J Ind Microbiol Biotechnol (2004) 31: 29–34
DOI 10.1007/s10295-004-0111-3

ORIGINAL PAPER

**Ana Eusébio · Maurizio Petruccioli
Manuela Lageiro · Federico Federici
José Cardoso Duarte**

Microbial characterisation of activated sludge in jet-loop bioreactors treating winery wastewaters

Received: 23 March 2003 / Accepted: 3 December 2003 / Published online: 21 January 2004
© Society for Industrial Microbiology 2004

Abstract Jet-loop reactors (JLR) used as biological waste treatment processes introduce an additional selective pressure on the natural microbial flora of the incoming effluent. Several high-performing microbial inocula were tested for winery wastewater treatment and the microbial composition was analysed. A microbial consortium was enriched and selected for use with a new type of aerobic JLR. The reactor was operated continuously for more than 1 year using winery wastewaters collected in different seasons. Chemical oxygen demand (COD) removal efficiency was on average greater than 80%, with retention times of 0.8–1 day. Microbial populations were sampled for characterisation after 6 months and at the end of the study. Isolates were identified at genus and/or species level. Almost all isolates belonged to the genera *Pseudomonas* and *Bacillus*. *Saccharomyces cerevisiae* was also found but no filamentous fungi. These results show that a highly adapted population develops in JLRs treating winery effluents as compared to other bioreactors. Aerobic JLRs impose a stringent selective criterion on the composition of the microbial biomass.

Keywords Jet-loop reactors · Selective pressure · Microbial consortium characterisation · Winery wastewater · COD removal

Introduction

Wine production is one of the most highly represented agro-industries in Mediterranean countries, and its importance has also extended to a large number of countries in other parts of the world (e.g. Australia, Chile, United States, South Africa and China) with increasing impact on the economy of these countries. The high amount of effluent produced (usually 1–2 l water/l wine) and the seasonal nature of these industries raise specific problems for the treatment process in terms of volume and composition of the incoming effluent. Consequently, treatment plants must be versatile in relation to the loading regimen and must be able to cope with successions of start-ups and closedowns, and even with intervals of inactivity [17].

The average values for chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) of winery effluents resulting from the vinification process, wash of tanks and racking, are 7–40 g l^{-1} and 5.5–20 g l^{-1}, respectively [5,6,16]. Many conventional treatment methods—chemical, physical or biological—have been used to reduce the organic load of these effluents. Most are relatively expensive and difficult to apply and are unable to withstand fluctuations in the hydraulic and pollution loads [6,7,12]. Thus, effluents with levels of toxicity/colour too high to be discharged into the environment remain [2].

Traditional wastewater treatment plants are based on the use of selected mixed microbial flocs using recycling of settled biomass, resulting in the development of high performance reactors by increasing biomass concentration inside the reactors. Improvements in this technology have resulted in more efficient and compact reactors able to perform better treatment with shorter retention times. Some of these alternative technologies are based on fixed bed [3,8,10,13], fluidised bed [3,9,13], up-flow anaerobic sludge bed (UASB) [4,15] and expanded bed [13] reactors and rotating biological contactors [11]. All of them use biomass flocs or granules, free or immobilised, as process catalysts, with the objective of increasing the biomass concentration inside the reactor for faster removal of organic matter.

Application of these technologies for aerobic reactors is limited by their capacity to supply oxygen at low cost

A. Eusébio · M. Lageiro · J. C. Duarte (✉)
Biotechnology Department, INETI,
Estrada do Paço do Lumiar 22, 1649-038 Lisbon, Portugal
E-mail: jose.duarte@ineti.pt
Tel.: +351-217164211
Fax: +351-217166966

M. Petruccioli · F. Federici
Dipartimento di Agrobiologia e Agrochimica,
University of Tuscia, Via San Camillo De Lellis 1,
01100 Viterbo, Italy

for the oxidation of organic matter at the high rates required. Often, other restrictive criteria result from the availability of space, mainly in densely populated zones. The use of vertical reactors could become an interesting alternative if good oxygen transfer and high biological conversion capacity are attained. The application of jet-loop reactor (JLR) types is an interesting alternative since it is claimed that they have higher oxygen transfer rates at lower energy costs. Also, due to the reduced reactor volumes needed for treatment, small areas are required resulting in significant savings in installation and maintenance costs [1]. However, JLRs have not yet achieved great application in the wastewater treatment industry.

