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ORIGINAL ARTICLE

Production of biogas and ethanol from stationery wastes using a microbial consortium isolated from soil as starter culture

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Abstract

The conventional pretreatments used during the valorization of paper waste in renewable energies are expensive, long, slow, require high temperatures and particularly not eco-friendly. However, the application of microbial cultures with cellulolytic capabilities becomes an attractive and low-cost strategy. Therefore, the aim of this study was to screen an efficient microbial culture and its evaluation as a starter culture during hydrolysis process of biogas and bioethanol production. Our results indicated that from 18 isolates, two bacteria (identified as *Pseudomonas horyzihabitans* and *Serratia liquefaciens*) and one consortium (CS2, predominated by *Enterobacteriaceae*) had an important cellulosic hydrolysis activity. The application of the selected consortium as a starter culture during the hydrolysis process of biogas and bioethanol production improved yields. Indeed, the application of CS2 enhanced the biogas and bioethanol yields to 9.4 mL g^{-1} and 78.2 µL g^{-1} ($P < 0.05$) respectively. Also, starter culture CS2 addition reduced the time needed for cellulosic hydrolysis to 21 days, respect to 24 days in control sample, during biogas production under psychrophilic temperature. Thus, this low cost and practical procedure can be used as an efficient strategy to release sugars from paper waste, to reduce the time needed for cellulosic biodigestion, and to enhance the biogas and bioethanol recovered.

Keywords: anaerobic biodigestion; cellulosic biomass; renewable energy; paper waste.

1. Introduction

The depletion of fossil fuel resources, global warming, environmental degradation and pollution of biosphere are becoming a major concern. Facing the significant increase in energy demand, the world is seeking for new sources of energy, particularly, the renewable energies. As a result, research for sustainable and less polluting alternatives becomes essential (Besnard, 2019).

Renewable energies (solar, hydraulic, biomass, etc.) constitute a set of energy solutions, making possible the reduction of dependence on petroleum and also eco-friendly technology (Caglar, 2020). Morocco is now progressing towards more renewable energy production. Actually, Noor Power Station—construed at Ouarzazate (in the South of Morocco)—, with production of 580 MW, has set the target of generating 52 % of electricity in 2030. In addition, 711 MW and 1220 MW are produced by several solar and wind energy parks respectively. Likewise, Tunisia (as other North Africa country) also plans to increase its renewable energy production from 3 % to 30 % in 2030. Currently, 620 MW and 120 MW are produced with solar and wind energy respectively (Climate investment funds, 2018; Qadir *et al.*, 2021). Moreover, China is leading the renewable energy production in world with 758 626 MW, followed by the United States (with 264 504 MW), India

(with 128 323 MW) and Germany (with 125 386 MW) (Qadir *et al.*, 2021). Unfortunately, the renewable energies depend a lot on environmental, technical and climatic factors such as sun, wind, temperature, pretreatments, high investment, etc. (Mahjabeen *et al.*, 2020).

Currently, lignocellulosic biomass—as an abundant, inexpensive and sustainable solution—is considered one of the most promising alternatives for fossil energy (Lynd *et al.*, 2008). In fact, this biomass can be valorized by biodigestion for biogas and biofuels production (Makhuvele *et al.*, 2017; Young *et al.*, 2018).

The office paper waste, as cellulosic biomass, is a complex mix predominated by cellulose (64.7 %), hemicellulose (13 %) and lignin (0.93 %) (Chen *et al.*, 2004). This mixture requires chemical pretreatment (acid or alkaline catalyze) and/or enzymatic hydrolysis steps to convert it to reducing sugars. However, these treatments are expensive, require high temperatures and are not eco-friendly. In addition, the enzymatic hydrolysis is essential but the production of hydrolytic enzymes is expensive and it often needs higher levels of cellulases to enhance yields, which considerably increase the cost production (Ramos and Malcata, 2017). Also, to achieve complete hydrolysis, the degradation of cellulosic biomass requires synergistic action of many cellulolytic enzymes (Warren, 1996).

As a result, extensive biological strategies are developed, such as the application of microbial culture with cellulolytic ability. Several studies on microbial degradation of the lignocellulosic biomass are published. They mainly concern microorganisms screening, genetic improvements and culture conditions (Liu and Qu, 2019; Tahir *et al.*, 2019; Claes *et al.*, 2020). Therefore, the aim of the present study is to screen an efficient microbial culture and its evaluation as a starter culture during hydrolysis process of biogas and bioethanol production from paper waste (generated by various academic departments at the university as cellulosic biomass).

2. Material and Methods

2.1. Bacterial strain and culture conditions

Saccharomyces cerevisiae BZ18, from our collection, was used as production strain of bioethanol. This strain was cultivated in Malt extract agar (ME, Biokar Diagnostics, France) at 30 °C during 24 h and maintained at 4 °C.

2.2. Isolation and screening for cellulase microbial cultures

The screening of cellulase microbial cultures was performed using paper waste generated by various academic departments of our university. Isolation of cellulose-producing strains was carried out using different soil samples (S1, S2 and S3) taken from the top 10 cm layer of the sampling located in Ouad-Fez forest. Also, the gut microbiota of desert locust *Schistocerca gregaria* (G1) was used.

