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DETERMINATION OF THE MICHAELIS-MENTEN KINETIC PARAMETERS WITH SINGLE CONCENTRATION ASSAYS

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Results from a number of studies in brackish water and freshwater environments were used to examine the relationship between heterotrophic activity parameters derived from kinetic and single concentration assays. The results showed good agreement between the kinetic and the single concentration parameters. The concentration sum, (K + S) from the kinetic assay may be used to determine approximate concentration levels for the single concentration assays: one tenth of (K + S) for the turnover rate ($1/T_s$) determinations and tenfold (K + S) for the heterotrophic potential (V_s) determinations, provided that the (K + S) value has been determined over a wide range of substrate concentrations. In oligotrophic conditions, 0.1 μg glucose l^{-1} appeared to be low enough for the $1/T_s$ determinations and 30 μg glucose l^{-1} high enough for the V_s determinations. In eutrophic waters, glucose concentrations in excess of 100 μg l^{-1} may have to be used for the V_s determinations. In oligotrophic waters and in calculating substrate flux rates, the single concentration assay should be used for turnover rate determinations. On the other hand, in highly eutrophic and polluted waters, the kinetic assay seems to be the best approach for heterotrophic potential determinations.

Index words: Heterotrophic activity, glucose assimilation, kinetic assay, single concentration assay, oligotrophy, eutrophy.

1. INTRODUCTION

The enzyme kinetic method, which was introduced to aquatic ecology by Parsons and Strickland (1962), developed by Wright and Hobbie (1966) and further improved by Hobbie and Crawford (1969), has been widely used to determine microheterotrophic activity in both fresh water and marine environments. The kinetic method yields three parameters: the maximum uptake velocity of the given substrate (V), the turnover time (T) and the concentration sum (K + S), which is a combination of the halfsaturation constant (K) and the naturally occurring substrate concentration (S).

All of the three kinetic parameters can be used to describe the activity or the state of heterotrophic micro-organisms in the environment.

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Maximum uptake velocity (V), also known as heterotrophic potential, is a measure of activity at the saturation concentration of the substrate. It is a function of population size and potential activity and therefore, when used alone, is interpreted as a relative indicator of the heterotrophic activity of the environment.

Turnover time (T) expresses the time in which the whole amount of substrate would be removed if the uptake continued unchanged and no extra supply of the substrate was added. This parameter is a function of population size and actual activity, but also of the ambient substrate concentration (s). By taking the inverse of the turnover time and multiplying it by 100, we get turnover rate (1/T), which is a measure of the percentage of available substrate taken up per unit of time (cf. Kuparinen et al. 1984). This parameter is of particular interest in heterotrophic activity studies, since in conjunction with the knowledge of the natural substrate concentration, the flux rates of the substrate can be calculated. Even if the natural substrate concentration is unknown, the parameter can be used to describe and compare the heterotrophic activities of different water bodies since the concentrations seem to vary only slightly in time and space (Andrews and Williams 1971, Dawson and Gocke 1978, Cavari and Hadas 1979) compared to the variation in the turnover times (cf. Hoppe 1978).

The third parameter, (K + S) is not as straightforward to interprete. If neither of the constituents in the sum is determined separately, the sum may be interpreted as an upper limit to either K or S. In substrate flux rate calculations, the sum value may be used as a rough estimate of the natural substrate concentration if the kinetic data indicates high affinity for the substrate (K<<S). In conjunction with the kinetic data, the value may sometimes be clearly interpreted as an indicator of the affinity of the population for the substrate, or more exactly a weighted mean of individual affinities of the organisms in the population (Williams 1973).

In spite of its wide application in heterotrophic activity studies, the kinetic method has been seriously critizised. The criticism has been focused on the application of the method in ecological studies (Vaccaro and Jannach 1967, Williams 1973, Krambeck 1979) and on the labour involved in the assay. The question: "is the kinetic assay worth the trouble?" was raised since two of the kinetic parameters, the turnover time (Williams and Askew 1968, Azam and Holm-Hansen 1973, Wright 1974, Gocke 1977) and the heterotrophic potential (Kadota et al. 1966, Griffiths et al. 1977, Wright 1978), could be approximated with the single concentration assays with considerably less work.

