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Principles of Respirometry in Activated Sludge Wastewater Treatment

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1. Introduction

Respirometry is the measurement and interpretation of the biological oxygen consumption rate under well-defined experimental conditions. Because oxygen consumption is directly associated with both biomass growth and substrate removal, respirometry is a useful technique for monitoring, modelling and control of the activated sludge process. In the early years application of the technique was mainly focused on measurement of the Biochemical Oxygen Demand (BOD) of (waste)water. At that time, respirometry was seen as an instrumental alternative to the original BOD-test which needed chemical analysis of oxygen concentration. Later, starting in the sixties, respirometry was developed further and the technique began to generate much interest in process control. During the past decade respirometry increasingly is being employed to obtain biokinetic characteristics, and it is considered one of the most important information sources in activated sludge process modelling.

Since the discovery of the activated sludge process in the beginning of the 20^{th} century it has been recognised that the rate at which activated sludge consumes oxygen, the *respiration rate*, is an important indicator of the process condition. Consequently, efforts have been made to measure this variable. Respiration rate usually is measured using ***respirometers***. These range from very simple manually operated BOD-bottles to fully self-operating instruments that automatically perform sampling, calibration, and calculation of respiration rate. All respirometers are based on some technique for measuring the rate at which biomass takes up ***dissolved oxygen*** (DO) from the liquid. This can be done directly by measuring DO or indirectly by measuring ***gaseous oxygen***. Electrochemical DO measurements (e.g. based on the Clark-cell) are almost uniquely applied in DO-based respirometers. Gaseous oxygen concentration can be measured by physical techniques such as the paramagnetic method. Other physical techniques such as manometric and volumetric methods measure the change of gaseous oxygen concentration. In the early years all respirometers were based on gaseous measuring methods. After the introduction of the electrochemical Clark-type DO measuring cell in 1959 this type of sensor became more and more common in respirometers. Currently about 50% of the commercial respirometer brands are based on a DO-sensor. While commercial respirometers are chiefly being used in wastewater treatment practice, a considerable number of home-made respirometers, of which the majority is of the DO-sensor type, is operational in research environments.

The respiration rate itself can be used as the variable of interest. For example the actual or endogenous rate may be maintained at a certain value by manipulating some process variable, or the measurement may be used to indicate disturbances or activate alarms. However, often respirometry is used to extract information with a particular biological significance from the measurements. In these cases not respiration rate itself but another variable is the true variable of interest. Although specifying these two approaches is not based on rigorous fundamental differences it may be helpful in realising that respirometry is not restricted to monitoring the respiration rate of activated sludge.

2. Biochemical background

Strictly, in biochemistry terms, ***respiration*** is the adenosine triphosphate (ATP) generating metabolic process in which either organic or inorganic compounds serve as electron donor and inorganic compounds such as O_2, NO_2^-, NO_3^-, SO_4^{2-} etc. serve as the ultimate electron acceptor. If oxygen is the ultimate electron acceptor, the process is called ***aerobic respiration***. ATP is generated as electrons removed from the substrate are transferred along the ***electron transport chain*** from one metabolic carrier to the next and, ultimately, to oxygen. The biomass, in this way, converts the energy of intramolecular bonds in the substrate to the high-energy phosphate bonds of ATP. The energy then is used to synthesise the various molecular components required for cell growth and reproduction. The overall process of aerobic respiration by heterotrophic biomass

is depicted schematically in Fig. 1. ***Heterotrophic biomass*** (including not only bacteria and their storage materials, but also protozoa and other higher organisms), uses substrate consisting of carbonaceous material. Only a portion (1-Y_H) of the consumed organic substrate is oxidised to provide energy. The remainder of the substrate molecules (the yield, Y_H) is reorganised into new cell mass. Typically about half of the substrate (on a weight/weight basis) is converted into new biomass.

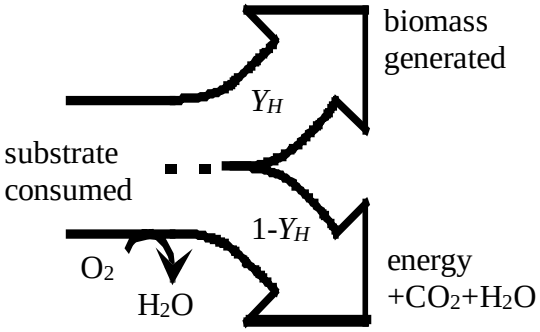


Figure 1. Schematic representation of aerobic respiration by heterotrophic biomass.

Carbonaceous substrate removal is not the only oxygen consuming process. In addition, oxygen is consumed in other bacterial processes such as the oxidation of inorganic compounds by nitrifiers and other autotrophic bacteria, and specific microbial oxidation reactions catalysed by oxidases and mono-oxygenases.

Nitrifying bacteria include only a minor part of the substrate ammonia into new biomass while most of the substrate is oxidised for energy production. These autotrophic bacteria use dissolved carbon dioxide as a carbon source for new biomass. In comparison to heterotrophic biomass, nitrifiers need more oxygen for their growth. Nitrification occurs in two steps: the oxidation of ammonia to nitrite and the oxidation of nitrite to nitrate.

In addition to the oxygen consumption by heterotrophic and nitrifying biomass there are some other biological processes that may contribute to the respiration of activated sludge. Like nitrifiers, the autotrophic ***sulphur bacteria*** and ***iron bacteria*** utilise inorganic compounds instead of organic matter to obtain energy and use carbon dioxide or carbonate as a carbon source. Sulphur bacteria are able to oxidise hydrogen sulphide (or other reduced sulphur compounds) to sulphuric acid. Iron bacteria oxidise inorganic ferrous iron to the ferric form to obtain energy.

Finally, some inorganic electron donors like ferrous iron and sulphide can be chemically oxidised utilising oxygen and contribute to the observed respiration of biomass.

All oxygen consuming processes contribute to the observed total respiration rate of the biomass. Respirometry is usually intended to measure only biological oxygen consumption and sometimes it is attempted to distinguish between different biological processes such as heterotrophic substrate removal and nitrification. However, in many cases it is difficult to distinguish between specific microbial processes and to identify chemical oxygen consumption.

