Comparing different schemes of agricultural wastewater lagooning: depuration performance and microbiological characteristics

Water, Air, & Soil Pollution

COMPARING DIFFERENT SCHEMES OF AGRICULTURAL WASTEWATER LAGOONING: DEPURATION PERFORMANCE AND MICROBIOLOGICAL CHARACTERISTICS

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Abstract:	Since the number of factors influencing depuration efficiency and energy requirement is high, the choice of the most suitable aeration scheme in aerated lagooning systems treating agricultural wastewater is a difficult task. This study provides technicians and researchers with guidelines on air diffuser position, presence of inoculum and characteristics of microbial mass, focusing organic matter degradation and energy performance of aerated ponds treating citrus processing wastewater. Four experimental batch tanks were set up: three tanks were subject to low air flow rates and times, while a fourth tank was not aerated. The organic matter degradation in aerated tanks was on average six times higher compared to the non-aerated tank. Positioning the air diffuser at mid-depth and consequent separation of tank contents into two layers determined a decrease in the depuration performance, presumably due to reduced transfer of oxygen to wastewater. Although the ability of spontaneous microflora to adapt to essential oil concentrations up to 1400 mL L-1 was detected, the effect of inoculum (50% of wastewater with spontaneous microflora already adapted to high essential oil concentration) was not noticeable. The results of the investigation confirm the suitability of aerated lagoons subjected to fine bubble aeration also for agricultural wastewater depuration.
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	(line 45-50), as well as to change style of citation in this sentence "wastewater of different origin e.g. agricultural wastewater (Kimball, 1999), livestock wastewater (Trias et al., 2004),". Authors' reply: we have shortened the sentence and changed the style of citations. 2.Referee's comment: line 64: it is not needed to write inoculum in italics, after this word, I recommend to drop brackets and word "that is" and after word inoculum continue with comma and characterization of inoculum. Authors' reply: We have removed italics from the word "inoculum", dropped the words "that is" and explained shortly characterization of an inoculum after comma. 3.Referee's comment: Chapter results and discussion should be more discussed. Authors' reply: we have enlarged this section, with more discussion about performance of lagooning systems in depurating citrus wastewater (also by comparison with activated sludge plants, commonly used for this kind of agro-industrial wastewater) and analisys of the microbial mass behaviour in depuration tanks. Finally, we have added other bibliographic citations to support the further discussion.
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COMPARING DIFFERENT SCHEMES OF AGRICULTURAL WASTEWATER LAGOONING: DEPURATION PERFORMANCE AND MICROBIOLOGICAL CHARACTERISTICS

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Abstract

Since the number of factors influencing depuration efficiency and energy requirement is high, the choice of the most suitable aeration scheme in aerated lagooning systems treating agricultural wastewater is a difficult task. This study provides technicians and researchers with guidelines on air diffuser position, presence of inoculum and characteristics of microbial mass, focusing organic matter degradation and energy performance of aerated ponds treating citrus processing wastewater. Four experimental batch tanks were set up: three tanks were subject to low air flow rates and times, while a fourth tank was not aerated. The organic matter degradation in aerated tanks was on average six times higher compared to the non-aerated tank. Positioning the air diffuser at mid-depth and consequent separation of tank contents into two layers determined a decrease in the depuration performance, presumably *due to reduced transfer of oxygen to wastewater. Although the ability of spontaneous microflora to adapt to essential oil concentrations up to 1400 mL L^{-1} was detected, the effect of inoculum (50% of wastewater with spontaneous microflora already adapted to high essential oil concentration) was not noticeable. The results of the investigation confirm the suitability of aerated lagoons subjected to fine bubble aeration also for agricultural wastewater depuration.*

Keywords: Wastewater treatment; aerated lagooning; agro-industrial wastewater; energy efficiency; essential oils; microbial population.

1. Introduction

In treating agricultural wastewater, the depuration performance of activated sludge plants is negatively conditioned by characteristics of the effluents, such as qualitative and quantitative variability, high acidity, lack of nutrients, presence of inhibiting compounds, high energy consumptions (Zema et al. 2012); moreover, the economic sustainability of these plants is limited by the high energy requirement. Aerated lagooning systems have been proposed as a suitable alternative to activated sludge plants for treatment of urban wastewater (e.g. Juanicó & Milstein 2004; Salgot et al. 2006). The use of lagooning is suitable also for wastewater of different origin (e.g. agricultural, industrial and livestock wastewater, Kimball 1999; Trias et al. 2004; Chan et al. 2009). However, little work has been done about lagooning of agro-industrial effluents (Tamburino et al. 2007; Jail et al. 2010) and, in particular, wastewater produced by citrus industries.

In aerated ponds, wastewater is usually stored in wide and deep ponds, with long retention time and pneumatic or mechanical aeration. Thanks to these characteristics, the microorganisms (especially bacteria) are able to adapt to the high variability of wastewater physico-chemical properties. In fact, the high volumes of wastewater, previously depurated in the pond, dilute influent wastewater, lowering the concentration of organic matter and nutrients, and increasing pH. The long retention time allows slow and gradual bio-chemical processes, whose oxidation efficiency is further enhanced by aeration. Aerated ponds do not require the constant settleability of large sludge flocs, which is instead reduced by the presence of inhibiting compounds (as essential oils in citrus wastewater) in activated sludge plants.

