Journal of Environmental Management 345 (2023) 118643

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Respirometric tests in a combined UASB-MBR system treating wastewater containing emerging contaminants at different OLRs and temperatures: Biokinetic analysis

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ARTICLE INFO

Handling Editor: Jason Michael Evans

Keywords:
Bio-supports
Oxygen consumption respirometry
UASB-MBR
OUR

ABSTRACT

This research focuses on the application of respirometric techniques to provide new insights into the biokinetic behaviour of bacterial species developed in an Upflow Anaerobic Sludge Blanked -UASB reactor combined with a membrane bioreactor -MBR, treating urban wastewater with emerging contaminants frequently found in this kind of effluents. The lab-scale pilot plant was operated at different metabolic and operational conditions by limiting the organic loading rate- OLR of the influent. In a first stage, the MBR was performed with suspended biomass, while in a second stage bio-supports were introduced to operate coexisting suspended and fixed biomass. From the results of the microscopic monitoring of sludge, it was concluded that the decrease in OLR resulted in a greater disintegration of the floc structure, more dispersed growth, and a low presence of inter-floccular bonds. However, no effect of toxicity or inhibition of microorganisms caused by the presence of emerging contaminants -ECs was determined. Kinetic modelling was carried out to study the behaviour of the system. The results showed a slowing down of biomass degradative capacity at low OLR stages and operating at low temperatures of mixed liquor. In addition, a decrease in oxygen consumption was observed with decreasing biodegradable substrate, resulting in lower degradation of organic matter. Mean values of specific oxygen uptake rate and heterotrophic biomass yield at low OLR were $SOUR_{end} = 1.49$ and 1.15 mg $O_2 \cdot$ g $MLVSS^{-1}$ h^{-1} and $Y_{H, MLSSV\ end} = 0.48$ and 0.28 mg MLVSS· mg $COD^{-1}_{substrate}$ at stage 1 (suspended biomass) and stage 2 (suspended and supported biomass), respectively. From the analysis of the endogenous decomposition constant (k_d), a higher cell lysis was determined operating with suspended biomass ($k_d = 0.03\ d^{-1}$) in comparison to the operation coexisting suspended and supported biomass ($k_d = 0.01\ d^{-1}$). Heterotrophic biomass yield values ($Y_{H, MLVSS} = 0.48 \pm 0.06$, 0.40 ± 0.01 and 0.29 ± 0.01 mg MLVSS· mg $COD^{-1}_{substrate}$ at high, medium and low OLR) showed lower sludge production at low OLR due to the influence of substrate limitation on cell growth.

1. Introduction

Conventional wastewater treatment plants -WWTP were not designed for the removal of emerging contaminants- EC. Consequently, it is expected an early implementation of advanced wastewater treatment systems based on the combination of different technologies able to achieve a better removal of these compounds (Moya-Llamas et al., 2018; Qiu et al., 2013). UASB-MBR combined biological system is a robust and promising technology that can improve the removals of organic matter, nutrients, and emerging contaminants -EC in urban and industrial wastewater due to the synergies between both reactors. Table 1 briefly summarizes the main research on this topic.

The use of the UASB anaerobic reactor as a first stage of partial degradation of organic matter and ECs together with a complementary treatment by means of the MBR, results in a treated effluent of excellent quality suitable for reuse. Recirculation between the two reactors and high sludge retention times -SRT lead to a significant increase in the bacterial diversity developed in the reactors (Kim et al., 2012). Furthermore, according to authors such as Rios-Miguel et al. (2023), the operational parameters and redox conditions of wastewater treatment

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https://doi.org/10.1016/j.jenvman.2023.118643
Received 20 April 2023; Received in revised form 6 July 2023; Accepted 15 July 2023

Table 1
Selection of studies using the combination UASB-MBR for ECs removal.

Configuration	Type of wastewater	Contaminant	Reference
UASB-MBR	Urban wastewater	Organic micropollutants (3 PPCPs, 4 antibiotics, 3 hormones and 10 pharmaceuticals)	Alvarino et al. (2016)
	Urban wastewater	Antibiotic (berberine)	Qiu et al. (2013)
	Urban wastewater	Organic micropollutants (5 PPCPs, 3 hormones, 1 plasticizer and 2 surfactants)	Bernal et al. (2018)
	Urban wastewater	Organic Micropollutants (3 hormones, 1 plasticizer and 2 pharmaceuticals	Moya-Llamas et al. (2018)
	Industrial wastewater	Solvents, oils and chemicals	Niwa et al., 2018
	Industrial wastewater	Purified terephthalic acid (PTA)	Yen et al. (2016)
	Industrial wastewater	11 pharmaceuticals	Abdel-Shafy and Mansour (2017)

systems can also determine the ECs removal efficiencies.

However, there is a significant lack of knowledge about the behaviour of biomass growth in biological systems combining aerobic and anaerobic conditions. Therefore, it is essential to determine the main kinetic and stoichiometric parameters as well as the influence of physico-chemical parameters such as temperature or the organic load of the influent on their activity.

