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Halophytes and salt tolerant wild plants as a feedstock for biogas production

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Article info:

Received: 2019
Accepted: 2019

ABSTRACT

This paper describes the ability of wild plants to be investigated as feedstock in biogas production. Anaerobic degradation of four wild halophytes and salt-tolerant plants (*Avicennia marina*, *Tamarix nilotica*, *Zygophyllum album*, and *Zygophyllum coccineum*) collected from the Red Sea coast in Egypt was studied. Lab-scale reactors were fed with dried and milled plant biomass.

Obtained results showed that the highest biogas production result from *A. marina* 487.862 ml/Vs (403.385 ml/TS) followed by *T. nilotica* 441.30 ml/Vs (333.278 ml/TS) while 291.28 ml/Vs (206.21ml/TS) and 127.923 ml/Vs (81.272 ml/TS) for *Z. album* and *Z. coccineum* respectively. The chemical structure of these plants was the main factor controlling the variation in biogas production especially cations (Ca^{2+} , Na^+ , K^+ , Mg^{2+}) and organic fractions (volatile solids, crude fiber, crude protein). At high volatile solids with high protein content and low salt content, there was the highest biogas production in *A. marina*. on the other hand, increasing salt content decreasing biogas as in *Z. coccineum*. This indicates that the wild plants can represent a promising source for renewable energy and their solid digestate fraction can be used as biofertilizer.

Key words: Halophyte biomass, anaerobic biogas production potential, Antagonistic effect, digestate

Introduction

The need to produce safe and cheap energy is necessary to overcome the increased demand for energy. Organic resources are the main provider to produce safe energy. Renewable energy is a clean and cheap source of energy and has a low impact on the environment (Panwar et al., 2011). Natural plants can be considered as a cheap source for biofuel production (Baute, 2015). They grow naturally depending on the prevailing environmental conditions and don't represent an economic problem especially in developed and poor countries. The expected decrease in electricity in Egypt especially after the complete construction of the Ethiopian dam will affect the different activities from the house uses until the great industrial economics.

Several plant species have been tested as feedstock for biogas production, but there is a low concern on halophytic plant species (Turcios et al., 2016). Halophytes are known as plants that survive in environments with high concentrations of salts (Ungar, 1991). In Egypt, halophytes occupy inland salt marshes in the desert area, and littoral salt marshes along the Mediterranean and the Red Sea coasts (Salama et al., 1999). Halophytes and salt-tolerant species can be surviving in salt-affected, degraded and inhibited unproductive lands.

Perennial plants biomass represent one of most promising source for bioenergy and also, biogas as they can be harvested successional for several years without reseeding and give high biomass yield with sufficiently biomass quality (Lewandowski et al., 2003; Tilvikiene et al., 2012).

The most productive halophytic plant species yield ranges from 10 to 20 tons/ha of biomass when irrigated with seawater (Glenn et al., 1999). Generally, the cultivated halophytes productivity comparing with traditional crops is high (Jaradat, 2003). Therefore, halophytes considered a valuable feedstock for bioenergy production. It must take into concern the influence of their chemical structure on the anaerobic digestion process. The high salt content in their structure can influence the methanogenesis process (De Baere et al., 1984). Halophytes tend to accumulate a high concentration of salts in their tissues to adjust the osmotic pressure and prevent toxicity effects on metabolism (Flowers et al., 2014). It is crucial to determine the concentration of salts in the plant tissue used for anaerobic digestion.

Many researchers studied the possibility of using natural halophytic plants as bioenergy resources such as *Tamarix sp* (Debez et al., 2017; Sharma et al., 2016; Sun & Norman, 2011), *A. marina* (Almardeai et al., 2017), *Zygophyllum sp* (Cybulska et al., 2014).

The anaerobic process transforms the added feedstock into biogas and digestate that can be used as a fertilizer which is high in nitrogen, potassium and phosphorus contents (Walsh et al., 2012).

To study the possibility of producing biogas using anaerobic digestion (AD) from the biomass of some halophytic plant species.

