The Use of the Walkley-Black Method for Organic Carbon Determination as a Procedure for Estimating Algal Yields

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Abstract

The Walkley-Black method for the determination of organic carbon was successfully adapted for estimating algal biomass in cultures containing sediment. Comparison of this method with the estimation of biomass by measurement of suspended solids indicated that a similar level of precision is obtainable. The Walkley-Black method gave equally good estimations of biomass in the presence or absence of sediment in algal cultures. An advantage of the Walkley-Black method is that inexpensive and relatively simple instrumentation is required and staff without advanced training may be used.

Introduction

Sediment may be a source of nutrients to algae (Porcella, Kumagai and Middlebrooks, 1970; Sagher, Harris and Armstrong, 1975; Cowen and Lee, 1976; Golterman, 1976; Grobler and Davies, 1979). It is, therefore, important to measure the availability of nutrients associated with sediments in aquatic systems (Lee, Rast and Jones, 1978). If bioassays with sediment as the only source of a nutrient are used to study the algal availability of nutrients, some measure of the algal yield in cultures containing sediment is needed. It was found that the accepted procedures for yield estimates namely biomass by suspended solids or measuring the absorbance at 600 nm (Toerien, Huang, Radimsky, Pearson and Scherfig, 1971) or by chlorophyll *a* determinations (Standard Methods, 1976; Grobler and Davies, 1978) could not be used when sediment was present in the cultures. Several attempts were made to overcome this problem. Fitzgerald (1970) added sediment in dialysis bags to cultures. The algae could, therefore, easily be separated from the sediment and the biomass estimated by any of the above methods. Golterman (1976), however, after investigating this procedure, concluded that the lack of physical contact between the sediment in the dialysis bags and the algae, resulted in the unavailability of the sediment phosphate to the algae.

Sagher, Harris and Armstrong (1975) measured adenosine triphosphate (ATP) levels, using a modification of the method of Lee, Harris, Williams, Syers and Armstrong (1971) to estimate algal biomass in the presence of sediment. Grobbelaar (1979) used organic carbon determinations carried out on a carbon analyser to estimate biomass in algal cultures containing sediment. Both these methods involve the use of expensive apparatus and skilled operators. Organic carbon determinations with a carbon analyser were initially tried out by us but problems were experienced with sample injection of cultures containing coarse sediments.

Therefore, this study was undertaken to evaluate the suitability of an adaption of the Walkley-Black method (Jackson, 1958) for the determination of organic carbon as a means of estimating algal yields in cultures containing sediment.

Materials and Methods

Analyses of Sediment

The sediment used in this study was analysed for cation exchange capacity (CEC) (Loveday, 1974) and organic carbon content (OC) (Jackson, 1958).

Adaption of the Walkley-Black Method

The Walkley-Black method was developed for the determination of organic carbon in soils. Its principles, uses, limitations and possible interferences are discussed in detail by Jackson (1958). In the original method the amount of organic carbon present is estimated by a determination of the amount of dichromate consumed by the organic carbon by back titration of the excess dichromate with a ferrous solution. Jackson (1958) also suggested the use of a colorimetric procedure whereby the amount of green chromium-III-ion produced in the oxidation reaction can be measured at 645 nm, as a direct measure of the amount of organic carbon present. The latter option was used in the adaption of the Walkley-Black method to estimate algal yields.

Reagents

Dichromate solution

Exactly 49,04 g of $K_2Cr_2O_7$ was dissolved in distilled water and made up to 1 dm^3.

Ferrous solution

$Fe(NH_4)_2(SO_4)_2.6H_2O$ (196,1 g) was dissolved in 800 cm^3 distilled water containing 20 cm^3 of concentrated H_2SO_4 and made up to 1 dm^3. This solution was standardised against the dichromate solution, according to the procedure given by Jackson (1958) before preparation of standards.