The development of activated sludge models -ASM has greatly contributed to the understanding and control of biological processes in wastewater treatment plants -WWTP based on conventional activated sludge systems -CAS. Particularly relevant is the Activated Sludge Model No.1. -ASM1 (Henze et al., 1987), which includes the estimation of the main kinetic parameters of aerobic biomass (Karlikanovaite-Balikci and Yagci, 2019; Espinosa-Rodríguez et al., 2012).

Mainardis et al. (2021) and Spanjers and Vanrolleghem (2016) defined respirometry as a measurement and interpretation of the biological consumption rate of an inorganic electron acceptor under well-defined experimental conditions. These techniques allow to reproduce at laboratory-scale aerobic biological treatment processes through the determination of the dissolved oxygen -DO consumed by micro-organisms to oxidize of a given organic substrate (Di Trapani et al., 2018; Espinosa-Rodríguez et al., 2012; Garcia-Ochoa et al., 2010). The oxygen uptake rate -OUR is the amount of DO per unit volume and time that is consumed by the consortium of microorganisms. Consequently, respirometry can become a key tool to assess the biodegradability of wastewater while monitoring biomass viability. (Garcia-Ochoa et al., 2010; Spanjers et al., 1996).

Two main biochemical processes are directly related to the oxygen demand of microorganisms: biomass growth and substrate consumption, both strongly dependent on the temperature of the liquor-mixture and the organic load of the influent. Temperature is a key parameter with an important impact on biomass activity. Indeed, authors such as Antiñolo Bermúdez et al. (2023), Chen et al. (2022) or Li et al. (2019) reported the close correlation of temperature with the main sludge kinetic and physicochemical parameters. Likewise, the organic loading rate -OLR of the influent has a great influence on the microbial metabolism performance of aerobic and anaerobic biomass (Moya-Llamas et al., 2018; Buntner, 2013).

The aim of this research was to provide new insights on the biomass behaviour of combined aerobic and anaerobic systems under substrate limitations (OLR) and temperature variations in the presence of emerging contaminants. For this purpose, the degradative activity of biomass was analyzed in a combined UASB-MBR pilot plant using respirometric techniques, which have widely demonstrated their great potential in the evaluation of kinetic and stoichiometric parameters by analysing DO profiles (DO concentration throughout time) (Mainardis et al., 2021).

The experimentation was designed addressing two operational stages: in the first one the aerobic biomass was suspended, while in the second one the aerobic reactor was operated with both suspended and supported biomass through the addition of biosupports. This second stage is highly relevant since the coexistence of suspended and supported aerobic biomass can modify the kinetics of both biomasses given their competition for the available substrate and oxygen (Madan et al., 2022; Di-Trapani et al., 2018; Martín-Pascual et al., 2012).

2. Materials and methods

2.1. Description of the UASB-MBR combined pilot plant

This pilot plant consisted in a UASB reactor followed by a MBR in external submerged configuration. The filtration was carried out through a hollow fiber membrane module (Micronet Porous Fibers) (pore size 0.4 μm filtration surface 0.2 m^2). The flowrate of the combined pilot plant was 0.67 L h^{-1} and was determined by the flux (J) of the microfiltration membrane unit (5.35 L $m^{-2}{\cdot}h^{-1}$). The plant was equipped by measurement and control instruments which were governed by a Programmable Logic Controller -PLC where a specific software developed by the research group was installed.

2.2. Experimental design

The sludge used in the experimental stage came from the UASB-MBR lab-scale pilot plant operated at different organic loading rates –OLR to evaluate its effect on the removal of selected emerging contaminants from urban wastewaters (Moya-Llamas et al., 2021). Organic contaminants used in this study and scheme of UASB-MBR combined pilot-plant, and its main operational parameters (start-up, inter-stabilization stage and performance) are provided as a Supplementary Information.

To run the combined system under controlled conditions, the temperature of the UASB reactor was maintained at 30 °C, while the MBR was operated at ambient temperature. The effect of temperature on aerobic biomass activity was evaluated by repeating the respirometric tests on the same sample at three different temperatures set by the equipment operator on the control panel.

Regarding the aerobic biomass, in a first stage of operation the MBR reactor worked with suspended biomass, while in a second stage plastic bio-supports (K1 type AnoxKaldnes®) were introduced in the aerobic tank to simulate an integrated fixed film activated sludge –IFAS system where suspended and attached biomass coexist. According to Martín-Pascual et al. (2012), the filling ratio of bio-supports was 35% and the evaluation of the density of biofilm (the amount of solids attached to bio-supports) was determined by centrifugation (Martín-Pascual et al., 2012; Sriwiriyarat and Randall, 2005).

The microbial communities in the sludge were monitored and observed using a trinocular microscope mod. BA210 equipped with a digital camera (Moticam, Motic), which was controlled by the software Motic Images Plus 2.0.

