Active heterotrophic and autotrophic biomass distribution between fixed and suspended systems in a hybrid biological reactor

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Abstract This paper presents the results obtained when modifying sludge retention time (SRT 8, 5, and 3.7 days during phases A, B and C respectively) for a hybrid biological reactor (HR) compared with a classical activated sludge process. The study was conducted by following active biomass evolution and distribution for two lab-scale pilots plants operating with the same conditions, one acting as HR and the other as the control reactor (CR) without support material. At the end of phase C, support material was split into two fractions between both reactors to study the effect of support to reactor volume ratio (Fr). Active biomasses in suspended and fixed systems were calculated using respirometric techniques. Evolutions of active autotrophic and heterotrophic biomasses for both reactors are presented during all the operational periods and it is observed that in the HR biomass concentrations are up to double that in the CR, mainly due to the presence of support material. When studying biomass distribution in HR, autotrophic biomass is mainly located over the support material (from 95% to 99% during periods A and C respectively) while only about 60% of heterotrophic biomass is located over the support.

Keywords Autotrophs; biomass distribution; heterotrophs; hybrid reactor; OUR; oxygen uptake rate

Introduction
Combining suspended and fixed biomass systems has arisen as an alternative to classical activated sludge systems to improve plant performance. Such systems, known as hybrid systems, are able to improve nitrification potential by increasing cellular retention time independent of purge flows. An important aspect to enable high performance in these configurations is the carrier support characteristics (Odegaard, 2000). Using support material with high specific surface area, total active biomass inside the reactor is highly increased and thus higher volumetric substrate conversion rates are possible, in addition, some effects on sludge quality, such as reducing bulking phenomenon (Andreottola et al., 2000), have been observed.

Distribution of microorganisms in fixed systems (biofilm) could be studied by means of microbiological techniques or activity measurements. Some authors studied the effect of C:N ratio in the feed medium on spatial distribution of nitrifiers and heterotrophs using microslicer and FISH techniques (Okabe et al., 1995; Lazarova et al., 1998; Satoh et al., 2000, Gieseke et al., 2001), or the utilization of oxygen microelectrodes for the measurement of activity in biofilm on small suspended particles (Van Loosdrecht et al., 1995). The use of activity measurement techniques are based on the measurement of oxygen uptake rate (OUR) and have been proved to be a reliable method for determination of viable biomass in wastewater treatment, (Jorgensen et al., 1992; Riefler et al., 1998).

In this paper the biomass distribution between the liquid (suspended biomass) and the support (fixed biomass) as well as their composition (heterotrophic and autotrophic...
fractions) are presented for a hybrid reactor and compared to an activated sludge reactor. Active biomass for both heterotrophic and autotrophic biomass was calculated using respirometric techniques for both suspended and fixed biomass. The study started with 8 days of SRT and successively reduced to 5 and 3.7 days. When working at 3.7 days of SRT, colonised support from the hybrid reactor was split into two fractions for the hybrid and the control reactor to study the effect of support volume in the hybrid reactor, as well as the effect of adding a colonised support to a classical activated sludge system.

Material and methods

Experimental set-up

Two pilot plants (Figure 1), one with a mobile plastic support (HR) and an other without, acting as control reactor (CR), were operated for this study (22 litres reactor and 2 litres settler). Sludge recycle flow from settler to reactor was constant at a ratio of 1.5 times the influent flow. A sequenced aeration (45 min. aerobic + 45 min. anoxic) operation was selected in order to perform nitrification and denitrification in the same reactor.

In HR, a plastic support (polyethylene, density 900 g·litre⁻¹, spherical shape, equivalent diameter between 2–3 mm and specific surface about 2,000 m²·m⁻³) was used to permit the development of a biofilm over the support surface (Figure 2a). Support homogenisation into the reactor was conducted by means of mechanical agitation using two marine-helix devices (Figure 2b) and retained inside the reactor by reducing reactor outflow velocity as show in Figure 1(9). Thus, ascending velocity of support material was higher than descendent liquid velocity due to a support density lower than liquid suspension. Used filling ratio (Fr), calculated as support real volume to total biological reactor volume, was initially set at

![Figure 1 Diagram of the biological reactor.](https://iwaponline.com/wst/article-pdf/46/1-2/397/476862/397.pdf)

![Figure 2 (a) Support media used in the experiments; (b) Agitation system, constant speed to keep plastic support under suspension with reduced superficial oxygen transfer](https://iwaponline.com/wst/article-pdf/46/1-2/397/476862/397.pdf)
0.2 (20%). Both pilot plants were operated with controlled water temperature at 16 ± 1°C by means of a cryogenic unit and monitoring dissolved oxygen concentration and pH with two CONSORT® units. Aeration was conducted by diffusion of compressed air.

