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Evaluation of Methane Production of Six Varieties of Date Pulp Waste (Phoenix Dactylifera L.)

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Anaerobic digestion is the biodegradation of the waste materials with the aid of microorganisms which grow in the absence of oxygen. Fruits and vegetable wastes are a good substrate with potential to produce biomethane and are abundantly available. The present study investigated the biogas production of 6 different varieties of date pulp (Phoenix Dactylifera L.) and the possible correlation with their bio-chemical compositions (total solids, volatile matter, lipids, proteins, ash, sugars and polyphenols content). The methane production curves were fitted to the modified Gompertz model to extract the kinetic parameters related to the methane production. The specific methane production potential of the variety Kenichi alone was the lowest with 252 NL $CH_4 \cdot kg\ VS^{-1}$. However, the variety Deglet Nour had a maximum methane production potential with 334 NL $CH_4 \cdot kg\ VS^{-1}$. The data were subjected to Pearson correlation test and Principal Component Analysis. Results show that the total sugars contributed the most to the methane potential and the methane production rate, which indicates that date pulps with more total sugars could bring about more and faster methane yield.

1. Introduction

Anaerobic digestion (AD) of solid organic waste has gained increased attention as a means of producing energy-rich biogas, destructing pathogenic organisms and reducing problems associated with the disposal of organic waste (Sonakya et al., 2001). Fan et al. (2017) reported that life cycle assessment is important to understand the environmental impact of the AD processes with many factors affecting the design and performance of AD. Some studies are related to the evolution of the substrates concentration and the characterization of the physico-chemical parameters of AD digesters in real time for the purpose of kinetic modeling of methane production. Physical and chemical characteristics of the organic wastes are important for designing and operating digesters, because they affect the biogas production and the process stability during AD. They include moisture content, volatile solids, nutrient contents, particle size, and biodegradability. Especially, detrimental effects of polyphenols on AD process were reported by Battista et al. (2013) concerning the wastes derived from olive oil production. They found that the polyphenols in the olive oil inhibited the activity of methanogenic bacteria. Similar result was also observed by Sayadi et al. (2000) who stated that for a higher molecular mass of polyphenols, the aerobic and anaerobic biodegradability of the olive mill wastewater decreased. The anaerobic biodegradability of a feed is interpreted by the percentage of organic matters that are converted in methane during AD. The biogas or methane yield is measured by the amount of biogas or methane that can be produced per unit mass of volatile solids contained in the feedstock after AD treatment.
The date palm *Phoenix dactylifera L.* has played an important role in the daily life of the Tunisian people over the last 7,000 y. Today the worldwide production, the utilization and the industrialization of dates are drawing increasing attention since date fruits have earned great importance in human nutrition owing to their rich content of essential nutrients (Faostat, 2010). However, this booming date-processing industry is accompanied by a considerable rejection of dates of secondary class. Over 2,000,000 t of date palm fruit wastes in the world are

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discarded annually, leading to severe environmental problems (Mrabet et al., 2012). Thus, there is an urgent need to search for suitable solutions to this issue. The prospects of the valorization of these date-processing by-products and wastes can be found through the technology towards total utilization of this valuable commodity for the bio-production of biofuels, biopolymers, bio-surfactants, etc.
In this context, the present study seeks to investigate the biogas production from the waste of date pulp. To the best of our knowledge, no previous study has examined the AD of the waste of the date pulp. Therefore, this study is to evaluate the anaerobic biodegradability and carry out a comparative analysis of AD kinetics of various wastes of date pulp. The main objectives of this study were to determine the main chemical components of the 6 pulp dates varieties (total solids, volatile matter, total chemical oxygen demand, lipids, proteins, ash, sugars and polyphenols content) and to evaluate the anaerobic biodegradability and the methane potentials of the samples. The possible correlations between the parameters were investigated using the statistical methods.

2. Materials and methods

2.1. Raw materials

The 6 different Tunisian varieties of dates fruit (*Phoenix Dactylifera L.*), Deglet Nour, Alig, Ammari, Bejou, Kenta and Kentichi, were harvested in October 2017 in the Tozeur region (Tunisia). These dates of secondary class, not dedicated to commercial market, were usually treated as food wastes pending valorization.
The anaerobic digestate used as inoculum for AD process has been collected from the mixed economy company LIGER (Locminé, France).

