New sustainable bioconversion concept of date by-products (Phoenix dactylifera L.) to biohydrogen, biogas and date-syrup

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Nesrine Ben Yahmed [a], Kévin Dauptain [b],Imen Lajnef [a], Hélène Carrere [b], Eric Trably [b], Issam Smaali [a]

[a] Laboratoire LIP-MB INSAT, LR11ES24, Université de Carthage, INSAT-BP 676, Centre urbain nord, 1080 Carthage Cedex, Tunisie

[b] INRAE, Univ Montpellier, LBE, Avenue des Etangs, 11100 Narbonne, France

* Corresponding author: Nesrine Ben Yahmed

Tel.: +216 71 703 829; Fax: +216 71 704 329, e-mail: nesrine.benyahmed@gmail.com

Address: LR11ES24 - INSAT, Centre Urbain Nord, University of Carthage, BP 676, 1080 Tunis cedex, Tunisia.

Abbreviations: crude fibers extract (CFE), biochemical hydrogen potential (BHP), biochemical methane potential (BMP), total solids (TS), volatile solids (VS)

Abstract

The dates production is usually accompanied by considerable loss of fruit byproducts. The chemical analysis showed that 'Deglet Nour' discarded flesh is rich in soluble sugars (79.8 % ± 0.8%) and fibers (12.3% ± 0.4%). A processing approach was implemented to permit the production of biohydrogen from the flesh and biogas from the crude fiber fraction after soluble sugars extraction. This approach showed interesting results since the obtained biochemical hydrogen potential and the maximum methane yield were 292 $mLH_2/gVS_{initial}$ and 235 $mLCH_4/gVS_{fibers}$ respectively. Parallelly, the "hot water" soluble sugar fraction (date syrup) was of interest for agro-alimentary applications and showed a high sucrose, glucose and fructose content of 33.5 %, 11.8% and 13.17% respectively. This study presents a proof of concept allowing an efficient sustainable energetic conversion of the date by-products biomass to biohydrogen via dark fermentation or to soluble sugars fraction and biogas via a biorefinery approach.

Keywords: Date by-products, dark fermentation, anaerobic digestion, biohydrogen, date-syrup, biogas

1. Introduction

Date palms (*Phoenix dactylifera L.*) cultivars represent an important agriculture crop in the arid and semi-arid regions. In the Middle East and the North Africa, they are considered as indispensable fruits owing to their rich content of essential nutrients [1]. The world production of dates reached 8 166 014 tons in 2017 [2]. About 2000 date cultivars are known but a small amount is valued for their performance and their fruit quality [3]. In Tunisia, the average annual production of dates has improved remarkably and has increased from 193 000 tons in 2012 to 260 000 tons in 2017 [2] dominated by the variety 'Deglet Nour' (65% of total production), which has a much-appreciated sensory quality and a great commercial value. Despite this progress, the production and marketing of dates are unfortunately accompanied by considerable loss of fruit, whether directly on the palm grove or during the process of gathering, storage and packaging [4]. As a result, a significant loss of 25 000 tons, is recorded each year in Tunisia [5]. Besides, this production is also associated with a considerable raised loss in secondary dates varieties (approximately 30 000 tons for Tunisia and 2 000 000 tons worldwide) [6]. Due to their soft texture and deteriorated organoleptic qualities these by-products of dates are not edible and are often discarded. Currently, they are used for limited purposes such as animal feed [5]. This discarded biomass is mainly composed by cellulosic compounds which makes it a good candidate for biofuel production such as biogas and bioethanol [7].

In fact, dates flesh is rich in soluble sugars (81-88%) mainly fructose, glucose and sucrose, dietary fiber DF (5−8.5%), and small quantities of proteins, fats and ashes [8-10]. Soluble sugars are usually used for fructose rich syrup production [5, 11]. Previous studies focused on the chemical characterization and technological applications of these dates by-products especially the soluble sugars and dietary fibers DF in the agro-alimentary field [6, 8, 12, 13].

Biogas production via the anaerobic digestion of agriculture crops, residues and wastes is of increasing interest in order to reduce the greenhouse gas emissions and to facilitate a sustainable development of energy supply [14, 15]. Anaerobic digestion can be divided in four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Depending of the substrate composition and its structure, hydrolysis or methanogenesis can be considered as limiting steps [16].

Besides, the biological hydrogen production processes including biophotolysis (direct and indirect), photo-fermentation, dark fermentation and two-stage fermentation have received a considerable attention [17, 18]. Among them, the dark fermentation appears as promising technology using anaerobic bacteria which freely and efficiently produce H_2 with no need of light and low operation costs compared to the photo-production [19-21]. This cost advantage could be maximized when the biohydrogen is produced from waste such as agriculture by-products, agro-industry and food waste.

To date, limited data are available regarding the energetic bioconversion of dates fleshes via biogas [22, 23], bioethanol [24] and biohydrogen [25] production.

