Respirometry in Activated Sludge

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Abstract

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The purpose of the study was (1) to develop a respiration meter capable of continuously measuring, using different procedures, the oxygen uptake rate of activated sludge and (2) to expand knowledge about respiration related characteristics of wastewater and activated sludge.

A newly-developed respiration meter is described. The meter consists of a closed, completely mixed respiration chamber of 0.5 to 1 litre through which activated sludge is continuously pumped. The characteristic feature of this meter is that the dissolved oxygen concentration in the sludge entering the chamber and in the sludge leaving the chamber is measured with one single probe, located at one opening. This is realised by changing the direction of the flow through the chamber. The respiration rate is calculated from the dissolved oxygen mass balance over the respiration chamber. Because the derivative of the mass balance is included in this calculation, the respiration rate can also be calculated during dynamic conditions. An improved method for calculating the respiration rate is described, which accounts for the time lag of the DO probe. An additional result of this improvement is that it yields the time constant of the probe response, which provides a diagnosis of the probe condition.

Experimental research was performed using a continuous pilot activated sludge plant with a completely mixed aeration tank of 0.475 m^3, fed with domestic wastewater, and a batch reactor with an aeration tank of 1.5 to 2 litres.

A strategy is described for measuring four types of respiration rate of the same sludge under different conditions: endogenous, instantaneous, actual and maximum respiration rate. Emphasis is given to the actual respiration rate. The actual respiration rate is defined as the oxygen uptake rate of the sludge in the aeration tank. It is demonstrated that this rate is measured if the respiration chamber and the aeration tank are equally loaded with wastewater. An improved method is described which does not involve addition of wastewater to the respiration chamber. Instead, the transient respiration rate during two modes of operation which are alternately executed is used to calculate the actual respiration rate.

The measurement of the maximum respiration rate is discussed in some detail with emphasis on the partition of readily biodegradable matter into two components. The maximum respiration rate is measured if wastewater is continuously fed into the respiration chamber so that the loading exceeds a certain critical loading. Batch respirometric tests are used to verify the continuous measurement of this maximum rate. An application of the developed measurement is described in which the effect of the influent flow rate on the maximum respiration rate of nitrifying sludge was investigated.

Methods are described for continuous estimation of the short-term biochemical oxygen demand (BOD_{st}) of influent and effluent by using respirometry. BOD_{st} values of the influent are verified with batch measurements. The BOD_{st} of the examined wastewater appears to be mainly caused by ammonium being oxidized by nitrifiers.

Batch respiration measurements have been used for identifying a mathematical nitrification model. The investigation was focused on finding an optimal experimental design and a good model validation method.

Keywords: activated sludge, biochemical oxygen demand, process control, dissolved oxygen, dissolved oxygen probe, endogenous respiration rate, experimental design, fouling, kinetics, maximum respiration rate, modelling, monitoring, Monod, nitrification, nonlinear regression, oxygen uptake rate, respiration meter, respiration rate, respirometry, wastewater

Stellingen

1. De aanbeveling van de American Public Health Association om na de bemonstering van het aktiefslib uit de beluchtingtank de respiratiemeting zo snel mogelijk te beginnen impliceert dat de meting van de aktuele respiratiesnelheid op deze manier principieel onjuist is.

   APHA (1989) *Standard Methods for the Examination of Water and Wastewater*, 17th edition. American Public Health Association, Washington, D.C.

2. Sherrard *cum suis* konkluderen dat de respiratiesnelheid geen geschikte variabele is voor de regeling van het aktiefslibproces. Deze konklusie is niet gerechtvaardigd want zij is gebaseerd op foutieve metingen van de aktuele respiratiesnelheid.

   Sherrard J.H. (1980) Communication: oxygen uptake rate as an activated sludge control parameter. *J. Wat. Pollut. Control Fed.* **52**, 2033.

3. Sollfrank en Gujer houden er bij de bestudering van de nitrifikatiekinetiek ten onrechte geen rekening mee dat de vorming en oxidatie van nitriet een rol speelt.

   Sollfrank U. and Gujer W. (1990) Simultaneous determination of oxygen uptake rate and oxygen transfer coefficient in activated sludge methods by an on-line method. *Wat. Res.* **24**, 725-732.

4. De bedenkelijke gewoonte om parameters van een niet-lineair model te schatten na een linearisatie kan worden afgeschaft nu programmatuur voor niet-lineaire regressie in ruime mate verkrijgbaar is.

5. De in de literatuur veel voorkomende gewoonte om een kinetiekvergelijking in een differentiaalvergelijking op te nemen is principieel fout omdat kinetiek een stationaire situatie veronderstelt.

6. Het opleidingsnivo van de moeder is de meest bepalende faktor voor de gebitsgezondheid van een kind.

7. De verspreiding van tuberculose via het huishoudelijke rioolwater is een reëel gevaar.

8. Het toepassen van echt rioolwater bij zuiveringstechnologisch onderzoek vergroot de kans op serendipiteuze vondsten.

9. Het noemen van het principe van een instrument in een wetenschappelijke publikatie moet worden beschouwd als het citeren van de bedenker van dat principe.

10. Door de overmaat aan wielerwedstrijden in Nederland op een kort en bochtig parcours (de zogenoemde 'rondjes rond de kerk') wordt het aanwezige wielertalent niet volledig ontwikkeld.

11. Als we in Nederland alle honden als varkens in stallen zouden huisvesten dan hadden we er een mestprobleem bij.

12. Om misverstanden over de goede werking van het zuiveringsproces te voorkomen verdient het aanbeveling de tussen-s in het woord "beluchtingstank" weg te laten.

Stellingen bij het proefschrift "Respirometry in activated sludge" van Henri Spanjers
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Contents

Aan mijn vader

1

General introduction

1.1 Purpose of this study

Respirometry is the measurement and application of respiration rate, a key variable in the activated sludge process. The respiration rate can be used to increase our understanding of the process, which is essential for good control. The purpose of this study is to (1) develop a respiration meter capable of continuously measuring, using different procedures, the oxygen uptake rate of activated sludge and (2) expand knowledge about respiration related characteristics of wastewater and activated sludge.

1.2 Background of respiration

The activated sludge process is a widely used biological method of wastewater treatment. In this process the wastewater is brought in contact with an aerated suspension of activated sludge. Activated sludge is a flocculent mixture of an aerobic heterogenous population of micro-organisms and particulate organic materials. This sludge removes soluble and particulate matter from the wastewater. The activated sludge is separated from the treated wastewater by sedimentation and in this way retained in the system. Because the amount of sludge increases as a result of biomass growth and entrapment of particulate materials, surplus sludge is discharged from the system.

Although also non-biodegradable material is removed in the activated sludge process, this study was restricted to the removal of biodegradable material. The biodegradation process is performed by a mixed population of micro-organisms degrading multiple substrates along

several reaction pathways. The incoming wastewater is characterised by a varying substrate composition and concentration and varying flow rate. This means that the properties of the biomass and the wastewater, and the kinetics of the activated sludge process are difficult to identify. There is clearly a need for measurement procedures that provide information about the wastewater and biomass properties and the process kinetics for two reasons:

First, the information can be used to extend fundamental knowledge, for instance by identifying mathematical models of the activated process. Second, the information is in the public interest because it can be used for monitoring and controlling the process, thereby saving energy costs.

In characterising the process, the biodegradable matter plays a dominant role. However, because of its heterogeneous constitution, the concentration of this material can only be expressed by a general variable. Oxidation is the main mechanism of removal of biodegradable matter. Therefore, the oxygen uptake per unit of volume and unit of time, or respiration rate, is the key variable that characterises the process and the associated removal and degradation of the biodegradable matter. Dold *et al.* (1980) identify the respiration rate as a most sensitive variable on the basis of which the activated sludge process theory can be validated.

1.3 Measurement of the respiration rate

Measurement of the respiration rate of activated sludge has been the subject of many studies. Three methods can be distinguished:

- estimator method,
- exhaust gas method and
- instrumental method.

The estimator method, based on a model of the dissolved oxygen (DO) dynamics in a completely mixed aeration tank, uses on-line measurements of the DO concentration and the air flow rate to calculate the respiration rate (Holmberg, 1982; Howell *et al.*, 1984; Goto and Andrews, 1985; Howell and Sopido, 1985; Bocken *et al.*, 1989; Hamamoto *et al.*, 1990; Holmberg, 1990). The exhaust gas method is based on measuring the difference between the oxygen concentrations in the air entering and the air leaving the aeration tank (Kubota *et al.*, 1981; Redmon *et al.*, 1983; Boyle *et al.*, 1989; Stenstrom *et al.*, 1989). The instrumental method uses a respiration chamber where the oxygen uptake takes place and some device

intended to measure the uptake of DO. This work deals with the latter category. The combination of respiration chamber and device for measuring the DO uptake is called respirometer or respiration meter.

Various respiration meters have been described in literature. They can be divided into two categories: manometric meters and meters using an electrochemical DO sensor (DO probe). The manometric technique measures the volume of oxygen used by the bacteria (Montgomery, 1967; Jenkins, 1960; Umbreit, 1972; Tebbut and Berkun, 1976; Huang and Cheng, 1984). A special version is a respiration meter which replenishes the oxygen used by the bacteria. This oxygen can be supplied from a tank (Cadena *et al.*, 1989; Jacquez *et al.*, 1990) or can be generated by electrolysis (Clark, 1960; Hickey and Nagels, 1985; Tabak *et al.*, 1990; Hill, 1991). Most manometric respiration meters are primarily designed for determination of the biochemical oxygen demand (BOD) of wastewater or other substrates, although certain meters are capable of measuring the respiration rate itself as well (Therien and Ilhan, 1983; Huang and Cheng, 1984; Mueller and Stensel, 1990).

At present, most meters use a DO probe which measures the dissolved oxygen concentration. The respiration rate is then calculated from changes in the DO. The meter developed in this study is an example of this technique. Therefore, it will be considered further in this section. Two types of meters using a DO-probe can be distinguished: the batch respiration meter and the continuous flow-through respiration meter.

Batch meters can be either closed or open. The closed respiration meter is operated by withdrawing a sample of activated sludge from a plant, transferring it into a small vessel, aerating it, ceasing the aeration and then monitoring the decline of DO concentration with time. The respiration rate is derived from the slope of the DO decline (Lamb *et al.*, 1964; Eye and Ritchie, 1966; Vernimmen *et al.*, 1967; and others). One type of open respiration meter is operated in the same manner (Farkas, 1969; Takamatsu *et al.*, 1981; Randall *et al.*, 1991), however, this type requires prevention of the entry of oxygen from the air. Other open respiration meters are continuously aerated (Blok, 1974; Ros *et al.*, 1988a; Vanrolleghem *et al.*, 1990). They have the advantage that higher sludge concentrations can be used, because there is a continuous input of oxygen. Using this approach, the aeration coefficient and the saturation DO concentration under the operating condtions have to be known to calculate the respiration rate. This applies as well to a type of open batch respiration meter proposed by Ciaccio (1992). This meter consists of an aerated vessel with water and a closed reactor containing a microbial population on a carrier. It is evident that this meter cannot be used to determine the respiration rate of a particular activated sludge suspension. Although batch respiration meters are inherently discontinuous, they can be automated to operate in a semi-

continuous way (Fujimoto *et al.*, 1981; Takamatsu *et al.*, 1981; Kaneko *et al.*, 1985; Sekine *et al.*, 1988a; Randall *et al.*, 1991).

Continuous flow-through meters measure the DO concentration at the inlet and at the outlet of a closed respiration chamber through which sludge is pumped continuously. The respiration rate is calculated from the difference of the two DO measurements and the residence time. The device developed in this study is a flow-through meter of this type. Edeline *et al.* (1978) described a respiration meter consisting of a coiled tube and two DO probes at both ends of the tube. Activated sludge is pumped through the tube and the respiration rate is calculated from the DO difference. This method is in fact a plug flow version of the closed batch respiration meter described above. Other meters use a completely mixed respiration chamber through which the sludge is continuously pumped (Reynolds, 1969; Clarke *et al.*, 1978; Chen *et al.*, 1980; Hisset *et al.*, 1982; Sollfrank and Gujer, 1990). In all these cases one DO probe is placed in the aeration basin while the other is located at the outlet of the respiration chamber. In the calculation of the respiration rate steady state with respect to the DO concentration is assumed. Heckershoff and Wiesmann (1986) suggested a method using two parallel respiration chambers with a closed aeration system, which are both supplied with activated sludge from an aeration tank. Additionally, one is fed with wastewater while the other is fed with tap water. The respiration rate is calculated from mass balances of oxygen over the chambers. The authors claim that this technique also allows the calculation of the wastewater BOD.

Some continuous flow-through respiration meters are not particularly designed for measuring the oxygen uptake rate. Siepmann and Teutscher (1984) proposed a meter restricted to the measurement of BOD. In this meter wastewater is continuously diluted with tap water and the mixture is led through a chamber with immobilised micro-organisms. The DO difference over the chamber is kept constant by manipulating the dilution ratio. After calibration with a standard of known BOD, this ratio is used as a measure for the wastewater BOD (Riegler, 1984; Kalte, 1990). Several continuous toxicity meters are based on the change in respiration rate but do not provide respiration rate values (Solyom *et al.*, 1976; Pagga and Günther, 1981).

From the literature it was concluded that only a continuous flow-through respiration meter is capable of real-time monitoring the rate of oxygen uptake of activated sludge in an aeration tank. Only continuous flow-through meters can be useful for state estimation and control of the activated sludge process (Holmberg, 1982). Therefore, in this study the attention was restricted to the development of such a meter.

1.4 Newly-developed respiration meter

The respiration meter developed in this study consists of a closed, completely mixed chamber of 0.5 to one litre through which the activated sludge is continuously pumped (Figure 1). The characteristic feature of this meter is that the DO concentration in the sludge entering the chamber and in the sludge leaving the chamber is measured with one single probe, located at one opening. This is realised by changing the flow direction through the chamber. Initially, two probes were used and the flow direction was not changed (Spanjers, 1983; Spanjers and Klapwijk, 1987). This version, however, was unreliable, because when the probe sensitivities changed and there was a small difference between the two signals, relatively great errors resulted. The flow direction is changed using four solenoid valves excited, two by two.

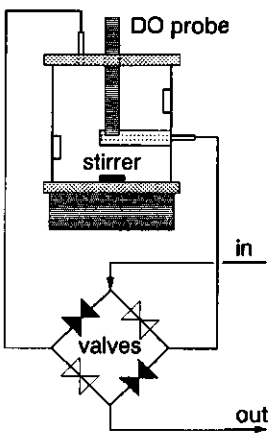

***Figure 1:** Design of the respiration meter.*

Two problems are associated with this strategy. First, the DO concentration can only be measured when the signal of the DO meter, after changing the flow direction, has reached its end value. With the DO meter employed, it takes about 10 - 30 seconds to attain 95% of the full response. This means that the measuring frequency for the DO concentrations is limited by the response time of the DO meter. For accurate continuous measurement of the respiration rate this frequency must be increased. Therefore, a method is needed to find the end value, even when the full response has not been attained. Chapter 6 of this thesis describes a method for estimating the end value by fitting a response model of the probe to the DO measurements. The second problem is that at a specific time instant only one

measurement is available: either the DO concentration of the sludge entering the chamber or the DO concentration of the sludge leaving the chamber. Hence, in the calculation of the respiration rate the missing DO concentration at that time instant has to be estimated as well. For this an interpolation method is used.

The respiration rate is calculated on the basis of the DO mass balance over the respiration chamber:

$$\frac{dc}{dt} = \alpha (c_a - c) - r \qquad (1)$$

where α=the dilution rate ($time^{-1}$). Given the discrete time measurements of the DO concentration, equation (1) has to be approximated by a difference equation. In this study, two numerical approximations of (1) were applied: an analytical method and the trapezoidal rule (Appendix A). Generally, with flow-through meters, steady state is assumed and the derivative is assumed to be zero. In this study, the derivative in (1) is determined so that the respiration rate can also be calculated during dynamic conditions.

1.5 Different respiration rates

Actual respiration rate

Reviewing the literature reveals that there is general consensus on the validity of using respiration rate for supervision and control of the activated sludge process (Benefield *et al.*, 1974; Haas, 1979; Holmberg, 1982; Allsop *et al.*, 1990; Tur *et al.*, 1990). However, there has been some opposition as to the utility of the respiration rate in controlling the activated sludge process, notably pursued by Sherrard's group (Sherrard, 1980; Khararjian, 1980; Edwards and Sherrard, 1982; Chandra *et al*, 1987). In my opinion this controversy is partly due to an inadequate technique used by Sherrard's group for measuring the respiration rate of the activated sludge in the aeration tank, in this work denoted as the **actual respiration rate**. Their technique, the BOD bottle method as recommended in 'Standard methods', is not suitable for measuring the actual respiration rate in an aeration tank, because it tends to underestimate this rate. In 'Standard Methods' (APHA, 1989) this notion is reflected in the statement that the determination of the respiration rate (using, for example, the BOD bottle technique) 'is sensitive to the time lag between sample collection and test initiation'. It is therefore recommended to start the measurement 'immediately' after sampling the activated

sludge suspension. Moreover, it is stated that 'because test conditions are not necessarily identical to conditions at the sampling site, the observed measurement may not be identical with actual oxygen consumption rate'. Several workers reported this inaccuracy of the batch technique in determining the actual respiration rate (Allsop *et al.*, 1990; Mueller and Boyle, 1990; Mueller and Stensel, 1990). Pfeffer *et al.* (1968) reported a significant decline in oxygen uptake of mixed liquor samples as the time lag between sampling and initiation of the measurement increases. Several workers using the batch technique were aware of this change in respiration rate and stated that the measurement should start as quickly as possible after sludge collection (Reinnarth and Rüffer, 1983; Suschka and Ferreira, 1986; Chandra *et al.*, 1987; Tur *et al.*, 1990). Yet some results are doubtful because of the delay between sampling and measurement (Duggan and Cleasby, 1976; Chandra *et al.*, 1987). Duggan and Cleasby, for example, reported that there is a direct response of the DO concentration to load variations, while there is a dampening of the mixed liquor oxygen uptake response.

The actual respiration rate in the aeration tank (r_{act}) is a function of the concentration of biodegradable matter. The concentration of biodegradable matter in the aeration tank is the net result of the inflow of biodegradable compounds and the biodegradation and outflow of these compounds. When a sample of sludge is collected from the aeration basin, only the biodegradation continues, resulting in a decreasing concentration of biodegradable matter. Hence, the observed rate will be lower than r_{act}. Sampling of sludge from the aeration tank is, in this regard, comparable to terminating the influent flow: Dold *et al.* (1980) observed a precipitous decrease in respiration rate at the instant of feed termination. Hence, the batch technique results in erroneous r_{act} values, even when the measurement is started 'immediately' after sampling as stated in Standard Methods (APHA, 1989). The same holds for continuous flow techniques: the loading of biodegradable matter from the influent falls off when the sludge flows through the respiration chamber. The difference between the measured rate and r_{act} depends on the kinetics and mean hydraulic residence time in the chamber.

In this work the condition is derived which makes it possible to measure r_{act} in one completely mixed aeration tank, using a continuous flow respiration meter. To obtain a respiration rate in the chamber equal to the rate in the aeration tank, the chamber has to be loaded with the same wastewater. It is demonstrated (Appendix B) that the respiration rate in the chamber equals the actual rate in the aeration tank if simultaneously with the sludge, a sample of influent is continuously led into the chamber in such a way that the ratio of sample flow rate and chamber volume equals the ratio of influent flow and aeration tank volume.

Endogenous respiration rate

The endogenous respiration rate (r_{end}) has generated some interest as a variable for characterising the activated sludge process. It is considered to be a measure for the active biomass concentration (Brouzes, 1979; Arthur, 1982; Allsop *et al.*, 1990; Young, 1981; Jørgensen *et al.*, 1992). Weddle and Jenkins (1971) stated that r_{end} divided by the volatile suspended solids concentration reflects the viable organisms content. Vandebroek (1986) used r_{end} as an indicator for toxicity. Kappler and Gujer (1992) reported a coefficient for the production of inert COD, derived from r_{end}. In order to determine the respiration rate due to the oxidation of soluble substrate, r_{end} has to be subtracted from the measured rate (Dietrich and Burris, 1967; Hartmann, 1968). In some publications dealing with substrate respiration rate, the results can be questioned because r_{end} was not subtracted (Takamatsu *et al.*, 1981; Huang *et al.*, 1985; Chandra *et al.*, 1987). Duggan and Cleasby (1976) reported that the endogenous respiration rate is not useful for monitoring the effect of influent loading, because it responds only gradually to changes in waste loading.

There is little consensus in literature as to the definition of the endogenous respiration rate. In microbiological literature r_{end} is defined in terms of maintenance: endogenous respiration occurs when there is no observed substrate consumption and when maintenance ATP is obtained from biomass degradation (Herbert, 1958). One definition in the wastewater literature is: 'metabolic respiration of a living cell using the contents of the cell as a substrate; occurs usually when there is an absence of any other substrates' (Patry and Chapman, 1989). Another is: 'the amount of oxygen utilized per unit of MLVSS in the reactor in the absence of exogenous substrate' (Suschka & Ferreira, 1986). Bhatla *et al.* (1966) stated that r_{end} is related to the auto-oxidation of cell material and is not related to substrate from the wastewater. A residual substrate concentration remains that is due to the endogenous reaction in which end products of cell lysis become substrate again and are available for the synthesis of new cell material. However these definitions are not entirely satisfactorily because they do not clearly say what is meant with substrate in this context. Dold *et al.* (1980) formulated the 'death regeneration hypothesis' as opposed to the classical endogenous respiration concept. This hypothesis implies that maintenance energy per se (oxygen requirement for maintenance) is considered to be so small that it can be lumped with, and completely swamped by, the oxygen demand for the synthesis of new cell mass from the lysed substrate.

As a result of the different concepts of endogenous respiration, the opinions of how to measure r_{end} are also different. Several workers observed that r_{end} as measured is not constant (Suschka and Ferreira, 1986; Ros *et al.*, 1988a) but depends on the loading

conditions (Bucksteeg, 1969) and on the sludge age (Holmberg and Ranta, 1982). Dietrich and Burris (1967) showed that r_{end} does not necessarily stays constant when substrate is added to a seed, and concluded that the usual practice of subtracting the oxygen consumption of the seed from that of the test suspension may not always be valid.

Most workers reported that the respiration rate approaches a more or less constant value (r_{end}), a certain aeration time after feed termination (continuous or batch). Authors reported various aeration times: 30 min (Mueller and Stensel, 1990; Stenstrom and Song, 1991), 1 hour (Cech *et al.*, 1984; Jφrgensen *et al.*, 1992), 2 hours (Heckershoff and Wiesmann, 1986), 4 hours (Suschka and Ferreira, 1986), 5 hours (Dold *et al.*, 1980). Sometimes the achievement of the endogenous respiration state was determined only qualitatively by observing a sharply decreasing rate followed by a more or less constant level (Bhatla *et al.*, 1966; Lijklema, 1971; Therien and Ilhan, 1983; Vasel *et al.*, 1991; Drtil *et al.*, 1993). In case of continuous operation the respiration rate of return sludge (Arthur, 1982; Therien and Ilhan, 1983) or even, during low loading, of mixed liquor in the aeration tank (Brouzes, 1979; Stephenson *et al.*, 1983) was considered to approach r_{end}.

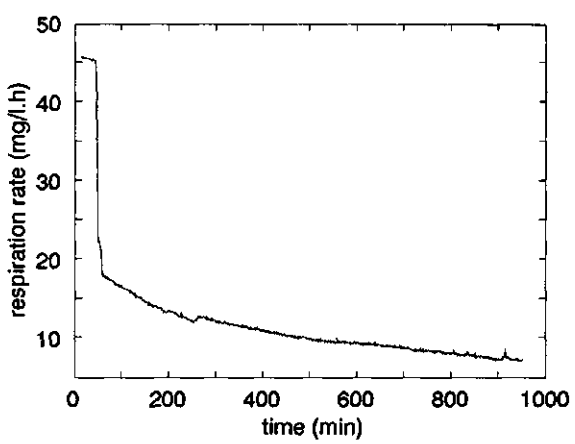

***Figure 2:** Respiration rate after sampling activated sludge from the aeration basin. Readily biodegradable matter was still present in the sludge at the start of the measurement.*

In this study the operational definition of r_{end} is: the oxygen uptake per unit of volume and unit of time in the absence of readily biodegradable matter in the solution. It is assumed that the rate is associated with the oxidation of readily biodegradable matter produced by (1) hydrolysis of slowly biodegradable matter, (2) lysis of dead cells and, (3) the release of

substrate for maintenance. In the next chapter the readily biodegradable matter will be specified further. In case of continuous operation the measurement of r_{end} is realised by measuring the rate of sludge from an unfed bypass tank through which the sludge is continuously recirculated. In case of batch operation r_{end} is measured by determining the rate after the sharp decrease which is typically observed as soon as the readily biodegradable matter has been oxidised. After this moment the respiration rate continues to decrease gradually. This is illustrated in Figure 2.

Maximum respiration rate

Measuring the maximum respiration rate can be useful for monitoring, control (Takamatsu *et al.*, 1981), assessing toxicity (Temmink *et al.*, 1993), estimation of biomass concentration and viability (Takamatsu *et al.*, 1981; Huang *et al.*, 1985; Vargas-Lopez, 1988) and determination of maximum aeration capacity (Huang *et al.*, 1985). There is reasonable consensus that the maximum respiration rate is to be measured in the presence of excess substrate. However there seems to be limited understanding of the conditions under which an excess of substrate is attained. In this thesis the measurement of the maximum respiration rate is explicitly discussed in some detail with special emphasis on the partition of readily biodegradable matter into several components.

1.6 Short-term biochemical oxygen demand

History

The biochemical oxygen demand (BOD) is a widely used and important test in the measurement of organic pollution. Rather arbitrarily an incubation period of 5 days was adopted as the time required for oxidation of most of the organic material (Leblanc, 1974). Initially the BOD_5 test was designed to assess the effect of pollutants on the oxygen content of receiving waters. Later its use was extended to the design and control of treatment plants. Because of its arbitrary character the BOD_5 test represents a varying part of the ultimate BOD of different wastewaters. This observation and the fact that a period of five days is unsuitable for activated sludge process operation purposes have led to efforts to develop a quicker BOD test. In evaluating various rapid BOD test methods, Leblanc (1974) concluded that a short-term BOD test would be useful for controlling treatment plant operation. Vernimmen *et al.* (1967) introduced the concept of short-term biochemical oxygen demand. Short-term biochemical oxygen demand will be denoted as BOD_{st} in this work. Much

attention has been payed to BOD_{st} in relation to process dynamics, modelling and control.

BOD_{st} as opposed to BOD_5

Contrary to the BOD_5 method, the BOD_{st} test uses the same micro-organisms and biomass concentration as those of the activated sludge plant under consideration. The BOD_{st}, therefore, shows better the effect of wastewater on an activated sludge plant. Moreover, the BOD_{st} represents the oxygen demand within the time limits of the process, so that it is more amenable to implementation in control strategies than the BOD_5. The BOD_5 test is known to give unreliable results. Köhne *et al.* (1986), comparing BOD_{st} with the BOD_5 method, concluded that the first has a better reproducibility.

Several authors tried to correlate BOD_5 and a short-term BOD with varying success (Vernimmen, 1967; Arthur and Hursta, 1968; LeBlanc, 1974; Farkas, 1981; Therien and Ilhan, 1983; Arthur, 1984; Harita *et al.*, 1985; Vandebroek, 1986; Suschka and Ferreira, 1986; Ciaccio, 1992). The goodness of correlation often appeared to be dependent on the type of wastewater. Vernimmen *et al.* (1967) admitted that their technique would be of doubtful value for accurate BOD prediction of wastes such as certain sewages, containing varying proportions of rapidly and slowly oxidizable substrates. Correlation was employed in the application of BOD-meters, such as the BOD probe, which thus need to be calibrated against standards with known BOD_{st} (Strand and Carlson, 1984; Riegler, 1984; Riedel *et al.*, 1988; Lenk *et al.*, 1990; Princz and Olah, 1990).

I do not consider it useful to investigate possible relationships between BOD_5 and BOD_{st}, because the BOD_{st} itself provides valuable information for the regulation of the activated sludge process.

Definition and nature of BOD_{st}

In the literature there is inconsistency as to the definition of BOD_{st}. One definition is: amount of oxygen that is consumed until all the substrate of a waste sample is converted into bacterial mass (Farkas, 1981). Another is: total amount of oxygen utilized for the bio-oxidation of substrate (Suschka and Ferreira, 1986). Vernimmen *et al.* (1967) defined the BOD_{st} in operational terms as the amount of oxygen utilised in addition to the endogenous oxygen consumption after introduction of a limited volume of a sample into a batch respiration meter containing activated sludge. The latter definition depends strongly on the

endogenous respiration rate. However, the authors did not specify r_{end}.

In this thesis BOD_{st} is defined in a similar operational way, but irrespective of the respiration meter: the BOD_{st} is the amount of oxygen utilised in addition to the endogenous oxygen consumption. Since in this thesis r_{end} is defined as the oxygen uptake per unit of volume and unit of time in the absence of readily biodegradable matter, the BOD_{st} is due to the oxidation of readily biodegradable matter. In this study, nitrification, if occurring, is considered to be included in the BOD_{st}. Henze (1992) found that the readily biodegradable matter includes acetic acid (42%), higher volatile fatty acids, lower amino acids, simple carbohydrates (each 17%) and alcohols (8%). However, he excluded ammonium.

The definition given above is in agreement with the approach of Dold *et al.* (1980). The authors suggested to make a distinction, based on biological response, not on physical separation, between readily and slowly biodegradable organic matter. They hypothesized that readily biodegradable substrate consists of small simple soluble molecules that can pass through the cell wall. Slowly biodegradable substrate consists of larger complex molecules and particulate matter that cannot pass the cell wall but are adsorbed and hydrolysed into readily biodegradable substrate that passes directly to the organisms. The hydrolysis is slow and rate limiting.

Respirometric methods for the determination of BOD_{st}

In most cases batch BOD_{st} tests use the same biomass concentration as that of the activated sludge plant. Because the tests imply higher oxygen demands, there are special requirements for the oxygen supply and hence the BOD botlle technique generally does not satisfy. Respirometric BOD tests were reviewed by Jenkins (1960), Montgomery (1967), LeBlanc (1974) and Vasel *et al.* (1991). Because of inconsistency, as mentioned before, these tests do not yield comparable BOD values at all. In some cases a "rapidly determined BOD_5" is meant, in other cases the BOD_{st} is considered to be an individual measure of waste concentration, in agreement with my own viewpoint.

Ekama *et al.* (1986) described three methods for estimating the readily biodegradable chemical oxygen demand. The first method involves feed termination in a flow-through activated sludge process and subsequent monitoring of the respiration rate. Knowledge of several stoichiometric constants is required to calculate the BOD_{st}. The second method consists of mixing wastewater and sludge and subsequently monitoring the respiration rate in a batch reactor. The readily biodegradable chemical oxygen demand is then calculated

from the area between endogenous and substrate respiration rate. The third method, also described by Kristensen *et al.* (1992), is identical to the second except for the utilisation of nitrate as electron acceptor instead of oxygen.

The second method has been used by far most frequently (see section 'Measurement of the respiration rate') and it is also used in this work. Other methods are based on a continuous flow-through respiration meters and BOD probes as described above. Additionally, in this work, a method is developed for measuring on-line the influent BOD_{st}.

Wastewater utilised in this study

All results (except one) of this study are based on the operation with presettled domestic wastewater from the village of Bennekom and activated sludge grown on this wastewater. The major fraction of the wastewater is of domestic origin; a small portion (7%) originates from a hospital. Compared with the COD, the BOD_{st} of this wastewater is relatively high. This is illustrated in Table 1, where a comparison is made with data from literature. The oxygen needed for nitrification is responsible for the high ratio found in this study.

Table 1: *Ratio of BOD_{st} to total wastewater COD (readily biodegradable matter expressed as a percentage of total COD). Data in this work represent averages of duplicate measurements. References refer to BOD_{st} tests with suppressed nitrification.*

type of wastewater	% BOD_{st}	reference
municipal	14	Sollfrank & Gujer (1991)
municipal and domestic		Henze (1992)
raw	7-32	
primary	16-33	
pre-precipitated	56	
presettled domestic (nitrification included)	26[1]	this work
	56[2]	

[1]wet weather conditions: low COD (236 mg l^{-1}) and N-Kjeldahl (28 mg l^{-1})
[2]average weather conditions: medium COD (312 mg l^{-1}) and N-Kjeldahl (60 mg l^{-1})

The BOD_{st} of the wastewater utilised in this study originates from soluble matter. This is illustrated in Figure 3, where the analysis results in relation to various filtrates of the wastewater are compared. The BOD_{st} turned out to be independent of filtration: the raw wastewater and the 0.45 μm filtrate had equal BOD_{st}. Assuming that the 0.45 μm filtrate only contained dissolved matter (Gujer, 1980), it is concluded that the readily biodegradable matter in the wastewater used consisted of dissolved substrate. This is in agreement with the conclusions of others (Dold *et al.*, 1980, 1986; Henze *et al.*, 1987).

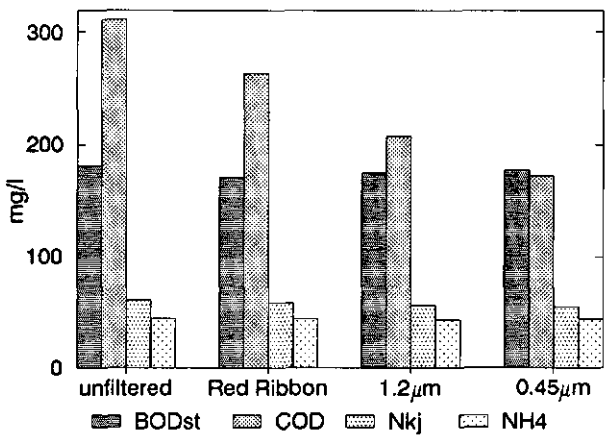

***Figure 3**: BOD_{st} (mgO_2l^{-1}), COD (mgO_2l^{-1}), N-Kjeldahl ($mgN\,l^{-1}$) and ammonium ($mg\,N\,l^{-1}$) of various filtrates of the presettled wastewater. Filters used (all Schleicher & Schuell): Red Ribbon 589[5], membrane ME 28 1.2 µm and membrane BA 85 0.45 µm.*

1.7 Outline of this thesis

The chapters in this thesis are all presented as publications (published or to be published), each of which can be read independently. Necessarily this involves some repetition of theories and methods. They do not follow the chronological order of the publications but are arranged so that they form a systematic structure.

The experimental research described in chapters 2, 3, 4 and 5 was performed using a pilot plant with one single completely mixed aeration tank of 475 m^3. Next to the experiments described in these chapters batch experiments were performed using an aerator of 1.5 to 2 litre. The batch reactor was used exclusively in the research described in chapters 6 and 7.

Chapter 2 presents the respiration meter used along with measuring procedures for obtaining information on the activated sludge process in one single completely mixed tank. The procedures consist of on-line measurement of four types of respiration rate of the same sludge under different conditions: endogenous, instantaneous, actual and maximum respiration rate. A method is presented for monitoring the influent and effluent BOD_{st}. It uses three of the four rates mentioned above. The importance of nitrification as part of the BOD_{st} is discussed. Control schemes, based on the different types of respiration rate, are suggested.

In chapter 3 an improved method for measuring the actual respiration rate is introduced. In contrast to the method in chapter 2, this method also makes it possible to measure the actual respiration rate in the second compartment of a plug flow reactor or further downstream. The method involves the measurement of the transient respiration rate during two different modes of operation of the measuring system, which are alternately executed. The BOD_{st} in the completely mixed aeration tank is estimated by choosing predetermined boundary conditions. The measured respiration rate and the estimated BOD_{st} in the respiration chamber are used to find the kinetic relationship between these two variables. Finally, this relationship is used to calculate the actual respiration rate. The result of this procedure is compared with the actual respiration rate as measured according to the method presented in chapter 2.

Chapter 4 describes *mutatis mutandis* a technique and estimation algorithm similar to those described in chapter 3, to estimate the influent BOD_{st} on-line. In order to verify the estimated values, the BOD_{st} is also independently determined in batch tests.

In chapter 5 the measurement of the maximum respiration rate as described in chapter 2 is explained in some detail. Especially the conditions under which this rate needs to be measured are discussed. An application of the measurement is described. It concerns the effect of the influent flow on the maximum respiration rate.

Chapter 6 describes an improvement of the evaluation of the respiration meter results which accounts for the time lag of the DO probe. The real DO concentration at the end of each response is estimated by fitting the DO measurements to a first-order response model of the probe. An additional result of this strategy is that it yields the time constant of the probe response, which provides a diagnosis of the probe condition.

Chapter 7 is an application of using the respiration rate for identifying a mathematical model of a subprocess in the activated sludge. Nitrification is chosen because there is need for a better model of this process including both nitrification steps (Grady, 1989). Since nitrification involves a high uptake of oxygen, respiration rate is a useful variable for

identifying this process. Because an optimal experimental design and a good model validation method are needed, the investigation described in this chapter was focused on these items. The batch experimental procedure described in chapters 2, 4 and 5 was used in this investigation.

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2

On-line meter for respiration rate and short-term biochemical oxygen demand in the control of the activated sludge process

2.1 Abstract

A meter, based on a novel principle, is described for monitoring of the respiration rate of activated sludge and, as a derivative, the short-term biochemical oxygen demand (BOD_{st}). Operational implications and reliability are discussed. A method is presented for measuring four respiration rates of the same sludge under different conditions. These rates are: the endogenous, the instantaneous, the actual and the maximum respiration rate. Theoretical and experimental conditions for the measurement of the different rates are discussed. It is shown that the BOD_{st} of both influent and effluent can be calculated from three of the four respiration rates. The method is applied to a completely mixed continuous-flow activated sludge pilot plant. Results from two experimental set-ups are shown and discussed. There is some evidence that the maximum respiration rate is dependent on the loading. It is concluded that the measurements provide relevant information on the activated sludge process and in addition that the influent and effluent BOD_{st} can also be calculated. The BOD_{st} of the examined wastewater appears to be predominantly caused by ammonium being oxidized by nitrifiers. The measurements can be used to control activated sludge plants with respect to sludge loading, dissolved oxygen concentration and sludge balance. Control schemes are suggested.

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2.2 Introduction

The respiration rate of activated sludge and the short-term biochemical oxygen demand (BOD_{st}) are valuable variables for the control of activated sludge processes. In contrast with traditional variables like BOD_5 and TOC, these variables are related to the biological process itself. The respiration rate is one of the few directly measurable variables. It provides information as to the loading to the plant (Holmberg, 1982) and it can also indicate toxic effects on the activated sludge (Vandebroek, 1986). The respiration rate can also be used as a quantity from which otherwise poorly accessible variables such as the biomass concentration can be estimated (Takamatsu *et al.*, 1981). In the work described here we used the respiration rate to estimate the BOD_{st} of both the influent and the effluent. Such information can be used for effective control of the activated sludge process.

The respiration rate can be estimated in situ using the measurement of dissolved oxygen concentration in the aeration tank (Holmberg *et al.*, 1989). Holmberg (1982) showed that a dynamic model of the activated sludge process can be used for on-line estimation of the influent BOD-loading and the effluent BOD.

Another approach is to use an on-line respiration meter. Such a meter is often operated by withdrawing a sample of activated sludge into a small vessel, aerating it, and then monitoring the decline in the dissolved oxygen concentration with time (Fujimoto *et al.*, 1981; Kaneko *et al.*, 1985). The difficulty with this batch method is that at the actual time of measurement, the readily biodegradable compounds may have been oxidized already, resulting in the respiration rate being close to the endogenous rate. The state within the respiration meter would then differ substantially from that in the aeration tank itself.

Continuous flow-through meters which measure the oxygen concentration at the inflow and at the outflow of a respiration chamber do not have this problem (Edeline *et al.*, 1978; Sollfrank and Gujer, 1985). Flow-through meters with two oxygen probes may be subject to problems when the probe sensitivities change and a small difference between two signals exists.

Respiration measurements can be combined with measurement of the BOD. In batch procedures a sample of water is added to sludge which is either sampled from the aeration tank (Vernimmen *et al.*, 1967) or has been grown separately (either fixed or not) (Vandebroek, 1985) and which has the endogenous respiration rate. The respiration rate is

then monitored continuously until the endogenous level is reached again. The (short-term) BOD is the excess amount of oxygen additionally used to the endogenous oxygen consumption. The disadvantage of this method is that it is not continuous. In the case of separately grown sludge also the state of the sludge may differ substantially from that in the aeration tank. The measurements should be done under conditions as close as possible to those in the aeration tank.

In continuous procedures water is conducted to either a separate sludge system (Köhne *et al.*, 1986) or is mixed with sludge from the aeration tank in an aerated reaction vessel (Heckershoff and Wiesmann, 1986). The last method is complicated, the measurement time of 1 up to 4 hours is lengthy and the calculation of the effluent BOD_{st} is not obvious.

In this chapter we describe a meter for monitoring the respiration rate of activated sludge from which the BOD_{st} can be derived. The device consists of a pump, a respiration chamber through which the sludge flows continuously, and one single oxygen probe. Contrary to other systems the same probe is used for measuring the oxygen concentration at the inlet and at the outlet of the respiration chamber.

In addition we propose a method for measuring four respiration rates of the same sludge under different conditions, at a continuous flow activated sludge plant, namely the endogenous respiration rate, the instantaneous respiration rate, the actual respiration rate and the maximum respiration rate.

Furthermore, we demonstrate that, at a continuous flow activated sludge plant, the BOD_{st} can be calculated from three respiration rates: the endogenous, the instantaneous and the actual respiration rate. BOD_{st} mass balance equations for the respiration chamber are used for these calculations.

It is concluded that the measurements supply relevant information on the activated sludge process and that the influent and effluent BOD_{st} can also be calculated. The BOD_{st} of the examined wastewater appears to be predominantly caused by the oxidation of ammonium by nitrifiers.

2.3 Measurement of the respiration rate

Respiration meter

The respiration meter (Figure 1) is commercially available (Manotherm, The Netherlands) and consists of a closed, completely mixed respiration chamber of one litre through which the activated sludge is continuously pumped. Dissolved oxygen concentration is periodically measured with one and the same probe at the inlet as well as at the outlet of the respiration chamber. This is achieved by alternating the flow direction. In this design the varying flow direction has no impact on the process in the respiration chamber, hence the flow can be assumed to be continuous.

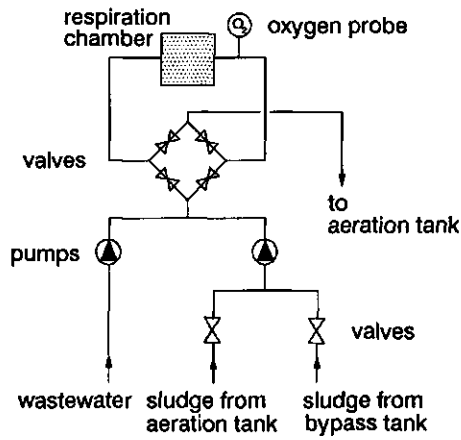

***Figure 1:** Respiration meter*

For the completely mixed respiration chamber under continuous flow conditions the dissolved oxygen mass balance is:

$$\frac{ds_O(t)}{dt} = \frac{q_{res}}{v_{res}}\left(s_{OI}(t) - s_O(t)\right) - r(t) \qquad (1)$$

$s_O(t)$ = oxygen concentration at outlet at time t (kg m^{-3}).
$s_{OI}(t)$ = oxygen concentration at inlet at time t (kg m^{-3}).

q_{res} = volumetric flow through the respiration chamber (m^3h^{-1}).
v_{res} = volume of the respiration chamber (m^3).
$r(t)$ = respiration rate at time t (kg $m^{-3}h^{-1}$).

For numerical analysis of the on-line measured oxygen concentration, equation (1) is integrated over one sampling interval T. Assuming that $s_{OI}(t)=s_{OI}(t-T)$ and $r(t)=r(t-T)$:

$$r(t) = \frac{\alpha}{1-e^{-\alpha T}}\left[(1-e^{-\alpha T})s_{OI}(t-T)+e^{-\alpha T}s_O(t-T)-s_O(t)\right] \quad (2)$$

$$\alpha = \frac{q_{res}}{v_{res}} \quad (3)$$

For accurate measurements, T should be as short as possible. The lower limit of the time interval T for the calculation of $r(t)$ is set by the response time of the oxygen probe. Typical values of T are between 30 and 60 seconds. For increasing values of T the response time of the respiration measurement increases. Because the oxygen concentration is measured periodically in the inflow and in the outflow, s_{OI} cannot be measured at time t-T and must be calculated from $s_{OI}(t-½T)$ and $s_{OI}(t-1½T)$. The measuring uncertainty is less than 5% of the reading for respiration rates greater than 20 g $m^{-3}h^{-1}$.

