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Biomass activity assessment in a membrane bioreactor operated at high SRT and low COD/N using respirometric analysis and modelling

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ABSTRACT

In this paper, respirometric analysis and Activated Sludge Model (ASM) have been evaluated to identify active biomass fraction of activated sludge in a submerged Membrane Bioreactor (sMBR). X_{BH} (Heterotrophic biomass), X_{BAI} (Autotrophic biomass responsible of nitrite production) and X_{BAA} (Autotrophic biomass responsible of nitrate production) have been quantified within activated sludge fed with soluble and easily biodegradable substrate (ethanol/sodium acetate (1:1)). This influent, containing no hardly biodegradable organic or inorganic particulate matter, has led to the generation of a sludge constituted of essentially two fractions: X_{BH} and X_{BA} (Autotrophic biomass which correspond to the sum of X_{BAI} and X_{BAA}). Specific respirometric measurements of endogenous and exogenous activities allow to ensure the integrity of ASM and to develop equations allowing the quantification of active biomass in these models. Results highlight the growth of this specific biomass under high SRT and substrate limited conditions. Additionally, Soluble Microbial Products (SMP) have been characterized in order to evaluate the fouling propensity of sludge in sMBR. Finally, SMP kinetic coefficients (production and consumption) were calculated.

KEYWORDS: *membrane bioreactor, heterotrophic bacteria, autotrophic bacteria, respirometric analysis, activated sludge, soluble microbial products.*

INTRODUCTION

Conventional wastewater treatment systems have been improved by introduction of membranes as solid/liquid separation devices and these combined systems, which are called membrane bioreactors

(MBRs), have been widely used in recent years for urban wastewater treatment (Judd, 2008). It enables to increase by a significant way the biomass concentration inside the biological reactor and by this way, permits to reduce the volumes and to reach a water quality much higher than those obtained with conventional activated sludge (CAS) (specifically in terms of solid matter removal and effluent disinfection). Specific features of the MBR technology are that hydraulic and sludge retention times (HRT and SRT, respectively) could be uncoupled and a better biomass retention, by comparison to CAS, could be offered. Higher SRTs are favorable to slow growing micro-organisms such as autotrophic bacteria. Then, it is possible to work with more diverse and efficient biomass (Khongnakorn et al., 2007) for the treatment of ammonium (Ouyang et al., 2009) but also for the biodegradation of some micro-pollutants by cometabolism (Delgado 2009; Clouzot et al., 2011). However, membrane fouling during operation has greatly hindered their application (Drews et al., 2010; Aslam et al., 2017).

Extracellular Polymeric Substances (EPS) and Soluble Microbial Products (SMP) released by biomass are regarded in general as one of the main cause of membrane fouling in submerged MBR (sMBR), (Drews et al., 2006; Nataraj et al., 2008; Rosenberger et al., 2002) but other authors are more reserved on this assumption (Guglielmi et al., 2007; Pollice et al., 2005). However, they all agree that there are strong links between membrane fouling and activated sludge structure and activity. To optimize sMBR functioning, a thorough understanding is needed of the impact of the operational parameters and conditions on the viability and activity of the overall biomass community.

In a sMBR, a modification in biomass activity and viability is often noticed compared to CAS. In this context, respirometric analysis is presented as a reliable tool in order to evaluate the representative biomass kinetic parameters, to be inserted in mathematical models during the design phase, as well as to monitor the biomass viability and activity, especially when these processes operate at high SRT values (Di Trapani et al., 2011). The most common biological kinetics models are the ones developed by the International Water Association (IWA): the series of Activated Sludge Models (ASM). ASM simulations allow describing the global oxygen and energy consumptions, the produced biomass, and the pollution removal efficiency. Notwithstanding the fact that a large number of ASM applications have been reported in MBRs (Janus et al., 2015), some key factors still have to be further investigated and considered in wastewater characterization. One of these relates to the assessment of the active heterotrophic biomass (X_H) in the influent wastewater that, although usually neglected in CAS modeling, needs to be better addressed when modeling membrane bioreactors. In fact, from a theoretical point of view, longer SRT lead to decrease the fraction of active biomass within MLVSS (Ramdani et al., 2012; Zuthi et al., 2015). This contribution is influenced by the characteristics of the influent.

A combination of well-known respirometric methods for X_H, X_{BAI} and X_{BAA} determination has been used to investigate the performance and biomass activity of a laboratory-scale sMBR operated at high

SRT and a low COD/N ratio in the influent. The biological activity was monitored as a result of respirometric assessments by focusing on heterotrophic and autotrophic microorganism activities. The equations of Oxygen Uptake Rate (OUR) have been extracted from ASM equations and were adjusted according to experimental conditions. Simulations done with Mantis2lib model (GPS-X) allow following active biomass growth under this specifically operating conditions.

MATERIAL AND METHODS

Experimental set up and operating condition

The study was achieved using a laboratory-scale sMBR which consisted of two main compartments, the anoxic tank and the aerobic one with a working volume of 30 L each (Figure 1). Filtration module consists in flat sheet membranes (Microdyn-Nadir, Germany) with a pore size of 0.04 µm and a surface area of 0.34 m^2. The module was directly immersed into the aerobic tank: membrane filtration was carried out by a volumetric pump. The trans-membrane pressure (TMP in kPa) was defined by the difference between the atmospheric pressure and the permeate pressure which is measured on the membrane downstream side. The bioreactor was inoculated with seed sludge taken from a sewage WWTP working under low organic loading rate to remove nitrogen compounds. Internal recycle (400% of the input flow) between aerated and anoxic tanks was performed at constant flow using peristaltic pumps (Watson Marlow). In order to study the effect of high SRT on membrane fouling, a SRT of 60 days was applied by a daily sludge withdrawal of 1 L. The sMBR was operated for a period of 90 days after 150 days of biomass acclimatization to the operating conditions and feeding influent. The substrate feed was prepared with a mixture of ethanol/sodium acetate (1:1) and ammonium chloride, keeping a constant COD/N ratio of 3.5. The experiments were carried out at ambient temperature (20±5°C) with a pH adjusted at 7.25±0.25 thanks to the addition of a sodium hydroxide solution (2 $mol.L^{-1}$) or sulphuric acid solution (1 $mol.L^{-1}$) by a pH controller and a dosing pump. A blower assured the air flow for membrane scouring and aeration of the aerobic reactor where Dissolved Oxygen (DO) was always maintained between 4 and 6 $mgO_2.L^{-1}$. This aeration strategy was designed to produce hydraulic shearing forces and delay membrane fouling. Moreover, membrane fouling was limited by air injection and a specific filtration strategy: a 10 min operational cycle included 8.75 min of filtration with a permeate flow rate of 17 LMH, 0.5 min of relaxation and 0.75 min of backwashing at 8.8 LMH. So, the average effective rate was close to 14.7 LMH (Table 1).