Respirometric test were carried out using a respirometer mod. BM-EVO of Surcis, S.L., Spain (Fig. 1). This laboratory-scale analyzer consists of a batch system equipped with a recirculation pump that allows three tests to be performed: static OUR and cyclic OUR (both tests to determine of the rate of oxygen consumption of the biomass), and dynamic R_S test (which analyses the evolution of the dynamic exogenous respiration rate, referring exclusively to the oxygen demand to degrade a biodegradable substrate), being the static test the one used in this study.

The respirometer was equipped with a pH and temperature control, and an oximeter which sent the signals of the measurements in progress to the computer where, through the specific BM-Respirometer software, they were processed to carry out the automatic generation of respirograms and the calculation of respiration rates, oxygen consumption and

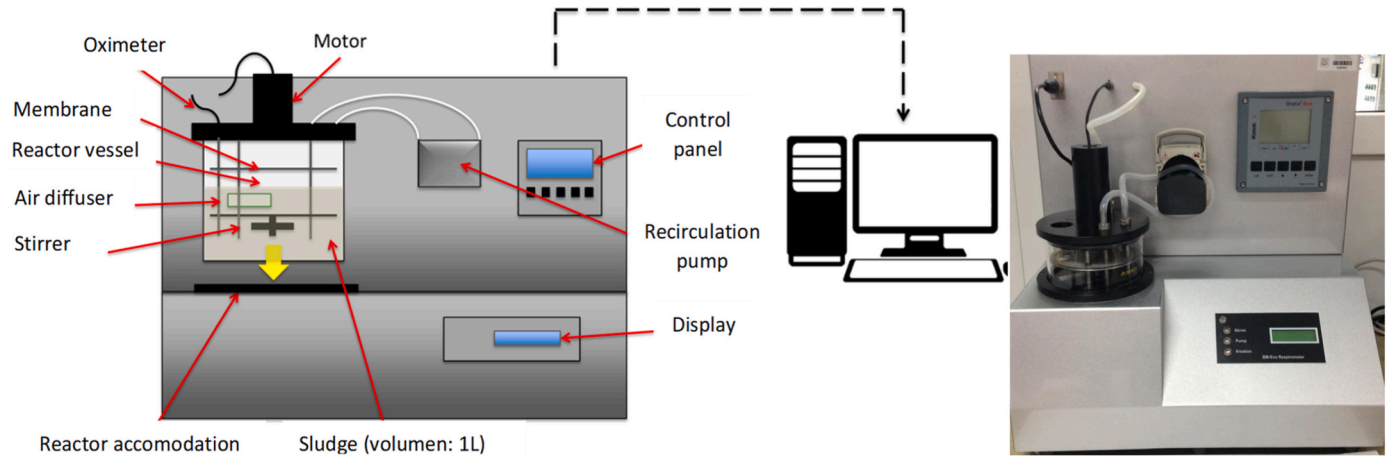

Fig. 1. Scheme (left) and image (right) of the respirometer mod. BM-EVO (Surcis, S.L.).

COD biodegradable fraction. This software also allowed the creation and saving of the generated files as well as their export with the respective respirograms.

Static respirometric test were performed periodically at three different temperatures (20 °C, 23 °C and 26 °C) for each loading rate evaluated. In a second stage, bio-supports were introduced in the aerobic tank and respirometric tests were carried out at the same operational conditions.

2.3. Analytical methods

The concentration of suspended solids in the mixed liquor -MLSS was determined by gravimetric methods, according to Standard Methods for the Examination of Water and Wastewater (WPCF et al., 1992). This concentration was maintained under 4000 mg/L in the three OLR steps analyzed, therefore no dilution was required. Chemical Oxygen Demand -COD was determined using colorimetric methods (WPCF et al., 1992) by tube-test and spectrophotometer (NANOCOLOR® Machery-Nagel GMBh & Co., Düren).

The endogenous conditions of an activated sludge are those in which the rapid loss of microbial activity is due to the non-addition of external substrate. Therefore, the biomass only has as available substrate the one resulting from the microbial processes of decomposition and hydrolysis. Consequently, the main kinetic parameters, such as the endogenous decomposition constant (k_d), must be determined under endogenous conditions. Therefore, during experimental period samples were taken in duplicate. One was analyzed immediately (under normal or exogenous conditions) while the other one was subjected to aeration and agitation for 24 h To stablish fully endogenous conditions (Mainardis et al., 2021; Rodriguez et al., 2011).

To prevent other processes such as nitrification from occurring spontaneously and interfering with the oxygen consumption results, the autotrophic communities were inhibited by adding Allylthiourea -ATU (3 mg ATU-g-1 SSV) to the samples before testing. The volume of sludge established for running the tests was 1 L, and this was introduced into the respirometer reactor vessel with a continuous oxygen supply (aeration) until a stable concentration of dissolved oxygen -DO was reached in the sample. Once the oxygen saturation conditions were achieved and the sludge reached the first of the established temperatures, aeration and recirculation were stopped, maintaining the stirring of the sample. Then the membrane dividing the upper and lower enclosure in the reactor vessel was automatically closed, and the lower part was automatically insulated from the outside during the respirometry test. From that moment on, the respirometer automatically recorded the oxygen consumption of the microorganisms and represented it by a curve of the dissolved oxygen –DO concentration (mg $O_2 \cdot L^{-1}$) versus time –t (h). The slope of this curve or respirogram indicated the Oxygen Uptake Rate –OUR (mg O_2 consumed$\cdot L^{-1} \cdot h^{-1}$) (Equation (1)).

$$OUR = \frac{\Delta DO}{\Delta t} \quad (1)$$

Since the activity of the autotrophic bacteria was inhibited, there was no oxygen consumption due to the nitrification processes, and this was only due to that consumed by heterotrophic bacteria.

