Report No. PETC 22-91

DOE/PC/90051--T2

DE92 010844

ANAEROBIC BIOPROCESSING OF LOW-RANK COALS

CONTRACT NO. DE-AC22-90PC90051

PROGRESS REPORT

October 1 - December 31, 1991

Mahendra K. Jain
Ramani Narayan
Ohantaek Han

Michigan Biotechnology Institute
3900 Collins Road
P. O. Box 27609
Lansing, Michigan 48909

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

JAN 3 0 1992

QUARTERLY PROGRESS REPORT

Anaerobic Bioprocessing of Low Rank Coals

Contract No. DE-AC22-90PC90051

October 1 - December 31, 1991

INTRODUCTION

The overall goal of this project is to find biological methods to remove carboxylic functionalities from low-rank coals under ambient conditions and to assess the properties of these modified coals towards coal liquefaction. The main objectives for this quarter were: (i) continuation of microbial consortia development, (ii) evaluation of the isolated organisms for decarboxylation, (iii) selection of best performing culture (known cultures vs. new isolates), and (iv) coal decarboxylation using activated carbon as blanks. The project began on September 12, 1990.

PROGRESS REPORT

Continue Coal Decarboxylation and Development of Microbial Consortia

1. Batch Fermentation System

Fresh rumen fluid was obtained from a dairy farm of Michigan State University. Three batch fermentor systems were started using the rumen inoculum to obtain new microbial consortia and to decarboxylate coal in PBB medium containing 0.05% of yeast extract. Nitrogen gas was purged through #7 fermentor while a mixture of 5% H_2 and 95% N_2 was purged through #8 fermentor to minimize possible loss of hydrogen during the decarboxylation of coal and to find any beneficial effects of H_2 enrichment on the quality of biotreated coals. When compared to adapted inoculum in terms of total gas production, rumen fluid itself may not be a good inoculum for coal decarboxylation (Figures 1 and 2). The presence of H_2 gas in batch #8 also reduced total CO_2 production compared to batch #7 without H_2 gas. This could be due to formation of CH_4 by methanogens present in fresh rumen inoculum using CO_2 and H_2. Since rumen fluid was found to contain 3.84% of dry matter, it is expected that elemental composition of the rumen inoculum could affect the final elemental composition of biotreated coals. Therefore, we

analyzed the CHN contents of dried rumen inoculum for C (41.55%), H (6.02%), and N (4.98%) which showed about five times higher level of nitrogen than that of coal. These results indicate that increase in final nitrogen content of the biotreated coal is mainly due to rumen inoculum because it was difficult to remove the rumen solid particles from the biotreated coal before analysis. As seen in Table 1, mixed gas environment gave a slightly higher H/C ratio than only N_2 gas environment, suggesting possible beneficiary effect of 5% H_2 gas on coal quality during anaerobic biotreatment. Therefore, two additional batch fermentor systems, #10 and #11, containing 5% coal (m.f.), 0.2% sodium succinate and 0.1% yeast extract were completed under the same gas phase conditions used in #7 and #8, respectively, to verify this result and to maximize the coal decarboxylation using an adapted microbial consortium (RW71C-5A) instead of rumen inoculum. Characterization of these biotreated coals is in progress.

Table 1. CHN Contents and H/C Ratios of Biotreated Coals from #7 and #8 Fermentor Systems.

