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RESEARCH ARTICLE

Connection between oxygen uptake rate and carbon dioxide evolution rate in aerobic thermophilic sludge digestion

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Abstract

The main aim of studying the relation of carbon dioxide evolution rate CER to oxygen uptake rate (OUR) is the possible application of CER in mathematical modelling of aerobic biodegradation processes instead of OUR. Biodegradation tests using glucose and sewage sludge as feed were performed to compare the OUR and CER.

The respiratory quotient (RQ) was 0.9 mol $CO_2 \cdot$ *mol* O_2^{-1} *in endogenous stage while its value was increased to 1.2 mol* $CO_2 \cdot$ *mol* O_2^{-1} *during glucose degradation. At higher F/M ratios and high respiration rates RQ values up to 2.95 were observed which may indicate the appearance of anaerobic degradation pathways.*

These results prove that there is no direct, simple relation between OUR and CER in case of sewage sludge degradation so direct substitution of OUR with CER in modelling studies is not feasible.

Keywords

respiratory quotient · CER · thermophilic aerobic · sludge

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1 Introduction

The progress of the biological sewage sludge and other high-strength wastewater handling processes appears on one hand in the permanent improvement of anaerobic sludge digestion. On the other hand, the strong expansion of thermophilic aerobic treatment technologies is also observable. In smaller treatment plants this means the use of autothermal thermophilic aerobic digestion [5] while in larger ones composting technologies are being introduced [8].

Along with this, methods for evaluating the thermophilic aerobic metabolism are increasingly coming into focus. One of the most suitable methods for monitoring aerobic metabolic processes is respirometry. It has a wide application area e. g. in investigations of microbial processes in soil [2], different fields of wastewater treatment [16] and in performing ecotoxicological tests [21].

In the thermophilic case the determination of oxygen and carbon dioxide from the gas phase is favored because of the elevated temperature and high concentrations of substrates in the liquid/solid phase which limits the accuracy and life span of liquid phase sensors. Especially high attention is devoted to CO_2-measurement from gas phase [2] because high measuring sensitivity can be reached with relatively cheap instrumentation.

However, CO_2 measurement has drawbacks too: first, the liquid-phase pH affects the CO_2 concentration in the reactor off-gas very strongly, and in the second place the direct application of carbon dioxide evolution rate measurements assumes a direct, tight relation between microbial oxygen consumption and carbon dioxide evolution.

Gas-phase CO_2 measurement is especially popular in the monitoring of composting processes [6, 7] where the measurement in the solid phase is practically impossible. However, these measurements usually over-simplify the actual situation as they do not consider the effect of pH and CO_2 transfer limitations. Applications of classical respirometry, i. e. measurement of oxygen in the liquid phase after suspending the sludge in water can also be found [11] but this intervention proved to enhance the respiration rate substantially.

Spérandio and Paul [17] presented the applicability of

respirometry based on carbon dioxide measurements in the field of classical activated sludge processes. In addition to outlining a model which takes into account the effect of mass transfer and the pH (i. e. the reactions of carbon dioxide with water), they presented the application of this model concerning the kinetic investigation of denitrification processes [18]. The approach however, they presented for their kinetic investigation assumes that carbon-dioxide is produced in equimolar amounts to the consumed oxygen.

Actually, this CO_2/O_2 ratio, or the respiratory quotient (*RQ*) plays an important role in aerobic respirometric investigations as its value depends on the substrate, on the microorganisms present and on the environmental conditions too [2]. *RQ* is used especially often in the context of soil microbial studies as different *RQ* values can indicate the presence of different metabolic pathways (e. g. nitrification, denitrification, and anaerobic metabolic routes cause characteristic changes in *RQ* [3]).

The goal of our study was to make clear whether the CO_2-based respirometry can replace the conventional, oxygen-based respirometric methods in the kinetic analysis of thermophilic aerobic degradation processes. This prerequisite is met when the respiratory quotient is 1 or at least a safely constant value during the kinetic experiments this way allowing a simple conversion of carbon dioxide evolution rates to oxygen uptake rates.

