# LIMITATIONS OF SHORT-TERM EXPERIMENTS DESIGNED FOR IDENTIFICATION OF ACTIVATED SLUDGE BIODEGRADATION MODELS BY FAST DYNAMIC PHENOMENA

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Abstract: Experimental results obtained in a batch reactor are presented showing different fast dynamic phenomena. All measured OUR-profiles show a 'start-up' phase upon substrate addition. Neither the response time of the DO-electrode, the mixing characteristics in the reactor or the extracellular transport limitations could explain this behaviour. It is hypothesised that intracellular transport and conversion processes are responsible for the transient response. Second, dynamics induced by regulation processes of the macromolecular cell composition could be observed. Finally, the adaptation of the mixed culture population to changed operating conditions is demonstrated. The implications for modelling activated sludge biodegradation with simple structured models identified from such experiments are discussed.

Keywords: Biotechnology, Environmental engineering, Identification, Models, Time constants

## 1. INTRODUCTION

In many biokinetic studies batch experiments are conducted to obtain quantitative information on the behaviour of a biosystem (Vanrolleghem & Coen, 1995). It is important to assess whether the conditions in these experiments are representative of the model one is trying to identify because this information is subsequently summarised in mathematical models of the full-scale system and used for basic understanding of the process, for process design purposes or the design of control strategies. In this paper phenomena will be described that cannot be explained or described using the simple, unstructured kinetic models in use today.

If a system is studied in which a large number of relevant processes take place, each of these processes will contribute to the dynamics of the system's behaviour at a rate which is characterised by the relaxation time of the mechanism considered. The concept of relaxation times provides insight in the required degree of complexity of the description of a bioengineering system (Roels, 1983).

A bacterial cell circulating in a bioreactor is exposed to a micro-environment that is changing depending on chemical and physical parameters (e.g. mixing properties, substrate feeding profile, pH, temperature, light,…). In principle the behaviour of a culture of organisms is the result of a number of internal mechanisms which regulate the organism's behaviour in response to changes in the environment. In Figure 1 a comparison of the relaxation times of the mechanisms inside organisms and those of the relaxation times of the environment in a bioreactor is made.

Examples of the effect of rapid changes in environmental parameters on waste water treatment processes are common.

- Toxic compounds in waste water affect the microbiota of activated sludge.
- Selectors which impose short periods (minutes) of high substrate concentrations to the mixed liquors, are used to improve the settleability of the sludge by influencing the composition of the different microbial communities.
- Operation of sequencing batch reactors (SBR) are preferred to systems which work more or less continuously because of their ability to sustain a rich, diverse and effective microbial population.

The process studied in this paper is the aerobic respiration of bacteria in activated sludge under batch operation. To get more insight in the different aspects of the complex nature of the aerobic respiration process an approach is followed based on the internal mechanisms of the biosystem and their so-called relaxation times.

## 2. PROBLEM DEFINITION

Respirometry is increasingly used to model the degradation of pollutants present in wastewater and to characterise the carbon oxidizing and nitrifying activity of the activated sludge. As a standard tool for monitoring and control of waste water treatment plants (WWTPs) respirometry becomes more and more important (Spanjers, *et al.*, 1996). Batch respirometric experiments are typically performed. It has been shown that such experiments give information on model structure and model parameters of the activated sludge in the full scale WWTP (Vanrolleghem & Van Daele, 1994). However, the respirometric experiment for extraction of this information must be designed in such a way that it does not influence the representativeness of the results for full-scale behaviour.