The respiration meter is connected to a modular I/O processor which collects the oxygen concentration data, calculates the respiration rate and controls the process flows.

Reliability of the respiration meter

The reliability of the respiration meter was tested by means of batch experiments. The meter was connected to a reactor of 1.5 litre containing activated sludge. Temperature and pH were kept constant. When the sludge was in the endogenous phase of respiration a certain amount of ammonium chloride was added which caused an increase in the respiration rate until the ammonium was oxidized. Figure 2a shows the response when the same dose was repeated four times.

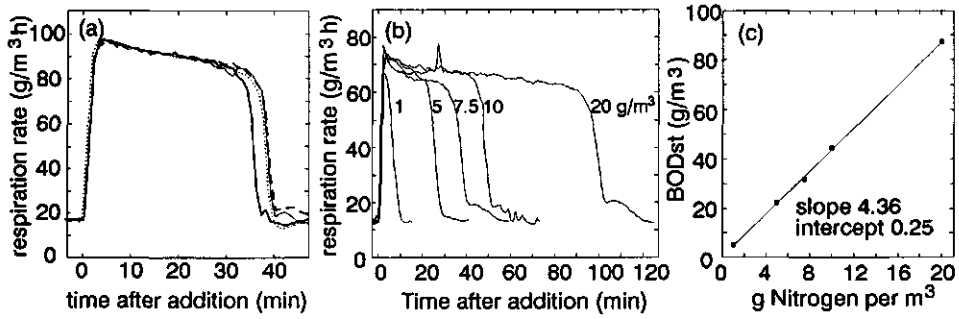

***Figure 2:** Respiration rates after addition of ammonium chloride to activated sludge (pH 7.5, temperature 20 °C). (a) same dose repeated four times. (b) increasing amount of ammonium: 1, 5, 7.5, 10 and 20 gram nitrogen per m^{-3} (sludge MLSS 3.34 kg m^{-3}, MLVSS 2.61 kg m^{-3}) (c) calculated BOD_{st} versus amount of ammonium-nitrogen added.*

It is concluded that the reproducibility is good. In Figure 2b the course of the respiration rate following the addition of increasing amounts of ammonium is presented. From the data, the total amount of oxygen additionally used to the endogenous oxygen consumption, defined as BOD_{st}, was calculated. In Figure 2c this BOD_{st} is plotted against the amount of ammonium-nitrogen. The slope of the regression line, representing the oxygen-nitrogen ratio, is 4.36 kg O_2 per kg N, which is in accordance with the value cited in the literature (Sharma and Ahlert, 1977) and close to the stoichiometric oxygen demand.

Different respiration rates

For on-line monitoring activated sludge plants, we propose the use of the four different respiration rates mentioned before (Figure 3).

The endogenous respiration rate (r_{end}) is defined as the oxygen uptake rate of activated sludge that has been aerated for two hours without feeding. The measurement is practically realised by measuring the rate of activated sludge from a completely mixed bypass tank of 0.028 m^3 through which sludge from the aeration tank is continuously recirculated.

The instantaneous respiration rate (r_{ins}) is defined as the oxygen uptake rate of activated sludge flowing directly from the completely mixed aeration tank through the respiration chamber. This rate is lower than the oxygen uptake rate in the aeration tank: the actual

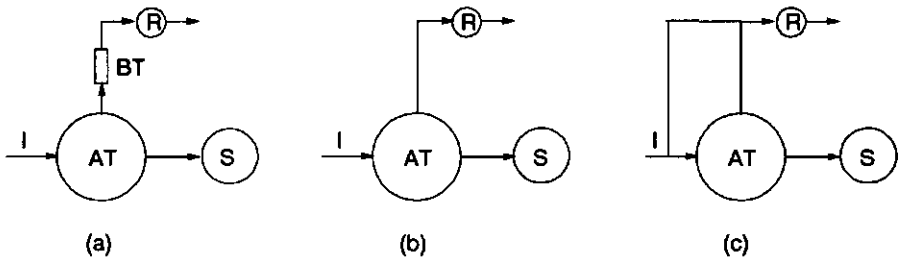

***Figure 3:** Measurement of four different respiration rates. (a) endogenous rate; (b) instantaneous rate; (c) actual and maximum rate. AT = aeration tank, BT = bypass tank, I = influent, R = respiration meter, S = secondary settler.*

respiration rate. The absolute value of r_{ins} depends on the detention time in the respiration chamber.

The actual respiration rate (r_{act}) can be measured provided that the sludge loading in the respiration chamber equals the loading in the aeration tank. Therefore influent is continuously added to the sludge flowing into the respiration chamber in the proportion (Spanjers and Klapwijk, 1987):

$$q_{sam} = \frac{v_{res}}{v_{at}} q_{in} \qquad (4)$$

q_{sam} = influent sample flow to respiration chamber (m^3h^{-1})
q_{in} = influent flow (m^3h^{-1})
v_{res} = volume respiration chamber (m^3)
v_{at} = volume aeration tank (m^3).

The maximum respiration rate (r_{max}) is measured when an excess of influent is continuously added to the sludge flowing into the respiration chamber. It was established experimentally that under normal operating conditions, an excess of influent is guaranteed when the ratio of influent flow to sludge flow is at least 0.03.

In the study described here, we used only one respiration meter, which meant that the different respiration rates could only be measured sequentially.

2.4 Calculation of the BOD_{st}

Both the influent BOD_{st} and the effluent BOD_{st} can be calculated from three respiration rates: the endogenous, the instantaneous and the actual respiration rate. When measuring the actual respiration rate (r_{act}) the mass balance of BOD_{st} for the respiration chamber is (steady state assumed):

$$s_{in} - s_{ef} - \frac{v_{at}}{q_{in}}(r_{act} - r_{end}) = 0 \qquad (5)$$

s_{in} = influent BOD_{st} (kg m^{-3})
s_{ef} = effluent BOD_{st} (kg m^{-3}).

The effluent BOD can be calculated from the mass balance of BOD_{st} for the respiration chamber when measuring the instantaneous respiration rate (r_{ins}):

$$s_{ef} - s_{res} - \frac{v_{res}}{q_{res}}(r_{ins} - r_{end}) = 0 \qquad (6)$$

s_{res} = BOD_{st} in respiration chamber during measurement of r_{ins} (kg m^{-3})
q_{res} = activated sludge flow through respiration chamber (m^3h^{-1}).

To calculate s_{res} an assumption with regard to the reaction kinetics has to be made. In preliminary studies (batch experiments) it was found that the oxidation of BOD_{st} can be modelled as a ½-order reaction as is usual in practice for biofilm kinetics:

$$r_{act} - r_{end} = kx(s_{ef})^{\frac{1}{2}} \qquad (7a)$$

$$r_{ins} - r_{end} = kx(s_{res})^{\frac{1}{2}} \qquad (7b)$$

k = rate constant ($(m^3kg^{-1})^{½}h^{-1}$).
x = ML(V)SS (kg m^{-3}).

From equations (7) s_{res} can be calculated:

$$s_{res} = \left(\frac{r_{ins} - r_{end}}{r_{act} - r_{end}}\right)^2 s_{ef} \qquad (8)$$

Because the different respiration rates are measured sequentially, on-line calculation of the BOD_{st} is carried out after each respiration measurement by estimating the missing respiration rates from linear interpolation of two values.

2.5 The experimental procedure

The activated sludge pilot plant was composed of a completely mixed aeration tank (0.475 m^3) and a settler (0.159 m^3). The plant was fed with pre-settled domestic sewage. To prevent bulking sludge, influent and return sludge were mixed in an unaerated contact tank consisting of four 2 litre compartments in series. Control of the valves and pumps (see Figure 1), collection of dissolved oxygen data and calculation of respiration rate and BOD_{st} was done by a modular I/O processor. The data collection was commenced after the activated sludge process had reached the steady state. In this chapter we present the results of two different experimental set-ups.

Table 1: Relevant process conditions

process variable	1st set-up May 1988	2nd set-up March 1988
influent flow (m^3h^{-1})	0.020 (a.m.)	0.038
averaged COD loading	0.08 (a.m.)	0.20
(kg per kg MLSS per day)	0.27 (p.m.)	
averaged N loading	0.049 (a.m.)	0.025
(kg per kg MLSS per day)	0.016 (p.m.)	
recirculation factor	1	1
solids retention time (day)	23	15
MLSS (kg m^{-3})	4.0-5.0	3.1-3.4
MLVSS (kg m^{-3})	3.3-4.2	2.4-2.7
temperature (°C)	16-20	11-14
pH	7.1-7.4	7.1-7.4

During the first experiments the influent flow rate was varied according to a square wave pattern as follows: 0.020 m^3h^{-1} during the morning and 0.060 m^3h^{-1} during the afternoon. In the second set-up the influent flow rate was kept constant, but the influent was taken alternately from the sewer (during the morning) and a stock (during the afternoon). The stock was daily refilled with fresh wastewater. Table 1 summarizes the relevant process conditions.

2.6 Results

During the first experiments the greater part of the variation in loading was due to the varying influent flow rate (see Table 1) which dominated the natural variation in influent strength. Figure 4 shows the sludge loading during the first experimental period.

The course of the measured respiration rates can be seen in Figure 5. The endogenous respiration rate (r_{end}), although relatively constant, shows a variation which depends on the loading. This is probably caused by the varying concentration of slowly biodegradable compounds resulting in a concomitant variation in oxidation.

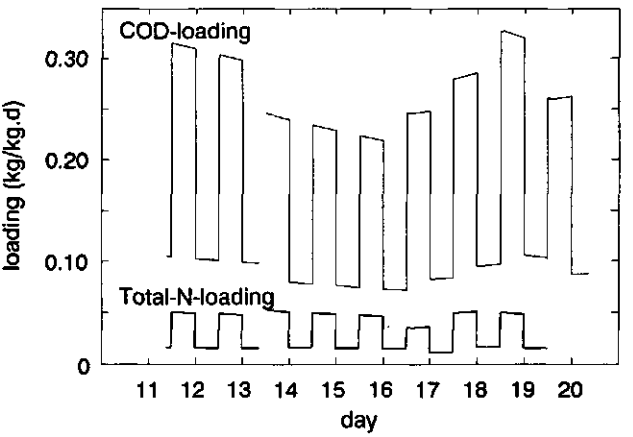

Figure 4: *COD and total nitrogen sludge load during the first experiments. COD, N and MLSS are based on 24 and 72 hour averages.*

The instantaneous respiration rate (r_{ins}) is an indicator for the presence of readily biodegradable compounds in the effluent: when r_{ins} is close to r_{end} the concentration of these compounds will be very low; when r_{ins} is close to the actual respiration rate (r_{act}) the concentration is likely to be high. In Figure 5 r_{ins} lies between these extremes.

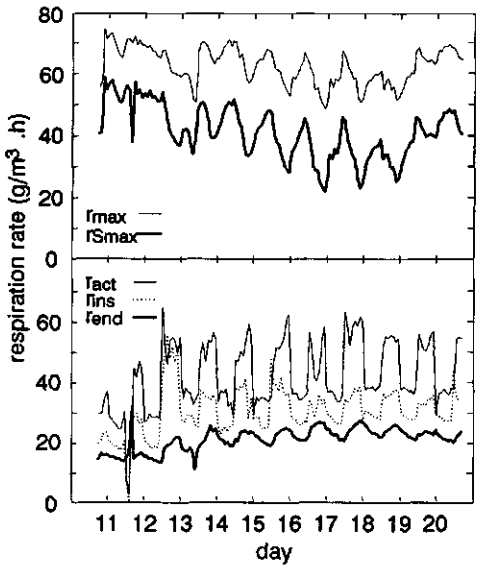

***Figure 5:** Respiration rates during the first experiments.*

r_{act} depends strongly on the loading. The dip on 11 May was due to problems with the aeration: the oxygen concentration in the aeration tank decreased below 1 g m^{-3} during one hour and the nitrification ceased. The readily biodegradable compounds thus accumulated in the aeration tank and were subsequently oxidized in the bypass tank (Figure 1), resulting in a failure of the measurement of r_{end} (peak in Figure 5).

The maximum rate for the oxidation of readily biodegradable compounds, r_{Smax}, is calculated by subtracting r_{end} from the maximum respiration rate r_{max} (Figure 5). Since the BOD_{st} of the used wastewater is chiefly caused by ammonium, r_{Smax} mainly represents the nitrification rate. It was expected that r_{Smax} would be relatively independent of short time variation in the loading. In Figure 5, however, it can be seen that this rate depends on the loading in an unexpected way: at high loading it tends to decrease and at a low loading it tends to increase. At present we are looking into this phenomenon.

Sometimes r_{act} is close to r_{max}, which means that the loading with readily biodegradable compounds is close to the maximum loading. A serious risk of an increased concentration of readily biodegradable compounds occurs if this maximum loading is exceeded.

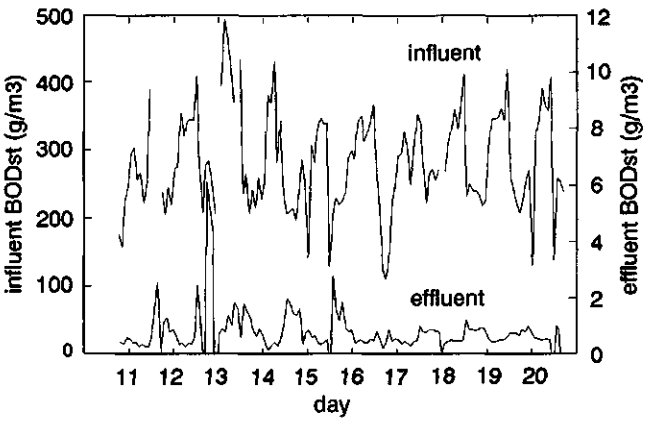

***Figure 6:** Influent and effluent BOD_{st} calculated from the respiration rates during the first experiments.*

Both the influent BOD_{st} (s_{in}) and the effluent BOD_{st} (s_{ef}) are calculated from the respiration rates (Figure 6). s_{in} shows mostly higher values in the morning which is to be expected for the domestic wastewater used. In the afternoon of 16 May there is a remarkable decrease in s_{in}. From the influent COD (although a 24 hour average) and from other variables (rainfall, influent temperature) it was found that the influent strength was low during that time. s_{ef} shows higher values during the afternoon when the loading is high. Nevertheless, s_{ef} is low and the removal of readily biodegradable compounds always exceeded 99%.

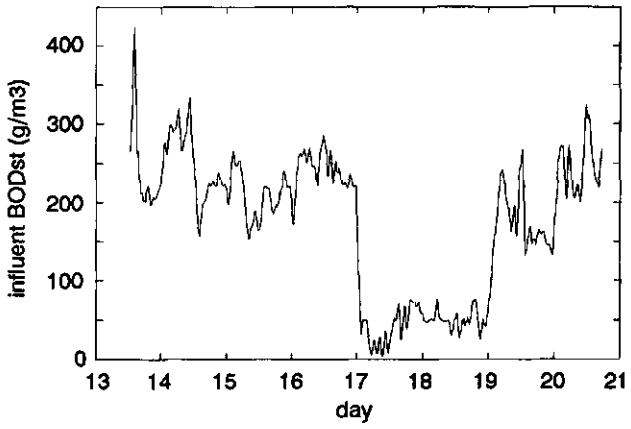

***Figure 7:** Influent BOD_{st} during the second experimental period.*

It should be emphasized that the influent BOD_{st}, as measured by the method described here, must be independent of the loading. It is a measure of the concentration of readily biodegradable compounds in the influent. To check whether the measured BOD_{st} is constant while the plant is fed with influent of constant quality, the results of the second experimental period can be analyzed (Figure 7). During this period (see also Table 1), the measured BOD_{st} should be constant during the afternoon. The BOD_{st} indeed showed significantly less variation during the afternoon: 7% to 26% as compared with 8% to 90% during the morning, though the variation exceeded the measuring uncertainty for (batch) measurement (5 - 8%).

Two components in wastewater may contribute to the BOD_{st}: ammonium, which is oxidized by nitrifiers, and readily biodegradable organic compounds, which are oxidized by heterotrophic organisms. In Figure 8, the BOD_{st} of the second experimental period has been plotted against the influent ammonium concentration (both 12 hours averages).

Linear regression yields a slope of 4.42 kg O_2 per kg nitrogen which is close to the value obtained in batch experiments (see section Reliability of the Respiration Meter). This suggests that ammonium contributes to a great extent to the BOD_{st} of the used wastewater. This corroborates results from batch experiments where wastewater was added to activated sludge without nitrifying organisms (Spanjers and Klapwijk, 1987) or with inhibited nitrification (Figure 9). Addition of wastewater induces only a limited increase in respiration rate during a short period.

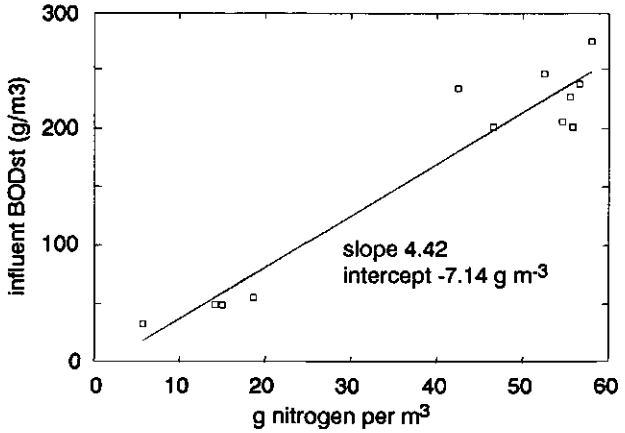

Figure 8: *Measured BOD_{st} against influent ammonium nitrogen concentration (both averaged over 12 hours) in the second experimental period.*

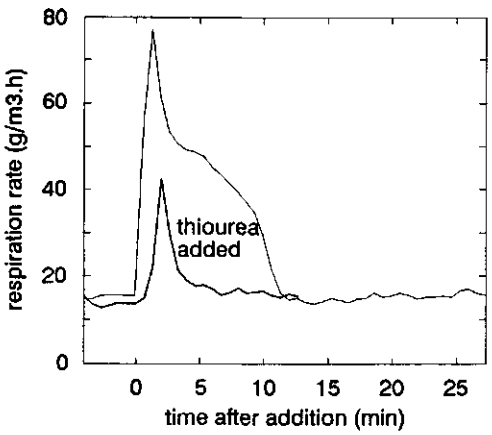

Figure 9: *Effect of inhibition of nitrification. Conditions: batch experiment, dosage of 0.1 litre wastewater to 3.1 litres activated sludge with and without inhibition by 10 mg thiourea per litre.*

2.7 Discussion

The respiration meter is capable of monitoring the respiration rate of activated sludge continuously and trouble free. Crucial factors determining the accuracy of the measurement are the oxygen measurement and the volumetric flow of activated sludge through the respiration chamber. During the experimental periods daily calibration of the sample pumps and the oxygen meter was sufficient. No interruption of the measurements was required for calibration of the pumps. Calibration of the oxygen meter caused an interruption for 2 - 5 minutes. At present we are considering the possibility of a less frequent calibration because the calculated respiration rate is less sensitive to calibration drift than the absolute oxygen concentration.

The shortest realizable response time of the respiration meter to a stepwise input is one up to two minutes for 95% of the reading. This is sufficiently short for monitoring and control purposes at a wastewater treatment plant and for most batch experiments. In the experiments we used one meter for sequentially measuring four different respiration rates. The measurement of one rate took at least 8 minutes when fulfilling the condition of steady state in the respiration chamber for calculation of the BOD_{st}. Consequently, the BOD_{st} could be calculated every 8 minutes. This will be sufficient for adequate control in most plants. Nevertheless the calculation method needs to be improved. The results of the second experimental period show that the accuracy of the BOD_{st} was not satisfactory, because the calculation method was susceptible to noise in the respiration rates. A dynamic estimation method for the BOD_{st} which does not require a steady state condition is a research topic at present.

When considering the control of an activated sludge plant one can envisage three aims:
1. sufficient degree of BOD (organics and nitrogen) removal;
2. production of sludge with good settling properties;
3. keeping energy costs at a minimum.

These aims can be achieved by controlling the sludge loading, the oxygen concentration and the sludge balance.

The reasons for controlling the loading are: maintenance of nitrification and good sludge quality, minimization of aeration costs and dealing with toxic inputs. The loading can be influenced by adjusting the sludge concentration and, if storage is available, the influent flow rate. The difference between r_{act} and r_{end} (substrate respiration rate) is a measure of the concentration of readily biodegradable compounds whereas the ratio of the substrate

respiration rate to r_{end} is a measure of the loading with these compounds. When r_{ins} is close to r_{act} and also r_{act} is close to r_{max}, there is a risk of overloading the plant. A toxic input may be recognized by a sudden decrease of r_{max} caused by an inhibition of nitrifiers.

The objectives for controlling the oxygen concentration are: saving of aeration costs, maintenance of nitrification and prevention of bulking sludge. The oxygen concentration can be controlled by adjusting the air flow rate. The concentration must be as low as possible to minimize aeration costs. On the other hand it must be high enough to maintain nitrification and to prevent bulking sludge (Hermanowicz, 1987). The oxygen concentration in the bulk fluid gives insufficient information on the activity of the sludge as does the respiration rate. Moreover, in a completely mixed reactor, the concentration varies with the depth, whereas the respiration rate does not. The oxygen transfer rate can be calculated continuously from the oxygen concentration and r_{act}, and can be used as an indicator for clogging of air diffusors.

The reasons for controlling the sludge balance are: adjustment of loading, maintenance of the composition and quality of waste sludge, and cost saving. Control can be exerted on the return sludge flow rate and on the waste sludge flow rate. The sludge balance is mainly considered on the basis of ML(V)SS. This variable does not provide meaningful information on the active biomass concentration as r_{end} and r_{max} do. Therefore control of the sludge balance ought to be based on these two variables. When r_{end} is high in relation to r_{act} a relatively large part of the oxygen is consumed for maintenance. As this is uneconomic the action of control would be to waste sludge.

2.8 Conclusions

The respiration meter is capable of continuous and trouble free monitoring of the respiration rate with satisfactory accuracy. The method for continuous measuring four different respiration rates at a completely mixed activated sludge plant provides relevant information on the process; furthermore the influent and effluent BOD_{st} can be calculated as well. The calculation algorithm for the BOD_{st} still needs to be improved. The respiration rates measured can be used as a basis for process control. The BOD_{st} of the examined wastewater appears to be predominantly caused by the oxidation of ammonium.

2.9 Notation

k rate constant $((m^3kg^{-1})^{1/2}h^{-1})$
q_{in} influent flow (m^3h^{-1})
q_{res} volumetric flow through the respiration chamber (m^3h^{-1})
q_{sam} influent sample flow to respiration chamber (m^3h^{-1})
r respiration rate (kg $m^{-3}h^{-1}$)
r_{act} actual respiration rate (kg $m^{-3}h^{-1}$)
r_{end} endogenous respiration rate (kg $m^{-3}h^{-1}$)
r_{ins} instantaneous respiration rate (kg $m^{-3}h^{-1}$)
$r$max maximum respiration rate (kg $m^{-3}h^{-1}$)
r_{Smax} maximum rate for the oxidation of readily biodegradable compounds (kg $m^{-3}h^{-1}$)
s_{ef} effluent BOD_{st} (kg m^{-3})
s_{in} influent BOD_{st} (kg m^{-3})
s_{res} BOD_{st} in respiration chamber during measurement r_{ins} (kg m^{-3})
s_O oxygen concentration at outlet (kg m^{-3})
s_{OI} oxygen concentration at intlet (kg m^{-3})
T sampling interval (h)
v_{at} volume aeration tank (m^3)
v_{res} volume of the respiration chamber (m^3)
x mixed liquor (volatile) suspended solids (kg m^{-3})
α $= q_{res}/v_{res}$ dilution rate (h^{-1})

2.10 References

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3

Determining short-term biochemical oxygen demand and respiration rate in an aeration tank by using respirometry and estimation

3.1 Abstract

For good control of the activated sludge process, accurate measurement of the short-term biochemical oxygen demand (BOD_{st}) in the aeration tank (s_a) and the actual respiration rate of the activated sludge (r_{act}) is required. A previously described measurement technique for these variables requires the measurement of three types of respiration rate of which one rate, the actual respiration rate, involves continuous addition of influent sample to a respiration chamber. The technique is only applicable to a plant with one single aeration tank or to the first compartment of a plug flow reactor.

In this chapter, a method is proposed for the estimation of s_a and r_{act} in a completely mixed aeration tank, no matter whether this is a single reactor or a compartment of a plug flow reactor. In contrast to the other technique, this method does not involve addition of influent sample to the respiration chamber. Instead, the transient respiration rate is measured during two modes of operation which are alternately executed. In one mode, sludge from the aeration tank flows through the respiration chamber, whereas in the other mode, sludge having the endogenous rate is directed through the chamber. The s_a is obtained by integrating the mass balance equations for each cycle of two different modes. The measured respiration rate and the calculated BOD_{st} during each cycle of two modes are used to find the kinetic relationship in order to calculate r_{act}.

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The proposed method has been verified using simulated and experimental data. The estimated s_a and r_{act} from experimental data are in agreement with the diurnal variation typical for the wastewater used. The pattern of r_{act} is in agreement with the one obtained by the previously described method. There is, however, a significant difference between the average values of the two methods. A possible explanation for this bias is given. Compared to the previously described method, the proposed method is simpler to use in practice.

3.2 Introduction

The activated sludge process is aimed to achieve, at minimum energy costs, a sufficiently low concentration of biodegradable matter in the effluent, under conditions of variable loading, along with a low sludge production. Therefore, the process has to be controlled. For good control accurate measurement of process variables is required. Two of these variables are the short-term biochemical oxygen demand (BOD_{st}) of the mixed liquor in the aeration tank and the actual respiration rate.

The BOD_{st} includes readily biodegradable ammonium and organic matter which are oxidized within the operating time of the activated sludge process. Thus, it is closely related to the current reaction rates of the process. In dynamic modelling of the activated sludge process the bacteria are assumed to utilize only readily biodegradable matter (Henze *et al.*, 1987). The BOD_{st} in relation to the readily biodegradable COD as used in the IAWPRC model (Henze, 1987) and the BOD_5 is discussed elsewhere in this work. Since the BOD_{st} includes nitrification it is a good measure of the oxygen demand in a nitrifying activated sludge plant.

The actual respiration rate in the aeration tank is directly related to the biological activity and to the BOD_{st}. It provides information on the BOD-loading to the plant (Duggan and Cleasby, 1976; Chen *et al.*, 1980) and on toxic effects (Temmink *et al.*, 1993). In combination with other types of respiration rates it can be used to display the performance of the process (Spanjers and Klapwijk, 1990).

Few on-line monitoring techniques for the BOD_{st} in the aeration tank have hitherto been proposed. One application would be in using a microbial probe (Princz and Oláh, 1990). However, the use of such a probe for the BOD_{st} in the aeration tank has not been pursued. Spanjers and Klapwijk (1990) have shown that the BOD_{st} in a completely mixed aeration tank can be derived from the measurement of three types of respiration rate,

including the actual rate.

Two methods for the measurement of the actual respiration rate in the aeration tank can be distinguished: the estimator method and the instrumental method. The estimator method, based on a model of the DO dynamics in the completely mixed aeration tank, estimates the respiration rate and the oxygen mass transfer coefficient. One variant of the method only uses the measurement of the DO concentration in the aeration tank and on-line measurements of the air flow rate (Hamamoto *et al.*, 1990; Goto and Andrews, 1985). In another variant, the estimator is part of an adaptive controller for the DO concentration (Holmberg and Olsson, 1989; Howell and Sopido, 1985). In evaluating the estimator method, Bocken et al. (1989) proposed refinements to overcome offsets of the estimated respiration rate. Holmberg (1990) concludes that these offsets still remain; there is still an undetermined bias in both the respiration rate and the oxygen mass transfer coefficient, which is not identifiable.

The instrumental method uses a respiration chamber in which activated sludge from the aeration tank is sampled. In the discontinuous respiration meter, a sample is aerated, the aeration is stopped and the decline in the DO concentration is recorded (Duggan and Cleasby, 1976; Kaneko et al., 1985). The respiration rate is then calculated from the slope of the DO with time. The discontinuous respiration meter is incapable of measuring the actual respiration rate under normal operating conditions. The continuous flow-through meter measures the difference of the DO at the inflow and at the outflow of either the air (Heckershoff and Wiesmann, 1986) or the activated sludge (Sollfrank and Gujer, 1990; Spanjers and Klapwijk, 1990). Some continuous flow-through meters (Reynolds, 1969; Clarke *et al.*, 1978; Chen *et al.*, 1980), that just sample sludge from the aeration tank, do not directly measure the actual respiration rate in the aeration tank because, due to the detention time in the chamber, the concentration of readily biodegradable matter will have decreased. Hence, the respiration rate in the chamber will be lower than the actual rate in the aeration tank.

For the measurement of the actual respiration rate with a continuous flow-through meter it is crucial that the respiration chamber is loaded with wastewater in the same proportion as the aeration tank (Spanjers and Klapwijk, 1987, 1990; Sollfrank and Gujer, 1990). Then the concentration of readily biodegradable matter in the chamber equals that of the aeration tank. Therefore, simultaneously with the sludge, a sample of influent is introduced continuously into the respiration chamber such that the ratio of the sample flow and the chamber volume equals the ratio of the influent flow and the aeration tank volume. Hence, during operation, the influent sample flow has to be adjusted in relation

to the influent flow to the plant. A problem associated with this measurement technique is that the measurement of the actual respiration rate in subsequent compartments of a series of well-mixed tanks is not obvious.

In this chapter, we propose and test an alternative method for the determination of the BOD_{st} and the actual respiration rate in a completely mixed aeration tank, no matter whether this tank is a single reactor or a compartment of a plug-flow reactor. Furthermore, the method avoids the addition of influent to the respiration chamber for the measurement of the actual respiration rate. The method which is analogous to a method for the determination of the influent BOD_{st} (Spanjers *et al.*, 1993), uses the transients between two modes of respiration measurement. To verify the r_{act}, determined with the proposed method, (estimated r_{act}), we also measure this variable (measured r_{act}) according to the previously described method (Spanjers and Klapwijk, 1990).

It is concluded that the proposed method allows the estimation of the BOD_{st} and the actual respiration rate. However a significant bias exists between the measured and estimated actual respiration rate.

3.3 Measurement of the respiration rate

The principle of the respiration measurement (Spanjers and Klapwijk, 1990; Spanjers and Olsson, 1992) will be reviewed briefly here. The respiration meter consists of a closed, completely mixed chamber of 0.75 litre through which the activated sludge is continuously pumped. The DO concentrations at the inlet (c_a) and at the outlet (c) of the chamber are measured by using one and the same probe. This is made possible by four valves that switch the flow through the chamber, typically every 30 seconds (the probe response period).

Figure 1 shows a typical recording from the respirometer DO probe, the signal oscillating between c_a and c. The DO concentrations c_a and c are calculated from each response (Spanjers and Olsson, 1992), resulting in two series of DO concentrations, one of the inlet and one of the outlet, with sampling interval h. These series are shifted ½h time units with respect to each other.

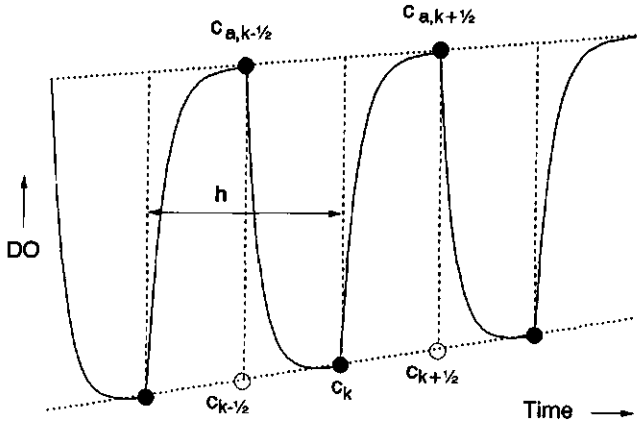

***Figure 1**: Recording from the respirometer DO probe. The respiration rate is calculated from the DO measurements. Dotted lines: real DO concentration at the inlet and at the outlet, c_a and c respectively. Solid line: measured DO concentration. Filled rounds: known DO concentrations, necessary for the calculation of the respiration rate at time instant t_k. Open rounds: interpolated DO values. Probe response period ½h, typically 30 s. Calculation interval, h, for respiration rate, typically 60 s.*

From the DO concentrations, the respiration rate can be calculated using a numerical approximation of the DO mass balance over the respiration chamber. In previous work an analytical method was used to calculate the respiration rate (Spanjers and Klapwijk, 1990). Here we apply numerical off-line calculation and use the trapezoidal rule.

$$\frac{dc}{dt} = \alpha(c_a - c) - r \tag{1}$$

$$r_k = \frac{1}{2}\left[\alpha\left(c_{a,k+\frac{1}{2}} + c_{a,k-\frac{1}{2}}\right) - \left(\alpha+\frac{2}{h}\right)c_{k+\frac{1}{2}} - \left(\alpha-\frac{2}{h}\right)c_{k-\frac{1}{2}}\right] \tag{2}$$

The respiration rate is calculated at each sampling time of c_k, hence with a period of h. From Figure 1 it can be seen that $c_{k-½}$ and $c_{k+½}$ can be found by linear interpolation from two adjacent c values.

3.4 Two modes of respiration measurement

In this work, two sludges of the same origin (the aeration tank) but with different respiration rates are measured consecutively such that we obtain two modes of operation (Figure 2). In the *endogenous* mode (e-mode), sludge having the endogenous respiration rate is pumped through the respiration chamber. This sludge is produced in a bypass tank. In the *instantaneous* mode of measurement (i-mode), sludge from the aeration tank directly flows through the respiration chamber. The measuring period for one mode is typically 10 or 15 minutes and is sufficiently long to let the respiration rate in the chamber converge to an ultimate value. Figure 3 shows an example of a part of an experimental run.

At the end of each i-mode, the measurement is changed to the e-mode and the sludge in the respiration chamber is gradually replaced by endogenous sludge. In the endogenous sludge the concentration of readily biodegradable matter is considered negligible, meaning that the BOD_{st} of the endogenous sludge is zero. Hence, in the e-mode, the BOD_{st} in the respiration chamber remaining from the i-mode will be washed out and the respiration rate will decrease. Thus, at the end of each e-mode the BOD_{st} in the respiration chamber is zero and thus the rate measured is the endogenous respiration rate.

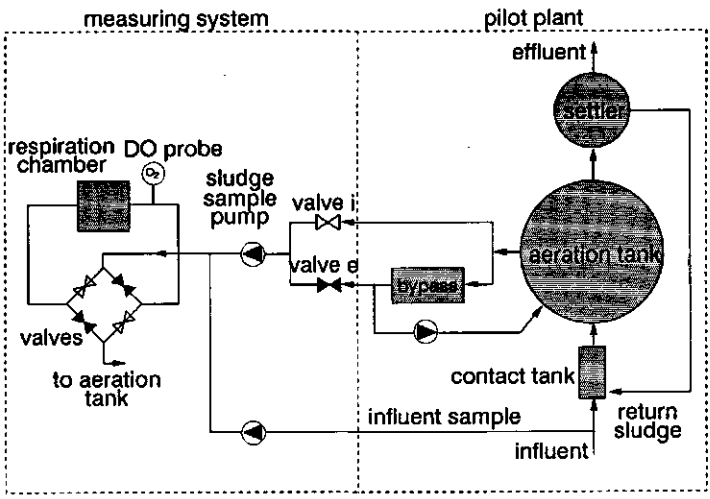

Figure 2: *Schematic of the pilot plant and the respiration measuring system. Valve i is open in the i-mode, valve e in the e-mode.*

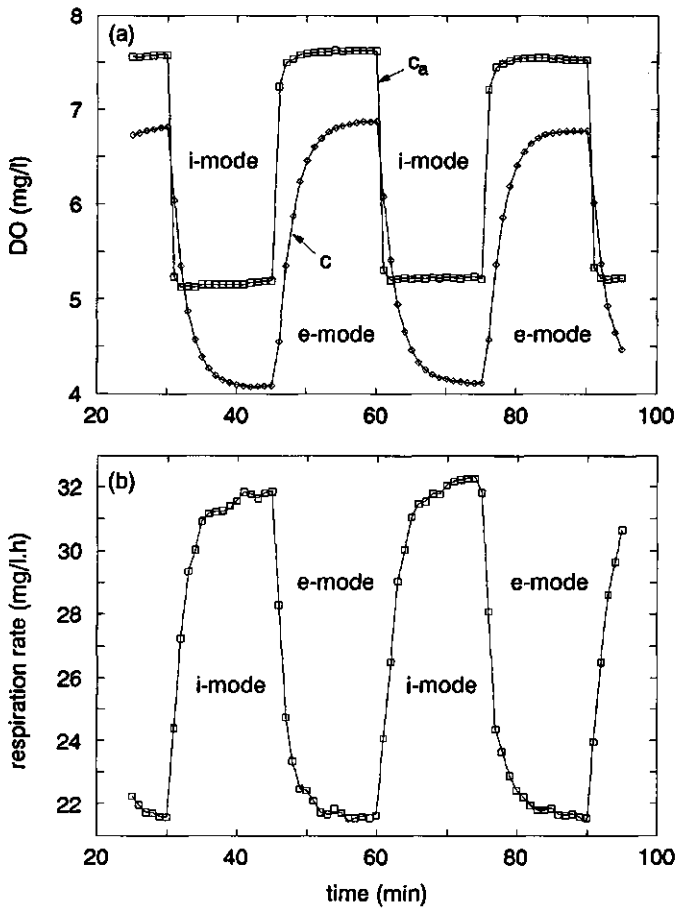

***Figure 3:** Example of the measurement in the two consecutive modes of operation: e-mode and i-mode. (a) Measured DO concentration from the respiration meter probe. Each point corresponds to a point in Figure 1. (b) Respiration rate calculated using equation (2).*

At the end of each e-mode, the measurement is changed to the i-mode and the sludge in the chamber is gradually replaced by sludge that comes directly from the aeration tank. This sludge contains a certain amount of readily biodegradable matter, in other words: this sludge has a finite BOD_{st}. This BOD_{st} (s_a) is the driving force behind the increase of

the BOD_{st} in the chamber. At the end of the i-mode, a steady state is reached with respect to the BOD_{st} and the respiration rate in the chamber. The resulting BOD_{st} is lower than s_a and is a function of the hydraulic retention time in the chamber and of the biological activity. Since the respiration rate is a function of the BOD_{st}, the rate measured at the end of an i-mode is lower than the actual respiration rate in the aeration tank.

In the next section we will show how the respiration rate measurement in the transients during the two modes can be used for the estimation of the BOD_{st} and the actual respiration rate in the aeration tank.

3.5 Estimation of the BOD_{st} and the actual respiration rate

The BOD_{st} in the aeration tank (s_a) and actual respiration rate (r_{act}) are calculated from cycles of two successive modes: one i-mode and one e-mode, meaning that the calculation interval of these variables is typically 20 - 30 minutes. The estimation procedure involves five steps executed for each cycle:

(1) assessment of the endogenous respiration rate (r_{end});
(2) calculation of the substrate respiration rate (r_s);
(3) calculation of the initial BOD_{st} in the e-mode (s_0);
(4) calculation of the BOD_{st} in the aeration tank (s_a);
(5) calculation of the actual respiration rate in the aeration tank (r_{act}).

We will now explain the estimation procedure according to these steps. Figure 4 schematically outlines the calculation procedure.

(1) Endogenous respiration rate

It is assumed that, at the end of each e-mode, the concentration of readily biodegradable matter is zero, so that the endogenous respiration rate (r_{end}) is measured. It is further assumed that r_{end} is constant during the whole cycle. To obtain an operational r_{end} for the whole cycle, the last measured rates of the current and previous cycle are averaged (Figure 4a).

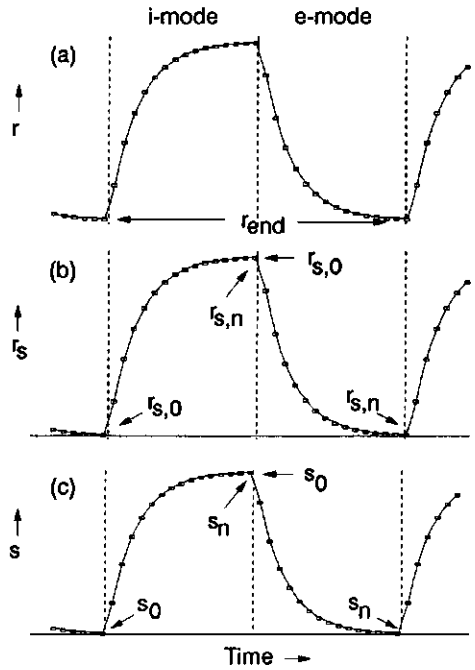

Figure 4: *Schematic representation of calculations within a cycle of one i-mode and one e-mode. (a) respiration rate measured (step 1); (b) substrate respiration rate (step 2); (c) BOD_{st} (step 3 and 4).*

(2) Substrate respiration rate

The rate of the oxidation of readily biodegradable matter, the substrate respiration rate (r_s), can be calculated from each respiration measurement (r). This is done for both the i-mode and the e-mode (Figure 4b).

$$r_{s,k} = r_k - r_{end} \tag{3}$$

where k = 1, 2, ... n (n typically 10 or 15).

For both modes, the concentration readily biodegradable matter (s), expressed by BOD_{st}, can now be derived from r_s using the mass balance of s over the respiration chamber (steps 3 and 4).

(3) Initial BOD_{st} in the e-mode

In the e-mode, the mass balance of s over the respiration chamber is:

$$\frac{ds}{dt} = -\alpha s - r_s \tag{4}$$

where: α = dilution rate ($time^{-1}$)

The magnitude of s is known at the end of the e-mode. The mass balance (4) is integrated backwards to find s at the beginning of the e-mode. This value is equal to s at the end of the i-mode. Approximating the derivative with the trapezoidal rule we get for the e-mode:

$$\frac{s_k - s_{k-1}}{h} = \frac{1}{2}\left[-\alpha s_k - \alpha s_{k-1} - r_{s,k} - r_{s,k-1}\right] \tag{5}$$

or:

$$s_{k-1} = \frac{1}{\frac{1}{2}\alpha h - 1}\left[-\left(\frac{1}{2}\alpha h + 1\right)s_k - \frac{1}{2}h\left(r_{s,k} + r_{s,k-1}\right)\right] \tag{6}$$

Using equation (6) and the boundary conditions $s_n=0$ (and consequently $r_{s,n}=0$) and $r_{s,0}$ (e-mode) = $r_{s,n}$ (i-mode), the BOD_{st} at the beginning of the e-mode (s_0) can be found. This is used in the i-mode to find s_a (step 4).

(4) BOD_{st} in the aeration tank

In the i-mode, the mass balance of s over the respiration chamber is:

$$\frac{ds}{dt} = \alpha (s_a - s) - r_s \tag{7}$$

The initial condition of the i-mode is already known: s=0. The end condition of the i-mode was calculated in step 3, and is identical with the initial condition of the e-mode. Using these conditions we can calculate the unknown s_a. Approximating the derivative of (7) with the trapezoidal rule we get for the i-mode:

$$\frac{s_k - s_{k-1}}{h} = \frac{1}{2}\left[2\alpha s_a - \alpha s_k - \alpha s_{k-1} - r_{s,k} - r_{s,k-1}\right] \tag{8}$$

or:

$$s_k = \frac{1}{\frac{1}{2}\alpha h + 1}\left[-\left(\frac{1}{2}\alpha h - 1\right)s_{k-1} - \frac{1}{2}h\left(r_{s,k} + r_{s,k-1}\right) + \alpha h s_a\right] \tag{9}$$

Using equation (9) and the initial condition $s_0=0$ (and consequently $r_{s,0}=0$) the value s_n at the end of the i-phase can be calculated. The only unknown, s_a, can be found explicitly from equation (9) so that s_n (i-mode) = s_0 (e-mode).

(5) Actual respiration rate in the aeration tank

Once the BOD_{st} in the aeration tank (s_a) is known, the actual respiration rate in the aeration tank (r_{act}) can be calculated by using a kinetic model that describes the relation between r_s and s. In this work two models are tested, first-order model (10) and half-order model (11):

$$r_s = b_1 s \tag{10}$$

$$r_s = b_{0.5} s^{0.5} \tag{11}$$

where b_1 and $b_{0.5}$ are kinetic constants. The first-order model is a reasonable approximation of Monod kinetics for small substrate concentrations. This is a common case in activated sludge systems. The half-order model has a theoretical basis in biofilm kinetics (Harremoës, 1977). The kinetic constants are found by fitting equations (10) and (11) to the $[r_{s,k}, s_k]$ values from both modes. The actual respiration rate, r_{act}, can be calculated from:

$$r_{act} = r_{end} + b_1 s_a \tag{12}$$

$$r_{act} = r_{end} + b_{0.5} s_a^{0.5} \tag{13}$$

The procedure, to estimate s_a and r_{act} was verified using both simulated data and experimental data from a pilot plant.

