Effect of different carbon sources on aerobic storage by activated sludge

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Abstract A study of substrate removal by real activated sludge with several synthetic substrates (acetate, ethanol, glutamic acid) and wastewater (raw and filtered) was carried out. Substrate, stored compounds (polyhydroxyalkanoates, PHA and internal carbohydrates), ammonia and oxygen uptake rate (OUR) were analytically determined. Polyhydroxybutyrate (PHB) was stored when the substrate was acetate or ethanol, while no appreciable formation of storage compound was detected using glutamic acid. A low amount of PHB was also formed in tests with raw and filtered wastewater which was probably mainly due to its acetate content. As far as the sum of storage and growth (indirectly estimated through ammonia consumption) did not match the overall solids formation, other unidentified mechanisms of substrate removal were likely to occur (biosorption, accumulation and/or storage of unidentified compounds). ASM3 and two derived models were used in the interpretation of experimental data with reference to synthetic substrates. With reference to synthetic substrates ASM3 can well describe the experimental data only assuming a stored product formation much higher than the analytically detected one, whereas the model that assumes a parallel growth and storage on the substrate can well describe the observed stored product profile only assuming a direct contribution of growth much higher than estimated from ammonia consumption. The model that assumes an accumulation/biosorption stage as first step of substrate removal can better describe the whole experimentally observed behaviour. However as well as in ASM3 this implies that some fraction of removed COD is still unidentified. With reference to real wastewater where the different phenomena were mixed up due to the presence of several substrates, the different models gave similar results.

Keywords Acetate; activated sludge; ASM3; ethanol; glutamic acid; modelling; storage

Introduction

Activated sludge processes are often operated under non steady-state conditions, because of frequent changes in feed flow rate and composition. Moreover, even if the overall process can be considered in steady-state, biomass grows under dynamic conditions, being continuously recycled among zones with different redox environments and substrate concentrations. The microbial response to dynamic conditions can be different from a simple increase in cell number ("growth"), and include other substrate removal mechanisms like sorption, accumulation and storage (Majone et al., 1999). It is important to distinguish among these different contributions to the overall COD removal, in order to better understand the dynamics of the process and to build up a useful basis for activated sludge process designing and modeling.

These dynamic phenomena have been widely studied in the laboratory on ad hoc cultivated cultures fed with synthetic substrates (Majone et al., 1999; Beun et al., 2000), showing the relevance of storage during the substrate uptake under dynamic conditions. However current knowledge about substrate removal mechanisms by real activated sludge in wastewater treatment plants (WWTPs) and about the fate under dynamic conditions of substrates other than acetate and glucose is still scarce. The limited knowledge of substrate removal mechanisms with other substrates is particularly relevant considering that wastewater is a complex mixture of many different substrates (Henze et al., 1994). A previous study (Carucci et al., 2001) has shown that storage phenomena are relevant also in the case
of COD removal from a real wastewater by a real activated sludge, even though the main contribution to the only detected stored product (polyhydroxybutyrate, PHB) was still due to acetate. Similar results, confirming the relevance of PHB storage on acetate removal by activated sludge systems, have been reported in a recent work by Dircks et al. (2001).

A better knowledge of substrate removal mechanisms in activated sludge plants is particularly needed for modelling purposes. In this regard it is noteworthy that in the first model set up by the IAWPRC Task Group on Mathematical Modelling for Design and Operation of Activated Sludge Processes, ASM1 (Henze et al., 1987), removal of the soluble substrate is fully described in terms of growth and related energy needs. Even though this model (with its numerous modified versions) has been extensively used for many years, it completely neglects storage processes, which has led to the formulation of ASM3 (Gujer et al., 1999), in which soluble substrates are removed only by storage and then growth occurs only on internally stored polymers. Even if ASM3 has already been used in activated sludge simulations (Koch et al., 2000) and respirometric methods have been proposed for the calculation of storage yield (Karahan-Gul et al., 2001), the fundamental assumptions of this model (storage and growth in series) have not been extensively tested yet on data obtained on real plants.

Thus the aims of the paper are essentially two: to provide a deeper insight about substrate removal mechanisms by a real activated sludge of a municipal WWTP with some synthetic substrates and with wastewater (raw and filtered), with the main focus on the role of storage, and to verify how ASM3 and some simple derived models can be applied to the obtained data.

Methods

Sludge and wastewater source
All experimental work has been performed by using sludge and wastewater from Rome Nord (Italy) activated sludge plant (780,000 p.e.). Influent wastewater (354,000 m³/d) is almost entirely domestic, with a small industrial fraction. The plant consists of preliminary treatments, primary settling, an oxidation tank, secondary settling and a final disinfection stage. The plant is operated at a load of 0.24 mgBOD₅/mgSS/d and a sludge age of 10 days.

