Effects of pretreatment methods on solubilization of beet-pulp and bio-hydrogen production yield

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ABSTRACT

Sugar processing wastewater and beet-pulp are two major waste streams of sugar-beet processing plants. Contrary to wastewater, beet-pulp is generally used as animal feed in cattle-raising industry. However, it can serve as a substrate for bio-hydrogen production which corresponds to a higher valorization of beet-pulp. Moreover, pretreatment of lignocellulosic materials like beet-pulp is needed in order to improve overall energy efficiency and enable economic feasibility of bio-hydrogen production. Therefore, the effect pretreatment methods (alkaline, thermal, microwave, thermal-alkaline and microwave-alkaline) on bio-hydrogen production from sugar beet-pulp through dark fermentation were investigated in this study. Reactors pretreated with alkaline, microwave-alkaline and thermal-alkaline methods yielded significant solubilization of beet-pulp compared to others. Therefore, in the second phase of the study, they were used to pretreat the beet-pulp which was then subjected to dark fermentation for bio-hydrogen production. Maximum bio-hydrogen production yield of 115.6 mL H_2/g COD was observed in reactor which contained alkaline pretreated beet-pulp.

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1. Introduction

Hydrogen is a clean and environmentally friendly fuel since the combustion of hydrogen produces only water vapor instead of greenhouse gases like CO_2. Furthermore, hydrogen has a high energy yield of 122 kJ/g, which is about 2.75 times greater than that of hydrocarbon fuels [1]. Therefore, hydrogen is considered as a viable alternative fuel and "energy carrier" of future because of its clean, efficient, renewable, and non-polluting characteristics.

Hydrogen can be obtained via biological, thermochemical and electrochemical processes. However, hydrogen production technologies need to utilize renewable sources instead of non-renewable energy sources for sustainability. Thermochemical and electrochemical processes use fossil fuels as source for hydrogen production. Thus, biological hydrogen production processes are considered to be more environment friendly and less energy intensive as compared to thermochemical and electrochemical processes [2,3].

From the perspective of global environmental impacts, such as greenhouse effect and resource recovery, biological hydrogen production from renewable biomass reduces dependence on fossil fuel, decrease carbon dioxide emission and recovers bio-energy. Moreover, in order to establish

a sustainable and cost-effective hydrogen production process, hydrogen should be produced from wastes. Hydrogen production from organic wastes not only enables a partial stabilization of waste, but also it is an attractive process because organic wastes are free or low cost carbon sources [4]. However, the yield and rate of bio-hydrogen production are still low at present. Yield improvement is necessary for improving the economic feasibility of bio-hydrogen production [5].

Numerous studies have been conducted to produce hydrogen by using organic wastes as substrate such as crude cheese whey [6], wastewater sludge [7], rice winery wastewater [8], food waste [9], molasses [10], sugar factory wastes [11,12], bean curd manufacturing waste [13,14], cattle wastewater [15], cassava starch manufacturing wastewater [16], sweet potato starch residue [17,18], domestic wastewater [19], potato industry wastewater [19], organic fraction of municipal solid waste [20]. According to these studies, hydrogen production from organic wastes was feasible. However, in order to improve overall energy efficiency and enable economic feasibility of bio-hydrogen production from wastes, additional treatment processes need to be done [5].

Several pretreatment methods have been investigated for conversion of lignocellulosic wastes into ethanol or methane [21]. On the other hand, since it is still in the research and development phase [22]; a limited number of studies have been conducted on pretreatment of lignocellulosic wastes for hydrogen production [23–27]. Therefore, the effect of pretreatment methods on bio-hydrogen production from beet-pulp, a sugar-beet processing waste, through dark fermentation was investigated in this study.

2. Materials and methods

2.1. Waste characteristics

Pressed beet-pulp used in the experiments was obtained from Amasya Seker Fabrikasi, a private beet-sugar factory located near Amasya, Turkey. Characterization of the beet-pulp was carried out and the results were tabulated (Table 1). After the characterization, beet-pulp was kept frozen at $-20\,^{\circ}C$ in order to inhibit biological activity prior to the use in the experimental studies.

In order to achieve physical homogeneity, first the frozen beet-pulp was thawed at room temperature and further dried at $105\,^{\circ}C$ for 24 h. Then, the dried pulp particles were grinded by the help of a pestle and the homogenized powdered pulp was used for reactor feeding.

Table 1 – Pressed beet-pulp characteristics.

