ISSN : 0974 - 7435 Volume 11 Issue 11

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 11(11), 2015 [405-412]

Impact of amino-acids and their derivatives on biogas generated from anaerobic digestion of rice straw

Aboul-fetouh E.Mourad*, Osman A.Osman, Kamal M.El-Shaieb, Rasha M.Tony
Department of Chemistry, Faculty of Science, Minia University, El-Minia, (EGYPT)
E-mail: mouradaboulf@yahoo.com

ABSTRACT

Anaerobic digestion of rice straw treated with three amino-acids (tyrosine, taurine and L-cysteine) as well as their isatin derivatives was carried out on a laboratory scale monitored through 14 weeks. The results indicated that fluctuation of the ambient temperature and amino-acids and their derivative treatments affected markedly the quantity of biogas produced. There was a significant increase in the yield of biogas produced in case of treatment of rice straw with these compounds that reached about 104% in case of taurine treatment relative to the sample free. Also, the results showed that these treatments resulted in a continuous generation of biogas even at low temperature. The findings indicated that the methane percentage in the biogas produced ranged from 21 to 67%. Fluctuation of biogas production was observed through the study as a result of climate changes.

KEYWORDS

Anaerobic digestion;
Methane;
Rice straw;
Amino-acids;
Biomethanization.

INTRODUCTION

Anaerobic treatment of the agricultural wastes is the use of a biological process in the absence of oxygen for breaking down of organic matter, and the stabilization of these materials by conversion to methane and carbon dioxide and nearly stable residues.

Large[1] illustrated the mechanism of CO-$_2$ reduction to methane, in which co-enzyme F420 accepts two electrons and works as a carrier beside $NADP^+$ and FAD. Many investigators interpreted the acetic acid degradation into methane.[2-4] They concluded that acetate yields about 70% of methane.

An anaerobic–phased solids digester system was applied for conversion of rice straw into biogas.[5] In this system ammonia was used as a supplemental nitrogen source for rice straw digestion in addition to mechanical and thermal effects, which resulted in increasing the biogas yield by about 17.5% with respect to untreated whole straw. It has also been reported that addition of ammonia and/or amino-acids such as threonine, histidine and methionine in suitable concentrations during the biogas process increases the growth of methanogenic bacteria, which is responsible for biogas production[6].

Banik and Nandi[7] reported that supplementation of rice straw with biogas residual slurry manure has strong impact in improving the yield potential of protein and mineral nutrient contents of *Pleurotus saju* mashroom in Indian Subcontinent or similar climate conditions. Improvement of biogas

TABLE 1 : Amount of biogas (ml) generated for 500 ml fermenters charged with rice straw treated with tyrosine, taurine and L-cysteine as well as their derivatives (accumulative production)

Treatment / Fermentation Period (week)	Temperature (°C)	Control	Tyrosine	Taurine	L-Cysteine	Temperature (°C)	Control	Tyrosine	Tyrosine derivative	Temperature (°C)	Control	Taurine	Taurine derivative	Temperature (°C)	Control	L-Cysteine	L-Cysteine derivative
1st	31-34	131	195	251	301	22-25	12	14	16	26-30	100	108	168	21-24	10	24	14
2nd	32-35	274	257	553	479	21-24	40	47	46	26-28	204	214	348	21-24	39	67	53
3rd	33-37	386	499	772	847	20-23	76	89	101	27-30	289	390	541	20-23	76	120	106
4th	33-37	496	813	953	1050	23-25	160	182	208	24-26	449	656	626	24-26	188	190	167
5th	34-37	596	916	1118	1332	23-26	213	231	267	24-26	627	820	826	23-26	241	227	276
6th	35-39	727	1257	1281	1544	24-28	313	396	381	22-24	827	1074	1082	25-28	326	326	593
7th	35-39	905	1785	1578	1920	23-26	425	600	504	22-25	959	1338	1434	23-26	463	362	758
8th	35-40	1271	2480	2318	2552	25-28	605	797	614	21-23	1071	1786	1779	25-28	643	517	949
9th	32-36	1623	2927	2831	2891	27-31	721	907	773	19-22	1231	2088	2007	27-31	762	732	1184
10th	31-36	1980	3535	3512	3440	28-31	832	1087	952	19-22	1231	2552	2425	28-31	927	913	1357
11th	31-35	2332	4048	4383	3804	25-28	919	1177	1152	12-16	1231	2808	2809	25-28	1014	1048	1425
12th	31-35	2657	4442	5012	4046	24-28	980	1270	1293	11-15	1231	3304	3195	24-28	1075	1220	1496
13th	32-36	2979	4918	5815	4148	28-30	1044	1368	1379	13-15	1231	3570	3430	24-28	1176	1339	1663
14th	32-35	3205	5163	6530	4223	29-32	1087	1426	1447	13-15	1231	3742	3659	28-32	1219	1432	1718