Thus, the objective of this study is to demonstrate the feasibility of using these discarded dates in a processing approach aimed at producing biofuels namely biohydrogen and biogas while recovering the residual soluble sugars (date syrup) as high added value product. Chemical composition of 'Deglet Nour' discarded biomass (sorting gap) as well as of their by-products such as the soluble sugars and the crude fibers extract (CFE) were analyzed. Associated biochemical hydrogen potential (BHP) and biochemical methane potential (BMP) tests were assessed to evaluate the energetic potentials of this discarded biomass. The kinetic and efficiency of the bioconversion were also investigated.

2. Materials and methods

2.1. Biological material

97 ‘Deglet-Nour’ date coproducts including discarded and trashed fruits were obtained from
98 local market from the region of Tozeur oasis (southern of Tunisia). The seeds of the dates
99 were manually removed. The date fleshes were rinsed with water to eliminate sand and dust,
100 dried for 24 h at 60 °C, milled and preserved at room temperature until use.

101 **2.2 Bioconversion concept based on biohydrogen / soluble sugars / biogas production**

102 Figure 1 illustrates the overall methodology followed in this study. It consisted on a cascade
103 conversion aimed at the production of soluble sugars ‘dates syrup’ and biofuels namely
104 biohydrogen by a dark fermentation process and biogas by anaerobic digestion from ‘Deglet-
105 Nour’ by-products.

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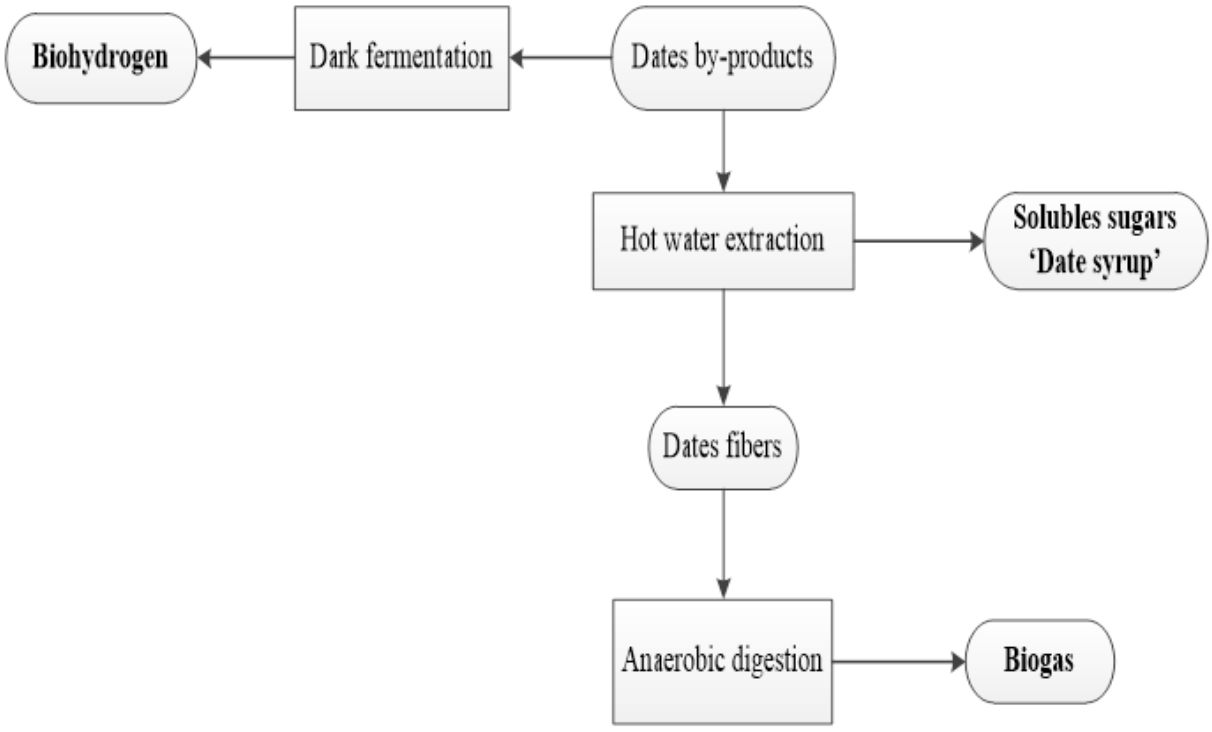

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108

109 **Fig.1.** Processing concept aimed at the production of biohydrogen, biogas and date syrup

110

111 ***2.2.1 Biohydrogen production (BHP tests), final metabolites and microbial communities’***
112 ***analysis***

Biohydrogen production was carried out in batch Biochemical hydrogen potential test (BHP test) in 550 ml plasma bottle (200 ml of working volume) with 2.66 g of milled dates' fleches. The medium was composed of 12.5 ml of minimal nutrient solution (Table S2 in Supplementary material) and 100 mM of MES (2-[N-morpholino] ethane sulfonic acid buffer). BHP test was performed in triplicate according to the standardized protocol [26] adapted without inoculum addition as described by [20]. Nitrogen gas was flushed into the airspace of each bottles to maintain anaerobic conditions. The bottles were sealed using butyl-rubber stoppers and incubated at 37 °C. During incubation, the gas production was monitored with an automatic micro-gas chromatograph (SRA l-GC R3000) equipped with two columns: a Molsieve 10 m/PPU at 80 °C with Argon as vector gas and a VAR 8 m/PPU at 70 °C with Helium, for O_2-CH_4-H_2-N_2 and CO_2 analysis, respectively. The TCD temperature was set at 90 °C. The fermentation was stopped when the hydrogen production stabilized.