The purpose of this study was to compare the parameter values derived from single concentration assays with those from the kinetic determinations and to present a technique for determination of the correct concentration levels of substrate for single concentration assays. The kinetic parameters were chosen for the basis of comparison although the substrate uptake was not in all cases in accordance with Michaelis-Menten kinetics. The validity of the application of the single concentration and kinetic assays in different water bodies is discussed.

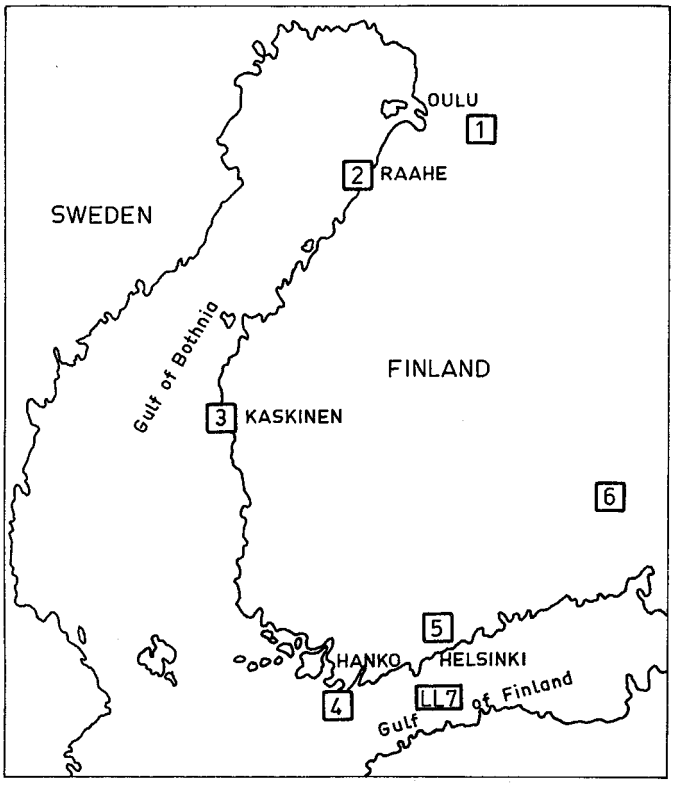
2. MATERIALS AND METHODS

The data for this study was collected from a number of kinetic studies in different brackish and fresh water environments (Fig. 1). The basic methodology for both the single concentration and kinetic assays is presented in Kuparinen et al. (1984). The concentration levels used in the assays ranged from 0.005 to 120 μg glucose l^{-1}. All results presented here are net values of glucose uptake (respiration is not taken into account).

Calculations

The kinetic parameters (T, V and K + S) were calculated over the range of substrate additions according to the Lineweaver-Burk transformation (Wright and Hobbie 1965). From each of the added glucose concentrations involved in the kinetic assay, a value for the turnover rate ($1/T_s$), and glucose uptake velocity (V_s), was calculated. In other words, each substrate addition in the kinetic assay was considered as a single concentration assay (Griffiths et al. 1977). In this paper, the kinetic parameters obtained with the single concentration calculations are indicated with the index "s". For the correlation analysis, $1/T_s$ was obtained from the lowest and V_s from the highest glucose additions. From the data in which glucose concentrations varied from tracer to saturation concentration, an approximation to the sum parameter K + S was calculated. The calculation was performed according to Wright and Hobbie (1965):

$$(K + S) = T \cdot V \qquad (1)$$



1.	27° 35′ N	64° 16′ E
2.	24° 20′ N	64° 40′ E
3.	21° 10′ N	62° 20′ E
4.	23° 10′ N	59° 50′ E
5.	25° N	60° 25′ E
6.	28° 10′ N	61° 15′ E
LL7	24° 50′ N	59° 51′ E

