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

3

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

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24

25 **Abstract**

26 The dates production is usually accompanied by considerable loss of fruit byproducts. The
27 chemical analysis showed that ‘Deglet Nour’ discarded flesh is rich in soluble sugars (79.8 %
28 \pm 0.8%) and fibers (12.3% \pm 0.4%). A processing approach was implemented to permit the
29 production of biohydrogen from the flesh and biogas from the crude fiber fraction after
30 soluble sugars extraction. This approach showed interesting results since the obtained
31 biochemical hydrogen potential and the maximum methane yield were 292 mLH₂/gVS_{initial}
32 and 235 mLCH₄/gVS_{fibers} respectively. Parallely, the “hot water” soluble sugar fraction (date
33 syrup) was of interest for agro-alimentary applications and showed a high sucrose, glucose
34 and fructose content of 33.5 %, 11.8% and 13.17% respectively. This study presents a proof
35 of concept allowing an efficient sustainable energetic conversion of the date by-products
36 biomass to biohydrogen via dark fermentation or to soluble sugars fraction and biogas via a
37 biorefinery approach.

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

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48 **1. Introduction**

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

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

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

78 Besides, the biological hydrogen production processes including biophotolysis (direct and
79 indirect), photo-fermentation, dark fermentation and two-stage fermentation have received a
80 considerable attention [17, 18]. Among them, the dark fermentation appears as promising
81 technology using anaerobic bacteria which freely and efficiently produce H₂ with no need of
82 light and low operation costs compared to the photo-production [19-21]. This cost advantage
83 could be maximized when the biohydrogen is produced from waste such as agriculture by-
84 products, agro-industry and food waste.

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

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

95 **2. Materials and methods**

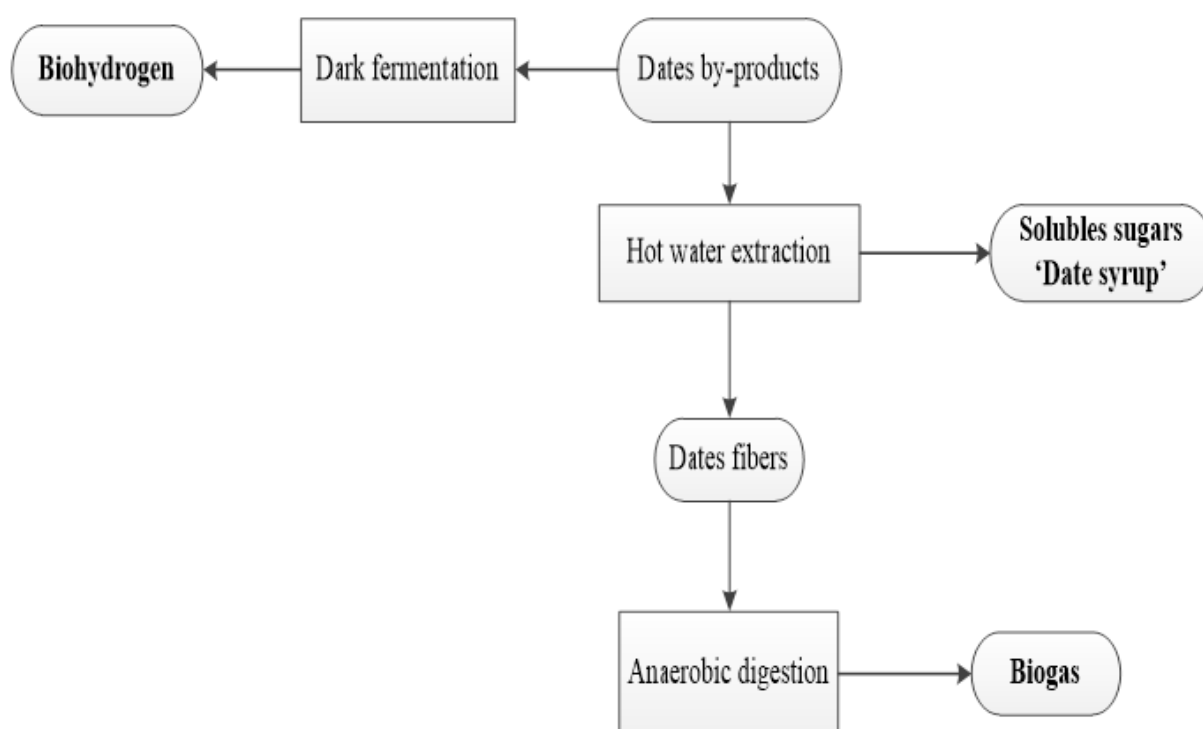
96 **2.1. Biological material**

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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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*