3.6 Materials and methods

In simulations, executed with SIMNON (Elmqvist *et al.*, 1986), measuring data were generated using a simple mathematical model of the pilot plant and the measuring system (Figure 2). This model simulates the respiration rate in the respiration chamber during the alternating modes (i-mode and e-mode) of operation. Noise, having uniform distribution with standard deviation 0.5 mg $l^{-1}h^{-1}$, was added to the simulated respiration rate. First-order kinetics for BOD_{st} was employed. Linear regression was used to find the estimated first order kinetic constant. The values of the parameters and variables (Table 1) were in accordance with the experimental conditions in the pilot plant. The data generated with the simulation model were saved in a file to be processed according to the procedure proposed (equations (3)-(13)) in order to calculate s_a and r_{act}. These were compared with the original values.

In the experiments, the measurements were carried out at a pilot plant equipped with a respiration measurement system (Figure 2). This system was capable of measuring consecutively the respiration rate of sludge in the i-mode and in the e-mode.

The pilot plant, fed with presettled domestic sewage, was composed of an unaerated contact tank (four 2 litre compartments in series), a single compartment completely mixed aeration tank and a settling tank. The activated sludge was found to fully nitrify the ammonium in the influent. The air flow rate was maintained constant using a flow controller. Sludge with the endogenous respiration rate was produced in a bypass tank, consisting of five 5 litre aerated compartments in series, through which activated sludge from the aeration tank was continuously circulated. In previous research it was verified that, under normal conditions, the sludge in this tank was free of readily biodegradable matter.

Table 1: Simulation and experimental conditions. Irrelevant condition indicated with "-".

	simulation	experiment	
		run 1	run 2
sampling interval c_a, c and r(s)	60	60	60
duration one mode (min)	15	10	15
volume aeration tank (l)	475	475	475
influent flow (l h^{-1})	40	36-40	36-40
return sludge flow (l h^{-1})	-	39-40	39-40
waste sludge flow from aerat. tank (l h^{-1})	-	1.43	1.43
air flow (m^3h^{-1})	-	2.4	2.4
recirculation flow bypass tank (l h^{-1})	-	22	22
volume respiration chamber (l)	0.731	0.731	0.731
flow through respiration chamber (l h^{-1})	21	21.45	21.70
influent sample flow (l h^{-1})	0.0616	0.0615-0.0616	0.0615-0.0616
influent BOD_{st} (mg l^{-1})	180 $0<time<4$ h	200-300	200-300
	300 $4\leq time<8$ h		
MLSS (mg l^{-1})	-	2.8	2.4
MLVSS (mg l^{-1})	-	2.20	1.89
sludge volume index (ml l^{-1})	-	320	355
temperature sludge (°C)	-	16.5-17.5	16.5-17.5
pH	-	7.2-7.3	7.2-7.3
endogenous respiration rate (mg $l^{-1}h^{-1}$)	20	19-23	19-23
endogenous sludge BOD_{st} (mg l^{-1})	0	assumed 0	assumed 0
kinetics	1^{st}-order 10 h^{-1}	unknown	unknown

The measuring system consisted of a respiration meter (prototype of RA1000, Manotherm) having a respiration chamber, an influent sample pump and two valves. To operate alternately in the i-mode and in the e-mode, two valves were used to select sludge from the aeration tank and the bypass respectively. To verify the estimated r_{act} (see Introduction), this variable was also measured, at regular intervals, according to the method described by Spanjers and Klapwijk (1990). Therefore, in the i-mode, a predetermined sample of influent was continuously added to the sludge flowing through the respiration chamber. Table 1 lists the average process conditions during the measuring period.

The control of valves and pumps, collection of data and the basic calculations were performed by a modular I/O processor (μMac 6000, Analog Devices).

During the course of the measurements sludge samples were taken between 9:00 and 10:00 a.m. for the analysis of MLSS, MLVSS and sludge volume index (dutch standard NEN).

3.7 Estimation of s_a and r_{act} from simulated data

The procedure to estimate s_a and r_{act} was tested using simulated respiration measurements. The mode measuring period was 15 minutes and the sampling interval (h) was 1 minute. At t=4 hours there was a step increase of the influent BOD_{st} from 180 to 300 mg l^{-1}. Figure 5 shows the estimated values along with the originally simulated values.

Figure 5 shows that the estimation procedure slightly overestimated s_a and underestimated b_1 and r_{act}. These biases apparently emanated from the differential approximation of the mass balances and from the boundary assumptions (see eqs. (6) and (9)).

Because the estimation procedure relies on r_s, the sensitivity of s_a, r_{act} and b_1 to this variable was examined. It was found that especially the calculated initial BOD_{st} in the e-mode, s_0, is quite sensitive to r_s. This is shown in Figure 6a where the simulated and calculated s(t) in the e-mode are plotted. In this simulation, r_s was given a noise with standard deviation of 0.5 mg $l^{-1}h^{-1}$, a reasonably practical measurement error. The calculated values of s were higher than the originally simulated s, and s_0 was overestimated with 12%. In addition, the standard deviation of s_0 was unacceptable for practical situations because it was greater than the estimated s_0 itself. The high variance of s_0 results from the application of equation (6) to the very small or, because of the noise, even negative values of r_s at the tail end of the e-mode. To avoid the build up of noise from the tail end of the e-mode, the calculation of s_0 was repeated with another starting point for equation (6) (Figure 6b).

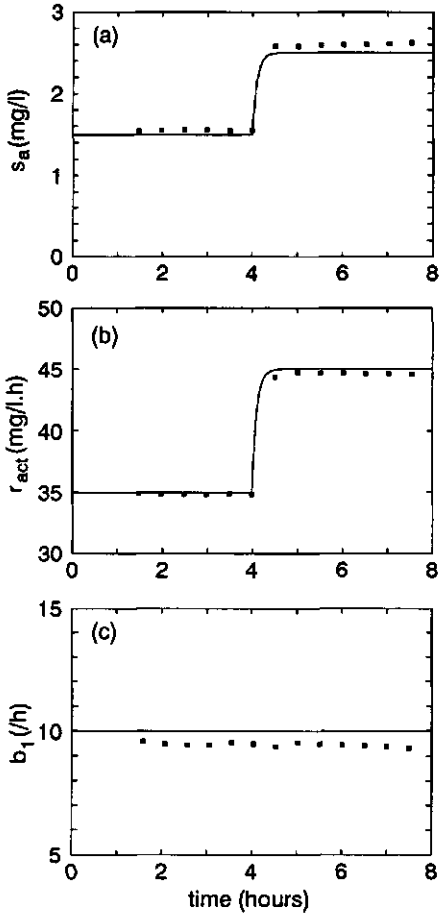

***Figure 5**: Verification, using simulations, of the procedure to estimate s_a and r_{act}. At t=4 hours there is a stepwise increase of the influent BOD_{st}. Original values (lines) and estimated values (points) of s_a, r_{act} and b_1. Conditions: see Table 1.*

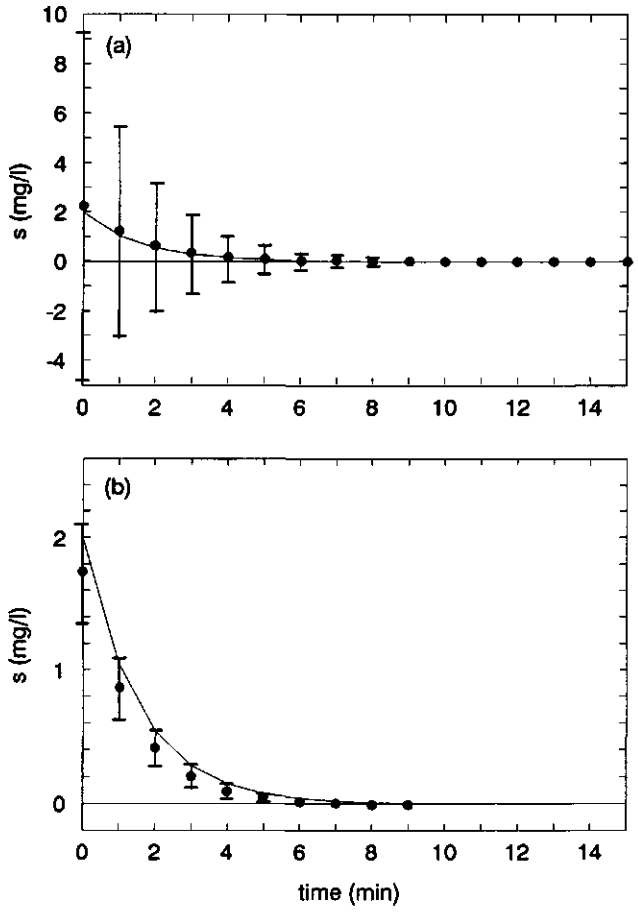

Figure 6: Simulated (lines) and calculated (markers) values in the e-mode (compare Figure 3) for different starting points of calculation. Standard deviation simulated r_s: 0.5 mg $l^{-1}h^{-1}$. The standard deviation for the calculated values is indicated in the figure. Direction of calculation: from the right to the left. (a) Calculation started at the last point (at 15 minutes); (b) Calculation started at 11 minutes. (note the different s-scale).

Figure 6b shows that the calculation, starting with the end condition of the e-mode defined at an earlier point in time, keeps the bias of s_0 on 12% and reduced the variance

though the latter was still high. The effect of the choice of the starting point on the estimates of s_a, b_1 and r_{act} was studied for the simulation of r_s during the two modes (Figure 7).

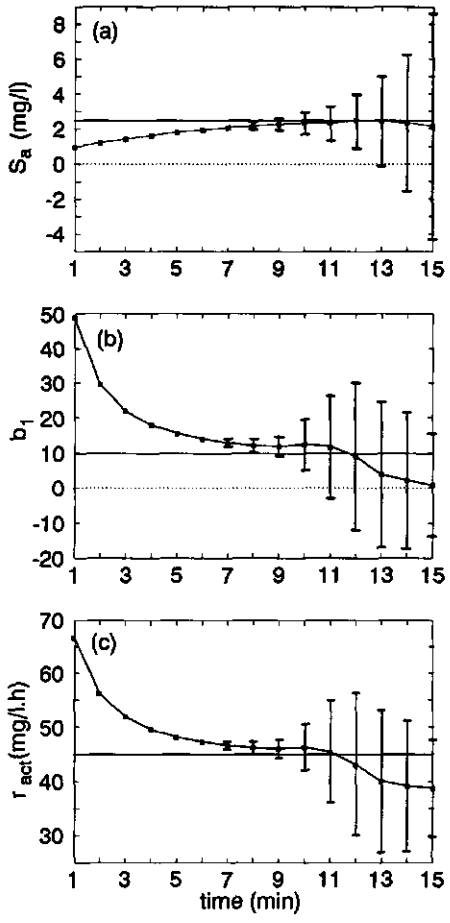

***Figure 7**: Effect of the choice of the starting point of the backward calculation in the e-mode, for the calculation using equation (6), on (a) s_a, (b) b_1 and (c) r_{act}. The starting point in the calculation is displayed on the x-axis (15 minutes means that the calculation is started at the end of the e-mode, meaning that all the points are involved). Original value (line), calculated values (markers) and standard deviation of the calculated values is indicated.*

Figure 7 demonstrates that, for the simulation, the best starting point (low variance and low bias) was found to be at 8 or 9 minutes. The figure also shows that the estimated values were more or less constant on a certain range of starting points. Finally, while s_a had a standard deviation of 260% in the worst case (when the calculation was started at the ultimate point, Figure 7a), the variability of r_{act} was no more than 27%. When the calculation was started at 8 minutes, s_a and r_{act} deviated respectively -11% and 2.5% from the originally simulated value with standard deviations of respectively 10% and 2.3%.

It must be stressed that the precise shape of the graphs in Figure 7 depends on process conditions such as the magnitude of s_a, the kinetic characteristics of the biological system and the hydraulic properties of the measuring system. For real measurements, the operational starting point has to be found experimentally. In this work the starting point was defined as, counting from the last measurement of the e-mode, the first point where r_s exceeds the standard error of measurement (0.5 mg $l^{-1}h^{-1}$). In the above simulation this occurred at 8 minutes (r_s not shown in the figures).

From the simulations it was concluded that the proposed method allowed the estimation of s_a and r_{act}, although the estimated variables were quite sensitive to the respiration rate measured in the e-mode. However, when the backward calculation of s_0 in the e-mode was started at a point earlier in the response, instead of at the last point, the variance and bias of the estimated variables could be reduced to a satisfactory level. In the next section, the ability of the method to estimate these variables will be investigated experimentally.

3.8 Estimation of s_a and r_{act} from experimental data

At the pilot plant, during two experimental runs of 48 and 72 hours, the respiration rate was measured according to the strategy proposed in this chapter. The measuring period for one mode in these runs was 15 and 10 minutes respectively. Figure 8 shows a detail of the pattern of the respiration rate in the first measuring run (cf. Figure 3). In the figure it can be seen that the respiration rate was measured in consecutive modes of operation of the measuring system. From each cycle, of one i-mode and one e-mode, s_a and r_{act} were estimated according to the calculation procedure proposed. To verify the estimated r_{act}, this variable was also measured, according to the previously described method, at regular intervals of five cycles.

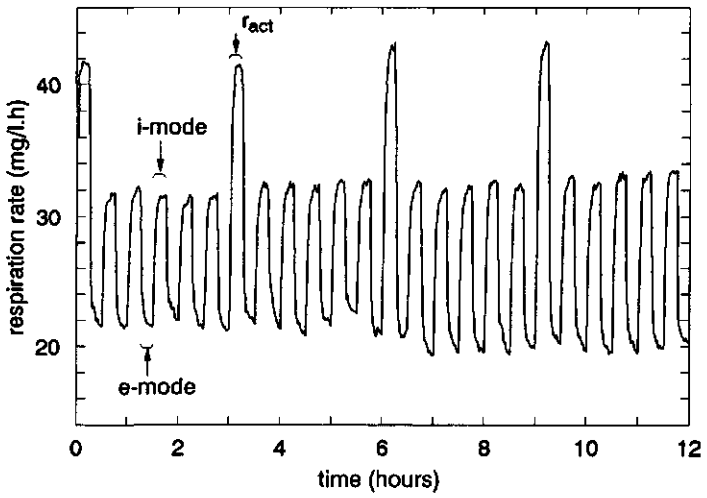

***Figure 8:** Part of the respiration rate measurement of the first run. After five cycles of one i-mode and one e-mode, one actual respiration rate is measured according to the previously described method (Spanjers and Klapwijk, 1990; see also Introduction).*

The estimated s_a is plotted in Figure 9a. The starting point for the initial condition for the backward calculation in the e-mode for run 1 and 2 was at 5 and 8 minutes, respectively. s_a showed a significant variation corresponding to a variation of the DO concentration in the aeration tank (Figure 9b). At the end of run 1 and the beginning of run 2, the influent to the aeration tank was obstructed due to troubles with the wastewater supply. s_a immediately dropped to a low value and the DO increased, likely because of the low oxygen demand in the aeration tank. During the interval 12 - 24 hours of run 2, no measurements were available because of a technical failure of the measuring system. During both runs s_a clearly showed a diurnal peak around 12:00 h, corresponding with a dip in the DO concentration, because of a higher influent BOD_{st} concentration.

The substrate concentrations (s) and the corresponding substrate respiration rates (r_s) of each cycle (not shown) were used to fit the kinetic models (9) and (10). On the whole, there was no significant difference in the goodness of fit for the two models. In examining the data points of a representative cycle (Figure 10) we found a hysteresis as there was a difference in sludge activities during the two modes. Therefore we decided to use, as a compromise, the first order model for the rest of this chapter.

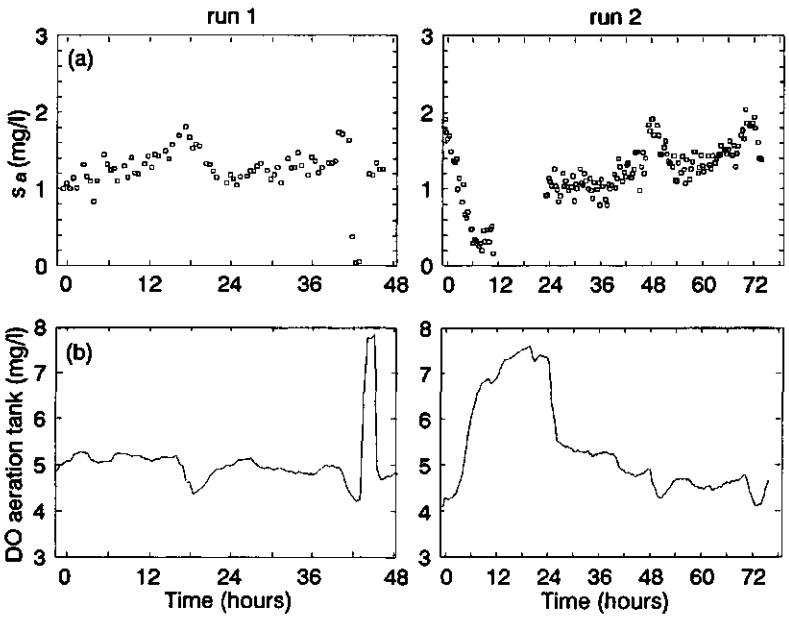

***Figure 9**: Result of two measuring runs. (a) Estimated s_a and (b) measured DO in the aeration tank.*

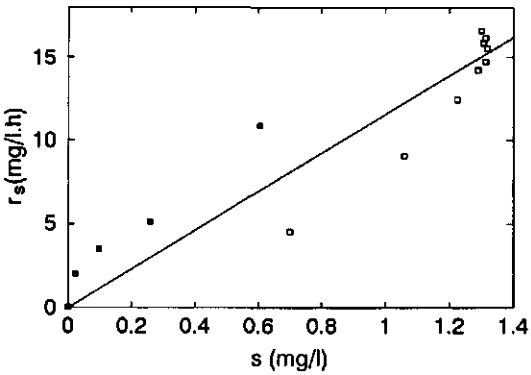

***Figure 10**: Kinetic relationship from a representative cycle of one i-mode and one e-mode (run 2 at 12:00 h). Filled markers indicate points of the e-mode. The first order regression line is also shown (b_1 = 11.4 h^{-1}).*

The estimated b_1 during both runs is given in Figure 11. Taking into account the variance assessed in the simulation, it was found that b_1 remained relatively constant. The peaks which showed up in s_a, did not have a counterpart in b_1. It was expected that the kinetic properties of the sludge did not change substantially because of a relatively constant operation (except for the beginning of run 2) with respect to the influent flow and the MLSS of the pilot plant. Nevertheless, at 9 hours (run 2) there was a decrease of b_1 indicating a lower activity, possibly caused by the absence of influent during 6 hours and a decrease of the MLSS from 2.4 to 2.3 g l^{-1}.

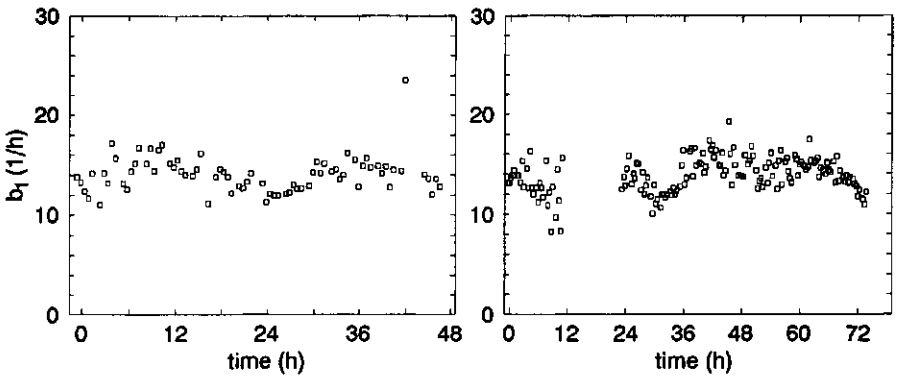

***Figure 11:** Estimated first order kinetic constant.*

The estimated r_{act} along with the measured r_{act} and r_{end} are shown in Figure 12. There was a good agreement between the patterns of the estimated and measured r_{act}. However, the estimated values were systematically lower than the measured ones. The simulation showed that, under the assumed conditions and with optimal choice of the starting point, r_{act} was slightly underestimated with reasonable variance. This cannot explain the observed bias in the experiments. An explanation for the lower values for the estimated r_{act} may be that r_{end} does not represent the respiration rate in full absence of readily biodegradable matter. r_s would then have been underestimated and so may r_{act}. However, r_{end} remained fairly constant during both experimental runs and was not significantly affected by the observed variation of s_a (Figure 12). This supports the presumption that the concentration of readily biodegradable matter in the endogenous sludge was approximately zero.

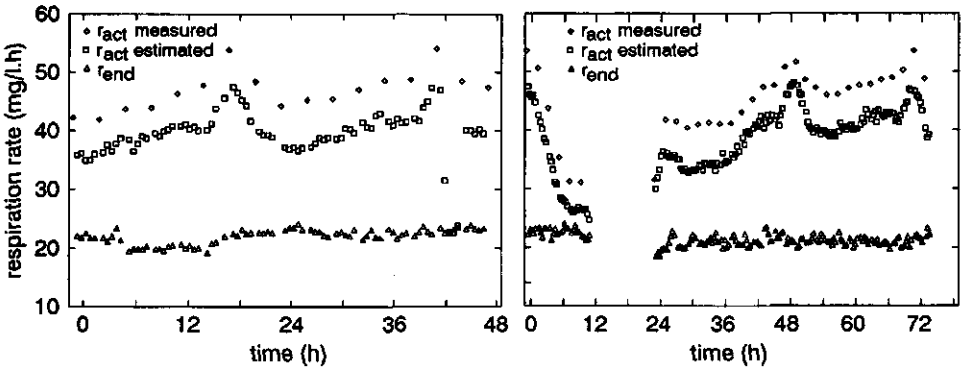

***Figure 12**: Estimated r_{act} compared with measured r_{act}. Measured r_{end} is also shown.*

A second explanation may be that the measured r_{act} turns out too high. This would occur when the ratio of the influent sample flow into the respiration chamber and the flow into the aeration tank is greater than the ratio of the corresponding volumes. In the operation of the pilot plant the aim was to maintain the influent flow at 40 l h^{-1}. Therefore, the influent sample flow was fixed at $40 \cdot 0.731/475 = 0.0616$ l h^{-1}. This flow was daily verified and found to vary within an acceptable range (Table 1). However the influent flow varied between 36 and 40 l h^{-1} due to clogging in the influent line. From the mass balances of s for the aeration tank and the measuring system, it can be estimated that an influent flow of 36 l h^{-1} in combination with the applied influent sample flow would cause an overrating of the measured $r_{s,act}$ ($=r_{act}-r_{end}$) of about 5%.

Another circumstance that may have contributed to an overrating of the measured $r_{s,act}$ is the adoption of too small an aeration tank volume in the calculation of the wastewater sample flow to be employed. For instance, when the oxidation of readily biodegradable matter also occurs in the contact tank and in the settling tank, (a part of) the volumes of these tanks should also be included in the aeration tank volume (see Figure 2). From the mass balances of s for the aeration tank and the measuring system, it can be estimated that a true reactor volume of 642 litre in combination with the applied influent sample flow would cause an overestimate of the measured $r_{s,act}$ of about 11%. Hence, in the worst case the total overrating of the measured $r_{s,act}$ would be about 16%. The correct $r_{s,act}$ would then be about 0.16/1.16 or 14% lower than the measured value. This error of the measured $r_{s,act}$ can explain the difference between the estimated and measured r_{act} (or $r_{s,act}$) for most of the points in Figure 12.

From the measurements it was concluded that the estimated s_a and r_{act} showed the typical diurnal variation usually found in the COD of the wastewater studied. A break in the influent supply was clearly reflected in a decrease of the two variables. The estimated r_{act} was significantly lower than the measured reference value. Accounting for the maximum (worst case) overrating of this measured r_{act}, the estimated and measured values matched approximately.

3.9 Discussion

The purpose of this work was to develop a method for the measurement of the mixed liquor BOD_{st} (s_a) and the actual respiration rate (r_{act}) in a completely mixed aeration tank no matter whether this is a single reactor or a compartment of a series of well-mixed tanks. Contrary to another method developed earlier, this method does not involve continuous addition of influent sample to the respiration chamber. Instead, the transient respiration rate is measured during two modes of operation which are alternately executed. In the i-mode sludge from the aeration tank flows through the respiration chamber, whereas in the e-mode sludge having the endogenous rate is directed through the chamber. s_a is obtained by integrating the mass balance equations holding for each cycle of both modes. In order to be able to do this, it is necessary to assume that the BOD_{st} in the respiration chamber is zero at the end of the e-mode. Finally, the measured respiration rate and the calculated BOD_{st} during each cycle of two modes are used to find the kinetic relationship in order to calculate r_{act}.

From the simulation it was found that the calculated s in the e-mode was quite sensitive to the measured respiration rate, which led to considerable errors in the estimated s_a and r_{act}. This problem was solved for the greater part by starting the numerical calculation procedure in the e-mode at a point earlier than the end point of this mode. This point was chosen where r_s exceeded the standard error of measurement for the respiration rate. For the experiments, this point was determined and fixed as an average optimum after examining the data. Another approach would be to find the best starting point for each individual e-mode. Instead of using the standard deviation of the respiration rate, the variables could also be found where they show minimum sensitivity to the choice of the starting point (Figure 7). Finally, an alternative numerical approximation could be tried to reduce the sensitivity of s_a and r_{act}.

In the procedure applied, the respiration rate was first calculated from the DO measurements (equation (2)). Next, the substrate respiration rate, r_s, was calculated using

the endogenous respiration rate r_{end}. Then, r_s was used to solve the mass balances for s. An alternative procedure is to eliminate r_s using equations (1), (3) and (4) or (7). This will allow to calculate s_a directly employing the DO measurements carried out with the respiration meter. Applied to the simulated data as well as the measured data, this alternative procedure did not yield significant differences in the estimates compared to the procedure presented here. Nevertheless, for the calibration of the kinetic model, used to calculate r_{act}, r_s is needed after all.

In the evaluation of the measuring data to assess the kinetic order of the process, a hysteresis was observed in the kinetic relationship between r_s and s, which complicated the choice of a kinetic model. A decline of the s-curve at the end of the i-mode (not shown) caused a scattering of the r_s values at high s values (Figure 10). Therefore, the data were modelled with, as a compromise, a simple first order relationship. Since, for kinetic modelling, it is assumed that diffusion of substrate through the sludge flocs is not limited, it may be useful to examine if this assumption is violated by the dynamic operation of the measuring system. One possibility would be to investigate the effect of different hydraulic retention times on the relationship between r_s and s.

The measurements showed a good agreement between the pattern of the estimated r_{act} and the one obtained from the previously described method (the measured r_{act}). However the estimated values were significantly lower than the measured ones. A probable explanation is that the measured r_{act} turned out too high because of too high an influent sample flow based on wrong assumptions on influent flow and reactor volume. One verification would be to use an influent with constant composition and known BOD_{st} so that the plant can be operated at steady state. This would allow to calculate s_a from the mass balance for the aeration tank. Special attention should then be paid to the possible elimination of BOD_{st} in the settling tank. Next, the estimated r_{act} could be verified using information from separate kinetic experiments under plant condition.

For real time control, on-line monitoring of process variables is essential. In this chapter, the estimation the BOD_{st} and r_{act} in the aeration tank was executed off-line. However the method is suitable for on-line implementation.

3.10 Conclusions

The method proposed allows the estimation of the mixed liquor BOD_{st} (s_a) and the actual respiration rate in the aeration tank (r_{act}), provided that too large a build up of measuring

errors is avoided during the integration. This is achieved by choosing the starting point in the numerical integration at a point where the measured substrate respiration rate has not reached zero. The estimated variables from experimental data are in agreement with the diurnal variation typical for the wastewater used and r_{act} is in agreement with r_{act} obtained by the previously described method. However a significant bias exists between the two results. This is possibly caused by an overrating of the traditionally measured r_{act}. Compared to the previously described method for the measurement of s_a and r_{act}, the proposed method is simpler in practice because the addition of influent sample to the respiration chamber is not necessary to assess r_{act} and only two instead of three modes of respiration measurement are needed to find s_a. Another advantage of the proposed method, in contrast with the other method, is that estimation of s_a and r_{act} is still possible, even if the influent supply would be interrupted. The method can function until s_a becomes too small.

3.11 Notation

BOD_{st}	short-term biochemical oxygen demand
b_1	first-order kinetic constant (time^{-1})
$b_{0.5}$	half-order kinetic constant (mass^{½} volume$^{-½}$ time^{-1})
c_a	DO concentration at the inlet of the respiration chamber (mass volume^{-1})
c	DO concentration at the outlet of the respiration chamber (mass volume^{-1})
DO	dissolved oxygen
h	sampling interval for c, c_a and r (time)
ML(V)SS	mixed liquor (volatile) suspended solids
q	activated sludge flow through respiration chamber (volume time^{-1})
r	respiration rate in the respiration chamber (mass volume^{-1} time^{-1})
r_s	$r-r_{end}$ = substrate respiration rate in the respiration chamber (mass volume^{-1} time^{-1})
r_{act}	actual respiration rate (mass volume^{-1} time^{-1})
r_{end}	endogenous respiration rate (mass volume^{-1} time^{-1})
s	BOD_{st} in the respiration chamber (mass volume^{-1})
s_a	BOD_{st} in the aeration tank (mass volume^{-1})
s_0	initial BOD_{st} in the e-mode in the respiration chamber (mass volume^{-1})
v	volume respiration chamber (volume)
α	q/v = dilution rate (time^{-1})

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4

Determining influent short-term biochemical oxygen demand by combining respirometry and estimation

4.1 Abstract

This chapter describes a method for the continuous estimation of the influent short-term biochemical oxygen demand (BOD_{st}) of a wastewater treatment plant. The method uses the dissolved oxygen (DO) concentration in the inlet and outlet of a closed, completely mixed respiration chamber during the transients between two modes of respiration measurement which are alternately executed. In one mode, sludge having the endogenous respiration rate is directed through the respiration chamber. In the other mode, sludge from the aeration tank directly flows through the respiration chamber and, in addition, a sample of influent is continuously introduced in the chamber. The influent BOD_{st} is estimated for each cycle of both modes by integrating the mass balances of BOD_{st} and DO over the respiration chamber. Knowledge of the degradation kinetics of readily biodegradable matter is not required. The proposed procedure is verified using both simulated and experimental data from a pilot plant. It is concluded that the procedure enables the estimation of the influent BOD_{st} with a relative error ranging from 2 - 6%.

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4.2 Introduction

Because activated sludge plants receive wastewater with varying flow and waste concentration, the process is seldom in steady-state. In order to operate the activated sludge process (have a sufficient degree of waste removal and production of sludge with good settling properties) control is needed. By measuring the influent waste concentration, useful information for control is obtained. Biochemical Oxygen Demand (BOD) is the most widely used measure of the waste concentration, because it is related to the biochemical process of the activated sludge itself.

Unfortunately, the BOD is unsuitable for control purposes, as it is mostly expressed in BOD_5, which requires a five days analysis. This time span is too long for real-time control purposes. To overcome this problem other measures like chemical oxygen demand (COD) and total organic carbon (TOC) are used, however these do not predict how the activated sludge would respond to changes in the waste concentration. Another possibility is to seek a correlation between the BOD_5 and variables, like influent flow, that can be measured continuously.

Considerable efforts have been spent to develop methods that enable continuous or batchwise measurement of the BOD for monitoring purposes. All these methods are based on a respirometric principle, using a low substrate to biomass ratio, and they yield a short-term BOD (BOD_{st}) that is not equivalent with the BOD_5. Several authors correlated the BOD_{st} with the conventional BOD_5, with more or less success (Vernimmen *et al.*, 1967; Riegler, 1984; Strand and Carlson, 1984; Vasel *et al.*, 1991). However, the BOD_{st} is, in contrast to the BOD_5, a more valuable variable for control purposes, because it represents the oxygen demand within the operating time of the activated sludge process and it is, in most cases, intimately related to the specific organisms of the activated sludge plant. The BOD_5 is more appropriate to characterise the impact on the receiving water.

We propose to classify the methods for monitoring the BOD_{st} into three categories: state estimation, BOD probe method and respirometer method.

Holmberg (1982) proposed a state estimation method to assess the influent BOD from a simplified model of the activated sludge process. In fact this method uses the aeration tank as a respiration chamber. No further experimental verification of the approach has been presented.

A BOD probe consists of immobilized living cells, a membrane and a dissolved oxygen

electrode. The signal of the probe is a measure of the activity of the cells, which is, in turn, a measure of the substrate concentration in the water. Probes with both activated sludge organisms (Strand and Carlson, 1984; Princz and Oláh, 1990) and yeast cultures (Harita *et al.*, 1985; Riedel *et al.*, 1988) are reported in the literature. The performance of the probe, calibrated in a certain solution, is dependent on the composition of wastewater. Another disadvantage is that the linear range of BOD probes is below the BOD_{st} of most wastewaters, so that samples have to be diluted, which is unsuitable for continuous measurement.

Respirometric BOD procedures can be divided into batch methods and continuous methods. Typical of batch methods is that the BOD is calculated from a respirogram obtained after the addition of a wastewater sample to a respirometer. Closed respirometers, which are not aerated, use a DO-probe or a manometer to record the respirogram (Vernimmen *et al.*, 1967; Tebbutt and Berkun, 1976; Cadena *et al.*, 1988). These meters are laborious to operate and unsuited for continuous measurement. Open aerated respirometers need either a calibration, using a substrate with known BOD (Farkas, 1981; Vanrolleghem *et al.*, 1990), the aeration constants need to be known (Suschka and Ferreira, 1986; Ros *et al.*, 1990; Ciaccio, 1992) or two DO measurements at a separated closed respiration chamber are needed (Spanjers and Klapwijk, 1990a). When applying a sampling device for sludge and wastewater, most batch methods can be automated to obtain a discontinuous monitor of the influent BOD.

In continuous respirometric BOD methods, the wastewater sample is continuously fed into a flow-through reactor having a volume of at most a few litres. Heckershoff and Wiesmann (1986) suggested a method which measures the difference of the oxygen consumption between two parallel reactors, both continuously fed with sludge. Additionally, one is fed with influent sample while the other is fed with tap water. The influent BOD is calculated from DO and BOD mass balances over the reactors. For this calculation it is necessary to assume that the BOD in the activated sludge supplied is equal to zero and that the DO concentrations in both reactors are equal. Riegler (1984) described a method which is based on calibration with a standard with known BOD. The sample is continuously diluted with tap water and the mixture is directed through a reactor with immobilized microorganisms. The DO concentration difference of the flow entering the reactor and the flow leaving the reactor is measured and kept constant by manipulating the dilution ratio. From the comparison with a standard with known BOD_5, the BOD_5 of the wastewater can be determined. In fact, the measurement uses the correlation between BOD_{st} and BOD_5 so that the result is dependent on the type of wastewater. Moreover, since the bacteria are separately grown, the population of microorganisms in the reactor may not have the same properties as the activated sludge in the plant.

Spanjers and Klapwijk (1990a) proposed a method to calculate the influent BOD_{st} from three types of respiration rate: the endogenous, the instantaneous and the actual rate. The endogenous respiration rate is the oxygen uptake rate of sludge free of readily biodegradable matter. The instantaneous rate is the oxygen uptake rate measured when sludge directly flows from the aeration tank through a respiration meter. The actual respiration rate, defined as the real oxygen uptake rate in the aeration tank, is measured if the respiration chamber is equally loaded with BOD_{st} as the aeration tank. The authors showed, using a mass balance over the respiration chamber, that the influent BOD can be calculated from these three rates. The model is based on the assumptions that the degradation of readily biodegradable matter can be described by half order kinetics and that steady state is attained during the respiration measurements.

In this chapter, we propose and test a procedure for the continuous estimation of the influent BOD_{st}. The method uses the DO concentration in the inlet and outlet of a closed respiration chamber during the transients between two modes of respiration measurement. Knowledge of the degradation kinetics of readily biodegradable matter is not required. Other advantages are that a BOD calibration is not needed and that the aeration constants need not to be known. The proposed procedure is verified using both simulated and experimental data from a pilot plant. It is concluded that the procedure enables the estimation of the influent BOD_{st}.

4.3 Principle of the measurement

For the continuous estimation of the influent BOD_{st}, a measuring system is developed which is fed with sludge of the plant under consideration. This means that the measuring system is closely linked to the activated sludge plant. Figure 1 shows a schematic diagram of the measuring system along with the activated sludge plant.

The measuring system consists of a closed, completely mixed respiration chamber, a DO probe, sample pumps for activated sludge and influent and six solenoid valves. By using four valves that switch the flow through the respiration chamber, typically every 30 seconds, the DO concentration is successively measured in the inlet and outlet of the chamber. From the DO-concentrations, the respiration rate can be calculated using a numerical approximation of the mass balance over the respiration chamber (Spanjers and Klapwijk, 1990a).

In the present work, we calculate the respiration rate for the assessment of the endogenous respiration rate. Furthermore, the influent BOD_{st} is estimated from the DO concentration in the inlet and outlet of the respiration chamber during the transients between two modes of

operation. In the endogenous mode (e-mode), sludge having the endogenous respiration rate is directed through the respiration chamber. This sludge is produced in an aerated bypass tank (Spanjers and Klapwijk, 1990a). In the mode where the actual respiration rate is measured (a-mode), sludge from the aeration tank directly flows through the respiration chamber and, in addition, a sample of influent is continuously introduced in the chamber. It can be demonstrated (Spanjers and Klapwijk, 1987; Sollfrank, 1988) that the BOD_{st} in the chamber equals the BOD_{st} in the aeration tank if the ratio of the influent sample flow to the chamber volume equals the ratio of the influent flow to the aeration tank volume. The measuring period for one mode is typically 15 minutes and is sufficiently long to let BOD_{st} and respiration rate in the chamber converge to an ultimate value.

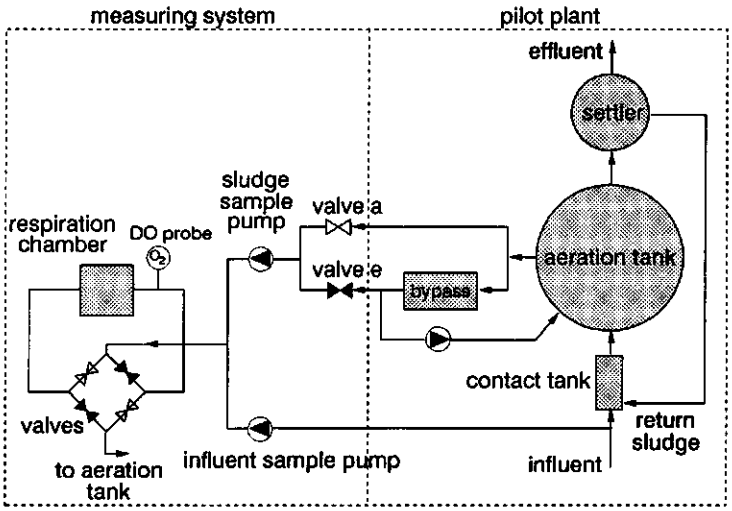

Figure 1: *Scheme of activated sludge plant and measuring system.*

At the end of each a-mode, the measurement is changed to the e-mode and the sludge in the respiration chamber is gradually replaced by endogenous sludge. The endogenous sludge contains no readily biodegradable matter, so the BOD_{st} of the endogenous sludge is assumed to be zero. Hence, in the e-mode, the BOD_{st} in the respiration chamber remaining from the a-mode will be washed out and the respiration rate will decrease. At the end of each e-mode the BOD_{st} in the respiration chamber is zero and the rate measured is the endogenous respiration rate.

At the end of each e-mode, the measurement is changed to the a-mode and the sludge in the chamber is gradually replaced by sludge from the aeration tank, supplied with influent sample. Since, in the a-mode, the respiration chamber is equally loaded as the aeration tank, the BOD_{st} in the chamber will converge to a value equal to the BOD_{st} in the aeration tank (s_a).

In the next section we shall show how the DO measurements in the inlet and outlet of the respiration chamber during the transients can be used to estimate the influent BOD_{st}.

4.4 Estimation of the influent BOD_{st}

The influent BOD_{st} (s_i) is calculated from cycles, each of them consisting of two successive modes: one a-mode and one e-mode. Because each mode takes 15 minutes, the calculation interval of s_i is typically 30 minutes. Figure 2 schematically illustrates the estimation procedure. This involves three steps, executed for each cycle:

1. assessment of the endogenous respiration rate (r_{end});
2. calculation of the BOD_{st} in the aeration tank (s_a);
3. calculation of the influent BOD_{st} (s_i).

1. Endogenous respiration rate

It is assumed that, at the end of each e-mode, the BOD_{st} in the respiration chamber is zero, so that the endogenous respiration rate (r_{end}) is measured. It is further assumed that r_{end} is constant during the whole cycle of 30 minutes. To obtain an operational r_{end} for the whole cycle, the last measured rates of the current and previous cycle are averaged (Figure 2b).

2. BOD_{st} in the aeration tank (s_a)

In the e-mode, the mass balances of DO and BOD_{st} over the respiration chamber are respectively:

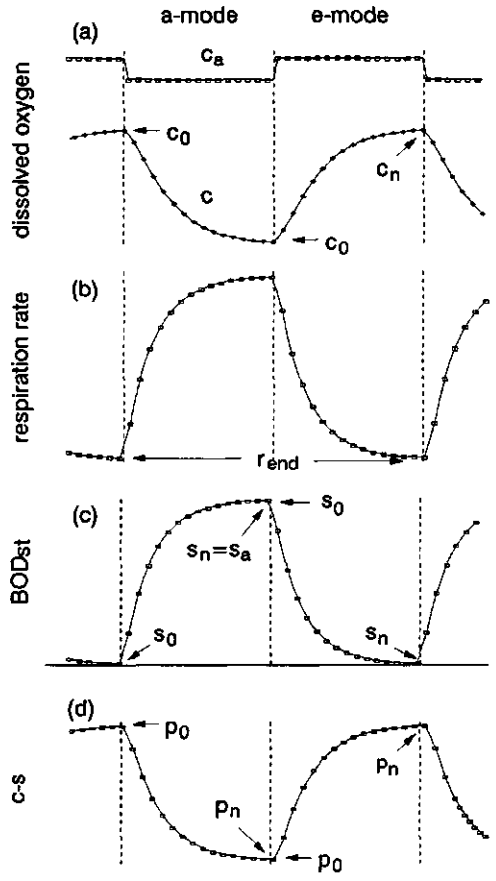

Figure 2: *Schematic representation of the procedure for the estimation of s_i. One cycle, consisting of one a-mode and one e-mode is shown. (a) Measured DO concentration at the inlet, c_a, and outlet, c, of the respiration chamber. (b) Respiration rate calculated from the DO concentrations. (c) Pattern of the BOD_{st} in the respiration chamber. (d) Difference between c and s.*

$$\frac{dc}{dt} = \alpha(c_a - c) - r_s - r_{end} \tag{1}$$

$$\frac{ds}{dt} = -\alpha s - r_s \tag{2}$$

The BOD_{st} in the respiration chamber is known as being zero at the end of the e-mode (Figure 2c). The BOD_{st} during the e-mode can now be calculated directly from the DO concentrations. From the observation that r_s can be eliminated from (1) and (2), we introduce a help variable $p=c-s$ (Figure 2d):

$$\frac{dp}{dt} = \alpha(c_a - p) - r_{end} \tag{3}$$

The variable p is known at the end of the e-mode, so that its value at the beginning of the e-mode can be calculated. Given the discrete time measurements, equation (3) has to be approximated by a difference equation. Approximating the derivative with the trapezoidal rule and solving for p_{k-1} we get for the e-mode:

$$p_{k-1} = \frac{1}{\frac{1}{2}\alpha h - 1}\left[-\left(\frac{1}{2}\alpha h + 1\right)p_k + \alpha h c_{a,k-\frac{1}{2}} - h r_{end}\right] \tag{4}$$

where $k=1, 2,\ldots, n$ (n typically 15).

Using the end condition $s_n=0$, so that $p_n=c_n-s_n=c_n$, p_0 can be calculated. This value is used to find the influent BOD_{st} from the a-mode.