Influent wastewater characterization
The wastewater used for kinetic experiments was the effluent of primary settling. Average total COD was about 150 mgCOD/l while filtered COD was about 70 mgCOD/l (in the typical range of diluted wastewaters). With reference to single substrates, propionate, lactate, glutamic acid and ethanol were never found, while acetate was found only occasionally and at low concentrations (always less than 20 mg/l).

Respirometric batch tests
Preliminary respiration tests were carried out aerating the sludge without any substrate and measuring the OUR at regular time intervals in order to establish the time needed to reach the endogenous level of respiration. On the basis of these tests it was decided to aerate the sludge for 24 hours before adding the selected substrate. In order to characterise the sludge behaviour with wastewater, simple and short-time aerobic batch tests (Ekama et al., 1986) were performed with a pre-selected proportion of sludge drawn from the oxidation tank and five different types of substrate: i) acetate; ii) ethanol; iii) glutamic acid; iv) filtered wastewater; v) raw wastewater (three replicates for each synthetic substrate, four replicates both for raw and filtered wastewater). The wastewater samples which were used were collected particularly during the morning peak hour of load. All respirometric tests were carried out at F/M (food to microorganisms) ratio of about 0.05 gCOD/gVSS. At the beginning of the
preliminary aeration period thiourea (20 mg/l) was added to inhibit nitrification. Before adding the selected substrate biomass was sampled for VSS determination (APHA, 1995). During the preliminary aeration phase and then during the respirometric test, samples were taken at regular intervals from the batch reactor (1 litre volume) for substrate or soluble COD, ammonia (immediately filtered), carbohydrates, copolymer consisting of 3HB (3-hydroxybutyrate), 3HV (3-hydroxyvalerate) and other HA units determination. Before and during the test, the oxygen uptake rate (OUR) was also measured: aeration was interrupted at intervals and the dissolved oxygen decrease was measured as a function of the time. OUR was calculated by also taking into account oxygen transfer through the air-medium interface. Slides for Nile Blue staining for PHA granules were taken at the beginning and at regular intervals during the tests. Standard Methods (APHA, 1995) were followed for the analytical determination, but PHB and PHV were measured according to Braunegg et al. (1978).

Calculation of rates
Specific removal rates for each substrate (\(-q_S\)) were calculated by linear regression of experimental substrate profile (in the batch tests with raw wastewater this rate was calculated only on the soluble COD, which was only a fraction of the overall removed COD) and by dividing the obtained slope by the initial biomass concentration (converted to COD units according to the conversion factor of 1.33 mgCOD/mgVSS, default value of ASM3, Henze et al., 2000). Initial biomass concentration was also considered in calculation of all the following rates. Specific net storage rates (\(q_{STO}\)) were calculated by linear regression of the only detectable stored product (PHB). Specific net growth rates (\(q_{GRO}\)) were estimated by linear regression of ammonia profile by assuming (according to ASM3 default values) a biomass composition of 0.07 gN/gbiomassCOD (not applied for glutamic acid and wastewater, due to their nitrogen content). Average specific oxygen uptake rates (\(-q_{O2}\)) during the high-OUR phase were calculated by integrating the OUR profile (subtracted the endogenous value) vs. time and dividing it by the time necessary for substrate depletion.

Results and discussions
Summary of batch test results
Table 1 reports specific rates of substrate removal obtained in batch tests with single substrates and wastewater, as well as other specific rates in terms of ratio with respect to substrate uptake rate (the ratios \(q_{GRO}/(-q_S)\) were not calculated for nitrogen containing compounds, as glutamic acid and wastewater, while the ratio \(q_{O2}/(-q_S)\) was not calculated for raw wastewater, as not meaningful, because of the particulate COD removed which was not measured). Time profiles for typical batch tests are reported in Figures 1–5 for substrate and PHB (parts A) and OUR and ammonia (parts B). As far as biomass carbohydrates, no appreciable change was observed during batch tests under all conditions, so excluding

