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A biotechnological valorization and treatment of olive mill waste waters by selected yeast strains

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RESUMEN

Valoración biotecnológica y tratamiento de aguas residuales de la molturación de la aceituna por cepas de levaduras seleccionadas.

Aguas residuales de la molturación de la aceituna se diluyeron en la proporción 1/10, se le añadió un 2% de urea y se inoculó con cepas de levaduras. 20 cepas de levaduras aisladas de aguas residuales de la molturación de la aceituna (OMW) se seleccionaron por su producción de biomasa, reducción DQO y actividades de bioconversión de polifenoles. Se llevaron a cabo cultivos puros de levaduras en matraces erlenmeyer de 100 ml. Se tomaron 50 ml de cultivos y los matraces se incubaron a temperatura ambiente (22°C) en un agitador. Se siguió la producción de biomasa, la reducción de DQO (demanda química de oxígeno) y la bioconversión de polifenoles en las aguas residuales de la aceituna. Los resultados mostraron que el suministro de urea mejoró significativamente la producción de biomasa en relación al control. Esta alcanzó en algunos ensayos el 2.06% expresado como g de peso seco de biomasa por 100 ml de agua residual de la aceituna. La eliminación de polifenoles se estimó en torno al 50% y la DQO disminuyó desde 54.14 g/Kg a 21.56 g/Kg. Este tratamiento aeróbico condujo a la producción de biomasa y también a un efluente pretratado por la DQO y la eliminación de los compuestos polifenólicos que inhiben la metanización.

PALABRAS-CLAVE: Alpechín — Biomasa — DQO — Levadura — Polifenol.

SUMMARY

A biotechnological valorization and treatment of olive mill waste waters by selected yeast strains.

Olive mill waste waters were diluted to 1/10, supplied with 2% urea and inoculated with yeast strains. 20 yeast strains isolated from Olive Mill Waste (OMW) water were screened for their biomass production, COD reduction and polyphenols bioconversion activities. Pure cultures of yeasts were realized in 100 ml erlen-meyer flasks. 50 ml cultures were used and the flasks were incubated at room temperature (22°C) on a shaker. Biomass production, COD (chemical oxygen demand) reduction and Polyphenols bioconversion were followed up in the inoculated OMW waters. Results showed that the urea supply improve significantly the biomass production relatively to the control. This reached in some assays 2.06% expressed as g of biomass dry weight per 100 mL of OMW water. Polyphenols removal was estimated to around 50% and the COD was decreased from 54.14 g/Kg to 21.56 g/Kg. This aerobic treatment lead to the biomass production and also to a pretreated efluent by the COD and the removal of the methanization inhibiting polyphenolic compounds.

KEY-WORDS: Biomass — COD — Olive mill waste water — Polyphenol — Yeast.

1. INTRODUCTION

Olive mill waste (OMW) waters are produced in huge amounts in Morocco. These efluents are still discarded in natural water streams or collected in pits near the olive mill (maasra). OMW waters are highly contaminated with polyphenols [Mouncif et al, 1993] which may render the efluent hard to treat biologically by inhibiting the microorganisms responsible of the organic matter decomposition.

In a recent study, the authors demonstrated that the microbiota of OMW waters is made of yeasts, moulds and to a lesser extent lactic acid bacteria. These microorganisms may survive or grow in presence of a high concentration of polyphenols since they were isolated in high numbers from the OMW waters. Borja-Padilla et al (1991) and Maestro-Durán et al (1991) used an aerobic pretreatment to eliminate part of the polyphenolic compounds to improve the anaerobic treatment. Hamdi (1993) reported that polyphenolic compounds may render the single cell biomass non utilizable by the adsorption of polyphenols. Hamdi et al (1992) used *Aspergillus niger* to remove the polyphenols in an integrated biological treatment. Borja-Padilla et al (1992a,b) used *Geotrichum candidum* for the aerobic treatment of the OMW waters prior to the biomethanization process. The use of OMW waters as a medium for yeast cultures was not investigated enough to improve the growth and consequently the biomass production.

In the present work, yeast growth was improved supplying the medium with urea to correct the nitrogen deficiency of OMW water. Biomass, COD and polyphenols removal were studied.

