COMBINED ELECTROCOAGULATION AND FUNGAL PROCESSES FOR THE TREATMENT OF OLIVE MILL WASTEWATER

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ABSTRACT

Olive mill wastewater (OMW) is characterized by its high suspended solids content, chemical oxygen demand (COD) concentration up to 100 g/l and toxic phenolic compounds concentration up to 10 g/l. This work evaluates the improvement in effectiveness in the treatment of raw olive mill wastewater when the fungal treatment is enhanced with electrocoagulation pre-treatment. The electrocoagulation was found to be a suitable pre-treatment for the biological processes, and it was found to be useful as a complete treatment for four-fold-diluted OMW. Under the following conditions: electrolysis time 15 minutes, NaCl concentration 1 g/l, initial pH 4.57 and current density 250 A/m^2, the electrocoagulation was able to decrease the COD and phenol content of the 25% OMW by more than 80 % and the color by more than 90%. The strain *Aspergillus niger* van Tieghem (DSM 24787) was more efficient to reduce chemical oxygen demand, phenol content and color when the OMW was prior diluted or pretreated. The results show that the fungus is capable of reducing all parameters analyzed (COD, phenol content and color) by at least 60%, after 7 days of growth, from 25% OMW. No significant growth in term of biomass increase was observed for the strain on 75% and 100% OMW. The biological treatment alone was not effective for the treatment of the raw effluents, but in combination with the electrocoagulation process it led to a high-quality effluent and it reduces the processing time and the supply of water for dilution.

Keywords: Olive mill Wastewater; Electrocoagulation; *Aspergillus niger* van Tieghem; Combined treatment

1. INTRODUCTION

One of the major environmental problems caused by agro-food industry in Mediterranean area is the treatment of the olive mill wastewaters. These are the main by-product of olive oil production and are characterized by high concentrations of several organic compounds, such as organic acids, sugars, tannins, pectins, polyphenolic substances, that make them difficult to treat (Vlyssides et al., [1]; Borja et al., [2]; Azbar et al., [3]). The presence of these compounds makes olive mill wastewaters phytotoxic and inhibits bacterial activity (El Hajjouji [4], D'Annibale [5]). Besides, they contain also inorganic compounds such as chloride, sulfate and phosphoric salts of potassium as well as calcium, iron, magnesium, sodium, copper and traces of other elements.

Finally, they are produced in a limited period of time (from October to February) and in very large quantities and their physical and chemical characteristics vary according to cultivars, harvesting time, type of olives and the technology used in the extraction process (pressing or centrifugation). For all the above-mentioned reasons, flexible and efficient treatment plants are needed.

It has been shown for example that OMW should be diluted 70 times in order to keep the concentration of toxic compounds at a biologically tolerable level for anaerobic treatment (Jaouani [6]). Furthermore, the anaerobic biological treatment showed limited efficiency in removing aromatics particularly high molecular weight fraction (Ergüdera [7]). Only few microorganisms, mainly white rot fungi, are capable to decolorize the effluent and so to act on the high molecular weight phenolic fraction (Asses et al., [8], Ergül [9]). Nevertheless, relatively low initial COD (OMW should be diluted prior treatment) and in some cases elaborated cultivation media are needed, which preclude any industrial application.

A possible solution would be the pre-treatment of OMW, prior to its biological treatment. Therefore, a combination of physicochemical and biological methods appears constitute a promising alternative to purify OMW effluent i.e. to decrease the COD and to remove the phenolic fraction (El-Gohary [10], Lafia [11], Badawy [12]).

In this work the electrocoagulation using aluminum electrodes (as cathode and anode) and biological treatment by a newly isolated potent strain of *Aspergillus niger* van Tieghem (DSM 24787*)* was applied to treat undiluted and slightly diluted OMW. The performance of separate single-step electrocoagulation and biological processes was evaluated. However, the results suggest that none of these processes could be employed alone for the efficient treatment of this undiluted or slightly diluted wastewater. In contrast, a combined electrocoagulation-biological treatment system results in high organic matter removal efficiency with a better reduction of phenolic compounds and color and it reduces the processing time and the supply of water for dilution.