The choice of the most suitable depuration scheme is a difficult task, because many factors must be considered in order to optimise the removal efficiency and energy requirement. Some of these factors are: concentration of inhibiting compounds, air flow rate, aeration time, aerator position, presence of inoculum, a microbial mass already adapted to wastewater, and characteristics of microbial mass. A previous study (Zema et al. 2012) investigated the first three of the above mentioned factors, monitoring depuration processes of citrus wastewater with high concentration of essential oil (EO) in aerated lagoons. The results highlighted the possibility of increasing efficiency by limiting aeration to the 12 night hours, with a cost saving of 20%, or by halving the night air flow rate to 7 L h^{-1} m^{-3}, with a further reduction of 40% of energy costs. Depuration processes in aerated lagoons showed tolerance to a concentration of EO up to 1400 ppm, presumably due to microbial adaptation to the terpenes contained in the EO.

On the basis of the previous outcomes, the other factors playing an important influence on depuration efficiency and energy requirement of aerated lagoons are analysed here, also in comparison with the performance of activated sludge plants. In more detail, this study compares the main physico-chemical parameters, the removal rates of the organic load and the microbial population characteristics of citrus wastewater treated in the following lagooning schemes: (i) a tank subjected to fine bubble aeration from an air diffuser placed above its bottom; (ii) a tank aerated at the bottom and containing a microflora already adapted to high EO concentrations; (iii) a tank with mid-depth aeration, in which anaerobic and aerobic processes were specialised by separating wastewater layers; (iv) an anaerobic tank without aeration. The study provides guidelines for technicians and researchers in management of aerated lagooning systems treating agricultural wastewater.

2. Material and methods

Citrus processing wastewater (water from washing fruits, machinery, floors as well as from the extraction of juice and essential oils, peel drying and cooling, Kimball 1999) was treated from December to June in four cylindrical batch tanks (volume of 1 m^3): three tanks were subject to fine bubble aeration through a diffuser fed by a blower (Ferplast Airfzz) and another one was not aerated. During the seven months of treatment the temperature of the wastewater ranged from 7.4 °C (February) to 29.5 °C (June).

Fresh wastewater was collected in a citrus processing factory and poured into the tanks at the start point of the cycle treatment. The following experimental schemes were adopted and tested:

1) a tank, indicated as “T_0”, where the air diffuser (flow rate 7 L h^{-1} m^{-3}) was placed 0.05 m above the bottom (0.85 m below the wastewater surface) (Fig. 1);
2) a tank (“T_{IN}”), similar to “T_0”, where 50% of fresh citrus industry effluent was initially mixed with citrus wastewater coming from a previous treatment cycle (in aerated ponds

simulating the upper aerobic layer of aerated lagoons treating citrus wastewater, Zema et al. 2012), containing a spontaneous microflora already adapted to EO concentration within the range of 600-1000 mL L^{-1} (600-1000 ppm, Fig. 1);

3) a tank ("$T_{1/2}$") where the diffuser was placed at mid-depth, in order to separate two layers, the upper aerobic, the lower anaerobic; the lower layer was partially separated from the upper one by a wooden disk, thus limiting wastewater mixing due to air bubble lift with the aim of adapting microflora (Fig. 1); this tank simulates the integrated anaerobic-aerobic bioreactor proposed by Chan et al. (2009);
4) a tank ("T_{NA}") without aeration (Fig. 1).

In the aerated tanks T_0, T_{IN}, and $T_{1/2}$ the air flow rates (7 L h^{-1} m^{-3}) and times (12 night hours: 7 pm – 7 am) were chosen on the basis of a previous study (Zema et al. 2012), which highlighted the possibility of increasing energy costs by limiting the aeration to the 12 night hours (since in Italy energy is cheaper at night) and reducing the night air flow rate to 7 L h^{-1} m^{-3}. The power per unit volume (0.6 W m^{-3}) of the experimental tanks was set according to the lowest values adopted in aerated aerobic-anaerobic ponds at full scale (Tamburino et al. 2007; Bombino et al. 2009). Daily air flow rate was equal to 84 L m^{-3} d^{-1}. In 210 days, a total supply of 6.3 kg of oxygen was measured and 7.7 kWh of electrical energy was estimated to have been consumed.

The initial COD concentration of wastewater was close to 6600 mg L^{-1} and the ratio COD:N:P to 600:5:1. During the experiment, COD was kept within the range of 3.5-7.7 (tank T_{NA}), 3.0-8.5 (T_0), 5.4-11.4 ($T_{1/2}$), 5.7-11.8 (T_{IN}) g L^{-1}, typical of citrus wastewater (Kimball 1999; Tamburino et al. 2007), restoring the organic matter consumed by microorganisms. To this purpose, saccharose was added in late January and early April for a total amount of 3150 (tank

T_{NA}), 16520 (T_0), 10950 ($T_{1/2}$), and 12870 (T_{IN}) mg L^{-1} of COD.

The initial EO concentration was close to 610 mL L^{-1} and constantly maintained within the range of 500-625 mL L^{-1} by periodical additions: a total amount of EO of 2400 mL L^{-1} per tank was added. EO concentration in this range slows down biological processes and thus reduces depuration efficiency of treatment plants (Kimball 1999; Zema et al. 2012).

