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Winery Wastewater Treatment - Evaluation of the Air Micro-Bubble Bioreactor Performance

Margarida Oliveira[1,2] and Elizabeth Duarte[2]
[1]*Instituto Superior Politécnico de Santarém, Escola Superior Agrária de Santarém*
[2]*Technical University of Lisbon, Instituto Superior de Agronomia*
Portugal

1. Introduction

The wine sector has faced increasing pressure in order to fulfill the legal environmental requirements, maintaining a competitive position in a global market. The rising costs associated have stimulated the sector to seek sustainable management's strategies, focussing on controlling the demand for water and improving its supply. These can be accomplished by defining the best practical techniques, using technological means (Best Available Technologies) (Duarte *et al*., 2004). Some EU Directives were implemented concerning water protection and management. These included in particular the Framework Directive in the field of water policy and environmental legislation about specific uses of water and discharges of substances. The disposal of the untreated waste from the wine sector is considered an environmental risk, causing salination and eutrophication of water resources; waterlogging and anaerobiosis and loss of soil structure with increased vulnerability to erosion (Schoor, 2005). The winery wastewater is seasonally produced and is generated mainly as the result of cleaning practices in winery, such as washing operations during crushing and pressing grapes, rinsing of fermentations tanks, barrels washing, bottling and purges from the cooling process. As a consequence of the working period and the winemaking technologies, volumes and pollution loads greatly vary over the year. Each winery is also unique in wastewater generation, highly variable, 0.8 to 14 L per litre of wine (Schoor, 2005; Moletta, 2009). Consequently, the treatment system must be versatile to face the loading regimen and stream fluctuation. During the peak season (vintage), the winery wastewater has a very high loading of solids and soluble organic contaminant, but after this period, contaminant load decreases substantially. The high concentration of ethanol and sugars in winery wastewater justifies often the choice of a biological treatment (Bolzonella & Rosso, 2007). But the different wine processing method of each winery generates wastewater with specific properties, causing the impossibility to meet a general agreement on the most suitable cost-effective alternative for biological treatment of this wastewater.

Several winery wastewater treatments are available, but the development of alternative technologies is essential to increase their efficiency and to decrease the investment and exploration costs (Coetzee *et al*., 2004). So criteria should be considered in the selection of the adequate technology, such as maximization of removal efficiency, flexibility in order to deal with variable concentration and loads, moderate capital cost, easy to operate and maintain, small footprint, ability to meet discharge requirements for winery wastewater and also low sludge production. On the other hand, small producer with relatively modest financial capacity are interested in simple treatment systems with low maintenance and manpower requirements (Andreottola *et al.*, 2009).

Most treatment systems have been designed with large oxidation tanks and oversizing the aeration system to deal with the peak load with a very high oxygen demand, during the vintage period. As a result, wastewater treatment plants are quite large and difficult to manage. One of the most promising technologies appears to be the vertical reactors characterised by high oxygen mass transfer improving the biological conversion capacity. To optimise the mass transfer, a highly efficient Venturi injector coupled with multiplier nozzles were developed (AirJection®), in order to increase the treatment efficiency.

The main goals of the present paper are the comparison of different biological treatment systems, in particular fixed and suspended biomass, operating under aerobic conditions. Since the accurate design of the bioreactor is dependent on many operational parameters, aspects related to hydraulic retention time; oxygen mass transfer and contact time, energetic costs; sludge settling and production; response time during startup, flexibility and treated wastewater reuse, in crop irrigation, with the aim of closing the water cycle in the wine sector, will be addressed. A new treatment system will be presented as a case study, an air micro-bubble bioreactor (AMBB), that will highlight the advantages and constraints on its performance at bench-scale and full-scale, in order to fulfill the gaps associated with the implemented winery wastewater treatment systems. The data presented was collected during four years monitoring plan and used to develop a tool to support the selection of the best available technology. The present study will also contribute to the implementation of an integrated strategy for sustainable production in the wine sector, based on a modular and flexible technology that will facilitate compliance with environmental regulations and potential reuse for crop irrigation. This approach will contribute to the development of a bio-based economy in the wine sector that should be integrated in a Green Innovation Economy Cycle.

2. Comparison of different biological treatment systems

2.1 Biological treatments in winery wastewater

Several treatment systems, both physico-chemical and biological, have been assayed to reduce the organic load of the winery wastewater. Some of these technologies are based on membrane bioreactors (MBRs), sequencing batch reactor (SBR), upflow anaerobic sludge blanket (UASB), anaerobic sequencing batch reactor (ASBR) and jet loop reactors (JLR). However, most of these methods have some characteristics in common: they are relatively expensive, they are not applicable in all situations, and they are not always able to deal with fluctuations in the hydraulic and pollution load. In order to overcome some of these problems, research efforts have been made towards the development of novel bioreactors as alternatives or to improve, the above-mentioned conventional methods. Although the high organic load of this wastewater would recommend the application of an anaerobic treatment for removing its polluting content, several problems have been found in the application of anaerobic processes due to its seasonal nature, its variable volumes and compositions and the difficulties in the monitoring and process control by specialized personnel (Malandra *et al.*, 2003). The anaerobic treatments such as UASB and ASBR have successfully been used to treat a variety of effluents including those from wineries. The chemical oxygen demand (COD) removal efficiency is greater than 90%, but a specific microbial community is required. However, normally after this reactor there is an aerobic post-treatment, to return the treated water to environment (Moletta 2005) in addition, the process control needs specialized personnel (Malandra *et al.*, 2003).

The MBRs are very compact systems and offer an alternative to conventional activated sludge processes. The COD efficiency achieved is above 97%. The electricity consumption and the operating life of the membranes are higher than those associated with traditional activated sludge systems (Artiga et al., 2005), what may constitute a constraint to its application.

The subsurface-flow constructed wetland is described as suitable for treating these wastewaters, but frequently wineries do not have available area for setting up such plants (Grismer *et al.*, 2003). However, phytotoxicity bioassays carried out with *Phragmites, Juncus and Schoenoplectus* at different wastewater dilutions showed that at greater than 25% wastewater concentration all the macrophytes died (Arienzo *et al.*, 2009a). Nevertheless, the same authors showed that this system when combined with a previously sedimentation/ aerobic process could be used for small wineries located in rural areas, achieving 72% of COD removal rate (Arienzo *et al.*, 2009b).

The wastewater treatment of small wineries (less than 15,000 hL of wine per year) can be also performed using a SBR, fed once a day. The SBR system is a modified design of conventional activated sludge process and it has been widely used in industrial wastewater treatment. The COD removal efficiency is between 86-99% (Torrijos & Moletta, 1997). The on-line monitoring of dissolved oxygen concentration appeared as a good indicator of the progress in the COD biodegradation (Andreottola *et al.*, 2002). Some modifications have been done in order to improve the reactor performance. The opportunity of combining the advantages of the SBR with fixed biomass was investigated (Andreottola & *et al.*, 2002). This system permits the treatment of high organic loads, 6.3 kg COD m^{-3} d^{-1} with high biofilm grown (4-5 kg TSS m^{-3}), allowing the reduction of the required volume for biological treatment and avoiding bulking problems. However, the degradation of organic matter present in a winery wastewater sometimes require the addition of extra nutrients, to balance the C/ N/ P ratio and some oxygen efficiency transfer problems were detected when higher organic loads were applied (Lopez-Palau & Mata-Alvarez, 2009).

