# Biodegradation Kinetics of Winery Wastewater from Port Wine Production

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Original scientific paper
Received: April 19, 2011
Accepted: November 8, 2011

Winery wastewaters are characterised by seasonality and variable volume and organic load. This fact together with their high content of biodegradable compounds often results in problems in the operation of biological systems as they may lead to poor sludge settleability, floc disintegration and increased presence of solids in the treated effluent. Biodegradation of winery wastewater from a Port Wine production industry was studied in aerobic batch assays, varying substrate and biomass concentrations. More than 90 % of COD was removed in all cases, in a short period when biomass concentration was higher than 3 g VSS $L^{-1}$. Data was correlated to several kinetic models, and Haldane model best fitted the experimental data, particularly for lower biomass concentrations. Therefore, an initial high biomass concentration should be present in aerobic treatment of winery wastewater, in order to cope with the large fluctuations in their organic loads. These batch assays are valuable for winery wastewater treatment, as they may simulate typical start-ups after short and long shutdown periods often observed in the winery industry.

*Key words:*
Winery effluent, aerobic treatment, batch assay, kinetic modelling, Haldane model

## Introduction

Port Wine is a widely consumed type of wine produced in the Portuguese Douro region that was recently recognised as a world heritage by UNESCO. Its production plays an almost exclusive role in local economics, involving harvesting, winery processing, bottling and trading. In the past, winery effluents (WE) were not treated, even though they are characterised by high organic content and responsible for high pollution loads in the water bodies of the region, especially the international basin of Douro River. Therefore, environmental impacts of WE discharge must be carefully assessed.

The wine industry is characterised by its high water consumption at specific stages of the vinification process, leading to high amounts of pollutant load. In general, COD content of WE varies from 3 to 30 g $L^{-1}$.[1,2] In addition, the production of WE is seasonal, which leads to significant variations in volume and organic load produced throughout the year, according to the phase of the production cycle (high season: vintage production and racking; low season: bottling and cleaning), to the type of wine produced (red or white, sparkling, sweet, etc.) and to the technology used.[2–5] Therefore, the treatment of WE must be versatile regarding the loading regimen, and able to cope with a succession of start-ups and closedowns, including periods of inactivity.[6]

Typical compounds present in grapes and wine (organic acids, sugars, alcohols, phenolics, anthocyanins, tannins, etc.) are commonly found in WE, as well as some residues from the vinification processes (yeasts, bacteria, clarification and filtration agents) and the products used for cleaning and sterilization of the washing vats and enological equipment.[7,8] Due to these ecological threats, many countries have recently limited these effluents discharge and tried to develop more efficient treatment technologies, such as specific biological or physical-chemical processes, thus reducing the pollutant load through organic substances degradation or their transformation into more biodegradable residues.

In general it can be said that the treatment techniques applicable to winery effluents are similar to those developed for other wastewaters, although requiring specific adaptations, due to the high fluctuations in hydraulic and organic loads and to the lack of nutrients, namely nitrogen and phosphorus.[9] In addition, some refractory compounds present in WE, such as polyphenols, have been identified as toxic for non-acclimated microorganisms if present in high concentrations.[10] These compounds may cause WE to impart strong antibacterial effects in

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biological treatment systems.[11] Higher efficiencies for WE bio-treatment are still needed, and should result from the evaluation of several parameters such as the effluent characteristics (volume and pollutant load of wastewater produced), geographical location, climate, investment and operating costs as well as the technological aspects.[12] Several processes, such as coagulation-flocculation-aeration,[1] sequencing batch reactors with granular sludge,[13] or zeolite packed anaerobic reactors,[7] are some of the recent approaches for WE treatment using microbial mixed cultures. These leading technologies have proven to remove recalcitrant compounds of WE. Alternative approaches may include the dilution of WE with domestic wastewater followed by treatment at municipal wastewater treatment plants.[14,15]

Microbial growth and pollutant biodegradation kinetics aids in proper prediction and optimisation of biological treatment processes at an industrial scale.[16] Kinetic modelling studies are usually carried out in batch lab-scale assays, and they usually aim to relate the rate of biodegradation of an organic compound (per unit of biomass) to its concentration. Such models can be used to estimate both the cycle time for batch treatment processes, such as sequencing batch reactors, as well as the adequate hydraulic retention time for continuous processes such as activated sludge reactors.[17]

The main objective of this study was to provide experimental data on the biodegradability of winery wastewaters and to evaluate the specific kinetic parameters for aerobic biodegradation, which can be useful for the design and operation of biological systems treating this type of effluent. In addition, the physical-chemical characteristics of typical WE from Douro Region were also examined, in order to provide data on the variability and seasonal pollutant load of this wastewater.

