A study of chemical and microbiological characteristics of olive mill waste water in Morocco

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RESUMEN

Estudio de las características químicas y microbiológicas del alpechín en Marruecos.

Se ha realizado un estudio bacteriológico y físico químico de alpechín de diferentes almazaras tradicionales. Se han investigado los siguientes parámetros físico químicos: pH, salinidad, densidad, conductividad, alcalinidad, acidez, materia seca total, materia seca volátil, materia en suspensión, carbono orgánico, nitrógeno amoniacal, nitrógeno orgánico, grasa, polifenoles, fosfatos, sodio, potasio, BOD y COD.

Las determinaciones microbiológicas efectuadas son: recuento de aerobios mesofilos, coliformes totales y fecales, enterococos, *Staphylococcus, Salmonella,* esporulados (*Clostridium* y *Bacillus*), levaduras, mohos y microorganismos lipolíticos.

Los valores de los parámetros físico químicos son superiores comparados con los obtenidos por otros autores en otros países. La carga microbiana es débil y está representada principalmente por levaduras, mohos y microorganismos lipolíticos.

Se observan claras diferencias entre las muestras de almazaras tradicionales y las procedentes de industrias en todos los análisis.

PALABRAS-CLAVE: Alpechín - Estudio físico-químico - Estudio microbiológico - Marruecos.

SUMMARY

A study of chemical and microbiological characteristics of olive mill waste water in Morocco.

Olive mill waste water samples from different traditional mills were analyzed for their microbiota and the physicochemical characteristic. A survey of the most frequent microorganisms including standard plate count (SPC), counts of the indicator microorganisms (total and fecal coliforms and enterococci) staphylococci, *Salmonella,* sporeforming bacteria (*Clostridium* and *Bacillus*), yeasts and molds and the lipolytic microorganisms. The physicochemical analysis grouped the determination of pH, salinity, density, conductivity, alkalinity, acidity, total dry matter, non volatile dry matter, suspended solids, organic carbon, ammoniacal nitrogen, organic nitrogen, fat, polyphenols, polyphosphates, sodium and potassium and the BOD and COD. Results showed that the physicochemical factors were higher than the reported values in other countries. The microbial load was low and mainly made of yeasts and molds as well as the lactic acid bacteria. A net difference between the traditional mill samples and the industrial samples was observed for both analyses.

KEY-WORDS: Marocco - Microbiological study - Olive mill wastewater - Physico-chemical study.

1. INTRODUCTION

In Morocco, the traditional manufacture process of olive oil is still used. This yields small amounts of olive mill black water (OMW) relatively to the industrial high production. The mills or «maasras» cannot treat high amounts of olives but they are numerous and dispersed in the olive production area which are far from the sea. The OMW is not rejected in the sea nor in any water stream as it is the case in other countries in the mediterranean area. Every maasra may have a pit for collecting its own black water. It is not used for any purpose, so it may evaporate leaving a solid residue.

Some studies were recently carried out on the possibility of using the OMW for the production of methane (Curi et al, 1982; Lombardo et al, 1988; Martín et al, 1991; Rigomi-Stern et al, 1988), butanol and butanediol (Wachner et al, 1988) and for alcohol (Bombalov et al, 1989). The OMW were also used as feed (Denosa, 1979) or as an organic fertilizer for the soil (Levi-Menzi et al, 1992; Ranalli, 1991) were studied. Some chemical, physical and biological treatment processes were summarized by Fiestas Ros de Ursinos and Borja Padilla (1992). It can be pointed out from the studies mentioned that works are still needed to elucidate more and/or to enhance the OMW by biological means prior to their use in some treatment processes.

So far, no study had been carried out on the characterization of the OMW in Morocco to investigate the enhancement of the chemical and/or the microbiological properties.

In the present study the physicochemical properties and the microbiota of Moroccan OMW were studied to characterize the environmental factors of the effluent and their effects on the microbiota for further studies on its treatment or on its reuse for other biotechnological purposes.

2. MATERIAL AND METHODS

2.1. Samples collection

OMW samples (5 liters each) were collected from olive mills in two regions: a southern region around Marrakech and an eastern region around Taza. Some samples were also taken from an olive processing plant. The samples were stored at 4°C until analyses.

2.2. Physicochemical methods

The physicochemical characteristics were carried out according to the guidelines of the standard methods for the examination of water and waste water (Apha, 1987). They included pH, alkalinity, acidity, chlorides, density, total dry matter, non volatile dry matter, volatile dry matter, suspended solids, COD, BOD_5, organic carbon, Nitrogen (Kjeldahl), Ammoniacal nitrogen, organic nitrogen, fat, polyphenols, polyphosphates, sodium and potassium.

