Dark Fermentative Bio-hydrogen Production from sugar-beet processing wastes

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Summary
The bio-hydrogen generation potential of sugar-beet processing wastes (sugar-beet processing wastewater and beet-pulp) through dark fermentation was investigated in this study. To this purpose, the bio-hydrogen production from only beet-pulp and co-digestion of beet-pulp and sugar-beet processing wastewater at high COD values were tested. The results of this study revealed that the reactor fed by 20 g/L COD beet-pulp provided the highest bio-hydrogen production yield (95.6 mL H₂ /g COD) and bio-hydrogen production efficiency of beet-pulp is higher than co-digestion of beet-pulp and sugar-beet processing wastewater.

Keywords: biohydrogen, anaerobic digestion, bioprocessing, bioreactors.

Introduction
The worldwide energy need has been increasing while the reserves of fossil fuels have been decreasing. Moreover, the utilization of fossil fuels result in environmental pollution problems due to the emission of pollutants like COₓ, NOₓ, SOₓ, CₓHₓ, soot, ash, etc. In response, researchers investigate sustainable energy production options from renewable sources. Hydrogen is considered as a viable alternative fuel and “energy carrier” of future because of its clean, efficient, renewable, and non-polluting characteristics.

Some of the conventional hydrogen gas production methods are steam reforming of natural gas, gasification of coal and electrolysis of water (Nath and Das, 2004). These methods use non-renewable energy sources to produce hydrogen. However, bio-hydrogen production from renewable biomass by bacteria assumes paramount importance as an alternative energy resource because it has significant potential to meet the increasing energy needs in the future (Chowdhury et al., 2007).

Bio-hydrogen production can be realized by anaerobic (dark fermentation) and photosynthetic microorganisms (photofermentation) using carbohydrate rich and non-toxic feedstocks including wastes. However, the latter method is hard to be applied due to the low light utilization efficiency and difficulties in designing light reactors (Liu et al., 2008). The former possesses the ability to generate hydrogen without photoenergy. Moreover, bio-hydrogen production by dark fermentation, compared to alternatives such as bio-photolysis of water or photofermentation, is advantageous because of its ability to produce H₂ at higher rates (Magnusson et al., 2008). Dark fermentation has the flexibility to convert a wide range of reduced carbon sources, which are found in many industrial effluents and agriculture residues, into bio-hydrogen (Ray et al., 2008). This is advantageous because not only a partial waste stabilization is achieved, but also valuable metabolites such as acetic, butyric and lactic acids are produced (Kim et al., 2006) from cheap and even free carbon sources which makes it more attractive from the economical point of view. So, dark fermentation is a viable alternative to the aforementioned methods for hydrogen gas production.

Sugar production has considerable potential in Turkey which is one of the major sugar producing countries in the world (Zuhal and Kemal, 2004). Sugar-beet processing industry consumes large amount of energy and produces considerable amount of wastes. Therefore, several measures have been investigated to reduce energy consumption and enable waste minimization (Krajnc et
Sugar-beet processing wastes can be considered a suitable source of renewable energy and bioproducts via different biological processes.

Earlier studies indicated that mainly pure carbohydrate substrates (such as glucose, sucrose) have been widely used for H₂ production (Chowdhury et al., 2007; Ray et al., 2008). On the other hand, bio-hydrogen production from organic wastes not only provide a partial waste stabilization but also it is an economically feasible process because organic wastes are free or very cheap carbon sources. Therefore, bio-hydrogen production from low cost substrates is a very promising hydrogen production method to meet the current renewable energy demand (Chowdhury et al., 2007; Liu et al., 2008; Ozkan et al., 2010). Sugar-beet processing wastes have significant potential as a renewable source of fuel (Zuhal and Kemal, 2004; Farhadian et al., 2007). Bio-hydrogen production from sugar-beet processing wastes by dark fermentation can not only enable waste minimization but also contribute to sustainability via generation of a bio-based product, namely bio-hydrogen. There are some studies on bio-hydrogen production from sugar mill wastewater, sugar-beet wastewater, and molasses in the literature (Logan et al., 2002; Wu and Lin, 2004; Hussy et al., 2005; Vatsala et al., 2008; Ozkan et al., 2010). However, there is not any study investigating bio-hydrogen generation potential of both sugar-beet processing wastewater and beet-pulp.

