

Acknowledgements

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Abstract

The energy consumption of wastewater treatment processes had become an important tool parameter for designers and engineers in the wastewater treatment industry, and efforts to improve the energy efficiency of the equipments represent a great challenge. The present study proposes the use of the Air Micro-Bubble Bioreactor (AMBB), with a working volume of 14 dm³ and a hydraulic retention time of 14 days, operating in continuous mode to treat winery wastewater from second racking period, for seven different runs, during 167 days.

The organic loads fluctuations, usual in the winemaking industry, and the aeration time rate (ATR), both related to energy costs, were the studied variables. The chemical oxygen demand (COD) was selected as a key parameter to monitor the AMBB performance. The total polyphenol compounds were also followed. The effect of the organic loading rate OLR was assessed by adjusting feed substrate concentration between 0.16-0.44 kg COD m⁻³ d⁻¹. The ATR varied between 1, 5 and 15 min h⁻¹. Each run was operated until steady state was reached with respect to COD concentration. All runs showed a positive answer for COD removal, having a minimum efficiency of 70%. Also the COD removal efficiency showed to be not dependent on the applied OLR and the decrease in the ATR to 5 min h⁻¹ was accompanied by a nearly three-fold reduction in energy consumption without relevant changing on the final COD removal efficiency (93-96%). However, the total polyphenol compounds removal was highly affected by decreasing the ATR from 15 to 1 min h⁻¹, leading to a decrease in the total polyphenols removal efficiency from 94% to 4%.

In the present study, we report for the first time the impact of oxygen-limited conditions in the AMBB performance with emphasis on the efficiency energy use. The polyphenols as a performance indicator, the energy costs of treated wastewater and COD removal efficiency achieved in pilot scale show the technical feasibility of the process, giving a sustainable solution for this important sector of activity.

Keywords: Aeration time rate, Air Micro-Bubble Bioreactor; COD removal efficiency; polyphenol removal efficiency; winery wastewater

Resumo

O consumo energético decorrente dos processos de tratamento de águas residuais, tornou-se um parâmetro importante para projetistas e engenheiros na indústria de tratamento de águas residuais, e os esforços para melhorar a eficiência energética dos equipamentos representam um grande desafio para os profissionais que actuam neste sector de actividade. O objetivo do presente trabalho é a avaliação do desempenho do reator *Air Micro-Bubble Bioreactor* (AMBB), com um volume útil de 14 dm³ e um tempo de retenção hidráulico de 14 dias, operando em modo contínuo para o tratamento de águas residuais vinícola, provenientes do período segunda trasfega, durante sete ciclos diferentes, e durante 167 dias. As flutuações de carga orgânica, habituais no sector vinícola, e a taxa de arejamento do reator, ambos relacionados com os custos de energia, foram as variáveis estudadas. O desempenho reator AMBB foi avaliado através da monitorização da carência química de oxigénio (CQO). Os polifenóis totais também foram monitorizados. O efeito da carga orgânica de alimentação foi analisado, efetuando-se um ajuste na concentração de entrada do substrato entre 0,16-0,44 kg CQO m⁻³ d⁻¹. A taxa de arejamento do reator variou entre 1, 5 e 15 min h⁻¹. A duração de cada ciclo, foi o tempo necessário até se atingir o estado estacionário no que diz respeito à concentração de CQO. Todos os ciclos mostraram uma resposta positiva para a remoção de CQO, com uma eficiência mínima de 70%. Além disso, a eficiência de remoção de CQO não mostrou ser dependente da concentração de entrada do substrato aplicada, e a diminuição da taxa de arejamento para 5 min h⁻¹, foi acompanhada por uma redução de aproximadamente três vezes no consumo de energia, sem alteração relevante sobre a eficiência de remoção de CQO final (93 - 96%). No entanto, a remoção de compostos polifenóis foi muito afectada pela diminuição da taxa de arejamento de 15 para 1 min h⁻¹, levando a uma diminuição da eficiência total da remoção de polifenóis de 94% para 4%.

No presente estudo, relata-se pela primeira vez o desempenho do reator AMBB em condições limite de oxigénio, dando ênfase à questão da eficiência energética. Os polifenóis como um indicador de desempenho, os custos de energia associados ao tratamento do efluente, e a eficiência da remoção de CQO alcançada em escala piloto, mostram a viabilidade técnica do processo, contribuindo para uma solução sustentável para este importante sector de actividade.

Palavras chaves: Air Micro-Bubble Bioreactor; taxa de tempo de arejamento, eficiência de remoção de CQO; eficiência de remoção de polifenóis; águas residuais vinícolas.

Extend Abstract

O processo de vinificação, possui operações padronizadas bem-definidas (colheita, esmagamento, prensagem, operações de fermentação, clarificação, trasfega e engarrafamento), gera águas residuais muito poluentes e o respectivo tratamento e eliminação (ou a reutilização) deste efluente, requer adequadas opções sustentáveis de gestão em conformidade com a legislação e regulamentos em vigor.

Como consequência do carácter sazonal desta actividade e das tecnologias de vinificação, os volumes e cargas orgânicas do efluente têm variações acentuadas ao longo do ano. As águas residuais provenientes da segunda trasfega (o que ocorre quando o processo de fermentação está completo), são consideradas uma das mais poluentes devido ao alto teor de carga orgânica total e da concentração de sólidos em suspensão que podem atingir valores elevados, os quais resultam da precipitação dos tartaratos. Estes sólidos são problemáticos não só devido à elevada carga orgânica mas, também devido aos compostos fenólicos adsorvidos. De acordo com o método de vinificação a produção de efluentes pode apresentar características específicas, tornando-se seleccionar a alternativa mais adequada e de baixo custo para o respectivo tratamento.

O consumo energético decorrente dos processos de tratamento de águas residuais, tornou-se um parâmetro importante para projetistas e engenheiros na indústria de tratamento de águas residuais, e os esforços para melhorar a eficiência energética dos equipamentos representam um grande desafio. Para isso, é necessário quantificar a energia gasta por cada metro cúbico de água residual tratada combinando os custos de energia vs eficiência obtida.

Trabalhos desenvolvidos por outros investigadores sobre o desempenho do reator *Air Micro-Bubble Bioreactor* (AMBB) demonstraram que este pode ser uma boa opção para o tratamento de efluente vinícola, tendo sido a eficiência avaliada pela carência química de oxigénio (CQO), como também a reutilização do efluente tratado para irrigação. No entanto, os custos operacionais e gestão poderiam ser potencialmente otimizados.

O presente estudo teve como principal objetivo a avaliação do desempenho do reator AMBB em modo contínuo para o tratamento de águas residuais vinícola geradas durante o período da segunda trasfega. As amostras do efluente vinícola foram recolhidas durante a operação de segunda trasfega, entre Janeiro e Março de 2012, numa adega com uma produção de cerca de 100 mil litros de vinho tinto por ano (Quinta da Casaboa, Runa, Torres Vedras).

A eficiência do reator AMBB foi avaliada tendo em conta a remoção do CQO e dos compostos fenólicos, sendo este último um indicador de desempenho. O AMBB tem um volume de trabalho de 14 dm³ e um tempo de retenção hidráulico de 14 dias e operou durante sete ciclos diferentes, durante 167 dias. As flutuações da carga orgânica do efluente, habituais na indústria vinícola, e o tempo de arejamento imposto ao reator, ambos relacionados com os custos de energia, foram as variáveis monitorizadas durante o período de pesquisa. O efeito da carga orgânica de entrada foi analisada, efectuando-se um ajuste na concentração de alimentação do substrato entre 0,16-0,44 kg CQO.m⁻³.d⁻¹. A taxa de arejamento do reator variou entre 1, 5 e 15 min⁻¹ h. A duração de cada ciclo, foi o tempo necessário até se atingir o estado estacionário no que diz respeito à concentração de CQO que se pretende alcançar.

Os resultados deste estudo revelaram que a eficiência de remoção do CQO foi independente da taxa de carga orgânica aplicada e da taxa de arejamento (5 e 15 min h⁻¹). Apesar da remoção dos polifenóis ter sido muito afectada pela diminuição da taxa de arejamento de 15 para 1 min⁻¹ h, ainda assim, o reator AMBB mostrou-se adequado para remover CQO (93-96%), com apenas 5 min⁻¹ h de taxa de arejamento, o que permitiu a redução dos custos de energia em aproximadamente três vezes.

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Index of Acronyms

AMBB – Air Micro-Bubble Bioreactor

ASBR – Anaerobic Sequencing Batch Reactor

AOPs – Advanced Oxidation Process

ATR – Aeration Time Rate

BOD – Biochemical Oxygen Demand

COD – Chemical Oxygen Demand

FBB – Fluidized Bed Bioreactor

FBBR – Fixed Bed Biofilm Reactor

HEVI - High Efficiency Venturi Injector

HRT – Hydraulic Retention Time

INE – Instituto Nacional de Estatística

IVV – Instituto da Vinha e do Vinho

JLR – Jet Loop Reactor

MTM – Mass Transfer Multiplier

MBBR – Moving Bed Biofilm Reactor

MBRs – Membrane Bioreactors

Mha – Thousands of hectares

Mha – Millions of hectares

OIV – Organisation Internationale de la Vigne et du Vin

OLR – Organic Loading Rating Rate

PI – Performance Indicators

SBBR – Sequencing Batch Biofilm Reactor

SBR – Sequencing Batch Reactors

TS – Total Solids

TSS – Total Suspended Solids

UAF – Up flow Anaerobic Filter

UASB – Upflow Anaerobic Sludge Blankets

USBF – Up Flow Sludge Blanket Filter

WW – Winery Wastewater

WWTP – Wastewater Treatment Plants

Chapter 1.