The fixed bed biofilm reactor or the air-bubble column bioreactor using self-adapted microbial population either free or immobilized can achieve 90% of COD removal (Petruccioli *et al.*, 2000). In order to overcome the energetic costs associated with the aeration systems, a Venturi injector was used in the JLRs. This system achieves COD removal efficiency near 90%. Though, the high shear stress applied on the Venturi influence the composition of the microbial population (Petruccioli *et al.*, 2000; Eusébio *et al.*, 2005) leading to settling sludge problems. A similar technology that utilizes also a Venturi injector is the AMBB. This technology is very promising because it consists in a vertical reactor with good oxygen transfer and high biological conversion capacity. To optimise the mass transfer, a highly efficient Venturi injector coupled with multiplier nozzles was patented (AirJection®) and was applied in a lagoon system (Meyer *et al.*, 2004) and in a vertical reactor (Oliveira *et al.*, 2009), at pilot scale, to treat winery wastewater with a treatment efficiency of 90 %.

The maintenance and enhancement of a biological reactor is highly dependent on the microbial population that changes with time and winery activity (Jourjon *et al.*, 2005). A deep understanding on the microbial population involved in the process is crucial to address any strategy for treatment system management (Tandoi *et al.*, 2006). Although some researchers have been developed (Eusébio *et al.*, 2004; Eusébio *et al.*, 2005; Jourjon *et al.*, 2005), the understanding of the microflora dynamics inside the bioreactor will be of utmost importance for the treatment system optimisation. Moreover, in the aerobic bioreactors the microorganisms are dependent on aeration oxygen supply. Knowledge of mass transfer coefficients between the different phases together with reaction dynamics is utmost importance to design gas-liquid-solid reactor and to predict the microbial metabolism pathway.

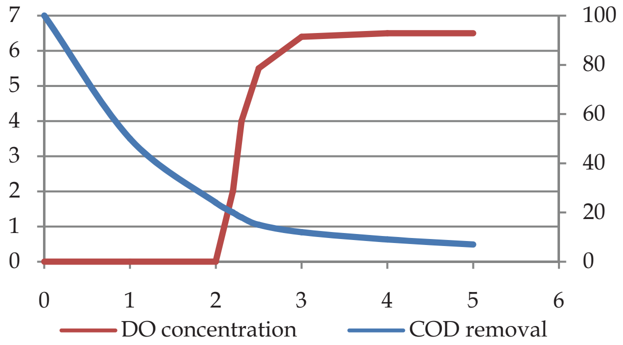
2.2 Optimization of operational parameters in aerobic reactors

The optimization of operational parameters in bioprocesses is based essentially on reducing the volume and footprint, oxygen mass transfer and contact time, energetic costs, sludge settling and production and response time during start-up, while maintain a high removal efficiency of organic matter.

The SBR system has been widely applied to organic carbon removal in municipal and industrial wastewater treatments, as this system presents different advantages such as space reduction and the ability to make operational changes, during the treatment cycle.

In the SBR system the sludge settlement occur in the same tank as oxidation, so in order to optimize the sludge settling time, the formation of granules could be performed based on feast-famine periods (Lopez-Palau *et al.*, 2009). The start-up were performed with the increasing of the COD loading (2.7-20 kg COD. m^{-3} day^{-1}) in order to reach the feast period. After ten days of operation, the first aggregates were observed. But, the use of a high organic load promotes microbial growth and the reactor reached solids concentration of around 6 g VSS L^{-1}. Consequently, some problems of aeration appeared, and the air supply had to be increased from 13.5 L min^{-1} to 20 L min^{-1}. This study showed that is possible to cultivate aerobic granular sludge in SBR, improving the sludge settleability. Nevertheless, the aeration must be proportional to the COD load.

The combination of the SBR with fixed biomass SBBR (Sequencing Batch Biofilm Reactor) to treat winery wastewater was studied by Andreottola *et al.* (2002) and revealed the possibility of treating higher organic loads without increasing the required treatment volume, as the biomass grown on plastic media. However, this type of reactor needs a separated settler, as the biomass settlement worsens in the presence of the plastic material. In order to optimize the energetic costs and the SBBR performance, a strategy based on dissolved oxygen (DO) monitoring was developed.



Adapted from Andreottola *et al.* (2002)

Fig. 1. DO concentration and COD dynamic during a typical SBBR cycle

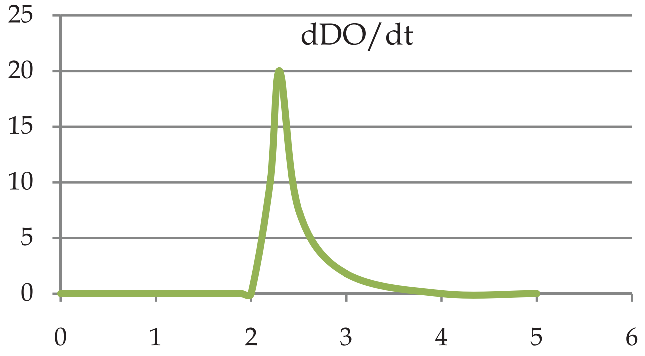
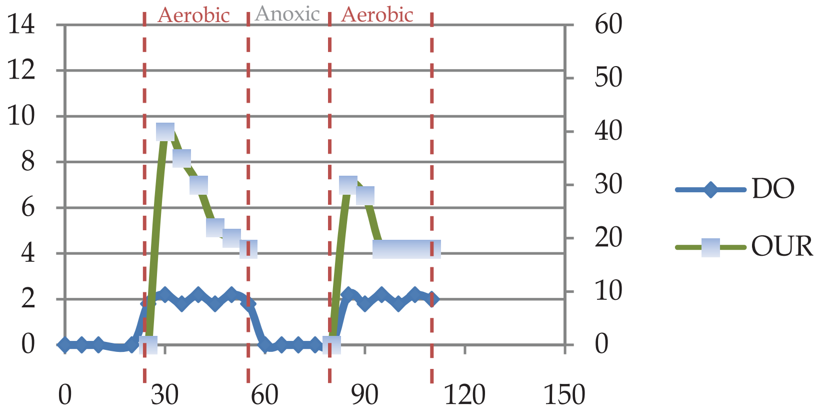


Fig. 2. Time derivative of DO concentration

During the assays the DO control was the key to the COD removal, because this treatment was carried out with constant aeration (up to 4.5 hours). When the treatment started the COD decreases as the DO concentration is maintained at low levels (Figure 1). Once the microbial activity decreases by diminishing the organic load, the DO concentration begins to rise until reaching a plateau. At this stage, the process is complete and the cycle can be stopped. The end of each cycle can be calculated based on the first derivative function of the DO concentration *vs* time (Figure 2). With this strategy it was possible to reduce the hydraulic retention time in about three times, which has allowed the treatment of a higher flow with a similar effluent quality.

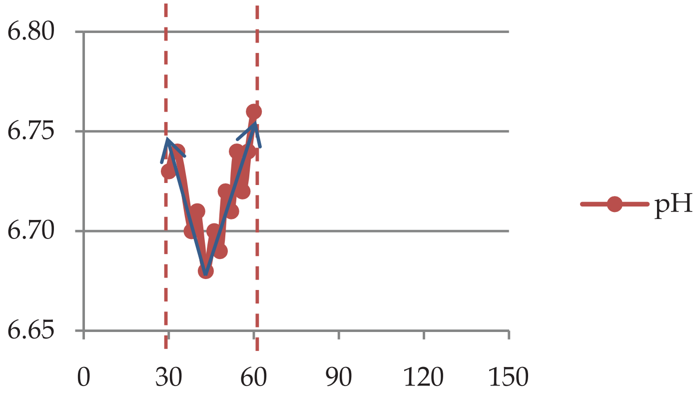
Another approach based on dissolved oxygen control was carried out to optimize a SBR cycle for total organic carbon and ammonia removal (Puig *et al.*, 2006). In this treatment the aerobic phases of the SBR cycle were initially operated using an On/ Off dissolved oxygen control strategy.

The cycle was divided in reaction phase, under aerobic and anoxic conditions, settling and discharge.



Adapted from Puig *et al.* (2006).