COD and EO concentrations of wastewater were periodically evaluated using standard methods (Scott & Veldhuis 1966; APHA, AWWA & WEF 1998). Sample collection was performed every month by taking, after mixing, 500 mL of wastewater in duplicate. Timing of the sampling operations was similar to the values of the hydraulic retention time (longer than 3-4 weeks) of lagooning systems, where wastewater is subject to slow and gradual changes in its physical and chemical properties.

Dissolved oxygen (DO), temperature, pH and oxidation-reduction potential (ORP) of wastewater (0.2, 0.45 and 0.8 m below the wastewater surface, Fig. 1), were weekly measured using a digital multimeter (HACH Lange HQ40).

Since the efficiency of oxygen transfer to the wastewater depends on the oxygen deficit (OxDef), the latter was calculated as the difference between the saturation concentration at the measured temperature "T" ($DO_{sat}(T)$) and the actual DO (DO_{act}) concentration ($OxDef = DO_{sat}(T) – DO_{act}$). The ratio between the supplied energy (7.7 kWh, equal for the tanks T_0,$T_{1/2}$, T_{IN}) and the COD removal rates was calculated to estimate the energy efficiency in the aerated tanks. The COD removal rate was quantified measuring monthly the COD degraded by the biological processes and the amount of saccharose added (converted to COD equivalent) in each tank.

To identify the microorganism class based on oxygen requirement in the wastewater differently treated, the total microbial count (TMC) was carried out at 30, 60 and 210 days from the start of the treatment both in aerobic and in anaerobic conditions at 25°C for three days, either with or without the addition of 1400 mL L^{-1} of EO to Plate Count Agar (BioKar Diagnostics, Beauvais, France). Different types of colonies were selected from the plates and were observed by optical microscope (Zeiss Standard 20).

3. Results and discussions

3.1. Evolution of the main physical-chemical properties of wastewater

The evolution of pH, averaged on the tank vertical profile, showed small differences among the investigated tanks (T_{NA}, T_0, $T_{1/2}$ and T_{IN}). The initial acidity of wastewater (pH = 5.1-5.5) was due to the organic acid components of citrus fruits and fermentation of sugars and other carbohydrates, which produce organic acids under oxygen shortage. In activated sludge plants, where pH must be in the range 6.0-8.5 with very gradual temporal variations, the acidity of wastewater could have negative effects on biological processes (Masotti 2002) with slowdown of block of depuration capacity.

During the investigation the pH increased up to values close to 6.5 in the aerated tanks, due to degradation of organic acids in the oxidation processes; the highest increase of pH was detected in tank $T_{1/2}$. A lower increase was recorded in tank T_{NA} (from 5.5 to 5.7), due to less intense oxidation processes in the absence of aeration. When the organic matter was added in late January and early April, a pH reduction (-0.9) was noted, presumably due to the fermentation of sugars. Nor compounds (as, for instance, lime) were added to limit the negative effects of high acidity of wastewater, as usually operated in activated sludge plants

(Tamburino et al. 2007), neither nutrients. Nutrient scarcity of citrus wastewater produces serious troubles in depuration processes and especially in activated sludge plants (due to the difficult sedimentation of sludge). In such kind of depuration plants, a COD/N/P ratio of about 200/5/1 is normally suggested (Masotti, 2002), which can be obtained by adding nutrients. Nitrogen and phosphorous supply should be made with care to avoid to exceed the discharge limits of acceptance in water bodies. Vice versa, in biological ponds (anaerobic and aerated lagoons) a ratio of 400/5/1 can also be tolerated (Masotti, 2002). In the lagooning systems nutrient supply can be very limited (or even null, as in our experience) thanks to the nitrogen and phosphorous recycling from sludge digestion.

EO content was quickly removed in a few weeks. The tolerance of biological ponds to high EO concentrations (whose antimicrobial activity is well known, particularly in activated sludge plants, Lane 1983; Ratcliff 1990; Kimball 1999) may be presumably attributed to microbial adaptation to terpenes contained in EO and to the low airflow rate (Zema et al. 2012).

The values of ORP in the aerated tanks, averaged on the vertical profile, decreased from initial values of 93-103 mV to 21-82 mV. In wastewater treated in tank T_{NA} the ORP fell in a narrower range (75-85 mV), because of the slowness of the anaerobic biochemical processes and reduced variations of pH. As noted for pH, a decrease in ORP was detected after organic matter supply, presumably due to the oxygen demand of the degradation processes, followed by a quick increase after the organic degradation. Values of ORP were strongly correlated ($r^2 = 0.93$, Fig. 2) to pH and weakly ($r^2 = 0.11$) to DO concentration.

Values of DO (Table 1), averaged on the vertical profile of the tanks, were within the range of 0.01-0.8 mg L^{-1}. These values are much lower than the typical DO concentrations needed by activated sludge plants (more than 2 mg L^{-1}, Masotti 2002; Metcalf & Eddy 2006), in order to assure the presence of settleable sludge flocs. This means that in aerated lagooning systems the electrical energy can be supplied with lower power equipment (1-2 W m^{-3}, Zema et al. 2012), thus with higher money savings compared to activated sludge plants (in which electric power of about 10 W m^{-3} is usual, Masotti et al. 2002).