Parameter	Value
Moisture (%)	85 ± 0.1
TS (%)	15 ± 0.1
VS (%TS)	94 ± 0.01
COD (g/g dry weight)	1.22 ± 0.15
TKN (%TS)	7.28
P_{Total} (%TS)	1.0 ± 0.28

2.2. Inoculum

The mixed anaerobic cultures used as the seed were obtained from the anaerobic sludge digesters at the Ankara Wastewater Treatment Plant. Before being used as inoculum, the mixed anaerobic culture was concentrated by settling 24 h period of time. Then, concentrated sludge was filtered through a screen with a pore size of 1 mm before use. The volatile suspended solids concentration of the concentrated seed cultures was 18730 ± 189 mg/L.

2.3. Basal medium

Basal Medium (BM) contains all the necessary micro- and macro-nutrients for an optimum anaerobic microbial growth. So in order to supply adequate nutrients for an optimum microbial growth, reactors were fed by basal medium (BM). In this study, the medium composition was as follows (concentrations are given in parentheses as mg/L): NH_4Cl (1200), $MgSO_4\cdot7H_2O$ (400), KCl (400), $Na_2S\cdot9H_2O$ (300), $CaCl_2\cdot2H_2O$ (50), $(NH_4)_2\cdot HPO_4$ (80), $FeCl_2\cdot4H_2O$ (40), $CoCl_2\cdot6H_2O$ (10), KI (10), $MnCl_2\cdot4H_2O$ (0.5), $CuCl_2\cdot2H_2O$ (0.5), $ZnCl_2$ (0.5), $AlCl_3\cdot6H_2O$ (0.5), $NaMoO_4\cdot2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2\cdot6H_2O$ (0.5), $NaWO_4\cdot2H_2O$ (0.5), Cysteine (10) [28].

2.4. Analytical methods

Daily biogas production in each bottle was periodically measured using a 10 mL gas-tight syringe (Sanitex, Italy). Gas compositions were periodically analyzed using a gas chromatograph (Thermo Electron Co., Thailand) equipped with a thermal conductivity detector (TCD) using helium as a carrier gas at a constant pressure of 100 kPa. The inlet and detector temperatures were set to $50\,^{\circ}C$ and $80\,^{\circ}C$, respectively. Pure gases were used as calibration standards. Hydrogen (H_2), carbon dioxide (CO_2), oxygen (O_2), methane (CH_4) and nitrogen (N_2) gases were detected by the help of two columns in series (CP-Moliseve 5A and CP-Porabond Q) at a fixed oven temperature of $45\,^{\circ}C$.

For volatile fatty acid (VFA) measurements, samples were filtered by 0.22 μm pore sized filters. Then, the filtered samples were acidified with 98% formic acid to a pH less than 2.5, in order to convert the fatty acids to their undissociated forms (i.e. acid forms). Acidified samples were measured in a gas chromatograph (Thermo Electron Co., Thailand) for their VFA contents (acetic, propionic, butyric, iso-butyric, valeric, iso-valeric, caproic, iso-caproic and heptanoic acids) with a Nukol column (Model 25326, 15 m × 0.53 mm). The flame ionization detector (FID) was operated at $280\,^{\circ}C$. Helium was used as a carrier gas with a constant flow rate of 6 mL/min and the inlet temperature was kept at $250\,^{\circ}C$. The oven temperature was initially set to $100\,^{\circ}C$ with 2 min holding time and then increased to $200\,^{\circ}C$ with $8\,^{\circ}C$/min ramping. The concentrations of separate VFAs were expressed in terms of acetic acid equivalents by dividing the concentration value by its molecular weight and multiplying with the molecular weight of acetic acid. Total volatile fatty acid (tVFA) was determined by the sum of concentrations of all VFA species expressed as acetic acid equivalents.

Solids, total chemical oxygen demand (tCOD) for beet-pulp, total kjeldahl nitrogen (TKN), P_{Total} and alkalinity measurements were done in accordance with Standard Methods for the examination of water and wastewater [29]. pH measurements were conducted with a pH meter (HI 8314, Hanna Instruments, Italy) and a pH probe (HI 1230, Hanna Instruments, Italy). Total and soluble chemical oxygen demand (tCOD and sCOD, respectively) were performed for wastewater with the help of a spectrophotometer (SN 05827, PC Multidirect, Germany) and vials specifically prepared for the COD range of 0–1500 mg/L sCOD was measured after filtering by 0.45 μm pore sized filters. ORP values of the reactors were measured with a pH meter (PH 510, Eutech) equipped with an ORP electrode (Recorder S-500C, Sensorex, Italy).