<table>
<thead>
<tr>
<th>Substrate uptake rate ((-q_S, \text{mgCOD/gCODh}))</th>
<th>Acetate</th>
<th>Ethanol</th>
<th>Glutamic acid</th>
<th>Filtered wastewater</th>
<th>Raw wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>(q_{STO}/(-q_S))</td>
<td>0.45 (0.07)</td>
<td>0.25 (0.04)</td>
<td>0.00 (0)</td>
<td>negligible amounts of PHB stored</td>
<td></td>
</tr>
<tr>
<td>(q_{GRO}/(-q_S))</td>
<td>0.13 (0.05)</td>
<td>0.26 (0.04)</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>(-q_{O2}/(-q_S))</td>
<td>0.16 (0.04)</td>
<td>0.27 (0.06)</td>
<td>0.34 (0.06)</td>
<td>0.46 (0.16)</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^1\) referred to soluble COD, – not available
any storage of glycogen or other polysaccharides (data not shown). Lines in the figures represent calculations according to the three different models sketched in Figure 6. Discussion of the results given by the three models are reported in the Modelling subsection, while a detailed description of their structure (kinetic expressions, stoichiometric matrix and parameter values) is given in the Appendix.

**Batch tests with acetate**

Acetate is removed at a constant rate, OUR increases immediately and remains more or less constant until acetate is present in the medium, PHB is stored (45% of the overall acetate...
removed), ammonia slightly decrease (corresponding to an active biomass formation of 13% of the overall acetate removed). After acetate depletion PHB is consumed as internal carbon and energy source. Among the three synthetic substrates, acetate is the one removed at the highest specific rate, and the one in which storage contribution is most relevant. On the other hand, the sum of growth, storage and oxidation for energy needs in COD balance (Table 1) accounts for no more than 75% of the overall COD removed, so probably other unknown mechanisms of solids formation occur. Because of the low oxygen consumption during all the tests, these unidentified mechanisms are low-energy mechanisms, like biosorption (Grau et al., 1982) or internal accumulation (Cech and Chudoba, 1983 and Dionisi et al., 2001a).

**Batch tests with ethanol**

Biomass behaviour on ethanol is similar to that on acetate: the substrate profile is linear, associated oxygen consumption is low, PHB is formed in significant amounts, and ammonia profile indicates the presence of growth during substrate removal. On the other hand, the contribution of PHB storage in substrate removal is lower than in the test with acetate (25% vs 45%), whereas the growth is higher (26% vs 13%). Also in this case the sum of growth, storage and oxidation for energy needs in COD balance (Table 1) accounts for no more than 80% of the overall COD removed.

**Batch tests with glutamic acid**

After glutamic acid addition in the medium a lag phase of about 30 minutes is observed, where neither substrate is removed nor oxygen in excess of the endogenous requirement is consumed. After this lag phase, glutamic acid is consumed and the corresponding OUR increases. No significant storage occurs for PHB or other polyhydroxyalkanoates (PHAs) that could confirm a more relevant role of growth. Conversely, ammonia profile is practically flat: even though nitrogen content of glutamic acid is in excess of that needed for active biomass formation (0.1 gN/gCOD in glutamic acid vs. 0.07 gN/gCOD in the assumed composition of activated sludge biomass), the excess nitrogen is not released in the medium. An hypothesis to explain this behaviour is that a fraction of the removed glutamic acid is not used for active biomass synthesis but it is stored inside the cell in a form other than PHA (e.g. a polyaminoacid, as already reported under anaerobic conditions by Satoh et al., 1998) or simply accumulated inside cells. On the other hand, glutamic acid removal caused an intense fluorescence (after Nile Blue staining) in the cells. Fluorescence without corresponding PHA storage has already been reported in the literature for some strains of *Acinetobacter* (Tandoi et al., 1998; Fixter et al.; 1986), and could be attributed to the formation of wax-like substances. As a consequence, the fate of glutamic acid is mostly unidentified.

**Batch tests with wastewater (raw and filtered)**

The batch tests with the filtered and raw wastewater show a similar OUR pattern: a first phase of high and quickly decreasing OUR, a second phase of lower and more or less constant OUR, and finally a third phase in which the OUR comes back to the endogenous values. The main differences in the OUR profiles are the length of the second phase at constant OUR (which is clearly longer with raw wastewater, corresponding to the higher COD) and the decrease to the endogenous value which is much slower in the test with raw wastewater. The presence of a second OUR phase in the test with filtered wastewater seems to indicate that a significant fraction of the analytically soluble COD is actually slowly biodegradable, as already reported in previous studies (Carucci et al., 2001). This second phase could in principle be attributed to consumption of storage product (Dircks et al.,
1999; Goel et al., 1999) under the assumption that all soluble COD is also readily biodegradable. Under this assumption, raw wastewater should show a higher second-phase OUR when compared to the filtered one, due to the additional presence of slowly biodegradable COD. Because this is not observed, it is likely that storage does not play an important role, as confirmed by the very little amount of PHB stored at the beginning of both tests (probably due to the low amount of acetate present in the wastewater, 5 mg/l). Ammonia profile remains virtually unchanged. However, in this case the interpretation of ammonia profile is not so straightforward as for synthetic substrates, as far as the hydrolysis process of N-containing substrates gives a positive contribution to ammonia profile. A more quantitative evaluation of the role of growth in substrate removal can be done only by using a mathematical model, as described in the next section.
Modelling