Method

The stoppers were removed from the 100 cm^3 conical flasks containing the algal cultures and the cultures were evaporated to dryness at 80 °C in a forced draft electrical oven. Dichromate solution (10 cm^3) was added followed by 20 cm^3 of concentrated sulphuric acid whilst gently swirling the contents. The mixture was left to cool for 30 min before making up to 50 cm^3 in a volumetric flask. Turbidity was caused by presence of sediment in the cultures. The mixtures, however, could be cleared by either leaving them to stand overnight or by centrifuging at 2 000 r/min for 20 min. The concentration of the chromium-III-ion was determined by measuring the intensity of the green colour, at a wavelength of 649 nm using either a spectrophotometer or a colorimeter, against a range of standards prepared with known concentrations of chromium-III-ion present. Organic carbon present in the sediment (if sediment is present in the cultures) or organic carbon present in the nutrient solution was compensated for by preparing blanks containing nutrient solution or nutrient solution and sediment and were treated as above. The organic carbon figure obtained for the blanks were then subtracted from those obtained for the cultures to give a net figure of organic carbon due to the algae in the culture. This also compensated for interferences caused by chloride which might have been present in the nutrient solutions or sediment. Standards were prepared by adding 0; 1; 2; 3; 4; 5; 7,5 and 10 cm^3 of dichromate solution and 20 cm^3 of concentrated sulphuric acid and made up to 50 cm^3. The ferrous ions are oxidized to ferric ions producing chromium-III-ions, the same as organic carbon would. The range of standards allowed organic carbon contents to be determined between 0 and about 300 $mg.dm^{-3}$. If more sensitivity was required a range of standards allowing for organic carbon concentrations between 0 and 100 $mg.dm^{-3}$ was used. The concentration of organic carbon present was calculated from the formulae given by Jackson (1958).

The concentrated sulphuric acid present in the final solution proved to be hazardous to the operator and instruments when the colour measurements had to be done manually on a spectrophotometer. A Technicon Autoanalyser II was used instead, where a sampler, pump, colorimeter and recorder were used for the colour measurement. The wash-out solution used was prepared by making up a solution of dichromate and concentrated sulphuric acid, in the proportions used for the organic carbon determinations. A flow diagram for the Autoanalyser system is given in Figure 1.

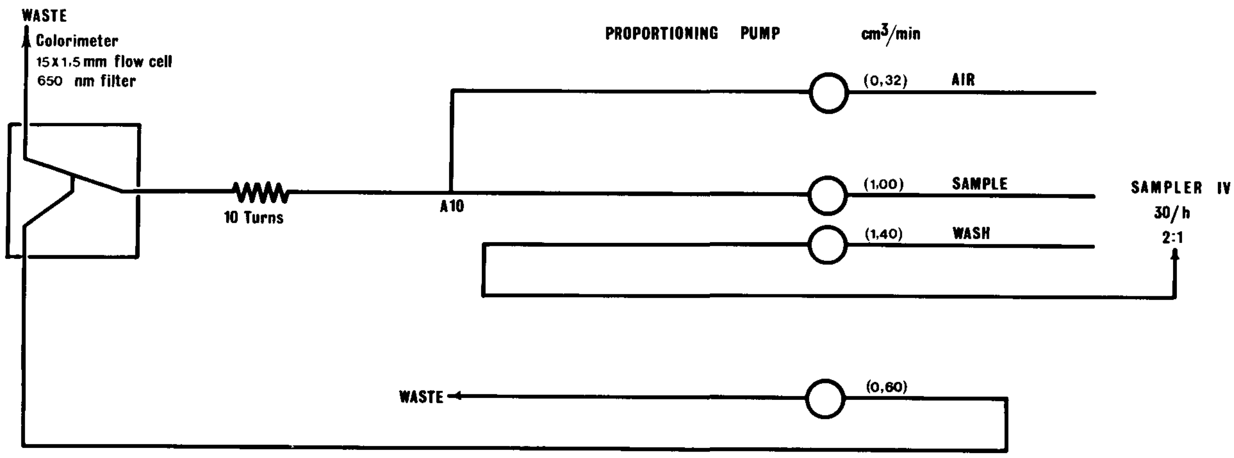

Figure 1
Organic carbon manifold

TABLE 1
SUSPENDED SOLIDS AND ORGANIC CARBON (OC) VALUES DETERMINED ON SAMPLES CONTAINING DIFFERENT CONCENTRATIONS OF *SELENASTRUM CAPRICORNUTUM* OR *MICROCYSTIS AERUGINOSA* CELLS. THE MEANS AND COEFFICIENTS OF VARIATION ARE GIVEN