At the end of BHP tests, final fermentation metabolites were analyzed by high-performance liquid chromatography (HPLC) coupled to a refractometer (Waters R410). Conditions were identical to those previously detailed by Rafrafi et al. (2013) [27].

Besides, microbial communities of one replicate were measured after dark fermentation (the closest to the average H_2 yield). The procedure was as cited by Dauptain et al. (2020) [28]. Briefly, after sampling, the Eppendorf tube (2 mL) was centrifuged for 15 min at 13,400 g. DNA was extracted with a DNA isolation kit (PowerSoil™ - MoBio Laboratories) according to the instructions of the manufacturer. The sequencing of the purified PCR products was performed in Toulouse, France (get.genotoul.fr). A bioinformatic procedure was applied to gather sequences into operational taxonomic units (OTU) with a similarity of 97%. To identify the OTUs, a blast search was performed (www.ncbi. nlm.nih.gov/BLAST).

2.2.2 Crude fibers extraction

A hydrothermal extraction using hot water at 100 °C for 10 min was released to extract 'Deglet Nour' crude fibers. After solubilization of the sugars, the crude fibers extract (CFE) was recovered by centrifugation (6500g, 10 min). Five successive rinsings with water at 40 °C followed by five centrifugations were achieved to concentrate the fibers until it was free of sugars. The obtained residues were dried then stored for biogas production.

2.2.3 Soluble sugars characterization

After hot-water extraction, sucrose, fructose and glucose contents of 'Deglet Nour' syrup were analyzed using high-performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) on a Dionex system (Dionex Corporation, CA, USA) equipped with a CarboPac PA-1 column (Dionex, 250 × 4.5 mm). Conditions were similar to those previously described by Smaali et al.(2012) [5].

2.2.4 Methane production (BMP tests)

Biochemical methane potential tests (BMP tests) were performed to access the methane production. These batch tests were carried out in duplicate, in mesophilic conditions (35°C) as described by Jard et al. [29], using the 'Deglet Nour' crude fibers extract (CFE) as substrate. The used inoculum was recuperated from the outlet of an up flow anaerobic sludge blanket reactor (UASB) treating wastewater from a sugar industry. The applied Substrate/Inoculum ratio was 0.5 gVS of 'Deglet Nour' fibers per gVS of inoculum. Hence, each bottle was consisted of 2 gVS of ground dry dates fibers and 4 gVS of inoculum. Bottles were filled to 400 mL with a bicarbonate buffer complemented with nutrients (Table S1 in Supplementary material). Control tests containing a fully biodegradable substrate (ethanol) and a blank (without sample) were achieved. Ethanol control was used to check the inoculum activity and the blank control to measure endogenous methane production which was subtracted from the methane production of each sample. Each bottle was flushed with nitrogen to create anaerobic

conditions. Bottles were then capped with a rubber stopper and shaken thoroughly to be incubated at 35 °C with continuous agitation.

All along incubation, biogas production was followed by measuring the pressure of the headspace. The methane concentration in biogas was determined by gas chromatography (PerkinElmer, Clarus 480). BMP was accomplished until biogas production stopped [30].

The volume of methane produced ΔV_{CH4} (mL) between the dates j and j-1 was calculated following Eq (1):

$$\Delta V_{CH4} = \left(\left[y(j)P1(j)\frac{V}{RT}\right] - \left[y(j-1)P2(j-1)\frac{V}{RT}\right]\right)\frac{RT^{\circ}}{P^{\circ}} \quad (1)$$

Where y(j-1) et y(j) are CH4 contents in biogas at dates j-1 and j, respectively

P1(j) (Pa) is the bottle head space pressure before sampling at the date j,

P2(j-1) (Pa) is the bottle head space pressure after gas release at the date j-1,

V (mL) is the bottle head space volume

R is the ideal gas constant (8.314 J.(mol.K)$^{-1}$),

T is the bottle temperature (K),

T° et P° are normal condition of temperature and pressure (273,15 K, 1013 hPa).

CH_4 yields were calculated by dividing the corrected methane volume (standard pressure and temperature) by the weight of sample VS added to each bottle.

2.3 Chemical composition of dates fleshes and fibers extract

Total Solids (TS) and Volatile Solids (VS) of dates fleshes and dates extracted fibers were analyzed for in accordance with APHA standard methods [31].The carbohydrates and uronic acids were determined using the two-stage acid hydrolysis protocol adapted from Effland (1977) [32] as described by Ben Yahmed et al. (2017) [33]. The analysis of monosaccharide sugars was carried out by high performance liquid chromatography (HPLC) using combined Water/Dionex system supplied with BioRad HPX-87H column at 50 °C. The solvent

186 consisted of 0.005 M H_2SO_4 was run at a flow-rate of 0.3 mL/min. A refractive index detector
187 (Water R410) was used to quantify carbohydrates. The system was calibrated with glucuronic
188 acid, galacturonic acid, glucose, xylose, sucrose, arabinose (Sigma–Aldrich). The cellulose
189 content was calculated following Eq (2):

