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ORIGINAL PAPER

Biodegradation of olive-mill pomace mixed with organic fraction of municipal solid waste

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Abstract This study investigated the effects of organic fraction of municipal solid waste (OFMSW) addition on the anaerobic treatment of the olive-mill pomace. Biodegradability of olive-mill pomace mixed with OFMSW was examined in anaerobic bioreactors. Only OFMSW was loaded in the first (control) bioreactor, while run 1 and run 2 bioreactors included different ratio of OFMSW and olive-mill pomace. COD, BOD_5, NH_4–N, pH, VFA, CH_4 quantity and percentage in anaerobic bioreactors were regularly monitored. In addition, inert COD and anaerobic toxicity assay (ATA) were measured in leachate samples. The results of the study showed that 70% of OFMSW addition to olive-mill pomace has an advantage in terms of pollution parameters and methane generation. Since olive-mill pomace is not easy biodegradable, addition of high proportion of OFMSW promotes biodegradability of olive-mill pomace. Decreasing in BOD_5/COD ratios in the run 1 and run 2 reactors carried out as 62 and 52%, respectively.

Keywords Olive-mill pomace · Biodegradation · Anaerobic digestion · Municipal solid wastes

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Introduction

Olive-mill wastes represent an important environmental problem in Mediterranean where they are generated in huge quantities in short periods of time. Their high phenol, lipid and organic acid contents turn them into phytotoxic materials, but these wastes also contain valuable resources such as a large proportion of organic matter and a wide range of nutrients (Roig et al. 2006). Being in the Mediterranean region, Turkey is also one of the leading countries in the production of olives (600–700 kt per year) and olive-mill pomace (100–120 kt per year). Here the term "olive-mill pomace" stands for the solid residue remaining after solvent extraction from the cold-presses olives (Tekin and Dalgıç 2000). Olive-mill pomace, namely prina, comprises significant portion (30%) of the olive-mill wastes and consists of olive pulp, stones, water and a remaining quantity of oil and is further treated by drying and solvent extraction to recover valuable oil (Ergüder et al. 2000; Mavros et al. 2008). Olive-mill pomace is characterized by a high concentration of organic matter and volatile fatty acids, low pH, the presence of inhibitory compounds as phenols and large quantities generated (Rincon et al. 2009).

Olive-mill wastes are a significant source of potential or existing environmental pollution in the olive growing countries, like Turkey. The difficulties of treatment of olive-mill effluents are mainly related to high organic loading, seasonal operation, high territorial scattering and presence of organic compounds which are hard to biodegrade such as long chain fatty acids and phenolic compounds (Ergüder et al. 2000). Composting of organic wastes is well established method to obtain their chemical stabilization, biological maturation and sanitization before application to agricultural land (Plaza et al. 2008). Although physical and chemical properties of olive-mill pomace are rather unsuitable for composting (very low porosity, high moisture and low contents of N and P), many complementary residues are usually generated in these areas, such as animal manures (source of N and P), olive leaves, cereal straws, almond shells or pruning wastes (bulking agents). The results of the study realized by Canet et al. (2008) demonstrate the feasibility of composting olive-mill pomace and other organic residues from Mediterranean areas using simple and affordable techniques and long composting times. Elevated pH and salinity of the final products are the main disadvantages.

The seasonal production and high organic loading of olive-mill wastes make anaerobic treatment a very attractive option for these wastes. Furthermore, much less biosolids (sludge) and biogas as a valuable end product, which may offset the associated treatment costs, further add to the positive aspects of anaerobic treatment. Ergüder et al. (2000) showed that anaerobic treatment efficiency of olive-mill residual solids (olive-mill pomace) alone was found to be low. However, when olive-mill pomace was mixed with olive-mill wastewater in certain ratios, olive-mill pomace could be treated efficiently under anaerobic conditions. In addition, Borja et al. (2005) informed that the anaerobic digestion process is a good and attractive alternative for the economical reduction of the organic matter concentration in vegetable solid wastes. They performed kinetic model study of the hydrolysis, acidogenic and methanogenic steps of the anaerobic digestion process of two-phase olive-mill pomace. Another study related to anaerobic treatment of olive-mill pomace was carried out by Tekin and Dalgıç (2000). In this study, biogas production from slurry obtained by mixing finely ground olive-mill pomace in water was investigated using anaerobic digesters. The maximum rate was found to be 0.70 l of biogas per liter of digester volume per day. Besides, in this study, landfill leachate was used as inoculum due to consists of numerous sources of waste and therefore, the possibility of finding all types of microorganisms (hydrolyzing, acidogenic and methanogenic bacteria) contributing to anaerobic digestion, is much higher in landfill areas than that in a "single source" such as cow manure and other plant wastewaters.