3. Modelling respiration

Modelling is important for the design and control of the activated sludge process, as well as for understanding the basics of respiration. In the ***traditional*** modelling approach respiration is associated with growth and decay of micro-organisms. In the ***death-regeneration*** approach, adopted in the IAWQ Activated Sludge Model No.1, respiration is associated only with aerobic growth of heterotrophic and nitrifying biomass. Fig. 2 schematically shows the main processes for heterotrophic growth and biodegradation for the two approaches.

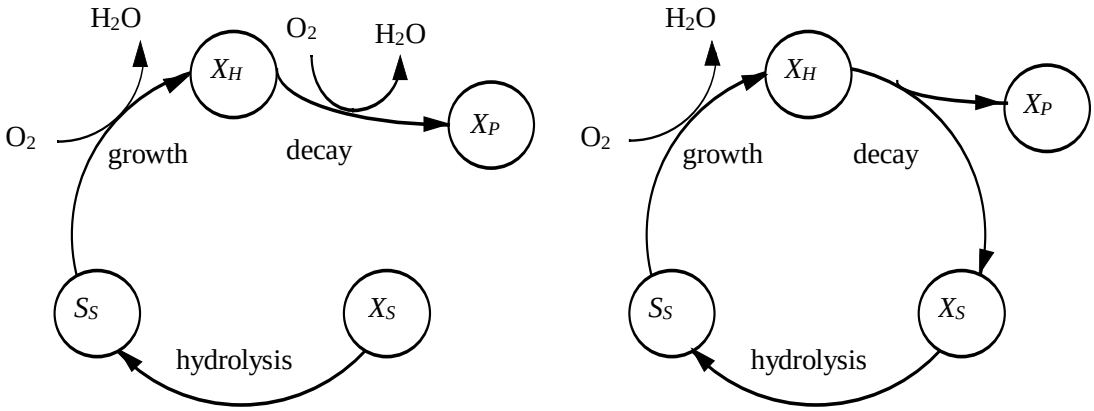


Figure 2. Two modelling approaches for the activated sludge process: Traditional (left) and death-regeneration (right).

Both approaches describe growth of biomass (X_H) as a process where oxygen is consumed. However, the traditional approach considers biomass decay as an additional oxygen consuming process in which decaying biomass is oxidised while inert matter (X_P) is formed. The model implies that, when the activated sludge has run out of readily biodegradable substrate (S_S) and slowly biodegradable matter (X_S) from the wastewater, the remaining oxygen consumption is associated with biomass decay only.

According to the death-regeneration approach decaying biomass is split into two fractions: inert matter and slowly biodegradable matter. The latter is subsequently hydrolysed into readily biodegradable substrate. This process does not involve any consumption of oxygen (or another electron acceptor). The death-regeneration model implies that, even when all the substrate originating from the wastewater is oxidised, there remains an oxygen consumption associated with the growth on substrate released from decay and hydrolysis. The amount of new biomass formed from released substrate is always less than the amount of biomass lost.

Both models imply that if biomass is left on its own without input of biodegradable matter from wastewater the respiration rate will gradually decrease until all the biomass has decayed. The respiration rate during this process is called the ***endogenous respiration rate***. The endogenous respiration rate of activated sludge can be defined in operational terms as the oxygen consumption rate in the absence of substrate from external sources. According to this definition the endogenous respiration not only includes decay of bacteria (and concomitant growth in case of death-regeneration) but also oxygen consumption by protozoa. Note that in the microbiological literature the maintenance concept is also used as another description of microbial behaviour. In this concept external substrate is oxidised to maintain the biomass in its current state. No new biomass is produced, distinguishing maintenance from growth, and substrate is only oxidised for energy generation. A consensus is growing that from a modelling and measuring point of view both maintenance and endogenous concepts are able to represent this specific process behaviour. The endogenous respiration rate is practically independent of the substrate concentration and, as such, indicative for the concentration of active biomass.

Usually in the activated sludge process a continuous input of biodegradable material exists from the influent. This results in a net growth of biomass and an associated respiration rate which is higher than the

endogenous rate. This ***actual respiration rate*** is a function of the concentration of biodegradable matter in the aeration tank, which is in turn the net result of three processes: input from the influent, loss via the effluent and biodegradation. It is obvious that this balance is disturbed when activated sludge is sampled from the aeration tank and that, hence, the respiration rate measured in the sample is likely to be biased to lower values.

If the concentration of biodegradable matter is very high the biomass will grow at its maximum rate and the rate of oxygen consumption will approximate its maximum value: the ***maximum respiration rate***. In the real world of an activated sludge plant treating sewage, respiration is the result of the oxidation of multiple substrates by a heterogeneous population of micro-organisms. This means that the true maximum respiration rate is only reached if all the individual substrates are present in excess. In an activated sludge plant this condition is not very likely to happen. In a well designed respirometric experiment, however, the appropriate condition for the measurement of the maximum respiration rate may be created and the measurement can be used for model identification. Like the endogenous respiration rate, the maximum respiration rate is practically independent of substrate concentration and, therefore, is indicative of the active biomass concentration.

Using the model concepts depicted in Fig. 1, respiration rate can be mathematically expressed in terms of growth and decay. The traditional model (growth and decay of nitrifiers included) is:

$$r_O = \frac{1-Y_H}{Y_H}\mu_H(S_S,S_O)\,X_H + \frac{4.57-Y_A}{Y_A}\mu_A(S_{NH},S_O)\,X_A + \left[1-f_P'\right]\left[b_H'(S_O)\,X_H + b_A(S_O)\,X_A\right] \quad (1)$$

and the death-regeneration model is:

$$r_O = \frac{1-Y_H}{Y_H}\mu_H(S_S,S_O)\,X_H + \frac{4.57-Y_A}{Y_A}\mu_A(S_{NH},S_O)\,X_A \quad (2)$$

The specific growth rates $\mu_H(S_S,S_O)$ and $\mu_A(S_{NH},S_O)$ are dependent on the substrate concentrations S_S and S_{NH}, respectively, and on the dissolved oxygen concentration S_O:

$$\mu_H(S_S,S_O) = \frac{\mu_{mH}S_S}{K_S+S_S}\,\frac{S_O}{K_{OH}+S_O} \quad (3)$$

$$\mu_A(S_{NH},S_O) = \frac{\mu_{mA}S_{NH}}{K_{NH}+S_{NH}}\,\frac{S_O}{K_{OA}+S_O} \quad (4)$$

