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Soluble microbial products and performances assessment during OMW biological treatment by an activated sludge pilot

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Abstract

Olive mill wastewater (OMW) is currently one of the most serious environmental pollution problems, because of its high potential to contaminate surface and ground waters. Untreated OMWW is a major problem for the wastewater treatment plants (WWTP) based on activated sludge because of its phenolic compounds content which inhibit microbial activity and growth. This paper aims to assess the soluble microbial products (SMP) and treatment performances in activated sludge pilot plant (ASPP) depending the increasing OMW mass ratio F/M. The results highlight optimal performances of the system during the experiment. Indeed, the biomass concentration in bioreactor of ASPPwas high and reached5.71 $g_{MLSS}.L^{-1}$ for mixed liquor suspended solids (MLSS) and 4.86 $g_{MLVSS}.L^{-1}$for mixed liquor volatile suspended solids (MLVSS),the respirometric activity was very promising of about 9.95 $mg_{O2}.g_{mlvss}^{-1}.h^{-1}$which indicates good promptness of the biomass against the OMW increasing feedings. Theresults showalso an important abatement of COD and polyphenols which both reached more than 90%. Moreover, HPLC analysis shows an important abatement of the most toxic polyphenols present in OMW, hydroxtyrosol and tyrosol.The SMP have a crucial importance for biological wastewater treatment systems because of their significant impacts on both treated effluent quality and treatment efficiency. In this study, their concentrations were significantly influenced by OMW increasing mass ratio F/M. Consequently, they reacheda stable concentrations at end of the experimental study of 8 $mg.L^{-1}$ for proteins and 17 $mg.L^{-1}$ for polysaccharides.

Keywords: *Activated Sludge; Olive mill Wastewater; Soluble microbial products; Performances*

1. Introduction

Olive oil industries are vital source for the economy and social life of the Mediterranean region; however, the other effect of the olive-oil mill activity is the generation of huge quantities of a contaminant and toxic olive mill waste (OMW).Discharge of OMW is of serious environmental concern due to its high acidity and its high content of organic matter with phytotoxic properties, namely phenolic compounds. The estimation of phenolic compounds in this effluent is about 53%while it doesn't exceed 1-2% in olive oil [1,2]. Currently, the principal destinies of OMW either their direct spreading in agricultural soils under controlled conditions with convenient doses[3,4], or their discharging in artificial big ponds into which OMW is stored awaiting for its natural evaporation. However, this later elimination method encountered a wide range of drawbacks accurately:slow speeding evaporation, requiresrelatively large evaporation surfaces, with high investment costs, and causessubsequent unpleasant environmental pollution linked to generation of bad odors, infiltration and insect proliferation.The reduction of the extremely high organic load of OMW to environmentally acceptable levels for disposal in aquatic environments necessitates the development of new solutions for its treatment. The available treatment technologies for OMW treatment include physic chemical and biological processes[5-8].The first includes coagulation, flocculation, filtration, sedimentation, electrochemical oxidation, electrocoagulation, and the combinations of these processes[9].Regarding the biological processes, several batch studies were carried out using pure bacteria cultures for OMWbiodegradation[10,11]. Also, the application of specific bioreactors to treat this effluent has been performed. Among aerobic treatments based on bioreactors is activated sludge processwhich uses a mixture of microbial consortium to degrade the polluting effluents charge[12]. However, the treatment of OMW by activated sludge process can inhibit the growth of microorganisms and halt the functioning of the process due the high toxic effect of this effluent[13,14]. As few experiences have showed[15,16], the best way to deal with this complex effluent biodegradation is go through the biomass adaptation during the aerobic treatment.In such situation, a new generation of specified biomass is developed which is able to manageeasily the stress of the surrounding environment by releasing extra polymeric substances called soluble microbial products (SMP). These substances are composed of different organic compounds (e.g. polysaccharides, proteins, humic substances) and are secreted by microorganisms in their environment during their growth, cells lysis or in response to harsh conditions[17-19]. Containing of the low number of dynamic researchesmade related to OMW treatmentby aerobic treatment and the lack of studies analyzing SMP released by adapted OMW microorganisms;the present study aims to assess the soluble microbial production and treatment performances during OMW biological treatment by activated sludge process. In fact, the effect of OMW on the biomass growth, system performances and SMP production, were measured depending tothe increasing of OMW food to microorganism mass ratio (F/M)during this experiment.

