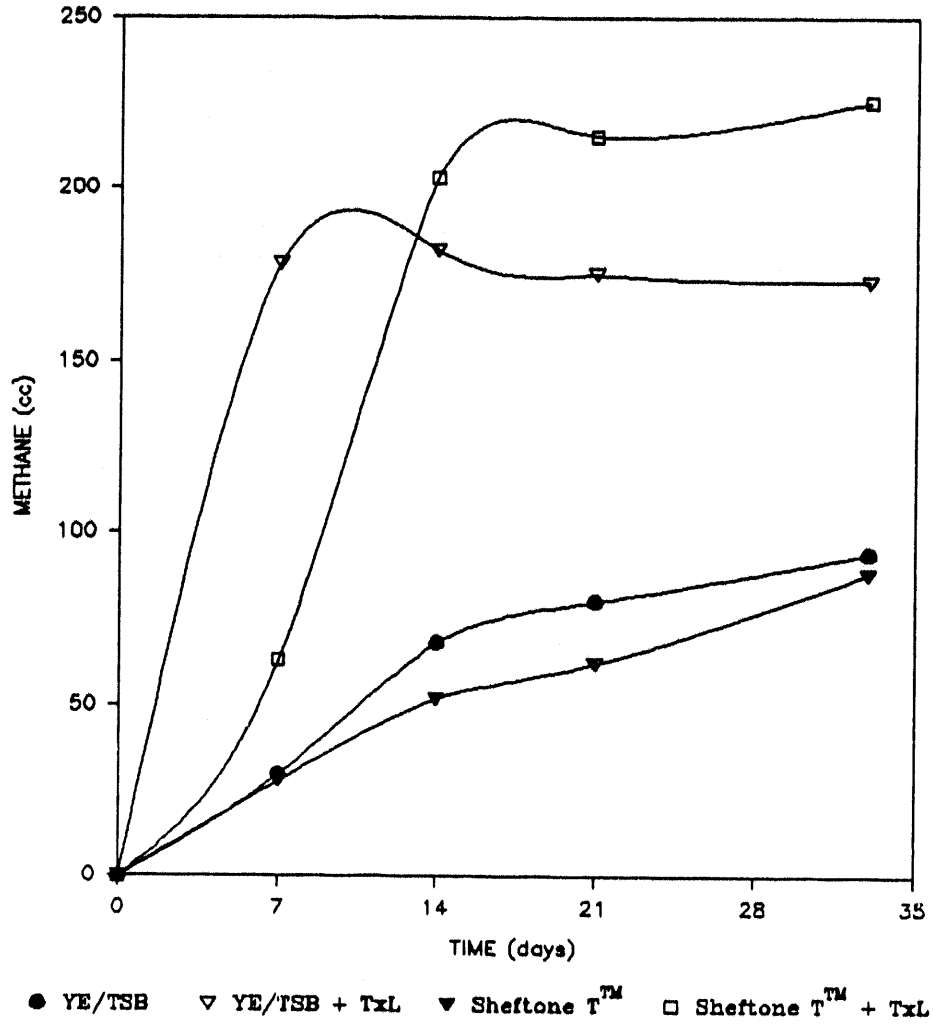

Figure 25. Effect of Yeast Extract/Tryp Soy Broth (YE/TSB) and Sheftone T™ on Biomethanation of Texas Lignite by Mic-1 Consortium.

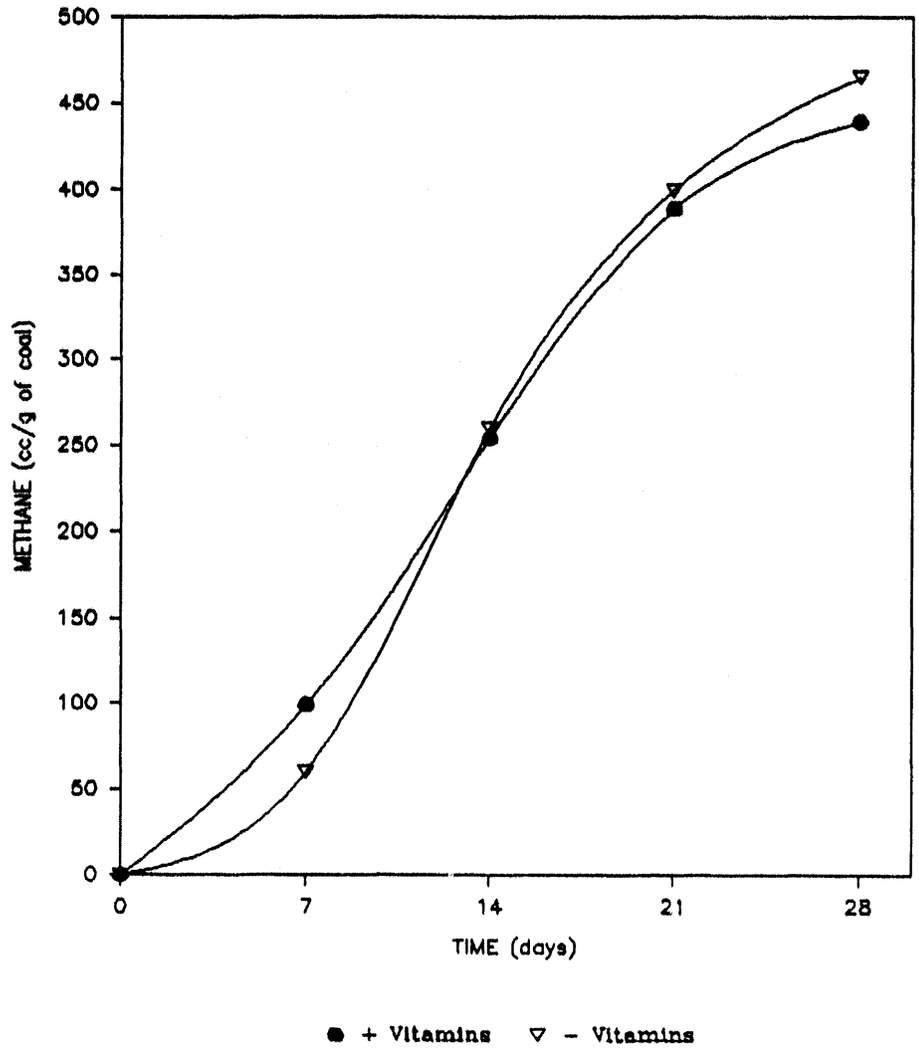

Figure 26. Effect of B-Vitamins Solution on Methane Production by Mic-1 Consortium in 0.2% SNTM + Texas Lignite.

Sheftone T™ is used at 0.2%. Subsequent to confirmation of these results in a later study the current formulation of SNTM does not contain B-Vitamins solution. Comparative ingredient composition of NTM and SNTM medium are presented in Table 11.

Table 11. Comparative Composition of NTM and SNTM Media

Major Components	Initial Medium (NTM + YE/TSB)	Present Medium (SNTM)
Organic Nitrogen Source	Yeast extract + TSB	Sheftone-T™
Macronutrients (Mineral salts)	NH_4Cl, $CaCl_2 \cdot 2H_2O$, NaCl, $MgCl_2 \cdot 6H_2O$, KCl, KH_2PO_4	Same
Micronutrients (Trace minerals)	H_3BO_3, $CaCl_2 \cdot H_2O$, $CoCl_2 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, $MnCl_2 \cdot 4H_2O$, $NiCl_2 \cdot 6H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$	Same
Vitamins	B_{12}, Nicotinic acid, PABA, Pantothenate, Pyridoxine, Thiamin	Not required
Other Components	$NaHCO_3$, Na_2SeO_3, resazurin	Same

In order to further economize the process cost the SNTM was supplemented with 4 x (32 g/ L compared to 8 g/ L) concentration of NH_4Cl. This was based on the fact that for the production of industrial enzymes, such as protease or lipase, the production cost can be reduced by increasing the inorganic nitrogen constituents of culture medium to compensate for minimizing the organic nitrogen amendment such as YE. Results obtained from the experiments conducted in serum vials were verified in simulated tank reactors. The data indicated that additional NH_4Cl inhibited biomethanation of TxL by Mic-1 consortium (Figure 27).

g. Effect of Pretreatment of Texas Lignite on Biomethanation. Although several fold increase in the biomethanation of TxL resulted from some of the above described studies, the inhibition of biomethanation of TxL at higher TxL solids loadings was evident. It has been postulated that lignites have a micro- and a macromolecular component. Based on the studies at ARCTECH, specially at higher solids loadings, it is conjectured that the Mic-1 population was capable of utilizing only the micromolecular and not the macromolecular fraction of TxL. This postulation was verified by removing the micromolecular fraction either by biological or chemical treatment. The biomethanation of residual TxL was carried out as described above.