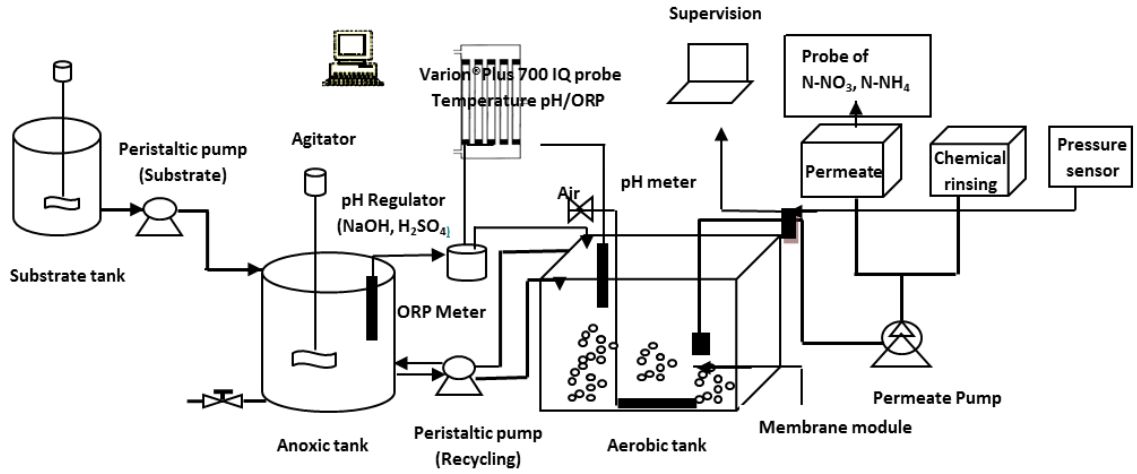


Figure 1 Submerged membrane bioreactor (sMBR) set-up

Table 1 Operating conditions of the sMBR

Q ($L.d^{-1}$)	SRT (d)	HRT (h)	J_W ($L.h^{-1}.m^{-2}$)	OLR (kg $COD.m^{-3}.d^{-1}$)	NLR (kg $N.m^{-3}.d^{-1}$)	COD/N
120	60	12	14.7	0.56	0.16	3.5

Analytical methods

The supernatant was sampled twice a week and filtrated through a dead end filtration (Whatman GF/C filter). Nitrogen compounds (NH_4^+-N, NO_2^--N and NO_3^--N) were quantified by spectrophotometric analysis (Method salicylate by Amver 26069-45 Test N'Tube ™ and cadmium reduction method by NitraVer ® 5 tests, Hach, Loveland, CO, USA). Total organic carbon was measured in permeate and supernatant using TOC meter (SHIMADZU (V CSH/SCN)). Chemical Oxygen Demand (COD), MLSS and MLVSS were measured according to the Standard Methods (APHA 1998).

In addition, Varion®Plus 700 IQ probes were used to continuously measure pH, temperature, ammonium and nitrate ions in the mixed liquor (Wissenschaftlich-Technische Werkstätten GmbH, Germany).

To measure the respirometric activities, activated sludge from mixed liquor was transferred into sealed bottles (250 mL) where the DO was monitored using a DO sensor (WTW 340i, Germany) allowing Oxygen Uptake Rate (OUR) calculations. The Specific Oxygen Uptake Rate (SOUR) was obtained by dividing the OUR by the MLVSS concentration.

Concerning the sludge activity simulation, GPS-X (Hydromantis, Canada) modeling tool was used. The default stoichiometric and kinetics values were used and the chosen models were the activated sludge models n°1 and 3 (ASM 1 and ASM 3). In fact, the Activated Sludge Model n°1 (ASM 1),

published by the IWA task Group on Mathematical Modeling for Design and Operation of Biological Wastewater Treatment, is the most widely recognized tool for aerobic biological wastewater treatment modeling (Henze et al. 2008). Membrane separation was considered as a perfect particles separator.

RESULTS AND DISCUSSIONS

Carbon/organic matter removal

Figure 2a shows the measured COD concentration. During the experiment, the averages COD concentration in permeate (COD_P) was around 15 $mg.L^{-1}$, allowing an overall COD removal of 95%. COD and COT concentrations in permeate also show the efficiency of the membrane to enhance organic matter removal during the operation (Figure 2b). If most similar studies chose to report COD removal based on the difference between influent and effluent concentrations, the attention may be drawn on Soluble Microbial Products (SMP) generation. In fact, SMP (part of COD) are defined as soluble cellular components that are released during cell lysis or associated with the substrate metabolism and biomass growth (Iacopozzi et al. 2007, Daigger et al. 1977, Orhon et al. 1089, Heran et al. 2012, Di Bella et al. 2013, Philippe et al. 2013). So, this study provided the experimental evidence that the overall COD reduction would not reflect the level of substrate removal or utilization, mainly because all biodegradable influent COD are soluble and wholly used under our selected operating conditions. Because of that, the effluent COD essentially included generated SMPs. In order to decrease effluent COD or sludge fouling propensities, mass balance were performed on SMP fractions (Eq. (1), Eq. (2)). It should be noted that the SMP fraction with high molecular weight (SMP_{HMW}) have been accumulated in the same way as biomass, and at least leaving the reactor only as part of excess sludge. If it is assumed that SMP with low molecular weight (SMP_{LMW}) and SMP with high molecular weight (SMP_{HMW}) are produced in the same rate (proportional to Organic Loading rate), then the relationship between SMP_{HMW} and SMP_{LMW} or between the effective SMP fraction retained in the reactor (COD_{SR}) and the observed COD concentration in permeate (COD_P), can be expressed as follows:

$$COD_{SR}/COD_P = SMP_{HMW}/SMP_{LMW} = SRT/HRT \qquad \text{(Eq.1)}$$

$$\begin{cases} r_{S_{SMP_{LMW}}} V = Q\, SMP_{LMW} + V \dfrac{dSMP_{LMW}}{dt} \\ r_{S_{SMP_{HMW}}} V = Q_W\, SMP_{HMW} + V \dfrac{dSMP_{HMW}}{dt} \end{cases} \Leftrightarrow \begin{cases} r_{S_{SMP_{LMW}}} = \alpha\, OLR = \dfrac{SMP_{LMW}}{HRT} + \dfrac{dSMP_{LMW}}{dt} \\ r_{S_{SMP_{HMW}}} = \alpha\, OLR = \dfrac{SMP_{HMW}}{SRT} + \dfrac{dSMP_{HMW}}{dt} \end{cases}$$

$$\Leftrightarrow \begin{cases} \dfrac{SMP_{HMW}}{SMP_{LMW}} = \dfrac{SRT}{HRT} = C_F \\ SMP_{HMW}(t) = SMP_{LMW}\, e^{-\frac{t}{SRT}} + \alpha C_F S_S \left(1 - e^{-\frac{t}{SRT}}\right) \end{cases} \qquad \text{(Eq. 2)}$$

Where SRT is sludge retention time (d), HRT is hydraulic retention time (d), $r_{S_{SMP_{LMW}}}$ is the rate of SMP_{LMW} consumption (mgSMP.L^{-1}.d^{-1}), $r_{S_{SMP_{HMW}}}$ is the rate of SMP_{HMW} consumption (mgSMP.L^{-1}.d^{-1}), V is the volume of the tank (m^3), Q is the effluent flow rate (m^3.d^{-1}), Q_w is the sludge waste flow rate (m^3.d^{-1}), t is the time(d), OLR is the Organic Loading Rate (kgCOD.m^{-3}.d^{-1}), C_F is the concentration factor (-), and S_S is the soluble substrate (mg.L^{-1}).

The α value was found around 0.07 which is close to the f_p (fraction of X_I generated in biomass decay) factor found in ASM1 (f_p = 0.08).

The layout of the ratio COD_{SR}/COD_p illustrated in Figure 2c underlines two major assumptions: (i) more SMP_{LMW} are generated, (ii) the components with high molecular weight disappear due to hydrolysis (biodegradation) or adsorption on the membrane surface. In this case, by adding a simple « production – consumption» SMP model, the equation (2) becomes:

$$\alpha\, OLR - r_D = \frac{SMP_{HMW}}{SRT} + \frac{dSMP_{HMW}}{dt} \qquad \text{(Eq.3)}$$

Where r_D is the SMP_{HMW} consumption rate (mgSMP.L^{-1}.d^{-1}). This rate was assumed to be (i) zero order kinetics ($r_D = k_{SMP}$). In this way, SMP_{HMW} consumption rate was found equal to 1.38 mg SMP.L^{-1}.h^{-1}. Moreover, a last point should be considered before drawing conclusions from SMP assumption. This point concerns the evolution of SMP concentration versus time. In fact the dynamical response for SMP_{HMW} is low (Figure 2c) confirming the simple « production – consumption» SMP model (Eq. (3)).