A new type of JLR has been developed by our team and used for the treatment of winery wastewaters [14]. A major and less studied problem in the use of JLR concerns the type of microbial flora that develops with this technology. High flow velocities of liquid through the venturi nozzle could impose a selective criterion on the type of microbial population, e.g. filamentous microorganisms have not been isolated in such reactor systems. Furthermore, the energy that is dissipated at the nozzle also raises the fluid temperature and could create an additional selective pressure.

Our previous work indicated that a restricted population without capacity for floc formation developed in a JLR [15]. This advantageous characteristic of the JLR system should be investigated further in order to clarify a possible relationship between the observed population behaviour and the project design features of the reactor. In this paper we report the development of high-performing inocula and microbial flora evolution in a JLR during 1 year of use for winery wastewater treatment.

Materials and methods

Winery effluent

Winery effluent from white grapes was collected from the Winery Research Station at Dois Portos (Estação Vitivinícola Nacional, Dois Portos, Portugal), and was used without addition of further nutrients (including phosphorous or nitrogen). Its analytical characterisation is presented in Table 1. The composition of the winery effluent changed over the course of the year, the highest COD values being observed on effluents from vintage and rackings.

Microbial inocula comparison

Microbial inocula were sampled from different origins: (1) activated sludge from the wastewater treatment plant of the Cooperative Winery at Pegões, Portugal; (2) activated sludge from a pulp and paper industry treatment plant at Constância, Portugal; (3) biomass from the site of a thermal natural spring (50°C) from the Viterbo area, Italy; (4) an activated sludge from the winery treatment plant of the Cooperativa Vitivinicola of Orvieto (Orvieto, Italy); (5) a commercial liquid starter Liquibat N12 (Lamberti, Milan, Italy); and (6) a freeze-dried commercial inoculum, Micropan Complex (Eurovix, Brescia, Italy). Microbial inocula samples were frozen and stored at −20°C until used. A mixed consortium enriched from a mixture (3:1) of biomass (1) and biomass (2) was used to inoculate the JLR and was maintained there during the 6 months of reactor operation. Thereafter, the resulting microbial biomass (7) was tested and characterised.

Table 1 Analytical characterisation of the crude winery wastewater used to feed the jet-loop reactor (JLR) prototype during biotreatment experiments. These values were obtained over the 390 days of JLR operation

Character	Unit	Range
pH	–	4.00–4.99
Chemical oxygen demand (COD)	$g.l^{-1}$	3.10–27.2
Biochemical oxygen demand (BOD_5)	$g\ l^{-1}$	0.21–8.00
Total suspended solids (TSS)	$g\ l^{-1}$	0.17–0.49
Volatile suspended solids (VSS)	$g\ l^{-1}$	0.13–0.42
Total phosphorous	$mg\ l^{-1}$	16.6–65.7
Nitrogen (Kjeldhal)	$mg\ l^{-1}$	21.3–64.0
Phenols (folin index)	–	2.80–20.0
Organic matter	(%)	94.9–95.9

In order to compare the degradation efficiency of the different microbial consortia, 1,000-ml flasks containing 800 ml winery effluent (COD: $6.3\ g\ l^{-1}$)—a volume ratio identical to the JLR—were inoculated with 200 ml each consortium (1–7), simultaneously and under the same conditions. The effluent was used after neutralisation to pH 6.8. Native microflora from winery effluents were used as controls. Assays were performed in duplicate and incubation was at 40°C, using a forced aeration of 1 vvm. From each culture, samples were taken after the 4th and the 6th day of incubation for COD analysis.