113 Biohydrogen production was carried out in batch Biochemical hydrogen potential test (BHP
114 test) in 550 ml plasma bottle (200 ml of working volume) with 2.66 g of milled dates' fleches.
115 The medium was composed of 12.5 ml of minimal nutrient solution (Table S2 in
116 Supplementary material) and 100 mM of MES (2-[N-morpholino] ethane sulfonic acid
117 buffer). BHP test was performed in triplicate according to the standardized protocol [26]
118 adapted without inoculum addition as described by [20]. Nitrogen gas was flushed into the
119 airspace of each bottles to maintain anaerobic conditions. The bottles were sealed using butyl-
120 rubber stoppers and incubated at 37 °C. During incubation, the gas production was monitored
121 with an automatic micro-gas chromatograph (SRA 1-GC R3000) equipped with two columns:
122 a Molsieve 10 m/PPU at 80 °C with Argon as vector gas and a VAR 8 m/PPU at 70 °C with
123 Helium, for O₂-CH₄-H₂-N₂ and CO₂ analysis, respectively. The TCD temperature was set at
124 90 °C. The fermentation was stopped when the hydrogen production stabilized.

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

128 Besides, microbial communities of one replicate were measured after dark fermentation (the
129 closest to the average H₂ yield). The procedure was as cited by Dauplain et al. (2020) [28].
130 Briefly, after sampling, the Eppendorf tube (2 mL) was centrifuged for 15 min at 13,400 g.
131 DNA was extracted with a DNA isolation kit (PowerSoil™ - MoBio Laboratories) according
132 to the instructions of the manufacturer. The sequencing of the purified PCR products was
133 performed in Toulouse, France (get.genotoul.fr). A bioinformatic procedure was applied to
134 gather sequences into operational taxonomic units (OTU) with a similarity of 97%. To
135 identify the OTUs, a blast search was performed (www.ncbi.nlm.nih.gov/BLAST).

136 ***2.2.2 Crude fibers extraction***

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

142 ***2.2.3 Soluble sugars characterization***

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

148 ***2.2.4 Methane production (BMP tests)***

149 Biochemical methane potential tests (BMP tests) were performed to access the methane
150 production. These batch tests were carried out in duplicate, in mesophilic conditions (35°C) as
151 described by Jard et al. [29], using the ‘Deglet Nour’ crude fibers extract (CFE) as substrate.
152 The used inoculum was recuperated from the outlet of an up flow anaerobic sludge blanket
153 reactor (UASB) treating wastewater from a sugar industry. The applied Substrate/Inoculum
154 ratio was 0.5 gVS of ‘Deglet Nour’ fibers per gVS of inoculum. Hence, each bottle was
155 consisted of 2 gVS of ground dry dates fibers and 4 gVS of inoculum. Bottles were filled to
156 400 mL with a bicarbonate buffer complemented with nutrients (Table S1 in Supplementary
157 material). Control tests containing a fully biodegradable substrate (ethanol) and a blank
158 (without sample) were achieved. Ethanol control was used to check the inoculum activity and
159 the blank control to measure endogenous methane production which was subtracted from the
160 methane production of each sample. Each bottle was flushed with nitrogen to create anaerobic

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

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

166 The volume of methane produced ΔV_{CH_4} (mL) between the dates j and $j-1$ was calculated
167 following Eq (1):

$$168 \quad \Delta V_{CH_4} = \left(\left[y(j)P1(j) \frac{V}{RT} \right] - \left[y(j-1)P2(j-1) \frac{V}{RT} \right] \right) \frac{RT^\circ}{P^\circ} \quad (1)$$

169

170 Where $y(j-1)$ et $y(j)$ are CH_4 contents in biogas at dates $j-1$ and j , respectively

171 $P1(j)$ (Pa) is the bottle head space pressure before sampling at the date j ,

