Wat. Sci. Tech. Vol. 25, No. 7, pp. 275–284, 1992.
Printed in Great Britain.

0273–1223/92 $15·00

TREATMENT OF LEES VINASSES OF RED WINE BY METHANOGENIC FERMENTATION IN PRESENCE OF TANNINS AND SULPHIDES

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ABSTRACT

A two-stage anaerobic fermentation of red wine distillery wastes with high tannin concentrations (2-3 g/l) and high sulphate concentrations (3-8 g/l) was investigated. Tannins did not inhibit acid production and sulphate reduction in the acidogenic phase. Even with a concentration of 800 mg/l, 30% of the tannins were degraded in the acidogenic reactor.
A concentration of 500 mg/l tannins in the methane reactor progressively limited the methanogenic biological activity by 20%. A strong inhibition effect was detected towards sulphate-reducing bacteria in the methanogenic reactor.

KEYWORDS

Anaerobic fermentation; red wine vinasses; tannins; sulphate; inhibition.

INTRODUCTION

Over the last 15 years, it has been generally admitted that methanogenic fermentation is better suited to the treatment of high strengh effluents than aerobic treatments. In the case of distillery wastes, this technique demonstrated its ability to reduce high concentrations of organic pollutants while producing biogas which could cover a large part of energy requirements (Oleszkiewicz and Olthof, 1982). Nevertheless, methanogenic fermentation cannot prove a satisfactory efficiency if toxic compounds are present in the feed. Many authors presented results on the anaerobic treatment of distillery wastes of white wine, whiskey, cognac (Szendrey, 1983; Racault, 1990) but very little information is available on red wine distillery effluents.

We decided to study the anerobic fermentation of such an effluent and try to understand the main phenomena resulting from such a treatment. Lees vinasses of red wine are in fact characterized by high concentrations of soluble Chemical Oxygen Demand (COD) and suspended solids. A large part of the COD is composed of tartaric acid which can be recovered. After recuperation of tartaric acid by precipitation with calcium sulphate, some concentrations of sulphate in the range of 3-8 g/l are often registered in the vinasses. Phenolic compounds are extracted from grapes (Singleton and Esau, 1969) and are also found in lees, marc and wine vinasses. Phenolic compounds are likely to be toxic and the toxicity results in a slow loss of biological activity. The toxicity can be attributed to two causes :
- penetration of small phenolic compounds into the bacteria through the membrane (Field, 1989)

- "tanning" effect of phenolic compounds by interaction with membrane proteins (Haslam, 1974).

Romero et al. (1988) mentioned a non-biodegradable fraction of wine vinasses but did not mention the influence on methanization. Therefore, distillery wastes of red wine are likely to present two of the most toxic compounds inhibiting methanisation. In spite of extensive knowledge of the influence of sulphides on anaerobic fermentation, the concomitant influence of sulphides and tannins towards biological mechanisms remains unknown. Thus, the aim of the study was to find the most suitable process for the treatment of such a complex effluent and to establish operating conditions for persistent treatment efficiency.

MATERIALS AND METHODS

Reactor. During the preliminary study, the pilot plant consisted of a two-phase system. The acidogenic phase was carried out in a 20 litre completely mixed reactor. The methanogenic phase took place in an 8 litre fluidized-bed reactor. The support was a fine porous granular material with a specific density of 2 and a specific surface area of 5000 m^2/m^3. The reactors were maintained a 37°C by a heated water jacket. The seeded support of the methanogenic reactor came from another fluidized bed having treated the same effluent for 5 months. Acidogenic biomass came from a fermentor having treated synthetic distillery wastes.

In the running test period a 2 litre column filled with decolorizing resin and a 5 litre column filled with small PVC rings (8 mm diameter) for air stripping of sulphides were installed.

Influent Characteristics. Raw water consisted of a mixture of lees vinasses of red wine and vinasses of wash water of marc. As there was a high concentration of suspended solids (20 g/l), calcium hydroxide had to be used to remove them. Average characteristics of the feed after decantation are shown in Table 1.