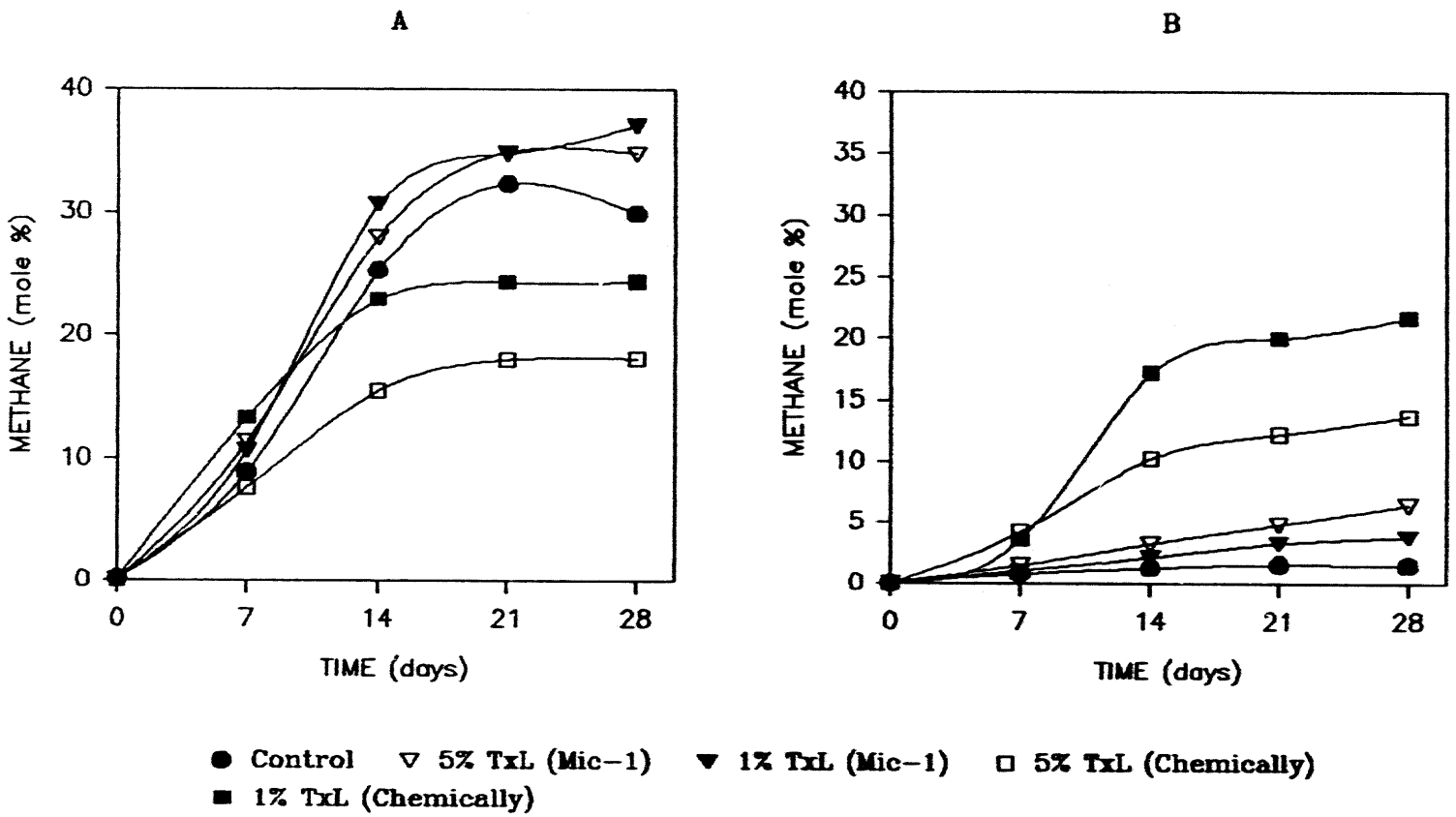

Figure 27. Methane Production from Mic-1 Treated and Chemically Treated Texas Lignite Using 0.2% SNTM (A) and 0.2% SNTM + 4 x NH_4Cl (32 g/L) (B).

The data indicates that methane production was 1.5 fold higher in experimental vessels containing residue from biologically pretreated TxL than those containing residues from chemically (THF) pretreated TxL (Figure 27). However, biomethanation was inhibited in the THF soluble (mobile) fraction even though the THF was removed from the mobile fraction (Figure 28). The most encouraging data was that methane production was not inhibited at higher (1% vs. 5%) TxL solids loadings (Figure 27). These results are a significant achievement towards enhancing biomethanation of TxL. This would also provide better process economics because the residual TxL can be recycled. Furthermore, this information might be a prelude towards unfolding the mechanism of biological attack on the TxL. In the absence of a detailed molecular fractionation of TxL, the data provides preliminary support to our hypothesis that the initial population of Mic-1 could not attack the macromolecular fraction of TxL. Whereas biological pretreatment makes the macromolecular fraction of TxL more amenable to Mic-1 consortium.

h. Isolation of Individual Bacterial Components of Mic-1 Consortium. Thirteen bacterial isolates were obtained from the Mic-1 and Mic-4 biogasification consortia. In order to determine whether these isolates are primary TxL degraders, they were grown in medium containing TxL. The degradation of lignite would produce an increase in soluble carbon in the liquid phase of the reaction mixture. Therefore, bacteria producing a significant increase in soluble carbon in reactions containing TxL could be considered potential primary TxL degraders. However, there was no significant change in COD levels in the culture broth from these bacteria (Table 12). As CH_4 and other gaseous products were not measured, it is quite possible that the bacteria utilized the soluble carbon for their metabolism for CH_4 production which could explain the reduction in COD. This possibility is supported from the data on VFA analysis (Table 13). Alternatively, the anaerobic biodegradation of TxL may also be postulated to be the result of synergistic activity (i.e. activity of two or more different bacteria). Nevertheless, the capability of these isolates to biodegrade TxL needs further investigation.

Volatile fatty acid analyses (Table 13) indicates that each isolate (except for M4-2 and M4-8) produced acetate at a concentration greater than 100 ppm. The greatest concentration of acetate was produced by M4-1 (404 ppm), an isolate from Mic-4. The greatest concentration of acetate produced by an isolate from Mic-1 was 298 ppm, produced by M1-2. Several other VFAs were produced in smaller quantities by various isolates. The conclusion from these data is that most isolates are acedogens.

It was interesting to note that four isolates (M1-4, M4-2, M4-4 and M4-8) produced detectable amounts of ethanol. Certain isolates produced similar amounts of the same VFAs. Based on comparisons of the production of various VFAs, as well as other characteristics (eg. cell morphology, Gram stain, etc.), it appears that the 13 isolates represent seven different bacterial strains. Although more comprehensive

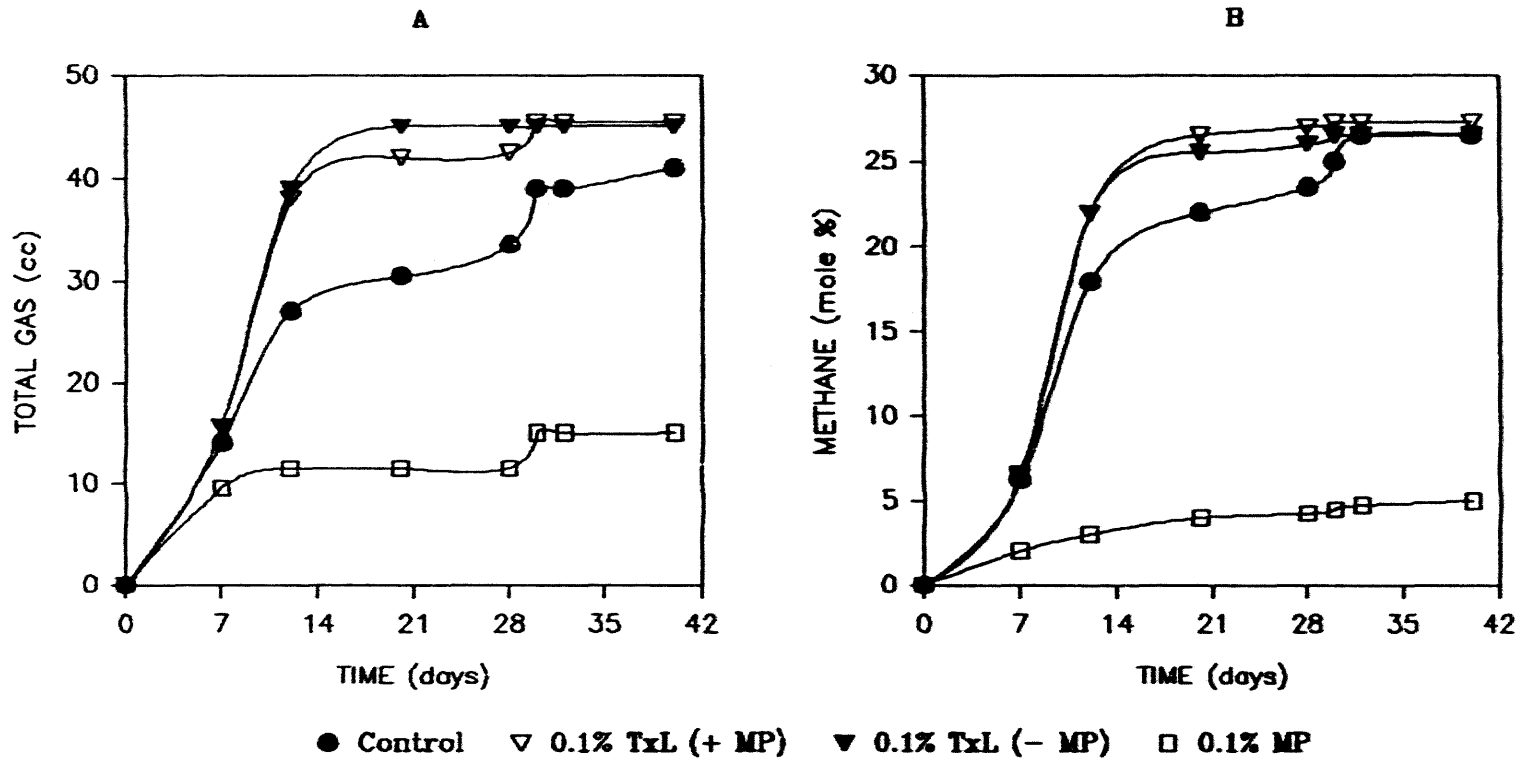

Figure 28. Biogas (A) and Methane (B) Production from Chemically Treated (THF Extracted) Texas Lignite and Mobile Phase (MP) by Mic-1 Consortium.