	S. capricornutum		*M. aeruginosa*	
	mg.dm^{-3}			
	Suspended solids	OC	Suspended solids	OC
	50,80	24,30	66,60	23,09
	50,00	23,09	65,20	23,09
	50,60	24,90	65,20	23,09
	50,00	23,70	64,80	22,48
	51,40	24,30	66,00	22,48
Mean	50,56	24,06	65,56	22,85
CV	1,2%	2,9%	1,1%	1,4%
	98,60	47,39	133,00	42,53
	98,20	48,00	128,80	43,75
	98,80	47,79	132,40	43,75
	99,60	47,39	132,60	43,75
	98,20	48,00	133,80	45,57
Mean	98,68	47,51	132,12	43,87
CV	0,6%	1,1%	1,5%	2,5%
	148,20	71,09	199,20	65,62
	149,20	71,09	198,40	65,62
	149,40	70,79	195,20	65,62
	150,20	71,39	196,80	65,01
	151,00	70,57	196,40	65,62
Mean	149,60	70,97	197,20	65,50
CV	0,7%	0,5%	0,8%	0,4%
	199,40	94,18	235,60	82,63
	202,40	91,75	263,80	84,46
	202,40	93,57	268,80	85,67
	201,40	93,57	262,80	87,49
	203,20	93,57	253,00	86,28
Mean	201,76	93,33	260,40	85,31
CV	0,7%	1,0%	2,6%	2,2%

Algal Cultures

Organic carbon determination, as an estimate of algal biomass, was evaluated by comparing it with the estimation of biomass by the measurement of the suspended solids concentration (Toerien, Huang, Radimsky, Pearson and Scherfig, 1971). Two algal species, *Selenastrum capricornutum,* cultures in BG-11 nutrient medium (Stanier, Kunisawa, Mandel and Cohen-Bazire, 1971), and *Microcystis aeruginosa,* cultured in the nutrient solution as suggested by Volk and Phinney (1968), were used.

Preparation of Samples

Samples covering a range of concentrations of each of the algal species were prepared by dilution of 10, 20, 30 and 40 cm^3 of the original cell suspensions to 50 cm^3. Ten samples of each concentration were prepared, replicated 10 times, 5 of which were used for organic carbon determinations and the rest for suspended solids determinations.

The influence of the presence of sediment in the samples of the determination of organic carbon was evaluated by pre-

