Yield determination by respirometry - The possible influence of storage under aerobic conditions in activated sludge

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Abstract

In this study we found that the oxygen uptake rate (OUR) response from activated sludge to the addition of a single organic substrate can be divided into two phases. The first phase reflects the primary metabolism of the added substrate, while the second presumably originates from metabolism of stored polymers like polyhydroxyalkanoate (PHA) and/or glycogen produced during the metabolism of the exogenous substrate. This shift is due to the depletion of exogenous substrate. The obtained yield on acetate of 0.71 g COD/g COD correlates very well with experimental results in literature where addition of excess acetate to a pure culture led to extensive formation of polyhydroxybutyrate (PHB). Furthermore the highest obtained yield on glucose of 0.91 g COD/g COD is very close to the theoretical yield for formation of glycogen from glucose (0.96 g COD/g COD). This indicates that the response of activated sludge to a substrate addition is to store the substrate instead of an immediate growth response.

Introduction

The use of oxygen uptake rate (OUR) measurements for control purposes and as an experimental tool is rapidly increasing (Brouwer et al. 1994; Spanjers and Vanrolleghem, 1995; Smolders et al. 1997). By using it we can get more information on the biological wastewater treatment processes and the wastewater (Henze, 1992). The direct registration of the oxygen consumption rate in a biological process allows us to get a first insight into the metabolism of the micro-organisms. The consumption rate of the electron donor has for many years been the primary objective of these measurements, but a more detailed analysis of the data obtained allows us to extract more information from the OUR measurements. The coupling between oxygen and substrate consumption can be used to calculate the amount of substrate consumed. A key question in this calculation of the substrate consumption is the conversion factor, normally called the yield coefficient. If the yield coefficient used in the calculation is incorrect, then the calculated substrate consumption is false. Since many OUR experiments expose the biomass to significant substrate dynamics, the metabolic response need not be the traditional growth and oxidation only. There is thus a need for a more detailed analysis of OUR curves and the associated yields in order to learn more details of the fate of the substrate in the metabolic process. This paper analyses the yield of activated sludge from full-scale treatment plants being subjected to OUR experiments with pure substrates.

Respirometric measurements

Empirically an OUR curve for a batch culture, to which an amount of substrate is added, can look like the one shown in Fig. 1.

When interpreting the OUR curve it is essential to know the respiration due to the biomass itself, called the endogenous respiration. This respiration is normally assumed to be caused by maintenance of the biomass. The concept is then to subtract this respiration from the measured respiration in order to determine the true yield coefficient. In this work the endogenous respiration used in all experiments is the original start respiration, the one measured in the sludge sample just before substrate is added.

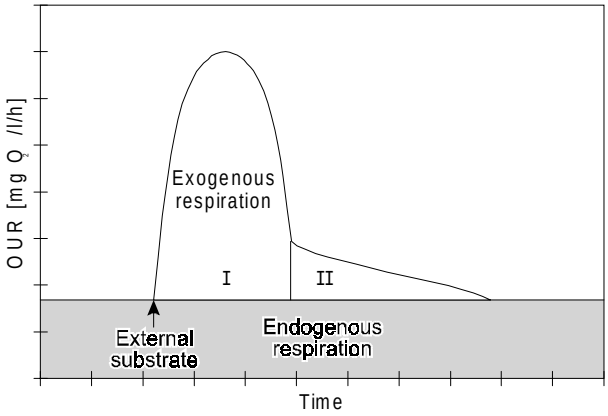

Figure 1

Example of a respirogram, where a pulse of organic substrate is added. If certain experimental conditions are met, the peak Phase (I) and the tail Phase (II) will be seen.

It is obvious that the operation and design of the wastewater treatment plant (WWTP), including the composition of incoming wastewater and sludge age from where the activated sludge sample is taken, must play an important role for the experimental results obtained. This is demonstrated in Fig. 2 where the change in the endogenous respiration over time in the activated sludge used in an experiment is shown. The figure shows that the time before a relatively constant level of respiration is reached is very different for the two types of sludges investigated.