1. Introduction

The world wine production remained nearly constant between 2010 and 2011, with values of the order of 265×10^{10} L (Figure 1.1). Europe, with a total production of 175×10^{10} L, leads the sector with 66% of production, face competitors from America (19%), Asia (6%), Oceania (5%) and Africa (4%). In the period of 2000-2011, Europe was the only continent where there was a decrease in wine production (Figure 1.2). Countries like France, Italy, Spain and Portugal, the production was respectively 28.4%, 23.8%, 19.1% and 3.4% (Castellucci, 2012).

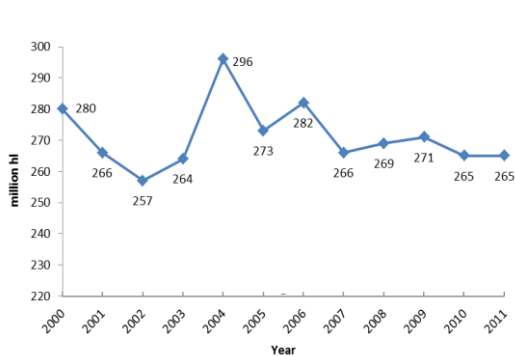


Figure 1.1 – World production trend of wine (source:www.oiv.int)

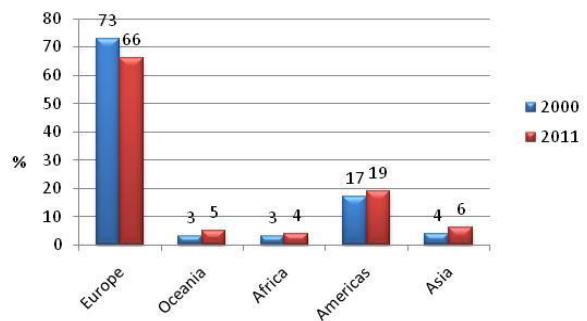


Figure 1.2 - Percentage distribution of the world wine production (source:www.oiv.int)

In 2011, the total area of vineyards worldwide was 7.585 mha which represents a reduction of area at 1%, 79 mha between 2010 and 2011. Analyzing the evolution of the last 10 years (2000-2011), it was found that there was an increase in global surface of vineyards in all continents except Europe (Figure 1.3). In Europe this tendency with principal focuses on Bulgaria, Hungary and Spain respectively with a decrease of 22%, 13% and 12%. France with a reduction in the area under vines in the order of 7%, Greece and Italy 6%, Turkey 4%, and Portugal reduced its area by 3%.

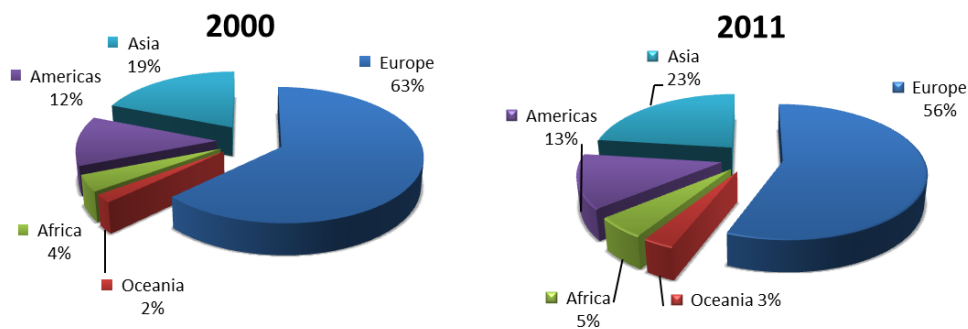


Figure 1.3 - Evolution of the last 10 years of the total area of vineyards worldwide (source:www.oiv.int)

The world production of grapes was 69,2 million tons (Figure 1.4), showing an increase, although the area have decreased since 2000. This situation can be explained by an increase in efficiency, more productive varieties and the existence of more favorable climatic conditions and techniques of continuous improvement, as well as winemaking practices. In Figure 1.5, it can be observed that for example Europe with a total of 41% of world production of grapes, get a percentage of 66% in relation to world wine production (Figure 1.2), which confirms somehow increased efficiency in wine production.

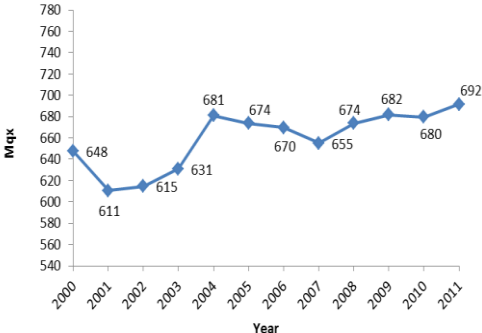


Figure 1.4 – World production trend of grapes. (source:www.oiv.int)

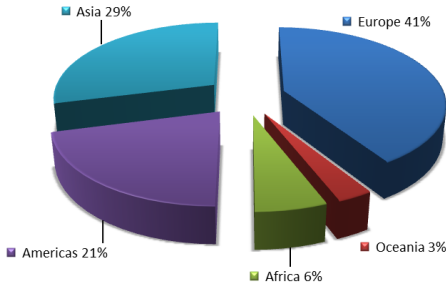


Figure 1.5 - Percentage distribution of the world grape production in 2011 (source:www.oiv.int)

In Portugal winemaking is one of the most traditional industries. The total amount of grapes in Portugal at 2011 was about 722 791 tones, covering the 174 Mha of the total cultivated winery area. The wine production is about 61% red and 39 % white wines, corresponding to a total of 7.132 million hL of wine (INE, 2012).

According to the Instituto da Vinha e do Vinho (IVV), the highest yields of wine are in the wine regions of Douro, Alentejo and Lisbon. (Figure 1.6).

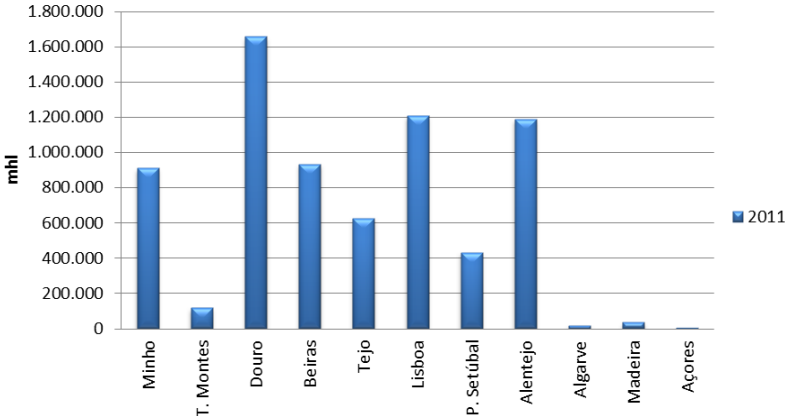


Figure 1.6 - Regional production of wines in Portugal in 2011 (Source: Institute of Vineyard and Wine.)

The wine sector in Portugal represents a highly significant economic activity and is the most dynamic within the Portuguese agricultural exports. The current wine consumption in Portugal is above $5\,925 \times 10^5$ L per year (Castellucci, 2012), which according to INE in 2007 it contributes for the high relevance of the wine sector to the country economy, because wine production represented about 16% of all agricultural output Portugal (INE, 2007).

During the last decade, the perceived higher quality of the Portuguese wines has been recognized worldwide and despite a small decrease of area under vines (-3%) and of wine production (-2%), Portugal is actually included in the Top 10 list of wine exporter countries in the world (Figure 1.7) (Castellucci, 2012).

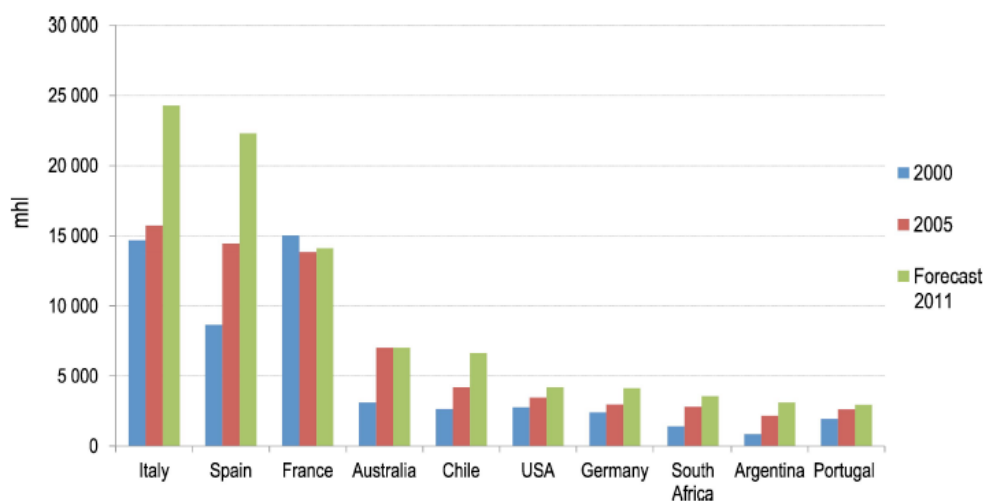


Figure 1.7 - Top 10 wine exporters - Trends 2000-2011 (source:www.oiv.int)

The winemaking process generates high pollutant wastewaters, and the treatment of this wastewater requires appropriate and sustainable management options in compliance with legislation and regulations. The wastewaters are normally result from the cleaning operations of washes production equipment, pavements and bottles (Petruccioli *et al.* 2000; Petruccioli *et al.* 2002), fermentation tanks and barrels (Malandra *et al.* 2003) as well as the sterilization of treatment tanks and storage of wines.