2 Materials and Methods

2.1 Respirometric Experiments

During the experiments the response of an adapted thermophilic culture to different kinds of feed was monitored. An aerated and thermostated (55°C) fermenter with effective volume of 6 L (Braun Biostat, Braun Melsungen AG, Germany) served for maintaining the thermophilic culture. The operating conditions of the maintenance reactor were similar to those of full-scale sludge digestion plants i. e. it was fed once a day with sewage sludge of 1/6 of the effective volume in a semi-continuous manner (=1 L sludge feed daily, originating from WWTP Biatorbágy, with a 20 $g \cdot L^{-1}$ volatile solids (*VS*) concentration). This feeding pattern resulted in an average sludge retention time of 6 d which is common among full-scale plants.

Batch experiments were performed using the culture described above with two kinds of feed: glucose was chosen to investigate the degradation of a simple, well defined organic compound. To model a realistic case, the sewage sludge used for maintenance reactor was used as feed in respirometric experiments too.

The experiments were performed in a stirred (600 rpm) aerated (720 $L \cdot d^{-1}$) fermenter with a total volume of 2 L (Applikon Biotechnology B. V, Netherlands). The fermenter was thermostated at 55±1°C. The liquid volume in the reactor was 1.2 L in all tests. The pH was monitored by a WTW SenTiX-81 pH-electrode (WTW GmbH, Weilheim, Germany). The respiration rate was measured by two methods: oxygen uptake rate (*OUR*) was measured by a closed respirometry loop equipped with a WTW TriOxMatic EO 200 oxygen electrode while carbon dioxide evolution rate (*CER*) was calculated from CO_2 concentrations measured in the exhaust gas.

A special instrument with a measuring principle based on membrane separation and catalytic reduction of carbon dioxide to methane from the gas phase was used to monitor CO_2 in the reactor off-gas [14].

As mentioned in the introduction, convenient methods for measuring oxygen uptake rate by oxygen electrodes from the concentrated liquid phase at elevated temperatures suffer from problems of poor accuracy and strongly limited life span of the sensor, however a frequent cleaning and replacement of the electrode membrane along with electrode regeneration provided a solution for these problems.

3 Model for Carbon Dioxide Evolution Rate Calculation

The slightly simplified model incorporating the reactions of carbon dioxide with water and the limitations of CO_2 transfer introduced in [17] was used for calculating *CER* from CO_2 concentrations in exhaust gas. The graphical schema of the processes incorporated in the model can be seen in Fig. 1.

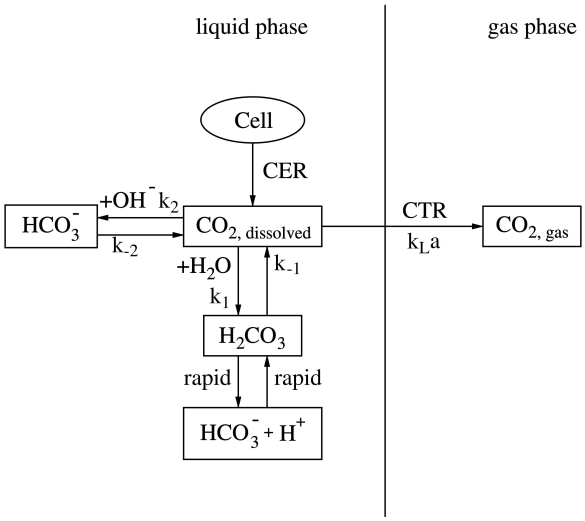

Fig. 1. Reactions of carbon-dioxide in water according to the model in [17]

The applied simplification affects the mass transfer description: in [17] the logarithmic mean concentration difference is introduced as the driving potential of the mass transfer because under usual operating conditions of bioreactors with low stirring rates a plug flow gas phase is often observed. On the other hand, in our lab-scale fermenter the gas phase can be treated perfectly mixed so the simple description using only the concentration difference as the driving potential is sufficient. With this modification the model equations take the following form (Eqs 1-5):

$$\frac{d[CO_2]}{dt} = \frac{CER}{M_{CO_2}} - k_L a_{CO_2} \cdot \Delta C - \left(k_1 + k_2 \cdot 10^{pH-14}\right) \cdot [CO_2] + \left(k_{-2} + k_{-1} \cdot 10^{-pH}\right) \cdot [HCO_3^-] \quad (1)$$

$$\frac{\mathrm{d}[\mathrm{HCO_3^-}]}{\mathrm{d}t} = \left(k_1 + k_2 10^{\mathrm{pH}-14}\right)[\mathrm{CO_2}] - \left(k_{-2} + k_{-1} 10^{-\mathrm{pH}}\right)[\mathrm{HCO_3^-}] \quad (2)$$