If the endogenous respiration is known then the exogenous respiration, which is due to added substrate, can be calculated. The amount of added substrate expressed as COD, which has been oxidised, is then equal to the white area in Fig. 1. In Fig. 1 the first part of the curve the peak (Phase I) has a similar shape to what has been reported in Kong et al., 1996. The OUR curve can then,

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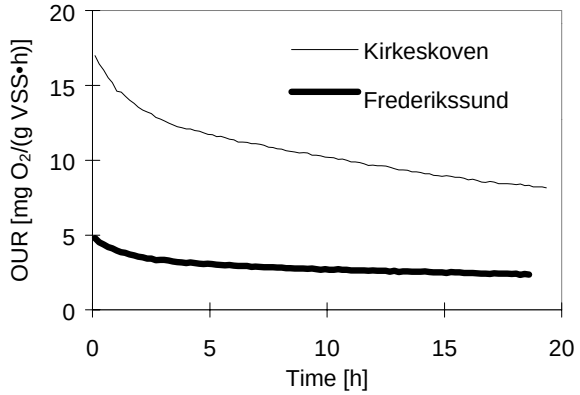

Figure 2
Example of the endogenous respiration of the activated sludges from two different full-scale treatment plants (Kirkeskoven- and Frederikssund WWTP)

according to the results presented in this paper, have a secondary phase i.e. the tail phase (defined as Phase II in Fig. 1) before the original level of endogenous respiration is reached. This second phase could be the result of adsorption to the activated sludge, but it is presumably caused by a metabolic shift to a more "slowly" readily biodegradable COD (RBCOD) (Xu and Hasselblad, 1996). This more "slowly" RBCOD could be a storage polymer like polyhydroxyalkanoate (PHA) or/and glycogen produced from the added substrate.

Materials and methods

Sludge

The activated sludge was obtained from the aeration tanks of two different wastewater treatment plants in Denmark. Frederikssund WWTP, Frederikssund Municipality, has a sludge retention time (SRT) of 16 d without preclarification. The plant operates according to the BIODENITRO-system, with alternating nitrification-denitrification treatment of the wastewater without an anaerobic tank in order to enhance biological removal (more system details can be found in Henze et al., 1995). Kirkeskoven WWTP, Søllerød Municipality, has an SRT of 7 d and presettling with addition of $FeCl_3$. The plant operates solely for the aerobic removal of COD without nitrification.

Sludge preparation

The sludge was settled and the supernatant was then replaced with tap water in order to reduce the initial concentration of RBCOD in the sludge. This washing procedure was repeated until the theoretical concentration of soluble matter was reduced by more than 95%.

The following nutrients were added to the sludge in order to avoid any nutrient limitation: $MgSO_4$:166 mg/ℓ, $FeCl_3$: 2.3 mg/ℓ, $CaCl_2$: 412 mg/ℓ, NH_4Cl-N: 6.8 mg/ℓ and phosphate buffer (pH 7.24) - $H_2PO_4^-$: 90 mg/ℓ, HPO_4^-: 410 mg/ℓ. To inhibit nitrification N-allylthiourea was added to a concentration of 12 mg/ℓ. The sludge was then aerated for at least 24 h at room temperature before it was used for experiments.

Measurements

Respiration was measured in an experimental set-up consisting of an open aeration batch reactor (volume 1.064 ℓ sludge) and a closed measuring batch reactor (volume 136 mℓ sludge), as shown in Fig. 3. The system was kept at a temperature of 20 ± 0.5°C and mixing was obtained by magnetic stirring. Oxygen was measured every 6 s in the measuring batch with an oxygen electrode (TriOxmatic 300 connected to Oxi 539 oxygen measuring unit from WTW). When the oxygen level dropped below 2 mg O_2/ℓ or the measuring period lasted more than 13 min the sludge in the measurement batch was replaced by pumping aerated sludge from the aeration batch into the measuring batch for 2 min (~300 mℓ/min). At the same time the sludge in the measuring batch was recycled back to the aeration batch. Oxygen measurements were logged by a computer and respiration rates were then calculated by linear regression of all the obtained dissolved oxygen (DO) data.

