



PII: S0045-6535(98)00569-4

EVALUATION OF A RESPIROMETRIC TEST METHOD TO DETERMINE THE HETEROTROPHIC YIELD COEFFICIENT OF ACTIVATED SLUDGE BACTERIA

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(Received in Germany 14 October 1998; accepted 16 November 1998)

Abstract

The heterotrophic yield coefficient of activated sludge bacteria is an important parameter in the field of wastewater degradation kinetics and the determination of degradation kinetics of defined chemicals. With the help of easily biodegradable organic compounds like glucose and acetate the respiration kinetics of degrading activated sludge bacteria was studied. The results of the respiration analysis were transformed to examine the degradation and respiration kinetics of municipal wastewaters at different food to microorganism ratios (F/M ratios). It was proved that the heterotrophic yield coefficient of aerobically degrading activated sludge bacteria could reliably be determined and that the heterotrophic yield coefficient was independent of the F/M ratio over a wide range.

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Keywords

Respirometry, heterotrophic yield coefficient, biodegradable COD, wastewater degradation, degrading bacteria, activated sludge bacteria

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1. Introduction

Respirometric methods are widely used in the field of aerobic wastewater treatment processes. They are used to determine the biochemical oxygen demand (BOD) of wastewaters or the toxicity of chemical compounds [1; 2]. In the recent years an automation of several methods has been performed in order to develop on-line measurement methods to determine continuously possible toxic effects of wastewater and the short-term BOD [3; 4; 5]. Especially the short-term BOD proved to be a parameter which represents the readily biodegradable fraction of chemical oxygen demand (COD) in wastewater. This fraction is assumed to serve as a preferred carbon source for denitrification processes and therefore the estimation of this fraction is of increased interest. The fraction of readily biodegradable COD can only be determined if the heterotrophic yield coefficient (Y_H) of the degrading bacteria is known. Therefore, the heterotrophic yield coefficient of activated sludge bacteria is an important parameter for modelling degradation processes by activated sludge [6; 7].

The heterotrophic yield coefficient is also of importance as an additional information for biodegradability studies with chemical compounds. A number of methods exist with the help of which biodegradability can be determined. For these test methods it is advantageous to determine the heterotrophic yield coefficient of the degrading bacteria to get an accurate impression of the flow of carbon during the degradation processes. This determination is either necessary for test systems based on oxygen consumption [8] and also for test systems based on the production of carbon dioxide [8; 9].

The purpose of this work was (I) to optimize respirometric methods to easily determine the heterotrophic yield coefficient of activated sludge bacteria degrading easily degradable organic compounds, (II) to determine the variation of the heterotrophic yield coefficient under different loading conditions and (III) to use the heterotrophic yield coefficient to estimate the readily biodegradable COD fraction in wastewater.

2. Materials and Methods

Respirometric methods

Oxygen consumption processes were determined with an oxygen electrode (OXI 3000, WTW, Weilheim, Germany). The oxygen consumption was measured in a closed test vessel (total volume: 1.5 l) containing 20 mM KH_2PO_4 as a buffer substance to maintain a pH of 7.0. Allylthiourea (ATU; final conc. 10 mg/l) was added as an inhibitor of nitrification processes. The activated sludge suspension used for the experiments was from the municipal wastewater treatment plant in Borken, Germany, and had been starved out for about 16 hours prior to use in the test. The concentration of activated sludge in the test system was 1 to 3 g of mixed liquid suspended solids (MLSS) per litre. Before adding substrate or wastewater the endogenous respiration rate R_{end} was recorded for 20 to 40 minutes. Organic compounds were added as pure compounds or as wastewater samples. Directly after the addition of substrate or wastewater the respiration rate increased to its maximum value (R_{max}). The spiking factor (SF) was an indicator for the increase of the respiration rate and was calculated according to the following equation:

$$(1) \quad \text{SF} = \frac{R_{\text{max}}}{R_{\text{end}}}$$

Respiration rates were always indicated in $\text{mg O}_2 \text{ l}^{-1} \text{ h}^{-1}$. The incubation temperature was 30°C. The incubation was performed until the respiration rate decreased to R_{end} . The respiration rates at different times were stored in a computer and used to calculate the short-term biochemical oxygen demand (BOD_{ST}). The BOD_{ST} is represented by the area under a respiration peak and was calculated with a computer (see Fig. 1A, Fig. 2A). The chemical oxygen demand was also followed during the test period and determined with test kits (Dr. Lange, Düsseldorf, Germany).