TABLE 1: Characteristics of the Raw Water

total COD	(mg/l)	=	17 000
soluble COD	(mg/l)	=	15 000
total BOD_5	(mg/l)	=	10 200
soluble BOD_5	(mg/l)	=	9 100
suspended solids (SS)	(mg/l)	=	1 700
calcium	(mg/l)	=	1 800
magnesium	(mg/l)	=	100
potassium	(mg/l)	=	4 100
sodium	(mg/l)	=	20
chloride	(mg/)	=	540
nitrogen (NTK)	(mg/l)	=	300
P (PO_4)	(mg/l)	=	300
Mn	(mg/l)	=	0.3
iron	(mg/l)	=	4
cobalt	(mg/l)	=	< 0.01
nickel	(mg/l)	=	< 0.01
copper	(mg/l)	=	0.5
UV adsorbed compounds	(g/l)	=	(0.5 - 1.5)

Analysis. pH, COD, SS, SO_4, sulphide, tannins were analysed once a day.

Sulphate was precipitated in the presence of $BaCl_2$ and Tween 20 in acidic conditions following the nephelometric method (Analyse de l'Eau - J. RODIER - Editions DUNOD).

The sulphide concentration was estimated spectrophotometrically by spectroquant E. MERCK Reagents (ref. 14779) Frankfurter Str250 - Darmstadt.

The tannins concentration was estimated by ultraviolet absorption of the sample after filtration (0.2 μm) at 275 mm. The reference used was oak tannins. Volatile fatty acids were measured by gas chromatography using a Pye-Unicam chromatograph with a column filled with Chromosorb NPGA 25%, H3PO4 3%, 100-120 Mesh, nitrogen as the gas vector and a flame ionisation detector.

RESULTS

During a preliminary study, the flow sheet of the pilot plant was simple i.e an acidogenic phase plus a methanogenic phase. Up to the 30th day, the applied organic load (Figure 1) on the fluidized bed reached 15 kg COD/m^3.d with a COD removal of 80%. From then on, the reactor became unstable and in spite of a rapid

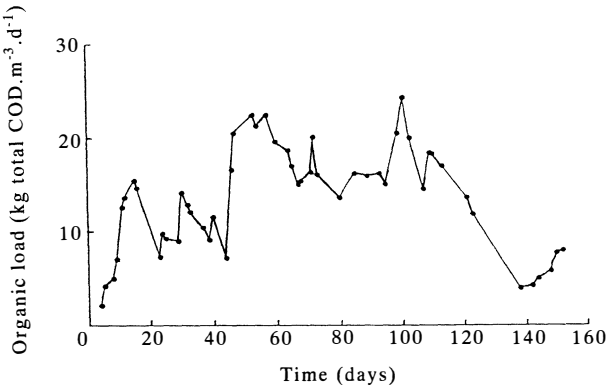

Fig. 1. Evolution of the applied organic load

drop of the inlet COD, the COD removal did not increase. Taking into account the variability of the COD concentration in the feed, we observed a slow decrease of the organic load and the COD removal down to 4 kg COD/m^3.d and 50% respectively. The pH remained steady (pH 7.5 in the fluidized bed). The free H_2S concentration was around 50 mg/l which was below toxic concentration (Karhadkar et al., 1987). Nevertheless, the tannins concentration in the feed was quite high, between 2 and 3 g/l which is the range of toxicity of numerous monomeric and oligomeric phenolic compounds (Field, 1989).

The conclusion of these experiments was that a large part of the toxicity was due to the presence of tannins in the vinasses. Therefore, the aim of the study was to verify this hypothesis. Subsequent experiments were performed to optimize the three main biological phases i.e acidogenic, sulphate-reduction and methanogenic phases during the control of the tannins concentration. During this running test period, the same flow-sheet was used but a device was installed between the acidogenic reactor and the fluidized bed. This device was necessary to remove the sulphides from the effluent of the acidogenic phase. 99% of the sulphide was removed. To study the influence of tannins, the influent was treated by a decolorizing resin before entering the acidogenic reactor.