production from rice straw has also been achieved by co-digestion of rice straw with kitchen waste and pig manture[8]. Furthermore, pretreatment of rice straw with N-methylmorpholine-N-oxide to anaerobic digestion resulted in a significant increase of methane production[9].

The effect of phosphate supplementation on methane production from rice straw with acclimated anaerobic sludge indicated that an adequate level of phosphate addition (465 mg-P/L) could accelerate the biogasification process 7-13 days earlier appearance of the two peaks and shorter time needed for complete biogasification of rice straw[10].

In the light of these findings and knowing that nitrogen is preferred as a simple source of the essential nutrients for methanogenic bacteria, we used in this study rice straw as an agricultural waste in the presence of some amino-acids treatments such as tyrosine, taurine and L-cysteine as well as their isatin derivatives 1a-c in a laboratory–scale aiming to study the impact of these nitrogen rich compounds on biogas production.

RESULTS AND DISCUSSION

Amino-acid derivatives 1a-c were synthesized according to Mannich reaction, and their structures have been elucidated as described in the experimental part.

TABLE 1 includes the accumulative biogas volumes generated from rice straw affected by amino-acids treatments (e.g. tyrosine, taurine and L-cysteine) as well as their derivatives 1a-c by anaerobic digestion in 500 ml laboratory-scale experiment monitored through 14 weeks. It is clear from the data in this Table that addition of amino-acids to rice straw led to an increase in the amount of biogas produced in comparison to control sample along the period of study. From the accumulative data of biogas production it is observed that the anaerobic digestion of rice straw treated with taurine gave the highest volume of biogas followed by tyrosine and finally L-cysteine treatment. The percentage of volume increase was decreased in the following sequence: 104, 61 and 32 % relative to control respectively.

The present study is also concerned with a comparison between the effect of each amino-acid and its derivative on biogas production. The data in TABLE 1 indicated that addition of tyrosine and its derivative 1a into rice straw at ambient temperature