Knowing that s at the beginning of the e-mode is equal to s at the end of the a-mode, and recalling that the BOD_{st} in the respiration chamber at the end of the a-mode is equal to s_a, the latter can be calculated as an intermediate:

$$s_a = s_0 = c_0 - p_0 \tag{5}$$

3. *Influent BOD_{st} (s_i)*

In the a-mode, the mass balances of DO and BOD_{st} over the respiration chamber are respectively:

$$\frac{dc}{dt} = \alpha c_a - \alpha_r c - r_s - r_{end} \tag{6}$$

$$\frac{ds}{dt} = \alpha s_a + \alpha_i s_i - \alpha_r s - r_s \tag{7}$$

The BOD_{st} in the respiration chamber is known as being zero at the beginning of the a-mode. Using the same help variable, p, to eliminate r_s, we obtain:

$$\frac{dp}{dt} = \alpha(c_a - s_a) - \alpha_r p - \alpha_i s_i - r_{end} \tag{8}$$

The variable p is known at the beginning of the a-mode, and its value at the end of the a-mode can be calculated. Approximating the derivative and solving for p_k we get for the a-mode:

$$p_k = \frac{1}{\frac{1}{2}\alpha_r h + 1}\left[-\left(\frac{1}{2}\alpha_r h - 1\right)p_{k-1} + \alpha h c_{a,k-\frac{1}{2}} - \alpha h s_a - \alpha_i h s_i - h r_{end}\right] \tag{9}$$

Using the initial condition $s_0=0$, so that $p_0=c_0-s_0=c_0$, p_n can be calculated. Finally, s_i can be found explicitly from equation (9) so that p_n (a-mode) = p_0 (e-mode).

The procedure for the estimation of s_i was verified using both simulated data and experimental data from a pilot plant.

4.5 Materials and methods

In the simulations, executed with SIMNON (Elmqvist *et al.*, 1986), measuring data were generated using a model of the pilot plant and measuring system (Figure 1). This model simulates, for a known s_i, the DO at the inlet and at the outlet of the respiration chamber during the alternating modes of operation (a-mode and e-mode). The DO was given a noise

having a normal distribution with a standard deviation of 0.005 mg l^{-1}. The values of the parameters and variables (Table 1) were in accordance with the experimental conditions of the pilot plant. First order kinetics for BOD_{st} was employed. The DO measurements generated with the simulation model were saved in a file and processed according to the procedure proposed in this chapter to calculate s_i. The result was compared with the simulated, known s_i.

In the experiments, the measurements were carried out at a pilot plant equipped with a respiration measuring system (Figure 1). This system was capable of measuring consecutively the respiration rate of sludge in the a-mode and in the e-mode. The control of valves and pumps, collection of data and the basic calculations were performed by a modular I/O processor (μMac 6000, Analog Devices).

The pilot plant, fed with presettled domestic sewage, consisted of an unaerated contact tank (four 2 litre compartments in series), a completely mixed aeration tank and a settling tank. The air flow rate was maintained constant using a flow controller. Sludge with the endogenous respiration rate was produced in a bypass tank, consisting of five 5 litre aerated compartments in series, through which activated sludge from the aeration tank was continuously recirculated. It was verified that, under normal loading conditions, the sludge leaving this tank was free of readily biodegradable matter (Spanjers *et al.*, 1993).

The measuring system consisted of a respiration meter (prototype of RA-1000, Manotherm) having a respiration chamber, an influent sample pump and six valves. To operate alternately in the a-mode and in the e-mode, two valves were used to select sludge from the aeration tank and the bypass, respectively. During the a-mode, a sample of influent was continuously added to the sludge flowing through the respiration chamber. Table 1 lists the average process conditions during the measuring period.

To verify the proposed estimation procedure, the following strategy was used. During the period 12:00 h to 24:00 h, the influent was taken from a slowly stirred 500 litre storage tank. Because the concentration of readily biodegradable matter of the stored wastewater was assumed constant, it was expected that the estimated s_i during this period was constant. In addition, the BOD_{st} of the wastewater was independently determined using a batch respiration measurement system (Spanjers and Klapwijk, 1987, 1990b), equipped with a RA-1000 respiration meter. During the period 0:00 h to 12:00 h the storage tank was refilled with wastewater from the sewer while the reactor was simultaneously fed with the same water. During this period, there was a varying concentration of readily biodegradable matter in the influent and consequently s_i was expected to vary.

***Table 1:** Conditions of simulations and experiments for estimation of s_i. Irrelevant conditions indicated with "-".*

	simulation	experiment
sampling interval c_a, *c and r* (s)	60	60
duration one mode (min)	15	15
volume aeration tank (l)	475	475
influent flow (l h^{-1})	40	40-42
return sludge flow (l h^{-1})	-	41-43
waste sludge flow from AT (l h^{-1})	-	1.43
air flow (m^3h^{-1})	-	2.4
volume bypass tank (l)	-	25
recirculation flow bypass (l h^{-1})	-	26
volume respiration chamber (l)	0.731	0.731
sludge flow through resp. chamber (l h^{-1})	21	21.78
influent sample flow (l h^{-1})	0.0616	0.0616
influent BOD_{st} (mg l^{-1})	100 if 0 < time < 6 h 250 if 6 ≤ time < 12 h	100-350
influent ammonium-N on day 1 (mg l^{-1})	-	23.5
influent total nitrogen (mg l^{-1})	-	34.5
influent COD on day 1 (mg l^{-1})	-	243
MLSS (g l^{-1})	-	2.42-2.52
MLVSS (g l^{-1})	-	1.92-1.99
temperature sludge (°C)	-	17.8-19.2
pH	-	7.2-7.3
endogenous respiration rate (mg $l^{-1}h^{-1}$)	20	17-21
endogenous sludge BOD_{st} (mg l^{-1})	0	assumed 0
biodegradation in settler	none	assumed 0
biomass growth	none	unknown
kinetics with respect to BOD_{st}	1^{st}-order 10 h^{-1}	unknown

For the batch experiments, a sludge sample from the aeration tank was transferred to the batch system and the respiration rate was recorded. When the rate reached the endogenous level, a known volume of wastewater from the storage tank was added. The rate was recorded until the endogenous level was reached again. The BOD_{st} of the wastewater sample

was calculated from the total amount of oxygen additionally used to the endogenous oxygen consumption. This procedure was repeated several times with another addition of the same water sample. During the batch experiments, pH and temperature were maintained at 7.5 and 20 °C.

4.6 Estimation of s_i from simulated measurements

To verify the proposed procedure and to examine the error in the estimated s_i, this variable was estimated from simulated DO data. These data were generated from a mathematical model which was given a known input step function of s_i. Normally distributed noise was added to the simulated DO. In this simulation, any deviation of the estimated values from the original ones resulted from the noise added to the DO and the assumptions associated with the estimation procedure. Figure 3 shows the original and estimated values. Since r_{end} and s_a, as intermediates in the estimation procedure, have an influence on s_i, these variables are also given in the figure.

As required by the method, the estimated r_{end} did not depend on the influent BOD_{st}, was close to the original value of 20 mg $l^{-1}h^{-1}$ and showed a small standard deviation (Figure 3a). As dictated by the model, the simulated s_a was a function of the influent BOD_{st}. The estimated s_a (Figure 3b) also showed the step response. However, the average values of this variable (calculated from repeated simulations) deviated from the simulated ones. Moreover, the estimated s_a showed a substantial standard deviation. Before the step increase, when the influent BOD_{st} was 100 mg l^{-1}, the error was even greater than the estimated value itself. When the influent BOD_{st} was 250 mg l^{-1}, considered to be a moderate value for the wastewater, the relative error was 53%.

Because of its large value the estimated s_i was much less sensitive to noise on the DO. The average s_i before and after the step change, 99 ± 3.8 mg l^{-1} and 250 ± 3.4 mg l^{-1} respectively, were close to the simulated values.

The simulation leads to the conclusion that the procedure proposed here allows the estimation of the influent BOD_{st} (s_i). The BOD_{st} in the aeration tank (s_a), an intermediate in the estimation of s_i, shows a substantial relative error. This is due to the low value of s_a.

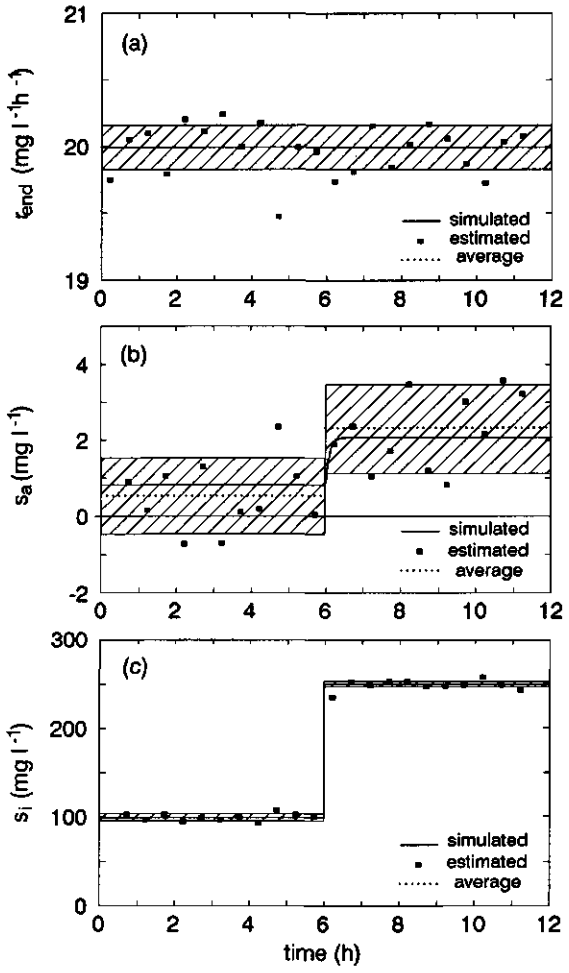

Figure 3: *Result of the estimation of (a) r_{end}, (b) s_a and (c) s_i from simulated DO measurements. The simulated s_i was given a step change from 100 mg l^{-1} to 250 mg l^{-1} at 6 h. Noise with standard deviation 0.005 mg l^{-1} was added to the simulated DO. Solid lines: simulated value, points: estimated values; dotted lines: averaged estimate; dashed zones: standard deviation estimation.*

4.7 Estimation of s_i from experimental data

During a period of several days, the pilot plant was fed alternately with wastewater from a storage tank and wastewater directly from the sewer system. The respiration measuring system was operated alternately in the two modes of operation. The DO concentration, meanwhile measured at the inlet and outlet of the respiration chamber, was used to estimate the BOD_{st} of the influent (s_i). An illustrative example of the DO measured is given in Figure 4(a). It is recalled that the measurement is done with one and the same probe. Figure 4(b) shows the respiration rate calculated from the DO measurements. The peaks at the end of each mode are a consequence of the numerical approximation used in the calculation of r. The magnitude of these errors was dependent on the extend of the jump in c_a, being the difference between the DO in the bypass tank and the aeration tank. The peaks have been excluded from the calculation of r_{end} in order to minimize the error.

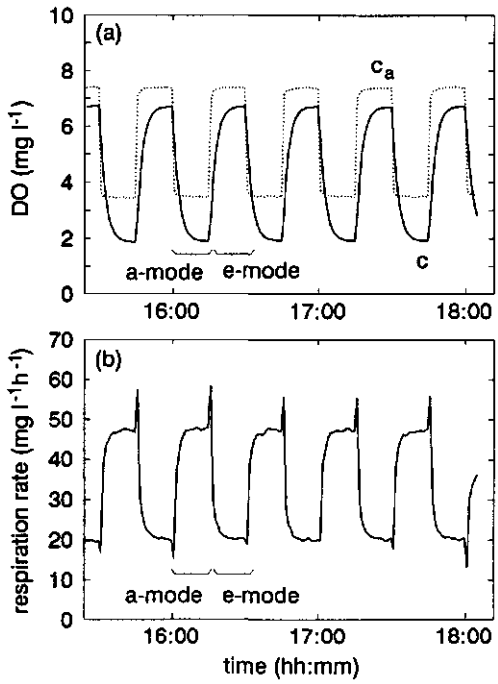

Figure 4: *Part of (a) the measured DO concentration and (b) the calculated respiration rate. Calculation interval respiration rate: 60 seconds.*

The estimated variables, for a measuring period of 53 hours, are given in Figure 5. r_{end}, having relatively little noise, shows a gradual variation, probably caused by varying activity and biomass concentration. The decrease in the night of day 2, is partly caused by a loss of biomass. The MLSS of grab samples of activated sludge, taken at about noon from the aeration tank was 2.50 g l^{-1} and 2.42 g l^{-1} on day 2 and 3, respectively.

As expected from the simulation, s_a showed considerable relative variation (Figure 5b). Assuming that the real s_a was constant during the periods with influent from the storage tank, the standard deviation of the estimated s_a in these periods would be a indication of the estimation error. For the first and the second period we got 2.51 ± 1.12 mg l^{-1} or $\pm 45\%$ and 4.40 ± 0.80 mg l^{-1} or $\pm 18\%$, respectively.

Figure 5(c) shows that the estimated s_i was relatively constant during the periods with influent from the storage tank. For the first and second period, assuming constant BOD_{st} during storage, we obtained for s_i: 104 ± 6 mg l^{-1} or $\pm 5.8\%$ and 287 ± 7 mg l^{-1} or $\pm 2.6\%$, respectively. To verify the estimated s_i during the periods with influent from the storage tank, the BOD_{st} of samples of the stored water was also determined using the batch respirometric method. These BOD_{st} values are also shown in Figure 5(c).

Both methods matched reasonable well, indicating that the method proposed enables the estimation of s_i. Table 2 summarises the values of both methods. The wastewater usually shows a diurnal peak in the waste concentration at about noon (Spanjers and Klapwijk, 1991). A part of this peak can be seen in Figure 5(c), on day 2 at the end of the period with influent from the sewer system. On day 2, both methods indicated a decrease in the influent BOD_{st} during storage. An explanation is that there was biochemical oxidation in the storage tank. A higher biochemical activity during storage of the wastewater in the second period, compared with the first one, was not unlikely, because of higher wastewater temperature (1.0 to 1.5 °C) and waste concentration.

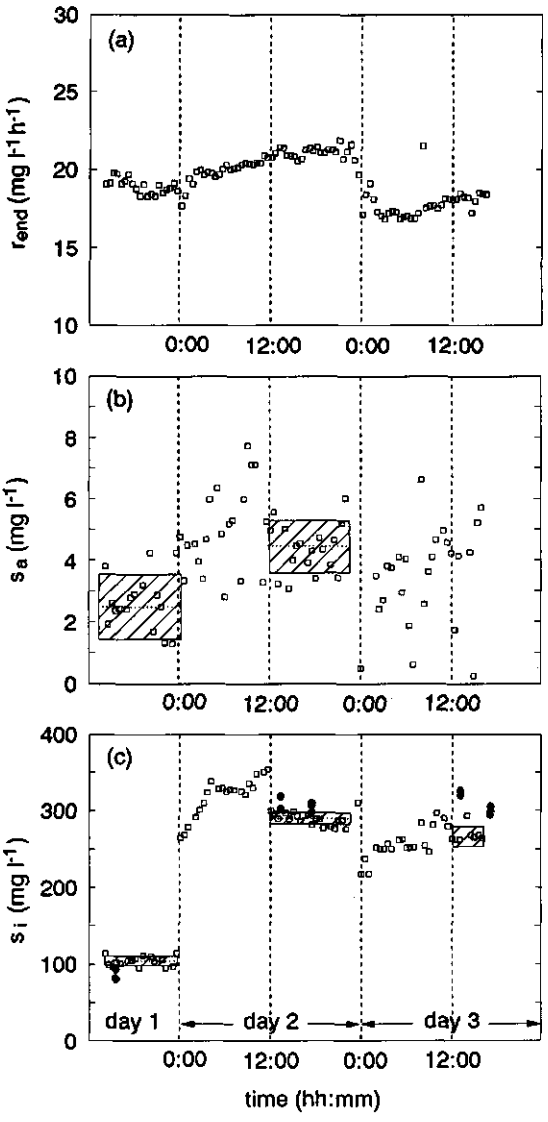

Figure 5: *Results of the estimation procedure applied on experimental measurements. (a) r_{end}, (b) s_a and (c) s_i. Open markers: estimated values; dotted lines: average; dashed zones: standard deviation; filled markers: BOD_{st} determined in batch experiments. Influent was taken from the storage tank from 12:00 to 24:00 h.*

Table 2: Influent BOD_{st} from estimation procedure and batch experiments compared. avg = averaged BOD_{st}, std = standard deviation of BOD_{st}.

	estimation		batch				
	avg	std	time	repeated measurements			avg
day 1, 12:00 h - 24:00 h	104	6	15:00	94	82	80	85
day 2, 12:00 h - 24:00 h	287	7	13:00	303	317	-	310
			16:00	310	298	311	306
day 3, 12:00 h - 17:00 h	264	11	12:30	319	313	318	317
			16:00	300	294	293	296

Examples of respirograms obtained with the batch method are given in Figure 6. In agreement with earlier results (Spanjers and Klapwijk, 1990a), each respirogram shows an initial peak, a first shoulder and, at the end, a second shoulder. During these additions, the respiration rate at the baseline only decreased from 22 to 20 mg $l^{-1}h^{-1}$ in 110 minutes, indicating that the endogenous respiration rate, as defined in this work, was reached. However this rate was still higher than r_{end} measured at the pilot plant: 18.5 mg $l^{-1}h^{-1}$. This is probably caused by the higher temperature in the batch experiments: 20 °C compared with 18.7 °C in the pilot plant.

Further experimental evidence, using ammonium concentration in the wastewater (Table 1), has confirmed that the BOD_{st} of the wastewater considered is mainly caused by ammonium being oxidised by nitrifiers.

From the experimental results it is concluded that the proposed method enables the estimation of the influent BOD_{st}. The BOD_{st} in the aeration tank, estimated as an intermediate variable, however, shows substantial lack of precision.

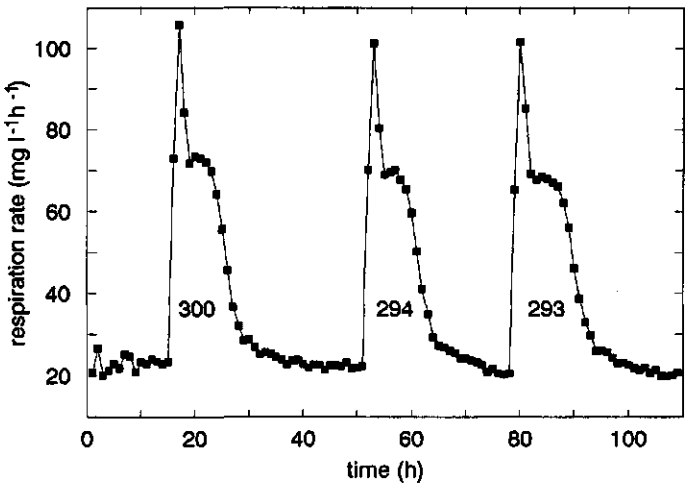

Figure 6: *Batch respirograms of one wastewater sample from the storage tank in the afternoon of day 3. Activated sludge and wastewater were sampled at 15:30 h and 16:00 h, respectively. The volume of wastewater added was 50 ml for the first and second respirogram and 60 ml for the third one. The wastewater BOD_{st} obtained is indicated in the figures.*

4.8 Discussion

The purpose of our investigation was to develop and test a procedure for the continuous estimation of the influent BOD_{st}. The proposed procedure used the DO concentration in the inflow and outflow of a respiration chamber during the transients of two modes of operation: a-mode and e-mode. Advantage of the procedure, as compared with a previously published method (Spanjers and Klapwijk, 1990a), was that an assumption on the biodegradation kinetics of readily biodegradable matter was not needed. However, an important condition was that, at the end of the e-mode, the BOD_{st} in the respiration chamber was zero. The procedure was tested in a simulation and verified in an experiment.

The simulation showed that the estimation procedure for the model considered was correct and the assumptions were reasonable. A typical error of the simulated DO led to an acceptable relative error of the estimated s_i. However, the relative error of the estimated s_a, an intermediate in the estimation procedure, was too large to assess this variable with reasonable accuracy.

The measurements showed similar results with regard to the errors of the estimated variables. Assuming that, during the periods with influent from the storage tank, s_a and s_i were constant, their relative errors could be determined as being 20-45% and 2-6%, respectively. Under the plant conditions in this work, s_a was only 1.5 to 2.5% of s_i, meaning that s_a could have been neglected in the estimation procedure. However, in a higher loaded plant, this may not be allowed.

Because the calculation of s_a started at the end of the e-mode, with the last c, the numerical integration was much affected by the inaccuracy of this DO value. This may be considered in the algorithm, and features can be added to the integration to minimize this sensitivity. One possibility would be to start the calculation where the slope of p (Figure 2d) becomes significantly different from zero. A critical part of the estimation is the filtering and integration of the measurement values. This may be subject for further investigations.

Because, in the proposed method, the respiration rate is measured as a function of a continuously varying BOD_{st} in the respiration chamber, the kinetic relationship between these variables may be determined for each cycle. Steady state with regard to the transport of substrate must then be assumed. From eqs. (1) and (6), r_s can be calculated. Then, from eqs. (2) and (7), s is calculated using the boundary conditions $s_0=0$ (a-mode) and $s_n=0$ (e-mode). Finally, the kinetic relationship can be fitted on the corresponding points r_s and s.

Our experience was that the proposed method was easily applicable in practice. Most attention had to be paid to correctly measuring the a-mode, since here it was necessary that the ratio of the influent sample flow rate to the chamber volume matched the ratio of the influent flow rate to the aeration tank volume. Since the influent flow was constant in the experiment described here, the sample flow could be fixed. A varying influent flow, common in full scale plants, would require an accurate control of the sample flowrate.

The experiments showed that the BOD_{st}, as defined in this work, is an important variable for characterising the dynamics of the oxygen demand in the activated sludge process. In this work the BOD_{st} was defined as the oxygen demand in excess of the demand for endogenous oxygen consumption. The endogenous respiration rate was practically defined as the oxygen uptake rate of sludge after a mean residence time of 1 hour in an aerated reactor consisting of five 5 litre compartments in series. The batch experiments clearly illustrated (Figure 6) that this was a reasonable definition since the r_{end} level changed only slightly during a period of 2 hours after sampling and, after the addition of wastewater, the rate rapidly returned to this level. The endogenous rate includes the oxidation of slowly biodegradable matter. The gradual increase of r_{end} on day 2 (Figure 5) partly indicates an enlarged oxygen uptake rate

for the degradation of slowly biodegradable matter caused by the higher loading compared to day 1.

4.9 Conclusions

The method proposed here allowed the estimation of the influent BOD_{st}. When it was assumed that, during the measurement with influent from the storage tank, the concentration of readily biodegradable matter was constant, the relative error of the estimated BOD_{st} could be determined: 2 to 6 %. There was a reasonable agreement between the estimated BOD_{st} and the BOD_{st} determined in batch experiments.

4.10 Notation

BOD_{st} short-term biochemical oxygen demand
c DO concentration in respiration chamber (mass volume^{-1})
c_a DO concentration in aeration tank (mass volume^{-1})
DO dissolved oxygen
h sampling interval DO concentration (inlet or outlet) (time)
p $= c\text{-}s$ help variable (mass volume^{-1})
q sludge flow through respiration chamber (volume time^{-1})
q_i influent sample flow through respiration chamber (volume time^{-1})
r $= r_s + r_{end}$ respiration rate (mass volume^{-1} time^{-1})
r_{end} endogenous respiration rate (mass volume^{-1} time^{-1})
r_s substrate respiration rate (mass volume^{-1} time^{-1})
s BOD_{st} in the respiration chamber (mass volume^{-1})
s_0 BOD_{st} in the respiration chamber at the beginning of a measuring mode (mass volume^{-1})
s_a BOD_{st} in the aeration tank (mass volume^{-1})
s_i BOD_{st} of the influent (mass volume^{-1})
s_n BOD_{st} in the respiration chamber at the end of a measuring mode (mass volume^{-1})
v volume respiration chamber (volume)
α $= q/v$ dilution rate (time^{-1})
α_i $= q_i/v$ dilution rate (time^{-1})
α_r $= \alpha + \alpha_i$ dilution rate (time^{-1})

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5

Maximum respiration rate of activated sludge

5.1 Abstract

The maximum respiration rate (r_{max}), defined as the oxygen uptake rate when there is an excess of readily biodegradable substrate, is a variable that can be measured with a respiration meter. It is useful in operating the activated sludge process. Respiration is the result of the oxidation of multiple substrates by a heterogenous population of micro-organisms. This implies that, theoretically, r_{max} can only be measured if all the individual substrates are present in excess.

This work discusses under which condition on-line measurement of r_{max} can take place. The discussion is restricted to nitrifying sludge fed with domestic wastewater from a particular source. The readily biodegradable part of this wastewater can be represented by two components. It is shown that r_{max} can be measured if wastewater is continuously fed into a respiration chamber, so that the loading exceeds a certain critical loading. This critical loading is a function of the kinetic parameters of the components and of the fractions in which the components occur in the wastewater concerned. It is shown that batch tests can be used to verify if the condition for on-line measurement of r_{max}, an excess of substrate, is met.

An application of the developed measurement is described: an investigation of the effect of the influent flow of a nitrifying activated sludge plant on r_{max}. It is concluded that, under the experimental conditions applied, there is no significant effect. The batch tests are useful for checking the on-line measurements. However, the results are not fully satisfactory. Suggestions for improvements are made.

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5.2 Introduction

In the activated sludge process, besides the conversion into cell material, oxidation is the main process for removal of biodegradable matter. Therefore, the oxygen uptake per unit of volume and unit of time, or respiration rate, is an important variable for monitoring and controlling the activated sludge process and performing kinetic studies .

Respiration rate has been proposed and used as a variable for **monitoring** the activated sludge process (Duggan and Cleasby, 1976); Brouzes 1979; Young, 1981; Spanjers and Klapwijk, 1990; Tur *et al.*, (1990). Variables which cannot easily be measured like the biochemical oxygen demand, have also been derived from the respiration rate (Holmberg, 1982; Holmberg and Ranta, 1982; Therien and Ilhan, 1983; Spanjers *et al.*, 1993).

Since the electrons available in a substrate undergoing oxidation all must be transferred to oxygen or incorporated into new biomass, the respiration rate can provide the same information as either substrate conversion or biomass growth (Grady *et al.*, 1989). Also the IAWPRC task group (1986) proposed measurement of growth constants based on respiration rate rather than cell growth or substrate removal. In many studies, respiration rate values were used to obtain **kinetic** and growth parameters (Cech *et al.*, 1984; Huang *et al.*, 1985; Ekama *et al.*, 1986; Vargas Lopez, 1988; Grady *et al.*, 1989; Tabak *et al.*, 1990; Ossenbruggen *et al.*, 1991; Kappler and Gujer, 1992; Wentzel *et al.*, 1992; Vanrolleghem, 1993).

In early papers the respiration rate was already considered to be a useful **control** variable (Brouzes, 1969; Andrews, 1977; Haas, 1979). Subsequently, several control strategies based on respiration rate were proposed (Sϕrensen, 1980; Addie, 1981; Stephenson *et al.*, 1983; Takamatsu, 1981; Allsop, 1990; Hamamoto, 1990).

The actual respiration rate of activated sludge is dependent on the substrate concentration and reflects the loading of the activated sludge plant. It ranges between the endogenous respiration rate (no readily biodegradable matter is present) and the maximum respiration rate (there is an excess of readily biodegradable substrate). The endogenous rate is a measure of the active biomass concentration (Brouzes, 1979; Allsop, 1990). However, endogenous rate has not often been used as such as it also depends on the duration of substrate depletion (Lijklema, 1971). The maximum respiration rate is independent of the substrate concentration. However, since it is the sum of the respiration rates resulting from the oxidation of several components, it is dependent on the substrate composition. For a given substrate composition the maximum respiration rate is also a measure of the active biomass concentration. In the literature, several applications of the maximum respiration rate are reported.

Takamatsu *et al.* (1981) used the maximum respiration rate, as measured in batch operation, to estimate the mixed liquor suspended solids (MLSS) concentration and the substrate concentration in activated sludge. These variables were shown to be useful for the control of a treatment plant. The authors first determined the relationship between maximum respiration rate and MLSS concentration, which they assumed to be linear, and the relationship between maximum respiration rate and substrate concentration (expressed as COD). The unknown MLSS concentration and substrate concentration were then estimated on the basis of the respiration rate of an activated sludge sample of the plant. The endogenous respiration rate was not considered in this method. The authors claimed that the maximum respiration rate was measured after adding "some" extra wastewater. However, they did not present experimental evidence showing that saturation with respect to the substrate concentration had been achieved. Further, they did not address the effect of varying wastewater composition on the maximum respiration rate and how often the predetermined relationships had to be determined.

Huang *et al.* (1985) presented a method which uses the specific maximum respiration rate (maximum respiration rate divided by MLSS concentration), measured in batch operation, for estimating the microbial activity and viability. They stated that the viability determined in this way would represent realistically the activity of bacteria. They found a functional relationship between this specific maximum respiration rate and the mean solids retention time. They also concluded that the specific maximum respiration rate can be used to find the maximum aeration capacity requirement. In order to determine the maximum respiration rate, they added two types of substrate to a batch reactor with activated sludge and measured respiration rate and COD in relation to time. The maximum rate was then derived from these variables, using the Monod function. The method of Huang *et al.* easily leads to underestimation of the respiration rate because of the delay between sludge sampling and measurement. Furthermore, the endogenous respiration rate was not subtracted from the measured respiration rates, so they overestimated the maximum respiration rate. Because the substrates used were not representative for wastewater, the results cannot be related directly to plant conditions.

Vargas-Lopez (1988) used the method of Huang (1985) to derive the viability and active fraction of biomass from the maximum respiration rate. He developed a mathematical model of the activated sludge process, which consideres the active fraction as opposed to the MLSS, and showed that MLSS is not appropriate to represent active biomass when using kinetic constants that truly reflect the observed phenomena in activated sludge. The maximum respiration rate was derived according to the method of Cech *et al.* (1984), which consists of injecting different doses of substrate into a closed respiration meter filled with activated

sludge and measuring the decline of the dissolved oxygen concentration. For each dose the respiration rate is calculated on the basis of the tangent to the initial DO decrease. The calculated initial substrate concentrations and the corresponding initial respiration rates are evaluated by using the Monod model in order to obtain the maximum respiration rate. A disadvantage of this method is that in determining the tangent the respiration rate is easily underestimated, particularly if a limiting substrate concentration is applied. Because, again, a specific substrate was used, conclusions based on the measured maximum respiration rate may not be valid for activated sludge fed with wastewater.

Jørgensen *et al.* (1992) related the maximum respiration rate to viable biomass concentration by means of a conversion factor assessed in exponentially growing cultures. This factor (300 mg O_2 per g dry weight per h) was used for *in situ* determination of the viable biomass concentration in wastewater and activated sludge. According to their findings the biomass ranges from 67 to 177 mg dry weight per g suspended solids for activated sludge. They concluded that measurement of the maximum respiration rate provides a reliable method for assessing the viable biomass concentration in wastewater and activated sludge. In order to standardize the conditions for measuring the maximum respiration rate, a complex carbon source (mixture of sodium acetate and yeast extract) was added to all samples. Maximum respiration was assumed to occur during the period of exponential growth, and this period was defined as the time interval in which the optical density of the suspension increased linearly. The authors did not present evidence showing that the maximum respiration rate was attained with respect to all substrates in the complex medium.

Temmink *et al.* 1993 used the maximum respiration rate as an indicator of toxic wastewater by monitoring the change in maximum respiration rate of activated sludge. For that purpose a test vessel through which activated sludge was recirculated was continuously fed with wastewater at a high loading. The critical loading above which the maximum respiration rate was measured, was determined in preliminary experiments under treatment plant conditions.

Spanjers and Klapwijk (1990) suggested a control strategy for activated sludge plants, utilising the continuous measurement of different types of respiration rate of activated sludge from a completely mixed aeration tank, including endogenous respiration rate and maximum respiration rate. The endogenous rate was defined as the oxygen uptake rate in the absence of readily biodegradable matter. The maximum respiration rate was defined as the oxygen uptake rate in the presence of an excess of readily biodegradable matter. The latter was measured by continuously feeding wastewater into sludge flowing through a respiration chamber, while maintaining a wastewater to sludge ratio of at least 0.03. In their measurements it was observed that the maximum respiration rate was related to the influent

flow in an unexpected way: at a high flow it decreased and at a low flow it increased. They found that this was not due to changes in MLSS concentration or temperature of the sludge. An explanation of this phenomenon is that at high organic load the nitrification was temporarily inhibited (Stover *et al*, 1976; Beck, 1982; Stenstrom and Song, 1991). This may be due to oxygen limitation within the activated sludge flocs at high respiration rate. No evidence was presented showing that there was an excess of readily biodegradable matter and a constant influent composition during the measurements.

Literature shows that measuring the maximum respiration rate can be useful for monitoring, controlling, assessing toxicity, estimation of active biomass and determination of the needed aeration capacity. However, questions arise from the evaluation of the respiration rate measurements. In particular when the maximum respiration rate was assessed by measuring with an excess of substrate, it is not clear if there was an excess of substrate indeed in all cases. Furthermore, when in experiments of others the Monod-model was used to determine the maximum respiration rate, the endogenous respiration rate was not always accounted for. Finally, the effect of a varying substrate composition on the maximum respiration rate is not clear.

In this research we studied the experimental **conditions** under which on-line measurement of the maximum respiration rate of sludge from a completely mixed activated sludge tank fed with domestic wastewater from a particular source is possible. It was presumed that the wastewater consists of two biodegradable components. The ultimate objective of the measurements is to obtain a control variable which can be used to determine overloading of the activated sludge plant. Special emphasis was placed on the **effect of the influent flow** on the maximum respiration rate, observed in earlier research (Spanjers and Klapwijk, 1990). The results of a two weeks measuring period are presented. In this period the maximum respiration rate of sludge in a completely mixed pilot plant fed with domestic wastewater was measured. By means of batch respirometric experiments we **checked** if the condition for measuring the maximum respiration rate was met.

It will be shown that on-line measurement of the maximum respiration rate is indeed possible, provided that a certain critical loading of the respiration chamber is reached. However, an effect of the influent flow on the maximum respiration rate, as mentioned above, was not found.

5.3 Concept of maximum substrate respiration rate

Biological background

The respiration rate (r) of activated sludge is defined as the oxygen uptake in mass of dissolved oxygen per unit of volume mixed liquor and per unit of time. This variable can be considered to be the sum of the endogenous respiration rate (r_{end}) and the substrate respiration rate (r_s):

$$r = r_{end} + r_s \tag{1}$$

In this work we define the endogenous respiration rate as the oxygen uptake rate in the absence of readily biodegradable substrate. Contrary to the more biological definition, which states that the endogenous metabolism results from the degradation of endogenous reserves (Pirt, 1965), this definition is operational. The substrate respiration rate, r_s, is the oxygen uptake rate due to the oxidation of readily biodegradable substrate. The total amount of oxygen needed for the degradation of all the readily biodegradable substrate is the short-term biochemical oxygen demand (BOD_{st}). This BOD_{st} is equivalent with the concentration of readily biodegradable substrate.

If we express the substrate concentration (s) in terms of BOD_{st}, we can represent the relationship between substrate respiration rate and substrate removal rate as follows:

$$r_s = -\frac{ds}{dt} \tag{2}$$

The substrate removal rate is related to the growth of micro-organisms as follows:

$$\frac{ds}{dt} = -\frac{x\mu}{y} \tag{3}$$

In contrast to the customary use, here y represents the amount of biomass synthesised per mg of oxygen used for the oxidation of readily biodegradable matter. Combining (2) and (3) results in:

$$r_s = \frac{x\mu}{y} \tag{4}$$

Thus the substrate respiration rate is a function of the biomass concentration (x), the biomass yield coefficient (y) and the specific growth rate (μ). The specific growth rate is a function of the substrate concentration. A number of models have been formulated to describe the kinetics of microbial growth. There is general agreement that the growth rate is dependent on s under substrate-limited conditions and practically independent of it under substrate-saturated conditions. To describe the kinetics under both conditions, the model should make a transition between these conditions. In this chapter we consider a combined first-order / zero-order model to substantiate the concept of maximum respiration rate:

$$\begin{aligned} \mu &= \frac{\mu_{max} s}{s_c} \qquad if \quad s<s_c \\ \mu &= \mu_{max} \qquad if \quad s \geq s_c \end{aligned} \tag{5}$$

Using equation (4) we get:

$$\begin{aligned} r_s &= \frac{r_{smax} s}{s_c} \qquad if \quad s<s_c \\ r_s &= r_{smax} \qquad if \quad s \geq s_c \end{aligned} \tag{6}$$

In model (6):

$$r_{smax} = \frac{x\mu_{max}}{y} \tag{7}$$

Hence, r_{smax} is dependent on the biomass characteristics represented by μ_{max} and y, and the biomass concentration. The dependency of micro-organisms on temperature and toxicity is included in μ_{max}. From the above it follows that r_s reaches its maximum value, r_{smax}, if s exceeds the critical concentration. It means that in practice sufficient influent sample should be fed into the sludge. In the following the consequence of multiple substrates for the

measurement of r_{smax} will be discussed.

Multiple substrates

In the activated sludge process r_s is the overall result of the oxidation of multiple substrates by a heterogenous population of micro-organisms. We assume that the individual substrates are independently oxidised (Argaman, 1991). This means that theoretically r_{smax} can only be measured when all the individual substrates are present in excess. In previous investigations we found that the readily biodegradable part of the wastewater under consideration can be represented by two components. This is illustrated in Figure 1, where two batch respirograms are compared. The first one shows the result of adding a sample of wastewater to nitrifying activated sludge and the second one shows the result of adding a mixture of ammonium chloride and sodium acetate to the same sludge. The initial peak in the latter corresponds with the oxidation of acetate. The figure shows that the main features of the readily biodegradable part of the wastewater are reasonably represented by such a mixture of acetate and ammonium.

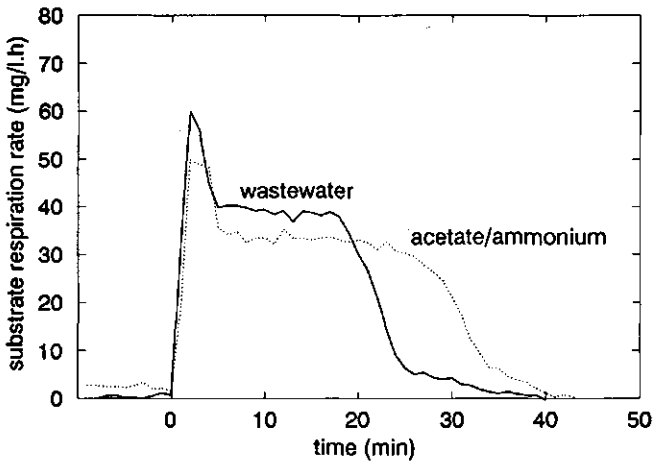

Figure 1: *Respirograms of wastewater (initial BOD_{st}: 14.7 mg l^{-1}) and a synthetic medium consisting of two readily biodegradable compounds (initial concentrations: 3.2 mg NH_4-N l^{-1} and 6.0 mg acetate per litre). The MLSS concentration in the measurement with synthetic medium was lower than with the wastewater.*

In the following we assume that the total BOD_{st} (s) of the wastewater can be divided into two components with a BOD_{st} of s_1 and one of s_2 so that:

$$s = s_1 + s_2 \tag{8}$$

and

$$s_1 = f_1 s \quad \wedge \quad s_2 = f_2 s \tag{9}$$

where f_1 and f_2 are fractions of the total BOD_{st}. The total substrate respiration rate is the sum of the individual rates:

$$r_s = r_{s1} + r_{s2} \tag{10}$$

The condition for measuring r_{smax} of the wastewater is met if both components are present in excess. It can be shown that r_{smax} is only attained if $s \geq s_c$ or:

$$s \geq \max(s_{c1} + s_2 \ , \ s_1 + s_{c2}) \tag{11}$$

Equation (11) implies that s_c is governed by the critical BOD_{st} of one component and the actual BOD_{st} of the other. The values of s_1 and s_2 depend on f_1 and f_2, on the kinetic characteristics of the components and on the process conditions. (In the next section we will go into details concerning the process conditions.)

Figure 1 suggests that both components represent quite different fractions of the total BOD_{st}. This is better illustrated in Figure 2a, where a respirogram is shown which was recorded after equal volumes of wastewater and activated sludge were mixed. The surface under the curve and above the endogenous respiration rate represents the total BOD_{st} of the wastewater. The surfaces 1 and 2, representing the two components, constitute fractions of 0.09 and 0.91 respectively of the total BOD_{st} of 314 mg l^{-1}.

From the values in the respirogram the remaining BOD_{st} in the activated sludge can be calculated (see further eq. 19). This variable is plotted against time for the first component and for the tail-end of the second component (Figure 2b). The figure suggests s_c values of about 0.8 and 3.5 mg l^{-1} for component 1 and 2 respectively. Component 1 seems to follow

more closely a zero order kinetic above s_c than component 2. From the slope of the substrate depletion curves (Figure 2b and 2c) the maximum substrate respiration rates of component 1 and 2 were derived: 18 and 23 mg $l^{-1}h^{-1}$ respectively.

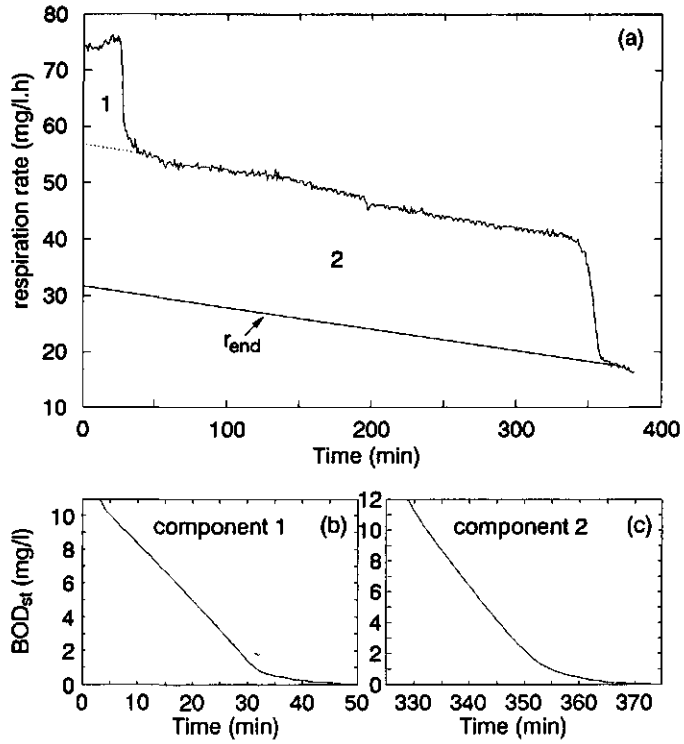

Figure 2: *(a) Respirogram after mixing equal volumes of activated sludge (MLSS=2.8 g l^{-1}) and wastewater. Surfaces 1 and 2 represent the two readily biodegradable fractions of the wastewater. (b) BOD_{st} in the activated sludge. Only the end part of the oxidation of component 2 is shown.*

Using a model of the measuring system, equations (8-10), the assumption that both components follow combined first/zero-order kinetics (eq. (6)) and the parameter values found in Figure 2, the relationship between r_s and s can be simulated (Figure 3). Figure 3b demonstrates that the condition for the measurement of r_{smax} is particulary sensitive to the kinetic parameters of component 1, because this component is found in an inferior fraction of the total BOD_{st}. In case of the example discussed here, the lower boundary for equation (11) is set by the value of $s_{c1}+s_2$.

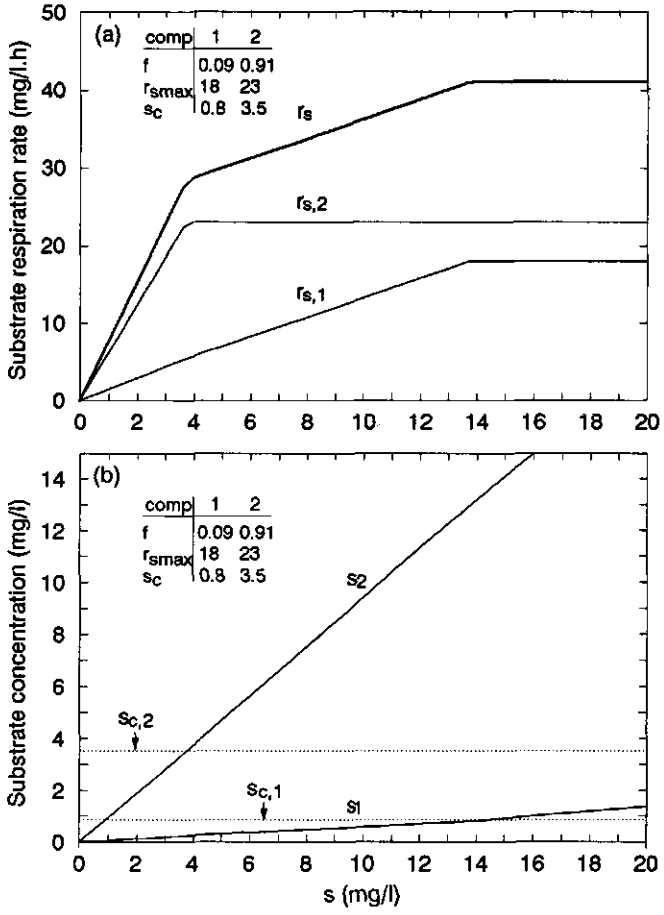

***Figure 3:** Simulation of substrate respiration rate as a function of the BOD_{st} for critical substrate concentrations found in Figure 2, and for s_{c1} equal to s_{c2}.*

In reality the kinetic parameters are not exactly known. For the measurement of r_{smax} it would be favourable, in this particular case (with component 1 as an inferior fraction), if $s_{c1} << s_{c2}$ because component 2 would then be expected to govern s_c. On the other hand, if $s_{c1} >> s_{c2}$ then the s needed to achieve saturation with respect to component 1 is beyond the span which can be realised. The rate measured will be the r_{smax} of component 1 plus a

contribution of component 2. If s_{1c} is large enough, this contribution may be negligible, meaning that a reasonable variation in s would not lead to significant changes in the r_{smax} measured.