The aim of this research is to operate two pilots Air Micro-Bubble Bioreactor (AMBB) in continuous mode in seven different experimental periods (cycles) during 167 days treating winery wastewater from 2nd racking stage, which is considered one of the most polluting winery wastewater. The results obtained from different combinations concerning the organic

loading rating (OLR) and the aeration time rate (ATR) provide key information needed to assess the efficiency of the system. The performance indicator (PI), energy costs of treated wastewater and COD removal efficiency achieved in pilot scale show the technical feasibility of the process, giving a sustainable solution for this important sector of activity.

The present Dissertation is structured in four chapters:

In Chapter 1, a global overview of the world wine production is presented. The importance of the wine sector in Europe and especially in Portugal is described. The quality improvement of the Portuguese wines on the last decades contributes to increase the exports and a positive contribution for the national budget.

In Chapter 2, describes the winery production process and sources of wastewater, as the environmental concerns of the winery industry. Also compare the different wastewater treatment, with special focus on the AMBB technique.

In Chapter 3, the data used, methodology, main results and their discussion are presented in the format of scientific journal submission.

Chapter 4 covers the main conclusions resulting from the work.

Chapter 2.

2. State of the art

2.1 Production process and sources of wastewaters

The red winemaking process, which includes well-defined and standardized operations (harvest, followed by crushing and pressing operations, fermentation, clarifying, racking and bottling), generates high pollutant wastewaters and the treatment and disposal (or reuse) of this wastewater requires appropriate and sustainable management options in compliance with legislation and regulations. The main stages of the production process of a winery are as follows (Pirra, 2005; Vlyssides *et al.* 2005).

The process begins with the transport of the grapes to the winery, where they are discharged to a grain tank.

The process proceeds to the stalk removal (destemmer), and it follows the crushing. During this process the squeeze of the berries, the break of the skins (Figure 2.1), and the consequent extraction of juice take place. The wastewater generated from this stage comes from the washing of the machinery and of the production room.

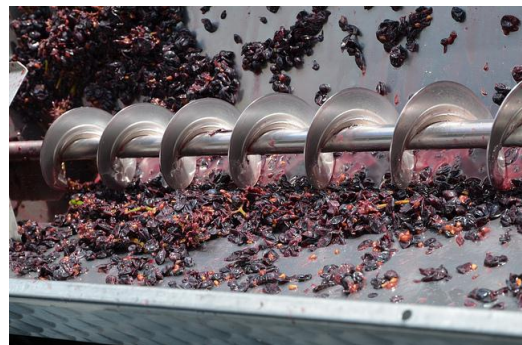


Figure 2.1 - Grape transport. (source: www.flickr.com/photos/28771820@N03/6972693979)



Figure 2.2 - Fermentation vats

(source: www.posters.co.uk/Wineries-Cellars/104048)

The fermentation is a process, which lasts 15 days, and run through biochemical reactions, checking for the conversion of the sugars into alcohol and carbon dioxide as to obtain energy, and occurs for example in fermentations vats (Figure 2.2). In fact in the production of wine, may be considered that this reaction is the main processing taking place in the process. During this stage, no wastewater is produced.

After fermentation occurs the separation of grape marcs. The produced wastewater from this stage comes from the washing of the tanks and production room, from the cleaning of the equipment's, and from wine losses.

The racking is effected in two steps: The first transfer occurs after the fermentation process, and the second separation also occur after the fermentation, but in this case is the malolactic fermentation (Duarte, J. M., 2006). Fermentation of this kind is intended to act in the smoothing of the palate and to reduce the feeling of acidity which characterizes many wines, particularly red wine coming from the cooler regions. It can also add complexity and delicacy to most red and white wines, even from the relatively warm climates, resulting in more balanced wine, round and harmonious.

After malolactic fermentation takes place the 2nd racking, and follow-up processes of conservation, maturation, stabilization.

Maturation is a process used in winemaking to reduce tartrate crystals in wine. It takes place in the barrels (Figure 2.3), and lasts about 15 days, with no wastewater produced



Figure 2.3 – Maturation (source: www.posters.co.uk/Wineries-Cellars/104048)

Subsequently, the wine is subjected to a bonding process which is a process the filtration of wine, whose function is to remove phenolic compounds (Ricardo-da-Silva, *et al.* 2003), clarification and microbial stabilization. This process contribute to decrease the precipitation of particles not removed in racking, and avoid any precipitation inside the bottles (Ricardo-da-Silva, *et al.* 2003).The produced wastewater of this stage comes from the washing of the tanks, production room, pre-washing of the storage tanks, from the cleaning of filters, as well from wine losses during its transfer.



The last process is the bottling and packaging. The produced wine is now transferred from the tanks to packing units (Figure 2.4), and the produced wastewater comes from the washing of tanks, transportation pump and of packaging room.

Figure 2.4 – Bottling (source: <http://asiabottling.com/>)

As a resume, Figure 2.5 schematizes the process of production of red wine, as the winery wastes and their sources.

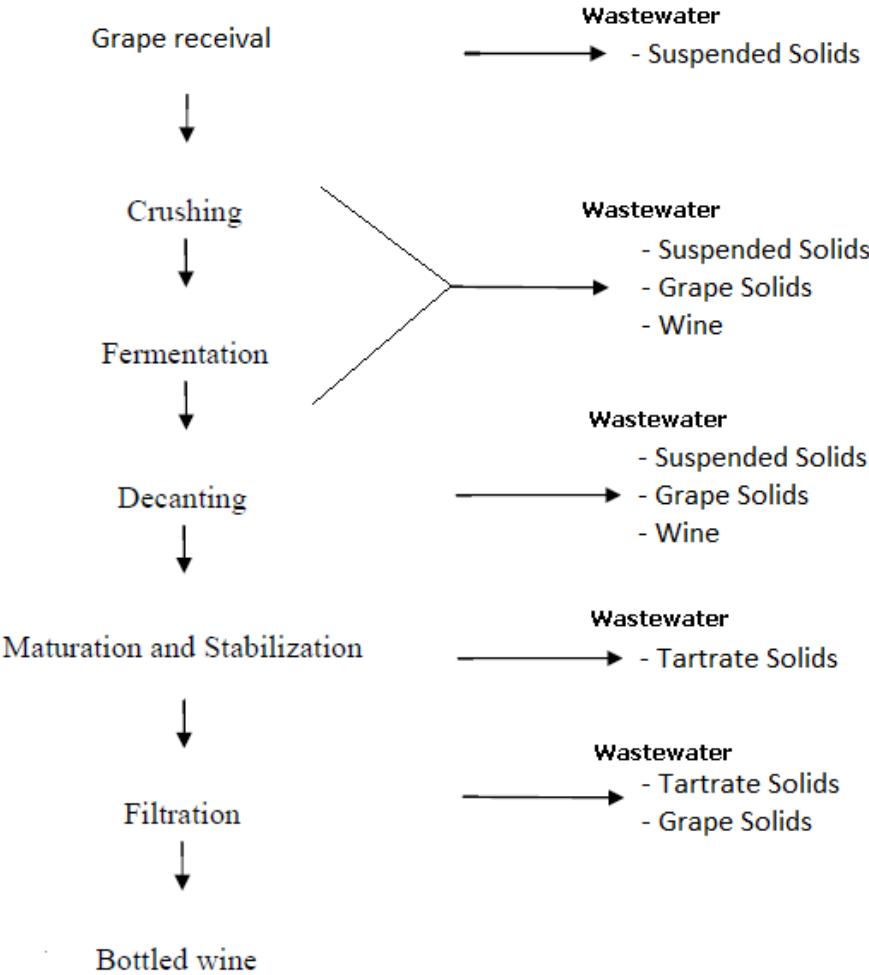


Figure 2.5 – Typical winery wastes and their sources

2.2 Environmental concerns of winery industry.

The winery wastewater is seasonally produced and is generated mainly as the result of cleaning operations, rinsing of fermentations tanks, barrels washing, bottling and purges from the cooling process. As a consequence of the seasonal working period and the winemaking technologies, volumes and pollution loads greatly vary over the year.

The wastewater from the second racking, which occurs when the fermentation activity is complete, is considered one of the most polluting due to the high content of organic load and total suspended solids concentration which may reach huge values resulting from the presence of tartrate precipitates. These solids are often problematic due to the high phenolic load adsorbed (Oliveira and Duarte, 2011). So, according to the processing method, each winery generates wastewater with specific properties, thus preventing the possibility to meet a general agreement on the most suitable and cost-effective alternative for treatment.

The winery wastewater is characterized for a high content of organic compounds, consisting of highly soluble sugars, alcohols, acids and recalcitrant high molecular weight compounds, such as polyphenols, tannins, and lignins (Arienzo *et al.* 2009; Pirra, 2005). The chemical analysis of this type of wastewater indicates that the COD is mainly dependent on the total sugar content, unlike phenolic compounds which represent only a small fraction. This wastewater can have a negative effect when discharged into the wastewater treatment plants (WWTP) as well as to the environment, without treatment (Malandra *et al.* 2003; Strong and Burgess, 2008).