In all the experimental tanks DO decreased from surface (0.7-0.8 mg L^{-1}) to the bottom, where the lowest values were recorded (0.01-0.02 mg L^{-1}). The lowest mean DO concentration was measured in tank T_{NA} (0.4 mg L^{-1}). Tanks T_0 and $T_{1/2}$ showed DO concentrations about 10% higher than 0.5 mg L^{-1}. In all tanks the mean DO deficit was close to 9.4 mg L^{-1}, thus 95-98% of the saturation values, which confirms that DO saturation deficit is not a limiting factor of oxygen transfer to wastewater. This very high DO deficit, which induces a very high efficiency of oxygen transfer to the wastewater, is over 20% more than standard activated sludge plants, in which an oxygen deficit around 80% is usually recorded.

Moreover, the low aeration supplied to wastewater has avoided unpleasant smells, typical of anaerobic processes (often beyond the tolerable limits). Sludge accumulation in the deep tank layer (that is, under the air diffuser) was in a small amount, confirming what found in the previous experimental tests (Zema et al., 2012) and reported also by Juanicò and Milstein (2004).

3.2. Organic matter degradation and energy efficiency

The organic load of citrus processing wastewater is very high (COD in the range 0.05-170 g L^{-1}, Di Giacomo & Calvarano 1987) and variable in time. This parameter is one order of magnitude higher than urban wastewater treated in activated sludge plants (e.g. Masotti 2002;

Tchobanoglous et al. 2002).

In our experience, on average, the COD monthly removal rate in the aerated tanks (2.17 g L^{-1} $month^{-1}$) was six times higher (statistically significant at $P < 0.05$) compared to tank T_{NA} (0.36 g L^{-1} $month^{-1}$). The highest COD monthly removal rate (2.64 g L^{-1} $month^{-1}$) was measured in tank T_0 (Fig. 3). The effect of inoculum (tank T_{IN}) was not noticeable, since COD removal rate (2.29 g L^{-1} $month^{-1}$) was 13% lower than in tank T_0 (not significant at $P < 0.05$).

The COD monthly removal rate detected in tank $T_{1/2}$ (1.58 g L^{-1} $month^{-1}$) was 40% lower than in tank T_0 and the difference between these values was significant at $P < 0.05$ (Fig. 3). This may be due to the fact that the lower organic load removal rate of the strictly anaerobic processes in the deep wastewater layer (where DO concentration was close to zero) was not balanced by an increase in the upper layer (where the oxygen supply rate per unit volume of wastewater is two times the value of tank T_0). Since the DO saturation deficit was similar (with mean differences lower than 1%), we deduced that part of the oxygen supplied in the upper layer was not transferred to wastewater, presumably to the halved water depth (compared to tank T_0) that reduces noticeably the contact time among air bubbles and wastewater. This confirms the influence of diffuser submergence on oxygen transfer efficiency, as reported in other studies (e.g. Hodkinson et al. 1998) and suggests to maximise the depth of the aerated layer.

In Fig. 4 the correlations between COD removal rates and temperature of tank T_{NA} are compared to the literature model by Mara & Pearson (1998), analysing the influence of temperature to the rate of organic load degradation per volume and time units. This comparison showed that the organic load removal rate in the experimental tank, well correlated to the temperature ($r^2 > 0.88$), is much lower especially at temperatures of 20-30 °C (0.4-0.6 g L^{-1} $month^{-1}$ versus 11.2-14.7 g L^{-1} $month^{-1}$, Fig. 4). Moreover, in the tank T_{NA} the increase of COD removal rate with temperature (i.e. the slope of the regression line, 0.015 g L^{-1} $month^{-1}$ °C^{-1}) is much lower compared to the reported model (0.78-0.84 g L^{-1} $month^{-1}$ °C^{-1}). The reasons of the reduced anaerobic activity could be identified in the presence of inhibiting compounds (as EO), nutrient scarcity and acidity of wastewater, which slowdown biological processes if falling out of the range 6-8 (Burak & Orhan 2002; Ruiz & Flotats 2014).

In relation to the other tanks (i.e. T_0, $T_{1/2}$ and T_{IN}), given that the simultaneous presence of both aerobic and anaerobic processes in the aerated tanks (due to the limited air flow rates and times), we hypothesized that the COD removal rate of the depuration process is the sum of two components, of which one is aerobic and the other is anaerobic (this latter equal to the COD removal rate measured in tank T_{NA} at the same temperature). The residual COD removal rate due to the aerobic process showed a low correlation ($r^2 < 0.28$) with the temperature. These results are consistent with those of other experiences, which highlighted the rather limited influence of temperature in aerobic ponds (e.g. Tchobanoglous et al. 2002).

Weak correlations ($r^2 < 0.21$) were also noted between COD removal rate and DO (data not shown), which may be due to the low variability of DO concentrations. In the experimental tanks the air flow rates are limited and, as a consequence, the supplied oxygen is quickly utilised in the aerobic degradation of the organic matter, thus constantly keeping low concentrations of DO. This confirms the high efficiency of oxygen exploitation shown by the lagooning systems compared to activated sludge plants, in which conversely a significant part of the air supplied is not consumed by microorganisms.