Both biological reactors were operated with 45 l·d⁻¹ of influent flow and an external recycling flow that were maintained constant during all the operational periods. External carbon source was added during 15 minutes in the middle of the anoxic period to ensure a dissolved oxygen concentration near to zero, and to focus the use of external carbon mainly for denitrification purposes.

**Analytical methods.** Chemical Oxygen Demand (COD), ammonium (NH₄⁺), Total Kjeldahl Nitrogen (TKN), total (TSS) and volatile (VSS) suspended solids were measured according to Standard Methods (1995).

**Wastewater characteristics.** Real urban wastewater was collected from local sewer of Toulouse (France), settled for 45 minutes, and stored in a stainless steel tank at 4°C (COD: 350 mg·l⁻¹, soluble COD: 250 mg·l⁻¹, TSS: 200 mg·l⁻¹, VSS/TSS: 0.84, TKN: 75 mg N-TKN·l⁻¹, Ammonium: 65 mgN-NH₄⁺·l⁻¹). Wastewater was renewed every 3–4 days to minimize composition degradation. The C/N ratio of fresh wastewater was between 3–5 g COD/g N-TKN, and in order to improve denitrification during anoxic periods, a synthetic external carbon source (COD: 5000 mg·l⁻¹, flow rate: 1.5 l·d⁻¹) was added during the anoxic phases to reach C/N ratios between 6 and 7. Applied loading rate was between 0.9–1.2 kg COD/m³·d.

**Sludge Retention Time (SRT).** The operational conditions for the CR and HR systems were identical (i.e. influent, recycling rate, external carbon, air flow, reactors and settler volumes and purge flows). Setting the purge flow from the biological reactor, SRT could be calculated as reactor to purge flow ratio. However the presence of the carrier inside HR involves a reduction of liquid volume and in consequence, the SRT for the suspended biomass in the HR was lower than for the CR.

**OUR determination.** Oxygen Uptake Rate (OUR) was measured using a closed respirometric unit. The respirometer was composed of a stirred reactor (0.25 l of liquid) in which dissolved oxygen concentration was measured by means of an electrode CellOx 325 WTW and a microprocessor oximeter Oxi 538 WTW. These values were continuously monitored on a 486 PC. A water jacket was used to keep the temperature of the system constant. The oxygen uptake rate (OUR) was determined by linear regression from the slope obtained from the plot of dissolved oxygen concentration versus time.

The OUR was calculated under three different conditions in order to obtain 1) endogenous OUR (OUR₁), 2) oxygen consumption during nitrification of ammonia without carbon source (OUR₂), and 3) exogenous respiration with carbon source and inhibited nitrification with Allylthiourea (ATU) as presented in Table 1 (OUR₃).

In the case of suspended biomass, a mixed liquor sample was removed directly from the reactor (250 ml), centrifuged for 10 minutes and rinsed with distiller water. For fixed biomass, 33 ml of colonised plastic support from the reactor were rinsed three times with distilled water.

<table>
<thead>
<tr>
<th>OUR</th>
<th>Ammonia</th>
<th>Carbon source</th>
<th>ATU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous, OUR₁</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Autotrophic, OUR₂</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Heterotrophic, OUR₃</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Afterwards the sample (suspended or colonised support) was introduced into the respirometric cell and filled with an aerated nutrient solution (Standard Methods for the Examination of Water and Wastewater, 1995) without the presence of carbon source or ammonia.

From the experimental values obtained following Table 1 experiments, oxygen uptake rates for the endogenous process ($OUR_{End} = OUR_1$), autotrophic nitrification ($OUR_{A\text{-MAX}} = OUR_2 - OUR_1$) and organic carbon oxidation ($OUR_{H\text{-MAX}} = OUR_3$) were calculated and referred to total reactor volume (mg O$_2$·h$^{-1}$·litre reactor$^{-1}$) according to the used sample volume and the volume of liquid or support material inside the reactor.

