Evaluation of the biogas potential of a lignocellulosic residue

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ABSTRACT

Anaerobic digestion (AD) or methanization is a biological process that allows the treatment of organic wastes and the production of renewable energy as biogas. This research describes assessment and optimization of dates pedicels rich in organic components under the conditions of mesophiles in co-digestion in association with the cattle manure and sludge of the wastewater treatment plant (WWTP). To realize this objectives, we investigated the impact of substrate load (date pedicel) on the co-digestion anaerobic of date pedicels and the sludge in wastewater treatment plants. The different parameters of the digester stability such as the pH, VFA, CAT, ammoniacal nitrogen, and the volume of biogas produced were followed. The results show that by increasing the mass of the pedicels and therefore the percentage of the substrate in the reactor, from 0.5 to 1% (W/V), the volume of produced biogas increases by five times. However, an increase in the percentage of the substrate introduced into the digester from 1 to 2% resulted in a slight decrease in the volume of biogas produced.

The use of fresh cattle manure as inoculum shown a significant improvement (40%) in the production of biogas, which can be attributed to the nature of the inoculum. Indeed, cattle manure fresh is more adapted for the fermentation of lignocellulosic materials unlike sludge of wastewater treatment plants which may contain toxic substances that limit their use in AD.

Key words: anaerobic digestion, biogas, dates pedicels, inoculum, mesophile, methanization

HIGHLIGHTS

- Pedicels of dates mixed with cattle manure and sludge from the waste water treatment plant (WWTP) were effective feedstocks for biogas production.
- Production optima of biogas are 1395 mL for WWTP and 1953.4 mL for cattle manure.
- Load maximum admissible in a digester depends on the nature of the substrate.
- Use of fresh cattle manure as the inoculum is more adapted for the fermentation of lignocellulosic materials.
INTRODUCTION

Algeria is ranked as one of the world’s important date producers, as the fourth one, by its world production of around 14%. It is important to note that its production is growing increasingly. Statistics announced by Algerian Chamber of Commerce and Industry (CACI) reveal that the production had risen from 600,096 tons, in 2012, to about 11,000,000 tons, in 2017, all varieties combined. (Allalou et al. 2018). The recovery of this waste has several objectives: economic, technical and environmental. Their availability in abundance in nature, their properties physicochemical, makes it possible to classify them among the strategic resources of renewable energies which can be enormously exploited (Jaafar 2010). Researchers and industries explore technological solutions allowing an efficient and less expensive treatment of these wastes. One of the technologies allowing the treatment of the organic fraction of these wastes is anaerobic digestion (AD) (biomethanization), which consists of biological degradation, in the absence of oxygen, of organic matter into a mixture of methane (CH₄) and carbon dioxide (CO₂) called ‘biogas’. Anaerobic digestion provides environmental benefits such as reducing odors, reducing the amount of waste to combustible or putting it in the landfill.

In addition, the energy recovery of the biogas obtained makes it possible to avoid greenhouse gas emissions and to serve as a potential source of energy that can replace fossil fuels and be used in the production of electricity and heat. Through anaerobic digestion, waste becomes a source of wealth. (Jaafar 2010; Hajji & Rhachi 2013; Anjum et al. 2016; Hajji & Rhachi 2016; Maamir et al. 2017; Mahajan et al. 2017; Wu et al. 2019). During the AD process, only part of the organic matter is
completely degraded, the rest is an excellent fertilizing agent for agricultural land and which can be used as such. (Kerroum et al. 2014). In view of the diversity of microbial populations present in a digester, controlling the process of a methane fermentation requires the choice of a number of physico chemical parameters, making it possible overall to ensure the metabolism of different bacterial species in satisfactory conditions. Among these parameters include temperature, pH, redox potential, and anaerobiosis. The production of biogas also depends on the nature and composition of the substrate. As with all biological reactors, the proper operation of a digester is related to the adequacy of the amount of incoming pollution, the treatment capacity of the microbial population. If this is too low, the reactor is undernourished, if it is too strong, the reactor is in overload. To identify the beginning of a change that might lead to organic overload, for example, some parameters are more relevant than others. For liquids these are: the decrease in pH, the decrease in alkalinity due to bicarbonate, the increase in VFA concentration and therefore the increase in Total Soluble Organic Carbon. For biogas, these are generally the increase of its hydrogen content, the decrease of the flow rate, the decrease in its CH₄ content with an increase in CO₂ (Williams 2013). This study aims to evaluate and optimize the biogas potential of date pedicels by examining the effect of substrate loading and the type of inoculum on biogas production. In order to properly control and examine the methanation process we were interested in the following parameters: pH, VFA, CAT, ammoniacal nitrogen and the quantity of biogas produced.