Sample	% C	% H	% N	H/C ratio (% increase)
Control coal	66.68±0.27	4.85±0.078	0.88±0.072	0.872 (-)
#7 Fermentor	63.34±0.23	4.73±0.028	1.21±0.21	0.895 (+2.6)
#8 Fermentor	63.56±0.17	4.76±0.014	1.20±0.035	0.899 (+3.0)

2. Enrichment of Microbial Consortium in Anaerobic Vials

In addition to bioreactor systems, anaerobic vials were also used to enrich and develop various microbial consortia for coal decarboxylation in media containing different carbon and nitrogen sources with or without 5% coal. The CO_2 and CH_4 production by enrichment cultures for new microbial consortia on day 48 is shown in Table 2. In the presence of benzoate, the gas production was greatly reduced due to severe growth inhibition of microbial population and possibly due to low density of benzoate utilizing organisms. Large increase in gas production from coal was observed in only one condition (named as "microbial consortium LC"), where the consortium utilizes lactate as carbon/energy source and NH_4Cl as nitrogen source. The results indicate that this new consortium utilizing coal as the substrate could be grown in a chemically

Table 2. Enriched Microbial Consortia Obtained from Fresh Rumen Inoculum in Anaerobic Vials.

Medium*	CO_2 (%)	CH_4(%)	Total (%)
Control (PBBM + inoculum)	2.14	5.07	7.22
+ 5% Coal	2.10	3.31	5.41
+ 0.2% Sodium Benzoate + BES**	1.11	0	1.11
+ Benzoate + BES + Coal	1.39	0	1.39
+ Benzoate	0.89	0.62	1.51
+ Benzoate + Coal	1.69	1.50	3.19
+10% H_2 gas	2.22	5.33	7.55
+ H_2 + Coal	1.69	6.86	8.55
+ 0.2% Sodium Lactate	10.92	40.37	51.29
+ Sodium Lactate + Coal	**12.87**	**41.25**	**54.11**
+ 0.2% NH_4Cl	1.69	3.69	5.38
+ NH_4Cl + Coal	2.04	2.83	4.87
+ 0.2% S. Succinate + 0.05% Yeast Extract	2.19	4.83	7.03
+ Succinate + Yeast Extract + Coal	2.39	3.78	6.18

* Nitrogen source was 0.2% NH_4Cl except where 0.05% of yeast extract was added.
** Antimethanogenic agent.

NOTE: No succinate and yeast extract were added to the control tubes.

defined medium. Therefore, microbial consortium LC is currently being subcultured as one of main inocula for coal decarboxylation.

Evaluation of Isolated Organisms for Decarboxylation and Selection of Best Performing Culture (Known vs. New Isolates)

A total of 11 isolates with high decarboxylation activity were selected for examination of their coal decarboxylation potential. These were subcultured in PBB medium containing 0.4%

glucose and 0.2% yeast extract to get high cell density to allow substantial coal decarboxylation. Two of these isolates, NHC and LC, were obtained recently from new microbial consortia enriched from fresh rumen inoculum. Among these 11 isolates, six isolates showed good growth after three days and the remaining took more than 5-7 days. In parallel, two known cultures, *Veillonella alcalescens* and *Propionibacterium acidipropionici*, were also grown in their respective medium. Three-day old cultures of two knowns and six fast growing isolates were used as the inoculum for coal decarboxylation in PBB medium containing 0.4% sodium succinate and 0.2% yeast extract. The results presented in Table 3 indicate that *Veillonella alcalescens* exhibited highest net decarboxylation activity, followed by isolates #8 and LC. Five slow-growing isolates were also compared for their coal decarboxylation ability in the same manner (Table 4). As observed in previous reports, total gas or net gas production from coal by known cultures or isolates was much less than that by microbial consortia, suggesting that decarboxylation of coal probably involves coordinated activities of various microorganisms in a manner similar to that observed in anaerobic degradation of organic materials. Therefore, *V. alcalescens* was selected as the primary candidate for enzyme (decarboxylase) study and isolates #8 and LC will be used for comparison experiment, if necessary.

Table 3. Comparison of Net % CO_2 Production from Coal by Two Known Cultures and Six Isolates.

