

## Microbiological treatment of oil mill waste waters

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### RESUMEN

#### Tratamiento de los alpechines de almazaras con fermentos seleccionados.

Han sido efectuadas pruebas de tratamiento biológico de alpechines, provenientes de sistemas continuos, con fermentos seleccionados adaptados a condiciones de toxicidad muy elevadas.

Han sido utilizadas las formulaciones microbianas SNKD, LLMO y PSBIO; las dos últimas son suspensiones líquidas, constituidas por microorganismos vivos, los cuales a diferencia de los liofilizados o congelados, no deben ser revitalizados antes del uso; estos tienen una fase «lag» más breve y entran antes en completa actividad.

Las pruebas con la preparación biológica SNKD han sido efectuadas en los alpechines filtrados (tipo A) con DQO inicial alrededor de 43 g/l, y también con alpechín filtrado «defenolado» con ácido de Caro ( $H_2SO_3$ ) (tipo B), con DQO igual a 30 g/l; los complejos LLMO y PSBIO se utilizan en alpechines provenientes de la elaboración de otras variedades de aceitunas, filtradas y diluidas en la relación 1:0,5 (tipo C) con DQO inicial igual a 44 g/l, y también en alpechín filtrado y sometido previamente a criotratamiento (tipo D), con DQO inicial de 22 g/l aproximadamente.

La DQO residual, con la formulación microbiana SNKD, ha resultado igual a 15 g/l (Tipo A) y a 5 g/l (tipo B), con el PSBIO a 7 g/l (tipo C) y a 1,5 g/l (tipo D); con la formulación microbiana LLMO a 6 g/l (tipo C) y a 1,3 g/l (tipo D).

**PALABRAS- CLAVE:** Alpechín - Fermento seleccionado - Tratamiento biológico.

### SUMMARY

#### Microbiological treatment of oil mill waste waters.

Experiments of the biological treatment of the oil mill waste waters, deriving from continuous system, have been carried out with selected mutant ferments, adapted to rather forced toxic conditions.

The commercial microbial formulations SNKD, LLMO and PSBIO have been utilized; the last two are liquid suspensions, constituted by living micro-organisms that, in contrast to those frozen or lyophilized, do not need be revitalized before their use and became completely active in short time.

The experiments with the SNKD biological preparation were carried out both on filtered oil mill outflows (type A) with an initial COD of approximately 43 g/l and on waste water dephenolized by Caro-acid (type B) with a COD equal to 30 g/l. The experiments with LLMO and PSBIO complexes were conducted both on oil mill outflows filtered and diluted (ratio 1:0.5) with an initial COD equal to 44 g/l (type C), and on waste water that were filtered and preventatively subjected to a cryogenic treatment (type D), with an initial COD of approximately 22 g/l.

The residual COD with the microbic formulation SNKD, was about 15 g/l (type A) and 5 g/l (type B); with the PSBIO it was about 7 g/l (type C) and 1.5 g/l (type D); with the microbic formulation LLMO it resulted in 6 g/l (type C) and 1.3 g/l (type D).

**KEY-WORDS:** Biological treatment - Olive mill wastewater - Selected ferment.

### 1. INTRODUCTION

The COD/BOD5 ratio of oil mill waste waters is approximately 2.5; this means that the natural microflora is able to remove only a part of the organic material contained in it (Ranalli, 1989).

As it is known the above-mentioned waste waters are characterized by an elevated load of organic substances and by the presence of molecules with a complex structure and that can be degraded with difficulty (Ranalli, 1989).

Some of these substances, as catacolmelaninic pigment (Ranalli, 1987) of phenolic nature, and mostly the free phenols, are moreover strong inhibitors of the biodegrading microflora.

Owing to the quantity, variety, and chemical nature of the organic substances present in the oil mill outflows, their removal by simple or single treatments is not possible and so a multisteps treatment is necessary.