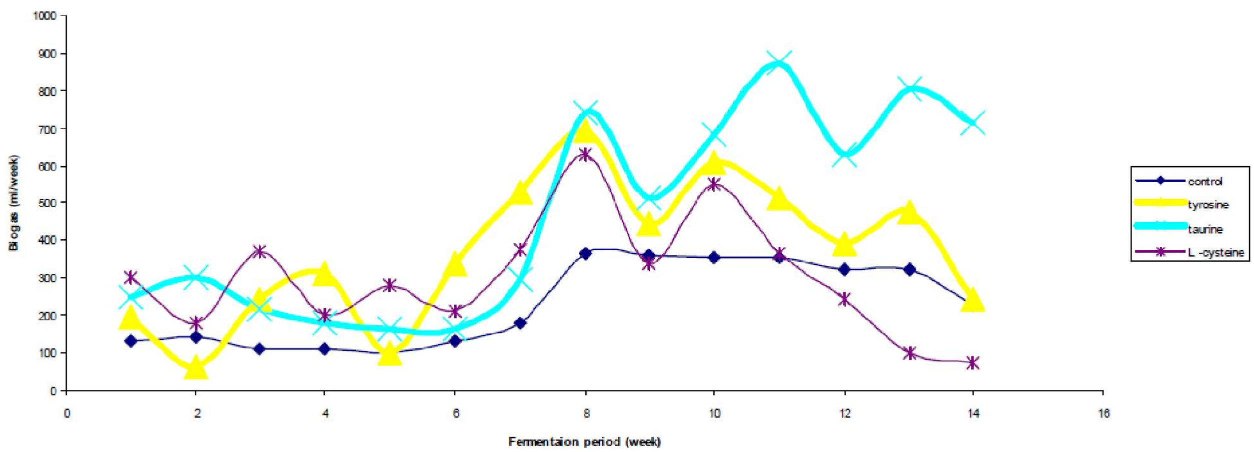


Figure 1 : Weekly volumes of biogas generation of control, tyrosine, taurine and L-cysteine

resulted in increasing the accumulative volume of biogas by about 31 and 33 % with respect to the sample free respectively. Interestingly, in case of treatment of rice straw with taurine and its derivative 1b, the percentage of biogas increase was 204 and 197% respectively with reference to the sample free in spite of undergoing this experiment at relatively low temperature in comparison with the other experiments undertaken with tyrosine and L-cysteine as well as their derivatives. This low temperature tends to stop the biogas generation in the 9th week in case of control sample. Finally, addition of L-cysteine and its derivative 1c to rice straw increased the yield of biogas by about 17% and 41% with respect to the sample free respectively.

TABLE 2 and Figure 1 include the weekly volumes of biogas produced from reference sample and its treatments with the amino-acids under investigation as well as their derivatives 1a-c. It is evident from the data in this Table that these volumes reached their maximum values for control, tyrosine, taurine and L-cysteine treatments in the 9th, 8th, 11th and 8th week respectively. The data also confirm that the weekly production of biogas suffer a markedly fluctuation, which is probably due to a fluctuation of the ambient temperature. This result is in agreement with that reported by Pyle[11].

It is also clear from the TABLE 2 that L-cysteine derivative 1c the earliest one which gave its maximum gas production at 6th week, while taurine treatment, which gave the highest volume of biogas among other treatments, reached its maximum value at the 12th week. This may be due to the high content of soluble nitrogen in case of taurine in the medium, which was composed mainly of ammonia. The high concentration of ammonia at the earliest stages could delay or retard the growth of methane bacteria[12].

The maximum production for tyrosine and its derivative treatments was in the 7th and 11th week respectively. This may be attributed to the fact that tyrosine is a bio-activator for many groups of bacteria.

Total volatile acids (TVA)

It is well known that the higher the concentration of TVA the larger number of acid forming bacteria, which in turn will decrease the survival of the methanogenes and will affect the amount of biogas produced[13]. TABLE 3 includes the amount of TVA produced at the end of digestion period. It is clear from the data that both tyrosine and taurine as well as their derivative treatments have the same concentration of TVA, which is higher than that of control in case of tyrosine and is lower than in case of taurine treatments respectively. On the other hand, in case of L-cysteine the TVA value is decreased in the following sequence: Control > L-cysteine > L-cysteine derivative.

N, P, K content

N, P and K are considered the most important micro nutrient for all the plant stages of growth. The percentage values of N, P and K were estimated after digestion period and given in TABLE 3. Comparison between the effect of the three amino-acids under investigation will reference to the control

FULL PAPER

TABLE 2 : Weekly volumes (ml) of biogas for control and its treatments with rice straw treated with tyrosine, taurine and L-cysteine as well as their derivatives

