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Caractérisation des Consortia Anaérobies développées dans un filtre anaérobie employé dans le traitement d'effluents des caves vinicoles

Characterization of the Anaerobic Consortia developed in an anaerobic filter applied to the treatment of winery effluents

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Résumé - On a évalué l'application d'un filtre anaérobie au traitement des effluents des caves vinicoles, en utilisant des effluents synthétiques et réels produits pendant les périodes de la vinification et des soutirages. Le réacteur a été sans alimentation pendant un an avant le démarrage de ce travail. La composition et la qualité du consortium, aussi bien que son évolution au long du temps ont été déterminées par des tests d'activité des groupes trophiques : syntrophiques, acétoclastiques et hydrogénophiliques. L'activité méthanigène spécifique de quelques groupes trophiques étudiés a subi une augmentation significative après trois mois d'opération. Cependant, au long de toute la période de l'expérience, on a constaté l'évidence d'une inhibition, soit dans les bactéries méthanigènes acétoclastiques soit dans les syntrophiques. L'activité méthanigene spécifique avec l'éthanol a augmenté et l'activité hydrogénophilique s'est maintenue élevée pendant toute l'expérience. En même temps, on a fait des tests batch de biodégradabilité de l'effluent réel avec différentes concentrations, en employant l'effluent filtré et non-filtré. L'objectif de ces tests a été l'évaluation de la dégradabilité anaérobie soluble et totale. La présence des solides dans l'effluent du collage n'a pas eu d'influence évidente sur les résultats. Par contre, on a remarqué que la présence des solides dans l'effluent de la défécation statique du moût a diminué la limite de concentration à partir de laquelle l'inhibition était évidente.

Abstract *- In the present work, the application of an anaerobic filter for winery effluent's treatment was evaluated, using synthetic and real effluent from the vintage and wine-racking periods and after one year without feeding. The composition of the anaerobic consortium and its evolution with operating conditions were determined with the use of methanogenic activity tests of the syntrophic, acetoclastic and hydrogenophilic trophic groups. The methanogenic activity of some trophic groups suffered a significant increase after three months of operation. However, during the total trial period, there was evidence of inhibition in the acetoclastic and syntrophic bacteria. The methanogenic activity against ethanol was strongly enhanced and the hydrogenophilic remained high. Batch biodegradability tests of filtered and total effluent were carried out in order to evaluate the soluble and total anaerobic degradability. The presence of solids had no significant influence in the racking effluent and decreased the threshold concentration after which inhibition was evident in the static sedimentation effluent.*

Mots clés : filtre anaérobie, activité méthanigène, caves, inhibition

Keywords : *anaerobic filter, methanogenic activity, winery, inhibition*

INTRODUCTION

Pollution in the winery industry is mainly originated in the washing operations of the process equipment during the vintage period. Its most important characteristics are: seasonal and daily fluctuations, acid nature, high concentration of organic matter (1000-10000 mg COD/l) and suspended solids (1000-16000 mg/l), nutrient deficiency, presence of possibly-inhibitory sulphur compounds. When rejected to the aquatic media it causes perturbations in its biological balance. A technique based in the **anaerobic digestion** using the **Anaerobic Filter**, is very well placed in terms of available treatment options as it complies with the criteria of efficiency, reliability, economical costs and simplicity of operation and maintenance and has a proven efficiency in the winery effluent's treatment.

Anaerobic digestion is a complex process that involves the co-ordinated and differentiated activity of a big number of bacterial trophic groups. It can be divided in several phases and occurs in the absence of oxygen. The end-products are CO_2, CH_4, H_2O and NH_3. The phases can be divided in four: *hydrolysis* (breakdown of complex organic matter in simpler components), *acid fermentation* (production of acetate, butyrate, propionate and lactate), *acetogenesis* (conversion of VFA in acetate, CO_2 and H_2 through the acetogenic bacteria) and *methanogenesis* (production of methane through the acetoclastic (from acetate, 70 %) and hydrogenophilic (from H_2, 30 %) bacteria).

The use of methodologies for anaerobic sludge characterisation is one of the major requisites for the operational control of anaerobic digesters. Of the great variety of procedures presented in literature, with the objective of determination of the specific methanogenic activity (SMA) of anaerobic sludges, the procedure put forward by Colleran and Pistilli (1994) was used in the present study.

