Development of a high-rate thermophilic UPBR reactor for the treatment of three-phase olive mill wastewater (OMW)

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ABSTRACT

Purpose: Olive mill wastewater (OMW) is a by-product of the three-phase olive oil extraction process, displaying a serious environmental riskdue to its high content in organic constituents and phenolic compounds. This work was focused on the development of a high-rate thermophilic (55°C) anaerobic digester, able toremove a high percentage ofthe OMW contained organic load by efficiently converting it to valuable biogas.

Methods: The proposed bioreactor is an UpflowPacked Bed Reactor(UPBR) with recycling stream, filled with commercial biomass carriers appropriate for colonization by anaerobic consortia. A hydrodynamic study of the UPBR was performed under abiotic conditions, using NaF solution as tracer, in order to simulate its hydraulic behavior to idealtheoretical continuous reactor models. An anaerobic microbial inoculumwas acclimatized under thermophilic conditions in a draw-and-fill reactor operating at a hydraulic retention time (HRT) of 30 days. The acclimated culturewas then filled into the UPBR reactorand its performance was tested against a feed with diluted (1:1) twice-centrifuged OMW under thermophilic conditions (55°C).

Results: The reactor's hydrodynamic characterization exhibited its CSTR operational tendency, except in the case of using packing material without recycling. During the acclimatization period a constant biogas production was observed. The acclimated culture was filled in the UPBRand thermophilic anaerobic treatment of diluted solids-free OMW was realized operating at HRT of 25 and 14 days.

Conclusion:A UPBR reactor was hydrodynamically characterized and successfully operated with an acclimated anaerobic microbial culture under thermophilic conditions using a solids-free OMW feeding stream. The maximum measured CH_4 production at the HRTs of 25d and 14d was 0.50 L/L_R.d and 0.70 L/L_R.drespectively.

Keywords : Hydrodynamic characterization, olive mill wastewater, thermophilic anaerobic digestion, upflow packed bed reactor, tracing

INTRODUCTION

Olive mill wastewater is generated as the main by-product of the three-phase olive oil extraction process.This waste product displays a serious environmental risk, especially in the Mediterranean, due to its high content in organic constituents, phenolic compounds, chemical oxygen demand (COD),suspended solids,lipids and their recalcitrance to biodegradation as well as acidic pH and possibility of pathogens' existence [1].The lack of efficient and cost-effective technologies to treat OMW has resulted to direct discharges of enormous amounts of this agro-waste being disposed of directly into sewer systems and water streams or concentrated in ponds until itsdrying throughout the summer season. This discharge causes not only phytotoxicity to the land but also pollution and anoxic phenomena to the water bodies, despite the fact that such disposal methods are prohibited in many Mediterranean countries [2].

Up-to-date various systems have been proposed in the international literature for OMW treatment employing biological, physicochemical, and combined processes [3]. Such methods include aerobic [4] and anaerobic [5] bioprocesses, physicochemical methods (flotation and settling, coagulation, flocculation, filtration, sedimentation, dilution, oxidation using ozone and Fenton reagent, open evaporating ponds and incineration) [6], use of membrane systems [7], chemical and electrochemical treatments [8] and manufacturing into animal food [9] among others. However, because of its high organic content, the majority of research conducted on biological (since it's more environmental friendly) OMW treatment has been focused on the development and use of anaerobic processes and bioreactors that are promising to remove high organic loads, while a gaseous biofuel (biogas) is simultaneously produced. High-rate processes are usually aimed at [10] in view of reducing the anaerobic reactor size and thus capital costs, while a better utilization of the useful volume is attempted. Among the high-rate processes developed in recent years, the UpflowPacked Bed Reactorwith recycling stream (UPBR) is probably one of the most commercially successful ones having seen worldwide application, especially for the wastewater treatment [11]. Microorganisms are forming dense biofilm layers on the filling material into the UPBR reactor, succeeding thus increased biomass concentrations with high bioactivity. Nevertheless, most of the systems are designed based on empirical parameters in order to achieve a form of biomass self-control and stability of operation. Studies of basic parameters,transport phenomena and hydrodynamic characteristics can contribute to simulate a reactor to the known standard continuous reactors, such as the Plug Flow Reactor (PFR) and the Continuous Stirred Tank Reactor, also known as CSTR, in order to succeed a more precise design of the up-scaled reactor.

The aim of this study was at first the development of a UPBR reactor, its hydrodynamic characterization and then the study of its anaerobic performanceunder thermophilic conditions (55^{o}C) when treating solids-free OMW originating from a three-phase olive oil extraction process. Thermophilic conditions were targeted to because of the anticipated acceleration of biochemical reactions at higher than ambient temperatures and the higher efficiency in organic matter degradation and destruction of possible existing pathogenic organisms compared to conventional systems [10]. The results of the present study would be then compared to the performance of a similar reactor operating under mesophilic conditions which was also successfully deployed in treating 3-phase OMW [12].

MATERIALS AND METHODS

Experimental set-up

The UPBR reactor was designed according to [13] and constructed, using Plexiglas, with a double wall through which hot tap water was being circulatedserving as heating jacketfor maintaining the reactor's temperature at thermophilic conditions (55$\pm$0.5^{o}C). The total and working volume of the reactor was 6.2 L and 6.0 L respectively.However, the working volume decreasedto 5.0 L when the reactor was packed with K5 biomass carriers (with protected surface of 800 m^2/m^3) kindly provided by Anoxkaldness (Lund, Sweden). Whenever recirculation of treated effluent was applied the recycling flow rate was set at 6L/h. The schematic diagram of the experimental set-upincorporating the UPBR is depicted in Fig. 1.

