from 20.9 mg/L to 14.9 mg/L, indicating the solubilisation of the suspended solids (Gomes et al., 2013; Ariunbaatar et al., 2014). The solubilisation released the suspended COD into the aqueous phase leading to the observed increase in DOC and soluble COD from 1 700 to 2 300 mg/L and 155 to 245 mg/L, respectively. On the other hand, the total COD slightly decreased from 24 500 to 21 600 mg/L, ensuring adequate substrate retention for the ensuing anaerobic process. Through ozonolysis, the hard cell walls contained in WAS were ruptured, and the extracellular polymeric substances (EPS) solubilised, releasing the cellular contents. The increased concentration of sulphates (18 to 75 mg/L) and nitrates (0 to 115 mg/L) in the supernatant confirmed the release of cellular contents (Otieno et al., 2019). The BOD_5 of the pre-treated WAS increased from 1 520 to 3 520 mg/L contributing to an overall 2.6-fold increase in biodegradability in the BOD_5:COD ratio.

Table 1: Characteristics of WAS before and after ozonolysis.

Parameter	Value	
	Before ozonolysis	After ozonolysis
pH	6.7	6.1
TSS (mg/L)	20.9	14.9
COD_T (mg/L)	24 500	21 600
COD_S (mg/L)	1 700	2 300
DOC (mg/L)	155	245
BOD_5 (mg/L)	1 520	3520
BOD_5:COD	0.06	0.16
Sulphate (mg/L)	18	75
Phosphate (mg/L)	67	35

Table 2: Characteristics of distillery wastewater before and after ozonolysis.

Parameter	Value	
	Before ozonolysis	After Ozonolysis
pH	5.55	5.01
COD_T (mg/L)	15 000	14 400
COD_S (mg/L)	13 500	14 000
DOC (mg/L)	5 580	6 200
BOD_5 (mg/L)	7 250	8352
BOD_5/COD	0.48	0.58
Absorbance at 254 nm (a.u.)	3.85	1.75
Absorbance at 475 nm (a.u.)	12.88	16.38

Key: COD_T – Total COD COD_s – Soluble COD DOC – Dissolved organic carbon

For the DWW, ozonation pretreatment increased biodegradability, as indicated by an increase of 21% in the BOD_5:COD ratio. The complex colour-causing biorecalcitrant aromatic compounds such as melanoidins are broken down to biodegradable acidic intermediates and simple aliphatic compounds through ozonation. The reduction of the aromatics contributed to the reduced absorbances at 254 nm for aromaticity (3.85 to 1.75 a.u.) and 475 nm for colour (16.38 to 12.88 a.u.). Also, the ozonation process achieved solubilisation of suspended organic solids, releasing them into the aqueous face, as shown by the reduction in total COD but with an increase in soluble COD. However, the total COD reduction was very low (15 000 to 14 400 mg/L), ensuring adequate biomass retention for the ensuing AD.

3.2. Effect of ozone pretreatment on anaerobic digestion of WAS

The COD and TSS reductions during anaerobic digestion of raw WAS and ozone pretreated WAS are given in Figures 2a and b, respectively. The pre-treated WAS had better COD and TSS reductions during anaerobic digestion than the raw WAS. Ozonolysis pretreatment led to the rupture of the hard cell walls and partial solubilisation (as shown by the 29% reduction in TSS in Table 1) of the sludge, availing the cellular contents and leading to improved degradation by the microorganisms. The most significant effect of the pretreatment process on AD was observed in biogas production, as shown in Figure 3. A comparison of the biogas production profiles (Figure 3a) showed that the pretreated WAS had more than double daily biogas production than the raw WAS. The daily biogas production was higher for the pretreated WAS at the beginning of digestion and remained relatively high with continued digestion than the raw WAS, which had a significantly diminished biogas production after the first day. The pre-treated WAS had a higher cumulative biogas production of 4.75 L after six days of digestion than the raw WAS which had 1.48 L (Figure 3b). Ozonolysis pretreatment solubilised part of the organic matter, which was then easily converted to biogas. The significant increase in biogas production indicates that a major fraction of the solubilised matter was biodegradable (Kim et al., 2013; Sosnowski et al., 2008).