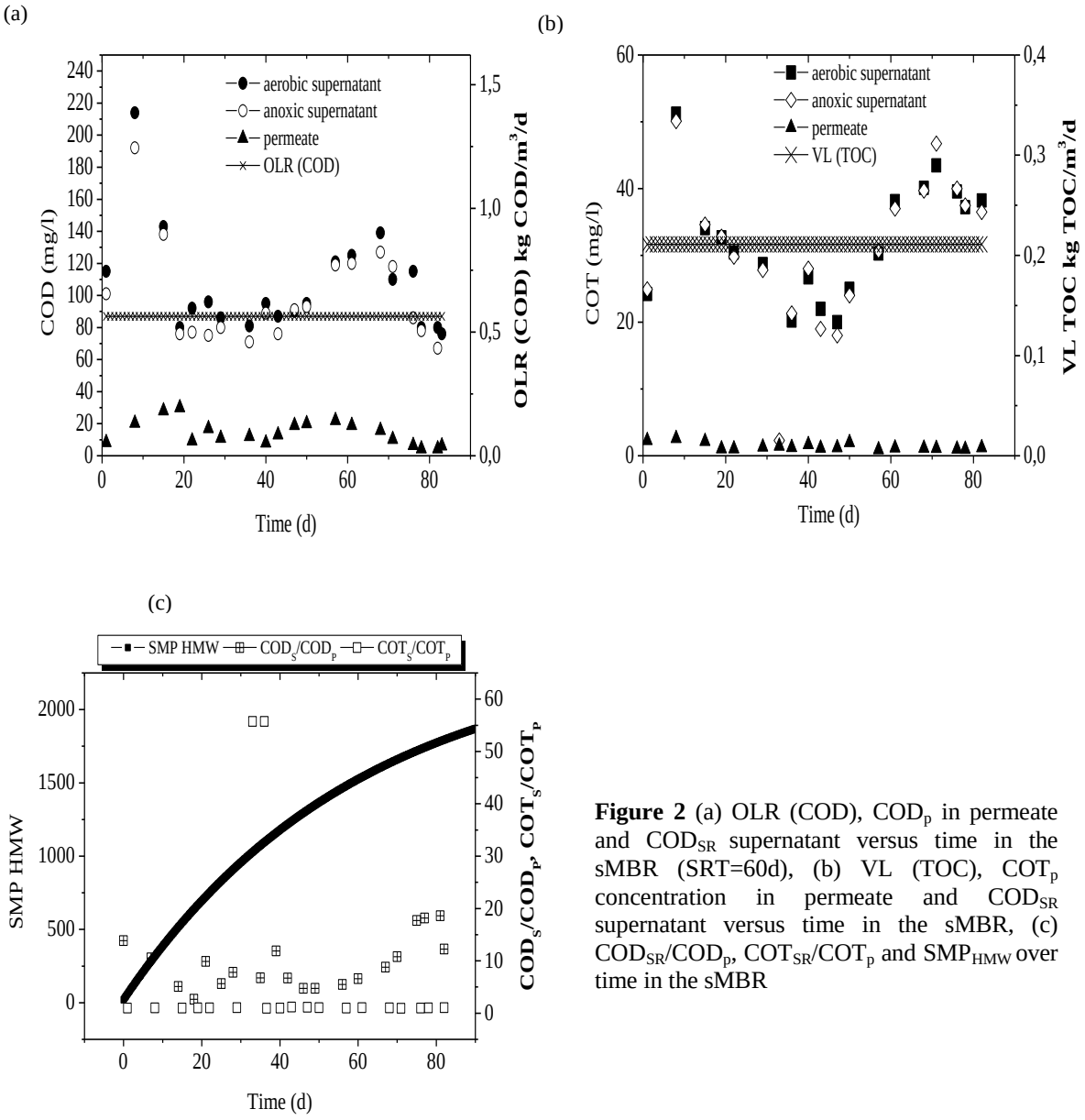


Figure 2 (a) OLR (COD), COD_p in permeate and COD_{SR} supernatant versus time in the sMBR (SRT=60d), (b) VL (TOC), COT_p concentration in permeate and COD_{SR} supernatant versus time in the sMBR, (c) COD_{SR}/COD_p, COT_{SR}/COT_p and SMP_{HMW} over time in the sMBR

Biological activity

Biomass activity was evaluated by means of the oxygen consumption rate measurement determined in a respirometer. Endogenous respiration is defined in conditions of deficiency of the exogenic substrate when the micro-organisms consume their endogenous cellular material to sustain vital function (after 24h aeration without addition of exogenous substrate). Then, addition of toxics or specifics substrates allows the determination of respirometric activities of both heterotrophic and autotrophic micro-organisms (Fenu et al. 2010, Lobos et al. 2009) (Figure 3). Advanced Carbon-Nitrogen library CN2LIB was used in this study. It is characterized by the two steps nitrification, which requires state variables for ammonia and nitrite oxidizers, as well as the division of NO_X into nitrite and nitrate. Table 2 shows the biological state of active biomass: Heterotrophic Biomass (X_{BH}), Autotrophic Biomass nitrite producer (X_{BAI}) and Autotrophic Biomass nitrate producer (X_{BAA}) according to the biological condition

tested. Then specific oxygen uptake rate (OUR) equations were determined through the CN2LIB model.

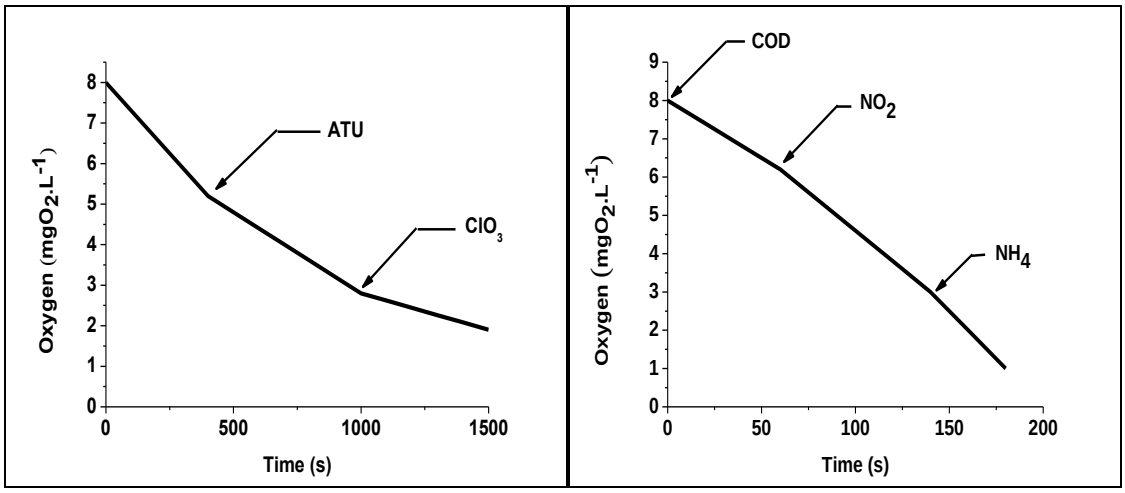


Figure 3 Profile of O_2 versus time for determination of biomass activity

Global approach

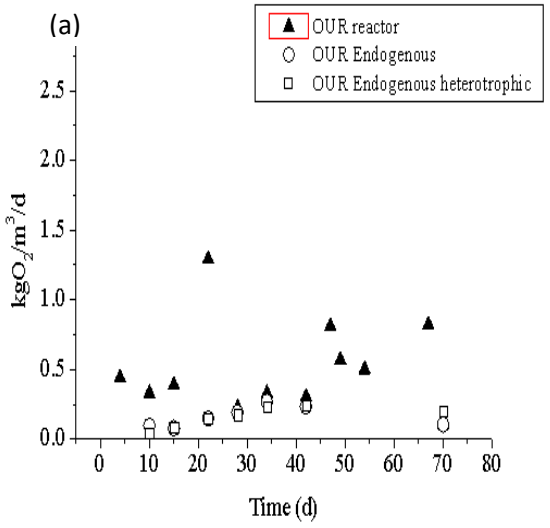
Endogenous respiration of heterotrophic and autotrophic microorganisms was also investigated during the acclimatization (Figure 4a). The autotrophic respiration decreased while the heterotrophic activity increased because the micro-organisms were in a growth state. An increase in the endogenous respiration would indicate a protective state of the biomass (Rodríguez et al. 2011). The proposed hypothesis is that a longer acclimatization with a high SRT may reveal a better development of autotrophic biomass in sMBRs. In a "stabilized" state, endogenous respiration of single heterotrophic populations represents a large part, if not apparently the entire one of endogenous respiration. During the same periods, the respirometric activity directly measured within the reactor, show our values fairly close to those ones identified in endogenous conditions after 24h aeration without addition of exogenous substrate. This proves that, at a significant SRT (60 d), the environmental Conditions for active population in the sMBR are close to endogenous ones.