Treatment / Fermentation Period (week)	Temperature (°C)	Control	Tyrosine	Taurine	L- Cysteine	Temperature (°C)	Control	Tyrosine	Tyrosine derivative	Temperature (°C)	Control	Taurine	Taurine derivative	Temperature (°C)	Control	L- Cysteine	L- Cysteine derivative
1st	31-34	131	195	251	301	22-25	12	14	16	26-30	100	108	168	21-24	10	24	14
2nd	32-35	143	62	302	178	21-24	28	33	30	26-28	104	106	180	21-24	29	43	39
3rd	33-37	112	242	219	368	20-23	36	42	55	27-30	85	176	193	20-23	37	53	53
4th	33-37	110	314	181	203	23-25	84	93	107	24-26	160	266	185	24-26	112	70	61
5th	34-37	100	103	165	282	23-26	53	49	59	24-26	178	164	200	23-26	53	37	109
6th	35-39	131	341	163	212	24-28	100	165	114	22-24	200	254	256	25-28	85	99	317
7th	35-39	178	528	297	376	23-26	112	204	123	22-25	132	264	352	23-26	137	36	165
8th	35-40	360	695	740	632	25-28	180	197	110	21-23	112	448	345	25-28	180	155	191
9th	32-36	362	447	513	339	27-31	116	110	159	19-22	160	302	228	27-31	116	215	235
10th	31-36	357	608	681	549	28-31	111	180	179	19-22	0	464	418	28-31	165	181	173
11th	31-35	352	513	871	364	25-28	87	90	200	12-16	0	256	384	25-28	87	135	68
12th	31-35	325	394	629	242	24-28	61	93	141	11-15	0	496	386	24-28	61	172	71
13th	32-36	322	476	803	102	28-30	64	98	86	13-15	0	266	235	24-28	101	119	167
14th	32-35	226	245	715	75	29-32	43	58	68	13-15	0	172	229	28-32	43	93	55

TABLE 3 : Analytical data of the digested slurry

Treatment	T. V. A meq/L as acetic acid	% N	% P	% K	% T.S	% V.S	% Organic Matter	% Organic Carbon	C:N
Control	24.2	1.35	0.16	0.67	4.1	30.21	69.79	40.49	30.29:1
Tyrosine	36.4	1.47	0.31	0.86	2.8	34.22	65.78	38.15	25.95:1
Taurine	24.2	1.44	0.12	0.40	4.3	31.19	68.81	39.91	27.72:1
L-cysteine	36.4	1.54	0.16	0.78	4.5	31.71	68.29	39.61	25.72:1
Control	12.2	1.22	0.62	0.23	2.52	11.40	88.60	51.39	42.12:1
Tyrosine	24.2	2.18	0.65	0.48	0.31	48.70	29.16	16.91	7.76:1
Tyrosine derivative	24.2	1.81	0.81	1.00	0.96	34.07	65.93	38.24	21.13:1
Control	36.4	1.50	0.16	0.35	3.00	32.30	72.00	41.70	27.80:1
Taurine	24.2	2.36	0.27	0.56	5.32	33.71	66.29	38.45	16.29:1
Taurine derivative	24.2	1.58	0.31	0.91	4.01	29.95	70.05	40.63	40.63:1
Control	48.7	1.22	0.62	0.23	0.31	11.40	88.60	51.39	42.12:1
L-Cysteine	36.3	3.17	1.08	1.09	3.70	40.19	59.81	34.69	10.94:1
L-Cysteine derivative	12.1	1.18	0.56	0.37	2.00	34.36	65.64	38.07	32.26:1

sample on the value of K, N and P percentage. It is clear from the data in this Table that L-cysteine gave the highest value for N % followed by tyrosine then taurine and finally the control sample. On the other hand, in case of both K % and P %, the order is decreased as follows: tyrosine > L-cysteine > taurine. In a comparative determination of N, K and P percentages for treatments of the amino-acids and their derivatives the data given in TABLE 3 indicates that both tyrosine and L-cysteine have the same behaviour for each of the elements, however in case of L- cysteine the behaviour is similar in case of N% and P%.

