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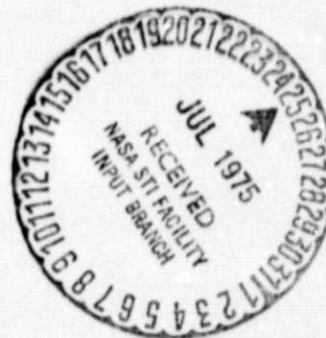
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BIO-CONVERSION OF WATER HYACINTHS INTO METHANE GAS: PART I

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16. ABSTRACT Bio-gas and methane production from the microbial anaerobic decomposition of water hyacinths (<i>Eichhornia crassipes</i>) (Mart) Solms was investigated. These experiments demonstrated the ability of water hyacinths to produce an average of 13.9 ml of methane gas per gram of wet plant weight. This study revealed that sample preparation had no significant effect on bio-gas and/or methane production. Pollution of water hyacinths by two toxic heavy materials, nickel and cadmium, increased the rate of methane production from 51.8 ml/day for non-contaminated plants incubated at 36°C to 81.0 ml/day for Ni-Cd contaminated plants incubated at the same temperature. The methane content of bio-gas evolved from the anaerobic decomposition of Ni-Cd contaminated plants was 91.1 percent as compared to 69.2 percent methane content of bio-gas collected from the fermentation of non-contaminated plants. Use of trade names or names of manufacturers in this report does not constitute an official endorsement of such products or manufacturers, either expressed or implied, by the National Aeronautics and Space Administration or any other agency of the United States Government.					
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BIO-CONVERSION OF WATER HYACINTHS INTO METHANE GAS: PART I

Two of the most pressing problems facing the United States and other industrial nations today are rapid depletion of vital natural resources and pollution of the environment. One important factor in the rise of the United States to its present high industrial level has been an abundance of fossil fuel resources. Presently, available coal, oil, and large reservoirs of underground natural gas are all produced through natural decomposition of prehistoric forms of life. Modern society is depleting these resources at an alarming rate, and renewable sources must be developed within the near future for continued industrial growth.

As we deplete our natural resources, we are also polluting our environment at the same alarming rate. Fortunately, a large number of the minerals with which we are polluting our water systems have the potential of being recovered through natural biological processes.

Recently, the ability of vascular aquatic plants to remove organic chemicals, heavy metals, pesticides, and nutrients from polluted waters has been demonstrated (1, 2, 3, 4, 5, 6). Harvested plant material from these experiments is a potential source of renewable resources, such as natural gas, fertilizers, and other valuable minerals. Vegetation can be fermented anaerobically and made to release bio-gas containing a high percentage of methane (7, 8).

Many factors can affect the actual amount of gas and fertilizer produced from the digestion of plant material. One of the most important of these factors is the carbon to nitrogen (C/N) ratios of the material used. For maximum bio-gas production the C/N ratio should be approximately 30:1.

Water hyacinths (Eichhornia crassipes) (Mart.) Solms, were chosen for this study because they have demonstrated the most promise in removing chemicals from polluted waters and producing large quantities of harvestable plant material possessing a desirable C/N ratio for maximum methane gas production. This aquatic plant has the potential of producing over 240 Kg (529 lbs.) of dry plant material per 0.40 hectare (acre) per day while removing undesirable chemicals from waste waters.

MATERIALS AND METHODS

Water hyacinths used in these experiments were grown by vegetative reproduction inside a greenhouse maintained between 25°C and 30°C. Several plants were selected for each experiment whose total wet mass ranged from 300g to 878g. In one of the fermentation studies, water hyacinths were contaminated with nickel and cadmium by exposing them to a known concentration of cadmium and nickel prior to fermentation. The 542 g wet mass of water hyacinths absorbed 5.40 mg of nickel and 6.87 mg of cadmium from 2.5 liters of Ni-Cd contaminated distilled water. Metal concentration was monitored by atomic absorption.

For four of the five fermentation units, the plants were chopped into approximately one-inch long pieces. Water hyacinths were blended into a slurry form for the other fermentation study. The chopped or blended water hyacinths were transferred into three liter Erlenmeyer flasks covered with aluminum foil to prevent exposure to light.