In the traditional model the decay rates $b_H'(S_O)$ and $b_A(S_O)$ are only dependent on the dissolved oxygen concentration:

$$b_H'(S_O) = b_{mH}'\,\frac{S_O}{K_{OH}+S_O} \quad (5)$$

$$b_A(S_O) = b_{mA}\,\frac{S_O}{K_{OA}+S_O} \quad (6)$$

It is seen from (1) that the respiration rate is governed by the growth of both heterotrophs and nitrifiers, and the decay of both types of biomass. At high substrate concentrations (S_S, S_{NH} and S_O) the first and second term approach saturation and so does the respiration rate (maximum rate). If all the substrate is oxidised then the first and second terms become zero and the lower - endogenous - respiration rate is governed by

the biomass concentration. In the death-regeneration model (2), even when external substrate is absent, the concentration will never approach zero since substrate is regenerated from decay and subsequent hydrolysis (see Fig. 2). In nitrifying biomass part of the nitrogen released from decay will be nitrified during endogenous respiration.

Note that in the description above heterotrophic growth is associated with only one substrate (S_S). In other model approaches heterotrophs may grow on more substrates with concomitant oxygen consumption. In addition, nitrification may be explicitly modelled as a two-step process: oxidation of ammonium to nitrite and subsequent oxidation of nitrite to nitrate.

4. Linking respirometry to substrate removal and growth

In wastewater treatment, waste removal and biomass activity are important processes which need to be monitored for good process control. On a cellular level these correspond with substrate conversion and biomass growth. It was shown above that respiration is linked to these key metabolic processes that take place in the cell. Because it is not possible to measure respiration rates within the biomass itself we have to recur to a measurement of the oxygen uptake rate from the bulk into the biomass. However, since a rate measurement is done (i.e. time is involved), the dynamics of the dissolved oxygen concentration are important. In other words, it is necessary to assess the dissolved oxygen accumulation (positive or negative) in the bulk liquid, and the inputs and outputs as well. We may also have to consider removal of oxygen through chemical oxidation reactions and eliminate this to calculate biological oxygen consumption. Alternatively, as oxygen is typically supplied from the gas phase, the disappearance of gaseous oxygen also can be measured and related to biomass growth and substrate removal. Obviously, this additional phase complicates the interpretation of the measured gaseous oxygen uptake rate, for the interphase transport process should be characterised.

Fig. 3 illustrates that linking respirometry to substrate removal and growth may involve three phases (biomass, liquid phase and gas phase) and that respiration rates can be assessed from either the liquid or the gas phase.

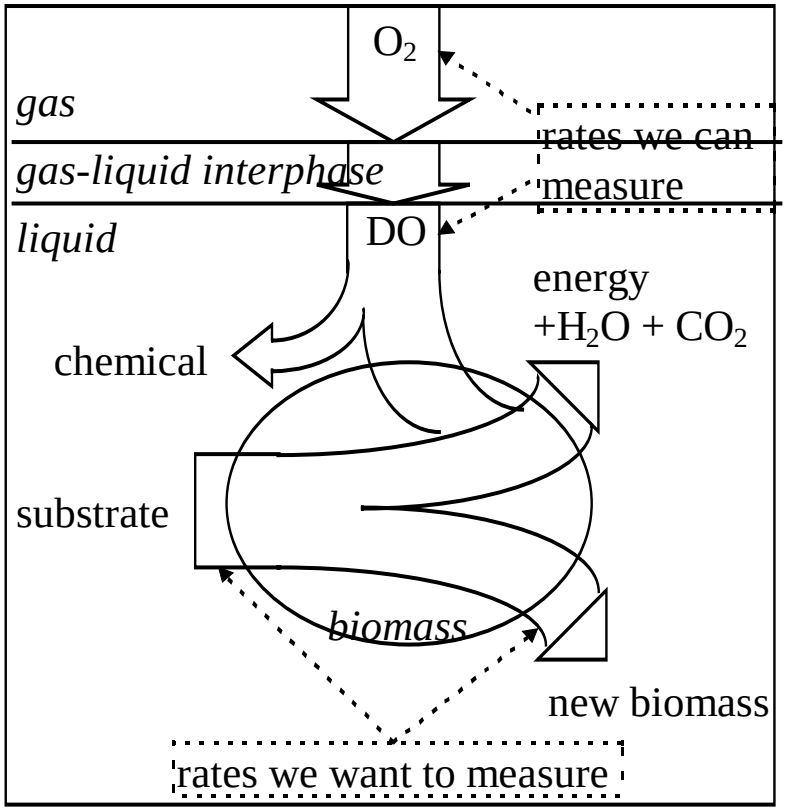


Figure 3. Relationship respiration, substrate utilisation and growth.

5. Respirometric measurement: General principle

In the description of respirometers there is, probably more than with any other measuring instrument, confusion about operating principles. Operation is characterised with terms like in-line, on-line, in-situ, continuous, semi-continuous, batch, etc. The reason is that respirometers are often small activated sludge reactors by themselves of which the operation is more open to the users allowing them to make their own interpretation of the measuring results. The characterisation can pertain to the operation of the respirometer or to the way it interacts with the treatment plant.

A ***respirometer*** is an instrument for measurement of the respiration rate, that is the mass of oxygen consumed per (unit of volume and) unit of time. Instruments that are specifically designed to measure Biochemical Oxygen Demand are sometimes called respirometers too. However, these instruments often cannot provide rates (measurements expressed per unit of time) without major modifications in operation and calculation procedures. Therefore, they should be denoted ***BOD-meters***. The same holds for respirometry-based ***toxicity-meters*** which are designed to provide a measure of toxicity. Some instruments are capable to operate in different modes in such a way that they comprise all the three meters above.