On the basis of the COD removal rates detected in the aerated tanks, the lowest oxygen and energy requirement per unit of COD removed was detected in tank T_0 (0.59 kgO_2 kg_{COD}^{-1} and 0.16 kWh kg_{COD}^{-1}). These values were respectively 20% and 50% lower compared to tanks

T_{IN} (0.72 kgO_2 kg_{COD}^{-1} and 0.2 kWh kg_{COD}^{-1}) and $T_{1/2}$ (1.14 kgO_2 kg_{COD}^{-1} and 0.32 kWh kg_{COD}^{-1}) (Fig. 3). The energy requirements detected in the experimental aerated tanks are quite low compared to the typical values of activated sludge plants. These plants show specific energy consumption of about 0.5 kWh kg^{-1}_{COD} when treating urban wastewater (Masotti 2002; Tchobanoglous et al. 2002). This energy efficiency is very difficult to achieve in the case of agricultural wastewater, due to the fact that little organic matter is settleable. Therefore, depuration processes of citrus wastewater in activated sludge plants show energy requirement which can reach 2.0 kWh kg^{-1}_{COD} (Cheng et al. 2011).

3.3. Analysis of microbial population characteristics

In a previous study, Zema et al. (2012) studied the microflora of citrus wastewater. It was mainly composed of immobile bacteria; the cells were spherical in shape, single and also arranged either in tetrads or in chains. These bacteria were mainly aerobic with nearly 81% resistant to EO. In this study, the above described pool of bacteria spontaneously evolved were used as starting point of the process.

Table 2 reports the evolution of the microflora during the treatment. Both in aerobic and anaerobic test conditions, the microbial population showed different trend of growth in the four tanks. Concerning the aerobic microflora (Table 2a), the count observed in T_{NA} can be explained considering the growth of facultative aerobic microorganisms; similarly for the anaerobic microflora (Table 2b) the count observed in T_0 e T_{IN} can be explained considering the growth of facultative anaerobic microorganisms. Both in aerobic and anaerobic test conditions, the presence of EO induced a decrease in microbial count probably due to the EO concentration used. The effect of inoculum (50% of wastewater with spontaneous microflora already adapted to high EO concentration) in the tank T_{IN} was not significant.

At the end of the cycle treatment (210 days), the wastewater showed a different microbial composition in each tank. In tank T_{NA} the aerobic microflora was prevalently filamentous rod and spherical-rod shaped, immobile bacteria, often arranged in chains; the strictly anaerobic microflora was spherical-rod shaped, mobile or immobile bacteria. The prevalent aerobic microflora naturally adapted to 1400 mL L^{-1} of EO was rod-shaped, immobile bacteria, often arranged in chains; the strictly anaerobic microflora naturally adapted to 1400 mL L^{-1} of EO was single rod-shaped immobile bacteria. In tank T_0 the aerobic microflora was mainly composed by short rod-shaped immobile bacteria, often arranged in chains; the strictly anaerobic microflora was spherical-rod shaped, mobile bacteria, also arranged in chains. The prevalent aerobic microflora naturally adapted to 1400 mL L^{-1} of EO was spherical-rod shaped, mobile bacteria; the strictly anaerobic microflora naturally adapted to 1400 mL L^{-1} of EO was rod or spherical-shaped, immobile bacteria. In tank $T_{1/2}$ the aerobic microflora was prevalently rod-shaped, mobile bacteria; the strictly anaerobic microflora was large rod-shaped, immobile bacteria. The prevalent aerobic microflora naturally adapted to 1400 mL L^{-1} of EO was rod-shaped immobile bacteria; the strictly anaerobic microflora naturally adapted to 1400 mL L^{-1} of EO was rod-shaped, immobile bacteria. In tank T_{IN} the aerobic microflora was prevalently filamentous rod-shaped, immobile bacteria; the strictly anaerobic microflora was spherical-rod shaped, immobile bacteria. The prevalent aerobic microflora naturally adapted to 1400 mL L^{-1} of EO was spherical-rod shaped, mobile bacteria; the strictly anaerobic microflora naturally adapted to 1400 mL L^{-1} of EO was thin filamentous rod-shaped, immobile bacteria, also arranged in chains.

In general, the evolution of the spontaneous microflora was somewhat in accordance with the aeration scheme of the tanks: (a) the aerobic microflora was stable in tank T_{NA}, decreased both in tanks $T_{1/2}$ and T_{IN}, while increased in tank T_0; (b) the anaerobic microflora was stable in tank T_{NA}, while decreased, as expected, in the other three tanks. Depending on the oxygen availability in the tanks, part of the microbial population (aerobic and anaerobic in tank T_{NA}, aerobic in T_0 and $T_{1/2}$ and anaerobic in T_{IN}) showed a greater ability to adapt to high EO concentration (1400 mL L^{-1}), whose explanation would require further studies. Essential oils affect, at the same time, different functions of the microbial cell, thus the microbial mass finds difficulty in surviving in an environment rich in EO (Ruiz & Flotats 2014). Although the exposure of the microbial population, sampled from each tank, to high EO concentration (1400 mL L^{-1}) resulted in a decrease in microbial load, our results confirm the bacteria ability to adaptation, modifying their cell structure (Ruiz & Flotats 2014). This ability is in accordance to Bakkali et al. (2008).