Later, 1 g of sample was taken, aseptically transferred into 9 mL of sterile Ringer solution, and homogenized for 30 min. Subsequently, 1 mL was added to the tube containing strip of paper (approximately length 8 cm × width 1 cm) immersed (maintained 4 cm above liquid level) in 9 mL of the mineral liquid medium MLM previously sterilized (containing 0.025 % K_2HPO_4, 0.0125 % $MgSO_4$, 0.0125 % NaCl, 0.1 % NH_4NO_3, and 2.5 mg L^{-1} for the rest of components: $Fe_2(SO_4)_3$,

K_2MoO_4, $Na_2B_4O_7$, $CdSO_4$, $Co(NO_3)_2$, $CuSO_4$, $ZnSO_4$, $MnSO_4$, and $FeCl_3$, pH 7). Also, a negative control without inoculation (containing strip of paper immersed in MLM) was prepared. Finally, all test tubes were incubated at 30 °C for 15 days.

Culture tubes that showed evidence of cellulolytic activity after 15 days of incubation were serially 10-diluted and plated on Luria-Bertani agar (LB, Biokar Diagnostics). All plates were incubated at 30 °C for 48 h to 72 h. Then, individual isolates were taken with sterilized loop, purified using streaking on same culture media, and repicked on liquid medium.

2.3. Selection of cellulolytic isolates

Approximately 1×10^6 CFU mL^{-1} of individual isolate liquid cultures (prepared above) were added to test tubes containing strip of paper immersed in 10 mL of MLM previously sterilized (as described above). Also, negative control without inoculation was prepared. All test tubes were incubated at 30 °C for 20 days.

The selection of cellulosic isolates was performed by 3,5-dinitrosalicylic acid (DNS) assays for reducing sugars, after 10 and 20 days of incubation at 30 °C, as described by Wood *et al.* (2012). Indeed, bacterial isolates/culture with highest carbohydrase activities were selected and submitted to morphological characterization (by colonial characteristics and Gram's reaction) and biochemical identification (catalase test (Solvapur, France), oxidase test (Sigma-Aldrich, Germany).

Finally, the bacterial isolates selected were identified using API 20E and API 20NE (Biomérieux, France). However, the consortium culture selected was submitted to the taxonomic study using different culture media (all from Biokar Diagnostics). Indeed, LB, Tryptose Sulfite Cycloserine agar (TSC, incubated under anaerobic condition), MacConkey agar (MC) and ME—supplemented with chloramphenicol (0.1 mg mL^{-1})—were used for total aerobic mesophilic flora (TAMF), clostridia, enterobacteria and fungi enumerations. All plates were incubated at 30 °C for 2 days.

2.4. Evaluation of selected cultures as a starter culture during hydrolysis process of biogas production

500 g of cutpapers were added to 4.5 L of MLM and inoculated with the selected culture at 1×10^9 CFU mL^{-1}. The biodigestion of paper waste was carried out using a conventional batch bioreactor connected to the gas collector by norprene tubing and provided with a sample collector. This bioreactor, manually assembled and hermetically sealed, was designed to ensure anaerobic conditions.

The bioreactor was stirred and incubated at room temperature for 51 days. The biogas recovered in the collector was directly measured (mL) and yields (v/w) were determined using the following formula:

$$\text{Yield (Biogas)} = \frac{\text{Volume of biogas produced}}{\text{Mass of paper used}}. \quad (1)$$

Also, pH and room temperature (maximum and minimum values) were measured at selected times by pH-meter (Hanna Instrument, Portugal) and Auriol-thermometer (Auriol, Germany) respectively. In addition, negative control, without culture inoculation, was prepared under the same conditions.

2.5. Evaluation of selected cultures as a starter culture during bioethanol production

500 g of cutpapers were added to 4.5 L of MLM and inoculated with the selected culture at 1×10^9 CFU mL^{-1}. The biodigestion of paper waste was carried out under anaerobic conditions using two methods:

- **Method 1 (P1).** The bioreactor was stirred and incubated at 30 °C for 15 days. Then, the culture was supplemented with *S. cerevisiae* BZ18 (at 1×10^9 CFU mL^{-1}) and subsequently reincubated at 30 °C for 2 days under anaerobic conditions. Also, negative control, without yeasts, was prepared under the same conditions.
- **Method 2 (P2).** Co-culture containing cellulolytic starter culture (at 1×10^9 CFU mL^{-1}) and *S. cerevisiae* (at 1×10^9 CFU mL^{-1}) was incubated at 30 °C for 15 days under anaerobic conditions. Also, negative control, without yeasts, was prepared under the same conditions.

  Ethanol was recovered by distillation, using Buchi rotary evaporator, and dehydrated using water-absorbing polymers.