## Materials and methods

With the purpose of studying typical WE from the Douro region, three wineries with different sizes were selected: (A) small size; (B) medium size; (C) large size. For each one, the WE production and physical-chemical properties were evaluated once per week for a 2-year period. A probe was used to measure dissolved oxygen (DO), pH and temperature. All the analyses were carried out according to Standard Methods:[18] Chemical Oxygen Demand – COD (method 5220 C), Biochemical Oxygen Demand – $BOD_5$ (method 5210 B), total suspended solids – TSS (method 2540 D) and volatile suspended solids – VSS (method 2540 E).

For the biodegradability assays 3 L glass reactors equipped with mixing and oxygenation devices (ceramic diffusers) were used. Wastewater collected in the larger size winery (C) during the first raking period was used as substrate, given that this is the most representative wastewater concerning hydraulic load and seasonality. These tests will gain us better knowledge on the activity of microorganisms dealing with WE.

Aerobic biomass used as inoculum was collected in a conventional activated sludge plant treating domestic wastewater and acclimatized to WE at 3-day cycles for a period of 30 days. At the end of each cycle, the sludge was allowed to settle and the supernatant removed. Then it was spiked with a small amount of winery effluent (c.a. 0.5 L) once every 3 days and the mixture continuously aerated. Dissolved oxygen was permanently monitored, thus following the substrate exhaustion at the end of the 3-day cycle. Soluble COD concentration was measured at the end of the acclimatisation period thus ensuring that all substrate was consumed prior to the beginning of the batch experiments. The biomass was allowed to settle and the supernatant removed. Then the biomass was characterised (TSS, VSS) in order to determine volumes to be used in the experiments.

Initial organic matter concentration in the batch assays varied between 1 and 7 g COD $L^{-1}$, while biomass concentrations varied between 1.5 and 4.5 g VSS $L^{-1}$, corresponding to initial F/M ratios in the range 0.22 – 4.67 g COD $g^{-1}$ VSS. Nutrient solutions consisting of $KH_2PO_4$ and $NH_4Cl$ were added in the ratio COD:N:P of 100:7:1. DO was permanently monitored to maintain concentrations above 1.5 g $L^{-1}$, ensuring that DO was not a limiting factor for microorganisms growth. All the assays were carried out for 48 h. Every 2 hours, 7.5 mL of the mixed liquor were collected and centrifuged (5 min at 4000 rpm.). The pellet of sludge was reintroduced inside reactors and the supernatant was filtered to measure soluble COD thus obtaining detailed COD uptake profiles. Finally, these biodegradation profiles were fitted to several kinetic models, using Micromath Scientist®, a specialised software package for experimental data fitting and parameter evaluation.

## Results

### Characteristics of wine effluent (WE)

Characteristics of COD, $BOD_5$ and TSS for each selected winery are presented in Table 1. Due to the seasonality of the wine processing that is widely reported,[1,2,13,19,20] only the average values are provided is this paper. The largest winery

Table 1 – *Average values ± standard deviation of COD, $BOD_5$ and TSS achieved in the winery effluents during different periods of wine production (g $L^{-1}$)*[2]

| Winery | Vintage/first racking | | | 2nd racking | | | 3rd racking | | |
|---|---|---|---|---|---|---|---|---|---|
| | COD | $BOD_5$ | TSS | COD | $BOD_5$ | TSS | COD | $BOD_5$ | TSS |
| A – small | 70±10 | 18±8 | 21±3 | 30±5 | 15±3 | 6±0.8 | 10±3 | 9±2 | 1±0.5 |
| B – medium | 14±2 | 6±2 | 1.5±0.3 | 14±3 | 6±2 | 6±1 | 120±20 | 80±10 | 9±0.9 |
| C – large | 14±4 | 10±3 | 2±1 | 6±2 | 4±1 | 0.5±0.2 | 11±2 | 8±2 | 1.2±0.5 |

(C) produces the wastewater with the lowest pollutant loads, achieving COD values between 6 and 15 g COD $L^{-1}$, although it is variable during the several phases of the wine production cycle. Seasonal peaks were observed (data not shown) and corresponded to specific operations, such as filtrations, centrifugations, washing of vats, etc.[2]