Culture	On Day 1	On Day 5	On Day 10
P. acidipropionici	-0.07	0.22	0.19
V. alcalescens	-0.05	**2.18**	**2.24**
#1	0.28	0.15	0.16
#8	0.74	**1.01**	**1.17**
#9	0.13	-1.33	-0.65
NHC	-0.05	0.32	0.71
LC	0.26	**0.98**	**1.04**
MIX*	0.24	0.41	0.51

* Mixed culture of 11 isolates

Table 4. Comparison of Net % CO_2 Production from Coal by Five Slow Growing Isolates

Isolates	On Day 1	On Day 6
R623	0.11	-0.24
#3	0.15	-1.16
CON	-0.23	0.08
A2	0.34	0.03
E4	0.19	0.69

Decarboxylation Experiments Using Activated Carbon as Blanks in Anaerobic Tubes Using Microbial Consortia

During the meeting with Dr. Farcasiu and Dr. Rao (November 12-13, 1991) at MBI, several suggestions were given to modify the work plan of this project. One of the suggestions was regarding use of activated carbon as the blank in order to
(i) find out if there is really a decarboxylation of coal, (ii) to provide increased surface area, and (iii) to provide carbon for CO_2 formation. Accordingly, we initiated the experiment using activated carbon (100 mesh, Aldrich Chemical Cat. # 24,2276) as blanks for coal decarboxylation in anaerobic tubes. Two microbial consortia, RW71C-5B and #34C-2, were used as the inoculum @ 5%. To PBB medium, 0.2% of sodium succinate and 0.1% of yeast extract were supplemented in the presence or absence of coal or activated carbon. Tubes containing no coal and no activated carbon were used as controls after inoculation with the consortium. As seen in Figures 3 and 4, the gas production from medium containing activated carbon was significantly lower than the control (no coal) and the coal containing microbial consortia throughout the biotreatment period. Highest net gas production with microbial consortium #34 was achieved on day 14, while with consortium RW on day 28. These results also indicate that these two consortia probably have distinct populations and, therefore, exhibit differences in the time required for decarboxylation activity. A second set of experiments using acid (3N HCl) washed activated carbon also showed similar results (Figures 5 and 6). These results show that these microbial consortia produced CO_2 from coal probably by decarboxylation as almost insignificant

amount of CO_2 was produced from inert substances such as activated carbon. Decarboxylation experiment using proper model polymeric materials with carboxyl groups is being initiated.

SIGNIFICANT ACHIEVEMENTS

1. Completed the evaluation of available known cultures and new isolates for their coal decarboxylation potential.
2. Selected *Veillonella alcalescens* as the best decarboxylating culture along with isolates #8 and LC as secondary candidates.
3. Demonstrated that adapted microbial consortia produced CO_2 only from coal and only insignificant amount from activated carbon.
4. Enriched a new microbial consortium LC that can be grown in a chemically defined medium.