Microorganisms' acclimatization to thermophilic conditions

The effluents from a mesophilic (37^{o}C) UASB reactor, which was also successfully deployed in treating 3-phase OMW were centrifuged twice at 4.000 rpm for 15 minutes. Afterwards, the settled sludge was put into a 3L Erlenmeyer flask which was filled with effluent liquid from the mesophilic UASB reactor, was closed with a rubber stopper and maintained at constant thermophilic temperature (55$\pm$0.5^{o}C) in a water bath. For the first two days the Erlenmeyer flask was kept without any feeding, although biogas production was observed. A periodical feeding of the flask with diluted (1:1 v/v with tap water) twice-centrifuged OMW was then initiated maintaining an HRT of 30 days in order to avoid any microbial acidic stress which could be caused by accumulated VFAs due to incomplete fermentation and unbalanced anaerobic digestion. The flask was continuously operated as draw-and-fill reactor by removing the respective volume from the reactor and then feeding the same diluted OMW volume until steady-state conditions were achieved. The volume of produced biogas was measured using a tailor-made device, while its composition was determined by gas chromatography (7890A GC system by Agilent technologies).

Feedstock

Fresh OMW was used in this study obtained from a three-phase olive mill located in Patras (Achaia, Western Greece). Due to the seasonal production of OMW and its natural tendency for fermentation the collected OMW was stored in plastic vessels in the freezer (-18^{o}C). After thawing, the raw OMW was centrifuged twice for 15 min at 4.000 rpm followed by removal of the precipitated sludge. The supernatant was diluted with tap water (1:1) and used as feeding substrate in the UPBR reactor.

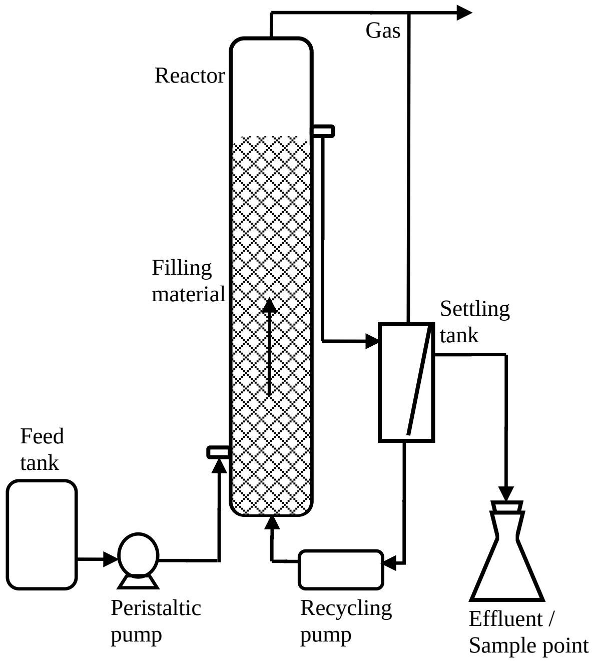

Fig. 1: Schematic diagram of the experimental set-up used in this study

Reactor start-up and operation

During start-up the UPBR reactor was seeded up with all the acclimated sludge from the draw-and-fill reactor, was filled with the treated effluent from a similar mesophilic UASB reactor treating the same diluted OMW and left for two days without any feeding.The recycling pump was however activated aiming to assist the added microorganisms to distribute as uniformly as possible within the reactor's internal space and adhere to the packing material. The system's operation was then initiated using an HRT of 25d. Afterreaching steady-state conditions the HRT was decreased to 14 days, while samples were taken periodically throughout its operation and chemical analyses were performed.

Analytical methods

The physico-chemical characterization of raw and twice-centrifuged OMW as well as the chemical analyses of the reactor samples were performed according to APHA [14]. For the determination of carbohydrates, a colored sugar derivative was produced through the addition of L-tryptophan, sulfuric and boric acid, which was subsequently measured colorimetrically in a Cary 50 UV/VIS spectrophotometer (Varian) at 520 nm [15]. Total phenolic compounds were determined spectrophotometrically in centrifuged and filtered samples according to the Folin–Ciocalteu method [16], while pH measurements were taken using an Orion 3-Star electrode.The biogas production in the biomass acclimatization reactor as well as in the UPBR was measured separately by a tailor-made hybrid gas meter configuration, based on the description given by Angelidaki et al [17]. Biogas composition and volatile fatty acids (VFA) analysis were performed throughout the experimentation period as reported by Dareioti et al. [18].

Fluoride ions (F^-) that were used as tracer in the hydrodynamic study were measured in a DIONEX IC-3000 ion chromatography system using a thermostated (30ºC) DionexIonPac analytical column (AS19 length 4x250 mm and 7.5 mm I.D), a guard column (4x50 mm length and 12 mm I.D.) and an electron conductivity detector (Dionex). Analysis was performed by elution gradient with KOH solution as mobile phase at flow rate of 1 mL/min. The eluent gradient was programmed to result in a 20 mM KOH solution during equilibration and analysis and a 50 mM KOH solution during column regeneration. The total running time of analysis was 20 min, and the gradient programme was scheduled as follows: 20 mM KOH for 8 min, 50 mM KOH in 3 min and maintained for 4 min and 20 mM KOH in 0.5 min until the end of running (20 min). The injection volume was 10 μL.