In order to relate the activity of the sludge to the amount of active biomass in the respirometer reactor the Specific Oxygen Uptake Rate –SOUR (mg $O_2 \cdot$ g $MLVSS^{-1}$ h^{-1}) was determined as the quotient between the OUR and the concentration of volatile suspended solids –Xvss (mg VSS$\cdot L^{-1}$) in the sludge (Equation (2)).

$$SOUR = \frac{OUR}{X_{VSS}} \quad (2)$$

Based on the SOUR, a sludge is more active if, for the same amount of substrate and the same community of microorganisms, its endogenous respiration and synthesis of organic matter is faster and, as a result, the SOUR is higher. The test was completed when the DO available in the sludge had been consumed by the biomass and the DO concentration remained constant.

The heterotrophic biomass yield coefficient and the biomass decay rate are two kinetic parameters of great relevance in respirometry. Heterotrophic biomass yield represents the production of biological sludge produced per unit mass of total substrate consumed.

$$Y_{H,MLVSS} = \frac{Y_{H,COD}}{f_{cv}} \quad (3)$$

Being:

$Y_{H,\ MLVSS}$ (mg MLVSS·mg $COD^{-1}_{substrate)}$: heterotrophic biomass yield coefficient related to microorganism concentration.

f_{cv}: 1,48 (stoichiometric adjustment factor for reactions involved in the degradation process of organic matter).

$Y_{H,\ COD}$ (mg $COD_{microorgs}$·mg $COD^{-1}_{substrate)}$: coefficient of heterotrophic biomass yield related to oxygen consumption to synthesize a biodegradable substrate.

$$Y_{H,COD} = 1 - \frac{\Delta DO}{COD} \quad (4)$$

As for the endogenous decomposition constant -k_d, it is indicative of heterotrophic biomass mortality under endogenous conditions.

$$k_d = \frac{SOUR_{end}}{1,42} \quad (5)$$

According to Mainardis et al. (2021) and Martín-Pascual et al.

(2012), the main kinetic parameters of the process were determined using sodium acetate was used as a readily degradable substrate. The oxygen consumed (ΔDO) by the active biomass to metabolize this substrate was measured using the respirometer (dynamic R assay) and the test was replicated at four different and known substrate concentrations (100, 200, 400 and 600 mg COD/L) to define a calibration line.

These tests at three different temperatures (20 °C, 23 °C and 26 °C) were repeated for the three organic loading steps (high, medium and low) to which the combined plant was subjected.

In order to study possible toxicity effects of emerging contaminants on the active biomass, the varitation of respiration rate -($R_{S,p}$) was determined by respirometry according to OECD Method 209. (OECD, 2010). The evolution of $R_{S,p}$ was monitored during the biodegradation of a readily assimilable substrate (100 mg of NH_4Cl and 500 mg of sodium acetate dissolved in 10 mL of distilled water), added to a 1 L of mixed liquor together with consecutive doses of the studied organic contaminants mixture ($D_1 = 4$ μg/L, $D_2 = 10$ μg/L, $D_3 = 30$ μg/L and $D_4 = 60$ μg/L). The percentage of microbial inhibition was calculated by means of equation (3):

$$\%\text{Inhibition} = \left(1 - R_{S,p} \,/\, R_{S,p\,max}\right) \cdot 100 \quad (6)$$

$R_{S,p}$ (mg $O_2 \cdot g^{-1}_{MLVSS} \cdot h^{-1}$): Variation of the oxygen consumption of the sample, depending on the concentration of microorganisms in the mixed liquor or Saturation rate of oxygen consumption.

$R_{S,p\ max}$ (mg $O_2 \cdot g^{-1}_{MLVSS} \cdot h^{-1}$): Variation of the maximum oxygen consumption, depending on the concentration of microorganisms in the mixed liquor or Saturation rate of oxygen consumption.

3. Results and discussion

3.1. Biokinetic study

During the first 55 days of experimentation, the UASB-MBR system was stabilised at high OLR. Table SI2 summarizes the main operating parameters, as well as the start and end day of each OLR period (high, medium and low) for both stage 1 and stage 2. For the introduction of bio-supports and growth of the attached biomass, inter-stage stabilization was carried out from day 182 to day 265 of operation (grey area in Fig. 2 (left and right)).