$$\frac{\mathrm{d}C^b_{\mathrm{CO_2}}}{\mathrm{d}t} = \frac{1}{\epsilon_G V_L}\left(V_L k_L a_{\mathrm{CO_2}} \Delta C + Q_a C^a_{\mathrm{CO_2}} - Q_b C^b_{\mathrm{CO_2}}\right) \quad (3)$$

$$\frac{dC_{\mathrm{CO_2}}}{dt} = \frac{Q_b}{V_G} \cdot \left(C^b_{\mathrm{CO_2}} - C_{\mathrm{CO_2}}\right) \quad (4)$$

where

$$\Delta C = [\mathrm{CO_2}] - \frac{C^b_{\mathrm{CO_2}}}{H} \cdot 10^3 \cdot R \cdot T_b \quad (5)$$

The model's physico-chemical parameter values were obtained from different sources in the literature. The Henry's law constant was calculated from solubility data published in [10], with a value of $5.836 \cdot 10^6$ Pa $\cdot$ mol$^{-1}\cdot$ L^{-1} at 55°C. The rate constants k_1 and k_2 were extrapolated from the data of Pinsent et al. [15] with a value of $1.203 \cdot 10^4$ d^{-1} and $4.919 \cdot 10^9$ L $\cdot$ mol$^{-1}\cdot$ d^{-1} respectively. The equilibrium constant K_1 and the dissociation constant of water (K_w) necessary for calculation the rate constants k_{-1} and k_{-2} according to [17] were from [10]. Values of k_{-1} and k_{-2} were $2.349 \cdot 10^{10}$ L $\cdot$ mol$^{-1}\cdot$d^{-1} and $6.866 \cdot 10^2$ d^{-1} respectively.

For calculation of carbon dioxide evolution rates from exhaust gas CO_2 concentrations the method presented in [1] was adopted. It contains the application of extended Kalman filtering and the correction of the filter's results by the Bryson-Frazier formulas. The tuning of the filter was made by adjusting the covariance matrix of the process noise (for details see [1]) which reduces actually to a scalar value in this case since we are estimating only one state variable of the model (the *CER*).

The state estimator algorithm was implemented in GNU Octave [4]. The code allowed the dynamic change of the process noise covariance matrix during the experiments thus allowing the accurate reconstruction of *CER* even in case of sudden changes in CO_2 concentration in the exhaust gas. To investigate the performance of the estimator algorithm, it was applied to data that were generated by simulating the model with known *CER* and pH values. The results of this estimation are given in Fig. 2. As it can be seen the model is able to reconstruct the known *CER* curve even in the case of sudden pH or CO_2 concentration change.

Only the $k_L a_{\mathrm{CO_2}}$ and the ϵ_G parameters were to calibrate to our specific reactor setup. This calibration was made by injection of $NaHCO_3$ into the reactor under the usual operating conditions listed above. The reactor contained tap water, with pH set below 5. At this pH the injected HCO_3^- ions instantly transform to CO_2 thus measuring the CO_2 concentration in the exhaust gas allows the determination of the mass transfer parameters. The $k_L a_{\mathrm{CO2}}$ and ϵ_G were determined fitting the model output to the CO_2 curve. Results of fitting can be seen in Fig. 3.