4. Summary and conclusions

This paper investigated organic matter degradation and energy performance of aerated ponds treating citrus processing wastewater during a period of seven months.

The highest COD monthly removal rate was measured in the tank with air diffuser just above the bottom. The depuration efficiency in the aerated tanks was on average six times higher compared to the non-aerated tank; this latter showed COD removal rates much lower compared to the values reported in literature for the treatment of urban wastewater. Positioning the air diffuser at tank mid-depth determined a decrease of the COD removal rate, presumably due to a reduced oxygen absorption by the wastewater.

The spontaneous microflora differently evolved in the tanks and their variability is in accordance with the aeration scheme of the tanks. In general, the microflora exposed in the tanks to EO concentration within the range of 500-650 mL L^{-1} showed the ability to adapt up to 1400 mL L^{-1}. From the experimental tests it emerges that the effect of *inoculum* (that is, 50% of fresh citrus industry effluent with citrus wastewater already depurated as far as much lower organic matter and EO concentrations) was not noticeable.

Considering that in depuration plant design, both effluent characteristics and bacterial cultures should be taken into account (Moura et al. 2009), the results of this investigation could be considered as guidelines for choosing the most suitable scheme allowing the maximum reduction of energy needs in depuration processes of citrus wastewater. Nevertheless, more research is expected about treatment of agricultural wastewater of different origin (e.g. olive oil mill wastewater, winery effluents) also by tests in plants at full scale, in order to confirm the suitability of aerated lagoons subjected to fine bubble aeration for agricultural wastewater. If so, the adoption of extensive technologies, as lagooning, seems to be a valid alternative to the most common activated sludge plants, since COD and EO are removed from agricultural wastewater more efficiently and with lower energy costs.

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TABLES

Table 1. Values of dissolved oxygen (DO) and its saturation deficit of citrus wastewater depurated in tanks simulating aerated ponds.

	DO (mg L^{-1})$^{(*)}$				**DO saturation deficit (mg L^{-1}) $^{(*)}$**			
	T_{NA}	T_0	$T_{1/2}$	T_{IN}	T_{NA}	T_0	$T_{1/2}$	T_{IN}
Minimum	0.01	0.01	0.02	0.02	7.88	7.85	7.84	7.91
Maximum	0.74	0.75	0.79	0.73	10.79	10.78	10.80	10.79
Mean	0.39	0.46	0.44	0.41	9.42	9.34	9.37	9.40
Standard deviation	0.22	0.21	0.23	0.24	1.17	1.15	1.17	1.16
Coefficient of variation	0.57	0.46	0.52	0.57	0.12	0.12	0.13	0.12

(*) Averaged on three values measured over the vertical profile of the tank.

Table 2a, b. Evolution of the total microbial count (values measured in $mL^{-1} \times 10^{6}$) of citrus wastewater depurated in tanks simulating aerated ponds:

(a) aerobic microflora; (b) anaerobic microflora.

Time after treatment start (days)	Aerobic microflora				Aerobic microflora in the presence of 1400 mL L^{-1} of EO			
	T_{NA}	T_0	$T_{1/2}$	T_{IN}	T_{NA}	T_0	$T_{1/2}$	T_{IN}
30	8.1	1.4	93.0	16.0	0.6	0.4	18.0	0.4
60	100.0	4.0	15.0	72.0	6.2	0.4	4.4	4.5
210	10.0	33.0	30.0	7.5	2.1	0.3	0.4	1.0

(a)

Time after treatment start (days)	Anaerobic microflora				Anaerobic microflora in the presence of 1400 mL L^{-1} of EO			
	T_{NA}	T_0	$T_{1/2}$	T_{IN}	T_{NA}	T_0	$T_{1/2}$	T_{IN}
30	14.0	24.0	120.0	44.0	1.1	1.1	39.0	3.2
60	87.0	3.6	24.0	31.0	6.6	0.4	8.0	4.3
210	10.0	1.7	0.2	7.6	2.4	0.1	0.2	1.9

(b)

FIGURE CAPTIONS

Fig. 1 Scheme of the tanks (volume of 1 m^3) simulating aerated ponds (measures in metres)

Fig. 2 Correlation between pH and oxidation-reduction potential (ORP) in citrus wastewater depurated in tanks T_{NA}, T_0, $T_{1/2}$, T_{IN}

Fig. 3 Means of COD removal rate as well as of oxygen and energy supply per unit of COD removed in tanks T_{NA}, T_0, $T_{1/2}$, T_{IN} (different letters indicate significant differences in the values, Tukey's test, $p < 0.05$)

Fig. 4 Variation of COD removal rate with temperature in tank T_{NA} in comparison to the model of Mara & Pearson (1998)