Therefore, the main objective of this study was to investigate bio-hydrogen generation potential of sugar-beet processing wastes (sugar-beet processing wastewater and beet-pulp) through dark fermentation. The bio-hydrogen production potentials of only beet-pulp and co-digestion of beet-pulp and sugar-beet processing wastewater at high Chemical Oxygen Demand (COD) values (20, 25, 30 g/L COD) were investigated.

**Materials and Methods**

**Waste Characteristics**

Sugar-beet processing wastewater used in the experiments was obtained from Ankara Beet-Sugar Factory. Characterization of the sugar-beet processing wastewater was carried out and the results are provided in Table 1. After the characterization, wastewater was kept frozen at –20 °C in order to inhibit biological activity prior to the use in the experiments. Prior to the characterization and the use in the study, sugar-beet processing wastewaters were settled for 2-hour to remove the suspended materials which are very common for sugar-beet processing wastewater. The time period of 2-hour was chosen to represent the typical hydraulic retention time of primary sedimentation before the secondary treatment systems (Metcalf and Eddy, 2003).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tCOD</td>
<td>3418 ± 94.2</td>
</tr>
<tr>
<td>sCOD</td>
<td>3168 ± 237.3</td>
</tr>
<tr>
<td>TS</td>
<td>4516 ± 25.1</td>
</tr>
<tr>
<td>VS</td>
<td>2110 ± 12.2</td>
</tr>
<tr>
<td>TSS</td>
<td>483 ± 14</td>
</tr>
<tr>
<td>VSS</td>
<td>160 ± 5.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.08</td>
</tr>
<tr>
<td>Alkalinity (as CaCO₃)</td>
<td>1120</td>
</tr>
<tr>
<td>TKN</td>
<td>10</td>
</tr>
<tr>
<td>P_Total</td>
<td>3.4</td>
</tr>
<tr>
<td>tVFA (as H-Ac)</td>
<td>578 ± 6</td>
</tr>
<tr>
<td>H-Ac</td>
<td>329 ± 5</td>
</tr>
<tr>
<td>H-Pr</td>
<td>149 ± 4</td>
</tr>
<tr>
<td>H-Bu</td>
<td>28 ± 1</td>
</tr>
</tbody>
</table>
Pressed beet-pulp used was obtained from a private beet-sugar factory located near Amasya. Characterization of the beet-pulp was carried out and the results are tabulated (Ozkan et al., 2011). After the characterization, beet-pulp was kept frozen at –20 °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 °C for 24 hours. Then, the dried pulp was grinded and the homogenized powdered pulp was used for reactor feeding.

Experimental Set-up and Procedures

Six different reactors (Table 2) were used. Total and effective volume of the reactors were 250 mL and 180 mL, respectively. R3, R5, R6 were run as duplicates. The mean values and standart deviations of duplicate reactors were used in all tables. All reactors were inoculated with mixed anaerobic sludge, establishing a VSS concentration of 1800 mg/L. Basal Medium (BM) was added into the reactors to supply adequate macro- and micro-nutrients and initial pH values of the reactors were adjusted to 6 (Mohan et al., 2007; Lee et al., 2008; Pakarinen et al., 2008) by 2M NaOH and 2M HCl solutions.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Including</th>
</tr>
</thead>
</table>
| R1      | - 20 g/L COD of sugar-beet processing wastewater and beet-pulp  
- Mixed anaerobic culture  
- Basal Medium |
| R2      | - 25 g/L COD of sugar-beet processing wastewater and beet-pulp  
- Mixed anaerobic culture  
- Basal Medium |
| R3      | - 30 g/L COD of sugar-beet processing wastewater and beet-pulp  
- Mixed anaerobic culture  
- Basal Medium |
| R4      | - 20 g/L COD of beet-pulp  
- Mixed anaerobic culture  
- Basal Medium |
| R5      | - 25 g/L COD of beet-pulp  
- Mixed anaerobic culture  
- Basal Medium |
| R6      | - 30 g/L COD of beet-pulp  
- Mixed anaerobic culture  
- Basal Medium |