The results achived for the main biokinetic parameters of the aerobic heterotrophic biomass (OUR, SOUR, $Y_{H,\ COD}$, $Y_{H,\ MLSS}$ and the decay coefficient -K_d) throughout the experimental period are shown below (see Fig. 3).

The mean values of the Specific Oxygen Uptake Rate by microorganisms -SOUR decreased progressively throughout the experimentation. The decrease in OLR in the influent (less available substrate) at the different OLRs steps analyzed, as well as the ageing of the sludge (high SRT), since no sludge purges were performed, except those due to sampling for analytical determinations (200 mL per day), led to a slowing down of the sludge's degradative activity. This is consistent with previous research (Li et al. (2019) which confirms a lower capacity for contaminants removal at lower OLRs. De Oliveira et al. (2018) stated that operating at high SRT the decrease of biomass activity may be linked to biomass ageing and the subsequent decrease in the heterotrophic active fraction. This is consistent with the results obtained for low organic loads -OLR in both stages of operation (Table 2) where, despite the increase in the concentration of active biomass in the reactor (X_{MLVSS}) in stage 2, there is a decrease in both the respiratory activity of the microorganisms ($SOUR_{end}$) and their degradation activity ($Y_{H,\ MLVSS}$), which also results in sludge production (k_d) (Table 2):

According to Abdel-Shafy and El-Khateeb (2011) this progressive slowing down of microbial activity could also be caused by the accumulation of emerging contaminants adsorbed onto sludge during the whole experimental stage. In fact, most of the analyzed compounds have an octanol-water partition coefficient or hydrophobicity coefficient -K_{ow} equal or higher than 3.2, thus, they are highly hydrophobic and have a high sorption potential (Bernal et al., 2018; Jones et al., 2005).

For the endogenous decomposition constant (k_d), the results indicated that operating with supported biomass the microbial decay (death of microorganisms) per day was about 3% of the total amount of biomass contained in the reactor, considerably higher than operating with suspended and supported biomass (around 1%) ($k_d = 0.03$ and $0.01\ d^{-1}$, respectively). These results are in agreement with those obtained for $Y_{H,\ MLVS}$ (Fig. 3), which was also more stable in stage 2. According to Li et al. (2019), highest values of k_d in the first operation steps could be due to cell lysis in those early stages of operation resulting from accomodation of the mixed liquor from a full-scale plant used as the inoculum of the laboratory-scale MBR reactor. However, these decay constant or mortality values were significantly lower than those reported in previous research with real wastewater (Leyva-Díaz et al., 2013).

The results confirmed that the presence of emerging contaminants in the effluent at trace concentrations (10 μg/L) did not produce toxic effects in the sludge, which is in accordance with previous research such as that of Rios-Miguel et al. (2023) on biomass adaptation to the presence of ECs.

3.2. Effect of temperature on the DO variation versus time

Concerning the variation of dissolved oxygen over time, repeating

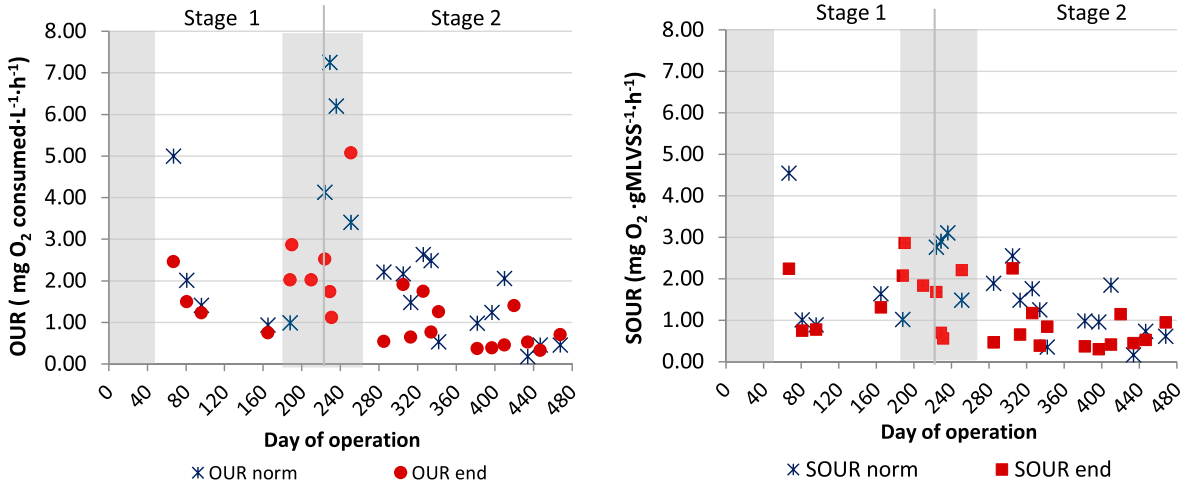

Fig. 2. Evolution of the OUR (left) and SOUR (right) under normal and endogenous conditions along the experimental period (stage 1: suspended biomass, stage 2: suspended and supported biomass).