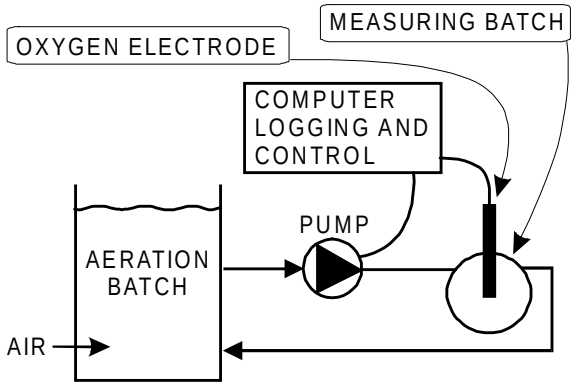

Figure 3
Principle of the OUR measuring system

Organic substrate

Organic substrate was added to the sludge in the form of dissolved sodium acetate, D-glucose or 96 vol. % ethanol.

Results and discussion

All the obtained values for the yield coefficients are summarised in Table 1 and the unit of the yield coefficient throughout this paper is g biomass COD/g substrate COD.

The substrates used, acetate, glucose and ethanol are all considered as belonging to the RBCOD fraction (Henze, 1992).

OUR of acetate

The OUR response by adding acetate (53.5 mg Ac COD/ℓ) is shown in Fig. 4.

At first the OUR measurement gives a very fast response and then after a short period, the respiration rate levels off. The first increase of the OUR curve can be explained by the adaptation of the organisms to this sudden much higher concentration of organic substrate or it could be caused by growth of the biomass. Then the curve exhibits a sudden drop, indicating that the added substrate has been removed from the water phase. The sudden drop of the OUR curve is then followed by a constantly decreasing

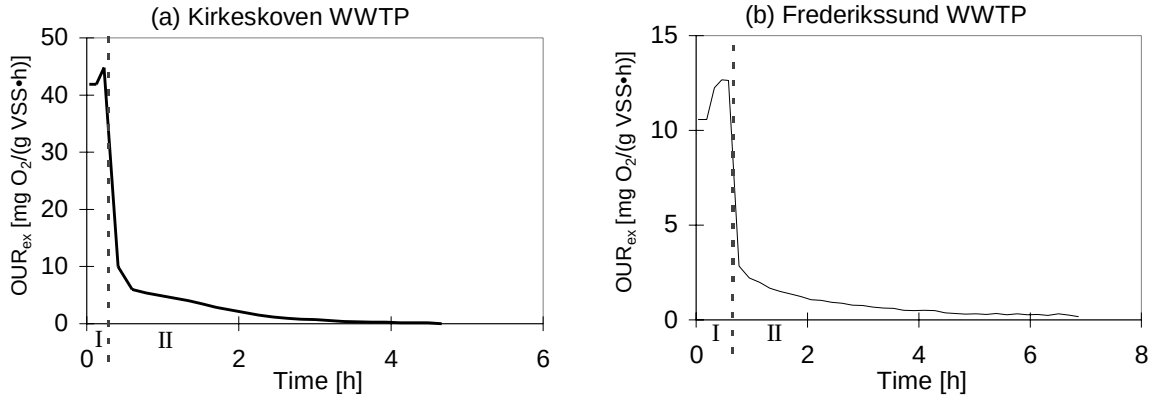

Figure 4

Exogenous oxygen response when adding acetate to sludge from WWTP Kirkeskoven (a) and WWTP Frederikssund (b) (53.5 mg acetate COD/ℓ is added to both). Area below the curve is divided into Area I and II. Endogenous respiration has been subtracted from the original measurement.