2.2. Physico-chemical characterization of the inoculum and the substrates

Total Solids (TS) content was determined by dry weight in oven with forced air circulation at 105 °C until constant weight. Afterwards, Volatile Solids (VS) based on TS were determined in a muffle furnace after 2 h at 550 °C and then cooled in a desiccator and weighed. Total Chemical Oxygen Demand (COD) was measured using Merck COD Spectroquant test (Merck KGaA, Darmstadt, Germany) and by a spectrophotometer NOVA 60 (Merck, Darmstadt, Germany). The samples were put into the thermo-reactor and heated for 2 h at 148 °C.

2.3. Automatic Methane Potential Test System (AMPTS)

Four hundred grams (400 g) of the fresh inoculum and the substrates with different quantity were added to 500 mL incubation bottles respectively. A ratio VS substrate / VS inoculum at 0.4 was fixed to start the AD and all experiments were duplicate. The specific CH_4 yield of each substrate was determined using an Automatic Methane Potential Test System (AMPTS, Bioprocess Control, Sweden). AMPTS was developed for the automatic real time measurement of the methane production during the AD of organic biomass. The system was designed to carry out 15 analyses at one experiment. The system operation was described in Badshah et al. (2012). Bottles were incubated at 37 °C. All tests and blanks were carried out in duplicate, and the net values of methane production were obtained by subtracting the methane production of the blank bottles. The experimental biochemical methane potential (BMP) was calculated from the volume of methane produced divided by the VS of the substrates introduced and expressed in (NL $CH_4 \cdot kg\ VS^{-1}$).

2.4. Determination of biochemical compositions

The protein content of each sample was quantified using the method described by Bradford (1976). Under heat and with the sulphuric acid, the lipids formed, in the presence of vanillin and orthophosphoric acid, a complex pink. In 100 µL of lipid extracts, 1 mL of sulphuric acid (96 %) was added. After five minutes cooling, 2.5 mL of sulfo-phospho-vanillin reactive were added to 200 µL of this mixture (Goldsworthy et al., 1972). The total amount of carbohydrates was determined using the Roy method modified by Duchateau Flokin (1959). This technique consists in adding 0.5 mL of the sample as well as 4.5 mL of the anthrone reagent, and then heating the mixture at 80 °C for 10 min. The absorbance was read at 620 nm. Total phenolic compound were quantified after liquid extraction (solid-liquid ratio of 1:5 w:w using methanol solvent) according to the method described by Allaith (2008) using the Folin-Ciocalteu reagent. The absorbance against blank was read at 760 nm. The total polyphenolic content was expressed as mg of Gallic Acid Equivalents (GAE) per 100 g of fresh weight (FW). The samples were dried in an oven set at 70 °C. One gram of sample is placed in a previously weighed porcelain dish and was introduced into a muffle furnace at 550 °C for 4 h. After calcination of the plant extract, the capsules were weighed to determine the mass of the ash content (Chaira et al., 2009).

2.5. Data processing

The methane production kinetics BMP(t) were fitted using the Gompertz model (reviewed by Bong et al., 2017) This equation has been identified as a good non-linear regression model and commonly used in the simulation of methane accumulation using R studio software (Massachusetts, US):

$$BMP\,(t) = BMP_0 \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{BMP_0}(\lambda - t) + 1\right]\right\} \quad (1)$$

where BMP_0 is the Biochemical Methane Potential (NL CH_4·kg VS^{-1}), R_m the maximum methane production rate (NL CH_4·kg VS^{-1}·d^{-1}), e the exp(1), λ the lag time (d) and t the time (d).
The Pearson correlation (with dimensionless parameters p-value and Pearson correlation coefficient) was performed between different groups of physicochemical compositions and kinetic parameters of Gompertz model related to 6 date varieties. Principal Component Analysis (PCA) was performed to investigate the interaction among the studied parameters that took place behind the AD of date pulp wastes. All of the statistical tests were conducted using IBM SPSS 20 (IBM Co., New York, US).

3. Results and discussion

3.1. Anaerobic digestion (AD)

The results of the biochemical compositions and the physico-chemical characterisation with regard to the AD of the substrates tested are presented in Table 1 and Table 2. The inoculum had a pH value of 8.1, a TS content of 5.3 %, a VS/TS ratio of 63.8 % and a COD of 58.7 g COD·kg^{-1}.