Fig. 3. DO and OUR evolution during aerobic and anoxic phases



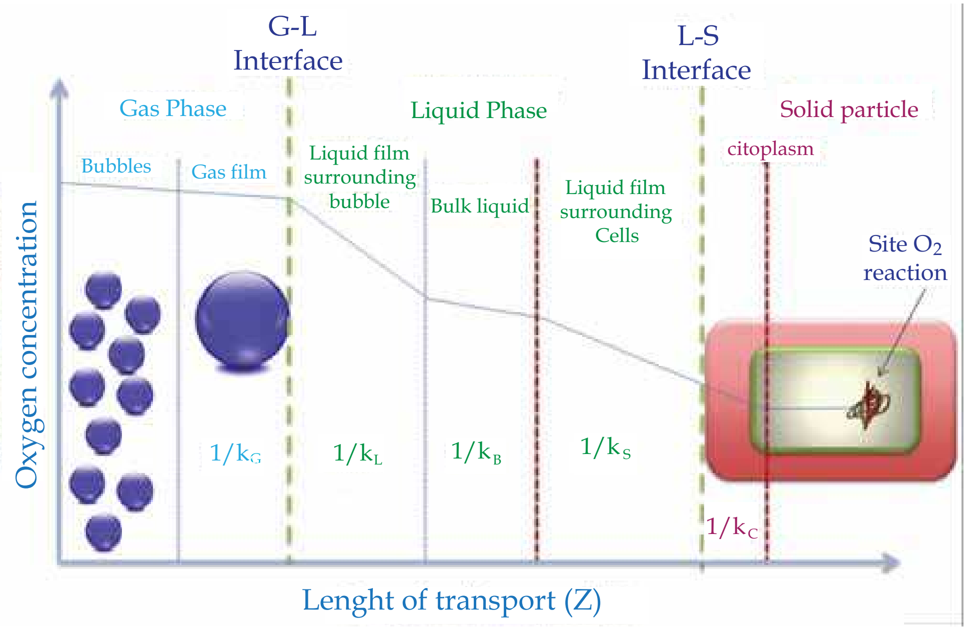
Adapted from Puig *et al.* (2006).

Fig. 4. Detection of the ammonia valley in the pH evolution during aerobic phase

During the aerobic phase a fixed DO set-point of 2.0 mg DO L^{-1} was applied, as a simple On/ Off control. The system optimization was based on pH, DO and OUR evolution. This strategy allows the detection of the ammonia valley in the pH profile and also the end of nitrification, through the OUR outline (Figure 3 and Figure 4). The analysis of the OUR profile shows a plateau in the OUR value, in the end of the aerobic phase, which may indicate that the microbial populations are under endogenous conditions and that organic matter and ammonia has been completed degraded.

In fact, one of the most important aspects in many biological systems is the aeration supply. The wastewater treatment is one of these processes that require proper aeration to maintain the growth of the microorganisms responsible for the biodegradation of the organic matter. Most wastewater treatments are aerobic and are carried out in aqueous medium containing inorganic salts and organic substances which can give viscosity to the broth, showing a non-Newtonian behavior. In bioprocessing it is very important to ensure an adequate oxygen distribution to the gas stream and to the fermentation broth. Some of the systems used to supply the oxygen are sparging, free-jet flow and bubbling column, among others. Also the different nozzle geometry, the liquid phase properties, the jet length and diameter influences the oxygen distribution to the system that in many cases is a limiting factor to the success of the treatment process. In this sense, it is important to estimate the mass transfer characteristics in order to predict the kinetic growth reaction constant, and control and optimize the aerobic fermentation processes (Choi *et al.*, 1996; Fakeeha *et al.*, 1999; Tojabas & Garcia-Calvo, 2000; Garcia-Ochoa & Gomez, 2009). The volumetric mass transfer coefficient, k_La, is the parameter that characterizes the gas-liquid mass transfer in bioreactors. However, this value can vary substantially from those obtained for the oxygen absorption in water or in simple aqueous solutions, and in static systems with invariable composition of the liquid media along time. The transfer rate is very influenced by the nature of pollutants present in the wastewater, for example glucose increases the medium viscosity causing a decrease in the k_La value while the low foam surfactants enhances this value (Fakeeha *et al.*, 1999; Tojabas & Garcia-Calvo, 2000). Thus, it is necessary to know the composition of the fermentative broth, at least some of the major compounds, to understand the effect of combination of different pollutants for proper design and operation of aerobic process. Many strategies have been proposed to determine the volumetric mass transfer coefficient, empirical equations and also theoretical prediction, most of them developed for bubble columns and airlifts (Garcia-Ochoa & Gomez, 2009).

The bioprocesses involves simultaneous transport and biochemical reactions, the oxygen is transferred from a rising gas bubble to the liquid phase and then to the place of oxidative phosphorylation within the cell, considered as a solid particle. The steps related to this mass transfer processes can be represented according to the film theory model for mass transfer, which describes the flux through the film based on a driving force (Figure 5).



Adapted from Garcia-Ochoa & Gomez (2009)

Fig. 5. Steps and resistances for oxygen transfer from gas bubble to cell, in three phases reactors

The oxygen mass transfer rate per unit of reactor volume is obtained by a solute mass balance for the liquid phase (Fakeeha *et al.*, 1999):

$$OTR = k_L a \times (C^* - C_L) \qquad (1)$$

As k_L and a are difficult to measure separately, usually the k_La is evaluated together and this parameter is identified as the volumetric mass transfer coefficient that characterizes the gas-liquid mass transfer. The driving force is the gradient between the oxygen concentration at the interface and in the bulk liquid. This gradient varies with the solubility and microbial activity. Also, the gas solubility depends on temperature, pressure, concentration and type of salts present in the system.

In bioreactors it is essential to determine the experimental k_La to set the aeration efficiency and to quantify the effects of the operating variables on the dissolved oxygen supply. To select the appropriated method, some factors should be taken into account, such as aeration system; bioreactor type and its mechanical design; the composition of the fermentation broth and the possible effect of the microorganisms (Xu *et al.*, 2010).

The mass balance for the dissolved oxygen in the well-mixed liquid phase can be established as (Garcia-Ochoa & Gomez, 2009; Irizar *et al.*, 2009):

$$\frac{dC}{dt} = OTR - OUR \tag{2}$$

Where dC/ dt is the accumulation oxygen rate in the liquid phase, OTR is the oxygen transfer rate from the gas to the liquid phase, described by equation (1) and OUR is the oxygen uptake rate by microorganisms. The methods that can be applied for the oxygen transfer rate measures can be classified depending on whether the measurement is done in the absence of microorganisms or with dead cells or in the presence of biomass that consumes oxygen at the time of measurement. When biochemical reactions do not take place, OUR=0, then the equation (2) can be simplified to:

$$\frac{dC}{dt} = k_L a \times (C^* - C_L) \tag{3}$$

The dynamic method used to measure the k_La value is based on the dissolved oxygen consumption and supply. In this method the change in the dissolved oxygen concentration is analyzed supplying air until the oxygen saturation concentration in the liquid phase is reached. The oxygen decreasing is then recorded as a function of time. Under these conditions the equation (2) can be expressed as equation (4), but, after the decreasing phase, the oxygen is again supplied and the equation (2) can be written as equation (5). In these cases the k_La values can be determined from the slope of the *ln f(C_L) vs* time.

$$ln\left(\frac{C_{L0}}{C_L}\right) = -k_L a \times t \tag{4}$$

$$ln\left(1 - \frac{C_L}{C^*}\right) = -k_L a \times t \tag{5}$$

Furthermore k_La is usually expressed at standard conditions of temperature and pressure, 20ºC, 1atm (equation 6).

$$k_L a_{20} = k_L a_T \times 1.024^{20-T} \tag{6}$$

The determination of the oxygen uptake rate OUR can also be carried out using a dynamic method which measures the respiratory activity of microorganisms that grow in the bioreactor. When the air supply is switching off, the dissolved oxygen concentration will decrease at a rate equal to oxygen consumption due to the microorganisms respiration rate. In this situation the OUR is determined from the slope of the plot of dissolved oxygen concentration *vs* time. The biomass concentration should be known in order to determined the specific oxygen uptake rate (SOUR).