2.5.   Experimental set-up and procedures

This study consisted of two parts: Effects of pretreatment methods on solubilization of beet-pulp and effects of pretreatment methods on bio-hydrogen production yields. In the first part, the effects of five different pretreatment method (alkaline pretreatment, thermal pretreatment, microwave pretreatment, thermal-alkaline pretreatment, microwave-alkaline pretreatment) on solubilization of beet-pulp were investigated. In the second part, three out of five pretreatment methods (Alkaline, microwave-alkaline, thermal-alkaline pretreatment) were used to compare effects of pretreatments on bio-hydrogen productivity.

2.5.1.   Effects of pretreatment methods on solubilization of beet-pulp
Beet-pulp was pretreated by five different pretreatment methods (alkaline, thermal, microwave, thermal-alkaline and microwave-alkaline). Control reactor was also run to determine initial sCOD and VFA of beet-pulp. Reactors were run as duplicates and the mean values of duplicate reactors were used in reporting. Each reactor contained 36 g/L COD of beet-pulp. After pretreatments, sCOD and VFAs of the reactors were measured.

For alkaline pretreatments, the pH was set at 12 by the addition of 2M NaOH then mixed with magnetic stirrer for 30 min [30]. Alkaline pretreatment duration was extended to 30 min in order to achieve a more homogenous distribution of alkaline agent and application of NaOH was continuously added for 30 min to keep the pH at the desired value. Thermal pretreatments were done with autoclave at 121 °C, 1.5 atm for 30 min. Similarly, Wang et al. [7] pretreated wastewater sludge at 121 °C for 30 min and Kim et al. [30] pretreated waste activated sludge at 121 °C and 1.5 atm pressure for 30 min to increase soluble COD. Microwave pretreatments were achieved with Berghof, MWS-2 Microwave System, having a maximum temperature of 220 °C, maximum power of 1000 W and maximum pressure of 40 bar and the frequency of 2450 MHz. For microwave pretreatment 700 W; 170 °C; 30 min were chosen. For thermal-alkaline pretreatments, alkaline pretreated beet-pulp (at pH 12 for 30 min) was exposed to thermal pretreatment (autoclave at 121 °C, 1.5 atm for 30 min). In addition, for microwave-alkaline pretreatments, alkaline pretreated beet-pulp (at pH 12 for 30 min) was exposed to microwave pretreatment (700 W; 170 °C; 30 min).

2.5.2.   Effects of pretreatment methods on bio-hydrogen production yields
Before used in bio-hydrogen production, beet-pulp was pretreated with alkaline, microwave-alkaline and thermal-alkaline methods. Since their performance in increasing the initial sCOD were superior compared to thermal and microwave methods.

250 mL glass reactors with effective volume of 180 mL were used in bio-hydrogen production experiment. Four different types of reactors (S1, S2, S3 and S4) were set-up and run as duplicates. The mean values and standard deviations of duplicate reactors were used in reporting. All reactors contained 20 g/L COD of beet-pulp. The beet-pulp added to Reactors S1, S2, S3, S4 was subjected to no, thermal-alkaline, alkaline and microwave-alkaline pretreatment, respectively. All reactors were inoculated with mixed anaerobic sludge. The VSS concentration in the reactors was adjusted to 1800 mg/L. BM was added into the reactors in order to supply adequate nutrients for an optimum microbial growth. Initial pHs of the reactors were adjusted to 6 [31,32] by 2M NaOH and 2M HCl solutions.

Reactors were flushed with nitrogen gas for 3 min at the start of cultivation in order to maintain anaerobic conditions and then sealed with natural rubber stoppers and plastic screw-caps. Prepared reactors were placed in a mechanical shaker and stirred at 175 rpm in a constant temperature room (35 °C ± 2). To avoid sunlight, reactors were covered with aluminum foil.

For all of the reactors, gas productions and compositions were measured daily during digestion period. In addition, initial and final pH, VFA, tCOD, sCOD measurements were carried out.

3.   Results and discussion

3.1.   Effects of pretreatment methods on solubilization of beet-pulp

3.1.1.   Soluble COD concentrations
sCOD values increased from 4266 ± 125 mg/L (control) to 4920 ± 81, 5990 ± 471, 6739 ± 587, 19129 ± 660 and 20884 ± 818 mg/L at microwave, thermal, alkaline, microwave-alkaline, thermal-alkaline pretreatment reactors, respectively. In terms of the solubilization ratio (or percentages of soluble to total COD), the ratio increased from 11.8% (control) to 13.7% in microwave, 16.6% in thermal, 18.7% in alkaline, 53.1% in microwave-alkaline, 58.0% in thermal-alkaline pretreatment reactors (Fig. 1). Thus, the highest sCOD concentration (20884 mg/L) and solubilization ratio (58.0%) were observed in thermal-alkaline pretreatment reactor (Fig. 1).