The medium size winery (B) produces a wastewater with a much more variable and seasonal pollutant load during the several phases of the wine production cycle, achieving maximum values in the period from February to June (up to 120 g COD $L^{-1}$). As it was observed for the previous winery, there are also peaks associated with the same specific operations previously mentioned.[2]

The smallest winery (A) also produces a wastewater with variable and seasonal pollutant loads during the several phases of the wine production cycle and the activity of the winery. Generally, the pollutant load here generated is the highest among the three studied wineries, and it is associated with the extremely red-coloured high-tannin wines that are produced in this winery. Maximum values for COD were achieved immediately after the vintage period (up to 70 g COD $L^{-1}$), sometimes prolonging these peaks until March (end of the malolactic fermentation), as previously reported.[2]

The $BOD_5$/COD ratio for the larger size producer effluent (C) varied much less than the ratios for the others. In addition, average values of this ratio were significantly higher for that effluent than for the other two wineries, meaning that the large size winery effluent is more biodegradable than the others.

## Biodegradability

Biodegradability tests in batch mode enabled determination of COD uptake profiles as a function of time, in order to find the optimal conditions for biodegradation of this wastewater. For a better comparison, COD concentration profiles were normalised to COD removal efficiencies and their evolution with time is presented in Fig. 1.

The plateau of the biodegradation curve that reflects a COD removal above 80 % was reached

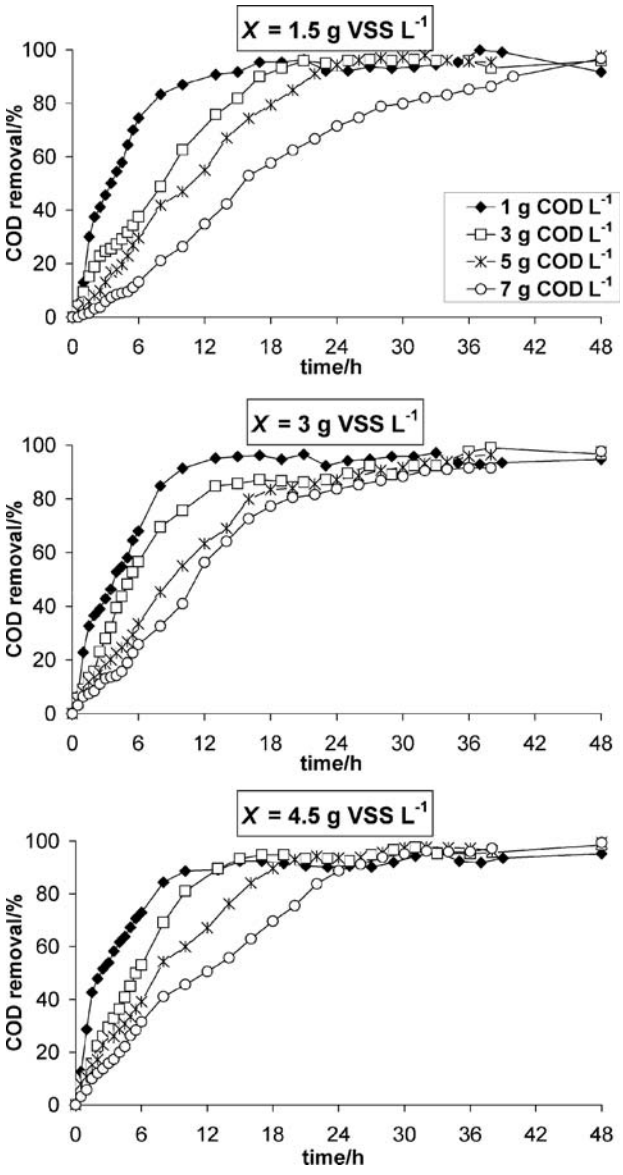


Fig. 1 – *Evolution of COD removal efficiency*

up to 24 h for almost all cases (Fig. 1). It is notable that there was a sharp drop of COD concentration (higher degradation) at the beginning of the biodegradation study, followed by a gentle drop, possibly indicating an intermediate adsorption of some compounds before biodegradation. For lower initial COD concentrations, adsorption is an im-

portant factor that may affect the biodegradation process and lead to exaggerated degradation slopes.[21] For the lowest initial substrate concentration (1 g COD $L^{-1}$), higher values for the maximum slopes were also observed in the first part of the assays reflecting a faster COD removal, regardless of the biomass concentration. Therefore, it can be stated that the higher pollutant load applied to the reactor, the higher is the time needed for its removal.