172 $P2(j-1)$ (Pa) is the bottle head space pressure after gas release at the date $j-1$,

173 V (mL) is the bottle head space volume

174 R is the ideal gas constant ($8.314 \text{ J} \cdot (\text{mol} \cdot \text{K})^{-1}$),

175 T is the bottle temperature (K),

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

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

179 **2.3 Chemical composition of dates fleshes and fibers extract**

180 Total Solids (TS) and Volatile Solids (VS) of dates fleshes and dates extracted fibers were
181 analyzed for in accordance with APHA standard methods [31]. The carbohydrates and uronic
182 acids were determined using the two-stage acid hydrolysis protocol adapted from Effland
183 (1977) [32] as described by Ben Yahmed et al. (2017) [33]. The analysis of monosaccharide
184 sugars was carried out by high performance liquid chromatography (HPLC) using combined
185 Water/Dionex system supplied with BioRad HPX-87H column at 50 °C. The solvent

186 consisted of 0.005 M H₂SO₄ 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):

$$190 \text{ Cellulose (\%TS)} = \text{Glucose (\%TS)} / 1.11 \quad (2)$$

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)):

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

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 H₂ / gVS), R_m is the

207 maximum hydrogen production rate (mL H₂ / gVS /day), λ is the lag phase (days) and e is exp

208 (1). The values of P, R_m 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:

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

211 where M (mL CH₄/g VS) is the cumulative specific methane production, M_{max} (mL CH₄/g
 212 VS) is the ultimate methane production, K (days⁻¹) is the specific rate constant or apparent
 213 kinetic constant and t (days) is the time. The adjustment by non-linear regression of the
 214 experimental data (M, t) using the Sigmaplot software (version 14.0) allowed the calculation
 215 of the parameters K and M_{max}.

216 3. Results and discussion

217 3.1 Chemical composition of date flesh and crude fibers extract

218 Prior to the energetic bioconversion, the approximate chemical composition of dates fleshes
 219 as well as of the extracted fibers was determined. Table 1 shows that ‘Deglet Nour’ flesh is
 220 rich in soluble sugars (79.8 ± 0.8%) mainly sucrose, fructose and glucose, total fibers (12.3 ±
 221 0.4%) with small quantities of proteins and lipids based on total solids. These results are in
 222 agreement with Elleuch et al.’s study (2008) which demonstrated that ‘Deglet Nour’ flesh was
 223 characterized by the predominance of sugars with low percentages of ash and proteins. It is
 224 worth noting that substrates rich in soluble sugars are interesting for hydrogen production by
 225 dark fermentation [38, 39]. ‘Deglet Nour’ could be also considered as a good source of fibers
 226 [8]. However, the biochemical composition of dates depends on the culture conditions such as
 227 the growth zone and the harvest period (ripeness stage) and it varies significantly among
 228 cultivars [40, 41].

229 **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

230

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

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

235 For ‘Deglet Nour’ Flesh the cellulose content was calculated taking into the account the soluble
 236 glucose which was subtracted from the total glucose of ‘Deglet Nour’ Flesh

237 ^c The protein content was calculated by using a nitrogen conversion factor of 6.25