Figure 1. Profile of carbon dioxide production from #7 batch fermentor system

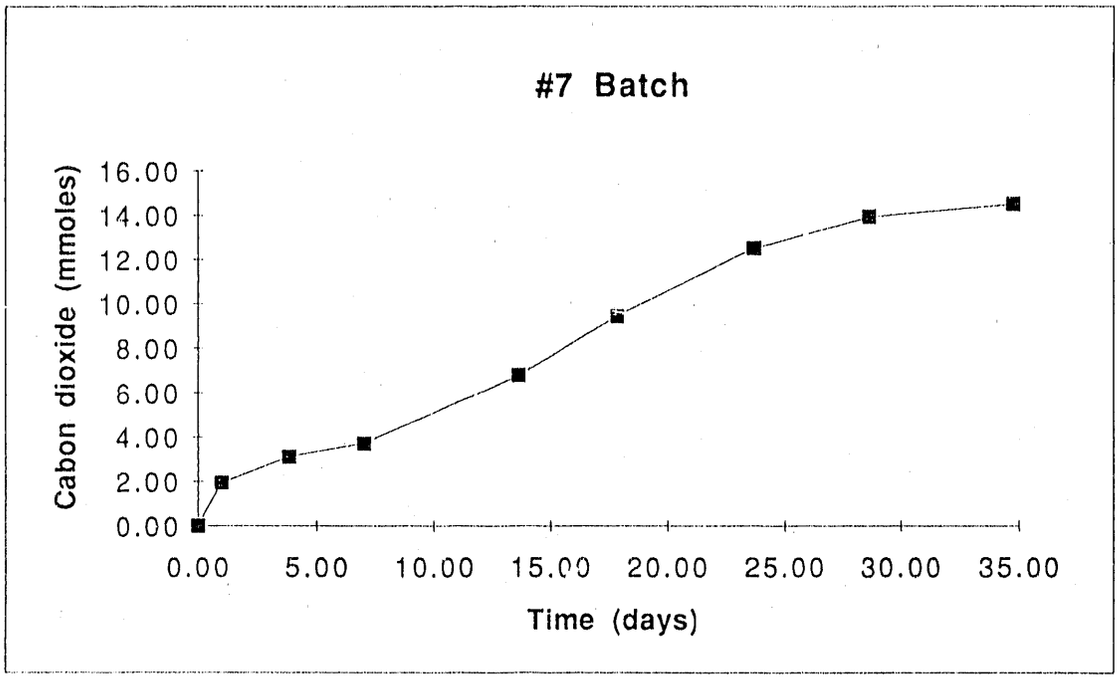


Figure 2. Profile of carbon dioxide production from #8 batch fermentor system

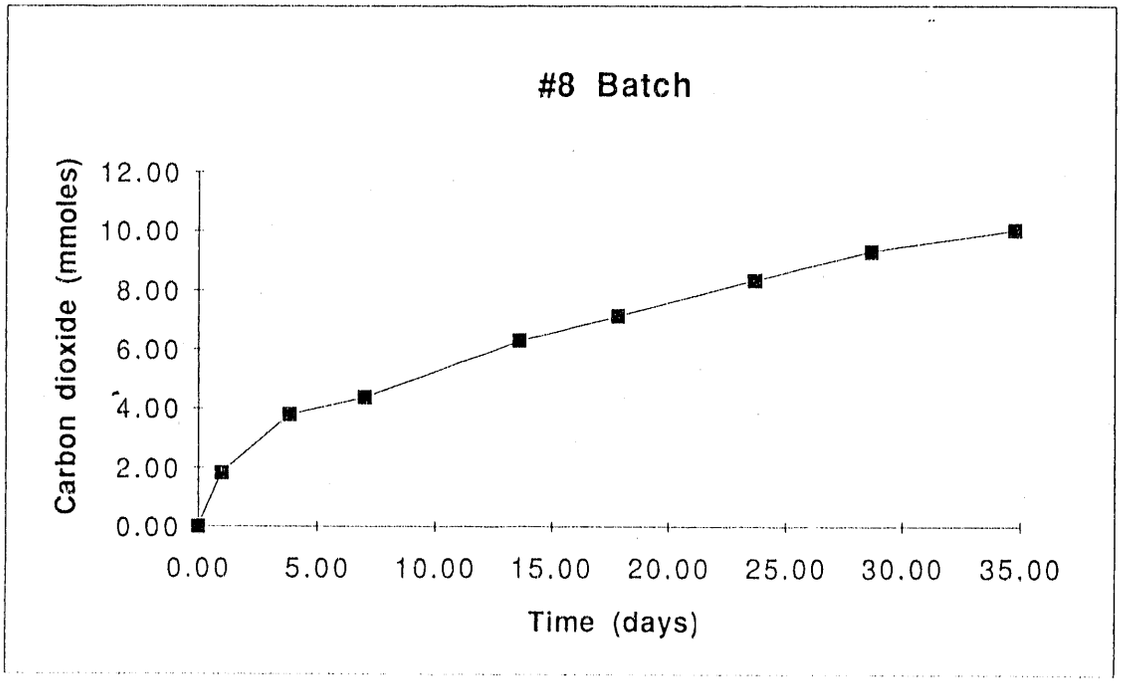


Figure 3. Profiles of gas production from coal and activated carbon in PBB medium containing 0.2% sodium succinate and 0.1% yeast extract using microbial consortium RW71C-5B