Kim et al. reported that initial soluble COD (8.1%) increased to 51.8% after thermal-alkaline pretreatment (at 121 °C for 30 min, pH 12) [30]. Similarly, the initial sCOD (11.8%) was increased to 58.0% after thermal-alkaline pretreatment (at 121 °C for 30 min, pH 12). Therefore, the result of combined effect of thermal and alkaline pretreatments was in line with the result of Kim et al. [30] in terms of the amount of soluble COD release (solubilization ratio).
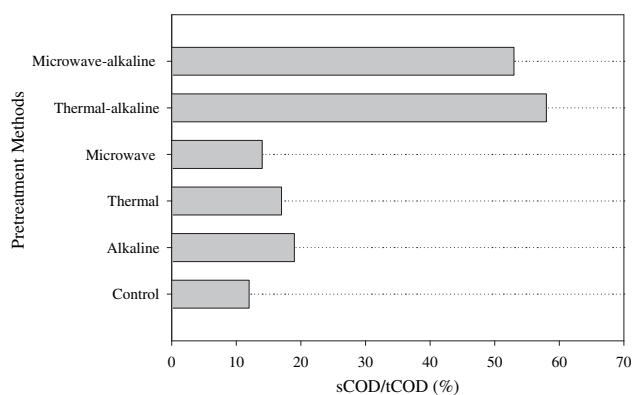
Fig. 1 – sCOD/tCOD ratios of pretreatment reactors.

Synergistic effect was observed in microwave-alkaline and thermal-alkaline pretreatment reactors in terms of sCOD values. Thus, it was observed that the percent sCOD release values of combined thermal and alkaline pretreatment were well above the releases achieved by each individual method. Similarly, the reactor at which microwave pretreatment combined with the alkaline pretreatment had higher solubilization ratio (53.1%) than individual microwave (13.7%) or alkaline (18.7%) pretreatment. The results of this study were in agreement with findings such as Kim et al. [30] who reported that COD solubilization efficiencies of pretreatment methods as follow; thermal and alkaline pretreatment > alkaline pretreatment > thermal pretreatment.

3.1.2. Volatile fatty acid productions

In all pretreatment reactors, tVFA concentrations (199–1153 mg/L) was higher than that of control reactor (190 mg/L). tVFA values increased from 190 mg/L (control) to 199 mg/L, 248 mg/L, 951 mg/L, 981 mg/L and 1153 mg/L at microwave, thermal, alkaline, microwave-alkaline, thermal-alkaline pretreatment reactors, respectively (Fig. 2). In addition, the results revealed that the major acidification products were H–Ac, H–Pr and H–Bu in all of the reactors. Moreover, there is a direct relationship between tVFAs and sCOD concentrations (Fig. 2). This indicated that the organic
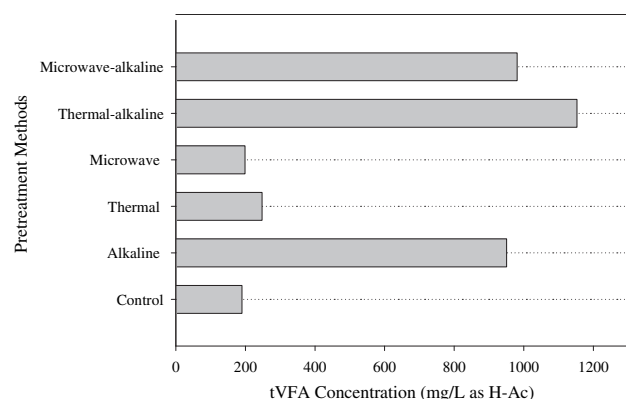
particles in beet-pulp were liquidized to soluble carbohydrates and acids after pretreatment.

3.2. Effects of pretreatment methods on bio-hydrogen production yields

3.2.1. Solubilization ratios

The initial solubilization ratio was calculated as the percentages of initial soluble CODs of reactors before incubation for bio-hydrogen production to total COD. The final solubilization ratio was calculated as the percentages of final soluble CODs of reactors after incubation for bio-hydrogen production to total COD.