The analysis of experimental profiles and COD balance (at the single and specific moment of substrate depletion) can only give a qualitative description of the different phenomena occurring in series and/or in parallel. In order to have a more quantitative description of the experimental behaviour, three different kinetic models have been compared, whose conceptual sketches are presented in Figure 6. Details on processes, material balances and rates for each model are listed in Appendix, as well as regression procedures for parameter adjustment and related values.

The three models only differ in the fate of readily biodegradable substrate (\(S_s\)). The model ASM3 (Gujer et al., 1999) assumes that \(S_s\) is removed only by storage; growth then occurs only on internal stored polymers. The model MOD1 adds one mechanism for substrate removal, by assuming that growth can also occur directly on \(S_s\), in parallel with storage, as already reported in the literature (Novak et al., 1995; Van Aalst-van Leeuwen et al., 1997). The MOD2 (Dionisi et al., 2001b) assumes that the first step of substrate removal is always a sort of internal accumulation (i.e. the substrate is transported into the cell and maintained inside as such or slightly metabolised) or simply of “biosorption” (Grau et al., 1982). Then the accumulated compound can be used for growth either directly or through previous storage and subsequent use of the stored product, as in MOD1. In ASM3, there is no assumption on the chemical nature of stored compounds which are assumed to not correspond to any particular detectable polymer; hence in ASM3 calibration, stored compounds were not fitted to experimentally detected PHB and were only calculated on the basis of the fitting of remaining experimental data (substrate, OUR and ammonia). Conversely, in MOD1 and MOD2, it was assumed that experimental PHB should correspond to stored compounds and so the model was fitted to experimental PHB.

On the other hand, it was clear from preliminary tests that MOD1 was not able to simultaneously represent ammonia and PHB profile, so the model was not fitted to ammonia data. The only model which was fitted to all experimental data (substrate, ammonia, PHB and OUR) was MOD2.

From Figures from 1 to 5, it can be seen that ASM3 describes acceptably well all experimental profiles that were used for calibration, but the ammonia profile in the glutamic acid test. On the other hand, the estimated profiles for storage compounds are strongly overestimated with respect to the experimental ones, in all tested conditions. Even though this does not necessarily indicate a failure of the model for real wastewater where most COD has an unknown chemical nature, overestimation is quite evident in tests with acetate and ethanol where PHB is likely to be the only stored compound. Overestimation of storage is also likely in the glutamic acid test, in that in ASM3 storage of N-rich glutamic acid should cause an ammonia release that is not experimentally found. Comparison of experimental and calculated profiles clearly shows that glutamic acid is not stored at all or that it is stored in a N-rich polymer. The presence of parallel growth and storage, as assumed in MOD1, could in principle help in avoiding overestimation of storage compounds and, indeed, good correspondence between experimental PHB and calculated storage compounds is obtained in all conditions. On the other hand, the presence of much faster growth in MOD1 with respect to ASM3 causes ammonia consumption to be overestimated in tests with acetate and ethanol. Moreover, in the case of glutamic acid, MOD 1 calculates an ammonia release (because of the nitrogen content of the substrate which is in excess with respect of that needed for synthesis of cellular constituents) which is not detected. MOD2 gives the best overall representation of experimental profiles, in that both PHB and ammonia profiles can be simultaneously represented acceptably well. The main feature of MOD2 is that the third component is assumed to have the same chemical form as the removed substrate, thus it does not contribute to PHB formation during storage or to ammonia removal or release during growth.
Differences among models are somewhat less evident with filtered and raw wastewater (MOD1 and MOD2 practically give the same results), which probably indicates that different phenomena are lumped together, also depending on the presence of several different substrates with different removal mechanisms. In that sense, ASM3 is the simplest way to represent experimental profiles, under the assumption of no correspondence of storage compounds with the analytically detectable ones. On the other hand, MOD1 and MOD2, both including parallel growth and storage can well represent data, under assumptions that are likely to be more close to the true behaviour. It is also noteworthy that with all models, OUR profiles for filtered wastewater are well represented only under the assumption that a relevant part of soluble COD is slowly biodegradable. The models could in principle describe experimental OUR profiles in the second phase (constant OUR) also assuming that all the biodegradable COD is removed in the first phase and in the second phase the removed COD is consumed. However with no values of stoichiometric and kinetic parameters the experimental OUR profile could be fitted and that indirectly confirms the presence of the slowly biodegradable COD. This is also confirmed from comparison of OUR profiles for filtered and raw wastewater: these perfectly correspond for the first 2.5 hours, and then OUR with unsettled wastewater slowly decreases to the endogenous level. This indicates that SBCOD in soluble and raw wastewater are quite similar in utilization rate.