Finally, bioethanol yields (in µL g^{-1} of paper) were determined by potassium dichromate method (Khalil *et al.*, 2015). Ethanol recovered was centrifuged at 6000 rpm for 10 min at 4 °C. Later, 1 mL of the supernatant was makeup to 5 mL with distilled water and 5 mL of chromic acid reagent was added (prepared by dissolving 34 g of $K_2Cr_2O_7$ in 400 mL of distilled water, 325 mL of H_2SO_4 and making up the volume to 1000 mL). Afterwards, the reaction mixture was heated at 60 °C for 20 min and cooled to room temperature. The absorbance was measured at 600 nm on BK-UV1000 spectrophotometer. Also, ethanol standard curve was prepared using ethanol absolute solutions (Merck, Germany) under similar conditions.

Bioethanol yields were calculated using the following formula:

$$\text{Yield (Bioethanol)} = \frac{\text{Volume of bioethanol recovered}}{\text{Mass of paper used}}. \quad (2)$$

2.6. Statistical analyses

The results are expressed on average of duplicate independent experiments. Statistical analyses were performed using trial SPSS-PC v-17.0 (SPSS, Chicago, IL., USA). Data related to reduced sugars content, pH, and biogas/bioethanol production along the incubation period were subjected to ANOVA. The presence of starter culture was used as factor respect to other cultures. Differences were considered significant at $P < 0.05$.

3. Results and Discussion

3.1. Isolation and screening for cellulase microbial cultures

Isolation of cellulase-producing strains was carried out using different biotopes: S1, S2, S3 and G1. Our results indicated that the cellulolytic activity was detected after 9 days—and through 15 days—of incubation at 30 °C, with slight turbidity of MLM and morphological changes on the immersed strip of paper (**Figure 1**). These observations can be attributed to microbial activities and secretion of different extracellular cellulolytic enzymes (Warren, 1996). Indeed, microbial adhesion to cellulosic support—strip of paper—facilitates enzymes induction and mechanisms of action involved with cellulosic degradation. Thus, cellulytic enzymes are more active when microbial cells are immobilized on substrate (Bond and Stutzenberger, 1989).

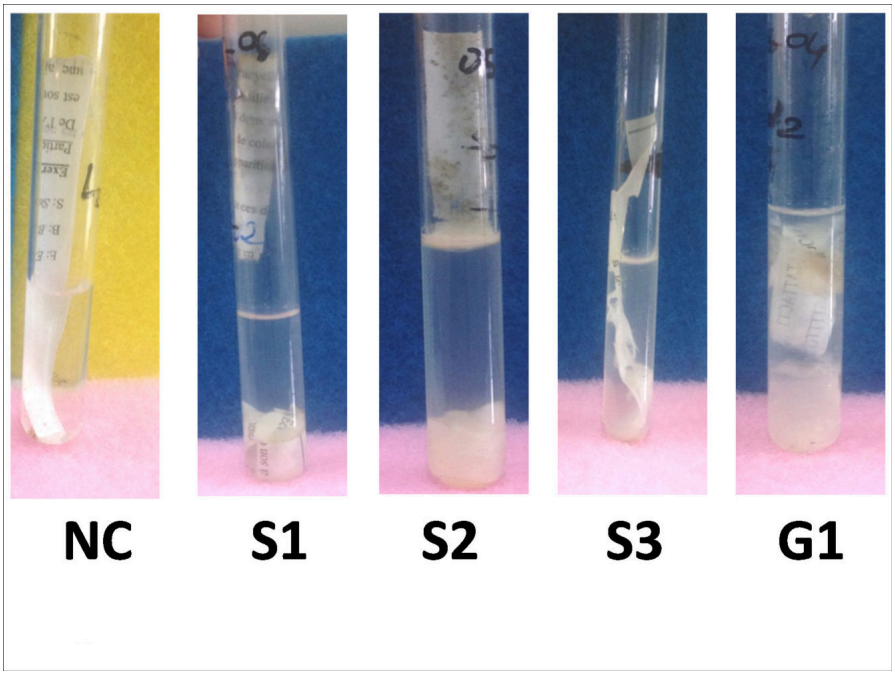

Figure 1. Morphological changes detected on immersed strip of paper after 15 days of incubation at 30 °C. **NC)** Negative control without inoculation; **S1)** Sample inoculated with soil 1; **S2)** Sample inoculated with soil 2; **S3)** Sample inoculated with soil 3; and **G1)** Sample inoculated with gut microbiota of desert locust.

Among 4 biotopes tested, culture S2 (CS2) showed a strong cellulolytic performance at 15 days and caused rapid and continuous deformation/degradation of paper strip (Figure 1). The microbial source plays an important role in the isolation of performed microorganisms (Feng *et al.*, 2011). Previous studies reported the presence of potential cellulolytic microorganisms in different soils, which some were characterized as important sources of cellulolytic strains. Much of this can be attributed to forest vegetation, soil fertility, organic matter and animal waste. Likewise, the abundance of cellulolytic, amylolytic and pectinolytic microorganisms in the gut of desert locust (*Schistocerca gregaria*)—facilitating the degradation of cellulose and other types of biomass—was also referred (Brune, 2009). This microbiota, predominated by *Enterobacteriaceae* and enterococci (Dillon and Charnley, 2002), was submitted to several cellulose degradation studies (Nelson *et al.*, 2021).