Bioreactor equipment

The JLR prototype had a total volume of 25 l. A schematic drawing of this prototype is shown in Fig. 1. The reactor consisted of a cylindrical column with a central aeration tube and a cylindrical degassing tank with refrigeration systems. The mixed liquor was pumped through an ejector venturi, where the air was drawn into the liquid through the aeration tube. The aerated mixed liquor passed from the column reactor to the degassing tank, from where it was recycled, passing again through the nozzle of the ejector venturi into the column reactor. The degassing tank was connected to a settling tank (volume = 12 l) where the displaced mixed liquor was collected. Probes for measurement of temperature, pH and dissolved oxygen (O_2) were placed on the top of the degassing tank. The reactor was fed through a peristaltic pump from a reservoir containing the wastewater to be treated.

Reactor operation and control

After a start-up phase, a feeding flow rate of $9.6\ l\ day^{-1}$ was fixed for continuous winery effluent treatment operation. The aeration rate was 0.7 vvm. Temperature, pH and dissolved O_2 were monitored and registered daily using a Modular control unit from SGI-Inceltech-LH (MOD 7F) connected to the sensors of temperature, pH and dissolved O_2. The inoculum used was a mixture (3:1) of biomass 1 and biomass 2. The reactor was operated continuously for 390 days.

Determination of winery effluent parameters and analytical methods

COD was determined on the winery wastewater samples before and after treatment. Analyses of COD and mixed liquor volatile suspended solids (MLVSS) were carried out following *Standard Methods for the Examination of Water and Wastewater* [18]. For

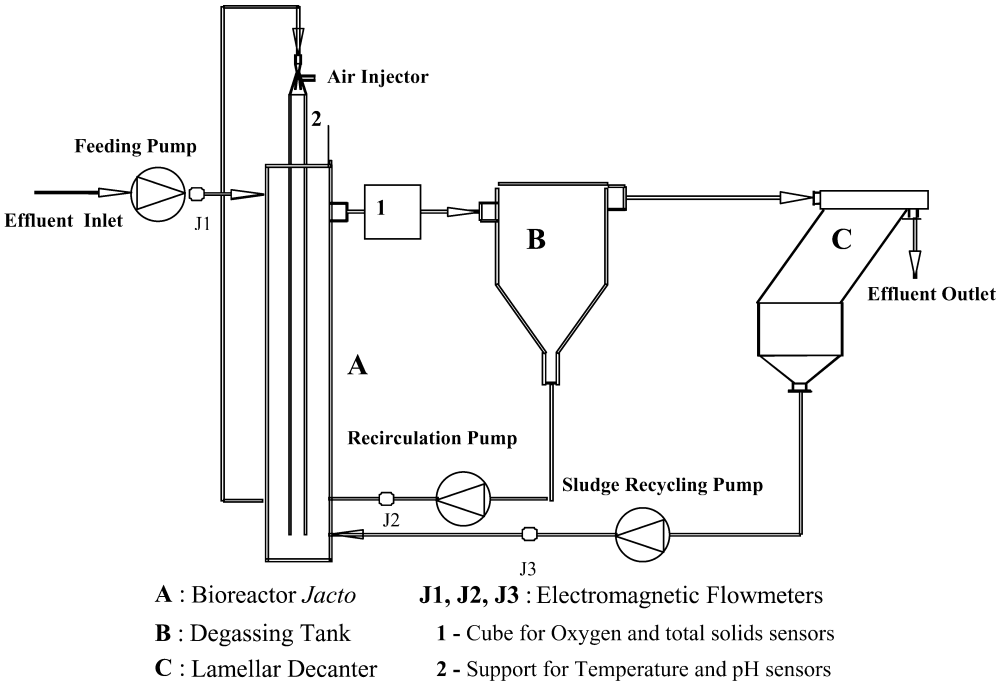

Fig. 1 Schematic drawing of the jet-loop reactor (JLR) designed and tested at INETI's laboratory. *A* Column reactor, *B* degassing tank, *C* settling tank

COD determination, samples were previously acidified to a pH lower than 2.0 using sulphuric acid, stored at −20°C and de-frosted at room temperature (RT) before analysis.

