

## Quantification of kinetic parameters for heterotrophic bacteria via respirometry in a hybrid reactor

Daniele Di Trapani, Giorgio Mannina, Michele Torregrossa and Gaspare Viviani

### ABSTRACT

Over the last decade new technologies are emerging even more for wastewater treatment. Among the new technologies, a recent possible solution regards Moving Bed Biofilm Reactors (MBBRs) that represent an effective alternative to conventional processes. More specifically such systems consist in the introduction of plastic elements inside the aerobic reactor as carrier material for the growth of attached biomass. Recently, one of the mostly used alternatives is to couple the Moving Bed Biofilm Reactor (MBBR) process with the conventional activated sludge process, and the resulting process is usually called HMBBR (Hybrid MBBR). In the MBBR process the biofilm grows attached on small plastic elements that are kept in constant motion throughout the entire volume of the reactor. Indeed, in such a system, a competition between the two biomasses, suspended and attached, can arise for the availability of the substrates, leading, as a consequence, to a modification in the biokinetic parameters of the two biomasses, compared to that of a pure suspended or attached biomass process. This paper presents the first results of a study aimed at estimating the kinetic heterotrophic constants in a HMBBR pilot plant using respirometric techniques. The pilot plant was built at the Acqua dei Corsari (Palermo) wastewater treatment plant and consisted of two parallel lines realized in a pre-anoxic scheme, in one of which the carrier material was added to the aerobic reactor with a filling ratio of 30%.

**Key words** | hybrid moving bed biofilm reactor, MBBR, organic carbon removal, pilot plant experiments, respirometric analysis

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### INTRODUCTION

Nowadays, due to the increasing awareness about environmental impact of discharges, it is necessary to realize biological processes that allow complete treatment of the wastewater; however, this fact must face with a complex situation in which a lot of difficulties arise when someone wants to build a new treatment plant or to upgrade an existing one. The growing increase of urbanization, together with the social and economic implications, make this problem topical. Indeed, the space request for the traditional activated sludge treatment (CAS) would be excessive in case of very high pollutant removal efficiency requirements. In this context, the technical and scientific

community in the last years showed a growing interest in developing innovative treatment processes that, together with very high removal efficiencies, can lead to a very low space and volume request.

A possible solution is represented by the combination of attached and suspended biomass systems, introducing plastic elements inside the aerobic reactor as carrier material for the growth of attached biomass. In recent years many studies have been carried out on hybrid systems, with the aim of investigating the process performances and also to compare different carrier media, obtaining very interesting results and highlighting the effectiveness of

such systems both for carbon and nitrogen removal (Morper & Wildmoser 1990; Müller 1998; Gebara 1999; Hamoda & Al-Sharekh 2000; Münch *et al.* 2000; Germain *et al.* 2007). In particular, one of the mostly used alternative is to couple the Moving Bed Biofilm Reactor (MBBR) (Ødegaard 2006) process with a conventional activated sludge process, and the resulting process is usually called HMBBR (Hybrid MBBR) (Mannina *et al.* 2007; Di Trapani *et al.* 2008a; Di Trapani *et al.* 2009; Mannina & Viviani 2009). Originally, the MBBR is a pure attached biomass process, where the biofilm grows attached on small plastic elements that are kept in constant motion throughout the entire volume of the reactor (Ødegaard *et al.* 1994; Rusten *et al.* 1995). The carriers are kept within the reactor through a sieve arrangement at the reactor outlet. The typical advantages of MBBR systems is represented by the low head loss, no filter channelling and no need of periodic backwashing (Pastorelli *et al.* 1999). When used in a hybrid process, the carriers can be kept in the whole or in a part of the activated sludge volume, depending on the main aim of treatment. Recently Mannina & Viviani (2009) demonstrated the effectiveness of the HMBBR systems for the upgrading of WWTPs that are overloaded. Specifically, employing this new technology, the authors concluded that it was possible to upgrade an Italian overloaded WWTP from 480,000 up to one million of equivalent inhabitants.

However, Hybrid Moving Bed Biofilm Reactor (HMBBR) processes are relatively new and there are still some uncertainties concerning the kinetic behaviour of the system; indeed, in such a system, a competition between the two biomasses, suspended and attached, can arise for the availability of the substrates, leading, as a consequence, to a modification in the biokinetic parameters of the two biomasses, compared to that of a pure suspended or attached biomass process. Further, the system behaviour is strictly related to the effect that some “key parameters” such as organic load, ammonium load, dissolved oxygen, temperature, SRT (Sludge Retention Time), have on the process (Di Trapani *et al.* 2008b). In this context, respirometry seems to be a useful device to characterize the biokinetic behaviour of such a system.