Starter seed for the fermentation studies was prepared by allowing water hyacinths to decompose under water and mud approximately six months in an anaerobic condition. For each fermentation unit incubated at 36°C, approximately 20 g of this seed was blended with 350 ml of distilled water. Fifty grams of seed and 800 ml of distilled water were used for each experiment at room temperature. The sediment from the seed and water mixtures was allowed to settle 30 minutes, and the supernatant liquid was then decanted into each flask containing water hyacinths.

The Erlenmeyer flasks were sealed to the atmosphere with two-hole rubber stoppers. One outlet was fitted with a rubber septum for gas chromatographic sampling, and the other outlet was connected with rubber hose to a sealed container filled with water acidified with sulfuric acid. The displacement of water in the second container by the bio-gas produced in the fermentation flask provided a convenient method of monitoring the volume of bio-gas production. Mixing of the water hyacinths was accomplished by shaking the fermentation flask once each day.

Samples for gas chromatographic analysis were taken through the rubber septum. Matheson Gas Products c.p. grade methane was used as the methane standard. The methane content of the bio-gas was analyzed by gas chromatography using a Varian 2100 GC with a flame ionization detector. Gas chromatographic conditions were:

Column:	6' x 1/4" i.d. glass
Packing:	Porapak [®] Q 150-200 mesh

Flow Rates:	Nitrogen 60, Hydrogen 35, and ml/minute Air 235
Temperature:	Detector 155°C, injection 150°C, Column 55°C
Carrier Gas:	Nitrogen
Sample Size:	5 μ l and 10 μ l

RESULTS AND DISCUSSION

Five laboratory experiments were conducted in order to evaluate the effect of temperature, toxic metal contamination, and plant preparation on the production of bio-gas and/or methane from the microbial anaerobic decomposition of water hyacinths, (Eichhornia crassipes) (Mart.) Solms.

Two of the three experiments were conducted at room temperature ($25^{\circ}\text{C} \pm 5^{\circ}\text{C}$) and contained water hyacinths chopped into one-inch long pieces. The other experiment was conducted under the same conditions except the water hyacinths were blended into a slurry form. According to the data presented in Tables 1 and 2, there was no significant difference in the results of these three experiments. The chopped water hyacinths produced 11.0 and 6.4 ml methane per gram wet weight, as compared to a production of 7.9 ml methane per gram weight for the blended water hyacinths. The methane content of the total bio-gas produced by the anaerobic decomposition of the slurried water hyacinths was 61.1%. This value was comparable to the 57.2% and 61.5% methane content of the bio-gas produced in the other two experiments with chopped water hyacinths at room temperature.

Temperature played an important role in the rate of bio-gas and methane production. The time lag between the production of bio-gas and the production of methane gas was reduced from an average of eight days for those maintained at $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ to approximately one day for experiment 4 incubated 36°C . The methane content of the total bio-gas produced in experiment 4 at 36°C was 69.2%. This percent methane was higher than the average methane content of 59.9% for the three experiments conducted at room temperature.

Comparison of the data for experiments 4 and 5 in Table 1 in which chopped plants were incubated at 36°C showed that nickel and cadmium contamination of the water hyacinths at concentration levels of 9.95 and 12.66 mg/kg wet weight nickel and cadmium, respectively, had no adverse effect on the percent methane content, volume of methane produced per unit wet weight, or the rate of bio-gas and/or methane production. In fact, the Ni-Cd contaminated plants produced bio-gas with a 91.1% methane content, as compared to the lower value of 69.2% for the other experiment incubated at 36°C .

The total volume of bio-gas produced from the Ni-Cd contaminated plants was less than the volume produced from the non-contaminated plants; however, due to the much higher percentage of methane in the bio-gas, the Ni-Cd contaminated plants yielded 11.3 ml methane per gram wet weight, as compared to only 8.9 ml methane per gram wet weight for the non-contaminated plants. Also, the average rates of bio-gas and methane production (87.5 and 81.0 ml/day, respectively) for the Ni-Cd contaminated water hyacinths for the first 65 days of incubation was significantly higher than the rates for the non-contaminated plants (76.8 and 51.8 ml/day bio-gas and methane production, respectively) incubated at the same temperature.