Table 12. Total Soluble Carbon, Measured as Chemical Oxygen Demand in the Culture Broth of Individual Bacterial Isolates from Mic-1 and Mic-4 Consortia Grown With and Without Texas Lignite

Isolate[a]	COD (ppm)[b]					
	Day 0		Day 7		Day 14	
	With TxL	Without TxL	With TxL	Without TxL	With TxL	Without TxL
M1-1	2,509	2,660	2,553	2,612	2,714	2929
M1-2	2.292	2,354	2,359	2,302	2,207	2,335
M1-3	2,316	2,259	2,288	2,316	2,155	2,264
M1-4	2,297	2,368	2,288	2,316	2,155	2,264
M1-5	2,283	2,420	2,146	2,420	2,127	2,345
M4-1	2,578	2,665	2,412	2,621	2,397	2,568
M4-2	2,273	2,396	2,226	2,382	2,113	2,311
M4-3	2,587	2,699	2,465	2,241	2,470	2,607
M4-4	2,582	2,587	2,241	2,485	2,558	2,568
M4-5	2,660	2,699	2,456	2,641	2,456	2,802
M4-6	2,383	2,675	2,763	2,734	2,480	2,831
M4-7	2,514	2,714	2,251	2,490	2,392	2,458
M4-8	2,555	2,568	2,051	2,646	2,420	2,733

[a] Isolates derived from Mic-1 are designated as M1, and those derived from Mic-4 are designated as M4

[b] Data are average of 2 replicates

Table 13. Concentration of Volatile Fatty Acids and Ethanol in the Culture Broth of Individual Bacterial Isolates from Mic-1 and Mic-4 Consortia Grown on 0.1% Texas Lignite

Isolate	Concentration (ppm)[a] After 14 Days of Incubation				
	Acetic Acid	Butyric Acid	Propionic Acid	Ethanol	Other VFAs
M1-1	268	78	99	ND	204
M1-2	298	ND	32	ND	149
M1-3	291	73	96	ND	211
M1-4	166	88	ND	29	179
M1-5	293	76	92	ND	213
M4-1	404	ND	55	ND	195
M4-2	58	22	ND	27	ND
M4-3	258	ND	120	ND	197
M4-4	176	84	ND	46	152
M4-5	357	ND	38	ND	173
M4-6	272	87	93	ND	215
M4-7	129	104	36	ND	ND
M4-8	64	24	ND	31	ND

[a] Isolates derived from Mic-1 are designated as M1, and those derived from Mic-4 are designated as M4

ND = Non detectable levels

testing is required to confirm this, for the purpose of this project they will be considered as seven distinct bacterial strains (Table 14).

Table 14. Strains Designation to Isolates Obtained from Mic-1 and Mic-4 Consortia

Strain	Isolates[a]	Strain #	Isolates and Sources
1	M1-1, M1-3, M1-5 , M4-6	5	M4-2, M4-8
2	M1-2	6	M4-3
3	M1-4, M4-4	7	M4-7
4	M4-1, M4-5	[a]- Isolates derived from Mic-1 are designated M1, and those derived from Mic-4 are M4-	

Several of the strains were found to be aerotolerant (able to survive exposure to air) rather than strict anaerobes (killed by exposure to air). In order to test whether the TxL biodegradation in to substrates for methanogens is brought about by these, a fresh culture of Mic-1 consortium was aerated. This culture was used to inoculate medium with and without TxL. It was expected that aerating the culture for the inoculum would have eliminated the strict anaerobes, especially the methanogens. This would result in an increase in soluble carbon in the liquid phase because the carbon would not be converted to methane. However, there was no increase in soluble carbon in the cultures containing TxL (Table 15). Surprisingly, methane was produced during incubation. Epifluorescence microscopy confirmed that viable methanogens were still present in the culture. This indicates that the aeration did not completely oxidize the inoculum. Therefore, complete selection of aerotolerant bacteria did not occur.

Table 15. Soluble Carbon Measured as Chemical Oxygen Demand (COD) in the Culture Broth of Aerated Mic-1 Consortium Grown in Presence and Absence of Texas Lignite

Treatment	Chemical Oxygen Demand (in $\mu g\ O_2/L$)		
	Day 0	Day 7	Day 14
With Texas Lignite	1,077.2	1,063.1	537.5
Without Texas Lignite	1,261.8	1,280.8	935.2

During the course of this experiment, in several of the isolated cultures TXL formed large clumps. Although TxL was not degraded, it is possible that TxL was modified by the bacteria. To verify this phenomenon mixed sub-populations, rather than pure isolates need to be incubated with TxL.

D. Bench Scale Bioreactor Studies

The bench scale bioreactor studies were initiated to determine the potential of scaling up the results obtained in the serum vial. At the same time data obtained from these studies were helpful in obtaining the preliminary economic analysis of the MicGAS Process. As outlined in the Materials and Methods Section, four bioreactor configurations were evaluated.

1. Rotating Biological Contactor (RBC). The maximum rate of methane production began at day 4 and proceeded through day 15 (Figure 29). Rates of CH_4 production over this time period ranged from 496 to 603 cc CH_4/ day. Control reactions produced very little methane or CO_2. Total gas production reached 11 liters within 25 days. Methane concentration in the reactor headspace reached 72 mole% and methane accounted for more than 60% of the total gas produced.

In a study by Fluor Daniels, it was pointed out that in order for the MicGAS Process to be commercially feasible, it will be necessary to use solids loadings of 20% or greater. In previous studies, biogasification of TxL at 1% and 5% was demonstrated using a rotating biological contactor (RBC). In a latter experiment this RBC was charged with 10% TxL solids loadings. Initially, methane production rates were high (4800 cc produced during the first 10 days). However, methane production decreased over the next month (Figure 30). Possibly, this decrease in methane production could be due to a loss of methanogens. To test this, fresh Mic-1 was added to the reactor as a source of viable methanogens, and methane production was monitored for another month. However, methane production continued to decline. Operation of this reactor was discontinued after approximately two months.

2. Upflow Fluidized Bed Reactor (UFBR). Initially, significant methane production was observed in all three plexiglass reactors containing 1% TxL (Figure 31). Methane concentrations of 51.96 mol% in the control and 73.21 mole% in the reactor containing 1% TxL were obtained. Higher levels of butyrate and caproate (Figure 32) were observed in the reactor containing 1% TxL. However, similar amounts of acetate (Figure 33A) were measured in both the reactors, and trace amounts of heptanoic acid in the one with 1% TxL. Furthermore, biomass concentration was lower in the control reactor (Figure 33B). Another important observation was that YE/ TSB addition along with TxL was necessary to maintain the CH_4 production. However, addition of all three resulted in an immediate response. On the other hand, addition of YE/ TSB to the control UFBR did not result in any substantial enhancement of CH_4.

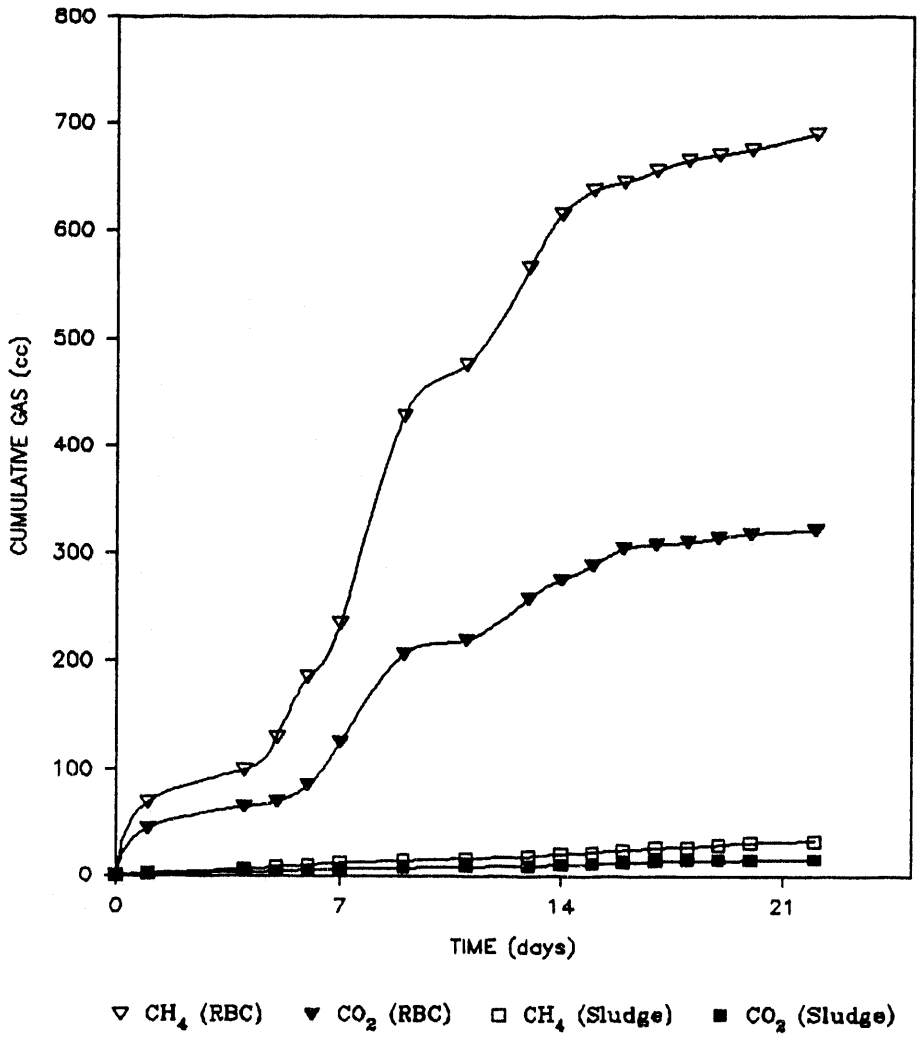

Figure 29. Methane Production in a Rotating Biological Contactor (RBC) with 1% Texas Lignite.