Table 1: Biochemical Compositions of the 6 varieties of date pulp tested

Variety	Polyphenol content (mg GAE·100 g FW^{-1})	Ash content (g·100 g FW^{-1})	Total sugars (g·100 g FW^{-1})	Lipids (g·100 g FW^{-1})	Proteins (g·100 g FW^{-1})
Alig	120.6	1.23	62.7	1.79	3.71
Ammari	108.0	1.36	61.6	3.72	2.32
Bejou	114.6	1.13	55.8	2.15	2.00
Deglet Nour	124.1	1.31	73.9	2.70	2.54
Kenta	116.4	1.36	55.6	2.72	2.19
Kentichi	116.9	1.20	45.2	1.97	3.29

The biogas production curves for different varieties of the date pulp are present in Figure 1. The AD of the date pulp terminated in 18 d. Little daily methane production was observed after the 6^{th} day which indicated that the digestion could be considered close to the end. The experimental methane yield of the varieties of Deglet Nour, Alig, Ammari, Bejou, Kenta and Kentichi was observed at 341.5, 326.9, 316.1, 283.8, 297.8 and 257.7 NL CH_4·kg VS^{-1} respectively. Deglet Nour, with the highest methane potential, produced nearly 32.5 % more methane than Kentichi, the lowest one. Speaking of the biodegradability, the methane yield normalised by COD was of 334.4, 320.2, 310.0, 277.2, 290.5 and 252.1 NL CH_4·kg COD^{-1} for the varieties of Deglet Nour, Alig, Ammari, Bejou, Kenta and Kentichi respectively, corresponding to an anaerobic biodegradability from 85.7 % to almost 100 %. The results of the modelling given by modified Gompertz equation was presented in Table 2.

Table 2: Physico-chemical characteristics of 6 varieties of date pulp tested for AD and their methane potential (BMP_0), lag time (λ), the maximum methane production rate (R_m) given by the modified Gompertz model

Variety	TS (%)	VS/TS (%)	COD (g O_2·kg FW^{-1})	BMP_0 (NL CH_4·kg VS^{-1})	R_m (NL CH_4·kg VS^{-1}·d^{-1})	λ (d)
Alig	74.5	96.6	756.1	320.2 ± 11.7	100.7 ± 3.3	0.29 ± 0.05
Ammari	66.7	96.9	611.0	310.0 ± 0.0	94.8 ± 0.5	0.26 ± 0.03
Bejou	73.0	97.1	619.0	277.2 ± 14.0	96.9 ± 10.6	0.22 ± 0.09
Deglet Nour	77.9	96.9	730.7	334.4 ± 10.2	113.2 ± 1.0	0.25 ± 0.02
Kenta	79.2	97.2	719.5	290.5 ± 1.2	97.8 ± 1.6	0.26 ± 0.00
Kentichi	85.8	97.1	711.4	252.1 ± 6.2	92.8 ± 0.5	0.25 ± 0.03

