CLXXXIII. ON THE ORGANIC MATTER GIVEN OFF BY ALGAE.

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ACCORDING to the hypothesis first proposed and maintained by Pütter [1909], aquatic animals of all groups up to and including fishes live largely on the organic substances found dissolved in natural waters. These substances are taken to be mainly derived from the photosynthetic processes of the plankton algae, it being assumed that the products of these processes become fixed in the bodies of the algae only to a slight extent, and are mainly given off to the surrounding water.

One of us [Krogh, 1930, 1] has described methods for a fairly accurate determination by combustion of organic substances dissolved in fresh water. These methods utilise the fact that organic substances belonging to the classes of proteins, fats and carbohydrates (as well as several others), can be oxidised by combustion in nitrogen by means of cupric oxide, liberating CO_2 and N_2 as gases. If a measured volume of oxygen be added, while the mixture is still at a red heat, the reduced copper is again oxidised and it is possible by gas analysis to determine the carbon dioxide and nitrogen liberated and the residue of oxygen, left over after the oxidation. The amount of substance suitable for such a determination in its micro-form is 3–15 mg., and the volume of water supposed to contain a quantity between these limits is concentrated to a small bulk; certain ions (Mg and Ca) which interfere with the combustion are removed and the solution is finally evaporated to dryness in the combustion tube. For the technical details the reader is referred to the paper quoted above.

In a separate paper [Krogh, 1930, 2] experiments on the absorption of dissolved organic substances by animals are described and here we shall deal with the alleged liberation of such substances by algae. Our experiments fall into two groups. In one series we have worked on pure cultures of *Scenedesmus obliquus* in artificial media, while in a second we have taken natural water from a lake and brought the algae to a luxuriant growth.

Scenedesmus experiments. We are greatly indebted to Prof. Chodat of Geneva, who supplied us with pure cultures of the algae *Scenedesmus obliquus*, *Stichococcus bacillaris*, *Chlorella vulgaris*, and *Chlorella rubescens*, and gave us

many useful hints regarding their cultivation. After a number of trials we selected *Scenedesmus* as the form showing the best rate of growth under the conditions imposed by the limitations of our combustion analysis. We could not use nitrate as a source of nitrogen, because it would give rise to error in the combustion, and had therefore to experiment with urea and ammonia. Urea can be retained in the final evaporation only if the temperature is kept below 70° , while ammonia can be driven off entirely at p_H 9.5. In our earlier experiments we did not drive off the ammonia but determined it in the acid concentrate before the final evaporation.

Our final substrate was made up by diluting a mixture of 1 cc. of M CaCl_2 , 0.15 cc. of M MgCl_2 , 0.3 cc. of M KH_2PO_4 , 0.05 cc. of M Na_2HPO_4 , and 0.01 cc. of M Fe_2Cl_6 to 1 litre with water re-distilled from hard glass and was distributed in suitable flasks with 250 cc. in each and autoclaved for 1 hour at 125° . After the autoclaving the solution was sometimes a little cloudy but could be cleared with a few drops of M HCl . The reaction was brought to the optimum of p_H 8.4 by the addition of sterile M NaOH , about 0.1 cc. being required for each bottle. A molar solution of $(\text{NH}_4)_2\text{CO}_3$ was sterilised by filtration and a suitable amount, usually 0.15 cc., was added to each flask. To insure a rapid growth the bottles were usually inoculated with 1–2 cc. of an older culture.

The culture flasks plugged with cotton wool were placed in a white box, exposed to strong artificial light (two 75 c.p. reflector lamps at a distance of about 30 cm. above the flasks) and incubated at a temperature of about 25° for 18–45 days. To insure a liberal supply of CO_2 a slow current of the gas was constantly flowing into the box through a wash-bottle. At the end of the experiment the algae were filtered off by a membrane filter as described by Krogh [1930, 1] and the filtrate, usually from 2 flasks (500 cc.), was taken for analysis. The algae were quantitatively removed from the filter, dried, weighed, and in some cases analysed.