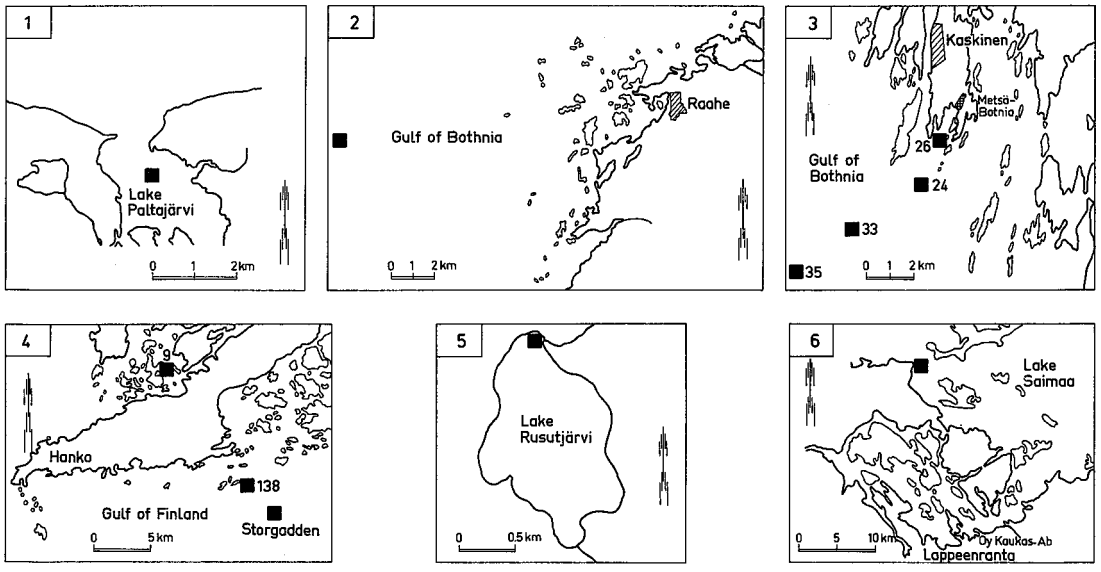


Fig. 1. Location of the sampling sites.

As described above, T (or 1/T) and V were approximated from single concentration assays, thus

$$(K + S)_s = T_s \cdot V_s \quad (2)$$

3. RESULTS

Results from the correlation analysis are presented in Figs. 2 and 3. The analysis showed good agreement between the kinetic and the single concentration parameters. The best quantitative relationship was obtained with the turnover rate (1/T), which was slightly underestimated by the single concentration assay when rates were high and overestimated when rates were low. The heterotrophic potential (V) was clearly underestimated with the single concentration assay. Although the underestimations in $1/T_s$ and V_s values accumulate in the concentration sum $(K + S)_s$, the correlation between $(K + S)$ and $(K + S)_s$ was high ($r = 0.962$, see Fig. 3). This suggests that it is possible to obtain a realistic value for $(K + S)$ from the two single concentration assays, by using the regression equation in Fig. 3.

The yields of the single concentration parameters as percentages of the kinetic yields are summarized in Table 1 and in Fig. 4. In all the study sites presented in Table 1, about 90 % of the kinetic 1/T value was obtained with the single concentration assay at glucose concentrations lower than 0.2 $\mu g\ l^{-1}$. Over 80 % of the kinetic V value was obtained with the single concentration assay at above 7 $\mu g\ l^{-1}$ glucose concentration in the open sea and above 20 $\mu g\ l^{-1}$ concentration in the coast of the Bothnian Sea. In the case of Tvärminne (Table 1c) it was not possible to point out the concentration level at which the uptake approaches maximum velocity because the difference between the two highest concentrations was too large, almost an order of magnitude. The