190 Cellulose (%TS) = Glucose (%TS)/1.11 (2)

191 with 1.11 the conversion factor for glucose-based polymers (glucose) to monomers [34].
192 For 'Deglet Nour' Flesh the cellulose content was calculated taking into the account the
193 soluble glucose which was subtracted from the total glucose of 'Deglet Nour' Flesh.
194 Pectin content was determined using the colorimetric method described by Englyst et al. [35].
195 Total fibers were extracted and measured according to the AOAC enzymatic-gravimetric
196 method of Prosky et al. [36]. Protein content was determined using the Kjeldahl method and
197 applying a factor of 6.25 to convert the total nitrogen (TKN) into protein content. The content
198 of lipid was measured using the protocol described in the standard NF V 03-713 [37]. The
199 results of different component of dates fleshes and dates fibers were expressed in percent of
200 total solids and were presented as mean ± SD (standard deviation of triplicates).

201 **2.4 Kinetic models of hydrogen and methane productions**

202 A modified Gompertz model was used to assess hydrogen production kinetic parameters (Eq.
203 (3)):

204 $$H(t) = P .\exp [- \exp [(R_m.e /P) (\lambda -t) +1]] \quad (3)$$

205 where H is the cumulative volume of hydrogen production (mL/gVS) along the incubation
206 time (days), P is the maximum cumulative hydrogen production (mL H_2 / gVS), R_m is the
207 maximum hydrogen production rate (mL H_2 / gVS /day), λ is the lag phase (days) and e is exp
208 (1). The values of P, Rm and λ were estimated using grofit R package (v 3.5.1).
209 For methane production, a first-order exponential model was used following this equation:

210 $$M = M_{max} .(1 – \exp (-K.t)) \quad (4)$$

where M (mL CH_4/g VS) is the cumulative specific methane production, M_{max} (mL CH_4/g VS) is the ultimate methane production, K ($days^{-1}$) is the specific rate constant or apparent kinetic constant and t (days) is the time. The adjustment by non-linear regression of the experimental data (M, t) using the Sigmaplot software (version 14.0) allowed the calculation of the parameters K and M_{max}.

3. Results and discussion

3.1 Chemical composition of date flesh and crude fibers extract

Prior to the energetic bioconversion, the approximate chemical composition of dates fleshes as well as of the extracted fibers was determined. Table 1 shows that 'Deglet Nour' flesh is rich in soluble sugars (79.8 ± 0.8%) mainly sucrose, fructose and glucose, total fibers (12.3 ± 0.4%) with small quantities of proteins and lipids based on total solids. These results are in agreement with Elleuch et al.'s study (2008) which demonstrated that 'Deglet Nour' flesh was characterized by the predominance of sugars with low percentages of ash and proteins. It is worth noting that substrates rich in soluble sugars are interesting for hydrogen production by dark fermentation [38, 39]. 'Deglet Nour' could be also considered as a good source of fibers [8]. However, the biochemical composition of dates depends on the culture conditions such as the growth zone and the harvest period (ripeness stage) and it varies significantly among cultivars [40, 41].

Table 1. Chemical composition of 'Deglet Nour' flesh, crude fibers and syrup

Component	'Deglet Nour' Flesh	'Deglet Nour' Fibers	Deglet Nour' syrup [d]
TS (%wet weight)	76.7 ± 0.1	86.2 ± 0.1	ND
VS (%TS)	98.6 ± 0.1	79.3 ± 0.1	ND
Total carbohydrates (%TS) [a]	79.8 ± 0.8	36.6 ± 0.8	58.5 ± 0.9
Glucose (%TS)	15.2 ± 0.1	12.5 ± 0.3	11.8 ± 0.3
Fructose (%TS)	15.8 ± 0.1	ND	13.2 ± 0.4
Sucrose (%TS)	48.8 ± 0.5	ND	33.6 ± 0.9

Xylose (%TS)	ND	19.2 ± 0.5	ND
Arabinose (%TS)	ND	4.9 ± 0.3	ND
Cellulose [b]	6 ± 0.1	11.3 ± 0.3	-
Pectin	ND	2.3 ± 0.13	-
Total fibers (%TS)	12.3 ± 0.4	-	-
Uronic acids (%TS)	ND	17.8 ± 0.2	ND
Proteins (%TS) [c]	2.6 ± 0.1	9.1 ± 0.3	ND
Lipids (%TS)	0.24 ± 0.02	ND	ND

[a] Total carbohydrate content was quantified as the sum of each individual sugar (glucose, fructose and sucrose for 'Deglet Nour' Flesh; glucose, xylose and arabinose for 'Deglet Nour' Fibers) measured in duplicate using the strong acid hydrolysis protocol

[b] The cellulose content was calculated following the Eq (2): Cellulose (%TS) = Glucose (%TS)/1.11

For 'Deglet Nour' Flesh the cellulose content was calculated taking into the account the soluble glucose which was subtracted from the total glucose of 'Deglet Nour' Flesh

[c] The protein content was calculated by using a nitrogen conversion factor of 6.25

[d] Sugars content of 'Deglet Nour' syrup was expressed in relative % on dry weight basis.