The highest initial solubilization ratios were observed in reactors which contained thermal-alkaline pretreated beet-pulp (S2: 43.6%) and in reactor which contained microwave-alkaline pretreated beet-pulp (S4: 36.9%). Lower solubilization ratio (12.6%) was achieved with alkaline pretreatment (S3) when compared to other pretreatments. Initial solubilization ratios were consistent with the results which were observed in the first part of the study. In reactors which contained microwave-alkaline and thermal-alkaline pretreated beet-pulp (S2 and S4), a synergistic effect was observed in terms of sCOD/tCOD values. Solubilization ratios of these reactors are much higher than reactor which with alkaline pretreated beet-pulp. Initial sCOD/tCOD values determined (12.6–43.6%) were comparable with the values reported in the literature; 51.8% after thermal-alkaline pretreatment [30], 26.2% after alkaline-gamma ray irradiation [30], 40.7% after thermal-alkaline pretreatment [33].

During fermentation, the ratio of sCOD/tCOD increased further. Final solubilization ratios were 19.5% in reactor which contained beet-pulp without pretreatment (S1), 47.7% in reactor which contained thermal-alkaline pretreated beet-pulp (S2), 27.0% in reactor which contained alkaline pretreated beet-pulp (S3), 40.1% in reactor which contained microwave-alkaline pretreated beet-pulp (S4). These results revealed that not only pretreatment but also fermentation was effective for solubilizing organic matters from beet-pulp.

3.2.2. Soluble and consumed COD concentrations

Initial sCOD values increased from 1711 ± 37 mg/L (S1) to 8721 ± 115 mg/L, 2528 ± 45 mg/L, and 7382 ± 86 mg/L in S2, S3 and S4, respectively. Thus, COD solubilization efficiencies of pretreatment methods are as follows: thermal-alkaline pretreatment > microwave-alkaline pretreatment > alkaline pretreatment. COD solubilization efficiencies of pretreatment methods depicted that the pretreatment could destroy cellular wall, and release dissoluble matters efficiently. Maximum initial sCOD was observed in S2 (8721 ± 115 mg/L) which contained thermal-alkaline pretreated beet-pulp. Initial soluble COD in S1 (8.5%) increased to 43.6% after thermal-alkaline pretreatment of beet-pulp. The result of thermal-alkaline pretreatment was in line with the result of Kim et al. who stated that initial soluble COD (8.1%) increased to 51.8% after thermal-alkaline pretreatment [30].

Final sCOD concentrations were 3900 ± 141, 9546 ± 398, 5400 ± 28 and 8020 ± 255 mg/L in S1, S2, S3 and S4, respectively. Thus, final sCOD concentrations of reactors were

Fig. 2 – tVFA concentrations in pretreatment reactors.

higher than the initial sCOD concentrations. sCOD concentrations increased further during fermentation due to production of organic acids (VFA). These acids are assumed to catalyze the further hydrolysis of the hemicellulose [34]. These results revealed that both pretreatment and fermentation were effective for solubilizing organic matters from beet-pulp.

Highest sCODs were observed in reactors which contained thermal-alkaline and microwave-alkaline pretreated beet-pulp (S2 and S4). However, the maximum bio-hydrogen production yield (115.6 mL H_2/g COD) was observed in S3. Hence, it can be stated that not all organic matter released from beet-pulp is readily anaerobically fermented to bio-hydrogen. Moreover, thermal-alkaline and microwave-alkaline pretreatment includes risk on solubilization of phenolic compounds besides the solubilization of hemicelluloses [21]. Thus, toxic effects of these compounds might reduce bio-hydrogen production yields.

Consumed COD (cCOD) concentrations of the reactors were depicted in Fig. 3. As can be seen from Fig. 3. in reactors which contained pretreated beet-pulp (S2, S3 and S4) higher COD consumptions were observed when compared to reactor which contained beet-pulp without pretreatment (S1). cCOD concentration of S1 (5900 mg/L) increased to 7700 mg/L in S2, 6500 mg/L in S3, 9250 mg/L in S4. In terms of the COD removal efficiency (percentages of consumed to total COD), the efficiency increased from 29.5% in S1 to 32.5% in reactor which contained alkaline pretreated beet-pulp, 38.5% in reactor which contained thermal-alkaline pretreated beet-pulp, 46.3% in reactor which contained microwave-alkaline pretreated beet-pulp. In other words, the COD removal efficiency of reactors varied between 29.5 and 46.3%.