Phenolic compounds in general are harmful pollutants that are toxic to human, animals and many microorganisms, even at relatively low concentration (Nair *et al.* 2008). They are particularly resistant to degradation, but some bacteria (Melamane *et al.* 2007; Pepi *et al.*, 2010; Saravanan *et al.* 2008) and fungi (Asses *et al.* 2009; Strong and Burgess, 2008) can tolerate and degrade those compounds. If high levels of biological oxygen demand (BOD) in untreated wastewater are allowed to flow to streams, rivers, ponds, lakes and other surface water, the dissolved oxygen in the waterways may be quickly consumed. As the dissolved oxygen in the waterways is depleted, aquatic and amphibious life suffocates (Chapman *et al.*, 2001).

2.3 General Characterization of winery wastewater

Important point to focus before the qualitative characterization is the quantitative characterization of the wastewater to be treated. Reject volumes per volume of produced wine vary from one wine cellar to another, with extreme values comprised between, $0.8 \text{ m}^3/\text{m}^3$ and $14 \text{ m}^3/\text{m}^3$ (Moletta, 2009; Van Schoor, 2005).

The wine industry has a seasonal production, and is considered high season, the harvest and the 1st racking that occurs in October and as low season, the corresponding storage and bottling, which takes place during the rest of the year (Table 2.1). The weather can also determinate when the harvest his made, with the threat of heat, rain, hail, and frost. Harvest season typically falls between August and October in the Northern Hemisphere and February & April in the Southern Hemisphere. With various climate conditions, grape varieties, and wine styles the harvesting of grapes could happen in every month of the calendar year somewhere in the world (Jancis Robinson, 2006).

So the wastewater varies in its quality, homogeneity and flow rate, depending on the working period (Artiga, *et al.* 2005; Rodrigues, *et al.* 2006). The 2nd racking, in particular, is responsible for producing the largest amount of suspended solids and therefore the greater the pollutant load, which by volume, reaches about 20% of the capacity of the tanks.

Table 2.1 - Wineries activities over a year of operation at Portugal. (Duarte, E. A., *et al.* 2006)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Harvest									■	■		
1 st Racking										■	■	
2 nd Racking	■	■										
3 rd Racking			■									
Filtration				■	■	■						
Bottling				■	■	■	■					

The organic content of winery wastewater consists of highly soluble sugars, alcohols, acids and recalcitrant high-molecular-weight compounds (e.g., polyphenols, tannins and lignins). They are easily biodegradable elements, except polyphenols that not easily removable by physical or chemical means alone (Duarte E., *et al.* 1998).

Polyphenols are a monitoring parameter used as a performance indicator. The ratio BOD₅ / COD ratio is about 0.15, which demonstrates the great difficulty of degradation of these compounds (Duarte, E., *et al.* 1998). Microorganisms are sensitive to these compounds, occurring inhibition of microbial activity and thus, degradation of the wastewater occurs with slower kinetics (Duarte, E., 2004; Heredia, *et al.* 2005). The wineries wastewater are very biodegradable (CBO₅/CQO between 0.43 e 0.93), and the period where the ratio BOD₅ / COD is higher is the vintage, by the presence of molecules such as sugar and ethanol (Fernández, *et al.* 2007).

The wastewater has usually a much accentuated deficiency in nitrogen and phosphorus, with a relation of BOD/N/P near of 100/1/0.3 (Andreottola *et al.* 2002), for this reason, it is often required the addition of nutrients, as urea and orthophosphoric acid, to guarantee the process of cellular synthesis.

Table 2.2 – Physico-chemical characterization of winery wastewater (Source: Oliveira *et al.* 2009)

Parameter	Labor Period			
	Vintage	1 st Racking	2 nd Racking	Bottling
pH	5-7	4-8	5	8
Conductivity ($\mu\text{S cm}^{-1}$)	1885-2110	1145-2260	2030-2400	1265
COD (mg L^{-1})	5360-10170	4460-7260	1580-5930	1805
BOD ₅ (mg L^{-1})	1770-8085	2250-4360	250-900	580
TS (mg L^{-1})	2160-10270	2555-3210	2170-4470	2200
TSS (mg L^{-1})	340-550	730-1010	160-2060	185
Surfactants (mg L^{-1})	10-30	4-20	3-7	2
Phenolic compounds (mg L^{-1})	6-32	20-35	5-29	2

From the analysis of the Table 2.2 It is possible to point out that during the vintage period, the total solids (TS) concentration is greater than total suspended solids (TSS), since organic matter that is mainly present in soluble form although even though a significant fraction is easily settleable (seeds, tartaric salts). During 2nd racking, denotes a higher concentration of TSS because of tartrate, which could also be associated with a greater adsorption of phenolics compounds. These compounds, because they are poorly biodegradable, can remain in the final wastewater, giving a reddish color. The wineries wastewaters are normally acids, but may vary with the vinification process, the time of year and the volume of water consumed. The wastewater generated by cleaning process equipment, which is made with alkaline compounds and organochlorine, shows pH values that can reach 12 Scale Sorensen (Pirra, 2005).

2.4 Wastewater treatment

The wastewaters can be treated as they are produced, or they can be stored (generally after good sieving) and treated within several months. The objectives of wastewater management are to be protective of the receiving environment and enhance water reuse opportunities.

The treatment system must be versatile to face the loading regimen and stream fluctuation. Attending to the different wine processing method implemented in each winery, it 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. So some decision 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 (Malandra *et al.*, 2003). Wineries have traditionally disposed of wastewater through storage and biological treatment, some form of land disposal or alternatively through the sewage treatment system, but this last option is often impractical in large scale due the inadequate cities infrastructure capacity. Several processes are available to reduce the organic load of the winery wastewater, namely physicochemical processes, biological aerobic processes, biological anaerobic processes and ecological treatment process. Besides the main treatment processes, one can and should include a pre-treatment, and a final adjustment treatment, depending on the final destination of the effluent.

It is important to have an equalization tank for storing the wastewater generated along the cycle of wine activity production, because the seasonal character of this activity, leads to a large variation in the wastewater flow. The storage allows the homogenization of effluent from different periods of peak production, and the times of low or zero wastewater production (Pirra, 2005) which contribute to the increment of the biological treatment efficiency. The suspended solids are removed by physical treatment such as grids, grit chambers, floaters and decanters (Pirra 2005). The importance of this procedure has to do with the protection of mechanical equipment such as pumps and aerator Venturi (Rodrigues *et al.* 2006), to avoid colmatation, partially reduction of pollutant load (the pollutant load can increase the level of organic contamination COD values until 500 000 mgO₂ / L (Commission UE, 2003)), and improve the effectiveness of subsequent treatment (Pirra 2005). Winery wastewaters have a low content of colloidal fraction and fine particulate matter, which limits in general the action of the physical-chemical treatments and are replaced by biological treatment (Pirra, 2005).

2.4.1 Physicochemical treatments

The chemical processes in conjunction with various physical operations have been developed for the treatment and management of different type of effluents. There are several technologies, such as sorption / adsorption, coagulation / precipitation, oxidation, evaporation and membrane filtration, which can reduce the organic loading and mainly the final color of the effluent (98%) (Pirra, 2005). According to some researchers (Jourjon *et al.* 2001; Pirra, 2005) the applicability of these technologies allows the removal of organic matter from the wastewater achieving the required quality to be discharged on the water bodies or reducing drastically the effluent flow rate (zero discharge).

Innovative and emerging technologies as the advanced oxidation process (AOPs) (Beltran de Heredia *et al.*, 2005; Gimeno *et al.* 2007; Lucas *et al.* 2009), are technologies that can be applied for the treatment of winery wastewaters. For example, photocatalytic different systems were tested, combining the hydrogen peroxide with titanium dioxide (TiO₂) reaching COD efficiency removal of 52-58% (Navarro *et al.* 2005). Mosteo *et al.* (2007) applied the photo-Fenton technology as a pretreatment to enhance the biological winery wastewater treatment. The organic matter degradation rates, applying photo-Fenton homogeneous and heterogeneous process, are 80% and 55% respectively.

There are organic compounds resulting from wine production and present in the wastewater as polyphenols, which are not effectively removed by biological processes (Benitez, *et al.* 1999; Benitez *et al.* 2000). These authors achieved removal efficiency of phenolic close to 100%, through the ozonization of wineries effluent, and for this reason ozonation has been seen as a pre-treatment desired for the biological treatment of wastewater wineries. The ozonation is capable of converting the complex molecules into simpler molecules, more easily biodegradable, attenuating inhibitory and toxic effects on microorganisms.

2.4.2 Ecological treatments

Constructed wetlands and the use of land application or irrigation are an interesting technology with a good (77-88%) performance in the post-treatment of the winery wastewaters. In this type of process, the wetlands act as a biofilter very effective in removing BOD, nutrients, sediments and pollutants such as heavy metals from the water, because different aquatic plants and their microorganisms are used to degrade the organic matter in the wastewater. This system uses a variety of floating, emerged and submerged plants. The advantages over other technologies, is that they do not need energy, are rely on self-maintaining and self-regulating biological processes (Grismer *et al.* 2003; Mulidzi, 2007).

2.4.3 Biological treatments

Biological treatment can be classified in two main processes: aerobic and anaerobic. These two bio-processes are related to the type of microorganisms involved in the organic matter biodegradation and to operating conditions of the bioreactor.