This paper presents the first results of a study aimed to the quantification of kinetic heterotrophic parameters in a HMBBR pilot plant using respirometric techniques.

## MATERIALS AND METHODS

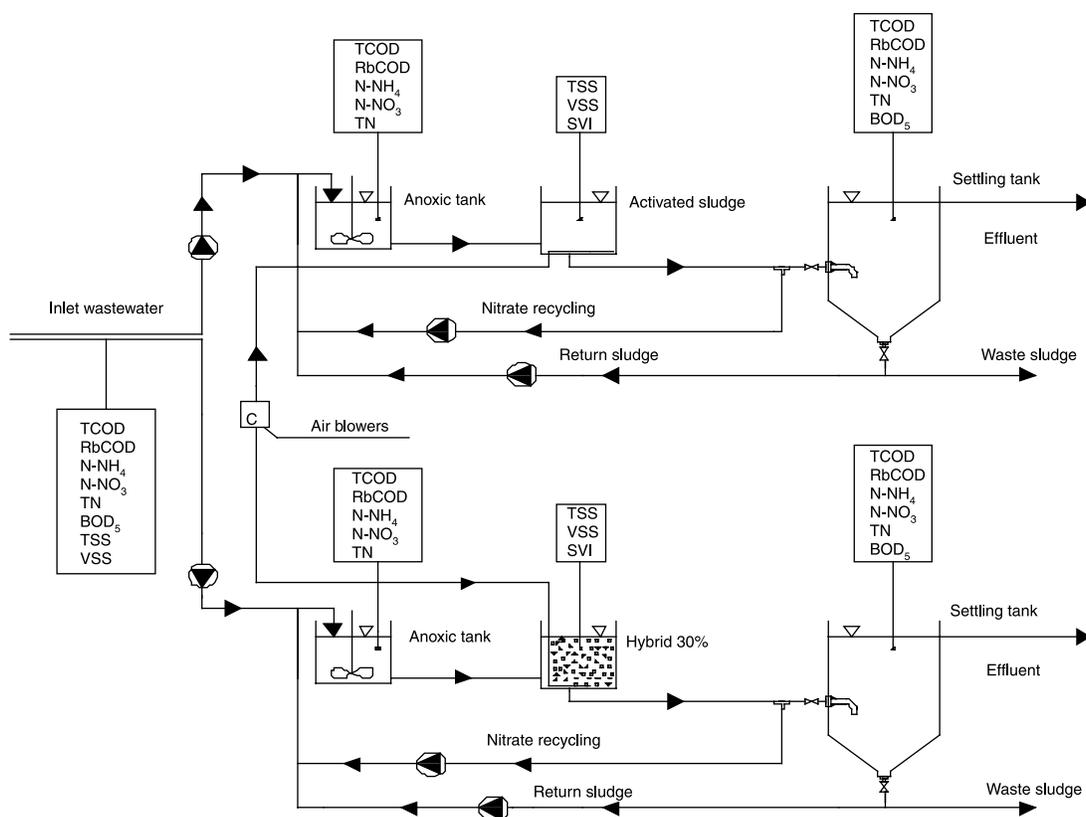
### Description of the pilot plant

The pilot plant was built at Palermo (IT) municipal WWTP (Acqua dei Corsari), and consisted of two parallel lines, each in a pre-anoxic scheme, in one of which the suspended carriers (AnoxKaldnes™ K1) were added to the aerobic reactor with a 30% filling ratio. Each line consisted of a 6 litres anoxic reactor, a 12 litres aerobic one and a 3.5 litres final clarifier. In Figure 1 the pilot plant layout with the sampling sections is reported.

Mixing in the aerobic reactors was provided by the medium-coarse bubble aeration systems installed at the bottom of the tanks, while in the anoxic tanks mixing was accomplished by mechanical stirrers. Special sieve arrangements were adopted to retain the carriers inside the aerobic reactors. The support carrier material used was the AnoxKaldnes™ K1, which characteristics are summarised in Table 1.

The experimental campaign lasted for a period of approximately three months. However, in order to allow microbial communities in the wastewater to adapt to the configuration used as well as to enhance the biofilm growth on the carriers, the pilot plant lines were kept under the same conditions in terms of influent load during approximately 40 days. The pilot plant was continuously fed with municipal wastewater taken immediately downstream the fine screen of the WWTP.