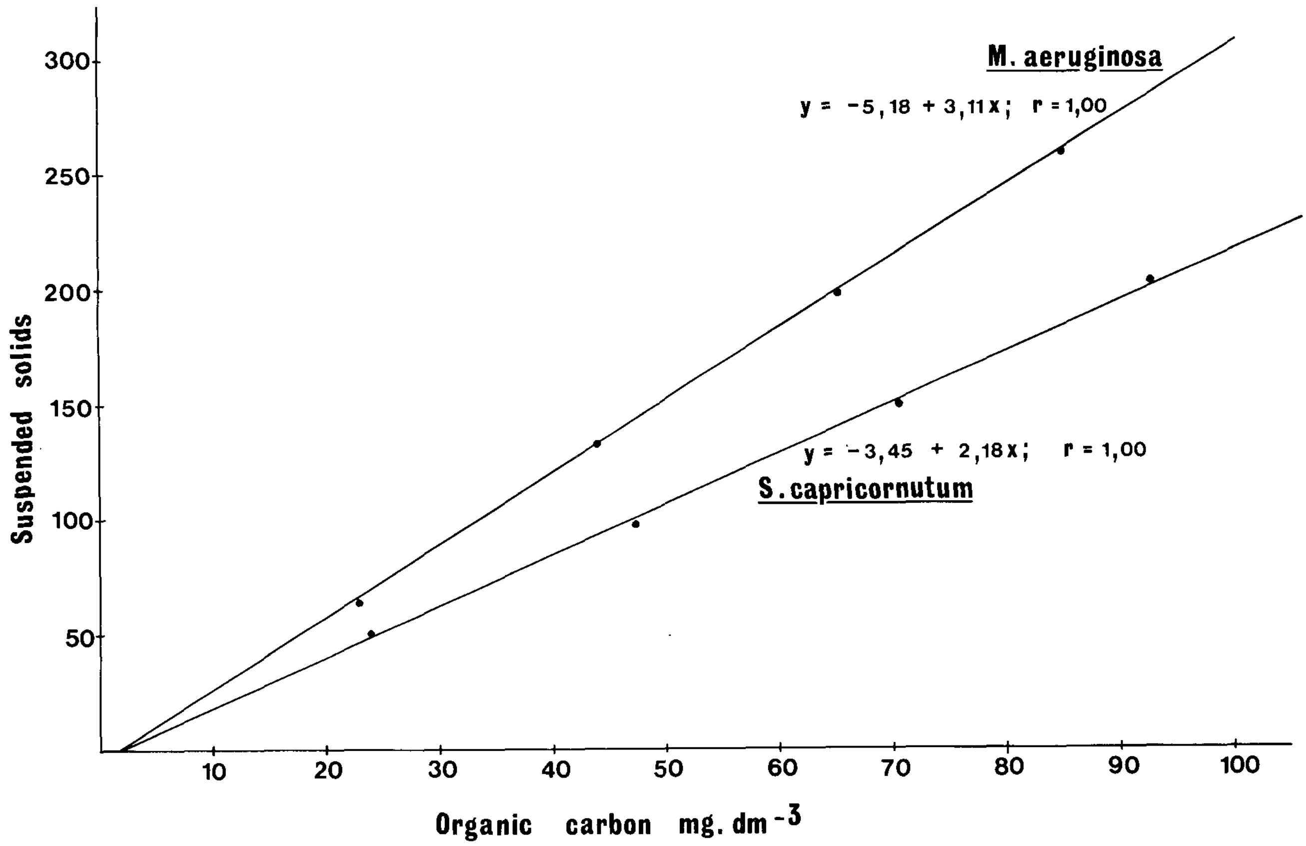

Figure 2
The relationship between mean suspended solids and mean organic carbon values for ***Selenastrum capricornutum*** *and* ***Microcystis aeruginosa***

paring a second range of samples with different algal concentrations using a culture of *S. capricornutum*. Ten replicates of each concentration were made. Five contained sediment at a concentration of 0,2% by mass, and the rest contained no sediment. Organic carbon analyses were done on all samples. A blank was prepared by adding the same mass of sediment to BG-11 nutrient medium containing no algal cells.

Statistical Analyses

Means and coefficients of variation (CV) (Snedecor and Cochran, 1956) were calculated for each group of replicates analysed by the different methods. Linear regression equations, between the different methods of estimating biomass, were calculated with the aid of the computer program P/REPOL available at the Biometrics section of the Department of Agricultural Technical Services. The correlation coefficients (r) were calculated and used as estimates of the significance of the regression equations using the table given by Snedecor and Cochran (1956). Confidence intervals about the regression coefficients were also calculated (Steel and Torrie, 1960). Means were compared for significant differences using the t-test (Snedecor and Cochran, 1956).

Results

Relationship Between Suspended Solids and Organic Carbon

The individual values obtained for each replicate by both the organic carbon and suspended solids methods are given in Table 1.

The coefficients of variation for suspended solids varied between 2,6% and 0,6% for means between 260 and 50 $mg.dm^{-3}$.

The relationship between the suspended solids determination and the organic carbon determinations were obtained by calculating the regression equation for each algal species using the means given in Table 1. The regression lines and the correlation coefficients (r) are given in Figure 2. For both algal species r = 1,00, and were therefore significant at the 1% test level.

TABLE 2
REGRESSION COEFFICIENTS AND THEIR 95% CONFIDENCE INTERVALS FOR THE REGRESSION EQUATIONS REPRESENTING THE RELATIONSHIP BETWEEN SUSPENDED SOLIDS AND ORGANIC CARBON DETERMINATIONS FOR BOTH ALGAL SPECIES

Algal Species	Intercept	Slope
Selenastrum capricornutum	−3,45 ± 12,17	2,18 ± 0,19
Microcystis aeruginosa	−5,18 ± 6,19	3,11 ± 0,10

TABLE 3
ORGANIC CARBON VALUES DETERMINED ON 5 REPLICATES OF SAMPLES CONTAINING THE SAME CONCENTRATIONS OF *SELENASTRUM CAPRICORNUTUM* CELLS BUT WITH AND WITHOUT SEDIMENT PRESENT IN THE SAMPLES. MEANS, COEFFICIENTS OF VARIATION (CV) AND t-VALUES ARE GIVEN

	Organic carbon in $mg.dm^{-3}$		t-value
	Sediment present	No sediment	
	15,1	15,1	
	14,5	14,5	
	15,4	14,5	
	14,5	14,5	
	15,4	14,2	
Mean	15,0	14,6	1,67
CV	3,03%	2,25%	
	28,4	26,1	
	27,8	28,7	
	25,4	24,4	
	27,8	28,9	
	26,7	25,5	
Mean	27,2	26,7	0,48
CV	4,37%	7,47%	
	40,3	40,0	
	41,8	39,4	
	40,3	38,3	
	41,2	41,3	
	39,2	39,4	
Mean	40,6	39,68	1,33
CV	2,44%	2,76%	
	57,4	56,3	
	55,7	54,4	
	57,4	59,2	
	56,4	56,8	
	58,4	55,1	
Mean	57,1	56,4	0,74
CV	1,82%	3,28%	