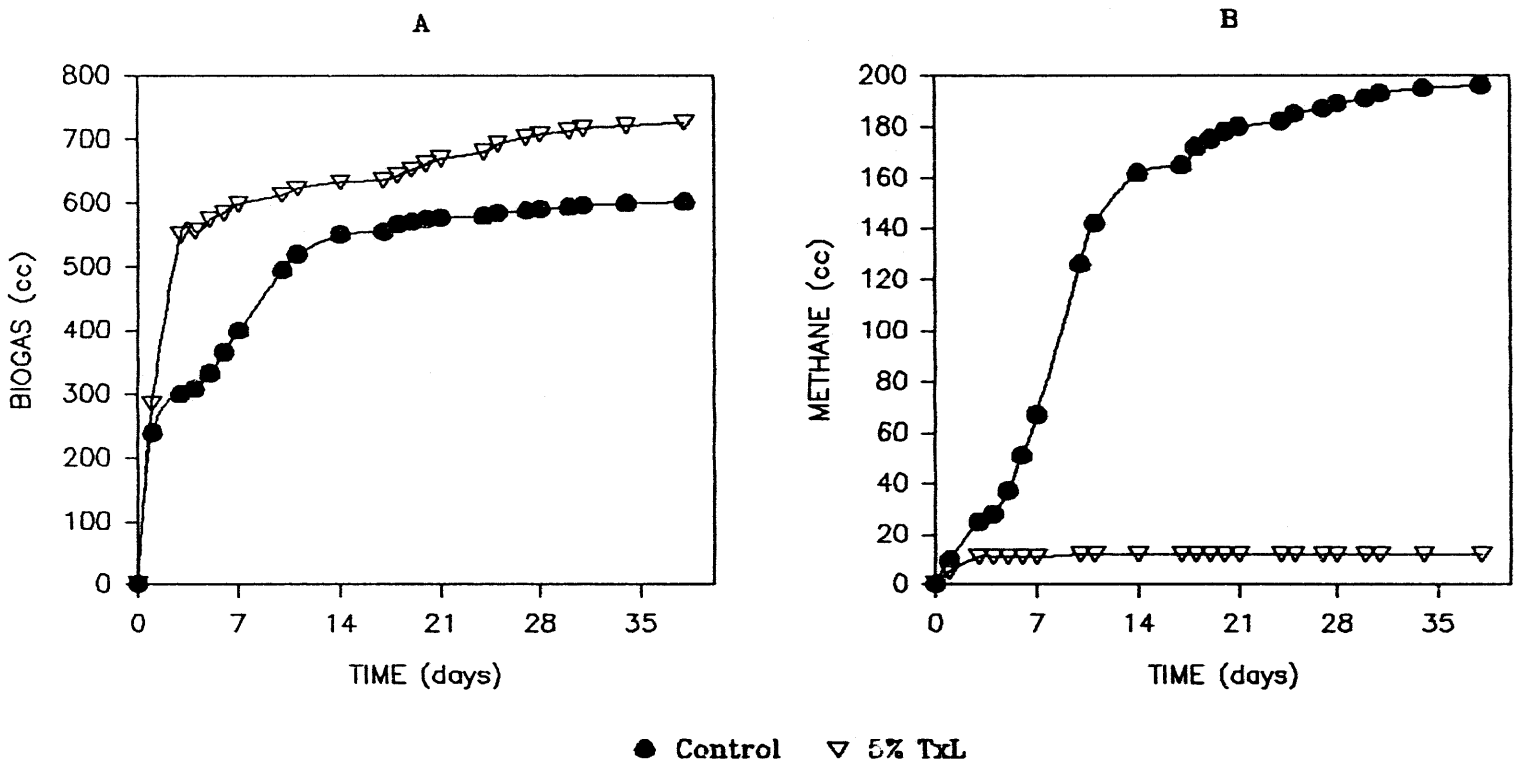

Figure 30. Cumulative Biogas (CH_4 + CO_2) Production (A) and Cumulative Methane Production (B) During Biomethanation of 5% Texas Lignite in a Rotating Biological Contactor (RBC) with 0.2% SNTM.

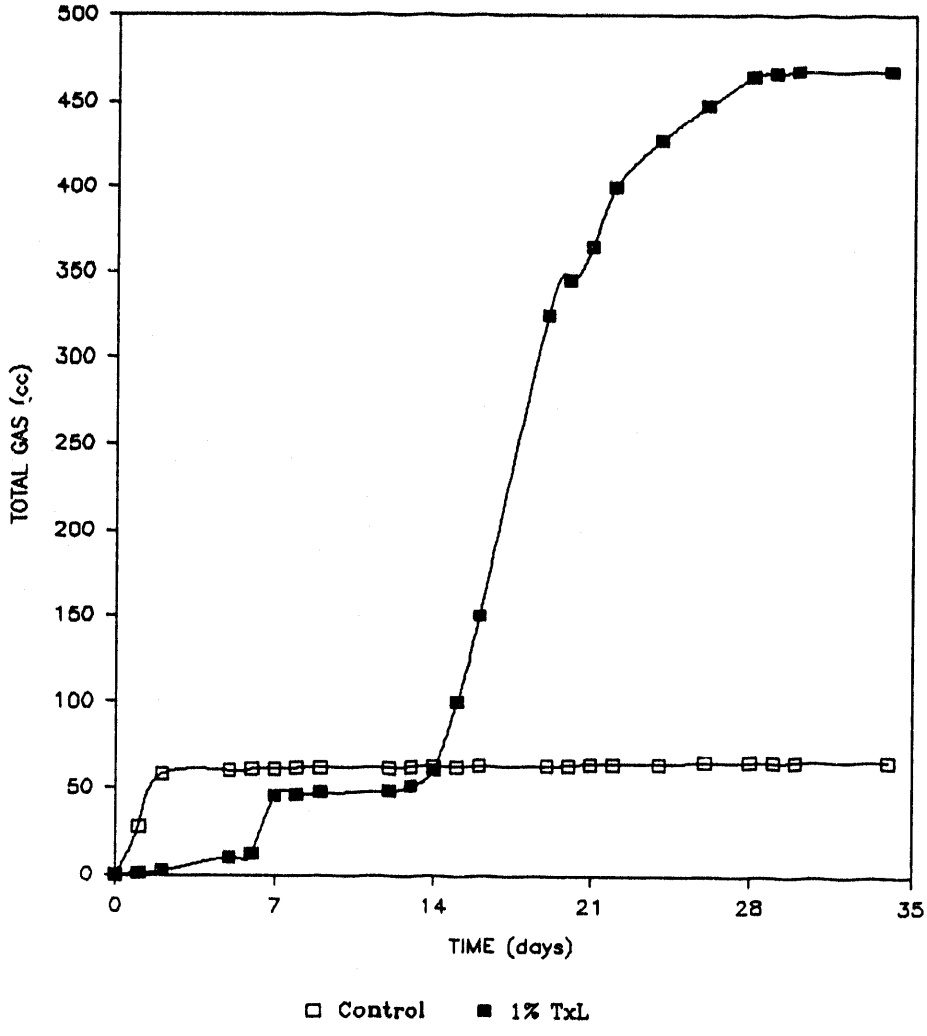

Figure 31. Biomethanation of 1% Texas Lignite in an Upflow Fluidized Bed Reactor (UFBR).

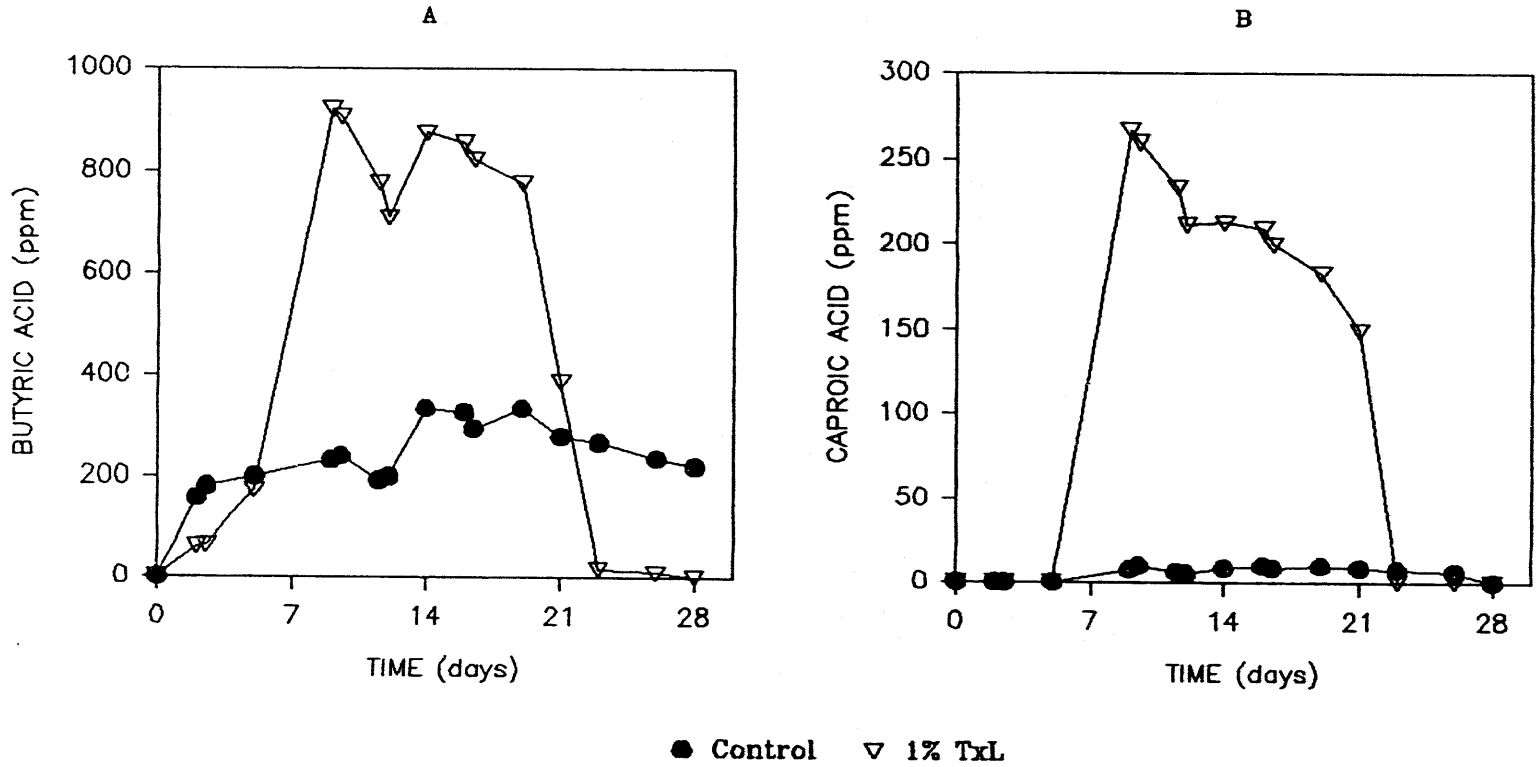

Figure 32. Time Course of Butyric Acid Production (A) and Caproic Acid Production (B) During Biomethanation of Texas Lignite in an Upflow Fluidized Bed Reactor (UFBR).