Subsequently, 18 isolates from CS2—different morphologically and microscopically—were selected for their cellulolytic activity (on the basis of DNS assays for reducing sugars). Finally, the selected isolates (named A, B, C, D, E, F, G, H, I1, I2, J, K, L1, L2, M, N, O and P) were identified as 15 Gram negative bacteria (83.3 %) and 3 Gram positive bacteria (16.7 %), which 50 % of them were Bacilli, 33.3 % Cocci and 16.7 % Coccobacilli (**Table 1**).

In addition, the results indicated that after 10 days of incubation at 30 °C, the best bacterial isolate was F, followed by isolate L2, and isolates I1 with 0.278 µmol, 0.257 µmol and 0.247 µmol of reducing sugars, respectively. After 20 days, the best isolate was L2 with 0.261 µmol of reducing sugars, followed by isolate I1 with 0.216 µmol of reducing sugars, and isolate I2 with 0.195 µmol of reducing sugars (**Figure 2**). Moreover, reducing sugars levels decreased at 20 days for several isolates (A, B, C, F, G, H, I1, I2, J, K, L1, N, O and P), probably due to utilization of reducing sugars by itself, lack of nutrients, cells lysis, end-product's inhibition, and/or absence of enzymes synergism (Sandgren *et al.*, 2005).

Table 1. Morphological and biochemical characters of the selected cellulosic isolates (A, B, C, D, E, F, G, H, I1, I2, J, K, L1, L2, M, N, O and P).

Isolates	Origine	Type	Gram's staining results	Catalase test	Oxidase test
A	S1	Cocci	–	–	–
B	S1	Bacilli	–	+	+
C	S1	Cocci	–	–	–
D	S1	Cocci	–	+	+
E	S2	Cocci	–	+	+
F	S2	Bacilli	–	+	–
G	S2	Bacilli	+	–	–
H	S2	Coccobacilli	–	+	+
I1	S3	Bacilli	–	+	–
I2	S3	Bacilli	–	+	–
J	S3	Cocci	+	–	–
K	S3	Bacilli	–	+	+
L1	S3	Cocci	+	–	–
L2	S3	Bacilli	–	+	–
M	C1	Bacilli	–	+	–
N	C1	Bacilli	–	+	–
O	C1	Bacilli	–	+	–
P	C1	Coccobacilli	–	+	–

However, culture/consortium CS2 was able to reach significantly higher levels of reducing sugars: 0.371 µmol and 0.585 µmol after 10 and 20 days of incubation respectively (Figure 2). This could be due to synergistic interactions among cellulolytic microorganisms forming the consortium (Wongwilaiwarin *et al.*, 2010; Tantayotai *et al.*, 2017; Wang *et al.*, 2020).

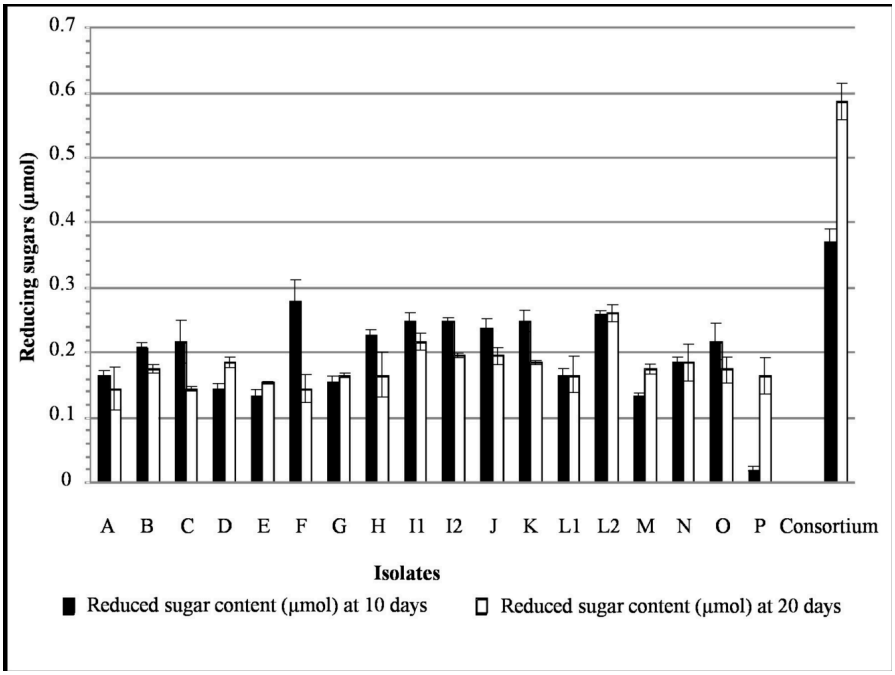

Figure 2. Reduced sugar contents (µmol) obtained with the selected isolates (A, B, C, D, E, F, G, H, I1, I2, J, K, L1, L2, M, N, O and P) and consortium CS2 after 10 and 20 days of incubation at 30 °C.

Then, bacterial isolates F, L2 and consortium culture CS2 were selected, identified and characterized. Indeed, isolate F—which exhibited, rough, wrinkled, adherent and yellow pigmented colonies—were characterized as Gram-negative rods, catalase-positive, oxidase-negative and identified as *Pseudomonas oryzihabitans* (with 97 % of percentage identity, using API 20NE). The negative-oxidase of this species is unique among *Pseudomonas* spp. (Brady and Leber, 2018). Also, this strain was characterized as glucose+, mannitol−, sucrose−, sorbitol−, arabinose−, inositol+, and melibiose+.