C/N Ratio

It has been reported that it is important to main-

TABLE 4 : Cumulative and weekly volumes (ml) of biogas for control and different concentrations of taurine and its derivative

Treatment	Control		Taurine						Taurine derivative					
			Concentration g/digester						Concentration g/digester					
			0.05		0.1		0.2		0.05		0.1		0.2	
Fermentation Period (week)	Cumulative	Weekly	Cumulative	Weekly	Cumulative	Weekly	Cumulative	Weekly	Cumulative	Weekly	Cumulative	Weekly	Cumulative	Weekly
1st	80	80	105	105	146	146	155	155	85	85	130	130	124	124
2nd	154	74	200	95	346	200	335	180	198	113	246	116	254	130
3rd	254	100	341	141	544	198	593	258	298	100	400	154	366	112
4th	391	137	456	115	837	293	816	223	443	145	504	104	446	80
5th	494	103	666	210	1022	185	1143	327	593	150	880	376	571	125
6th	646	152	1053	387	1222	200	1527	384	763	170	1203	323	704	133
7th	813	167	1251	198	1480	258	1872	345	941	178	1390	187	873	142
8th	953	233	1391	140	1703	223	2172	300	1095	154	1562	172	958	121
9th	1152	172	1477	86	1915	212	2384	212	1205	110	1730	168	1158	200
10th	1340	215	1567	90	2101	196	2553	169	1315	135	1876	146	1368	210
11th	1499	159	1662	95	2191	90	2842	174	1467	152	1966	90	1548	180
12th	1599	100	1812	150	2291	100	2992	150	1627	160	2036	70	1648	100
13th	1653	54	1922	110	2376	85	3077	85	1792	165	2131	95	1738	90
14th	1675	22	2007	85	2448	72	3159	82	1884	92	2181	50	1803	65

TABLE 5 : Analytical data of the digested slurry for taurine and its derivatives at different concentrations

Treatment	T.V.A meq/L as acetic acid	% N	% protein	% K	% T.S	% P	% V.S	% Organic Matter	% Organic Carbon	C:N
Control	19	1.70	10.63	0.60	3.50	0.85	31.03	68.97	40.00	23.53 : 1
Taurine 0.05	18	1.75	10.94	0.96	5.20	1.33	33.68	66.32	38.47	21.98 : 1
Taurine 0.1	14	1.81	11.31	0.48	5.00	0.70	36.25	63.75	36.98	20.43 : 1
Taurine 0.2	10	2.05	12.81	1.03	4.70	0.75	38.66	61.34	35.58	17.36 : 1
Taurine derivation 0.05	11	1.46	9.13	0.85	4.82	1.03	38.80	61.20	35.50	24.32 : 1
Taurine derivation 0.1	10	1.57	9.81	0.90	4.54	1.00	36.17	63.83	37.02	23.58 : 1
Taurine derivation 0.2	10	1.53	9.56	0.94	4.30	1.80	33.93	66.07	38.32	25.05 : 1

tain the C/N ratio close to 30/1 to achieve an optimum rate of digestion[14]. The data in TABLE 3 indicate that the C/N values for control and other treatments are ranged from about 8/1 to 42/1.

Effect of concentrations of an amino-acid and its derivative on biogas production

The aim of this experiment is to elucidate the effect of applying different concentrations of taurine, which gave the highest biogas yield in the first experiment, and its derivative on the quantitative determination of the amount of biogas. The accumulative and weekly yields of biogas resulting from control sample as well as other treatments of different concentrations of taurine and its derivative are given in TABLE 4. It is clear from the data obtained that the amount of biogas produced depends on the concentrations of amino-acid and its derivative added. It was observed that addition of 0.2 g of taurine into 500 ml digester gave the optimum biogas product in

TABLE 6 : Methane content of different concentrations of taurine and its derivative

Treatment	Amino acid concentration g/500 ml	Methane concentration %
Control	-	50
Taurine	0.05	67
Taurine	0.1	41
Taurine	0.2	21
Taurine derivation	0.05	58
Taurine derivation	0.1	54
Taurine derivation	0.2	49

comparison to other concentrations. On the other hand, 0.1 g of taurine derivative was preferable among other concentrations. Despite adding larger concentrations of taurine derivative, it gave lower yields of biogas. This may be due to the toxicity caused by higher concentrations.