CONCLUSION

This study on the anaerobic decomposition of water hyacinths revealed that sample preparation, either chopped or blended, had no significant effect on bio-gas and/or methane production. Incubation of the experimental units at 36°C increased not only the rate of bio-gas production, but also the methane content of the total bio-gas produced in these experiments. Pollution of the water hyacinths by two toxic heavy metals, nickel and cadmium, actually increased the rate of methane production and improved the methane content of the bio-gas evolved in the anaerobic decomposition of the contaminated plants.

Further studies are planned in this area utilizing the preliminary information from these experiments. Temperature controlled, continuous fec . fermentation chambers with efficient stirring devices are presently being developed by NASA at the National Space Technology Laboratories.

Table 1. Bio-gas Data From the Anaerobic Decomposition of Water Hyacinths

EXPERIMENT 1				EXPERIMENT 2				EXPERIMENT 3							
Wet Mass: 300g Form: Chopped Temp: 25°C ± 5°C Vol. H ₂ O: 800 ml				Wet Mass: 754 g Form: Chopped Temp: 25°C ± 5°C Vol. H ₂ O: 800 ml				Wet Mass: 800 g Form: Blended Temp: 25°C ± 5°C Vol. H ₂ O: 800 ml							
No. Days Elapsed	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)	No. Days Elapsed	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)	No. Days Elapsed	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)	No. Days Elapsed	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)
4	325	0	1,082	4	600	0	798	4	600	0	750	4	600	0	0
7	1,150	0	3,330	10	1,465	0	1,943	10	1,250	0	1,563	10	1,250	0	0
12	1,550	43	5,493	12	1,745	56	2,314	12	1,450	11	1,813	12	1,450	11	14
41	2,461	846	5,155	19	2,745	356	3,641	21	2,050	47	2,563	21	2,050	47	59
67	2,861	1,206	9,527	21	3,345	692	4,436	27	2,475	247	5,094	27	2,475	247	309
83	3,911	1,994	13,024	25	4,095	1,097	5,431	31	3,775	897	4,719	31	3,775	897	1,121
97	4,961	2,939	16,520	27	4,495	1,497	5,962	35	4,850	1,972	6,063	35	4,850	1,972	2,465
126	5,361	3,299	19,986	35	5,485	2,487	7,275	39	5,460	2,192	6,825	39	5,460	2,192	2,740
				30	6,010	2,891	7,971	45	6,300	3,032	7,875	45	6,300	3,032	3,790
				45	6,750	3,491	8,966	52	6,660	3,392	8,325	52	6,660	3,392	4,240
				52	7,460	4,051	9,894	64	7,660	4,392	9,575	64	7,660	4,392	5,490
				68	8,110	4,571	10,756	79	9,310	5,547	11,638	79	9,310	5,547	6,934
				95	8,410	4,811	11,154	122	10,310	6,297	12,888	122	10,310	6,297	7,871

EXPERIMENT 4				EXPERIMENT 5			
Wet Mass: 500 g Form: Chopped Temp: 36°C Vol. H ₂ O: 350 ml				Wet Mass: 500 g Form: Chopped Temp: 36°C Vol. H ₂ O: 350 ml			
No. Days Elapsed	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)	No. Days Elapsed	Cum. Bio-gas (ml)	Cum. Methane (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)
3	665	1,330	86	3	575	1,150	1,036
22	1,425	2,850	413	20	2,350	4,700	4,302
27	2,920	5,840	1,833	22	2,825	5,650	5,234
34	3,900	7,800	2,470	27	3,750	7,500	7,048
50	4,525	9,050	3,064	34	4,100	8,200	7,510
65	4,990	9,980	3,366	50	4,350	8,700	7,986
101	6,260	12,520	4,319	65	5,690	11,380	10,532
103	6,460	12,920	4,469	101	6,210	12,420	11,312

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Table 2. Calculated Data of Bio-gas and Methane Production for Experiments 1-5

Experiment #	% Methane in Total Bio-gas	ml Bio-gas per Gram Wet Weight	ml Methane per Gram Wet Weight
1	61.5	17.9	11.0
2	57.2	11.1	6.4
3	61.1	12.9	7.9
4	69.2	12.9	8.9
5	91.1	12.4	11.3