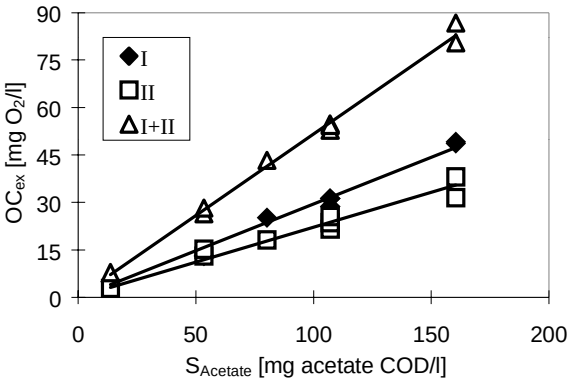

Figure 5

Total OC$_{ex}$ vs. the start concentration of acetate (sludge from WWTP Kirkeskoven). The oxygen consumption is calculated from the areas I, II and I + II, related to the principle shown in Fig. 1. The linear regression of the data is shown and is forced to go through (0,0).

respiration (Phase II). The respiration in Phase II is probably due to the transformation of a secondary substrate (maybe a storage polymer like PHA or glycogen) or it could be due to more difficult access to the remaining primary substrate caused by an accumulation of the substrate in the flocs.

The maximum respiration rate on a certain amount of acetate in Phase 1 is highest in the sludge from Kirkeskoven, also when expressed per grams VSS. This indicates, with reservation for the fact that grams of VSS is not equal to grams of living biomass, that there is a higher activity per g of VSS of micro-organisms capable of oxidising acetate in the sludge from Kirkeskoven than in the one from Frederikssund.

The accumulated exogenous oxygen consumption (OC$_{ex}$) for Phases I, II and I + II as function of the start concentration of substrate used, is shown in Fig. 5 (with sludge from WWTP Kirkeskoven). According to the theory used, OC$_{ex}$ must be zero when there is no substrate added. The regression lines for the OC$_{ex}$ as a function of the start concentration of substrate are therefore forced through (0,0).

The yields can then be calculated from $OC_{ex} = (1 - Y_H) \cdot S$ meaning that the Y is 1 minus the slope of one of the lines in Fig. 5. This procedure means that one yield coefficient is assumed

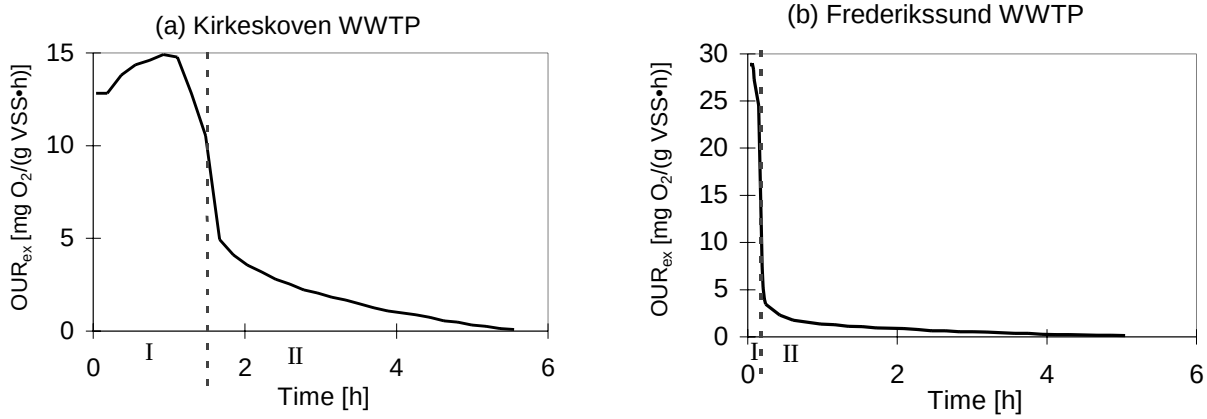

Figure 6

Exogenous oxygen response when adding glucose to sludge from WWTP Kirkeskoven (a) and WWTP Frederikssund (b) (107 mg glucose COD/ℓ is added to both). Area below the curve is divided into the Areas I and II.