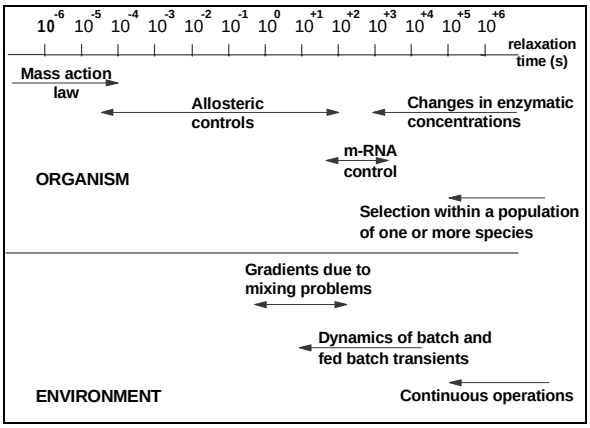


Figure 1. Mechanisms of biosynthesis and the comparison of the relaxation times of these mechanisms compared to the relaxation times of the environmental processes in a bioreactor (Roels, 1983).

Substrate degradation is typically modelled using the classic Monod rate equation. Aim of the respirometric batch experiments is to obtain values of the Monod parameters. To introduce the problems encountered in this exercise, the following experiment is described.

A pulse of acetate was added to a 10 litre sludge sample in a continuously aerated and mixed vessel. The Dissolved Oxygen (DO) concentration is measured in time and Oxygen Uptake Rates (OUR) are calculated from the oxygen mass balance equations. A typical DO and OUR profile measured with the respirometer are shown in Figure 2. It is seen that only after several minutes a maximum OUR is reached. The classic Monod rate equation, however, is not able to describe the transient increase in OUR upon substrate addition as it predicts an immediate maximum OUR after substrate addition. If the amount of substrate would have been rather low, the substrate would have been depleted from the solution before the maximum uptake rate was attained and this evidently would make identification of the maximum specific growth rate impossible. The objectives of this work were to examine the possible reasons for this 'start-up' behaviour upon substrate addition and to study the influence of the sludge history on the results of a respirometric experiment (and, as such, evaluate whether the experiment is representative for the full-scale biological behaviour).

First, attention is paid to phenomena characterised by time constants in the order of seconds. To explain the 'start-up' delay three mechanisms are studied : dynamics of the DO-electrode, mixing in the respirometric vessel and diffusion limitation in the sludge floc.
Second, at the level of ten minute time constants, respirometric results will be given that can be described by the dynamics of regulation processes of the macromolecular cell composition, like enzyme activation or m-RNA control.
Finally, respirometry is used to illustrate the adaptation of biomass to changing operating conditions. This adaptation effect causes changes in the biokinetics with time constants in the range of hours.

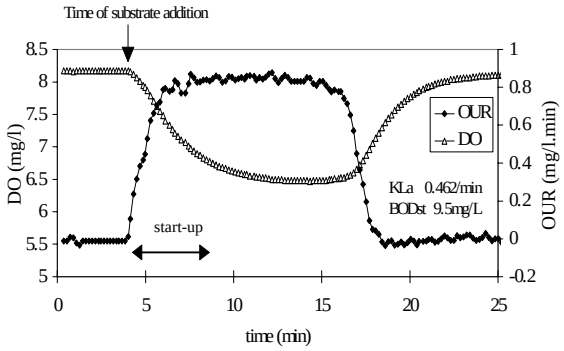


Figure 2. DO and OUR-profile recorded by the RODTOX.

## 3. MATERIALS

The respirometric sensor RODTOX (Kelma bvba, Niel, Belgium) consists of a biological unit interfaced to a microprocessor. A reactor vessel, filled with 10 litres of activated sludge is constantly aerated, stirred and thermostated. In the cover of the bioreactor, dissolved oxygen and pH probes are installed. The RODTOX is a respirometer working in batch mode (Vanrolleghem, *et al.*, 1994).
After injection of a pulse of substrate into the reactor, the substrates are oxidised by the activated sludge. The changing DO concentrations are collected on a personal computer and oxygen uptake rates can be calculated, resulting in the so-called respirogram given in Figure 2. The temperature was controlled at 25°C and the pH at 7.5. In this paper two Endress and Hauser DO electrodes are tested, i.e. the Conducta 905 and the Conducta 905 S.

## 4. RESULTS

### *4.1 Time constant < 1 minute*

Three hypotheses were evaluated to explain the 'start-up' phenomenon with a relaxation time of approximately 2 minutes: probe response, mixing and substrate diffusion.