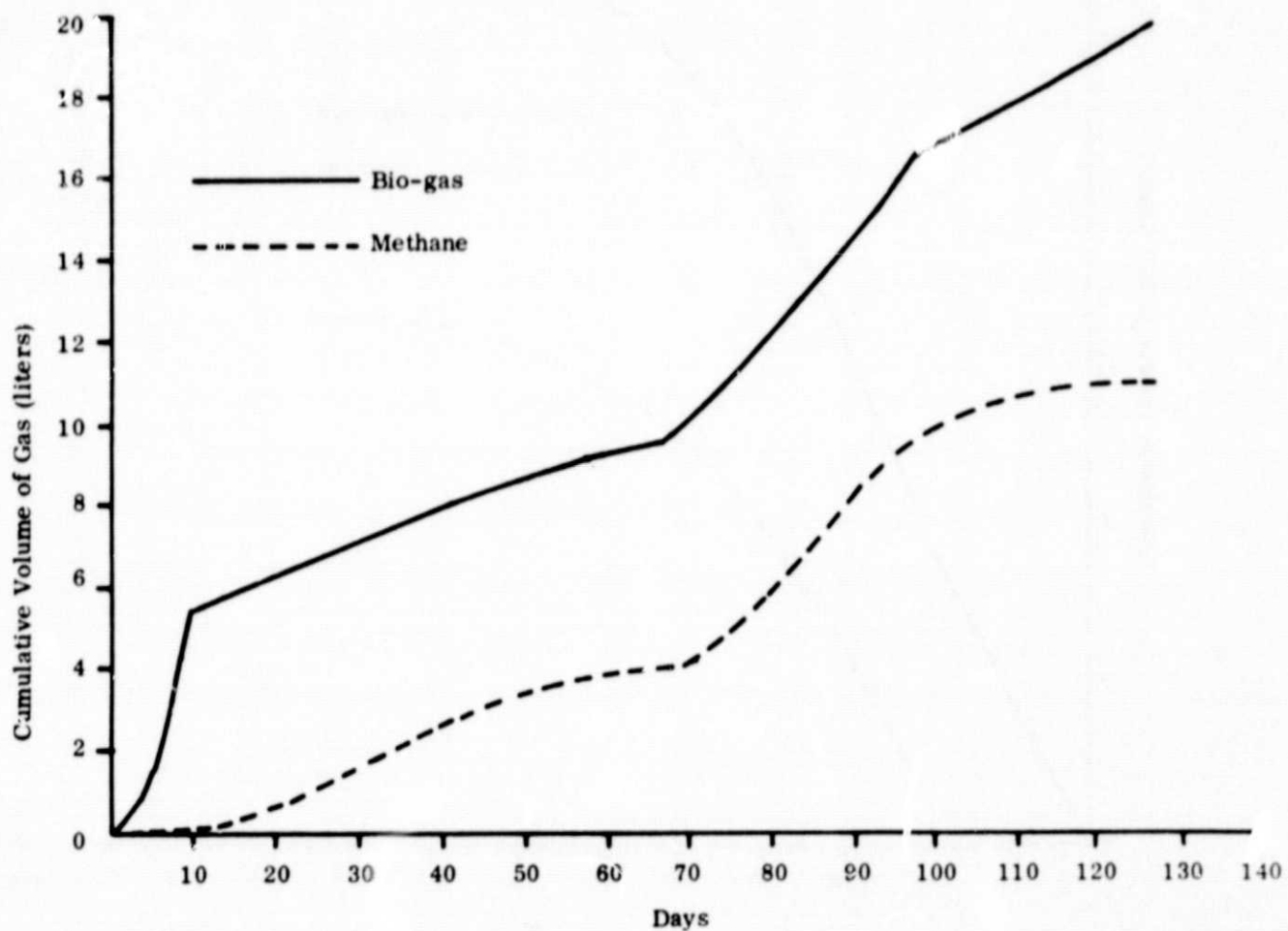


Figure 1. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of chopped water hyacinths at $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ versus number of days elapsed since initiation of anaerobic fermentation.