The experimental campaign was divided into three different phases. At the beginning of the experimental period, the influent flow was the same for both lines, with a constant value of 1.5 L/h, corresponding to a hydraulic retention time (HRT) in the biological reactors of almost 12 hours and a nitrate recycle of 6 L/h (first phase); a second phase during which the flow rate of the HMBBR line was increased up to 3 L/h, corresponding to a 6 hours HRT, and a nitrate recycle of 12 L/h, in order to better highlight the difference for the respect to the AS system. In order to better single out the attached biomass contribution of the HMBBR system, the MLSS concentrations of the two systems were kept as similar as possible. In this phase of the study it was decided to keep the AS line under the same operative conditions of the previous one in order to



**Figure 1** | Configuration of the pilot plant.

investigate only the performance of the HMBBR process under the new operative conditions. It has to be stressed that in order to increase the influent organic load to the plant it should also have been possible to add synthetic substrates. Such choice was discarded due to the fact that the idea was to study a pilot plant fed with real wastewater. Indeed, this latter choice is more representative of a real WWTP characterised by the increase of the influent organic load. Finally, in the third experimental phase, the influent flow to the HMBBR line was reduced to the original value; therefore, in the last experimental days, the two lines have worked still under the same operative conditions. In **Figure 2**, the influent organic loads, in terms of TCOD, during the three phases are reported. In particular, the average influent

organic load in the first and latter phase is approximately 14 gTCOD/d and it rises up to 30 gTCOD/d in the second phase for the HMBBR due to the increase of the flow rate. The influent organic load generally shows a sensibly variability assuming a maximum peak of 50 and 100 g TCOD/d, respectively, for the AS and HMBBR system.

During the whole experimental period, samples from the influent and the effluent have been taken three times a week for the chemical analysis, while periodical samples have been taken from the anoxic tank to check if there was some anomaly. TSS and VSS, TCOD,  $\text{NH}_4\text{-N}$ ,  $\text{NO}_5\text{-N}$ , TN, were analysed according to the *Standard Methods* (APHA 1995), while the readily biodegradable COD (rbCOD) was determined according to Mamais *et al.* (1993). Periodical sludge volume index (SVI) tests have been also carried out in order to evaluate the sludge settleability properties for the two lines. Test on carrier samples were carried out, in order to establish the amount of biomass attached on the carriers. The assessment of attached biomass was carried out considering the TSS on the support carriers. To evaluate

**Table 1** | Characteristics of the K1 carriers, filling ratio and effective surface in the reactor

Diameter (mm)	Heigh (mm)	Density ( $\text{kg}/\text{m}^3$ )	Protected surface ( $\text{m}^2/\text{m}^3$ )	Filling ratio (%)	Reactor surface ( $\text{m}^2/\text{m}^3$ )
9.1	7.2	0.95	500	30	150

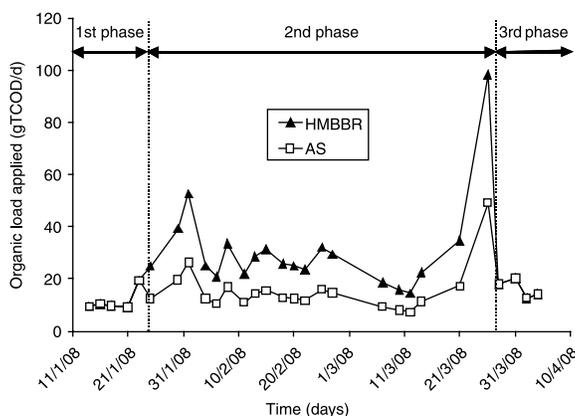


Figure 2 | HMBBR and AS influent organic loads in the three sub-periods.

the latter, a sample of carrier elements was taken from the aerobic reactor, dried at 105°C for 24 hours and weighted. Then, this value was compared with an average “zero” weight determined at the beginning of the experimental period, thus obtaining the biomass weight in a single carrier element. So, as the number of carrier was known for each reactors, it was calculated the total attached biomass in the reactor and consequently, through the filling ratio, the grams of TS per litre of reactor volume.

### Kinetic parameter estimation using respirometry

Respirometric batch tests were carried out with the aim to characterise the process behaviour and to detect the different roles played by the biomass species in carbon removal. Respirometric experiments were conducted using a “flow-gas/static-liquid” type as batch respirometer (Spanjers *et al.* 1998). The samples (1.5 litres) were taken from the aerated reactor of HMBBR line, containing 30% carrier concentration, moved into a 2 litres beaker and finally aerated until endogenous conditions were reached. The samples were maintained at a constant temperature of 20 ± 1°C with a thermostatic cryostat (JULABO). Agitation was provided by a magnetic and a mechanical stirrer (FALC), and the sample was aerated intermittently using an aeration pump. The dissolved oxygen concentration was measured with an oxygen sensor (WTW mod. MULTI 340i), while the aeration control and data acquisition were provided by the OURsys software. The aeration intervals were set from 3 to 6 mgDO/L. The OURsys software

provided the generation of the respirograms, that feature the characteristic biomass endogenous and exogenous respiration phases. Starting from the OUR data, the estimation of the kinetic parameters was carried out. In Figure 3, the respirometric set-up layout is reported.