3.2.3.   pH values

The final pH ranged from 4.49 to 4.60 in all reactors. It indicated that, H decreased at all reactors due to fermentation. These low final pHs values indicated successful acidification in the reactors. The final pH values were consistent with Zhang et al. who reported that the final pH in the batch tests were about 4.6 [35] and Ren et al. who observed that the final pHs in reactors were between 4.5 and 5 [36]. Lowest final pH value (4.49) was observed in reactor which contained alkaline

pretreated pulp (S3) due to effective acidification. It can be supported by the highest bio-hydrogen yield (115.6 mg/L COD).

3.2.4.   Volatile fatty acid productions

Initial tVFAs for S1-S4 were 116 ± 23, 446 ± 18, 360 ± 13, 375 ± 19 mg/L, respectively. Initial VFAs of the reactors which contained pretreated beet-pulp (S2, S3 and S4) were higher than that of reactor which include beet-pulp without pretreatment (S1). In addition, the initial VFA concentrations in the reactors increased parallel with the increased initial sCOD concentrations as organic particles in beet-pulp were liquidized to soluble carbohydrates, acids with pretreatment.

The results indicated that, the soluble metabolites of initial VFAs included mainly H−Ac, H−Pr and H−Bu in all of the reactors (S1, S2, S3 and S4). H−Ac was the most abundant product, which accounted for 69.8–87.8% of tVFA for all of the reactors.

Furthermore, the production of H−Bu and H−Pr was also significant, which accounted for 1.4–5.2% and 6.4–12.9% of tVFA, respectively. H−Ac percentage in S1 increased from 69.8% to 84.9% in reactors which contained thermal-alkaline (S2), 87.8% in reactor which contained alkaline pretreated beet-pulp (S3) and 84.8% in reactors which contained microwave-alkaline pretreated beet-pulp (S4). On the other hand, H−Pr percentage in S1 decreased from 12.9 to 9.2% in reactor which contained thermal-alkaline beet-pulp (S2), 6.4% in reactor which contained alkaline pretreated beet-pulp (S3) and 8.3% in reactor which contained microwave-alkaline pretreated beet-pulp (S4). Similarly, H-Bu percentage in S1 decreased from 5.2 to 2.0% in reactors which contained thermal-alkaline (S2), 1.4% in reactor which contained alkaline pretreated beet-pulp (S3) and 1.9% in reactors which contained microwave-alkaline beet-pulp (S4). Wang and Wan stated that H−Bu and H−Pr might repress the process of fermentative bio-hydrogen production [37]. In addition, Wang et al. stated that the rate of converting propionic acid to hydrogen is very slow, resulting in the accumulation of propionic acid that can repress the activity of hydrogen-producing bacteria and decrease the hydrogen production [38]. Thus, the decrease in H−Pr and H−Bu percentages at all reactors which contained pretreated beet-pulp might lead to higher bio-hydrogen productions (Fig. 4).
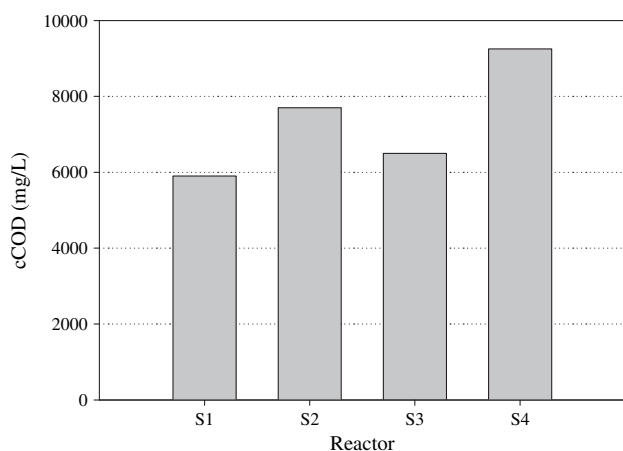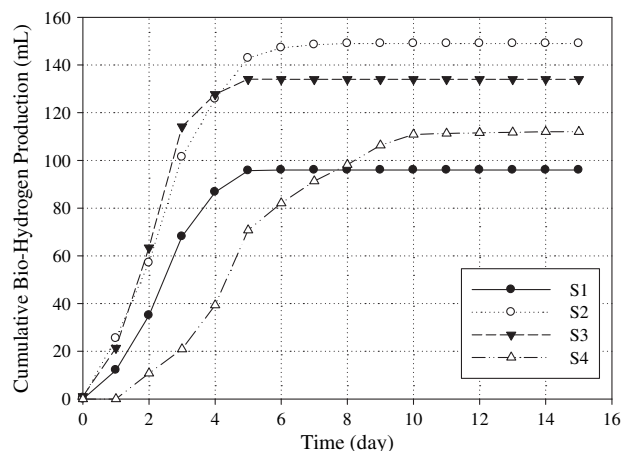

Fig. 3 − cCOD concentrations of the reactors.