In the anaerobic process, occurs the transformation of organic matter in biogas (Moletta, 2009), composed by methane, carbon dioxide, carbon monoxide, di-hydrogen and di-hydrogen sulphur (Moletta, 2002). Several technologies like the upflow anaerobic sludge blankets (UASB) (Moletta, 2005); anaerobic sequencing batch reactor (ASBR) (Ruíz *et al.* (2002), hybrid upflow sludge blanket filtration (USBF) (Molina *et al.* 2007), anaerobic filters (Moletta 2005) and fluidised bed reactors (Moletta, 2005) are being used. The UASB systems have frequently been used to treat wastewater (Seghezzo *et al.* 1998) and can offer an inexpensive and simple solution to winery wastewater management with 80-98 % removal of COD load (Moletta, 2005), but have some disadvantages like other anaerobic systems such as the slow start up time to the process, the efficiency of the treatment which can be limited due to the variable nature of the composition of the effluent and the generation of malodors (Keyser *et al.* 2003).

The advantage of using anaerobic treatments are the low need of energy (no aeration), the low sludge production, the low need for nutrients and the methane produced that can be regarded as a renewable energy source. As disadvantages, these reactors, due to slow anabolism characteristic of anaerobic bacteria in the reactor startup takes longer to reach the ideal amount of biomass to its full operation. They allow the removal of nitrogen and phosphorus, but are more sensitive to toxic substances and changes in temperature and produce malodors. For higher levels of quality of the final effluent, it is requiring subsequent aerobic treatment (Metcalf and Eddy 2003).

The biological aerobic treatments can be based on technologies like the membrane bioreactors (MBRs) (Bolzonella, *et al.* 2010); sequencing batch reactors (SBR) (Torrijos & Moletta, 1997; Andreottola *et al.* 2002); sequencing batch biofilm reactor (SBBR) (Andreottola *et al.* 2002); conventional activated sludges (Bruculeri, *et al.* 2005); jet loop reactors (JLR) (Petruccioli, *et al.* 2002; Eusébio, *et al.* 2005); air micro-bubble bioreactors (AMBB) (Meyer, *et al.* 2004; Oliveira *et al.* 2009).

The microorganisms that carry out the purification of organic matter, utilize the nutrients and oxygen present in the wastewater to convert organic matter into more simple compounds releasing CO₂ and water. The growth rate of the biomass in aerobic processes is very high, which means that it is a process from the outset, with high efficiencies (Metcalf and Eddy

2003). Many aerobic have been used to treat winery wastewater with greater than 90% removal rate of COD (Petruccioli *et al.* (2000); Brucculeri *et al.* (2005); Oliveira *et al.* (2009); Artiga *et al.* (2005,2007); Torrijos *et al.* (1997)).

Biological treatment has proved to be a viable and cost effective method to remove dissolved organic material from winery, due to the content of organic matter to be quite soluble and readily biodegradable (about 80% of total COD; Brucculeri *et al.* (2005)), and because oxygen is readily available in air, the use of air as a supplier is the method of choice.

Aerated systems, such as the AMBB bioreactors (Oliveira *et al.* 2009) and JLR (Petruccioli *et al.* 2002), offers new advances in aeration wastewater treatment systems showing a good efficiency, not only in COD removal, but also in polyphenol compounds. Due to the lack of results of polyphenols removal efficiency, it is important to compare the operating conditions of these two studies.

In the case of the AMBB bioreactor (Oliveira *et al.* 2009), the samples of winery wastewater were composite samples representative of each phase of the process (vintage, 1st racking and 2nd racking), the bioreactor had a working volume of 15 dm³, the OLR range was 0.45-1.00 kg COD m⁻³d⁻¹ with maximum efficiency obtained after 15 days of 99% and 94%, to COD and polyphenols respectively. The ATR was 20 min hour⁻¹. The JLR reactor had the same working volume, operated in continuous aeration conditions, with winery wastewater from different wineries and from different periods (vintage and racking's). The OLR ranged between 0.4-5.9 kg COD m⁻³d⁻¹, with HRT of 2.1-4.4 days, the maximum efficiency obtained were 94-98% to COD and 65-75% to polyphenols (Petruccioli *et al.* 2002). It is important to report that besides the lower HTR, in this case the recirculation of the mixed liquor was continuous.

The Table 2.3 show the comparison of different biological treatments efficiency applied to winery wastewaters.

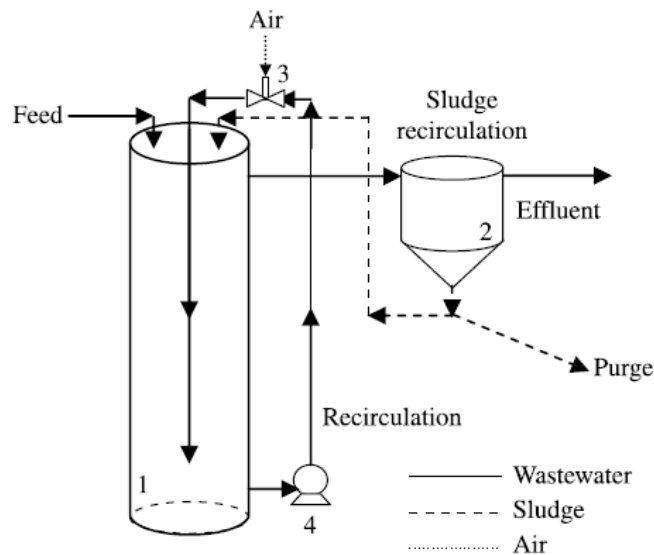
Table 2.3 – Efficiency comparison of different biological treatments applied to winery wastewaters

	Biological treatment	Applied COD Load kg COD m ⁻³ d ⁻¹	HRT	Total COD reduction (%)	Total Polyphenols reduction (%)	References
Aerobic Processes	Activated Sluged	0,81	---	90	---	Bruculeri <i>et al.</i> (2005)
	JLR	0.4-5.9	2.1-4.4d	94-98	65-75	Petruciolli <i>et al.</i> (2002)
	AMBB (ATR: 20 min hour ⁻¹)	0.45-1.00	15d	99	94	Oliveira <i>et al.</i> (2009)
	MBR	0.5-2.2	1.8d	>97	---	Artiga <i>et al.</i> (2005,2007)
	SBR	0.8-1.3	24h	>90	---	Torrijos, <i>et al.</i> (1997)
Biofilm	FBBR	0.4-5.5	---	91	---	Andreottola <i>et al.</i> (2005)
	SBBR	4.6-9.0	10h	86-99	---	Andreottola <i>et al.</i> (2002)
Anaerobic Processes	UASB	2-15	43-48h	80-98	---	Andreottola <i>et al.</i> (1998); Moletta (2005)
	ASBR	8.6	2.2d	>98	---	Ruiz <i>et al.</i> (2002)
	Hybrid USBF	5-13	> 20h	85-98	---	Molina <i>et al.</i> (2007)
	Anaerobic Filters	5-20	20-38h	88-98	---	Moletta (2005)
	Fluidised bed reactors	15-30	---	80-98	---	Moletta (2005)
	Constructed wetlands	---	14d	77-88	---	Mulidzi (2007)

The great advantage of some oxygen injection techniques in wastewater treatments is the increment of the contact time between the dissolved oxygen with the biomass and the dissolved organic material. When comparing different aeration techniques, the efficiency removal, oxygen transfer efficiency and energy spent are important operational key parameters to select the most appropriate technology, because it is difficult to get dissolved oxygen into the bioreactor a lot of energy is lost in the process of oxygen mass transfer from the gas to the liquid phase. The more oxygen transferred per kilowatt hour, the more energy efficient the system.

Biological treatment of winery effluents requires a power supply, proportional to the organic loading rate, 3 kWh per kg COD d⁻¹ (ITV, 2000). Previous research on the AMBB, revealed to be a good option for the seasonal WW treatment assessed by the COD removal efficiency in addition to the suitability of the treated effluent to be used for irrigation (Oliveira *et al.* 2009). Taking in account that the experimental work developed in this dissertation seems important

to describe in more detail the bioreactor AMBB. This is composed of a high efficiency Venturi injector (HEVI) coupled with mass transfer multiplier nozzles (MTM), one circulated pump, and with a cylindrical shape. The MTM nozzles discharge the air/water mixture from the HEVI into the bottom of the bioreactor (Oliveira *et al.* 2009). The flow diagram of the AMBB is shown in Figure 2.6.



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

Figure 2.6 – Flow diagram of the AMBB (Source: Oliveira *et al.* 2009).

In the present study, we report for the first time the impact of oxygen-limited conditions in the AMBB performance with emphasis on the efficiency energy use. The results obtained from different combinations concerning the OLR and the ATR provide key information needed to assess the efficiency of the system. The PI polyphenol, energy costs of treated wastewater and COD removal efficiency achieved in pilot scale show the technical feasibility of the process, giving a sustainable solution for this important sector of activity.

Chapter 3.

3. Materials and methods. Results and discussion

Optimization of the Air Micro-Bubble Bioreactor for the Winery Wastewater Treatment under Oxygen-Limited Conditions

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Abstract

The present study proposes the use of the pilot Air Micro-Bubble Bioreactor (AMBB) under continuous mode of operation to treat winery wastewater from the second racking period. The organic loads fluctuations, usual in the winemaking industry, and the aeration time rate, both related to energy costs, were the studied variables. Different combinations concerning organic loading rate ($0.16\text{-}0.44 \text{ kg COD m}^{-3} \text{ day}^{-1}$) and aeration time rate (1, 5 and 15 min h^{-1}) were presented. The study revealed that COD removal efficiency was not dependent on the applied organic loading rate. Also, the decrease in the aeration time rate was accompanied by a nearly three-fold reduction in energy consumption without relevant changing on COD removal efficiency (93-96%). These results represent a great challenge and may contribute to satisfying all requirements for the success of the AMBB with lower environmental footprint.