Generally, COD removals higher than 95 % were achieved at 38 h for the assays containing lower biomass concentrations (1.5 and 3 g VSS $L^{-1}$), and 30 h for the assays containing higher biomass concentrations (4.5 g VSS $L^{-1}$). For the lowest biomass concentration (1.5 g VSS $L^{-1}$), the maximum COD degradation (higher than 90 % of COD fed) occurred at 8, 18, 20 and 38 hours for 1, 3, 5 and 7 g initial COD $L^{-1}$, respectively. When biomass concentration is increased from 1.5 to 3 or 4.5 g VSS $L^{-1}$, a significant decrease is observed on the time needed to achieve the same COD removal, particularly for the highest load applied (24 h for 7 g COD $L^{-1}$). Moreover, the COD removal curve presented the smallest slope for the highest biomass concentration series, which means that COD uptake occurred more gradually in this case, reflecting slower biodegradation behaviour.

The results show that COD removal occurred more gradually as the pollutant load was increased, regarding the effects of both adsorption and biodegradation. For substrate concentration of 1 g COD $L^{-1}$, maximum removal efficiencies were achieved during 12 h. However, when substrate concentration increased, the rate of biodegradation decreased, and maximum removal (94 %) of COD at a concentration of 7 g COD $L^{-1}$ was achieved at 30 h, for a biomass concentration of 4.5 g VSS $L^{-1}$. Comparing removal profiles for 3 and 4.5 g VSS $L^{-1}$ at the highest substrate concentration assays, it was noticed that COD removal at 4.5 g VSS $L^{-1}$ was lower than 3 g VSS $L^{-1}$. At 18 h, only 69 % of COD was removed at the highest biomass concentration assay, whereas 77 % of COD was removed at the intermediate biomass concentration assay. This fact provides clues that increasing biomass concentration does not lead to faster biodegradation, and even resulted in an antagonistic effect (uptake curve slowing down) probably due to further difficulties in substrate diffusion within the biomass.

The COD removal efficiencies obtained at the end of the batch experiments were similar to those reported by Fernández *et al.*[19] (in the range 93–96 %), for a continuous aerobic treatment of winery effluent, regardless of the F/M ratios tested. Petruccioli *et al.*[22] reported COD removal efficiencies in the range 89–92 % for the treatment of winery effluent in immobilized cells aerobic reactors, which were slightly lower than those achieved in this study.

## Kinetic study

Biological reactors treating winery effluents may often have several problems due to either variable organic load or inhibition caused by the presence of polyphenolic compounds.[10,11,23] Therefore, the assessment of the biodegradability characteristics of these effluents should always be coupled with a kinetic study of the biodegradation process, thus allowing a critical evaluation when implementing strategies to improve the performance of the treatment systems.

The most significant period in the growth cycle of a batch cultivation is the exponential growth phase, when the population of biomass is perfectly acclimatised to the substrate.[11,21] In this situation, the first-order model provides accurate simulations of the biomass ($X$) growth. Since the biomass production is proportional to the substrate ($S$) depletion, a first-order model can also be expressed by eqs. 1 and 2:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu X \tag{1}$$

$$-\frac{\mathrm{d}S}{\mathrm{d}t} = \nu X \tag{2}$$

where $\mu$ and $\nu$ represent the specific rates for biomass growth and substrate consumption respectively. Several relationships between these two variables can be found in kinetic models, which allow modelling the growth dependence on substrate concentration. Simplistic first-order relations by themselves may not be a good approach when modelling complex biological systems such as the aerobic process. Therefore, different kinetic models are widely applied to emphasise the effect of several factors such as substrate inhibition.