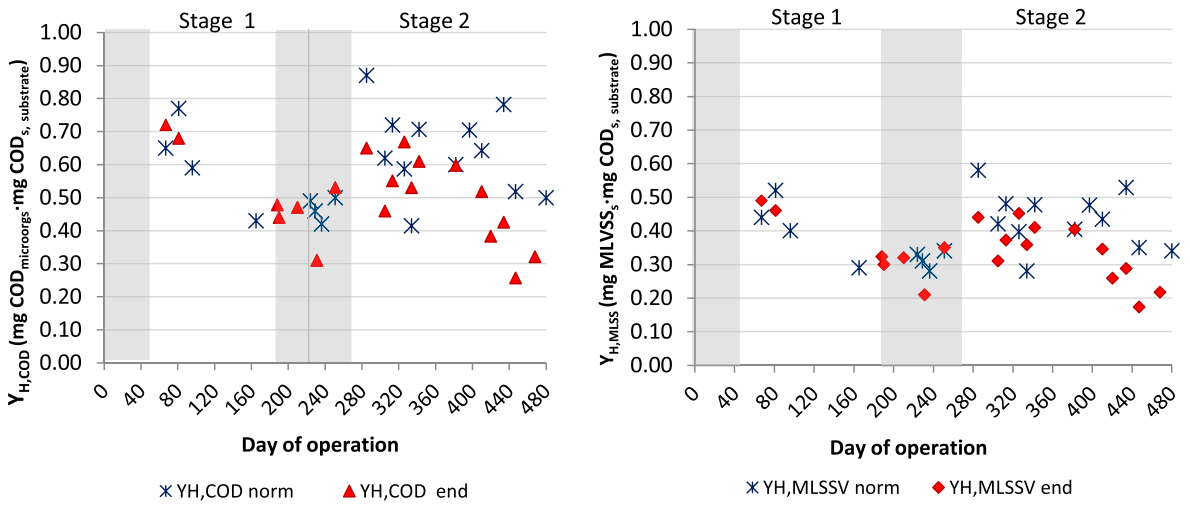

Fig. 3. Evolution of the heterotrophic biomass yield coefficient –Y_H related to oxygen consumption (left) and related to microorganism concentration (right) under normal and endogenous conditions along the experimental period (stage 1: suspended biomass, stage 2: suspended and supported biomass).

Table 2
Results for the main kinetic parameters of biomass at low OLR.

		Stage 1: Suspended aerobic biomass	Stage 2: Suspended and supported biomass
X_{MLVSS}	$mg{\cdot}L^{-1}$	1,55	2,2
$SOUR_{end}$	mg O_2· g $MLVSS^{-1}$ h^{-1}	1,49	1,15
$Y_{H,MLVSS_{end}}$	mg MLVSS·mg $COD^{-1}_{substrate}$	0,48	0,28
k_d	d^{-1}	0,03	0,02

OUR static test for the same samples at three different temperatures through the control panel, a decrease in DO consumption by the aerobic biomass was measured as the temperature increased for every respirometric test performed at each organic load step analyzed. It thus demonstrates the great effect of sludge temperature on the efficiency and speed of reactions occurring in aerobic biomass, particularly regarding the biodegradability of organic matter and main operational parameters such as oxygen solubility in water (Ferrai et al., 2010). A graphical example of the effect of temperature shocks on the endogenous respiration of heterotrophic communities when the influent of the combined system was at medium OLR is provided by Fig. 4.

Regarding endogenous and exogenous operating conditions, under endogenous conditions (substrate limitation) the DO consumed by the biomass was higher than operating without these limitations (exogenous conditions). A graphical example of DO consumption operating at 20 °C, medium OLR, and for both biomass conditions is provided in Fig. 5.