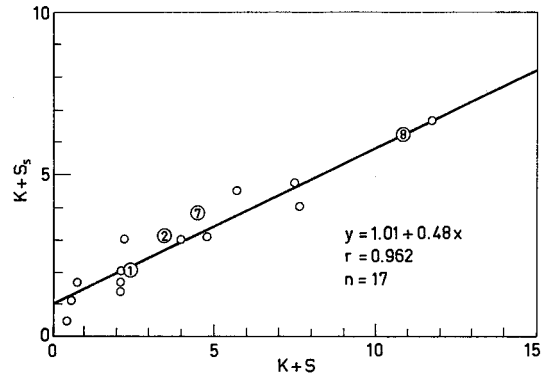
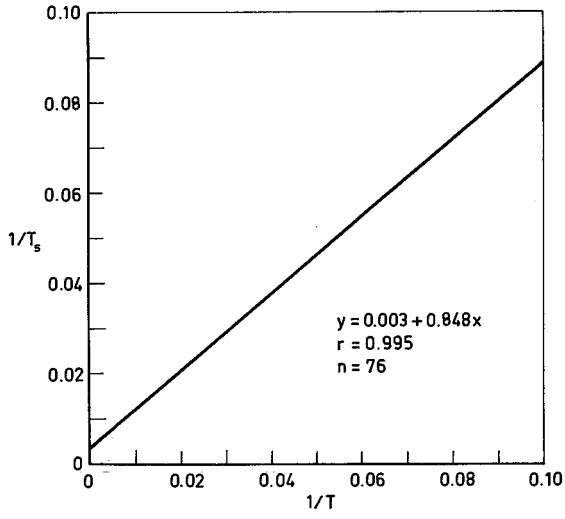


Fig. 3. Relation between the kinetic and the single concentration sum concentration (K+S vs. $K+S_S$). The numbered circles refer to the data in Table 2.



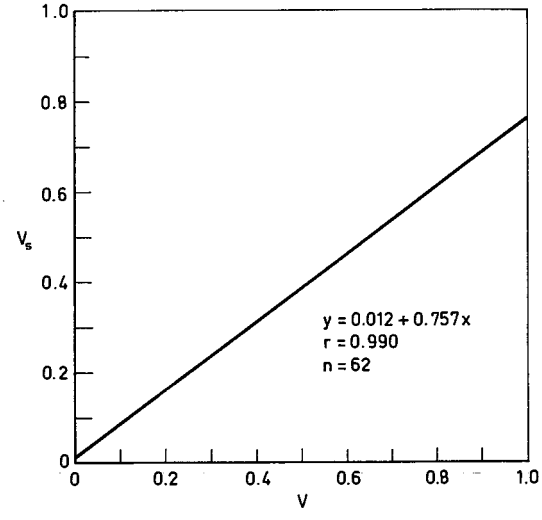


Fig. 2. Relation between the kinetic and the single concentration turnover rate (1/T vs. $1/T_S$) and the heterotrophic potential (V vs. V_S).

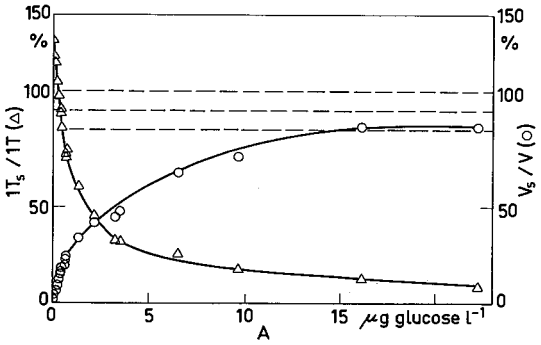


Fig. 4. Distribution of the single concentration values as percentages of the kinetic parameter value. The kinetic value is marked as 100 %.

extremely high percentage yield (159) in Table 1c is due to the fact that in kinetic calculations the data from the highest concentration level (40—47 μg l^{-1}) were not included because they caused marked nonlinearity in the regression line. If these data points had been included in the kinetic assay, the V values would have increased and the pecentage yield would have been 96.