Table 2 Active biomass statement and its associated biological conditions

	Active biomass

		X_{BH} : Heterotrophic Biomass	X_{BAI} : Autotrophic Biomass nitrite producer (*Nitrosomonas*)	X_{BAA} : Autotrophic Biomass nitrate producer (*Nitrobacter*)
No Substrate	1-	Endogenous	Endogenous	Endogenous
	2- + ATU	Endogenous	No activity	Endogenous
	3- + ClO_3	Endogenous	Endogenous	No activity
Substrate injection	4-+DCO	Exogenous	Endogenous	Endogenous
	5- + NH_4Cl	Endogenous	Exogenous	"Pseudo Exogenous"
	6- + Nitrite	Endogenous	Endogenous	Exogenous

(a)



(b)

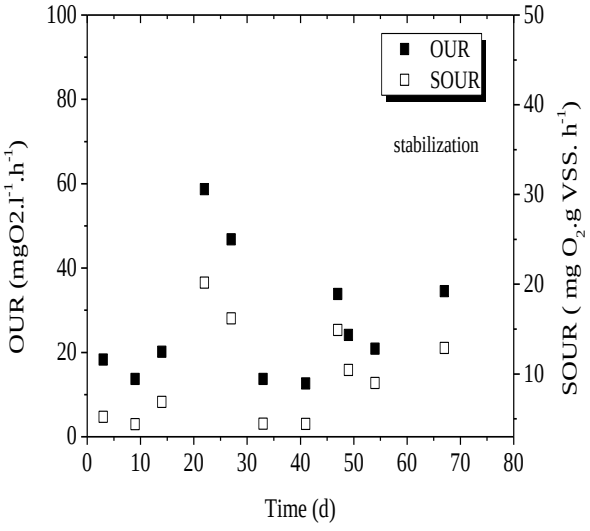


Figure 4 (a) OUR endogenous of bacteria and OUR reactor versus time (b) OUR and SOUR based on MLVSS of activated sludge in the sMBR versus time

Considering endogenous respirometric activity of the total population and the average concentration of volatile suspended solids in the bioreactor, these results allow calculation of the respirometric coefficient (Eq. (4)).The value of this coefficient is 0.063 d^{-1}; close to 0.06d^{-1}.

$$\text{OUR endogenous} = b'.\ X_{VSS} \qquad \text{(Eq.4)}$$

Exogenous respiration of heterotrophic and autotrophic microorganisms was also followed during the acclimatization phase to characterize the microbial responsiveness. Indeed, exogenous respirations were measured by adding ammonium chloride or sodium acetate in the batch reactor (Table 2, Figure 5). Heterotrophic behavior continues to increase through the acclimatization to reach the average values of 72 $mgO_2.L^{-1}.h^{-1}$ and F/M of 0.18 $kgCOD.kgVSS^{-1}.d^{-1}$ to a SRT stabilized at 60 days. The autotrophic bacteria were submitted to an adaptation period in which the activity always remained lower than the initially obtained with activated sludge AS of the sewage treatment plant. Villain et al. (2013) showed the autotrophic biomass is defined by a few species with slow growth and high sensitivity to the medium conditions, which can explain the slower adaptation to the MBR technology than heterotrophic microorganisms. Moreover, the high organic F/M ratio characteristic of WWTP conditions

(0.2 kg COD./kgVSS.d) was not well-adapted to autotrophic micro-organisms (inorganic carbon required). Figure 4b shows the OUR and SOUR based on MLVSS of activated sludge in the sMBR versus time. Autotrophic bacteria seems to oscillate average value of

0.4-0.6 $kgO_2./m^3.d$, this one significantly increases from the end of acclimatization in where nitrification seems complete. For heterotrophic bacteria population, the values where in a range of 1 to 2 $kgO_2/\ m^3$ d. The activities, measured in batch, with instantaneous flows of exogenous substrate are very much higher than the respiration requirements measured during operation in the reactor. This confirms, that under high sludge retention time (SRT= 60 days) imposed on the system, the populations develop within the sMBR in partial deficiency conditions of substrate.

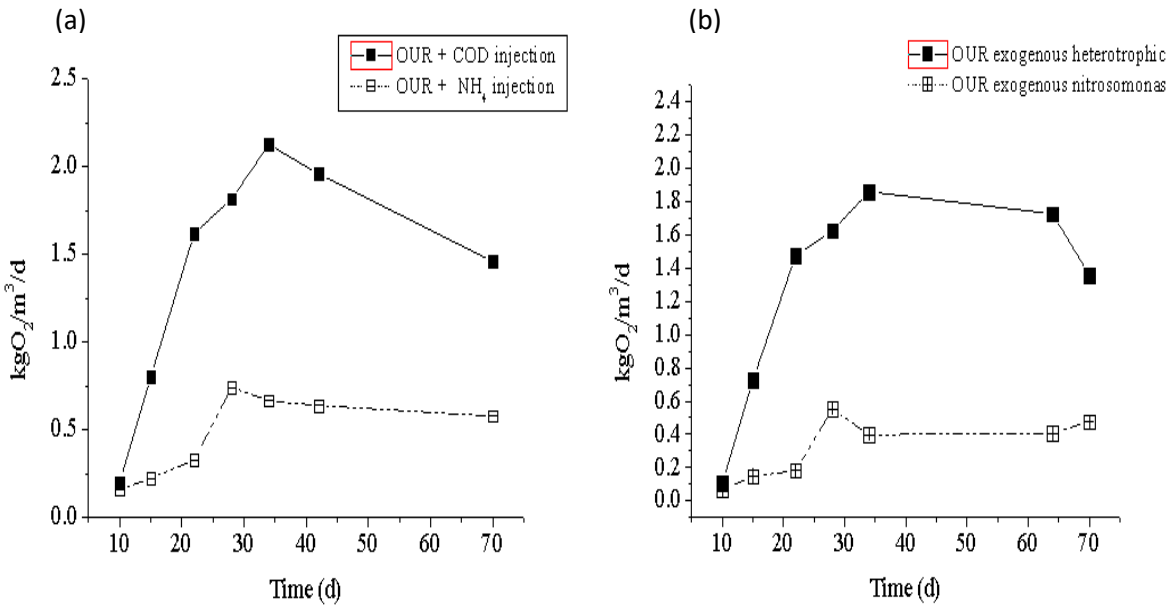


Figure 5 (a) Exogenous population needs: OUR COD and OUR NH_4 versus time (b) OUR exogenous of autotrophic and heterotrophic bacteria versus time