2. MATERIAL AND METHODS

2.1. Yeast strains isolation

Appropriate dilutions (10-1 to 10-5) of OMW samples were pour plated on Potato-Dextrose Agar (PDA) (BioKar, France). The plates were incubated at 28°C for 72 hours. The colonies grown were re-isolated on the same medium and pure cultures were examined for their shape and size by microscopic observations. This is to check the typical cell size and shape of yeasts.

2.2. Screening tests

The isolate strains were screened for growth on OMW water as a medium prepared as follow. OMW water was centrifuged to remove the fatty portion and the solids in suspension, supplied with 15 g/L of agar and sterilized at 120°C for 20 min. The medium was poured into plates and allowed to solidify. The plates were spot-inoculated by the strains and incubated at 28°C. The growth was examined every day for 5 days. Strains that had been grow were selected to be tested for growth and bioconversion of polyphenols in OMW waters.

2.3. Cultures in flasks

The OMW water was diluted to 1/10 with distilled water, centrifuged at 2000 g for 15 min and dispensed in 100 mL erlen-meyer flasks (45 mL/flask). The flasks were autoclaved at 120°C for 20 min and allowed to cool to room temperature (24°C). The prepared medium was supplied with urea so the final concentration was 2%. Urea solution (20%) was sterilized by filtration and amounts of 5 ml were added aseptically in each flask which were then inoculated with the selected yeast strains and incubated on a shaker (type IKA, Germany). Polyphenols and biomass were determined in the initial cultures, and after 3 and 7 days.

2.4. Biomass determination

10 mL of the medium were poured in a weighed centrifuging tube and centrifuged at 2000 g for 20 min. The obtained solids were weighed, dried at 105°C and reweighed. The biomass yield was calculated as the dry matter per Kg of OMW water.

2 5. Polyphenols determinations

Total polyphenols were determined by a spectrophotometric method using the Folin-Denis reagent as described by Maestro-Durán et al (1991). A calibration curve was realized in the same conditons using tannic acid as standard at the concentrations: 0, 1, 2, 2.4, 3.6, 4.8, 6 and 7.2 ppm. The absorbance was measured against a blank containing 0 mL of tannic acid.

2.6. COD

The COD was determined according to the APHA standards for the examination of water and waters (APHA, 1989).

3. RESULTS AND DISCUSSION

3.1. Screening of the strains

Growth of yeasts and yeast-like fungi G. *candidum* strains on OMW waters are reported in table I. Data indicated that several strains could grow even slightly on the solid OMW water medium. Only few isolates were inhibited and could not then grow on the medium. The strains that showed heavy growth were screened on liquid OMW waters to select the most eficient strains to be used furtherly. 20 isolates were grown on the liquid medium incubated under shaking conditions at ambient temperature. All the strains could grow on the OMW water medium but slightly. This is most probably due to the low nitrogen content of the OMW waters. The C/N ratio is about 138.92 according to the data reported by Mouncif et al (1993). The dilution to 1/10 may lower the polyphenols level in the culture medium made of OMW waters and in the same time the nutrients are also diluted, so there is a lack of nitrogen as it was observed in a previous study (Mouncif et al, 1993).

Table I
Growth of the yeast strains isolates on OMW waters

Nb	Growth solid	Growth liquid
1	–	–
2	+	–
I	++	++
3	+	+
4	–	–
5	++	++
6	–	–
7	++	++
8	–	–
9	–	–
11	–	–
A	++	++
B	++	++
E	++	++
G	++	++
T	–	–
16	–	–
17	++	++
51	+	–
61	+	–

3.2. Biomass production

Growth on olive mill waste waters was evaluated by the biomass production. All the cultures showed a net improvement of the biomass production on the OMW waters supplied with urea relatively to the OMW water (Figure 1). This is most probably due to the low nitrogen content of the efluent. The C/N ratio of OMW water in Morocco is about 138.92 according to the data reported by Mouncif et al (1993). This may show a low level of nitrogen which must be corrected. High yield biomass production may tell about the valorization degree for the use of OMW waters as a medium for the single cell proteins production by some adapted strains of yeasts and/or moulds. There is a direct application of these results in the field of feed processing and not only the biomass production would be a convincing approach for the use of OMW waters in feed production but also the removal of polyphenols which may occur as digestive inhibitors of feed made of untreated OMW waters.