In this study, four kinetic models commonly used (Table 2) were fitted to the experimental data calculated from the batch experiments, and they were chosen because each one reflects a different effect on the biodegradation process. Monod equation is usually more suitable for fitting microbial process under substrate-limited conditions, and it relates the growth rate of microorganisms to the concentration of a single growth controlling substrate.[24] When Contois equation fits the experimental data, it indicates that the microbial growth is limited by the available surface area, causing mass transfer limitations. Hence, as the population density of biomass increases there is an increasing obstruction in the substrate uptake and growth of any microorganism.[25] On the other hand, Powell equa-

Table 2 – *Biodegradation kinetic models fitted to the experimental data*

| Kinetic model | Equation No. | Function | Parameters |
|---|---|---|---|
| Monod | (3) | $\nu = \nu_{max} \frac{S}{K_S + S}$ | $\nu_{max}$ – Maximum specific substrate utilisation rate (g COD $g^{-1}$ VSS $d^{-1}$)<br>$K_S$ – Saturation constant (g COD $L^{-1}$) |
| Contois | (4) | $\nu = \nu_{max} \frac{S}{K \cdot X + S}$ | $\nu_{max}$ – Maximum specific substrate utilisation rate (g COD $g^{-1}$ VSS $d^{-1}$)<br>$K$ – Contois saturation constant (g COD $L^{-1}$) |
| Powell | (5) | $\nu = \nu_{max} \frac{S}{K_S + S + H}$ | $\nu_{max}$ – Maximum specific substrate utilisation rate (g COD $g^{-1}$ VSS $d^{-1}$)<br>$K_S$ – Saturation constant (g COD $L^{-1}$)<br>$H$ – Mass transfer resistance constant (g COD $L^{-1}$) |
| Haldane | (6) | $\nu = \nu_{max} \frac{S}{K_S + S + \frac{S^2}{K_i}}$ | $\nu_{max}$ – Maximum specific substrate utilisation rate (g COD $g^{-1}$ VSS $d^{-1}$)<br>$K_S$ – Saturation constant (g COD $L^{-1}$)<br>$K_i$ – Inhibition constant (g COD $L^{-1}$) |

tion points out that a differential resistance to substrate transfer through the medium could affect the growth rate, where the specific parameter $H$ depends on the diffusional resistance around the cell.[26] Whereas these models do not consider substrate inhibitory effects, the Haldane equation models the so-called self-inhibition, in which the biodegradation rate is slowed down because of the high concentration of the substrate.[27] Indeed, the Haldane model is one of the most commonly employed kinetic models for biodegradation of organic substances.[16]

Experimental COD concentration profiles obtained in the experimental tests were correlated with the kinetic equations presented in Table 2. For model fitting, the degradation term ($-dS/dt$) was calculated from the experimental curves ($t$, $S$). Polynomial expressions were fitted to the substrate concentration profiles by a regression technique, and then the maximum slope was calculated by deriving the polynomial equation with respect to time domain. Specific substrate consumptions ($\nu$) were calculated by dividing the slope by the biomass concentration present in each experiment, and were plotted against $S$ in order to fit the kinetic models by a least-squares fitting method. The parameter used to compare the different models was based on the least-squares root minimisation of the sum of the differences between experimental points and model simulations. For each model, the estimated kinetic parameters as well as the sum of the squared errors (SSE) obtained for each biomass concentration series are listed in Table 3.

The values of specific substrate consumption rates ($\nu$) for each kinetic model as well as the experimental values were plotted against initial COD concentration in Fig. 2. From this figure it can be observed that the values for specific substrate uptake rates are much higher for low biomass concentration series and tend to peak at a COD concentration of around 2 g $L^{-1}$. This finding may highlight the substrate adsorption as an important effect on substrate removal, which leads to exaggerated specific degradation rates, mainly at low initial biomass concentrations, as also stated in the literature.[21]

The Haldane model best fitted the experimental data for the lowest biomass concentration series (1.5 g VSS $L^{-1}$), regarding the low SSE value obtained (Table 3) when compared to the other models. The values of $K_S$ for the Haldane model are in

Table 3 – *Fitting of the kinetic models to the experimental biodegradation data*