From the foregoing it can be concluded that initial assumptions on the wastewater composition and kinetic coefficients must be critically reevaluated each time a change in the measured r_{smax} is observed. In the next section we will outline the conditions for the measurement of r_{smax} at an activated sludge plant and subsequently we will describe the use of batch tests to check the on-line measurement of r_{smax}.

On-line measurement of r_{smax} at the plant

The respiration measurements should be done with a continuous flow-through respiration meter. To determine r_{smax}, the variables r_{end} and r_{max} have to be measured continuously (eq. 1). The endogenous rate, r_{end}, is measured while activated sludge from a small aerated bypass tank flows through the respiration chamber (Figure 4). The residence time in this tank is long enough to allow all the readily biodegradable matter to be oxidised. r_{max} is measured while activated sludge from the aeration tank flows through the respiration meter. Meanwhile an excess of readily biodegradable matter is maintained in the respiration chamber; this is achieved by sufficiently loading the respiration chamber with wastewater.

During the measurement of the respiration rate, the mass balance of BOD_{st} for component 1 over the respiration chamber is as follows (steady state is assumed):

$$q_w f_1 s_w - (q_w + q_a) s_1 - \nu r_{s,1} = 0 \qquad (12)$$

In equation (12) the BOD_{st} in the sludge from the aeration tank is neglected, because it has only little bearing on the mass balance. According to equation (6) the substrate respiration rate (r_s) is a function of s. Using the condition

$$r_{s,1} = r_{smax,1} \quad if \quad s_1 \geq s_{c,1} \qquad (13)$$

and equation (12), we obtain:

$$\frac{q_w f_1 s_w - v r_{smax,1}}{q_a + q_w} \geq s_{c,1} \qquad (14)$$

or:

$$q_w s_w \geq \left((q_a + q_w) s_{c,1} + v r_{smax,1}\right) \frac{1}{f_1} \qquad (15)$$

For component 2 we can formulate a similar equation. The required loading is governed by only one component. Hence, in a way analogous to equation (11) the condition for the measurement of r_{smax} can now be formulated as follows:

$$q_w s_w \geq \max\left[\left((q_a + q_w) s_{c,1} + v r_{smax,1}\right) \frac{1}{f_1} \; , \; \left((q_a + q_w) s_{c,2} + v r_{smax,2}\right) \frac{1}{f_2}\right] \qquad (16)$$

Equation (16) demonstrates that the critical loading of the respiration chamber depends on the individual kinetic parameters (r_{smax} and s_c) and fractions (f) and on process constants (v, q_a and q_w). Because r_{smax} is a function of x (eq. 7), the critical loading is also a function of the activated sludge concentration. Using the parameters given in Figure 3 and the process constants of the measuring system, the critical loading may be calculated. However, because of uncertainty about the values of f, s_c and r_{smax}, the critical loading was determined experimentally in this study. Equation (16) was used *a posteriori* to verify the measurements.

Batch tests

In a batch test a known volume of influent sample, or another solution, is added to activated sludge and the respiration rate is recorded. Contrary to the continuous measurement where substrate is supplied continuously, the substrate concentration will decrease until all the substrate is entirely depleted. Provided that the initial concentration is high enough, the kinetic parameters can be derived from a batch test. The initial concentration expressed in terms of BOD_{st} is the total amount of oxygen used for the oxidation of the readily biodegradable matter:

$$s_0 = \int_{t_0}^{t_e} r_s(t)\,dt \tag{17}$$

This can be approximated by:

$$s_0 = \sum_{k=0}^{k_e} r_{s,k}\Delta t \tag{18}$$

If the total volume and the added volume are known, the BOD_{st} of the influent sample (s) can be calculated. Once the initial concentration is known, the BOD_{st} in the sludge during the batch test can be calculated:

$$s(t) = s_0 - \int_{t=0}^{t} r_s(t)\,dt \tag{19}$$

Using an Euler approximation:

$$s_k = s_0 - \sum_{k=0}^{k} r_{s,k}\Delta t \tag{20}$$

If a plot of s_k versus time yields a straight line above some critical concentration, the reaction is zero-order. The slope of the line represents r_{smax}. In the case of a sample with two components the individual r_{smax} and s_c can only be determined if both components have initial concentrations sufficiently higher than the critical concentrations and if the kinetic parameters of one component can be measured separately (e.g. Figure 2). The condition for the measurement of r_{smax} is:

$$s_0 \geq \max\left[\frac{s_{c,1}}{f_1}\ ,\ \frac{s_{c,2}}{f_2}\right] \tag{21}$$

In practise s_0 must be sufficiently greater than the minimum value, because the concentrations of both components decrease immediately after addition. Equation (21) illustrates that if one component occurs in a relatively small fraction, the initial total concentration needs to be relatively high. Provided that the batch test is conducted at an identical temperature and biomass, the total r_{smax} should be equal to the on-line measured value at the pilot plant.

5.4 Materials and methods

Activated sludge pilot plant

The activated sludge pilot plant (Figure 4) consisted of an unaerated contact tank including four 2 litre compartments in series, a completely mixed aeration tank (0.475 m^3) and a cylindric settler (0.159 m^3). The air flow to the aeration tank was set to a fixed value (2.4 m^3h^{-1}, later 3 m^3h^{-1}) using a Brooks mass flow controller (series 245191/1E). The DO concentration in the aeration tank was maintained above 2.5 mg l^{-1}. The pH was maintained at 7.5 using a WTW pH-191 pH meter and a hydroxide dosing pump. The temperature of the sludge was monitored and was found to vary between 15 and 18 °C. Sludge with endogenous respiration was produced in an aerated bypass tank, with a total volume of 24 litre, consisting of five compartments in series through which sludge from the aeration tank was continuously recirculated at a rate of 28.0 l h^{-1}). In batch experiments it was checked if this bypass tank produced endogenous sludge indeed.

Respiration measuring system

The respiration meter (prototype of RA1000, Manotherm) consisted of a closed, completely mixed respiration chamber of 0.731 litre through which activated sludge was continuously pumped at a rate of 21.5 l h^{-1}. The respiration rate was measured with an interval of 1 minute (Spanjers and Klapwijk, 1990).

The measuring system was connected to the pilot plant as shown in Figure 4. To measure alternately r_{end} and r_{max}, two valves were used to select sludge from the bypass tank and the aeration tank respectively. For the measurement of r_{max} influent sample was continuously fed into the sludge flowing into the respiration chamber. The influent sample loading required was determined experimentally and verified using equation (16). The measuring period for one type of respiration rate was 15 minutes which is sufficiently long to attain steady state.

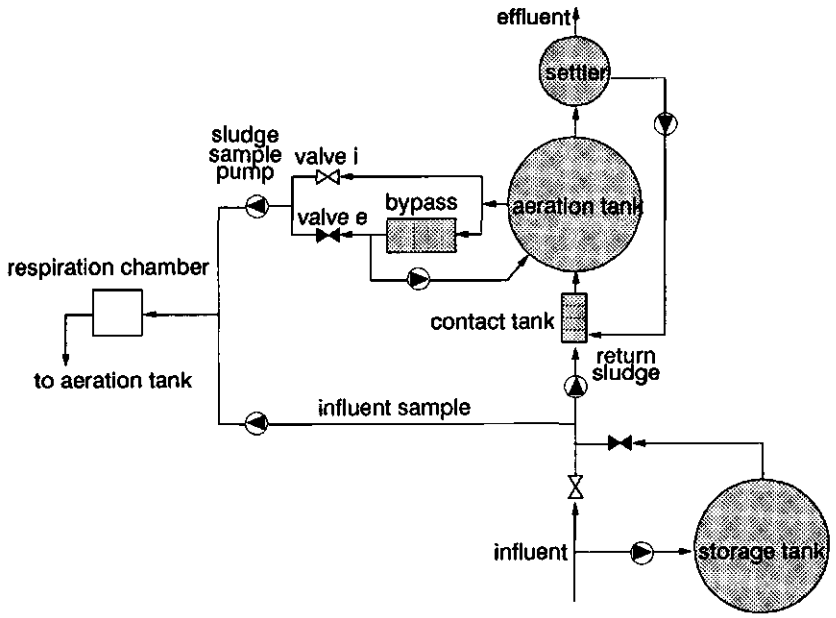

***Figure 4:** Scheme of the pilot activated sludge plant.*

The respiration rate was calculated as the average of the last three values. The control of valves and pumps, collection of data and the primary calculations were done by means of a modular I/O processor (μMac 6000, Analog Devices).

Influent

The plant was fed with presettled domestic wastewater. To study the effect of the influent flow on r_{smax}, the influent flow to the aeration tank was varied according to a square wave pattern as follows: 57 l h^{-1} from 6:00 h to 18:00 h and 16 l h^{-1} from 18:00 h to 24:00 h. The recycle ratio was maintained at one. To exclude the possibility that a change in influent quality would affect r_{smax}, wastewater with constant quality was used. Constant quality was ensured by taking water from a slowly stirred storage tank of 500 litres. Because of the limited capacity of this tank it could only serve as an influent source from 12:00 h to 24:00 h. The period from 0:00 h to 12:00 h was used to refill the tank with wastewater while the plant was simultaneously fed with the same water.

To check if the wastewater quality remained constant during storage, samples were taken at irregular intervals and analyzed for BOD_{st}, COD and ammonium concentration. As these variables did not change significantly during the first 6 hours of the storage period and the shape of the respirogram remained the same, we assumed that this also applied to the second half of the storage period.

To examine the effect of an absence of varying concentration of slowly biodegradable matter on r_{smax}, we only varied the loading with readily biodegradable matter during two days. Therefore, in addition to a constant wastewater flow of 23 l h^{-1} from the storage tank, a varying flow (zero from 18:00 h to 6:00 h and 35 l h^{-1} from 6:00 to 18:00 h) of a solution containing 53 mg of ammonium nitrogen and 50 mg of acetate per litre was fed into the plant. During this phase the influent sample for measuring r_{smax} was taken from the storage tank.

Batch tests

To check the measurements at the pilot plant, batch experiments were performed using sludge from the aeration tank. The sludge (1.5 litres) was transferred to an aerator which was connected with a respiration meter (Manotherm RA1000). The pH was kept constant at 7.5 using a WTW pH 96 meter and a dosing unit for 0.2 M HCl or 0.2 M NaOH. The temperature was maintained at the value of the temperature of the sludge in the pilot plant, averaged over the last six hours, using a Braun Thermomix 1441 / Frigomix 1496 water bath. Wastewater samples or other solutions were added to the sludge when the respiration rate had reached the endogenous level.

Measuring programme

Before the measurements were started, the plant was run 2 weeks to bring about steady state with respect to the endogenous respiration rate and the MLSS concentration.

First, it was determined experimentally what influent sample loading to the respiration chamber was needed to ensure maximum respiration rate under normal operating conditions. For this purpose a stepwise increasing flow of influent sample was applied to the respiration chamber after measuring the endogenous respiration rate. The influent was taken from a reservoir with wastewater. The BOD_{st} of this water was determined in a batch test. After each step the respiration rate was measured at steady state. Is was assumed that during this

experiment there would be no significant change in endogenous respiration rate, MLSS concentration and wastewater characteristics. The critical loading was defined as the loading above which no increase in respiration rate would be observed.

During the measuring period of 2 weeks r_{max} and r_{end} were monitored on-line, while the influent flow to the plant was varied according to a square wave pattern. In addition, the influent was alternately taken from the storage tank and the sewer. During the last three days the flow was varied only by adding a solution of readily biodegradable matter composed of ammonium and acetate.

From time to time, when influent was withdrawn from the storage tank, samples of sludge were taken from the aeration tank and transferred to the batch system to check the on-line measurements. r_{end}, the wastewater BOD_{st}, the fractions and the kinetic parameters (r_{smax} and s_c) of the two components were assessed. For comparison also ammonium and acetate/ammonium mixtures were added now and then.

During the measuring period sludge samples were frequently taken from the aeration tank for MLSS analysis (Dutch standard NEN 3235 4.1). One reason for this analysis was to check if the MLSS concentration remained constant after a change in influent flow rate. At irregular time intervals influent and effluent grab samples were taken for the analysis of COD, Kjeldahl nitrogen, ammonium, nitrite and nitrate (NEN 6472, 6474 and 6440 respectively, adapted by Skalar).

5.5 Results and discussion

Influent sample loading required for maximum respiration

The influent sample loading to the respiration chamber needed to ensure maximum respiration rate under normal operating conditions was determined. For this purpose an increasing influent sample flow was applied to the respiration chamber, after measuring the endogenous respiration rate. Further, the respiration rate was measured at steady state. Figure 5 shows the substrate respiration rate (r_s), calculated using equation (1), as a function of the influent sample loading applied.

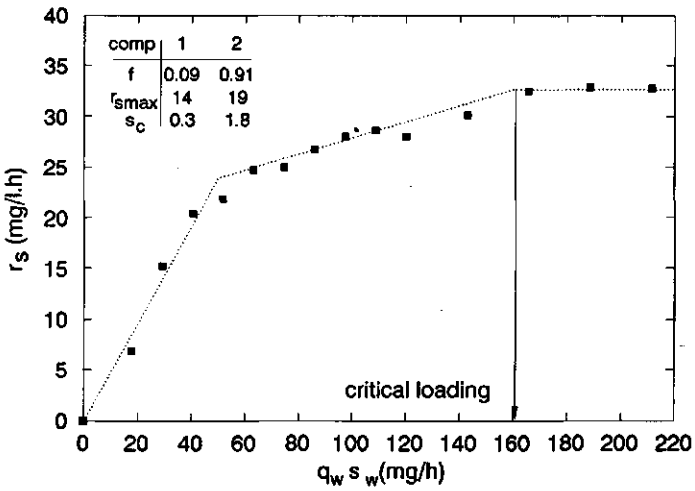

***Figure 5:** Substrate respiration rate as a function of the influent sample loading. Influent BOD_{st}=295 mg l^{-1}, MLSS=2.0 g l^{-1}. Dotted line: model fit.*

The figure also shows a fit of the model based on equations (6) and (12), using values of f_1 and f_2 obtained in the batch test of Figure 2. The kinetic parameters obtained are also listed in the figure. The critical loading found in this way is 160 mg h^{-1}. Because under normal operating conditions the BOD_{st} of the wastewater used is higher than 145 mg l^{-1} in 90% of the cases, the influent sample flow rate (q_w) was fixed at 1.1 l h^{-1} during the entire measuring period of two weeks.

The experiment described above was repeated several times during another measuring period. It was found that the individual curves did not always show two distinct break points corresponding with the achievement of saturation with one of the components.

Therefore, the statement that the BOD_{st} of the wastewater utilised typically can be represented by two components was not always confirmed by these experiments. However, a critical influent sample loading, where r_s approached r_{smax} could be determined in most cases.

Effect of the influent flow on r_{smax}

During a period of two weeks the maximum respiration rate (r_{max}) and the endogenous respiration rate (r_{end}) were measured while the influent flow to the plant varied according to a square wave pattern with a period of 24 hours. The objective of these measurements was to investigate the effect of the influent flow on r_{smax} during the phases with constant influent quality. Figure 6 represents typical results of three consecutive days of measurement. Figure 7 shows a summary of the measurements performed during the phases with influent from the storage tank, r_{smax} and r_{end} for the whole measuring period. The maximum substrate respiration rate, r_{smax}, was calculated according to equation (1). Table 1 lists the relevant process conditions.

Table 1: *Process conditions of the pilot plant. The influent characteristics refer to the stored wastewater; the effluent data are from grab samples taken at 15:00 h. Activated sludge samples for MLSS were taken between 9:00 h and 19:00 h. BOD_{st} and O/N (mg of oxygen needed for the oxidation of 1 mg NH_4-N) were determined in batch tests.*

	influent				effluent		act. sludge	
day	BOD_{st}	COD	N_{Kj}	NH_4	COD	NH_4	MLSS	O/N
	(mg l^{-1})	(mg l^{-1})	(mgN l^{-1})	(mgN l^{-1})	(mg l^{-1})	(mgN l^{-1})	(g l^{-1})	
1	-	306	57.1	39.0	53	0	2.38	-
2	172[1]	276	48.1	32.6	36	0.3[2]	2.26	4.3
3	89	192	31.6	22.1	43	0	2.23	3.7
4	-	285	42.0	30.7	42	1.5	2.29	-
7	-	-	-	-	-	-	2.42	-
8	217	463	88.3	51.0	73	2.8	2.39	-
9	295	474	72.9	75.9	68	1.5	2.34	4.4
10	-	471	68.3	63.4	55	1.1	2.09	-
11	309	471	63.8	65.3	27	1.3	2.3	4.4
12	382	590	81.0	-	34	-	2.26	4.3
14	-	-	-	-	-	-	2.05	-

[1] standard deviation (n=5): 5 mg l^{-1}

[2] 2.43 mgN l^{-1} at 19h00

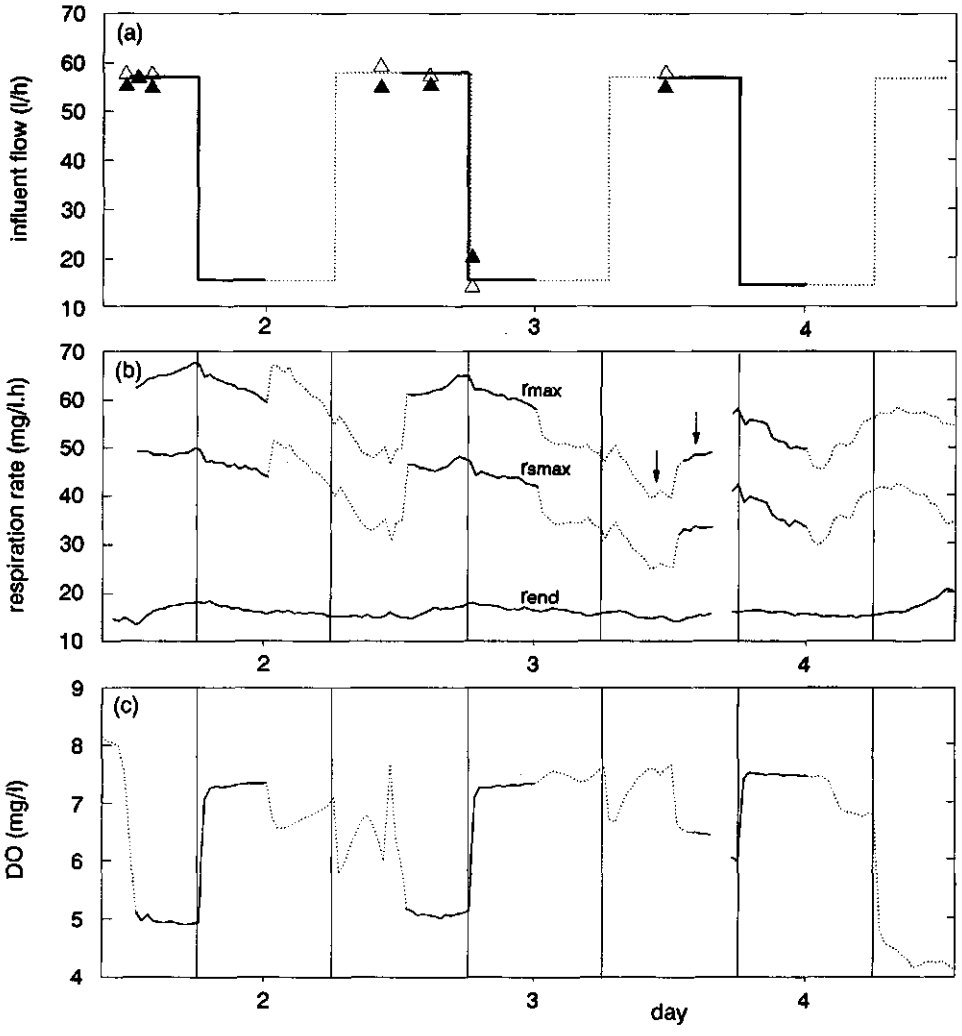

Figure 6: *Representative selection from a two weeks measuring period. Solid lines indicate phases with influent from the storage tank; dotted lines refer to phases with influent from the sewer. (a) Influent and return sludge flow; markers indicate flow measurements; filled: influent, open: return sludge. (b) Respiration rates; arrows indicate short duration increases of influent sample flow from 1.1 to 1.3 l* h^{-1}. *(c) DO concentration in aeration tank.*

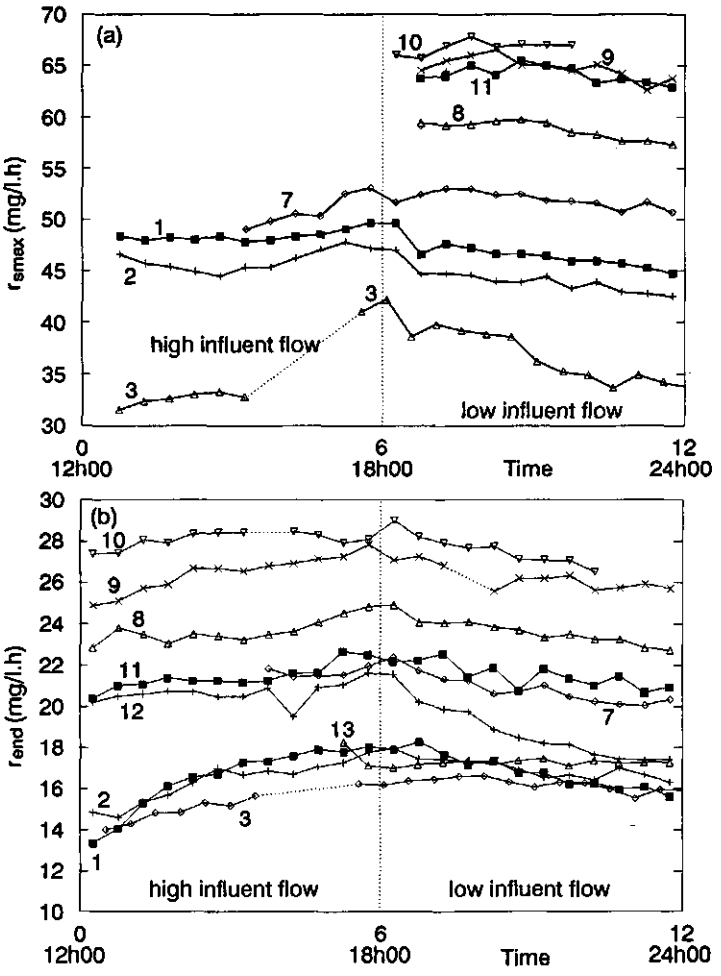

Figure 7: *(a) r_{smax} and (b) r_{end} during the phases of wastewater feeding from the storage tank. Day numbers indicated in the figure.*

Is was verified that the MLSS concentration in the aeration tank did not change significantly in the period from 8:00 h to 12:00 h (not shown in Table 1). Figures 6b and 7 show that, when the influent flow was abruptly changed from a high value to a low value, there was no

instantaneous change in r_{smax}. This was expected for a variable being only dependent on wastewater composition and sludge properties. The DO concentration in the aeration tank, which is a function of the actual respiration rate, immediately responded to the change of influent flow (Figure 6c). When the plant was fed with wastewater from the storage tank, r_{smax} and r_{end} (Figure 6b and 7) slightly increased at high influent flow and decreased at low influent flow, whereas the DO concentration remained constant (Figure 6c). The variation of r_{end} was probably due to changes in the concentration of slowly biodegradable matter and in active biomass concentration. When the influent was taken directly from the sewer, both r_{smax} and DO varied considerably (Figure 6b and 6c).

During the last four days, starting on day 11 at 9:00 h, a high influent flow was applied with a solution of readily biodegradable matter (ammonium and acetate) instead of wastewater, while a small influent flow from the storage tank was maintained. On day 11 r_{smax} was still measured while introducing influent into the respiration chamber. This strategy would make it possible to reduce a possible effect on r_{smax} caused by slowly biodegradable matter. Unfortunately during the period of high loading with acetate and ammonium the DO concentration in the respiration chamber dropped below 1.5 mg l^{-1}, being obviously a limiting value for the oxidation process. Therefore, the respiration rate measured during this period could not be used. Figure 7a shows that after this period, when a low influent flow was applied and when no DO limitation occurred, r_{smax} did not change significantly.

On the days 12 to 14 r_{smax} was measured by introducing a solution of ammonium chloride instead of influent sample into the respiration chamber, in order to measure r_{smax} due to nitrification. Figure 8 shows the respiration rates when no DO limitation occurred.

During both periods of low influent flow r_{smax} decreased significantly (12 mg $l^{-1}h^{-1}$ in 12 hours) after termination of the acetate/ammonium supply. Although a reliable r_{smax} could not be monitored during the periods with acetate/ammonium supply, it was obvious that its value increased. From the moment the feeding with a acetate/ammonium mixture was started, r_{end} gradually decreased from 26 mg $l^{-1}h^{-1}$ on day 11 to a constant value of 17 mg $l^{-1}h^{-1}$ on day 14. This is partly visible in Figure 8.

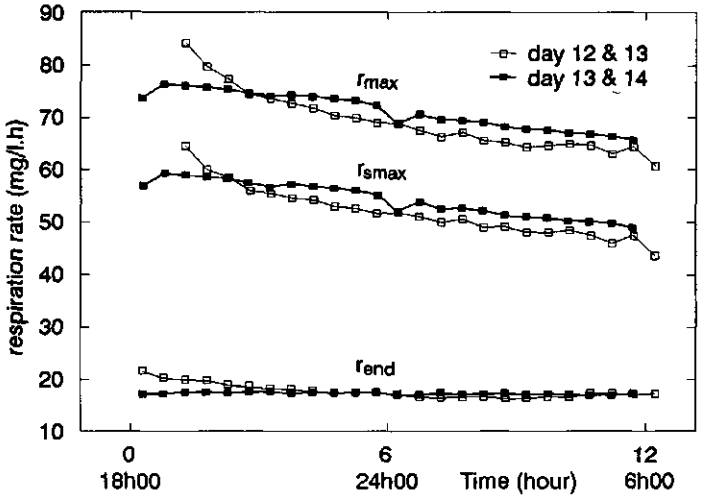

Figure 8: *Respiration rates during periods with low influent flow after high loading with an acetate/ammonium mixture.* r_{smax} *(measured during addition of ammonium) represents the nitrification.*

Batch tests

A sample of activated sludge from the aeration tank was transferred to the batch system. After the endogenous respiration was reached, a known volume of wastewater from the storage tank was added and the respiration rate was recorded until the endogenous rate was reached again. Addition was repeated with another sample of wastewater and, in most cases, with an ammonium solution and an acetate/ammonium mixture. The aim of the batch tests was to verify the on-line measured r_{smax}. For this purpose the following information was derived from the respirograms: r_{end}, wastewater BOD_{st}, fractions (f) and kinetic parameters (r_{smax} and s_c) of the two components.

In all the respirograms obtained two components could be detected: one being a minor fraction (component 1), the other (component 2) being a major fraction of the total BOD_{st}. This is illustrated for the batch tests on day 12 (Figure 9). Figure 9 also shows respirograms obtained after the addition of ammonium and acetate/ammonium mixture to the same activated sludge. These respirograms will be discussed later in this section.

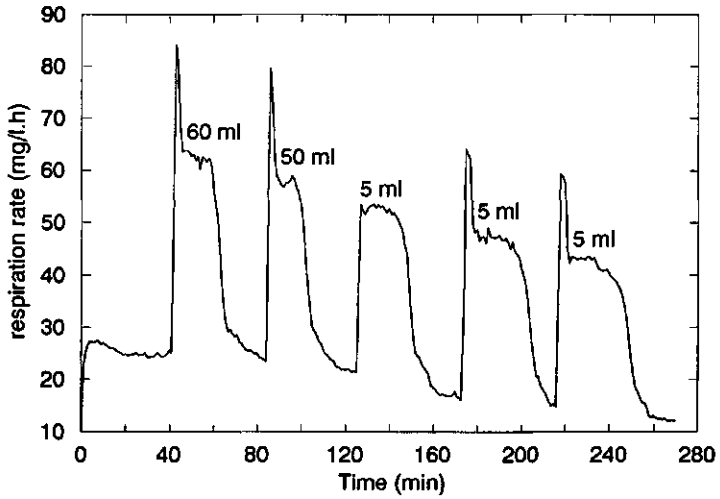

***Figure 9:** Batch tests on day 12. Respirograms of: wastewater (twice), ammonium chloride (1 gN l^{-1}) and (twice) a mixture of acetate (1.95 g of acetate per litre) and ammonium (1.05 g N l^{-1}). Initial volume of activated sludge 1.5 litre. The volume of additions is indicated in the figure.*

The BOD_{st} of the wastewater samples is listed in Table 1 and reproduced in Table 2. Since the influent sample flow rate during the continuous measurement was 1.1 l h^{-1}, the minimum influent BOD_{st} needed to achieve the critical loading of 160 mg h^{-1}, was 145 mg l^{-1}. This condition was not met on day 3 (Table 2).

Using equation (21) and the values 0.09 and 0.8 for f_1 and $s_{c,1}$ respectively (Figure 2), we obtain the minimum initial concentration required for the measurement of r_{smax} in the batch tests: $s_0 \geq 8.8$ mg l^{-1}. According to Table 2 this condition was not met in the batch tests of days 2 and 3. The other tests were used to assess f, r_{smax} and s_c (Table 2).

The fractions were calculated by using equation (18) for the individual components in the respirogram. Due to the low quantity of component 1 in all the additions (cf. Figure 9) together with the limited measuring frequency of the respiration meter, the precision of the calculated fraction was low and f_1 was probably underestimated. The value of r_{smax} was derived from the peak height in the respirograms. Although the precision was low for the reason mentioned above, the duplicate values did not differ more than 4%. The value of $r_{smax,2}$ was derived from the slope of s (eq. 20) as a function of time. An example of this

function is given in Figure 11. The slope represents $r_{smax,2}$, because component 1 was already oxidised in the very beginning of the test. The critical concentration $s_{c,2}$ was found at the point where s diverged more than 0.1 mg l^{-1} from the optimum regression line (Figure 11).

Initially, it was attempted to estimate f and the kinetic parameters r_{smax} and s_c by fitting a dynamic model, based on first/zero order kinetics (eq. 6), of the batch system to the respirogram data. Figure 10 gives an example using the data of the batch test on day 12. However, the parameters could not be calculated with reasonable accuracy due to the small number of measuring points representing oxidation of component 1 and an initial time lag of the respiration rate which was not included in the model. Therefore, this method was not used.

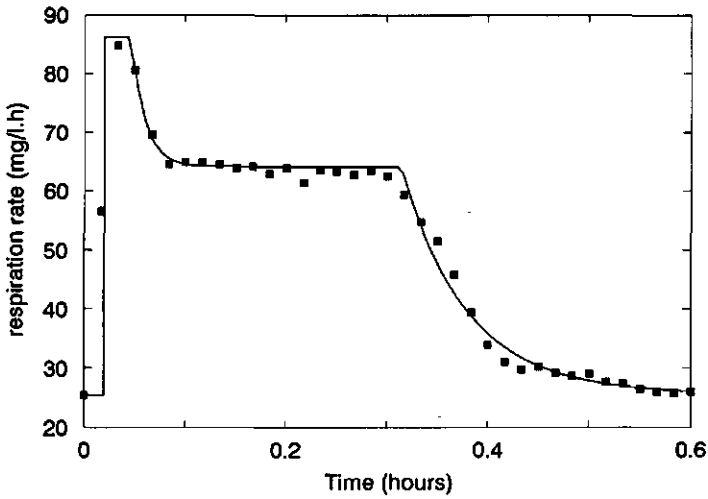

Figure 10: *Dynamic optimisation of a model for the batch system, based on first/zero order kinetics. Points: measurements on day 12; line: model prediction.*

Comparison of both measurements of r_{smax} (Table 2), reveals that the batch tests yielded values below the range of the on-line measured values on four days. On the days 2 and 3 this may have been due to s_0 being too low, as mentioned before. However, this could not be said of day 9 and 11. On two days the batch tests yielded values above the on-line range. On day 12 this was obviously caused by DO limitation during the on-line measurement of r_{smax}.

Table 2: *Kinetic parameters and fractions derived from batch experiments. Batch values of r_{smax} are compared with the ranges of the on-line measured values (constant influent quality). Only the last column applies to on-line measurements.*

day	s_w (mg l^{-1})	s_0 (mg l^{-1})	f_1 (-)	f_2 (-)	$s_{c,2}$ (mg l^{-1})	$r_{smax,1}$ (mg $l^{-1}h^{-1}$)	$r_{smax,2}$ (mg $l^{-1}h^{-1}$)	r_{smax} (mg $l^{-1}h^{-1}$) batch	r_{smax} (mg $l^{-1}h^{-1}$) on-line[1]
2	172	6.6	0.06	0.94	1.4	10	28	38	34-47
3	89	3.4	0.02	0.98	1.1	4	26	30	31-41
8[2]	218	8.8	0.07	0.93	1.4	26	44	70	57-60
9	295	11.3	0.06	0.94	2.0	22	34	56	63-66
11	309	11.9	0.05	0.95	2.1	21	40	61	63-65
12	382	14.7	0.05	0.95	2.5	21	37	58	36-39[3]

[1] high and low influent flow

[2] temperature of batch test 8°C higher than in pilot plant

[3] r_{smax} under DO limitation

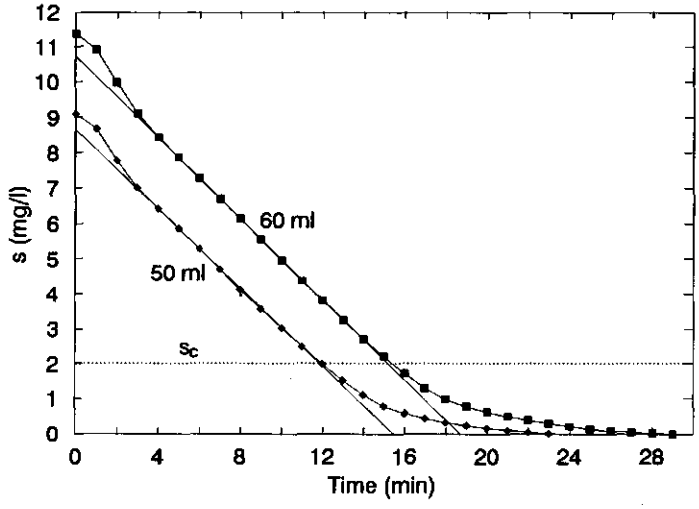

Figure 11: *BOD_{st} (s) as a function of time in the batch test on day 9. Two additions with different volumes of wastewater are presented. Addition volume and linear regression lines are indicated. The slope of the regression line represents $r_{smax,2}$.*

It should be noted that the parameters f and s_c are not necessarily constant during on-line measurement. A change in these parameters will influence the critical loading. Table 3 shows values of critical loading, calculated according to equation (16) using the parameters in Table 2, next to the loadings applied. Because f_1 was probably underestimated in Table 2, the value from Figure 2 (0.09) was used. As expected, component 1 governed the critical loading.

According to Table 3 the calculated critical loading was satisfied on the days 2, 9, 11 and 12. However, r_{smax} was measured under DO-limitation on day 12. Compared to Figure 5, showing a critical loading of 160 mg h^{-1}, the values in Table 3 are higher. This is due to the higher values of $r_{smax,1}$ during the measuring period.

***Table** 3: Critical loading (eq. 16) next to loading applied on the basis of the BOD_{st} of the wastewater and q_w=1.1 l h^{-1}.*

	loading (mg h^{-1})	
day	critical	applied
2	156	189
3	108	98
8	286	240
9	254	325
11	246	398
12	246	420

Figure 9 shows that the oxidation of component 2 and the nitrification result in approximately equal values of r_{max} and that the composition of the readily biodegradable part of the wastewater was reasonably simulated by the mixture of acetate and ammonium, although there was some evidence showing that this mixture (calculated fractions in BOD_{st} equivalents: 0.88 and 0.12) contained a somewhat higher fraction of component 1. To be able to compare $r_{smax,2}$ of wastewater and r_{smax} of ammonium, we calculated activities by dividing maximum substrate respiration rates by MLSS concentrations (Table 4).

Table 4 shows that, with one exception, component 2 of the wastewater was oxidised with higher activities than ammonium. In a mixture of acetate and ammonium the activity of ammonium oxidation was lower than when pure ammonium was added.

Since r_{end} is essential in the calculation of r_{smax} and BOD_{st}, its meaning was studied in some detail. Most batch respiration measurements were started as quickly as possible after sampling sludge from the aeration tank of the pilot plant. In all cases, also when the influent

BOD_{st} was high (e.g. on day 12; see Table 1), the respiration rate was at the endogenous level within 15 minutes after sampling. Since the hydraulic residence time in the bypass tank was 0.9 hours, it was concluded that this time was long enough to obtain sludge with the endogenous respiration rate. Continuing the measurement revealed that r_{end} gradually decreased (e.g. from 25 to 8 mg $l^{-1}h^{-1}$ in 17 hours). However, within the time scale of a BOD_{st} measurement, this decrease was negligible (cf. Figure 9).

***Table 4:** Activities with regard to the oxidation of component 2 and ammonium, measured in batch tests. Activity (in mg $g^{-1}h^{-1}$) is calculated by dividing maximum substrate respiration rate by MLSS concentration.*

day	wastewater component 2	ammonium	ammonium/ acetate mixture
2	12.2	11.0 (11.5)[1]	-
3	12.4	12.6	-
8	19.6	-	-
9	15.8	13.5	-
11	18.0	14.7	-
12	17.3	16.1	14.6

[1] repetition of measurement with a fresh sludge sample

DO limitation

During a number of measuring periods with high influent flow, r_{max} was (unintentionally) limited by the DO concentration in the respiration chamber. Therefore, these measurements were not used in examining the effect of influent flow on r_{max}. Figure 12a gives an example of this DO limitation.

The fact that a peak in DO concentration (Figure 12b), probably due to a change in the effluent quality, corresponds with an increase in respiration rate (Figure 12a) suggests that r_{max} was limited by the DO concentration. Influent supply from the storage tank was started at 12:00 h. At 15:00 h the air flow to the aeration tank was increased in order to raise the DO concentration in the tank and the respiration chamber respectively. This resulted in a higher but still limited r_{max}. At 18:00 h the influent flow was lowered, resulting in an increase in DO concentration and a r_{max} non limited by DO.

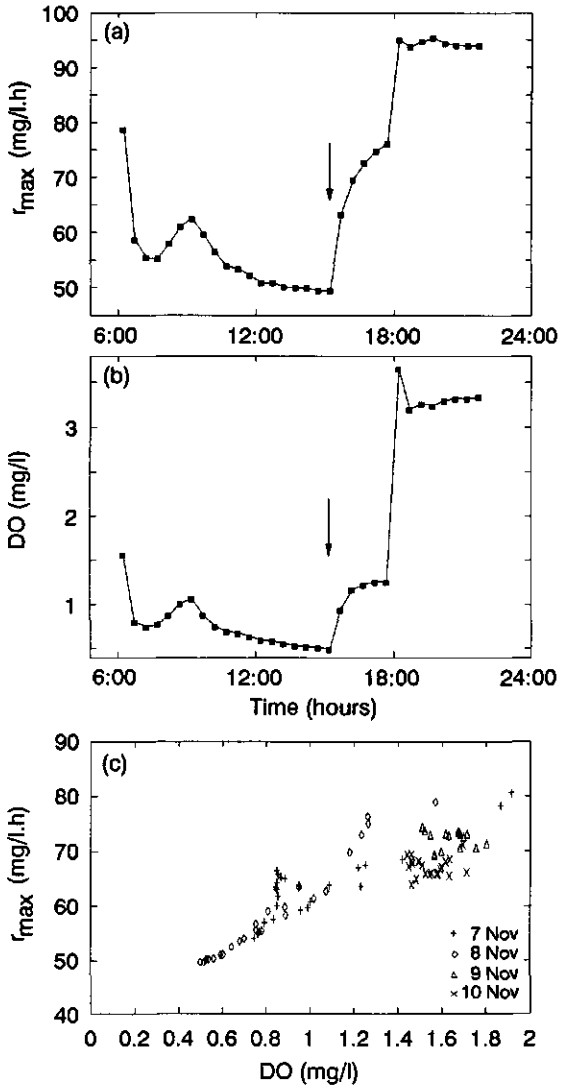

Figure 12: *Effect of DO limitation on r_{max}. (a) r_{max} during a measuring period with high influent flow on day 10. (b) DO in respiration chamber during the same period. Arrows: increase in air flow to aeration tank. (c) r_{max} as a function of DO concentration during several periods with high influent flow.*

Figure 12c summarises several measuring periods with DO-limitation. Although the figure represents days with potentially different wastewater and sludge characteristics it shows a strong relationship between r_{max} and DO for DO concentrations below 1.3 - 1.4 mg l^{-1}. Above this value, r_{max} seems to be independent of DO concentration.

5.6 General discussion

The purpose of this work was to (1) specify the condition to be met during on-line measurement of the maximum substrate respiration rate (r_{smax}) of activated sludge from a plant fed with wastewater from a particular source; (2) study the effect of the influent flow on r_{smax}, and (3) indicate how batch tests can be used to check the on-line measurement of r_{smax}.

We assumed that the readily biodegradable part of the wastewater is made up of two components, one of them (component 1) forming a small fraction of the total BOD_{st}. This was found to be typical of the wastewater considered. The condition to be met for measuring r_{smax} is that both components are present in excess in the activated sludge flowing through the respiration chamber. It was demonstrated that this condition for measuring r_{smax} particulary depends on the kinetic parameters of component 1, because it was found to form an inferior fraction of the total BOD_{st}. Knowing the fractions and kinetic parameters of both components, the required loading of the respiration chamber can be calculated. In reality the fractions and kinetic parameters are not exactly known. It is even possible that one component is completely absent. For example, in another investigation (Chapter 1) carried out with wastewater of the same origin we observed only one component in the respirogram.

In our study of the effect of the influent flow rate on r_{smax} we determined the critical loading prior to the measurements by increasing the influent sample loading of a wastewater with constant BOD_{st} (Figure 5). Next, the influent sample flow was fixed at a value that would ensure the measurement of r_{smax} under normal operating conditions. The ratio of influent sample flow to sludge flow (q_w/q_a) was 0.05. In an earlier paper (Spanjers and Klapwijk, 1990) we reported a ratio of 0.03. There are two reasons to believe that this value was too low. First, the possible occurrence of a second component in the form of an inferior fraction was not considered, so that saturation with respect to only one component was achieved. Second, the MLSS concentration was almost twice as high as the one used in the study reported here, resulting in a higher oxidation rate and consequently a lower BOD_{st} in the respiration chamber.

During the measurements with influent of constant quality we generally did not observe, on a short term, an effect of the influent flow on r_{smax} (Figures 6b and 7), as was seen in previous research (Spanjers and Klapwijk, 1990). If any, the effect was opposite: respectively a slight increase and slight decrease during high and low influent flow. Since BOD_{st}, MLSS concentration and temperature remained constant during these periods, this effect has to be attributed to changes in active biomass concentration. On a longer term r_{smax} changed significantly (Figure 6 and Figure 7). During the days 1 to 4, this was probably due to a decreasing concentration of active biomass, caused by the low influent strenght. In some cases r_{smax} changed instantaneously when the influent source was changed from one source to the other and r_{smax} varied more when the influent was taken from the sewer. See for instance day 2 (Figure 6). In this particular case it was unlikely that the loading was much lower than in case of wastewater from the storage tank. One explanation of this varying r_{smax} is that the fractions of the components vary substantially so that saturation is not always achieved. Because in practise the influent is not of constant quality, the on-line measured r_{smax} would be difficult to interpret.