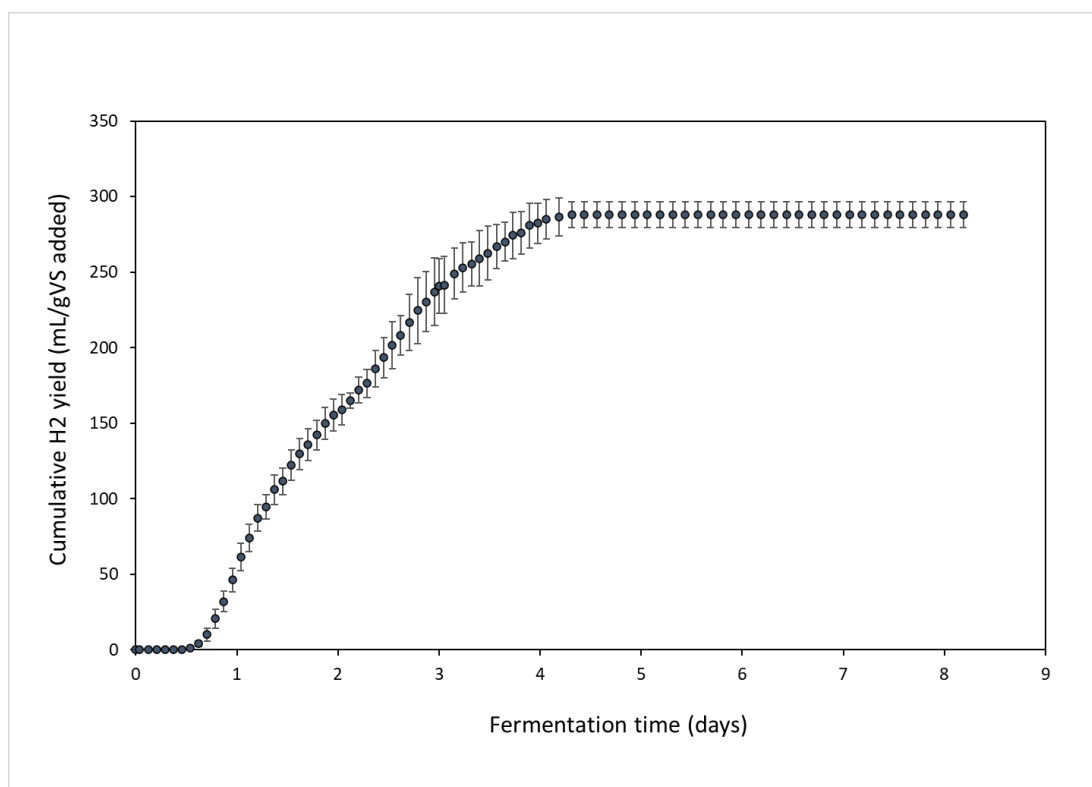
238 ^d Sugars content of ‘Deglet Nour’ syrup was expressed in relative % on dry weight basis.

239 ND: not determined

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

251 3.2 Biohydrogen production from date flesh

252 ‘Deglet Nour’ sorting gap represents a complex organic waste. This discarded biomass was
253 used for biohydrogen production without inoculum addition. In fact, this organic solid waste
254 generally contains abundant indigenous microflora [20, 42]. Hydrogen production via dark
255 fermentation is of great interest thanks to its double action of waste reduction and clean
256 energy production. BHP tests of this waste lead to an hydrogen yield of 292 mLH₂/gVS_{ini}
257 (Fig. 2) which is high compared to other organic substrates such as rice bran (61 mLH₂/gVS)
258 [43] and food waste (96.9 mLH₂/gVS) [20] (Table 2).



259
260 **Fig. 2.** Cumulative biohydrogen H₂ production, expressed as mL H₂/g VS added, obtained
261 during the BHP tests carried out with ‘Deglet Nour’ fleshes. The standard deviation was lower
262 than 10%.

263 To better evaluate and discuss the biohydrogen production, a comparison study was also
264 carried out with other date feedstocks such as date seeds and rotten date fruits (Table 2).
265 Although the H₂ production in the present work was performed without pretreatment and

266 inoculation, the obtained H₂ yield of 292 mLH₂/gVSini corresponding to an hydrogen
 267 production efficiency of 2.47 mol H₂/mol eq.hexose ini, was comparable with those using
 268 mixed cultures fermentation (1-3 mol H₂/mol hexose) reported in the literature [44]. In fact, a
 269 maximum biohydrogen yield of 224 mL/gTS (1.87 mol H₂/mol eq.hexose ini) was reached
 270 using acid-pretreated date seed as substrate and *Clostridium thermocellum* ATCC 27405 as
 271 inoculum [45]. Besides, the dark fermentation of rotten dates fruits using *Clostridium*
 272 *acetobutylicum* ATCC 824 carried out by Abd-Alla et al (2011) allowed a cumulative
 273 H₂ yield of 399.4 mLH₂/gVS (4 mol H₂/mol eq.hexose ini) [25]. This high H₂ yield could be
 274 explained by the fact of the utilization of a pure culture for the inoculation unlike our study
 275 where no inoculum was employed.

