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ORIGINAL PAPER

Tertiary treatability of molasses secondary effluents for color and organics: performance and limits of ozonation and adsorption

C. Fall[1] · L. M. Barrón-Hernández[1] · M. T. Olguín-Gutierrez[2] · K. M. Bâ[1] · M. Esparza-Soto[1] · M. Lucero-Chávez[1]

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Abstract

Molasses wastewaters (MWWs) such as baker's yeast and distilleries effluents are usually pretreated by anaerobic, followed by aerobic biodegradation. This removes almost all the BOD, enough for meeting the current discharge standards in many low- and middle-income countries. However, as shown in the present work, the biotreated effluent still contains high levels of recalcitrant COD, color (melanoidins) and inorganic salts that end up in rivers (approx. 1000 mg/L COD, 2850 Pt–Co color units, 5000 mg/L TDS and 5400 µS/cm conductivity). To address this global problematics, and given the lack of proven cost-effective advanced treatment trains for MWW, this study assessed the performance and limits of ozonation and activated carbon (AC) adsorption (contrasting with O_3 applications on raw MWW). The applied versus the reacted O_3 doses were quantified, allowing also to estimate the ozone uptake rate as a new tool for characterizing the reactivity of the wastewaters. The effects of the treatments on different key parameters were studied: COD, color, aromaticity ($UV_{254\ nm}$), toxicity (Microtox) and biodegradability (by respirometry). O_3 reduced the color (> 95%), but causing low COD mineralization (< 35%) and biodegradability enhancement (only 8% more). Meanwhile, adsorption was efficient on both COD and color (97–91%), but needing high AC dosage. In consequence, a more sustainable treatment train was suggested, i.e., upgrading the activated sludge with aerobic granular sludge technology and transforming the granules into AC.

Keywords Activated sludge · Bakery yeast · Melanoidins · Ozone · Sorption · Vinasse

Introduction

Molasses, a sugar industry by-product generated in large quantities, is widely used as feedstock by fermentation industries, such as for baker's yeast and for bioethanol production. So, molasses containing wastewater (MWW) results from different industrial processes, mainly sugar mills (e.g., cane-based), ethanol distilleries (industrial grade or biofuels) and baker's yeast manufactures, being the latter of most interest in the present study. A great increase in MWW generation is expected with the foreseeable increase in bioethanol production in the world. Regardless of the specific source, raw MWW is a high-strength effluent characterized by an elevated soluble organic load, mostly biodegradable, but with considerable amounts of recalcitrant chemical oxygen demand (COD) very difficult to remove. It also has a persistent dark brown color attributable to the pigments of melanoidins (Hoarau et al. 2018; Zhang et al. 2019) and great quantities of total dissolved solids (TDS) and inorganic salts. Vinasse residuals from the fabrication of different beverages (Robles-González et al. 2018) bear similar characteristics; so, the problematic wastewater corresponds to a large volume of heavily contaminated effluents throughout the world. Meanwhile, nowadays treatment technologies and agricultural management practices of MWWs are very deficient, causing serious pollution threat to many surface waters and soils, more specially in low- and medium-income countries.

Raw wastewater rejected by molasses-fed yeast fermenters contains high COD levels that are easily reduced from

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✉ C. Fall
c-fa-ll@hotmail.com

[1] UAEM, IITCA (ex CIRA), Universidad Autónoma del Estado de México, col. Centro, C.P. 50000 Toluca, Mexico

[2] Dep. de Química, Instituto Nacional de Investigaciones Nucleares (ININ), Ocoyoacac, Mexico

10,000–30,000 to 2000–3000 mg/L by anaerobic digestion (Fall et al. 2012). Subsequent aerobic treatment by activated sludge follows usually, providing an effluent quality in the range of 800–2000 mg/L COD (Gengec and Kobya 2013). Most of the organic matter, almost all the biodegradable fraction, is eliminated thus by the biological processes. However, the salts, the melanoidins (complex and recalcitrant high molecular weight polymers) and other inert CODs go straight through the reactors, with almost no reduction (Blonskaja and Zub 2009). So, in addition to the recalcitrant COD it contains, yeast secondary effluents are strongly colored and present an elevated conductivity which typically end to the rivers.

Residual contamination in molasses effluents (after biological treatment) is detrimental to aquatic life in rivers, as well as to irrigation (Chandra et al. 2008; Fuess et al. 2018); additionally, a potential source of reclaimed water is being lost. Color hinders the sunlight penetration, photosynthesis and oxygen production in rivers. The inert organic matter is recalcitrant and bio-cumulative; moreover, the organics can suffer complexation reactions with metals or adsorb other toxic chemicals, amplifying the bio-accumulation and mobility of all these toxins. Residual organics combine easily with chlorine (during disinfection in plants), producing carcinogenic by-products such as trihalomethanes and haloacetic acids. Moreover, the dissolved salts increase the salinity of the water bodies and soils. Therefore, the biological treatment that commonly is the only treatment offered by yeast industries in emerging countries is not enough. Tertiary treatments are needed to better protect the environment and provide reuse opportunities. Generally, multinationals of the yeast (and ethanol) sector bypassed this requirement, localizing a majority of the industries in developing and emerging countries with relatively permissive discharge standards, such as in Estonia, Russia, India, China, Turkey, Mexico, Brazil, Iran, Syria and Tunisia (Balcioglu and Gonder 2018; Rahimpour et al. 2014; Kalyuzhnyi et al. 2005).

Many treatment processes have been tested to improve the quality of molasses effluents (Ghosh Ray and Ghangrekar 2019). These are ozonation (Jing and Li 2016), advanced oxidation, adsorption (Hadavifar et al. 2016), chemical coagulation and electrocoagulation (Martinez et al. 2018; Sahu et al. 2019; Blonskaja and Zub 2009), enzymatic decolorization (Zhang et al. 2019), membrane bioreactor, ultrafiltration and reverse osmosis (Balcioglu and Gonder 2018; Singh et al. 2018). Mostly, even when the processes seemed efficient, the studies did not go beyond the step of laboratory tests (Chandra et al. 2008); main limitations for large-scale application are the associated high doses and costs of the reactants, elevated sludge volumes and/or accelerated membrane fouling (Hoarau et al. 2018). The adequacy of the biological pretreatment is not in doubt (Gengec and Kobya 2013; Marcato et al. 2019; dos Reis et al. 2019); now, it remains to determine how to combine it cost-efficiently with the most promising advanced post-treatments, such as ozonation and/or adsorption.

Chemical coagulation (Fe and Al) was able to fulfill the effluent disposal standards in Russia (COD and Color); however, the required amounts (> 275 mg/L) of coagulant were relatively high (Kalyuzhnyi et al. 2005). Nguyen et al. (2010) compared different kinds of treatment processes (coagulation, ultrafiltration and ozonation) where ozonation gave the greatest reductions in color (86%) and COD (51%). Manganese oxide sorbents evaluated by Arimi et al. (2015) removed the color (up to 90%), but with less success on the dissolved organic carbon. Post-treatment by nanofiltration was evaluated by Rahimpour et al. (2014), which allowed to lower the COD below the Iranian river disposal limit of 200 mg/L, but very soon after, the permeate flux decreased steadily from 2300 to 1250 L/day. So, it appears that up to now reasonably cost-effective technology is not available for MWW advanced treatment. Seeking for innovative treatment trains, there is a need to gain more insight into the capacity and complementarity of the advanced processes, when dealing with biologically pretreated molasses effluents.