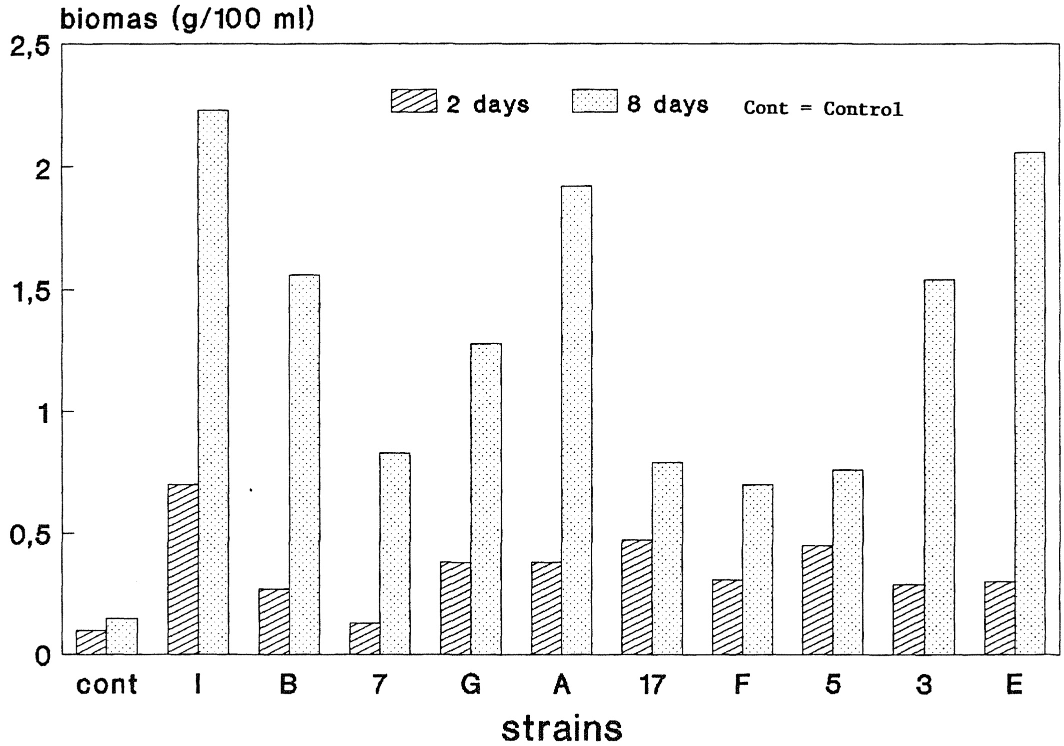

Figure 1
Biomass production by the selected strains of yeasts on OMW supplied with urea (results expressed as g of dry matter per 100 mL OMW)

3.3. COD

The COD was decreased from 0.54 to 0.21 by the strain 17 and to 0.23 by the strains A and I (Figure 2). Most of the isolates showed a reduction of the COD. This is due to the activity of the yeast strains inoculated to the efluent. Our results are in concordance with those reported by some authors. Hamdi et al (1992) removed 58.7% of the COD of OMW waters in an aerobic treatment system by the use of A. *niger.*

The COD reduction may suggest a possible uptake of the organic matter by the microorganisms (oxydation). This uptake may result in biomass formation by the inoculated strains. This phenomenon was observed in most assays and there is a correlation between the COD reduction and the biomass production.

3.4. Polyphenols

Results reported in Figure 3 showed also the effect of some yeast strains belonging to the genera *Candida* sp and *G. candidum* on the removal of polyphenolic compounds from OMW waters. According to data reported by Mouncif et al (1993), these waste waters are highly contaminated with polyphenols (64.7g/l) which may inhibit the microbiota during aerobic and/or anaerobic treatment as it was stated by the same authors. Strains of yeasts and *Geotrichum* were isolated from OMW waters were incubated in pure cultures to study their growth and their effect on the polyphenols degradation. Several cultures showed an activity on these compounds compared to the control. There was a need for the adjustment of the medium by urea addition to increase the nitrogen proportions and encourage the growth of the strains.