The respiration rate usually is measured with a respirometer. Respirometers range from a simple, manually operated bottle equipped with a dissolved oxygen (DO) sensor to complicated instruments that operate fully automatically. In some cases the aeration tank of the treatment plant itself can serve as a respirometer. Except for the latter case, a feature common to all respirometers is a reactor, separated from the activated sludge tank, where different components (biomass, substrate, etc.) are brought together. The operation of all respirometers involves some technique for assessing the rate at which the biomass takes up DO from the liquid. Many techniques have been developed in the past. However, the Task Group found that all measuring techniques for the respiration rate can be classified into only eight basic principles according to two criteria: (1) the phase where oxygen concentration is measured (gas or liquid, G and L, respectively) and (2) whether or not there is input and output of liquid and gas (flowing or static, F and S, respectively). The operation of all existing respirometers can be explained in terms of these criteria. Fig. 4 shows a generic scheme of a respirometer. Note that the gas phase also includes bubbles dispersed in the liquid phase. In the subsequent sections, the principles will be discussed according to the above criteria.. We will not discuss the usefulness of the different measuring techniques, because we believe that any technique has its merits, depending on the specific application, provided that the correct measuring conditions are satisfied.

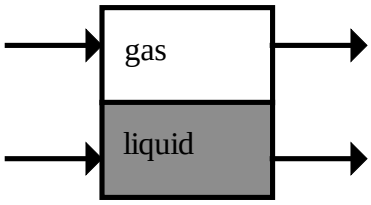


Figure 4. ***Generic scheme of a respirometer.***

6. Respirometry based on measuring oxygen in the liquid phase

The majority of the techniques based on the measurement of oxygen in the liquid phase use an electrochemical DO-sensor. The DO-sensor generally consists of two or three electrodes in an internal electrolyte solution separated from the liquid by a semi-permeable membrane. Dissolved oxygen molecules diffuse from the liquid through the membrane into the internal solution. The molecules are reduced on the cathode, generating an electrical current. This current is proportional to the diffusion rate of the oxygen molecules through the membrane which, in turn, is proportional to the DO concentration in the solution. The relationship between electrical current and DO concentration is established by calibrating the DO sensor. For a DO sensor, water saturated air is equivalent to oxygen saturated water. This is used to calibrate the DO sensor in water saturated air at 100% DO, i.e. saturation DO concentration in the liquid. A reliable respiration rate measurement is only possible if the DO sensor is correctly calibrated and if a number of environmental variables, such as temperature and pressure, is accounted for. DO sensors also have a response time that must be accounted for in some respirometric set-ups.

Respirometers that are based on measuring DO concentration in the liquid phase use a DO mass balance over the liquid phase. Consider a system consisting of a liquid phase, containing biomass, and a gas phase both being ideally mixed and having an input and output (Fig. 5). It is assumed that the DO concentration in the liquid phase can be measured. The DO mass balance over the liquid phase is:

$$\frac{d\left(V_L S_O\right)}{dt} = Q_{in} S_{O,in} - Q_{out} S_O + V_L K_L a\left(S_O^* - S_O\right) - V_L r_O \qquad (7)$$

where:

S_O = DO concentration in the liquid phase
S^*_O = saturation DO concentration in the liquid phase
$S_{O,in}$ = DO concentration in the liquid phase entering the system
K_La = oxygen mass transfer coefficient (based on liquid volume)
Q_{in} = flow rate of the liquid entering the system
Q_{out} = flow rate of the liquid leaving the system
r_O = respiration rate of the biomass in the liquid
V_L = volume of the liquid phase

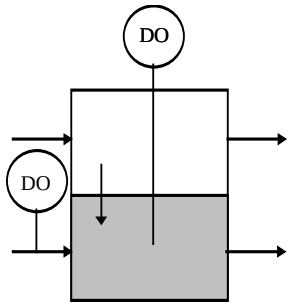


Figure 5. Liquid phase principle, Flowing gas, Flowing liquid (LFF).

Notice that, since it is a mass balance over the liquid phase, Eq. (7) does not contain gas flow terms. The first and second term on the right hand side represent advective flow of DO in the input and output liquid streams. In most systems Q_{in} and Q_{out} will be equal so that the liquid volume is constant. The third term describes the mass transfer of oxygen from the gas phase to the liquid phase. The last term contains the respiration rate to be derived from the mass balance. Therefore, S_O must be measured and all other coefficients be known or neglected. In practice, the determination of r_O can be simplified in several ways. In what follows it is assumed that the liquid volume is constant, so that the terms in Eq. (7) can be divided by V_L.

6.1. Static gas, static liquid (LSS)

One approach is to use a method without liquid flow and oxygen mass transfer (Fig. 6). Then the first three terms on the right hand side of Eq. (7) fall away and the mass balance reduces to:

$$\frac{dS_O}{dt} = -r_O \qquad \textbf{(8)}$$

Hence, to obtain the respiration rate only the differential term has to be determined. This can be done by measuring the decrease in DO as a function of time due to respiration, which is equivalent to approximating the differential term with a finite difference term: $\Delta S_O/\Delta t = -r_O$. Typical of this principle is that the DO may become exhausted after some time so that for continued measurement of r_O reaeration is needed to bring the DO concentration back to a higher level. DO and substrate are limiting the respiration (see Eqs. 1 and 2) when their concentrations become too low, causing a non-linear DO decrease complicating the assessment of the differential term. Note that in Fig. 6 there is a gas phase. However, there is no mass transfer from the gas phase into the liquid phase. In practice, in order to prevent input of oxygen into the liquid, the gas phase may be absent. The procedure for the determination of r_O according to "Standard Methods" (American Public Health Association, Washington D.C., 18th edition, 1992) is based on this principle. The principle is often used for manually measuring r_O but it is also implemented in automatic respirometers which sample activated sludge from an aeration basin and do one or more measurements of the DO decrease.