Microbial count and identification

Cell counts were performed using a Neubauer chamber under phase contrast microscopy (Olympus). Operating the reactor in continuous feeding conditions for more than 1 year, high concentrations of microbial biomass were observed (6.0 g l^{-1} and 3.0×10^{10} cell ml^{-1}), as well as the colonisation of the inner surfaces of the bioreactor by a thick microbial film (biofilm). Samples of mixed liquid were collected from the bioreactor after 180 days of treatment. Samples of mixed liquid and biofilm were collected from the bioreactor after 360 days of treatment. Heterotrophic plate counts and isolation of bacteria and fungi were carried out on Plate Count Agar and Rose-Bengal Chloramphenicol Agar Base (at 37°C). Before plating, biofilm samples (1.0 g, wet weight) were suspended in 99.0 ml physiological salt solution (0.9% NaCl) in 500-ml flasks and kept under agitation (150 rpm) for 1 h at RT. Identification of the isolates, mostly at genus level, was performed on the basis of cell and colonial morphology, Gram staining, motility, presence of endospores, catalase and oxidase tests, as well as the following API tests (Bio-Merieux, Rome, Italy): API 10S, API 20NE, API 20E and API CORYNE for bacteria and API 20C AUX for yeasts and yeast-like fungi.

Results and discussion

Comparison of microbial inocula

The biodegradation activities of several microbial consortia (1, 2, 3, 4, 5, and 6 as described in Materials and methods), were compared by measuring the effluent COD reduction (%). A non-sterile control was used without any addition of inoculum, allowing growth of the native microflora. The biodegradation activity of the different inocula was compared with that of the JLR (consortium 7).

Table 2 Chemical oxygen demand (COD) reduction (%) by several microbial inocula after 4 and 6 days of winery effluent treatment. Inocula *1–7* are described in Materials and methods. Values represent means of two determinations ±SD

Sample (day)	COD reduction (%)							
	Control	Microbial consortia						
		1	2	3	4	5	6	7
4	51±0	63±5	80±5	92±1	42±1	20±2	40±1	83±3
6	75±0	84±6	87±3	96±2	86±0	73±1	90±0	87±2

During this experiment, samples were collected at time zero, and at 4 and 6 days. Samples were analysed for COD (Table 2). Significant COD reduction levels (%) were observed even for the control. At 4 days of treatment, COD reduction activity was higher for microbial inocula obtained from a winery (1) and pulp and paper (2) treatment plants, the thermal water enriched isolate (3) and the reactor microbial mass representative sample (7). Inocula 4–6 did not show any improvement over the non-inoculated flasks.

The microbial population of the JLR (7) performed the second best, and slightly better than the two inocula from which it originated (1 and 2). The experiment shows that microbial biomass adapted over 6 months under JLR conditions maintains its biodegradation activity at an optimal level even when compared with the original inoculum and/or commercial selected inocula. Surprisingly enough, bearing in mind its origin, inoculum 3 performed best and it will be of interest to evaluate its activity under JLR conditions in future studies.

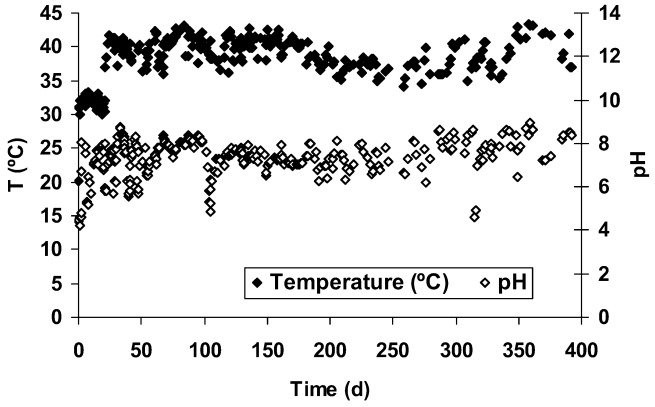

Fig. 2 Data acquisition during winery effluent biotreatment using the JLR: temperature and pH