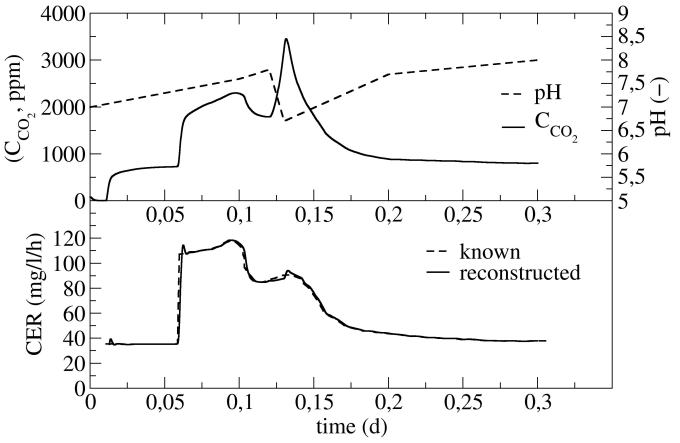

Fig. 2. Testing the *CER*-reconstruction process by known *CER* and pH-data

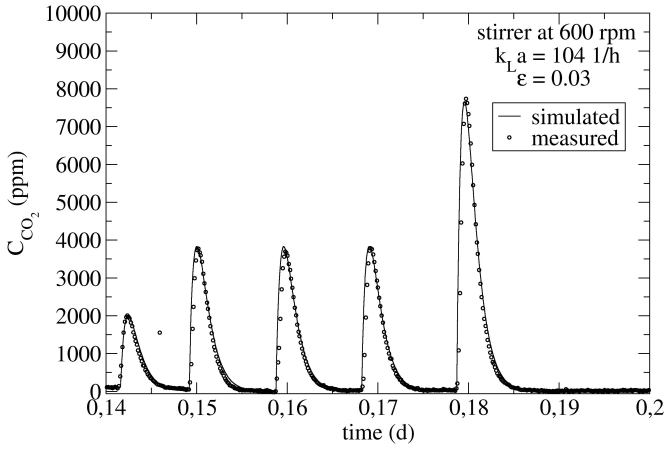

Fig. 3. Experimental response of the system to $NaHCO_3$ spikes. The injected $NaHCO_3$ amounts were 10, 20, 20, 20 and 40 mg respectively.

4 Results and Discussion

4.1 Respiratory Response to Glucose – Low F/M Ratio

At higher *F/M* ratios the enrichment of faster growing species in microbial cultures is difficult to avoid during the intensive growth phase, i. e. the structure of the original culture is changing significantly. Maintaining a low *F/M* ratio during respirometric experiments is therefore desirable because it makes possible the investigation of the microbial culture in its original state [19]. Typical respirogram obtained from glucose feed experiments with low *F/M* can be seen in Fig. 4. Two glucose feeds were applied to the endogenous thermophilic culture at 0.1 and 0.27 d with a glucose amount of 0.22 and 0.10 g respectively. The *F/M* ratios were 0.018 and 0.008 mg $COD_{glucose}$/mg VSS.

As it can be seen in Fig. 4, the oxygen uptake and carbon dioxide evolution rates change synchronously; a rapid increase can be observed right after the feed in both cases indicating the intensive degradation of the glucose and after the depletion of the substrate they fall back to their initial, endogenous values. The increase of the *CER* and *RQ* curves at the second feed is faster than in the first case which can be attributed to microbial adaptation to the glucose.

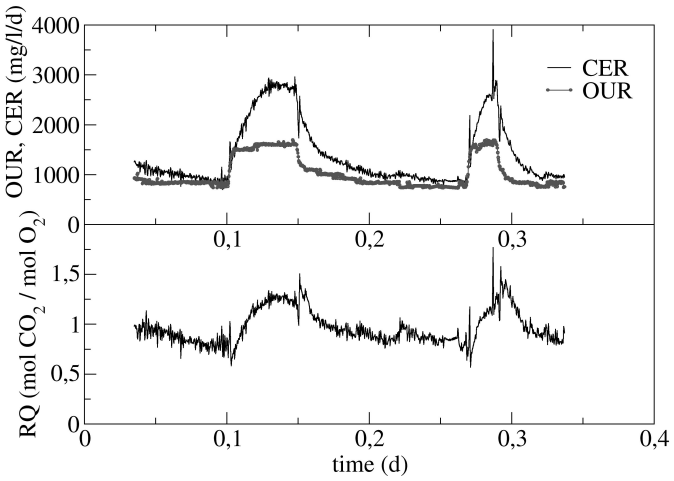

Fig. 4. Respiration profiles during experiments with low *F/M* ratio

Analyzing the respiratory quotient during the experiment it can be observed that it has two, well separable characteristic values: at the endogenous stages the value drifts between 0.8 and 1 mol $CO_2 \cdot$ mol O_2^{-1} while during the intensive phase it rises to 1.2-1.25 mol $CO_2 \cdot$ mol O_2^{-1}. This can be explained by the following stoichiometric considerations:

Previous investigations of Mihály et al. [13] provided a well applicable elementary composition of the same sewage sludge we also used in these experiments. From their data the $CH_{1.80}O_{0.49}N_{0.12}$ chemical formula can be calculated. Considering the fact that in the endogenous phase the microbes degrade their own cellular structures in the lack of substrate to obtain energy, the following stoichiometric relation (Eq. 6) can be determined:

$$CH_{1.80}O_{0.49}N_{0.12} + 1.115\,O_2 \longrightarrow CO_2 + 0.72\,H_2O + 0.12\,NH_3 \quad (6)$$

This gives $RQ = 0.89$ which is in good agreement with the endogenous *RQ* values from the experiment.