| Kinetic model | Parameter | Biomass concentration (g VSS $L^{-1}$) | | |
|---|---|---|---|---|
| | | 1.5 | 3 | 4.5 |
| Monod | $\nu_{max}$ (g COD $g^{-1}$ VSS $d^{-1}$) | 3.48 | 2.26 | 2.40 |
| | $K_S$ (g COD $L^{-1}$) | 0.35 | 0.60 | 0.55 |
| | SSE | 2.74 | 0.12 | 0.06 |
| Contois | $\nu_{max}$ (g COD $g^{-1}$ VSS $d^{-1}$) | 3.76 | 2.39 | 2.29 |
| | $K$ (g COD $L^{-1}$) | 0.41 | 0.28 | 0.21 |
| | SSE | 3.32 | 0.15 | 0.11 |
| Powell | $\nu_{max}$ (g COD $g^{-1}$ VSS $d^{-1}$) | 4.32 | 2.62 | 2.41 |
| | $K_S$ (g COD $L^{-1}$) | 1.17 | 1.12 | 1.17 |
| | $H$ (g COD $L^{-1}$) | 0.18 | 0.18 | 0.18 |
| | SSE | 4.91 | 0.22 | 0.16 |
| Haldane | $\nu_{max}$ (g COD $g^{-1}$ VSS $d^{-1}$) | 4.15 | 2.85 | 2.41 |
| | $K_S$ (g COD $L^{-1}$) | 0.64 | 1.03 | 0.55 |
| | $K_i$ (g COD $L^{-1}$) | 9.09 | 26.83 | 17000 |
| | SSE | 0.37 | 0.11 | 0.06 |

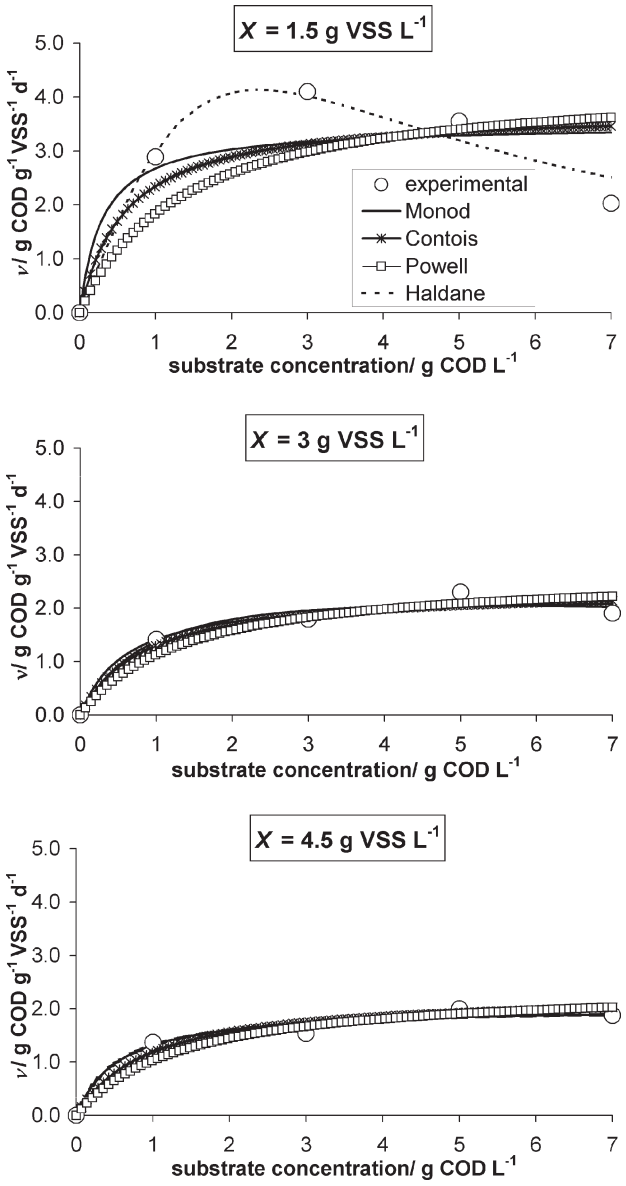


Fig. 2 – *Kinetic models fitted to experimental uptake rates*

the range 0.55 – 1.03 g COD $L^{-1}$ for all biomass concentrations, which indicates a high affinity of biomass to the substrate. The effect of a toxic compound on its biodegradation is quantified by the Haldane's inhibition constant ($K_i$).[16] This term increased as the biomass concentration increased, meaning that those effects may be overcome by the presence of higher amounts of biomass. Indeed, for the highest biomass concentration ($X$ = 4.5 g VSS $L^{-1}$), $K_i$ presented a very high magnitude (17000 g $L^{-1}$), which turned the Haldane model into a Monod type equation.