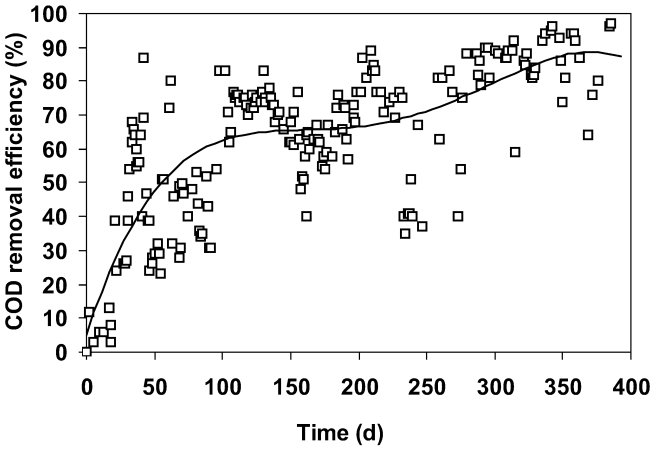

Fig. 3 Chemical oxygen demand (COD) removal efficiency (%) during winery effluent biotreatment using the JLR. Trendline added as type polynomial 4 order

At 4 days, only inocula 2, 3 and 7 degraded enough COD in winery effluent to attain a satisfactory level to be disposed of by municipal wastewater treatment plants in accordance with municipal water regulations in most European countries. However, if enough time was allowed (6 days), all inocula reached that performance level, confirming the well-adapted characteristic of the JLR microbial flora.

Winery wastewater treatment

As described in Materials and methods, microbial inocula 1 and 2 were mixed (3:1) to inoculate a 25-l JLR system (Fig. 1) used for winery wastewater treatment. Figure 2 shows temperature and pH profiles during the 390 days of continuous bioreactor operation. Figure 3 shows the COD removal efficiency.

During the whole year, the temperature inside the JLR was maintained at a relatively constant level, with an average value of 39.0 ± 3.2°C (Fig. 2) in spite of the effects of incoming effluent addition (at RT) and external temperature variations (RT ranged from 15 to 25°C). The pH also showed a very stable and self-controlled behaviour at an average value of 7.0 ± 0.9. This is due to the neutralising effect of the combination of the rate of incoming acidic medium with the rate of biodegradation of organic acids in the reactor. The initial pH of the effluent (4.0–4.9) did not affect treatment performance, and neutralisation of the incoming effluent was not necessary. Dissolved oxygen measured inside the JLR maintained itself between 75–90% of saturation during operation (data not shown).

As shown in Fig. 3, COD removal efficiency increased continuously after the start-up phase over more than 1 year of continuous operation, reaching high yields (80–90%) of organic matter removal corresponding to a maximum productivity of 20 kg COD m^{-3} day^{-1}. The trend of increase in efficiency in effluent treatment is clear (Fig. 3). Some problems during operation (pump breaks, sporadic foaming events) and changes of effluent composition—considered normal for this type of effluent during a whole yearly cycle—are responsible for some of the variations observed concerning COD removal efficiency (40–95%). This behaviour demonstrates the good performance of the JLR microbial population and its ability to respond to variations in pH, temperature, loading charge, and even mechanical breakdown, resulting in characteristics that are very attractive for winery wastewater treatment.

Microbial inocula characterisation

At 180 and 360 days of continuous functioning of the 25-l JLR system, a sample of the microbial population (no. 7 in Materials and methods) was collected for characterisation. Table 3 shows the results of the identification tests, while Table 4 reports results of plate counts.