The isolate L2—which exhibited smooth and cream pigmented colonies—was characterized as Gram-negative rods, catalase-positive, oxidase-negative and identified as *Serratia liquefaciens* (with 95 % of percentage identity, using API 20E). Also, this strain was characterized as glucose+, mannitol+, sucrose+, sorbitol+, arabinose+, inositol+, and melibiose+. Moreover, the ability of *Pseudomonas* and *Serratia* to degrade several complex sugars (such as cellulose, xylan, etc.) was frequently referred (Anand and Sripathi, 2004; Palleroni, 2010; Talia *et al.*, 2012; Qin *et al.*, 2013; Haq *et al.*, 2016).

On the other hand, the consortium CS2 was predominated by *Enterobacteriaceae* ((78.00 ± 26.68) %), followed by *Clostridia* ((3.6 ± 13.7) %), fungi ((0.07 ± 0.02) %), and other microorganisms ((18.33 ± 7.32) %) (**Figure 3**). Several microbial consortia—with cellulolytic activity—were isolated and selected from different ecosystems, such as soils, straws and composts (Haruta *et al.*, 2002; Feng *et al.*, 2011; Gao *et al.*, 2014; Young *et al.*, 2018; Da Silva *et al.*, 2019). Moreover, the identification of the cellulolytic consortia—degrading filter papers, newspapers, printing papers and cotton—found a great microbial diversity, including several genera: gram-positive bacteria (*Brevibacillus* and *Clostridium*), gram-negative bacteria (*Enterobacteriaceae*, *Pseudoxanthomonas*), obligate anaerobes (*Clostridia, Bordetella*, etc.), and obligate aerobes (molds, *Pseudoxanthomonas, Brevibacillus*, etc.) (Haruta *et al.*, 2002; Wongwilaiwarin *et al.*, 2010; Haq *et al.*, 2016; Tantayotai *et al.*, 2017; Wang *et al.*, 2020).

3.2. Evaluation of selected cultures as a starter culture during hydrolysis process of paper waste

During the use of selected *Pseudomonas oryzihabitans* and *Serratia liquefaciens* cultures alone—as a starter culture during hydrolysis process of biogas or bioethanol production—no significant differences were observed with respect to negative controls ($P > 0.05$) (data not shown). However, the use of consortium CS2 affected positively the hydrolysis process. These results suggest that CS2, showed great microbial diversity, exhibits community-intrinsic properties and synergistic interactions, allowing it to be a good starter culture for cellulosic degradation.

The use of microbial consortia in lignocellulosic biodegradation was already described by several researchers (Wongwilaiwarin *et al.*, 2010; Tantayotai *et al.*, 2017; Wang *et al.*, 2020). Due to the complexity of substrate and environmental conditions, the use of complex microbial communities—consortiums—is certainly favored (Wongwilaiwarin *et al.*, 2010; Tantayotai *et al.*, 2017; Wang *et al.*, 2020). However, little information is available about real interactions among microbial actors involved in consortia.

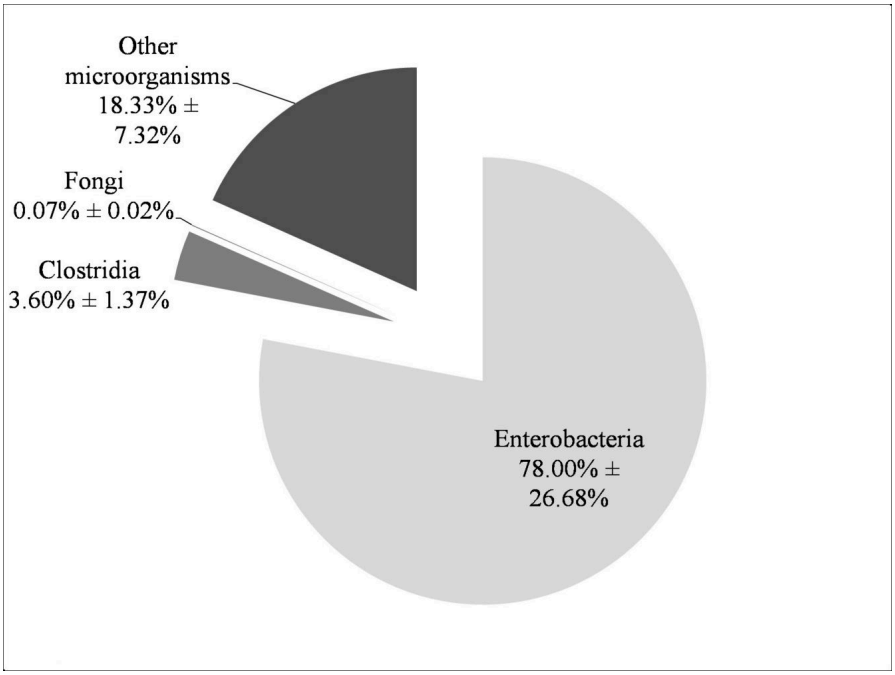

Figure 3. Diversity and taxonomic composition (in %) of consortium CS2. Values are the average ± SD of three independent experiments.