Calculations

The heterotrophic yield coefficient (Y_{H}) was calculated from the BOD_{ST} and the readily biodegradable chemical oxygen demand (COD_{DEG}) according to the following equation:

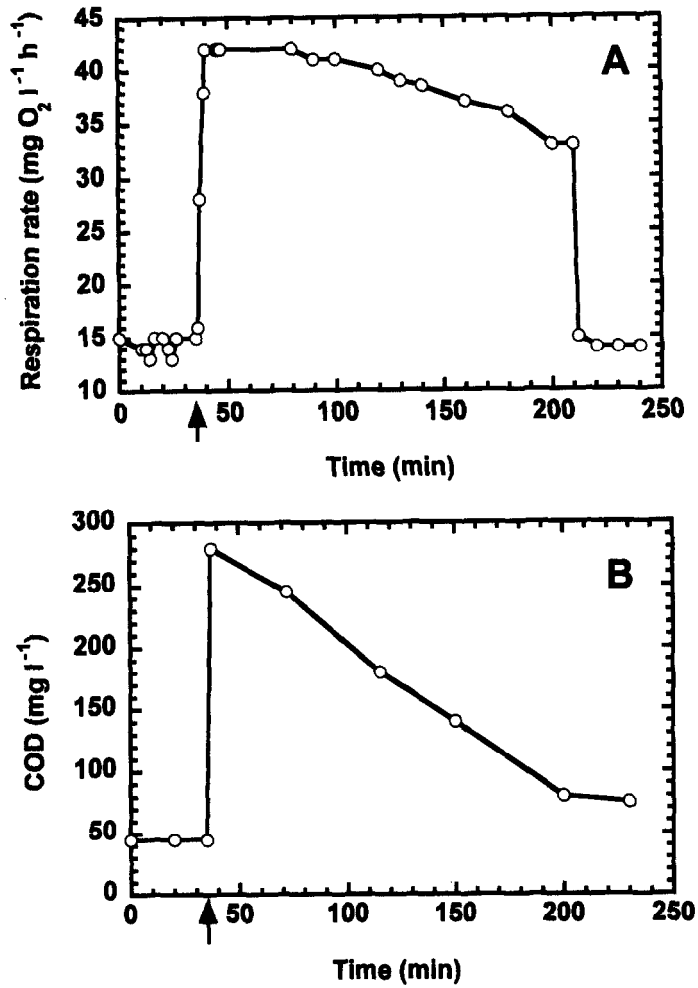


Fig. 1. Addition of acetate to starved activated sludge. The respiration rate (A) and the COD degradation (B) were followed. The arrow indicates the addition of the substrate. The F/M value was 0.07 g COD g⁻¹ MLSS.