Biological model

Approach with ASM1

Activated sludge model can be used to determine specific biomass concentration (X_{BH}, X_{BAI}, X_{BAA}) according to the OUR measurement. The following equations Eq. (6) to (11) have been extracted from the CN2LIB Petersen matrix and were adjusted according to experimental conditions (Table 2). Figure 6 demonstrate the ASM1 biological pathway for both nitrifiers and Heterotrophs. Thus for ammonium nitrogen, $1/Y_a$ fraction of S_{NH} is converted into nitrates and ixb fraction is integrated within the autotrophic population (X_{BA}) to meet the growth requirements of this population that allows nitrification, from even, an i_{BN} fraction of S_{NH} is used for growth requirements of heterotrophic population (X_{BH}) which allows the organic compounds oxidation. Oxygen requirements are related to two complementary processes: The first one is linked to the oxygen uptake rate by the bacteria placed in an endogenous state, in the absence of an available exogenous substrate, according to the concept of death-regeneration. This request is noted OUR_{endo}, it represents the required oxygen for substrate oxidation emitted by the lysis bacterial. The second one is related to the oxidation of S_{NH} to S_{NO}, approximately 3.42 gO_2/gS_{NH} for S_{NH} to S_{NO2} and 1.14 gO_2/gS_{NH} for S_{NH} to S_{NO3}. The dynamics of this oxygen demand is directly related to the dynamics of the oxidation substrate. It can be translated by an oxygen consumption rate, it is noted OUR_{Exo} (Eq. (5)).

$$OUR_{total} = OUR\ Endo + OUR_{EXO} \qquad (Eq.5)$$

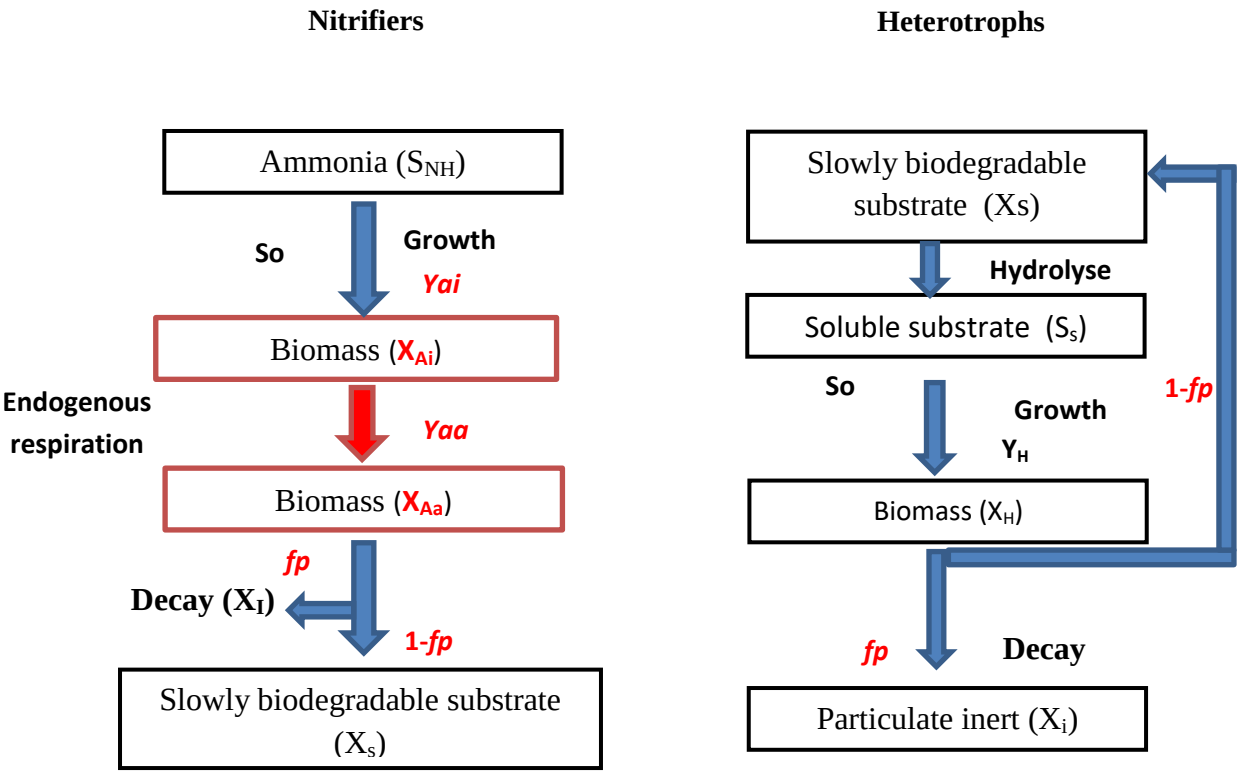


Figure 6 ASM1 biological pathway

The concentrations of autotrophic and heterotrophic biomass can be calculated using the measured values of total endogenous oxygen uptake rate OUR_{Endt} and heterotrophic endogenous oxygen uptake OUR_{endhet} (Eq. (6))

1-

$$\overbrace{OUR_{Endo} = (1 - f_p)(1 - Y_H)[b_H X_{BH} + b_{AI} X_{BAI} + b_{AA} X_{BAA}]}^{OUR_{endhet}} + \overbrace{[(3.42 - Y_{AI}) + (1.14 - Y_{AA})] i_{bn}(1 - f_p)[b_H X_{BH} + b_{AI} X_{BAI} + b_{AA} X_{BAA}] - i_{bn}[Y_H b_H X_{BH} + Y_{AI} b_{AI} X_{BAI} + Y_{AA} b_{AA} X_{BAA}]}^{OUR_{endaut}} \quad \text{(Eq. 6)}$$

Moreover, in order to differentiate autotrophic activity from heterotrophic one, specific inhibitors of autotrophs ought to be employed. An allylthiorea (ATU) solution is added for Nitrosomonas inhibition and a sodium chlorate ($NaClO_3$) is injected to exclude Nitrobacter 's activity . OUR expressions can be deducted as:

2-

$$OUR_{Endo+ATU} = (1 - f_p)[(1 - Y_H)][b_H X_{BH} + b_{AA} X_{BAA}] \quad \text{(Eq. 7)}$$