Studying the main factors which affect biogas production from halophytes.

Determine the possibility of digestate for use as fertilizer.

Materials and Methods

Raw materials for fermentation

Shoots of four perennial halophytic plant species were collected at Red Sea coast between (25°43'33.35"N - 26°37'4.33"N and 34°32'38.56"E - 34° 0'31.57"E) for use as raw materials for fermentation experiment. They were *Avicennia marina* (Forsk.) Vierh, *Tamarix nilotica* (Ehrenb.) Bunge, *Zygophyllum album* L and *Zygophyllum coccineum* L. The samples were dried using drying oven at 60°C for 72 hours, then ground to be powder ready for use.

Chemical analyses

Digests were prepared using 0.5 gm. of the grounded plants in 10 ml of H₂SO₄ and 2 ml of perchloric acid (Chapman and Pratt, 1961) to determine the percentage of total phosphorus spectrophotometrically (Jackson, 1958) using JENWAY 6305 UV/Visible Spectrophotometer and total nitrogen by Kjeldahl methods (Bremmer & Mulvaney, 1982). Organic carbon content was determined using the Walkley-Black wet combustion method (Tan, 1996).

Plant extract (w/v) was prepared according to (El-Sharkawi and Michel, 1977) to estimate the chlorides according to (Jackson, 1958), electric conductivity (EC) and total dissolved salts (TDS) according to (Jackson, 1967) using a conductivity meter (model 4520 JENWAY UK Bibby Scientific Ltd, Dunmow, Essex). Potassium and sodium were determined using the flame photometer (model PFP7 JENWAY stone, staffs, UK, ST15 OSA) method described by (Knudsen et al., 1982). Calcium and magnesium were determined volumetrically by the versene titration method described by (Johnson & Ulrich, 1959).

Crude fibers were determined according to (AOAC., 2005). Total solids (TS) and volatile solids (VS) were determined according to (Chandra, 2009).

Inoculum

The ruminal fluid used as inoculum for anaerobic digestion, due to its very high content of anaerobic bacteria (Aurora, 1983). It is brought from Qena public slaughterhouse. The total solid (TS) of the inoculum was

3.64%±0.13, and volatile solids (VS) of the inoculum was 61.19%±0.6.

Anaerobic digestion

Biogas potential and methane production rates were estimated using the batch method (Hansen et al., 2004). The used apparatus for anaerobic fermentation illustrated in Figure 1.

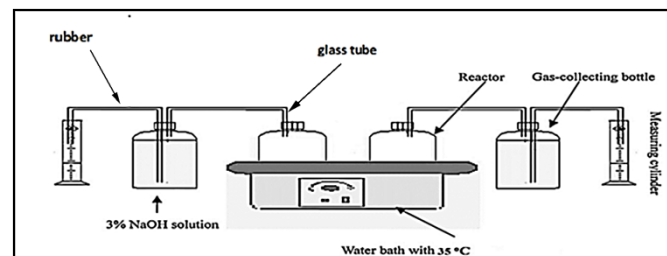


Figure 1. The apparatus used for biogas production.

The volume of the used reactor was 1120 ml, the gas was collected in a 1000 ml bottle and the displaced NaOH solution was collected in a 500 ml measuring cylinder (Guo, 2011; You et al., 2003). The fermentation carried out in the water bath at 37±2°C.

Three replicates of the suitable weight of digesting substrate containing (2.22 g VS). organic matter (VS) with 200 ml of inoculum (4.45 g VS) was added to three reactors. This proportion [2 inoculums (VS_i) / 1 digesting substrate (VS_s)] lead to minimize accumulation limitation and avoid toxicity inhibition (Rico et al., 2014). Each blank reactor (control) contained 200 ml of inoculum only. Then, the volume increased up to 800 ml with tap water. The fermentation process was continued for 10 weeks when the production became negligible. Daily biogas production was recorded by computing the increase in the volume of displaced alkaline solution. The released CO₂ during fermentation process dissolves in the alkaline solution without any effect on the volume of biogas (Esposito et al., 2012).