Hydrodynamic study

The UPBR reactor hydrodynamic characterization was carried out under abiotic conditions by implementing stimulus-response experiments, using the apparatus schematically shown in Fig. 1, for evaluating the residence time distribution (RTD) curve of a tracer and the parameters of simple hydrodynamic mathematical models. Tracer studies under biotic conditions are usually subjected to interferences due to toxicity and tracer retention or consumption by the biomass [19] and thus were avoided, at least in this part of the study.ANaF solution with standard concentration (39.8 g/L) was used as tracer. The selected tracer fulfills the characteristics suggested by [20], i.e. should not affect the flow, be characterized by a low molecular diffusivity, be injected quickly and be conveniently analyzed by a suitable method, stay inert, stable and non-absorbable throughout its stay inside the reactor. Prior to tracer addition the UPBR reactor was filled with ultrapure water and kept under thermophilic conditions (55^{o}C) throughout the experimentation period operating at constant HRT of 1.4 days. The volume of tracer solution was selected to be small enough (10 ml) in respect to the total liquid volume of the reactor so that its pulse injection (within approximately 3 seconds) into the feed stream of the UPBR reactor to simulate an ideal pulse. Moreover, the measured fluoride ion concentrations using an ionic chromatograph was thus above its detection limit and within its calibration curve enabling the recording of the response from the stimulus.Samples from the effluent stream were collected at regular time intervals (1h) to measure the concentration of F^- ions.The reactor's hydrodynamic behavior was tested both in the absence and presence of biomass carriers, with and without the operation of recycling stream. The different modes of operation that were testedare summarized in Table 1.

Table 1: Experimental conditions applied during the hydrodynamic study of the UPBR reactor

Experiment	A	B	C	D
Experimental conditions	– recycling stream – biomass carriers	+ recycling stream – biomass carriers	– recycling stream + biomass carriers	+ recycling stream + biomass carriers

The measured fluoride concentrations were then transformed into experimental functions of residence time distribution (RTD) versus the dimensionless time (θ) following the determination of the terms shown in Table 2. Afterwards, the RTD curves were compared to the corresponding functions of twotheoretical reactor models of PFR, i.e. with high and low dispersion, and the N-CSTR in series model in order to identify the closest theoretical model to the UPBR reactor. The term 'dispersion', was used to represent the combined action of all phenomena, namely molecular diffusion, turbulent mixing and non-uniform velocities [20], while the N-CSTR in series model simulated the reactor by N ideal stirred tanks in series [19]. The three theoretical single-parameter models used for the hydrodynamic study of the UPBR are shown in Table 3.

RESULTS

Hydrodynamic study

The concentration curves of the fluoride ion against time obtained from the UPBR reactor, when each one of the experimental conditions shown in Table 1 were implemented, are depicted in Fig. 2. Long tail experimental curves were observed, reflecting the slow decrease of the tracer concentration in the effluent. As could be expected there is a close correlation between the long tailing at the response curve and the fitting of the theoretical models to the data.

Despite the use of biomass carriers in experiment D, it is important to notice that a similar response curve was also obtained in experiment B (no biomass carriers present), the graph of which differs from graph D only at the point of maximum concentration. Specifically, the maximum F^- concentration observed in experiment B (no biomass carriers) was 23.9 mg/L whereas the maximum fluoride concentration observed in experiment D was

29.9 mg/L. The ratio of these maximum concentrations is closely related to the ratio of the reactor's working volume in these two cases, i.e. 5L working volume in the presence of biomass carriers versus 6L in their absence (empty reactor), respectively. It can thus be concluded that a uniform solution of the tracer in the whole reactor volume is rapidly created into the reactor in both experiments because of the intense recycling.The maximum concentration of the tracer gradually decreases as pure water enters the reactor via its feed. As far the A and C experiments are concerned, similar response curves were also obtained but of different magnitude roughly proportional to the working volume ratio with and without the presence of biomass carriers into the reactor.

Table 2: Definition of the variables used for obtaining residence time distribution function (E_θ) against dimensionless mean residence time (θ) [19]

Variable	Definition	Variable	Definition
S	$\sum Ci * \Delta ti$	E_θ	$t_R * Ei$
E_i	Ci/S	σ^2	$\frac{\sum ti^2 * Ci * \Delta ti}{\sum Ci * \Delta ti} - t_R^2$
t_R	$\frac{\sum ti * Ci * \Delta ti}{\sum Ci * \Delta ti}$	σ_θ^2	σ^2 / t_R^2
θ	t/t_R		

Table 3: Single parameter hydrodynamic theoretical models [19]

Model	Parameter	Equation
PFR with low dispersion	$Pe_l = 2/\sigma_\theta^2$	$E_\theta = \frac{1}{\sqrt{4\pi/Pe_l}} e^{\left[\frac{-Pe_l(1-\theta)^2}{4}\right]}$
PFR with high dispersion	$\sigma_\theta^2 = \frac{2}{Pe_h} + \frac{8}{Pe_h^2}$	$E_\theta = \frac{1}{\sqrt{4\pi\theta/Pe_h}} e^{\left[\frac{-(1-\theta)^2}{4\theta/Pe_h}\right]}$
N-CSTR in series	$N = 1/\sigma_\theta^2$	$E_\theta = \frac{N(N\theta)^{N-1}}{(N-1)!} e^{-N\theta}$