2. EXPERIMENTAL

2.1. Characteristics of olive mill wastewater

Olive mill wastewater was collected from an olive extraction plant which uses a classic process located in south of Morocco (Marrakech). OMW was stored in a closed plastic container at 4°C. The main characteristics of this OMW are presented in

Table 1. Before electrocoagulation, OMW sample was decanted and filtered by vacuum filter and glass - microfiber filter (Whatman GF / D (porosity 2.7 µm)).

Table 1 – Characteristics of the undiluted OMW, used in this study

Parameter	Value
pH	4.57
Conductivity (EC) (mS/cm)	3.6
Chemical oxygen demand (COD) (g d'O_2/l)	114
Polyphenols (g/l)	18
Ammoniums (NH_4^+)(g/l)	0.160
Orthophosphates (PO_4^{3-})(g/l)	0.110
Chlorides (Cl^-) (g/l)	4.160
Sodiums (Na^+) (g/l)	3.195
Potassiums (K^+)(g/l)	2.325
Suspended solid (SS)(g/l)	10.6
Color (Abs at 395 nm)	16

2.2. Electrochemical cell

The electrochemical cell has two aluminum plates, one serving as a cathode and the other as anode. The total effective electrode area was 18 cm^2 (4.5 cm x 4 cm) and the spacing between electrodes was 2.8 cm. The electrodes were connected to a digital DC power supply (4A, 30V). For each run, 100 cm^3 of OMW were placed into the electrolytic cell and a gentle stirring rate of about 200 rpm (revolutions per minute) was applied to allow the chemical precipitate to grow large enough for removal (with a stir bar of ø 6 x 15mm long).Thereafter, the samples were decanted for 24 h before being subjected to vacuum filtration through Millipore membrane filters with a pore size of 0.45 µm. In the sample filtrated: COD, phenolic content, dark color intensity and pH were measured.

2.3. Strain and cultures conditions

The strain used in the OMW treatment experiments was isolated from Moroccan pomace using the agar plate technique and classified by DSMZ (Braunschweig, Germany) as *Aspergillus niger* van Tieghem (DSM 24787). A fungus was maintained through periodic transfer at 4°C on potato-dextrose (2.4%) agar plates in the presence of 0.5% yeast extract.

2.4. Inocula Preparation

Before using in the biodegradation experiments, the strain was first precultivated during 2 days at 28°C under agitation (rotary shaker-150 rpm) in 50 ml of the liquid version of 25% OMW-based medium supplemented with 0.35% $(NH_4)_2SO_4$ and 0.065% KH_2PO_4 (w/v), contained in 250 ml Erlenmeyer flasks. The fungus was grown in the form of pellets.

2.5. Liquid cultures

Liquid cultures were conducted in duplicate, in 250 ml Erlenmeyer flasks containing 50 ml of an OMW (25, 50, 75 and 100% (v/v)) sterile supplemented uniformly with 0.35% $(NH_4)_2SO_4$ and 0.065% KH_2PO_4 (w/v), and adjusted to the final volume with tap water. The flasks were inoculated with 0.4 g/l dry weight fungal biomass and incubated in a rotary shaker at 150 rpm and 28°C. Samples were taken daily for analysis.

2.6. Biomass estimation

Biomass produced during cultivation of *A. niger* on the OMW media was estimated by filtration of the culture medium on glass microfibres (GF/A Whatman Inc.). The retained biomass was washed twice with 5 ml distilled water and dried overnight at 105°C. Growth yield was expressed as gram of dry weight per 50 mL of culture.