3.3. Evaluation of consortium CS2 as a starter culture during biogas production using paper waste

The biodigestion of cellulosic biomass was carried out under anaerobic conditions for 51 days. The biogas produced is the result of the anaerobic biodigestion of papers waste, with and without starter culture CS2, under ambient temperature oscillating between 3 °C and 30 °C (**Figure 4**A).

This production process takes place through four stages: hydrolysis, acidogenesis, acetogenesis, and methanation. Indeed, during hydrolysis step, cellulosic polymers were broken down into monomers and oligomers. While during acidogenesis, products of hydrolysis were broken down. Our results indicated that pH medium without starter culture CS2 decreased from 7.7 to 7.5, 7.0, 6.5, 6.1, 5.7 and 5.5 respectively at 4, 8, 11, 17, 21 and 24 days (Figure 4B). However, pH values decreased with starter culture CS2 to 7.3, 6.4, 6.0, 5.6, 5.5 and 5.5 respectively at 4, 8, 11, 17, 21 and 24 days. After 24 days, no significant differences were observed between cultures with and without starter culture ($P > 0.05$).

Furthermore, starter culture CS2 addition resulting in significant reduction of the biodigestion (hydrolysis/acidogenesis) time of cellulosic biomass to 21 days (respect to 24 days in control without CS2) affected positively biogas production despite psychrophilic conditions. Indeed, higher volume of biogas was achieved (300 mL, $P < 0.01$) after 24 days of storage, compared to control culture (140 mL) (Figure 4C). Moreover, biogas production incremented to 3000 mL and 4700 mL (yields of 6 mL g^{-1} and 9.4 mL g^{-1} of paper) in culture with CS2, compared to 2500 mL and 4000 mL (yields of 5 mL g^{-1} and 8 mL g^{-1} of paper) in culture without starter, respectively after 48 and 51 days (Figure 4C), enhancing gas production with 17.5 %. At 51 days, the biodigestion was still in exponential biogas production and the stationary phase was not

reached. Indeed, several studies reached stationary phase of biogas production, using different substrates (several types of spent livestock bedding and cow-dung) after 60 to 80 days (Igoud *et al.*, 2002; Ounnar *et al.*, 2012; Riggio *et al.*, 2017).

Thus CS2 may be an efficient microbial culture starter, with hydrolytic performance of cellulosic biomass, adapted to the production of biogas under ambient temperatures (carried out under psychrophilic conditions). In fact, the reaction temperature is considered a major factor that affects the anaerobic biodigestion of lignocellulosic biomass (biodigestion rates, methane formation, process duration and stabilization of biogas production) (Bekkering *et al.*, 2010). Indeed, psychrophilic