At 180 days, 40 isolates were collected and identified at the genus and/or species level. The bacterial isolates belonged mostly to the genera *Bacillus* and *Pseudomonas*. This predominance led us to suggest that these microorganisms possess a greater resistance to the high shear forces that develop in a JLR at the level of the nozzle. The absence of protozoa might be due to the same reason. Although these genera are common in most effluent treatments, a recent study does not attribute them with any significant role in COD reduction in winery wastewater treatment [11]. This is most probably not the case in our JLR, which is different from the system used in that study (rotating biological contactor—RBC), and also concerning the microbial population in the biofilm developed during the 50 days of RBC operation. As for fungi, nine isolates of *Candida* sp. and one isolate of *Blastoschizomyces capitalis* were identified. The absence of isolates belonging to the genus *Spirillum*, well known as a microaerophyl, confirms the good aeration rate obtained in the JLR as measured by the dissolved oxygen levels.

Table 3 Characterisation of the residential microbial population after 180 and 360 days of continuous winery wastewater treatment in the JLR. The results presented by our Italian project partner are shown for comparison [15]

Microorganisms	180 days	360 days		[15]
	Liquid[a]	Liquid[a]	Biofilm[a]	
Bacteria				
Acinetobacter sp.	+ (3)[b]	+ (1)	+ (1)	+ (2)
Bacillus sp.	+ + (12)	+ + (3)	+ (1)	+ (3)
Hyphomicrobium sp.	+ (1)	–	–	–
Moraxella sp.	+ (2)	–	–	–
Pseudomonas sp.	+ + (5)	+ (3)	+ (2)	+ + (5)
Pseudomonas cepacia	–	+ + (3)	+ (1)	–
Pseudomonas stutzeri	+ (2)	–	–	–
Sphingomonas paucimobilis	–	+ (1)	–	+ + (4)
Agrobacterium radiobacter	–	–	–	+ + (3)
Not-identified	+ (5)	+ + (4)	+ + (3)	+ (2)
Fungi				
Blastoschizomyces capitalis	+ (1)	+ (1)	+ (1)	+ (2)
Candida sp.	+ + (9)	+ + (2)	+ + (2)	+ (2)
Pichia fermentans	–	+ (1)	+ (1)	–
Saccharomyces cerevisiae	–	+ + (3)	+ + (3)	+ + (3)
Not-identified	–	+ (1)	+ + (4)	–

[a]– Absence, + presence (from 1 to 40% colonies per plate), + + high frequency (> 40% colonies per plate)
[b]Numbers in parentheses are the numbers of isolates: colonies for isolation were selected proportionally to their presence as gathered from the macroscopic colonial morphology

Table 4 Plate counts of the heterotrophic microbial population after 180 and 360 days of continuous winery wastewater treatment in the JLR. Values represent means of three determinations ± SD

Organisms	180 days	360 days	
	Liquid (cfu/ml $\times 10^6$)	Liquid (cfu/ml $\times 10^7$)	Biofilm (cfu/g wet weight $\times 10^8$)
Bacteria	212.5 ± 12.5	585.0 ± 25.0	106.5 ± 5.5
Fungi (yeasts)	5.15 ± 0.15	4.85 ± 0.55	5.7 ± 0.6

Determinations of MLVSS and cell counts also confirm the good microbial growth obtained in the JLR. Values of 0.5 g MLVSS l^{-1} and 0.2×10^{10} cell ml^{-1} measured at start-up increased significantly during biotreatment in the JLR, reaching values of 6.0 g MLVSS l^{-1} and 3.0×10^{10} cells ml^{-1} after 160 days of continuous process. In addition to the high concentrations of microbial biomass observed in the liquid, a biofilm also formed on the reactor inner surfaces after 1 year of operation. The polysaccharide-producing ability of some of the microorganisms present in the bioreactor, such as *Pseudomonas* [15], could be responsible for the formation of this biofilm, although hyphal growth of some genera (e.g. *Blastoschizomyces*) could also help with biofilm formation. Both the biofilm and the mixed liquid were rich in bacteria and also in yeasts, after the first year of operation (Table 3). No protozoa were present in the mixed liquid, while a certain number of ciliates were observed on the surface of the biofilm, highlighting the effect of the shear-forces at the Venturi ejector on the microorganisms present in the liquid phase.