**MATERIALS AND METHODS**

**Materials**

**Preparation of substrate**

Date pedicels represent an important quantity of agricultural by-products that it would be good to value; their use in the production of biogas constitutes a contribution to the efforts towards recovery of this waste. The date pedicels are washed with tap water, dried in the oven at 70 °C for 48 hours, cut into small pieces and finally ground until a fine powder is obtained.

Date pedicels are lignocellulosic materials, their physicochemical characteristics are shown in Table 1.

Date pedicels present a strong proportion in organic matter made of their composition of large and varied organic matter. The mass percentages of cellulose, hemicellulose, lignin, total sugars (TS), total nitrogenous matter (TNM) and phosphorus in date pedicels are presented in Table 2.

We note that the percentages of cellulose, hemicellulose and TS are important. Therefore, the presence of assimilable sugar resulting from their hydrolysis will promote the development of the bacterial communities responsible for biogas production.

**Inoculum**

The addition of a seeding inoculum during the start-up of the biological reactor makes available the active microbial biomass which makes it possible to avoid inhibition related in particular to an accumulation of VFA and decrease in pH and promotes an equilibrium state of all AD processes. The sludge from the Barraki (Algiers) wastewater treatment plant from the digester

**Table 1 | Physico chemical characteristics of the pedicels of crushed dates**

| Parameter                          | Value              |
|------------------------------------|--------------------|
| Mean particle diameter (μm)        | 447.95 ± 0.01      |
| Humidity (%mass)                   | 14.74 ± 0.01       |
| Bulk density (g/cm³)               | 0.44 ± 0.02        |
| Volatile matter (VM in % mass)     | 98.00 ± 0.01       |

**Table 2 | Results of the chemical analysis (%) of date pedicels (Yazid et al. 2013)**

| Parameters | Cellulose | Hemicellulose | Lignin | TS   | TNM | Phosphorus |
|------------|-----------|---------------|--------|------|-----|------------|
| % mass     | 18.76     | 18.97         | 9.54   | 41.84| 3.9 | 0.003      |
and cattle manure are used in our experiments as inoculum. The characteristics of cattle manure and sludge from the WWTP are shown in Table 3.

Experimental protocol
The experiments were carried out under mesophilic conditions in a ruled incubation chamber at 35 °C. Substrate samples are placed in 500 mL glass vials containing an inoculum-medium nutrient mixture. The vials are hermetically closed with a rubber septum. In addition to the carbon source provided by the substrate, a nutrient medium with a ratio \( C/N/P = 700/5/1 \) (Dinova et al. 2018) is added to provide supplementary essential minerals for microorganisms development. Generally, the nutrient medium contains phosphate or carbonate ions which give it buffering capacity to reduce pH variations. Samples are taken every other day for tracking the pH, VFA, CAT, the ammonia nitrogen. The determination of the amount of biogas produced is followed (Figure 1).

Analytical methods
VFA and CAT are considered as parameters for the proper functioning of AD, their following has been realized using the method given in the SUEZ degremont water handbook (online).

Table 3 | Characteristics of cattle manure and sludge from wastewater treatment plant

| Cattle manure            | Sludge from the WWTP          |
|--------------------------|------------------------------|
| **pH**                   | **pH**                       |
| 6.87 ± 001               | 7.30 ± 0.01                  |
| **DM (%)**               | **VFA**                      |
| 20                       | 192.00 ± 0.01                |
| **H (%)**                | **CAT**                      |
| 80                       | 3,300.00 ± 0.01              |
| **VFA/CAT**              |                              |
| 0.048 ± 0.001            |                              |

**Figure 1** | Experimental device; 1. Syringe for taking the samples, 2. plastic tube immersed in the reaction mixture allowing taking liquid samples, 3. leak-proof stopper, 4. anaerobic digester, 5. flexible tube for carry the biogas to the second reactor, 6. water-filled reactor, 7. flexible tube bound to an immersed capillary allowing for water displacement, 8. reactor for the recovered vacated water. The volume of water discharged represents the volume of biogas produced.
The determination of ammoniacal nitrogen was done according to the Nessler method (Nollet & de Gelder 2013). The pH was measured with a pH meter (HANNA).