2.7. Analytical methods

Before analysis the samples were filtered on glass microfibres (GF/A Whatman Inc.). In the sample, COD, phenolic content, dark color intensity and pH were measured.

a. **Chemical oxygen demand (COD).** COD were determined according to APHA standards methods.
b. **Color.** The dark color intensity was determined by measuring the sample's absorbance at 395 nm respectively using a CARY 1E VARIAN spectrophotometer, in 1 cm path-length cells (Ongen et al., [13]).
c. **Phenol content.** Determination of the concentration of total phenolic content was carried out with the official spectrophotometric procedure (720 nm) in which the reagent Folin-Ciocalteu was used as a selective reagent for polyphenols (Hanafi et al., [14]) using a CARY 1E VARIAN spectrophotometer, the results were expressed as gram of gallic acid per liter.
d. **pH.** A digital calibrated pH-meter (Jenco 6173) was used to measure the pH of the OMW samples.

The accuracies of all parameters measurements were better than 4%.

3. RESULTS AND DISCUSSION

3.1. Olive mill wastewater characteristics

The range of concentrations for the final olive mill effluents (OMW = vegetation plus washing waters) is quite wide, more than tenfold, depending on the extraction process used and on the operating conditions. Most of the olive mills in Morocco use a three-phase extraction process. Manual mills are still present but to a limited extent. As can be seen from **Table 1**, the OMW samples were slightly acidic with pH values around 4.57. It has a significant amount of COD (114 g/L) and of total solid matter (10.6 g/L). It is characterized by a large phenolic content (18 g/L), which is considered to be harmful for plants, hence, an early plant growth inhibitor for different vegetables and a source of objectionable odor when contacted with chlorinated waters (formation of chlorinated phenols). Capasso et al. [15] showed that some polyphenols, like methylcatechol and o-quinone, which are naturally present in olive oil vegetation waters, have toxic effects on some strains of gram-positive and gram-negative bacteria. Moreover, this wastewater is characterized by its black color (16 Absorbance at 395 nm) that is considered another undesirable property. The major components in the colored fraction are substances of polymeric nature, derived from several low molecular weight phenolic compounds, chemically related to lignin and humic acids (Ayed et al., [16]; Sánchez [17]). Furthermore, this OMW sample contains relatively higher amounts of Cl^- (4.160 g/l). For electrocoagulation, the presence of Cl^- in OMW solution was reported to increase the anodic dissolution rate of Al, either by the incorporation of Cl^- to the oxide film or by the participation of Cl^- in the metal dissolution reaction (Wang et al., [18]).

3.2. Fungus treatment

In a previous work Hanafi et al. [19] a broad screening was carried out on 32 fungi. *Aspergillus niger* van Tieghem was retained for their capacity to tolerate the polyphenols and to decolorize OMW. Initial experiments were conducted to determine the wastewater concentrations that were not toxic to *Aspergillus niger* van Tieghem by testing shake-flask cultures in 100%, 75%, 50% and 25% concentrations. With OMW the COD, phenolic compounds and color removal are crucial to bioremediation. The COD results for fungal treatment after seven days are shown in **Fig. 1.**