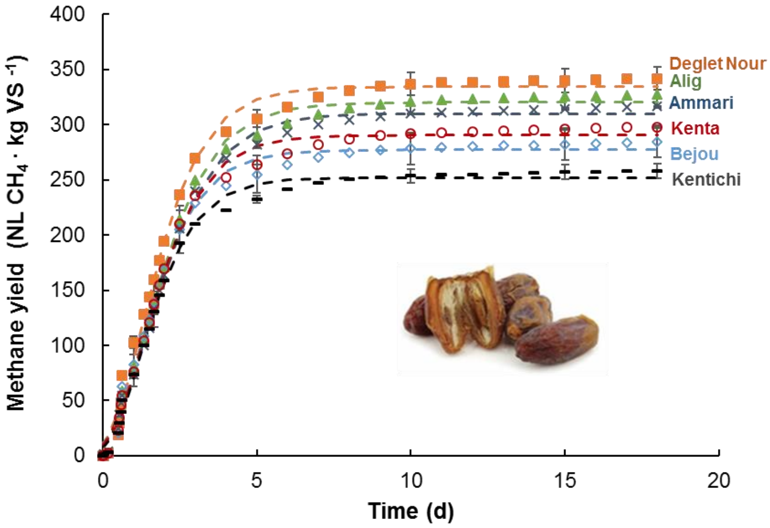

Figure 1: Cumulative methane production for 6 varieties of date pulps

3.2. Pearson Correlation Test

The Pearson correlation tests were performed for every two variables described in Tables 1 and 2. The results were presented in Table 3. Polyphenol is a well-known anti-oxidant substance and COD describes the reductive organic matters of the substrates, which could possibly explain a strong correlation between the polyphenolic content and the COD of the date pulps (with a Pearson correlation coefficient $r = 0.833$, $p < 0.05$). Besides, the total sugars were significantly correlated to the BMP_0 and R_m and this can be explained by the high degradability and abundance in methane potential of the sugars. It has to be mentioned that the AD lag time of the date pulps was related to the protein ($r = 0.693$ but not statistically significant, $p = 0.127$). This could indicate that the hydrolysis of protein is to some extent the limiting process to start the methane production. Our study investigating the methane production from the waste of pulp of dates demonstrates that Pearson correlation coefficient between polyphenol content and methane production of all the varieties was not significant ($r = -0.35$, $p = 0.50$). This result is in agreement with a study from several tropical tree leaves (Pal et al., 2015). Contrariwise, correlation coefficients were found significant in another study evaluating the dependence of the methane production potential of tropical plants on their phenolic fractions (Jayanegara et al., 2011). The polyphenols are generally known as inhibitors of AD systems by limiting the microorganism's activity as the consequence of biostatic effects (Obied et al., 2009). Many studies also suggest that the strength of the inhibition or deactivation effect of the polyphenols is a function of their polymerization levels, the dominant microbial species found in anaerobic systems and the type of hydrolytic enzymes (Jayanegara et al., 2011). The further interaction of the variables was interpreted in Section 3.3.

Table 3: Results of Pearson correlation test (dimensionless parameters) of selected groups of variables

	Polyphenol content vs. COD	Total sugars vs. BMP_0	Total sugars vs. R_m	Protein vs. λ
Pearson correlation coefficient	0.833*	0.960**	0.870*	0.693
p value	0.040	0.002	0.024	0.127

** Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).*

3.3. Principal component analysis (PCA)

The ultimate dataset obtained for the pulp date was analysed through PCA in order to evaluate the quantitative characteristics affecting the methane production and the biodegradability of the selected varieties. PCA is used for the description and visualization of the samples in relation to the studied parameters: TS, VS, methane potential based on VS introduced (BMP_0), maximum methane yield rate (R_m), lag time (λ), proteins, lipids, total sugars, ash content and polyphenol content. PCA is dedicated to interpreting the multidimensional information using the minimized number of factors. Three principal components F1 (37.0 %), F2 (33.3 %) and F3 (17.0 %) were extracted for analysis according to their weight on the total Eigenvalues of the factors. F1 and F2 explained almost 70.4 % of the total variance of the samples and up to 87.4 % with F3 (Figure 2-A). The rest of the components, F4 and F5, explained 10.7 % and 1.9 % of the total variance respectively. The initial Eigen vectors related to F1, F2 and F3 were presented with their corresponding variables in the form of equations in Eq(2), Eq(3) and Eq(4). The loadings of the ten variables on the factorial plane described by F1 × F2 (Figure 2-B) illustrates the internal relationships between the variables during the AD of date pulps.

F1:

$$C_1 = 0.234 \times \text{Polyphenol Content} + 0.256 \times \text{Ash Content} + 0.443 \times \text{Total Sugars} + 0.125 \times \text{Proteins} + 0.112 \times \text{Lipids} - 0.153 \times TS - 0.338 \times VS + 0.227 \times COD + 0.478 \times BMP_0 + 0.378 \times R_m + 0.309 \times \lambda \quad (2)$$

F2:

$$C_2 = -0.409 \times \text{Polyphenol Content} + 0.223 \times \text{Ash Content} + 0.110 \times \text{Total Sugars} - 0.365 \times \text{Proteins} + 0.458 \times \text{Lipids} - 0.426 \times TS - 0.142 \times VS - 0.434 \times COD + 0.104 \times BMP_0 - 0.102 \times R_m - 0.133 \times \lambda \quad (3)$$