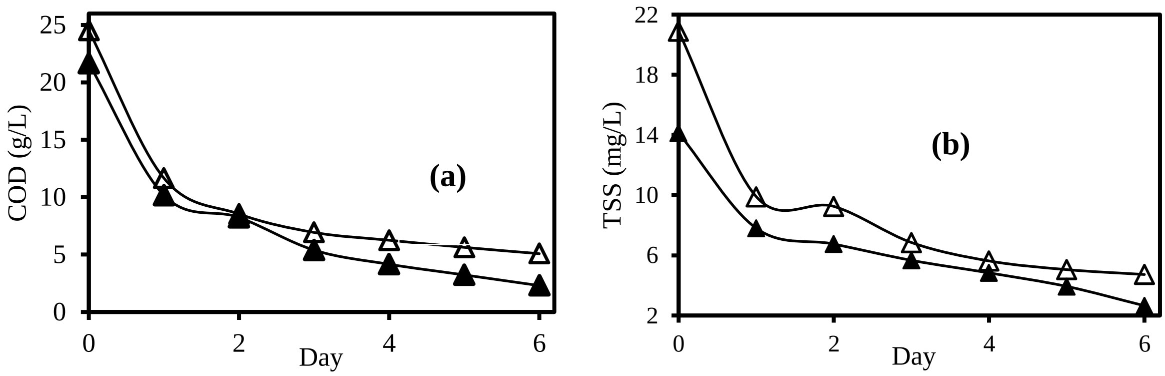

Figure 2; (a) COD and (b) TSS reductions during anaerobic digestion of raw (∆) and ozone pretreated (▲) waste activated sludge.

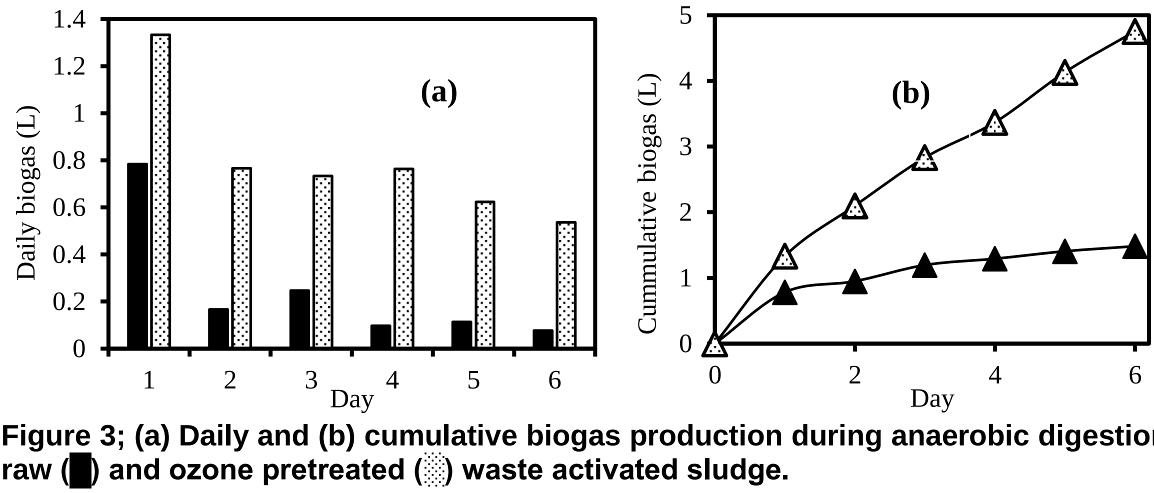

Figure 3; (a) Daily and (b) cumulative biogas production during anaerobic digestion of raw (█) and ozone pretreated (░) waste activated sludge.

3.3. Effect of ozone pretreatment on anaerobic digestion of DWW

The non-pre-treated DWW and the ozonated DWW were subjected to AD, and the changes in COD (Figure 4a), biogas production (Figure 4b), and colour (Figure 4c) were monitored. The COD removal during the digestion of raw DWW increased from 32% on day 34 to around

63% by the 37th day. The low COD reductions observed during the initial stages (days 34 to 36) were partly due to the acclimatisation of the microorganisms to the new feeding regime (semi-continuous feeding from batch). The highest reduction in COD averaged around 70% from the 42nd to 53rd day. To investigate the effect of pretreatment, ozone pretreated DWW was fed into the reactor from day 55 to 74 at the same OLR of 15 kg/m^3/d. During this period, the average COD removal remained constant at 71%, indicating that ozone pretreatment did not significantly affect the AD process, despite the significant increase in biodegradability of the pretreated DWW (Table 2). A similar observation was made with the daily biogas production, which averaged 10 L/day for raw and ozone pretreated DWW (Figure 4b). The lack of observable differences in the anaerobic digestion of the raw and pretreated substrates could be because of the relatively high biodegradability of the raw DWW, with only 2% being biorecalcitrant (Chavan et al., 2006).