To the water, concentrated to about 20 cc., we added excess of ammonia and sufficient phosphoric acid to form $\text{Ca}_3(\text{PO}_4)_2$ and MgNH_4PO_4 . After 24 hours the precipitate was filtered off and the excess of ammonia and CO_2 were driven off in a vacuum, as described by Krogh, before the fluid was finally evaporated down in the combustion tube. In some experiments we used a natrolite filter to get rid of calcium, magnesium and ammonia, but this proved less satisfactory.

Exp. 1.

Culture 15. ii. 29—1. iv. 29. Two flasks 500 cc. Weight of algae dried at 110° for 5 hours 80 mg.

Analysis of substrate after the culture (gas volume reduced to 770 mm. and 0°): CO_2 5.08 cc., O_2 4.45 cc., N_2 0.46 cc.

This analysis is not very reliable, the oxygen value being certainly too low and the nitrogen possibly a little too high. The pure substrate was not analysed but may very well have contained organic material corresponding to 2 cc. of CO_2 . The result shows that the organic substance possibly given off to the water is at most a small fraction of that retained in the algae.

Exp. 2.

Culture 20. iv. 29—8. v. 29.

Two flasks 500 cc. Dry weight of algae 50 (–60) mg. CO_2 2.58 cc., O_2 3.25 cc., N_2 0.16 cc., $\frac{\text{CO}_2}{\text{O}_2}$ 0.80. Two other flasks: CO_2 1.35 cc., O_2 1.67 cc., N_2 0.10 cc., $\frac{\text{CO}_2}{\text{O}_2}$ 0.81. Dry weight of algae from 6 flasks 145 mg. Average for 2 flasks 48 mg. Filtrate 1500 cc. 500 cc. analysed after filtration through natrolite gave CO_2 2.25 cc., O_2 2.42 cc., N_2 0.38 cc., $\frac{\text{CO}_2}{\text{O}_2}$ 0.93.

This analysis is less reliable than the first two, since it was found later that the natrolite would give off traces of organic substances.

Analysis of dry algae per 10 mg.: CO_2 6.48 cc., O_2 8.18 cc., N_2 0.53 cc., $\frac{\text{CO}_2}{\text{O}_2}$ 0.79.

The substrate incubated without algae gave 1.17 cc. CO_2 and 0.17 cc. N_2 . The oxygen determination was lost, but may be assumed as 0.7.

We find therefore that the algae in 1 litre gave off to the water organic substances yielding 2.76 cc. CO_2 and 0.09 cc. N_2 with a combustion quotient of about 0.8. In the organisms organic substance was stored to the extent of 96 mg. yielding 62 cc. CO_2 and 5.1 cc. N_2 with a quotient of 0.79.

Calculated as protein, carbohydrate, fat and gram-calories the figures are, for 1 litre:

	Protein mg.	Fat mg.	Carbohydrate mg.	Calories
Water	0.7	0.85	1.05	16.5
Algae	40.0	13.0	5.5	374.0

The total weight of the organic substances calculated for the algae

$$40 + 13 + 5.5 = 58.5 \text{ mg.}$$

falls far short of the 96 mg. dry weight and determinations of the ash content were therefore made on samples left over, but unfortunately not dated. The ash was found to be 30.3 % and 41.4 % respectively in two samples. The above calculation would leave 37.5 mg. or 39 % for the ash. The results show that in the conditions of the experiment these algae give off less than 5 % of the organic matter formed by photosynthesis to the water and store at least 95 % in their own cells.

Natural water experiments. Samples of water were taken from the surface of the lake Furesö on Nov. 28. A sample of this water was filtered through 30" collodion filters as described by Krogh [1930, 1], which hold back all organisms from bacteria upwards, and 1 litre of the filtered water was concentrated, precipitated with NH_4OH and H_3PO_4 , and filtered after standing for 24 hours in the ice chest. From the filtrate the NH_3 and later CO_2 were driven off and the fluid evaporated down in the combustion tube. It yielded 8.41 cc. CO_2 , 0.50 cc. N_2 and used 8.58 cc. O_2 .