Fig. 1

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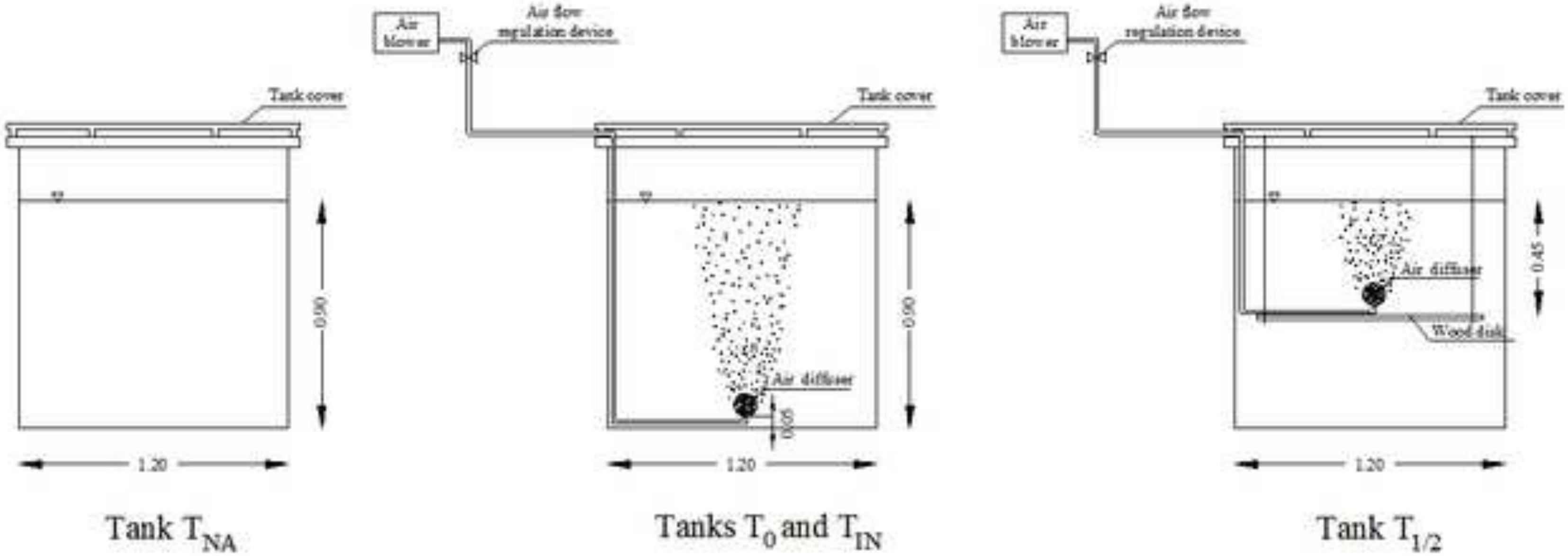