*Probe response.* The dynamics of DO-electrodes have been described most often as a first order process (Lee & Tsao, 1979). Therefore, upstep-responses of the RODTOX electrode have been recorded with an experimental set-up as described by Philichi & Stenstrom (1989). The RODTOX was filled with tap water and the DO was stripped to a concentration below 1 mg/l. A cap filled with this water was placed on the DO-electrode and the aeration in the reactor vessel was switched on to attain the normal saturation level. Then, the cap was suddenly removed from the DO-electrode and the response to this step change in DO was measured. Figure 3 shows the response for the Conducta 905 electrode. The time constant for the Conducta 905 was 55 s while for the Conducta 905S it was 12.5 s. Knowledge of the electrode model and its parameters allows to calculate the actual dissolved oxygen concentration $S_{O2}$ from the electrode output E.

$$S_{O2} = \tau \frac{dE}{dt} + E \qquad (1)$$

Clearly, attention must be paid to noise elimination since taking derivatives enhances the effect of noise. Applying this probe model, it was found that the electrode dynamics are only partially explaining the observed transient 'start-up' phenomenon.

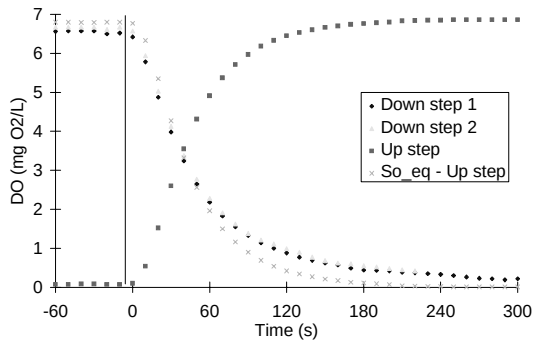


Figure 3. Up- and down step responses of DO-probe (Conducta 905, E+H).

*Mixing.* The OUR 'start-up' phenomenon may also be caused by inadequate mixing in the respirometer vessel. When the influent sample is injected into the respirometer the substrate concentration is high close to the injection point while remaining low for a while in other locations. This may result in different respiration rates depending on the local substrate concentration. The colouring-method was used to measure the mixing characteristics of the respirometric vessel. Phenolphthalein was added to the RODTOX vessel filled with 10 litre of water. Addition of an excess of base causes the colour to change from colourless to violet. Samples were taken automatically at 2 seconds interval and at two points in the reactor vessel, one close to the DO-electrode and the other at the bottom of the vessel. The colour intensity was analysed by spectrophotometry. Two series of mixing experiments were performed : one series with stirring and aeration (aeration intensity 15 l/min.) and another series with stirring but no aeration. Figure 4 shows the measurement of the colour intensity versus time at the position of the DO-electrode in case of aeration and no aeration.

The mixing time is defined here as the time necessary to reach 95 % of the end concentration. The mixing time is about 10 s in case of stirring only. With aeration the mixing is very fast as the sample taken after 2 seconds already exhibits the end concentration. Hence, one can conclude that mixing of the substrate injected in the batch reactor occurred significantly faster than the observed transient behaviour.

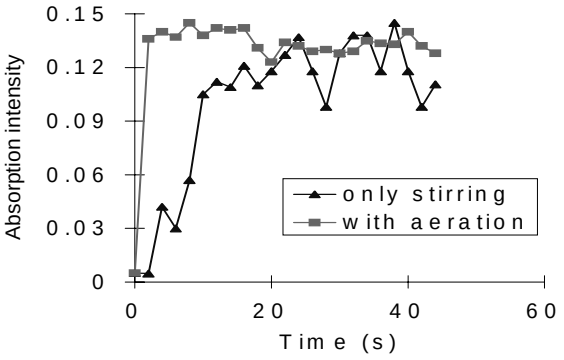


Figure 4. Absorption intensity at the position of the DO-electrode versus time.