2. Materials and methods

2.1. Activated sludge pilot plant

A pilot-scale automated activated sludge plant (TAE/3000) was used as prototype of a full-scale waste water treatment plant that is composed of an anoxic tank (18 L), an aerobic tank (60 L), a settling tank (30 L), a substrate vat (100 L), alimentation and circulating pumps, agitators and an aeration system (Figure1). The pilot system was equipped with various analytical instruments, including probes for oxygen, pH and redox potential. All equipment was connected to a central programmable logic controller (PLC) linked to a supervisory control and data acquisition (SCADA): PLC SOFREL that is a single program that manages the automation and supervision providing a direct access and data storage.

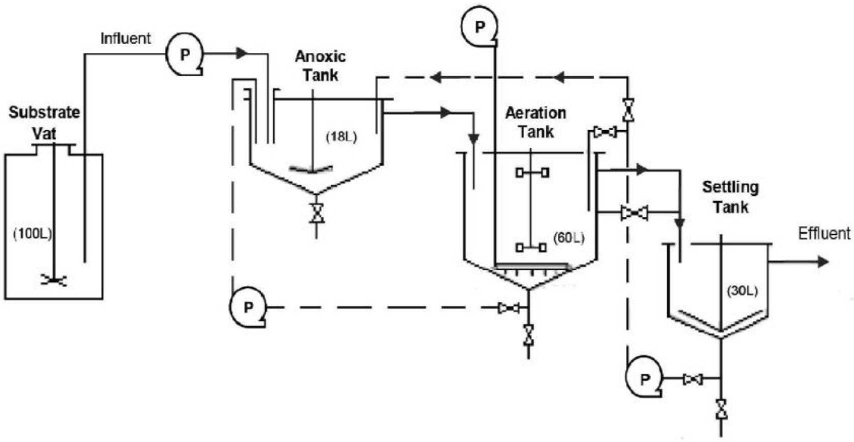

Figure1. Schematic diagram of activated sludge pilot plant (ASPP).

2.2. Operating conditions and experimental detail

The pilot plant has been operated for 70 daysand was fed first by a synthetic substrate prepared with mass ratios of 2.1 $C_6H_{12}O_6$, 0.2 KH_2PO_4, 0.4 $NaHCO_3$, 0.1 $MgSO_4$ and 0.02 $CaCl_2$. The F/M ratio was fixed in the beginning of the experiencearound 0.2$kg_{COD}.kg_{MLSS}.day^{-1}$and changed gradually to 0.3$kg_{COD}.kg_{MLSS}.day^{-1}$ to increase the biomass grow up.During the second step, the composition of the feeding was changed by a progressive adding of different mass ratio of OMW(5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) against synthetic substrate. The F/M ratio was maintained at 0.3 $kg_{COD}.kg_{MLSS}.day^{-1}$ and at every cycle of adaptation a new higher OMW mass ratio was added. The OMW effluent used in this experience was collected from a discontinuous extraction unit from Marrakesh, southern Morocco. During the operation period, recycling rate/flow rate ratio was set at 1 to ensure the presence of a significant amount of biomass in the reactor. The ambient temperature was approximately around 25°C. The hydraulic retention time (HRT) in the bioreactor was about 10 hours. Oxygen was provided by an air bump regulated to maintain residual dissolved concentration at 2-3$mgO_2.L^{-1}$.

2.3. Analytical methods

Samples were collected every working day from influent and effluent, as well as from biological tank, to investigate biomass evolution and the system performances.Mixed liquor total suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), nitrite (NO_2^-), Total phosphorus (TP), orthophosphates(PO_3^{2-}), chloride (Cl^-),calcium (Ca_2^+), sodium Na+ were determined according to standards methods[20,21,22]. Phenolic compoundswerequantified by means of the Folin–Ciocalteu colorimetric method according to Macheix et al, 1990[23]. Phenolic extracts were also analyzed by HPLC performed using Eurospher II 100-5 C-18 reversed phase column (Knauer-HPLC) equipped with a photodiode array detector and a software analysis. An efficient gradient of acetonitrile-o-phosphoric acidifiedbidistilled water (pH 2.6) was used and the elution consisted of linear gradient program of the acenotrile/water mixture over a detection time about 60 min. The identificationof phenolic compounds was executed on the basis of their spectra in comparison with nine phenolic standards (caffeic acid, ferulic acid, syringic acid, p-coumaric acid, tyrosol, quercetin, 2-hydroxycinnamic, 2-4 hydroxyphenylethanol, hydroxytyrosol). To measure the oxygen uptake rates (OUR) in every cycle of this experiment, an oxygen probeOxymax (COS61/COS61D) was directly implemented in the aeration tank. The Oxygen Uptake Rate (OUR) was determined through the slope of the linear portion of the DO versus time curve. The specific oxygen uptake rate (SOUR) was calculated by dividing the OUR by the MLVSS concentration[24].Carbohydrates in the SMP