The critical loading determined prior to the measurements (Figure 5) was only valid for the whole measuring period, if the fractions and kinetic parameters remained constant. Because this could not be guaranteed, the correct measurement of r_{smax} had to be checked in batch tests. The results of the batch test were used in three steps. First, the BOD_{st} of the wastewater was determined and it was checked if its value was high enough to achieve the critical loading determined in Figure 5. Second, r_{smax} was derived from the respirogram. Equation (21) was used to check if the initial BOD_{st} in the batch reactor was high enough to measure r_{smax}. Third, the respirograms were evaluated to determine the fractions and kinetic parameters. These were used the reevaluate the condition for measuring r_{smax}.

The first verification showed that on day 3 the BOD_{st} (Table 2) was not high enough to achieve the critical loading according to Figure 5, indicating that r_{smax} was not correctly measured. This is also suggested by Figures 6b and 7, where r_{smax} shows varying and relatively low values.

For the second verification the initial BOD_{st} required for deriving r_{smax} from the respirogram was considered to be high enough on the days 8, 9, 11 and 12 (Table 2). There was, however, a difference between the values of r_{smax} with regard to on-line and batch measurements. One explanation for this is that the number of points representing the oxidation of component 1 was too small to obtain a reliable value of r_{smax} from the peak height (cf. Figure 9).

In the third verification step the parameters f, s_c and r_{smax} of the individual components had to be derived from the respirograms. However, due to the low accuracy with which the oxidation of component 1 was measured, f and $s_{c,1}$ were derived from Figure 5. Using these parameters, the critical loading for each influent sample was calculated according to equation (16). All values, with the exception of that of day 3, were greater than the one obtained from Figure 5 (Table 3). The actual loading applied was greater than the calculated critical loading on the days 9, 11 and 12.

It is concluded that the second and third step of the batch verification tests did not provide consistent results. This can be attributed to the low accuracy in estimating the parameters of component 1. Since deriving BOD_{st} and comparing it with the experimentally determined critical loading (step 1) alone will not be sufficient, the parameters have to be determined anyway. The parameters can be determined more accurately in batch tests, if the added volume of wastewater is optimised. This volume may not become too large because of the difficulty in defining the endogenous respiration rate (cf. Figure 2). Additionally, the results of such a batch test can be used to identify a kinetic model for the oxidation of readily biodegradable matter (cf. Figure 10).

Comparative batch studies with addition of wastewater, ammonium and acetate/ammonium mixtures indicated that component 2 of the wastewater is probably nitrogen oxidised by nitrifiers (Figs. 1 and 9, Table 4). This was also found in a previous investigation (Spanjers and Klapwijk, 1990). This implies that r_{smax} essentially reflects the nitrification rate. There are two ways to evaluate r_{smax}: 1) the oxidation of both components is considered, 2) only the nitrification is considered. In this work we showed that a relatively high loading of the respiration chamber is required to achieve saturation with regard to component 1. In case the fraction of this component varies considerably and particularly in case it becomes very small, the required influent sample loading will be high. This loading may then be impracticably high because of the dilution of the activated sludge or inhibitory effects. When only the nitrification is of interest, a much smaller influent sample loading will suffice. In that case the rate measured will be the maximum nitrification rate plus a small contribution of component 1.

5.7 Conclusions

It has been shown that r_{max} can be measured if a sample of wastewater is continuously fed into a flow-through respiration chamber, so that the loading exceeds a certain critical loading. In the situation considered this critical loading particulary depends on the parameters of one

component, which constitutes an inferior fraction of the total BOD_{st}.

The effect of the influent flow on r_{smax}, reported in a previous paper, was not observed in this study. Batch tests proved to be useful to check the on-line measurement but they should be carefully executed.

5.8 Notation

BOD_{st} short-term biochemical oxygen demand
DO dissolved oxygen
MLSS mixed liquor suspended solids
f fraction of the total BOD_{st} (-)
q_a activated sludge flow through respiration chamber (volume $time^{-1}$)
q_w influent sample flow through respiration chamber (volume $time^{-1}$)
r respiration rate (mass $volume^{-1}time^{-1}$)
r_{end} endogenous respiration rate (mass $volume^{-1}time^{-1}$)
r_{max} maximum respiration rate (mass $volume^{-1}time^{-1}$)
r_s substrate respiration rate (mass $volume^{-1}time^{-1}$)
r_{smax} maximum substrate respiration rate (mass $volume^{-1}time^{-1}$)
s BOD_{st} in respiration chamber (mass $volume^{-1}$)
s_c critical BOD_{st} (mass $volume^{-1}$)
t time (time)
v volume respiration chamber (volume)
x biomass concentration (mass $volume^{-1}$)
y yield coefficient (mass $mass^{-1}$)
μ growth rate ($time^{-1}$)

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6

Modelling of the dissolved oxygen probe response in the improvement of the performance of a continuous respiration meter

6.1 Abstract

In the present technique for the measurement of the respiration rate, the DO-probe is repeatedly subjected to a step change of the oxygen concentration with a time interval of typically 20-30 s. The end value of each response is used to calculate the respiration rate of activated sludge. However, those end values can be inaccurate because of probe dynamics or probe response time. This chapter describes an improved method to measure the dissolved oxygen (DO) concentration with one and the same probe at the inlet and outlet of a respiration chamber. The real DO concentration at the end of each response can be estimated by fitting the DO measurements to a first-order response model of the probe. Furthermore, at each step response the time constant of the probe response can be estimated which provides a continuous diagnosis of the probe condition. The proposed method provides a reliable estimate of the real DO concentration and, as a result, the reliability of the calculated respiration rate is improved. The first-order probe response constant is a useful indicator for fouling of the probe membrane.

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6.2 Introduction

The respiration rate of activated sludge is an important variable for the control of the activated sludge process. It provides information as to the loading to the plant (Olsson and Andrews, 1978), the influent and effluent BOD (Spanjers and Klapwijk, 1990), the microbial activity (Vargas Lopez, 1988) and it can indicate toxic effects on the activated sludge (Temmink *et al.*, 1990). In batch experiments respiration rate measurements can be used for studies on biodegradation (Grady *et al.*, 1989), kinetics (Ossenbruggen *et al.*, 1991) and toxicity (Spanjers and Klapwijk, 1986).

Different respiration meters, measuring the uptake of oxygen by the activated sludge, are described in the literature. The manometric respiration meter is based on the measurement of the volume of oxygen gas used by the bacteria (Hisset *et al.*, 1982). Other meters use some oxygen sensing device which measures the oxygen concentration from which the respiration rate can be calculated.

The electrochemical oxygen sensor is based upon the electrochemical reduction of oxygen in an amperometric cell. The electrodes are immersed in an electrolytic solution which is separated from the bulk solution by a semi-permeable membrane. The electrode signal is determined by the diffusion of dissolved oxygen (DO) from the bulk through the membrane. The electrochemical sensor is used in the respiration meter described in this chapter. The probe-meter combination will be referred to as the "DO-probe".

Two types of respiration meters where the DO-probe is used can be distinguished: the batch respiration meter and the continuous flow-through respiration meter. A batch meter is operated by withdrawing a sample of activated sludge from the plant into a small vessel, aerating it and then monitoring the decline in the DO concentration with time. The respiration rate is calculated from the slope of the DO decline (Fujimoto *et al.*, 1981; Kaneko *et al.*, 1985). The continuous flow-through meter measures the DO concentration at the inlet and outlet of a closed respiration chamber through which the sludge is pumped continuously (Sollfrank and Gujer, 1985; Spanjers and Klapwijk, 1990). The respiration rate is calculated from the difference of the two DO measurements.

The respiration meter as described by Spanjers and Klapwijk (1987, 1990) has proven its usefulness and its reliability. The characteristic of this meter is that the DO concentration in the inlet and outlet is measured with one single probe, fixed at one opening of the chamber, by alternating the flow direction through the chamber (Figure 1).

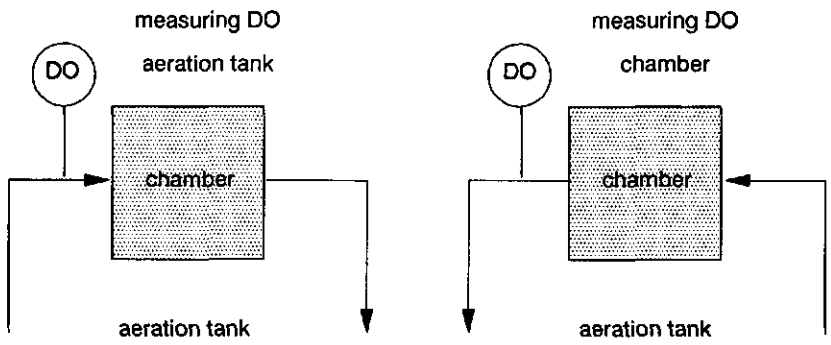

***Figure 1**: Scheme of the one-probe continuous respiration meter.*

When the DO probe is replaced by another sensor, such as a nitrate or chlorine probe, the measuring technique may also be applied to measure the production or utilization of those compounds. This application will not be considered here.

One problem with this principle is that a steady state DO reading is only available when the DO probe signal, after changing the flow direction, has reached its end value. Hence, the maximum measuring frequency for the DO concentration is limited by the response time of the DO probe. Till now, in practise, the measurement of the DO takes place when the signal has reached about 95% of its ultimate value. The time needed to attain this 95% response (response measuring period) is based on experience. As both the inlet and outlet concentration may be off the real value, a systematic error of about 10% may occur in the calculated respiration rate. Furthermore, a dirty membrane and a consequently slower DO response may result in an even higher error contribution.

A second problem is that at the end of each response measuring period only one concentration is available: either the DO at the inlet or the DO at the outlet. This means that in the calculation of the respiration rate the other DO value has to be estimated. Till now this has been done by means of linear interpolation. This problem will be discussed elsewhere in this thesis. For an accurate measurement of the respiration rate it is important to measure both DO concentrations as frequently as possible and to extract maximum information from the probe response.

A general problem with DO probes is that a probe failure caused by, for example, fouling of the membrane, may be interpreted as a change in the DO concentration. When the probe is applied in a respiration meter, the failure is interpreted as a change in the

respiration rate (Temmink, 1992).

In this chapter a first-order probe response model is proposed. Due to a short measurement time the steady state DO concentration has not been reached. Then the final DO concentration can be estimated from the model. Furthermore the first-order probe response time constant will be estimated after each response, which can be used for probe performance diagnosis. The estimation method is based on a least squares fit. Simulations and batch experiments are used to demonstrate the effectiveness of the method.

6.3 Model of the DO-probe response

It is assumed, and experimentally verified, that the DO probe signal can be modelled by a first-order dynamic system and that the real DO concentration during the response measuring period (typically 20-30 s) will change along a linear slope. The response of the DO probe after a change in the flow direction can then be considered as a combined step and ramp response of a first-order system (Figure 2).

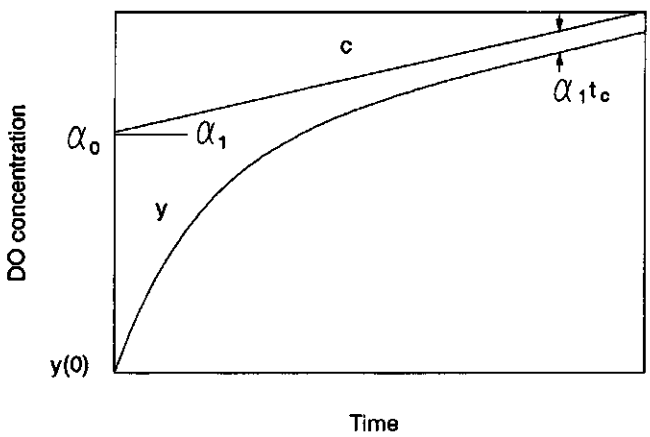

***Figure 2:** Combined step and ramp response of the DO probe after changing the direction of the sludge flow. First-order linear system assumed.*

The DO probe signal y is modelled by a first-order dynamic system:

$$t_c \frac{dy}{dt} = -y + C \tag{1}$$

Where the input C is the real DO concentration which is assumed to be a ramp function with offset α_0 and slope α_1:

$$C = \alpha_0 + \alpha_1 t \tag{2}$$

Combining (1) and (2) gives the response function:

$$t_c \frac{dy}{dt} = -y + \alpha_0 + \alpha_1 t \tag{3}$$

There is an analytical solution of (3) that can be written in the form:

$$y(t) = e^{-t/t_c}\, y(0) + \int_0^t e^{-(t-\tau)/t_c} u(\tau)\, d\tau \tag{4}$$

where y(0) = the initial condition of the probe signal at time t=0,
u = $(\alpha_0+\alpha_1 t)/t_c$.

Inserting the value of u gives the DO response as a function of time:

$$y(t) = \left[(y(0) - \alpha_0 + \alpha_1 t_c) e^{-t/t_c}\right] + \left[\alpha_0 + \alpha_1 t\right] - \left[\alpha_1 t_c\right] \tag{5}$$

It is recognized that the first term of (5) converges to 0 after some time, determined by the probe response time. The last term of (5) is then seen to show the lag between the real DO concentration and the probe response after the transient. Figure 3 shows the result of a simulation using (5) for different realistic values of the parameters. The simulation demonstrates that, when the real DO concentration changes along a linear slope, the

ultimate value of the DO signal (y) always deviates $\alpha_1 t_c$ from the real DO concentration (C).

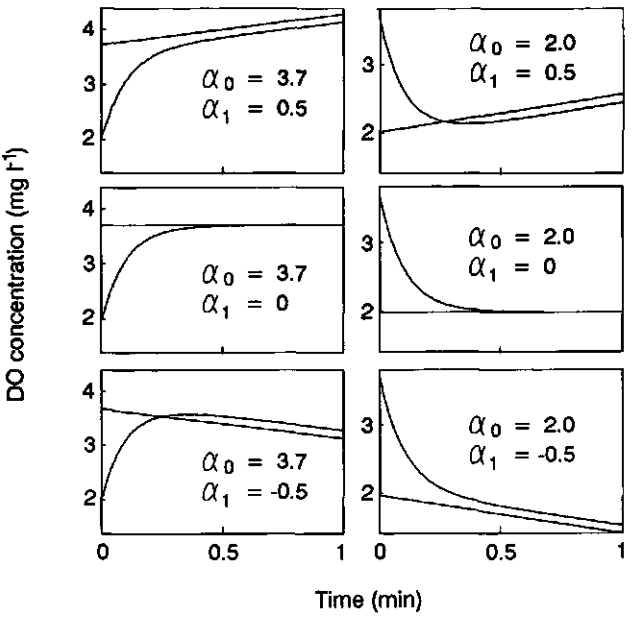

Figure 3: *Simulation, using equation (5), of the measured DO (y) after reversing the sludge flow direction, i.e. after a step change in the DO (C) followed by a time linear change of C. Probe response time constant* t_c*=0.10 min.*

6.4 Estimation of DO concentration and probe response time constant

All three parameters t_c, α_0 and α_1 in equation (3) are unknown and have to be estimated from the DO probe step response. Since the DO measurements are obtained in time discrete form it is straightforward to approximate the time derivative in (3) with finite differences. Here we choose the trapezoidal rule for integration:

$$t_c \frac{y_{k+1}-y_k}{h} = \frac{1}{2}\left(-y_{k+1}-y_k+\alpha_0+\alpha_1 kh+\alpha_0+\alpha_1(k+1)h\right) \qquad (6)$$

where k = 1, 2,...,K; K typically 10 or 15,
h = sampling interval DO concentration, typically 1 or 2 s.

The parameter α_1 can be estimated separately. Here we consider the last DO measurement of two consecutive step responses, called $y_K(m-1)$ and $y_K(m)$. The DO concentration slope is then estimated from:

$$\alpha_1 \approx \frac{y_K(m) - y_K(m-1)}{2Kh} \tag{7}$$

Now there remain two parameters, t_c and α_0, to be estimated. If equation (6) is applied to all K measurements (but the first) in one step response we obtain:

$$\begin{aligned} t_c(y_2-y_1)-h\alpha_0 &= \tfrac{1}{2}h(-y_2-y_1+3h\alpha_1) \\ t_c(y_3-y_2)-h\alpha_0 &= \tfrac{1}{2}h(-y_3-y_2+5h\alpha_1) \\ &\vdots \qquad\qquad \vdots \\ t_c(y_k-y_{k-1})-h\alpha_0 &= \tfrac{1}{2}h(-y_k-y_{k-1}+(2k-1)\alpha_1) \end{aligned} \tag{8}$$

This can be written in a vector form:

$$\begin{bmatrix} y_2-y_1 & -h \\ y_3-y_2 & -h \\ \vdots & \vdots \\ y_k-y_{k-1} & -h \end{bmatrix} \begin{bmatrix} t_c \\ \alpha_0 \end{bmatrix} = \begin{bmatrix} \frac{1}{2}h(-y_2-y_1+3h\alpha_1) \\ \frac{1}{2}h(-y_3-y_2+5h\alpha_1) \\ \vdots \\ \frac{1}{2}h(-y_k-y_{k-1}+(2k-1)h\alpha_1) \end{bmatrix} \tag{9}$$

or written in a more compact form:

$$\Phi\,\theta = y \tag{10}$$

The unknown parameter vector θ can be estimated by the least-squares method (Åström and Wittenmark, 1984):

$$\hat{\theta} = (\phi^T \phi)^{-1} \phi^T y \tag{11}$$

The estimate $\hat{\theta}$ makes it possible to calculate the DO concentration at the end of the sampling interval:

$$C(m) = \hat{\alpha}_0 + \hat{\alpha}_1 Kh \tag{12}$$

This is used for a new estimate of α_1:

$$\alpha_1 \approx \frac{C(m) - C(m-1)}{2Kh} \tag{13}$$

C(m-1) is the calculated real DO from the foregoing response. Then a new vector θ is calculated from the observations **y**. This procedure is repeated until a preset error criterion is met.

This method was tested by means of two simulations and an experiment. The application of the method was demonstrated in another experiment where the probe membrane was fouled intentionally.

6.5 Materials and methods

In the simulations, executed with SIMNON (Elmqvist *et al.*, 1986), the measuring data were generated using the first-order model (5) and chosen values of C and t_c. Then the method proposed was used to recalculate C and t_c. Any deviation of the result from the original C and t_c emanates from the assumption that the time derivative of (3) can be approximated by the trapezoidal integration method.

In the laboratory experiments a respiration meter RA-1000, Manotherm, equipped with a WTW dissolved oxygen meter (modified model OXY-219/R with sensor model EO90), was connected to an aerator, the total system having a content of 1.5 litres of activated

sludge. The sludge was sampled from a nitrifying activated sludge plant. The respiration meter which works according to the principle described by Spanjers and Klapwijk (1990) was operated in a mode such that the alternating DO signal could be sampled. Temperature and pH were kept constant at 20°C and 7.5, respectively.

In one experiment the assumption of the first-order model was tested. Therefore, a known amount of ammonium was added to the sludge and the DO signal was recorded. The DO concentration at the inlet and at the outlet were either calculated from 2 to 4 values at the end of the response measuring period (averaging method) or calculated according to the method proposed in this chapter (estimation method). From the DO concentrations, the respiration rate and the mass of oxygen used by the nitrifiers for the oxidation of one unit of mass nitrogen (O/N ratio) (Spanjers and Klapwijk, 1990) were calculated, respectively. The O/N ratio was compared for different situations.

In the other experiment the procedure to detect probe failure from an increasing t_c was tested. Probe fouling was imitated by covering a part of the probe membrane with ball-bearing grease. Ammonium was added to the sludge before and after contamination of the membrane. The effect on t_c and on the O/N ratio was studied.

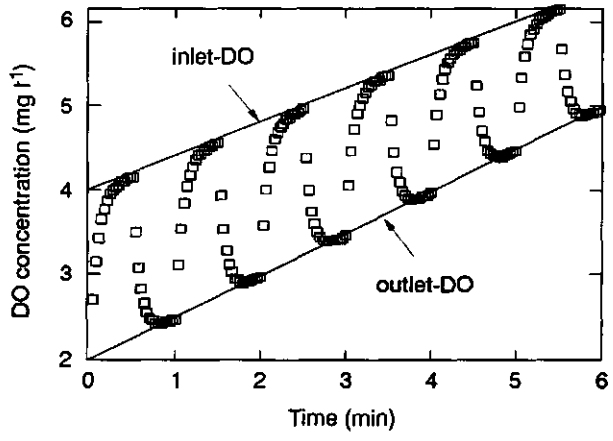

***Figure 4:** Simulation, using equation (5), of the measured DO concentration (points) during the operation of the respiration meter in comparison to inlet and outlet-DO (solid lines). h = 2 s, t_c = 0.08 min, t_r = 0.5 min, α_o = 4 and 2 g m^{-3}, α_I = 0.4 and 0.5 g $m^{-3}min^{-1}$ for, respectively, the inlet and outlet. Standard deviation noise: 0.008 g m^{-3}.*

6.6 Verification of the method

Linear change of the real DO concentration (simulation 1)

For given constant values of α_1, h and t_c, and an initial value of α_o, equation (5) was used to simulate the course of the measured DO during a short period of respiration measurement (Figure 4). In this simulation it was assumed that the respiration rate was decreasing linearly. Normally distributed noise was added to the calculated value of the DO.

The simulated DO values were used to estimate t_c and C employing equations (7)-(13). Figure 5 shows the result.

In case of an optimal estimation procedure, the resulting t_c and C would have been the original values. The figure shows that t_c (mean value 0.079 $\pm$ 0.002 min) and C are estimated well.

Non-linear change of the real DO concentration (simulation 2)

A batch experiment with the continuous flow-through respiration meter was simulated to test the assumption that, within the response measuring period, the change of the real DO concentration can be accurately represented by a linear relationship. In this experiment an amount of ammonium was added to the aerator connected to the respiration meter. The simulation model included Michaelis-Menten kinetics and a set of dynamic mass balances on DO and substrate for the aerator and the respiration chamber. The parameters were chosen from a fit of the simulation model on the experimental results of Spanjers and Klapwijk (1990). In this simulation, unlike simulation 1, the change of the simulated DO is not linear any longer. Even so, in the calculation of t_c and C, it is assumed to be linear within the response measuring interval.

Figure 6 shows the simulated DO concentration at the inlet and at the outlet of the respiration chamber as well as the simulated response of the DO probe.

The simulated DO probe measurement values were used to estimate t_c and C with the equations (9)-(13). Figure 7 shows the result.

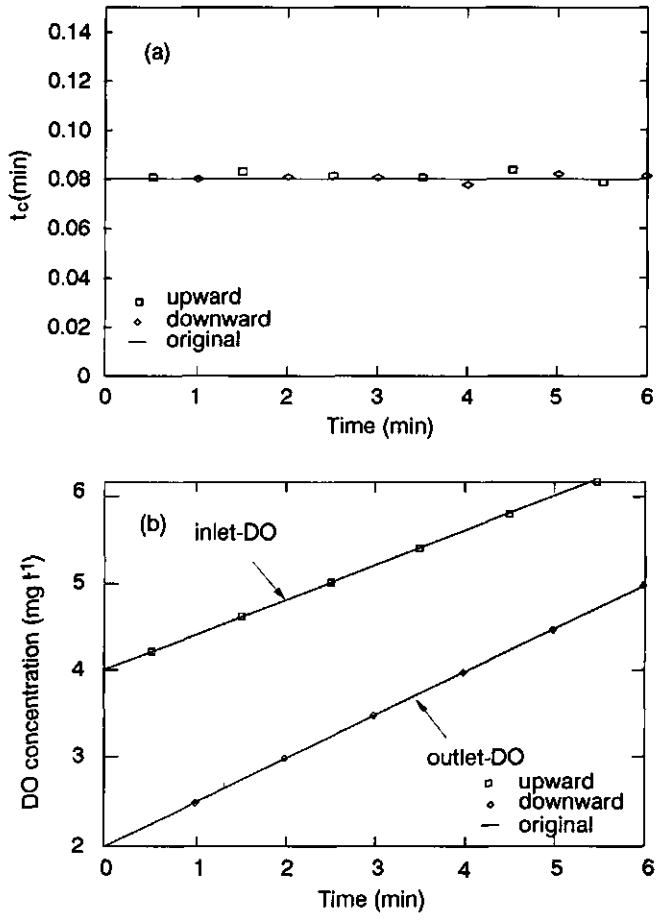

Figure 5: *Result of the estimation method applied on the simulated DO probe values (Figure 4). Calculated values (points) compared with originally simulated values (solid lines). (a) Probe response time constant (t_c) and (b) real DO concentration (C).*

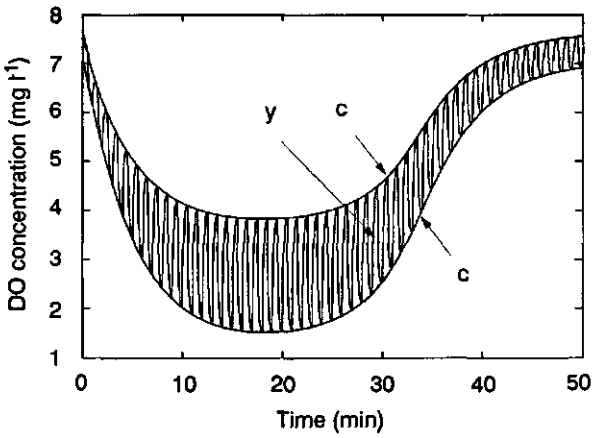

Figure 6: *Simulation of a batch experiment. Addition of 0.010 g ammonium-nitrogen to 1.5 l sludge. Michaelis-Menten constants: half-saturation constant = 1.5 g ammonium-nitrogen per m^3, maximum respiration rate = 65 g $m^{-3}h^{-1}$; probe time constant (t_c): 0.1 min; standard deviation noise: 0.01 g O_2 m^{-3}; response measuring period (t_r): 30 s.*

As in simulation 1, t_c and C are estimated well (Figure 7). t_c does not depend on the magnitude of the response. The estimated DO is very close to the originally simulated DO.

From the results of simulations it is concluded that the method proposed here allows the estimation of the first-order probe response time constant and the real DO concentration.

Experimental verification of the first-order model

To test the assumption of the first-order model, the experiment described and simulated in simulation 2 was carried out in the laboratory. In three experimental runs, the response measuring period (twice 15 s and once 20 s) was too short to attain the steady state response. Therefore this experiment was well suited to demonstrate the capability of the method to estimate the real DO concentration in the inlet and outlet of the respiration chamber. Figure 8 shows the DO-probe signal.

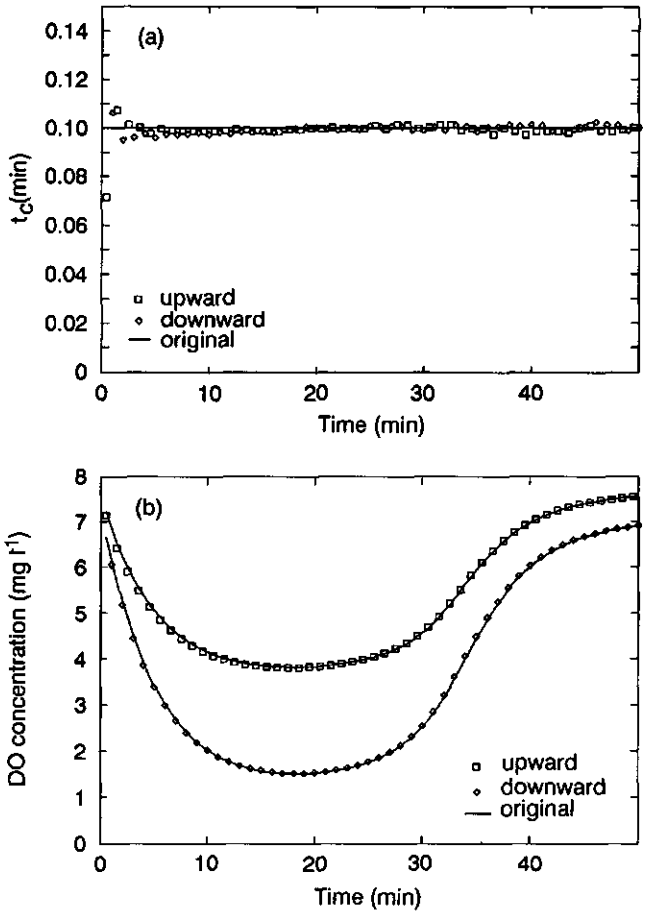

Figure 7: *Result of the estimation method for C and t_c applied on the simulated DO probe values (Figure 6). Estimated values (points) compared with the originally simulated values (solid lines). (a) Probe response time constant (t_c) and (b) real DO concentration (C).*

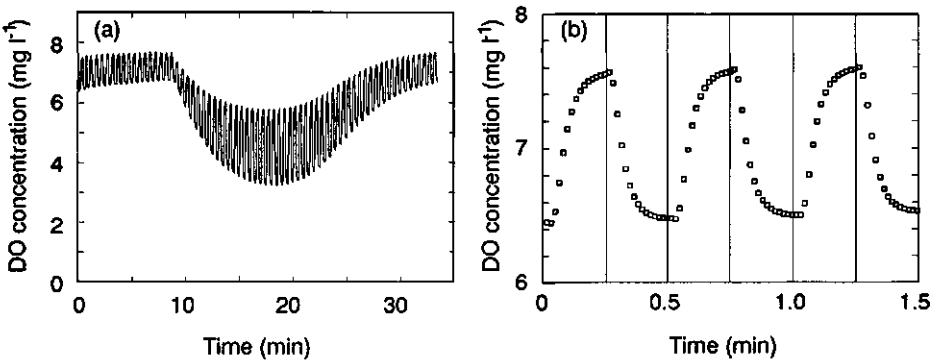

Figure 8: *DO-probe signal in a batch experiment: addition of 5 mg N as ammonium to activated sludge. (a) Full experiment and (b) first 1.5 min. of the experiment.*

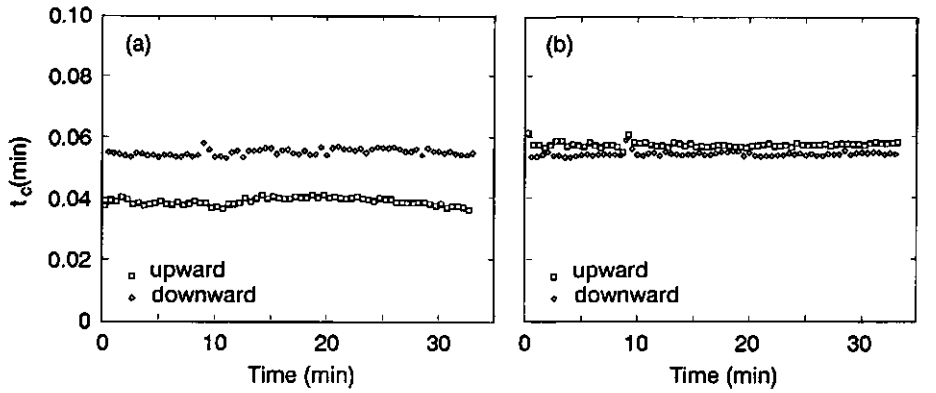

Figure 9: *t_c calculated from the DO-probe measurement. (a) without correction for dead time and (b) with correction for dead time.*

Figure 9a shows the estimated t_c for both the upward and the downward response. t_c from the upward response is higher than t_c from the downward response. Apparently, the model applied is not completely valid for the upward or downward response or both. After careful examination of the shape of the response curves (Figure 8b) in relation to the geometry of the respiration meter (there was a dead space in one flow direction) it was concluded that a dead time (t_d) had to be incorporated in the model (3):

$$t_c \frac{dy}{dt} = -y + \alpha_0 + \alpha_1 (t - t_d) \qquad (14)$$

t_d depends on the flow direction. In the experiments reported here, the values were found by trial and error to be 2 and 1 s for the measurement of the inlet DO concentration and the outlet DO concentration, respectively. Figure 9b shows that when the dead time is accounted for, both responses produce almost similar t_c which also show less variation. As in simulation 2, t_c is independent of the response amplitude.

The DO concentrations at the inlet and at the outlet of the respiration chamber were calculated by averaging three measurements at the end of each response (averaging method) and by the method proposed in this chapter (estimation method). Next, the respiration rate was calculated as described by Spanjers and Klapwijk (1990). The result is shown in Figure 10.

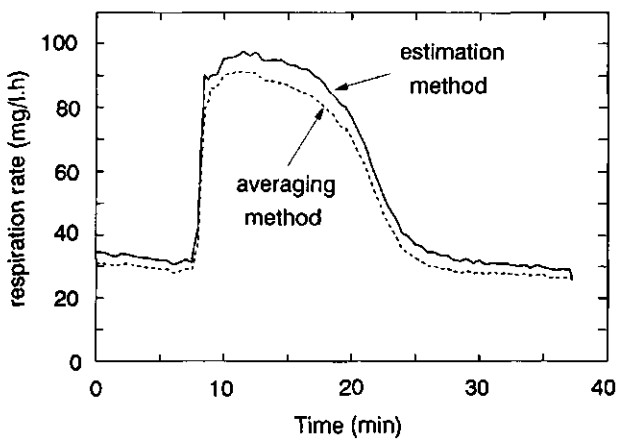

***Figure 10**: Respiration rate calculated from the DO probe signal in Figure 8. Comparison of the averaging method and the method proposed in this chapter.*

The estimation method results in higher values for the respiration rate, especially at a higher rate when the difference between inlet and outlet DO concentration is greater. It is difficult to verify the correctness of the absolute value of the respiration rate. Therefore, from the respiration rate, the total amount of oxygen additionally used to the endogenous oxygen consumption, defined as short-term biochemical oxygen demand (BOD_{st}), was

evaluated for both results. The BOD_{st} was calculated from the area under the respiration curves. This value divided by the amount of nitrogen added represents the mass of oxygen used by the nitrifiers for the oxidation of one unit of mass nitrogen (O/N ratio). Table 1 summarizes the results for the same experiment repeated with different response measuring periods.

***Table 1:** Comparison of the averaging method (average of three measurements at the end of the response measuring period) and the estimation method proposed in this chapter for different response measuring periods (t_r). Theoretical value O/N: 4.57; expected value: 4.4 (Spanjers and Klapwijk, 1990).*

		O/N		
h	t_r	averaging	estimation	
(s)	(s)	method	method	reference
1	15	3.91	4.26	this investigation
1	15	3.74	4.01	this investigation
2	20	4.29	4.39	this investigation
2	30	4.02	4.04	this investigation
2	30	4.33	4.38	this investigation
-	30	4.36	-	Spanjers and Klapwijk (1990)
-	-	4.33		Sharma and Ahlert (1977)

From Table 1 two conclusions can be drawn. Firstly, for a short response measuring period, the averaging method yields lower values for the O/N ratio while the estimation method produces equal values as compared to a large response measuring period. This means that the estimation method produces a better estimate of the real DO concentration. Two experiments yield a low O/N value from the estimation method compared to the value in the literature.

Secondly, the negligible difference between the two methods at a response measuring period of 30 s indicates that the effect of a changing real DO concentration on the probe response is marginal. For, in the determination of the O/N ratio, there is a sharp change of the DO at only two different occasions. Another reason for the small difference is that the shift in the endogenous as well as in the maximum respiration rate reduces the impact on the total amount of oxygen calculated from the area under the curve. Nevertheless, in kinetic experiments, when transients in the respiration rate and thus in DO concentration become important (Ossenbruggen *et al.*, 1991), the effect of the changing real DO will become stronger in the probe response.

From the experiments it is concluded that, for incomplete responses, the estimation method produces a better estimate of the real DO concentration and consequently a better result for the calculated respiration rate. Because of the actual geometry of the respiration meter, a correction in the response model for dead time is required.

6.7 Detection of probe fouling using the probe response time constant

In batch experiments is was attempted to detect probe failure from the estimated t_c. Therefore, the probe membrane was contaminated intentionally by covering part of the membrane with a thin layer of grease (approx. 10 - 25% of the surface).

First it was established that the contact of approximately the same amount of grease with the sludge in the aerator had no impact on either the DO concentration or the respiration rate. This means that the substance is not biodegradable or toxic on a short term and that it should neither provoke a change in the respiration rate when it is applied to the membrane surface.

Two experiments were carried out. In the first experiment an amount of grease was applied twice to the membrane, while the respiration meter was measuring the endogenous respiration rate. In Figure 11a the estimated t_c is shown and the applications of grease are indicated. The figure shows that a greater amount of grease results in an increasing t_c meaning that the probe becomes slower. Consequently, the DO concentration calculated from the end values of the response measuring period (averaging method) is erroneous and so is the calculated respiration rate (Figure 11b). The DO from the estimation method, and therefore the calculated respiration rate are affected to a lesser extent by the deterioration of the probe: after the first treatment the rate decreases from 20 to 15 mg $l^{-1}h^{-1}$; after the second treatment it remains at this level, although the noise has increased. This decrease may partly be caused by another incident, for example the real endogenous respiration rate may have decreased during the time that the membrane was being contaminated. This is supported by the check at the end of the experiment where the membrane was replaced by a new one and the respiration rate was found to be 18 mg $l^{-1}h^{-1}$.

Figure 11c illustrates the effect of the contamination on the individual response curves. In this figure the estimated DO is also indicated.

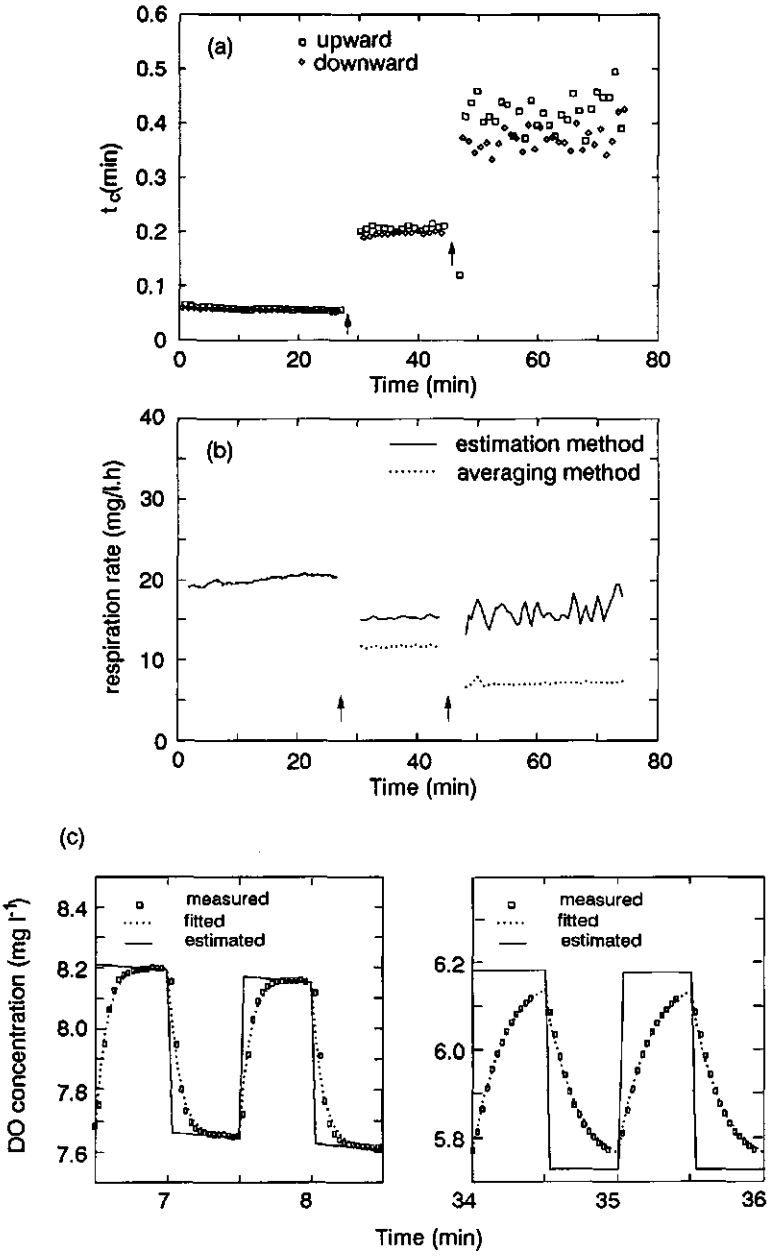

***Figure 11**: Effect of the contamination of the probe membrane. (a) Probe response time constant (t_c), (b) respiration rate; comparison averaging method and estimation method, and (c) part of the DO probe signal (points) before and after contamination (respectively left and right), in comparison with the estimated DO.*

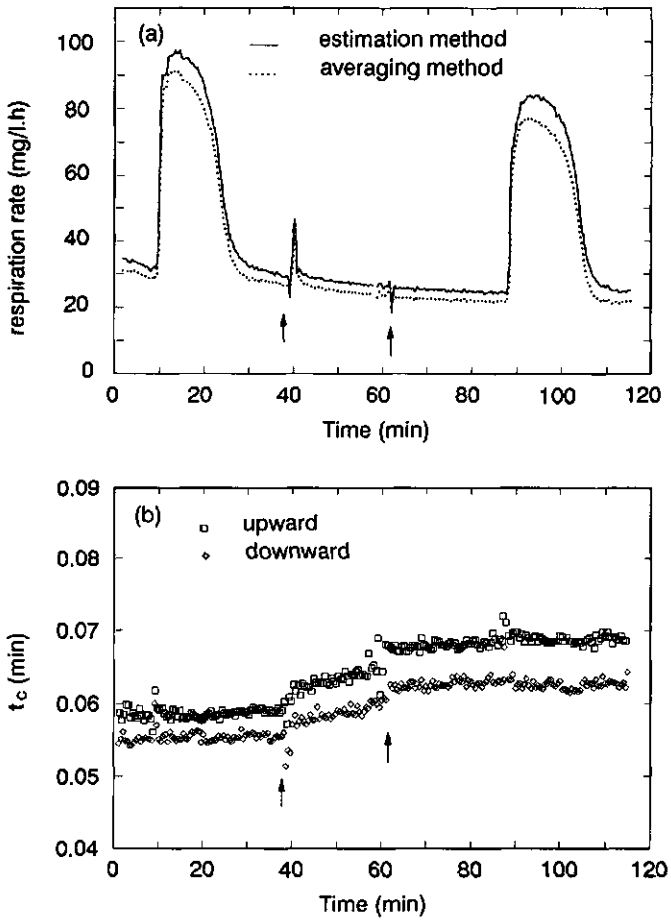

Figure 12: *Addition of 0.005 g N (as ammonium) to 1.5 l of activated sludge, before and after contamination of the probe membrane. (a) Respiration rate calculated from the DO concentration. Comparison of the averaging method and the method proposed here. (b) Probe response time constant (t_c). Arrows indicate contaminations.*

In the second experiment ammonium was added twice to activated sludge in the endogenous phase of respiration. The first addition is already reported in the previous section (Figure 10 and Table 1). The second addition was done after contamination of the

membrane. Figure 12a shows the respiration rate calculated from the DO concentration, whereas t_c is plotted in Figure 12b. The O/N ratio was calculated from the respiration rate (Table 2).

Table 2: *Effect of the contamination of the probe membrane on the calculated O/N value. Comparison of the averaging method (averaging three measurements at the end of the response measuring period) and the estimation method proposed here. h = 1 s, t_r = 15 s, theoretical O/N ratio: 4.57.*

	O/N	
	averaging method	estimation method
before contamination	3.91	4.26
after contamination	3.04	4.28

From Table 2 it can be concluded that the O/N ratio evaluated from the estimation method is not affected by the probe deterioration while the O/N ratio from the averaging method (already lower because of the short response measuring period, see Table 1) is decreased.

6.8 Discussion

The purpose of this investigation was to improve the measurement of the DO concentration with one and the same probe at the inlet and at the outlet of a respiration chamber. Inherent in the principle of the respiration meter is that the DO probe is repeatedly subjected to step changes in the DO concentration. This fact is employed, for the improvement of the DO measurement, by fitting a first-order response model to each measured response, which provides an estimation of the real DO concentration and of the first-order probe response time constant. The change of the real DO concentration during the response measurement is accounted for in the model.

The simulations show that the method allows the calculation of C and t_c. On the condition that the probe signal can be modelled by a first-order dynamic system, a correct estimation of C and t_c can be obtained from experimental data. The type of the response is chiefly determined by the manufacturer of the DO meter. However the response is also partly determined by conditions of the respiration meter in which the probe is mounted. The experiments show that, in this case, the assumption of a first-order response is reasonable, provided a dead time is accounted for. This dead time is fixed by the respir-

ation meter and can be determined exactly by measuring the dead space. The experiments also show that it is reasonable to assume that the change of the real DO concentration within one response measuring period can be approximated by a linear relationship. This can be clearly illustrated by looking at an enlarged portion of Figure 8a.

The DO concentration C can be estimated from only a part of the probe response, where the signal has not yet reached its steady state. The advantage of this, in the respiration measurement, is that the measuring frequency of the DOs at the inlet and outlet, and herewith the measuring frequency of the respiration rate, can be increased.

t_c indicates malfunction of the probe even in the case of severe deterioration (Figure 11a), when the probe becomes very slow and the estimation of C probably becomes unreliable.