Conclusions
The behaviour of activated sludge during the removal of synthetic substrates and of real wastewater has been investigated. Storage of PHB is an important removal mechanism when the substrate is acetate or ethanol, it does not occur with glutamic acid while only negligible amounts are found with the investigated raw and filtered wastewater. Moreover that amount was probably due to the little amount of acetate contained in the wastewater. Immediate growth exerts a minor but not negligible role during substrate depletion, while other unidentified mechanisms of solids formation are likely to occur. Of course, little storage from wastewater is not a general evidence: in a previous study with different wastewater and sludge (Carucci et al., 2001) storage played a more important role and evidence of PHB storage from substrates other than acetate was also found.

With reference to synthetic substrates, the obtained data have been interpreted using three models differing in the fate of substrate inside the cells. ASM3 can describe the observed behaviour (substrate, ammonia and OUR profiles) only assuming a formation of stored products much higher than the one experimentally observed. Actually, this is a basic assumption of ASM3, where the storage compound is not considered to correspond necessarily to well-known and detectable PHAs and glycogen. Nevertheless, discrepancy between calculated profile of storage compound and experimentally determined PHB profile for simple substrate like acetate and ethanol seems to indicate that storage is not the only mechanism removing the substrate. On the other hand, that discrepancy cannot be recovered by simply hypothesizing parallel growth and storage, as in the proposed model MOD1: it can well describe the experimental profile of PHB but calculates an ammonia consumption much higher than the experimental one. In order to well describe the whole range of observed experimental profiles (substrates, PHB, ammonia and OUR) a model was proposed (MOD2) that inserts a preliminary “internal accumulation” and/or “biosorption” step. However, the presence of such additional phenomena still require experimental confirmation, thus MOD2 is similar to ASM3 in its basic assumption: in COD balance, not all substrate depletion is recovered into components analytically detectable. ASM3 implicitly assumes that this “lacking” COD is a stored compound whereas MOD2 assumes it is accumulated or biosorbed. Moreover, both require an arbitrary assumption on the fate of nitrogen when dealing with nitrogen-containing carbon sources, which has relevant conse-
quences for calculated ammonia profile. ASM3 implicitly assumes that the storage compound does not contain nitrogen whereas in MOD2 it was assumed that the accumulated/biosorbed compound does. In the case of glutamic acid the latter assumption makes possible a better description of the experimental ammonia profile; however, this simply indicates that glutamic acid is biosorbed or accumulated or stored in some nitrogen-containing form.

Thus, further research is needed to better elucidate the fate of carbon sources in activated sludge processes, even for simple and single substrates.

Differences among models are somewhat less evident with filtered and raw wastewater (and MOD1 and MOD2 practically give the same results), this probably indicates that different phenomena are lumped into model flexibility, also considering that the mixture of several different substrates probably attenuates the differences in the relative importance of the different phenomena. In that sense, ASM3 is the simplest way to represent experimental profiles, under the assumption of no correspondence of storage compounds with the analytically detectable ones. On the other hand, MOD1 and MOD 2, both including parallel growth and storage can well represent data, under assumptions that are likely to be more close to the true behaviour. Whether this represents an unnecessary complication in calculation and designing of activated sludge processes remains an open matter and calls for further research on the presence of storage and other non-growth phenomena dealing with true wastewater and activated sludge.

Acknowledgements
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References


Appendix

Description of the models

The conceptual approach of the different models is represented in Figure 6 of the paper. Corresponding stoichiometric matrices showing relationships among variables and processes and the corresponding kinetic expressions are listed in Tables A1–A3.

Parameter values

In each case, model calibration was performed by multivariate regression (software Scientist, Micromath, 1994). Model calculations were fitted to experimental data through parameter adjustment, as reported below (the lists of the parameters adopted in each model in the five different tests are shown in tables A4–A6). With regard to parameter estimation, in ASM3 the parameters $K_{STO}$ and $K_S$ were assumed equal to ASM3 default values, the parameter $b_H$ was calculated in the preliminary endogenous tests and $b_{STO}$ was assumed to be equal to $b_H$. Parameter $Y_H$ was assumed to be the same in all the tests, as it was assumed that the undefined storage compound $X_{STO}$ was the same with every substrate. Thus, after a preliminary screening of the data, characterised by a low oxygen consumption, its value was fixed to 0.85. Parameters $k_{STO}$, $\mu_H$, $Y_{STO}$ were calibrated independently in each test on the basis of substrate, ammonia and OUR profiles (with the exception of raw wastewater where the soluble COD profile did not correspond of course to the total biodegradable COD and was not used for fitting). In the tests with raw and filtered wastewater the parameters relative to hydrolysis ($K_h$ and $K_s$) were also adjusted according to experimental profiles, while the initial values of $X_S$ and $S_S$ were manually adjusted on the basis of total and filtered COD data, on the soluble COD profile and on the OUR profile.