Another important parameter in the aerobic reactors optimization is the sludge settling and production. The large amount of excess sludge generated during activated sludge process is estimated to cost about 40-60 % of the operating cost (Chen *et al.*, 2001). This sludge contains volatile solids and retains about 95% of water resulting in a large volume of residual solids produced. The biological sludge production in conventional wastewater treatment plants can be minimized using different strategies (Pérez-Elvira *et al.* 2006), such as endogenous metabolism and maintenance metabolism. In this last approach part of energy source is used for maintaining living functions, in this phase the substrate consumption is not used for cellular synthesis. In the endogenous metabolism part of cellular components is oxidized to produce the required energy for maintenance functions, which leads to a decrease in the biomass production. The objective is to reach a natural balance between biomass growth and decay rates. The oxic-settling-anoxic activated sludge process, considered as a sludge feast/ famine treatment, is based on alternating exposure of sludge to oxic and anoxic environments. This working principle stimulate catabolic activity and make catabolism dissociate from anabolism. The sludge famine is related to an exposure of the settled sludge to anoxic conditions where the substrate concentration is low. Under these stressful conditions microorganisms are starving which may lead to a depletion of cell energy or nutrients storage. The sludge feasting means that fasted microorganisms return to an oxic environment with enough nutrients. As a consequence, the microorganisms growth may be limited by energy uncoupling (Chen *et al.*, 2001).

2.3 Diagnosis process

The selection of the most appropriate technology for the winery wastewater treatment is a difficult step that should be done after a diagnosis process. A proper diagnosis should conduct a survey report that includes all the information required for decision-makers. Regarding the production process, it should address all activities associated with it: vintage, racking and bottling (Figure 6). The knowledge of materials and supplies, as well as by-products generated during the process is essential in diagnosis. The water uses and water consumption are critical, both in terms of quantity or quality. The survey of sewers in the farm unit, particularly if the drainage system is separated or combined, and the points of wastewater discharge should also be covered. The wastewater flows should be evaluated through the installation of flow meters. The different streams of wastewater generated must be quantified in order to make an assessment, as rigorous as possible.

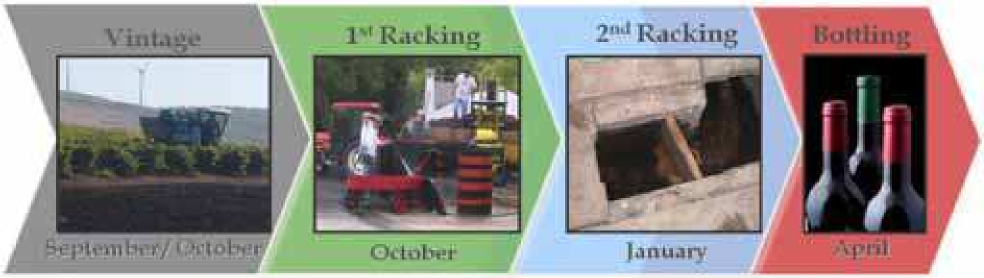
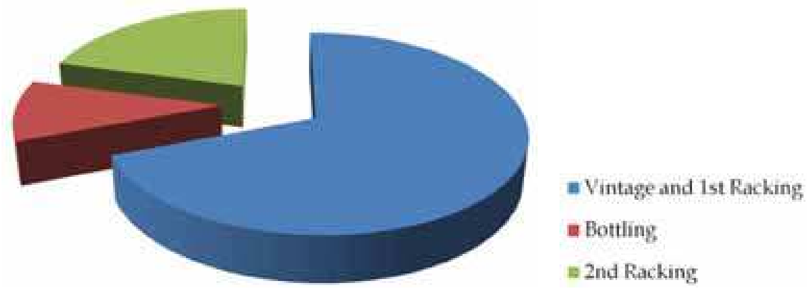


Fig. 6. Winery activities

The water consumption in two Portuguese wineries, one small and one medium size are quite different, with regard to quantity. However, the distribution of water consumption has a similar behavior throughout the year (Figure 7 and Figure 8). The data presented show that most water (60%-80%) is consumed in the vintage period that last about a month. So, the collection of water consumption associated with the physicochemical characterization of the wastewater is essential for the proper sizing of any treatment system. In addition, it is possible to understand the need for flexibility of the treatment system, because the system should allow good removal yields, during the vintage period, but has also to remain in operation during the rest of the year even at low loads. In small wineries often there is a minor stream of wastewater during several months, which may lead to bioreactor inefficient performance. To overcome this situation a feast/ famine strategy may be a challenge for future research at a full-scale.



Adapted from Duarte *et al*. (2004).

Fig. 7. Distribution of water consumption during the global period of processing at a medium dimension winery

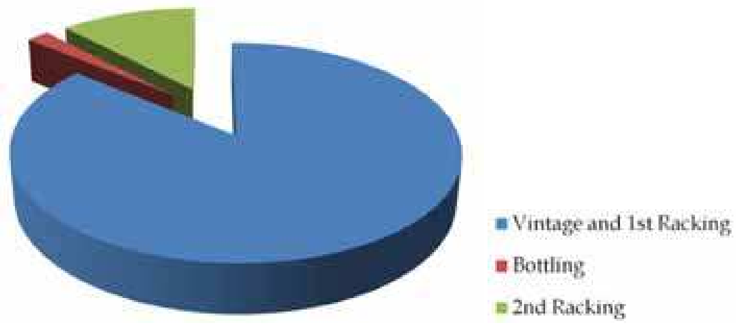


Fig. 8. Distribution of water consumption during the global period of processing at a small dimension winery

The physicochemical characterization assessment is carried out by determining specific parameters such as pH, electrical conductivity, dissolved oxygen, chemical oxygen demand, biochemical oxygen demand, total phosphorus, total nitrogen, total solids, suspended solids, total polyphenol compounds, anionic surfactants. In order to evaluate the fate of treated wastewater, it is also important to know the winery surroundings, in particular the existence of a sewage, the irrigation area, the type of structures and available areas, among others.

In wineries that intend to reuse the treated wastewater for irrigation, other concerns should be considered. The domestic wastewater flow containing high concentration of pathogenic microorganisms should not be mixed with the industrial wastewater stream. This flow should be treated separately or discharged in the sewage. This decision is extremely important, since the wastewater from winery operations does not contain pathogenic microorganisms. Thus, this separation reduces the costs of wastewater treatment and monitoring, which are associated with the disinfection process.

3. Winery wastewater treatment in the Air Micro-Bubble Bioreactor

3.1 Wastewater characterization

The winery wastewater was collected, during four years, from three wineries of different sizes and characteristics and located in different Regions of Portugal. The Casa Agrícola Quinta da Casa Boa, located at Runa, Lisboa Region, producing only red wines, has a small/ medium dimension with a production capacity of 200,000 L. The Catapereiro, located at Alcochete, Tejo Region, produces both white and red wines, has a medium dimension with a production capacity of 1 000,000 L of wine. The Herdade da Mingorra, located at Beja, Alentejo Region, has a medium dimension with a production capacity of 1 000,000 L of wine (Figure 9 to Figure 14).

Composite samples of the winery wastewater, representative of each phase of the process, were taken and maintained at 4ºC. A set of major key parameters were defined and analysed, according to *Standard Methods for the Examination of Water and Wastewater* (1998), in order to assess the winery wastewater pollutant charge: pH, conductivity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), volatile suspended solids (VSS), polyphenols, anionic surfactants, Na, K, Mg and Ca. The winery wastewater flows were evaluated from water consumption. With this propose the wineries installed general water counters to be daily read and register.