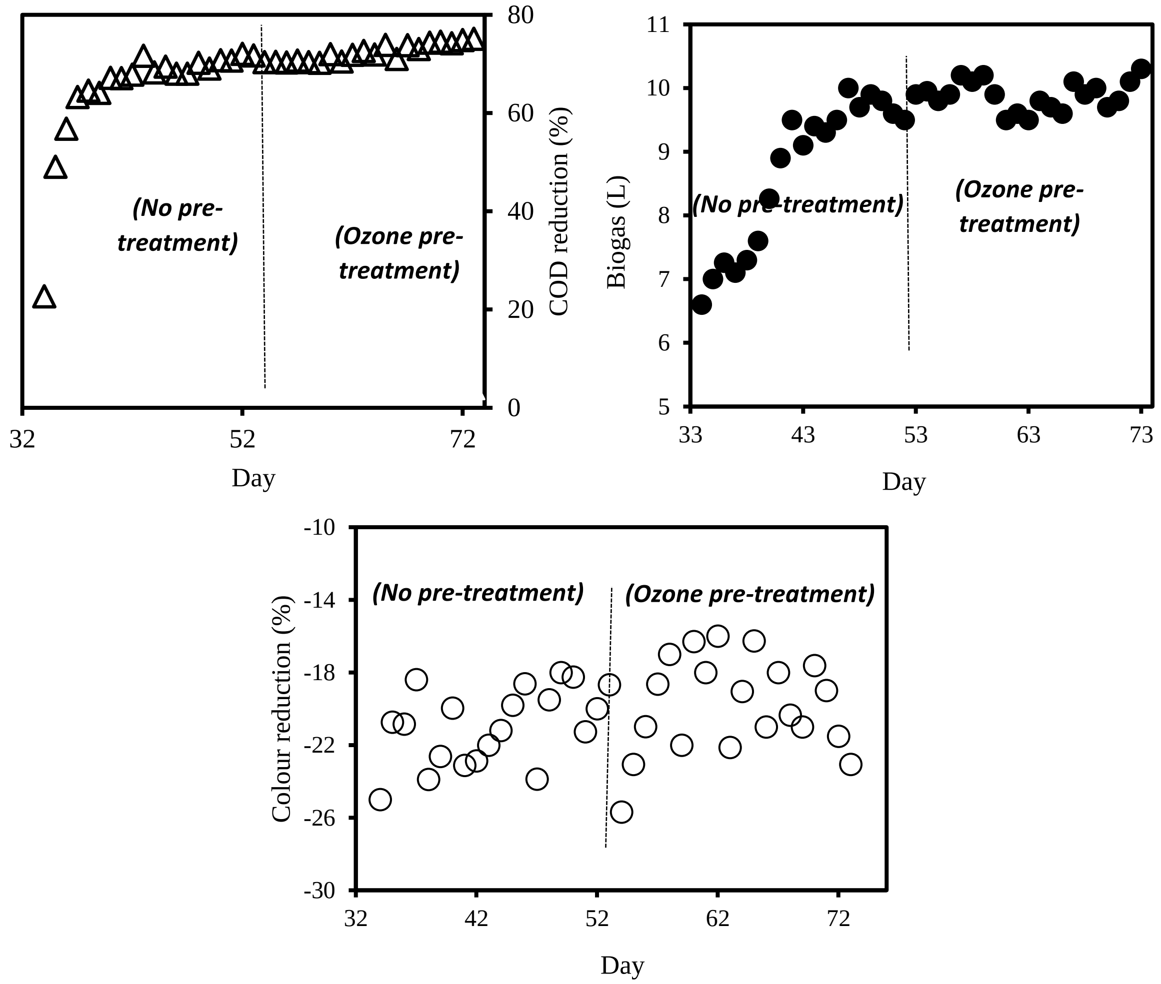

Figure 4; (a) Reduction in COD, (b) daily biogas production, and (c) daily colour reduction during anaerobic digestion of raw and ozone pretreated DWW.