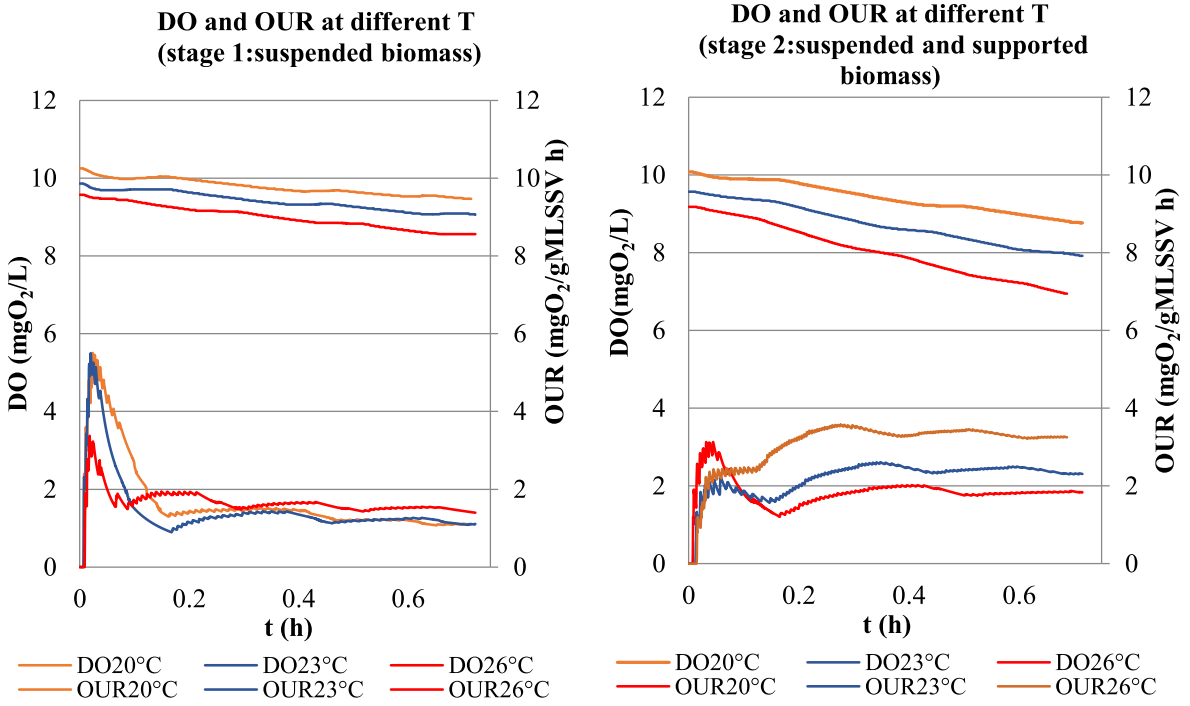

Fig. 4. Graphical example of the respirometric test at mean OLR during stage 1 (suspended biomass) (left) and stage 2 (suspended and supported biomass) (right) subjecting the sludge to different temperatures.

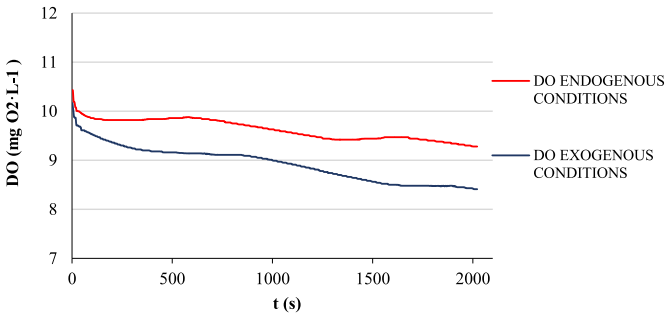

Fig. 5. Variation of DO concentration versus time for endogenous and exogenous conditions.

3.3. Effect of temperature on OUR at different OLRs

The average values of the Oxygen Uptake Rate -OUR of the aerobic biomass operating at high OLR of the influent were 1.16 ± 0.12, 1.96 ± 0.05 and 2.58 ± 0.39 mg $O_2 \cdot L^{-1}$ h^{-1} for mixed liquor temperatures of 20 °C, 23 °C and 26 °C, respectively. At medium OLR, OUR values were higher than at the previous organic loading stage (high OLR).

Operational conditions such as the accommodation of active biomass to the running conditions of the combined pilot plant and the stabilization of the combined pilot plant in the initial stage (provided as Supplementary Information, Table SI2) could affect the results of the high OLR stage (Li et al., 2019). Conversely, the active biomass of the pilot plant presented a complete stabilization in following OLR stages, as evidenced the OUR results for the medium OLR stage (1.65 ± 0.60, 2.04 ± 0.14 and 2.61 ± 0.19 for 20 °C, 23 °C and 26 °C, respectively).

The lowest OUR values were obtained when the UASB-MBR pilot plant operated in the low OLR range (1.09 ± 0.49, 1.59 ± 0.82 and 2.13 ± 1.1 mg $O_2 \cdot L^{-1}$ h^{-1}, respectively). The low available substrate in the last stage of OLR led to results with a significant standard deviation (Fig. 6).

The results indicated a slowdown of oxygen consumption by the aerobic microorganisms with decreasing the biodegradable substrate in the influent of the combined system. These findings are coherent with those reported in previous research such as the study of Krzeminski et al. (2012). Furthermore, authors such as Li et al. (2023) have reported that if oxygen and substrate become limiting, their influence on biofilm density and biofilm growth can affect the functioning of the system. Conversely, in accordance with Di Trapani et al. (2018), the increase of organic loads in the influent promoted a higher heterotrophic biomass activity in the sludge, which resulted in more intense organic matter degradation.