3-

$$OUR_{Endo+ClO_3} = (1 - f_p)[(1 - Y_H)][b_H X_{BH} + b_{BAI} X_{BAI}] + (3.42 - Y_{AI}) i_{bn} (1 - f_p)[b_H X_{BH} + b_{AI} X_{BAI}] - i_{bn}[Y_H b_H X_{BH} + Y_{AI} b_{AI} X_{BAI}] \quad \text{(Eq. 8)}$$

For exogenous respiration, OUR expressions can be deducted as:

4-

$$OUR_{Exo+DCO} = (1 - Y_H)\mu_H X_{BH} + OUR_{Endo} \quad \text{(Eq. 9)}$$

5-

$$OUR_{Exo+NH4} = [(3.42 - Y_{AI}) + (1.14 - Y_{AA})]\mu_{AI} X_{BAI} + OUR_{Endo} \quad \text{(Eq. 10)}$$

6-

$$OUR_{Exo+NO2} = (1.14 - Y_{AA})\mu_{AA} X_{BAA} + OUR_{Endo} \quad \text{(Eq. 11)}$$

Approach with ASM3 -2N

As an application of ASM3 model, the active biomass concentration could also be obtained by the OUR measurement. The following equations Eq. (12) to Eq. (14) have been extracted from the CN2LIB Petersen matrix and were adjusted according to experimental conditions.

1-

$$OUR_{Endo} = (1 - f_{Xi})[b_H X_{BH} + b_{AI} X_{BAI} + b_{AA} X_{BAA}] \quad \text{(Eq. 12)}$$

2-

$$OUR_{Endo+ATU} = (1 - f_{Xi})[b_H X_{BH} + + b_{AA} X_{BAA}] \quad \text{(Eq. 13)}$$

3-

$$OUR_{Endo+ClO} = (1 - f_{Xi})[b_H X_{BH} + b_{AI} X_{BAI}] \qquad \text{(Eq. 14)}$$

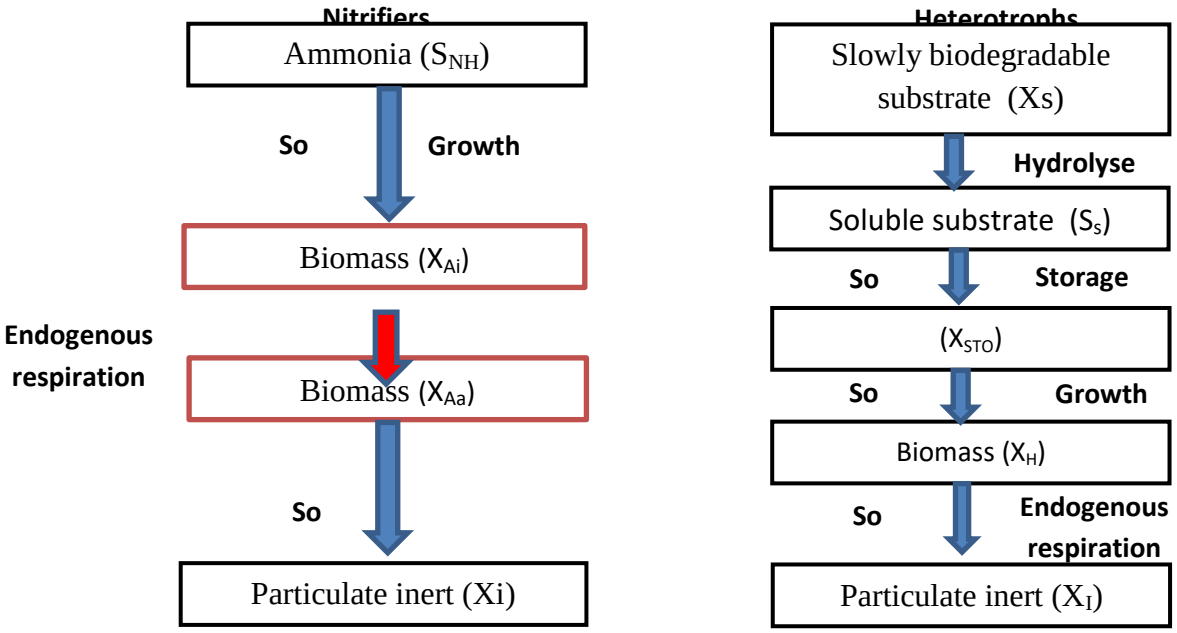


Figure 7 ASM3 biological pathway

Moreover, in sMBR systems operated at high SRTs, biodegradation of COD fractions, considered (X_S + X_I) or without inert fraction in (ASMs) as described in the table 3, induces a backwashing system of activated sludge for modeling sMBR. Changes in the parameters Y_H, b_H, ibn and fp can severely modify the predicted active biomass (X_{BH}, X_{BAI}, X_{BAA}). The estimation of these parameters has been attempted by GPS-X (default stoichiometric and kinetics values) and several research groups and the main outcomes are reported in table 4. MANTIS2LIB model was able to predict roughly the species biomass activity in endogenous respiration for this specific operation condition. The oxygen uptake rate with complete nitrification of biomass released ammonia of reactor is given by Eq. 15:

$$\text{OUR reactor} = \text{OUR endogenous} + \text{OUR Substrate} \qquad \text{(Eq.15)}$$

OUR substrates of heterotrophic biomass concentration: $(1\text{-}y_h)$. $Q.\frac{S_s - S_i}{V}$

And autotrophic soluble ammonium substrate S_{NH}: $(4.57\text{-}Ya)$. $Q.\frac{S_{snhi} - S_{inhe}}{V}$

The result from the endogenous respiration was performed. A good match between modeling and experimental results is observed, The Oxygen uptake rate (OUR Endo) seems to be close to the ASM simulation value. Backwashing play a major role in the process, an increase of active biomass was observed for both heterotrophic and autotrophic bacteria. The experimental and simulated biomass activity predicted by ASM1, ASM3 and Mantis2lib (GPS-X) of autotrophic and heterotrophic biomass represent only 40 to 60 % of the VSS composition. X_{BAI} concentration represents only 14% of the VSS, while X_{BAA} concentration is not stable during the whole acclimatization (very low), because of the competition between heterotrophic and Autotrophic Biomass nitrate producer bacteria. Indeed, NO_2^- was used both by X_{BH} and X_{BAA} (Figure 8).