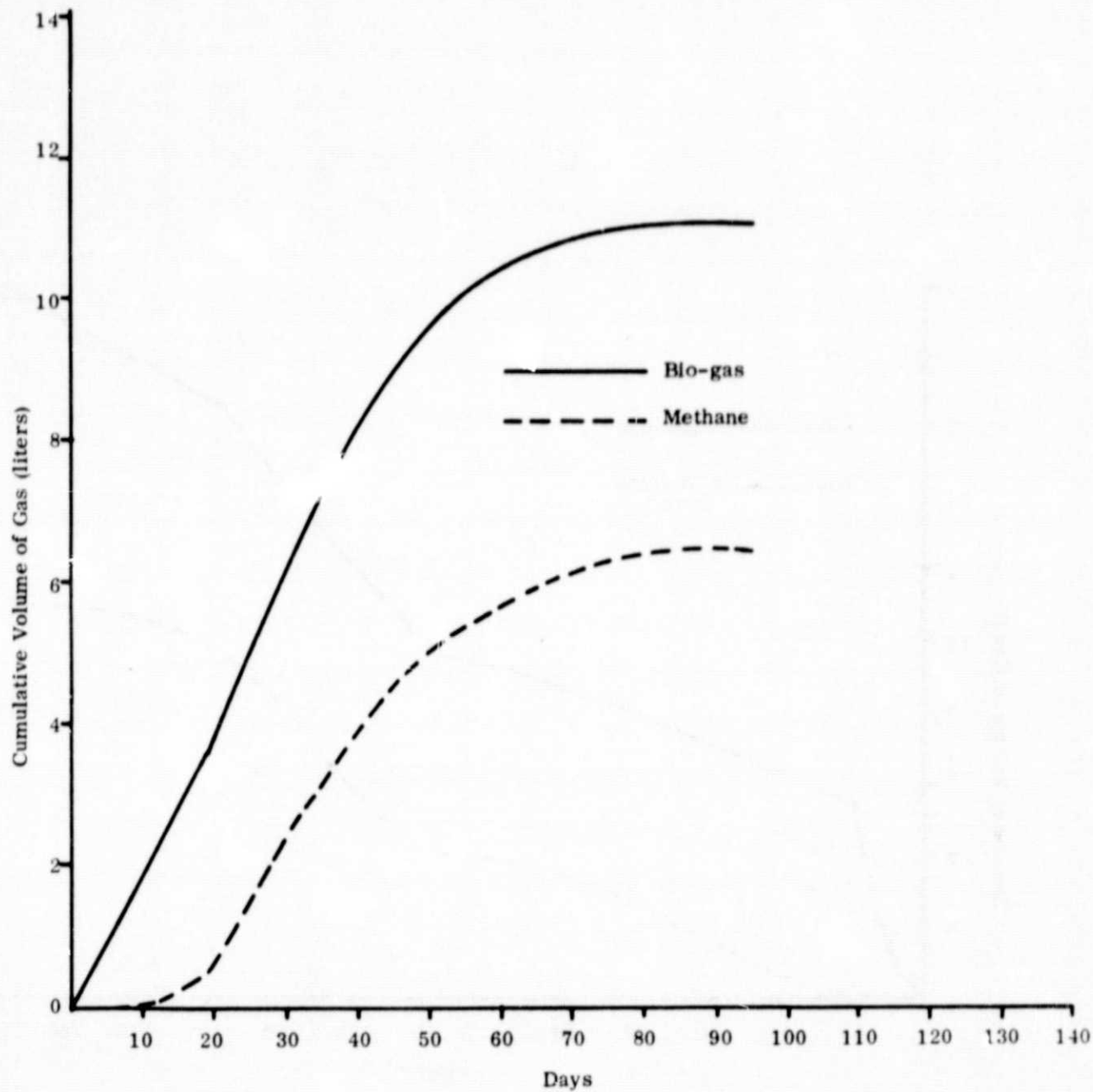


Figure 2. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of chopped water hyacinths at $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ versus number of days elapsed since initiation of anaerobic fermentation.