**Active biomass calculations**

From Henze et al. (1986) and assuming that inside the respirometric cell the saturation functions for dissolved oxygen and substrates are near to one, active heterotrophic biomass (gr. X$_H$·COD·m$^{-3}$) could be calculated from the OUR as presented in Eq. (2a). Considering the nitrification process, autotrophic biomass could be calculated from Eq. (2b). Used stoichiometric and kinetic parameter values for heterotrophic yield (Y$_H$) and maximum specific heterotrophic rate ($\mu_{H\text{-max}}$) are presented in Table 2.

\[
\text{a) } X_H = \frac{1}{\mu_{H\text{-max}}} \frac{Y_H}{1-Y_H} (OUR_{H\text{-MAX}}) \quad \text{b) } X_A = \frac{1}{\mu_{A\text{-max}}} \frac{Y_A}{4.57-Y_A} (OUR_{A\text{-MAX}})
\]  

Applying Eqs (2a) and (2b) to the experimental results obtained from suspended and fixed biomass samples, suspended, fixed, and total concentrations of active autotrophic (X$_{AR}$), heterotrophic (X$_{HR}$) and total (X$_{TR}$) biomass referred to the whole reactor volume could be calculated from Eq. (3).

\[
X_{AR} = [X_A]_{\text{fixed}} + [X_A]_{\text{suspended}} \quad X_{HR} = [X_H]_{\text{fixed}} + [X_H]_{\text{suspended}} \quad X_{TR} = X_{HR} + X_{AR}
\]

\[
X_{TF} = [X_A]_{\text{fixed}} + [X_H]_{\text{fixed}} \quad X_{TS} = [X_A]_{\text{suspended}} + [X_H]_{\text{suspended}}
\]

With sub indexes: A, autotrophic biomass; H, heterotrophic biomass; F, fixed; S, suspended; T, total; and R, reactor.

**Active biomass fractions**

In order to identify the biomass distribution between the fixed and the suspended system as well as the composition of both systems, several ratios or fractions (f) were defined following Eqs. (4).

\[
f_{AF/AT} = \frac{[X_A]_{\text{fixed}}}{X_{AR}} \quad f_{HF/HT} = \frac{[X_H]_{\text{fixed}}}{X_{HR}} \quad f_{TF/TR} = \frac{X_{FR}}{X_{TR}}
\]

\[
f_{AF/TF} = \frac{[X_A]_{\text{fixed}}}{X_{TF}} \quad f_{FS/TS} = \frac{[X_A]_{\text{suspended}}}{X_{TS}} \quad f_{AT/TR} = \frac{X_{AR}}{X_{TR}}
\]

Active biomass distribution is then presented as the i) fixed autotrophic biomass ratio to total autotrophic biomass ($f_{AF/AT}$), ii) fixed heterotrophic biomass ratio to total autotrophic biomass ($f_{AF/TF}$), and iii) heterotrophic biomass ratio to total biomass ($f_{FS/TS}$).

**Table 2** Used stoichiometric and kinetic parameters values in Eqs (2)

<table>
<thead>
<tr>
<th>Stoichiometric parameters</th>
<th>Temperature correction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y$_A$, g cell COD formed (g N oxidized)$^{-1}$</td>
<td>0.24</td>
</tr>
<tr>
<td>Y$_{H+}$, g cell COD formed (g COD oxidized)$^{-1}$</td>
<td>0.67</td>
</tr>
<tr>
<td>Kinetic parameters (20°C)</td>
<td></td>
</tr>
<tr>
<td>$\mu_{H\text{-max}}$, day$^{-1}$</td>
<td>0.8</td>
</tr>
<tr>
<td>$\mu_{H\text{-max}}$, day$^{-1}$</td>
<td>6.0</td>
</tr>
</tbody>
</table>
heterotrophic biomass (f_{HF/TR}) and, iii) fixed active biomass to total active biomass (f_{AT/TR}). Composition of suspended and fixed systems are presented as i) fixed active autotrophic biomass ratio to total fixed active biomass (f_{AF/TF}), ii) suspended active autotrophic biomass ratio to total suspended active biomass (f_{AS/TS}) and iii) total reactor active autotrophic biomass ratio to total reactor active biomass (f_{AT/TR}).