Keywords: Air Micro-Bubble Bioreactor; aeration time rate; COD removal efficiency; winery wastewater

1. Introduction

The wine sector in Portugal represents a highly significant economic activity and is the most dynamic within the Portuguese agricultural exports. The current wine consumption in Portugal is above $4.70 \times 10^5 \text{ m}^3$ per year (INE, 2008), which also contributes for the high relevance of the wine sector to the country economy. During the last decade, the perceived higher quality of the Portuguese wines has been recognized worldwide and despite a small decrease of area under vines (-3%) and of wine production (-2%), Portugal is actually included in the Top 10 list of wine exporter countries in the world (Castellucci, 2012).

The winemaking process, which includes well-defined and standardized operations (harvest, followed by crushing and pressing operations, fermentation, clarifying, racking and bottling), generates high pollutant wastewaters and the treatment and disposal (or reuse) of this effluent requires appropriate and sustainable management options in compliance with legislation and regulations. The winery wastewater is seasonally produced and is generated mainly as the result of cleaning operations, rinsing of fermentations tanks, barrels washing, bottling and purges from the cooling process. As a consequence of the seasonal working period and the winemaking technologies, volumes and pollution loads greatly vary over the year. Moreover, each winery is unique in wastewater generation, which is highly variable, 0.5 to 14 m^3 of water per m^3 of wine (Moletta, 2009; Van Schoor, 2005). The wastewater from the second racking period, which occurs when the fermentation activity is completed, is considered one of the most polluting due to the high content of organic load and total suspended solids concentration which may reach huge values resulting from the presence of tartrate precipitates. These solids are often problematic due to the high phenolic load adsorbed (Oliveira and Duarte, 2011). So, according to the processing method, each winery generates wastewater with specific properties, thus preventing the possibility to meet a general agreement on the most suitable and cost-effective alternative for treatment.

Winery wastewater is characterized for a high content of organic compounds, consisting of highly soluble sugars, alcohols, acids and recalcitrant high molecular weight compounds, such as polyphenols, tannins, and lignins (Arienzo et al., 2009; Pirra, 2005). The chemical analysis of such wastewater indicates that the chemical oxygen demand (COD) is mainly dependent on the total sugar content, unlike phenolic compounds which represent only a small proportion. Nevertheless, without treatment, this wastewater can have a detrimental effect when discharged into the treatment system, as well as into the environment (Malandra et al., 2003; Strong and Burgess, 2008).

Phenolic compounds in general are harmful pollutants that are toxic to human, animals and many microorganisms, even at relatively low concentration (Nair et al., 2008). They are particularly resistant to degradation, but some bacteria (Melamane et al., 2007; Pepi et al., 2010; Saravanan et al., 2008) and fungi (Asses et al., 2009; Strong and Burgess, 2008) can tolerate and degrade those compounds.

Several processes are available to reduce the organic load of the winery wastewater, namely physico-chemical processes, which include oxidation (Beltran de Heredia et al., 2005; Lucas et al., 2009) and adsorption to compounds (Rizzo et al., 2010); biological processes, based on upflow anaerobic sludge blankets (Moletta, 2009); membrane bioreactors (Bolzonella, et al., 2010); sequencing batch reactors (Andreottola et al., 2002; Torrijos & Moletta, 1997); conventional activated sludges (Bruccheri, et al., 2005); jet loop reactors (Eusébio, et al., 2005; Petruccioli, et al. 2002); air micro-bubble bioreactors (Meyer, et al., 2004; Oliveira et al., 2009) and constructed wetlands (Grismer et al., 2003; Mulidzi, 2007). The biological treatment of agro-industrial wastewater depends on the oxidative activities of competent microbes able to degrade carbon sources. The biodegradation of some recalcitrant compounds, such as polyphenols, is usually related to oxidoreductase enzymes that are dependent on the microbial strain, presence of inducers, composition of the culture medium and type of culture conditions (Luke and Burton, 2001; Minussi et al., 2007; Strong and Burgess, 2008). The dissolved oxygen concentration, considered a secondary selective

pressure of microbial communities (Quan et al., 2012), is also related to the COD removal efficiency and treatment performance (Ma et al., 2009; Zheng and Cui, 2012).

Previous research on the air micro-bubble bioreactor (AMBB) revealed the suitability of this system for the seasonal winery wastewater (WW) treatment assessed by the COD removal efficiency, in addition to the appropriateness of the treated effluent to be used for irrigation (Oliveira et al., 2009). In addition, the results showed that the operating and managing costs could be potentially optimized.

In the present study, we report the impact of oxygen-limited conditions in the AMBB performance with emphasis on the efficient energy use. The COD was selected as a key parameter to monitor the AMBB performance, during the second racking period, as a function of organic loading rate and aeration time. The total polyphenol compounds were also followed. The AMBB performance was compared between runs.

2. Materials and methods

2.1. Physico-chemical analyses of winery wastewater

The winery wastewater was collected during the 2nd racking operation, between January and March 2012, from Quinta da Casaboa winery (39° 3'45.77"N; 9°12'7.91"W, Portugal) that produces 100 000 dm³ of red wine per year. Three composite samples were taken and stored at 4 °C, until characterization. The physico-chemical characterization was carried out in duplicate, according to the *Standard Methods* (American Publication Health Association, 2005). A set of major parameters were evaluated: pH, electrical conductivity, total and soluble chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), volatile suspended solids (VSS), total phosphorus, total nitrogen, nitrate and total polyphenolic compounds (Folin-Ciocalteu Method). Total COD concentrations, total phosphorus, nitrate and total nitrogen were determined by spectrophotometric methods using the Spectroquant NOVA 30 A photometer (Merck). The

pH values and the conductivity were monitored using the HACH sension 4 pH meter and the HACH sension 7 conductivity meter. After the analytical evaluations, the wastewater samples were stored at -11 ° C.

2.2. AMBB set-up and work conditions

In this study, two AMBB bioreactors (AMBB1 and AMBB2) fabricated from rigid transparent PVC tube with a total volume of 15 dm³ were used. Each reactor consists of a vertical and cylindrical column having a highly efficient *Venturi* injector coupled with multiplier nozzles (AirJection®) equipped with a circulated pump, which supplies oxygen to the mixed liquor during recirculation, as previously described by Oliveira *et al.* (2009).

Both AMBB bioreactors were operated in continuous mode with a hydraulic retention time of 14 days and were inoculated with 14 dm³ of fresh winery wastewater, from the 2nd racking period. Acclimated biomass from other working periods was used to supplement AMBB1 and to achieve an initial mixed liquor volatile suspended solids (MLVSS) value of 2 kg m⁻³. The bioreactor AMBB2 was inoculated with biomass collected from the first, to attain a similar MLVSS concentration. Neither nutrients nor pH adjustments were done. The mixed liquor recirculation was held with a flow rate of 2.4 m³ h⁻¹ corresponding to an aeration flow rate of 0.12 m³ h⁻¹. The bioreactors were operated under room temperature (approximately 22 °C).

The AMBB feeding was performed by diluting the influent WW, in order to have the required organic loading rate (OLR). Different combinations of OLR and aeration time rate (ATR) were assayed (Table1). The effect of OLR was assessed by adjusting feed substrate to 0.16-0.44 kg COD m⁻³ day⁻¹, and the ATR varied between 1, 5 and 15 min h⁻¹. The AMBB1 bioreactor was evaluated throughout 113 days, by five different runs (R1 to R5) and AMBB2 was followed during 54 days, by two different runs (R1 and R2).

To assess de AMBB performance, samples were collected every two days to analyze COD, total polyphenols compounds, MLVSS, pH and conductivity.

2.3. AMBB statistical analysis

The data of COD and polyphenol removal efficiencies were subjected to analysis of variance (ANOVA) and the means compared by the Tukey's test at 5% significance level.

Agglomerative hierarchical clustering with Euclidean distance complete linkage was used to assign each run to a group, based on the observed variables representing the performance profile of the AMBB system. STATISTICA 7 software was used to compute statistical analysis of the data.

3. Results and Discussion

3.1 Winery wastewater characterization

Samples from the WW of the 2nd racking period were characterized to evaluate their polluting loads (Table 2). The WW analysis showed acidic characteristics and a conductivity similar to other working periods (Oliveira et al., 2009). The organic matter concentration, evaluated as COD, attained high values, thus revealing the high pollution load of this wastewater. However, these levels of COD are comparable to those reported by the literature for this type of wastewater (Eusébio et al., 2005; Montalvo et al., 2010; Oliveira *et al.*, 2009). Low nutrient levels were found in this WW as compared to the organic load, resulting in an imbalance COD:N:P ratio (100:0.10:0.05). This imbalance is usual for all operation periods, but becomes more noticeable at this stage.

The total content of polyphenols (Table 2) showed variation between samples (0.18 to 0.36 kg m⁻³), probably related to different TSS contents (Day et al., 2011). The polyphenol concentration range is however in accordance with data reported from other studies (Oliveira and Duarte, 2011; Petruccioli et al., 2002; Pirra, 2005).