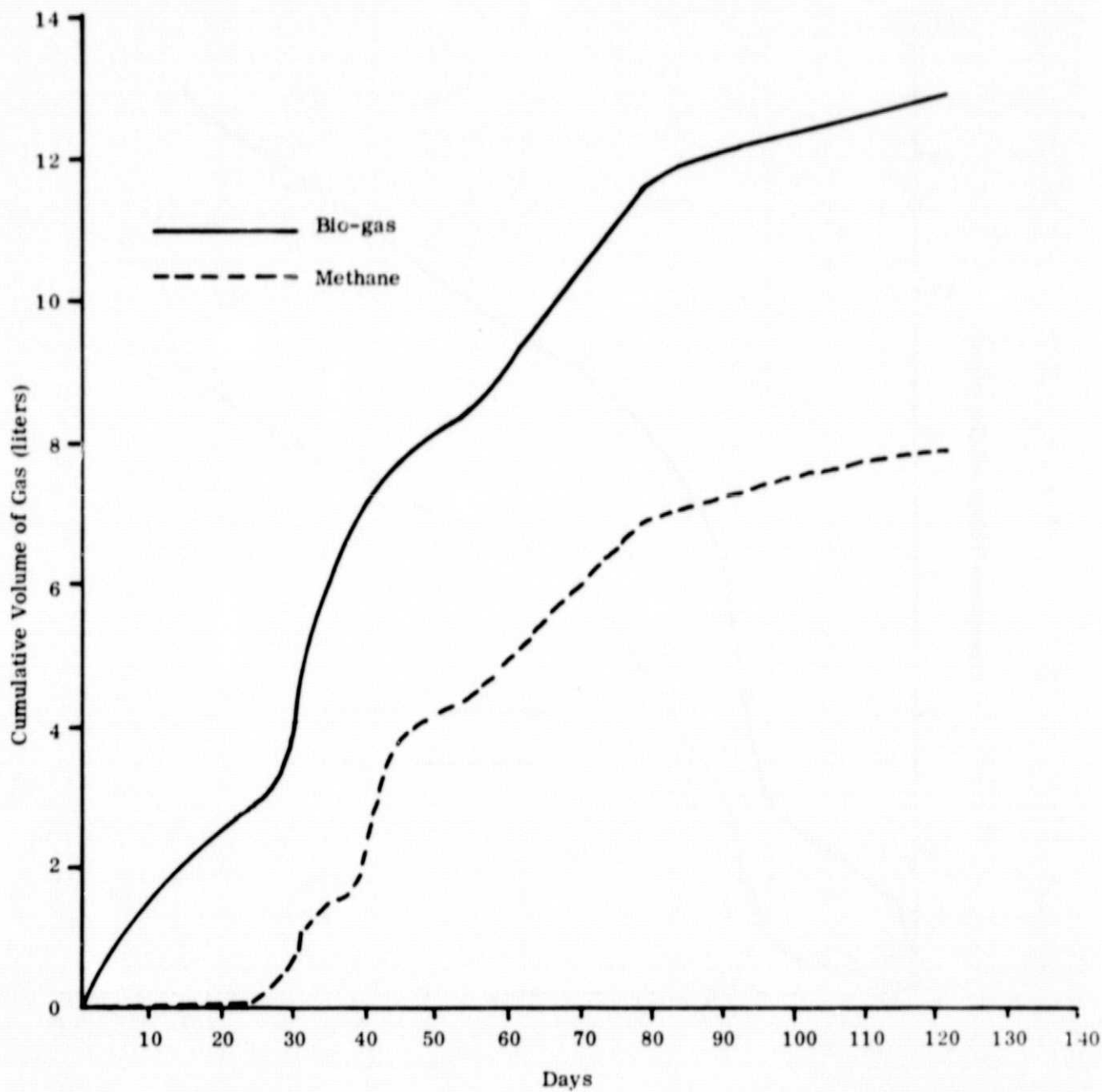


Figure 3. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of blended water hyacinths at $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ versus number of days elapsed since initiation of anaerobic fermentation.