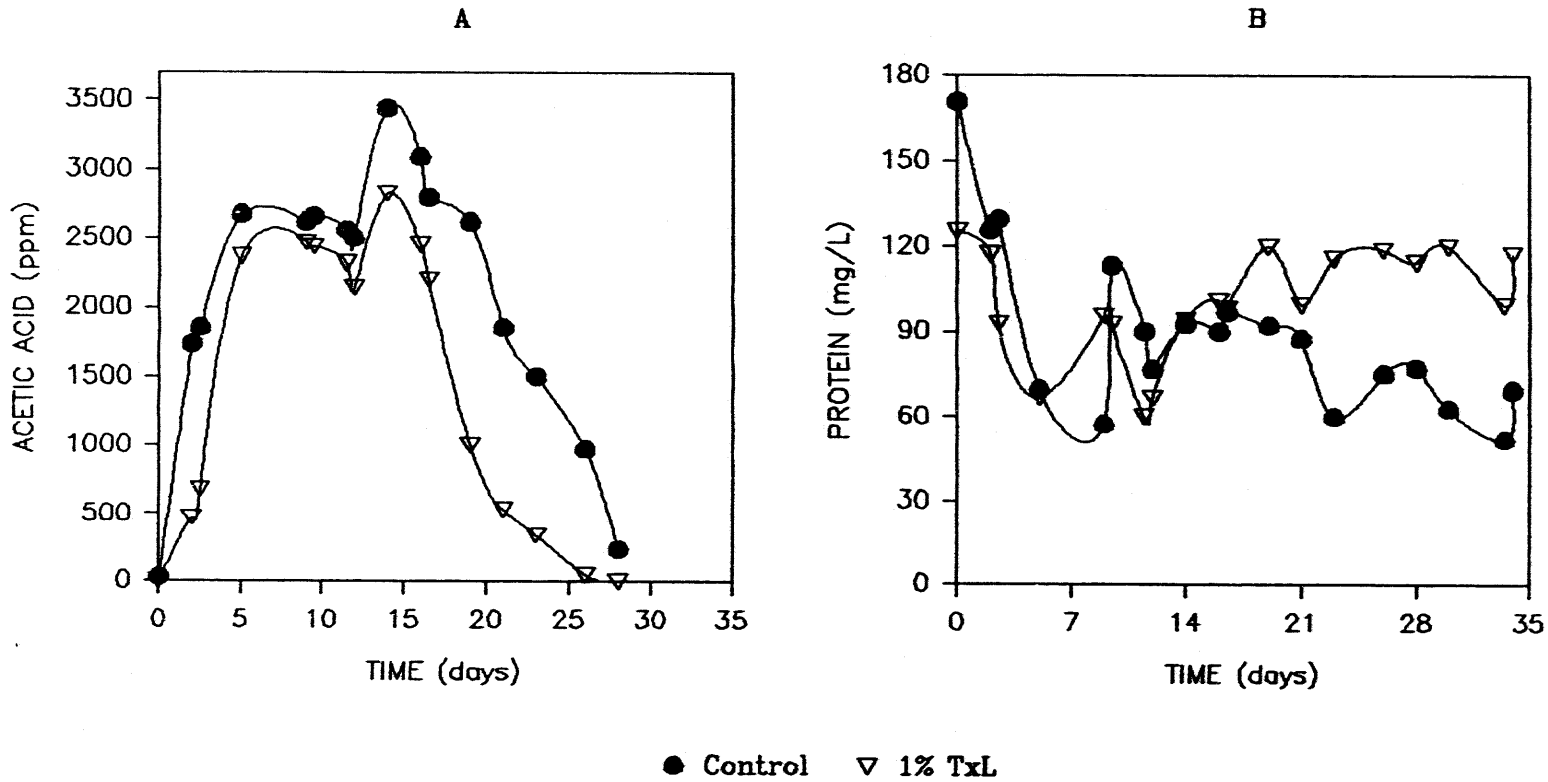

Figure 33. Time Course of Acetic Acid Production (A) and Biomass Production (B) During Biomethanation of Texas Lignite in an Upflow Fluidized Bed Reactor (UFBR).

In a later experiment, 5% TxL was loaded in to the glass UFBR. Methane concentrations of 48-50 mole% were present in the headspace gas after 4 days of incubation. However, gas production decreased rapidly after this time. It is hypothesized that the circulated headspace gas was not able to adequately mix the large mass of TxL. This was further evidenced by the fact that one of the reactors containing TxL had to be discontinued because the lower circulation port became clogged with TxL. After 1 month of operation, these bioreactors were stopped. These reactors were discontinued after approximately one month.

Influence of adding the fresh anaerobic sewage sludge on biomethanation of TxL with and without Mic-1 addition was also studied in these reactors. In general, enhanced methane production was observed by adding the TxL. The methane production was even higher when the headspace gas was recirculated (Figure 34). This set of data also brings out the point that for higher production of a given product, specifically developed microorganisms provide higher process efficiency than the natural microbiota. Further the complexity of TxL as a biomethanation substrate can be very well understood from this piece of information.

In the next experiments, influence of higher TxL solids loadings on biomethanation in UFBR was examined. There was little difference in total gas production between the two reactors containing TxL (Table 16). The methane concentration in the headspace was slightly higher in the reactor containing 5% TxL (18.2 mole%) than in the reactor containing 10% TxL (16.6 mole%). The carbon dioxide concentration was also highest in the reactor containing 10% TxL. The control (no TxL) reactor produced the lowest volume of total gas and contained the lowest concentrations of methane and carbon dioxide. The lower methane production in the reactor containing 10% TxL may be due to the lower pH (6.7) of the liquid phase, as mesophilic methanogens are generally inhibited by low pH. The low pH was probably due to the greater carbon dioxide concentration.

Table 16. Gas Production and pH in Upflow Reactors After 12 Days of Incubation

Treatments	Total Gas (cc)	CH_4 (mole %)	CO_2 (mole %)	pH
Without Texas Lignite (Control)	122	6.6	5.8	7.6
5% Texas lignite	228	18.2	9.7	7.3
10% Texas lignite	237	16.6	16.1	6.7

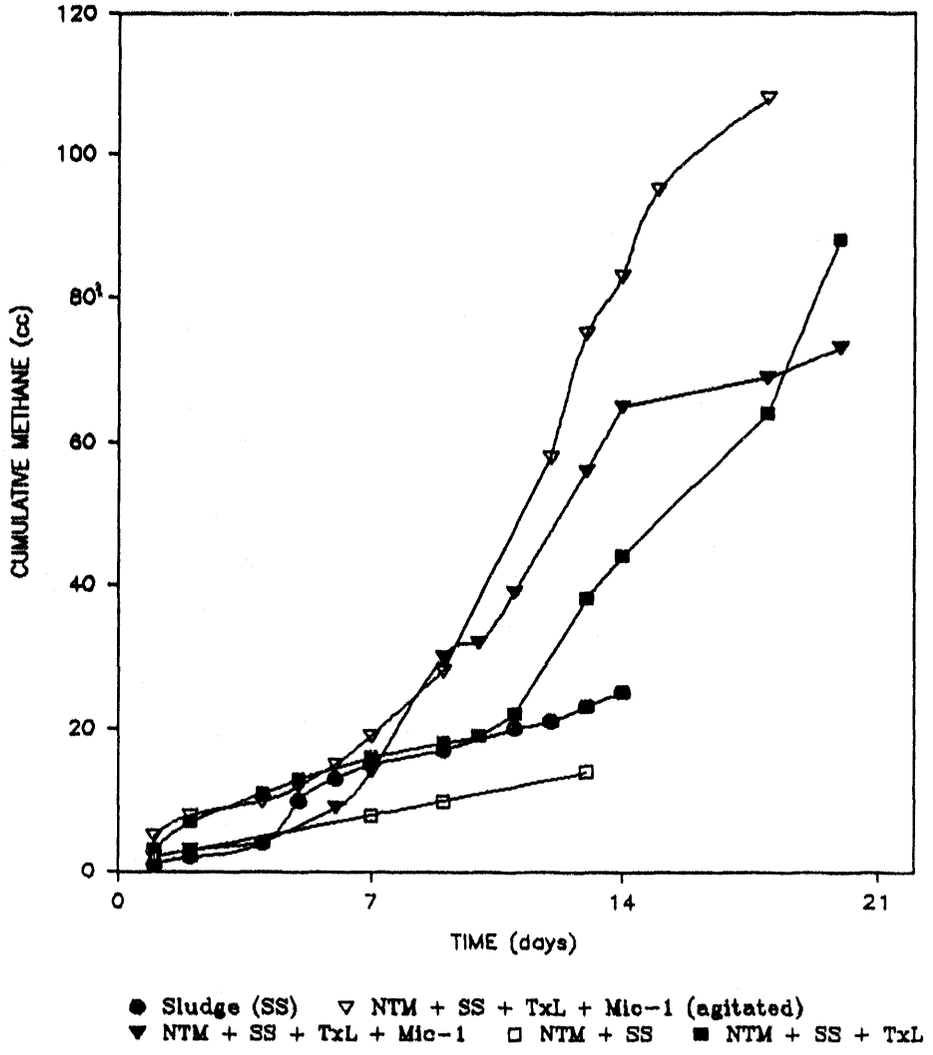

Figure 34. Influence of Anaerobic Sewage Sludge (SS) on Biomethanation of Texas Lignite in an Upflow Fluidized Bed Reactor (UFBR).