On the other hand, during glucose degradation the production of new biomass and the energy needs are covered from glucose. Herbert [9] gave the following elementary balance equation (Eq. 7) for complete glucose anabolism:

$$C_6H_{12}O_6 + 0.75\,O_2 + 0.19\,NH_3 \longrightarrow CH_{1.82}O_{0.47}N_{0.19} + 0.90\,CO_2 + 1.18\,H_2O \quad (7)$$

This equation corresponds to the *RQ* value of 1.2. As the biomass composition obtained from Mihály et al. [13] is very close to that in Herbert's equation it can be accepted that the *RQ* value of this sewage sludge is approximately equal to 1.2. This value is close to those found in Fig. 4.

The demonstrated change of the *RQ* depending on growth phases makes questionable the approach of Sperandio and Paul [18] concerning the direct usability of *CER* data for calibration of *COD*-based activated sludge models.

Respiration at high *F/M* ratios

Although low *F/M* ratios are desirable during respirometric experiments from the aspect of kinetic analysis, during usual operation of thermophilic aerobic sludge digestion the semi-continuous operation scheme is typical. Results of an experiment performed under realistic conditions (sludge spike into endogenous thermophilic culture with initial *F/M* 0.21 mg COD · $mgVSS^{-1}$) are shown in Fig. 5.

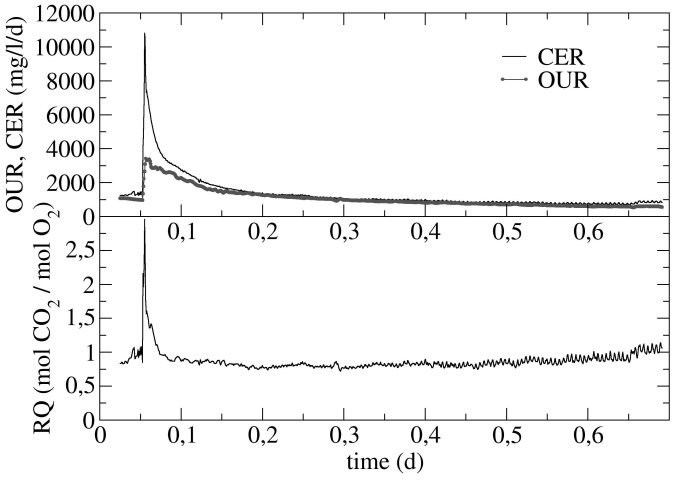

Fig. 5. Respiration profiles in case of feeding sewage sludge to the system

Again, the substrate degradation is indicated by intensive respiration. Before the feed and after the substrate depletion the endogenous respiration can be seen. These endogenous phases can be characterised by an average *RQ* value of 0.88 which is in good agreement with the glucose experiments and the theoretical considerations.

However, during the intensive degradation the *RQ* becomes very high (with a maximal value of 2.95) which cannot be explained by the pure aerobic degradation of sewage sludge. Plotting the *RQ* as the function of oxygen uptake rate (Fig. 6) shows a slight correlation of the respiratory quotient with *OUR*, especially at *OUR* values above 2500 mg/l/d.

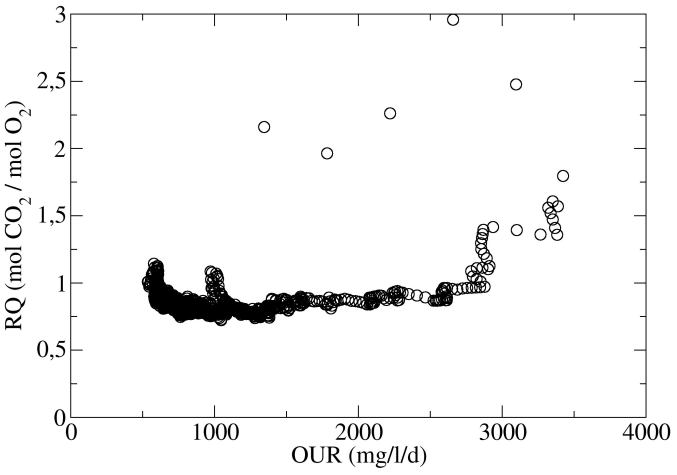

Fig. 6. Dependency of the respiratory quotient on oxygen uptake rate in case of feeding sewage sludge to the system