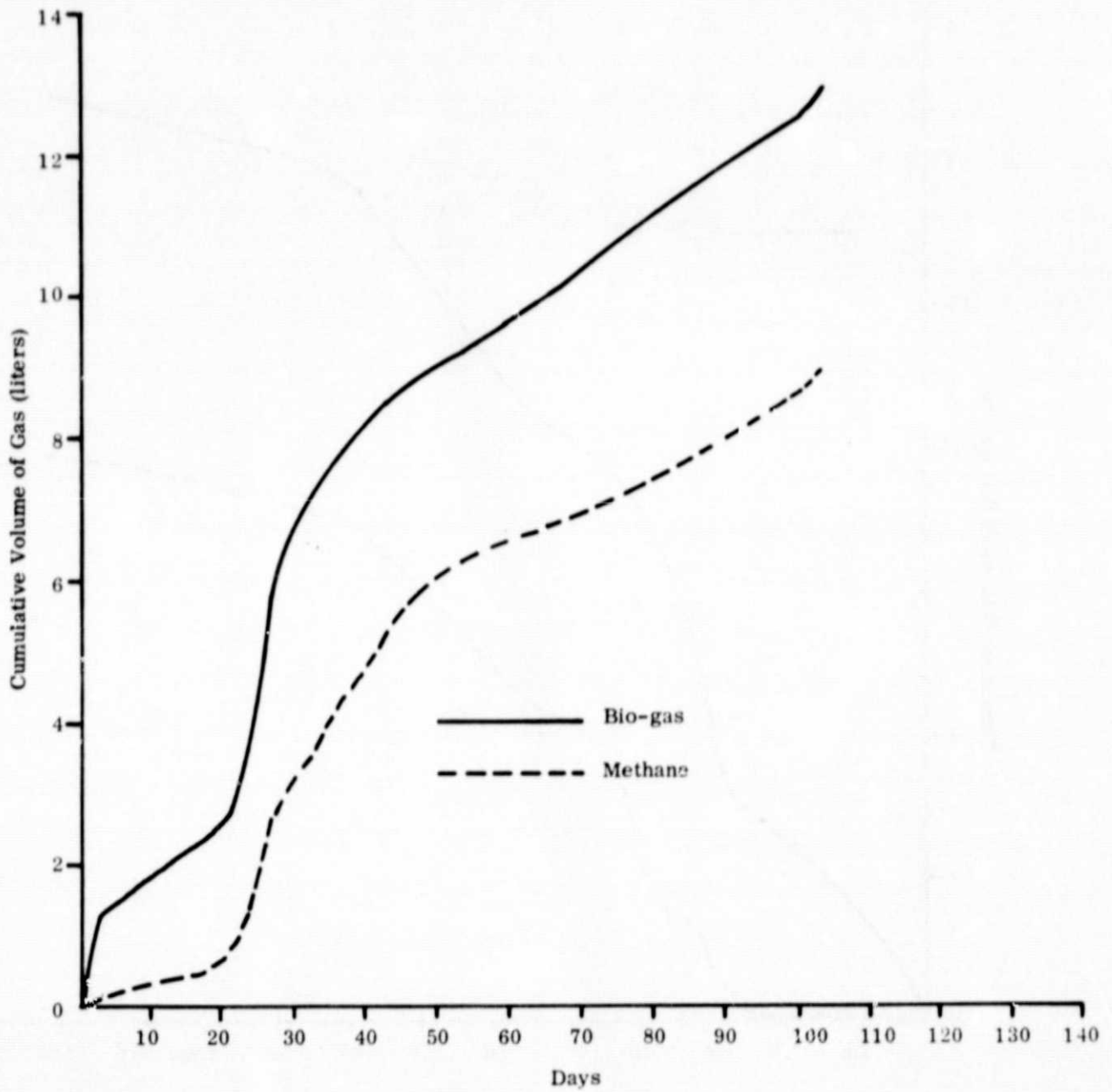


Figure 4. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of chopped water hyacinths incubated at 36°C versus number of days elapsed since initiation of anaerobic fermentation.