The data presented in Table 16 indicates that acetate was degraded into CO_2 but there was not enough hydrogen to carry out the reaction to convert CO_2 to CH_4. This data confirmed our previous results from laboratory scale reactors, that at TxL solids loadings higher than 1%, methane production does not enhance significantly. This phenomena could be due to the production of higher quantities of inhibitory compounds or depletion of factors necessary for methanogenesis.

The UFBR were recommissioned to study the effect of methanol (CH_3OH) as hydrogen donor. The data showed that total gas and cumulative methane production in the head space of reactor with CH_3OH were highest (2321 cc and 1118 cc, respectively). The peak in methane production during the first 10 days (Figure 35A) could be explained as the initial effect of addition of easy to metabolize co-substrate. Thus, in this reactor, the higher methane production observed during the latter part of the experiment was due to biogasification of TxL solids loadings. This was confirmed by comparing the volumes of cumulative methane produced in the reactor that did not contain CH_3OH and the one that did (Figure 35B). Total gas production was similar in reactors without TxL (containing only 0.2% SNTM) and the one that contained only 5% TxL in 0.2% SNTM, but methane production was observed only in the UFBR containing TxL. The net methane production was highest (1344 cc) in the one containing TxL + CH_3OH but significantly lower ($\sim$ 93 cc) in the one without CH_3OH. Differences in microbial growth and soluble carbon were not significant. As compared to other reactors, the addition of extra NH_4Cl to 0.2% SNTM showed significant increase in acetic, isobutyric, butyric and isovaleric acids production in the control reactor. Acetic and propionic acids were higher in the reactor with TxL + CH_3OH (Figure 36), while in the one without CH_3OH, the quantity of all VFAs was lower and isobutyric and butyric acids were not observed (Figure 37).

To evaluate the effect of solids loadings on survival of methanogens, fresh inoculum (10%, v/v) containing mixture of known methanogens was added on day 25 to provide viable methanogens. Methane production (337 cc) increased only in the reactor that contained 5% TxL (Figure 35B).

3. Trickle-Bed Reactor (TBR). Within five days of operation maximum (535 cc) gas production was observed in the reactor with constant recycle. After this time the gas production was not significant (Table 17). The control reactor, with no pumped recycle, produced 302 cc of gas over 5 days and continued to produce gas at low levels over 32 days with the headspace methane concentrations exceeding 56 mole%. These data indicate that pumping of the culture broth decreases biogasification efficiency and that a different pumping system must be put in place. It is noted, however, that biogasification does take place even at 30% TxL solids, suggesting that bioconversions at high solids loadings are possible.

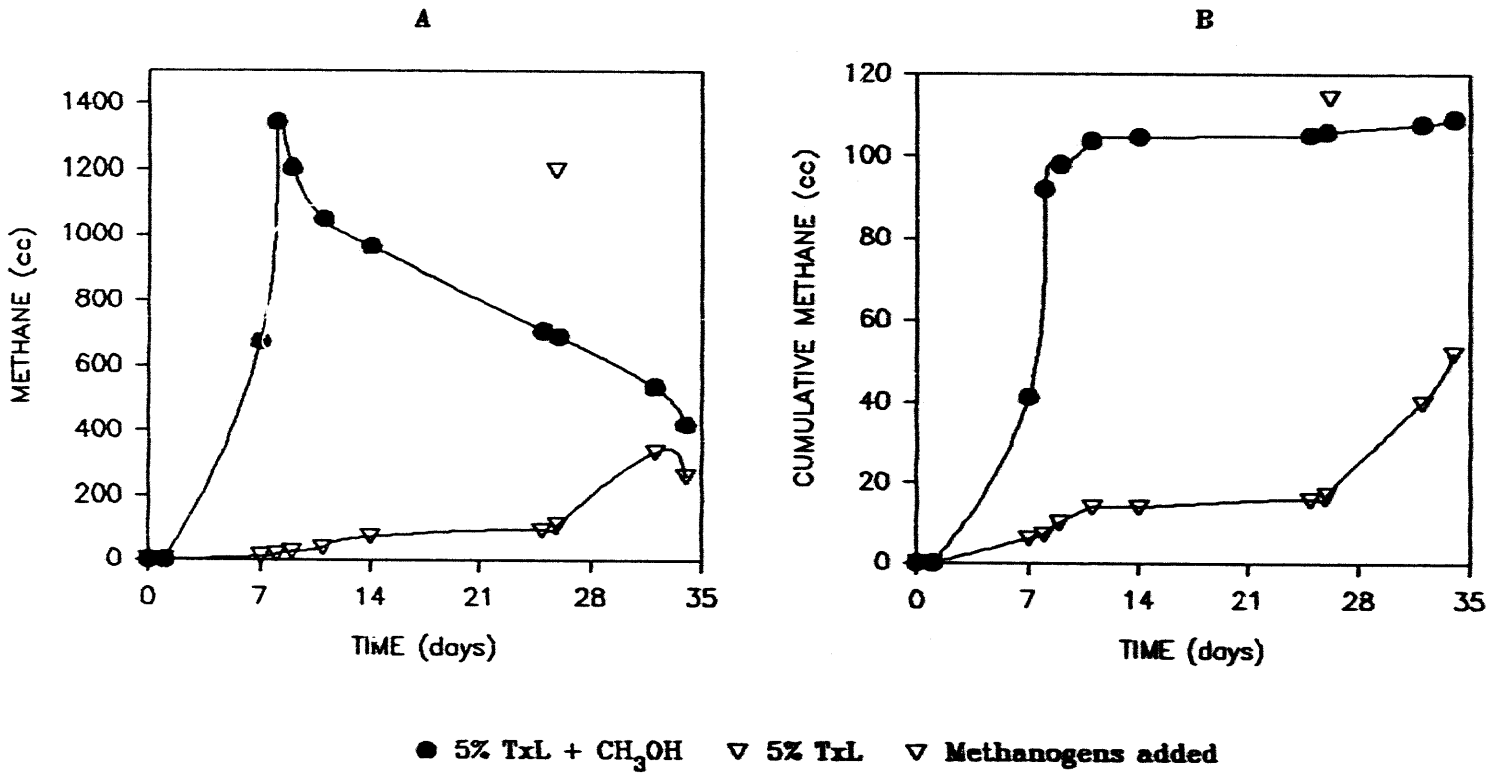

Figure 35. Actual (A) and Cumulative (B) Methane Production During Biomethanation of 5% Texas Lignite by Mic-1 Consortium in an Upflow Fluidized Bed Reactor (UFBR) with 0.2% SNTM + 4 x NH_4Cl (32 g/L). Methane Produced in Control Deducted.

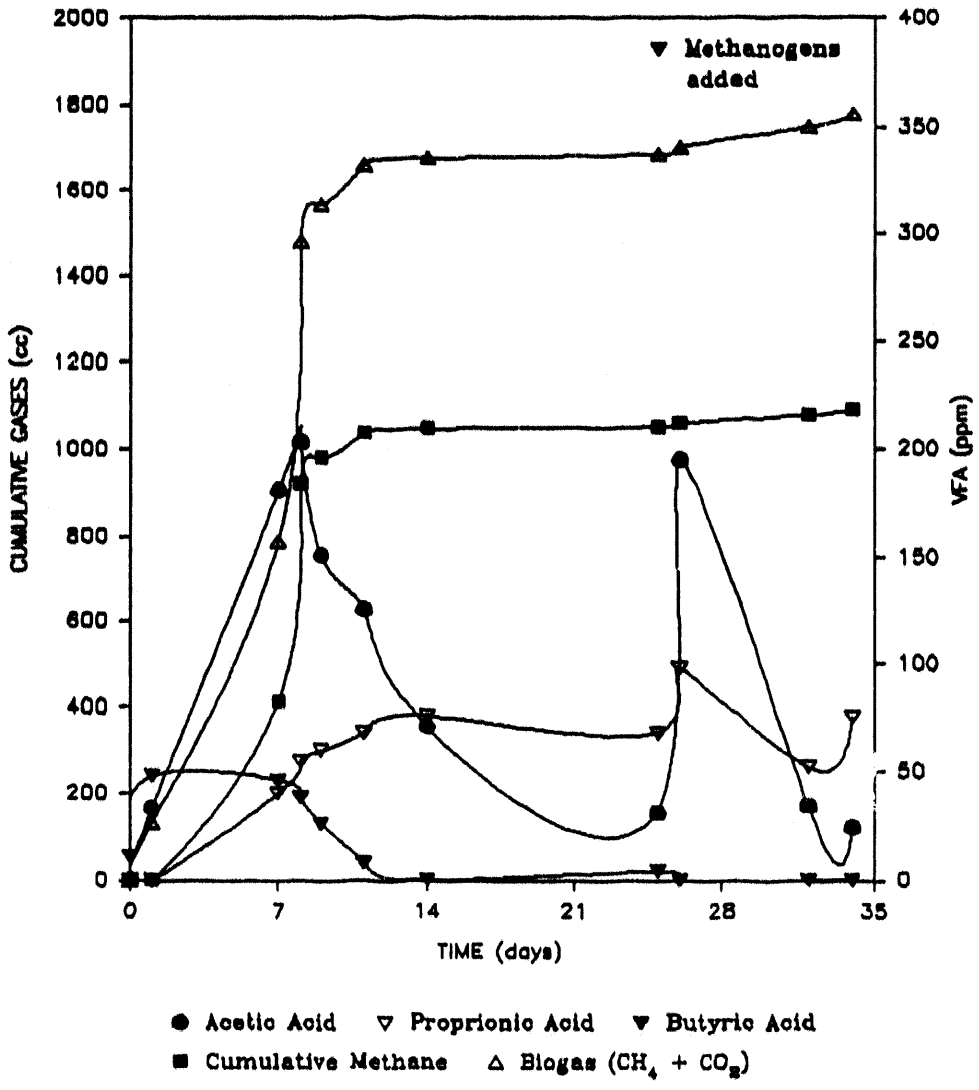

Figure 36. Biogas and VFA Production During Biomethanation of 5% Texas Lignite by Mic-1 Consortium in an Upflow Fluidized Bed Reactor (UFBR) with 0.2% SNTM + 4 x NH_4Cl (32 g/ L) + 0.5% CH_3OH.