The guidelines for the purification of oil-mill waste water involve, quite often, a biological treatment.

For some years new selected ferments were used for the purification of industrial and civil waste, to improve the efficiency of the biological treatment (Ranalli, 1989).

On this assumptions, experiments of biological treatment by selected microbic formulations, designated SNKD, PSBIO and LLMO and constituted by microbial species adapted to the difficult environmental conditions; were carried out on oil-mill waste waters, opportunely pre-treated to decrease the organic load and the amount of the inhibitory substances.

## 2. MATERIALS AND METHODS

The SNKD complex is constituted by 83 microbic species, related to the genres: *Saccharomyces*, *Bacillus*, *Candida*, *Endomycopsis*, *Cladosporium*, *Nocardia*, *Streptomyces*, *Clostridium*, *Azotomonas*, *Pseudomonas*, *Bacterium*, and *Basidiomycetes*.

The biological components of the PSBIO includes selected living organisms, in liquid suspension, related to the following families: *Rhodospirillaceae*, *Rhodopseudomonas*, *Chromatiaceae*, *Thiosarcina*, *Thiospirillum*, *Lamprocistys*, *Ectothiorhodospira*, *Amoebobacter*, *Chloropseudomonas*, *Clathrochloris*, *Chlorobaceae*.

The biological preparation LLMO (Liquid Live Micro Organisms) contains 7 microbic species, 5 of which are aerobic (*Aerobacter aerogenes*, *Bacillus subtilis*, *Nitrobacter winogradskyi*, *Pseudomonas stutzeri*, *Pseudomonas denitrificans*) and 2 anaerobic (*Cellulomonas biazotea* and *Rhodopseudomonas palustris*).

The experiments were conducted in aerobic environment (Ranalli, 1988).

The waste water utilized came from continuous extraction systems and was pre-treated in various ways, obtaining 4 types of effluents that were different as regards the initial organic material amount and inhibitory substances concentration, and indicated by the letters A, B, C, D.

The experiments with the SNKD biological preparation were carried out both on filtered waste water (type A) with an initial COD of approximately 43 g/l and on waste water de-phenolized by Caro-acid ( $H_2SO_5$ ) with a COD equal to 30 g/l (type B). The experiments with LLMO and PSBIO complexes were conducted both on waste water deriving from the culture of other varieties of olives (type C), filtered and diluted (ratio 1:0,5) with an initial COD equal to 44 g/l, and on waste water that were filtered and preventatively subjected to a cryogenic treatment (type D), with an initial COD of approximately 22 g/l.

For each of 4 types of effluent a control (not inoculated) was provided.

Each test was carried out, in triplicate, using 750 ml of waste water in 1 liter Erlenmeyer flasks in which air was bubbled during the experiment (50 l/h).

Before the inoculation the concentration of C,N and P was adjusted at corrected ratio 100:16:1 to provide the best growth of the microorganisms.

The pH of the waste waters was brought to the approximate level of 7 and corrections undertaken every day to maintain such value.

The temperature of the waste water was regulated at 30°C.

The lyophilized ferments were revitalized before their use (2).

On the waters, at the beginning, during, and at the end of the digestive process, the following parameters were determined: COD, total solids at 105°C, total solids at 180°C, volatile solids, reducing sugars, phenols and fats.

The analytical observations were carried out on the centrifugated liquid.

## 3. RESULTS AND DISCUSSIONS

The following are the mean percentage data concerning the final rate of removal of the single analytical parameters, attained by the microbic formulations under consideration, on the 4 types of waste water. The trend of the average variations during the digestive process of the single parameters (COD, total solids at 105°C, reducing sugars) is visualized in figures 1, 2, 3, 4, 5 and 6. The residual absolute values of those parameters are summarized in Table I.

COD (initial values: A= 42.8 g/l, B= 29.6 g/l, C= 43.6 g/l, D= 21.6 g/l).