ND: not determined

Furthermore, the crude fibers extract (CFE) was characterized. The approximate chemical composition of 'Deglet Nour' CFE after hydrothermal extraction was summarized in Table 1. The carbohydrate content (quantified as the sum of monosaccharides after strong acid hydrolysis) was found at 36.6 ± 0.8% containing 12.5 ± 0.3% of glucose based on total solids, making it a suitable substrate for the biogas production. In fact, soluble sugars are rapidly converted by microorganisms during the anaerobic digestion [33]. Moreover, as shown by table 1, 'Deglet Nour' CFE is rich on cellulose with low quantities of pectin which promotes the energetic valorization of this waste. It is also important to report that the crude fibers extract used in this work was characterized by a high protein content (9.1 ± 0.3%TS) which is similar to that reported by Elleuch et al. (2008) due to the presence of a portion of proteins that binds strongly to the fibers components (cell wall) [8].

3.2 Biohydrogen production from date flesh

'Deglet Nour' sorting gap represents a complex organic waste. This discarded biomass was used for biohydrogen production without inoculum addition. In fact, this organic solid waste generally contains abundant indigenous microflora [20, 42]. Hydrogen production via dark fermentation is of great interest thanks to its double action of waste reduction and clean energy production. BHP tests of this waste lead to an hydrogen yield of 292 mLH_2/gVS_{ini} (Fig. 2) which is high compared to other organic substrates such as rice bran (61 mLH_2/gVS) [43] and food waste (96.9 mLH_2/gVS) [20] (Table 2).

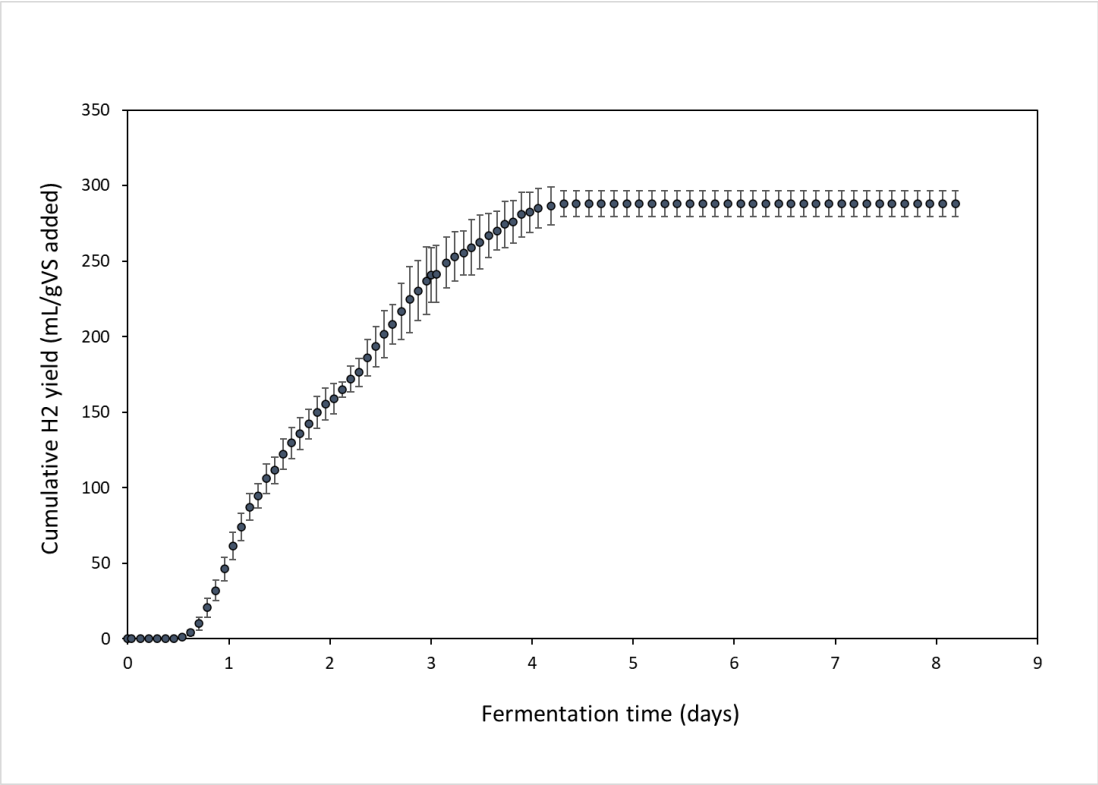


Fig. 2. Cumulative biohydrogen H_2 production, expressed as mL H_2/g VS added, obtained during the BHP tests carried out with 'Deglet Nour' fleshes. The standard deviation was lower than 10%.