The anaerobic biogas production potential (ABP) was calculated according to the following equation:

$$ABP = \frac{V_{(ino+substrate)} - V_{ino}}{mVS_{substrate}}$$

Where ABP (ml /g of added VS), V_{ino + substrate} is the volume (ml) of biogas produced from inoculum and substrate, V_{ino} is the volume of biogas produced by inoculum only and mVS_{substrate} is mass of volatile solids in the substrate (g).

N, P, K, and organic carbon were estimated, after the bio-digestion, to evaluate the fertilizer value of the slurry residue.

Statistical analyses

The standard error (SE) for chemical composition, Pearson's correlation coefficients to determine the correlation between the plant's chemical composition and the amount of produced biogas and the interrelations between plant chemical constituents were computed using SPSS 23.0 for Windows (SPSS, Chicago, IL, USA). Significant differences between the produced biogas from different plants versus blank tested using one-way analysis of variance (ANOVA).

Results and discussion

Chemical composition of the plants

The chemical composition of the substrate is the most important factor through the anaerobic digestion process to produce considerable amounts of biogas (Elsayed et al., 2015). Anaerobic digestion can convert any biomass to biogas with the exception of certain organic components as lignin (Tuomela et al., 2000; Turcios et al., 2016) or inorganic cations which can inhibit microbial activity (de Lemos Chernicharo, 2007).

The chemical structure of the selected plants (Table 1) showed clear differences between the different species. The VS content and crude fibers as the main source of biogas (Eboatu et al., 2006) were the highest in *A. marina*. The VS was 82.684% and 16.24% was crude fibers. Therefore, *A. marina* produced the highest amount of biogas about 487.862 ml/VS (403.385 ml/TS). On the other hand, *Z. coccineum* produced the lowest biogas outcome. It gave 127.923 ml/VS (81.272 ml/TS). This due to the low content of VS (63.53%) while the crude fibers were 4.193%.

In *T. nilotica*, the VS were 75.522% and the crude fibers were 14.74% and produced biogas was 441.30 ml/VS (333.278 ml/TS). *Z. album* had 70.793% VS and the crude fibers were 10.027% and produced 291.282 ml/VS (206.207ml/TS).

Statistically, there was a high positive significant difference between anaerobic biogas production potential ABP and volatile solids content ($R = 0.966$, $p = 3.3 \times 10^{-7}$). As reported by (Mayer et al., 2014) who pointed out the VS is the most controlling factor influencing the biomethane yield of maize silages. Also, there was a high positive significant correlation with crude fiber ($R = 0.940$, $p = 6 \times 10^{-6}$). That agrees with (Dugmore et al., 1986).

Anaerobic degradation depends essentially on the carbon/nitrogen ratio. (Kwietniewska & Tys, 2014) reported that the optimal C/N ratio for anaerobic degradation of organic waste is ranged 20-35. *A. marina* had the lowest C/N ratio, it was 36.728. Consequently, it produced the highest biogas amount (403.385 ml/TS). The increasing in C/N ratio means rapid depletion of the nitrogen in the medium, so, the reduction in biogas production is expected (Jingura & Kamusoko, 2017).

T. nilotica produced high amounts of biogas although its higher C/N ratio compared with *Zygothymum* sp. The high content of VS (75.5%), CF (14.74%) and CP (5%) with lower content of inorganic elements decreased the effect of the higher C/N ratio on the biogas production (Table 2). There was a high positive significant correlation between crude protein and ABP ($R = 0.815$, $p = 0.01$). Generally, a high percentage of Crude protein in both *A. marina* and *T. nilotica* increased the amount of biogas. Protein as a source for carbon causes a faster conversion rate to biogas (VDI, 2006).

The low biogas production produced from *Z. album* and *Z. coccineum* (Table 2) is due to the decrease of nitrogen in the medium. C/N ratio was 48.636 and 55.149 respectively. Statistical analysis showed a negative high significant difference between ABP and C/N ratio ($R = -0.788$, $p = 0.02$).