The method proposed in this chapter, for the estimation of t_c and C, can also be used, in combination with the measuring technique, in the measurement of the DO concentration in an activated sludge reactor. On the condition that the respiration meter is installed close to the reactor, the DO concentration in the inflow of the respiration meter is equal to the concentration in the reactor at the sampling point. Besides the measurement of the DO concentration and the optional measurement of the respiration rate this technique, through the estimated t_c, provides a continuous diagnosis of the probe condition.

6.9 Conclusions

The method proposed in this chapter provides a reliable estimate of the real DO concentration from the probe response signal when the probe is subjected, in the respiration measurement, to a repeated, stepwise changing DO concentration. As a result, the reliability of the calculated respiration rate is improved.

The first-order probe response constant, evaluated from the probe signal is a useful indicator for fouling of the probe membrane.

6.10 Notation

C real dissolved oxygen (DO) concentration (mass volume^{-1})
h sampling interval DO concentration (time)
K number of measuring points in one response

t time (time)
t_c first-order probe response time constant (time)
t_d dead time (time)
t_r response measuring period (time)
y DO probe signal (mass volume^{-1})
α_0 offset of real DO concentration at start of response (mass volume^{-1})
α_1 slope of real DO concentration change during one response (mass volume^{-1} time^{-1})
τ integration variable (time)

6.11 References

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7

Assessment of a two-step nitrification model for activated sludge

7.1 Abstract

The objective of developing empirical models with well determined parameters is to obtain a better understanding of the nitrification of activated sludge. The D-optimal experimental design criterion and nonlinear regression analysis were used to obtain precise parameter estimates. Our experiment consisted of a series of 14 batch runs where activated sludge was spiked with ammonium chloride and sodium nitrite individually and in combination. Specific respiration rate, the ratio of respiration rate to mixed liquor suspended solids concentration, was used as the model response variable. Empirical models for specific respiration rate were specified as piece-wise, Monod type functions of ammonium and nitrite concentration. Statistical methods were used to evaluate the effects of non-linearity and parameter correlation on the models. The effect of experimental design on model specification and on parameter estimation is discussed.

Submitted for publication by: Ossenbruggen P.J., Spanjers H. and Klapwijk A.

7.2 Introduction

Nitrification is a two-step reaction, by which ammonium is oxidized to nitrite and then the nitrite is oxidized to nitrate. The two oxidation reactions occur rapidly, both on a time scale of minutes. However, the two rates can be different. Therefore, concentrations of the two substrates are observed simultaneously. For simplicity, the process is often described with a one-step model with ammonium concentration used as a predictor variable (Lijklema, 1973; Henze *et al.*, 1987). The objective of this study was to develop empirical models assuming the process to be a two-step reaction, thereby obtaining a more fundamental understanding of the process involving both substrates of ammonium and nitrite.

Empirical evidence and theory suggests that a model depicting the process as a two-step reaction is reasonable (Quinlan, 1980; Beck, 1981; Ossenbruggen *et al.*, 1991). The respiration rate resulting from ammonium and nitrite oxidation was hypothesized to be the sum of two Monod or Michaelis-Menten type functions,

$$y = \frac{a_1 s_1}{a_2 + s_1} + \frac{a_3 s_2}{a_4 + s_2} \qquad (1)$$

where y = specific respiration rate (mg O_2 g^{-1}MLSS h^{-1}), r_s divided by the mixed liquor suspended solids concentration (g l^{-1});
r_s = $r - r_{end}$ = respiration rate (mg $O_2 l^{-1} h^{-1}$);
r = measured respiration rate (mg $O_2 l^{-1} h^{-1}$);
r_{end} = endogenous respiration rate (mg $O_2 l^{-1} h^{-1}$);
s_1 = ammonium (NH_4^+) concentration (mg N l^{-1});
s_2 = nitrite (NO_2^-) concentration (mg l^{-1});
a_1, a_3 = maximum specific respiration rates (mg $O_2 g^{-1}$MLSS h^{-1});
a_2, a_4 = half saturation constants for s_1 and s_2 (mg N l^{-1}).

The precision with which the parameters of a Monod or Michaelis-Menten function for a one-step model are known to depend upon the experimental design and the method of calibration (Curie, 1982). With simulation studies, it has been reported that, generally, the parameters of maximum specific respiration rate and half saturation constants can not be precisely estimated from experiments with noisy measurements (Holmberg, 1982). Statistics can help us to discover if a hypothesized model is adequate. Berthouex (1993) shows the need for proper experimental design, nonlinear regression and inference methods for nonlinear model building. Failing to use these methods can lead to misleading conclusions.

For example, he shows that the transformation of nonlinear models so that they can be fitted by linear regression (the Lineweaver-Burke plot and Thomas slope methods for fitting the Michaelis-Menten function) will give biased parameter estimates and that a few carefully selected data points are better than large amounts of data, as collected and recorded at treatment plants.

Our study required that models with two substrates be specified and evaluated. An experiment has been designed to test the hypothesis as given by model (1) and other steady state model forms. In a batch system using a continuous respiration meter (Spanjers and Klapwijk, 1990), the respiration rate of activated sludge and substrate concentrations were measured simultaneously. While a continuous run experiment may be desirable for calibration of a steady state model, it is laborious. The batch test method to obtain measurements has been successfully used for steady state model development (Cech *et al.*, 1984). It will be shown that the use of batch test data, where observations were taken in time intervals of minutes, satisfies the conditions for good experimental design. Nonlinear regression and statistical analyses were used for model calibration and assessment. These procedures give assurances that a model is correctly specified and that its parameters are well determined.

Parameter correlation and model non-linearity, which were suspected of affecting the model specification, calibration and assessment procedures, were given special attention in this study. The following steps were used:

1. conducting a series of 14 experimental runs at five different initial concentrations of s_1 and s_2;
2. calibrating a model with a data set formed by combining the data from the 14 experimental runs;
3. assessing a model by: (i) evaluating the adequacy of the model form using residual plots with the combined data set, (ii) determining the significance of model parameter estimates through statistical inference, and (iii) evaluating the goodness of fit of the model by using time series plots with data from each individual experimental run.

Steps 2 and 3 dealt primarily with issues related to model specification and parameter precision. If a model failed one or more of the tests of step 3, the model was rejected and a new model was specified, calibrated and assessed. This procedure will be described and discussed in this chapter.

7.3 Materials and methods

Nitrifying sludge was grown in a sequencing batch reactor fed with domestic sewage. The pH and temperature were fixed at 7.5 and 20°C, respectively. The average loading was 0.17 g COD per g MLSS per day. The dissolved oxygen concentration was maintained above 2.5 mg l^{-1} to ensure that the nitrifiers were not oxygen limited. The aeration intensity was kept as low as possible to prevent mechanical breakage of the sludge flocs, but it was high enough to ensure an aerobic condition and complete mixing of the liquor.

During the experiment and a week prior to the experiment, the sequencing batch reactor was run with an aim toward maintaining constant MLSS, temperature and pH. However, after each influent loading the temperature dropped to 15-16°C. It took 70 to 90 minutes to warm the mixture to 20°C with a heater. While the MLSS and MLVSS concentrations were assumed constant during individual experimental runs, the MLSS and MLVSS concentrations between experimental runs ranged between 2.3 and 3.5 g l^{-1} and 79.2 and 83.0 percent, respectively (see Table 1).

Two litres of sludge were transferred to the aerator of the measuring system and the respiration rate was recorded (RA-1000 Manotherm). When the respiration rate reached the endogenous level, a predetermined amount of ammonium chloride - sodium nitrite mixture was added. The respiration rate was recorded until the endogenous rate was reached again. During the measurements, filtered samples (Whatman glass microfibre 1 μm) were withdrawn from the aerator at 1 to 5 minute time intervals for analysis (Skalar autoanalyzer) for concentrations of ammonium (s_1) nitrite (s_2) and nitrate. The sample volume was small compared to the total volume; therefore, it was assumed that the amount of nitrogen withdrawn was negligible. A maximum of three individual experimental runs were made with the same sludge sample. A fresh sludge sample from the sequencing batch reactor was used for additional experimental runs. Each sludge sample was analyzed for MLSS concentration and mixed liquor volatile suspended solids (MLVSS) concentration.

7.4 Nonlinear regression analysis

The nitrification model is of the form $y = \mathbf{f}(\mathbf{a}, \mathbf{X}) + \varepsilon$ where y is a response, $\mathbf{X}$ is a matrix of predictor variables, $\mathbf{a}$ is a vector of model parameters, and ε is the error. The error is assumed to have a normal distribution with zero mean $E(\varepsilon) = 0$ and variance $V(\varepsilon) = \sigma^2$. For model (1) for example, $\mathbf{a} = [a_1\ a_2\ a_3\ a_4]^T$.

The model parameters were estimated with observations of $\mathbf{y}$, s_1 and s_2 where the response vector and predictor variable matrix were designated as $\mathbf{y} = [y_j]$ and $\mathbf{X} = [s_1\ s_2] = [s_{1j}\ s_{2j}]$ for observations numbered $j = 1, 2, \ldots, n$, respectively. A vector of elapsed times $\mathbf{t} = [t_j]$ was also recorded. The elapsed time t_j corresponds to the time of observation of y_j, s_{1j} and s_{2j} where $t_j \geq 0$ and $t_j = 0$, the time when an individual batch run was initiated. The same subscript index j and sample size n notation was used to identify individual observations and sample size for an individual sample run and for the entire and sorted portions of the combined data set (modelling step 2).

Nonlinear regression analysis employing a Gauss-Newton method was used to find the set of parameter estimates of $\mathbf{a}$, denoted as $\hat{\mathbf{a}}$, that minimizes the sum of the least square error,

$$S(\boldsymbol{a}) = \sum_{j=1}^{n} [y_j - f(\boldsymbol{a}, \boldsymbol{X}_j)]^2$$

Since the error vector $\mathbf{E}$ is assumed to have a spherically normal distribution or $\mathbf{E}=\mathbf{N}(\mathbf{0},\sigma^2\mathbf{I})$, the least square estimate of $\mathbf{a}$ is also the maximum likelihood estimate of $\mathbf{a}$. All observations used in the regression analysis are assumed to be equally reliable; therefore, all observations are given equal weight in calculating $\hat{\mathbf{a}}$.

7.5 The data set

In order to obtain a model with correct specification and well determined parameters, a series of 14 experimental runs were conducted (modelling step 1) and then combined into a single data set of $\mathbf{y}$ and $\mathbf{X}$ for the purpose of model calibration (modelling step 2). The reason for combining experimental runs at different initial concentrations of s_1 and s_2 was based upon proven practices for calibrating models. In this section, nitrification is described with the aid of scatter plots of individual experimental runs and the combined data set. These plots offer empirical evidence for describing nitrification as a two-step reaction and for specifying model (1).

Individual experimental runs

Each individual experimental run was distinguished by the initial concentration $s_0 = (s_{10}, s_{20})$ where s_{10} is the initial concentration of ammonium chloride and s_{20} is the initial

concentration of sodium nitrite. Fourteen experimental runs with five different initial concentrations were conducted: s_0 = (6.5, 6.1), (6.5, 3.1), (6.5, 0.0), (0.0, 6.2), (0.0, 3.1). With the exception of (0.0, 3.1), each experiment was repeated under the same conditions at least twice. In order to better understand the nitrite to nitrate oxidation process, the last four experimental runs shown in Table 1 were conducted in the absence of ammonium, $s_0=(0, s_{20})$ with $s_{20}>0$.

Table 1: Experimental variables

Run	Initial concentration	Biomass concentration			Maximum y	
	$s_0=(s_{10}, s_{20})$ mg l^{-1}	MLSS g l^{-1}	MLVSS % of MLSS	r_{end} mg $l^{-1}h^{-1}$	Step 1 $s_1>0$ and $s_2>0$ mg $g^{-1}h^{-1}$	Step 2 $s_1=0$ and $s_2>0$ mg $g^{-1}h^{-1}$
1	(6.5, 6.1)	2.32	79.2	16.56	18.56	5.05
2	(6.5, 6.1)	2.32	83.0	16.69	13.04	3.48
3	(6.5, 6.1)	3.21	82.0	23.46	15.34	3.94
4	(6.5, 3.1)	2.67	82.0	18.46	22.23	3.89
5	(6.5, 3.1)	2.55	82.2	20.05	18.35	4.02
6	(6.5, 3.1)	3.34	81.1	24.80	19.92	4.31
7	(6.6, 0.0)	3.46	81.6	24.30	21.51	1.42
8	(6.6, 0.0)	3.46	81.6	22.06	18.84	3.19
9	(6.6, 0.0)	3.26	81.1	25.37	18.44	11.87
10	(6.6, 0.0)	3.26	81.1	21.85	17.93	3.17
11	(0.0, 6.2)	2.55	81.2	16.37	-	4.12
12	(0.0, 6.2)	2.37	81.9	14.53	-	4.07
13	(0.0, 6.2)	3.34	81.1	21.95	-	3.77
14	(0.0, 3.1)	3.49	-	25.18	-	3.91

The time series plot of Figure 1 shows the observations of r, s_1 and s_2 and the calculated values of specific respiration rate y for experimental run 4 (Table 1) with initial concentration s_0 = (6.5, 3.1). It shows the ammonium concentration s_1 to steadily decrease from s_{10} = 6.5 to zero and nitrite concentration s_2 to increase from s_{20} = 3.1 to a maximum value and

then decrease to zero. This pattern was observed for all experimental runs with initial concentrations of $s_{10} > 0$ and $s_{20} > 0$. For all experimental runs with initial concentrations of $s_{10} = 0$ and $s_{20} > 0$, the values of s_2 were observed to steadily decrease from s_{20} to zero. The plots show nitrification as being distinguishable by: (stage 1) both ammonium and nitrite being oxidized; and then (stage 2) nitrite being oxidized only because ammonium has decayed to zero. During stage 1 or when $s_1 > 0$ and $s_2 > 0$, time series plots suggested that ammonium limits the specific respiration rate and nitrite tends to reduce this rate as its concentration increased.

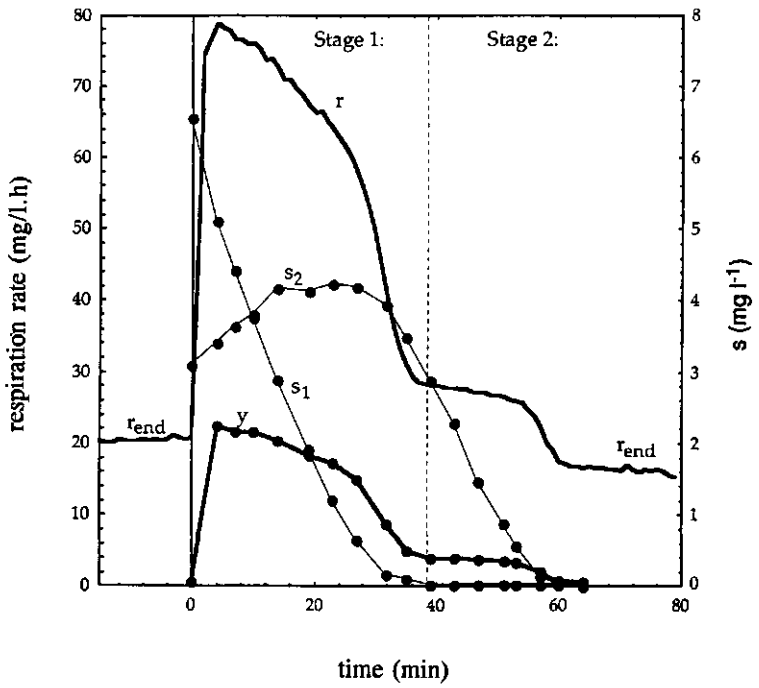

***Figure 1:** Observations for experimental run 4. Stage 1: $s_1 > 0$ and $s_2 > 0$; stage 2: $s_1 = 0$ and $s_2 > 0$*

The combined data set

Scatter plots of y versus s_1 and y versus s_2 for the combined data set (Figure 2) show some consistency among experimental runs having the same initial concentrations s_0. The plots show also that there is relatively little variability in specific respiration rate within an individual experimental run but significant variation in specific respiration rate among runs.

The transition from stage 1 to 2 seems to occur at a specific respiration rate of about y=4, as indicated in Figure 2b. The scatter plot of y and s_1 indicates that the ammonium concentration limits the specific respiration rate. The scatter plot of y and s_2 is not as clear in indicating the role of nitrite during stage 1. The scatter plots of y and s_2 for stage 2 and those for experimental runs 11 through 14 (not shown) exhibit a similar consistency. It indicates that nitrite s_2 limits the specific respiration rate. This empirical evidence suggests that model specification (1), where both s_1 and s_2 are rate limiting, is a reasonable choice.

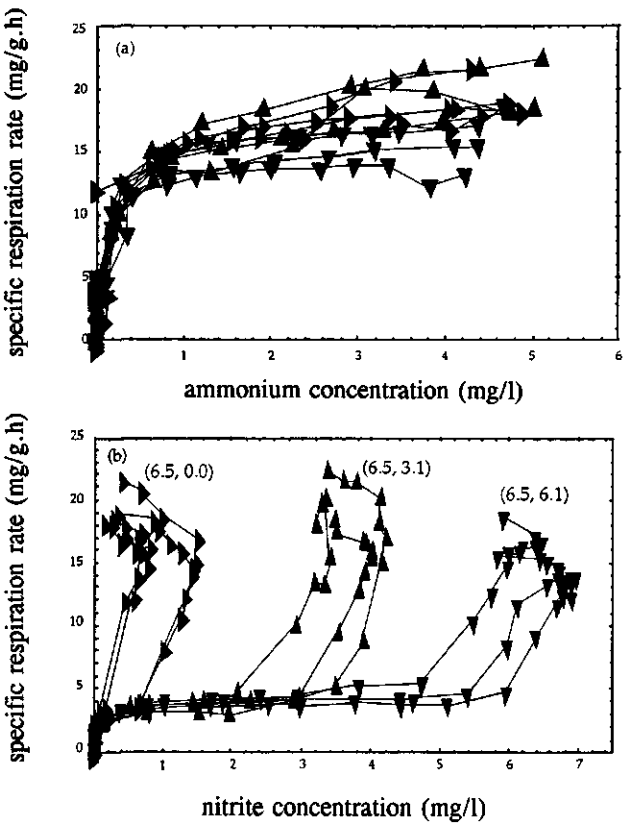

Figure 2: *Scatter plots for specific respiration rate for the combined data set excluding experimental runs 11-14. (a) s_1 versus y and (b) s_2 versus y.*

7.6 The experimental design

An important and primary objective for designing an experiment is to choose values of the **X** matrix that will lead to good parameters with small variances $V(a_p)$ (modelling step 2). The main diagonal members of covariance matrix,

$$V(a) = \sigma^2(V^T V)^{-1}$$

where

$$V = \left[\frac{\delta f(a,X)}{\delta a_1} \quad \frac{\delta f(a,X)}{\delta a_2} \cdots \frac{\delta f(a,X)}{\delta a_m}\right]$$

contain the parameter variance $V(a_p)$ terms and the off diagonal members contain the parameter covariance $cov(a_p, a_q)$ terms. The correlation coefficient ρ_{pq}, a measure of the linear dependency between parameters a_p and a_q, is calculated as

$$\rho_{pq} = \frac{cov(a_p, a_q)}{[V(a_p)\, V(a_q)]^{1/2}}$$

If the values of **X** are selected to maximize the determinant D = maximize $|\ \mathbf{V}^T\mathbf{V}\ |$, then the values of ρ_{pq} are minimized. Since $\rho_{pp} = V(a_p)$ is the parameter variance, both the parameter variances and correlations are minimized. This is called the D-optimality criterion (Box *et al.*, 1978; Cailas and Gehr, 1989). If the vectors of **V** are orthogonal, then the parameter estimates will not be correlated and changes in y will be associated with the appropriate changes in the causal factors of **X** (Bates and Watts, 1988). In other words, the suspected difficulties associated with parameter correlation are eliminated when $\rho_{pq} = 0$ and $p \neq q$.

Applying the D-optimality criterion to a one-step model for s_1, $y = a_1 s_1/(a_2+s_1)$ with $\mathbf{V} = [s_1/(a_2+s_1) - a_1 s_1/(a_2+s_1^2)]$ indicates that one-half the values of $\mathbf{X} = \mathbf{s}_1$, where $\mathbf{s}_1 = [s_{1j}]$ with $j = 1, 2, \ldots, n$ and n is the total number of observations, should be chosen such that the ammonium concentration is equal to a_2 and the other half of these values should be very large ammonium concentrations, theoretically approaching infinity. In practice, it is sufficient for the vector **X** to be long. The square root of the product $\mathbf{X}^T\mathbf{X}$ is a measure of distance;

therefore, a long vector of **X** is defined to be one that contains observations over a wide range of s_1 values. A good design for a one-step model of s_2, $y = a_3 s_2/(a_4 + s_2)$, is similar. The vector of $\mathbf{X} = s_2$ should be long and **X** should include observations of s_2 near a_4.

For model (1), which has a predictor matrix $\mathbf{X} = [s_1\ s_2]$, both s_1 and s_2 should be long vectors. By combining the 14 experimental runs with five different initial concentrations of s_0 into one data set, the D-optimality criterion is satisfied. Figure 3 shows the observations of s_1 and s_2 are both long, with s_1 ranging from 0 to 6.5 mg l^{-1}, and s_2 ranging from 0 to 7.0 mg l^{-1}.

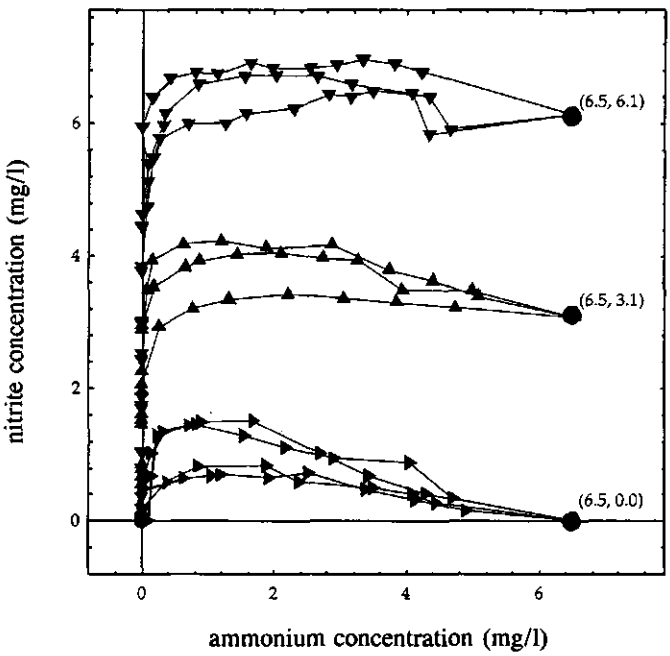

***Figure 3:** The combined data set excluding runs 11-14.*

Owing to non-linearity of the Monod type models being considered, parameter correlation ρ_{pq} can be minimized but not eliminated by the use of the $\mathbf{X} = [s_1\ s_2]$ values described in the preceding paragraphs. That is, the condition $\rho_{pq}=0$ is not possible for the one- and two-step models considered in this study. However, the design assures that the values of $V(a_p)$ and ρ_{pq} will be minimized; therefore, precise parameter estimates are expected.

Parameter correlation

While the parameter correlation coefficient ρ_{pq} can be a useful statistical variable, it can also be misleading when used exclusively for assessing a nonlinear model. The joint confidence region is preferred because both its orientation and shape are important in showing the dependency between parameter estimates as well as the relative precision of the parameters (Draper and Smith, 1981; Berthouex, 1993). A simple example will be used to illustrate the importance of using joint confidence region for model assessment and to demonstrate the importance of using data that satisfies the D-optimality condition.

Suppose nonlinear regression analysis is used to estimate the parameters a_1 and a_2 of a nonlinear model $y=f(a, x)$. The least square estimates of a_1 and a_2 of the model is shown as point A and its $(1-\alpha)$ joint confidence region is shown as an elliptical contour in Figure 4a. There is a chance or probability of $(1-\alpha)$, for example, 95% or 95 successes in 100 trials, that the true model parameters of a_1 and a_2 are enclosed within this region. The parameters are assumed to be correlated, thus $\rho_{12} \neq 0$. The extent of the dependency between parameters is reflected in the orientation angle ϕ of the elliptical contour. The dependency between a_1 and a_2 can be determined as a function of the angle ϕ. Since the shape of the joint confidence region is long and narrow, there is a relative lack of precision associated with the value of $\hat{a}_1\cos\phi+\hat{a}_2\sin\phi$, a measurement parallel to major axis of the ellipse. In contrast, the value of $-\hat{a}_1\sin\phi+\hat{a}_2\cos\phi$, a measurement perpendicular to the major axis of the ellipse, is well determined. Since the parameters are correlated, it is difficult to determine how well each individual parameter estimate is determined. As a result, the evaluation should consider parameter dependency as done here with the joint confidence region.

If the parameters were not correlated, then $\phi = 0$. Using Figure 4a, imagine that the ellipse is rotated clockwise so that the major axis is parallel to the a_1 axis. Under this condition, the estimates of $\hat{a}_1$ and $\hat{a}_2$ can be evaluated individually. In this case, estimate $\hat{a}_1$ is found to be poorly determined and $\hat{a}_2$ is found to be well determined.

When a strong parameter correlation exists, the use of the joint confidence region is preferable to the marginal confidence intervals for a_1 and a_2 because the joint confidence region accounts for parameter correlation. The parameter estimate, shown as point B in Figure 4a, is considered acceptable at the $(1-\alpha)$ confidence level because the point lies within the intersection of the marginal confidence intervals of both a_1 and a_2. Since this evaluation did not consider parameter correlation, the marginal confidence intervals are of questionable value because point B lies outside the boundary of the joint confidence region and model is considered inadequate and rejected.

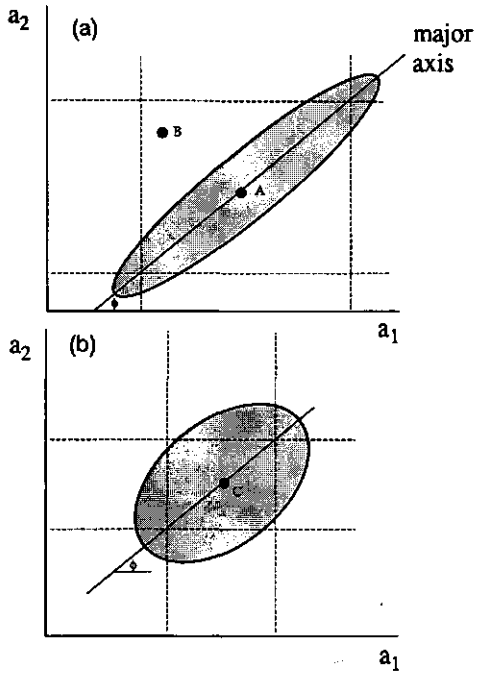

Figure 4: *(1-α) joint confidence region (shaded region) and the marginal confidence interval (dashed lines) for parameters a_1 and a_2 for model y=f(x). (a) Experimental design that led to strong parameter correlation. (b) Experimental design that led to weak parameter correlation.*

Suppose a second data set is obtained which satisfies the D-optimality criterion and which contains the same number of observations as the first data set. The vector $\mathbf{X} = [x_j]$ for the second data set is assumed to be longer than the first data set and it is assumed to contain observations in the vicinity of a_2. Since steps have been taken to minimize $V(a_1)$ and $V(a_2)$, the joint confidence region for data set two of Figure 4b will be smaller and more circular in shape than the one shown in Figure 4a. The angle ϕ is assumed to be the same in both figures; thereby, the parameter correlations for the two data sets are assumed to be the same. This assumption is conservative because the D-optimality criterion gives evidence that ρ_{12} will be minimized also. By visual inspection of the two (1-α) joint confidence regions, it shows that the parameter estimates for data set two are better determined than for data set one. In addition, when the second data set is used, the marginal confidence intervals provide useful information.

The D-optimality criterion for model (1) requires that both s_1 and s_2 be long vectors where both vectors contain concentrations of ammonium and nitrite in the vicinity of a_2 and a_4, respectively. These conditions were achieved by conducting batch runs with initial concentrations of s_0 = (6.5, 6.1), (6.5, 3.1), (6.5, 0.0) and then combining the individual runs form a single data set. The choice of s_0 lead to equally long vectors of s_1 and s_2, which can be seen by inspecting Figure 3. The correlation between s_1 and s_2 is weak, ρ_{s1s2} =-0.04.

There was no absolute assurance that this experimental design would eliminate problems associated with high parameter correlation, a major cause of modelling distortions and misleading conclusions about a model. The fact that models given in this chapter were subject to statistical inference tests as well as two goodness of fit tests give assurances that model specifications were adequate and parameters were well determined. For some nonlinear models it is important to note that their joint confidence region can be non-elliptical or even banana shaped; regardless, the principles presented here are still valid. Methods to evaluate non-linearity effects are discussed in section 7.8.

7.7 Model calibration

The model selection and development process is evolutionary. In fact, our first selection of model (1) was rejected and different models were proposed and analyzed. All proposed models used Monod type specifications in an attempt to describe the process as being ammonium and nitrite rate limiting during steps 1 and 2, respectively.

Model (1) was rejected because the Gauss-Newton algorithm failed to converge (modelling step 2). The numerical analysis showed that a_3, the maximum specific respiration rate, approached infinity. The rate therefore had no physical meaning and model (1) was rejected. The model is suspected of being improperly specified because of the $a_3s_2/(a_4+s_2)$ term. It was introduced to account for the fact that s_2 is rate limiting during stage 2 of nitrification. However, since s_2 is not rate limiting during stage 1, the model specification is considered improper and the cause of non-convergence.

It was necessary to select model forms that describe two distinctly different roles for s_2. It also proved essential to sort the combined data set as being in stage 1: $s_1 > 0$ and $s_2 > 0$ where both ammonium and nitrite are oxidized, or in stage 2: $s_1 = 0$ and $s_2 > 0$ where nitrite is oxidized. Data sorting is consistent with the interpretation of nitrification as being a two-step process. The following piece-wise nonlinear nitrification model was specified:

for $s_1 > 0$ and $s_2 > 0$,

$$y = \frac{a_1 s_1}{a_2 + s_1} - a_3 s_2 \qquad \textbf{(2a)}$$

and for $s_1 = 0$ and $s_2 > 0$,

$$y = \frac{a_4 s_2}{a_5 + s_2} \qquad \textbf{(2b)}$$

Inspection of the scatter plots of Figure 2 gave us confidence prior to performing model calibration calculations that model (2) was a reasonable form. The first term of the right-hand side of the equality sign in (2a) was chosen to describe s_1 as limiting y as shown in Figure 2a. The scatter plot of Figure 1 shows y decreasing with increasing s_2. Thus, the second term of $a_3 s_2$ was chosen to act as a corrective term and its sign was specified to be negative to reduce y as s_2 increases. These assumptions are challenged in the model assessment step of the analysis.

The specific respiration rates during stage 2 are typical of data representing a single substrate Monod type model, thus model (2b) was chosen. In addition, scatter plots for $s_{10} = 0$ and $s_{20} = 6.2$ and 3.1 gave further affirmation that the form of model (2b) was suitable.

The combined data set for all 14 experimental runs was sorted into two groups: $s_1 > 0$ for stage 1 and $s_1 = 0$ for stage 2. The data in each group was used to independently calibrate models (2a) and (2b). The D-optimality criterion is satisfied for these models and combined data set groupings. The calibrated model for stage 1 is:

for $s_1 > 0$ and $s_2 > 0$,

$$\hat{y} = \frac{19.5 s_1}{0.208 + s_1} - 0.333 s_2 \qquad \textbf{(2c)}$$

with residual mean square $s^2 = 1.78^2$ and $n = 91$ observations. The model for stage 2 is:

for $s_1 = 0$ and $s_2 > 0$,

$$\hat{y} = \frac{4.08 s_2}{0.0875 + s_2} \qquad \textbf{(2d)}$$

with $s^2 = 0.945^2$ and $n = 107$ observations.

The model calibration procedure did not place restriction on the signs of the parameters. Since the least square estimate of parameter a_3 for model (2c) was positive, the assumption that nitrite tends to reduce specific respiration rate as its concentration increases seemed justifiable.

7.8 Model assessment

Three different assessment procedures (modelling step 3) were performed. First, residual plots were used to determine the overall adequacy of the hypothesized model form $\mathbf{f(a, X)}$ for the combined data set of all experimental runs. The residual vector, $\mathbf{e} = \mathbf{y} - \mathbf{f}(\hat{\mathbf{a}}, \mathbf{X}) = \mathbf{y} - \hat{\mathbf{y}}$, was also used as error estimates to test assumptions about $\mathbf{E}$. Second, statistical inferences were used to test the significance of parameter estimates and to assess the significance of model non-linearity and parameter correlation (Bates and Watts, 1988). Third, expected value estimates for a model were evaluated for goodness of fit for each experimental run over time.

Residual plots

Residual plots are effective in validating model forms. When a model form is considered inadequate, residual plots often prove useful in suggesting a different model relationship or the absence of an important variable from the model. The residual error for the models of stages 1 and 2 was calculated as

$$e_j = y_j - \frac{19.5 s_{1j}}{0.208 + s_{1j}} + 0.333 s_{2j}$$

for model (2c), and

$$e_j = y_j - \frac{4.08 s_{2j}}{0.0875 + s_{2j}}$$

for model (2d). The residual plots of e as a function of s_1, s_2 and $\hat{y}$ (not shown) suggested that the model had a reasonable fit. Most importantly, the residual plots show no discernible trends suggesting an important variable may be absent from the model. For each residual plot, the error values were reasonably, uniformly distributed along the s_1, s_2, and $\hat{y}$ axes. The error values appeared to have a normal probability distribution with respect to s_1, s_2 and $\hat{y}$ axes. Therefore, there was no reason to suspect that the model form was inadequate or the distribution assumptions about the random variable **E** were violated.

Statistical inferences

Hypothesis tests were conducted by evaluating $(1-\alpha) = 95\%$ confidence regions and intervals. These graphical summaries were constructed using nonlinear analysis methods because they are considered effective for assessing the severity of non-linearity in an estimation situation and the effects of parameter correlation. The profile t function is a fundamental relationship used in these assessments (Bates and Watts, 1988). The profile t function for model parameter a_p is defined to be

$$\tau_p = \tau(a_p) = sign(a_p - \hat{a}_p)\sqrt{\tilde{S}(\hat{a}_p) - S(\hat{a})} \qquad (3)$$

where

$\hat{a}_p$ = minimum least square estimate of parameter p;
$\hat{\mathbf{a}}_p$ = matrix of parameter estimates conditioned on parameter a_p;
$S(\hat{a})$ = minimum sum of the least squares for estimate a;
$\tilde{S}(\hat{a}_p)$ = profile sum of squares for parameter a_p where $\tilde{S}(\hat{\mathbf{a}}_p) = \| \mathbf{e} \|^2$ with $\mathbf{e} = \mathbf{y} - \mathbf{f}(\hat{\mathbf{a}}_p, \mathbf{X})$.

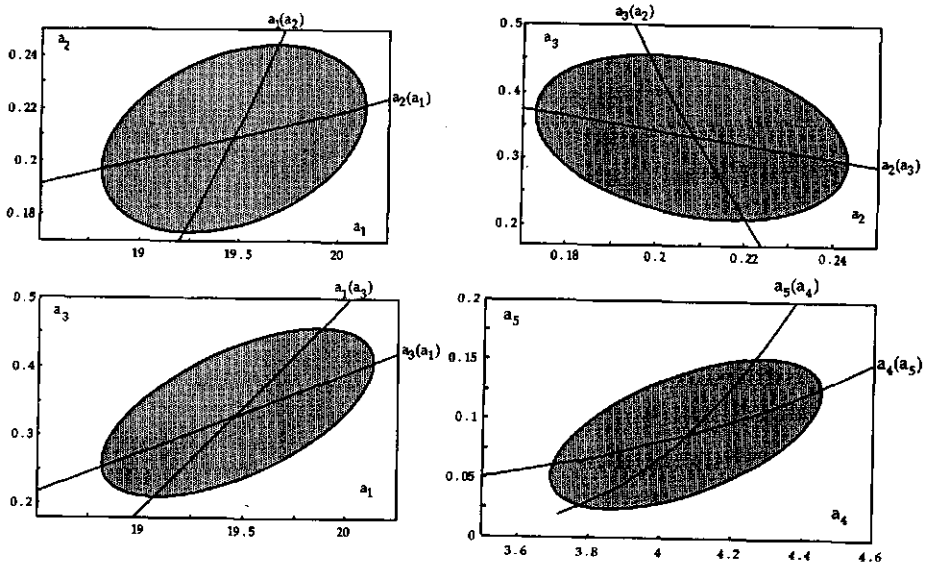

***Figure 5:** The conditional least square parameter estimates and 95% confidence regions for model (2).*

For each assigned or conditional value of a_p, the least square parameter estimates of all other model parameters $a_q(a_p)$ where $q \neq p$ of $\hat{\mathbf{a}}_p$ were calculated using the Gauss-Newton algorithm. For the two-parameter model (2d), a known value of a_4 was used to calculate $a_5(a_4)$ was calculated and was denoted as $\hat{\mathbf{a}}_4 = [a_4\ a_5(a_4)]$. Similarly, a known value a_5 of model (2d) was used to calculate $a_4(a_5)$ and was denoted as $\hat{\mathbf{a}}_5 = [a_4(a_5)\ a_5]$. For the three parameters of model (2c), a similar procedure was used to find $\hat{\mathbf{a}}_1 = [a_1\ a_2(a_1)\ a_3(a_1)]$, $\hat{\mathbf{a}}_2 = [a_1(a_2)\ a_2\ a_3(a_2)]$ and $\hat{\mathbf{a}}_3 = [a_1(a_3)\ a_2(a_3)\ a_3]$. The plots of a_p and $a_q(a_p)$ show the pair-wise dependency between parameters (Figure 5).

Knowing $\hat{\mathbf{a}}_p$, the value of τ_p was calculated with equation (3). A plot of τ_p versus a_p is called a profile trace. It provides exact likelihood intervals for individual parameters and the curvature of the trace gives information about the non-linearity of the model. If a model is linear, then τ_p is a straight line. Since all profile traces shown in Figure 6 are either linear or slightly curved, it suggests that non-linearity is slight for model (2).

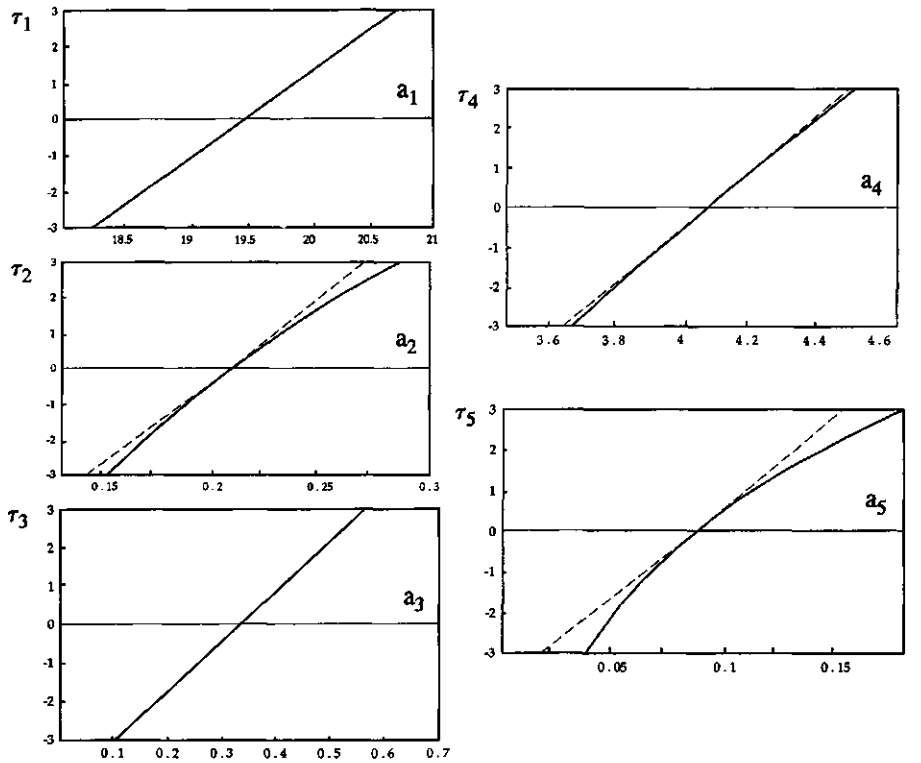

***Figure 6**: Profile traces for model (2).*

The 95% joint confidence regions for model (2) were constructed with the use of equation (3). The 95% joint confidence regions for model (2) are shown in Figure 5. The joint confidence regions are either elliptical or nearly elliptical in shape because non-linearity is slight.

The angle at the intersection of τ_p and τ_q is a measure of correlation. For zero correlation ($\rho_{pq} = 0$) the angle between them is 90° and for perfect correlation ($| \rho_{pq} | = 1$) the angle is 0°. The plots of a_p and $a_q(a_p)$ of Figure 5 serve as a good surrogate for the τ_p and τ_q plots for model (2). The parameter correlations were estimated to be $\rho_{12} = 0.35$, $\rho_{13} = 0.62$, $\rho_{23} = -0.31$ and $\rho_{45} = 0.58$. The plots of a_p versus $a_q(a_p)$ and the values of ρ_{pq} indicate weak to moderate correlation.

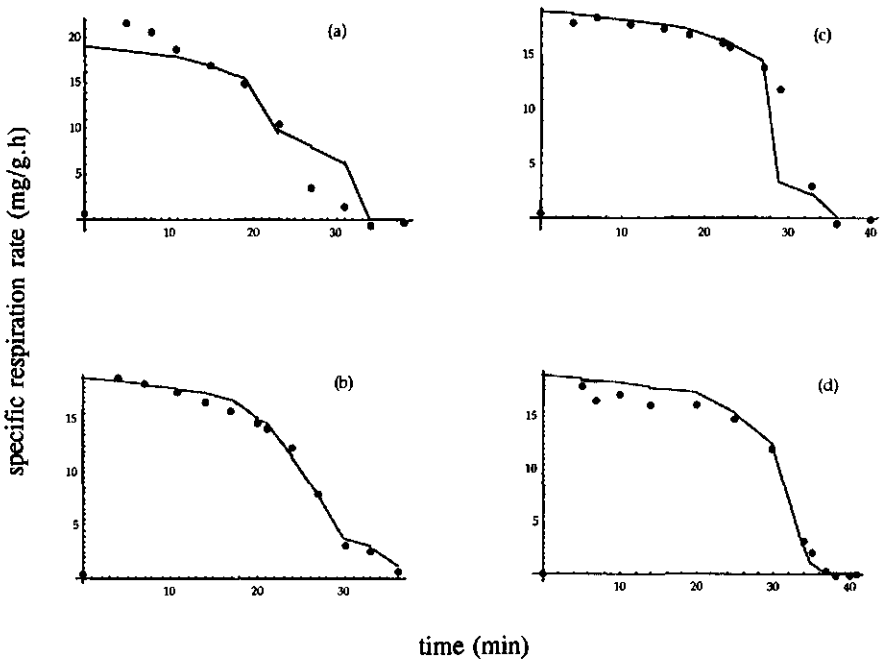

Figure 7: *Time series plots of model (2) for s_0=(6.5, 0.0) experimental runs. (a) run 7, (b) run 8, (c) run 9 and (d) run 10. The points are observed values and the lines are expected values.*

The interiors of the joint confidence regions were exclusive of zero; therefore, all parameter estimates of model (2) were considered to be statistically different from zero at the 95% level. Distortions caused by correlation between parameter estimates and model non-linearity were considered to be of little concern, thus all parameters and marginal confidence intervals should be well determined. The 95% marginal confidence intervals, which were determined with the profile traces of Figure 6, are:

$18.6 \leq a_1 \leq 20.3,$
$0.17 \leq a_2 \leq 0.26,$
$0.16 \leq a_3 \leq 0.48,$
$3.8 \leq a_4 \leq 4.4,$ and
$0.045 \leq a_5 \leq 0.13.$

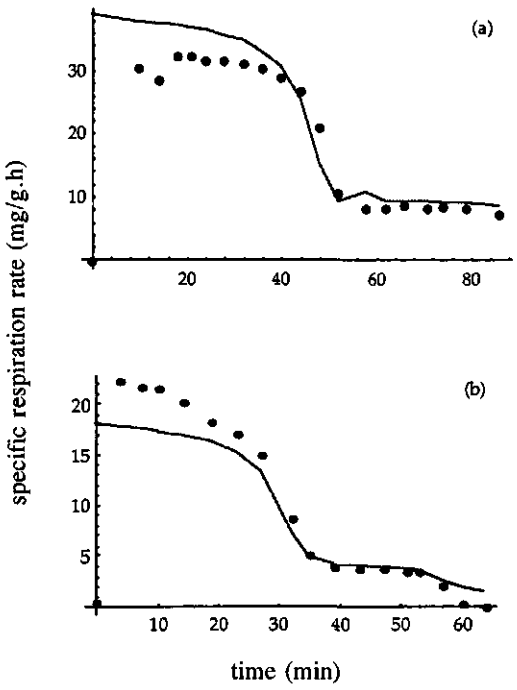

Figure 8: Time series plots for model (2) for experimental runs (a) run 2 and (b) run 4. The points are observed values and the lines are expected values.