TABLE 1
THE YIELD COEFFICIENTS

Substrate	Yield [g biomass COD/g substrate COD]			Reference
	Acetate	Glucose	Ethanol	
$Y_{H,I}$	0.71	0.79	0.66	WWTP Kirkeskoven
$Y_{H,I}$	0.72	0.91	0.67	WWTP Frederikssund
	0.71			Xu and Hasselblad (1996)
	0.71			Van Niel et al. (1995)
$Y_{H,I+II}$	0.48	0.71	0.55	WWTP Kirkeskoven
$Y_{H,I+II}$	0.52	0.82	0.55	WWTP Frederikssund
	0.5			Loosdrecht et al. (1996)*
	0.65	0.88		Chuboda et al. (1985) SE
	0.60	0.76		Chuboda et al. (1985) CM

SE - Selector system
CM - Completely-mixed system
* valid for a wide range of substrates

to be valid for the whole area of examined substrate concentrations. The values can be found in Table 1.

The yield coefficients for acetate for the activated sludge from WWTP Frederikssund are similar to the ones of WWTP Kirkeskoven (see Table 1).

The yield coefficient for acetate regarding Phase I is in good agreement with what can be calculated from the analytical work of Xu and Hasselblad (1996) and Van Niel et al. (1995) of $Y_{H, acetate} = 0.71$. In the latter the addition of excess acetate to *Thiosphaera pantotropha* led to rapid and massive formation of polyhydroxybutyrate (PHB) of 42% of the dry mass (Van Niel et al., 1995).

The value of the yield coefficient regarding both phases of 0.5 g COD/g COD seems to agree well with the value of 0.5 valid for biomass growth without substrate storage (Van Loosdrecht et al., 1997). This indicates that the part of the added acetate that was not oxidised after Phase II has been used for growth only.

OUR of glucose

The OUR response of activated sludge from Kirkeskoven and Frederikssund after the addition of glucose (107 mg glucose COD/ℓ) is shown in Fig. 6.

As seen in Fig. 6 the OUR response on glucose has a similar look as the OUR response on acetate. First a slow increase, then a fast decrease and after this a slow levelling out until the endogenous respiration level is reached again. Figure 6 also demonstrates the difference between the two activated sludges examined. This can be seen in and understood by the different areas below the two OUR curves and the difference in the maximum respiration rate on glucose.

The accumulated OC_{ex} for Phases I, II and I + II as a function of different concentration levels of substrate is shown in Fig. 7.

The high values of the yield coefficients (Table 1) indicate that glucose is stored in the sludge since $Y_{H,II}$ is much higher than the expected growth yield of 0.5 of Van Loosdrecht et al. (1997). The higher values for the yield on glucose in proportion to the yield on acetate is in good correlation with the results of Chudoba et al. (1985).