In order to evaluate the kinetic parameters of the process, known amounts of sodium acetate as rbCOD were added to the samples during the analysis, keeping the  $S_0/X_0$  ratio below 0.05 g COD g COD<sup>-1</sup>, with the aim to neglect the biomass growth during the batch test (Chudoba *et al.* 1992). Allylthiourea (ATU) was also added during the batch tests in order to inhibit the nitrifying activity (range 10–15 mg ATU/L).

The COD variations were calculated from O<sub>2</sub> consumption, stated that oxygen variation is equivalent to COD through the following expression:

$$\Delta\text{COD} = \frac{\Delta\text{O}_2}{1 - f_{cv} \cdot Y_H} \quad (1)$$

where  $f_{cv}$  is the conversion coefficient from COD to VSS (in the range 1.42–1.48 mg COD mg VSS<sup>-1</sup>) and  $Y_H$  the yield coefficient in this phase assumed to be equal to 0.45 mg VSS mg COD<sup>-1</sup>. The previous expression provides the COD measured.

On the other hand, the COD simulated was established solving the Monod-type kinetic expression with the finite differences method, estimating the parameters  $\nu_{H,\max}$  and  $K_S$  by fitting the following Equation (Metcalf and Eddy 2002):

$$\frac{\Delta S}{\Delta t} = \nu_{\max,H} \cdot \frac{S}{(K_S + S)} \cdot X_H \quad (2)$$

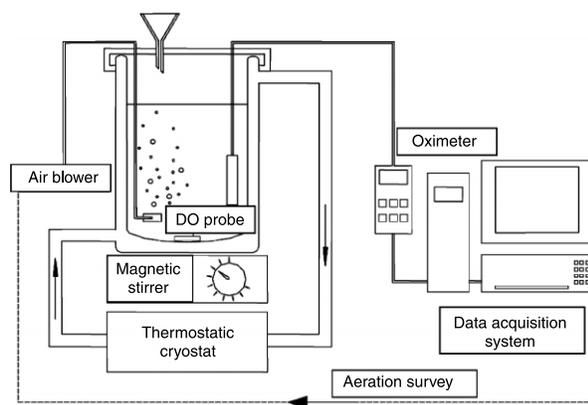


Figure 3 | Configuration of the respirometric system.

**Table 2** | Influent and effluent COD (total and readily biodegradable) and BOD<sub>5</sub> concentrations in the overall experimental period

	Inlet			Outlet			AS		
	TCOD mg/L	rbCOD mg/L	BOD <sub>5</sub> mg/L	TCOD mg/L	rbCOD mg/L	BOD <sub>5</sub> mg/L	TCOD mg/L	rbCOD mg/L	BOD <sub>5</sub> mg/L
Min	201	61	130	10	7	1	10	9	2
Max	1,365	368	250	103	64	14	96	60	20
Average	409	128	167	63.1	29.2	9.2	58.8	29.8	9.7

where  $\nu_{H,max}$  is the maximum removal rate,  $K_S$  is the half-saturation coefficient,  $S$  is the substrate concentration and  $X_H$  is the biomass active fraction. The kinetic coefficients were obtained by minimising the value of the root mean square error (RMSE) which represents the difference between measured and simulated data:

$$RMSE = \sqrt{\frac{\sum_{i=1}^N (X_{M,i} - X_{S,i})^2}{N}} \quad (3)$$

were  $X_{M,i}$  is the measured variable,  $X_{S,i}$  is the simulated variable and  $N$  is the number of available observations.

Further, other indices have been used to evaluate model adaptation to the measured data, in terms of COD removal: the Mean Error (ME), evaluated by means of Equation (3), the dimensionless mean error  $ME/\bar{X}_M$  (Equation (4)), the correlation coefficients ( $R^2$ ), between measured and simulated values and the Nash–Sutcliffe efficiency ( $E$ ) (Nash & Sutcliffe 1970):

$$ME = \frac{\sum_{i=1}^N (X_{M,i} - X_{S,i})}{N} \quad (4)$$

$$\frac{ME}{\bar{X}_M} = \frac{\sum_{i=1}^N (X_{M,i} - X_{S,i})}{N \cdot \bar{X}_M} \quad (5)$$

$$E = 1 - \frac{\sum_{i=1}^N (X_{M,i} - X_{S,i})^2}{\sum_{i=1}^N (X_{M,i} - \bar{X}_M)^2} \quad (6)$$

were  $\bar{X}_M$  is the mean value of the measured data.