Table 3 Values of stoichiometric and kinetic parameters

Parameters	Units	ASM1	ASM3
b_H	j^{-1}	0,12	0.1
b_{AI}	j^{-1}	0,04	0.061
b_{AA}	j^{-1}	0,04	0.061
Y_H		0,62	-
Y_{AI}	$gCOD.gN^{-1}$	0,2	-
fxi	-	-	0.2
Y_{AA}	$gCOD.gN^{-1}$	0,1	-
f_P	-	0,08	-
i_{XB}	-	0,0875	-
i_{bn}	-	0,08	-

Table 4 Simulation versus steady state experimental results of active biomass in endogenous respiration

Endogenous respiration	Respirometric measurement		Simulation		
	ASM1	ASM3	MANTIS2LIB		
			BOD5	S_s+S_{NH}	Backwashing
X_{BH} (g COD/L)	0.68	2.05	2.43	1.15	1.18
X_{BAI}(g COD/L)	0.38	0.7	0.22	0.18	0.176
X_{BAA}(g COD/L)	0.01	0.01	0.06	0.04	0.054

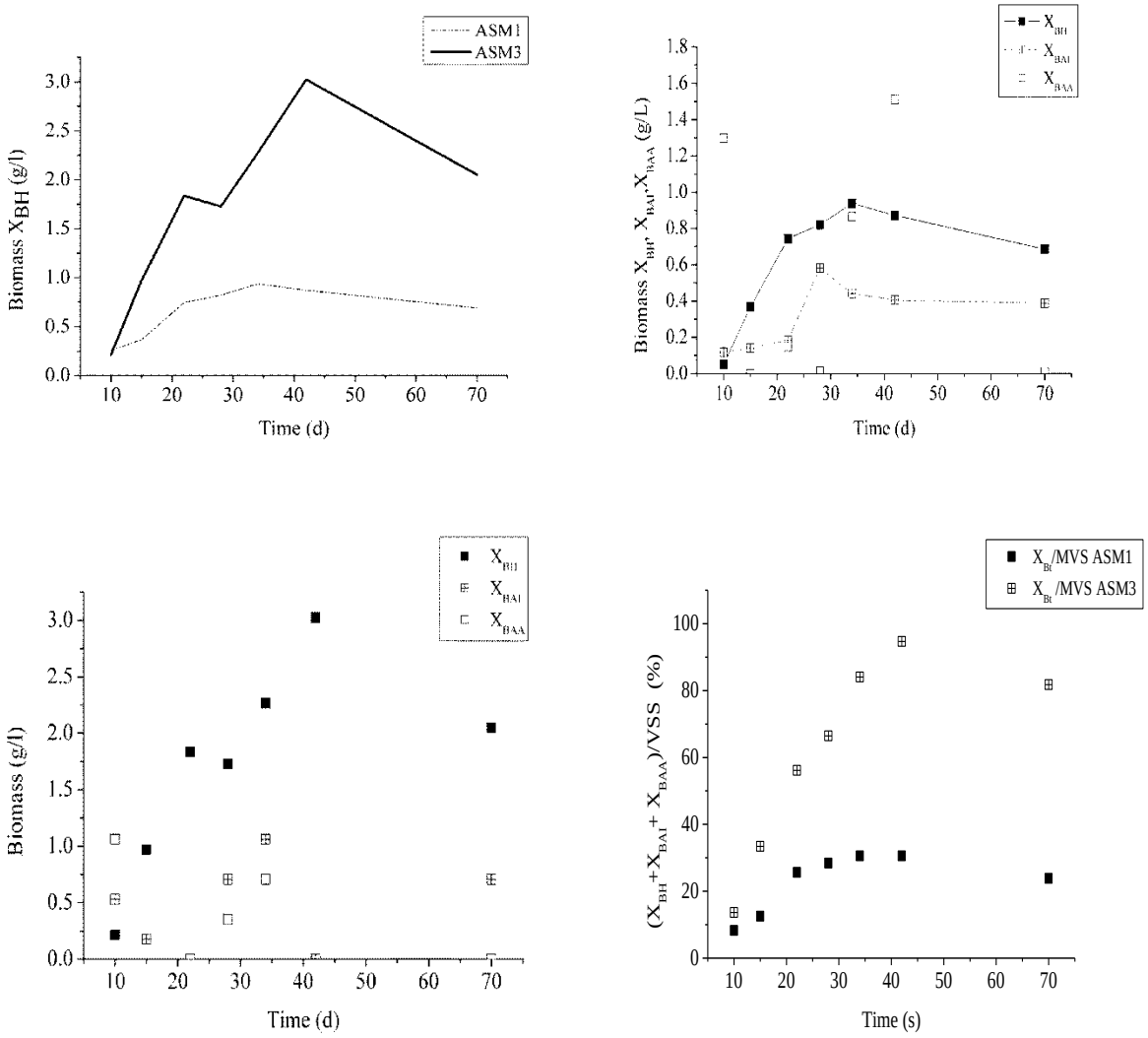


Figure 8 Biomass concentrations X_{BH}, $X_{BAI,}$ X_{BAA} with ASM1 and ASM3

CONCLUSION

An experimental campaign on sMBR pilot plant, conceived for biological nutrient removal, was performed. One of the main aims of the study was the evaluation of the active fraction of both heterotrophic and autotrophic (AOB and NOB) bacteria, as well as the SMP product, with the aid of respirometric batch tests. The development efficiency of equations at steady state which is fast and simple tool for quantifying the active biomass in the reactor. The experimental and simulation observations highlighted the important role of SRT in cell growth; at a significant SRT (60 d), the environmental conditions for active population in the sMBR are close to endogenous ones. Endogenous respirations represent 40% of VSS composition; X_{BAI} concentration represents only 14% of the VSS,

while X_{BAA} concentration is not stable during the whole acclimatization. The relatively low concentrations obtained allow a good control of dynamics clogging operations. The obtained results confirmed respirometry as a suitable tool for wastewater and biomass characterization, and that should provide a useful support in sMBR design and management, as well as in sMBR simulations.

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