To better evaluate and discuss the biohydrogen production, a comparison study was also carried out with other date feedstocks such as date seeds and rotten date fruits (Table 2). Although the H_2 production in the present work was performed without pretreatment and

inoculation, the obtained H_2 yield of 292 $mLH_2/gVSini$ corresponding to an hydrogen production efficiency of 2.47 mol H_2/mol eq.hexose ini, was comparable with those using mixed cultures fermentation (1-3 mol H_2/mol hexose) reported in the literature [44]. In fact, a maximum biohydrogen yield of 224 mL/gTS (1.87 mol H_2/mol eq.hexose ini) was reached using acid-pretreated date seed as substrate and *Clostridium thermocellum* ATCC 27405 as inoculum [45]. Besides, the dark fermentation of rotten dates fruits using *Clostridium acetobutylicum* ATCC 824 carried out by Abd-Alla et al (2011) allowed a cumulative H_2 yield of 399.4 mLH_2/gVS (4 mol H_2/mol eq.hexose ini) [25]. This high H_2 yield could be explained by the fact of the utilization of a pure culture for the inoculation unlike our study where no inoculum was employed.

Table 2. Comparison of biohydrogen and biomethane yields reported for different organic feedstocks in batch operating mode with present study.

Feedstock	Operating conditions	Hydrogen yield (mL H_2/gVS)	Methane yield (mL CH_4/gVS)	Reference
'Deglet Nour' sorting gap:				
- Date fleshes	No pretreatment + No inoculation	**292**		Present study
- Dates fibers	No pretreatment + Mesophilic conditions		**235**	
Rotten date fruits	No pretreatment + Three sequential fermentation stages (dark and photo fermentations)	399.4		[25]
Date seeds	Acid treatment + *Clostridium thermocellum* ATCC 27405 inoculum	224*		[45]
Rice bran	No pretreatment + Anaerobic microflora inoculum	61		[43]
Wheat bran	No pretreatment + Anaerobic microflora inoculum	43		[43]

Food waste	No pretreatment + No inoculation	4.4		[20]
	Heat treatment + No inoculation	96.9		
	Acid treatment + No inoculation	89.5		
	Alkali treatment + No inoculation	50.9		
Date palm fruit waste	No pretreatment + Mesophilic conditions		182	[22]
	No pretreatment + Thermophilic conditions		133	
	No pretreatment + Mesophilic conditions + Recycled digestate		203	
Iraqi date palm pulp waste	No pretreatment + Thermophilic conditions		579	[23]

* Hydrogen yield expressed in mLH_2/gTS corresponding to 30 mmol/L as mentioned by Rambabu et al. (2019) [45]

Actually, no data are available for date fleshes dark fermentation without inoculation. The bioconversion into biohydrogen without microorganism's inoculation represents an innovative efficient practically applicable method allowing a high H_2 production while degradation of dates biomass waste. Moreover, the dates by-products can be used both as substrate and inoculum source.

Regarding the kinetics of the biohydrogen production, experimental data were fitted to a modified Gompertz model whose parameters were presented in Table 3. Maximum of cumulated hydrogen production (P), maximum hydrogen production rate (Rm) and the lag phase (λ) were 300.80 ± 7.14 mLH_2/gVS, 113.38 ± 14.05 $mLH_2/gVS/day$ and 0.59 ± 0.09 day respectively. Compared to other substrates rich in soluble sugars such as watermelon waste (λ= 27.25 h) the dark fermentation without inoculation using 'Deglet Nour' fleshes as substrate represents a fast biohydrogen production with short lag phase [46].

Table 3. Kinetic parameters of hydrogen and methane production in batch tests determined from modified Gompertz equation and exponential model respectively with P the maximum cumulative hydrogen production (mLH_2/gVS), Rm the maximum hydrogen production rate ($mLH_2/gVS/day$), λ the lag phase (days), M_{max} ($mLCH_4/VS$) the ultimate methane production

296 and K ($days^{-1}$) the apparent kinetic constant. Values correspond to the average of replicates ±
297 standard deviation observed between these replicates.
298

Substrate	Hydrogen production			Methane production	
	Modified Gompertz equation parameter values			First-order exponential model parameters values	
	P (mLH_2/gVS)	Rm ($mLH_2/gVS/day$)	λ (days)	$M_{max}(mLCH_4/VS)$	K ($days^{-1}$)
'Deglet Nour' fleshes	300.80 ± 7.14	113.38 ± 14.05	0.59 ± 0.09		
'Deglet Nour' fibers				241 ± 11	0.17 ± 0.02

299

300 Furthermore, when taking into account the relative amount of COD converted to H_2, 23.3 %
301 of the initial equivalent COD was converted which is higher than to the theoretical value of
302 21% for mixed cultures indicated by Hawkes et al. (2007) [47]. Hence, the other metabolites
303 produced throughout fermentation should be also considered. Indeed, the analysis of the final
304 product profile is a good indicator of the metabolic pathways and fermentation efficiency
305 [45]. As shown by table 4, the soluble fermentation metabolites analyzed at the end of BHP
306 tests contained mainly acetate, butyrate and ethanol (Table 4). These final metabolites have
307 already been found in the mixed cultures for biohydrogen production [44]. Acetate and
308 butyrate are indeed the most common fermentation pathways for hydrogen production [47,
309 48]. The lactate was also detected at the end of a single replica of the BHP test (74.5
310 mg/gVS).

311 **Table 4.** Main metabolites accumulated at the end of the BHP tests and theoretical methane
312 potential calculated based on Buswell equation. Values correspond to the mean of three
313 replicates of independent values ± standard deviation.