A negative colour reduction indicated increased colour intensity (Figure 4c) during the anaerobic digestion of the raw and ozone pretreated DWW substrates. Under the mesophilic conditions of the UASB reactor employed, the melanoidin compounds were repolymerised into high molecular weight (usually > 5.0 kDa) long chain organics, thereby increasing the effluent's colour intensity (Liang et al., 2009; Liu et al., 2013). Ozone pretreatment was expected to eliminate the melanoidins before AD and improve colour reduction during AD. However, the increased colour intensity points to incomplete removal of the melanoidins during ozonation pretreatment. The melanoidins that remained after ozonolysis easily re-polymerised during AD, increasing the colour intensity. Ozone pretreatment is, therefore, ineffective in fully reducing the biorecalcitrant melanoidins into biodegradable compounds and ensuring complete colour removal during AD.

3.4. Ozonolysis Post-treatment of Anaerobically DWW

Table 3 shows that the DWW had a significant organic load before anaerobic digestion (BOD_5 7,200 mg/L, COD 15,000 mg/L). The BOD:COD ratio of 0.48 indicated that the DWW substrate was highly biodegradable (a ratio of 0.4 and above is recommended). Up to 75% of the COD and 95% of the BOD5 were eliminated during biodegradation, although the colour intensity was enhanced by 40%. After AD, there was still a sizeable quantity of COD present (3560 mg/L), responsible for the biorecalcitrant component, mainly melanoidin compounds that were the source of the intense colour of the AD effluent. The effluent had a BOD5:COD ratio of 0.05, confirming the elimination of all the biodegradable organics by AD. Sludge washout from the digester was indicated by the twofold increase in the total suspended solids. To remove the colour and solubilise the solids (sludge), ozonolysis posttreatment was applied to the anaerobically digested DWW effluent.

Table 3: Characteristics of DWW before and after AD

Parameter	Before	After	Change, %
pH	5.5	7.45	+62
COD (mg/L)	15000	3560	-76
DOC (mg/L)	5800	1700	-72
TSS (mg/L)	2.34	4.46	+110
BOD_5 (mg/L)	7250	190	-95
BOD_5:COD	0.48	0.05	-90
Colour/absorbance (a.u.)	3.20	4.50	+41

The ozonolysis posttreatment of the anaerobically digested AD resulted in up to 80% colour removal and a 14% reduction in DOC. The oxidation of the melanoidin molecules and the mineralisation of biorecalcitrant organic substances led to reductions in colour and DOC. Color-causing organic compounds with aromatic rings, functional groups like OCH_3 and OH, carbon double bonds (C=C), and atoms like N, P, O, and S (negatively charged) are selectively attacked by ozone (Bar Oz et al., 2018). The attack can lead to a rapid colour disappearance but with the formation of end products such as carboxylic acids and stable intermediates. The products formed are still detectable as DOC; thus, the observed low DOC reduction of 14%. Moreover, the products and intermediates are usually refractory to further oxidation by ozone (Peña et al. 2003). Ozone can also break down, resulting in the generation of hydroxyl radicals (OH●). The radicals can react (unselectively) with all organic compounds, potentially leading to total mineralisation via chain degradation reactions (Kasiri et al., 2013; Mecha et al., 2016; Wang et al., 2004).

Figures 6a and b show the changes in total (COD_T) and soluble (COD_S) COD and the BOD_5:COD ratio, respectively, during ozonolysis posttreatment of the AD effluent. Total, soluble, and suspended COD can be used to investigate the fate of sludge during ozonation. The COD_T reduced to 1250 from 1438 mg/L (13% reduction), while the COD_S was unchanged. The decrease in COD_T was attributed to the solubilisation of sludge washed out ((suspended COD) from the AD reactor. After one hour of ozonolysis, the COD_T and COD_S levels were nearly similar, indicating that up to 88% of the sludge had been solubilised. Previous investigations on the treatment of DWW with ozonolysis found low COD_T decreases within the first hour of treatment (Zeng et al., 2009; Sangave et al., 2007). The suspended COD (TSS) is dissolved during ozonolysis resulting in constant CODs, but with a reducing COD_T. After the posttreatment, the BOD_5:COD ratio of the AD effluent increased twofold (0.05 to 0.11), indicating improved biodegradability (Venkatesh et al., 2015).