Crude ash was associated parallel with total dissolved solids and electrical conductivity. *Z. coccineum* had the highest electrical conductivity (32.983 ms cm^{-1}) followed by *Z. album* (32.733 ms cm^{-1}), *T. nilotica* (19.2 ms cm^{-1}) and the lowest is *A. marina* (17.267 ms cm^{-1}).

Biogas production increased with the decreasing electrical conductivity, especially after 32 ms cm^{-1} . (Ogata et al., 2016) reported that the salt concentration of 35 mS cm^{-1} of EC (dilution 1:9) inhibited CH_4 generation. High salt concentration above 80 mS cm^{-1} of EC inhibits not only CH_4 and CO_2 generation but also the degradation of organic compounds. There was an extremely high negative correlation and highly significant difference between anaerobic biogas production potential ABP and electrical conductivity ($R = -0.909$, $p = 4.1 \times 10^{-5}$).

The increase of electrical conductivity reflects the increase in TDS and the concentration of individual ions, they increase parallel to each other. Several researchers investigated the effect of inorganic ions on the rate of biogas production. Na^+ is one of the familiar ions in the saline places and consequently in plant tissue. At high concentrations, sodium could readily effect on microorganisms activity and interfere with their metabolism (Balslev-Olesen et al., 1990; Gourdon et al., 1989; Kugelman and McCarty, 1964; Mendez et al., 1995; Rinzeema and Lettinga, 1988). The low concentration of Na^+ was detected in *A. marina* (3.08 mM) and increased gradually in *T. nilotica*, *Z. album*, and *Z. coccineum* respectively. The increase of sodium concentration increases the electrical conductivity causing a decrease in biogas production (Table 1). The rate of biogas production was 487.86 ml g^{-1} VS in *A. marina* decreased to 127.92 ml g^{-1} VS in *Z. coccineum* (Table 2).

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Table 1. *The chemical composition of the studied wild plants*

	<i>Avicennia marina</i>	<i>Tamarix nilotica</i>	<i>Zygophyllum album</i>	<i>Zygophyllum coccineum</i>
TS %	46.69± 0.26	63.15± 1.5	25.43±1.02	15.78±2.69
VS %	82.689±0.17	75.522±0.36	70.79±0.94	63.53±0.31
TN %	1.4	0.8	0.84	0.68
C/N	36.73±0.46	52.3±0.53	48.64±0.58	55.149±0.39
CP %	8.75	5	5.25	4.25
CF %	16.24±0.27	14.74±0.39	10.03±0.55	4.19±0.55
Na ⁺ %	8.86±0.33	9.37±0.22	11.11±0.22	12.04±601
K ⁺ %	1.013±0.004	0.496±0.004	0.45±0.004	0.6±0.006
Cl ⁻ %	3.605±0.102	5.260±0.27	8.81±0.37	10.05±0.45
Ca ²⁺ %	0.27±0.029	1.25 ±0.05	2.7±0.09	3.05 ±0.087
Mg ²⁺ %	0.45±0.06	0.91±0.017	0.72±0.08	0.81±0.03
CA %	17.32±0.169	24.48±0.36	29.21±0.94	36.47±0.36
TDS g/l				
Dilution(1:10)	10.42±0.62	11.5±0.69	19.52±0.4	19.85±0.61
EC ms cm ⁻¹				
Dilution(1:10)	17.27±0.95	19.2±1.15	32.73±0.38	32.98±0.96

Legend: Mean ± (SD); TS: total solid, VS: volatile solid, TN: total nitrogen, CP: crude protein, CF: crude fiber, Ca: calcium Cl: chloride, CA: crude ash, TDS: total dissolved solid, EC: electrical conductivity, Na: sodium K: potassium Mg: magnesium Note CP = TN*6.25.