1) Evolution of Tannins

The decolorizing resin was used up to the 70th day to remove phenolic pollutants. Figure 2 illustrates the evolution of tannins during the experiments for each treatment stage. During the 70 day period, the concentration of tannins in the pre-treated feed was below 90 mg/l. 85 % of the tannins were retained by the resin.

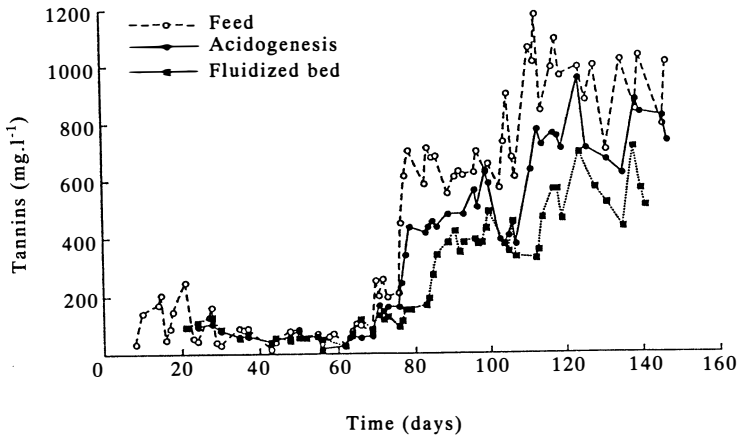

Fig. 2. Evolution of tannins in each reactor

No degradation of tannins was registered in the acidogenic and methanogenic reactors. Non-retained compounds generally have low molecular weight and it seemed that these compounds were not biodegradable.

After the 70th day, the resin was eliminated and tannins concentration in the feed increased up to 1 g/l. A degradation of the tannins concentration is observed in each reactor. About 45% of these compounds disappeared during the entire treatment. During this period, tannins in the feed of the fluidized bed gradually increased from 90 to 800 mg/l.

From these results, it is possible to deduce information on the nature of tannins in the vinasses. 55% are not biodegradable in such a two-phase process. 27% of the non-biodegradable part have a low molecular weight. It is generally admitted that there is an inverse correlation between polymer size and its anaerobic biodegradability (Field, 1989). So, we can assume that more than 70% of the non-biodegradable part of tannins has high molecular weight.

2) Acidogenic Phase

As shown in Figure 3, the pH of the acidogenic reactor remained constant around 6.8 from the 30th day on. The pH regulated naturally at this level due to the presence of a strong buffer capacity after decantation of suspended solids by $Ca(OH)_2$.

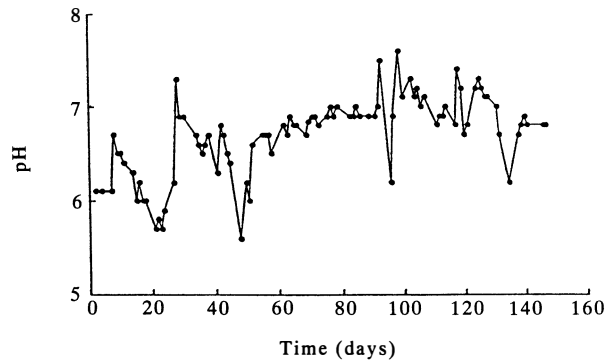

Fig. 3. Course of pH in the acidogenic reactor

The production of biogas was not measured but the composition was mainly CO_2 and H_2S. The elimination of COD during the experiment (Figure 4) was stable around 26%. Table 2 illustrates the influence of pH and residence time on organic acids production.