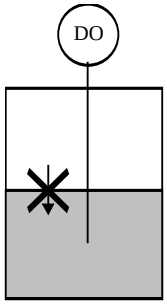


Figure 6. Liquid phase principle, Static gas, Static Liquid (LSS)

6.2. Flowing gas, static liquid (LFS)

The disadvantage of the need for reaerations can be eliminated by continuously aerating the biomass. Then, the oxygen mass transfer term $K_La(S^*_O-S_O)$ must be included in the mass balance (Eq. 9):

$$\frac{dS_O}{dt} = K_L a(S_O^* - S_O) - r_O \qquad \textit{(9)}$$

To obtain r_O both the differential term and the mass transfer term must be determined. To calculate the latter, the mass transfer coefficient (K_La) and the DO saturation concentration (S^*_O) must be known. These coefficients have to be determined regularly because they depend on environmental conditions such as temperature, barometric pressure and the properties of the liquid. The simplest approach is to determine these by using separate reaeration tests and look-up tables. Another approach is to estimate the coefficients from the dynamics of the DO concentration response by applying parameter estimation techniques. The advantage of the latter method is that the values of the aeration coefficients can be updated relatively easily. This respirometric principle allows the measurement of r_O at a nearly constant DO concentration, thereby eliminating the dependency of r_O on the DO concentration (provided DO » 0 mg l^{-1}). This principle can be implemented in a separate respirometer or directly in a batch aeration tank. Note that, while Fig. 7 shows an input and an output on the gas phase, there is no gas flow term in Eq (9). There is no need to consider gas flow terms provided S^*_O is known or determined (see section 3.4).

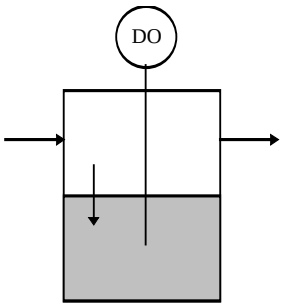


Figure 7. Liquid phase principle, Flowing gas, Static liquid (LFS).

6.3. Static gas, flowing liquid (LSF)

Repetitive aeration or estimation of oxygen transfer coefficients, as with the above principles, can be avoided when liquid with a high enough input DO concentration flows continuously through a closed completely mixed cell without gas phase (Fig. 8). The liquid flow terms now have to be included in the mass balance (6):

$$\frac{dS_O}{dt} = \frac{Q_{in}}{V_L} S_{O,in} - \frac{Q_{out}}{V_L} S_O - r_O \qquad \textbf{(10)}$$

Both DO concentrations, $S_{O,in}$ and S_O, must be measured continuously to allow calculation of r_O. In a respirometer Q_{in} and V_L are instrument constants and are, therefore, assumed known or calibrated. This principle is in fact the continuous counterpart of the one explained in Eq. (8), and it is as such also sensitive to the effect of substrate and DO limitation. However, the effect of limiting substrate can be eliminated by the continuous supply of substrate (wastewater) and DO to the respiration cell.

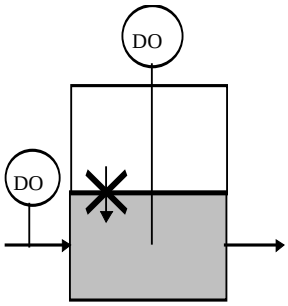


Figure 8. Liquid phase principle, Static (no) gas, Flowing liquid (LSF).

The principle described above and other principles with liquid flow are also applicable to a plug flow type cell. However, the exact respiration rate in the cell cannot be obtained from Eq. (10) because of the spacial distribution of r_O and S_O along the plug flow cell. In this case respiration rate can be calculated from the DO concentration in the liquid entering the cell and that in the liquid leaving the cell, obviously resulting in a measurement delay equal to the hydraulic residence time of the cell.

6.4. Flowing gas, flowing liquid (LFF)

Without the above simplifications the full mass balance (Eq. 7) holds for the principle depicted in Fig. 5. To obtain respiration rate measurements with this principle, a combination of the approaches mentioned for the above simplified principles is required. For instance, the flow rates and the inlet oxygen concentrations must be measured, while the coefficients K_La and S^*_O must be assessed, e.g. by estimating these from the dynamics of the DO concentration.

7. Respirometry based on measuring oxygen in the gas phase

Respirometric techniques based on measuring gaseous oxygen always deal with two phases: a liquid phase containing the respiring biomass and a gas phase where the oxygen measurement takes place. The main reason for measuring in the gas phase is to overcome difficulties associated with interfering contaminants common in the liquid phase (e.g. formation of sludge film on the sensor). Gaseous oxygen is measured by physical methods such as the paramagnetic method, or gasometric methods. Oxygen is one of the few gases that show paramagnetic characteristics, and so it can be measured quantitatively in a gaseous mixture by using the paramagnetic method. The method is based on the change of a magnetic field as a result of the presence of oxygen, and this change is proportional to the concentration of gaseous oxygen.

Gasometric methods measure changes in the concentration of gaseous oxygen. According to the ideal gas law $PV = nRT$, these can be derived from changes in the pressure (if volume is kept constant, ***manometric method***) or changes in the volume (if pressure is kept constant, ***volumetric method***). These methods are typically applied to closed measuring systems (no input and output streams), which may provoke a need for reaerations and thus temporary interruption of the measurements. This limits the possibility for continued monitoring of the respiration rate. However, interruptions because of reaerations are not needed if the consumed oxygen is replenished at a known rate, e.g. by supplying pure oxygen from a reservoir or by using electrolysis. The rate at which oxygen is supplied is then equivalent to the biological respiration rate (assuming infinitely fast mass transfer to the liquid). Because carbon dioxide is released from the liquid phase as a result of the biological activity, this gas has to be removed from the gas phase in order to avoid interference with the oxygen measurement. In practice this is done by using alkali to chemically absorb the carbon dioxide produced.

Respirometric principles based on measuring gaseous oxygen also use oxygen mass balances to derive the respiration rate. However, in addition to the mass balance on the liquid phase (Eq. 7), a balance on the (ideally mixed) gas phase must be considered (Fig. 9):

$$\frac{d}{dt}\left(V_G C_O\right) = F_{in} C_{O,in} - F_{out} C_O - V_L K_L a (S_O^* - S_O) \qquad (11)$$

where:

C_O = O_2 concentration in the gas phase
$C_{O,in}$ = O_2 concentration in the gas entering the system
F_{in} = flow rate of the gas entering the system
F_{out} = flow rate of the gas leaving the system
V_G = volume of the gas phase

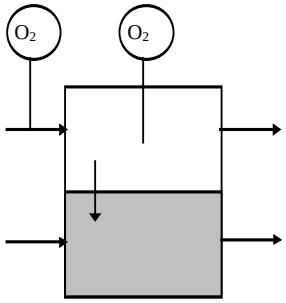


Figure 9. Gas phase principle, Flowing gas, Flowing liquid (GFF).