were determined according to the phenol-sulphuric acid method with the glucose as a standard[25]. Proteins and humic substances of SMP were measured bythe method described by Frolund et al 1995[26] with bovin serum albumin as a standard.

3.Results and Discussions

3.1. Physical chemical characteristics and HPLC analysis for raw OMW.

Table 1 shows the typical characteristic parameters of OMW used in this experiment. This OMW is characterized by an acidic pH value (4.07). Due to this relatively low pH value, biological treatment might be limited by the low ability of microorganisms to grow in like pH. The electrical conductivity was high (16 $mS.cm^{-1}$)which could be related to the occurrence of salting practices before olives extraction. OMW contains also high contents of substantial organic matter content (total COD 170 $g.L^{-1}$), phenolic compounds(8.07 $g\ L^{-1}$) and total suspended solids (5 $g.L^{-1}$)which are known to be highly phytotoxic.In addition, **Table 1**shows that this effluent possesses a great concentration of valuable nutrients phosphorus, calcium which would be useful in irrigation after effluent treatment. **Figure 2** represents the HPLC chromatograms obtained for the analyzed OMW. As the main phenolic compounds depicted in our effluent, we cited hydroxytyrosol (848 $mg.L^{-1}$), oleuropein (399 $mg\ L^{-1}$), quercetin (199 $mg.L^{-1}$), tyrosol (175 $mg.L^{-1}$) and caffeic acid (7.6 $mg\ L^{-1}$).

Table 1. Physicochemical characteristics of raw OMW

Parameters	Units	Content
pH		4.07
Electrical Conductivity	*$mS.cm^{-1}$ at 20 °C*	16.00
Total COD	$g_{O2}\ L^{-1}$	170.23 ± 5.36
DissolvedCOD	$g_{O2}\ L^{-1}$	51.07± 1.96
Total suspended solids (TSS)	$g.L^{-1}$	4.53± 0.06
TKN	$g\ L^{-1}$	2.60±0.10
NO_3^-	$mg\ L^{-1}$	251.67±3.06
NO_2^-	$mg\ L^{-1}$	14.37±0.55
Total phenols	$g\ L^{-1}$	8.07±0.38
Total P	$g\ L^{-1}$	0.25±0.02
PO_3^{2-}	$g\ L^{-1}$	0.11±0.04
Ca^{2+}	$g\ L^{-1}$	0.50±0.01
Na^+	$g\ L^{-1}$	1.02±0.01
Cl^-	$g\ L^{-1}$	1.50±0.01

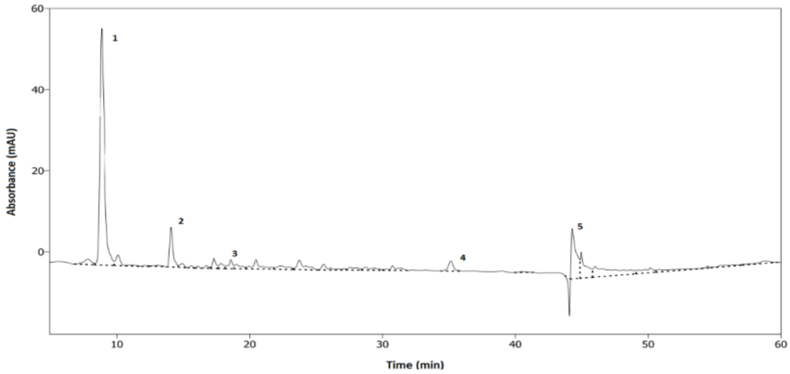

Figure 2. HPLC chromatograms of the phenolic compounds from raw olive mill effluent
(1: Hydroxytyrosol, 2:Tyrosol, 3:Caffeic acid, 4:Oleuropein, 5: Quercetin)