Final liquid-state metabolites	Unit	Acetate	Butyrate	Ethanol	Succinate	Total
Concentration	g/L	0.625 ± 0.07	2.017 ± 0.327	0.686 ± 0.066	0.370 ± 0.07	3.7 ± 0.12
	mg/gVS	61.94 ± 6.46	200.11 ± 28	67.95 ± 5.66	36.64 ± 6	366.64 ± 40
Theoretical methane potential	$mLCH_4/gVS$	23 ± 2	127 ± 20	49 ± 4	12 ± 2	212 ± 20

314

315 The final liquid state metabolites (34.2 % of the initial COD) may be converted in biomethane
316 to ensure a complete energetic bioconversion of this discarded dates biomass. Theoretical
317 BMP of 212 ± 20 mL/gVS (198 ± 18 ml/gCODinit,) is predicted based on the final liquid
318 chemical composition using Buswell equation [49]. Yield values of 23.3% and 66 % for H_2
319 and CH_4 respectively could be therefore obtained basis on COD conversion.

320 **Table 5.** The whole microbial communities detected after dark fermentation at the genus level

Genus level	Relative abundance	BLAST search	Identification percentage	Accession
Enterobacteriaceae_unclassified	9.7%	*Enterobacter tabaci*	98.82%	NR_146667.2
Escherichia-Shigella	20.7%	*Escherichia fergusonii*	99.06%	NR_074902.1
Lactobacillus (OTU 12)	39.4%	*Lactobacillus rhamnosus*	100.00%	NR_113332.1
Clostridium_sensu_stricto_5	26.7%	*Clostridium paraputrificum*	97.75%	NR_113021.1
Lactobacillus (OTU 29)	1.4%	*Lactobacillus fermentum*	100.00%	NR_104927.1
Enterococcus	0.1%	*Enterococcus hirae*	100.00%	NR_114783.2
Bacillus	0.1%	*Bacillus wiedmannii*	100.00%	NR_152692.1
Clostridium_sensu_stricto_12	1.9%	*Clostridium luticellarii*	100.00%	NR_145907.1

321

322 The hydrogen yield and other detected metabolites after dark fermentation can be explained
323 by Table 5, which shows all the microbial communities detected after dark fermentation.
324 Thanks to a BLAST program, the microbial communities were identified (closest match).
325 Soluble by-products distribution coincided generally with the microbial community analysis.
326 Nonetheless, *Lactobacillus* was, as unexpected, the main genus level (39.4%) followed by
327 *Clostridium sp.* (28.7%) and *Escherichia-Shigella* (20.7%). *Lactobacillus* are known to
328 produce lactate. However, no lactate was detected after dark fermentation (excepted for one
329 replicate), which suggests that lactate was further consumed by *Clostridium sp.* to produce
330 hydrogen and butyrate [28]. Due to a high abundance (26.7%), bacteria belonging to
331 *Clostridium_sensu_stricto_5* genus level (identified as *Clostridium paraputrificum*) are
332 probably able to consume lactate. Zhang et al.(2016) [50] studied corn stover fermentation by
333 *Clostridium paraputrificum* and detected succinate, lactate, acetate and ethanol in high

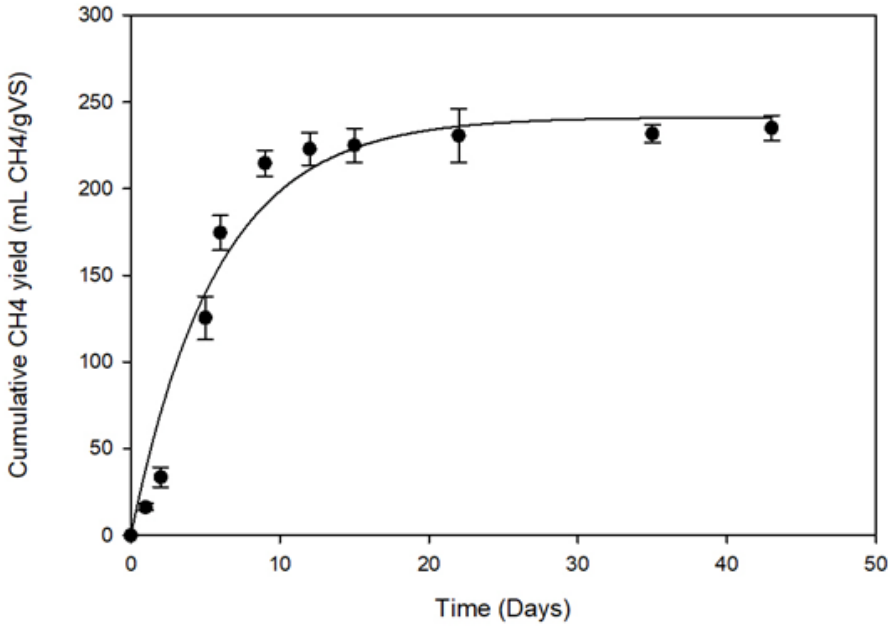
proportions (between 0.37 and 3.84 mM), which is consistent with the high concentrations (Table 4) measured in the present study (excepted for butyrate). Due to the high proportions in butyrate (Table 4), hydrogen production mainly occurred through the butyrate pathway (or after lactate consumption). The high ethanol amount can also be attributed to *Enterobacteriaceae* family (*Escherichia Shigella* and the unclassified *Enterobacteriaceae*) as reported previously [51]. The high hydrogen production is probably due not only to the high proportions of efficient H_2 producers as *Escherichia-Shigella*, *Clostridium sp.* [28] but also to *Enterobacter sp.* [52].