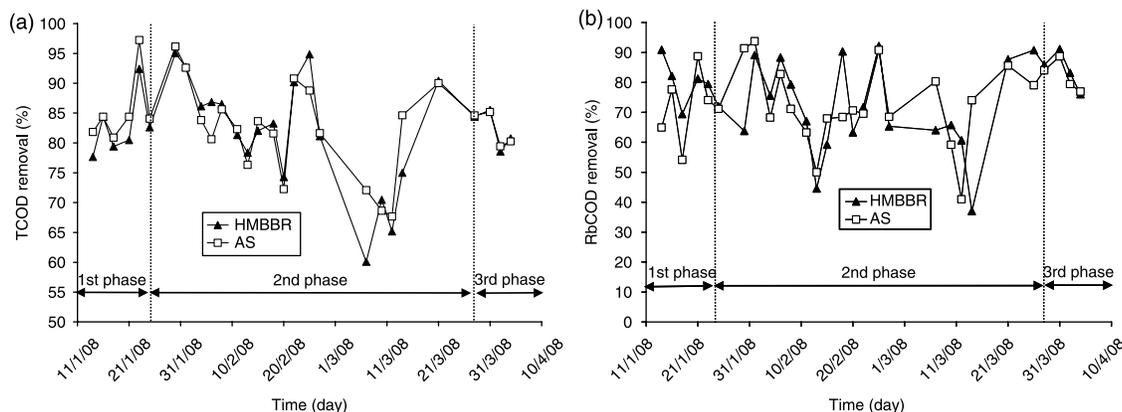
## RESULTS AND DISCUSSION

### Organic substrate removal

The influent and effluent characteristics, in terms of TCOD, rbCOD and BOD<sub>5</sub> are summarised in the following Table 2, where the minimum, maximum and average values are reported.

Both systems showed good removal efficiencies in each experimental phase, with very similar average TCOD removal efficiencies for the HMBBR and the AS, equal to 82.7 and 83.3% respectively in the overall experimental period.

In particular, in the first and in the third phase, both lines showed the same removal efficiency in terms of TCOD, with an average value of 82.6% (Figure 4(a)); such result

**Figure 4** | Total (a) and readily biodegradable (b) COD removal efficiencies during the experimental campaign.

**Table 3** | Average values of kinetic parameters

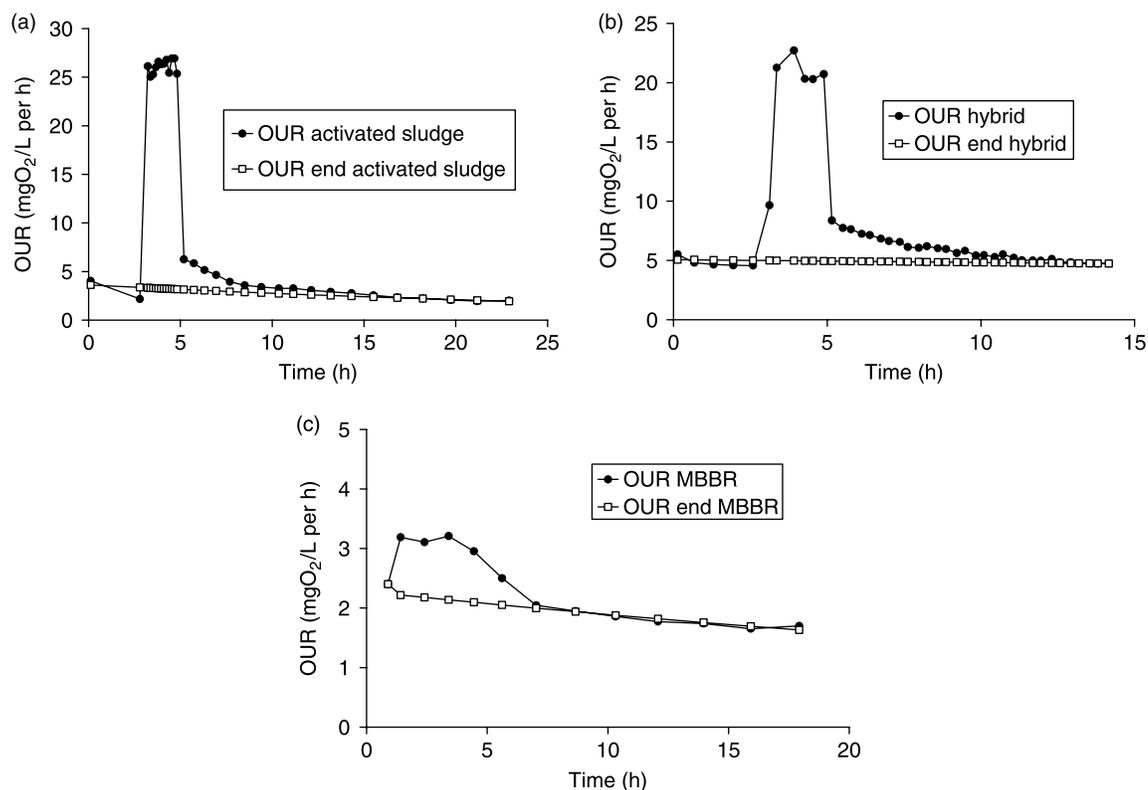
	$Y_{\text{obs}}$ kg COD kg COD <sup>-1</sup>	$Y_{\text{H}}$ kg COD kg COD <sup>-1</sup>	$K_{\text{s}}$ mg L <sup>-1</sup>	$\mu_{\text{H,max}}$ d <sup>-1</sup>	$\rho_{\text{H,max}}$ d <sup>-1</sup>	$b_{\text{H}}$ d <sup>-1</sup>
SB	0.81	0.79	12.41	2.37	2.97	0.098
SAB	0.92	0.88	8.82	2.06	2.24	0.056
AB	0.98	0.98	3.10	0.33	0.33	0.034