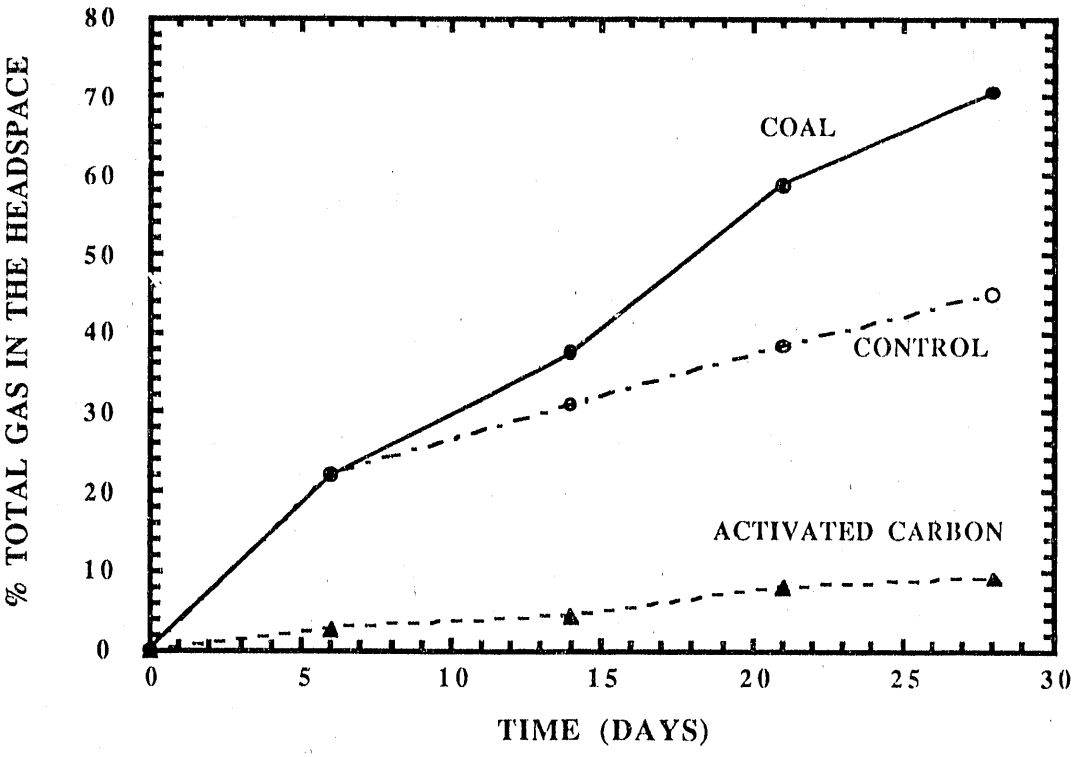


Figure 4. Profiles of gas production from coal and activated carbon in PBB medium containing 0.2% sodium succinate and 0.1% yeast extract using microbial consortium #34C-2