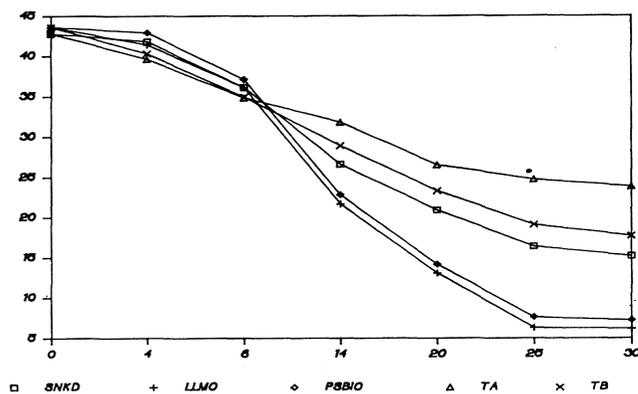


Figure 1

Time course (days) for the mean variations of the COD (g/l)

TA = control type A waste water (inoculated with SNKD biological complex).

TC = control type C waste water (inoculated with LLMO and PSBIO microbial formulations).

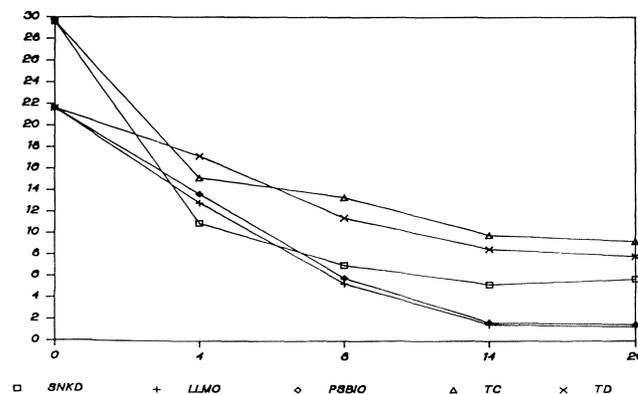


Figure 2

Time course (days) for the mean variations of the COD (g/l).

TB = control type B waste water (inoculated with SNKD biological complex).

TD = control type D waste water (inoculated with LLMO and PSBIO microbial formulations).

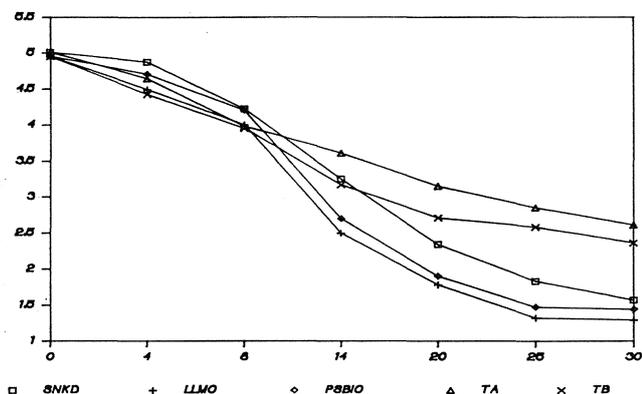


Figure 3

Time course (days) for the mean variations of the total solids (105°C)%  
TA= control type A waste water (inoculated with SNKD biological complex).

TC= control type C waste water (inoculated with LLMO and PSBIO microbial formulations).

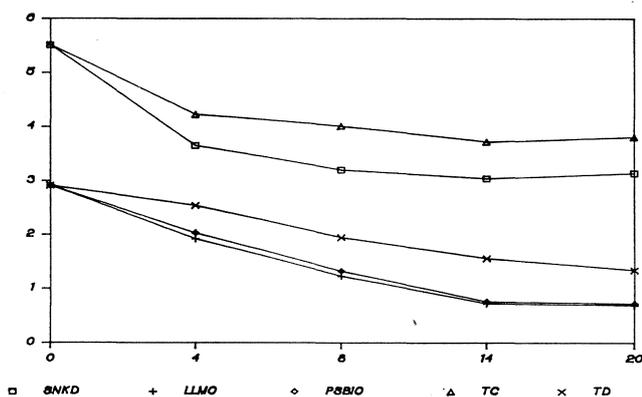


Figure 4

Time course (days) for the mean variations of the total solids (105°C)%.