3.2 Bioreactor set-up

The Air Micro-Bubble Bioreactor (AMBB) with a total volume of 15 dm^3 consists of a cylindrical bioreactor, equipped with a circulated pump and a settler (Figure 15). The aeration was conducted during the wastewater recirculation by a high efficiency Venturi injector (HEVI) in conjunction with mass transfer multiplier nozzles (MTM). The MTM nozzles discharge the air/ water mixture from the HEVI into the bottom of the bioreactor (Figure 14). The AMBB is equipped with an air flow meter and a monitoring probe (HANNA Instruments) able to on-line monitor pH, DO and temperature. Figure 16 shows a schematic overview of the bioreactor.

3.3 Bioreactor start-up and operating conditions

Several trails performed with the AMBB, under batch conditions were carried out during 15 days. The reactor was inoculated with 15 dm^3 of fresh winery wastewater, from the vintage period and with 0.15 dm^3 of acclimated biomass, obtained during the treatment of winery wastewater, in the previously year. Samples from the mixed liquor were daily taken for physico-chemical characterisation. The aerated flow was 2 dm^3 min^{-1}. The operating temperature was 20-30ºC. The recirculation of the mixed liquor started with 20 min hour^{-1}, with a flow of 40 dm^3 min^{-1} and then was changed to 5 min hour^{-1}.

3.4 Seed germination bioassays

Germination bioassays were performed following Fuentes *et al.* (2004), by using cress *Lepidium sativum L.* seeds, to evaluate the suitability of the treated wastewater in relation to crop irrigation and expressed as Germination Index. The treated wastewater and two dilutions in distilled water (25%, 50% v/ v) were tested (Oliveira *et al.*, 2009).

Fig. 9. Global view at Mingorra winery

Fig. 10. Mingorra winery unit

Fig. 11. Vintage at Quinta da Casa Boa

Fig. 12. 1st Racking at Quinta da Casa Boa

Fig. 13. Wastewater treatment system at Catapereiro

Fig. 14. Wastewater treatment system at Mingorra

Fig. 15. The air micro-bubble bioreactor filled with clean water

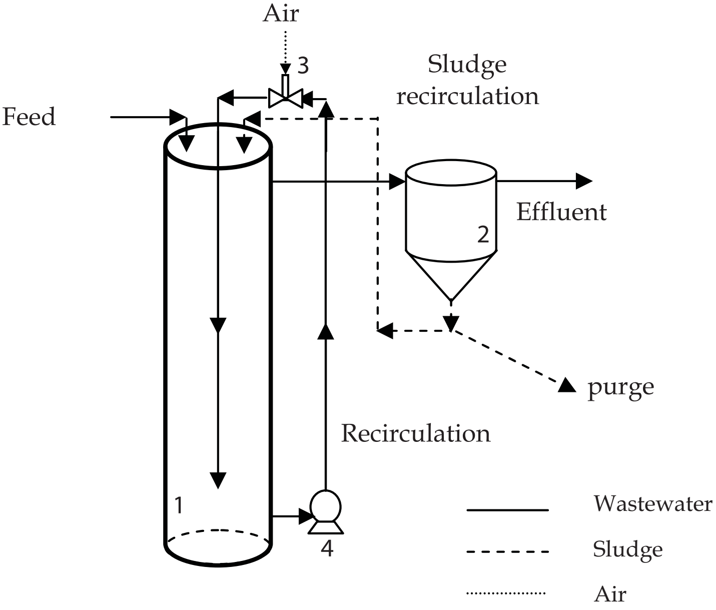


Fig. 16. Flow diagram of the air micro-bubble bioreactor. 1- Bioreactor; 2- Settler; 3- *Venturi* injector; 4- Recirculation pump

4. Results and discussion

4.1 Wastewater assessment

During the studying period, samples of winery wastewater were taken for laboratory characterization to evaluate their pollutant charge (Table 1). The values of pH ranged from 4 to 8, being this variation mostly dependent on the labor period. The electric conductivity of the wastewater showed no relevant variation in the different sampling periods and the range of registered values is not considered as inhibiting biomass growth.

The highest values of COD were reached during the vintage period, followed by the first racking. These results are in accordance to those previously reported by other authors (Petruccioli *et al.*, 2002). As expected, the highest values of biodegradability (BOD_5/ COD) were achieved during the vintage period, due to the high concentration of simple molecules, easily metabolized (sugars and ethanol) by microorganisms (Duarte *et al.*, 2004).

Concerning TS and TSS parameters, the results reveal a high variability during the vinification period. Moreover, the TS are significantly higher than TSS, which means that these wastewaters contain, mostly, dissolved organic pollutant charge. However, during 2nd racking the TSS concentration reach the maximum value derived from the presence of tartrate. These solids are often problematic due to the high phenolic load adsorbed.

Although polyphenols and anionic surfactants are important pollutants, it is not expected that they could influence the organic load, since they are present in low concentration. Nevertheless, after the wastewater treatment some compounds known as recalcitrant may remain in the treated effluent, such as the polyphenols that are responsible for colour and the residual COD, this can also be observed by the low biodegradability ratio presented in Figure 17.

Moreover, this type of wastewater has very low levels of nutrients that are essential to microbial growth. For this reason, it is often required the addition of nutrients to guarantee the process of cellular synthesis. Alternatively, it is possible to change some practices at the winery in order to balance this ratio (Oliveira & Duarte, 2010).

The assessment of the water consumption is another key parameter for the successful of the winery wastewater treatment. In one of the monitored wineries the water consumption was evaluated throughout the operation period for two consecutive years. Internal management strategies were implemented to increase efficient water use, such as cleaning methods that aim the water reuse (closed-loop) pressure washing machines, among others. These simple changes showed a saving in water consumption of about 40%.

4.2 AMBB treatment

In this type of seasonal industry, the treatment system must be able to treat the wastewater produced in the vintage period. For this reason, many reactors have an appropriate volume for this stage but over dimensioned during the rest of the year. On the other hand, the high organic load of these wastewaters may promote the excessive growth of biomass, that requires an increase in the air supply (López-Palau *et al.*, 2009) and creates problems of sludge generation and disposal.

The adopted strategy in this study is based on sludge reduction, as the production of excess sludge from the wastewater treatment plant is considered one of the serious problems encountered in the aerobic treatments (Liu & Tay, 2001). In this study, an aerobic step alternated with an anoxic one was adopted as a strategy.