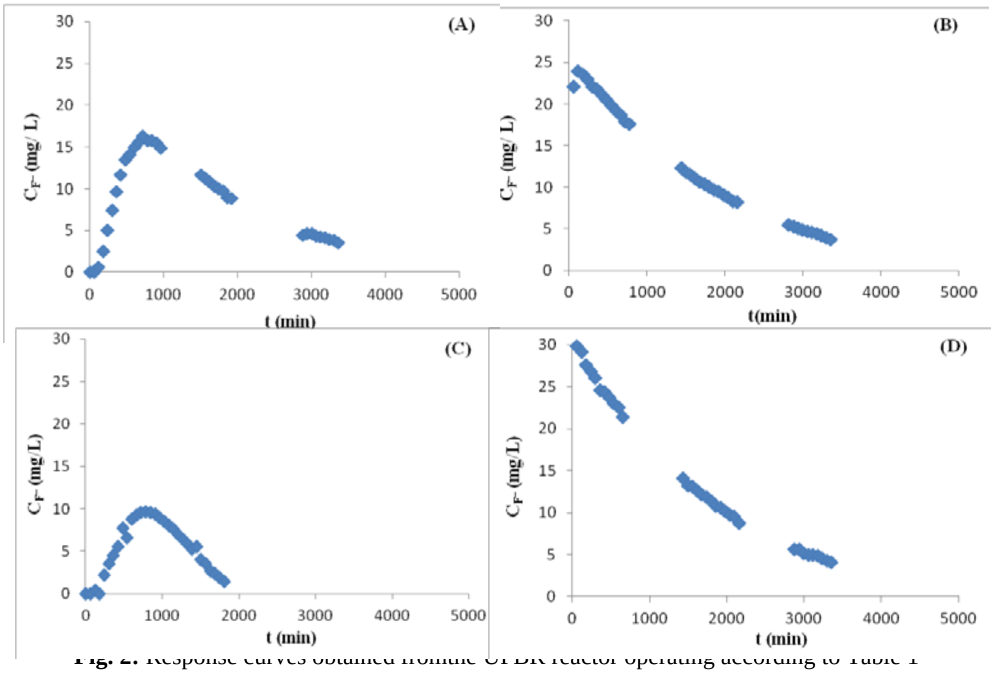

Fig. 2. Response curves obtained fromthe UPBR reactor operating according to Table 1

The experimental residence time distribution (RTD) curves obtained from each experiment as well as the adjusted single-parameter mathematical models are presented in Fig. 3. Pe_l and Pe_h represent the Peclet number for low and high dispersion respectively, while the parameter N shows the number of tanks in series in case of CSTR behavior. The single-parameter determination for each mathematical model was carried out according to Table 3 although bad correlation was obtained between the high and low dispersion theoretical models and experimental data for all the experiments. On the contrary, a simulation tendency to the N-CSTR in series model is obvious in all cases. Estimating the theoretical N according to Table 3 resulted to a close but very rough approximation of experimental data when compared with the response curves of N-CSTR in series model (Fig. 3).

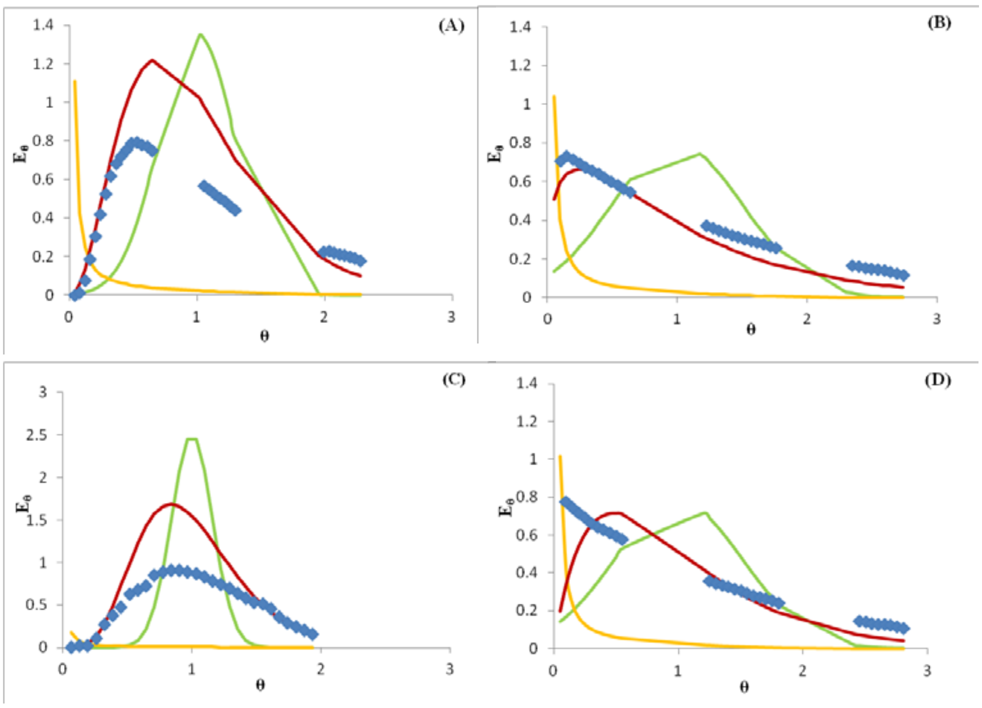

Fig. 3: RTD curves obtained experimentally usingNaF solution as tracer, Experimental E_θ, High dispersion, Low dispersion, N-CSTRs in series

However, when the number of N tanks in series was calculated as the integer number (single-parameter) that minimizes the residuals between the theoretical model of $E_\theta$versus the dimensionless time (θ), as describedin Table 3, against the experimental data then the obtained RTD curves of the adjusted single-parameter mathematical models exhibited an improved performance in all cases as illustrated in Fig. 4.Table 4 presents the mean residence time obtained from the RTD curves (t_R), the parameter values calculated from the theoretical mathematical models(Table 3), as well as the estimated N following the minimization of residuals (Least Squares Fitting, LSF) and the correlation coefficient for the last case.