From the weekly volumes given in TABLE 4 it is obvious that taurine, with 0.1 g concentration gave its maximum value in the 4th week earlier than all other treatments, while 0.2 g concentration, which gave the highest accumulative volume, its maximum value was in the 6th week. In case of taurine derivative, that gave the least accumulative value among all other treatments, its maximum value was in the 10th week, even after the control.

The analytical data of the digested slurry for taurine and its derivative at different concentrations are given in TABLE 5.

Biogas content

TABLE 6 includes methane content for biogas produced from control sample as well as other added concentrations of taurine and its derivative. It is observed from the data of gas quality (CH_4 content), that taurine with 0.05 g concentration gave the highest value. It means that trace concentration of the additive leads to higher concentration of methane content, so the higher concentration of the amino-acid or its derivative gives low concentration of methane. This may be attributed to the need of the methane producing bacteria to small concentrations of the added nutrient; however, the higher concentrations may lead to decrease its activity. This result confirms a high quality of biogas generated from rice straw treated with taurine and its derivative comparable to that produced from animal waste and olive product waste, which ranged from 57 to 65 %[15].

EXPERIMENTAL

Materials and methods

Melting points are uncorrected. The IR spectra were measured using Shimadzu 408 instrument. The mass spectra were recorded at Cairo University in the microanalytical center using Shimadzu GC MS-QP 1000 EX. Elemental analyses were performed in the Microanalytical Center at Cairo University.

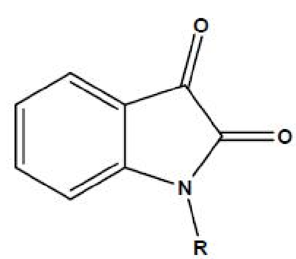
Amino-acids; tyrosine, taurine and L-cysteine (Aldrich) were used as commercial samples.

Preparation of amino-acid derivatives 1a-c:

General procedure:

A mixture of 25 mmol of isatin (3.675 g), 25 mmol of amino-acid and 37.5 mmol of paraformaldehyde in 25 ml of glacial acetic acid was heated on a boiling water bath for 3 h. The solvent was distilled off and the residue was purified by washing with ethanol and acetone.

2-[(2,3-Dioxo-2,3-dihydro-indol-1-ylmethyl)-amino]-3-(4-hydroxy-phenyl)-propionic acid (1a): Orange powder (651mg, 83.47 %), m.p. = 334-6



1

Tyrosine derivative a: R = $NHCH(COOH)CH_2$-C_6H_4-OH-*p*
Taurine derivative b: R = $NHCH_2CH_2SO_3H$
L-Cysteine derivative c: R = $NHCH(COOH)CH_2SH$

°C. IR (KBr): $\tilde{\nu}$ = 3445 (NH), 3333 (OH), 2934, 2930 (CH), 1733 (CO), 1609 (CO) cm^{-1}.

MS m/z (%) = 340 (M^+, 100), 233, 167, 139, 77, 55. Anal. Calcd. for $C_{18}H_{16}N_2O_5$ (340.34) C 63.53, H 4.74, N 8.23; Found C 63.39, H 4.68, N 8.11 %.

2-[(2,3-Dioxo-2,3-dihydro-indol-1-ylmethyl)-amino]-ethanesulfonic acid (1b): yellow powder (89 mg, 13.21%), m.p. 305-7 °C. IR (KBr): = 3210 (NH), 3052, 2970 (CH), 1725 (CO), 1617 (CO),1184 cm-1.MS m/z (%) = 284 (M^+, 100), 204, 139, 76, 55. Anal. Calcd. for $C_{11}H_{12}N_2O_5S$ (284.29) C 46.47, H 4.25, N 9.85, S 11.28; Found C 46.32, H 4.19, N 9.71, S 11.17 %.