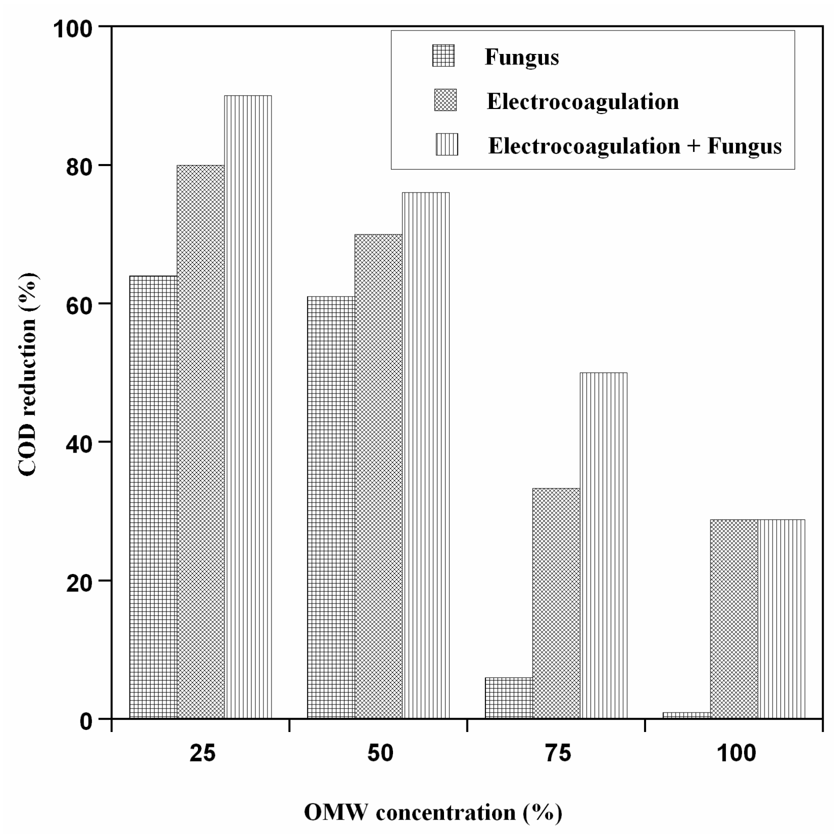

Fig. 1: Effect of OMW concentration on COD reduction by Fungus *Aspergillus niger* van Tieghem, Electrocoagulation and Combined Electrocoagulation + Fungus. Media containing respectively 25, 50, 75 and 100% (v/v) of OMW, corresponded to 4.5, 9, 13.5 and 18 g l^{-1} phenolic content

The effectiveness of the fungi to decolorize the effluent and to decrease phenolic content and COD was adversely affected in media containing slightly diluted OMW. No significant growth in term of biomass increase was observed for the strains on 75% and 100% OMW. These results are in agreement with the findings of previous workers (Aissam et al., [20]) and support that antimicrobial qualities of OMW must be diluted prior to treatment. Possibly this could result from a toxic effect of the OMW phenolic and/or other compounds, when present above a critical concentration. Aggelis et al. [21] used a white rot fungi Pleurotus ostreatus in bioreactor batch cultures to reduce phenolic content and toxicity of sterilized OMW. However, they reported that high OMW dilutions should be used, and/or additional treatment should be applied for complete detoxification and use of the OMW for irrigation. As a general rule it appeared that microbial growth and degradation of phenolics was adversely affected in media containing more than 20 to 25% (v/v) OMW. This was observed for Phanerochaete chrysosporium (Kissi et al., [22]), Pleurotus ostreatus (Olivieri et al., [23]), Aspergillus sp (Garcia et al., [24]), Geotrichum candidum (Assas et al., [25]) and aerobic and anaerobic bacterial communities (Fadil et al., [26]). Until now, only some strains, Pycnoporus coccineus, Pleurotus sajor caju, Coriolopsis polyzona and Lentinus tigrinus were reported as notably active in discoloration and COD removal of OMW above 50 g.l-1 COD (Jaouani et al., [8]).

In our experiments, for the medium containing 25% OMW (COD_0 = 28 g/l; $[PC]_0$ = 4.5 g/l) and 50% OMW (COD_0 = 57 g/l; $[PC]_0$ = 9 g/l) the percentages of total phenolic compounds removal was ranged between 73% and 55% respectively **(Fig. 2)**. The percentages of COD removal was ranged between 64% and 60% respectively **(Fig. 1)** and was accompanied by a biomass production of 0.18g/50mL dry weight for 25% OMW and 0.30g/50mL dry weight for 50% OMW. The color reduction was 64% for 25% OMW and it changed from black to light brown **(Fig. 3)**. A slight darkening of the mycelia pellets was also observed. The discoloration was probably due to the degradation and adsorption of some phenolic compounds on the fungal mycelium, these results were already shown by Ongen et al. [13]. This adsorption may be due to the hydrogen bound between phenolic compounds and proteins or to the chitin of the mycelial wall, which has a strongly coagulant effect (Aissam et al., [20]).