3.1. Physical chemical characteristics and HPLC analysis for raw OMW.

The **Figure 3** shows the change in MLSS during the increase of OMW percent in the bioreactor.At the first biomass contact with OMW (5% and 10%), the MLSS concentration was maintained at a similar concentration (2.59 g L^{-1}) likeduring the stage of synthetic substrate loading.As it can be seen the biomass was kept stable despite the change of the substrate composition to OMW. Simultaneously, the biomass activity was noted at 12 $mg_{O2}g_{MLVSS}^{-1}$ h^{-1},confirming the stable physiological state of the biomass at this stage of the treatment (**Table 2**).Afterwards, the MLSS levels increased slightly in the bioreactor from 2.62 $g.L^{-1}$to 4.32 $g.L^{-1}$and remained constant around this last value all over the stage of 20%, 30% and 40% of OMW loading. However, the biomass activity denoted a gradual decrease from 12 $mg_{O2}g_{MLVSS}^{-1}$ h^{-1} to 6 $mg_{O2}g_{MLVSS}^{-1}$ h^{-1}, showing that OMW at this period was less assimilated by the biomass. The low biomass activity indicated that the biomass was under endogenous respiration to provide the necessary energy for cells survivability and their protection from the harsh environment.After increasing the OMW loading between 50% and 70%, the biomass amount still important and stable (around 4 $g.L^{-1}$), with the occurrence of some temporary drop of its amount after each increase of OMWmass ratio.Even though the slight biomass drop obtained between the different OMW loading (50% to 70%), the values of respiration activity,which again restarted to increase reaching the SOUR control, showed that the biomass was in a good state and behavior. It could be saidthat the biomass developed as the adaptation took place enough resistance which enabled its more tolerance to this low biodegradable effluent.By the end of the experiment (90% to 100%), the MLSS concentration reached a high and constant value of 6.79 $g.L^{-1}$.

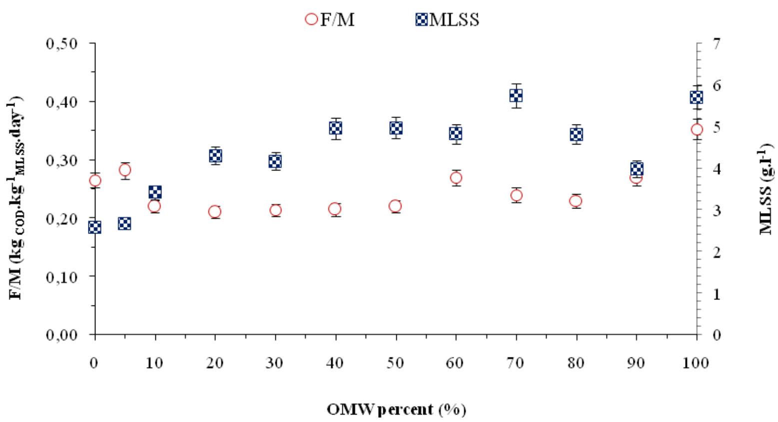

Figure 3. Evolution of MLSS and F/M versus OMW percent.

Table 2. Specific oxygen uptake rate evolution depending OMW percent

OMW% as mass ratio	COD influent (mg.L-1)	F/M ($kg_{COD}.kg_{MLSS}^{-1}.d^{-1}$)	SOUR ($mgO_2.gmlvss^{-1}.h^{-1}$)
0	466.47	0.27	11.65
5	499.69	0.28	11.17
10	449.63	0.22	12 .32
20	495.14	0.21	9.72
30	445.63	0.21	7.84
40	523.81	0.21	5.86
50	689.18	0.22	6.26
60	794.83	0.27	10.95
70	812.20	0.24	12.18
80	680.75	0.23	9.86
90	656.05	0.27	11.02
100	1171.05	0.35	11.58

3.3. Treatment performances

COD and phenolic compounds removal performances during the treatment are presented in **Table 3**. As regard to COD removal, the biomass showed generally a good response to the variation of OMW concentration during time leading to high treatment efficiency of this effluentwith an average of 86.81 %. However, slight drops (10%) in the percentage of COD removal have occurred during the high COD influent loading (between 40and 70% of OMWas mass ratio F/M) reflecting temporal stressful conditions due to the increase of OMW toxicityin the aeration tank.