Limited studies have been performed related to anaerobic treatment of olive-mill pomace. In addition, there is no study about anaerobic co-digestion of olive-mill pomace and organic fraction of municipal solid waste (OFMSW). This paper reports the results of biodegradation of olive-mill pomace mixed with OFMSW. The influence of OFMSW addition on biodegradation rate, methane generation and leachate characteristics of olive-mill pomace were investigated.

Materials and methods

Anaerobic bioreactor

Stainless-steel cylindrical bioreactors 10 cm diameter, and 30 cm high were constructed to treat the olive-mill pomace mixed with OFMSW and to determine biodegradation rate. The schematic configuration of the reactor is shown in Fig. 1. These bioreactors were operated in batch mode at a temperature of 35-40°C under anaerobic conditions. The leachate was collected at the bottom section of the bioreactor and it was recycled to the top of the reactor with a peristaltic pump. There are two separate ports on the top of the reactor for measurement of the methane and recirculation of the leachate.

Operating conditions

Anaerobic bioreactors were loaded with OFMSW and olive-mill pomace at different mixing ratios. All bioreactors were operated with leachate recirculation to enhance methane generation. Only OFMSW produced in the center kitchen of Pamukkale University was loaded in the first (control) bioreactor. To determine how effects of OFMSW on biodegradation rate of olive-mill pomace, the second (run 1) and the third (run 2) bioreactors were loaded with 70% of OFMSW + 30% of olive-mill pomace and 30% of OFMSW and 70% of olive-mill pomace, respectively. Table 1 shows operating protocol and loading of the anaerobic bioreactors through 170 days. The olive-mill pomace was supplied from a local olive processing plant. The characterization of raw olive-mill pomace is shown in Table 2.

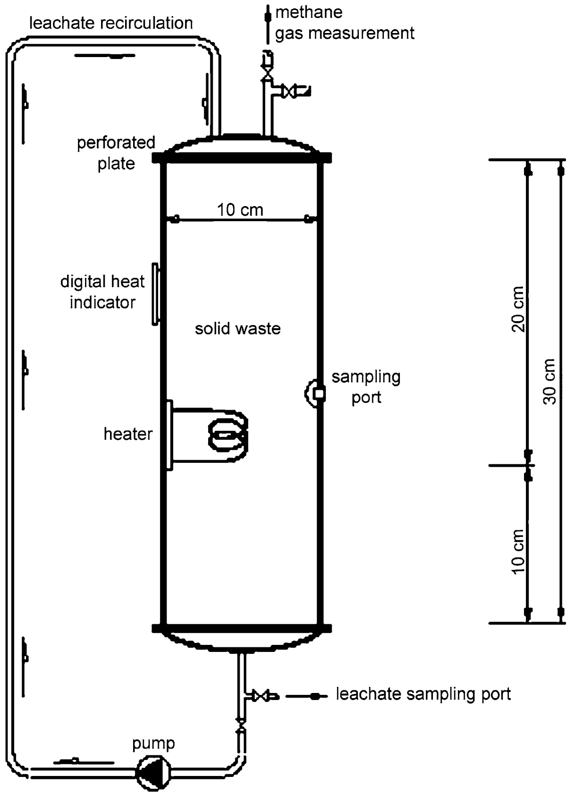

Fig. 1 Schematic configuration of anaerobic bioreactor

Table 1 Reactor loading and operating protocol for anaerobic bioreactors

	Control reactor	Run 1	Run 2
Quantity of waste (g)	1000	1000	1000
Ratio of OFMSW (%)	100	70	30
Ratio of olive pomace (%)	–	30	70
Water content (%)	88	65	60
Organic matter (%)	91	94	96
TN (mg/kg)	4250	4600	5250
NH_4–N (mg/kg)	450	625	784
PO_4–P (mg/kg)	2350	2675	3050
Operation time (day)	170	170	170