shows that the attached biomass basically does not provide an extra effective contribution to the TCOD removal; indeed, although the total biomass in the HMBBR system is higher respect to the AS one, due to the contribution of the attached biomass, the efficiencies are comparable.

Referring to the rbCOD, the HMBBR system showed a better performance than AS line in the first and latter phase, with average removal efficiencies of 82.16 and 76.8% respectively. In this case, the attached biomass gave a sensible contribution, suggesting that the rbCOD is removed on the basis of the total biomass amount in the reactor (Figure 4(b)).

Further, the higher removal capacity of the rbCOD operated by the HMBBR system respect to the AS and on the other hand the equal TCOD removal, lead to conclude that the particulate COD was less removed by the HMBBR system. This result suggests that a reduction of the hydrolysis and bioflocculation capacity that is generally operated by the suspended biomass (Andreottola *et al.* 2000) took place. This reduction was likely due to the presence of the carrier that prevented the formation of the flocs, as previously reported by other authors (Christensson & Welander 2004).

During the second experimental phase, the two systems showed almost equal removal performances; in terms of TCOD, the average removal efficiency was almost 82% for both lines, while for the rbCOD the removal efficiency was 70 and 72% for HMBBR and AS respectively. It has to be stressed that the HMBBR system in this phase was characterized by an influent organic load that was approximately two times higher, and by a half hydraulic retention time, respect to the AS one. These results suggest that the

**Figure 5** | OUR profiles for SB (a), SAB (b) and AB (c).

effectiveness of the HMBBR system is in the case of high organic loads corresponding to overloading conditions for the traditional AS. However a more reliable comparison between the two systems under high organic loads should be possible by increasing the influent load also in the AS line.

### Kinetic parameter estimation

Respirometric tests were carried out on three different samples taken from the HMBBR line:

1. samples having only suspended biomass (SB);
2. samples having both suspended and attached biomass (SAB);
3. samples having only attached biomass (AB).

The procedure described above was aimed to single out the different contribution of the biomass to the removal of the pollutant substances. The obtained respirograms for the different samples feature the typical endogenous and

exogenous respiration phases, as reported in previous studies (Plattes *et al.* 2007; Ferrai *et al.* 2008). In Table 3 the average values obtained for the kinetic parameters are reported.

### Yield coefficient $Y_H$

Yield coefficient for heterotrophic biomass was estimated from the integral of the exogenous oxygen uptake rate (Vanrolleghem *et al.* 1999), for the SB, SAB and AB samples. After the injection of acetate, oxygen was rapidly consumed and after substrate depletion, the endogenous phase was restored. It has to be stressed that for both SB and SAB samples, a tailing of the OUR-curve has been observed, suggesting that a storage phenomenon occurred, and the tailing phenomenon was probably due to the consumption of the stored products (Majone *et al.* 1999; van Loosdrecht & Heijnen 2002). This result led, as a consequence, that the obtained  $Y_H$  values were higher for the biofilm than for the SB, and both were higher than the default values proposed