Table 3. COD and phenolic compounds removal versus OMW percent

	COD (mg L^{-1})			Phenolic compound (g L^{-1})		
OMW%	**Influent**	**Effluent**	**Removal**	**Influent**	**Effluent**	**Removal**
0	466.47	60.20	87.1%	ND	ND	-
5	499.69	26.06	94.8%	0.003	ND	-
10	449.63	37.35	91.7%	0.0050	ND	-
20	495.14	46.91	90.5%	0.0090	0.0041	54.4%
30	445.63	83.39	81.3%	0.0091	0.0040	56.0%
40	523.81	49.51	90.5%	0.0297	0.0056	81.1%
50	689.18	88.60	87.1%	0.0267	0.0017	93.6%
60	794.83	143.33	82.0%	0.0270	0.0047	82.6%
70	812.20	163.59	79.9%	0.0270	0.0037	86.3%
80	680.75	105.54	84.5%	0.0320	0.0039	87.8%
90	656.05	90.937	86.1%	0.0440	0.0066	85.0%
100	1171.05	140.00	88.0%	0.0440	0.0061	86.1%

Table 3 shows also the removal of phenolic compounds over the whole of the experiment.During the operation time, phenolic compounds removal efficiency increased from 54% to 93% highlighting the development of a new generation of microorganisms during the adaptation periodable to degrade efficiently the toxic phenolic compounds of OMW. Furthermore, HPLC analysis performed onthe feed and on the permeate of AS outlet has showed a removal ofhydroxytyrosolandtyrosol the majorphenolic compounds present in the inlet. The obtained AS performance to treat OMWwas compared to those of other advanced processes in the literature.**Table 4**shows that the higher treatment performances were achieved using an AS process than by the others physicochemical [27-28]or biological processes[29]. In addition, the present study carried out in AS gave interesting results in term of COD removal comparedwith Submerged Bioreactor (SBR) from the same family of biological processes treatment[30].This higher COD removal rateobtained in the present could be as result of the application of a mixture of glucose easily biodegradable and COD from OMW slowly biodegradable which helped the grow up over the treatment of adapted OMWmicroorganisms with very speeding behavior toward the elimination of organic compounds. The use of only a diluted OMWin the case of SBR study could explain the difference between the two studies results.

Table 4. COD and phenol removals for OMWW treatment with different processes

Applied process	Initial COD ($g\ L^{-1}$)	COD removal (%)	Phenol removal (%)	references
Electrochemical	1.5	35	-	*[27]*
Photocatalytic	1.4	65	-	*[28]*
Photofermentation	2.8	53	-	*[29]*
SBR	0.4	60	-	*[30]*
AS	1.5	86	85	*This study*

3.4. Extracellular polymeric substance (EPS) production and composition

Soluble microbial products (SMP) produced by microorganisms are of great interest for biological wastewater treatment as they impacted the efficiencyof the treatment and the system stability[31]. Also, they play a crucial role in protecting the microorganisms by reducing chemical exposure and stabilizing the cells membrane against the harsh environment[18-19]. **Figure 4** shows theevolution ofsoluble microbial products (SMP) release depending OMWpercent input in the influent (substrate vat), in the biological reactor of ASPP (aeration tank) and in the effluent (output sedimentation tank). Polysaccharides, proteins, and humic substances represent the principalcomponents of SMP. The results showed that principals SMP released by the biomass in the aerobic tank are proteins and polysaccharides while the humic substances have as principal source OMW. **Figure 4** (a) and (b) illustrates the concentrations of proteins and polysaccharides SMP fractions in the aeration tank before and after adding OMW in progressive mass ratio. The concentration of SMP proteins released in the aeration tank before injecting OMW was high (17 $mg.L^{-1}$); after that they were stabilized at a value 8 $mg.L^{-1}$at the end of the period of synthetic substrate feeding (figure 4 (a)). The high concentration of proteins produced in the beginning of the experience indicated that the biomass was in a period of the adaptation to the surrounding environment [32].We can use the value of 8 $mg.L^{-1}$ as a control concentration in the presence of an easily biodegradable substrate. When the feeding substrate was changed from glucose to OMW.an increase of 25% was detected in the protein concentrations with increasing the OMW mass ratio from 5 to 70%; after that their concentration decreased significantly to 70%using 90 and 100% of OMW as mass ratio F/M. The results show an increase in the concentrations of SMP proteins as a result of OMW effects on