The results from an assay with a wide range of glucose concentrations (data from Kaskinen in 1978) are illustrated in Fig. 4. The kinetic parameters, 1/T and V, were calculated over the whole range of glucose concentrations (marked as 100 %). The single concentration parameters, $1/T_s$ and V_s, were plotted as percentages of the kinetic value. The single concentration assay gave markedly higher values for the turnover rate at concentrations below 0.3 μg glucose l^{-1} and about similar values for heterotrophic potential at concentrations above 16 μg glucose l^{-1}. The extent of the

Table 1. The values derived from the single concentration methods as percentages of the values of the kinetic method. "s" refers to the single concentration method. Data in "a" and "b" is from Kaskinen 1978, in "c" from Tvärminne 1980, and in "d" from the Gulf of Finland (open sea) 1979, n = number of samples. In the data "c" the kinetic parameters were calculated from the concentration range 0.1—5.8 μg glucose l^{-1} (see text for explanation)

	Glucose μg l^{-1}	label	$(1/T_s : 1/T) \cdot 100$	n	$(V_s : V) \cdot 100$	n
a)	.039	^3H	96	39	4	36
	.12	"	93	41	9	38
	.23	"	85	36	17	33
	.39	"	78	32	24	29
	.58	"	70	8	33	8
	.95	"	58	8	42	8
	1.50	"	45	8	55	8
b)	4.5	^{14}C	56	46	52	47
	12.0	"	29	46	68	35
	19.9	"	21	46	81	47
	27.5	"	16	46	87	47
	35.1	"	14	27	87	27
c)	0.10—.20	^{14}C	91	17	16	16
	0.48	"	77	19	27	17
	1.9	"	42	19	56	17
	3.8 —5.8	"	24	17	79	15
	40.0 —47.0	"	7	17	159	15
d)	0.09	^{14}C	98	18	8	18
	1.75	"	43	17	59	17
	3.50	"	28	18	71	17
	7.00	"	16	18	82	18
	8.75	"	14	18	88	18

difference between the single concentration and the kinetic 1/T value is due to the concentration range used in the kinetic calculation, as illustrated in Fig. 5. If lower concentrations had been used, the turnover times would have become shorter (as shown in Fig. 5), providing a better fit with the single concentration and kinetic 1/T value at the lower end of the concentration range. The effect of the concentration range used in the kinetic assay on the parameter values will be discussed further.

The results from kinetic studies, in which a wider range of glucose concentrations was used, are summarized in Table 2. An attempt to reveal the correct concentration levels for the single concentration assays was made by taking the highest substrate concentration (A_T) which gave 90 % or more of the kinetic 1/T value and the lowest concentration (A_V) which gave 90 % or more of the kinetic V value for the different water bodies. In all

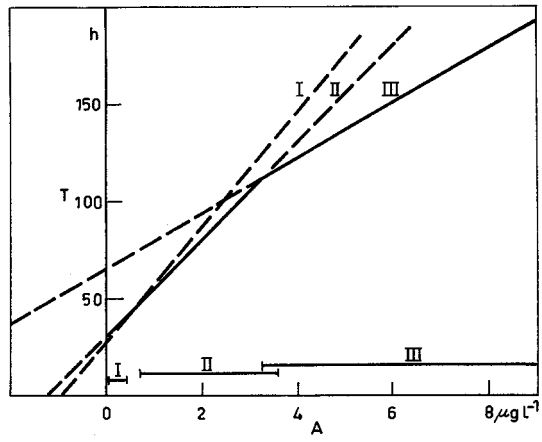


Fig. 5. Linear regressions calculated from the different concentration ranges (Tamminen 1980).

Table 2. Different kinetic studies in which a wide range of substrate concentrations has been used. A_T = the highest and A_V = the lowest concentration of glucose, in which about 90 % or more of the kinetic value was obtained by the single concentration assay. K + S = concentration sum (µg glucose l^{-1}) from the kinetic assay, K + S_s = concentration sum calculated from $1/T_s$ and V_s, n = number of subsamples involved in the kinetic assay.