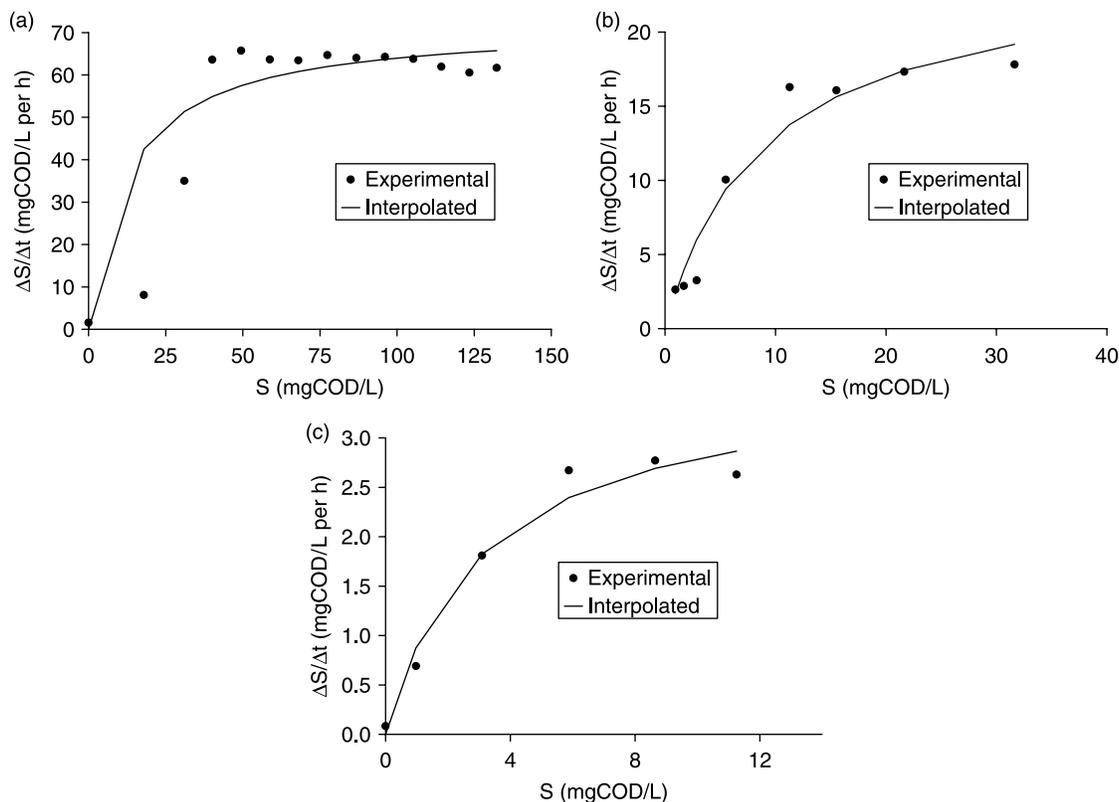


Figure 6 | Monod type curves for SB (a), SAB (b) and AB (c).

**Table 4** | Goodness of fit indices for the different biomasses

	<b>RMSE [mg/L]</b>	<b>ME [mg/L]</b>	<b>ME/<math>X_{M,AV}</math></b>	<b>E</b>	<b>R<sup>2</sup></b>
SB	6.18	1.51	0.03	0.90	0.83
SAB	1.48	-0.11	-0.01	0.56	0.95
AB	0.17	-0.002	-0.001	0.97	0.97

by the IWAPRC (Henze *et al.* 1987), in the range 0.38–0.75 for a conventional activated sludge system. The higher values obtained for the AB suggest that the storage phenomenon was predominant in biofilm; this behaviour can be explained in the following terms: since the organic load fed to the pilot in the last experimental phase, when respirometric tests were performed, was quite low, this led to a kind of starvation inside the biofilm, and when the acetate (a readily biodegradable substrate) was added to the sample during the respirometric tests, the biomass stored this substrate instead of using it for direct growth. In the following Figure 5, the respirograms for SB (a), SAB (b) and AB (c) are reported.

### Decay rate $b_H$

The decay rate of heterotrophic bacteria for both AB and SB, was established by taking the samples in endogenous conditions (not fed) for 1–2 days. Plotting the logarithmic endogenous rate vs time, the decay rate can be estimated by an exponential curve.

The values obtained in the batch tests with pure AB, were lower than the usual values reported in the technical literature for activated sludge systems, and also lower than the values obtained for the SB. This result is likely due to the high biofilm activity and consequently to a lower

decay rate. However, further research is needed to confirm such results.