MATERIALS AND METHODS

Reactor configuration: The anaerobic filter used in this study consisted in a conical cylinder with upflow flux, built in PVC. It had a operational total volume of 24.1 litres, (cylindrical part: 23.5 litres (internal diameter = 24 cm) and the conical 0.6 litres with a height of 4 cm). It was heated at a constant temperature of 35° C. The conical section had no support matrix while the cylindrical section was (randomly) filled with grape-stalk. It was connected to a gas counter and operated without recycle.

Inoculum & Feedstock: The inoculum was obtained from a municipal sewage sludge digester and 8 litres were added to the filter which operated for 6 months with a synthetic winery effluent. After one year without feeding it re-started with the present study. The feedstock was kept at 4° C and was divided in "Synthetic effluent" and "Real effluent". The synthetic effluent was used to acclimatise the biomass while the real effluent was not available from the winery. It consisted in an equal mixture of white and red wines, sucrose (simulating the sugars present in non-fermented wine) and a macronutrients solution (NH_4Cl:1.74 g/l, KH_2PO_4:28.3 g/l, $(NH4)_2SO_4$:28.3 g/l). Sucrose was added as 1 g/0.22 ml produced wine (Moreira, 1995) and the macronutrients solution was added as 0.6 ml/g COD fed. The real effluent consisted in a mixture of actual winery effluent (two origins: wash-water of static sedimentation of the must and racking operation) and the macronutrients solution. In both synthetic and real effluents, 6.0 g/l $NaHCO_3$ were added to supplement suitable buffer capacity. No micronutrients were added.

Experimental design: Two experimental trials were monitored: synthetic effluent and real effluent operation. Each of the trials were divided into 3 phases, as follows:

Table 1 - Operational conditions applied to the filter

Trial/phase		Days	Average COD (mg/l)	Average BV (g COD/l*day)	Average TS (g/day)
Trial I (synthetic)	Phase I[1]	0-36	1240	0.35	---
	Phase II[1]	36-125	5071	1.41	---
	Phase III[1]	125-159	2264	0.57	33.3
Trial II (real)	Phase I[2]	159-280	2133	0.43	29.7
	Phase II[2]	280-311	4292	1.30	56.4
	Phase III[3]	311- 342	5573	1.78	34.3

(1) synthetic effluent, (2) real effluent - wash-water of static sedimentation, (3) real effluent - wash-water of racking operation Bv-applied organic loading rate

The hydraulic retention time was kept at 4.2 days. **In Trial I**, Phase I was used to acclimatise the sludge to a winery-type effluent. In Phase II, COD was increased to 5000 mg/l, the expected value for the real effluent. However, it was not and the COD load was decreased to the actual concentration (Phase III). **In Trial II**, Phase I consisted in the application of the static must sedimentation effluent, in Phase II the COD load was increased in order to evaluate the filter's performance and in Phase III the effluent was shifted to the racking effluent.

Analytical determinations: Analyses were made according to Standard Methods. Biogas composition was measured by GC. SMA determinations on the trophic groups were carried out using propionate, butyrate and ethanol (syntrophic), acetate (acetoclastic) and H_2/CO_2 (hydrogenophilic) as substrates and according to Colleran and Pistilli (1994). The same procedure was used to assess the biodegradability of the static must sedimentation and racking wash-water with concentrations ranging from 0.2 to 10 g COD/l.

RESULTS AND DISCUSSION

A summary of the filter's performance is given in table 2:

Table 2 - Filter's performance data

Trial/Phase		total COD removal (%)	Methane (%)	L CH/gCOD removed	Solids removal (%)	
					TS	TVS
Trial I (synthetic)	Phase I	90.4	64.9	---	---	---
	Phase II	62.9	47.3	0.29	---	---
	Phase III	88.4	63.2	0.20	30.8	89.7
Trial II (real)	Phase I	92.3	72.3	0.23	32.3	82.8
	Phase II	91.5	63.8	0.15	44.6	84.4
	Phase III	89.0	74.1	0.07	32.9	77.3