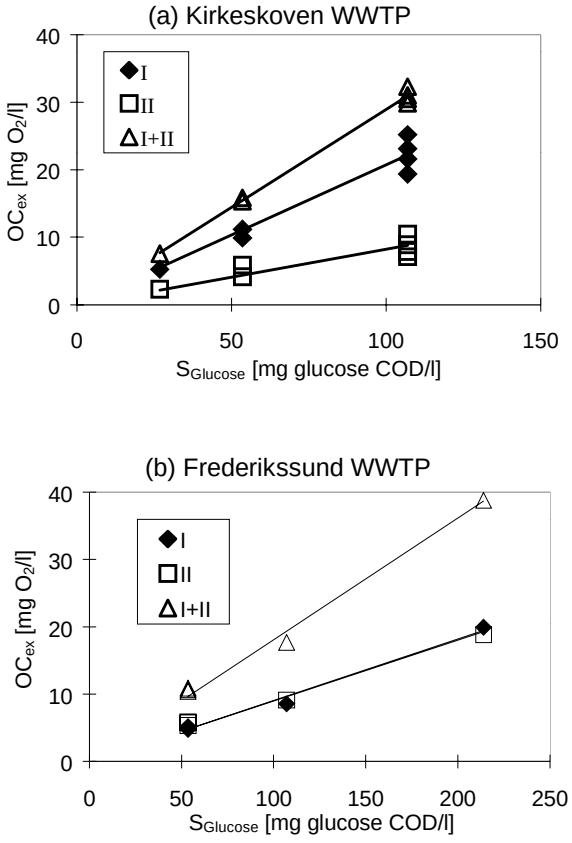

Figure 7
Total OC_{ex} vs. the start concentration of glucose (sludge from WWTP Kirkeskoven(a) and WWTP Frederikssund(b)). The oxygen consumption is calculated from the Areas I, II and I + II, related to the principle show in Fig. 1.

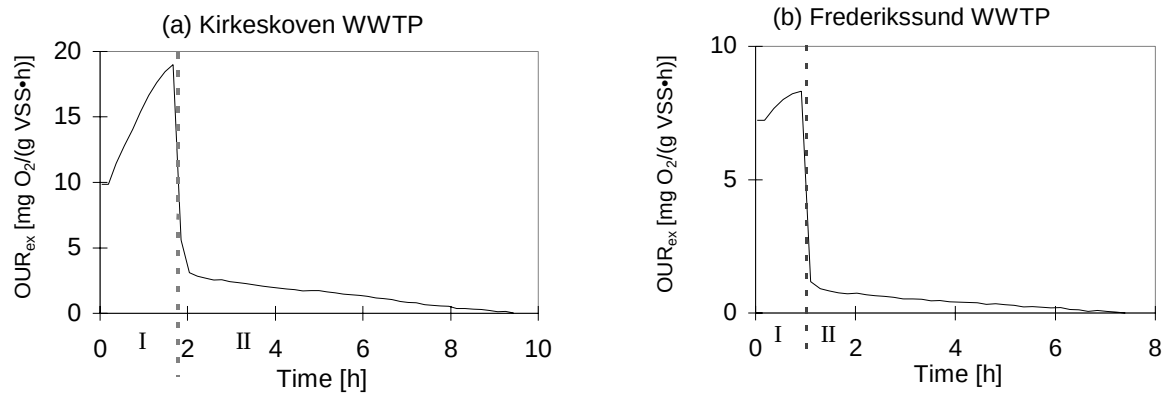

Figure 8

Exogenous oxygen response when adding ethanol to sludge from WWTP Kirkeskoven(a) (80 mg ethanol COD/ℓ added) and WWTP Frederikssund (b) (48 mg ethanol COD/ℓ added). Area below the curve is divided into the Areas I and II.

The highest level of storage is found in the sludge from Frederikssund. This is presumably caused by the presence of a fruit juice production company in the catchment area of the WWTP. Thus the raw wastewater could have a high content of carbohydrates, which then is causing a selection for micro-organisms specialised in metabolising carbohydrates. This hypothesis is supported by the fact that the activated sludge from Frederikssund has higher respiration rates in Phase I compared to the sludge from Kirkeskoven when the activated sludge is respiring on glucose. This indicates that the activity of micro-organisms per g VSS capable of oxidising glucose is higher in the sludge from Frederikssund than in the one from Kirkeskoven, where the highest activity of micro-organisms capable of oxidising acetate per g of VSS was observed.

OUR of ethanol

The OUR response of activated sludge from Kirkeskoven and Frederikssund upon the addition of ethanol is shown in Fig. 8.