The determination of the dry matter, organic matter, volatile dry matter and moisture was carried out according to the method described in the SUEZ degremont water handbook (online).

Test of flammability
Once the biogas is produced we must ensure that it is composed largely of methane. Thus, we carry out a test for flammability, the obtained blue flame confirms that a large proportion of this biogas is composed of methane.

RESULTS AND DISCUSSION

Study of the influence of substrate loading
Tests for biomethanization were carried out with different masses of dates pedicels and therefore substrate percentages in the reactors of 0, 0.5, 1 and 2% (W/V). These experiments were carried under mesophilic conditions (35 °C) with WWTP of the digester. The quantity of inoculum injected is 8 g per litre of the dry volatile matter (DVM). The pH, VFA, CAT and the production of biogas were followed for 28 days.

A control sample without substrate was also carried out, under the same conditions to evaluate the activity of the inoculum.

Evolution of pH
The pH is an essential parameter due to the sensitivity of the AD ecosystem, in particular, methanogenic organisms to variations of pH.

We admit that pH is the first indicator of faulty operation from a digester. It is known that AD works best in natural medium (pH = 7) (M’Salak & Ben M’barek 2015).

The evolution of the pH in the four digesters throughout of time is shown in Figure 2.

We noticed on the first day of the launch of AD, a slight decrease in pH due to the appearance of VFA in the digesters. Indeed, after the hydrolysis stage characterized by the degradation of complex organic molecules into monomers, comes the acidogenic stage where the monomers are fermented mainly into VFA (Hajji & Rhachi 2016). In order to avoid VFA from becoming toxic to microorganisms, we began adjusting the pH with a sodium bicarbonate solution on the fourth day. The pH values change afterwards around the neutral region for the four digesters. These results are similar to those obtained by Djaafrì (Djaafrì et al. 2014), Kainthola (Kainthola et al. 2019) and Elkhachine (Elkhachine et al. 2020).

Evolution of the CAT and the production of VFA
VFA and CAT are certainly first parameters for the follow of the anaerobic digestion. We know all that VFA and CAT are the variables that yield the quickest response to any disturbance of the AD.

Figure 2 | Variations of pH for the four digesters as a function of time.
Figure 3 illustrates the variations of CAT and VFA in the four digesters throughout time. Throughout time, we notice a decrease in the concentration of VFA in the reaction medium. In fact, during the first stage, carried out by hydrolytic and fermentative bacteria, the macromolecules are hydrolysed into monomers which are decomposed by acidogenic bacteria into a complex mixture rich in VFA. The latter are then transformed into acetate and carbon dioxide by the action of acetogenic bacteria (Park et al. 2018). The decrease in VFA is accompanied by an increase in alkalinity throughout time owing to the buffering power of bicarbonates, which makes it possible to ensure the proper functioning of the digesters (Raphique et al. 2010). Conversely, we note that the recorded VFA concentrations are proportional to the substrate load introduced into the digester. The accumulation of VFA’s leads to the breakdown of the equilibrium between methanogenic bacteria and acidogenic bacteria, resulting in the inhibition of methanogenesis (Park et al. 2018).

**Evolution of daily biogas production**

This study aims to examine the power of date pedicels to generate biogas. For this, the daily amount of biogas released while anaerobic fermentation of date pedicels was measured by the liquid displacement method.

The daily production of biogas in the four digesters is shown in Figure 4.

We report that no biogas production was recorded in the control digester. This might be the result of a lack of nutrients, particularly nitrogen. Indeed, nitrogen participates in protein synthesis and if its concentration in the reaction medium is insufficient, the microorganisms will not be able to use all the carbon present and the degradation process of the organic matter will not be complete (Tong 2019).

We observed from the first day of start-up the digesters a biogas production that increases during the first week of operation, which explained a very rapid adaptation of the microorganisms to medium rich in nutrient and soluble organic matter and easily biodegradable such as the TS (Zou et al. 2020).

From the seventh day of operation for digesters working with loads of 0.5 and 1%, we observe a stop of a few days of production which may be due to the exhaustion of the medium in easily biodegradable organic matter and the adaptation of microorganisms to the organic fraction of scarce biodegradable present in the date pedicels. During this adaptation phase, the bacteria synthesize the exoenzyme responsible for the degradation of complex organic matter such as cellulose and lignin (Zou et al. 2020).