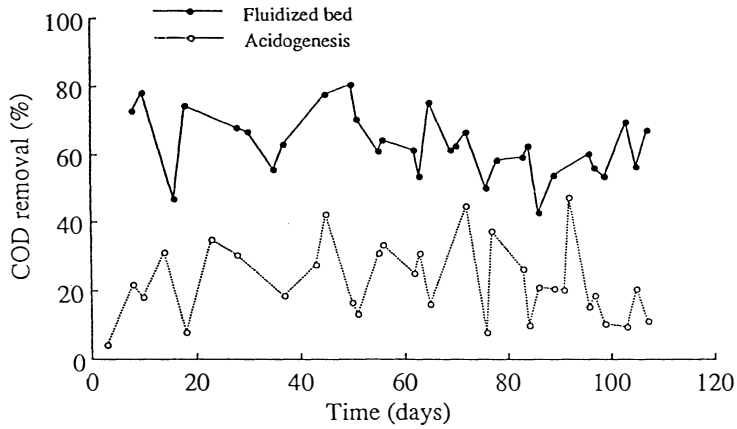

Fig. 4. COD removal in the two reactors

TABLE 2. Influence of pH, and HRT in the Acidogenic Phase

pH	Acetic	Propionic	Isobutyric	Butyric	Valeric	COD_S (g/l)	$\frac{VFA\ COD_S}{COD_S}$	HRT (h)
7.7	6 (36%)	1.66 (14%)	0.301	1.7 (17.5%)	/	17.7	$\frac{12.57}{17.7} = 71\%$	48
6.6	5.8 (29%)	2.89 (21%)	0.443	0.570 (5%)	/	21	$\frac{12.41}{21} = 59\%$	48
7.2	2.26 (30%)	0.49 (9%)	0.15	0.26 (6%)	0.27	8	$\frac{4.4}{8} = 55\%$	24
6.25	2.41 (28%)	1.31 (21%)	0.063	0.195 (5%)	0.153	9.15	$\frac{5.33}{2.15} = 58\%$	24

* Volatile fatty acid units are given in g/l. The related soluble COD (COD_S) is calculated and compared to the inlet soluble COD in percentage.

Organic acids were composed of three main acids, acetic, propionic and butyric acids. pH influenced the proportion and final production of volatile fatty acids (VFA). Whatever the hydraulic retention time (HRT) was, when the pH dropped, butyric acid tended to decrease while propionic acid increased.

At the same time, acetic acid concentration decreased. Between pH 6.2 and 7.2, the proportion of acids compared to COD was approximately the same (55 to 59%). In this range of pH, HRT did not seem to have any influence on the total production of acids. At extreme pH (7.7), and for HRT of 48 hours 71% of the COD was composed of VFA.

These results show that the production of acids related to COD does not vary significantly at a minimum hydraulic retention time of 24 hours and in a classical range of pH of an acidogenic reactor. Nevertheless, the observation of the changes in

metabolic pathways (Reis et al., 1988) is very useful. Propionic acid degradation which is the limiting step of the vinasse methanization (Segretain and Moletta, 1987) is produced at low pH.

So, for the following stages of the treatment, it would be better to enhance acetic and butyric acid concentrations by maintaining the pH very close to 7.

3) Sulphate-Reduction Phase

Sulphate reduction occurred both in the acidogenic and methanogenic phases. The hydraulic retention times were respectively 48 and 24 hours. Figure 5 illustrates the concentration of sulphates in the feed during the experiments. The average value of sulphate ranged from 1 to 4 g/l but some concentrations over 7 g/l were registered.

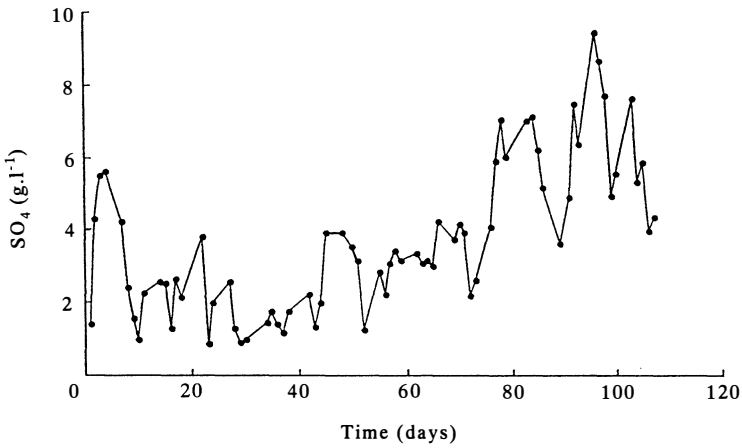

Fig. 5. Concentration of SO_4 in the feed

50% of sulphates were reduced (Figure 6) in parallel with the acidogenic phase. The concentration of H_2S in the gas phase of the acidogenesis reached 9% (V/V). So, the concentration of total sulphides in the liquid phase ranged from 30 to 360 mg/l with average values of 250 mg/l.