Time series goodness of fit test

The purpose of this test was to assess the goodness of fit of model (2) over time for each individual experimental run. The values of (t_j, y_j) and $(t_j, \hat{y}_j)$ were superimposed on the same time series plot. The overall goodness of fit for model (2) was considered good for all experimental runs. Figure 7 shows the results for experimental runs with initial concentrations of s_0 = (6.6, 0.0). Overall, the model fit for all experimental runs were better for stage 2.

The worse fits were observed for experimental runs 2 and 4. The lack of fit shown in Figure 8 was explained by investigating the individual experimental runs. During experimental run 2, the swivel of the DO probe was not firmly closed causing some loss of MLSS and a

reduction in the observed specific respiration rate. For experimental run 4, the stirrer was not engaged for the first 21 minutes of the run, possibly causing incomplete mixing. Since aeration seemed to be sufficiently intensive to cause complete mixing during the run, the data from experimental run 4 were introduced into the combined data set of fourteen experimental runs. In addition, unstable weather conditions, temperature, loading and MLSS, varied more than intended during the entire seven days period of the experiment. Since there was significant variability among experimental runs and the model fit was good for all 14 experimental runs, model (2) is considered to be robust.

7.9 Discussion

The experimental design and methods of model selection, calibration and assessment give assurance that model (2) is appropriately specified and the values of the parameter are well determined. However, there may be doubts that the statistical methods used in this study are necessary. After all, our experimental design used was expensive to conduct in terms of time and money. In this discussion, the use of the experimental design based on the D-optimality criterion and the two-step nitrification model will be challenged in a series of three related questions. Since the reaction is more complex during stage 1 of nitrification, the focus is on model (2c).

Is the model (2c) significant?

The first question deals with the significance of model (2c) in describing nitrification. Possibly a simpler, single substrate Monod model will be as effective in describing the process, $y = a_1 s_1/(a_2 + s_1)$. The following model was calibrated with the same stage 1 data set used for model (2c),

$$\hat{y} = \frac{18.4 s_1}{0.242 + s_1} \qquad (4)$$

with $s^2 = 1.94^2$ and $n = 91$ observations.

The model assessment tests showed that each model parameter is significantly different from zero; therefore, all terms of models (2c) and (4) were considered significant by this test. In addition, since model (2c) is of the same form as model (4) with the inclusion of an

additional term, an extra sum of squares analysis for nested models was used (Bates and Watts, 1988). It showed that the variance of model (4) is significantly larger than for model (2c) at $\alpha = 5\%$; consequently, it shows the importance of the additional term suggesting that the nitrite concentration s_2 is a significant factor during stage 1 of nitrification. From a descriptive point of view, model (2c) is a better model because it describes a cause-effect relationship between y and the predictor variables s_1 and s_2.

Ironically, both models (2c) and (4) give similar responses $\hat{y}$. Note that the residual mean square variance $s^2 = 1.94^2$ for model (4) is slightly greater than the variance $s^2 = 1.78^2$ for model (2c). In goodness of fit tests, the errors e_j for model (4) are slightly greater than for model (2c). The difference is considered minor so it is difficult to claim that one model is better than the other one based on goodness of fit testing. Even though model (4) has a simpler form, it fails to describe the role of nitrite; therefore it is not recommended. This point will be addressed again.

Is model specification (2c) unique?

The specification of model (2c) is based upon observed responses and no attempt has been made to explain the process with mechanistic or biological arguments. It should not be surprising that other model specifications could be used to describe the process. Furthermore, the respiration rates used to calculate the specific respiration rate vector y are based on total oxygen consumption measures by all nitrifying bacteria, not by species of *Nitrosomonas* or *Nitrobacter*. It was not possible through direct measurement to determine the portion of the specific respiration rate associated with either ammonium concentration s_1 or nitrite concentration s_2 during stage 1; therefore, model (2c) was specified without a valuable piece of information. In this study, the basis for specifying model (2c) is based heavily upon empirical evidence and statistical methods for model assessment.

In order to gain a different perspective on the role of s_2, the following Monod model was proposed, $y = \nu(s_2)s_1/(k(s_2) + s_1)$ where the maximum specific respiration rate is a function of nitrite concentration ($\nu(s_2) = a_1 - a_3s_2$), and the half saturation constant is a function of nitrite concentration ($k(s_2) = a_2 - a_4s_2$). This model, like model (2c), suggests that an increase in nitrite concentration s_2 tends to limit specific respiration rate y, and further suggests that nitrite concentration s_2 affects the half saturation rate. Here, the a_3s_2 and a_4s_2 terms act as correction factors for the maximum specific respiration rate constant a_1 and half saturation constant a_2. The calibrated model data set is

$$\hat{y} = \frac{(21.0 - 0.723 s_2) s_1}{(0.343 - 0.028 s_2) + s_1} \tag{5}$$

with $s^2 = 1.66^2$ and n = 91 observations. This model was calibrated with the same data, the stage 1 date set, used for models (2c) and (4).

Model assessment and extra sum of squares tests show the significance of all model parameters of model (5). It gives a slightly better fit than for model (2c).

Since model (5) is a four parameter model, there was the possibility of it being over-parameterized. An over-parameterized model generally leads to problems associated with ill-conditioning, that is poor parameter estimation and high parameter correlation where $| \rho_{pq} | \approx 1$. Model (5) did not suffer from this problem, thus there was no reason to reject it.

Is the experimental design important?

The third and final question deals with the effects of experimental design on modelling. In typical plant operation, mixed liquor does not have normally a high nitrite to ammonium ratio as the combined data set used in this experiment. In order to determine the effect of this experimental design, the data from the four experimental runs with initial concentrations s_0 = (6.5, 0.0) were used to calibrate one- and two-step models. The data were not sorted into stage 1 and 2 data sets as was done for models (2) and (4). The calibrated models are

$$\hat{y} = \frac{15.5 s_1}{0.132 + s_1} \tag{6}$$

with $s^2 = 5.81^2$ and n = 36 observations,

$$\hat{y} = \frac{12.2 s_1}{0.498 + s_1} + 7.01 s_2 \tag{7}$$

with $s^2 = 4.91^2$ and n = 36 observations.

Residual error plots for models (6) and (7) showed that there are no violations in the assumptions for error distribution. Time series plots for these models were satisfactory. Model (6), however, gave slightly biased results.

When comparing these models with models of the same specification calibrated with the combined data set of 14 experimental runs, the results are substantially different. For the one-step model specification, the mean square variance $s^2 = 5.81^2$ for model (6) is significantly greater than the mean square variance $s^2 = 1.94^2$ for models (4). For the two-step model specification, $s^2 = 4.91^2$ for model (7) is significantly greater than $s^2 = 1.78^2$ for model (2c). Model (6) and (7) explain little variation in the observed responses of y.

Additionally, the parameter estimates for models (6) and (7) are not well determined. For the one-step model, the best least square estimate of $a_2 = 0.132$ for model (6) has a standard deviation estimate of $s_2 = 0.96$. This estimate is not significantly different from zero at a 95% confidence level. For the two-step model, the parameter estimates of a_3 are opposite in sign. Model (7) suggests a different role than previously described for model (2c). Models (6) and (7) have parameter estimates of questionable merit.

Part of the problem associated with the poor parameter estimates for models calibrated with $s_0 = (6.5, 0.0)$ data set might be attributed to the size of the data set of $n = 36$. In our opinion, the major difficulties are associated with improper model specification and poor experimental design. The $s_0 = (6.5, 0.0)$ data set does not satisfy the D-optimality criteria for either model (6) or (7).

According to the D-optimality criterion for the model $y = a_1 s_1/(a_2 + s_1)$, observations of s_1 are needed in the vicinity of a_2. In other words, corresponding observations of s_1 and y that lie in the range of $0 < y < 10$. The upper range value of $y = 10$ was chosen to be about one-half the maximum specific respiration rate. Inspection of the $s_0 = (6.5, 0.0)$ data set shows no cause-effect relationship between y and s_1 in this range. The respiration was caused by the oxidation of nitrite not ammonium. This is stage 2 of nitrification, where $s_2 > 0$ and $s_1 = 0$. Model (6) is a poor specification in this range. Model (2), on the other hand, uses a piece-wise model where model (2d) is used to describe the process in this range.

Model (7) is a poor specification for the same reason as well as for other reasons. First, the discussion will focus on the inadequacy of the data set used to calibrate model (7) and then, in next paragraph, return to the issue of model specification for the overall process. According to the D-optimality criterion for the model $y = a_1 s_1/(a_2 + s_1) - a_3 s_2$, both s_1 and s_2 should be long vectors. The length of s_2 for the $s_0 = (6.5, 0.0)$ data set is short in

comparison to s_1. This is reflected in the correlation coefficient of s_1 and s_2. For the s_0 = (6.5, 0.0) data set, ρ_{s1s2} = -0.69. Strong parameter correlation is an expected problem in calibrating model (7). Secondly, inspection of the s_0 = (6.5, 0.0) data set shows a no discernible cause-effect relationship between y and s_2. These two facts offer explanations why the parameter estimates of model (7) are of questionable merit. One approach to increasing information in the data set is to conduct more experimental runs at s_0 = (6.5, 0.0). Repeat runs will increase the number of observations but there is no guarantee that this approach will lead to more confidence in model (7). More repeat runs will not lengthen s_2; therefore, it is not expected to decrease parameter variances $V(a_p)$ or parameter covariances ρ_{pq}. The D-optimality calling for long vectors of both s_1 and s_2 directly addresses the problem of parameter correlation. In hindsight, experimental runs s_0 = (6.5, 6.1) and (6.5, 3.1) of the combined data set for stage 1 provided valuable information in calibrating model (2c) with well determined parameters. It is also helpful for model specification.

The information contained in the s_0 = (6.5, 0.0) experimental runs do not clearly reveal the role of nitrite in nitrification; therefore, it provides a limited view of the process. In contrast, experimental runs s_0 = (6.5, 6.1) and (6.5, 3.1) coupled with experimental runs s_0 = (0.0, 6.2) and (0.0, 3.1) of the combined data set of 14 experimental runs provides important information about the role of nitrite as well as for describing nitrification by stages 1 and 2, and in turn, for specifying a piece-wise model or model (2). In summary, satisfying the D-optimality condition by providing long vectors of s_1 and s_2 provided valuable information for both model specification and parameter estimation.

A final comment about the goodness of fit test and model selection is in order. In this study, nonlinear regression analysis led to the calibration of models with good fits regardless of the data set or model specification. Basing a model on a goodness of fit test alone is considered insufficient evidence to accept a model. Statistical inference testing proved useful in giving added confidence in the model or in drawing attention to possible model inadequacies. A most important factor in specifying and calibrating models with well determined parameters proves to be a carefully designed experiment. Models (2c) and (5) satisfy these conditions. They are cause-effect models that help to describe the role of nitrite in nitrification. Further work is needed to determine whether empirical models (2c), (5) or some other model specification is justifiable in a mechanistic sense.

7.10 Conclusions

According to the statistical analysis of an experiment designed using the D-optimality criterion, the two-step nitrification can be modelled with piece-wise, nonlinear models containing ammonium and nitrite concentration. The study also suggests that questions of model specification and parameter precision associated with nonlinear models can be mitigated with careful experimental design and the use of nonlinear regression analysis.

7.11 Notation

a_1, a_3 maximum specific respiration rates (mg $g^{-1}h^{-1}$)
a_2, a_4 half saturation constants (mg N l^{-1})
n number of observations
r measured respiration rate (mg $l^{-1}h^{-1}$)
r_{end} endogenous respiration rate (mg $l^{-1}h^{-1}$)
r_s = $r-r_{end}$ substrate respiration rate (mg $l^{-1}h^{-1}$)
s_1 ammonium concentration (mg l^{-1})
s_{10} initial ammonium concentration (mg l^{-1})
s_2 nitrite concentration (mg l^{-1})
s_{20} initial nitrite concentration (mg l^{-1})
s^2 variance
y specific respiration rate (mg $g^{-1}h^{-1}$)
$1-\alpha$ confidence interval (%)
ρ_{pq} correlation coefficient parameters a_p and a_q

7.12 References

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Summary

There is a need for a technique capable of continuously measuring wastewater and activated sludge properties. The information obtained by means of such a technique can be used to expand the existing fundamental knowledge about the activated sludge process and to improve the operation of the process, thereby saving operating costs. The oxygen uptake per unit of volume and unit of time, or respiration rate, is the key variable of the activated sludge process. Therefore, this study was devoted to the measurement and application of this variable.

The purpose of this study was to (1) develop a respiration meter capable of continuously measuring, using different procedures, the oxygen uptake rate of activated sludge and (2) expand the existing knowledge about respiration related characteristics of wastewater and activated sludge.

From reviewing the literature on respiration meters it was concluded that only a continuous flow-through respiration meter is suitable for real-time monitoring the rate of oxygen uptake of activated sludge in an aeration tank. In addition, to be useful for state estimation and control of the activated sludge process, respiration meters should be operated continuously. Therefore, in this study the attention was focused on the development of such a meter.

In Chapter 1 the developed respiration meter is described and three different types of respiration rates that can be measured with this meter are presented. The actual respiration rate (r_{act}) is defined as the oxygen uptake rate in the aeration tank. It is argued that the controversy in literature about the usefulness of the respiration measurement can be attributed to inappropriate procedures for measuring r_{act}. In this study it was demonstrated that r_{act} can be correctly measured if simultaneously with the sludge a sample of the influent is fed continuously into the respiration chamber so that the ratio of sample flow rate and chamber volume equals the ratio of influent flow rate and aeration tank volume. The endogenous respiration rate (r_{end}) is defined as the oxygen uptake rate of activated sludge in the absence of readily biodegradable matter. It is conceived that this rate is limited by the rate of hydrolysis of slowly biodegradable matter. From literature it was concluded that there is no clear understanding of the conditions under which the maximum respiration rate (r_{max}) is to be measured. In this thesis the measurement of r_{max}, therefore, is explained separately in Chapter 5.

Biochemical oxygen demand is an important variable for characterising wastewater. In Chapter 1 the significance and nature of short-term biochemical oxygen demand (BOD_{st}) is discussed and substantiated. The BOD_{st} of wastewater (or another solution) is defined as the

amount of oxygen used in addition to the endogenous oxygen consumption. BOD_{st} is due to the oxidation of readily biodegradable matter including readily oxidizable nitrogen.

Finally it is shown in Chapter 1 that the BOD_{st} of the wastewater used in this study originates from soluble matter.

In Chapter 2 the procedures for measuring r_{act}, r_{end} and r_{max} are presented. It is shown that the BOD_{st} of both influent and effluent can be derived from r_{act}, r_{end} and the instantaneous respiration rate; the latter is measured when sludge from the aeration tank flows directly through the respiration chamber. Results from two experimental periods are presented and discussed. It is concluded that the measurements provide relevant information on the activated sludge process and the influent. The BOD_{st} of the examined wastewater appears to be predominantly caused by ammonium oxidised by nitrifiers. There is some evidence that r_{max} depends on the influent flow rate. This observation is amplified in Chapter 5.

The technique for the measurement of r_{act}, described in Chapter 2, is only applicable to a plant with one single aeration tank or the first compartment of a plug flow reactor. In Chapter 3 a method is presented for the estimation of r_{act} in a completely mixed tank, no matter whether it is a single reactor or any compartment of a plug flow reactor. As an intermediate, the BOD_{st} in the aeration tank is estimated simultaneously. In contrast to the other technique, this method does not require the addition of influent to the respiration chamber. Instead, the transient respiration rate is measured during two alternating modes of operation. In one mode sludge from the aeration tank is directed through the respiration chamber, whereas in the other mode sludge having the endogenous rate is used. The method was tested using simulated and experimental data. To verify the estimated r_{act} on the basis of experimental data, this variable was also measured, using the method described in Chapter 2. The pattern of the estimated r_{act} is in agreement with the measured one and with the diurnal variation typical for the wastewater used. There is, however, a significant difference between the average values of the two methods. It is suggested that the measured r_{act} turns out too high because of too high a flow rate of influent into the respiration chamber.

The experimental procedure and estimation technique described in Chapter 3 is applied in Chapter 4 to estimate the influent BOD_{st}. For this purpose, in one mode influent is added to the sludge flowing through the respiration chamber in order to measure r_{act}. In the other mode sludge with the endogenous respiration rate is used. The procedure was tested experimentally and the estimated influent BOD_{st} was compared with values obtained independently from batch respirometric tests. There is a reasonable agreement between the estimated and measured BOD_{st}. It is concluded that the procedure can be used to estimate

the influent BOD_{st} with a relative error varying from 2 to 6%.

In Chapter 5 the measurement and application of r_{max} is reviewed. It is concluded that measuring r_{max} can be useful for monitoring, controlling, assessing toxicity, estimation of biomass concentration and determination of aeration capacity. However, questions arise from the evaluation of the measurements. In particular when the maximum respiration rate is determined by measuring with an excess of substrate, it is not clear whether there was an excess indeed in all cases. The effect of a varying substrate composition on the maximum respiration rate has not been addressed in the literature. In this chapter, therefore, is discussed under which conditions it is possible to measure on-line the maximum respiration rate of sludge from a completely mixed activated sludge tank fed with domestic wastewater from a particular source. It is presumed that the readily biodegradable part of the wastewater can be represented by two components. It is shown that r_{max} can be measured if wastewater is continuously fed into the respiration chamber, so that the loading exceeds a certain critical value. This critical loading is a function of the kinetic parameters of the components and of the fractions in which these components occur in the wastewater. It is shown that batch tests can be useful in checking if the condition for on-line measurement of r_{max}, an excess of substrate, is met. However, the results were not fully satisfactory.

An application of the measurement is also described in Chapter 5. It concerns the effect of the influent flow rate of a nitrifying activated sludge plant on r_{max}. It is concluded that, under the experimental conditions applied, there is no significant effect. The batch tests are useful for verification of the on-line measurements.

Inherent in the principle of the respiration meter, described in Chapter 1 and 2, is that the DO probe is repeatedly subjected to step changes in the DO concentration. As described in Chapter 6, this fact has been employed for the improvement of the DO measurement and, consequently, the performance of the respiration meter by fitting a first-order response model to each measured response. This procedure provides an estimation of the real DO concentration, even when the full response is not attained, and of the probe response time constant. Simulations and nitrification batch tests were used to test the reliability of the method. It is concluded that the proposed method provides a reliable estimate of the real DO concentration and, as a result, the reliability of the respiration meter is improved. Moreover, the estimated first-order probe response time constant proves to be useful for indicating fouling of the probe membrane.

An application of the respiration meter for identifying a mathematical model for nitrification is presented in Chapter 7. Nitrification was chosen because there is a need for a better model

of this process, including both nitrification steps. Because an optimal experimental design and a good model validation method are needed, the investigation was focused on these two items. The investigation consisted of batch tests in which solutions of ammonium chloride and sodium nitrite were added to activated sludge. The respiration rate was monitored whereas ammonium and nitrite concentrations were measured at regular time intervals. Model identification was started by hypothesising a model consisting of two Monod type functions. Nonlinear regression was used for parameter estimation and for evaluating the effects of non-linearity. Residual plots and other tests were used for model assessment. It is demonstrated that nitrification can be modelled with two-step, nonlinear models using the ammonium and nitrite concentration as variables. A two-step model is considered to be better than a one-step model, as the latter considers the respiration rate to be dependent on the ammonium concentration only. It is also suggested that careful experimental design and the use of nonlinear regression are useful for model identification.

Samenvatting

Er bestaat behoefte aan een techniek waarmee kontinu eigenschappen van afvalwater en aktiefslib kunnen worden gemeten. De met een dergelijke techniek verkregen informatie kan worden gebruikt om de bestaande fundamentele kennis van het aktiefslibproces te vergroten en de werking van het proces te verbeteren, zodat bedrijfskosten worden bespaard. De zuurstofopname per volume-eenheid en per tijdeenheid, oftewel de respiratiesnelheid, is de belangrijkste variabele van het aktiefslibproces. Daarom was het onderhavige onderzoek gewijd aan het meten en toepassen van deze variabele.

Het doel van het onderzoek was (1) een respiratiemeter te ontwikkelen die kontinu de zuurstofopnamesnelheid van aktiefslib kan meten en (2) de bestaande kennis over eigenschappen van afvalwater en aktiefslib met betrekking tot respiratie te vergroten.

Het literatuuronderzoek naar respiratiemeters leidde tot de konklusie dat alleen een kontinu doorstroomde meter geschikt is voor het direkt registreren van de zuurstofopnamesnelheid van aktiefslib in een beluchtingtank. Bovendien moet een respiratiemeter kontinu werken om geschikt te zijn voor het schatten van toestandvariabelen en het regelen van het aktiefslibproces. Daarom is in het onderzoek gestreefd naar de ontwikkeling van een dergelijke meter.

In Hoofdstuk 1 wordt de ontwikkelde respiratiemeter beschreven en worden drie soorten respiratiesnelheid geïntroduceerd die de meter kan registreren. De aktuele respiratiesnelheid (r_{act}) wordt gedefinieerd als de zuurstofopnamesnelheid in de beluchtingtank. Verder wordt gesteld dat de kontroverse in de literatuur over de bruikbaarheid van de respiratiemeting is toe te schrijven aan inadekwate meetmethodes. Dit onderzoek laat zien dat r_{act} korrekt wordt gemeten als, gelijktijdig met het aktiefslib, een deelstroom van het aangevoerde afvalwater in het respiratievat wordt gebracht, waarbij het deelstroomdebiet en het respiratievatvolume zich tot elkaar verhouden als het influentdebiet en het beluchtingtankvolume. De basisrespiratiesnelheid (r_{end}) wordt gedefinieerd als de zuurstofopnamesnelheid van aktiefslib bij afwezigheid van snel-biodegradeerbare stoffen. Er wordt van uitgegaan dat deze snelheid afhankelijk is van de hydrolysesnelheid van langzaam-biodegradeerbare stoffen. Literatuuronderzoek leidde tot de konklusie dat er geen duidelijk inzicht is in de kondities waaronder de maximale respiratiesnelheid (r_{max}) moet worden gemeten. Daarom wordt in Hoofdstuk 5 van dit proefschrift de meting van r_{max} apart uiteengezet.

Het biochemisch zuurstofverbruik is een belangrijke grootheid voor het karakteriseren van afvalwater. In Hoofdstuk 1 wordt het belang en de aard van het korte termijn biochemisch zuurstofverbruik (BZV_{kt}) besproken en worden daarvoor gronden aangevoerd. Het BZV_{kt} van

afvalwater (of een ander medium) wordt gedefinieerd als de hoeveelheid zuurstof die naast het basiszuurstofverbruik wordt verbruikt, als dit water met aktiefslib in kontakt wordt gebracht. Het BZV_{kt} is het gevolg van de oxidatie van snel-biodegradeerbare stoffen, inklusief snel-oxideerbare stikstof.

Tot slot wordt in Hoofdstuk 1 aangetoond dat het BZV_{kt} van het in dit onderzoek gebruikte afvalwater het gevolg is van opgeloste stoffen.

In Hoofdstuk 2 worden de procedures voor het meten van r_{act}, r_{end} en r_{max} beschreven. Er wordt aangetoond dat het BZV_{kt} van zowel influent als effluent kan worden berekend uit r_{act}, r_{end} en de momentane respiratiesnelheid. De laatste wordt gemeten als aktiefslib van de beluchtingtank door de respiratiemeter stroomt. De resultaten van twee meetperiodes worden gepresenteerd en besproken. Er wordt gekonkludeerd dat de metingen relevante informatie geven over het aktiefslibproces en het influent. Het BZV_{kt} van het onderzochte afvalwater blijkt voornamelijk het gevolg te zijn van de oxidatie van ammonium door nitrificerende bakteriën. Er is een aanwijzing dat r_{max} afhankelijk is van het influentdebiet. Deze waarneming wordt besproken in Hoofdstuk 5.

De in Hoofdstuk 2 beschreven methode voor het meten van r_{act} geldt alleen voor een inrichting met een enkele beluchtingtank of voor het eerste kompartiment van een propstroomreaktor. In Hoofdstuk 3 wordt een methode beschreven voor het schatten van r_{act} in een volledig gemengde tank, ongeacht of dit een enkele reaktor is of een kompartiment van een propstroomreaktor. Het BZV_{kt} in de beluchtingtank wordt gelijktijdig als tussenprodukt geschat. In tegenstelling tot de andere methode is het bij deze methode niet nodig afvalwater aan het respiratievat toe te voegen. In plaats daarvan wordt de overgangsrespiratiesnelheid gemeten tijdens twee elkaar afwisselende modi operandi. In de ene modus wordt het slib vanaf de beluchtingtank door het respiratievat geleid, terwijl in de andere modus slib met de basisrespiratiesnelheid wordt gebruikt. De methode is beproefd met behulp van gesimuleerde en experimentele meetgegevens. Om de op basis van experimentele gegevens geschatte r_{act} te verifiëren, is deze variabele ook gemeten met behulp van de in Hoofdstuk 2 beschreven methode. Het verloop van de geschatte r_{act} komt overeen met dat van de gemeten r_{act} en met de dagelijkse variatie die voor het gebruikte afvalwater kenmerkend is. Er bestaat echter een signifikant verschil tussen de gemiddelde waarden van de twee methodes. Een mogelijke verklaring is dat de gemeten r_{act} te hoog is door een te hoog debiet van het afvalwater naar het respiratievat.

De in Hoofdstuk 3 beschreven experimentele procedure en schattingsmethode wordt toegepast in Hoofdstuk 4 voor de schatting van het influent BZV_{kt}. Daartoe wordt in de ene modus een

deelstroom van het influent toegevoegd aan het slib dat door het respiratievat stroomt. Hierbij wordt r_{act} gemeten. In de andere modus wordt aktiefslib met de basisrespiratiesnelheid gebruikt. De procedure is experimenteel getest en het geschatte influent BZV_{kt} is vergeleken met onafhankelijk in batchproeven gemeten waarden. Het geschatte en gemeten BZV_{kt} komen redelijk overeen. De konklusie is dat met deze procedure het influent BZV_{kt} kan worden geschat met een relatieve fout tussen 2 en 6%.

In Hoofdstuk 5 wordt de meting en toepassing van r_{max} besproken. Er wordt gekonkludeerd dat het meten van r_{max} nuttig kan zijn voor het registreren, het regelen, het vaststellen van de toxiciteit, het schatten van de biomassakoncentratie en het bepalen van de beluchtingkapaciteit. De evaluatie van de meetresultaten roept echter vragen op. In het biezonder wanneer r_{max} wordt bepaald door met een overmaat van substraat te meten, is het niet duidelijk of er werkelijk steeds een overmaat was. Het effekt van een variërende substraatsamenstelling op r_{max} wordt niet in de literatuur behandeld. Daarom wordt in dit hoofdstuk besproken onder welke omstandigheden kontinu r_{max} gemeten kan worden van aktiefslib uit een volledig gemengde tank die met afvalwater uit een bepaalde bron wordt gevoed. Er wordt ondersteld dat het snel-biodegradeerbare deel van het afvalwater kan worden voorgesteld door twee komponenten. Aangetoond wordt dat r_{max} kan worden gemeten als afvalwater kontinu in het respiratievat wordt geleid en wel zodanig dat de belasting een bepaalde kritische waarde overschrijdt. Deze kritische belasting is een funktie van de kinetische parameters van de komponenten en van de frakties waarin deze komponenten in het afvalwater voorkomen. Er wordt aangetoond dat batchproeven geschikt zijn om na te gaan of wordt voldaan aan de voorwaarde voor kontinue meting van r_{max}, namelijk een overmaat van substraat. De behaalde resultaten zijn echter niet volledig bevredigend.

Een toepassing van de meting wordt ook beschreven in Hoofdstuk 5. Bij deze toepassing wordt het effekt onderzocht van het influentdebiet van een nitrificerende aktiefslibinrichting op r_{max}. Er wordt gekonkludeerd dat er onder de toegepaste experimentele omstandigheden geen signifikant effekt optreedt. De batchproeven zijn geschikt voor verifikatie van de kontinumetingen.

Inherent aan het principe van de respiratiemeter zoals beschreven in Hoofdstuk 1 en 2 is dat de zuurstofsensor herhaaldelijk wordt onderworpen aan stapsgewijze veranderingen in de zuurstofkoncentratie. Zoals beschreven in Hoofdstuk 6, is dit gegeven toegepast om de zuurstofkoncentratiemeting te verbeteren ,en daarmee de prestatie van de respiratiemeter. Daartoe is een eerste-orde-responsmodel gefit op elke gemeten respons. Deze procedure levert, zelfs als niet de volledige respons wordt gemeten, een schatting op van de werkelijke

zuurstofkoncentratie en van de eerste-orde-tijdkonstante van de sensor. Simulaties en batchnitrifikatieproeven zijn uitgevoerd om de betrouwbaarheid van de procedure na te gaan. Er wordt gekonkludeerd dat de voorgestelde procedure een betrouwbare schatting oplevert van de werkelijke zuurstofkoncentratie en zo de betrouwbaarheid van de berekende respiratiesnelheid en daarmee die van de respiratiemeter vergroot. De geschatte eerste-orde-tijdkonstante van de sensor is bovendien bruikbaar gebleken om vervuiling van de sensormembraan aan te tonen.

In Hoofdstuk 7 wordt een toepassing van de respiratiemeter beschreven voor het identificeren van een mathematisch model voor de nitrifikatie. Nitrifikatie werd gekozen omdat er behoefte is aan een beter model voor de beschrijving van dit proces, inklusief de twee nitrifikatiestappen. Omdat een optimaal experimenteel ontwerp en een goede modelvalideringsmethode nodig zijn, stonden deze twee onderwerpen bij het onderzoek centraal. Bij het onderzoek zijn batchproeven uitgevoerd waarbij oplossingen van ammoniumchloride en natriumnitriet aan aktiefslib zijn toegediend. De respiratiesnelheid is kontinu geregistreerd terwijl de ammonium- en nitrietkoncentraties met regelmatige tussenpozen zijn gemeten. Als eerste stap voor de modelidentifikatie is uitgegaan van een model bestaande uit twee Monod-vergelijkingen. Niet-lineaire regressie is toegepast voor het schatten van parameters en het vaststellen van effekten van niet-lineariteit. Residual curves en andere statistische toetsen zijn toegepast voor het beoordelen van het model. In het hoofdstuk wordt aangetoond dat de nitrifikatie kan worden gemodelleerd met een twee-staps niet-lineair model met de ammonium- en de nitrietkoncentratie als variabelen. Een twee-stapsmodel wordt als beter beschouwd dan een een-stapsmodel, omdat in de laatste de respiratiesnelheid alleen een funktie is van de ammoniumkoncentratie. Er wordt in dit hoofdstuk ook aangevoerd dat een zorgvuldig experimenteel ontwerp en de toepassing van niet-lineaire regressie nuttig zijn voor de modelidentifikatie.

Appendix A: Calculation of the respiration rate

The respiration rate is calculated from the mass balance of dissolved oxygen (DO) over the respiration chamber. Figure A1 shows a scheme of the respiration chamber. The DO concentration at the inlet (c_a) and outlet (c) is measured with one single probe, fixed at one opening of the chamber. This is realised by alternating, typically every 30 seconds, the sludge flow through the chamber by using four valves.

The procedure results in a DO probe output, also shown in Figure A1, oscillating between c_a and c. The values of c_a and c are determined at the end of each response, resulting in two time series of DO concentrations, one of the inlet and one of the outlet. The respiration rate is calculated from these time discrete values by integrating the mass balance (A1) of DO over the respiration chamber.

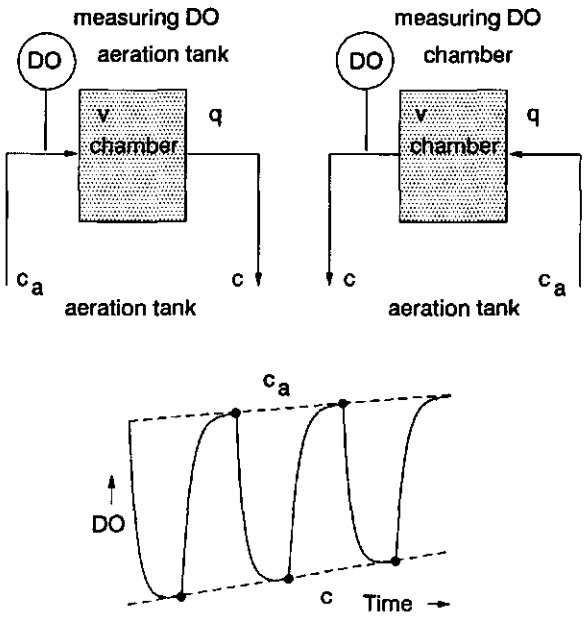

Figure A1: Scheme of the respiration chamber and typical DO probe output.

$$\frac{dc(t)}{dt} = \alpha\left(c_a(t)-c(t)\right) - r(t) \qquad \text{(A1)}$$

where α=q/v.

Given the discrete time nature of the data, equation (A1) has to be approximated by a difference equation. In this study two methods are used: an analytical method and the trapezoidal rule.

Analytical method

Figure A2 shows the DO measurements used for the calculation of the respiration rate at time instant k.

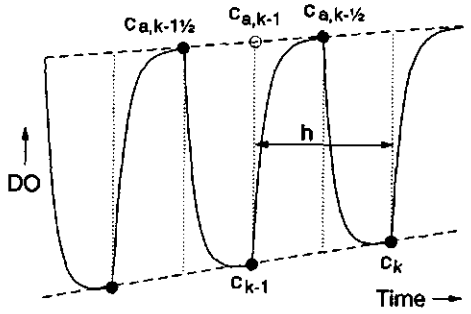

***Figure A2**: Signal from the respirometer DO probe. Solid circles: measured DO concentrations to be used for the calculation (analytical method) of the respiration rate at time instant k.*

From (A1):

$$\frac{dc(t)}{dt} = -\alpha\, c(t) + \alpha\, c_a(t) - r(t) \qquad \text{(A2)}$$

Consider the interval $[t_{k-1}, t_k]$, where c_a and r are constant and $t_k - t_{k-1} = h$ is the sampling interval for the DO concentration. The general solution of (A2) is

$$c(t) = e^{-\alpha(t-t_{k-1})}\left(c(t_{t-1}) + \int_{t_{k-1}}^{t} f(s)\, e^{\alpha(s-t_{k-1})} ds\right) \tag{A3}$$

where

$$f(t) = \alpha\, c_a(t) - r(t) \tag{A4}$$

or

$$c(t) = c(t_{k-1})\, e^{-\alpha(t-t_{k-1})} + \int_{t_{k-1}}^{t} f(s)\, e^{-\alpha(t-s)} ds \tag{A5}$$

Since f(t)=f is constant in the interval $[t_{k-1}, t_k]$ equation (A5) can be written:

$$c(t) = c(t_{k-1})\, e^{-\alpha(t-t_{k-1})} + \frac{f}{\alpha}\left(1 - e^{-\alpha(t-t_{k-1})}\right) \tag{A6}$$

With $t=t_k$ we get

$$c(t_k) = c(t_{k-1})\, e^{-\alpha h} + \frac{f}{\alpha}\left(1 - e^{-\alpha h}\right) \tag{A7}$$

At $t=t_{k-1}$, equation (A4) gives

$$f(t_{k-1}) = f = \alpha\left(c_a(t_{k-1}) - r(t_k)\right) \tag{A8}$$

Combining (A6) and (A7) and solving for r yields:

$$r(t_k) = \frac{\alpha}{(1-e^{-\alpha h})}\left((1-e^{-\alpha h})c(t_{a,k-1}) + c(t_{k-1})e^{-\alpha h} - c(t_k)\right) \qquad \textbf{(A9)}$$

As illustrated in Figure A2, the values of $c(t_{k-1})$ and $c(t_k)$ are measured. The value of $c_a(t_{k-1})$ can be found by linear interpolation:

$$c_a(t_{k-1}) = \tfrac{1}{2}\left(c_a(t_{k-0.5}) + c_a(t_{k-0.5})\right) \qquad \textbf{(A10)}$$

Trapezoidal rule

The respiration rate can also be calculated from (A1) by approximating the derivative by a piecewise linear function (trapezoidal rule):

$$\frac{c(t_{k+0.5})-c(t_{k-0.5})}{h} = \tfrac{1}{2}\left(\alpha\left(c_a(t_{k+0.5}) + c_a(t_{k-0.5})\right) - \alpha\left(c(t_{k+0.5}) - c(t_{k-0.5})\right) - 2r_k\right) \qquad \textbf{(A11)}$$

or

$$r_k = \tfrac{1}{2}\left(\alpha\left(c_a(t_{k+0.5}) + c_a(t_{k-0.5})\right) - \left(\alpha+\frac{2}{h}\right)c(t_{k+0.5}) - \left(\alpha-\frac{2}{h}\right)c(t_{k-0.5})\right) \qquad \textbf{(A12)}$$

Figure A3 illustrates that $c_a(t_{k-½})$ and $c_a(t_{k+½})$ are measured values, while $c(t_{k-½})$ and $c(t_{k+½})$ have to be found by interpolation:

$$\begin{aligned} c(t_{k-0.5}) &= \tfrac{1}{2}\left(c(t_{k-1}) + c(t_k)\right) \\ c(t_{k+0.5}) &= \tfrac{1}{2}\left(c(t_k) + c(t_{k+1})\right) \end{aligned} \qquad \textbf{(A13)}$$

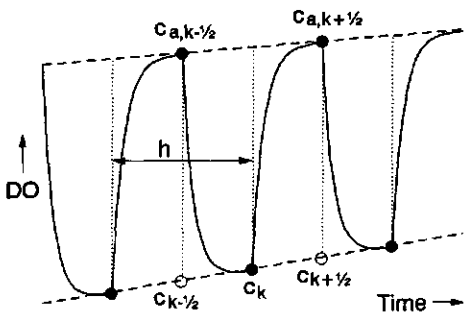

Figure A3: *Signal from the respirometer DO probe. Solid circles: measured DO concentrations to be used for the calculation (trapezoidal rule) of the respiration rate at time instant k.*

Notation

a, b = help variables
c = DO concentration at the outlet of the respiration chamber
c_a = DO concentration at the inlet of the respiration chamber
q = flow through respiration chamber
r = respiration rate
t = time
v = volume respiration chamber
α = dilution rate

Appendix B: Condition for measurement of the actual respiration rate

For the measurement of the actual respiration respiration rate (r_{act}, in this appendix denoted as r_a) with the flow-through respiration meter it is crucial that the activated sludge in the respiration chamber and the aeration tank are loaded with substrate in the same proportion. Therefore, simultaneously with the sludge, wastewater is continuously introduced into the chamber (Figure B1). In this appendix, the required influent sample flow (q_s) is derived.

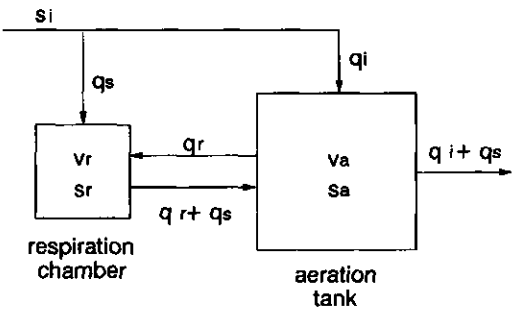

Figure B1: *Schematic diagram of the aeration tank and the respiration chamber (not on scale). The substrate concentration (s) is expressed in* BOD_{st}.

The following assumptions are made:

- The volume of the tubes can be neglected.
- No oxidation of readily biodegradable compounds takes place in the settling tank.
- Steady state exists with respect to the substrate concentration.
- The respiration rate is only a function of biomass concentration and substrate concentration.

The mass balances of s over the aeration tank and the respiration chamber are respectively:

$$q_i s_i + (q_r+q_s)s_r - q_r s_a - (q_i+q_s)s_a - x_a v_a a_a = 0 \qquad \textbf{(B1)}$$

$$q_s s_i + q_r s_a - (q_r+q_s)s_r - x_r v_r a_r = 0 \qquad \textbf{(B2)}$$

where the activity is a function of the substrate concentration:

$$a = \frac{r}{x} = f(s) \tag{B3}$$

Now, we choose the condition

$$a_r = a_a \rightarrow s_r = s_a \tag{B4}$$

Combining equations (B1), (B2) and (B3) and solving for q_s gives:

$$q_s = \frac{x_r v_r}{x_a v_a} q_i \tag{B5}$$

Because of dilution with wastewater, the biomass concentration in the respiration chamber is lower that that in the aeration tank. Using

$$\frac{x_r}{x_a} = \frac{q_r}{q_r+q_s} \tag{B6}$$

we obtain:

$$q_s = \frac{q_r v_r}{(q_r+q_s) v_a} q_i \tag{B7}$$

or:

$$q_s = -\frac{1}{2}q_r \pm \frac{1}{2}\sqrt{q_r^2 + 4\frac{v_r}{v_a}q_r q_i} \tag{B8}$$

From (B7) it follows that, if $q_s << q_r$, the influent sample flow can be approximated by:

$$q_s = \frac{v_r}{v_a} q_i \qquad \textbf{(B9)}$$

When q_s is calculated according to equation (B8) and set in the measuring system, the activity in respiration chamber will be equal to that in the aeration tank. However, the measured respiration rate (r_r) will differ somewhat from that in the aeration tank (r_a). Using (B3) and (B6):

$$r_r = \frac{q_r}{q_r+q_s} r_a \qquad \textbf{(B10)}$$

The error of r_r relative to r_a can be calculated as follows:

$$\frac{r_r-r_a}{r_a} = \frac{-q_s}{q_r+q_s} \qquad \textbf{(B11)}$$

Equation B2 demonstrates that the error depends on the ratio of q_s and q_r. Figure B2 shows the relative error as a function of q_s/q_r, on a practical range, according to equation (B11). In this study q_s/q_r is not larger that 0.007. Thus the error of r_r relative to r_a, caused by the dilution with wastewater does not exceed 0.7%.

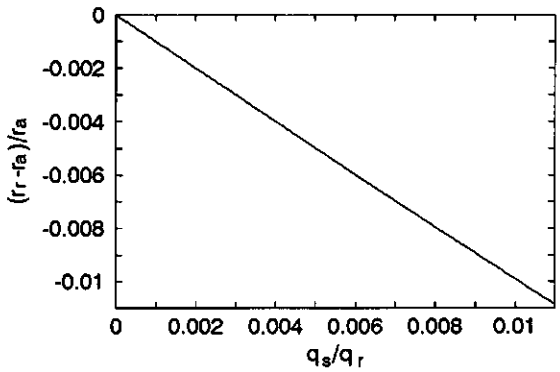

***Figure B2**: Error of the measured respiration rate relative to the respiration rate in the aeration tank.*

Notation

symbols

- a = activity
- q = flow
- r = respiration rate
- s = BOD_{st} in aeration tank
- v = volume
- x = biomass concentration

subscripts

- a = aeration tank
- i = influent
- s = influent sample
- r = respiration chamber

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Ik bedank iedereen die heeft bijgedragen aan het tot stand komen van dit proefschrift. Een aantal mensen wier aandeel is terug te vinden in dit boekje wil ik noemen:

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Paul Ossenbruggen (Department of Civil Engineering, University of New Hampshire, Durham, USA) kwam op het idee respirometrie toe te passen bij het valideren van een nitrifikatiemodel en de eksperimentele resultaten statistisch te verwerken. Het laatste hoofdstuk van dit proefschrift heb ik hem te danken. Het kommentaar van Bert Hamelers en Paul's kollega's Ernst Linder en Marie Gaudard is in dit hoofdstuk verwerkt.

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Curriculum vitae

Henri Spanjers is in 1957 geboren in Roermond. In 1978 begon hij met de studie Milieuhygiëne aan de Landbouwuniversiteit. Na het behalen van het kandidaatsdiploma in 1981 bracht hij de praktijktijd door aan het "Institut für Wasser-, Boden- und Lufthygiëne" in Berlijn en aan het "Département de Chimie Minérale et Analytique de l'Université de Genève". In 1985 behaalde hij het doktoraaldiploma met als vakken waterzuivering, fysische chemie en bodemverontreiniging. Vanaf 1985 is hij in dienst van de vakgroep Milieutechnologie van de Landbouwuniversiteit en heeft hij gewerkt aan diverse projekten op het gebied van de respirometrie. In 1988 is hij begonnen met het onderzoek dat tot dit proefschrift heeft geleid. Een gedeelte van het onderzoek is gedurende een half jaar aan de "Lunds Tekniska Högskola" in Zweden uitgevoerd.