The unit processes studied in the literature were tested mostly in isolated form, not in synergy for the entire treatment train, which is one of the merits of the present study. In many cases too, the physicochemical processes were evaluated directly as secondary treatment alternatives on raw MWWs, or after anaerobic degradation; the present research represents a comprehensive study of the ozonation process applied to biotreated MWW (instead of raw MWW). Moreover, the ozone uptake rate and a method for its determination were proposed as new tools for characterizing the reactivity and the ozone consumption rate of wastewaters.

The aim of this study was to determine whether ozonation and activated carbon adsorption can realistically contribute to tertiary treatment trains of biologically pretreated molasses effluents, for color and COD removal. Of special interest were the estimation of the ozone consumption rate, the reacted ozone doses and the amounts of adsorbent needed, as well as their differentiated effects on COD removal, color, aromaticity, pH, toxicity and biodegradability enhancement (the latter, for a possible second step of biotreatment after ozonation). The secondary MWW used in the study originated from the water resource recovery facility (WRRF) of an industrial park (Reciclagua, Toluca, Mexico, 2018–2019). Having a centralized activated sludge process, the WRRF receives the effluents (generally pretreated) of several small industries and a bigger discharge of molasses wastewater, that by far, determines the characteristics of the whole influent.

Materials and methods

Wastewater sampling

The molasses secondary effluent subjected to ozonation and adsorption was collected after the activated sludge process of the centralized WRRF, exactly at the outlet of the secondary settlers and before the chlorination tank. The influent was anaerobically pretreated at the industry, before its arrival at the centralized plant. Eight grab samples were collected over a 7-month period. Their characterization included the following parameters: total and soluble chemical oxygen demands (COD_T and COD_S), 5-day biochemical oxygen demand (BOD_5), color (Pt/Co), solids (total, dissolved and volatile fractions), total P, nitrates, alkalinity, pH, conductivity, turbidity and in situ temperature (in situ). Major cations and anions were measured occasionally. All analyses were performed according to standard methods (APHA 2012). In particular, the COD measurements were carried out with HACH dichromate-based COD test kits and spectrophotometer (DR2800, HACH, USA).

Ozonation tests

The O_3 treatments took place at around 20 °C in semi-batch reactors (batch addition of the MWW and continuous bubbling of the O_3 gas). A schema of the experimental setup is provided as *Online Resource 1*. The ozone generator (TG10, Ozone Solutions Inc., Hull, USA) was fed with pure oxygen, having a maximum capacity of 10 g O_3 per hour. For each run, 800 mL of secondary effluent was transferred to a glass reactor of 2L equipped with a magnetic stirrer. The reactor was closed with a silicone stopper having two holes for the incoming and the outlet gases. The inlet gas flow was brought into contact with the WW through a porous stone. A three-way bypass valve in stainless steel and Teflon was installed after the generator. At the end of preselected times, the O_3 generator was turned off, but still without interrupting the oxygen gas flow; this was to flush out the residual ozone in the reactor (if any) and in the headspace. During the ozonation, the off-gas transited in two serial washing bottles, filled each with 900 mL of potassium iodide solution (2% KI). For each WW grab sample evaluated, the duration of the ozonation was varied in five levels (0, 2, 5, 10 and 15 min, identified as the T0, T1, T2, T3 and T4 treatments), providing different O_3 doses.

The quantity of ozone consumed in the reaction (mg of O_3 per g of COD) was calculated for each run, by monitoring the O_3 mass entering and leaving the batch, according to recommended methods (Rakness et al. 1996). The gas flow rotameter was adjusted at 2 L/min. The analyzer (API 454) provided the pressure and the O_3 concentration in the inflow gas-phase. The actual pressure and temperature were registered during each run, being around 13.55 psi and 293 K (*Online Resource 1*). On other side, the residual unreacted O_3 mass leaving the reactor was quantified, based on the KI chemistry method. The dissolved O_3 in the bulk liquid was assumed to be negligible (Chu et al. 2008; Lee and von Gunten 2012); if not, anyway the final purge of the reactor with pure oxygen flushed out any potential O_3 residuals. Registering all the above-mentioned data and conditions allowed to precisely quantify the applied and the reacted ozone doses. Moreover, the efficiencies of the ozonation process were monitored through the changes on the COD, color, toxicity, aromaticity ($UV_{254\ nm}$), biodegradability, pH and conductivity, as a function of the ozone doses. The biodegradability and toxicity tests are described later.

Activated carbon adsorption

Batch adsorption tests were carried out to evaluate the activated carbon (AC) for polishing the biologically pretreated yeast wastewater. In view of different possible treatment trains, the tests included two types of secondary WWs, i.e., the non-ozonated (T0) and the pre-ozonated one (T1 and T4, respectively, at 2 and 15 min of O_3). Sigma-Aldrich provided the AC (Darco^R, 12–20 mesh). The sorbent is representative of the characteristics of typical commercial carbons and was used as a surrogate adsorbent. Different masses of AC from 0 to 6 g were put in contact with 35 mL of each WW, in six glass centrifuge tubes, at room temperature (18–21 °C). At the end of the equilibration time of 1 h (based on previous kinetic runs, data not shown), the tubes were centrifuged to decant the liquid and measure Ce and the residual concentrations on it (COD, color, toxicity and UV_{254} absorbance). For COD and color, the adsorbed quantities in the solid phase (qe) were recalculated through mass balancing, allowing to obtain the isotherms (qe vs. Ce). The isotherms data were analyzed to identify the best fitting models. These were extrapolated to estimate the carbon usage, depending on the position of the adsorption process in the overall treatment train. With respect to toxicity and UV measurements, their reduction was quantified (%).

Biodegradability enhancement testing through BOD and respirometry

Tests were carried out to quantify whether ozonation improves the biodegradability of the recalcitrant COD remaining in the secondary WW. Moreover, the tests aimed to know whether the bio-COD potentially produced was

readily or slowly biodegradable (S_S and X_S COD fractions, according to ASM1; Henze et al. 2000). Such knowledge informs about the pertinence of a second stage of biodegradation in the treatment trains (after ozonation). Firstly, the BOD time curves were obtained for the ozonated WW samples, allowing to estimate the BOD_5 values, the BOD_5/COD index, but also the total biodegradable COD content (bCOD). Secondly, the readily biodegradable COD fraction (S_S) was measured through batch respirometric tests. The counterpart, the slowly biodegradable COD fraction (X_S), remains as the bCOD minus S_S.

The BOD time measurements were carried out with a BODTrak™ equipment (HACH, USA) allowing the simultaneous analysis of up to six samples of pre-ozonated WW at different doses. The BOD bottles were filled with 160 mL of reaerated WW, inoculated with 0.5 mL of diluted mixed liquor from the WRRF plant and incubated at 20 °C for up to 10 days, together with the oxygen sensors. Nitrification was observed in the preliminary assays and was suppressed later in the following tests, by adding 15 mg/L of allylthiourea to the bottles. Concerning the S_S measurements, a respirometer having four reactor cells was used, providing the capacity of carrying out four simultaneous tests at a time (two replicates with T0 and two with T4). The experimental setup and the components of the respirometer are described more thoroughly elsewhere (Fall and Silva-Hernández 2017, Fall et al. 2018). A standard respirometric test procedure was followed (low F/M test; Henze et al. 2000): 550 mL of WW sample and 150 mL of mixed liquor from the WRRF were added to the respirometric cells, together with 15 mg/L of allylthiourea. Then, the oxygen uptake rate (OUR, mg/L h) was measured until reaching a quasi-plateau. S_S was calculated later from the area under the respirogram (OUR versus time), as explained in the results section.