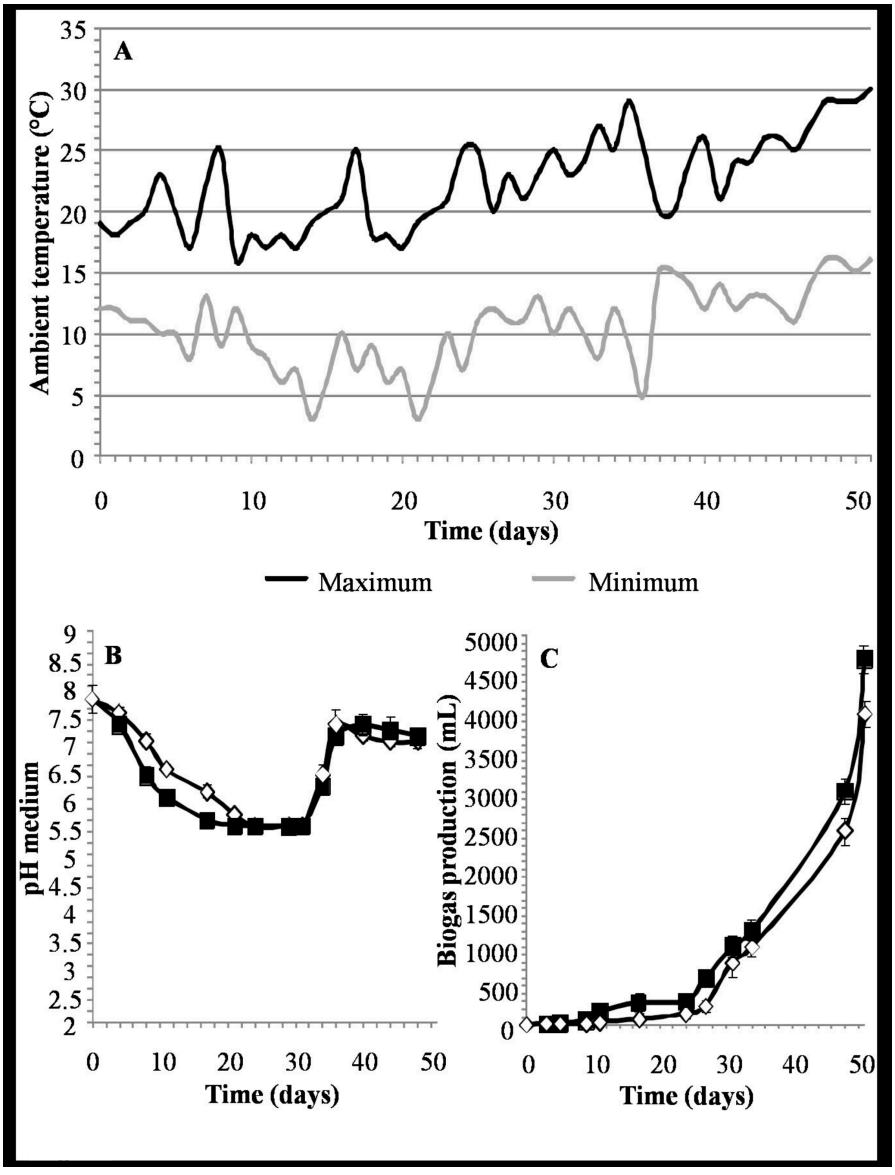

Figure 4. Anaerobic biodigestion of papers waste, with and without starter culture CS2, under ambient temperature. **A)** Ambient temperature oscillation (minimum and maximum); **B)** pH medium; and **C)** biogas recovered (mL). Control without CS2 (◇); containing CS2 (■).

biodigestion, below 25 °C, requires longer lag phase respect to mesophilic biodigestion (25 °C to 45 °C) and thermophilic biodigestion (45 °C to 55 °C), which allowed rapid decomposition of substrates and high gas yields (Bouallagui *et al.*, 2003; Gannoun *et al.*, 2009).

3.4. Evaluation of consortium CS2 as a starter culture during bioethanol production using paper waste

Our results indicated that 78.2 µL g^{-1} and 59 µL g^{-1} of bioethanol were recovered with P1 and P2 respectively (**Figure 5**). Hence, P1 was more efficient (with an improvement of 32.5 %) with respect to P2. In fact, with P1, the cellulosic polymers were broken down into monomers and simple sugars, easily assimilated by yeasts, which could improve bioethanol titers. With P2, the decrease on bioethanol production can be attributed to the competition between CS2 and yeasts and/or yeast metabolism alteration. Indeed, this co-culture can negatively affect the bioethanol production (Basso *et al.*, 2011; Della-Bianca and Gombert, 2013; Carvalho-Netto *et al.*, 2015). Moreover, ethanol titers depend on conversion efficiency into ethanol and the type of biomass (cellulose, hemicellulose and lignin contents) (Tye *et al.*, 2016). Many researchers recovered bioethanol from cellulosic materials at 5.87 %, 6.12 % and 6.91 % (v/v), using *S. cerevisiae, Cytophaga hutchnisonni* and other isolated culture respectively (Sharif-Hossain, 2015; Byadgi and Kalburgi, 2016). However, high bioethanol titers could be obtained under optimized conditions (16.9 % (v/v), using the combinations of *S. cerevisiae, Aspergillus foetidus* and *Fusarium oxysporum* during simultaneous saccharification and fermentation) (Chatanta *et al.*, 2008).

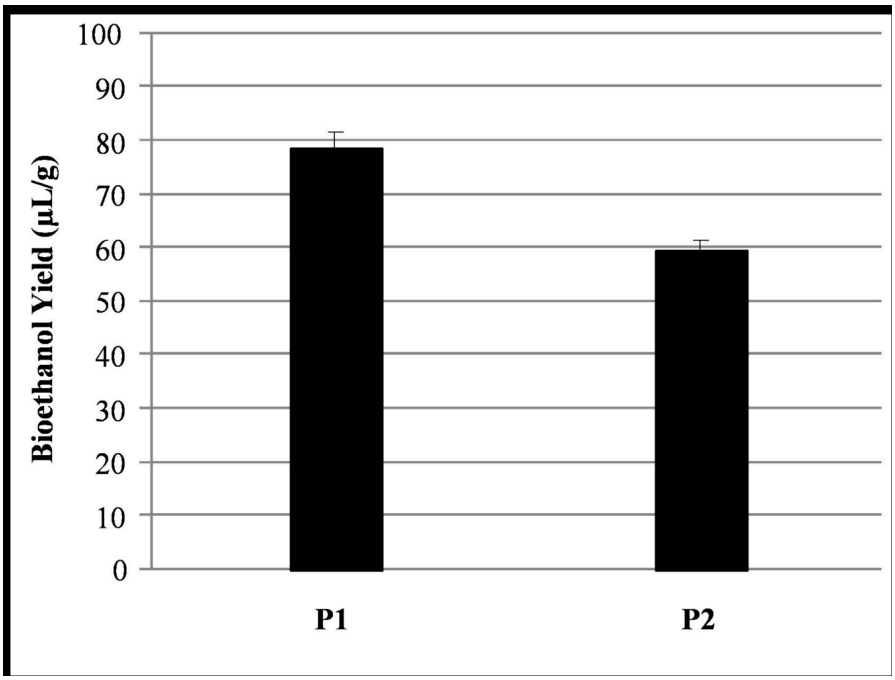

Figure 5. Bioethanol yields recovered with P1 (sequential process of CS2 and *S. cerevisiae*) and P2 (simultaneous process of CS2 and *S. cerevisiae*).