3.2 Influence of aeration supply, under low organic loading rate

The AMBB was operated for 20 days with an OLR of $0.16 \text{ kg COD m}^{-3} \text{ day}^{-1}$ and 15 min h^{-1} of ATR under saturated conditions ($6 \text{ g O}_2 \text{ m}^{-3}$), prior to the beginning of the oxygen deprivation study (AMBB1-R1). The COD removal efficiency was 82% within the first 5 days of operation. Moreover, the rapid decrease in COD concentrations occurred in parallel to the increase in biomass (Fig. 1 and Fig. 2). After 15 days of operation, the reactor reached the steady-state and a high-quality effluent was produced. Signs of stability of the effluent were observed until day 20. The COD and total polyphenols removal performance were $95.8\% \pm 1.3\%$ and $94.0\% \pm 0.7\%$, respectively (Table 3). In addition to a competent biomass able to degrade the WW organic matter, other ecological considerations related to the AMBB adaptation should be assessed, namely the oxygen supply. For this purpose, the ATR was changed to 5 min h^{-1} (AMBB1-R2). The decrease of the oxygen supply resulted in a temporarily high COD concentration in the effluent (Fig. 2), although complete organic matter removal was restored again within 4 days after operating. The COD removal efficiency regained the initial value and the total polyphenols removal efficiency decreased only 8%. There was no significant difference ($P \leq 0.05$) in COD removals, between runs. In this case, the decrease in the ATR was accompanied by a nearly three-fold reduction in energy consumption without relevant changing on the final effluent quality. This result is of utmost relevance but should be validated for higher OLR values, thus mimetizing more real operation conditions.

3.3 Influence of OLR

Aiming to verify the AMBB behavior under near-real conditions a 3rd run assay was carried out (AMBB1-R3). The OLR was approximately 2.5-fold increased and the ATR was maintained (5 min h^{-1}), since that aeration condition revealed suitability for the WW treatment in the previous run.

In response to the OLR shock, an immediate increase in the MLVSS concentration from 1.1 kg m^{-3} to 1.8 kg m^{-3} was detected (Fig. 1). This increase was probably related to the growth

of heterotrophic microorganisms, since the amount of available substrate for the biomass growth was larger than in the other runs. Nevertheless, the OLR shock led to a slight decrease (3%) of the COD efficiency, which stabilized after 4 days and remained stable throughout the run ($92.9\% \pm 0.9\%$). Concerning the total polyphenols, the removal efficiency was always declining during the treatment and stabilized at 27%, on day 28. Therefore, the results showed that although these conditions are favorable for the WW treatment, considering the COD removal, they proved to be in some extent detrimental to the removal of the total polyphenols. The pH remained stable during the runs (7.0 ± 0.5). The obtained results are similar to those obtained in a previous study (COD removal, 93% and OLR, $0.45 \text{ kg COD m}^{-3} \text{ day}^{-1}$) but where the ATR was 20 min h^{-1} (Oliveira et al., 2009). So, a comparable COD removal was obtained with a four-fold reduction in ATR inside the bioreactor. These unexpected results have led us to test the removal of COD still under more drastic conditions of oxygen supply, to better understand the AMBB flexibility, although the polyphenols removals had been lower than expected.

3.4 Influence of aeration supply, under conventional organic loading rate

In order to evaluate the AMBB flexibility, a 4th assay (AMBB1-R4) was carried out under oxygen-limited conditions. An ATR of 1 min h^{-1} was applied, while the OLR was remained constant ($0.44 \text{ kg COD m}^{-3} \text{ day}^{-1}$). The reduction of the ATR was accompanied by an increase of organic matter in the AMBB effluent. The COD removal efficiency was acceptable but dropped to $70.0 \pm 0.6\%$. The biomass, assessed as MLVSS concentration, was slightly affected (1.6 kg m^{-3} instead of 1.8 kg m^{-3}). However, under such operating conditions, the most negative effect was recorded on the polyphenol removal, which was remarkably affected by the oxygen decrease. As shown in Fig. 2, the reduction of the ATR led to an abrupt decline on the polyphenol removal, which was not degraded under limited-oxygen conditions. Under aerobic conditions, the polyphenol degradation is usually related to oxidoreductases enzymes produced by microorganisms (Luke and Burton, 2001; Minussi et al., 2007). The decrease in the polyphenol removal efficiency was most probably due to

enzymatic activity inhibition, as a result of oxygen depletion inside the reactor, as oxygen is essential for the biochemical degrading reaction (Mayer, 2002).

Since shock loads occur frequently in the winemaking industry, it is necessary to assess how long a recovery period is for the system regain stability. Therefore, a longer aeration run was carried out (AMBB1-R5) and, gradually, the effluent characteristics began to recover. Within 15 days, following the transient conditions, the COD removal was restored to values similar to those observed in AMBB1-R3 assay and the biomass concentration attained 2.2 kg m^{-3} (Fig. 1 and Fig. 2). On the day 24, the COD removal efficiency was already 94%. The main results showed that both COD and biomass had similar behaviors, the negative effects resulting from limited-oxygen conditions were short-term and reversible.

Concerning polyphenol removal efficiency, there was an increase (20%) after 2 days of treatment. However, an additional period of 13 days was required to increase the polyphenols removal to acceptable levels, and a new steady state was reached with a removal efficiency of $73.6 \pm 2.5\%$.

Overall, the obtained results revealed a positive effect of the AMBB either to shock loads or low aeration. These findings are of utmost importance, taking into account that the responsiveness of the treatment system is a major concern for the reactor design, particularly in agro-industries that are subjected to large organic load variation and flow fluctuations, due to seasonality (Cheong and Hansen, 2008; Moletta, 2009).

3.5 Evaluation of AMBB robustness

To validate the robustness of the AMBB treatment system a second startup was followed in an independent reactor (AMBB2) and its performance was compared in two different operating conditions (R1 and R2). The OLR was kept constant ($0.44 \text{ kg COD m}^{-3}\text{day}^{-1}$) and only the two best ATR conditions were tested (5 min h^{-1} and 15 min h^{-1}).

Under an ATR of 15 min h^{-1} , a good COD removal performance ($95.0 \pm 0.9\%$) was promptly reached after 5 days of operation and kept stable until the next run. This fast stabilization

may be related to biomass adaptation, as acclimated sludge from the first reactor (AMBB1) was used as inoculum for the second reactor (AMBB2).

Although some differences in the operation conditions of the reactors existed, most variables were kept constant to verify whether there was reproducibility between assays. The results for runs that took place in both reactors with ATR of 15 min h^{-1} were concordant. Under such conditions, the COD removals stabilized at $94.1 \pm 0.8\%$ (AMBB1-R5) and $95.0 \pm 0.9\%$ (AMBB2-R1) (Table 3). Concerning the polyphenols removal the obtained values for the AMBB1-R5 and AMBB2-R1 were $73.6 \pm 2.5\%$ and $69.5 \pm 3.6\%$ respectively. No significant ($P \leq 0.05$) differences were obtained between runs (Table 3).

With regard to runs under ATR of 5 min h^{-1} , it was found that polyphenol yields were very similar between reactors and no significant differences ($P \leq 0.05$) were recorded. The COD removal efficiency obtained in the AMBB2-R2 was lower than in the AMBB1-R3 and affords no easy explanation. Despite OLR have been set, the AMBB2-R2 was inoculated with a different wastewater sample. Moreover, the AMBB2-R2 was subjected to a different shock when compared to the AMBB1-R3. This unexpected result may be explained by these operational conditions. However, further studies are needed to better understand the obtained values.

In this designed experience different effects were recorded on the dependent variables, after varying an explanatory variable. The observed inter-runs relationships are supported by the dendrogram (Fig 3) where three clusters are observed with significant linkage distance. In cluster 1 a single run appears isolated, corresponding to AMBB1-R4. This run was carried out at ATR of 1 min h^{-1} and showed the worst performance, when compared to the others runs. Although there are some differences in the behavior of dependent variables, the AMBB1-R3 and AMBB2-R2 assays cluster together (cluster 2), revealing the similarity between runs. Cluster 3 shows four cases that are related to the best overall AMBB performance. Despite most runs has been conducted with an ATR of 15 min h^{-1} , the run AMBB1-R2 was carried out with 5 min h^{-1} of ATR, evidencing a strong mutual correlation to

form the primary cluster pairs with AMBB1-R2, which was designed with an ATR of 15 min h⁻¹. According to the cluster analysis these runs show similarity, which reinforce the evidence that AMBB could be efficiently operated under oxygen-limited conditions, thus contributing to diminish the energetic costs.

To develop efficient strategies for the WW treatment, several parameters should be considered in the selection of the adequate technology. The treatment system should maximize removal efficiency, improve the flexibility, meet discharge requirements for WW and concur to low sludge production and energetic costs. In addition, it should be easy to operate and maintain and should represent a moderate capital cost. The results of the present study reveal that most of these requirements could be achieved by the AMBB system, even operating under low oxygen supply.

4. Conclusions

The responsiveness of any wastewater treatment system to shock loads and lack of oxygen supply is one of the major concerns on planning and management of a winery wastewater treatment system. This study demonstrated that COD removal efficiency was independent on the applied OLR and ATR (5 and 15 min h⁻¹), beyond the cycle where the shock occurred. Albeit the total polyphenol removal has been highly affected by both OLR and ATR, the data revealed the suitability of 5 min h⁻¹ of aeration for COD removal with a three-fold reduction in the energy consumption.