Table 1. Physico-chemical characterization of winery wastewater

	Vintage			1st Racking			2nd Ra
pH	5.88	±	0.92	5.92	±	1.95	5.23
Conductivity (μS cm^{-1})	1714	±	279	2036	±	618	3260
COD (mg.L^{-1})	8942	±	5310	8025	±	4220	5993
BOD$_5$ (mg.L^{-1})	4107	±	2900	3104	±	817	1395
BOD$_5$/COD	0.66	±	0.32	0.47	±	0.26	0.23
Oxidized matter (mg.L^{-1})*	5988	±	2448	4461	±	842	1640
TS (mg.L^{-1})	6268	±	4274	3034	±	248	8313
TSS (mg.L^{-1})	523	±	190	866	±	199	3739
NH$_4^+$ (mg.L^{-1})	0.73	±	0.68	4.5	±	2.6	
Kjeldahl nitrogen (mg.L^{-1})	16	±	8	26	±	19	135
P (mg.L^{-1})	12	±	8	27	±	22	93
Na (mg.L^{-1})	347	±	46	218	±	305	234
K (mg.L^{-1})	117	±	8	71	±	72	264
Mg (mg.L^{-1})	12	±	1	9.4	±	3.0	10
Ca (mg.L^{-1})	39	±	15	27	±	31	18
Cu (mg.L^{-1})	0.13	±	0.01	0.21	±	0.13	0.09
Turbidity	209	±	138	635	±	214	3628
Total Phenols (mg.L^{-1})	28	±	11	32	±	15	30
SO$_4^{2-}$ (mg.L^{-1})	191	±	38	160	±	129	177
Cl$^-$ (mg.L^{-1})	130	±	62	185	±	129	113
Anionic surfactants (mgMBAS.L^{-1})	15	±	13	7	±	8	4.2

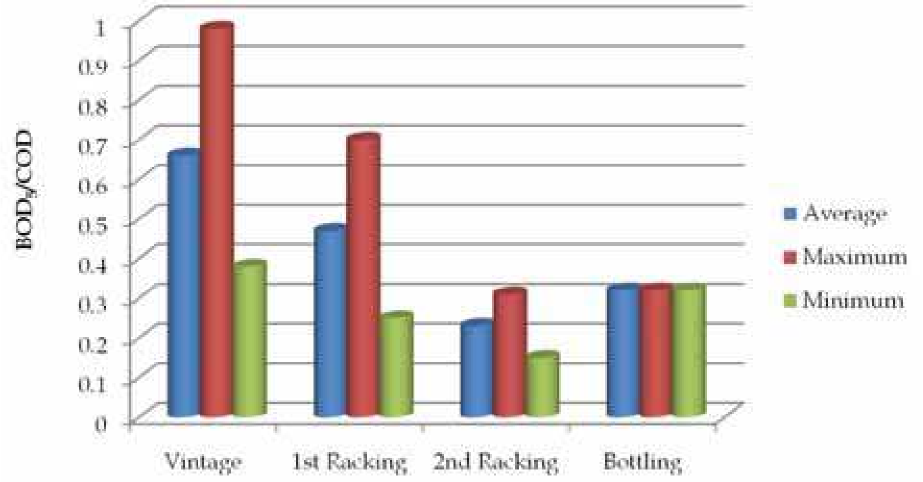


Fig. 17. Biodegradability indicators of the winery wastewater, in different labour periods

The bioreactor AMBB was tested in different phases of the wine process and it started in the vintage period (Figure 18-19). The biomass inoculated in this assay was already acclimated to winery wastewater and was maintained in aerobic/ anoxic conditions, with insufficient substract. During the AMBB operation the microorganisms grow as suspended biomass but also as biofilm adsorbed to the reactor walls.

Fig. 18. Inoculation of the AMBB with fresh winery wastewater

Fig. 19. AMBB in the beginning of the treatment

The evolution of COD concentration, biomass and dissolved oxygen was followed. Regarding the biomass evolution, a typical growth curve for batch cultivation was achieved (Figure 20). This curve does not show a lag phase, since biomass was already adapted. The recirculation of the mixed liquor was 20 min hour^{-1}.

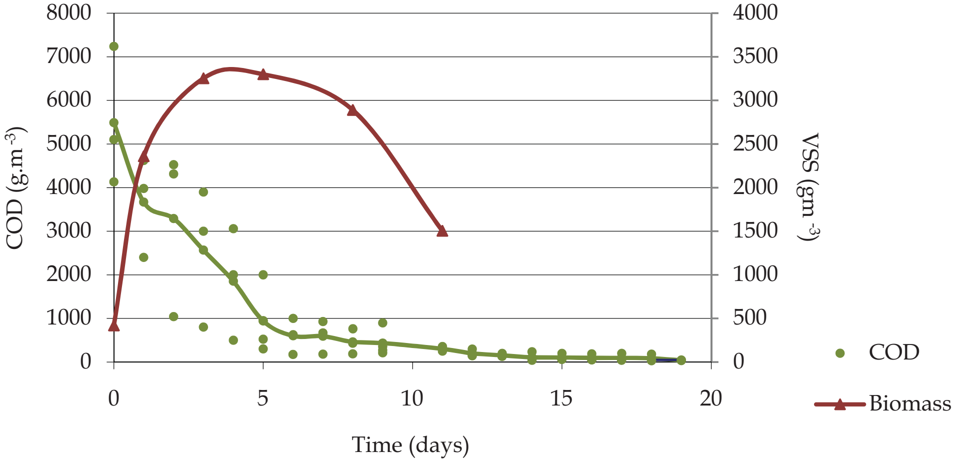


Fig. 20. Evolution on COD and biomass concentration in the AMBB

The COD of the winery wastewater ranged between 4.0-8.0 kg COD m^{-3} but the efficiency was similar for each batch, about 90.0±4.3%, after 6 days of operation. This period is related to the biomass exponential phase. The maximum efficiency obtained (98.6±0.4%) was achieved after 15 days of treatment. These results are comparable with those reported by Beltran de Herédia *et al*. (2005), where they achieve 75% of COD reduction, after 3 days of treatment.

In order to minimize the sludge production and the energetic costs during the recirculation of the mixed liquor, the aeration time was reduced. During this assay dissolved oxygen, pH, COD and biomass was evaluated.

Concerning the DO concentration the Figure 21 illustrate the dynamic change of this parameter in the AMBB. During the air supplying, the DO increases until it reaches saturation. The period of time required to reach saturation is directly related to the oxygen transfer rate. The estimation of OTR under different operational conditions has a relevant role to predict the metabolic pathway for microbial growth in aerobic treatments. So, this approach could be interesting for studying the influence of operational conditions on volumetric mass transfer coefficient.

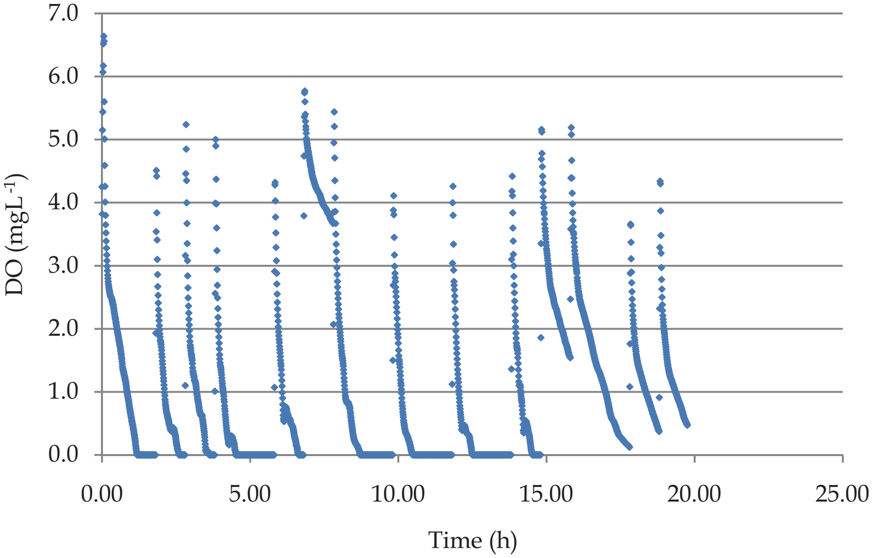


Fig. 21. Evolution on pH and DO concentration in the AMBB

A dynamic method was used to determine the volumetric mass transfer coefficient, k_La (Table 2). The k_La values were calculated by solving the Equation 2, during the aeration phase and considering that the gas flow and OUR were both constant. In these cases the slope of the *ln* f(DO) *vs* time allows the determination of the oxygen transfer parameter (Figure 22). The k_La values were corrected to 20 ° C, according to equation 6.