2.3. Microbiological methods

Ten ml of the sample were added to 90 ml of distilled water. The homogenous mixture was used as mother dilution for making serial dilutions for further microbiological determinations.

Standard Plate Count (SPC), coliforms counts, staphylococci, enterococci, *salmonella* spore-formers and, yeasts and molds were determined according to the method described by Asehrau et al, (1992). The yeasts isolates were identified according to the method described by Deak an Beuchat (1987).

Sixty five yeast isolates were picked at random from the Potato-Dextrose-Agar (PDA) plates used for the viable counts. The isolates were subcultured before being examined for: assimilation of glucose, sucrose, xylose, trehalose, galactose, maltose and ethanol (bacto yeast nitrogen base Difco) assimilation of nitrate and urea (Bacto yeast carbon base Difco), production of gas from glucose and sucrose growth at 37°C in malt extract, and formation of pseudomycelia and pigment on PDA, characteristics of vegetative cells (reproduction and morphology). All organisms were cultured at 28°C unless otherwise indicated.

2.4. Lipolytics

Dilutions from 10^{-2} to 10^{-5} were pour plated on Victoria blue B agar. Casein soy peptone agar supplied with a Victoria blue B butterfat mixture according to the method described by Alford (1976). Plates were incubated at 30°C for 48 to 72 hours and blue colonies were counted.

2.5. Lactic acid bacteria

Lactobacilli

One ml of each dilution was plated on MRS agar (Merck, Germany) for 48-72 hours at 30°C. The grown colonies were checked for Gram and catalase reactions before counting.

Leuconostoc

Leuconostoc species were counted on APT agar (Merck Germany). The inoculated plates were incubated at 28°C for 48 to 72 hours. The grown colonies were checked for Gram stain and catalase reactions.

3. RESULTS AND DISCUSSION

3.1. Physicochemical determinations

Table I showes the physicochemical characteristics of the OMW samples. It should be pointed out that the effluent had a high capacity values to induce a heavy pollution of the nature especially the samples from the traditional press mills or maasra. The COD was 224.1 g/L and the BOD_5 was 98 with a high content in chlorides and polyphenols. The factory OMW had lower values except for the chlorides than the traditional press mill OMW. The high content of organic carbon and nitrogen is well correlated to the BOD_5. This may tell about the heavy pollutant capacity of the waste. All the values are higher than those reported by Fiestas Ros de Ursinos and Borja-Padilla (1992) for the OMW in Spain.

Table I
Physicochemical characteristics of olive mill waste water from different maasras

Analyses	Units	Factory	Maasra
pH	–	4.50	4.73
Alkalinity at pH 3,7	g($CaCO_3$)/l	3.15	9.07
Acidity (volatile)	g(Ac.Act)/l	2.49	0.43
Conductivity	ms/cm	50.0	18.6
Salinity	g/l	32.0	11.9
Chlorides	g(cl)/l	21.48	2.56
Density	g/l	1.037.0	1.059.6
Total dry matter	%	7.3	16.9
Non volatile dry matter	%	3.8	2.5
Volatile dry matter	%	3.4	14.4
Suspended solid	g/l	3.12	4.99
COD	g/l	57.66	224.1
BOD_5	g/l	–	98.18
BOD_5/COD	–	–	0.44
Organic carbon	g/l	21.83	103.12
Nitrogen (Kjeldal)	g/l	0.46	0.74
Ammoniacal Nitrogen	g/l	0.06	0.06
Organic nitrogen	g/l	0.40	0.77
Lipids	g/l	9.0	6.5
Polyphenols (*)	g/l	–	64.78
Phosphates	g/l	–	1.82
Sodium (Na^+)	g/l	–	0.49
Potassium (K^+)	g/l	–	18.91

* as tanic acid.

The olive oil processing in Morocco is still made up of a large number of low capacity traditional press mills dispersed through out the country but concentrated in the olive producing regions. These units reject the OMW in pits they have built before starting the processing. The reject in water streams is seldom and the small quantities gathered by every maasra may evaporate leading a solid residue.

A modern olive oil processing industry is being installed in towns near the olive producing regions such as Beni Mellal, Marrakech, Fes and Mekenes where the OMW is to be rejected in water streams or with domestic waste waters without any treatment. The quantities are increasing with the high quantities that can be treated by a continuous extraction system and consequently pollution of the environment may occur.