TB= control type B waste water (inoculated with SNKD biological complex).

TD= control type D waste water (inoculated with LLMO and PSBIO microbial formulations).

Taking into account the type A waste water inoculated with the microbic formulation SNKD, the removal percentage of the COD at date of the last sampling, i.e., after 33 days, is 66.5% and for the control 44.4%; as regards the type B waste water inoculated with the microbic complex SNKD, the removal percentage of COD, after 14 days, beyond which variations are no longer statistically significant (Tukey test,  $P \leq 0.05$ ) was equal to 82.4% against the 66.9% of the control; on type C waste water, inoculated with the microbic formulation LLMO, the removal percentage of the parameter, after 30 days, corresponds to 85.8% and for the control 59.4%; as regards type C waste water treated by the microbic system PSBIO, the removal percentage of the COD, after 30 days, was 83.5% and for the control 59.4%; as regards the type D waste water inoculated with the LLMO formulation, the removal percentage of COD, after 14 days, was 93.1% against the

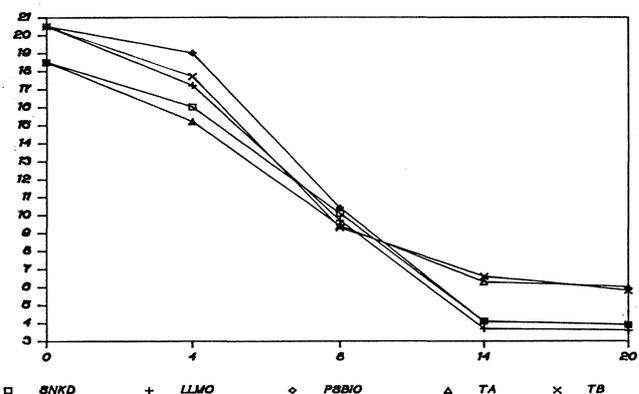


Figure 5

Time course (days) for the mean variations of the reducing sugars (g/l).TA= control type A waste water (inoculated with SNKD biological complex).

TC= control type C waste water (inoculated with LLMO and PSBIO microbial formulations).

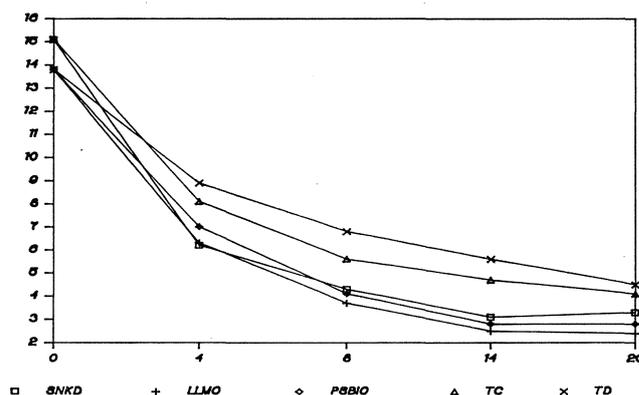


Figure 6

Time course (days) for the mean variations of the reducing sugars (g/l).TB= control type B waste water (inoculated with SNKD biological complex).

TC= control type C waste water (inoculated with LLMO and PSBIO microbial formulations).

60.6% of the control; as regards the type D waste water, treated by the biological preparation PSBIO, the removal percentage of the COD, after 14 days, was 92.1% against the 63.9% of the control.

\* TOTAL SOLIDS AT 105°C (initial values: A= 5.01%, B= 5.51%, C= 4.95%, D= 2.91%).