microorganisms which generate high amounts of these released compounds in the aeration tank as a response to the harsh environment. Several studies showed similarly a substantial production of extra polymeric substances during the biomass exposure to toxic wastewaters[33,34]. However, the significant decrease of these compounds despite the further increase of OMW mass ratio (90% and 100%) demonstrated that the biomass has developed more tolerance to this toxic effluent over the time. The produced SMP protein fraction seems have a positive impact in the formation of strong bioflocs enabling efficient pollutants elimination (i.e polyphenols and COD) and OMW treatment. This could be confirmed by the optimal COD and polyphenols removal obtained (**Table 3**). Concerning the fraction of SMP polysaccharide (figure 4 (b)), the great part measured of these compounds in the aeration tank has no longer as a source the biomass activity. Moreover, the results show all over the period of the OMW feeding a constant production of polysaccharides in the aeration tank (15 mg.L^{-1}) which is a similar to the obtained value (14 mg.L^{-1}) during the use of a biodegradable substrate.As it can be seen, the polysaccharide production remained constant at 15 mg.L^{-1}whatever the substrate composition glucose or OMW.

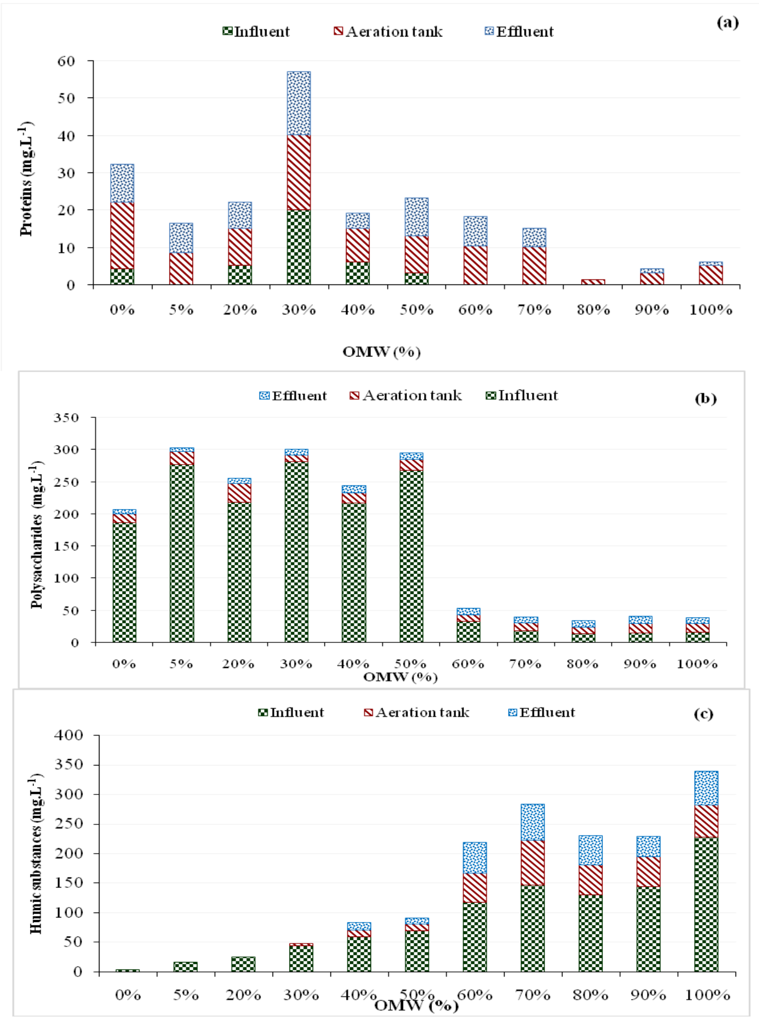

Figure 4. Soluble microbial products (SMP) release during time versus OMW%,

(a) Proteins. (b) Polysaccharides and (c) Humic substances

4. Conclusion

The research presented here assessesthe soluble microbial productsformation and treatment performances in activated sludge pilot plant treating OMW. The obtained results from this experimental study reveals an optimal response of the

biomass confirmed by the important growth, the respirometric test monitoring, treatment performances in COD and phenols abatement of about 95% and 93%, respectively. In addition, HPLC analysis confirms the important abatement of major phenolic compounds,hydroxytyrosolandtyrosol, present in high concentration in the influent. Furthermore, the analysis of SMP formation showed that the microorganisms released higher amounts of SMP (proteins and polysaccharides) as a response of the increase of OMW feedings percent F/M.

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