3.3. Electrocoagulation

Electrocoagulation is a process consisting of creating a floc of metallic hydroxides within the effluent to be cleaned, by electrodissolution of soluble anodes. Compared with traditional flocculation and coagulation, electrocoagulation has, in theory, the advantage of removing the smallest colloidal particles; the smallest charged particles have a greater probability of being coagulated because of the electric field that sets them in motion. It has also the advantage of producing a relatively low amount of sludge (Holt et al., [27]). The characteristics of electrocoagulation are simple equipment and easy operation, brief reactive retention period, decreased or negligible equipment for adding chemicals and decreased amount of sludge (Hanafi et al., [28]). The experiments were done on 100%, 75%, 50% and 25% concentrations of OMW. With the aim of COD removal and water purification, the pH of the solution was 4.57. Voltage 22 V and CD equal to 250 A m^{-2} were applied during 15 min. For each of OMW dilution, the percentage of the COD, phenolic compounds and color removal were measured. It has been observed that the percentage of all parameters removal depends immediately on the OMW concentration. In general, the removal efficiency of COD, phenolic compounds and color exceeded 70% in 25% and 50% OMW concentration. These results confirmed that electrocoagulation with aluminum electrode acted in a targeted way on aromatic colored compounds and on phenols as evidenced by the decrease of coloration. During 15 min electrocoagulation, higher OMW concentration (75% and 100%) led to lower efficiency of COD and phenolic compounds removal and no discoloration was observed, probably, due to a low formation of the metal hydroxide ions produced by hydrolysis of the metal ions generated in the electrochemical cell (eq. 1, 2). The solubility of aluminium hydroxide increases when the solution becomes either more acidic or alkaline. The typical pH of OMW is between 4 and 5.5, which allows it to be directly treated by electrocoagulation without further pH adjustment. Furthermore, the pH of OMW changes during the electrocoagulation process as observed by other investigators (Kobya et al., [29]). The final pH increase when the initial pH is low (<7) due to the OH^- ion accumulated in aqueous solution during the process. Yet, the recovered liquid fraction had a pH ranged between 6.6 and 7.6 and do not needs to be corrected for further biological treatment. After electrocoagulation, OMW can be filtrated more easily after decantation. Electrocoagulation seemingly adapted for a pre-treatment preceding biological treatments.