By SNKD microbic complex the removal percentage of this parameter in type A waste water, after 30 days, was 68.7% (control 47.9%) and in type B, after 20 days, 43.0% (control 30.9%); by LLMO bacteria, in type C waste water, it was 73.9% (control 53.2%) and in type D, after 14 days, 75.3% (control 46.4%); by PSBIO microbic formulation, in type C waste water, it was 70.9% (control 52.8%) and in type D 73.8% (control 46.4%).

VOLATILE SOLIDS (initial values: C= 4.17%, D= 2.09%)

Table I  
Mean residual values of the analytical parameters in the oil-mill waste waters.

Parameters	Effluents								
	A		B		C		D		
	Sample	Control	Sample	Control	Sample	Control	Sample	Control	
COD(g/l)	SNKD	15.2	23.8	5.7	9.2	-	-	-	-
	LLMO	-	-	-	-	6.2	17.7	1.3	7.8
	PSBIO	-	-	-	-	7.2	17.7	1.5	7.8
TS(%)	SNKD	1.57	2.61	3.14	3.81	-	-	-	-
	LLMO	-	-	-	-	1.29	2.36	0.69	1.34
	PSBIO	-	-	-	-	1.44	2.36	0.72	1.34
VS(%)	SNKD	-	-	-	-	-	-	-	-
	LLMO	-	-	-	-	1.00	2.00	0.56	1.15
	PSBIO	-	-	-	-	1.11	2.00	0.61	1.15
RS(g/l)	SNKD	3.9	6.0	3.3	4.1	-	-	-	-
	LLMO	-	-	-	-	3.6	5.8	2.4	4.5
	PSBIO	-	-	-	-	3.9	5.8	2.8	4.5
PH(g/l)	SNKD	2.7	3.0	0.12	0.15	-	-	-	-
	LLMO	-	-	-	-	1.7	2.0	0.53	0.67
	PSBIO	-	-	-	-	1.8	2.0	0.55	0.67

By the LLMO bacteria the removal percentage of this parameter, in type C waste water, after 30 days, was 76.0% (control 52.0%) and in type D, after 14 days, it was 70.3% (control 32.5%); by PSBIO microbic formulation, in type C waste water, it was 73.4% (control 52.0%) and in type D 68.4% (control 32.5%).

REDUCING SUGARS (initial values: A= 18.5 g/l, B= 15.1 g/l, C= 20.5 g/l, D= 13.8 g/l).

By SNKD biological complex the removal percentage of this parameter, in type A waste water, after 14 days, was 77.8% (control 65.9%) and in type B 79.5% (control 68.9%); by LLMO microbic formulation, in type D waste water, it was 82.0% (control 67.8%) and in type D 81.9% (control 59.4%); by PSBIO microbic formulation, in type C waste water, it was 80.0% (control 67.8%) and in type D 79.7% (control 59.4%).

PHENOLS (initial values: A= 3.6 g/l, B= 178 mg/l, C= 2.5 g/l, D= 862 mg/l)

By SNKD biological complex the removal percentage of this parameter, in type A waste water, after 20 days, was 25.0% (control 16.7%) and in type B 32.6% (control 16.9%); by LLMO bacteria, in type C waste water, it was 32.0% (control 20.0%) and in type D 35.6% (control 20.1%); by PSBIO microbic formulation, in type C waste water, it was 28.0% (control 20.0%) and in type D 35.3% (control 20.1%).

For the sake of brevity, the data relative to the level of removal of fat (oil) are omitted; the fat, was in any case, largely separated by preventatively operation of filtration.

The oil mill waste waters could be treated in higher

quantity in conjunction with urban liquid wastes in activated sludge plants after removal of a significant % of organic loads by means of previous treatment with one of the above-reported microbial formulations (Mascolo and Cucurachi, 1981). As alternative, the pretreated liquid wastes may be applied on the agricultural soils, but it is necessary its dilution to reduce further its contained inorganic and inorganic substances.

#### ACKNOWLEDGEMENTS

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