All data except operation time expressed in weight basis

Analytical procedure

Organic matter and water content in OFMSW and olive-mill pomace samples and COD concentrations in leachate samples were detected by using closed reflux colorimetric method following the Standard Methods (APPA-AWWA 1992). The BOD_5 was measured using the WTW Oxi Top IS 12 system. Total nitrogen (TN), phosphate phosphorus (PO_4–P) and ammonia–nitrogen (NH_4–N) were measured using spectroquant kits numbered 14537, 14546 and 14752 in a photometer Merck SQ 300. The pH was determined immediately after sampling to avoid any change due to CO_2 stripping, using a pH meter, type NEL 890. Volatile fatty acid (VFA) concentrations in the leachate samples were measured using Anderson–Yang method (1992). Methane gas productions were measured by liquid displacement method. Methane gas was detected using a liquid solution containing 3% NaOH (w/v) (Razo-Flores et al. 1997). Methane percentage was monitored with a digital methane meter (Drager Pac Ex). Inert COD content of leachate was determined with glucose comparison method developed by Germirli et al. (1990) in all soluble form of COD. Anaerobic toxicity assay (ATA) test was performed at 37°C using serum bottles with a capacity of 115 ml as described by Owen et al. (1978) and Donlon et al. (1995). Crude oil was analyzed by Soxhlet extraction with *n*-hexane (Horwitz 2000).

Table 2 Characteristics of olive pomace

Characteristics	Measured value
Water content (%)	51
Organic matter (%)	98.7
TN (mg/kg)	6650
NH_4–N (mg/kg)	960
PO_4–P (mg/kg)	4125
Crude oil (%)	2

All data expressed in weight basis

Result and discussion

COD variations in leachate produced from the anaerobic bioreactors

Daily variations in COD concentrations of the leachate taken at the bottom of the anaerobic bioreactors, called control (only OFMSW), run 1 (70% OFMSW + 30% olive-mill pomace) and run 2

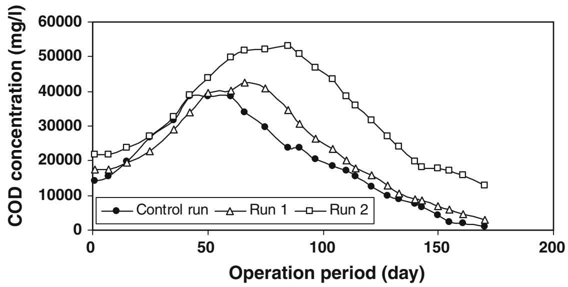

Fig. 2 Daily variation of COD concentrations in leachate for the control runs 1 and 2

(30% OFMSW + 70% olive-mill pomace) are given in Fig. 2. COD was monitored as indicator parameter of the leachate organic strength. The initial COD concentration in leachate samples collected from the control, run 1 and run 2 reactors were 14172, 17413 and 21694 mg/l, respectively. Since run 2 contains high organic matter, the initial COD value of the run 2 was higher than those of run 1 and control run (see Table 1). Lopez-Pineiro et al. (2007) indicated that olive-mill pomace contains high organic matter content. Macias-Corral et al. (2008) also showed that co-digested wastes had the highest initial COD values due to the high organic matter in the mix. Due to the rapid release and hydrolysis of organics from the municipal solid wastes into the leachate, the COD concentrations in control reactor (only OFSWM) increased to 38714 from 14172 mg/l. The highest COD concentrations were measured on day 42, 66 and 85 in control, run 1 and run 2 reactors, respectively. This result (high COD values of olive-mill pomace) is in consistent with other studies realized by Ergüder et al. (2000); Borja et al. (2005) and Rincon et al. (2009).

The final COD concentrations of control, run 1 and run 2 reactors were 890, 3100 and 12705 mg/l, respectively, on day 170. As seen in Fig. 2, the COD decreasing rate in the run 2 reactor which has high olive-mill pomace ratio is slower than those of run 1. This indicates that olive-mill pomace leachate is not readily biodegradable. Moreover, the OFMSW addition to olive-mill pomace (approximately OFMSW to olive-mill pomace ratio: 1:3) improved anaerobic biodegradation of olive-mill pomace. The result of this study showed that co-digestion of olive-mill pomace with OFMSW has a relatively positive effect in terms of decreasing of COD concentrations.