Table 4: Parameters of the adjusted theoretical models obtained for each experiment in the UPBR reactor subjected to the operating conditions shown in Table 1

Experiment	t_R(min)	Pe_l	Pe_h	N (theoretical)	N (LSF)	Correlation coefficient
A	1475	22.81	9.58	3.38	3	0.602
B	1226	7.82	6.42	1.98	1	0.289
C	911	26.70	10.18	3.65	5	0.277
D	1198	7.51	6.33	1.94	1	0.475

Analysis of the obtained results for the different values of N shows that N= 3, 1, 5 and 1 respectively, were the closest values for the N-CSTR in series theoretical model. Specifically, experiments B and D (both with recycling stream, with and without filling material respectively) exhibited an almost ideal CSTR operation. Although the reactor was packed with biomass carriers (experiment D), complete mixing of the reactor's working volume was taking place continuously, almost as it happened in experiment B. However, the main factor affecting the reactor's hydrodynamic behavior was the operation of the recycling stream. In the case of experiment A the estimated N value was 3. In this case, PFR tendency was predicted as the reactor exhibited a turbulent mixing behavior. Nevertheless the UPBR reactor could not be characterized as PFR yet due to the small N value, but mostly as a non-ideal mixer. However, the highest PFR tendency was observed during the experiment C, where an N value of 5 CSTR tanks in series was estimated. In this case, the presence of biomass carriers and the simultaneous absence of recycling resulted to a rather tubular flow scheme without internal mixing (low axial dispersion). In all experiments, however, the fitting of the N-CSTR in series theoretical model to the experimental data was not perfect as also indicated by the low correlation coefficient shown in Table 4 and the graphs in Fig. 4.

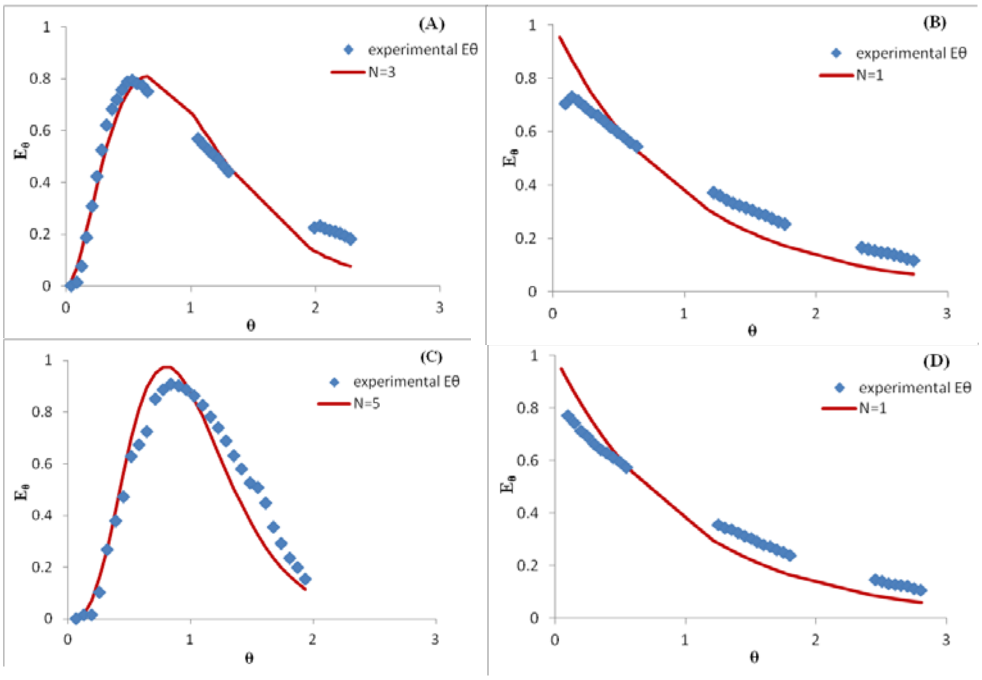

Fig. 4: Simulation of experimental data with the N-CSTR in series RTD theoretical model setting N values calculated via Least Squares Fitting.

Microorganisms' acclimatization to thermophilic conditions

A mixed microbial culture was collected via double centrifugation of the effluents from a mesophilic (37^{o}C) UASB reactor and was then kept into a 3L Erlenmeyer flask covered with effluent liquid from the mesophilic UASB reactor. The culture was maintained under constant heating at 55^{o}C without any feeding for two days. The aim was, at first, to identify the existence of a thermophilic inoculum and then monitor its behavior and verify its acclimatization to thermophilic conditions. The culture was fed for over 70 days, in a *draw-and-fill* mode, with 100 ml/d diluted (1:1) twice-centrifuged OMWin order to achieve an HRT of 30 days. The characteristics of the raw OMW and its derivative produced via double centrifugation are shown in Table5.