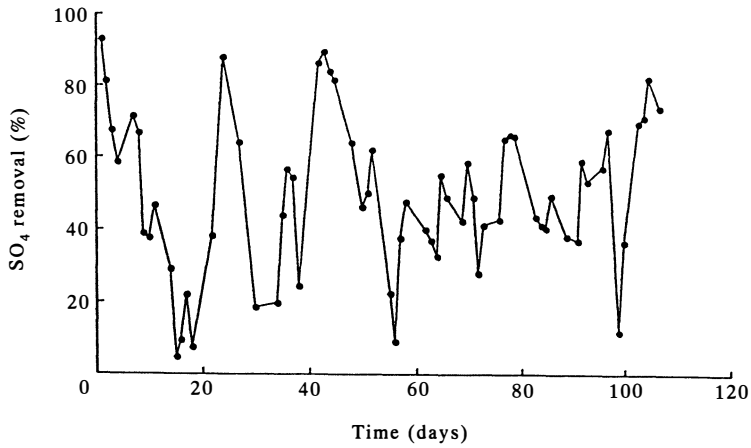

Fig. 6. Course of SO_4 removal in the acidogenic phase

Figure 7 illustrates the influence of pH on the sulphate-reduction rate in the acidogenic reactor. pH over 6.5 is necessary to obtain a minimum sulphate-reduction rate of 50%. In comparison with the acidogenic phase, it seems necessary to stabilize the pH at level realizing a good compromise between the production of organic acids and the degradation of sulphates.

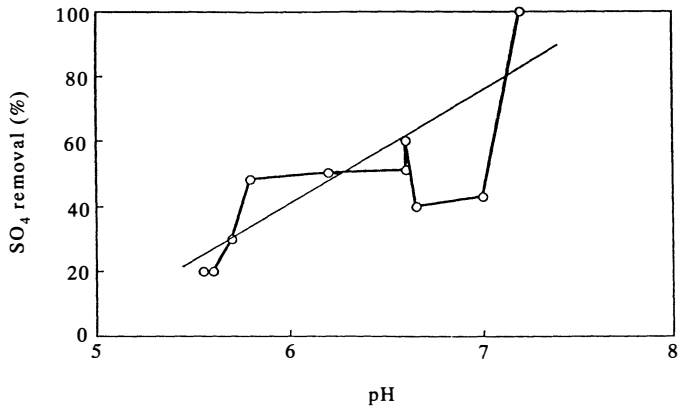

Fig. 7. Influence of pH on the sulphate-reduction rate in the acidogenic phase

To prevent the inhibition of the methanogenic phase by sulphides coming from the acidogenic phase, a stripping device by air was used between the two reactors. Over 95% of sulphides were removed from the liquid phase of the acidogenic reactor.

The sulphides in the fluidized bed were produced by the sulphate reduction occurring in the reactor. Figure 8 shows the evolution of the sulphate-reduction rate in the methanogenic phase. Up to the 70th day, 90% of the sulphates were reduced. Due to the high pH (7.5 - 8) average values of 20 mg/l of free H_2S were registered. After this period, sulphate reduction slowly decreased to below 10%. Apparently, this evolution was the result of a toxicity by tannins. This hypothesis will be discussed below.