Table 2. *Cumulative biogas production and biogas production rate of wild biomass plants*

Wild plants	<i>Avicennia marina</i>	<i>Tamarix nilotica</i>	<i>Zygophyllum album</i>	<i>Zygophyllum coccineum</i>
Cumulative gas production	1962±126.57	1636.67±248.34	1236.5±7.78	868.5±96.88
biogas production rate ml g ⁻¹ TS	403.39±38.95	333.28±82.92	206.21±2.68	81.27±34.23
biogas production rate ml g ⁻¹ VS	487.86±47.1	441.3±109.8	291.28±3.79	127.92±53.88

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The same behavior was observed with Ca^{2+} , the concentration of calcium increases from 0.05 mM in *A. marina* up to 0.61 mM in *Z. coccineum*. Ca^{+2} , Na^{+1} show a negative effect on biogas production and that due to their toxicity on methanogenic bacteria (Chen et al., 2008). There was an extremely high negative correlation with high significant difference between anaerobic biogas production potential ABP and sodium and calcium ($R = -0.916$, $p = 4.1 \cdot 10^{-5}$) and ($R = -0.948$, $p = 4.1 \cdot 10^{-5}$).

Except for *T. nilotica*, the concentration of Mg^{2+} increased gradually. The gradual increase in magnesium ions in the reactor stimulates the production of biogas. (Schmidt & Ahring, 1993) point out that cultures could be adapted to 300 mM Mg^{2+} without a change in growth rate, but growth ceased at 400 mg/L Mg^{2+} . Also, Mg^{2+} had an antagonistic effect which alleviates the inhibition effect resulted from the increase in ammonia concentration (Krylova et al., 1997) and Na^{+} toxicity (Kugelman and McCarty, 1964). Therefore, the high concentration of Mg^{2+} in the case of *T. nilotica* stimulated the biogas production than the two species of *Zygothylum*. *T. nilotica* produced 441.3 ml g^{-1} VS compared to the biogas volumes produced by *Z. album* and *Z. coccineum* which were 291.3 and 127.9 ml g^{-1} VS respectively.

Potassium is considered as a compatible solute preferred by plants that survived under saline conditions (Kamel, 2008). Therefore, *A. marina* accumulated approximately twofold the accumulated K^{+} in other species. The concentration of K^{+} was 0.21 mM. Potassium with Low concentrations (less than 400 mg/L) causes an enhanced performance in both the mesophilic and thermophilic ranges while at higher concentrations it showed an inhibitory effect especially in the thermophilic temperature range (Chen et al., 2008). The higher potassium concentration increased the protein content (Kamel & El-Tayeb, 2004). This lowered the C/N ratio enhancing the production of biogas in *A. marina* compared with other species. The produced biogas in the case of *A. marina* was 487.86 ml g^{-1} VS while *T. Nilotia*, *Z. album*, and *Z. coccineum* produced 441.3, 291.3 & 127.9 ml g^{-1} VS respectively.

Chlorides increase decreased biogas production (Table 2). The lower percent of Cl^{-} was found in *A. marina* (3.6%) produced 487.86 ml g^{-1} VS while the highest content (10%) in *Z. coccineum* produced 127.9 ml g^{-1} VS. The relative chlorophenols toxicity has been studied by many researchers and the results are slightly contradictory. Most of the halogenated aliphatic are strong inhibitors of methanogenesis (Chen et al., 2008).

Statistical computing showed a high negative significant difference between anaerobic biogas production potential ABP and chloride percentage. Person correlation between the chemical composition of plant species and the quantity of

biogas production and the interrelation among chemical constituents are computed in the Table 3.

Daily biogas production rate

The incubation period extended to 70 days under mesophilic conditions at $37 \pm 2^{\circ}\text{C}$. The quantity of biogas was measured at two days intervals. The production rate of biogas varies from one plant to another depending on its chemical structure (Wang et al., 2013).

The daily produced biogas is illustrated in Figure 2.