F3:

$$C_3 = -0.296 \times \text{Polyphenol Content} + 0.085 \times \text{Ash Content} - 0.264 \times \text{Total Sugars} + 0.454 \times \text{Proteins} - 0.014 \times \text{Lipids} - 0.124 \times TS - 0.396 \times VS + 0.040 \times COD + 0.085 \times BMP_0 - 0.443 \times R_m + 0.502 \times \lambda \quad (4)$$

For F1, BMP_0 and total sugars are strongly correlated to each other as described by Pearson correlation test, meaning that total sugars contributed most to the methane potential of the date pulps. The R_m is located near λ, however, they are statistically uncorrelated ($r = 0.094$, $p = 0.86$). In terms of F2, the protein, polyphenol content is close to COD showing their possible contribution to the measurement of the reductive organic content of the substrate. That the group of the polyphenol content and the group of BMP_0 are perpendicular to each other strengthens the comments given in section 3.2 (no significant correlation identified, $p = 0.35$). The factorial plane F1 × F3 further supported the comments stated above (not presented).
The Figure 2-B also gives the scores of the individual varieties when it comes to F1 vs. F2 indicating heterogeneity of the 6 substrates. It clearly separated the pulp dates varieties into many distinct categories. Axis F1 expresses the opposition between two categories of samples: one includes the most methane-productive varieties that contained the most amount of sugars (Deglet Nour and Alig), another includes Bejou and Kentichi, those that produced less methane and were less rich in sugars. Ammari situates at the centre of F1, demonstrating its average methane yield and the highest concentration in lipids. Kenta had nothing in terms of the biochemical properties that made itself stand out and consequently found itself at centre of the graph.

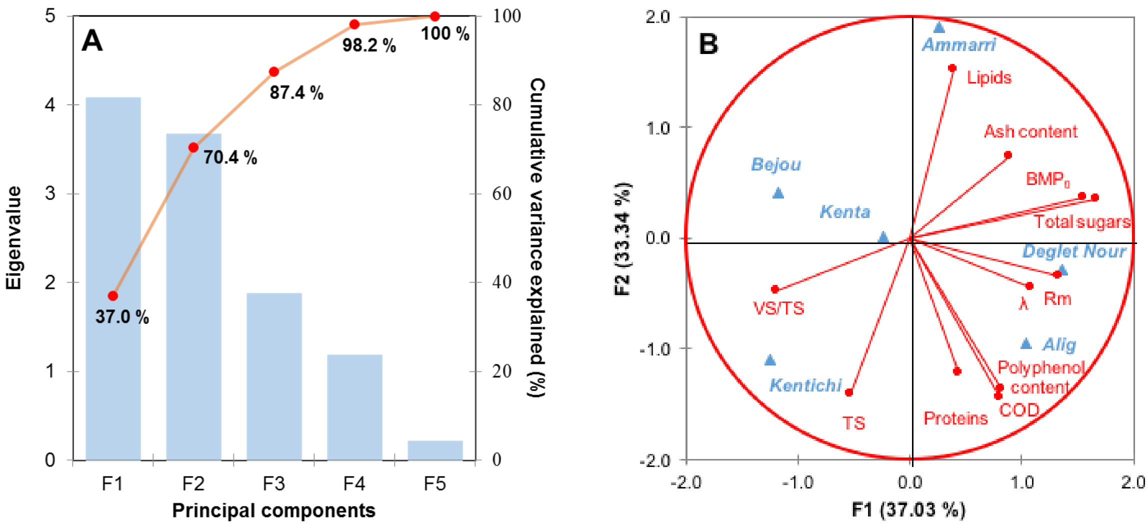

Figure 2: Results of the Principal Component Analysis (PCA), two principal components (F1 and F2) extracted which explain 70.4 % of the total variance. A) Scree plot of the Eigenvalues of the principal components B) Loadings of the variables and the scores corresponding to the individual date varieties for F1 vs. F2.

4. Conclusions

Six varieties of date pulps were tested for their relationship between the biochemical compositions and the kinetic parameters of methane production. The statistical analyses showed that the methane yield varied enormously depending on the varieties that consisted of different biochemical fractions. The correlation between methane yield and the total polyphenols content showed that no significant correlation was found. Generally total sugars contributed the most to the methane potential and the methane production rate, which indicates that date pulps with more total sugars could bring about more and faster methane yield.

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