*Diffusion in the sludge floc.* It was hypothesised that diffusion phenomena into the activated sludge floc may be a determining factor of the 'start-up' phenomenon. The hypothetical behaviour was qualitatively described as follows. Initially the sludge floc can be assumed fully penetrated with dissolved oxygen and lacking substrate. When substrate is added to the bulk liquid, substrate diffuses into the activated sludge floc. There aerobic micro-organisms convert the substrate consuming an amount of oxygen. This oxygen consumption induces a difference in oxygen concentration between the floc and the bulk, and oxygen diffuses from the bulk into the floc. Finally, the decrease in oxygen concentration in the bulk is measured by the DO-electrode. This means that the respirometric response to a pulse addition of substrate may be influenced by diffusion of either substrate or oxygen, or both, into the sludge floc.

In order to evaluate diffusion limitations the theoretical rate constants for diffusion of dissolved oxygen and acetate in a biofilm with a thickness of 200 µm are calculated with the formula :

$$\tau_D = \frac{\delta^2}{D_b}$$

$$\text{with } \delta = \text{biofilm thickness (mm)} \quad (2)$$

$$D_b = \text{diffusion constant (mm}^2\text{ / s)}$$

$$\tau_D = \text{diffusion time constant (s)}$$

Assume that the diffusion constant of dissolved oxygen and acetate in water at 25 °C is equal to respectively 2500 and 1240 $\mu m^2/s$ (Perry, 1984) and that the diffusion constant in a sludge floc is 80% of the diffusion constant in water. The time constant $\tau_D$ for the diffusion process of oxygen in the sludge floc is then equal to 16 seconds. The time constant for acetate diffusion is equal to 40 seconds. The formula above is normally used to calculate the time needed for an inert tracer to reach 90% of the biofilm/liquid interface concentration. Here also reaction takes place, which means that the calculated values should be regarded as maximum values. Nevertheless, this calculation shows that diffusion may be a possible explanation for the observed 'start-up' phenomenon.

An empirical approach to elucidate the importance of diffusion was also pursued. An experiment was set up in which the respiration response time of a single, non-flocculating *Pseudomonas* culture was compared to the response time of an activated sludge sample. In the *Pseudomonas* culture no diffusion limitations for substrate nor oxygen due to growth in flocs can exist. Addition of acetate to the *Pseudomonas* culture gave the respirogram in Figure 5. The time between addition of substrate and measurement of the maximum OUR is still 3 to 4 minutes. So the hypothesis of diffusion limitation in the sludge floc seems not adequate for the transient behaviour in microbial cultures.

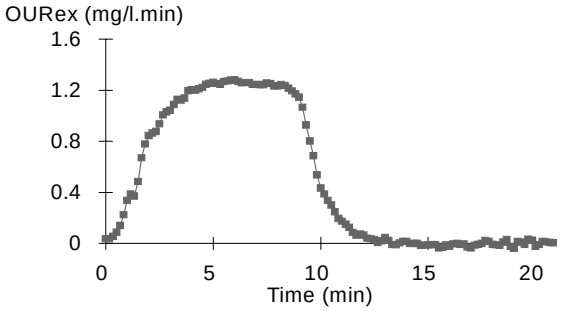


Figure 5. DO and OUR profiles for an addition of acetate to a *Pseudomonas aeruginosa* culture.

Although based on the theoretical calculation above one could expect that the diffusion process is contributing to the observed 'start-up' phenomenon, the experiment shows that the transient is not caused by transport limitations. According to Li & Ganczarczyk (1992), however, sludge flocs may be permeable, depending on size and operating conditions. They suggest that advective transport through channels in the sludge floc could be the main mechanism of mass transfer. This advective transport could then explain the observed absence of extra-cellular transport limitations.