Works are now focused on the use of the OMW for other purposes to enhance them by biotechnological methods or to treat them before a rejection in the nature. Experiments are now being carried out on the use of OMW as a carbon source for lactic fermentation of olives or to mix them with other solid wastes or by-products for animal feeds processing.

3.2. Microbiological characteristics

The microbiota of the olive mill waste water is reported in table II. The standard plate count values are unexpectedly high for some samples. This is related to the environmental conditions of this liquid effluent. Indeed the water used for the oil extraction came from naturel streams and wells. Moreover the effluent is usually collected in pits build near the mill press and may stay until a complete evaporation.

Table II
Microbial profiles of the OMBW samples

	T 10^5	M 10^4	D1 10^4	D2 10^3	F 10^6
SCP	44	60	1.2	1	24
Yeasts	1.6	52	38	57	15
Molds	80	40	20	80	1.4
Lipolytics	200	140	18	–	16
Lactics	1.5	20	3.2	1.2	2.9
Coliforms	<1	<1	<1	<1	<1
SP	<1	<1	<1	<1	<1

SPC : Standard Plate Count.
SP : Sporeforming Bacteria.
T : Region of Taza. M: Marrakech. D: Demnat.
F : Factory of olive processing in Mekenes.

But even if the microbial load is high the potentially hazardous microorganisms are not present at all. In fact, the indicator microorganisms were not found in any sample as well as *Clostridium* and enterococci this may suggest that the olive mill waste water cannot be involved in the potential microbial pollution of the environment.

Other microorganisms interesting by their properties were isolated from the olive mill waste water such as molds and yeasts. These microorganisms are active in organic matter decaying and they can play a role in the organic components degradation leading to a natural transformation of organic components to minerals. The lipolytics are represented by species of yeasts and molds as well as some other microorganisms which could not grow on the SPC Agar.

The yeasts, molds and lactic acid bacteria were the main microorganisms found in the olive mill waste water. These groups of microorganisms are known by their activities on some inhibitors in OMW such as polyphenols and can survive in the medium. In fact Borja-Padilla et al (4) used the yeast-like mould *Geotrichum candidum* in aerobic treatment of OMW prior to biomethanization process. Martínez-Nieto et al (1992) showed the activity of *Aspergillus terreus* in the biodegradation of polyphenolic compounds. The yeast counts in the different samples from 6 mills were high as reported in table II. This may suggest that the olive mill waste water would constitute a suitable liquid byproduct that can be used for yeasts and molds growth. This may help in aerobic treatment process of OMW.

The taxonomic criteria through the physiological and the biochemical properties of the yeast strains isolated from olive mill waste water showed the distribution of the species reported in table III. Some species are very common in the olive fermentations and may play a role in the technology of the fermented green olives. Among the genera widely distributed in high concentrations in brines, *Debaryomyces* and *Pichia* are the most common. As it can be seen the strain belonging to the genera *Debaryomyces* had some interesting properties including the growth in high sodium chloride concentrations up to 12-15% the growth at 37°C. The utilization of nitrate and urea the hydrolysis of lipids (lipase). The other species belonging to the genera *Pichia* are highly represented. This genera is known by the growth on the surface of brines and fermenting liquids.

Table III
Yeast and Molds strains distribution in olive mill waste water samples

Strain	Number	%
Yeasts		
Pichia sp	15	27.15
P. carsoni	1	1.85
Debaryomyces hansenii	16	29
D. castelli	2	3.7
Sacharomyces cerevisiae	7	12.95
Candida zeylanoides	3	5.55
C. oleophila	3	5.55
C. versatilis	2	3.7
Schizosaccharomyces malidevorans	2	3.7
Rhodotorula mucilaginosa	1	1.85
Molds		
Penicillium sp.	10	30.3
Aspergillus sp.	3	9.1
Geotrichum sp.	20	60.6

The microbiota of the olive wastes was also represented by molds and yeast-like molds. These microorganisms are resistant to unfavorable environmental conditions such as acidic pH, high salt concentrations and low nutritional compounds. *Penicillium sp, Geotrichum candidum* and *Aspergillus* sp were the most frequent (table III).

It should be emphasized here that the OMW are heavily contaminated by inhibitors from the olives especially polyphenols and tanins more than its acidic pH. These characteristics make them unfavorable for biological treatments. The aerobic treatment with some microbial species which can grow on the effluent would be the most suitable approach to alleviate the problem. Yeasts and molds can be used for this purposes. Results reported hereby gave the evidence that a bioconversion of the polyphenols as well as other inhibitors prior to other biological treatment of OMW would help developing a well monitored process.

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