Acute toxicity tests

Before and after ozonation, the toxicity of the samples was determined by using the Microtox™ system (Azur Environmental Inc., California, USA). The tests aimed to detect the toxicity of the intermediate products resulting from the oxidation. The manufacturer's standard basic test protocol was used, analyzing each WW sample at four dilution levels (from 5.6 to 45%). The reagent and materials (lyophilized *Vibrio fischeri* bacteria, osmotic adjusting solution, reconstitution solution, diluents, cuvettes and pipette tips), as well as the M500 luminescence analyzer, were from the local provider. Toxicity data analysis and reporting were carried out automatically with MicrotoxOmni™ 1.18 Software. The final result obtained was the half-effective concentration (EC50, causing a 50% biomass activity reduction) that was converted later into toxicity units (TU = 100/EC50). The samples showing hormesis (i.e., having an EC50 higher than 100% or TU < 1) were considered as non-toxic.

Results and discussion

Special characteristics of the secondary molasses effluent

Table 1 presents the average characteristics of the WRRF secondary effluent, as measured from eight grab samples. In general, the parameters were relatively stable, as indicated by the coefficients of variation (CV < 15% for pH, TDS and *T*; versus 25–40% for color and COD).

The suspended solids and turbidity were low; the contaminants were mostly in soluble form (> 98%). The BOD_5 was successfully reduced (< 45 mg/L). In contrast, the secondary effluent remained dark brown colored (2855 Pt–Co units), with warm temperature (28 °C), high conductivity (5387 µS/cm), high TDS (5042 mg/L) and high residual COD (979 mg/L). The inorganics predominated in the TDS (salinity), but the remaining 14% formed by the organics were too problematic, being responsible for the high and non-biodegradable COD (BOD_5/COD of 0.04). The pH of around eight remained very stable (CV of 5%), being consistent with the usual pH of the bakery yeast cultures (7.8–8.7; Gengec and Kobya 2013) and with the buffer capacity shown by the wastewater (around 1740 mg/L $CaCO_3$). Sulfate (1213 mg/L SO_4^-), sodium (1084 mg/L Na^+) and chlorides (871 mg/L Cl^-) were found in much more quantities, compared to calcium and magnesium (76 and 39 mg/L, respectively). The detected ions were the main causes of the

Table 1 Mean characteristic of the biologically pretreated MWW (secondary effluent)

Parameters	Mean* (mg/L)	CV (%)	Parameters	Mean* (mg/L)	CV (%)
Temperature (°C)	28	1	Cl^-	871	55
pH (–)	7.9	5	SO_4^{2-}	1213	37
Alk ($CaCO_3$)	1736	21	$N–NO_3^-$	15	–
Conductivity (µS/cm)	5387	51	Total P	7	48
Color (Pt–Co U.)	2855	25	Ca^{2+}	76	–
Total COD	979	38	Mg^{2+}	39	–
BOD_5	41	–	K^+	307	–
BOD_5/COD (–)	0.04	–	Na^+	1084	–
TDS	5042	4	TDS/TS (%)	> 99%	–
Turbidity (NTU)	15	35	VDS/TDS (%)	14%	–

*Units in mg/L, unless otherwise specified

CV coef. of variation (%), *Alk* alkalinity, *TS* total solids, *TVS* total volatile solids, *TDS* total dissolved solids, *TSS* total suspended solids, *VSS* volatile suspended solids

elevated conductivity and TDS, being part of the footprint of molasses WWs. Accessorily, it was also important to verify the chlorides levels (< 2000 mg/L Cl^- allowed), due to their potential interference on COD analyses.

The warm temperature of the WRRF effluent (28 °C, well above the average ambient *T* of 15 °C) was also in concordance with the mesophilic temperature range used in yeast culture (32–35 °C) and in the subsequent anaerobic digestion. From all points of view, the studied effluent was similar to the secondary yeast WWs depicted by different authors: by Jing and Li (2016), with 853 mg/L COD, 46 mg/L BOD_5, dark brown color, 14,300 mg/L TDS and pH 8.1; by Arimi et al. (2015) with 1500–1900 mg/L COD, 60 mg/L BOD_5 and 4650 µS/cm of conductivity; and by Rahimpour et al. (2014) with 2000 mg/L COD, 43 mg/L TSS, 6400 Pt–Co color units and 3200 µS/cm conductivity. Moreover, Balcioglu and Gonder (2018) reported the typical yeast WW dark brown color, along with the following values: 500 mg/L COD, 7430 µS/cm conductivity, 1950 mg/L Cl^-, 300 mg/L SO_4^-, pH 7.5, 24 mg/L TKN and 4 mg/L total P.

In general, the BOD_5, N and P contents of the biologically treated WW were successfully lowered. By this, the treated effluent (directed to a river) complies with the national discharge standards NOM-001 (DOF 1997; < 150 mg/L BOD_5, 40 mg/L total N and 20 mg/L total P). However, with its dark brown color, high salt content and conductivity, and large amounts of persistent organics, the discharge contributes a lot to the pollution of the Lerma River (Fall et al. 2007). This illustrates the situation of the huge amount of yeast industries (and distilleries) installed in the Third World. Moreover, with the foreseeable increase in bioethanol production in the world (Anwar Saeed et al. 2018; Ghosh Ray and Ghangrekar 2019), a limit is reached regarding the practice of ferti-irrigation as primary disposal method of vinasse: odors and salinization impacts and few available lands (Fuess et al. 2018; Coelho et al. 2017; Marcato et al. 2019). So, research of cost-efficient treatment trains for the MWWs is unpostponable.

In conclusion, yeast effluents disposal in rivers could be meeting the current standards in many developing and emerging countries, although the secondary treatment typically provided is clearly insufficient. Tertiary processes are needed to deal with the persistent color, recalcitrant COD and inorganic salts that reach the surface waters.

Ozone doses and reactivity of the wastewater

Specific ozone doses are usually reported in two different ways: the applied ozone dose (AOD) and the reacted ozone dose (ROD, also transferred). Both are represented in Fig. 1 for the studied wastewater. The applied doses are easily determinable from the inlet ozone gas-phase concentration

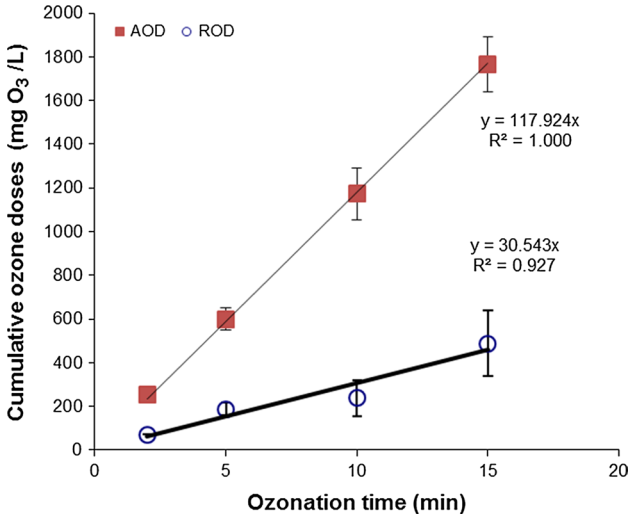

Fig. 1 Ozonation of the biologically pretreated yeast effluent: applied ozone dose (AOD) and reacted ozone dose (ROD) as a function of treatment time

and its flow rate (in average 2 L/min at 3.95 wt% of O_3 in O_2, knowing that 1 wt% is equivalent to 14.29 mg of O_3 per L of gas, STP). Hence, the supplied ozone mass flow was constant and equal to 5.7 g O_3/h. Five treatment times were used, ranging from 2 to 15 min of ozonation; this made the cumulative AODs to range approximately from 0.25 to 1.8 g O_3/L, increasing linearly with ozonation time (Fig. 1).