In MOD1 the same criteria were followed for parameter adjustment as in ASM3 (with one additional adjusted parameter, $Y_{HDIR}$, but the experimental data which were used for fitting were substrate, PHB and OUR.
In MOD2 all experimental data (substrate, PHB, ammonia, OUR) were used for parameter fitting. Parameter \( K_S \) was fixed as in ASM3 and MOD1, the value of \( K_{SHSTO} \) was not relevant and therefore fixed to 1. The parameter \( YACC \) was fixed in all the tests to 0.95, the hypothesised mechanism being low energy-requiring, while the maximum amount of biosorbed/accumulated compound (\( f_{maxacc} \)) was fixed in all the tests to 0.2, after a preliminary screening of the data. The parameters which were adjusted in each test were \( k_{STO}, \mu_H, Y_{STO}, Y_{HACC}, k_{ACC}, K_{SHACC}, K_{STOACC} \).

### Table A1  Stoichiometric matrix relative to ASM3

<table>
<thead>
<tr>
<th>O(_2)</th>
<th>S(_2) COD</th>
<th>S(_{am, N})</th>
<th>X(_S) COD</th>
<th>X(_H) COD</th>
<th>X(_STO) COD</th>
<th>Rates (mgCOD/lh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis</td>
<td>1</td>
<td>0.01</td>
<td>–1</td>
<td>( k_S \frac{X_S}{X_H} )</td>
<td>( K_S + X_S / X_H )</td>
<td>( X_H )</td>
</tr>
<tr>
<td>Storage of S(_S)</td>
<td>( (1-Y_{STO}) )</td>
<td>–1</td>
<td>X</td>
<td>( Y_{STO} )</td>
<td>( s_{STO} \frac{S_S}{K_S + S_S} )</td>
<td>( X_H )</td>
</tr>
<tr>
<td>Growth on X(_STO)</td>
<td>( (1-Y_H) / Y_H )</td>
<td>–0.07</td>
<td>1</td>
<td>( \mu_H \frac{X_{STO}}{X_H} )</td>
<td>( X_{STO} / X_H )</td>
<td>( X_H )</td>
</tr>
<tr>
<td>Endogenous respiration</td>
<td>–1</td>
<td>0.04</td>
<td>–1</td>
<td>( b_{STO} \frac{X_H}{X_{STO}} )</td>
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### Table A2  Stoichiometric matrix relative to MOD1

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<thead>
<tr>
<th>O(_2)</th>
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<th>S(_{am, N})</th>
<th>X(_S) COD</th>
<th>X(_H) COD</th>
<th>X(_STO) COD</th>
<th>Rates (mgCOD/lh)</th>
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<tbody>
<tr>
<td>Hydrolysis</td>
<td>1</td>
<td>0.01</td>
<td>–1</td>
<td>( k_S \frac{X_S}{X_H} )</td>
<td>( K_S + X_S / X_H )</td>
<td>( X_H )</td>
</tr>
<tr>
<td>Storage of S(_S)</td>
<td>( (1-Y_{STO}) )</td>
<td>–1</td>
<td>X</td>
<td>( Y_{STO} )</td>
<td>( s_{STO} \frac{S_S}{K_S + S_S} )</td>
<td>( X_H )</td>
</tr>
<tr>
<td>Growth on S(_S)</td>
<td>( (1-Y_{HDIR}) / Y_{HDIR} )</td>
<td>–0.07</td>
<td>X</td>
<td>( \mu_H \frac{X_{STO}}{X_H} )</td>
<td>( X_{STO} / X_H )</td>
<td>( X_H )</td>
</tr>
<tr>
<td>Growth on X(_STO)</td>
<td>( (1-Y_H) / Y_H )</td>
<td>–0.07</td>
<td>1</td>
<td>( \mu_H \frac{X_{STO}}{X_H} )</td>
<td>( X_{STO} / X_H )</td>
<td>( X_H )</td>
</tr>
<tr>
<td>Endogenous respiration</td>
<td>–1</td>
<td>0.04</td>
<td>–1</td>
<td>( b_{STO} \frac{X_H}{X_{STO}} )</td>
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### Table A3  Stoichiometric matrix relative to MOD2