Figure 5 presents the evolution of the biogas volume, the methane volume, the specific (per volume of feed) methane production as well as the culture's pH. Products of incomplete fermentation (acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic acid) were not detected throughout the whole experimentation period. These graphs verify not only the existence of thermophilic anaerobic microorganisms but also their constant activity towards OMW bioconversion to biogas. The experimental data depicted in Fig. 5 were considered promising for testing the culture's performance in a scaled-up reactor (5L UPBR) with decreased HRTs.

Table 5: Characterization of raw OMW and produced OMW after double centrifugation

Parameter	Unit	Raw OMW	Twice-centrifuged OMW
pH	-	5.13	5.23
Alkalinity	g $CaCO_3$ L^{-1}	0.750	0.725
Total Carbohydrates	g L^{-1}	37.5±1.70	24.95±0.07
Diss. Carbohydrates	g L^{-1}	25.95±0.32	23.12±0.28
Total phenols	g syringic L^{-1}	8.61±0.98	7.74±1.05
Total COD	g O_2 L^{-1}	169±9.24	128.92±10.66
Dissolved COD	g O_2 L^{-1}	89.23±3.93	74.79±2.65

Total Phosphorus	g L^{-1}	0.513±0.001	0.459±0.007
Diss. Phosphorus	g L^{-1}	0.309±0.004	0.2563±0.0005
TKN	g L^{-1}	0.735±0.01	0.0784±0.004
Ammonium N	g L^{-1}	0.086±0.005	0.030±0.002
TSS	g L^{-1}	40.56±0.59	9.9±0.99
VSS	g L^{-1}	39.93±1.11	9.86±0.92
TS	g L^{-1}	111.65±1.32	85.35±3.29
VS	g L^{-1}	82.32±6.77	63.195±2.64
Oils and Grease	g L^{-1}	21.98±4.02	11.53±2.26

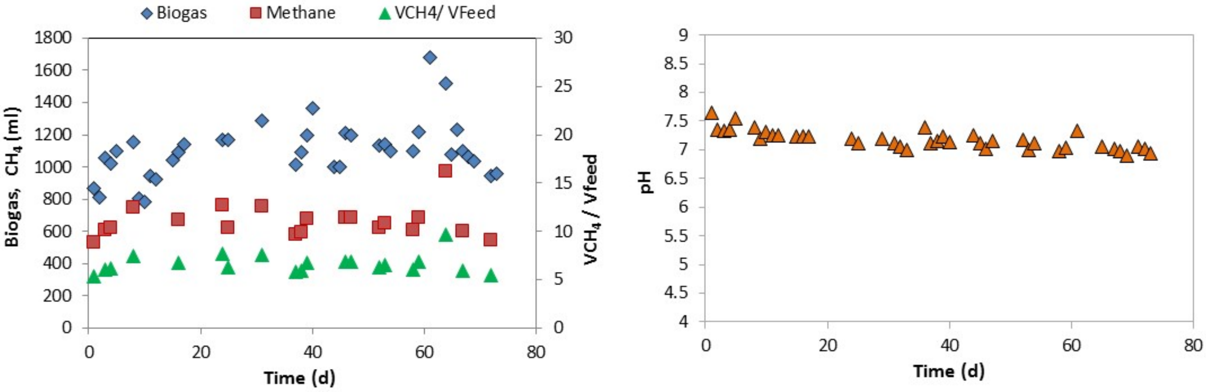

Fig. 5: Evolution of produced biogas and methane, the specific methane production and the pH of the culture during the microorganisms' acclimatization.

Thermophilic anaerobic treatment of OMW using the UPBR reactor

The acclimated thermophilic culture in the draw-and-fill reactor as well as an extra amount of new mesophilic sludge, which was concentrated in the same way as the acclimated inoculum (double centrifugation of the effluents from the same mesophilic UASB reactor), were inserted into the UPBR reactor. The reactor's HRT was set at 25 d and operated for a period of 72 days fed with diluted (1:1) twice-centrifuged OMW before reaching steady-state conditions (Fig. 6). Afterwards, the HRT was decreased to 14d for a period of 31 days and the system performance was monitored. At the beginning of the decreased HRT phase, there was an increase in biogas and methane production, although lower than anticipated, most probably due to the increased concentration of phenolics in the feed (Fig. 7). This could be also attributed to the fact that the microorganisms were facing a sudden increase of organic loading which caused a temporary overproduction of biogas while, afterwards, the same reason (increased loading) caused a mild inhibition realized as biogas production decrease. During the operation at HRT of 25d the maximum methane productivity reached up to 12.3 L CH_4/L_{feed} or 0.50 L $CH_4/L_{R.}$d. With the decrease of HRT to 14d, the methane productivity per fed volume of OMW decreased to 10.3 L CH_4/L_{feed} although the reactor volumetric productivity increased to 0.7 L CH_4/L_R.d.