Regarding the reacted ozone doses (RODs), it was determined as the difference between the AOD and the unreacted O_3 (captured in the KI traps) (*Online Resource 1*). The ROD increased linearly (Fig. 1, $R^2 = 93\%$) with ozonation time, varying between 0.07 and 0.50 g O_3/L (for 2–15-min treatment). Expressed in mg of O_3 per g of initial COD, for ozonation times from 2 to 15 min, the corresponding specific ROD doses varied between 40–80 and 300–550 mg O_3/g COD_{ini}, depending on the initial COD level of the secondary WW at each of the sampling campaigns.

Many studies only mention the AOD quantities (Watt et al. 1989; Ekblad et al. 2019); however, behind the ROD there is a powerful method to gain insights into the kinetics of the ozonation process and the reactivity of each wastewater. Firstly, the ozone transfer efficiency (OTE) can be assessed as the quotient ROD/AOD, being constant here at 26%. The OTE is reported for informative purposes, without any further action for optimizing the gas transfer system that was utilized; in real-scale systems, the choice of the diffusion system to maximize the OTE is important for economic reasons. Secondly, the linearity between ROD and time can provide the ozone uptake rate as explained hereafter. The linear trend was observed also in other studies (Watt et al. 1989; Paul and Debellefontaine 2007), but this

has rarely been used beyond the information it provides in terms of doses. The linearity suggests a zero-order reaction, instead of first order that is usually found in micro-contaminants ozonation. By this way, the slope of the ROD curve represents the O_3 consumption rate or ozone uptake rate (O_3UR, mg O_3/L h), a parameter that was introduced by Fall et al. (2018), as a way for characterizing and comparing the reactivity (ozone consumption rate) of different wastewaters or sludges. From Fig. 1, the ozone uptake rate of the studied MWW (1000 mg/L COD approx.) was estimated to 30.5 mg O_3/L min or 1.83 g O_3/L h. Normalizing the ozone doses with the COD is a common practice (Ekblad et al. 2019). So, to be able to compare with other wastewaters, and regardless of the COD content, the specific ozone uptake rate (SO_3UR) can be calculated as the ratio between the O_3UR and the initial COD concentration. The SO_3UR of the studied MWW was 1.5 g O_3/g COD h. This can be compared with the O_3UR of three mixed liquor suspensions (each at 1000 mg COD/L) that were evaluated by the latter author (sludge reduction context, Fall and Silva-Hernández 2017; Fall et al. 2018), being, respectively, 0.7, 1.1 and 3.8 g O_3/g COD h, varying with the kind of sludge. In a work of Jing and Li (2016), the reacted ozone quantities were measured for a yeast WW, reporting the overall consumption (around 5.5 g O_3/g COD), but not the rates (g O_3/g COD h).

In the literature, the effective ozone consumption rate (O_3UR or SO_3UR) has not been very often determined for wastewaters. Instead, the O_3 consumption rate is frequently of interest in trace micro-pollutants oxidation in clean waters. In the latter case, a pseudo first-order kinetic process is assumed (rate $= k_1 * O_3$) and the k_1 constant is obtained by monitoring the decay of the O_3 in time, in a batch reactor containing the trace contaminants (Lee and Von Gunten 2012). In the case of wastewaters (excess organic matter), the dissolved O_3 is generally near zero (Jing and Li 2016; Chu et al. 2008) and is hardly measurable. Moreover, as previously suggested, a zero-order reaction prevails (rate $= k_0$). Thus, another approach as the one used in the present study (ROD vs. time data) is needed to allow estimating k_0 (mg O_3/L min). The apparent zero-order constant corresponds to the O_3UR parameter, being the slope of ROD time curve, as shown in Fig. 1.

In summary, ozone consumption of the MWW increased linearly with treatment time (0.07 to 0.50 g O_3/L), revealing a zero-order reaction type and an OTE of 26%. Moreover, it is shown that the SO_3UR parameter provides a way for comparing the reactivity and the O_3 consumption among different WWs (1.5 g O_3/g COD h, for the studied MWW).

COD and color removal by ozone

Ozone considerably reduced the color, but just a little COD (Fig. 2). The error bars shown in Fig. 2 reflect the variability

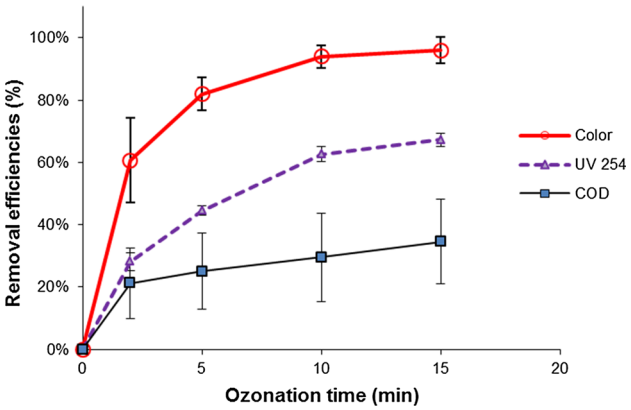

Fig. 2 COD, color and UV absorbance removal efficiencies with time

of the ozone effects on different samples of the same effluent (collected at different months). At the highest O_3 dose (15 min ozonation), the mean removal efficiency reached 96% (±4%) for color, against 35% (±14%) for COD; in average, this corresponds to decrease in color from 2855 to 114 Pt–Co units (Fig. 3a), against from 1230 to only 780 mg/L for COD, after 15 min of O_3. For color, as well as for COD, the removals increased with the treatment time (Fig. 2); however, most of the performance (two-thirds) was achieved in the first 2 min of treatment, i.e., with low ROD dose (0.07 g O_3/L). If 5 min of O_3 removed up to 80% of the color (28% of COD), doubling or tripling this time provided only an additional 15% removal when more (7% in COD). Apparently, beyond 15 min of treatment (ROD > 0.5 g O_3/L), removals will improve only marginally.