2-[(2,3-Dioxo-2,2-dihydro-indol-1-ylmethyl)-amino]-3-mercapto-propionic acid (1c): Orange powder (286 mg, 43.02 %), m.p. > 360 °C. IR (KBr):= 3427 (OH, NH), 2929 (CH), 2565 (SH), 1722 (CO), 1660 (CO), 1619 (CO) cm^{-1}. MS m/z (%) = 280 ($M^{+,}$ 80), 246, 201, 139, 77, 56. Anal. Calcd. for $C_{12}H_{12}N_2O_4S$ (280.30) C 51.42, H 4.32, N 9.99, S 11.44; Found C 51.29, H 4.26, N 9.86, S 11.34%.

A laboratory trails experiment were conducted to study the effect of some amino-acids and their derivatives on the amount of biogas produced. The experiment was carried out in a bench scale as follows:

Old digested slurry was used as starter (25 ml) mixed with (12.5 g) rice straw in 500-ml glass fermenters. The rice straw was first air dried, pulverized and mixed with starter. Then the different amino-acids were added (0.1g). The effect of 3 different concentrations of taurine and its derivative (0.05, 0.1, and 0.2 g) on the amount of gas produced was undertaken. The total volume is completed by water making total solid 8%. The pH was measured and C/N was adjusted by using ammonium sulfate (0.31g). The fermenters were kept under the same conditions at room temperature for 14 weeks. The generated biogas yield was collected and measured weakly by water displacement. Volatile solids were estimated before starting the digestion process to be 52%. At the end of each experiment, the following parameters were determined in the digesting slurry: total volatile acids, total nitrogen, potassium and phosphorus content, total solids, organic carbon, organic matter, total volatile solids. While C/N was calculated mathematically.

Measurement of the biogas

In the lab digesters, the generated biogas was measured by means of the displacement system using acidified water (1N H_2SO_4) to prevent solublization of carbon dioxide contained in the biogas mixture.

Chemical analyses

Quantitative determination of total volatile acids

Total volatile acids were determined by steam distillation according to Neish[16].

pH

The pH was measured directly in semi-solid samples using glass electrode pH meter.

Total Solids (TS)

Samples were dried in an electrical oven at 70 °C to constant weight. The loss in weight was the quantity of moisture percent.

Volatile solids (VS)

It was adopted by glowing the dried samples at 650 °C to constant weight[17].

Organic carbon

Organic carbon was estimated according to Black et al.[18]

Total nitrogen (%N)

Total nitrogen in dried samples was performed by Kjeldhal method recommended by Jackson[19].

Total phosphorus content (% P)

The total phosphorus content was determined according to a method recommended by Troug et al.[20]

Total potassium content (% K)

Potassium content was estimated by flame photometer. The previous parameters were estimated in central laboratory of Horticultural Research Center in Agricultural Research Center in Giza.

Methane content

Methane content was estimated using the Perkin Elmer Sigma 3B Gas Chromatograph installed with FID. Standard curves were prepared using natural gas with 70% methane content and used as reference for calculating methane concentration in the biogas produced.

CONCLUSION

It can be concluded from this study that treatment of rice straw with amino-acids and their derivatives has strong impact in improving the yield of biogas. It is anticipated that the information generated from this study will be used as a guideline in establishing small digesters to produce biogas from rice straw under the experimental conditions, especially rice straw produces twice the amount of biogas generated by conventional digestion of dung. Besides, rural pollution caused by burning of a valuable resource like rice straw can be eliminated.

REFERENCES

[1] J.P.Large; Methanotrophy and Methanogensis, Aspects of Microbiology, 8, Van Nostrand Reenhold Co.Ltd **(1983)**.