3.4. Effect of temperature on oxygen consumption depending on the typology of aerobic biomass

The DO rate required for the oxidation of organic matter was lower when the combined plant operated with suspended and supported biomass (stage 2) than operating without biosupports. In both phases of the investigation the amount of oxygen required to oxidize the same amount of organic matter was lower at lower loading stages, with mean $SOUR_{norm}$ values of 1.63 and 0.61 mg $O_2 \cdot g$ $MLVSS^{-1}$-h^{-1} in the low OLR stages of Phases 1 and 2 respectively, compared to average values of 1.63 and 0.94 mg $O_2 \cdot g$ $MLVSS^{-1}$-h^{-1} in the high OLR stages of these phases. It suggested that there was a saving of the energy required for biological degradation of the substrate in the lower organic load stages. This was probably due to the influence of substrate limitation on the growth of active biomass, resulting in lower sludge production at these loads, as derived from the values of the heterotrophic biomass yield in relation to microorganism concentration ($Y_{H,\ MLVSS}$), which average values were 0.48 ± 0.06, 0.40 ± 0.00 and 0.29 ± 0.01 mg $COD_{microorgs}$· mg $COD_{substrate}$ at hight, medium and low OLR, in accordance with previous research operating with combined anaerobic-aerobic configurations (de Oliveira et al., 2018). However, when the system was operated with suspended and supported biomass, the heterotrophic biomass yield, indicative of sludge production, was more stable ($Y_{H,\ MLVSS}$ = 0.31 ± 0.03, 0.43 ± 0.11, 0.43 ± 0.08 mg $COD_{microorgs}$· mg $COD_{substrate}$ at hight, medium and low OLR).

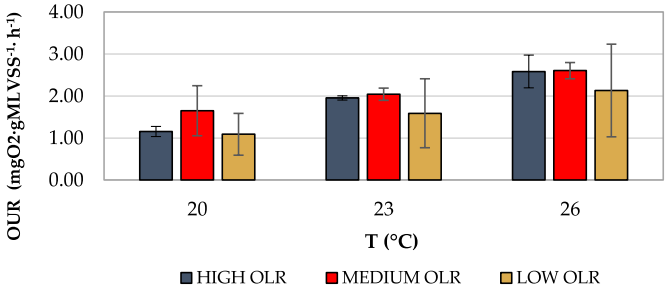

Fig. 6. Impact of temperature on the OUR at the three OLRs analyzed.

3.5. Future research needs

From the literature research it can be concluded that there is a lack of knowledge about the biomass behaviour of hybrid and combined biological systems in the treatment of wastewater containing ECs.

Recirculation between aerobic and anaerobic reactors and the introduction of bio-supports leads to an increase in the diversity of species developed. For this reason, it is necessary to deepen the microbiological characterization of the different species developed in combined aerobic and anaerobic biological systems and to quantify the different populations.

The effect of EC adsorption on sludge needs to be further investigated, both quantitatively and qualitatively.

The aerobic biomass activity of the UASB-MBR system at high cell retention times and under substrate restriction conditions, especially at low organic loads, should be further explored.

4. Conclusions

The results of the 480-day experimentation simulate the biomass behaviour in full scale combined biological systems treating wastewater with emerging contaminants. The conclusions of this research are summarized below:

- The active biomass of the UASB-MBR system can successfully tolerate the presence of emerging contaminants along the time, and the identified species are bio-indicators of a good functioning of the system. Nevertheless, their progressive maturation due to the high sludge retention time and the possible adsorption of the more hydrophobic emerging contaminants onto sludge reduces the microbial activity. However, further research is needed to determine the effect of EC adsorption on microbial communities.
- The results of the microscopic study of biomass as well as of the kinetic parameters SOUR and $Y_{H,\ MLVSS}$ confirm that operating under conditions of restricted available substrate (low OLRs) results in a deterioration of the floc quality and a lower quantity of oxygen used in the degradation of organic matter, suggesting an energy saving of the active biomass.
- Aerobic biomass increases its degradative activity ($Y_{H,\ MLVSS}$ mean values) with increasing temperature. However, the coexistence of different types of biomass (suspended and supported) the DO requirements (SOUR) for the oxidation of organic matter are lower. Although it is necessary to deepen this aspect, this is possibly due to the competition of the different communities for both the substrate and the available oxygen.

- The decrease of OLR in the influent (less available substrate) operating at low OLRs, together with the high SRT of operation, lead to the slowdown of endogenous respiration as well as degradative activity of the biomass.

The finding of this research may be promising for application to the development of new mathematical models for the design of UASB-MBR systems, and for the characterization, control and diagnosis, and prediction of the kinetic of the developed bacterial consortium under different metabolic and operational conditions.

Author contributions

This manuscript was written through contributions of all authors named and all of them have given their approval to the final version of the manuscript, **Moya-Llamas, M.J.**: Conceptualization, methodology, software, formal analysis, investigation, resources, writing original draft, review and editing, visualization, supervision, **Pacazocchi, M. G.:** Software, validation, formal analysis, resources, data curation, **Trapote, A.:** Investigation, supervision, project administration.

Funding

Spanish Ministry of Economy and Competitiveness through the projects "Combined System UASB + SMEBR + O_3/AC (CTM 2016-76910-R)" and "Combined treatments for the degradation and/or elimination of emerging pollutants in water" (CTM 2013-46669-R) and University of Alicante.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the paper entitled "Respirometric tests in a combined UASB-MBR system treating wastewater containing emerging contaminants at different OLRs and temperatures: biokinetic analysis".

Data availability

Data will be made available on request.

Acknowledgments

This study was developed at the University Institute of Water and Environmental Sciences. University of Alicante.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.118643.

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