Overlapping Figs. 1 and 2 allows seeing that other reactions, not resulting in COD and color changes, are affecting the O_3 balance. For up to 15 min, ozone was consumed at the same rate, while COD and color elimination slowed down more and more. The early plateauing and the low COD removal have been observed by other authors working on similar WWs. In the present study, the maximum efficiencies obtained were 96% for color against 35% for COD, compared to 86–96% of color versus 31–56% of COD in the following cases: Nguyen et al. 2010; Jing and Li 2016; Balcioglu and Gonder 2018. No improvement was able to increase significantly the COD removals in MWWs, e.g., by O_3/H_2O_2 advanced oxidation (Hadavifar et al. 2016; Jing and Li 2016). Moreover, catalytic ozonation was tested by other authors, provided only 51% COD removal (magnetic carbonaceous nanocomposite catalyst; Rahimi et al., 2018). In contrast, in the case of other types of contaminants and wastewaters, catalytic ozonation was always more efficient, e.g., with Ce-substituted goethite for degradation of dimethyl phthalate (Bai et al. 2017), or with iron-loaded zeolite for municipal WW (Ikhlaq et al. 2019). Similarly, in many other WW types (such as for pulp and paper; Munir et al.,

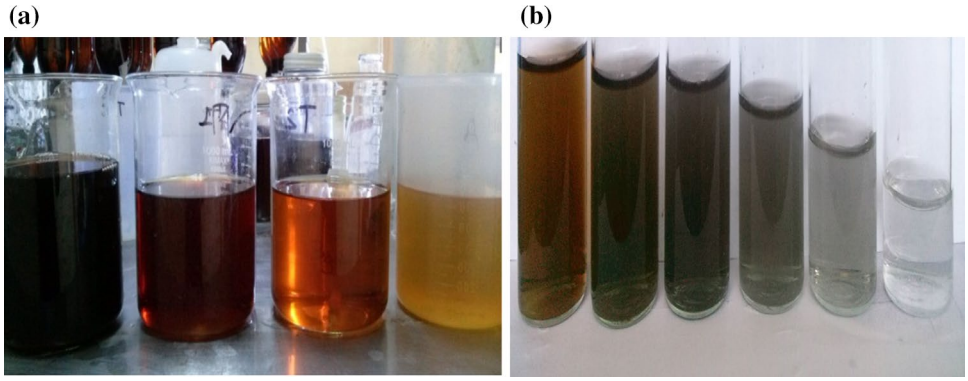

Fig. 3 Visual comparison of color removal: **a** at increasing ozone doses (0-, 5-, 10- and 15-min ozonated WW, left to right beakers); **b** and by AC adsorption at increasing carbon doses (left to right supernatants from treatments with 1 to 6 g of AC in 35 mL of WW)

2019), the advanced oxidation processes (AOP of O_3/H_2O_2 and O_3/H_2O_2) showed better performance than O_3 alone. Thus, there is a need to test more catalysts types and AOP options and to understand the limitations in molasses WWs. The fact that color was readily removed by O_3, unlike the COD, suggests that ozonation only modifies the unsaturated chemical bonds (chromophores) of the melanoidins, without actually mineralizing the organic matter.

In synthesis, O_3 can decolorize the secondary MWW but difficultly reduces the COD. Great part of the removals occurs at low O_3 doses, with little or no additional benefit at higher doses.

pH, conductivity, UV absorbance and toxicity changes due to ozonation

Apart from color and COD, changes on other parameters such as pH, conductivity, UV_{254} and toxicity were also followed. As in Jing and Li (2016) where pH decreased slightly from 8.1 to 7.8 (yeast wastewater), in the present study too, there was only a little decrease in the pH (0.2 units) after 15 min of ozonation. pH dropping within a small range indicates that hydroxide ions consumption was not high in the reaction, contrary to what would be occurring when ozone indirect-reaction pathway (via the OH* free radicals) becomes significant. So, apparently, O_3 reacted with the WW mainly through direct ozone oxidation pathway, probably due to scavenging of the OH* radicals by the bicarbonate ions present in the WW (Jing and Li 2016). Concerning the conductivity, no significant changes occurred when the ozone doses increased.

UV absorbance at 254 nm wavelength (UV_{254}) is an indicator of the amount of aromatic organic compounds (melanoidins) in WWs. After ozonation at different doses, UV absorbance was measured in all samples, using several dilution levels with each sample (1 + 0, 1 + 5, 1 + 10, 1 + 15 and 1 + 30, v/v of WW + distilled water). With 1 + 5 dilution and greater, the absorbance of all samples dropped below the preferred working range (< 2 cm^{-1}). When absorbance removal (expressed in %) was calculated as a function of the ozone doses, a single curve was obtained (Fig. 2, UV_{254}), independently of the dilution levels (1 + 5 to 1 + 30). As shown, the aromaticity of the compounds in the WW declined by 28% after 2 min of ozonation, and by 63 and 67% after 10 and 15 min of treatment. The maximum removal efficiencies were in the following order: color > UV_{254} > COD (96% > 67% > 35%), similar to the trend shown by Balcioglu and Gonder (2018) (96–98% color > 67% UV_{254} > 56% COD). Thus, ozonation affects the different chemical bonds unevenly: chromophores > aromatics > COD. All groups belong in organics, being the chromophores (melanoidins) a subgroup of the aromatics, and the aromatics a subgroup of the overall organics detected as COD; so, in terms of abundance, the sequence is in the reverse order. The aromatics (and even more its melanoidins subgroup made of colored high molecular weight polymers) are more prone to ozone attacks than the remaining organics (aliphatic). Ozone has the ability to directly attack the C double bonds in aromatic chromophoric molecules, leading to the formation of aliphatic "bleached" products (Sigge et al. 2007).

Concerning the Microtox test, it was used as screening tool for detecting the potential toxicity of the intermediate products from the ozonation process. This assessment was

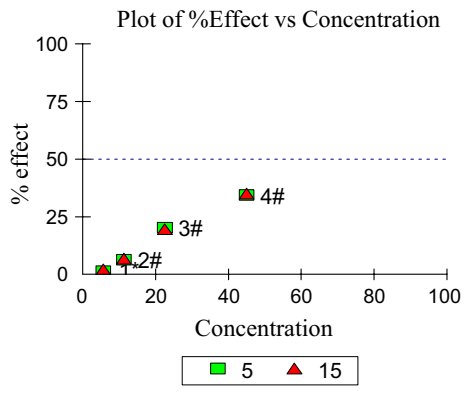

Fig. 4 Toxicity effects of the T2 sample diluted at different concentrations (in %) and incubated for 5 min (square markers) with the test bacteria (vs. 15 min, triangles)

important for the scenario of discharging the ozonated effluent in rivers, but also in the event of considering a second stage of biodegradation after ozonation. As an example with the T1 sample (2-min ozonated WW), Fig. 4 illustrates the results from the Microtox analyzer. The effect of the WW sample (at different dilution levels from 0 to 100%) on the test bacteria is quantified through the reduction of the luminescence emitted by the *Vibrio fischeri* species.

Toxicity readings were made after incubating the mixes (diluted WW samples + *Vibrio fischeri* bacteria) for 5 min and then for 15 min. The effects at 5 and 15 min resulted in similar values, as expected when toxicity is not due to metals. The half-effective concentration (EC_{50}, dotted line in Fig. 4) was obtained by extrapolating the dose–response curve of each ozonated sample and was converted then in toxicity units ($TU = 100/EC_{50}$). For instance, the EC_{50} for the T1 sample occurred at 65% dilution, which corresponds to 1.54 TU. Overall, for T0, T1, T2, T3 and T4, the toxicity levels detected were very low, being 1.27 and 1.54 TU for the first two samples (0- and 2-min ozonation), and decreasing even more (< 1 TU) in the remaining samples (i.e., for 5-, 10- and 15-min ozonation). This means that ozonation generally not increased the toxicity of the secondary yeast WW. Indeed, based on the scale proposed by Bennett and Cuttage (1992), the state changed from "lightly toxic" for T0 and T1 to "non-toxic" for T2, T3 and to T4. Gomes et al. (2013) also reported a case where ozonation did not result in toxic by-products either (3.08 TU for raw cork-boiling WW, against 1.24 TU after ozonation). Moreover, a wastewater which is not toxic to *Vibrio fischeri*, in principle, could not be toxic to activated sludge bacteria; as a proof concerning the relative sensitivity of the Microtox test, Fall et al. (2007) reported a micro-toxicity of 9.3 TU for a municipal WW that, afterward, was very well biotreated (< 1 TU). No chromatographic analysis was carried out to identity the intermediate compounds.