276 **Table 2.** Comparison of biohydrogen and biomethane yields reported for different organic
 277 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 + <i>Clostridium thermocellum</i> 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]

278 * Hydrogen yield expressed in mLH₂/gTS corresponding to 30 mmol/L as mentioned by Rambabu et
279 al. (2019) [45]

280 Actually, no data are available for date flesh dark fermentation without inoculation. The
281 bioconversion into biohydrogen without microorganism's inoculation represents an
282 innovative efficient practically applicable method allowing a high H₂ production while
283 degradation of dates biomass waste. Moreover, the dates by-products can be used both as
284 substrate and inoculum source.

285 Regarding the kinetics of the biohydrogen production, experimental data were fitted to a
286 modified Gompertz model whose parameters were presented in Table 3. Maximum of
287 cumulated hydrogen production (P), maximum hydrogen production rate (R_m) and the lag
288 phase (λ) were 300.80 ± 7.14 mLH₂/gVS, 113.38 ± 14.05 mLH₂/gVS/day and 0.59 ± 0.09 day
289 respectively. Compared to other substrates rich in soluble sugars such as watermelon waste
290 ($\lambda = 27.25$ h) the dark fermentation without inoculation using 'Deglet Nour' flesh as
291 substrate represents a fast biohydrogen production with short lag phase [46].

292 **Table 3.** Kinetic parameters of hydrogen and methane production in batch tests determined
293 from modified Gompertz equation and exponential model respectively with P the maximum
294 cumulative hydrogen production (mLH₂/gVS), R_m the maximum hydrogen production rate
295 (mLH₂/gVS/day), λ the lag phase (days), M_{max} (mLCH₄/VS) the ultimate methane production

296 and K (days^{-1}) the apparent kinetic constant. Values correspond to the average of replicates \pm
 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 ($\text{mLH}_2/\text{gVS}/\text{day}$)	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 \pm 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 H₂
 319 and CH₄ 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
<i>Enterobacteriaceae_unclassified</i>	9.7%	<i>Enterobacter tabaci</i>	98.82%	NR_146667.2
<i>Escherichia-Shigella</i>	20.7%	<i>Escherichia fergusonii</i>	99.06%	NR_074902.1
<i>Lactobacillus</i> (OTU 12)	39.4%	<i>Lactobacillus rhamnosus</i>	100.00%	NR_113332.1
<i>Clostridium_sensu_stricto_5</i>	26.7%	<i>Clostridium paraputrificum</i>	97.75%	NR_113021.1
<i>Lactobacillus</i> (OTU 29)	1.4%	<i>Lactobacillus fermentum</i>	100.00%	NR_104927.1
<i>Enterococcus</i>	0.1%	<i>Enterococcus hirae</i>	100.00%	NR_114783.2
<i>Bacillus</i>	0.1%	<i>Bacillus wiedmannii</i>	100.00%	NR_152692.1
<i>Clostridium_sensu_stricto_12</i>	1.9%	<i>Clostridium luticellarii</i>	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

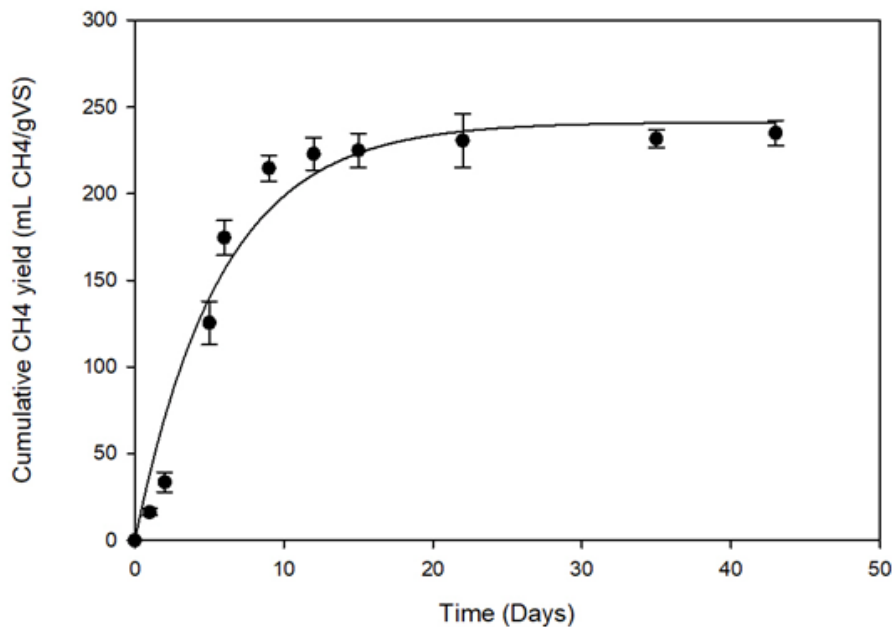
334 proportions (between 0.37 and 3.84 mM), which is consistent with the high concentrations
335 (Table 4) measured in the present study (excepted for butyrate). Due to the high proportions
336 in butyrate (Table 4), hydrogen production mainly occurred through the butyrate pathway (or
337 after lactate consumption). The high ethanol amount can also be attributed to
338 *Enterobacteriaceae* family (*Escherichia Shigella* and the unclassified *Enterobacteriaceae*) as
339 reported previously [51]. The high hydrogen production is probably due not only to the high
340 proportions of efficient H₂ producers as *Escherichia-Shigella*, *Clostridium sp.* [28] but also to
341 *Enterobacter sp.* [52].