[2] G.Gottschalk; Bacterial Metabolism (Editor M.P. Starr) Springer Verlag, N.Y.Berlin, **8**, **(1979)**.

[3] M.R.Smith, R.A.Mah; Acetate as sole carbon and energy source for growth of methanosarcina strain, Appl. Environ. Microbiol., **39**, 993 **(1980)**.

[4] J.Q.Yan, P.H.Liao, K.V.Lo; Methane production from cheese whey, Biomass, **17**, 185 **(1988)**.

[5] R.Zhang, Z.Zhang; Biogasification of rice straw with an anaerobic-phased solids digester system, Bioresource Tech., **68**, 235 **(1999)**.

[6] M.P.Bryany, S.F.Tzeng, I.M.Robinson, A.E.Joyner; Nutrient requirements of methanogenic bacteria, J.Adv.Chem.Ser., **105**, 23 **(1971)**.

[7] S.Banik, R.Nandi, Effect of Supplementation of Rice Straw with Biogas Residual Slurry Manure on the Yield, Protein and Mineral Contents of Oyster Mashroom, Industrial Crops and Products, **20**, 311 **(2004)**.

[8] J.Ye, D.Li, Y.Sun, G.Wang, Z.Yuan, F.Zhen, Y.Wang; Improved biogas production from rice straw by co-digestion with kitchen waste and pig manture, Waste Management, **33**, 2653 **(2013)**.

[9] Teghammar, K.Karimi, I.S.Horvath, M.J.Taherzadeh; Enhanced biogas production from rice straw, triticale straw and softwood spruce by NMMO pre- treatment, Biomass Bioenergy, **36**, 116 **(2012)**.

[10] Z.Lei, J.Chen, Z.Zhang, N.Sugiura; Methane production from rice straw with acclimated anaerobic sludge: effect of Phosphate supplementation. Bioresource Tech., **101**, 4343 **(2010)**.

[11] L.Pyle; Anaerobic digestion, The technical options, IDRC, 9-65 **(1978)**.

[12] A.ATroug, Meyar; Improvement indacineness, Calorimetric method for Phosphorus and Arsematic Imsl.Eng.Chem.Anl.Ed., **136** **(1939)**.

[13] E.J.Kroeker, D.D.Schulte, A.B.Sparling, H.M.Lapp; Anaerobic treatment process stability, J.Water Pollut.Control Fed., **51**, 718 **(1979)**.

[14] B.E.Ali; Bioenergy from organic residues for rural Egypt, ph.D. Thesis, Fac.of Agric., Ain Shams Univ., Egypt, **(1985)**.

[15] C.N.Acharya; Preparation of fuel gas and manure by anaerobic fermentation of organic materials. Indian Council of Agric.Res.Series, Indian Council of Agric.Res.Bull.No. 15 New Delhi, **(1958)**.

[16] M.Hammad, D.Badarneh, K.Tahboub; Evaluating variable organic waste to produce methane, Energy Conversion and Management, **40**, 1463 **(1999)**.

[17] A.C.Neish; Analytical methods for bacterial fermentations, Report No.46.8.3. NRC No.2952 **(1952)**.

[18] "APHA" American public health association, Standard Methods for Examination of Water and Waste Water, 14^{th} Edition, Washinton D.C, **(1976)**.

[19] C.A.Black, D.O.Evans, L,E,Ensminger, J.L.White; Methods of soil analysis, II-chem, and Microbio.Properties.Amer.Soc.Agron.Inc.Madison, Wisconsin, U.S.A **(1965)**.

[20] M.L.Jackson, Soil Chem.Analysis, Prentice Hall of Indian Private, New Delhi **(1973)**.

[21] A.A.Troug, Meyar; Improvement indacineness, Calorimetric method for Phosphorus and Arsematic Imsl.Eng.Chem.Anl.Ed., 136 **(1939)**.