In preliminary results of cultures on non supplied OMW water with urea, we could realize that almost all the isolates could grow weakly. We supposed that this phenomenon was not due to the inhibition but to the low C/N ration in the medium.

Polyphenols conversion was also higher for the cultures in OMW waters supplied with the urea than the non supplied OMW waters. The bioconversion of these compounds is related to the growth of the strains inoculated in the OMW waters. Polyphenols biodegradation reached 50% (strain 17). This value can be improved by studying deeply the growth conditions of the strains used in the present investigation. Our results approached those reported by Martínez-Nieto et al (1992) who reported a total biodegradation of polyphenols by A. *terreus* ranging from 42.08% to 68.90% respectively for non diluted OMW water and a 20% dilution. Maestro-Durán et al (1991) reported a similar reduction of the polyphenols by an aerobic treatment from 300 ppm to 103 ppm which may represent a 67% reduction.

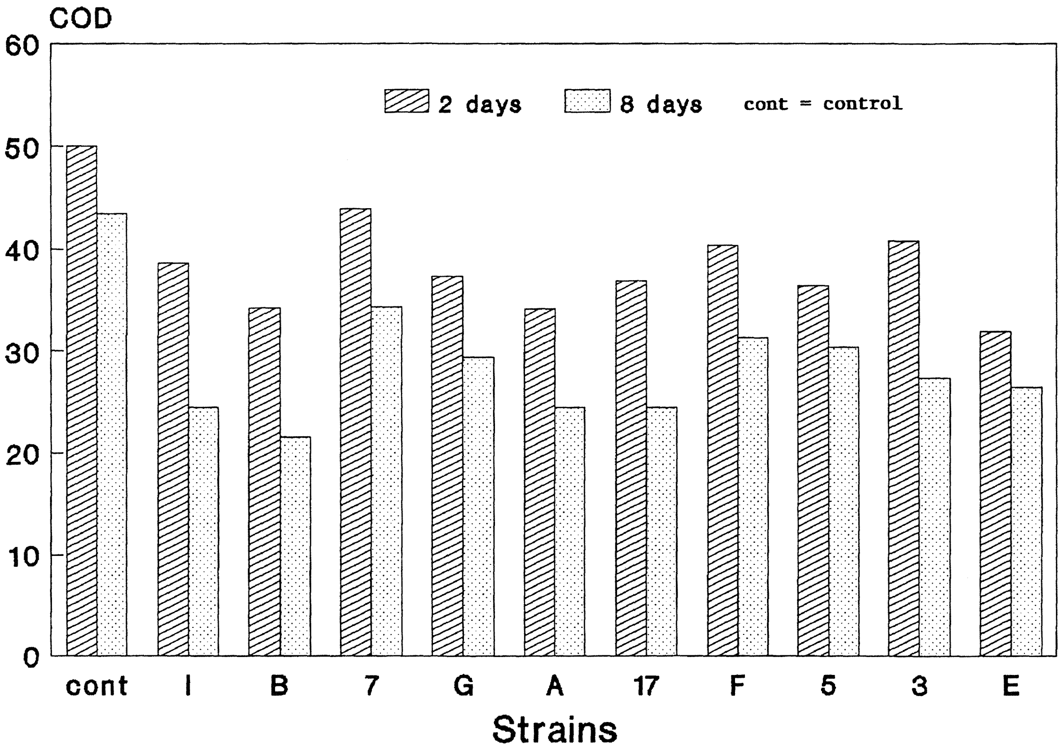

Figure 2
COD of the supernatent of OMW waters inoculated with the yeast isolates (results are expressed as g O_2 / L)