Fig. 4 − Cumulative bio-hydrogen productions of reactors.

Final tVFAs for S1-S4 were 1393 ± 272, 2455 ± 465, 2721 ± 176 and 2398 ± 267 mg/L, respectively. Bio-hydrogen production was accompanied with the formation of VFAs throughout the fermentation of beet-pulp. These phenomena were expected because hydrogen production appears to be usually accompanied with the formation of VFAs is the main by-products in the metabolism of bio-hydrogen fermentation. Final VFAs of the reactors which contained pretreated beet-pulp (S2, S3 and S4) were higher than that of reactor which include beet-pulp without pretreatment (S1). Highest tVFA concentration (2721 mg/L) was observed in S3 which contained alkaline pretreated beet-pulp. In addition, lowest final pH value (4.49) was also observed in S3 as higher tVFA productions lead to lower pH values.

In all of the reactors, main acidification products were H–Ac (65.3–75.2% w/w of tVFA), H–Pr (4.9–11.6% w/w of tVFA) and H–Bu (9.8–19.9% w/w of tVFA) comprising 91.1–96.6% of tVFAs. The higher molecular weight VFAs (valeric, caproic etc.) were produced with insignificant amounts. Ethanol production (219.6 mg/L) was observed only in reactor which contained microwave-alkaline pretreated beet-pulp (S4). Zhang et al. stated that high bio-hydrogen yields are associated with a mixture of acetate and butyrate as fermentation products, and low bio-hydrogen yields are associated with reduced resulting products (such as alcohols) [35]. H–Ac and H–Bu accounted for 85.0–87.0% of (w/w) tVFA. Thus, the dominance of H–Ac, H–Bu can also be associated with high bio-hydrogen production yields (66.7–115.6 mL/g COD). The results of VFA production part were in line with the results of Fan et al. reported that in bio-hydrogen fermentation from glucose, VFAs mainly consists of acetate and butyrate [39] and Fan et al. who stated that acetate and butyrate accounted for about 76–80% of VFAs in bio-hydrogen fermentation from wheat straw wastes [24].

3.2.5. Bio-hydrogen and methane productions

The operation of reactors was ceased when bio-hydrogen production stopped. Fig. 4 depicts the effects of the different pretreatment methods on bio-hydrogen productions at the fixed initial pH of 6.0 and substrate concentration of 20 g/L COD. As can be seen from Fig. 4, cumulative bio-hydrogen productions in all pretreatment reactors (111.7–148.5 mL) were higher than that of reactor which contained beet-pulp without pretreatment (95.7 mL). Maximum bio-hydrogen production of 148.5 mL was observed in the reactor which contained the thermal-alkaline pretreated beet-pulp (S2). Reactors which contained alkaline pretreated beet-pulp (S3) and microwave-alkaline pretreated beet-pulp (S4) achieved cumulative bio-hydrogen production of 134.0 and 111.7 mL, respectively. Maximum bio-hydrogen production achieved (148.5 mL or 50.3 mL/g beet-pulp) was comparable with that of Li and Chen et al. [23]. In that study, hydrogen produced from thermal pretreated corn straw was 68 ml H_2/g corn straw.

The longest lag time (2 days) was observed in reactor which contained microwave-alkaline pretreated beet-pulp (S4) when compared the others (1 day). In addition, the longest bio-hydrogen production was also observed in S4 (Fig. 4). Bio-hydrogen production in S4 ceased after 12 days of incubation while in reactors which contained beet-pulp without pretreatment (S1), alkaline pretreated beet-pulp (S3), thermal-

alkaline pretreated beet-pulp (S4) ceased after 5–7 days of incubation. However, less bio-hydrogen production was observed in S4 which contained microwave-alkaline pretreated beet-pulp than other reactors which contained pretreated beet-pulps (S2 and S3).