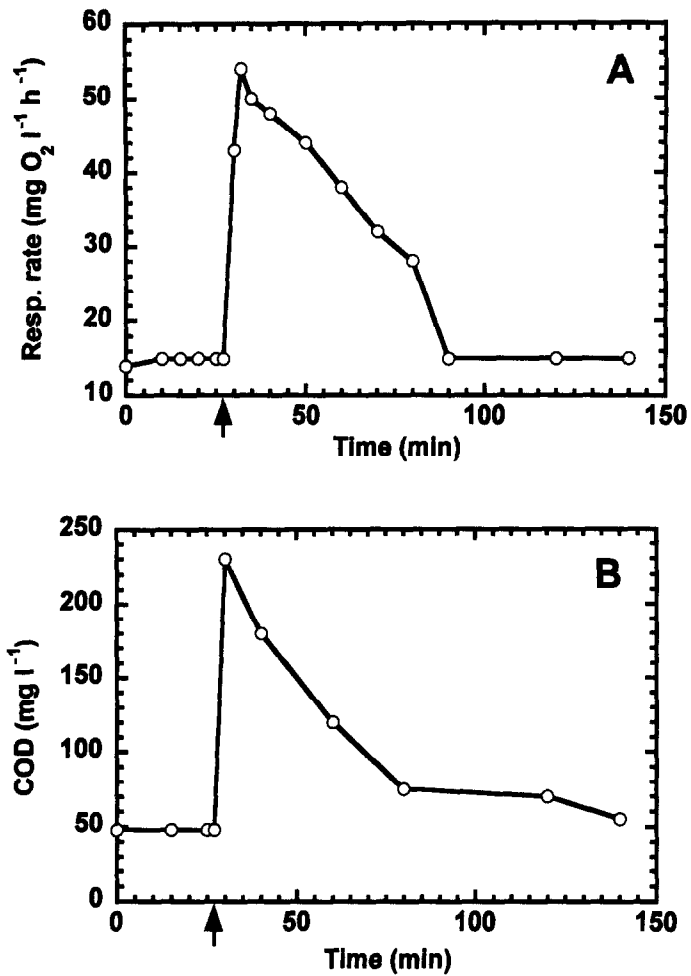


Fig. 2. Addition of glucose to starved activated sludge. The respiration rate (A) and the COD degradation (B) were followed. The arrow indicates the addition of the substrate. The F/M value was $0.05 \text{ g COD g}^{-1} \text{ MLSS}$.

$$(2) \quad Y_H = 1 - \frac{BOD_{ST}}{COD_{DEG}}$$

The readily biodegradable COD fraction was determined from the initial COD at the beginning of the test (COD_{init}) and the COD at the end of the test (COD_{end}) according to:

$$(3) \quad COD_{DEG} = COD_{init} - COD_{end}$$

If Y_H and BOD_{ST} were known from experimental data the readily biodegradable COD fraction could directly be determined by rearrangement of equation (2):

$$(2a) \quad COD_{DEG} = \frac{BOD_{ST}}{1 - Y_H}$$

3. Results

Degradation analysis of acetate and glucose

The endogenous respiration rate R_{end} of starved activated sludge ranged between 11 and 18 mg O₂ l⁻¹ h⁻¹. Directly after addition of acetate or glucose the respiration rate increased to the maximal respiration rate R_{max} which was in the range of 42 and 68 mg O₂ l⁻¹ h⁻¹ (Tables 1 and 3). The respiration data obtained for these substrates are summarized in Tables 1 to 4. The mean value for the spiking factor was 3.3 and 4.4 for acetate and glucose, respectively. The mean value for the heterotrophic yield coefficient obtained was 0.64 for acetate and 0.82 for glucose. It proved to be independent of the F/M ratio. The extent of degradation of acetate and glucose was in the range of 63 to 92 %.

Degradation analysis of municipal wastewater

The respiration profile of activated sludge after addition of municipal wastewater is illustrated in Fig. 3. At low F/M ratios a typical peak was formed whereas at high F/M ratios a plateau was formed. The data obtained from experiments at different F/M ratios are summarized in the Tables 5 and 6. In preliminary studies the mean value for the

spiking factor with municipal wastewater was 5.6 and the mean value for the heterotrophic yield coefficient was 0.79. The importance of adding an inhibitor of nitrification processes is illustrated in Fig. 4. Therefore, it is obvious that false Y_H data were obtained if the nitrification process was not effectively inhibited when testing wastewaters containing varying amounts of ammonium or easily degradable nitrogen containing organic compounds which did not provide a stable nitrification rate. The estimation of Y_H of wastewaters at different F/M ratios in the range of 0.01 to 0.48 g COD g^{-1} MLSS showed that Y_H was independent of the F/M ratio used. In extended studies the mean value of Y_H was determined to be 0.75 with a standard deviation of 0.0827 and a variation coefficient of 11%. The number of single estimations was 44 (Fig. 5).

Table 1. Respiration analysis after addition of acetate. Influence of different F/M-ratios on the duration of degradation processes, maximal respiration rate, and the spiking factor

F/M (g/g MLSS)	Duration (min)	R_{max} (mg/l*h)	R_{end} (mg/l*h)	Spiking factor
0.01	30	43	15	2.9
0.07	185	42	14	3.0
0.09	400	44	11	4.0
0.16	590	58	18	3.2

Table 2. Respiration analysis after addition of acetate. Influence of different F/M-ratios on short-term BOD, degraded COD and Y_H

F/M (g/g MLSS)	initial COD (mg/l)	degraded COD (mg/l)	COD degradation (%)	BOD _{ST} (mg/l)	Y_H
0.01	40	27	68	9.6	0.64
0.07	280	205	73	71.8	0.65
0.09	351	322	92	124.3	0.61
0.16	848	754	89	250.4	0.67

Table 3. Respiration analysis after addition of glucose. Influence of different F/M-ratios on the duration of degradation processes, maximal respiration rate, and the spiking factor