In Table 2 the regression coefficients and their 95% confidence intervals are given. In both cases the 95% confidence interval for the intercepts includes the origin. The 95% confidence interval about the slopes amounted to ± 9% of the value of the slope itself for *S. capricornutum* and to ± 3% for *M. aeruginosa.*

Organic Carbon Determinations in the Presence of Sediment

The sediment sample used in this study had a cation exchange capacity of 44 $mmol.100\ g^{-1}$ and an organic carbon content of 1,3%. These values indicated a highly chemically active sediment which, if present in a sample, would be likely to cause interferences with the estimation of algal biomass by organic carbon determinations. The organic carbon content of the sediment was subtracted from that of the cultures to measure the organic carbon content of the algae only. The organic carbon values obtained for samples in the presence or absence of sediment but containing the same concentrations of *S. capricornutum,* are given in Table 3. The means, coefficients of variation and t-values, used for testing for significant differences between means, are also given. The t-values varied between 0,48 and 1,67. None of these values indicated significant differences between means. The coefficients of variation ranged between 1,82% and 7,47%.

Discussion

Good precision was obtained by both the suspended solids and the organic carbon methods of estimating algal yields for both algal species, as indicated by the low coefficients of variation (Table 1). The coefficients of variation values of between 2,6% and 0,6% compare favourably with the coefficients of variation of 3,1% for a mean of 74 $mg.dm^{-3}$, for suspended solids determinations given by Toerien *et al.* (1971). The coefficients of variation for organic carbon determinations were between 0,4% and 2,9% for organic carbon means ranging from 23 to 93 $mg.dm^{-3}$. This value also compared favourably with coefficients of variation of 5% to 18% found by Porcella, Grau, Huang, Radimsky, Toerien and Pearson (1970) for suspended carbon measurements between 20 and >30 $mg.dm^{-3}$.

A good linear relationship (r = 1,00, Table 2) was found for both algal species between suspended solids and organic carbon determinations. It was also shown (Table 2) that the 95% confidence intervals for the intercepts of the regression equations included the origins; therefore, both intercepts did not significantly deviate from the origin. The 95% confidence interval about the slopes of the regression equations were ± 9% and ± 3% (Table 2) which were low values considering the limited number of values used in calculating the regression equations.

No significant differences were found between mean organic carbon contents of samples containing the same concentration of algae in the presence and absence of sediment.

The coefficients of variation (Table 3) were within an acceptable range for both cases where sediment was present and in the absence of sediment so that the lack of differences between means could not be ascribed to extraordinary variation between the replicates of one of the cases. It is therefore concluded that the presence of sediment in the cultures was unlikely to have a significant influence on the estimation of biomass by organic carbon determinations.

The Walkley-Black method offers a number of advantages over methods involving the measurement of organic carbon by means of a carbon analyser or the measurement of ATP.

Most laboratories have either a spectrophotometer or colorimeter available and no additional expensive instrumentation is needed. Less experienced personnel can use the Walkley-Black method and if an autoanalyser is available, colour measurement can be automated to overcome problems experienced when using samples containing concentrated sulphuric acid. Estimation of biomass by organic carbon determinations, using the Walkley-Black method proved to be comparable in precision to the measurement of suspended solids. The ratio of suspended solids to organic carbon for *S. capricornutum* of 2,18 ± 0,19 found in this study is in close agreement with the ratio of 2,04 to 2,17 found by Goldman (1972) for the same organism. The ratio of 3,11 ± 0,10 found for *M. aeruginosa* converted to 32 ± 1% carbon, falls in a range of between 29% and 50% carbon on a dry mass basis, reported for different algae by Goldman, Porcella, Middlebrooks and Toerien (1972).

Conclusions

1. The estimation of algal biomass by an adaption of the Walkley-Black method for organic carbon determinations gave results similar in precision to the measurement of suspended solids.

2. The Walkley-Black method can be used as an estimate of biomass on cultures containing sediment.

3. The Walkley-Black method requires less expensive and complicated instrumentation than either ATP measurements or organic carbon determinations with a carbon analyser. Requirements on the level of training of the operator are also much lower when applying the Walkley-Black method.

Acknowledgement

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