4. Conclusions

In conclusion, the paper waste generated by various academic departments at the university can be efficiently valorized in renewable energy production (biogas and bioethanol) using a starter culture—such as the successful consortium CS2—as novelty, ecological, low cost, fast and also eco-friendly alternative.

Despite psychrophilic temperatures, the starter culture CS2 addition reduced the hydrolysis and acidogenesis time from 24 days to 21 days and affected positively biogas production (with enhancement of 17.5 % of gas production). While ethanol production, the application of CS2 carried out efficiently the hydrolysis of paper waste and enhanced bioethanol yields to 78.2 $\mu L\, g^{-1}$. Specially, P1 procedure was more efficient and allowed an increase of 32.5 % in bioethanol production compared with P2. Thus, the primary function of this starter culture was to ensure the cellulosic biomass hydrolysis. However, other functions may be included (such as stimulation of biogas and bioethanol production).

This application can satisfy the demand to obtain biogas and bioethanol at low cost without the need to prior chemical hydrolysis step of cellulosic biomass, which is considered complex and expensive. However, future studies are required concerning the consortium characterization and the optimization of biogas/bioethanol production.

5. Conflict of interest

The authors report no conflict of interest.

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Producción de biogás y etanol provenientes de desechos de papelería usando un consorcio microbiano aislado de suelo como cultivo iniciador

Resumen: Los pretratamientos convencionales usados durante la valorización de residuos de papel en energías renovables son costosos, largos, lentos, requieren altas temperaturas y, particularmente, no son ecoamigables. Sin embargo, la aplicación de cultivos microbianos con capacidades celulolíticas constituye una estrategia atractiva y de bajo costo. Así pues, el objetivo de este estudio fue seleccionar un cultivo microbiano eficiente y evaluarlo como cultivo iniciador durante el proceso de hidrólisis en la producción de biogás y bioetanol. Nuestros resultados indicaron que, de 18 aislados, dos bacterias (identificadas como *Pseudomonas horyzihabitans* y *Serratia liquefaciens*) y un consorcio (CS2, dominado por *Enterobacteriaceae*) tuvieron una actividad importante de hidrolisis celulósica. La aplicación del consorcio seleccionado como cultivo iniciador durante el proceso de hidrólisis en la producción de biogás y bioetanol incrementó los rendimientos. De hecho, la aplicación de CS2 mejoró los rendimientos de biogás y bioetanol a 9.4 mL g^{-1} y 78.2 µL g^{-1} ($P < 0.05$) respectivamente. Además, la adición del cultivo iniciador CS2 redujo el tiempo necesario para la hidrolisis celulósica a 21 días, en comparación con los 24 días en la muestra control, durante la producción de biogás bajo temperatura psicrofílica. Así, este procedimiento práctico y de bajo costo se puede usar como una estrategia eficiente para liberar azúcares de desechos de papel, reducir el tiempo requerido para la biodigestión celulósica y aumentar la recuperación de biogás y bioetanol.

Palabras Clave: biodigestión anaerobia; biomasa celulósica; energía renovable; desecho de papel.

Produção de biogás e etanol a partir de resíduos de papelaria usando um consórcio microbiano isolado do solo como cultura inicial

Resumo: Os pré-tratamentos convencionais utilizados durante a valorização dos resíduos de papel em energias renováveis são caros, demorados, requerem altas temperaturas e não são amigáveis com o meio ambiente. Por tanto, a aplicação de culturas microbianas com capacidade celulolítica tem se tornado uma estratégia atrativa e de baixo custo. O objetivo deste estudo foi a triagem de uma cultura microbiana eficiente e sua avaliação como cultura inicial durante o processo de hidrólise na produção de biogás e bioetanol. Nossos resultados indicaram que de 18 isolados, duas bactérias (identificadas como *Pseudomonas horyzihabitans* e *Serratia liquefaciens*) e um consórcio (CS2, composto principalmente por *Enterobacteriaceae*) apresentavam uma atividade importante de hidrólise de celulose. A aplicação do consórcio selecionado como cultura inicial melhorou o rendimento do processo de hidrólise na produção de biogás e bioetanol. A aplicação de CS2 aumentou o rendimento de biogás e bioetanol para 9.4 mL g^{-1} e 78.2 µL g^{-1} ($P < 0.05$), respectivamente. Adicionalmente, a adição da cultura inicial CS2 reduziu o tempo necessário para hidrolisar a celulose durante a produção de biogás sob temperaturas psicológicas para 21 dias, comparado com 24 dias na amostra controle. Portanto, este procedimento prático e de baixo custo pode ser usado como uma estratégia eficiente para liberar açúcares dos resíduos de papelaria, para reduzir o tempo necessário para a biodigestão celulósica e para aumentar a quantidade de biogás e bioetanol recuperada.

Palavras-chave: biodigestão anaeróbica; biomassa celulósica; energia renovável; resíduos de papelaria.

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