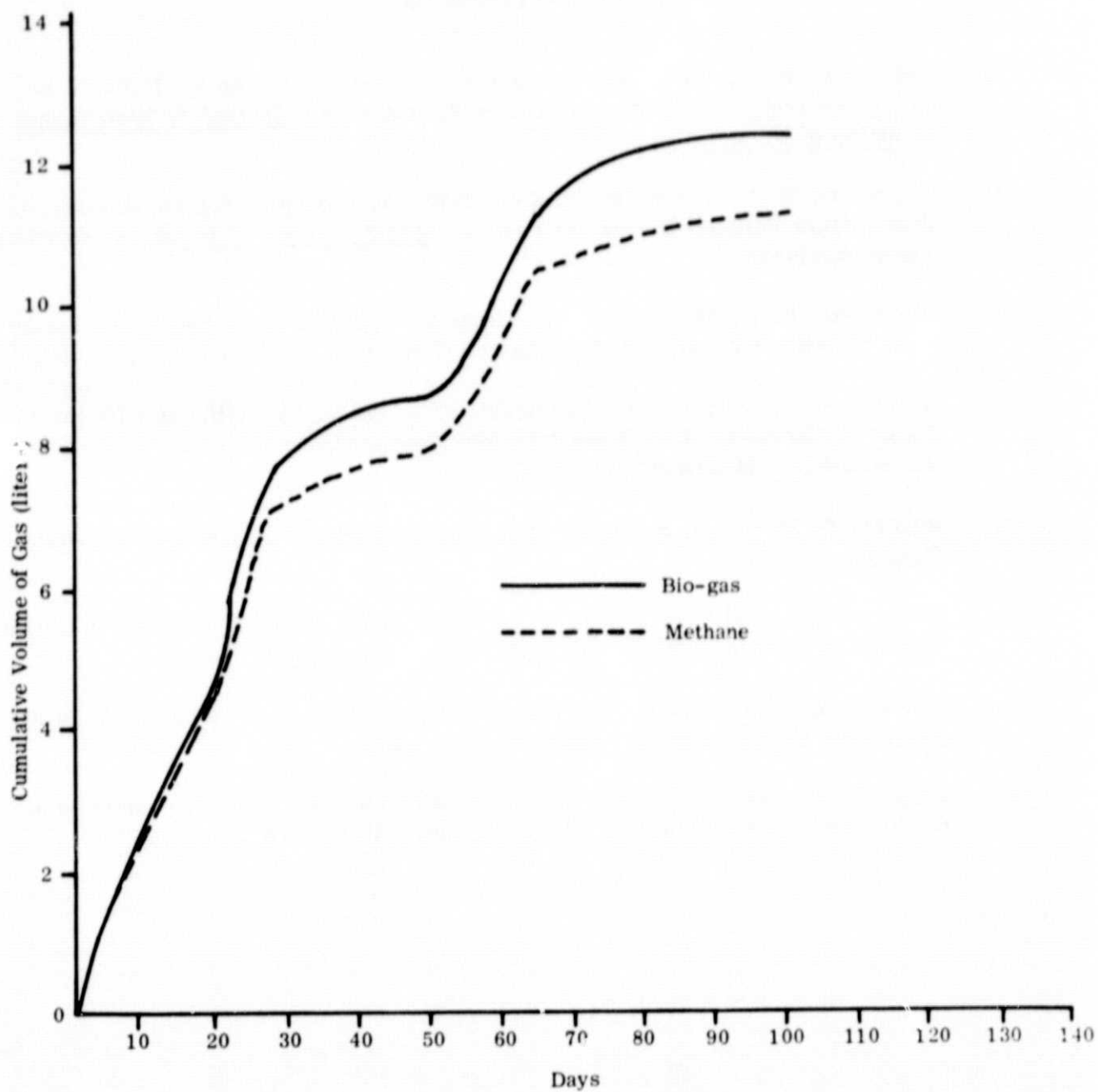


Figure 5. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of chopped water hyacinths incubated at 36°C versus number of days elapsed since initiation of anaerobic fermentation.

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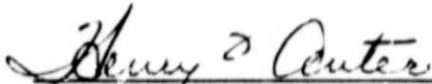
APPROVAL

BIO-CONVERSION OF WATER HYACINTHS INTO METHANE GAS: PART 1

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The information in this report has been reviewed for security classification. Review of any information concerning Department of Defense of Atomic Energy Commission programs has been made by the NSTL Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

This document has also been reviewed and approved for technical accuracy.



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