$$Al \rightarrow Al^{3+} + 3e^{-} \quad (1)$$

$$Al_3^{+}(aq) + 3H_2O \rightarrow Al\,(OH)_3(s) + 3H^{+}(aq) \quad (2)$$

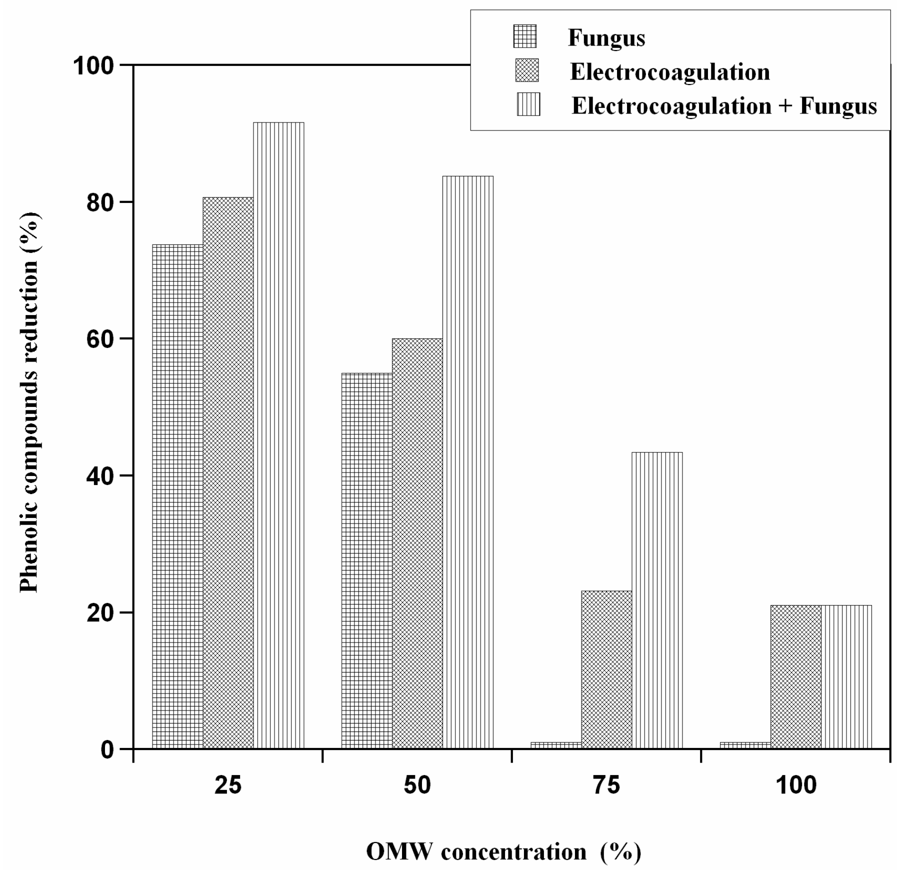

Fig. 2: Effect of OMW concentration on phenol content reduction by Fungus *Aspergillus niger* van Tieghem, Electrocoagulation and Combined Electrocoagulation + Fungus. Media containing respectively 25, 50, 75 and 100% (v/v) of OMW, corresponded to 4.5, 9, 13.5 and 18 g l^{-1} phenolic content

3.4. Combined treatment

It seems that although fungal treatment is ecofriendly and cost effective but it is time consuming and OMW must be diluted prior fungal treatment. This is probably due to the presence of toxic materials in the OMW. Coupling of an electrocoagulation and fungal treatment is considered as a new approach. The main objective of this strategy is the use of an electrocoagulation process to convert initial toxic and non-biodegradable components into by-products that can be assimilated by biomass especially for concentrated OMW where no fungal growth was observed. The OMW pretreated with electrocoagulation was supplemented with nitrogen and phosphorus. Previous works (Aissam et al., [20]) reported that additional nitrogen and phosphorus should be supplemented to enhance the growth of *A. niger.* **Figures 1, 2, 3** depict the percentage of COD, phenol content and color removal values obtained from each single treatment process as well as the total of all parameter removal achieved by the overall sequence. The obtained results regarding the effect of OMW pre-treatment with electrocoagulation, in order to reduce the COD, phenol content and color in OMW, prior to their subsequent fungal biodegradation, are very encouraging. The characterization of OMW showed indeed that this type of wastewater presents a very high organic and phenol load.