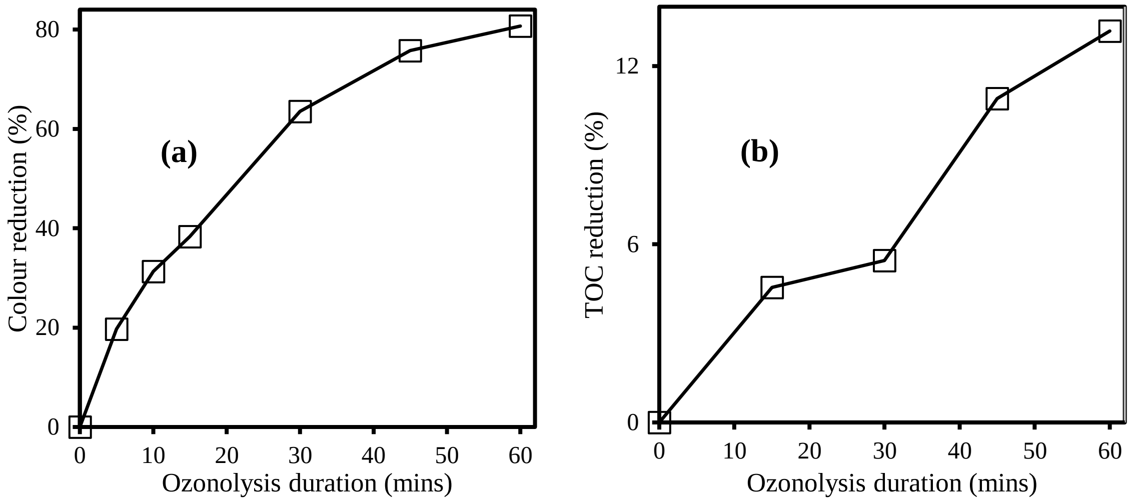

Figure 5; Colour (a) and DOC (b) reductions during ozonolysis of anaerobically digested DWW.

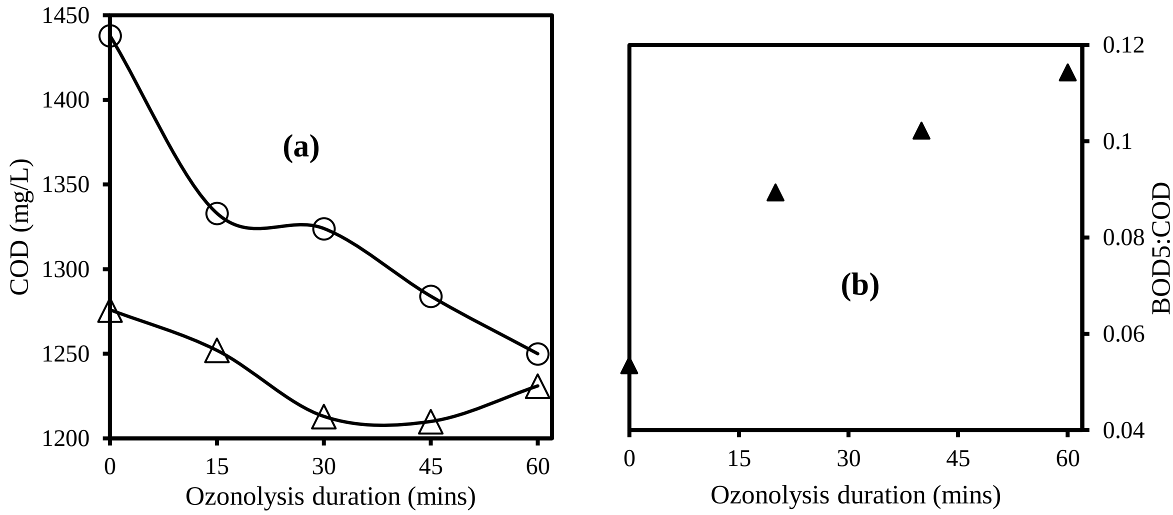

Figure 6; Change in (a) COD_T (○) and COD_S (Δ) and (b) BOD_5:COD ratio during ozonolysis of anaerobically digested DWW.

Conclusion and recommendations

An integrated AD-ozonolysis treatment system is a promising technique for treating distillery wastewater (DWW) and waste activated sludge (WAS). Ozonolysis pretreatment of WAS solubilised the sludge and led to a two-fold increase in the cumulative biogas production in the ensued anaerobic process. In the case of distillery wastewater, ozonolysis pretreatment did not significantly impact COD reduction or biogas production; however, when used for post-treatment, ozonolysis effectively removed the biorecalcitrant colour of the AD effluent and solubilised the TSS washed out from the AD unit. The configuration of the intergrade anaerobic digestion-Advanced oxidation process (AD-AOP) treatment system is thus substrate-specific. Ozonolysis should precede AD when treating WAS, while for DWW, ozonolysis should be applied as a posttreatment to AD. Kinetics and energy analyses should be determined to guide in designing an integrated AD-ozonation process for WAS and DWW treatment.

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