VFA variations in leachate produced from the anaerobic bioreactors

The concentration of VFA is an important parameter because of the degree of the stability of the anaerobic process. The VFA concentrations in the leachates of all reactors have similar properties with the COD concentrations. Figure 3 shows daily variations in VFA concentrations of the leachates originated from the anaerobic bioreactors. As shown in Fig. 3 the initial VFA concentrations in leachate samples were found to be 6504, 6995 and 7413 mg/l in control, run 1 and run 2 reactors, respectively. The highest VFA value occurred in run 2 reactor among three reactors since it contains high organic matter. Rincon et al. (2009) reported that olive-mill solid residue has high content of volatile fatty acids. In this study, organic matter degradation in initial phase of the fermentation caused high VFA concentration. Due to the slow degradation rate of olive-mill pomace, decreasing rate of VFA concentrations of run 2 reactor was low. At the end of the study, the final VFA concentrations in leachate samples were 666, 1310 and 4010 mg/l in control, run 1 and run 2 reactors, respectively. These results show that addition of OFMSW to olive-mill pomace provides an advantage in respect to organic matter degradation.

pH variations in leachate produced from the anaerobic bioreactors

The pH measured in the leachate samples are in accordance with the concentration of VFA monitored in all three bioreactors. The variation of the pH profiles over time is illustrated in Fig. 4. As shown in this figure, the initial pH of the leachates from the

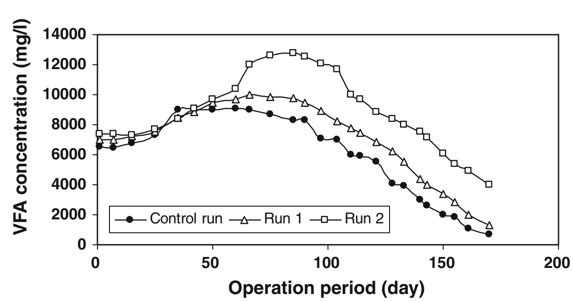

Fig. 3 Daily variation of VFA concentrations in leachate for the control runs 1 and 2

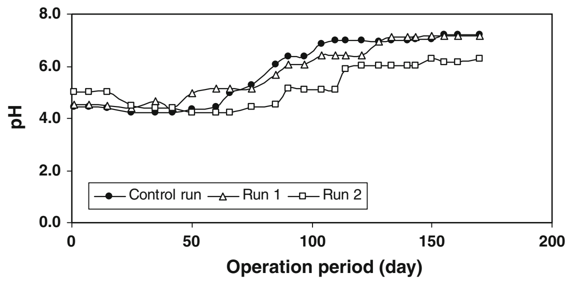

Fig. 4 Daily variation of pH levels in leachate for the control runs 1 and 2

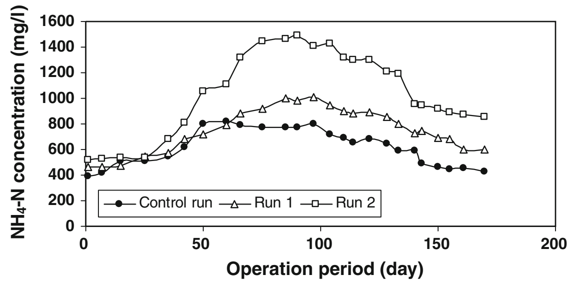

Fig. 5 Daily variation of NH4–N concentrations in leachate for the control runs 1 and 2

control, run 1 and run 2 reactors were 4.44, 4.51 and 5.01, respectively. The low pH value is caused by the high concentrations of VFA which are produced by acetogenic bacteria and inhibit the methanisation process (Ağdağ and Sponza 2007). Generally, the pH drops occurred rapidly at the beginning of experiment as the easily biodegradable fraction of organic matter was hydrolyzed and converted to short-chain organic acids and fatty acids. After the initial drop, the pH began to rise gradually as the fatty acids were transferred to the methane phase reactors and were consumed by methanogens (Macias-Corral et al. 2008). Although pH values of control and run 1 reactors dropped (until day 42), declining of pH values in the run 2 reactor lasted until day 110. The reason for this situation is hardly biodegradable organic matter quantity (70% of olive-mill pomace) in run 2 reactor is higher than control and run 1 reactor. At the end of the study, the final pH values in leachate samples were 7.2, 7.15 and 6.3 in control, run 1 and run 2 reactors, respectively. Borja et al. (2005) also reported that pH value of the olive-mill pomace was low (4.4–6.5). Low pH values of the run 2 reactor were resulted in the lower methane quantity. pH values of the run 1 reactor were almost equal to the control reactor. This result indicates that co-digestion of 30% of olive-mill pomace and 70% of OFMSW is appropriate for pH profile and methane generation.