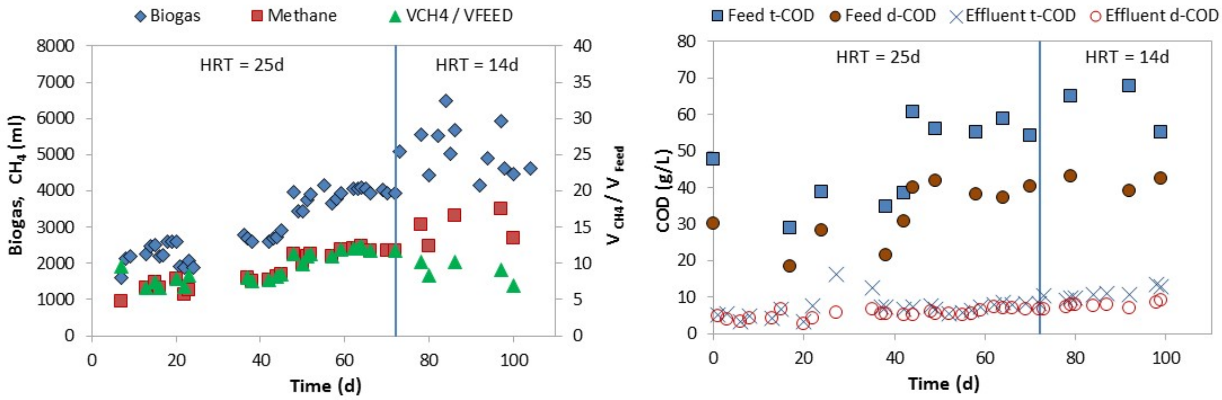

Fig. 6: Evolution of produced biogas and methane and specific methane production per liter of feed as well asCOD concentration in the influent and effluent for both HRTs.

In both operational phases, the pH of the reactor ranged in an average of 7.2-8.1, while $NaHCO_3$ was also added in some cases in order to sustain the reactor alkalinity close to 4 g/L. VFAs (acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic acid) were not detected throughout the reactor operation at the HRT of 25d, while the total carbohydrates fed in the reactor were practically completely removed. Low VFA levels (mainly of acetic and propionic acid, approximately 250 mg/L and 76 mg/L respectively) were occasionally detected during the operation at the HRT of 14d, while total carbohydrates were also fully removed (Fig. 7).

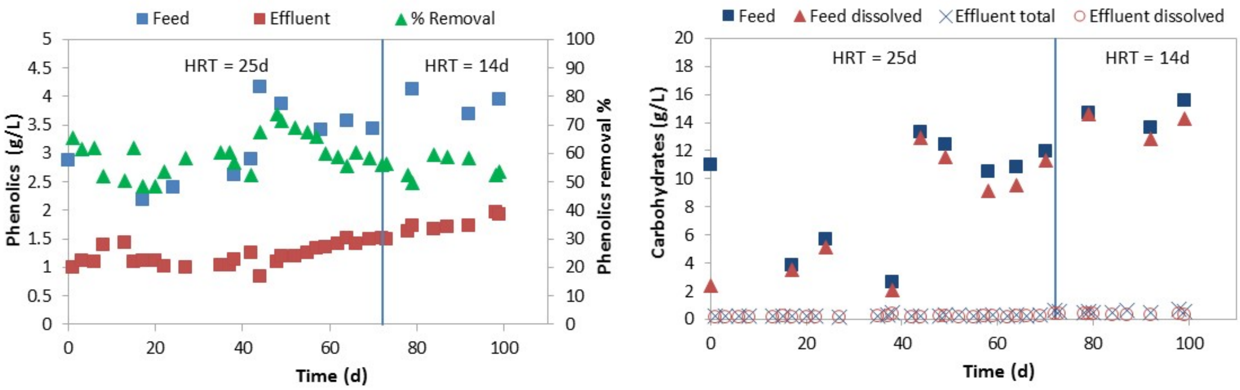

Fig. 7: Variation of phenolic compounds in the influent and effluent and their removal as well as carbohydrates concentration in the influent and effluent for both HRTs.

CONCLUSIONS

- **Hydrodynamic study**

The hydrodynamic characterization of the UPBR reactor exhibited mainly its non-ideal CSTR operation, which can however be simulated by 1, 3 or 5 N-CSTR in series, with reasonable accuracy. The UPBR's hydrodynamic behavior depends only on the recycling stream activity since the presence of filling material didn't seem to affect the characteristics of flow within the reactor. The hydrodynamic performance of the UPBR reactor could be representatively described by a CSTR under operating conditions similar to those applied in the subsequent biotic experiments.

- **Microorganisms' acclimatization to thermophilic conditions**

An active thermophilic cultureofanaerobic microorganisms originating from the effluents of a mesophilic UASB reactor could be efficiently acclimated to bioconvert diluted (1:1) OMW to biogas in a draw-and-fill system. The draw-and-fill reactor was operated at HRT of 30dexhibiting a stable performance in terms of OMW degradabilityand biogas productivity.

- **Thermophilic anaerobic treatment of OMW using the UPBR reactor**

A UPBR reactor was tested under thermophilic conditions against its treatment efficiency of diluted (1:1) OMW. The reactor was operated efficiently at two HRTs, namely 25dand 14 d. During the reactor operation sludge granulation was observed on the plastic biomass carriers (visual observation). Concerning the UPBR's efficiency in COD and phenolics removal the maximum achieved yields were 73.7% and 59.5% for the biodegradation of phenolic compounds and 93.0% and 84.9%for the total organicconstituents,when operated at the HRTs of 25d and 14d respectively. Simultaneously, the maximum measured CH_4 production at the HRTs of 25d and 14d was 0.50 L/L_R.d and 0.70 L/L_R.drespectively, while the average reached yields were 0.23 L CH_4/ g t-COD_{rem} and 0.16 L CH_4 /g t-COD_{rem}, measured at STP conditionsfor each HRT at steady-state.