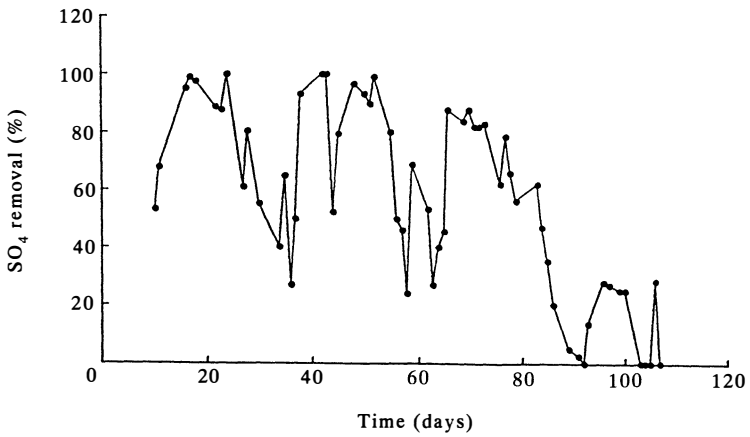

Fig. 8. Course of SO_4 removal in the methanogenic phase

All the sulphates of the feed were degraded by the association of acidogenic and methanogenic reactors. The difference of substrates between the two reactors did not prevent the transformation of sulphates into sulphides. Hilton and Oleszkiewicz (1988) explained this phenomenon by the presence of two groups of sulphate-reducing bacteria, complete and incomplete oxidizers. In both reactors no inhibition phenomenon of sulphate reduction was noted.

4) Influence of Tannins in the Acidogenic Reactor

No relation was found between acid production, reduction of sulphate in the acidogenic reactor and the tannins concentration in the feed.

The average COD removal of 26% was a sign of a satisfactory activity of the biomass. Before the 70th day, the concentration of tannins in the acidogenic reactor was below 90 mg/l. After the 70th day, tannins concentration gradually increased and we noticed a 30% degradation of tannins in this phase. 800 mg/l of tannins was the highest concentration detected in the reactor. Field and Lettinga (1987) have already mentioned degradation of some specific monomeric and polymeric phenolic compounds in batch conditions, but with methanogenic sludge. Our results demonstrate that a combination of tannins and sulphate did not influence the acidogenic activity of the biomass.

5) Influence of Tannins in the Methanogenic Reactor

a) Methanogenesis: Up to the 70th day, the applied organic load was 15 ± 2 kg $COD/m^3.d$ and the COD removal rate was 65% in the fluidized bed. The concentration of tannins was below 90 mg/l. From the 70th day on, the concentration of tannins in the feed of the fluidized bed gradually increased and we noticed a 20% degradation of tannins. Several tannins concentrations of 500 - 600 mg/l were detected in the methanogenic phase. COD removal began to decrease from 65% to 50% (Figure 9).

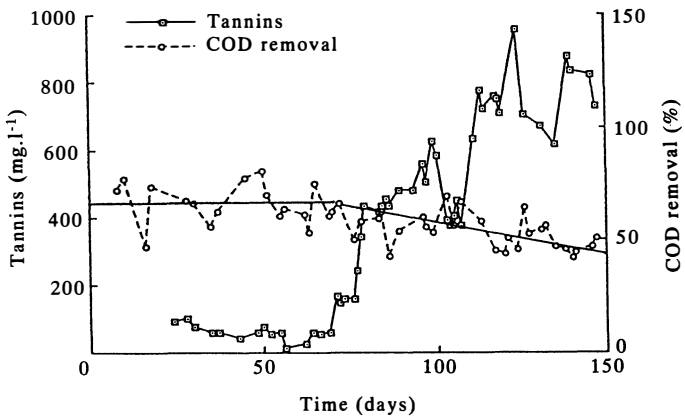

Fig. 9. Influence of tannins on methanogenesis

This inhibition phenomenon slowly took place in the reactor without complete loss of the bacterial activity. Field and Lettinga (1987) observed a 50% inhibition of the methanogenic activity with 700 mg/l of gallotannic acid with synthetic medium. This inhibition was not complete, the toxicity slowly appeared and remained a long time after the elimination of toxic compounds.

In our experiments, it was difficult to ascertain if the disappearance of tannins was due to real degradation or to adsorption on the biofilm.

b) Sulphate Reduction: Figure 10 illustrates the influence of tannins on the sulphate-reduction rate in the methanogenic reactor. During the first 70 days sulphate-reduction rate was in the range of 80-100%. Two days (30 and 60) were characterized by inferior data, which correspond to pH regulation and fluidization problems respectively.