The results show a decrease in the k_La value during the treatment period (Figure 23). Many factors could influence k_La, including air flow rate, air pressure, temperature, vessel geometry and fluid characteristics. All parameters were kept constant throughout the treatment, except the wastewater composition that varies during the treatment period. More readily biodegradable compounds such as sugars and ethanol are firstly assimilated by microorganisms; the more complex substrates are only degraded at a later stage. Previous studies indicate that the composition of the fermentation broth influences the oxygen mass transfer, such as glucose that can decrease the k_La, by increasing the viscosity of the medium but on the other surfactants increases this value (Fakeeha *et al.*, 1999). In fact, is practically impossible to determine the exact composition of wastewater, but the compounds mentioned above are always present in this type of wastewater. This decrease in k_La value means that some of the existing compounds in wastewater optimize the oxygen mass transfer during the initial phase of treatment. Indeed, even without being quantified, in all

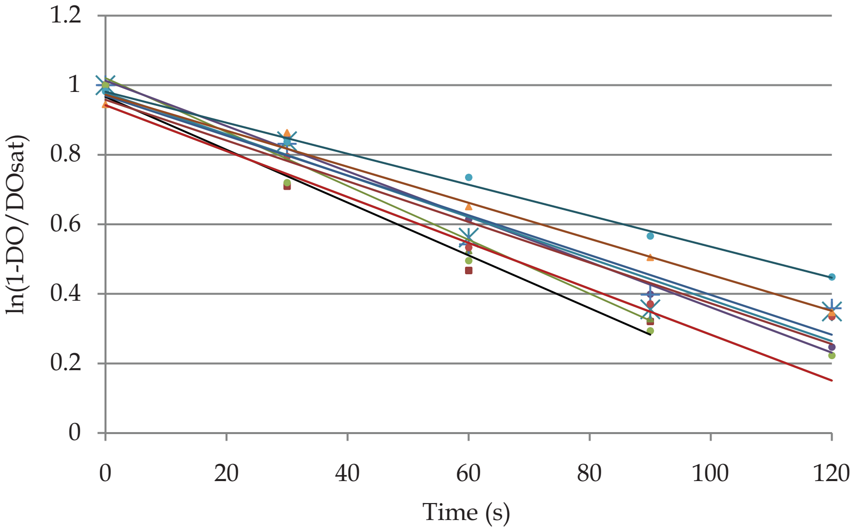


Fig. 22. Experimental determination of k_La based on DO concentration in the AMBB

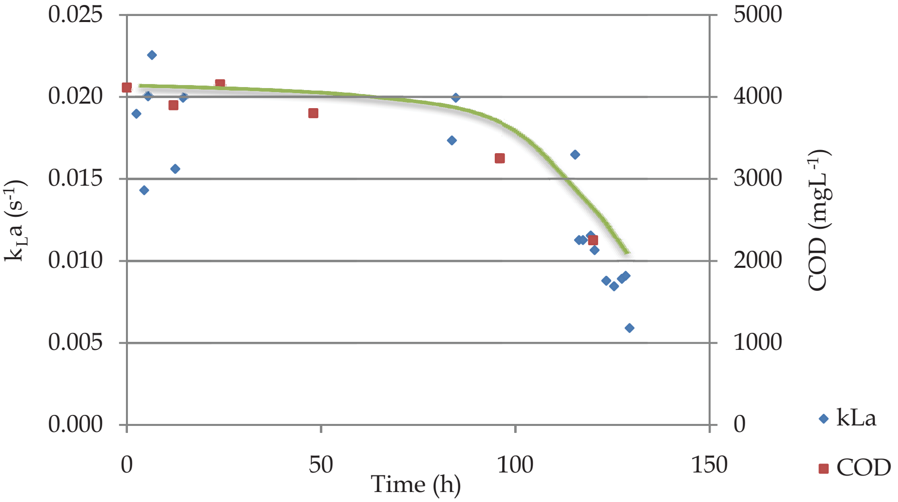


Fig. 23. k_La and COD dynamics during wastewater treatment in the AMBB

trials it was found that the size of the bubble formed, increased throughout the treatment period, which is in agreement with the obtained results. Moreover, it is interesting to observe that the k_La decline follows the degradation kinetics of organic matter, expressed as COD, which corroborate the obtained results. The k_La values obtained in these assays are in the same range that of values achieved by other authors in full-scale aeration tank equipped with fine bubble diffusers and jet loop reactor (Fakeeha *et al.,* 1999; Fayolle *et al.*, 2010).

In addition, the respirometric activity of microorganisms which are actively growing in the bioreactor can also be measured based on this dynamic method. When the gas supply to the bioreactor is turned off, the DO concentration decreases at a rate equal to oxygen consumption by the respiration process. In this situation the OUR can be calculated from the slope of the DO *vs* time (Figure 24).

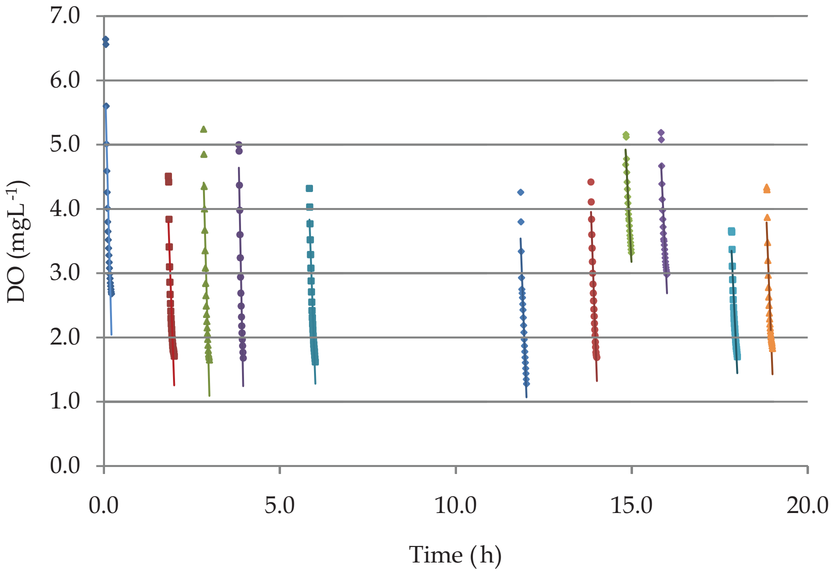


Fig. 24. Trendlines adjustment on DO concentration depletion to determine OUR in the AMBB

The specific oxygen uptake rate (SOUR) or respiration rate is expressed as milligrams of oxygen consumed per gram of volatile suspended solids per hour. The high SOUR values obtained (Table 2), indicate a high organic load to the existing suspended solids in the mixed liquor (MLSS).

The SOUR measurements throughout the wastewater treatment showed an initial increase in the SOUR values until reaching a plateau. The Figure 25 shows that after an adaptation period to the treatment system there is a removal of the organic load, expressed as COD rate corresponding to the increment of SOUR rate. This high SOUR rate is due to the high activity of the microbial population to oxidise substrates. These values may be induced by an increased energy requirement stimulated by a famine period, during sludge acclimatisation. The feast/ famine phenomenon has been reported by several authors as a strategy on sludge production (Chen *et al.*, 2001; Ramakrishna & Viraraghavan, 2005; López-Palau *et al.,* 2009). A similar behaviour was found by Chen *et al.* (2001) during the study of feast/famine growth on activated sludge cultures previously subjected to a famine treatment. This study also indicates that the COD removal ability of the fasted culture is higher than the non-fasted culture.