To better investigate this phenomenon, experiments were performed with glucose feed resulting the same *F/M* ratio but since glucose is a readily biodegradable substrate, its degradation allowed the development of *OUR* values being higher than in case of sewage sludge degradation. Typical respiration results are shown in Fig. 7. As it can be seen, the *RQ* value increases from the endogenous value of 0.8 to 1.1-1.2 right after the feed. This corresponds to the results from low *F/M* glucose experiments. However, approximately 5 hours after the feed the respiratory quotient increases rapidly and it falls back to the endogenous value only after the substrate depletion. This can be explained with the observation that high *RQ* values usually denote the appearance of anaerobic, fermentative metabolic pathways.

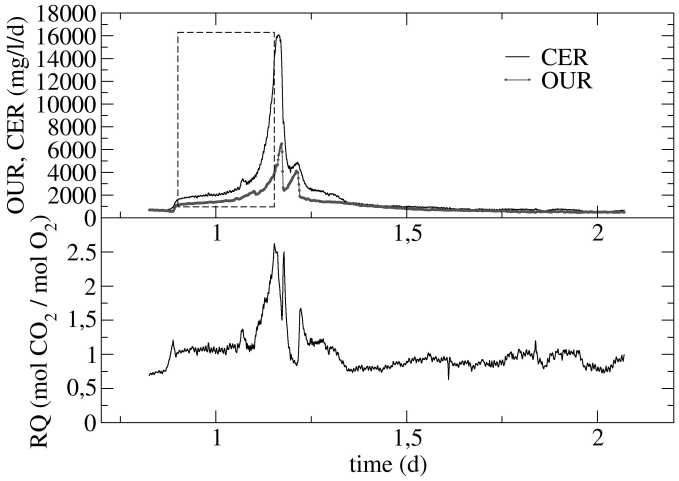

Fig. 7. Respiration profiles with glucose at high *F/M* ratio

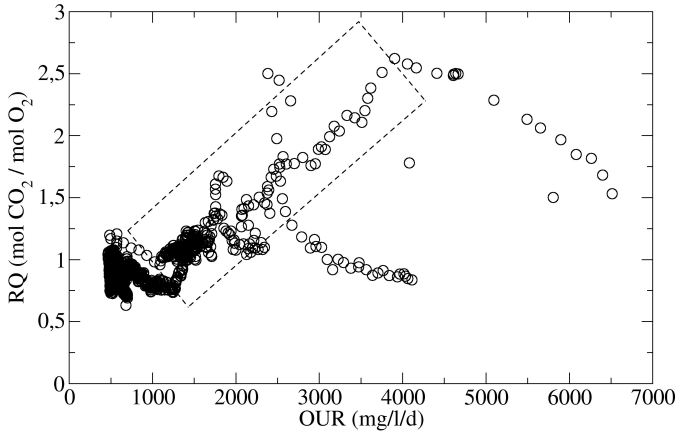

Fig. 8. Dependency of the respiratory quotient on oxygen uptake rate at high *F/M* ratio. The box denotes the intensive growth stage which corresponds to the area marked on Fig. 7

The lack of oxygen and presence of biodegradable substrate usually induce these anaerobic degradation pathways. It is known that oxygen transfer to the cells is hindered by many resistances [12]. Only one of these is the transport limitation through the gas/liquid interface which can be eliminated by efficient aeration. Further resistances are the oxygen diffusion within sludge flocs and through the cell membrane itself. The effect of mass transfer resistance of sludge flocs is illustrated by numerical simulations in [20]. It has been shown that dissolved oxygen concentrations can drop to very low levels in the core of the flocs, even under normal operating conditions.

Although our experiments were performed in an aerated reactor where the oxygen concentration in the bulk phase never dropped below 2 mg/L, it is likely that at higher oxygen uptake rates anaerobic zones form inside the sludge flocs.

As it can be seen in Fig. 8, the *RQ* is strongly correlated with the oxygen uptake rate. It exceeds the theoretical 1.2 value of glucose anabolism at approximately $OUR = 2500\,\mathrm{mg/l/d}$, indicating that this is the critical *OUR* value where the oxygen supply of the cells becomes insufficient.