Data source	Glucose µg l^{-1}	label	n	$(1/T_s : 1/T) \cdot 100$	A_T	K + S	$\frac{A_T}{K+S}$	$(V_s : V) \cdot 100$	A_V	$\frac{A}{K+S}$	K + S_s
1. Kaskinen -78 Station 33	0.005—22.3	^3H	78	92	0.44	2.4	0.18	92	16.0	6.6	2.1
2. Kaskinen -80 Station 35	0.095—51.6	^{14}C	30	104	0.19	3.5	0.06	103	17.2	5.0	3.0
3. Kaskinen -80 Station 26	0.095—51.6	^{14}C	30	91	1.40	23.0	0.06				
4. Bengtsår Station 9	0.003— .94	^3H	41	91	0.31	4.7	0.07				
5. Bengtsår Station 9	0.003—1.57	^3H	45	102	0.31	4.2	0.08				
6. Tvärminne -79 Station 138	0.003—2.51	^3H	45	91	0.07	1.3	0.06				
7. Tvärminne -80 Storgadden	0.048—45.7	^{14}C	41	92	0.95	4.5	0.21	87	27.9	6.3	3.3
8. Kajaani -81	26 — —105					96.1					
9. Lake Saimaa -78	5 —120	^{14}C	18			10.9		105	70.0	6.4	6.2
10. Lake Rusutjärvi	1.7 —120	^{14}C	16	100	8.30	79.4	0.11				
							$\bar{x} = 0.10$			$\bar{x} = 6.1$	

of the study sites included in Table 2, the 1/T value could be determined at glucose concentrations below 0.07 μg l^{-1}. In only four study sites the saturation level was reached with the concentration range used in the assays, lake Saimaa showing the highest concentration (70 μg glucose l^{-1}). The lake Kajaani and lake Rusutjärvi sites, which are both highly eutrophic, showed extremely low affinities for glucose and therefore concentrations close to 100 μg glucose l^{-1} were not sufficient for V_s determination. In the unpolluted brackish water sites a concentration of 30 μg glucose l^{-1} seemed to be high enough for V_s determination.

The ratios $A_T/(K + S)$ and $A_V/(K + S)$ indicated that the correct concentration levels could be calculated from the kinetic K + S value. Thus about an order of magnitude lower than the (K + S) concentration is enough for the $1/T_s$ and an order of magnitude higher than (K + S) for the V_s determinations. This, however, necessitates that the (K + S) value has been determined over a wide range of glucose concentrations, starting from a fraction of a microgram and ending in tens of micrograms per liter, for example in a series of 0.05, 0.1, 0.5, 1.0, 5.0, 10 and 50 μg l^{-1} (see discussion). For lakes Kajaani and Rusutjärvi the calculated concentration levels for V_s determinations were 960 and 790 μg glucose l^{-1}, respectively. These concentrations are, however, higher than the 500 μg l^{-1} above which Wright and Hobbie (1965) reported substrate diffusion into algae. Therefore these concentrations should not be used in the assay without studying the possibility of diffusion.

4. DISCUSSION

Results from the different study sites support the findings of Gocke (1977) and Griffiths et al. (1977) concerning the applicability of single concentration assays for the substrate turnover rate and heterotrophic potential determinations, provided that the correct concentration levels are used in the assays. The good correlations between the kinetic and single concentration parameters suggest that the single concentration assay could be used successfully to describe seasonal heterotrophic activities in different study sites. The quantitative difference between the two measurements reveals the tendency that the single concentration parameters give lower values than the kinetic when activities are high and slightly higher values when activities are low. Thus the single concentration values may be considered as "conservative" and they may safely be used in substrate flux rate calculations on an annual basis to provide lower limits to the flux rates. Turnover rate is thus used as a rate value and $(K + S)_s$ as an estimate for S if no other information about the natural substrate concentration is available (see e.g. Kuparinen et al. 1984).