Nomenclature	Symbol	Nomenclature	Symbol
Olive mill wastewater	OMW	Exit age distribution curve	E_i
Upflow packed bed reactor	UPBR	Residence time distribution function	E_θ
Plug flow reactor	PFR	Mean dimensionless time	θ
Continuous stirred tank reactor	CSTR	Exit age distribution function	E(t)
Upflow anaerobic sludge blanket	UASB	Mean residence time obtained from RTD curve	t_R
Residence time distribution	RTD	Variance	σ^2
Hydraulic retention time	HRT	Dimensionless variance	σ^2_θ
Chemical Oxygen Demand	COD	Number of tanks-in-series	N
Total Kjeldahl Nitrogen	TKN	Area under the concentration- time curve	S
Celsius degrees	^oC	Peclet number	P_e
Concentration	C	Revolutions per minute	rpm
Time	t	Days	d
Liter	L	Liter of the reactor's active volume	L_R
Volatile Fatty Acids	VFAs	Standard Temperature and Pressure	STP

REFERENCES

[1]. Sabbah, I., Marsook, T., Basheer, S.: The effect of pretreatment on anaerobic activity of olive mill wastewater using batch and continuous systems, *Process Biochemistry*, 39, 1947–1951(2004)

[2]. Stamatelatou, K., Kopsahelis, A., Blika, P.S., Paraskeva, C.A, Lyberatos, G.: Anaerobic digestion of olive mill wastewater in a periodic anaerobic baffled reactor (PABR) followed by further effluent purification via membrane separation technologies, *J ChemTechnolBiotechnol*, 84, 909-917 (2009)

[3]. Lafi, WK, Shannak, B, Al-Shannag, M, Al-Anber, Z, Al-Hasan,M.: Treatment of olive mill wastewater by combined advanced oxidation and biodegradation. *Sep.Purif. Technol.*70:141–146 (2009)

[4]. El Hajjouji, H, Fakharedine, N, AitBaddi, G, Winterton, P, Bailly J, Revel, J, Hafidi, M: Treatment of olive mill waste-water by aerobic biodegradation: an analytical study using gel permeation chromatography, ultraviolet–visible and Fourier transform infrared spectroscopy. *BioresourTechnol* 98:3513–3520 (2007)

[5]. Dareioti, MA, Dokianakis, SN, Stamatelatou, K, Zafiri, C, Kornaros,M : Biogas production from anaerobic co-digestion of agroindustrial wastewaters under mesophilic conditions in a two-stage process. *Desalination* 248:891–906 (2009)

[6]. Paraskeva, P, Diamadopoulos, E: Technologies for olive mill wastewater (OMW) treatment: a review. *J ChemTechnolBiotechnol* 81:1475–1485 (2006)

[7]. Paraskeva, CA, Papadakis, VG, Tsarouchi, E, Kanellopoulou, DG, Koutsoukos,PG: Membrane processing for olive mill wastewater fractionation. *Desalination* 213:218–229 (2007)

[8]. Un, UT, Altay, U, Koparal, AS, Ogutveren, UB. Complete treatment of olive mill wastewaters by electrooxidation. *ChemEng J.* 139:445–452(2008)

[9]. Alcaide, ME, Nefzaoui, A. Recycling of olive-oil by-products: possibilities of utilization in animal nutrition. *IntBiodeteriorBiodegrad*38(3):235–277(1996)

[10].Gannoun, H., Othman, N.B., Bouallagui, H., Moktar, H.: Mesophilic and Thermophilic Anaerobic Co-digestion of Olive Mill Wastewaters and Abattoir Wastewaters in an Upflow Anaerobic Filter, *Ind. Eng. Chem. Res.* 46, 6737-6743 (2007)

[11]. Rittmann B. E., McCarty P. L.: *Environmental Biotechnology: Principles and Applications*, McGraw-Hill, New York (2001)

[12]. Zakoura, M.,Kopsahelis, A., Kornaros, M.: Assessing the performance of high-rate anaerobic reactors treating three-phase olive mill wastewater (OMW), proceedings of the *13th World Congress on Anaerobic Digestion: Recovering (bio) Resources for the World (AD13)*,Santiago de Compostela, Spain (2013)

[13] Lettinga, G., Hulshoff, Pol.: UASB process design for various types of wastewater. *Wat. Sci. Technol.* 24(8), 87-107 (1991).

[14] APHA, AWWA, WEF,*Standard Methods for the Examination of Water and Wastewater*. 19th ed. American Public Health Association, Washington DC, USA (1995)

[15]. Joseffson, B.: *Rapid spectrophotometric determination of total carbohydrates*. In: Grasshoff, K., Ehrhardt, M., Kremling, K. (Eds.), Methods of Seawater Analysis, VerlagChemie GmbH, Berlin (1983)

[16]. Waterman P.G., Mole S.: *Analysis of phenolic plant metabolites*,J.H. Lawton, G.E. Likens (Eds.), Methods in Ecology, Blackwell Scientific Publications, Oxford (1994).

[17] Angelidaki, I., Ellegaard, L., Ahring, B.K. Compact automated displacement gas metering system for measurement of low gas rates from laboratory fermentors, *Biotechnology and Bioengineering* 39 (3), 351-353 (1991).

[18] Dareioti, A.M., Dokianakis, N.S., Stamatelatou, K., Zafiri, C., Kornaros, M.: Exploitation of olive mill wastewater and liquid cow manure for biogas production. *Waste Management* 30, 1841-1848 (2010)

[19] De Nardi, I.R., Zaiat, M., Foresti, E.: Influence of the tracer characteristics on hydrodynamic models of packed-bed bioreactors, *Appl. Bioprocess Engineering* 21, 469-476 (1999)

[20]. Kayode, C., KayodeCoker,A.:*Modeling of Chemical Kinetics and Reactor Design*,Gulf Professional Publishing (2001)