	OUR **(mg O_2 $(L.h)^{-1}$)**	**SOUR** **(mg O_2 (g MLSS. $h)^{-1}$)**	**$k_La.10^3$ (20º C)** **(s^{-1})**
	16.6	19.5	11.7
	19.8	23.3	19.0
	19.5	22.9	14.3
	17.6	20.7	20.0
Initial values	20.5	24.1	22.6
	20.2	23.8	13.0
	18.9	22.2	13.0
	19.5	22.9	15.6
	19.6	23.1	26.0
	21.4	25.2	19.9
	20.4	24.0	19.9
Maximum values	28.9	34.0	17.3
	32.8	38.6	19.9
	32.6	38.4	18.2
	31.8	37.4	16.5
	29.5	34.7	11.3
	32.8	37.4	11.3
	14.2	25.1	11.5
	21.4	17.9	10.7
Final values	15.2	23.4	8.9
	19.9	18.0	8.8
	15.3	17.4	8.5
	14.8	18.6	8.9
	15.8	12.4	9.1
	10.5	14.1	5.9
	12.0	13.4	7.7
	11.4	16.7	6.2

Table 2. Evolution of the mass transfer parameters OUR, SOUR and k_La values, throughout the treatment

In winery wastewater treatments systems the period prior to vintage is a non-productive period, without wastewater generation. In this sense, the existing biomass in the treatment system is subjected to a famine treatment. Moreover, during harvest the wastewater production has the highest flow rates and organic loadings. According to Chen *et al.*, 2001 after a famine period the microorganisms are starved and the substrate utilization rate increases. A treatment system based on this management model seems to be a good approach for winery wastewaters, with the additional advantage of keeping the low amount of sludge. The cause of sludge reduction in this process is not clearly known but the absence of oxygen reduces the growth of strictly aerobic populations and stimulates the facultative bacteria (unpublished results), which have lower specific growth rates. In this sense, as the dominant population is constituted of slow growers that may explain the low sludge yield production. Furthermore, the produced sludge shows low SVI values indicative of easy sludge settling.

The strategy based on low aeration time alternating with anoxic periods allows the treatment of the winery wastewater with lower sludge production but with lower efficiency. In fact, the MLSS achieved in this batch treatment, 1.2 g/ L was lower compared with the initial assay. In the management of a wastewater treatment of this nature is necessary to establish a compromise between operating costs and final quality of the treated wastewater, taking into account the final destination and the legal requirements.

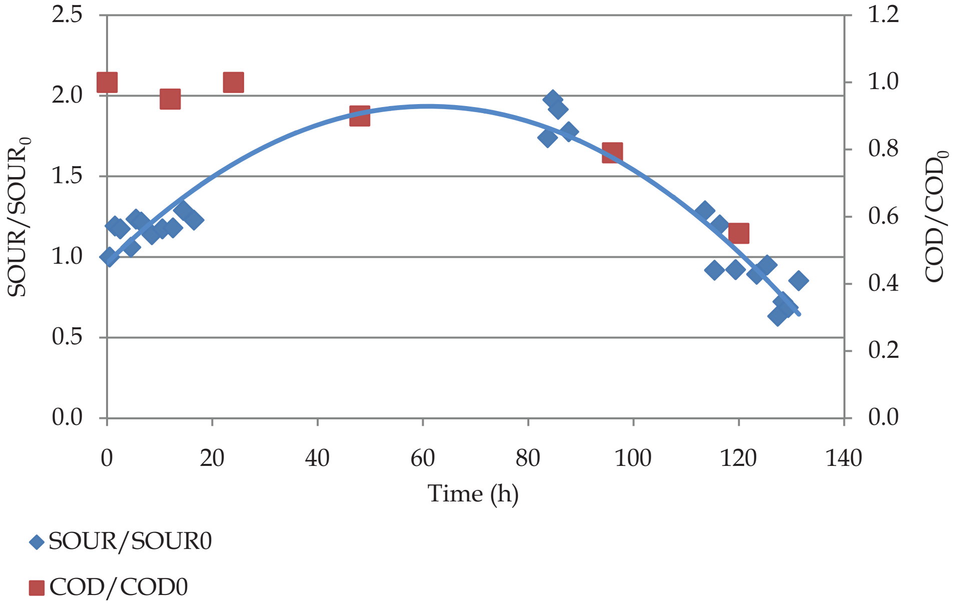


Fig. 25. Evolution of SOUR and COD rates during the batch treatment

In order to evaluate its suitability to be used in crop irrigation the treated wastewater from the AMBB batch assays was physico-chemical characterized. All the analyzed parameters except one were in agreement with EU and Portuguese Legislation (Directive 2000/ 60/ EC, DL n° 236/ 98) for irrigation use (Table 3). Of particular concern was the sodium adsorption ratio (SAR), the proportion of sodium to calcium and magnesium, which was higher than the permitted parametric value. Probably, some strategies can be applied in winery in order to reduce the problem. Nevertheless, the treated wastewater, normally, is used in irrigation systems to supplement the irrigation water, as an economic additional water supply. Also, seed germination assays carried out with *Lepidium sativum* were developed for evaluating the effects of water contaminants on germination and seedling growth. The adequacy of the treated wastewater for crop irrigation was evaluated with direct toxicity bioassays, by using cress seeds as indicator No significant differences (P=0.05) between batch experiments were registered on germination index (GI). As the cress bioassay is a standard procedure to evaluate the behaviour of crops to water contaminants, data (previously published) evidence the suitability of treated wastewater in relation to crop irrigation, thus minimizing water consumption (Oliveira *et al.,* 2009).

Parameter	**Treated wastewater**	**DL nº 236/98**
pH	8.0	4.5-9.0
Conductivity (μS cm^{-1})	920	-
COD (mg L^{-1})	140	-
N total (mg L^{-1})	2.0	-
P total (mg L^{-1})	0.6	-
Phenolic compounds (mg L^{-1})	0.5	-
TSS (mg L^{-1})	30	60
Cl^- (mg L^{-1})	60	70
SO_4^{2-} (mg L^{-1})	50	575
SAR	27	8

Table 3. Physical and chemical characterization of the treated wastewater and standard parameter

5. Conclusion

In this type of seasonal industry, the treatment system must be able to treat the wastewater produced in all labour period. A vertical reactor coupled with highly efficient Venturi injector and multiplier nozzles was used for winery wastewater treatment. Regarding mass transfer parameters, the estimation of OTR could be interesting for studying the influence of operational conditions on volumetric mass transfer coefficient. The results showed a decrease in the k_La value during the treatment period. This decrease in k_La value may evidence that some of the existing compounds in wastewater optimize the oxygen mass transfer during the initial phase of treatment.

The SOUR measurements throughout the wastewater treatment showed high values, which could indicate a high organic load to the existing suspended solids in the mixed liquor. This high SOUR rate is due to the high activity of the microbial population to oxidise substrates, in the begging of the treatment.

The implemented strategy, where an aerobic step alternated with an anoxic one was adopted, showed to be a good approach to minimize the sludge production and to reduce energetic.

However, further studies should be conducted in order to better understand the effect of winery wastewater composition in mass transfer coefficients. The use of two consecutive stages of treatment might improve the performance of this technology because it allows higher flexibility. The result of feast / famine treatment in sludge should also be exploited, as it is of interest in this type of seasonal industries.

Moreover, the treated wastewater revealed its suitability to be integrated in the irrigation systems as confirmed by direct toxicity bioassays. This study is expected to contribute to the implementation of an efficient wastewater treatment, intending the preservation of the water resource, the reduction of the wastewater sludge production and the energy safe.

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Mass Transfer - Advanced Aspects

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Our knowledge of mass transfer processes has been extended and applied to various fields of science and engineering including industrial and manufacturing processes in recent years. Since mass transfer is a primordial phenomenon, it plays a key role in the scientific researches and fields of mechanical, energy, environmental, materials, bio, and chemical engineering. In this book, energetic authors provide present advances in scientific findings and technologies, and develop new theoretical models concerning mass transfer. This book brings valuable references for researchers and engineers working in the variety of mass transfer sciences and related fields. Since the constitutive topics cover the advances in broad research areas, the topics will be mutually stimulus and informative to the researchers and engineers in different areas.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821