Reactors were purged with nitrogen gas for 3 min at the start of cultivation in order to maintain anaerobic conditions and then capped tightly with natural rubber stoppers and plastic screw-caps. Then, the reactors were incubated in a mechanical shaker at 175 rpm in a constant temperature room (35±2 °C). To avoid light, reactors were covered with aluminum foil. Gas productions and compositions of each reactor were measured daily during digestion period. Initial and final pH, VFA, total Chemical Oxygen Demand tCOD, Soluble Chemical Oxygen Demand (sCOD), ethanol measurements were carried out. Analytical methods, basal medium and inoculum used in the study are given elsewhere (Ozkan et al., 2011).

Results and Discussion

Effects of COD Values on Bio-Hydrogen Production Yields

The bio-hydrogen production observed in reactors R1-R6 was 89.5, 76.3, 57.5, 95.6, 81.8, and 54.2 mL/g COD. These results indicated an inverse relationship between substrate concentration and bio-hydrogen production yields. Increasing COD concentrations from 20 to 30 g COD/L resulted in decrease in bio-hydrogen yields. This might attribute to substrate concentration...
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inhibition. Substrate concentration has a significant effect on bio-hydrogen production. When a certain substrate threshold is exceeded, high substrate concentration would result in the accumulation of VFA and a drop in pH, which would inhibit bio-hydrogen producers (Pakarinen et al., 2008). According to results, higher substrate concentration (25 and 30 g COD/L) curtailed the bio-hydrogen production yield. This finding is consistent with Ginkel and Logan (2005) who reported that biological hydrogen production was increased with reduced organic loading. Increased substrate concentration, naturally increased the amount of acidification products (VFAs) (Table 3) which lead to a decrease in pH. Thus, the reduction in pH curtailed bio-hydrogen production in reactors which contained 25 and 30 g COD/L substrate (R2, R3, R5, R6) when compared the reactors which contained 20 g COD/L substrate (R1, R4). The optimum substrate concentration for bio-hydrogen production is dependent on several parameters such as the substrate used, type of reactor and HRT. The highest bio-hydrogen production yields (89.5 and 95.6 mL/g COD) were observed in reactors which contained 20 g/l COD substrate. Therefore, results indicated that, 20 g COD/L was the optimum substrate concentration for fermentative bio-hydrogen production from only beet-pulp and sugar-beet processing wastewater and beet-pulp in the range investigated. This is in agreement with Lin et al. (2008) who tested different substrate concentrations (5-60 g COD/L) to observe the effects on bio-hydrogen production from starch. Similarly, when starch concentrations were higher than 20 g COD/L, bio-hydrogen production yields decreased.

Table 3. Initial and final tVFAs.

<table>
<thead>
<tr>
<th>Substrate Concentration</th>
<th>mg/L as H-Ac</th>
<th>tVFA Initial</th>
<th>tVFA Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 20 g COD /L (ww+pulp)</td>
<td>388</td>
<td>2175</td>
<td></td>
</tr>
<tr>
<td>R2 25 g COD /L (ww+pulp)</td>
<td>395</td>
<td>2329</td>
<td></td>
</tr>
<tr>
<td>R3 30g COD /L (ww+pulp)</td>
<td>409</td>
<td>3271±252*</td>
<td></td>
</tr>
<tr>
<td>R4 20 g COD /L (pulp)</td>
<td>76</td>
<td>1490</td>
<td></td>
</tr>
<tr>
<td>R5 25 g COD /L (pulp)</td>
<td>107</td>
<td>1770±142*</td>
<td></td>
</tr>
<tr>
<td>R6 30 g COD /L (pulp)</td>
<td>105</td>
<td>2177±307*</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± standard deviation

Bio-hydrogen production efficiency of only beet-pulp was higher than co-digestion of beet-pulp and sugar-beet processing wastewater. The maximum bio-hydrogen production yield was calculated as 95.6 mL /g COD in R4 which contained 20 g/L COD of only beet-pulp. Maximum bio-hydrogen production yield (95.6 mL H₂/g COD) is higher than 89.2 mL H₂/g COD from sucrose (Sung et al., 2002) and 71.3 mL H₂/g COD from cassava starch manufacturing wastewater (Reungsang et al., 2004).