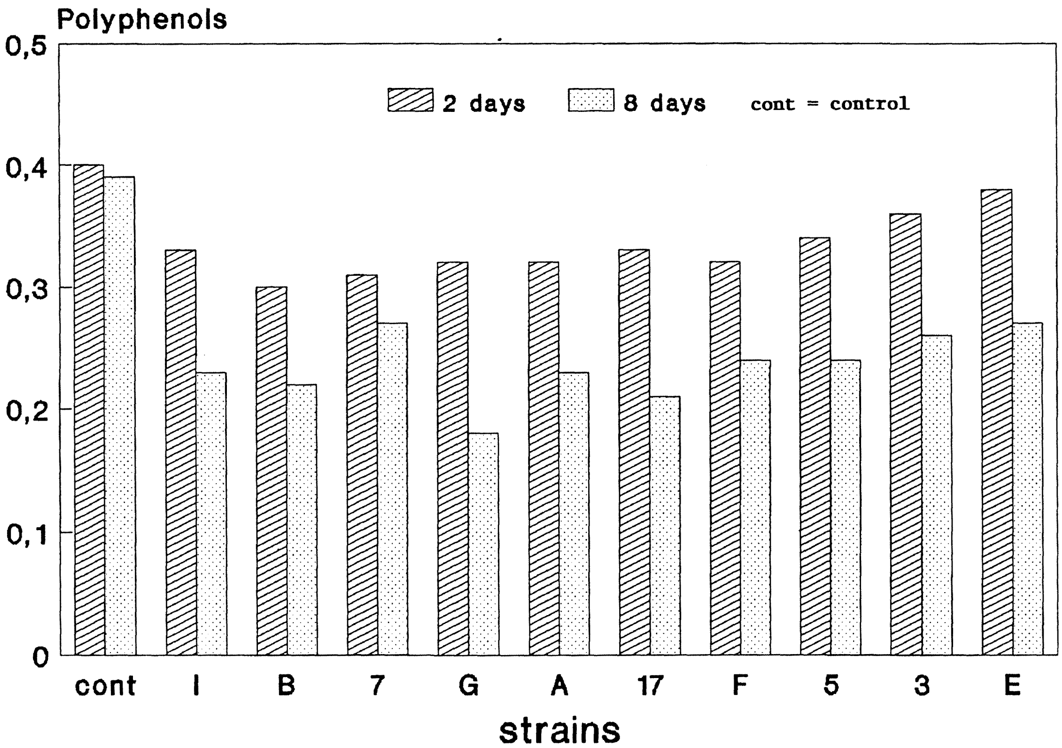

Figure 3
Polyphenols bioconversion by the yeast strains in OMW waters expressed as% reduction.

4. GENERAL DISCUSSION AND CONCLUSION

The strains I, B, 3 and E showed a high biomass yield while the strains I, B, A and 17 were the most active for the COD reduction. The strains I, B, G, A and 17 showed the highest activity in polyphenols biodegradation. 3 strains (I, B and A) were also active for the three parameters simultaneously. These strains showed a high growing activity on OMW supplied with urea (2%), a high activity in polyphenols as well as the COD reduction so the resultant efluent is not heavily polluted. Organic matter hydrolysis by these strains was assessed by the COD slow-down and the most interesting property of these strains was the destruction of polyphenols. These compounds may inhibit the microbiota of the efluent and render it hard to be treated. There is a correlation between the biomass production and the polyphenols conversion for some strains (17, A, B and I).

The preliminary results stressed the point that the growth of the inoculated yeast strains was reinforced by diluting to 1/10 to alleviate the inhibitory effect of polyphenols and by correcting the C/N ration by urea supply.

Up to now and to our knowledgement, no work has described the use of yeasts in OMW waters valorisation. Spanish investigators used G. *candidum* (Borja Padilla et al, 1992a) and *A. terreus* (Martinez-Nieto et al, 1992) to remove the polyphenols and to reduce the COD in a pretreatment system for the biomethanization process. Tunisian investigators (Hamdi et al, 1992) used *A. niger* in aerobic treatment in the same manner as in Spain. In Italy authors (Rannalli, 1991) used a mixture of various yeasts in the aerobic treatment of OMW waters. In the present work we demonstrated the eficiency of the yeasts in OMW waters treatment and valorization.

Donosa-Arce (1979) described some valorizations of OMW waters and pointed out to the inhibition of single cell proteins production by yeasts as well as its coloration. There is a lack of the ingredients used in animal feeds in Morocco, especially those with a high nitrogen content. This shortness can be overcome by the use of selected yeast strains in a semi-solid fermentation in which the OMW waters can be mixed with other solid wastes to produce animal feeds.

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