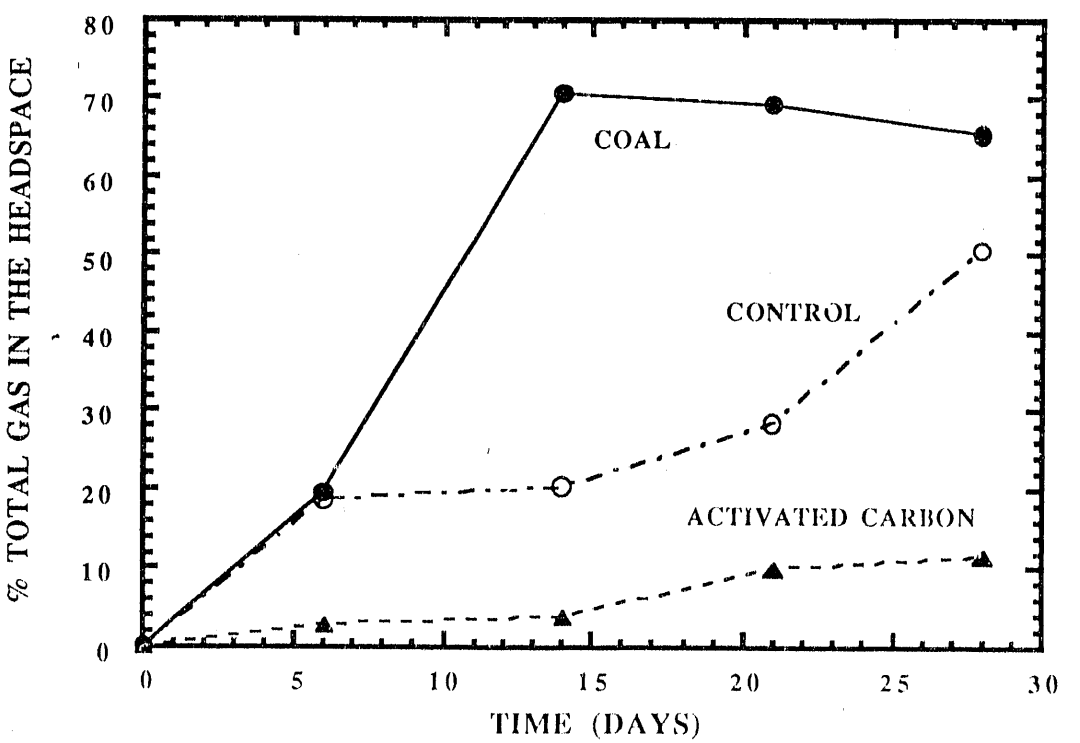


Figure 5. Profiles of gas production from coal and HCl washed activated carbon in PBB medium containing 0.2% sodium succinate and 0.1% yeast extract using microbial consortium RW71C-5B