After 1 year of biotreatment, we could observe the presence of a large number of *Saccharomyces cerevisiae* isolates in both the liquid and the biofilm, contrary to the microbiological characterisation carried out 6 months after start-up of reactor operation. The appearance of *S. cerevisiae* may be related to biofilm formation, and selection of *S. cerevisiae* strains more resistant to reactor stress. No significant differences were observed between the composition of the two microbial populations (in the liquid and in the biofilm). However, yeasts were relatively more abundant in the biofilm compared to the liquid. Selection of adapted microbial populations was also observed during treatment in the JLR, since the genera *Bacillus* and *Pseudomonas* were present until the end of the experiment although their relative distribution changed; other microorganisms like *Moraxella* and *Hyphomicrobium* disappeared. This survival ability of the predominant genera *Bacillus* and *Pseudomonas* could be explained by more than their metabolic versatility. Their presence after 1 year could also be an indication of the good mechanical stress resistance of these genera; a characteristic that will be further investigated. Microbial strains of the genus *Pseudomonas* are Gram (–), facultatively aerobic and grow at pH 7–9. They are active in biodegradation of the winery effluent due to their alkaline reactions from citric, malonic, and tartaric sodium salts. *Bacillus* strains are Gram (+), strictly aerobic or facultatively anaerobic, and grow at pH 5–8, being able to resist the acidic nature of that effluent. The winery effluent treatment occurred in the JLR in the pH range of pH 5–9 (Fig. 2), providing favourable conditions for growth and microbial activity (e.g. degradability) for both *Bacillus* and *Pseudomonas*. Anoxic conditions that normally develop in the deep layers of biofilms could also support the presence of both facultatively aerobic *Pseudomonas* and facultatively aerobic *Bacillus*.

As previously observed [14,15], *Bacillus* and *Pseudomonas* were also the most common genera of microorganism found during winery wastewater biotreatment, which suggests their importance in this efficient microbial consortium. The results presented by our project partner [15], shown for comparison in Table 3, are from a different type of experiment. Both studies corroborate the fact that the JLR is a versatile type of reactor. The microbial populations grown in the two reactors revealed significant quantitative differences (Table 3). Concerning qualitative microbial analysis, it is noteworthy that mostly the same genera were isolated from both populations even though they originated from different inocula and from Italian [15] and Portuguese winery wastewaters. This might influence the different performances of the two JLRs, resulting in a maximal COD removal efficiency of 5.3 kg COD m^{-3} day^{-1} [15] and of

20 kg COD m^{-3} day^{-1} (this paper). In addition, more significant differences between the microbial flora distribution at 180 and 360 days can also be inferred from Table 3, with a reduction in the relative frequency of the genera *Bacillus* and *Pseudomonas* and an increase in the frequency of *S. cerevisiae* and other, non-identified, fungi. This could be attributed either to the appearance and development of the biofilm or to the selection of more fastidious microorganisms, which are difficult to characterise and identify by conventional methods.

Conclusions

Operation of a JLR for the treatment of winery wastewaters for more than 1 year induced selection of the best-adapted microorganisms, maintaining a high degree of conversion and productivity, and revealing good adaptation of the microbial inocula initially developed. The resulting microbial consortium has a high capacity for COD removal (>80% biodegradation) in winery effluents. The results show that a specific microbial consortium was selected after a long operation time and strict bioreactor conditions. The predominant isolates found belong to the genera *Bacillus* and *Pseudomonas*. Later in the experiment, *S. cerevisiae*, a typical endogenous microorganism in the wastewater, was isolated, although its presence may be related to biofilm development. One characteristic of this population must be its resistance to the hydraulic shear stress developed at the nozzle.