The term $V_L K_L a(S^*_O - S_O)$ represents the mass transfer rate of oxygen from the gas phase to the liquid phase, and it is the connection between the two phases. From mass balances (7) and (11) it follows that, in order to calculate r_O, C_O must be measured (directly or using the gas law, see above) and knowledge of S_O is required. However, S_O is not measured in the gas phase principles. In these respirometric principles it is

assumed that the oxygen concentrations in the gas and liquid phases are in equilibrium, i.e. mass transfer is sufficiently fast ($K_La \rightarrow \infty$), so that $S_O \approx S^*_O$. Since, by definition, the saturation DO concentration is proportional to the O_2-concentration in the gas phase:

$$S_O^* = H\, C_O \quad \textbf{(12)}$$

it is reasonable to state that:

$$S_O = H\, C_O \text{, and} \quad \textbf{(13)}$$

$$\frac{dS_O}{dt} = H\frac{dC_O}{dt} \quad \textbf{(14)}$$

Hence, the measurement in the gas phase is a good representation of the condition in the liquid phase, provided the proportionality (Henry) constant *H* is known, e.g. from calibration or tables, and the mass transfer coefficient is high. Especially in full scale situations where the aeration tank is used as a respirometer, the validity of this equilibrium assumption should be critically evaluated.

7.1. Static gas, static liquid (GSS)

The simplest gas phase technique for measuring respiration rate is based on a static liquid phase and a static gas phase, i.e. no input and output (Fig. 10). In addition to the DO mass balance on the liquid phase, an oxygen mass balance on the gas phase must be considered:

$$\frac{dS_O}{dt} = K_L a(S_O^* - S_O) - r_O \quad \textbf{(15)}$$

$$\frac{d(V_G C_O)}{dt} = - V_L K_L a(S_O^* - S_O) \quad \textbf{(16)}$$

Hence, in order to calculate r_O, the change of the oxygen concentration in the gas phase, dC_O/dt, must be measured and knowledge of dS_O/dt is required (see above). dC_O/dt may be measured by using an oxygen sensor. If a gasometric method is used, dC_O/dt is related to the change in volume or the change in pressure (see above).

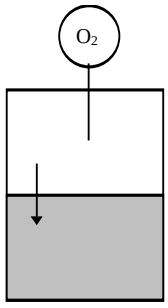


Figure 10. Gas phase principle, Static gas, Static Liquid (GSS).

With this principle the same restriction as with the simplest DO based principle exists: when the oxygen becomes exhausted it must be replenished by, for instance, venting the gas phase in order to continue the measurement of r_O.

7.2. Flowing gas, static liquid (GFS)

Another technique is based on a flowing gas phase, i.e. the biomass is continuously aerated with air (or pure oxygen) so that the presence of sufficient oxygen is assured (Fig. 11). In comparison to Eq. (16) two transport terms must be included in the mass balance on the gas phase:

$$\frac{dS_O}{dt} = K_L a(S_O^* - S_O) - r_O \quad \textbf{(17)}$$

$$\frac{d(V_G C_O)}{dt} = F_{in} C_{O,in} - F_{out} C_O - V_L K_L a(S_O^* - S_O) \quad \textbf{(18)}$$

In order to allow calculation of r_O, the gas flow rates, F_{in} and F_{out}, and the oxygen concentrations in the input and output streams, $C_{O,in}$ and C_O, must be known in addition to the variables of the previous technique. Of these, usually C_O is measured and the others are set or known. A gasometric method is not evident here, and the measurement of C_O is done for example with the paramagnetic method.

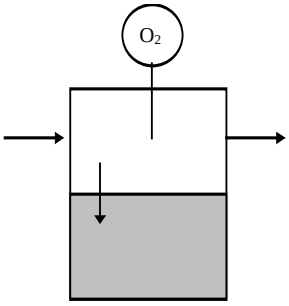


Figure 11. Gas phase principle, Flowing gas, Static liquid (GFS).

7.3. Static gas, flowing liquid (GSF)

Implementations of the gas phase principle with static gas and flowing liquid has not been found in literature and practice so far. With this principle the (change of) oxygen concentration in the liquid phase must be determined, e.g. as described above, in addition to the oxygen measurement in the gas phase:

$$\frac{d(V_L S_O)}{dt} = Q_{in} S_{O,in} - Q_{out} S_O + V_L K_L a(S_O^* - S_O) - V_L r_O \quad \textbf{(19)}$$

$$\frac{d(V_G C_O)}{dt} = - V_L K_L a(S_O^* - S_O) \quad \textbf{(20)}$$

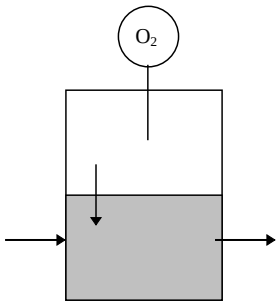


Figure 12. Gas phase principle, Static gas, Flowing liquid (GSF).

7.4. Flowing gas, flowing liquid (GFF)

The gas phase principle can also be applied to a full scale aeration tank. In this case there are liquid input and output streams for the tank, and transport terms must be added to the mass balance on the liquid phase (Eq. 7). The assumption on proportionality between C_O and S_O (Eq. 13) becomes more critical because, in addition, also the liquid outflow term depends on it. Additional measurement of dissolved oxygen may then be useful for a correct assessment of respiration rate. The technique then would no longer be a pure gas phase principle. Note, however, that in general combining L- and G-principles may lead to more reliable respiration rate measurements.

8. Summary of respirometric measuring principles

Table 1 summarises the measuring principles. The first column contains the names of the mass balance terms, and the second column the mathematical equivalents. The succeeding columns list the respirometric principles, the first four being liquid phase principles, and the others being gas phase principles. The mass balances for each principle are formed by multiplying the mathematical terms with the coefficients in the column of the appropriate principle. The sum of all terms must equal zero.

Table 1. Overview measuring principles respiration rate.