The production of biogas resumed afterwards with the daily quantities larger in the digester operating with a load of 1%. However, the production stops permanently in the reactor working with a 2% load from the third day of operation. This may be due to either an interruption of the equilibrium between methanogenic and acidogenic bacteria, led by the accumulation of VFA. As a result, the inhibition of the anaerobic process, or the presence of high concentrations of ammoniacal nitrogen. In fact, the nitrogen contained in large quantities in the waste from methanization (livestock effluents, agri-food waste, etc.) is reduced in the form of ammonia. Ammoniacal nitrogen is an inhibitor of methanation and more particularly of acetoclastic methanogenesis above a few grams (Kerroum et al. 2012).

![Figure 3](image-url) | Variation of CAT (a) and VFA (b) in the four digesters as a function of time.
An ammonia concentration of about 2 gN/L will have no inhibitory effect on acetoclast methanogenesis. However, the activity of methanogens is decreased with the increasing concentration of ammonia, and total inhibition is reached for a concentration of 4 gN/L (Krakat et al. 2017).

In order to detect the cause of disruption of the fermentation process in digesters operating with 0, 5, and 2% loads, we performed an analysis of the ammoniacal nitrogen concentration for two successive days at the end of the biogas production shutdown period. The results obtained are shown in Table 4.

We note that the ammoniacal nitrogen concentrations recorded in the reactors are relatively low and have no inhibitory effect on the methanation process, suggesting that the inhibition of the AD process in the 2%-sludge digester is probably due to the accumulation of VFA in the medium.

### Influence of the nature of the inoculum

The trials of fermentation were carried out with a substrate loading of 1% (W/V) in the inoculum composed of fresh cattle manure. A control digester that did not contain inoculum was launched under the same conditions. The production of biogas was followed for 30 days.

### Evolution of the pH

The pH is a very crucial indicator of the stability and the unfolding of the phenomenon. In addition, monitoring the evolution of the pH makes it possible to interpret the different stages of the degradation of organic matter.

In a fermenter, the degradation of fresh substrates leads to the production of metabolites such as VFA and CO₂ which tend to lower the pH, as seen in the two digesters (Figure 5) where the pH value decreases at the launching of the experiment further to the acidogenesis phase. The decrease in pH continues until the 15th day, which suggests that the buffering effect of the digester may be insufficient to maintain the pH at a correct value (Djaafri et al. 2014). To avoid process inhibition, we adjusted the pH, ensuring that it was maintained at optimal values (Riggio 2017).

### Table 4 | Concentration of ammonia nitrogen in digesters operating with 0.5 and 2% (W/V) loads

| Digesters                     | 0.5%            | 2%              |
|-------------------------------|-----------------|-----------------|
| Ammonia nitrogen concentration (mg/L) | 0.890 ± 0.001 | 0.685 ± 0.001  |
|                               | 0.304 ± 0.001  | 0.147 ± 0.001  |
Evolution of CAT and VFA

Figure 6 illustrates the variation in VFA and CAT in the two digesters. We noted very high concentrations in VFA and CAT compared to those recorded in the digesters operating with activated sludge.

The importance of the VFA concentrations recorded may be due to the high rate of organic matter from the date pedicels and cattle manure which is also rich in organic matter.

Moreover, the high alkalinity means that the process is suited to counteract the strong fluctuations of pH that could be due to significant acidification of the digester caused by the accumulation of the VFA.

The production daily of biogas

The amount daily of biogas produced was quantified daily by the liquid displacement method. The results are shown in Figure 7.

Figure 5 | Variation of pH in two digesters as a function of time.

Figure 6 | Variation of VFA (a) and CAT (b) as a function of time.
We noticed that the control digester produced nothing during the 30 days of operation. To determine the reason for the inhibition of the biological process, we measured the concentration of ammoniacal nitrogen, which is one of the inhibitors of methanation (Kerroum et al. 2012).

The results obtained are represented in Table 5. We noted that the ammoniacal nitrogen concentrations recorded in both digesters were relatively low and had no inhibitory effect on the methanation process, suggesting that the inhibition of the AD process in the control digester cattle manure is due probably to the accumulation of VFAs in the medium or lack of nutrients (Park et al. 2018).

Conversely, we observed from the first day of start-up of the digester of 1%-cattle manure a production of biogas that fluctuates during the first 11 days of operation, which explained a very rapid adaptation of the microorganisms to medium rich in nutrients and soluble organic matter and easily biodegradable such as TS.