**Results and discussion**

In order to know the effect of the SRT to biomass distribution and composition for both suspended and fixed systems, two pilots plants were operated with SRT values of 8 (Phase A), 5 (Phase B) and 3.7 (Phase C) days. In order to reach a near steady state, each phase was operating during 45, 20 and 20 days respectively for phases A, B and C. All SRT are calculated over the CR taking into account purge, analytical samples and effluent solids. During period C and after 3 weeks of operation, plastic support from the HR was split into two fractions between the HR and the CR in order to study the filling ratio for the HR and the performance improvement of CR by addition of a colonised support. Thus, at the end of the study both pilot plants operated like a hybrid reactor with a filling ratio of 10%.

**Active autotrophic biomass evolution**

In Figure 3A the evolution of $X_{AR}$ over all the operated SRTs for both reactors are presented together with the effluent ammonia concentration. It is noticed that during phase A (SRT, 8 days), $X_{AT}$ in HR was 2 times higher than in CR. When reducing SRT from 8 to 5 days, $X_{AR}$ in CR reduced from 93 to 31 mg $X_{AR}$–COD·l–1 (66%) obtaining worse nitrogen removal performances, while in HR, in spite of active autotrophic biomass being reduced from 212 to 181 mg $X_{AR}$–COD·l–1 (15%), nitrogen removal performance is not affected. When operating at a low SRT value (3.7 days, Phase C), the $X_{AR}$ levels in CR were near to the detection limits of the respirometric test while in the HR total active autotrophic biomass concentration remains at a value similar to phases A and B (between 181 and 245 mg $X_{AR}$–COD·l–1).

![Figure 3](https://iwaponline.com/wst/article-pdf/46/1-2/397/476862/397.pdf)

**Figure 3** Active biomass evolution over SRT in CR and HR. A) Active autotrophic biomass ($X_{AR}$) and effluent N-NH$_4^+$. B) Active heterotrophic biomass ($X_{HT}$) and effluent soluble COD
The reduction of active autotrophic biomass in CR during periods B and C concludes with an increase of effluent ammonium concentration with values between 20 and 50 mg NH₄-N·l⁻¹. This is explained by the washing effect of autotrophic biomass produced when reducing SRT in an activated sludge reactor (CR). Therefore in the HR, effluent ammonia concentration always presents values lower than 15 mg NH₄-N·l⁻¹ in spite of the low SRT values operated during phases B and C. For the HR, the best performances, with ammonia concentrations lower than 2 mg N-NH₄⁺·l⁻¹, are achieved during phase C.

After support splitting between HR and CR (Phase C, Fr 10%), active autotrophic biomass concentration in both reactors is similar (values around 150 mg Xₐ-COD·l⁻¹) and effluent ammonia concentration presents an increase for the HR and a reduction in CR to values around 20 mg N-NH₄⁺·l⁻¹.

Active heterotrophic biomass evolution

The evolution of Xₜₙₐₐₜ and the effluent soluble COD are presented for both reactors in Figure 3B. In spite of the reduction of SRT from phase A to C giving a reduction of total suspended solids in CR (results not shown), active heterotrophic biomass in CR presents a nearly constant value around 1,700 mg Xₜₙₐₐₜ-COD·l⁻¹. For the HR, the behaviour of active heterotrophic biomass concentration follows a different pattern during all the operational periods. It must be noticed that at the start of period B both reactors presented an increase of active heterotrophic biomass during 4 days mainly due to a high loading rate occurring at the influent flow (from 1.0 to 1.25 mg COD·l⁻¹·d⁻¹). This perturbation mainly affected the HR, where active heterotrophic biomass concentration increased from 2,350 to 3,000 mg Xₜₙₐₐₜ-COD·l⁻¹, while in the CR values similar to period A were achieved (1,750 mg Xₜₙₐₐₜ-COD·l⁻¹). During phase C, both reactors presented a reduction of active heterotrophic biomass with the HR showing a more perceptible reduction in Xₜₙₐₐₜ from 2,700 to 2,050 mg Xₜₙₐₐₜ-COD·l⁻¹ (24%). After HR support splitting between both reactors (Phase C, Fr 10%) and attending for four weeks, both reactors present similar values of Xₜₙₐₐₜ (1,850 mg Xₜₙₐₐₜ-COD·l⁻¹) and effluent soluble COD.