It can be concluded that the O_3 reaction with the organics apparently occurred through direct ozone oxidation pathway and did not result in toxic end products. Ozonation affects the different chemical bonds unevenly: chromophores > aromatics > overall COD.

Biodegradability enhancement due to ozonation (BOD and respirometry tests)

The BOD analysis was used for estimating the total biodegradable COD (bCOD), while the respirometric tests served for dividing the bCOD into readily (S_S) and slowly (X_S) biodegradable fractions. Figure 5 (left) shows the results of one of the BOD test series carried out with the secondary effluent, before ozonation (T0) and after 2, 5 10 and 15 min of O_3 (T1 to T4).

The higher the ozone dose, the greater the BOD generated. However, as for color and COD removals, here also the BOD increments were more perceptible between the lowest doses.

The BOD time curves were fitted to the BOD equation (Eq. 1), providing the two parameters of the model, i.e., the ultimate BOD (BODu) and the first-order kinetic constant (k), and all of them are reported in Table 2. The bCOD of each of the samples could then be estimated from the BOD_u values through Eq. 2 (Roeleveld and van Loosdrecht 2002), taking into account the fraction of biomass ($f_p = 0.15$) that is converted into inert COD with biomass lysis, as suggested in the activated sludge models (ASMs) (Henze et al. 2000):

$$\mathrm{BOD}(t) = \mathrm{BOD_u}\left[1 - \exp\left(-kt\right)\right] \quad (1)$$

$$\mathrm{bCOD} = \mathrm{BOD_u}/\left(1 - f_p\right) \quad (2)$$

Table 2 summarizes the different estimations: BOD_5 (5 days), BOD_u, k values and the total biodegradable COD,

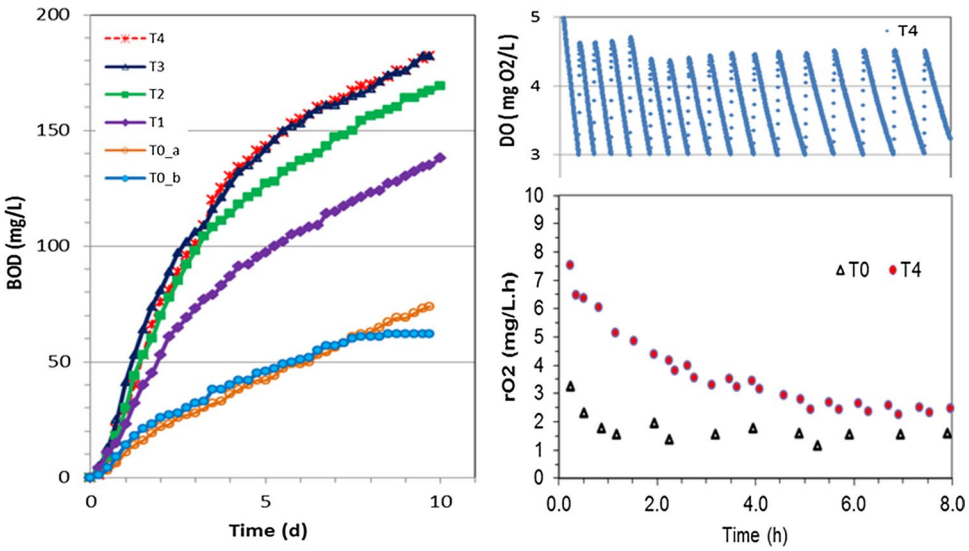

Fig. 5 Biodegradability enhancement by O_3: BOD (left) and respirometric (right) tests results

Table 2 BOD parameters of the secondary MWW treated at different ozone doses

Ozone treatment	BOD_5 (mg/L)	BOD_u (mg/L)	bCOD/COD_{tot} (%)	k (d^{-1})	R^2 (%)
T0	40 ± 4	85 ± 5	6	0.13 ± 0.04	> 99.1
T1	97	155	11	0.20	99.6
T2	127	184	13	0.23	99.4
T3	142	195	14	0.26	99.7
T4	148 ± 4	191 ± 8	14	0.33 ± 0.06	> 99.1

T0 = non-ozonated sample; T1 to T4 = ozonated samples for 2, 5, 10 and 15 min, respectively

± *SD* standard deviation of four replicates, R^2 coefficient of determination of the regressions

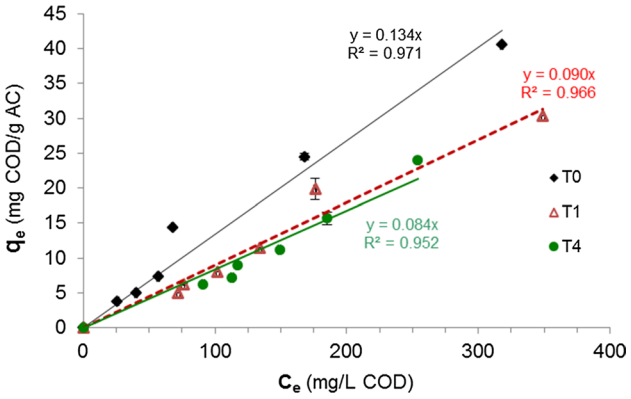

Fig. 6 COD adsorption isotherms by the activated carbon (AC)

expressed as a fraction (bCOD/CODtot). The rate constant k increased with the ozone dose, varying between 0.13 and 0.33 d^{-1}. No information was found in the literature about k for molasses WWs. For comparison, k values reported in Netherlands municipal wastewaters were between 0.33 and 0.70 d^{-1} (Roeleveld and van Loosdrecht 2002), showing a tendency of increasing with the S_S fraction present in the WWs.

The ozone treatment increased the BOD_5 of the samples, from 40 mg/L (non-ozonated WW) to a maximum of 148 mg/L (after 15 min of O_3). The biodegradability index (bCOD/COD_{tot}) shifted from 0.06 for raw secondary MWW to 0.014 at maximum after ozonation. This is only 8% more bCOD, which is comparable to the 10% increase reported by Robles-González et al. (2018), thus not too attractive for creating a second biodegradation step in the treatment train.

Concerning the respirometric tests, Fig. 5 (right) visualizes the raw data collected (DO conc. during the on–off aeration cycles) and the oxygen uptake rates (OUR or r_{O2}) calculated from them subsequently, for T0 and T4. The OURs decreased in time, reaching later a quasi-plateau that marks the depletion of the S_S substrate. The S_S of the samples was calculated by Eq. 3:

$$S_S = \frac{\Delta O_2}{1 - Y_H} * \text{Dilution} \quad (3)$$

where ΔO_2 represents the oxygen consumed in total (area under the OUR curve, up to the plateau; mg O_2/L). Y_H is the heterotrophic biomass yield, assumed as its default value in ASM1 (0.67 mg COD/mg COD). The dilution factor is the total volume of WW and sludge, divided by the WW volume. The S_S results were 2.5 mg/L COD in T0, shifting up to 50 mg/L COD in T4 (the highest ozone dose), which is not a considerable increase compared to the total COD of the WW used (893 mg/L). In percentage, the S_S in T0 was 0.3% of the total initial COD, compared to 5.6% in T4. Taking into account that the overall biodegradable fraction (bCOD) was 6% in T0 and 14% in T4, the slowly biodegradable fraction could be calculated as $X_S = \text{bCOD} - S_S$. Thus, from T0 to T4, the ozonation increased the X_S fraction from 5.7 to 8.4% of the total COD, compared to from 0.3 to 5.6% for the S_S fraction. Two-thirds of the additional bCOD produced by the ozonation were in S_S form, against one-third as X_S.