TS - Total solids; TVS - Total volatile solids

Although it was not an important goal the analysis of the performance of the filter in terms of organic matter removal, the COD removal efficiency had a minimum of 63 % but the overall efficiency was 90 %. The lower values obtained in phase II of the 1st trial was

attributed to the high COD input and the absence of micronutrients (necessary for several methanogenic bacteria, Archer (1984)) in the feedstock. The reason for the absence of micronutrients was that this experiment tried to simulate real field conditions, where their addition is not done due to high exploration costs. Bories and Moulon (1994) used an anaerobic filter which had 11 m^3 and grape-stalk as support matrix. Reported values of efficiency were higher than 90 % for loads of 0.7-1.7 kg COD/m^3*day, with effluent recycle and no nutrients added. The results obtained in this study are similar to the reported by these authors. After 342 days of continuous operation, there was no evidence of clogging problems despite the somewhat high solids input to the filter (Table 1). The following table summarises the results of the SMA tests:

Table 3 - Specific methanogenic activity results

Day	Effluent applied	Substrate/Activity (ml CH_4/g VSS*day)				
		Acet.	Prop.	But.	Eth.	H_2/CO_2
0	none	5.8	0.7	0	8.3	284.5
89	synthetic	0	0	0	70	569.7
159	synthetic	*1.4*	6.9	*0.4*	140	357.7
199	real (static sedim.)	*0.9*	11.0	*1.2*	124	352

Note: in *italic*, long Lag-phase before initial methane production

Prior to the start-up of the filter (day 0), a determination of the activity of the sludge inside the filter was carried out. Not surprisingly, the results of this first determination showed a very low methanogenic activity for all the substrates tested, specially for acetate, propionate and butyrate. The increase in the substrate concentration (2nd sample) induced an increase in the hydrogenophilic and the ethanol-syntrophic activity. The ethanol result was expected as the filter was in continuous operation with an ethanol-content effluent (both in the synthetic and real trials). Throughout the experimental period the acetoclastic activity was absent. In the 3rd and 4th samples a long Lag-phase was observed before the initial methane production from acetate, which put in evidence some sort of inhibition to this trophic group of bacteria. Simultaneously the syntrophic activity on propionate and butyrate were also affected presenting a similar pattern of methane production (although for propionate was less evident). Figure 1 presents an example of this situation, using the direct readings of the pressure transducer for the sample taken on the day 159.

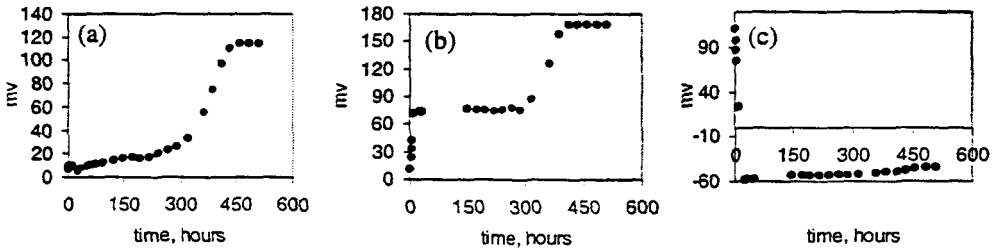

Figure 1 - Pressure curves for acetate (a), ethanol (b) and H_2/CO_2 (c)

A Lag-phase occurred for approximately 300 hours in the acetate test, as well as for butyrate (not shown). No Lag-phase was observed for H_2/CO_2. The degradation of ethanol to methane is a two-step process, involving a syntrophic association between ethanol degraders hydrogen (and acetate) producing bacteria and methanogenic hydrogenotrophic bacteria. By comparing figure 1 (b) and (c) it is suggested that the first increase in the ethanol curve consists in the degradation of the H_2 (syntrophic activity present, acetoclastic inhibited - Figure 1 (a)) until the extinction of H_2 and then the acetoclastic activity begins (after the referred Lag-phase of 300 hours). Considering that biomass samples were representative, it can be concluded that hydrogenotrophic bacteria were the major responsible for the methane production. The activity tests in the 4th sample were very similar to the 3rd sample, with the difference that the inhibition period lasted 150 hours (half than previously). Two explanations are possible: the presence of the inhibition factor was less important in the real effluent applied or, the acclimatisation of the biomass with continuous operation of the filter.