342 **3.3 Soluble sugars extraction and biogas production from crude fibers extract**

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

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

358 untreated CFE as feedstock, the maximum biomethane yield of 235 mLCH₄/gVS fibers
359 corresponding to 27.6 mLCH₄/gVS initial fleshes was obtained (Fig 3).



360

361 **Fig.3.** Cumulative methane yield, expressed as mL CH₄/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 $0.17 \pm 0.02 \text{ days}^{-1}$ and $241 \pm 11 \text{ mLCH}_4/\text{VS}$ 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

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

377 **Conclusion**

378 This study presents an innovative investigation regarding the energetic processing of ‘Deglet
379 Nour’ by-products. The dark fermentation of ‘Deglet Nour’ fleshes without inoculation,
380 newly explored in the present work, allowed a high biohydrogen potential (292 mLH₂/gVS_{ini}).
381 Besides, in another biorefinery approach, date syrup was extracted with a 0.73 g/g yield and
382 the crude fibers extract was submitted to anaerobic digestion. The methane potential reached
383 235 mLCH₄/gVS fibers. Hence, this work presents a proof of concept allowing an almost
384 complete bioconversion of the dates by-products. Optimization of some stages and a techno-
385 economic analysis are therefore needed to perform the scale-up of the proposed concept.

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Supplementary material

Macro nutriments		
NH ₄ Cl	26.2	g/L
KH ₂ PO ₄	10	g/L
MgCl ₂ , 6H ₂ O	6	g/L
CaCl ₂ , 2H ₂ O	3	g/L
Micro nutriments		
FeCl ₂ , 4H ₂ O	2	g/L
CoCl ₂ , 6H ₂ O	0.5	g/L
MnCl ₂ , 4H ₂ O	0.1	g/L
NiCl ₂ , 6H ₂ O	0.1	g/L
ZnCl ₂	0,05	g/L
H ₃ BO ₃	0,05	g/L
Na ₂ SeO ₃	0,05	g/L
CuCl ₂ , 2H ₂ O	0,04	g/L
Na ₂ MoO ₄ , 2H ₂ O	0,01	g/L
Bicarbonate buffer		
NaHCO ₃	50	g/L

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Table S1. Composition of BMP nutriments

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Macro nutriments		
NH ₄ Cl	32	g/L
KH ₂ PO ₄	20	g/L
Micro nutriments		
FeCl ₂ , 4H ₂ O	1.5	g/L
HCl	1.755	g/L
CoCl ₂ , 6H ₂ O	0.025	g/L
MnSO ₄ , H ₂ O	0.117	g/L
NiCl ₂ , 6H ₂ O	0.025	g/L
ZnCl ₂	0,07	g/L
H ₃ BO ₃ , H ₂ O	0,06	g/L
CuCl ₂ , 2H ₂ O	0,015	g/L
Na ₂ MoO ₄ , 2H ₂ O	0,025	g/L
MES buffer		
2-[N-morpholino] ethane sulfonic acid buffer	100	mM

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Table S2. Composition of BHP nutriments