Gocke (1977) found good agreement between the turnover rates calculated by the two methods in eutrophic waters. In oligotrophic waters a discrepancy was observed, although this was usually within the range of 25 %. As was suggested by Williams (1973), the great variation in individual transport constants (K) may cause nonlinearity in the Lineweaver-Burk plot in oligotrophic waters with mixed populations. In eutrophic waters, which usually show less diversity or dominance by one or only a few species with similar K-values, the departure from linearity is minor (Vaccaro and Jannasch 1967, Vaccaro 1969).

The results from Kaskinen (Table 1a and b and Fig. 5) are in agreement with the theoretical considerations of Williams (1973) concerning the difference between the single concentration and kinetic parameters and also with the findings of Gocke (1977). The decrease in percentage values of $1/T_s$ (Table 1a and b) shows a discontinuity over the 4,50 μg l^{-1} glucose concentration, due to the bending of the Lineweaver-Burk plot towards the x-axis at low substrate concentrations. The phenomenon is illustrated in Fig. 5. When the regression line (Fig. 5 line I) was determined from the values at the lower end of the concentration range, the slope was higher than if values at the higher end were used (Fig. 5 line III). Usually, the majority of the data points for the Lineweaver-Burk plot were obtained at high substrate concentrations, which means that the slope remained almost unaltered even though deviations from linearity occurred at low substrate concentrations (cf. Gocke 1977). This resulted in underestimation of the turnover rate by the kinetic assay, the percentage underestimation depending on the concentration range used in the assay. The error within the range of 25 % found by Gocke (1977) is acceptable in most ecological studies if all other error sources are considered, but it has significance in budget calculations based on fluxes of individual substrates.

A striking example of the effect of the concentration range chosen for the kinetic assay is seen in Table 1c. The high percentage yield (159) for the single concentration heterotrophic potential is due to the exclusion from the data of the highest substrate concentration in the kinetic assay. If all data points had been included in the

kinetic assay, there would have been a good agreement between the single concentration and the kinetic heterotrophic potential values but a marked overestimation of the turnover rate by the single concentration assay.

The transformation used in the kinetic assay for linearizing the data seemed also to have an effect on the quantitative relationship between the kinetic and the single concentration heterotrophic potentials. The single concentration potential activity value (V_s) did not seem to reach the 90 % level except in a few cases (Tables 1 and 2 and Fig. 4). This was possibly due to the application of the Lineweaver-Burk plot instead of alternative suggested linear transformations (cf. Tamminen 1984). As shown by Tamminen (1984), another linear transformation (1/T vs. A/T) would have resulted in full agreement between the kinetic and the single concentration values. Thus the potential activity value derived from the single concentration assay may be a more realistic representative of the heterotrophic potential of the water body than that derived from the most commonly used Lineweaver-Burk transformation of the kinetic assay. This, however, does not apply to highly eutrophic and polluted waters in which the concentration level for V_s determination exceeds 500 $\mu g\ l^{-1}$, such as lakes Kajaani and Rusutjärvi in this study. In such cases the kinetic assay seems to be a better approach for the determination of heterotrophic potential.

The results of the attempt to calculate correct concentration levels for the single concentration assays seemed promising. However, only one experiment was carried out at each study site and therefore it is not possible to say how representative the values are on a seasonal basis. It is thus recommended to check the K + S level at least twice a year, immediately after the phytoplankton spring maximum when the bacterial peak occurs and also in winter, and preferably more often, for example during mid- and late summer when the cyanobacterial bloom occurs.

The effect of the concentration range used in the kinetic assay on the parameter values (Williams 1973), the lesser applicability of the kinetic assay in oligotrophic conditions (Vaccaro and Jannach 1967, Williams 1973, Gocke 1977) and the differences in the parameter values due to the transformation applied (Tamminen 1984) seem to make the kinetic assay a tool with only limited value in ecological studies. However, the kinetic assay can be used successfully in highly eutrophic and polluted waters for heterotrophic potential determinations and for special applications, for example in order to study the affinities of groups of organisms or species for labelled substrates (Tamminen et al. 1984).