Beside the obvious conclusion concerning the limited applicability of *CER* measurements for direct estimation of oxygen uptake rates this phenomenon has an important consequence related to *COD*-based mathematical modelling too. In the field of mathematical modelling it is commonly accepted that measured oxygen uptake rates are applicable for *COD* change calculation. It has to be stressed that this is true only if there are no anaerobic zones in the monitored reactor, or in other words the oxygen uptake rate remains below the critical value (2500 mg/l/d in our case).

5 Conclusions

Thermophilic aerobic sludge digestion respiration tests were performed in order to investigate the relation between the carbon dioxide evolution and oxygen uptake rates.

The respiratory quotient was found to be 0.9 mol $CO_2\cdot$ mol O_2^{-1} in endogenous stage while its value was increased to 1.2 mol $CO_2\cdot$ mol O_2^{-1} during glucose degradation. At higher *F/M* ratios and high respiration rates ($OUR > 2500\,\mathrm{mg\cdot L^{-1}\cdot d^{-1}}$) *RQ* values up to 2.95 were observed. The *RQ* was strongly correlated with *OUR* which indicates that at higher respiration rates the oxygen supply of the active cells becomes insufficient and anaerobic degradation is induced.

These results prove that there is no direct, simple relation between *OUR* and *CER* in case of sewage sludge degradation so direct substitution of *OUR* with *CER* in modelling studies is not feasible.

Furthermore, the detected existence of anaerobic degradation at higher respiration rates requires more precaution when calculating COD balances from respiration data in classical *OUR* based modelling studies. However, *CER* can provide additional information which, if used together with *OUR*, provides an insight into reaction stoichiometry. This can be particularly relevant in view of the current trend to include more elemental balancing in contemporary models.

Nomenclature

C_{CO_2}	carbon dioxide amount in exhaust gas ($mol \cdot L^{-1}$)
$C^a_{CO_2}$	carbon dioxide amount in inlet gas ($mol \cdot L^{-1}$)
$C^b_{CO_2}$	carbon dioxide amount in the gas that passed through culture liquid ($mol \cdot L^{-1}$)
CER	carbon dioxide evolution rate mg $CO_2 \cdot L^{-1} \cdot d^{-1}$)
COD	chemical oxygen demand (mg $O_2 \cdot L^{-1}$)
CTR	carbon dioxide transfer rate (mg $CO_2 \cdot L^{-1} \cdot d^{-1}$)
$[CO_2]$	bulk phase carbon dioxide concentration ($mol \cdot L^{-1}$)
F/M	food-to-microbe ratio (mg COD/mg VSS)
H	Henry's law constant ($Pa \cdot L \cdot mol^{-1}$)
$[HCO_3^-]$	bulk phase bicarbonate ion concentration ($mol \cdot L^{-1}$)
k_1	forward rate constant for the reaction of CO_2 with water (d^{-1})
k_{-1}	backward rate constant for the reaction of CO_2 with water $L \cdot mol^{-1} \cdot d^{-1}$)
k_2	forward rate constant for the reaction of CO_2 with OH^- ($L \cdot mol^{-1} \cdot d^{-1}$)
k_{-2}	backward rate constant for the reaction of CO_2 with OH^- (d^{-1})
K_1	equilibrium constant of carbon dioxide hydration with water ($mol \cdot L^{-1}$)
K_w	water dissociation costant ($mol^2 \cdot L^{-2}$)
$k_L a_{CO_2}$	volumetric mass transfer coefficient for carbon dioxide (d^{-1})
M_{CO_2}	molecular weight of carbon dioxide ($mg \cdot mol^{-1}$)
OUR	oxygen uptake rate (mg $O_2 \cdot L^{-1} \cdot d^{-1}$)
Q_a	inlet volumetric aeration rate ($L \cdot d^{-1}$)
Q_b	outlet volumetric aeration rate ($L \cdot d^{-1}$)
R	ideal gas constant ($8.314\ J \cdot mol^{-1} \cdot K^{-1}$)
V_L	liquid volume in the reactor (L)
V_G	free gaseous space volume in the reactor (L)
ϵ_G	gas hold-up (dimensionless)
ΔC	driving potential ($mol \cdot L^{-1}$)

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