The OUR response for ethanol has an almost linear increase after the first rapid increase (this phenomenon was observed repeatedly after the addition of ethanol to the same sludge sample). This differs from the OUR responses of the other examined substrates and could be due to induction of enzymes.

If, however, the phenomenon is due to selected growth of the biomass it would mean that the biomass respiring on acetate has a doubling time of approximately 2 h and that its growth is linear, which is not very likely for micro-organisms from a wastewater treatment plant. The difference between ethanol and acetate in the yield regarding Phases I and II together is approximately 10%, similar to the difference in the free energy of the combustion of ethanol and acetate which corresponds very well with the theory presented by McCarty (1972). This means that the yield expressed as (g biomass COD formed)/(substrate energy utilised) is the same for acetate and ethanol. The phenomenon is probably caused by the fact that the metabolism of ethanol starts with the conversion of the ethanol into acetaldehyde (Alderete et al., 1993). It is therefore expected that the yield on ethanol is higher than the yield on acetate due to the release of extra energy from the oxidation of ethanol. This has also been observed in experiments with denitrification (Constantin and Fick, 1997).

Figure 9 shows that the precision in the determination of

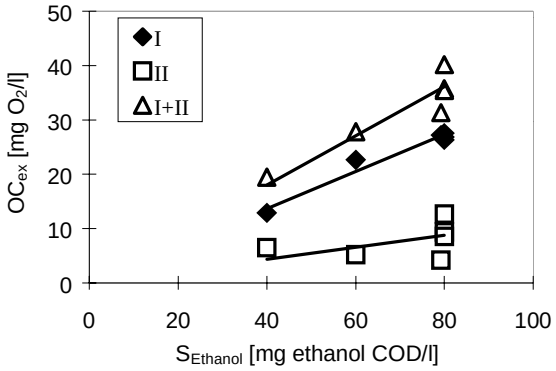

Figure 9

Total OC$_{ex}$ vs. the start concentration of ethanol (sludge from WWTP Kirkeskoven). The oxygen consumption is calculated from the Areas I, II and I + II, related to the principle show in Fig. 1.

Phase II in the case of ethanol is very small ($R^2 = 0.13$). This is presumably caused by the fact that the addition of ethanol can effect the endogenous respiration negatively either by inhibiting or even by poisoning certain bacterial species. This will especially influence the accuracy in the determination of Phase II.

This examination shows that if a wastewater containing monocarbohydrates is tested with the OUR method using sludge from WWTP Frederikssund or WWTP Kirkeskoven the obtained OC$_{ex}$ will differ. The determination of the RBCOD fraction will consequently also differ if the same yield coefficient is applied. This result is not correct according to the definition of Wanner (1994) which reads “Readily biodegradable substrates are organic compounds with small and simple molecules which can be directly metabolised inside the cells”, but for modelling purposes it is not necessarily wrong. It all comes down to the choice between a definition of the RBCOD fraction that can be measured analytically or a definition of the fraction of the COD that will give a certain response according to a model of the biological processes in the WWTP.

Conclusion

The OUR response on a single organic substrate can be divided into two phases. The first reflects the primary metabolism of the added substrate, while the second phase presumably originates from a shift to metabolism of storage polymers like PHA and/or glycogen produced during the primary metabolism of the added substrate, or it could be the result of absorbed substrate turn-over.

Oxygen consumption is a tool that allows a more detailed analysis of the metabolism of substrates added in an OUR test.

The yield coefficient found by respirometry is found to be independent of the concentration of substrate in the examined range.

There are substantial metabolic differences between the two activated sludges examined. This shows that there is potential for manipulating the activated sludge with respect to the utilisation of the substrate in full-scale plants. Furthermore the difference observed also implies that in the determination of the RBCOD fraction by respirometric methods, the yield coefficient should not be based on a standard value from literature but should be experimentally determined in the activated sludge used in the experiment.

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