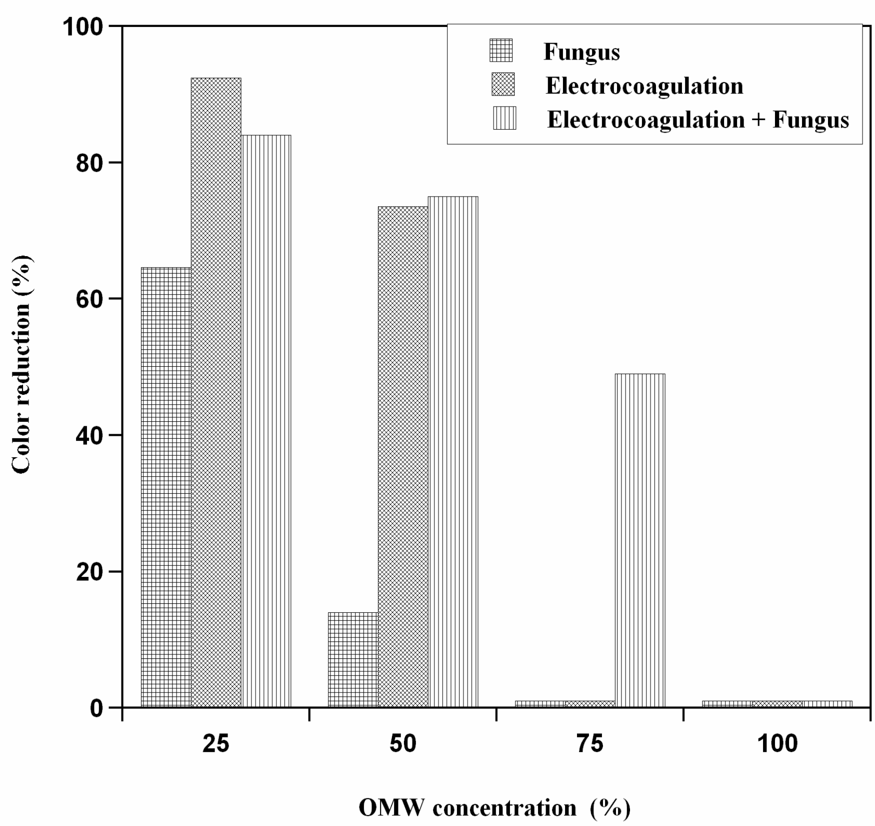

Fig. 3: Effect of OMW concentration on color reduction by Fungus *Aspergillus niger* van Tieghem, Electrocoagulation and Combined Electrocoagulation + Fungus. Media containing respectively 25, 50, 75 and 100% (v/v) of OMW, corresponded to 4.5, 9, 13.5 and 18 g l^{-1} phenolic content

The pretreatment of the OMW with electrocoagulation reduces considerably the COD and the phenolic compound concentration of the waste which is responsible for its biotoxicity. This fact is shown by an increase in the yield of treatment of high concentration of OMW. Combined treatment reduced the phenol content level much more significantly, which is about 92%, 84% and 44% for 25%, 50% and 75% OMW respectively **(Fig. 2)**. It also removes COD, and color remarkably compared to the treatment of electrocoagulation and *A. niger* alone. The combination treatment showed synergistic effect in the all parameter reduction and reduces the processing time and the supply of water for dilution. It has been found that with high concentration of OMW (75%) the percentage of COD, phenol content and color reduction increases significantly after 15 min electrocoagulation and 4 days fungal treatment. It is also observed (**Table 2**) that the final pH increased to 7.53, 6.6 and 5.27 for 25%, 50% and 75% respectively, which may be attributed to the reduction in phenols concentration, for phenols are acidic in solution, and their removal reduces the acidity of the solution. Whereas, for the medium containing 100% OMW, no significant growth in term of biomass increase was observed. It should be noted that in the undiluted medium the initial concentration of phenolic compounds was higher than in the diluted OMW medium. After a short time of electrocoagulation of undiluted OMW, the no fungus growth has been assigned to the oxidative polymerization of phenols and tannins originally present in the sample (Khoufi et al., [30]). For the medium containing 75% OMW with 85 g/l initial COD and 13.5 g/l initial phenolic content concentration; 49% discoloration, 50% reduction of COD and 44% reduction of phenolic content was considered to be a good result.

Table 2 – Final pH of treated OMW

OMW concentration (%)	Initial pH	Final pH		
		Fungal treatment	Electro-coagulation	Electro-coagulation + Fungus
25	4.68	6.23	5.24	7.53
50	4.61	5.75	5.68	6.6
75	4.59	4.49	4.87	5.27
100	4.57	4.48	5	4.7

4. CONCLUSIONS

From this work the following conclusions can be drawn:

1) The electrocoagulation is a suitable pre-treatment for the raw OMW. It decreases the phenolic content and color of the waste besides increasing the biodegradability of the sample considerably.
2) The electrocoagulation results can be explained by assuming that the enmeshment of pollutants into growing amorphous aluminum hydroxide precipitates (sweep coagulation) is the primary coagulation mechanism.
3) The fungal treatment is not an effective technique for the treatment of the undiluted OMW because of the large ratio of phenolic content.
4) Combined electrocoagulation and fungal processes leads to a high quality of slightly diluted effluent and it reduces the processing time and the supply of water for dilution.

Such combined process of electrocoagulation and fungal treatment proved to be efficient and presents potential application in industrial scale.

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