From the twelfth day of operation, we observed a halt in production of three days, which can be related to the exhaustion of the medium in easily biodegradable organic matter and to the adaptation of the microorganisms to the organic fraction of limited biodegradables contained in the pedicels of dates. Following that, biogas production resumes, with important daily quantities. These results are consistent with those obtained by others (Maamir et al. 2017; Neshata et al. 2017; Bote et al. 2019).

**Evolution of the production cumulative of biogas**

*Figure 8* represent the evolution of the cumulative biogas production in the digesters operating with a load of date pedicel load of 1% (W/V) in the presence the WWTP (1%-sludge) and cattle manure (1%-cattle manure) and the control digester (Witness-bovine manure).

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**Table 5 | Concentrations in ammonia nitrogen in mg/L recorded in digesters**

| t (days) | 17     | 18     | 23     | 25     | 30     |
|---------|--------|--------|--------|--------|--------|
| 1%- bovine manure | 1.66 ± 0.01 | 1.30 ± 0.01 | 2.15 ± 0.01 | 1.46 ± 0.01 | 3.22 ± 0.01 |
| Witness-bovine manure | 3.87 ± 0.01 | 2.24 ± 0.01 | 2.21 ± 0.01 | 1.53 ± 0.01 | 3.19 ± 0.01 |
The cumulative production of biogas increases over time until a bearing appears, which indicates the end of the methanation process after 27 days of operation with a total volume of biogas produced equal to 1,953.4 mL and 1,395 mL, for the digesters (1%-cattle manure) and (1% WWTP), respectively.

The digester (1%-bovine manure) presented the more important production, which can be justified by the nature of the inoculum (cattle manure) which is more suitable for the fermentation of lignocellulosic materials.

Test of flammability

The quality of this biogas also plays a very important role in its valorization later. In our study, the determination of the quality of the biogas produced is done in the traditional method, the gas is quite simply set alight. If the biogas is flammable, it means that has reached the flammability limitations, which are between 6 and 18 for biogas containing 70% CH4 (Tizé et al. 2015).

After the realization of the test for inflammability at the end of the experiment (on the 31st day of the experiment), the result was positive, we obtained a very clear blue flame in the digesters, which means that this biogas is composed mostly of methane.

For comparison, we present in Table 6 some values of the specific biogas production rates using some plant biomasses. These data show that the efficacy of date pedicels for biogas production is superior or nearly equivalent to that of other lignocellulosic materials.

Table 6: Specific rate of cumulative production from the lignocellulosic residues

| Substrate                        | Rate of biogas production (Nm³ de CH₄/Kg of MVS) | Reference                  |
|----------------------------------|-------------------------------------------------|----------------------------|
| Olive Mill Wastewater (OMW)      | 0.253                                            | Spyridonidis et al. (2020) |
| Municipal Solid Waste (MSW)      | 0.271                                            | Spyridon & Gerrit (2019)    |
| Food waste                       | 0.277                                            | Jingxin et al. (2019)      |
| Olive Mill Solid Waste (OMSW)    | 0.264                                            | Maamir et al. (2017)       |
| Papaya peels                     | 0.302                                            | Usa & Niramol (2017)       |
| Date pedicels                    | 0.309                                            | Our study                  |
CONCLUSION

The experiments carried out allowed us to determine the effect of the mass of the substrate used and the nature of the inoculum on the biogas potential of date pedicels. The monitoring of the stability parameters of the AD process of date pedicels in the mesophilic phase (35 °C) generally showed good progress of the process. The results obtained show that by increasing the mass of the pedicels and thus the percentage of substrate from 0.5 to 1%, the volume of biogas produced increased from 280 to 1,395 mL, respectively. However, it is necessary to respect the maximum load admissible in a digester, which depends on the nature of the substrate, when there is overloading of the digester, an accumulation of VFA then being able to inhibit the methanogenesis.

In fact, the increase of the substrate percentage fed into the digester from 1 to 2% decreases slightly changed the volume of biogas produced. Conversely, the optimal production of biogas is observed in the digester operating with a ratio (W/V) of 1% in the presence of cattle manure as inoculum (1%-cattle manure). The latter is more suitable for the fermentation of lignocellulosic materials unlike sludge of WWTP which may contain toxic substances limiting their usage in AD.

Perspective

• We designed a digester to study the influence of the agitation speed on the biogas production.
• Study of the temperature effect on biogas production.
• Trial for another waste like an urban waste for the improvement of biogas production.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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