### Half saturation coefficient $K_S$ and maximum growth rate $\mu_{H,max}$

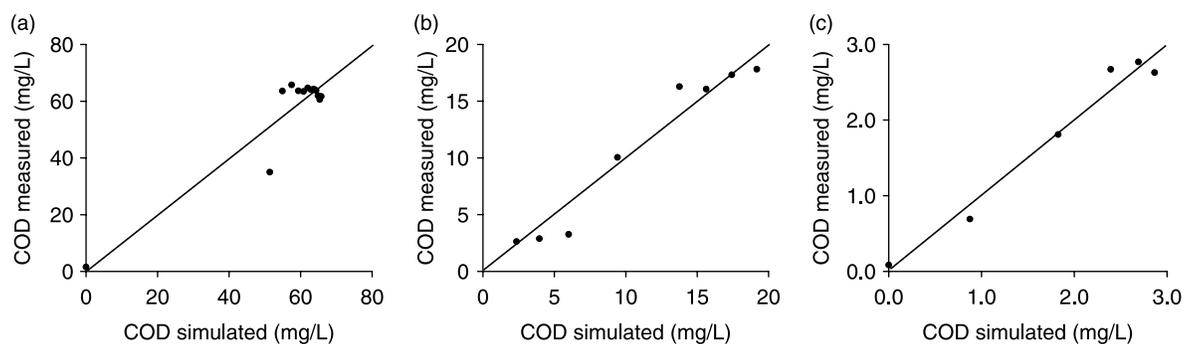
Figure 6 shows the measured data, together with the Monod type kinetic simulated data, for SB (Figure 6(a)), SAB (Figure 6(b)) and AB (Figure 6(c)) respectively.

Concerning the half-saturation coefficient  $K_S$ , the obtained values were well in the range reported in the technical literature for activated sludge systems (Henze *et al.* 1987); it has to be stressed that the higher values were recorded for the SB samples, equal to 30 mg COD/L: the suggestion is that mass transport limitations for the substrate were more important in the AB than in the SB one.

Further, referring to the maximum growth rate  $\mu_{H,max}$ , the obtained values were in the typical range for the SB, while, on the other hand, for the AB, they were slight lower than the values obtained in previous study on pure MBBR systems (Ferrai *et al.* 2008), maybe because of the competition of the two biomasses for the availability of soluble organic substrate in the reactor.

Globally, good results have been obtained as confirmed by the values of  $E$  ranging between 0.56 and 0.97; in addition the quite low values of ME ranging between -0.002 and 1.51 (mg/L) indicate that the model can be considered unbiased. The good agreement with the experimental data referring to COD removal is also demonstrated by the correlation coefficient values ranging from 0.83 to 0.97 (Table 4).

Figure 7 shows the results in terms of COD removal; indeed, the agreement between simulated and measured

**Figure 7** | Measured versus simulated values for SB (a), SAB (b) and AB (c).

values was satisfactory, with good correlation coefficients, as reported above.

## CONCLUSIONS

An experimental gathering campaign on a pilot-scale reactor, constituted by two parallel lines, a classical activated sludge system and a HMBBR one, was carried out. The aims of the study were to gain insight about the performances of the HMBBR process and to assess the biokinetic behaviour of heterotrophic biomass using respirometric tests.

In the first and later phases, the performances of the two systems were almost comparable in terms of TCOD, suggesting that the attached biomass did not give an extra contribution to the removal process. On the other hand, in the second phase, the HMBBR showed a better performance respect to the AS one, suggesting an employment of such systems for the case of high loaded WWTP.

In terms of rbCOD, the HMBBR system showed better performances than AS line in the first and latter phase, with average removal efficiencies of 82.2 and 76.8%, respectively. The lower particulate COD removal operated by HMBBR, suggests that a reduction of the hydrolysis and bioflocculation capacity occurred in HMBBR system; this reduction is probably related to the presence of the carriers in the reactor that can obstacle the formation of flocs.

Concerning the respirometric tests, they were performed on samples of AB, SAB and SB taken from the hybrid line only, in order to estimate the kinetic parameters.

The heterotrophic yield coefficient resulted higher for the pure MBBR samples than activated sludge ones, suggesting a better specialization of the AB in removing the soluble organic substrate. On the contrary, the heterotrophic maximum growth rate resulted higher for the SB, well inside the typical range of conventional activated sludge systems, while referring to the AB, the yield coefficient was slight lower compared to values previously obtained in pure MBBR pilot studies. Provided that the respirometric test were performed in the last experimental phase, when the organic load was decreased, this result is a

confirmation that for such operative conditions, the attached biomass basically does not provide an extra effective contribution to the TCOD removal. Concerning the half saturation coefficients, it has to be stressed that the higher values were obtained for the SB samples, thus indicating that mass transport limitations for the substrate were more important in the AB than in the SB.

The obtained results confirm that respirometry is a useful device to characterise the process behaviour, and that kinetic parameters obtained from OUR profiles can provide a support to attain HMBBR criteria design. However, further experimental studies are needed to confirm such results and to overcome some technical shortcomings when performing respirometric batch tests.

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