F/M (g/g MLSS)	Duration (min)	R_{max} (mg/l*h)	R_{end} (mg/l*h)	Spiking factor
0.05	64	54	15	3.6
0.14	220	47	11	4.3
0.27	660	51	11	4.6
0.71	1710	68	14	4.9

Table 4. Respiration analysis after addition of glucose. Influence of different F/M-ratios on short-term BOD, degraded COD and Y_H

F/M (g/g MLSS)	initial COD (mg/l)	degraded COD (mg/l)	COD degradation (%)	BOD _{ST} (mg/l)	Y_H
0.05	230	170	74	25.5	0.85
0.14	406	322	79	58.9	0.82
0.27	783	648	83	118.1	0.82
0.71	2627	1652	63	350.8	0.79

Table 5. Respiration analysis after addition of municipal wastewater. Influence of different F/M-ratios on the duration of degradation processes, maximal respiration rate, and the spiking factor

F/M (g/g MLSS)	Duration (min)	R_{max} (mg/l*h)	R_{end} (mg/l*h)	Spiking factor
0.02	130	16	3.8	4.2
0.06	270	34	5	6.8
0.125	240	19	4.5	4.2
0.242	360	28	4	7.0
0.393	250	30	4	7.5
0.468	250	23	6	3.8

Table 6. Respiration analysis after addition of municipal wastewater. Influence of different F/M-ratios on short-term BOD, degraded COD and Y_H

F/M (g/g MLSS)	initial COD (mg/l)	degraded COD (mg/l)	COD degradation (%)	BOD _{ST} (mg/l)	Y_H
0.02	70.6	47.8	68	14.3	0.70
0.06	229	147.3	64	28.5	0.81
0.125	165	102.1	62	19.9	0.81
0.242	276	193	70	47.2	0.76
0.393	488	295	60	56	0.81
0.468	533	333	62	44	0.87

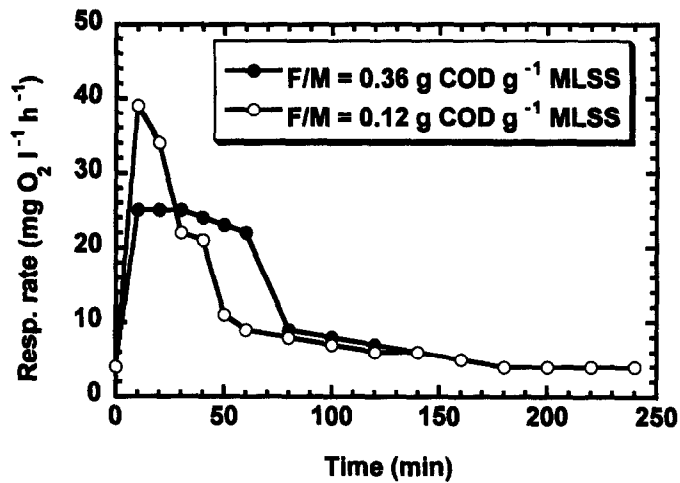


Fig. 3. Effect of different F/M ratios on the time course of the respiration rate. Municipal wastewater was added at time = 0 min. The data were obtained from preliminary experiments which are not contained in the data sets of Tables 5 and 6.

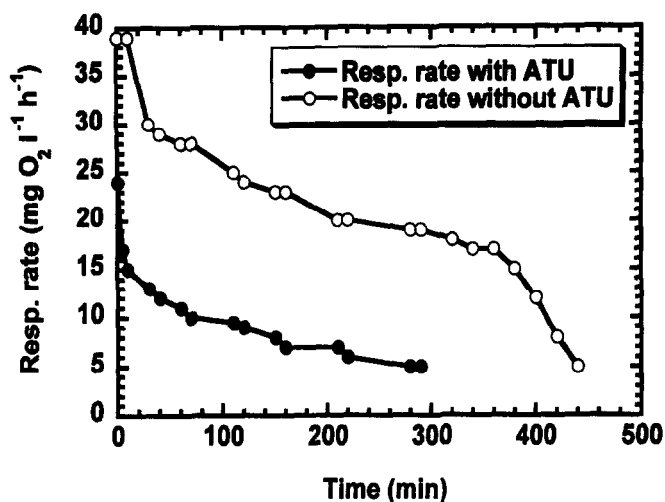


Fig. 4. Effect of allylthiourea (ATU) on the respiration rate of activated sludge. Municipal wastewater containing an ammonium concentration of 50 mg/l was added at time = 0 min.