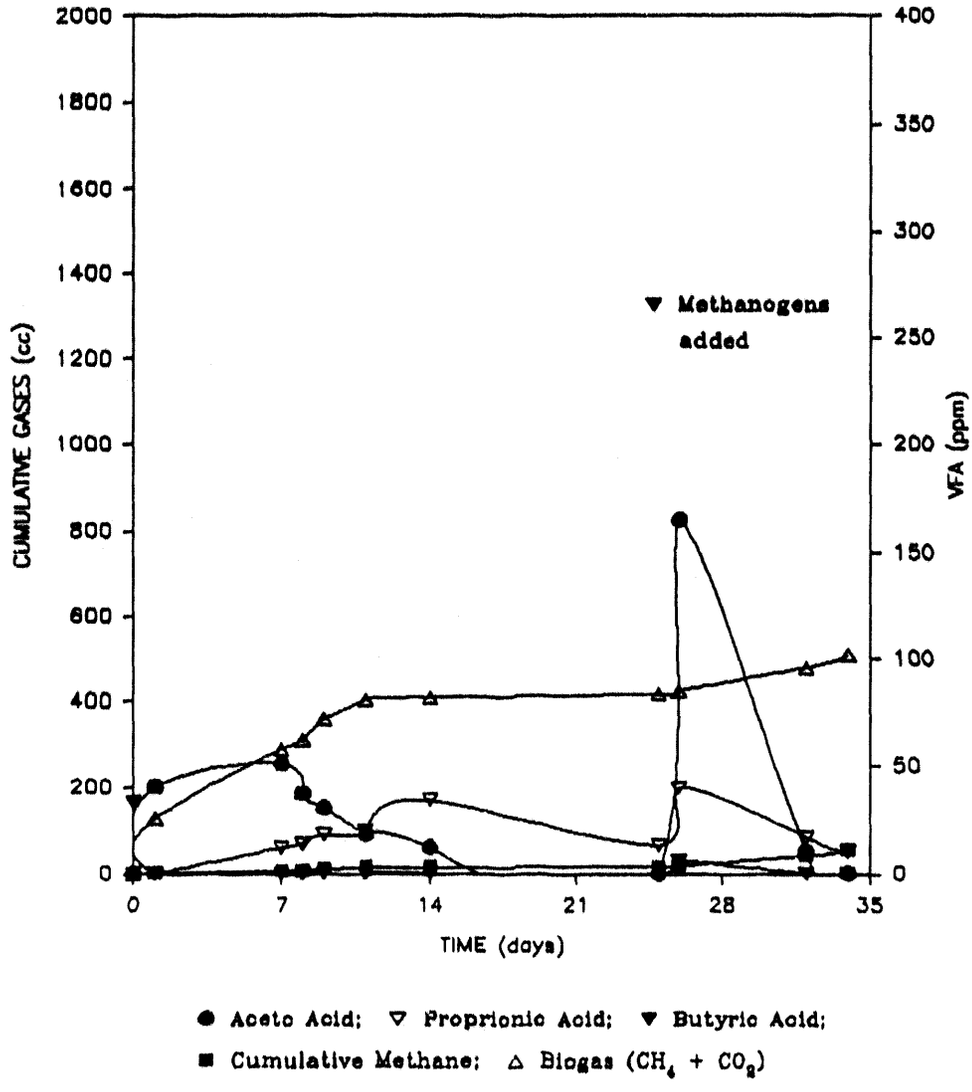

Figure 37. Biogas and VFA Production During Biomethanation of 5% Texas Lignite by Mic-1 Consortium in an Upflow Fluidized Bed Reactor (UFBR) with 0.2% SNTM + 4 x NH_4Cl.

Table 17. Methane Production and Texas Lignite Conversion During Operation of Various Bioreactor[a] Configurations

Bioreactor Type	Mode of Operation	Total Volume (L)	Head Space (L)	Total Gas Produced (L)	Total CH_4 (%)	Solids Loadings (%)	TxL Conversion (%)[c]
Rotating Biological Contactor	Batch	7.60	0.70	11.0	73.0	1	21.600
Upflow Fluidized Bed	Batch:						
	Liquid recycle	0.68	0.08	0.007	51.9	1	0.170
	Gas Recycle	0.68	0.14	0.465	73.2	1	8.410
Trickle Bed	Batch with Liquid Recycle	0.68	0.08	0.540	24.0	30	0.054
Continuously Stirred Tank	Batch	2.00	0.85	2.2[b]	57.7	1 or 5	23.100

[a] - All the reactors were run at 1% solids loadings
[b] - Calculated value based on the total head space volume and measured N_2
[c] - According to the data presented in Table 18

4. Tank Reactors. As outlined earlier, stirred tank, simulated tank, and simulated chemostat reactors were used.

a. Continuously Stirred Tank Reactor (CSTR). Parameters of methane production were a little bit difficult to measure in this reactor because of the multiple inlet and outlet lines. However, biomethanation of 1% TxL was calculated based on the total headspace volume and changes in gas composition over time. Nitrogen was used as the basis in all the determinations.

The data obtained showed the highest methane production in this reactor (Table 18). Nevertheless, this experiment needs further evaluation once all other parameters that are currently being evaluated have been completed.

b. Simulated Anaerobic Chemostats (SAC). Bottle reactors, simulating tank reactors were set up to monitor methane production from sewage sludge, 0.2% SNTM + sewage sludge and 0.2 % SNTM + sewage sludge + TxL by the indigenous sewage sludge microorganisms. In addition, bottle reactors were monitored for methane production when medium, sewage sludge, TxL and Mic-1 were incubated under static and agitated conditions. Data obtained from these experiments illustrated that little methane was produced from sewage sludge or from sewage sludge + 0.2% SNTM. Significant CH_4 production took place only in presence of TxL. The maximum rate of methane production (7.4 cc CH_4/ day) was observed between day 11 and day 20 when only sewage sludge microorganisms were present. The addition of Mic-1 resulted in earlier initiation of maximum methane production rates (7 days vs. 11 days) when TxL was present (data not presented). Agitation of the reaction

Table 18. Ultimate Analysis of Texas Lignite Solids Before and After Bioconversion to Methane by Mic-1 Consortium				
TxL Component	Amounts (%) Present		Change[a]	
	Before Biogasification	After Biogasification	Fold	%
Ash	14.0	31.8	+ 2.27	+ 127.0
Carbon	62.9	42.9	- 1.46	- 31.8
Hydrogen	4.5	4.0	- 1.13	- 11.1
Nitrogen	1.3	2.4	+ 1.85	+ 84.6
Oxygen (by difference)	16.1	18.1	+ 1.12	+ 12.4
Sulfur	1.2	0.9	+ 1.12	- 25.0

[a] - = decrease
+ = increase

mixture enhanced the rate of methane production from 7.4 to 8.6 cc CH_4/ day. Total methane as well as total gas produced increased by 41% and 35% respectively when the reaction mixture was incubated with agitation. These data indicate the need for gentle agitation to enhance microbial/ TxL contact to maximize methane production.

c. Simulated Tank Reactors (STR). The influence of NH_4Cl on microbial production of VFAs and CH_4 was studied in chemostat cultures containing 0% and 10% TxL (Figures 38 and 39). Gas analysis of the headspace showed high CO_2 production (513 cc) in experiments with 10% TxL and extra NH_4Cl (Figure 38A). However, lower methane production was observed compared to the ones that did not contain TxL (Figure 38B). This is yet another confirmation of our hypothesis that at higher TxL concentrations the CO_2 production is enhanced. The control (no TxL) reactor produced lower volume of total gas and contained low concentrations of methane and carbon dioxide (data not shown). The lower methane production in the 10% coal reactor may be due to the lower pH (6.5) of the liquid phase, as methanogens are generally inhibited by low pH. The low pH is probably due to the higher carbon dioxide concentration.