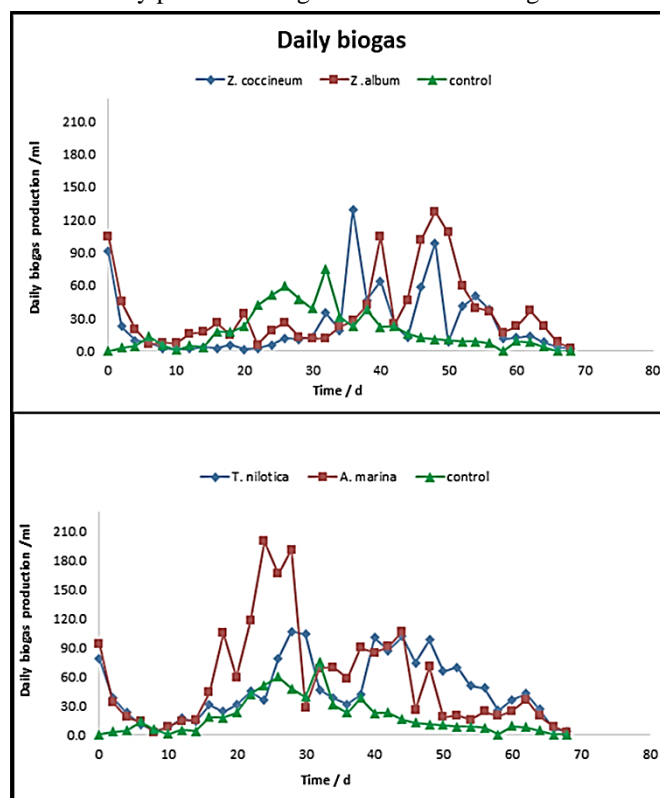


Figure 2. Daily biogas production during the digestion *Z. coccineum*, *Z. album*, *A. marina* and *T. nilotica*.

The control exhibited one peak only after 32 days, it produced 75 ml /g VS_{added}. *A. marina* showed the highest peak (200ml /g VS_{added}) after 24 days. This may be due to the high content of organic matter especially proteins that are more easily transformed into methane (Barbanti et al., 2014). *T. nilotica* came in the second order with two peaks; the first was 106.7ml /g VS_{added} after four weeks was 101.3ml /g VS_{added} after 44 days. The second peak may due to the conversion of complicated organic matter to low molecular weight organic matter (Khan & Ahring, 2019). (Ahring et al., 2001; El-Mashad et al., 2004) obtained two peaks during anaerobic digestion. Succulent species (*Z. album* & *Z. coccineum*) depends on the inorganic solutes to readjust their osmotic pressure to overcome the external salinity (Kamel, 2008).

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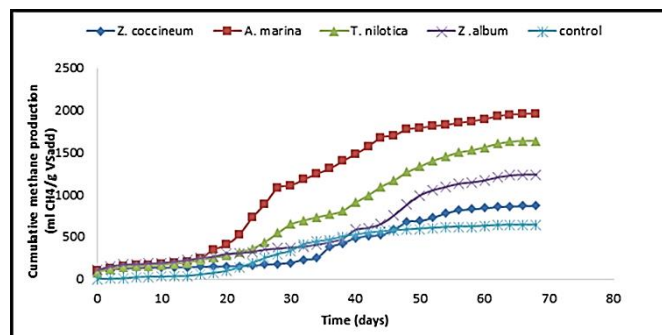
Table 3. Person correlation between the chemical composition of plant species and the quantity of biogas production and the interrelation among chemical constituents * $P < 0.05$, ** $P < 0.001$.

	VS	CA	CF	C/N	Cl ⁻	Ca ²⁺	Na ⁺	EC	TDS	Mg ²⁺	CP
ABP	.966**	-.966**	.940**	-.788**	-.943**	-.948**	-.916**	-.909**	-.912**	-.530	.815**
VS		-1.00**	.963**	-.854**	-.957**	-.959**	-.938**	-.884**	-.886**	-.604*	.875**
CA			-.963**	.854**	.957**	.959**	.938**	.884**	.886**	.604*	-.875**
CF				-.700*	-.946**	-.923**	-.948**	-.897**	-.902**	-.392	.719**
C/N					.731**	.775**	.674*	.601*	.599*	.909**	-.987**
Cl ⁻						.985**	.974**	.967**	.964**	.476	-.790**
Ca ²⁺							.938**	.966**	.966**	.525	-.837**
Na ⁺								.928**	.926**	.427	-.725**
EC									.998**	.317	-.680*
TDS										.330	-.677*
Mg ²⁺											.889**