Gossett et al. stated that pretreatment with temperatures of 160 °C and higher, causes, besides the solubilization of hemicellulose, also the solubilization of lignin [40]. The produced compounds are almost always phenolic compounds and have in many cases an inhibitory or toxic effect on bacteria, yeast and methanogens/archae. Microwave-alkaline pretreatment of beet-pulp was conducted at 170 °C for 30 min. Thus, this might be the one of the reason that less bio-hydrogen production in S4 when compared to S2 and S3. Not only microwave-alkaline pretreatment, but also thermal-alkaline pretreatment includes a risk on production of phenolic compounds. However, bacteria are capable of adapting to these compounds at least certain concentrations [21]. Microwave-alkaline pretreatment was conducted at severe pretreatment conditions (at 170 °C) when compared to thermal-alkaline pretreatment (at 121 °C). Moreover, microwave-assisted extraction has been developed for the extraction of a variety of toxic organic contaminants, such as phenols and pesticides from soils, sediments and other solid matrices [41]. Nakazato et al. also indicated that heavy metals in soil like Cd, Cr, Cu, Ni, Pb, and Zn could be extracted by microwave [42]. These toxic matters were deleterious for bio-hydrogen production bacteria. So, when the organic nutrients released, the toxic matters could be released simultaneously and they would restrain the bio-hydrogen production bacteria and this might be the other reason of less bio-hydrogen production observed in S4.

Cumulative total gas productions in all pretreatment reactors (S2, S3 and S4) were higher (258.2–289.0 mL) when compared reactor which contained beet-pulp without pretreatment (199.8 mL). The increase in cumulative total gas production was due to the transfer of organic matter from the particulate fraction to the soluble fraction. Highest total gas production was calculated as 289.0 mL in S2 which incubated with thermal-alkaline beet-pulp. This represents an increase of 44.6% in total gas compared with reactor which contained beet-pulp without pretreatment. Total gas composition consisted of bio-hydrogen, carbon dioxide and no or insignificant amounts of methane (0–3.5%). The bio-hydrogen and carbon dioxide percentage in the total gas was 43.4–51.6% and 48.4–56.6%, respectively. The bio-hydrogen and methane percentages were consistent with Zhang et al. who produced hydrogen from acid pretreated cornstalks and reported that the hydrogen percent in the total gas was 45–56% and there was no significant methane observed in the batch tests [26].

3.2.6. Bio-hydrogen production yields

Bio-hydrogen production yields for S1–S4 were determined as 90.1 ± 6.1, 108.2 ± 15.8, 115.6 ± 12.5 and 66.7 ± 10.1 mL/g COD, respectively. The results indicated that thermal-alkaline and alkaline pretreatment of the substrate improves the conversion of beet-pulp into bio-hydrogen by microorganisms. Bio-hydrogen production yields in thermal-alkaline and alkaline pretreatment reactors (S2 and S3) were higher than that of reactor which contained beet-pulp without pretreatment. Bio-hydrogen production yield of S1 (contained beet-pulp without

pretreatment) increased from 90.1 mL/g COD to 108.2 mL/g COD after thermal-alkaline pretreated beet-pulp and 115.6 mL/g COD after alkaline pretreatmented beet-pulp. On the other hand, microwave-alkaline pretreatment of beet-pulp (in S4) reduced bio-hydrogen production yield to 66.7 mL/g COD from 90.1 mL/g COD because of inhibitory or toxic effect of phenolic compounds or heavy metals. In S2 which contained thermal-alkaline pretreated beet-pulp higher bio-hydrogen production (148.5 mL) was observed than S3 (134.0 mL) which contained alkaline pretreated beet-pulp. However, the maximum bio-hydrogen production yield (115.6 mL H_2/g COD) was observed in S3 because of the difference in COD consumptions (Fig. 3).

Maximum bio-hydrogen production yield from beet-pulp was determined as 115.6 mL/g COD in this study. The energy yield of hydrogen is 122.0 kJ/g [5]. Thus, 1.3 kJ energy per gram of COD can be generated from hydrogen gas under standard temperature and pressure (1 atm and 0 °C).

4. Conclusions

This study investigated the effect of pretreatment methods on bio-hydrogen production from beet-pulp, a sugar-beet processing waste, through dark fermentation. From the results obtained, the following conclusions can be drawn:

- sCOD and tVFA concentrations increased in all pretreated reactors when compared to control reactor. Thermal-alkaline, microwave-alkaline and alkaline pretreatment methods increased initial sCOD and tVFA of beet-pulp significantly when compared to thermal and microwave pretreatments.
- COD solubilization efficiencies of pretreatment methods are as follows: thermal-alkaline > microwave-alkaline > alkaline. During fermentation, the ratio of sCOD/tCOD increased further. This revealed that not only pretreatment but also fermentation was effective for solubilizing organic matters from beet-pulp.
- The maximum bio-hydrogen production yield (115.6 mL H_2/g COD) was observed in reactor with alkaline pretreatment.

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