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<th>S(_2) COD</th>
<th>S(_{am, N})</th>
<th>X(_S) COD</th>
<th>X(_H) COD</th>
<th>X(_ACC) COD</th>
<th>X(_SHACC) COD</th>
<th>X(_STO) COD</th>
<th>Rates (mgCOD/lh)</th>
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<tr>
<td>Hydrolysis</td>
<td>1</td>
<td>0.01</td>
<td>–1</td>
<td>( k_S \frac{X_S}{X_H} )</td>
<td>( K_S + X_S / X_H )</td>
<td>( X_H )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accumulation of X(_ACC)</td>
<td>( (1-Y_{ACC}) )</td>
<td>–1</td>
<td>( Y_{ACC} )</td>
<td>( k_{ACC} \frac{X_{ACC}}{X_H} )</td>
<td>( X_{ACC} / X_H )</td>
<td>( f_{maxacc} )</td>
<td></td>
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<tr>
<td>Storage of X(_ACC)</td>
<td>( (1-Y_{STOACC}) )</td>
<td>–1</td>
<td>X</td>
<td>( Y_{STOACC} )</td>
<td>( k_{STOACC} \frac{X_{STOACC}}{X_H} )</td>
<td>( X_{STOACC} / X_H )</td>
<td></td>
<td></td>
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<tr>
<td>Growth on X(_ACC)</td>
<td>( (1-Y_{HACC}) / Y_{HACC} )</td>
<td>–0.07</td>
<td>X</td>
<td>( \mu_H \frac{X_{ACC}}{X_H} )</td>
<td>( X_{ACC} / X_H )</td>
<td>( f_{maxacc} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth on X(_STO)</td>
<td>( (1-Y_{HSTO}) / Y_{HSTO} )</td>
<td>–0.07</td>
<td>1</td>
<td>( \mu_H \frac{X_{STO}}{X_H} )</td>
<td>( X_{STO} / X_H )</td>
<td>( f_{maxacc} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endogenous respiration</td>
<td>–1</td>
<td>0.04</td>
<td>–1</td>
<td>( b_{HSTO} \frac{X_H}{X_{STO}} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In MOD2 all experimental data (substrate, PHB, ammonia, OUR) were used for parameter fitting. Parameter \( K_S \) was fixed as in ASM3 and MOD1, the value of \( K_{SHSTO} \) was not relevant and therefore fixed to 1. The parameter \( Y_{ACC} \) was fixed in all the tests to 0.95, the hypothesised mechanism being low energy-requiring, while the maximum amount of biosorbed/accumulated compound (\( f_{maxacc} \)) was fixed in all the tests to 0.2, after a preliminary screening of the data. The parameters which were adjusted in each test were \( k_{STO}, \mu_H, Y_{STO}, Y_{HACC}, k_{ACC}, K_{SHACC}, K_{STOACC} \).
With regard to stoichiometric parameters relative to soluble ammonia metabolism, needed in order to describe the ammonia profile, default values of ASM3 were generally assumed, with the exception of the ammonia released in endogenous metabolism, which was directly measured during preliminary endogenous respiration tests to be 0.04 (instead of 0.066, default values of ASM3). The parameter X was dependent on the nitrogen content of the substrate: it was set to 0 for acetate and ethanol, to 0.1 for glutamic acid (due to the molecular formula) and to 0.03 for raw and filtered wastewater (ASM3 default values).

### Table A4 Parameter values relative to ASM3

<table>
<thead>
<tr>
<th>Units</th>
<th>Acetate</th>
<th>Ethanol</th>
<th>Glutamic acid</th>
<th>Raw wastewater</th>
<th>Filtered wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_h$</td>
<td>mgX$_H$/mgX$_H$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.014</td>
</tr>
<tr>
<td>$K_X$</td>
<td>mgX$_X$/mgX$_H$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.00019</td>
</tr>
<tr>
<td>$k_{STO}$</td>
<td>mgS$_S$/mgX$_H$</td>
<td>0.030</td>
<td>0.014</td>
<td>0.011</td>
<td>0.044</td>
</tr>
<tr>
<td>$K_S$</td>
<td>mgS$_S$/l</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$\mu_H$</td>
<td>1/h</td>
<td>0.20</td>
<td>0.23</td>
<td>0.23</td>
<td>0.31</td>
</tr>
<tr>
<td>$K_{STO}$</td>
<td>mgX$_STO$/mgX$_H$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$b_H$</td>
<td>1/h</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
</tr>
<tr>
<td>$b_{STO}$</td>
<td>1/h</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
</tr>
<tr>
<td>$Y_{STO}$</td>
<td>mgX$_STO$/mgS$_S$</td>
<td>0.83</td>
<td>0.83</td>
<td>0.47</td>
<td>0.88</td>
</tr>
<tr>
<td>$Y_H$</td>
<td>mgX$_H$/mgX$_STO$</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
</tbody>
</table>