Volatile Fatty Acid Production

Initial and final tVFAs of the reactors were illustrated in Table 3. The VFA production in the reactors increased with the increased COD concentrations (from 20 to 30 g COD/L). In addition, higher tVFA concentrations were observed in reactors which contained beet-pulp and sugar-beet processing wastewater together (R1, R2, R3) when compared to reactors which contained only beet-pulp (R4, R5, R6). Sugar-beet processing wastewater is highly biodegradable with its soluble carbohydrates (Shore et al., 1984). However, ligno-cellulosic composition of beet-pulp results in difficulty in degradation when compared to wastewater. Thus, high rate acidification is observed in reactors which contained sugar-beet processing wastewater and beet-pulp together (R1, R2, R3) (38.7–42.0 mL/day) when compared with the reactors which contained only beet-pulp (19.9–28.6 mL/day). High rate acidification in R1, R2 and R3 resulted in a sharp drop of reactor pH and subsequent inhibition of bacterial hydrogen production. Therefore, higher tVFA concentrations were observed in R1, R2 and R3 as the metabolic pathway might be shifted to VFA production instead of bio-hydrogen production.
Acidification degree gives information about the production of VFAs due to anaerobic acidification of substrate. Acidification degrees of the reactors which were calculated as the ratio of COD-equivalent of acidogenic products (VFAs) and consumed COD concentrations for each of the reactors (Equation 4.1) are depicted in Figure 1. Dinopoulou et al. (1988) defined the acidification degree as the proportion of the substrate which is converted to VFAs. The COD equivalents of VFAs were acetic acid, 1.066; propionic acid, 1.512; butyric acid, 1.816; valeric, 2.036; caproic acid, 2.204 (Yilmaz and Demirer, 2008).

\[
\text{Degree of acidification (\%) = } \frac{S_i}{S_f} \times 100 \\
\text{Where: } S_i \text{ : Consumed substrate concentration, measured in COD (mg/L),} \\
S_f \text{ : Produced VFAs, expressed as theoretical equivalents of COD concentrations (mg/L).}
\]

Higher acidification degrees were observed in reactors which contained beet-pulp and sugar-beet processing wastewater together (R1, R2, R3) when compared to reactors which contained only beet-pulp (Figure 1). This is probably due to the shifting of the metabolic pathway to Volatile Fatty Acids (VFA). Moreover, increase in the substrate concentration decreased the acidification degree (Figure 1) which can be explained by stress on acidogenic bacteria with extra organic load (Oktem et al., 2006). Acidification degree of R1 (62.3%) was higher than that of R2 (55.4%) and R3 (44.9%). Similarly, R4 had higher acidification degree (39.0%) than R5 (33.0%) and R6 (27.6%). These results were consistent with Dinopoulou et al. (1988) who stated that the degree of acidification decrease with the increase in the influent substrate concentration. Acidification degrees estimated in this study (27.6–62.3%) were comparable with values stated in the literature; 60% for complex wastewater (Dinopoulou et al., 1988), 29.7–44.5% for solid waste (Raynal et al., 1998), 61% for wastewater (Fang and Yu, 2001), 40.3% for fruit and vegetable wastes (Bouallagui et al., 2004).

During an anaerobic digestion process, the formation of bio-hydrogen is usually accompanied by formation of soluble metabolites (such as VFAs) which reflects the metabolism of hydrogen-producing cultures. The distribution of metabolites formed during bio-hydrogen production is often a crucial signal in assessing the efficiency of hydrogen-producing cultures (Dinopoulou et al., 1988). In all of the reactors, main acidification products were Acetic (H-Ac), Propionic (H-Pr), and Butyric Acids (H-Bu) and these comprising 51.4–69.0; 4.0–13.9; 16.4–30.8% (w/w) of tVFA, respectively. The higher molecular weight VFAs (valeric, caproic etc.) and ethanol (8.2–60.4 mg/L) were produced with insignificant quantities. According to the literature, substrate characteristic is an important parameter on product distribution in an acidification reactor (Dinopoulou et al., 1988). The major products in bio-hydrogen production by anaerobic dark fermentation of carbohydrates are acetic, butyric and propionic acids (Kaptan and Kargi, 2006). The dominance of H-Ac, H-Pr and H-Bu can be associated with the carbohydrate fermentation as both sugar-beet processing wastewater and beet-pulp contain sugars (Shore et al., 1984; Hutnan et al., 2000). In addition, 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) (Zhang et al., 2005). To maximize the yield of bio-hydrogen, the metabolism of the bacterium must be directed away from alcohols (such as ethanol) towards VFAs. Thus, the dominance of H-Ac, H-Pr and H-Bu can also be associated with high bio-hydrogen production yields observed (54.2–95.6 mL/g COD).