In summary, ozonation increased the biodegradability of the secondary MWW, but not enough for justifying a second biodegradation step in the treatment train. Only 8% more COD was turned biodegradable, 2/3 of which as readily biodegradable form.

Sorption isotherms and carbon usage

The ozonation stage is indubitably useful for color mitigation (> 95%), but for more COD removal, other processes such as adsorption are needed. The activated carbon successfully reduced the COD (up to 97%) as well as color (up to 100%; Fig. 3b). Figure 6 shows the data of the batch adsorption tests, being adequately fit ($R^2 > 95\%$) with linear isotherm models (Eq. 4):

$$q_e = K_d * C_e \quad (4)$$

where C_e (mg COD/L) and q_e (mg COD/g AC) represent the equilibrium concentrations in the liquid and solid phases; the slope K_d is the distribution constant (L/g AC).

The K_d value was 0.13 L/g AC for the non-ozonated WW versus 0.08–0.09 L/g AC for the pre-ozonated samples. The corresponding adsorption capacities (q_e) were 120 and 71 mg COD/g AC, respectively, for the T0 and T1 (calculated at Ce equal to the influent conc., as in a column contactor). The AC adsorbed the organics of the WW, better in its original form (non-ozonated), than after ozonation. Moreover, K_d was practically similar for T1 and T4, being

a trend that was yet noted in many other parameters, with respect to the effects of the increasing ozone doses.

As for COD, the adsorbent reduced very well the color, aromatics and toxicity (data not shown). The removal efficiencies on T0 were similar for the aromatic fraction (UV_{254}) and for the overall organic matter (COD), at each of the AC doses. On the contrary, for the ozonated WW (T4), the aromatics were slightly better adsorbed than the whole COD fraction; this means that the aliphatic intermediates resulting from the ozone treatment were less adsorbed. This explains why K_d was smaller for the ozonated WWs (T1 and T4), compared to the non-ozonated one (T0).

By doing some additional hypotheses, the COD isotherms were extrapolated to estimate the AC masses needed for treating the WWs in continuous, in fixed beds (*Online Resource 2*). The carbon usage rate calculated per L of WW was 13 g of AC for T0 versus 20 g for T1–T4 (dynamic capacities). Despite the good adsorption capacity of the carbon, the great amount of COD present in the secondary effluent needs a relatively high adsorbent usage. Getting access to a cheap adsorbent is necessary for having a sustainable adsorption process.

For the same type of WW, the AC adsorption capacity was estimated to 70 mg COD/g AC by Hadavifar et al. (2016). Meanwhile, Gengec (2015) evaluated a cheap material (polyaniline-aluminosilicates) to polish a biologically pretreated molasses effluent; 88% color and 63% COD were removed with a dose of 8 g of adsorbent per L of WW. Vinasse itself was successfully converted into AC (Kazak et al. 2017). A Mn oxide sorbent was evaluated by Arimi et al. (2015), reaching 90 and 70% removals for color and for COD, but the adsorbent usage was very high. AC produced from a low-cost bio-waste material (bagasse fly ash) removed 60% of color and COD, needing 40 g of AC per L of spent wash (Nure et al. 2017).

In synthesis, recalcitrant organics in MWW are removable by AC adsorption, but better before, than after ozonation. Both COD and color are well removed, needing approx. 13 g AC/L WW.

Discussion (treatment train proposal)

Nowadays, treatment of yeast effluents in low- and middle-income countries is limited to anaerobic digestion (UASB), followed by conventional aerobic activated sludge (Gengec 2015; Blonskaja and Zub 2009). This removes a lot of BOD, meeting generally the discharge standards of the host countries. However, the secondary treatment does not deal with other important types of unregulated contaminants (color, recalcitrant COD and inorganic salts) that severely affect the rivers. Stricter disposal standards and sustainable tertiary treatment trains are needed for the molasses effluents (Singh et al. 2018).

Taking into account the strengths, weaknesses and complementarity of ozonation, adsorption and others advanced processes, as learnt from the present study (secondary effluent) and from the literature, an alternative treatment train is proposed for further researches on MWW treatment. It consists, first, in calling for replacement of the conventional activated sludge by an aerobic granular sludge (AGS) process. The second stage will be to transform the granular sludges (aerobic and anaerobic) into activated carbon (AC) by pyrolysis. Third, the color and the inert COD of the biotreated MWW will be removed by adsorption with the produced AC. Finally, reverse osmosis can be used to remove the salts, being preceded or no by low-dose ozonation. The O_3 after AC process, a fouling prevention measure, is aimed for disinfection, color polishing and for avoiding that the organics reach the membranes. The logic of the proposed train revolves around the complementarity of the units and the use of low-cost modalities. In particular, for the industrial WRRF under study, the sludge is currently treated by incineration; the energy of this process would go to pyrolysis in the new train.

The wastewater industry is currently shifting away from energy-intensive wastewater treatment, toward sustainable and energy-positive water resources recovery facilities (Metcalf & Eddy 2014; Hoarau et al. 2018). The aerobic granular sludge is much less expensive to operate (up to − 50%), having also a smaller footprint (up to 50% less) and sludge production. AGS is more tolerant to toxic shocks and is able to remove the BOD, as well as more nitrogen and phosphorus without additional costs (Pronk et al. 2017). In addition to this, a dense and granular aerobic sludge would be produced (adding to anaerobic sludge from the UASB), constituting a valuable raw material for activated carbon manufacturing (Kazak et al. 2017; Gutierrez-Segura et al. 2012).

As reported in the literature, granular activated sludge is being successfully used with several industrial wastewaters (Pronk et al. 2017), but not yet with molasses effluents. Anaerobically pretreated MWW has three specificities that may be challenging for aerobic granulation: temperature (Lopez-Vazquez et al. 2009), salinity (Ou et al. 2017) and the slowly biodegradable COD fraction (De Kreuk et al. 2010; Wagner et al. 2015), all of which are high. The molasses influent that reaches the activated sludge still contains a large amount of bCOD (approx. 600–1000 mg/L BOD_5), most of which is slowly biodegradable and in soluble state (Fall et al. 2012; Gengec and Kobya 2013). Besides AGS, melanoidins also need further studies. The structure and characteristics of these pigments are still not fully understood (Chandra et al. 2008; Nguyen et al. 2010; Zhang et al. 2019), and this has consequently hindered the development of new processes for their removal.

Conclusion

Disposal of molasses wastewaters (MWWs) in rivers is a common practice, while results showed that biotreatment typically provided is not enough (high residual inert organics, color and salts, of approx. 1000 mg/L COD, 2850 Pt–Co color units, 5000 mg/L TDS and 5400 µS/cm conductivity). Ozonation can decolorize molasses secondary effluent (> 95%) and does not lead to toxic by-products; however, it does not remove much COD (35% maximum) or significantly improve its biodegradability (only 8% more). The specific ozone uptake rate (SO_3UR) of the secondary MWW could be estimated (1.5 gO_3/gCOD h), using a new method that can be extended to ozone kinetic studies of other WWs. Recalcitrant organics and color in secondary MWW are removable by AC adsorption, but needing a relatively high carbon usage (approx. 13 gAC/L). To gain access to cheaper adsorbents and to shift toward sustainable water recovery facilities, an alternative treatment train is suggested for future research on molasses effluents: Upgrade activated sludge with AGS technology and produce AC from the granular sludges. Moreover, it is recommended to explore the development of new materials to improve the COD removal from MWWs by catalytic ozonation y/o by combined ozonation and adsorption.