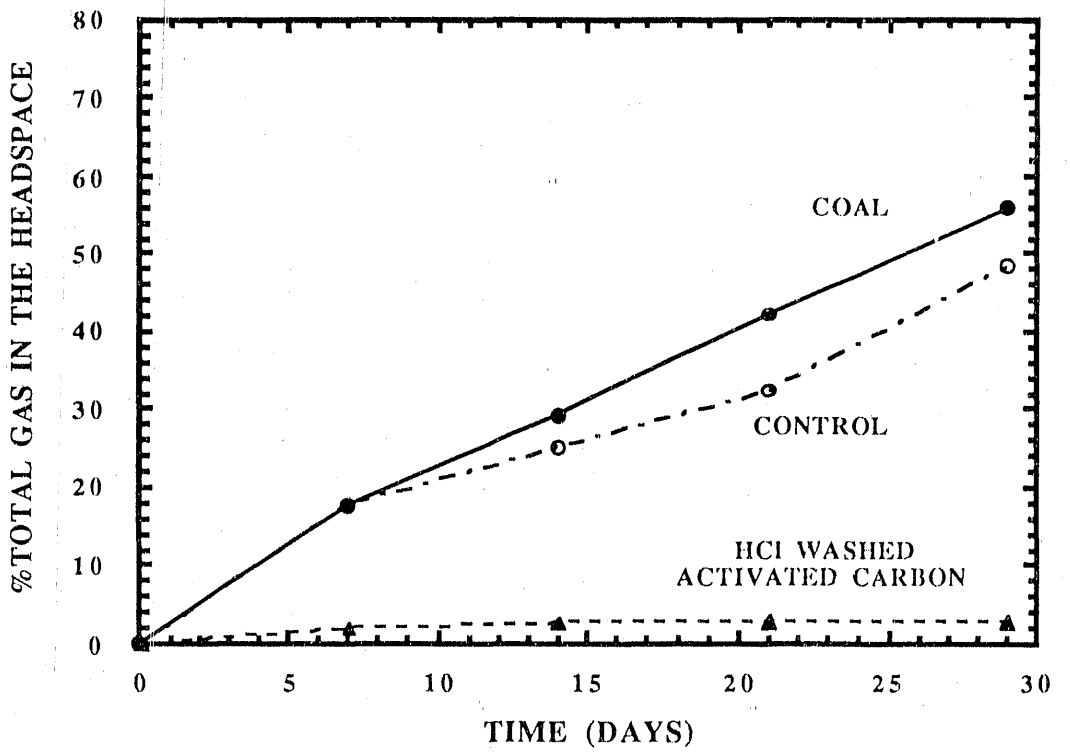
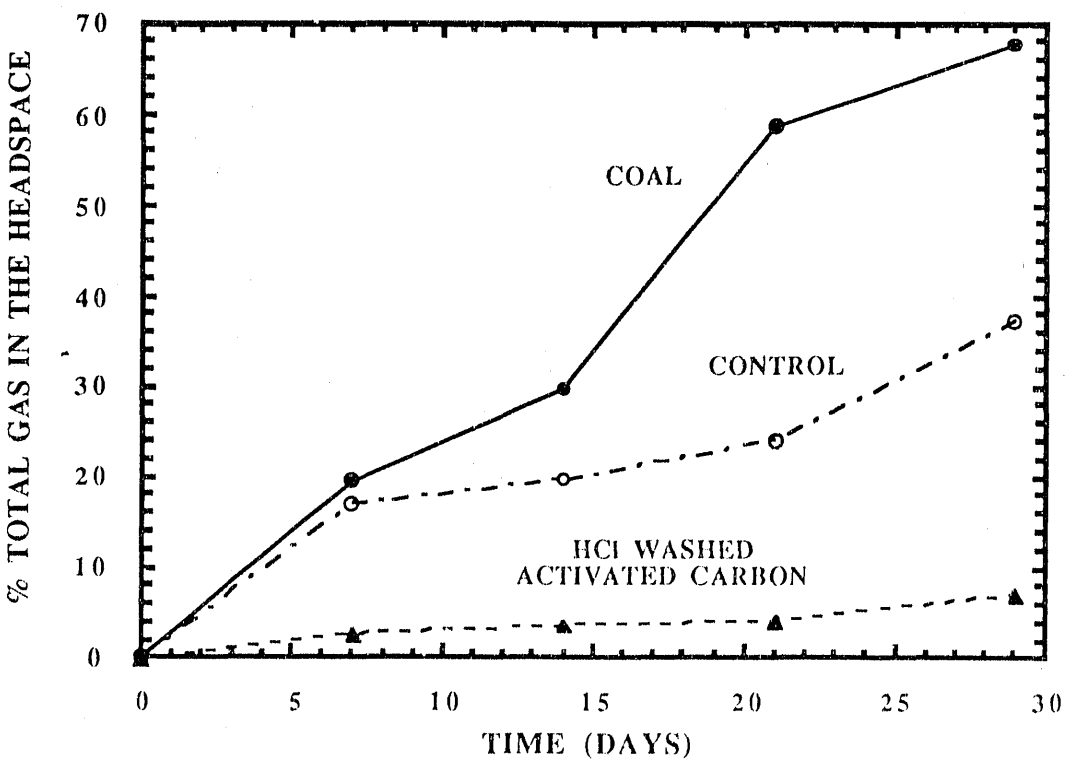


Figure 6. Profiles of gas production from coal and HCl-washed activated carbon in PBB medium containing 0.2% sodium succinate and 0.1% yeast extract using microbial consortium #34C-2



END

DATE FILMED

5/11/92