5. CONCLUSIONS

Kinetic parameters can be determined from single concentration assays with considerable accuracy, provided that the concentration levels in the single concentration assays are correct. The approximate concentrations for glucose additions can be calculated from the kinetic concentration sum, (K + S) by taking 10 % of the (K + S) value for the turnover rate measurement and multiplying the value by 10 for the heterotrophic potential measurement. However, the kinetic K + S must be determined over a wide range of substrate additions.

With the exception of extremely oligotrophic conditions, 0.1 μg glucose l^{-1} seems low enough for $1/T_s$ and 30 μg glucose l^{-1} high enough for V_s determinations. In eutrophic waters, glucose concentrations of about 100 $\mu g\ l^{-1}$ must be used for V_s determinations. In highly eutrophic and polluted waters the kinetic assay is a better approach for the determination of the maximum uptake velocity (V) because the required concentration level for V_s determination may exceed the value above which substrate diffusion into algae begins.

The amount of work saved by using the single concentration assays makes them useful in various ecological studies in which the knowledge of the distribution and fluctuation of the heterotrophic activity is essential. The single concentration assay should always be used for the turnover rate determination in oligotrophic waters and in calculating substrate flux rates in conjunction with the knowledge of the natural substrate concentration.

6. LOPPUTIIVISTELMÄ

Bakteeriplanktonin aktiivisuutta kuvaavia kineettisiä parametrejä mitattiin erilaisissa vesistöissä nk. yhden substraattilisäyksen menetelmällä ja tuloksia verrattiin perinteisen Michaelis-Menten -kinetiikan tuottamiin parametreihin. Tulokset osoittivat, että kineettiset parametrit voidaan määrittää riittävän tarkasti yhden lisäyksen menetelmillä edellyttäen,

että niissä käytetään oikeita lisäyspitoisuuksia. Oikeat lisäyspitoisuudet voidaan karkeasti laskea kineettisestä pitoisuussummasta (K + S), ottamalla 10 % luvusta kiertonopeuden määritykseen ja kertomalla luku kymmenellä, jolloin saadaan heterotrofisen potentiaalin määritykseen sopiva pitoisuusarvo. Em. menettelyn ehtona on kuitenkin se, että kineettinen pitoisuussumma on määritetty laajaa lisäysaluetta hyväksikäyttäen.

Erittäin oligotrofisia olosuhteita lukuunottamatta 0.1 μg glukoosia litrassa näyttää tarpeeksi alhaiselta pitoisuudelta yhden lisäyksen kiertonopeuden määritykseen ja 30 μg litrassa riittävän korkealta yhden lisäyksen heterotrofisen potentiaalin määritykseen. Eutrofisissa vesissä tarvitaan n. 100 μg l^{-1} glukoosipitoisuus yhden lisäyksen heterotrofisen potentiaalin määritykseen. Erittäin eutrofisissa ja likaantuneissa vesissä kineettinen menetelmä soveltuu parhaiten heterotrofisen potentiaalin määritykseen, sillä yhden lisäyksen menetelmän vaatima lisäyspitoisuus saattaa ylittää pitoisuusrajan jossa glukoosia alkaa diffundoitua leväsoluihin.

Yhden lisäyksen menetelmien helppous ja nopeus tekee niistä käyttökelpoisia erilaisissa ekologisissa tutkimuksissa, joissa tarvitaan tietoa heterotrofisen aktiivisuuden ajallisista ja paikallisista vaihteluista. Yhden lisäyksen menetelmää pitäisi aina käyttää kiertonopeuden määritykseen oligotrofisissa oloissa ja silloin kun lasketaan substraatin ottonopeuksia luonnon substraattipitoisuutta hyväksikäyttäen.

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