NH_4–N variations in leachate produced from the anaerobic bioreactors

The results of the NH_4–N concentrations measured in leachate samples over time from the control, run 1 and run 2 reactors were shown in Fig. 5. The highest NH_4–N concentrations were 819, 1010 and 1491 mg/l in control, run 1 and run 2 reactors, respectively, through the mineralization of organic nitrogenous compounds. It was observed that the NH_4–N concentrations in run 2 reactor is higher than those of control and run 1 reactors since the run 2 reactor contained more nitrogenous organic compounds such as proteins and amino acids. The TN concentrations in waste were higher in run 2 reactor as compared to control and run 1 (TN = 4250, 4600 and 5250 mg/kg in waste of control, run 1 and run 2 reactors, respectively, in the first operation day). Baeta-Hall et al. (2005) reported that the TN concentration was measured as 10500 mg/kg in olive-mill pomace sample.

Methane gas productions and percentages in the anaerobic bioreactors

The quantity of methane in control, run 1 and run 2 reactors were shown in Fig. 6. As summarized in this figure, the maximum cumulative methane gas production was recorded as 17.6, 13 and 9.7 l in control, run 1 and run 2 reactors, respectively at the end of 170 days. Daily methane generations (data not shown) showed that methane production in run 2

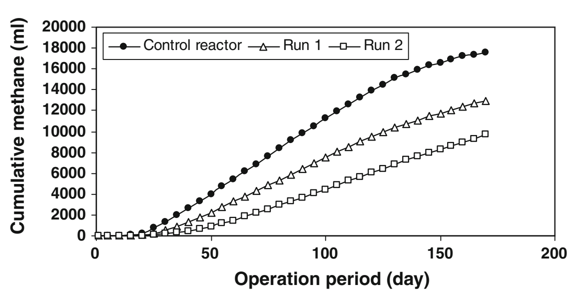

Fig. 6 Variation in cumulative methane gas productions in the control runs 1 and 2

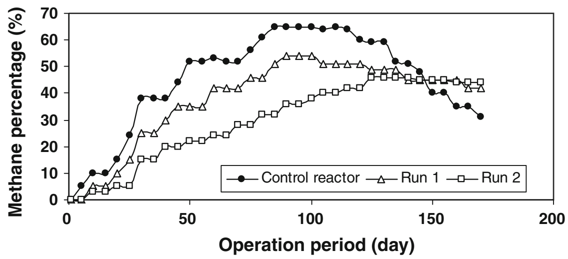

Fig. 7 Variation in methane gas percentages in the control runs 1 and 2

reactors was higher than control and run 1 reactor at the end of the last operation day. This situation also showed that methane production was not completed by day 170 in the run 2 reactor. Methane generation rate in high olive-mill pomace content reactor was slower than those of OFMSW and low olive-mill pomace content reactor. The reason for this situation could be "not easily degradable" properties of olive-mill pomace. Namely, as the olive-mill pomace content increases the resistance to digestion is also increases. In addition, relatively low pH and high VFA values in run 2 reactor may be also caused low methane generation rate. It is clearly observed that anaerobic digestion of olive-mill pomace alone is very difficult. Similar result was also obtained by Ergüder et al. (2000) in batchwise fermentation. The OFMSW addition (more than 50%) to olive-mill pomace increases methane production. Furthermore, Fig. 7 shows methane percentage in control, run 1 and run 2 reactors. The highest methane percentages were 65, 54 and 46% in control, run 1 and run 2 reactors, respectively. Methane percentage of the control, run 1 and run 2 reactors were 31, 42 and 44%, respectively, at the end of the 170 days of operation period. These results showed that, degradation period was continuing in the run 1 and run 2 reactors while the degradation periods of control reactor just about ended. Methane generation and percentage results showed that high ratio of OFMSW addition simplified to anaerobic digestion of olive-mill pomace.

Bioefficiency of the anaerobic process

The methane yield was found as 0.049 g CH_4-$COD_{removed}$/gVSS add in control reactor while the methane yields were 0.035 CH_4-$COD_{removed}$/gVSS add and 0.025 CH_4-$COD_{removed}$/gVSS add in run 1 and run 2 reactors. It was found that the maximum mass of removed COD was equal to 37.8, 39.6 and 40.3 g in control, run 1 and run 2 reactors on day 170. Using these values, and taking into consideration the methane gas produced, it was concluded that 85% of COD was removed from the control reactor was converted to methane and 83 and 61% of COD removed were converted to methane in run 1 and run 2 reactors, respectively on day 170.

The calculated methane yield was found to be higher for control reactor, since MSW primarily consists of easily biodegradable organic matter. But, methane yield in high olive-mill pomace content reactor (run 2) was the lowest. However, removed COD converted to methane in run 1 reactor was almost nearly identical with control reactor. This contributed to better biological efficiency compared to high olive-mill pomace content. This result shows that addition of OFMSW to olive-mill pomace (70% of OFSWM + 30% of olive-mill pomace) ensures an advantage in respect to methane yield.