The physiology of these microbial populations in relation to this external shear stress should be studied further. This type of bioreactor imposes a stringent selective criterion on the composition of a microbial biomass whose advantages and disadvantages for the process are still under evaluation. Poor settling ability of this biomass that eventually occurs in JLR systems is not an inconvenience per se since technological solutions have been developed for the downstream treatment of these effluents. A detailed knowledge of the microbial populations and their interactions with reactor design and operation, is becoming an important tool in promoting the use and selection of microbial inocula for effluent biotreatment.

Acknowledgements The authors thank Elsa Almeida-Vara and Gabriela Sena-Martins, at INETI, for review of the manuscript and their useful comments. The European Commission, DG Environment, within the Programme LIFE (Project 96ENV/P/00602/INDW), sponsored this research work.

References

1. Bloor JC, Anderson GK, Willey AR (1995) High rate aerobic treatment of brewery wastewater using the jet-loop reactor. Water Res 29:1217–1223
2. Borja R, Martín A, Maestro R, Luque M, Durán MM (1993) Enhancement of the anaerobic digestion of wine distillery wastewater by the removal of phenolic inhibitors. Bioresour Technol 45:99–104
3. Buffière P, Moletta R, Fonade C (1995) Continuous operations of a fluidized bed bioreactor for anaerobic digestion: residence time effect on degradation kinetics. Biotechnol Lett 17:833–838
4. Daffonchio D, Colombo M, Origgi G, Sorlini C, Andreoni V (1998) Anaerobic digestion of winery wastewaters derived from different wine making processes. J Environ Sci Health A33:1753–1770
5. Duarte EA, Martins MB, Ghira JP, Carvalho EC, Spranger I, Costa S, Leandro MC, Duarte JC (1998) An integrated approach for assessing the environmental impacts of wineries in Portugal. In: CEMAGREF (ed) 2nd International Specialized Conference on Wineries Wastewaters—Actes de Colloque. CEMAGREF, France, pp 61–69
6. Fumi MD, Parodi G, Parodi E, Silva A (1995) Optimisation of long-term activated-sludge treatment of winery wastewater. Bioresour Technol 52:45–51
7. Gonard B (1992) Les traitements de la pollution par procédés biologiques. Rev Fr Oenol 32:29–35
8. Henze M, Harremos P (1993) Anaerobic treatment of wastewater in fixed film reactors: a literature review. Water Sci Technol 15:1–101
9. Iza J (1991) Fluidized bed reactors for anaerobic wastewater treatment. Water Sci Technol 24:109–132
10. Lettinga G, Hulshoff Pol LW (1991) UASB—Process design for various types of wastewater. Water Sci Technol 24:87–107
11. Malandra L, Wolfaardt G, Zietsman A, Viljoen-Bloom M (2003) Microbiology of a biological contactor for a winery wastewater treatment. Water Res 37:4125–4134
12. Moletta R, Raynal J (1992) Procédés de dépollution innovants et recherches actuelles dans le domaine vinicole. Rev Fr Oenol 32:37–43
13. Pérez M, Romero LI, Sales D (1997) Thermophilic anaerobic degradation of distillery wastewater in continuous-flow fluidized bed bioreactors. Biotechnol Prog 13:33–38
14. Petruccioli M, Duarte JC, Federici F (2000) High-rate aerobic treatment of winery wastewater using bioreactors with free and immobilized activated sludge. J Biosci Bioeng 90:381–386
15. Petruccioli M, Duarte JC, Eusébio A, Federici F (2002) Aerobic treatment of winery wastewater using a jet-loop activated sludge reactor. Process Biochem 37:821–829
16. Racault Y, Lenoir A (1995) Evolution des charges polluantes de deux caves vinicoles du Sud-Ouest de la France sur un cycle annuel. Rev Fr Oenol 152:16–18
17. Sales D, Valcárcel MJ, Pérez L, Martínez de la Ossa E (1987) Activated sludge treatment of wine-distillery wastewaters. J Chem Tech Biotechnol 40:85–99
18. Standard methods for the examination of water and wastewater, 20th edn (1998) American Public Health Association/American Water Works Association/Water Environment Federation (eds) Washington D.C.