		Measurement in liquid phase				Measurement in gas phase			
respirometric principle→									
process ↓		LSS	LFS	LSF	LFF	GSS	GFS	GSF	GFF
respiration	$V_L r_O$	-1	-1	-1	-1	-1	-1	-1	-1
dissolved oxygen accumulation	$\frac{d}{dt}(V_L S_O)$	-1	-1	-1	-1	-1	-1	-1	-1
liquid flow	$Q_{in}S_{O,in} - Q_{out}S_O$			1	1			1	1
gas exchange	$V_L K_L a(S_O^* - S_O)$		1		1	1	1	1	1
gaseous oxygen accumulation	$\frac{d}{dt}(V_G C_O)$					-1	-1	-1	-1
gas flow	$F_{in}C_{O,in} - F_{out}C_O$						1		1
gas exchange	$V_L K_L a(S_O^* - S_O)$					-1	-1	-1	-1

9. Measured and Deduced Variables

Like any other measuring device, a respirometer can be considered to be a sensor. In fact, a respirometer can be constructed in such a compact and physically manageable way that it can easily be regarded as one unit consisting of a number of sub-units such as sampling port, sensing element, transducer, etc., that are concealed for the user. The user does not easily see the internal structure of the respirometer and, in fact, this is not necessary for operating the instrument.

On the contrary, it may be conceptually difficult to consider a respirometer as a sensor because it contains a (mini) reactor where experiments are done by combining different components or process streams, and in which the measurement conditions generally have a very large influence on the measurement results. Even the respirometer operating principle itself and its concomitant measurement condition (e.g. no oxygen mass transfer) can have an effect on the respiration rate of the biomass.

No matter where a respirometer is located, the conditions in the respirometer are decisive for the measurement results. Therefore, in this report these measurement conditions, not the measurement location, are specified as important characteristics for any measured respiration rate. In practice, however, the measurement conditions prevailing during respirometric experiments are not always clearly communicated.

In process operation, control, and research a biological interpretation is sought for the measured respiration rate. Therefore, the respirometric date are frequently converted to deduced variables that better characterise the biology of activated sludge. Again, it depends on the measurement conditions what variables can be deduced from the measured respiration rate. This section discusses possible deduced variables.

9.1. Measured respiration rates

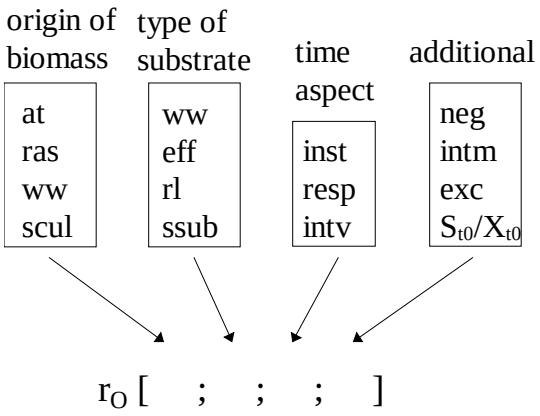
Most measured variables do not need additional information to be interpreted (e.g. dissolved oxygen or nitrate concentration). For respirometry this is not the case. A respiration rate value or a percentage inhibition calculated from respiration rate measurements cannot be interpreted without additional information about some measurement attributes. The Task Group has found that at least three attributes must be specified to interpret respiration rate measurements: (1) biomass source, (2) type of substrate and (3) time aspect (Fig. 13). Below, some examples are given for these attributes to indicate the diversity of respiration rates that can be obtained from respirometers.

Biomass

Several sources for the respirometer biomass exist: aeration tank, return activated sludge, wastewater from the treatment plant being monitored. While the choice of a biomass sampling point for a completely mixed aeration tank is trivial, for other reactor types the location from which the sludge is obtained (beginning, end, or which compartment) has a critical bearing on the measurement condition. Indeed, the state of the biomass itself and its environment (e.g. pH, dissolved oxygen and substrate levels) will partly determine the result of the respirometric measurement. Hence, the conditions at the sampling point are very important. The source of biomass also could be a specific culture grown separately, possibly on sewage or a synthetic substrate.

Activated sludge sampled from the aeration tank often contains dissolved oxygen and a varying and mostly unknown quantity of substrate. Return activated sludge has a higher biomass concentration and often low dissolved oxygen and substrate concentrations. On the contrary, the concentration of substrate is high and that of biomass is low when the sample comes from sewage, while dissolved oxygen is likely to be absent.

Biomass of a specific culture is grown separately from the activated sludge on sewage or a different substrate. Therefore, it resembles more or less the biomass in the treatment plant, but has the advantage that the characteristics of the culture are better known. Because of the limited production rate of biomass it is mostly kept in the measuring system by growing it on a carrier and the culture is as such an inherent part of the respirometer.



at	activated sludge tank	inst	instantaneous
at*k*	k^{th} compartment of plug flow tank	resp	respirogram
ras	return activated sludge	intv	interval
ww	wastewater	neg	negligible
scul	specific culture	intm	intermediate
eff	effluent	exc	excess substrate
rl	return liquor	S_{t0}/X_{t0}	initial substrate to biomass ratio
ssub	specific substrate		

Figure 13. Nomenclature of respiration rate (see text for explanation).

Substrate

Four substrate types have been considered in respirometry: wastewater (raw or settled), effluent, return liquors from the sludge treatment, and specific substrates. A specific substrate, such as acetate or ammonium, can be used to mimic the oxidation of (a) particular (group of) component(s) in wastewater.

Time aspect

Like many variables, respiration rate is a function of time. The measurement obtained with a respirometer can be executed in three modes: instantaneous, respirogram and interval. An instantaneous measurement assumes that the elapsed time between onset of the experiment and respiration rate measurement is zero, so that the initial condition is measured. A respirogram means that the respiration rate in the respirometer is tracked for some time in order to obtain a time series of respiration rate values. An interval type measurement denotes a (single) relevant respiration rate measurement after a specified time interval in the respirometer.

When sludge is transferred from an activated sludge tank into a respirometer the respiration rate in the meter is still a function of time (though not in the time frame of the treatment process but in a new time frame: that of the measurement) because of, for example, a change in the substrate concentration. If the rate is measured immediately, an instantaneous measurement is obtained which may closely reflect the condition in the activated sludge tank. Continuing the measurement yields a time course, i.e. a respirogram, which provides information on the biomass and substrate characteristics. After a predetermined or variable time interval the substrate may be exhausted, and the endogenous respiration rate is measured. At this time (or already immediately after sampling) an extra amount of substrate may be added in order to obtain a respirogram with sufficient dynamics to extract information about biomass and substrate characteristics.