Figure 4 Active biomass distribution evolution in HR. A) Fixed biomass to total biomass. B) Autotrophic biomass fractions in the biofilm and in the whole reactor.
When looking at effluent soluble COD, values obtained for both reactors are similar, with values slightly lower in HR than in CR. From Figure 3b it could be observed that, when a higher load rate occurs at the influent flow, effluent soluble COD in CR presents higher values than in HR. Observing the global pattern of effluent soluble COD during periods A, B and C, a slight increase could be observed when reducing SRT.

Biomass distribution

The distributions of active biomass between the biofilm and the mixed liquor in HR are presented in Figure 4. The values obtained for $f_{AF/AT}$ (Figure 4A) during all the operational periods indicate that active autotrophic biomass present in the reactor is mainly localised inside the biofilm. During period A, 95% of active autotrophic biomass present in the whole reactor is localised inside the biofilm. When reducing SRT, 98–99% of total autotrophic biomass, during phases B and C respectively, is localised inside the biofilm.

After starting phase B, $f_{HF/HT}$ falls from 70% to 55% due to the high influent loading rate explained above. When the influent loading rate increases, heterotrophic biomass grows faster over the biofilm. When returning to normal loading rate values, growth rate of heterotrophic biomass is reduced and then the detachment process increases due to shear stress forces at the support surface. In spite of influent loading rate, when the HR is operated with a Fr of 20%, the fraction of heterotrophic biomass fixed remains at values about 60%. At the end of phase C, with Fr 10%, only about 38% of active heterotrophic biomass is localised inside the biofilm. The percentage of fixed biomass in the whole reactor, $f_{TF/TR}$, follows the same pattern as the heterotrophic biomass because heterotrophic biomass represents about 85–90% of total active biomass as depicted in Figure 4B.

Composition of both biofilm and mixed liquor is presented in Figure 4B. At the end of phase A and during the whole phase B 10% of active biomass inside the biofilm ($f_{AF/TF}$) corresponds to autotrophic biomass. Taking into account the whole reactor ($f_{AT/TR}$), during phase B active autotrophic biomass corresponds to 6% of total active biomass. The main variation occurs during phase C, when SRT is reduced to 3 days and the fraction of autotrophic biomass reaches values up to 19% inside the reactor and 12% in the whole reactor. It is important to note that with an SRT of 3 days, active autotrophic biomass presents an increase in the whole reactor. This effect could be explained by the higher autotrophic growth rate occurring inside the biofilm and thus, because of the detachment process from biofilm to mixed liquor, autotrophic biomass present in the mixed liquor is increased.

After splitting the support material between the two operated reactors (Phase C, Fr 10%), the autotrophic biomass fraction inside the biofilm is reduced from 19% to 14%. This could be explained by the competition between the active species and the higher growth rate of heterotrophic biomass.

When observing the active biomass composition of mixed liquor (Figure 5), an impor-
tant reduction from 6% to 3% of $f_{AS/TS}$ could be observed in CR. This reduction is due to the wash out carried out when increasing the purge flow to achieve an SRT of 5 days during phase B. If purge flows are increased to achieve 3 days of SRT (phase C), the fraction of autotrophic biomass in the mixed liquor continues descending up to values near to zero (<0.5%). For the HR and during all the operational periods, the percentage of autotrophic biomass in the mixed liquor always remains under 2%. Nevertheless, when reducing SRT also a reduction in autotrophic biomass is observed. After splitting the support material between both reactors (phase C, Fr 10%), a sudden increase of autotrophic biomass could be observed in CR. This autotrophic increase could be explained by the biofilm detachment during the first operational days. After 30 days (day 237th) the mixed liquor in both reactors presents the same composition.

Conclusions
By means of respirometric techniques, active heterotrophic and autotrophic biomasses were measured for suspended and fixed biomass in a HR and a CR. These experiments proved useful to quantify active biomasses as well as the distribution between fixed and suspended systems. In HR, values obtained for $X_{AT}$ were nearly constant and were shown not to be highly affected by the operating SRT because nitrogen removal performances levels where directly related to available support surface. When reducing Fr from 20% to 10%, available active autotrophic biomass is reduced to a half and thus ammonia concentration at the effluent increases from values near to zero up to 20 mg N-NH$_4^+$/l. Concerning biomass distribution, autotrophic biomass is mainly localised over the plastic support (from 95% to 99% during phases A and C respectively).

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