3.3 Soluble sugars extraction and biogas production from crude fibers extract

Another way of 'Deglet Nour' by-products valorization was studied. It consisted on a biorefinery concept based on the extraction of soluble sugars (0.57 g/gVS) and the use of the residual crude fibers extract (0.11 g/gVS) for biogas production. The obtained 'Deglet Nour' syrup was characterized (Table 1). This aqueous extract is rich in sucrose (33.6 ± 0.9 %) which is characteristic of 'Deglet Nour' variety [5]. The high sugar content should justify their use as a source of liquid sugar suitable to many food products such as bakery products, ice cream and in confectionery [53]. It could be also used as a substrate for the enzymatic production of fructose rich syrups by invertase preparation [5, 11]. Date syrup was also employed as agricultural waste for xanthan production by *Xanthomonas campestris* [54]. Furthermore, the production of citric acid was investigated using pretreated dates syrup [55]. Recently, it has an increased interest view their potential health benefits and pharmacological activities [56, 57].

On the other hand, the date crude fibers extract (CFE) was also characterized. As shown by table 1 the crude fiber fraction is rich in cellulosic compounds and proteins making it a suitable substrate for the biogas production. After 45 days of anaerobic digestion using

358 untreated CFE as feedstock, the maximum biomethane yield of 235 $mLCH_4/gVS$ fibers
359 corresponding to 27.6 $mLCH_4/gVS$ initial fleshes was obtained (Fig 3).



360

361 **Fig.3.** Cumulative methane yield, expressed as mL CH_4/g VS added, obtained during the
362 BMP tests carried out with untreated 'Deglet Nour' fibers extract. The standard deviation was
363 lower than 10%. Exponential model (solid line) fitting to experimental data (solid points).
364

365 Besides, kinetic parameters were determined using a first-order exponential model. The
366 adjustment by non-linear regression of the experimental data using the Sigmaplot software
367 allowed the calculation of the parameters K and M_{max} for the methane production which were
368 0.17 ± 0.02 $days^{-1}$ and 241 ± 11 $mLCH_4/VS$ respectively (Table 3). Under the same conditions
369 (mesophilic conditions without chemical pretreatment), a study of biogas production from
370 date palm fruits was carried out by Lattieff et al. (2016). A maximum methane yield of
371 203 mL/gVS was reached using recycled digestate wastes [22]. It is important to note that
372 biomethane yields varied greatly according to the substrate composition and the experimental
373 conditions (Table 2). The proposed biorefinery approach based on crude fibers digestion after

soluble sugars extraction allows both biogas production as source of energy and sugar juice recovery as high added value product. It permits therefore a total utilization of the dates by-products.

Conclusion

This study presents an innovative investigation regarding the energetic processing of 'Deglet Nour' by-products. The dark fermentation of 'Deglet Nour' fleshes without inoculation, newly explored in the present work, allowed a high biohydrogen potential (292 mLH_2/gVS_{ini}). Besides, in another biorefinery approach, date syrup was extracted with a 0.73 g/g yield and the crude fibers extract was submitted to anaerobic digestion. The methane potential reached 235 $mLCH_4$/gVS fibers. Hence, this work presents a proof of concept allowing an almost complete bioconversion of the dates by-products. Optimization of some stages and a techno-economic analysis are therefore needed to perform the scale-up of the proposed concept.

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Supplementary material

Macro nutriments		
NH4Cl	26.2	g/L
KH2PO4	10	g/L
MgCl2, 6H2O	6	g/L
CaCl2, 2H2O	3	g/L
Micro nutriments		
FeCl2, 4H2O	2	g/L
CoCl2, 6H2O	0.5	g/L
MnCl2, 4H2O	0.1	g/L
NiCl2, 6H2O	0.1	g/L
ZnCl2	0,05	g/L
H3BO3	0,05	g/L
Na2SeO3	0,05	g/L
CuCl2, 2H2O	0,04	g/L
Na2MoO4, 2H2O	0,01	g/L
Bicarbonate buffer		
NaHCO3	50	g/L

Table S1. Composition of BMP nutriments

Macro nutriments		
NH4Cl	32	g/L
KH2PO4	20	g/L
Micro nutriments		
FeCl2, 4H2O	1.5	g/L
HCl	1.755	g/L
CoCl2, 6H2O	0.025	g/L
MnSO4, H2O	0.117	g/L
NiCl2, 6H2O	0.025	g/L
ZnCl2	0,07	g/L
H3BO3, H2O	0,06	g/L
CuCl2, 2H2O	0,015	g/L
Na2MoO4, 2H2O	0,025	g/L
MES buffer		
2-[N-morpholino] ethane sulfonic acid buffer	100	mM

Table S2. Composition of BHP nutriments