Additional attributes

The concentration of substrate, like the time aspect, is another important environmental condition in the respirometer reactor that is crucial for the information content of the obtained measurement results. Three levels of substrate concentration can be defined: negligible, intermediate and excess. Negligible substrate levels may be obtained when no substrate is added intentionally or sufficient removal of substrate is guaranteed before the onset of the experiment. It is expected or intended that the remaining substrate has no effect on the measured respiration rate. An intermediate level may be obtained if there is still a significant amount of substrate left in the sludge at the moment of sampling, or if a specific amount of substrate is brought together with the sludge in the respirometer. A condition with excess substrate is characterised by the fact that a small change of substrate concentration has no effect on the measured respiration rate. For some variables deduced from respiration rate data, the initial substrate to biomass ratio S_{t0}/X_{t0} at the onset may be crucial and should be reported.

In addition, specific components (e.g. ATU) which are not used as a substrate but which act as an inhibitor for part of the biochemical processes can be brought into the respirometer. Other environmental conditions like pH and temperature are also important for the measurement result. However, these factors are usually not a part of the measurement strategy. They are assumed similar to the conditions in the treatment plant and kept or assumed constant or of no influence on the result.

Nomenclature

In this report he respiration rate will be symbolically associated with the attributes presented above (Fig. 13). Some examples are as follows. The respiration rate measured immediately after sampling from a completely mixed aeration tank, the actual respiration rate in the tank, would be represented: r_O*[at;-;inst]*. The dash means that no substrate is added. If the sludge after sampling is aerated for a prolonged time in order to measure endogenous respiration rate, one would write: r_O*[at;-;intv]*. A respiration rate denoted r_O*[at1;ww;resp]* means the rate for sludge from the first compartment of an aeration tank in the presence of wastewater, measured for some time, i.e. a respirogram. It is not the intention to use the attributes as a symbolic notation, e.g. in equations, but merely as a way to concisely present and explain measured respiration rates.

9.2. Deduced Variables

The respirometric measurement result (the raw respiration rate value obtained under specific conditions in the respirometer) is usually not directly used in a control strategy. It must be first converted to a deduced variable which is relevant in the control strategy. A ***deduced variable*** is defined as a variable that results from a calculation performed with one or more measured respiration rate values and possibly other measured variables. Many deduced variables have been proposed. In Table 2 a selection of deduced variables is presented, together with the respiration rate(s) the variable is deduced from, the other measured variables used in the calculations, and the calculation method applied. For some deduced variables different methods have been proposed to calculate these.

Most often simple ***arithmetic*** calculations involving different types of respiration rates or involving respiration rate and some other measurement lead to a deduced variable. For instance, the specific respiration rate *R* is calculated as respiration rate divided by the mixed liquor suspended solids concentration *X*.

A second type of calculation involves the use of an ***integration*** step, i.e. the area under a time course of respiration rates (respirogram) is calculated $r_O[*;*;*]$. (The wildcard '*' means that different biomass sources, substrate types or time aspects are possible.) This is used in one approach for the assessment of the short term BOD (BOD_{st}), a measure of the substrate concentration. An alternative, arithmetric, method for BOD_{st} calculation uses a Monod relationship between the instantaneous respiration rate with a wastewater sample $r_O[at;ww;inst]$, and the wastewater BOD_{st}.

A third method used in deduced variable calculation consists of a ***comparison*** between respiration rates collected at different times or from different sources. For instance, the percentage inhibition (*%I*) of a wastewater can be assessed from a comparison between maximum respiration rates before and after a toxicant entered the wastewater. Basically, by performing such comparisons, the derivative of the maximum respiration rate with time is determined in a very simple way and the change of respiration rate is monitored. Another example where comparison is applied is in the assessment of the treatment time defined as the time needed to reach endogenous respiration in a batch experiment (time to endogenous, t.t.e.). Obviously, this requires the comparison of the measured respiration rates with a predefined endogenous rate.

Finally, in recent years a number of sophisticated algorithms for ***parameter estimation*** have evolved to deduce relevant variables. Respirometry is increasingly used to assess the parameters and components of the IWA Activated Sludge Model No.1. For instance, maximum growth rates and saturation coefficients of heterotrophs and nitrifiers, and the components in a wastewater, have been deduced from respirograms $r_O[*;ww;resp]$. In case of specific growth rate, a measure of biomass concentration is needed.

It is beyond the scope of this report to explain the underlying mathematics, but basically the parameter estimation algorithms try to find parameter values (maximum growth rates, saturation coefficients, component concentrations, etc.) that lead to the smallest deviation between model predictions and measured respiration rates.

In an alternative, arithmetic, approach the carbonaceous components of the ASM No. 1 S_S and X_S have been calculated using three different respiration rates with the same biomass and wastewater: $r_O[at;ww;inst;exc]$, $r_O[at;ww;inst]$ and $r_O[at;-;intv]$.

Table 2. Selection of deduced variables obtained from respiration rate measurements. For explanation of nomenclature, see Fig. 13. The wildcard '' means that different biomass sources, substrate types or time aspects are possible.*

Deduced variable	Respiration rates (and other measurements)	Method
R	$r_O[*,*,*]$ (and X)	arithmetic
BOD_{st}	$r_O[*,*,resp]$	integration
	$r_O[*,ww,inst]$	arithmetic
$\%I$	$r_O[*,ww,inst,exc]$	comparison
t.t.e.	$r_O[*,ww,resp]$	comparison
X	$r_O[*,-,inst]$	arithmetic
	$r_O[*,ssub,inst,exc]$	arithmetic
	$r_O[*,ssub,resp]$	parameter estimation
B_X	$r_O[at,ww,inst]$, $r_O[ras,-,inst]$ (and Q_{in}, Q_{ras})	arithmetic
$r_{NH,max}$	$r_O[at,*,resp]$, $r_O[at,ssub,resp]$	arithmetic
	$r_O[at,ssub,resp,exc]$	parameter estimation
ASM parameters	$r_O[*,ww,resp]$ (and X)	parameter estimation
ASM components	$r_O[*,ww,resp]$	parameter estimation
	$r_O[at,ww,inst,exc]$, $r_O[at,ww,inst]$, $r_O[at,-,intv]$	arithmetic