### Table A5 Parameter values relative to MOD1

<table>
<thead>
<tr>
<th>Units</th>
<th>Acetate</th>
<th>Ethanol</th>
<th>Glutamic acid</th>
<th>Raw wastewater</th>
<th>Filtered wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_h$</td>
<td>mgX$_H$/mgX$_H$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.012</td>
</tr>
<tr>
<td>$K_X$</td>
<td>mgX$_X$/mgX$_H$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.00019</td>
</tr>
<tr>
<td>$k_{STO}$</td>
<td>mgS$_S$/mgX$_H$</td>
<td>0.013</td>
<td>0.0027</td>
<td>0.00031</td>
<td>0.00011</td>
</tr>
<tr>
<td>$K_S$</td>
<td>mgS$_S$/l</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$\mu_H$</td>
<td>1/h</td>
<td>0.015</td>
<td>0.0089</td>
<td>0.0046</td>
<td>0.024</td>
</tr>
<tr>
<td>$K_{STO}$</td>
<td>mgX$_STO$/mgX$_H$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$b_H$</td>
<td>1/h</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
</tr>
<tr>
<td>$b_{STO}$</td>
<td>1/h</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
</tr>
<tr>
<td>$Y_{STO}$</td>
<td>mgX$_STO$/mgS$_S$</td>
<td>0.85</td>
<td>0.75</td>
<td>0.55</td>
<td>0.70</td>
</tr>
<tr>
<td>$Y_H$</td>
<td>mgX$_H$/mgX$_STO$</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>$Y_{HDIR}$</td>
<td>mgX$_H$/mgS$_S$</td>
<td>0.78</td>
<td>0.77</td>
<td>0.45</td>
<td>0.62</td>
</tr>
</tbody>
</table>

### Table A6 Parameter values relative to MOD2

<table>
<thead>
<tr>
<th>Units</th>
<th>Acetate</th>
<th>Ethanol</th>
<th>Glutamic acid</th>
<th>Raw wastewater</th>
<th>Filtered wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_h$</td>
<td>mgX$_H$/mgX$_H$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.012</td>
</tr>
<tr>
<td>$K_X$</td>
<td>mgX$_X$/mgX$_H$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.0014</td>
</tr>
<tr>
<td>$k_{STO}$</td>
<td>mgS$_S$/mgX$_H$</td>
<td>1.8</td>
<td>0.14</td>
<td>0.85</td>
<td>0.0025</td>
</tr>
<tr>
<td>$K_S$</td>
<td>mgS$_S$/l</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$\mu_H$</td>
<td>1/h</td>
<td>0.30</td>
<td>0.46</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>$K_{SHSTO}$</td>
<td>mgX$_STO$/mgX$_H$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$b_H$</td>
<td>1/h</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
</tr>
<tr>
<td>$b_{STO}$</td>
<td>1/h</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
<td>0.0017</td>
</tr>
<tr>
<td>$Y_{STO}$</td>
<td>mgX$_STO$/mgX$_ACC$</td>
<td>0.85</td>
<td>0.75</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>$Y_{HACC}$</td>
<td>mgX$_H$/mgX$_ACC$</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>$Y_{HDIR}$</td>
<td>mgX$_H$/mgS$_S$</td>
<td>0.77</td>
<td>0.81</td>
<td>0.45</td>
<td>0.64</td>
</tr>
<tr>
<td>$K_{ACC}$</td>
<td>mgX$_ACC$/mgS$_S$</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td>$k_{ACC}$</td>
<td>mgS$_S$/mgX$_H$</td>
<td>0.030</td>
<td>0.014</td>
<td>0.011</td>
<td>0.035</td>
</tr>
<tr>
<td>$K_{SHACC}$</td>
<td>mgX$_ACC$/mgX$_H$</td>
<td>0.21</td>
<td>0.58</td>
<td>1</td>
<td>0.00093</td>
</tr>
<tr>
<td>$K_{STOACC}$</td>
<td>mgX$_STO$/mgX$_ACC$</td>
<td>0.45</td>
<td>0.28</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$f_{maxacc}$</td>
<td>mgX$_ACC$/mgX$_H$</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>