BOD_5, ATA and inert COD in the anaerobic bioreactors

In order to study the proportion of biodegradable organic carbon in the leachate it was considered to determine the BOD_5/COD ratios. Table 3 shows BOD_5, COD concentrations and BOD_5/COD ratios in the leachate samples of the all reactors studied for days 25, 75 and 150. The BOD_5 values were ranging initially from 21300, 19300, 24350 mg/l to 650, 2200, 7700 mg/l at the end of anaerobic incubation in control, run 1 and run 2 reactors, respectively. The BOD_5 values decreased substantially as 97, 89 and 68% in the aforementioned reactors, respectively. Although the control reactor has high initial BOD_5 concentrations, the highest BOD_5 decreases were occurred in this reactor. Decreasing in BOD_5/COD ratios in the control, run 1 and run 2 reactors carried out as 81, 62 and 52%, respectively. The experimental results indicate that, higher olive-mill pomace content significantly retarded the decreasing of BOD_5 removals. But, co-digestion of low olive-mill pomace and high OFMSW has an advantage in terms of anaerobic biodegradation of olive-mill pomace.

Table 4 illustrates the results of the ATA tests indicating the quantities of methane produced from

Table 3 BOD_5, COD concentrations and BOD_5/COD ratios in the leachate samples of the bioreactors

	Control reactor			Run 1			Run 2		
	Day 25	Day 75	Day 150	Day 25	Day 75	Day 150	Day 25	Day 75	Day 150
BOD_5 (mg/l)	21300	11905	650	19300	22560	2200	24350	30650	7700
COD (mg/l)	26715	29615	4317	22681	41000	6877	27050	51935	17900
BOD_5/COD (mg/l)	0.8	0.4	0.15	0.85	0.55	0.32	0.9	0.59	0.43

the control (glucose) and leachate containing serum bottles through 3 days of anaerobic incubation. At the end of the ATA test, it was found that there is no significant toxicity in control and olive-mill pomace reactors. Methane quantity in the serum bottle prepared with glucose (easy biodegradable matter) was 32 ml/day. Methane quantities of the bottles prepared with leachate from control and run 1 reactor (different percentage of leachate ratio) were higher than those of glucose bottle (control: 55 ml/day and run 1: 39 ml/day). This situation indicated that leachate content of these reactors did not create anaerobic toxicity. On the contrary, rising leachate percentages in control and run 1 reactor increased the methane production. But, as can be seen in Table 4, the amount of methane production relatively decreased in run 2 reactor due to the slow biodegradability of the olive-mill pomace. However, this methane quantity decreasing in serum bottle prepared with leachate from run 2 reactor didn't substantially affect the anaerobic biodegradability.

Table 4 ATA test results in leachate samples of all reactors

	Quantity of methane (ml/day) ($n = 3$ mean value)	
	(After 3 days)	(After 6 h)
Glucose 0% leachate (Control)	32	33
Glucose 0% leachate (Control)	32	31
25% leachate (Control reactor)	35	36
50% leachate (Control reactor)	40	38
75% leachate (Control reactor)	46	44
100% leachate (Control reactor)	55	51
25% leachate (Run 1)	32	31
50% leachate (Run 1)	32	31
75% leachate (Run 1)	36	31
100% leachate (Run 1)	39	34
25% leachate (Run 2)	31	26
50% leachate (Run 2)	30	26
75% leachate (Run 2)	28	23
100% leachate (Run 2)	26	23

The results of inert COD assays in leachate samples showed that inert COD level was found to be approximately 10, 14 and 17% of the initial COD levels in control, run 1 and run 2 reactors, respectively. High inert COD levels in olive-mill pomace containing reactors could be attributed to high strength degradable organic matter content of olive-mill pomace. This indicates that the COD of leachate samples from run 2 could be treated up to approximately 83% which is the maximum COD removal efficiency reached under favorable anaerobic conditions.

Conclusion

The results of the study showed that olive-mill pomace mixed with high proportion of OFMSW could be treated anaerobically. But, biodegradability of high olive-mill pomace content reactor was found to be low. At the end of the study, the percentage of organic matter reduction in run 1 and run 2 reactors were found to be 40 and 21%, respectively. Similarly, waste quantity reduction in run 1 reactor (66%) is higher than run 2 (32%). Finally, it was concluded that anaerobic treatment efficiency of olive-mill pomace mixed with high proportion of OFMSW is high.

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