**pH Values**

It was reported that initial pH value 6.0 is the optimum value in anaerobic bio-hydrogen production (Mohan et al., 2007; Lee et al., 2008). Thus, initial pH values of all the reactors were adjusted to 6.0 in this study. Fan et al. (2006) stated that, acetate and butyrate producers are assumed to overcome propionate producers at the optimal pH, thereby increasing the bio-hydrogen yield. So,
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high bio-hydrogen production yields (54.2–95.6 mL/g COD) and distributions of acidic metabolites of the reactors in this study supported that the initial pH value 6.0 was suitable for fermentative bio-hydrogen production from sugar industry wastes.

The final pH values of the reactors were illustrated in Figure 2. It indicated that, pH decreased at all reactors due to fermentation. Figure 2 also revealed that the final pH in all tests were lower than the initial pH 6.0 due to fermentation. The decrease in pH during incubation is due to production of organic acids which depletes the buffering capacity of the medium resulting in low final pH (Khanal et al., 2004). In all reactors, the final pH ranged from 4.41 to 5.10. This observation illustrates that the microbial activity in all the bottles were typically of acidogenic nature. It can be supported by the high bio-hydrogen yields in reactors (54.2–95.6 mg/L COD).

![Figure 1: Acidification degrees in the reactors](image1)

Based on the results, it can be suggested that the increased substrate concentration (20 to 30 g/L COD) naturally enhanced the amount of acidification products (VFAs) (Table 5) which led to natural reduction of the pH. Final pH of R1 (5.10) was higher than R2 (4.93) and R3 (4.75). Similarly, final pH of R4 (4.58) was higher than R5 (4.43) and R6 (4.41).

**Figure 2: Final pHs of reactors**

![Figure 2: Final pHs of reactors](image2)

Soluble and Consumed COD Concentrations

sCOD concentrations of the reactors are given in Table 4. It is clear from Table 4 that, substrate addition increased sCOD concentration. Higher sCOD concentrations were observed for the reactors with higher initial substrate concentrations. It can be seen from Table 3 and 4 that, the rise in VFA concentrations was compatible with the increase in sCOD concentrations. In other words, tVFA concentrations in the reactors rose as the substrate concentration increased and this situation resulted in increases in sCOD concentrations for all reactors. Since, the hydrolysis of particulate organic matter occurs simultaneously during the acidification of soluble organics. Moreover, sCOD concentrations were higher (6765–8165 mg/L) in the reactors which contained beet-pulp and sugar-beet processing wastewater together (R1, R2, R3) since solubility of wastewater was higher than beet-pulp. Thus, highest sCOD concentration (8165 mg/L) was observed in R3.

cCOD (Consumed COD) concentrations of the reactors were depicted in Figure 3. Bio-hydrogen production in R1, R2 and R3 ceased after 2–3 days of incubation while in R4, R5 and R6 ceased after 4–5 days, because of faster pH drop. Thus, for the same COD value, cCOD concentrations of the reactors which contained only beet-pulp were higher than that of reactors which include sugar-
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beet processing wastewater and beet-pulp together. cCOD concentration of R4 was higher than R1 at an initial COD level of 20 g/L. Similarly, cCOD concentration of R5 was higher than R2 and R6 was higher than R2 at an initial COD level of 25 g/L and 30 g/L, respectively. In addition, there is a direct relationship between tCOD and cCOD concentrations. Increasing tCOD concentrations from 20 to 30 g COD/L resulted in increase in cCOD concentrations. cCOD concentration of R3 was higher than R1 and R2. Similarly, cCOD concentration of R6 (4.58) was higher than R4 and R5 (Figure 3).