For higher biomass concentrations (3 and 4.5 g VSS $L^{-1}$) all the models fitted well the experimental data, as SSE values were low and very close. These findings show that at all the tested conditions (initial COD concentration up to 7 g $L^{-1}$) with higher biomass concentration, only the substrate concentration affects significantly the biodegradation process. Therefore, it can be concluded that there is a lumped inhibition occurring in the aerobic degradation of WE that may be expressed by many kinetic models. Lumping several factors into a single one is a convenient mathematical approximation. Formally, the use of a model containing single substrates and single microorganisms can be justified if the overall process kinetics are controlled by a process-rate limiting step.[25] This so-called lumped inhibition accounts either for substrate properties or for mass transfer resistance or even a dilution issue.

Considering the Haldane model, the threshold substrate concentration, at which the maximum value of $\nu$ is achieved, can be calculated by taking the derivative of eq. 6 with respect to $S$ and setting the result equal to zero (eq. 7).

$$S_{threshold} = \sqrt{K_i K_S} \tag{7}$$

Threshold concentration achieves values of 2.41 and 5.25 g COD $L^{-1}$ for biomass concentration of 1.5 and 3 g VSS $L^{-1}$ respectively. These findings also confirm that inhibition may be overcome by higher biomass concentration, allowing treatment of higher organic loads.

The results achieved indicate that a potential design of a full-scale treatment plant for WE must follow some considerations, namely the use of a biomass concentration equal or higher than 3 g VSS $L^{-1}$, in order to obtain higher performance, regardless of the type of inhibition. This is of particular importance if significant concentrations of WE (higher than 3 g COD $L^{-1}$) are to be treated. On the other hand, regarding the COD removal profiles (Fig. 1), high biomass concentrations may have antagonistic effects on the performance of the biological system, possibly due to problems related to mass transfer resistance, and thus requiring higher retention times. Therefore, it should be considered a compromise between biomass concentration and hydraulic retention time.

## Conclusions

Biodegradability of WE was assessed by batch assays, using several substrate and biomass concentrations. The results confirmed that removal of the majority of the organic matter content (more than 90 %) is feasible. The increase on the organic load applied led to a progressive reduction of the treatment efficiency, being more evident for low biomass concentration assays. On the other hand, high biomass concentration may produce some antago-

nistic effect due to substrate diffusion difficulties, thus requiring longer times for removal. Biodegradation kinetics were then investigated by fitting several models to experimental removal rates. The Haldane inhibition model simulated best the assays with the lowest biomass concentration, where the specific substrate removal rate achieved a maximum (4 g COD $g^{-1}$ VSS $d^{-1}$) at a substrate concentration of 2 g COD $L^{-1}$. For higher biomass concentrations, all models fitted well the experimental data, suggesting that in these cases only the substrate concentration affected the biodegradation process (Monod type model). Biomass concentrations of 3 g VSS $L^{-1}$ proven to be suitable for an efficient COD removal without presenting the mass transfer resistance observed at higher biomass concentrations, which lead to higher retention times. In all cases, saturation constants are low, which emphasises high affinity of biomass to the substrate.

## *ACKNOWLEDGEMENTS*

*The authors highly acknowledge Quinta do Sol, Quinta da Gaivosa and Adega Cooperativa de Vila Real wineries for all the information and cooperation provided. This research was financially supported by the Programa AGRO, Medida 8 Acção 8.1, Projecto n° 693 "Desenvolvimento do conceito de leito misto no tratamento de efluentes vinícolas."*

## List of abbreviations and symbols

$BOD_5$ – Biochemical Oxygen Demand, g $L^{-1}$

COD – Chemical Oxygen Demand, g $L^{-1}$

DO – Dissolved Oxygen

F/M – food-to-microorganism ratio, g COD $g^{-1}$ VSS

$H$ – mass transfer resistance constant, g COD $L^{-1}$

$K$ – Contois saturation constant, g COD $L^{-1}$

$K_i$ – inhibition constant, g COD $L^{-1}$

$K_S$ – saturation constant, g COD $L^{-1}$

$S$ – substrate concentration, g COD $L^{-1}$

$X$ – biomass concentration, g VSS $L^{-1}$

SSE – sum of the squared errors

VSS – volatile suspended solids, g $L^{-1}$

WE – Winery Effluent

$\mu$ – specific rate of biomass growth, $d^{-1}$

$\nu$ – specific substrate utilisation rate, g COD $g^{-1}$ VSS $d^{-1}$

$\nu_{max}$ – maximum specific substrate utilisation rate, g COD $g^{-1}$ VSS $d^{-1}$

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