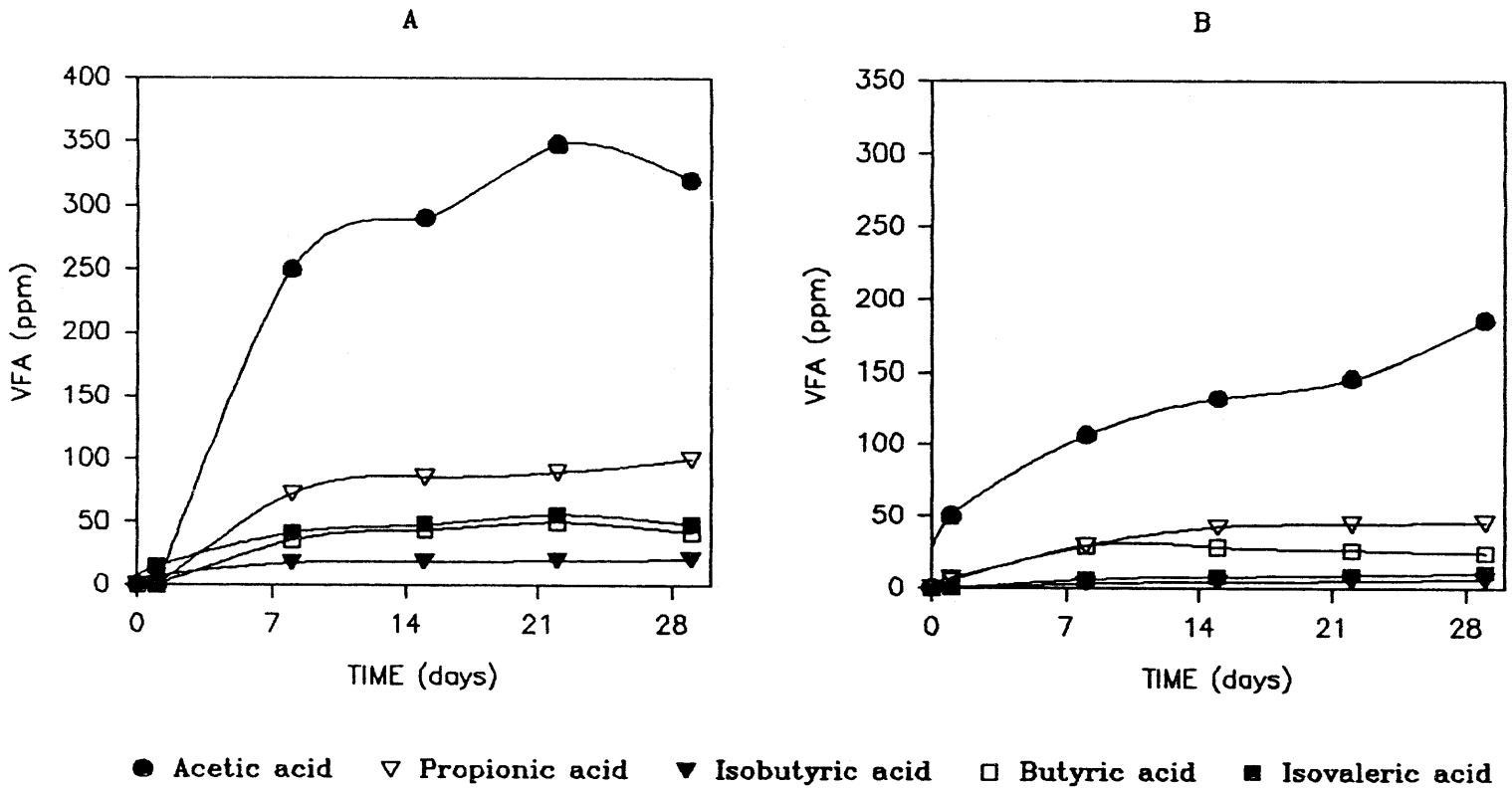

Figure 38. Production of VFA in Simulated Tank Reactors with 0.2% SNTM + 4 x NH_4Cl (32 g/ L) (A) and 0.2% SNTM + 4 x NH_4Cl (32 g/ L) + 10% Texas Lignite (B).

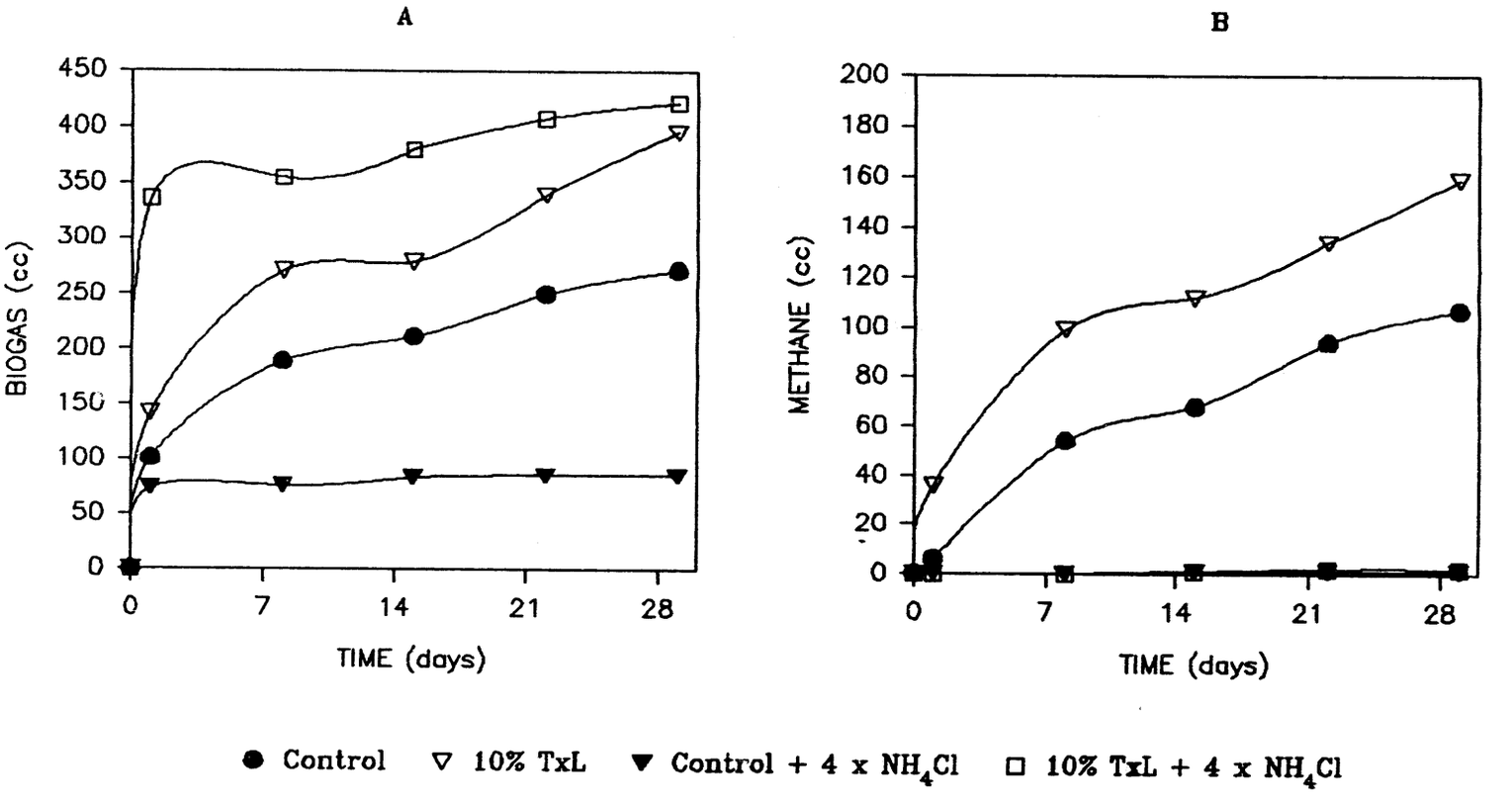

Figure 39. Biogas (A) and Methane (B) Production During Biomethanation of 10% Texas Lignite in Simulated Tank Reactors with 0.2% SNTM + 4 x NH_4Cl (32 g/L).

IV. MAJOR ACCOMPLISHMENTS

The Major accomplishments during this project period are summarized below:

- Enhanced metabolic activity of termite derived Mic-1 cultures for methane production. A five fold enhancement was observed.

- Reduced fermentation time from 60 days to 21-28 days for maximum methane production.

- Established that principal bacterial groups in Mic-1 consortium are: Primary coal degraders, Hydrocarbon degraders, acedogens and methanogens.

- Established that the limiting factors at solids loadings >1% are:
  - pH of the medium,
  - inhibitory product(s) formation, and
  - rate and extent of coal degradation.

- Sequestering agents (10 mM citrate, 1 mM ammonium oxalate) enhanced methane production by 23%, but at 10% solids loadings the methane production was relatively inhibited even in presence of citrate.

- Aluminum enhances, but magnesium inhibits biomethanation of lignites.

- Agitation of static cultures does not significantly enhance the methane production. However fluidization in a UFBR does.

- Addition of co-substrate like YE/ TSB required for higher reaction rates.

- Methane production at low rates from up to 20% solids.

- H_2S production was seldom observed, needs to be confirmed.

- Established a formula to evaluate the efficacy and cost effectivity of co-substrates like Sheftone-T™.

- A cost effective culture medium (SNTM) has been developed by replacing YE/ TSB in the NTM and omitting vitamin solution.

- Seven major group of acedogens have been isolated as homogeneous cultures from Mic-1 and Mic-4 consortia.

- Four bench scale bioreactor configurations (RBC, UFBR, TBR, and CSTR) were evaluated. Methane production was highest in CSTR and lowest in TBR.

- Highest coal carbon conversion (up to 36%) was obtained in the continuous stirred tank reactor.

- Methanol acts as a H_2 donor and enhanced five fold methane production when added at 0.5 % to a reactor containing 5% TxL solids loadings.

- The mass and electron balance calculations indicate a net enhancement in methane production from TxL carbon, not from other NTM or 0.2% SNTM components.

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VI. PLANNED RESEARCH

The extention tasks on this project will continue to examine:

1. Enhancement of methane production by improving Mic-1 consortium.

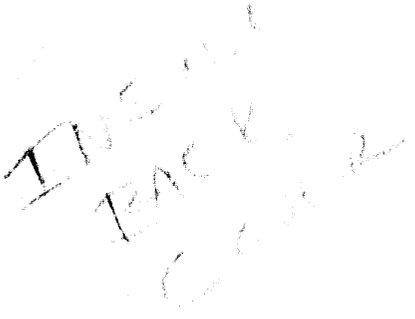
2. Develop an alternate culture as back-up for the MicGAS Process.

3. Effect of medium components (macronutrients, pH, hydrogen donor, etc.).

4. Effect of substantiating the SNTM with metal salts known to enhance biogasifcation.

5. Bench scale bioreactor studies.

6. Develop the solid residues from the MicGas Process into a value added product.



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