Table 4. Initial and final sCOD concentrations of the reactors.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Initial (mg/L)</th>
<th>Final (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>20 g COD/L (ww+pulp)</td>
<td>3668 6765</td>
</tr>
<tr>
<td>R2</td>
<td>25 g COD/L (ww+pulp)</td>
<td>3861 7698</td>
</tr>
<tr>
<td>R3</td>
<td>30 g COD/L (ww+pulp)</td>
<td>3957 8165±0 *</td>
</tr>
<tr>
<td>R4</td>
<td>20 g COD/L (pulp)</td>
<td>1958 3966</td>
</tr>
<tr>
<td>R5</td>
<td>25 g COD/L (pulp)</td>
<td>2112 6065±990 *</td>
</tr>
<tr>
<td>R6</td>
<td>30 g COD/L (pulp)</td>
<td>2496 7349±1154 *</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation

Bio-Hydrogen and Methane Productions

The operation of reactors was stopped when bio-hydrogen production ceased. Cumulative bio-hydrogen productions of reactors were depicted in Figure 4. Bio-hydrogen production in reactors which contained sugar-beet processing wastewater and beet-pulp together (R1, R2 and R3) ceased after 2–3 days of incubation. On the other hand, in reactors which contained only beet-pulp (R4, R5 and R6), bio-hydrogen production continued for 4–5 days (Figure 4).

The formation of organic acids as metabolic products causes a drop in pH (Figure 2). Gradual decreases in pH inhibit bacterial bio-hydrogen production since pH affects the activity of hydrogenase enzyme (Dabrock et al., 1992). Sugar-beet processing wastewater is more biodegradable than beet-pulp. For this reason, high rate acidification became in reactors which contained wastewater and beet-pulp together (38.7–42.0 mL/day) when compared with the
reactors which contained only beet-pulp (19.9–28.6 mL/day). High rate acidification in R1, R2 and R3 caused an abrupt decrease of reactor pH because of this acid/pH inhibition; bio-hydrogen production was inhibited and stopped in 2–3 days. In R4, R5 and R6, pH decreased slowly because of low biodegradability of waste so bio-hydrogen production continued for 4–5 days.

The direct relationship between substrate addition and bio-hydrogen production can be observed in Figure 4. Higher bio-hydrogen productions were observed in the reactors with higher substrate concentrations. In addition, for the same substrate concentration, in reactors containing only beet-pulp (R4, R5 and R6) more bio-hydrogen production was observed when compared to reactors containing beet-pulp and wastewater (R1, R2, R3) (Figure 4). High rate acidification in R1, R2 and R3 resulted in lower COD consumptions and lower bio-hydrogen productions, compared to R4, R5 and R6. Thus, highest bio-hydrogen production was calculated as 117.5 mL in R6.

Small amount of methane gas (0.7–6.1 mL) was observed at all reactors. pH of the reactors were set to 6.0 to suppress methanogenic activity as methanogens operate optimum at a range of 6.5 to 8.2 while acidogens prefer between 4 and 6.5 (Speece, 1996). High bio-hydrogen productions (81.4–117.5 mL) and low methanogenic activity (0.7–6.1 mL) confirmed the importance of pH in bio-hydrogen production. In addition, this observation indicated the inverse relationship between substrate concentrations and methane productions. It can be observed that, increased substrate concentrations caused decreases in methane productions because of acid/pH inhibition.

Conclusions

- Bio-hydrogen production efficiency of only beet-pulp is higher than co-digestion of beet-pulp and sugar-beet processing wastewater.
- The maximum bio-hydrogen production yield was determined as 95.6 mL/g COD in reactor which contained 20 g/L COD of only beet-pulp.
- Higher acidification degrees were observed in reactors which contained sugar-beet processing wastewater and beet-pulp together (44.9-62.3%) when compared with the reactors which contained only beet-pulp (27.6-39).
- Bio-hydrogen production from sugar-beet processing wastes via dark fermentation can not only enable waste minimization but also contribute to sustainability via valuable bio-based product formation from wastes.

Acknowledgements

This study was funded by The Scientific and Technological Research Council of Turkey through Grant Number 104I127.

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