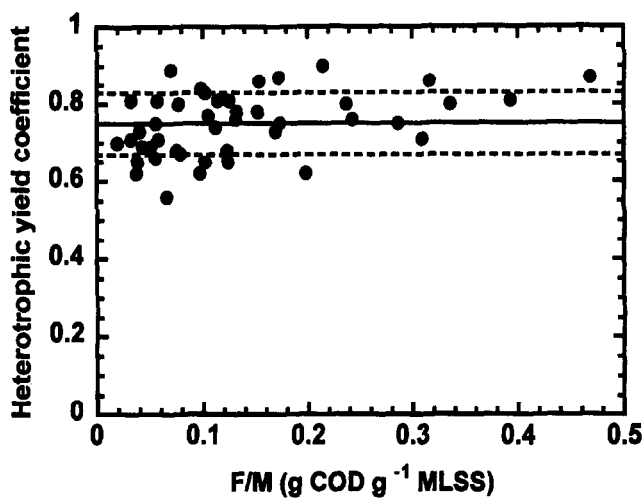


Fig. 5. Heterotrophic yield coefficients of activated sludge bacteria at different F/M ratios. The experiments were performed with municipal wastewater. The mean is indicated by a solid line and the standard deviation by dashed lines.

4. Discussion

The results obtained show that the the short-term BOD (BOD_{ST}) of single compounds and wastewaters could easily be determined by the method presented. The method included the inhibition of nitrification processes by the addition of allylthiourea. In the presence of a nitrification inhibitor the BOD_{ST} could be determined very reliably. This method has been controversely discussed in literature. A number of authors used similiar methods without reporting any problems [10, 11; 12; 13]. Other authors object using a nitrification inhibitor and recommend adding a sufficient amount of ammonium providing a stable nitrification rate over the test period [6; 14]. As in some cases we observed a pH shift during the respiration process the addition of a phosphate buffer seemed necessary to stabilize a pH of 7.0 during the test period . A preincubation period of the starved activated sludge was necessary to maintain stable steady state respiration conditions before adding organic substrate or wastewater.

An important point in the characterization of a respiration profile was the spiking factor. This factor had to be high enough ($SF > 3$) to enable a clear respiration profile. If the spiking factor was too high ($SF > 7.5$) the respiration profile showed a steep increase in respiration rate followed immediately by a fast decay. Due to the short time period for the respiration increase these profiles could cause problems in the calculation of the results. The shape of the respiration profile was dependent on the F/M ratio in the test system. These observations have also been found by other authors [6; 10].

The heterotrophic yield coefficient Y_H is an important parameter for modelling degradation processes with activated sludge [15]. It was stated that the heterotrophic yield coefficient is a striking parameter in the calculation of the readily biodegradable COD fraction in wastewater. Furthermore, it can also be used for the estimation of the growth rate constant of activated sludge bacteria [6; 7]. In the recent years the readily biodegradable COD fraction has gained an increased importance as the COD fractionation in the characterization of wastewaters was intensively studied [10; 12; 15; 16]. Therefore, the accurate determination of the heterotrophic yield coefficient is an important prerequisite for the subsequent modelling processes based on it. The

heterotrophic yield coefficient described for aerobic degradation processes varies over a wide range from 0.40 to 0.80 [10; 11; 12; 17; 18; 19; 20]. An estimated value which is frequently used in modelling processes is 0.67 [7]. The heterotrophic yield coefficients determined in this study were significantly different from this value and ranged from 0.79 to 0.85 for glucose, 0.61 to 0.67 for acetate and 0.75 to 0.79 for municipal wastewater. In order to avoid false calculations these observations imply the necessity for determining the heterotrophic yield coefficient for each wastewater and treatment plant studied separately.

In future research heterotrophic yield coefficients may also gain importance in the field of testing the biodegradability of chemicals by respirometric tests and test systems based on the production of carbon dioxide [8]. With the help of heterotrophic yield coefficients in combination with the determination of COD biodegradation the flow of carbon into catabolic and anabolic processes in bacteria can be estimated and thus the extent of degradation can be determined with an increased precision.

Acknowledgements

The authors thank D. Meng and D. Lefin for correcting the manuscript and helpful discussions.

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