Fig, 2

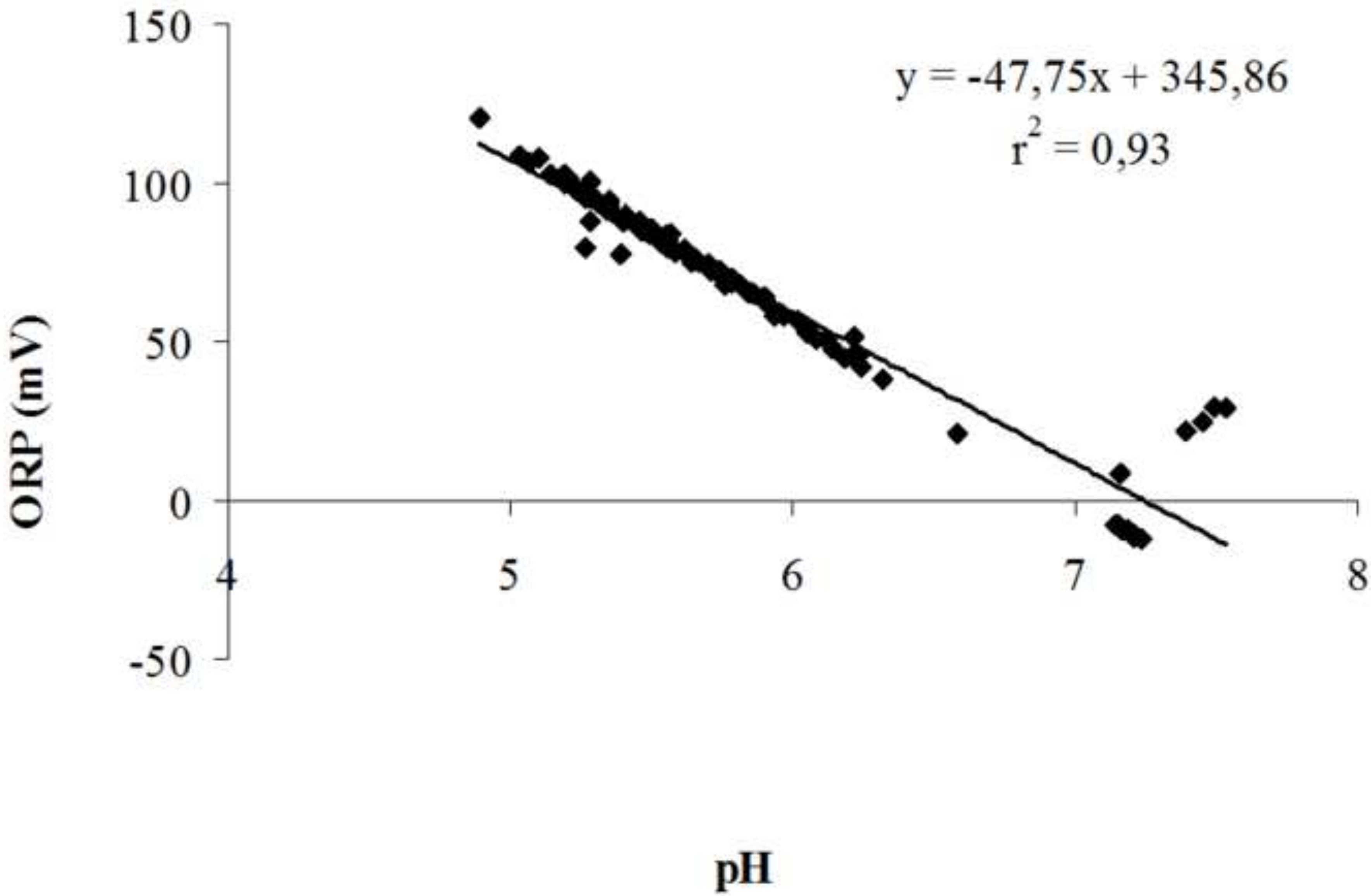

Fig. 3

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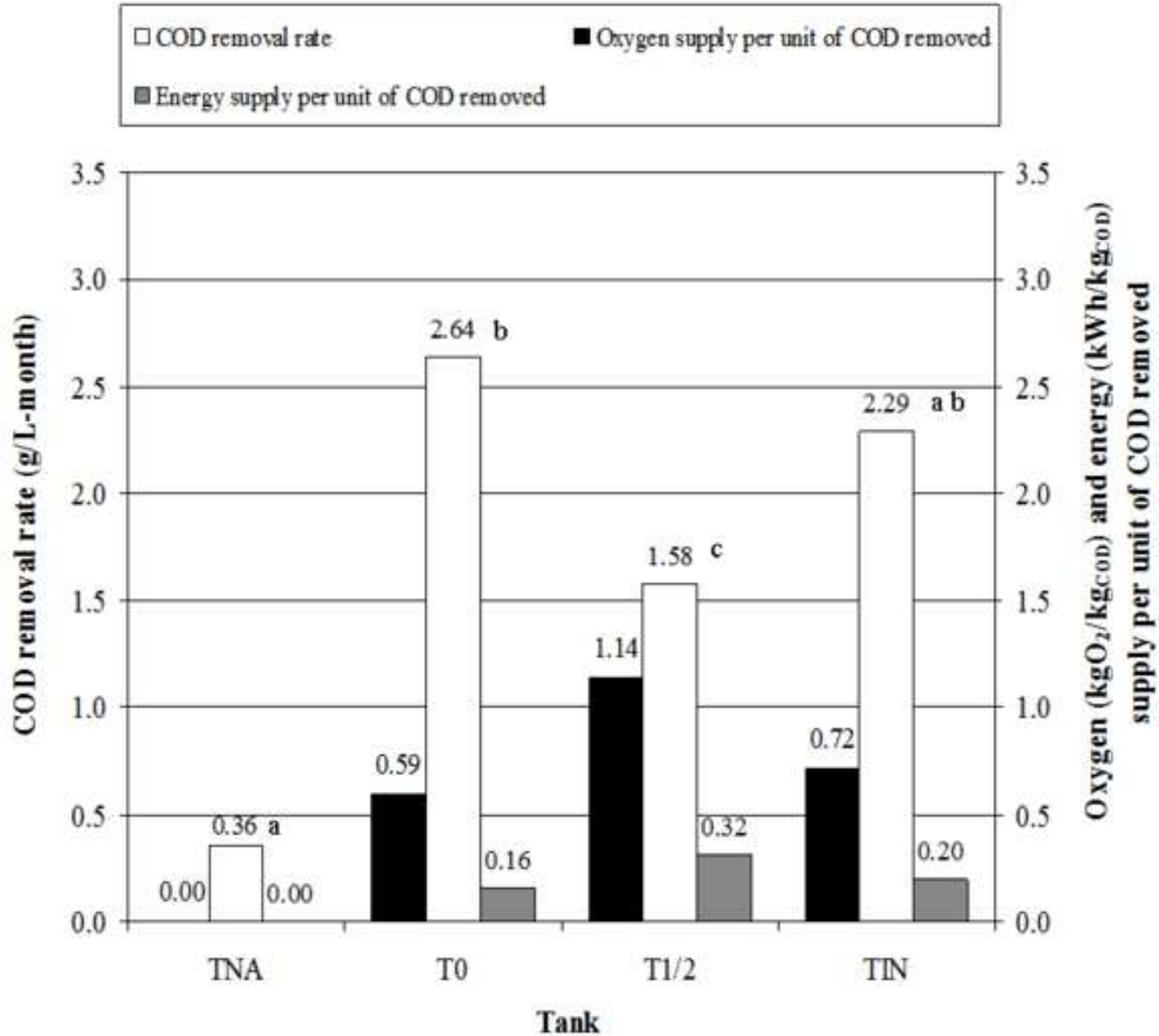

Fig. 4

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