*Conclusion*. The start-up phenomenon observed in the raw OUR-data (Fig. 2) can partially be explained by the delay in the DO-electrode response. The results above revealed that diffusion in the floc and mixing in the respirometric reactor are not responsible for the start-up phenomenon. It is hypothesised that transport and the sequence of metabolic reactions inside the cell are the cause of the transient with a time constant of approximately one to two minutes. This hypothesis is supported by results obtained with yeast cell cultures submitted to carbon shock-loads (Röhm, *et al.*, 1992; Rizzi, *et al.*, 1997). In these studies intracellular concentrations of intermediates of central metabolism are shown to undergo transients with time constants in the range of one minute and could therefore be the underlying mechanism of the start-up phenomenon found in the respirometric experimental results.

### *4.2 Time constant in the order of 10 minutes*

At the level of the ten minutes time constant the following phenomena were observed during respirometric experiments. Different acetate pulses were added sequentially to an activated sludge sample that had been starved for 12 hours. The OUR-profiles of the first three additions are shown on Figure 6. The first and second profile show a fast increase in respiration rate during the first four minutes (similar to the response studied above), while the maximum OUR is only reached after 12 minutes after a period of more gradual speed-up of respiration. In the third experiment the maximum respiration rate is reached immediately after the three minutes transient.

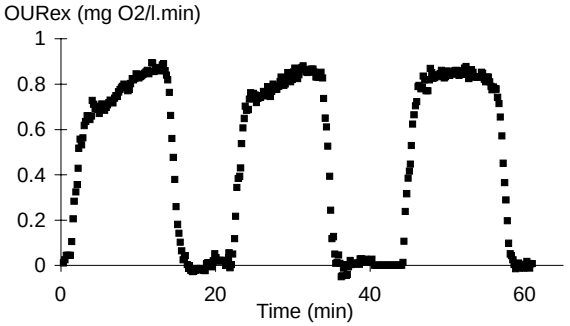


Figure 6. OUR-profiles obtained by addition of acetate to an activated sludge sample.

Although the three additions were performed each time under the same environmental conditions the OUR-profiles are obviously no replicates. This indicates that the history of the sludge has impact on the OUR-profiles obtained. Before the experiment the sludge was endogenously respiring for some hours. Then by addition of acetate electron donors became available. This availability of substrate induced a response at the level of enzymatic breakdown of the substrate.

It was also noted that leaving the sludge without acetate addition for another 12 hours resulted in a very similar response as the one depicted in Figure 6. Hence, it can be concluded that (some of) the organisms "forget" the

mechanism for acetate degradation. Note that such starvation periods may also occur in full-scale plants, warranting further research and a quantitative description.

According to Roels (1983) relaxation times in the order of 10 minutes are due to m-RNA control of the enzyme synthesis (Fig. 1). An induction process can explain the phenomenon noticed in the first two OUR-profiles. The added substrate can act as an inducer which binds to the repressor and removes it from the operator gene. Transcription thus increases and the enzyme amount increases. After two substrate additions the enzyme concentration has reached a level which makes that the m-RNA production is no longer rate limiting for the substrate removal process.

Alternatively, regulation and activation processes at the level of the enzyme itself, like allosteric control, can be responsible for the measured time constant. Allosteric controls are regulation mechanisms which allow to control the enzyme's activity. The modulation of the enzyme activity takes place by a so-called effector, which may be a compound closely related with the substrate or the end product of a given sequence of enzymatic reactions. The effector binds to one of a number of specific modulator sites on the enzyme molecule and affects the conformation of the enzyme molecule in such a way that the catalytic activity of the substrate-specific active site of the enzyme is influenced.

The Michaelis Menten equation for description of an enzyme-catalysed reaction can be used to model the OUR of the third respirogram after the initial 4 minutes 'start-up' phenomenon. This equation assumes that the intermediate enzyme substrate complex *ES* is in equilibrium. This assumption appears valid for respirogram 3 but it is not for respirograms 1 and 2.

Consequently, the unstructured Michaelis Menten equation can not be used to model the first 2 respirograms. In Roels (1983) two models are given that can be used to describe allosteric regulation, i.e. the model of Monod, Wyman and Changeux (Monod, *et al.*, 1965) and the model of Koshland, Némethy and Filmer (Koshland, 1966). Further study is, however, required.