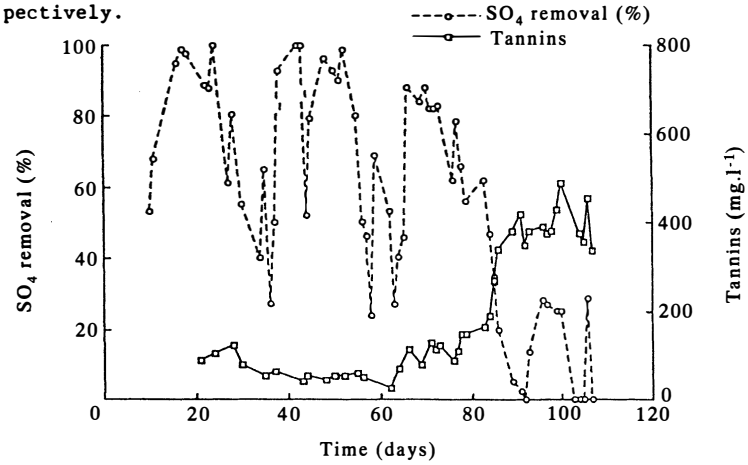

Fig. 10. Influence of tannins on sulphate-reduction rate

Several days after stopping the decolorizing resin pre-treatment, the sulphate-reduction rate progressively dropped from 90 to 0-20% without any reason other than presence of tannins in the feed. Figure 11 presents the correlation between tannins in the feed of the methanogenic reactor and the sulphate reduction (r = 0.81).

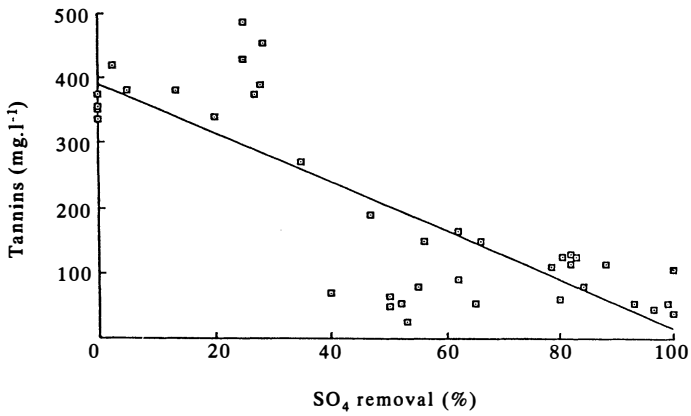

Fig. 11. Relation between tannins and SO_4 removal

These results are of great interest, as, to our knowledge, no publications have mentioned the inhibition of sulphate-reducing bacteria by tannins with acidified wine vinasses as a substrate. Compared to the influence of tannins on methanogenic bacteria, the toxic effect of tannins on sulphate reduction seemed to be very rapid (about 2 days). After 20 days, with increasing tannins concentration the sulphate-reduction rate approached zero.

CONCLUSION

With less than 90 mg/l of phenolic compounds, a two-stage anaerobic fermentation of a mixture of lees and washing water of marc vinasses permitted the removal of 70% of the COD and 100% of the sulphates.

In the acidogenic phase, the control of pH near neutrality was necessary to ensure high production of acids mainly composed of acetic and butyric acids, and sulphate reduction.

Sulphate reduction occurred in both acidogenic and methanogenic phases.

The presence of tannins around 800 mg/l did not disturb the acidogenic phase. 30% of tannins were degraded in this reactor.

In spite of a 20% tannins degradation in the methanogenic phase, tannins had a slight toxic effect on methanogenic bacteria. At 500 mg/l, the activity of methanogenic bacteria was reduced by 20%.

On the other hand, tannins showed a strong inhibitory effect towards sulphate-reducing bacteria which developed in parallel in the methane reactor. At 500 mg/l of tannins, no sulphate reduction occurred.

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