The high content of inorganic substrate especially Na⁺ and Cl⁻ affect the rate of biogas production (Alhraishawi & Alani, 2018). Therefore, they came after *A. marina* and *T. nilotica* in biogas production. The highest peak of *Z. album* (127.5ml /g VS_{added}) on day 48 while *Z. coccineum* peak was 129.5ml /g VS_{added} after 36 days. In summation, at the end of the 70-days digestion, the cumulative biogas yield obtained from *A. marina* was 487.86 mL CH₄/g VS_{added}, *T. nilotica* 441.3 mL CH₄/g VS_{added}, *Z. album* (291.28 mL CH₄/g VS_{added}) and *Z. coccineum* (127.92 mL CH₄/g VS_{added}) as presented in Table 2. The daily increase in cumulative biogas yield is illustrated in the Figure 3.

Slurry characteristics

At the end of the anaerobic process, two main products were formed. Biogas which used as a clean source of energy especially in the production of electricity. The second part is the digestate which can be used as a fertilizer. Digestate can be separated into fiber and liquor. The fiber can be sold or used as a good fertilizer or a soil conditioner, while the liquor contains various nutrients and could be used as a liquid fertilizer which could be sold or used on-site (Ortenblad, 2000).

**Figure 3.** Cumulative methane yields produced from the digestion of *T. nilotica*, *Z. album*, *A. marina* and *Z. coccineum*.

The Quality of solid digestate for using as biofertilizer depending on the ability of digestate to replace the inorganic fertilizers, according to its physicochemical properties. The residual slurry resulted from the current investigation contained a significantly higher content of N, P and K content (Table 4).

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Table 4. Physico-chemical properties of solid undigested slurry

Species	C %	C/N	P %	TDS g/l	K %	EC / mS cm ⁻¹
<i>Avicennia marina</i>	46.28±0.33	16.53±0.12	2.03±0.16	2.62	06.56±0.09	4.40
<i>Tamarix nilotica</i>	43.87±0.42	18.28±0.17	3.09±0.22	2.15	05.25±0.03	3.59
<i>Zygothymum album</i>	36.11±0.14	18.06±0.07	3.52±0.34	2.14	04.73±0.05	3.57
<i>Zygothymum coccineum</i>	40.43±0.74	20.22±0.37	5.57±0.13	2.46	04.00±0.13	4.12

Möller et al., 2008 pointed out the suitable carbon content ranges between 36-45%. (Fouda, 2011; Gutser et al., 2005; Möller et al., 2008) defined the preferred range of C/N ratio between 2-24.8 %. The optimal range of potassium detected by (Möller et al., 2010; Pötsch, 2004; Voća et al., 2005) lies between 1.9-4.3 %. Phosphorous ranges between 0.2-3.5 % according to (Pötsch, 2004; Teglia et al., 2011a, 2011b; Voća et al., 2005). The chemical composition of the produced digestate in this experiment as shown in Table 4, lies inside the boundaries detected by several pieces of research as mentioned above.

Conclusions

In arid regions, perennial xerophytes and halophytes can grow in severe conditions of drought and salinity. They need only a little attention to manage them as a considerable source of renewable energy production. Therefore, it is necessary to manage the desert and coastal areas to increase the biomass produced from plants that are adaptive to drought and salinity to use in biogas production as safe energy. It is helpful for developed and poor countries and decreases global warming.

Acknowledgments

We would like to express our deep gratitude to Dr. Ahmed Mahmoud Abass, Assistant professor of plant ecology, South Valley University for helping us in Statistical analysis, and Dr. Mohamed Owis Badry, lecture of plant systematics for his help in plant identification.

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