Batch effluent biodegradability tests were conducted using filtered and total effluent with concentrations of 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 g COD/l (both static sedimentation and racking - Figures 2 & 3).

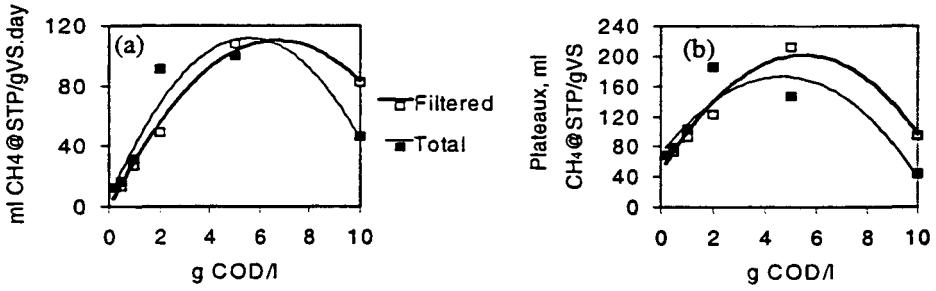

Figure 2a,b -Rate and maximum CH_4 production - static must sedimentation effluent biodegradability test

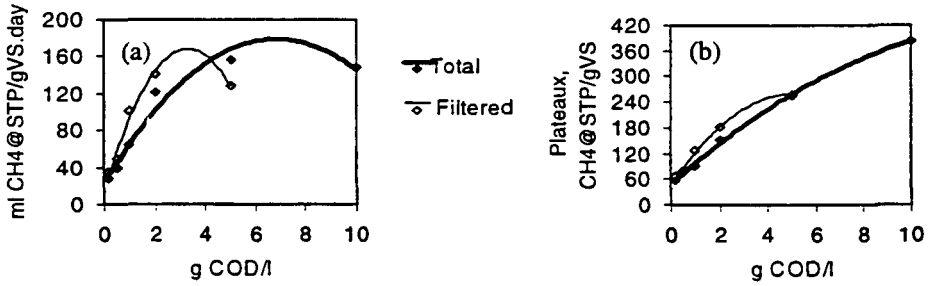

Figure 3a,b -Rate and maximum CH_4 production - racking effluent biodegradability test

No major differences were found for filtered and total static must sedimentation effluent (figure 2). There was an evident decrease in both the rate and the maximum methane production, indicating that this effluent was only partially degraded (at a lower rate) above 5.0 g COD/l. As referred earlier, this could be explained by the increased presence of an inhibitor or the lack of micronutrients possibly essential for concentrations above 5.0 g/l.

Considering the results for the racking effluent (figure 3), the maximum methane production occurred for total effluent at the highest COD concentration. However for the filtered effluent the maximum was reached at a lower COD, suggesting a possible inhibitory factor dissolved in the liquid, which is diluted when solids are present for the same COD levels. The linear increase of the maximum methane production with the initial COD present in the test vial, revealed that this effluent was biodegraded in the same extent for all the concentrations, suggesting a easier biodegradation.

CONCLUSIONS

The separate treatment of two types of winery effluents with a biomass let un-fed for 1 year and no micronutrient's addition presented interesting and unexpected results. If the application of loads of 0.43-1.30 gCOD/l*day presented no major problems in the filter performance, the characterisation tests of the consortia revealed the complete absence of acetoclastic activity. This activity was shown to suffer an inhibitory effect that lasted for 300 hours in batch acetoclastic activity tests. Several elements are known to cause methanogenic bacteria's inhibition: detergents, polyphenols, sulphur compounds, tannins (Boulenger *et al.*, 1994; Driessen *et al.*, 1994). The lack of micronutrients causes also lower methanogenic performance. The possible inhibitors referred were probably present in the effluents used (the tannins are constituents of grape-stalk) but in a very diminutive concentration due to the use of diluted effluents (simulation of a end-of-process effluent). The cause(s) for the inhibition phenomena observed are as yet not understood. The presence of solids in the racking effluent treatment (batch tests) were not detrimental to the methanogenic activity and were in fact beneficial in the static sedimentation effluent tests. Overall, racking effluent was found to be easier degradable. The results of this experiment poses several questions to be answered and directions for new studies.

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