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Table 1. Operating conditions of two Air Micro-Bubble Bioreactors (AMBB) at different runs

Reactor	Run	Aeration time rate (min h ⁻¹)	OLR (kg COD m ⁻³ day ⁻¹)
AMBB 1	R1	15	0.16
	R2	5	0.16
	R3	5	0.44
	R4	1	0.44
	R5	15	0.44
AMBB 2	R1	15	0.44
	R2	5	0.44

Table 2. Physico-chemical characteristics of winery wastewater from the 2nd racking period

Parameter	Units	Amount
pH		2.8-3.7
Conductivity	(mS cm ⁻¹)	1.5-2.5
Total COD	(kg m ⁻³)	23.2 – 48.9
Soluble COD	(kg m ⁻³)	20.0 – 30.8
BOD ₅	(kg m ⁻³)	6.99 – 16.4
Total nitrogen	(kg N m ⁻³)	0.014 -0.032
Total phosphorus	(kg P m ⁻³)	0.009-0.011
Total polyphenol	(kg m ⁻³)	0.18 – 0.36
TSS	(kg m ⁻³)	1.32 – 4.46

Table 3. AMBB performance under different operating conditions

	Run	Aeration time (min h ⁻¹)	F/M (kg BOD kg SSV ⁻¹ dm ⁻³)	COD _{in} (kg m ⁻³)	COD _{out} x10 ³ (kg m ⁻³)	COD Removal (%)	Total polyphenol _{in} x10 ³ (kg m ⁻³)	Total polyphenol _{out} x10 ³ (kg m ⁻³)	Total polyphenol Removal (%)
AMBB 1	R1	15	0.07	2.28	94.7±3.1	95.8 a	23	1.4 ± 0.1	94.0 a
	R2	5	0.06	2.28	97.7± 2.9	95.7 a	23	4.1 ± 0.1	82.3 b
	R3	5	0.10	6.00	437±45	93.3 a	59	43.1 ± 3.5	26.9 c
	R4	1	0.07	6.00	1812±28	70.0 b	59	55.7 ± 0.5	5.6 d
	R5	15	0.08	6.14	353±10	94.1 a	73	19.3 ± 1.8	73.6 e
AMBB 2	R1	15	0.07	6.00	300 ± 20	95.0 a	59	18.3 ± 2.9	69.5 e
	R2	5	0.07	6.14	914 ± 24	84.7 c	73	53.2 ± 3.0	27.4 c

AMBB1: R1 and R2 at OLR = 0.16 kg COD m⁻³ day⁻¹ and R3-R5 at OLR = 0.44 kg COD m⁻³ day⁻¹

AMBB2: R1 and R2 at OLR = 0.44 kg COD m⁻³ day⁻¹

Values marked with the same letter are not statistically different (P = 0.05), according to Tukey's test

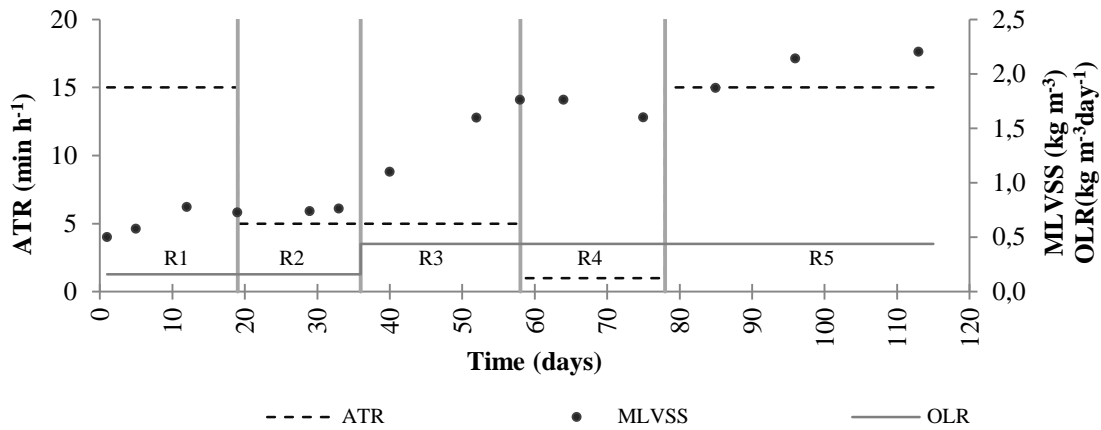


Fig. 1. Effect of OLR and oxygen supply in the biomass growth, during the AMBB1 assays:
 ● MLVSS (kg m^{-3}) concentration; --- ATR (min h^{-1}); ____ OLR ($\text{kg m}^{-3}\text{day}^{-1}$) affluent.
 R1- Run 1; R2- Run 2; R3- Run 3; R4- Run 4; R5- Run 5.
 R1 and R2 at $\text{OLR} = 0.16 \text{ kg COD m}^{-3} \text{ day}^{-1}$ and R3-R5 at $\text{OLR} = 0.44 \text{ kg COD m}^{-3} \text{ day}^{-1}$.

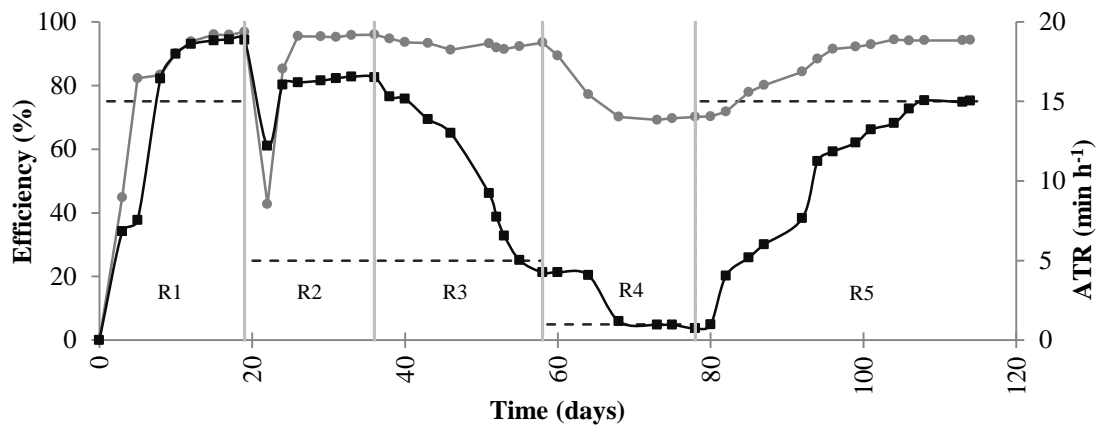


Fig. 2. AMBB1 removal efficiency during different runs:
 ● COD removal efficiency (%); ■ polyphenol compounds removal efficiency (%); --- ATR (min h^{-1});
 R1- Run 1; R2- Run 2; R3- Run 3; R4- Run 4; R5- Run 5.
 R1 and R2 at $\text{OLR} = 0.16 \text{ kg COD m}^{-3} \text{ day}^{-1}$ and R3-R5 at $\text{OLR} = 0.44 \text{ kg COD m}^{-3} \text{ day}^{-1}$

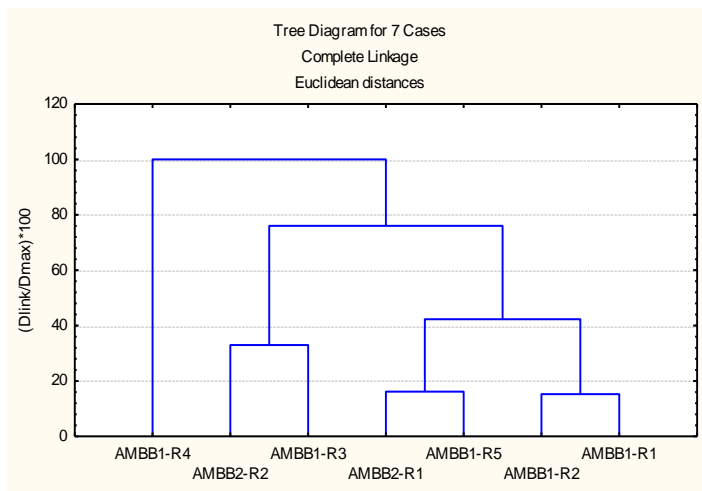


Fig. 3. Clustering using Euclidean distances complete linkage for AMBB performance.
 AMBB1: R1 and R2 at $\text{OLR} = 0.16 \text{ kg COD m}^{-3} \text{ day}^{-1}$ and R3-R5 at $\text{OLR} = 0.44 \text{ kg COD m}^{-3} \text{ day}^{-1}$
 AMBB2: R1 and R2 at $\text{OLR} = 0.44 \text{ kg COD m}^{-3} \text{ day}^{-1}$

Chapter 4.

4. Conclusions

Due to lack of studies using the winery wastewater from only the second racking period, it was important to understand the biodegradability of this particular wastewater.

In the present study, two AMBB bioreactors were used, with a working volume of 14 dm³ and a hydraulic retention time of 14 days, operating in continuous mode to treat winery wastewater from second racking period, for seven different runs, during 167 days.

The responsiveness of any wastewater treatment system to shock loads and lack of oxygen supply is one of the major concerns on planning and management of a winery wastewater treatment system. It is important to maximize the efficiency of a wastewater treatment plant, but never forget their energy costs. In the present study, the actual spent energy is one third of the equivalent aeration processes, and it operates with “near zero excess sludge”, thus the process turns extremely competitive in terms of sludge handling and disposal costs.

The results achieved demonstrated that the treatment of winery wastewater from the 2nd period using an AMBB bioreactor, is technically robustness and feasible, and certainly at a real scale the AMBB performance will comply with the legal requirements for reuse or discharge of the winery wastewaters, leading to a more environmentally sustainable and resource-efficient economy in the wine sector.

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