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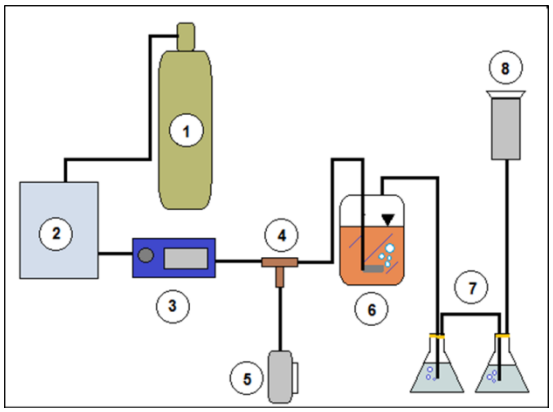
Online Resource 1: *Ozonation system and O_3 doses calculation.*

Paper Title: Tertiary treatability of molasses secondary effluents for color and organics: performance and limits of ozonation and adsorption.

Figure S1: Ozone generation, measurement and reaction systems:
1) pure oxygen tank, 2) ozone generator, 3) dry ozone meter, 4) three ways by-pass valve, 5) dry ozone destructor, 6) glass reactor, 7) KI wash bottles, 8) wet O_3 heated-destructor.



Reacted ozone doses calculation (example)

1°) Nomenclature:

C_{meas}: *Ozone concentration in the inflow gas (% w/w,* ***e.g. 4.15%****)*
C_{std} *: Ozone concentration in the inflow gas per Std L of gas (mgO_3/L_{std})*
COD_{ini}: *Initial total COD of the secondary wastewater (mg/L)*
Q_{meas}: *Actual inflow gas flowrate at actual T and P (2 L /min)*
Q_{std} *: Inflow gas flowrate (L/min) converted in standard conditions (STP = T_{std}, P_{std})*
P: *Actual pressure of the inflow gas (****e.g. 13.55 psi)***
P_{std}: *Standard pressure = 1 atm = 14.695* ***psi***
T_{std} *: Standard temperature = 273.15* ***°K (20°C)***
T: *Actual temperature of inflow gas and reactor (****e.g. 293.15 °K)***
Time: *Duration of the ozonation treatment* ***(e.g. 5 min)***
V_{WW}: *Volume of ozonated wastewater sample* ***(e.g. 0.8 L)***
V_{KI} *: Total volume of 2% KI solution in each of the two wash bottles (****e.g. 900 mL****)*
$V_{KI\ sub}$: *Volume of KI sub-sample titrated (****e.g. 150 mL),*** *from trap KI-1, only reached.*
V_{thios} *: Volume of sodium thiosulfate solution used in titration (****e.g. 15.3 mL****).*
N: *Normality of $Na_2S_2O_3$ solution used in titrations (****e.g.*** *0.155 mg/me, normalized)*

2°) Calculation (see at page 2)

Example of ROD calculation

Run: 5 min ozonation of the secondary WW (second titration, WW sample #4).
Local conditions of city: 2600 m altitude, atmospheric P of 10.94 psi (0.74atm).

- Standard gas inflow rate, $\mathbf{Q_{std}} = Q_{meas}$ x (P/P_{std}) x (T_{std}/T).
  = 2 L/min * (13.55psi/14.695psi)*(273 K/293K) = 1.718 std L/min.

- O3 conc in the inflow in mg per std L of gaz, $\mathbf{C_{std}} = C_{meas}$ * 14.287
  = 4.15% w/w * 14.287 mg O_3/std L O_2 = 59.35 mg O_3 /L std O_2
  (Note: 1% w/w O_3 in O_2 = 14.287 O_3 /L std O_2).

- Mass of ozone trapped in KI, $\mathbf{M_{O3\ trapped}} = 24 * N * V_{thios} * (V_{KI}/ V_{KI\text{-}sub})$
  = 24 x 0.155 x 15.3 x 900/150 = 341.58 mg O_3 (only Trap1 was reached by O_3).

- Mass of O_3 provided in the inflow, $\mathbf{M_{O3\ inf}}$ = Cstd * Qstd * Time
  = 59.35 mg/L x 1.718 L/min x 5 min = 509.9 mg O_3.

- Total reacted ozone mass, $\mathbf{MO_{3\ react}} = MO_3$ inf $- MO_{3\ trapped}$
  = 509.9 mg – 341.58 mg = 168.3 mg O_3.

- Reacted ozone dose in mg/L of WW, $\mathbf{ROD_v} = MO_{3\ react} /V_{WW}$
  = 168.3 mg/0.8 L = 210.4 mgO_3/L $_{WW}$.

- Reacted ozone dose in mg/g COD_{ini}, **ROD** = RODv * COD_{ini}
  = 210.4 mgO_3 L^{-1}/1.230 gCOD L^{-1})= 171.0 mgO_3/gCOD (sample #4 at WWTP).

- Applied ozone dose in mg/g COD_{ini}, **AOD** = $MO_{3\ inf}$ /(V_{WW}* COD_{ini}).
  = 509.9 mg O_3/L / (0.8 L * 1.230 gCOD/L) = 518.1 mgO_3/gCOD.

Online Resource 2: *Estimation of approximate carbon usage*

Paper Title: Tertiary treatability of molasses secondary effluents for color and organics: performance and limits of ozonation and adsorption.

By doing some additional hypotheses, the COD isotherms were extrapolated to estimate the AC masses needed for treating the WWs in continuous (column contactors).

Eq. A derived from the mass balance of the COD around the contactor at the end of the service time, allows calculating the carbon usage.

$$Carbon\ usage = {}^{M_{AC}}/_{V_{WW}} = \frac{C_{infl} - C_{effl}}{q_{\ actual}} \qquad \text{(Eq. A)}$$

The quotient M_{AC}/V_{WW} represents the carbon usage rate expressed in g of AC per L of WW, i.e., the total mass of AC needed (M_{AC}) divided by the total volume of MWW that can be treated (V_{WW}). C_{infl}, the COD of the influent was known (897, 791 and 633 mg COD/L, respectively for T0, T1 and T4). C_{effl} is the desired mean effluent quality, which was assumed as 100 mg COD/L for the simulations. $q_{\ actual}$ represents the adsorption capacity of the AC in the dynamic process (column). As it is the case in all adsorption columns, at the end of the service time, the concentration of the liquid phase in contact with the carbon bed is almost equal to the influent concentration. At this point (Ce = C_{infl}), the equilibrium adsorption capacity of the carbon (qe) can be derived by extrapolation from the isotherm equations and their respective K_d values. The corresponding qe values were 120 and 71 mg COD /g CA, respectively for T0 and T1 WWs. Due to much known reasons (Metcalf and Eddy, 2014), actual adsorption capacity in the columns ($q_{\ actual}$) is always lower (20 to 80%) than the theoretical equilibrium value (qe); 50% was utilized in the present estimation. Consequently, the carbon usage calculated per L of wastewater was 13 g of AC for T0, versus 20 g for T1 and T4.