### *4.3 Time constant in the order of hours*

The time constant of the growth processes encountered in waste water treatment is in the range of hours to days. To study these long-term processes in a respirometer one has to take into account that if the conditions pertaining in the respirometric experiment differ from the normal full-scale operating conditions, this will affect the sludge characteristics (the mixed culture population composition, the biokinetic properties, etc.) in the respirometer. This means that the respirometric results are no longer representative for the full-scale situation.

The following experiment was performed. Sludge from a municipal waste water treatment plant was taken. During the experiment the sludge concentration was kept constant. The sludge is batchwise fed at a high loading rate with the influent of the WWTP. After 6 batch additions of waste water a small and known amount of calibration substrate (acetate) is injected to the respirometer. Adaptation to the operating conditions in the respirometer was studied by measuring the change in maximum oxygen uptake rate upon acetate addition (Figure 7). After 5 days the sludge in the respirometer appears adapted to the new operating conditions and the maximum OUR is no longer changing.

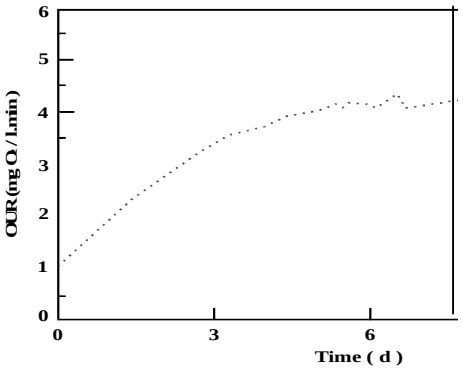


Figure 7. Evolution of the maximum OUR of a sludge sample adapting to the changed operating conditions of its environment.

## 5. DISCUSSION AND CONCLUSION

The process studied in this paper is the aerobic respiration of bacteria in activated sludge under batch operation. The aerobic respiration is measured by means of a respirometric device. Transient phenomena in the OUR data (the 'start-up' phenomenon, regulation processes and adaptation of biomass) were observed.

The fast transient of the 'start-up' phenomenon can only partially be explained by the dynamics of the DO-electrode response. Comparing the OUR-profile of a pure culture and activated sludge showed that the observed transient is not caused by transport of acetate or oxygen in the sludge floc. Also mixing in the respirometric reactor is not responsible for the start-up phenomenon. It is hypothesised that transport and the sequence of metabolic reactions inside the cell are the cause of the transient with a time constant of approximately one to two minutes. Further research is necessary to consolidate this hypothesis.

Respirometry is used for determination of model structure and model parameters (Vanrolleghem & Van Daele, 1994). This information enables the quantitative description of full-scale bioprocesses. Therefore, modelling of the observed 'start-up' phenomenon is essential to get a more realistic model for the measured OUR-profiles and, as such, to improve the quality of the kinetic information obtained by parameter estimation. In

addition, as in some full-scale configurations (e.g. with "selectors" for bulking control) the organisms are subjected to similar fast substrate concentration changes, accurate modelling of the biological response seems a mere necessity for full-scale application of a model.

The authors propose to model the 'start-up' phenomenon in a very simple but appropriate way by means of two first order models. One describes the observed probe response and the second first order model is an empirical model of the maximum specific growth rate which lumps fairly well the response of the unidentified intracellular processes. Further study may shed light on the underlying mechanisms.

Experimental results were presented showing the influence of regulation processes of the macromolecular cell composition on the respiration process. The time constant of the observed transient was in the order of ten minutes. Also, this experiment clearly showed how the system history can influence the degradation process.

Finally, the adaptation of the biomass to changing operating conditions was studied. These adaptation effects have time constants in the range of hours to days. For a good experimental set-up it is important to have and maintain a representative sludge in the respirometer. Otherwise, the respirometric results do not reflect the behaviour of the sludge of the full-scale installation. The results of the adaptation experiment show that only during the first hours representative experiments for determination of the sludge kinetics of the WWTP can be performed under the experimental conditions applied.

## ACKNOWLEDGEMENTS

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