Samples of the unfiltered water and of water filtered through paper were saturated with air at room temperature and put into 1 litre bottles. To each sample was added 0.05 cc. of $M \text{ H}_3\text{PO}_4$ and 0.15 cc. of $M \text{ NH}_4\text{OH}$. The stoppers were provided with wide tubes reaching down to the middle of the bottle and

through these 7 cc. samples were syphoned off at intervals for oxygen titrations. The amount of water removed was replaced from a small bottle treated in the same way and kept under the same conditions. The bottles were placed in a water-bath at a temperature of $23^{\circ} \pm 1^{\circ}$ and exposed to strong artificial light. The water-bath was painted white on the inside. The oxygen determinations gave in cc. per litre:

	Filtered	Unfiltered
Nov. 30	5.95	5.93
Dec. 5	2.75	3.70
„ 12	22.8	17.55
„ 28	28.5	33.6
Jan. 10	23.9	24.0

During the latter part of the experiment much air was given off in bubbles.

The organisms present in the paper-filtered water were a species of *Nitzschia* and a species of *Chlorella*, which were both very common, while *Scenedesmus quadricauda* was less abundant. In the unfiltered water *Scenedesmus quadricauda* was absolutely dominant, a *Chlorella* was common and several other forms were present.

On Jan. 13 the samples were filtered through 30" filters but most of the algae adhered to the glass and could not be recovered. The filtered samples were concentrated as before, evaporated and analysed with the following results:

	CO ₂ cc.	O ₂ cc.	N ₂ cc.
Paper-filtered	11.40	12.03	0.53
Unfiltered	9.95	10.59	0.40
Original water	8.41	8.58	0.50
Increase filtered	3.0	3.45	0
Increase unfiltered	1.55	2.0	-0.1

In the same period the production of organic substance corresponds at least to the maximum increase in oxygen content observed, 25.75 cc. in the filtered and 29.9 cc. in the unfiltered water. In this case again the organic substance going into solution in the water cannot be more than 10 % of the amount produced and is probably lower because the animals and bacteria must have given off a certain amount of organic material during the 44 days period. The influence of the animals must be slight since the paper-filtered water, from which animals must have been removed, gives the largest increase in organic material.

In a second experiment quantitative determinations were made on the organism developing during the experimental period. Samples of water were taken from the Furesö on June 5 at 2 m. depth. One sample was filtered through filter paper and another through plankton gauze (No. 20) to remove larger organisms. Each filtered sample was taken through a 30" collodion filter and put into 1 litre sterile flasks to which 0.05 cc. of *M* phosphoric acid and 0.15 cc. of *M* ammonia were added. The flasks were inoculated with plankton material from the collodion filters and cultivated in natural light at a window facing N.W. The sample inoculated with paper-passing material was cultivated in this way for 18 days and the sample inoculated with gauze-passing material

for 19 days. At the end of this period the plankton was filtered off, dried, weighed, and analysed, while the filtrate was subjected to the regular procedures leading up to a combustion analysis.

The sample inoculated with paper-filtered material gave the following results.

The organisms present were *Scenedesmus obliquus* Kg. (very abundant), *Ankistrodesmus Falcula* Brunnth. var. *tenellus* (abundant), *Ankistrodesmus Braunii* Lemm. var. *pusillus* Printz (common) and *Ankistrodesmus falcatus* Ralfs var. *spirilliformis* West (rare)¹.

The total dry weight of the algae in a 1000 cc. sample was 51.8 mg. with 22.6 % ash. The combustion gave per g. 726 cc. CO₂ and 43 cc. N₂ with an absorption of 919 cc. O₂ (R.Q. 0.79). Calculated as crude protein, fat and carbohydrate these figures give respectively 34.6, 20.3 and 13.8 %. The sum of the constituents as calculated is 68.7 leaving 31.3 % for ash and water. A sample dried further for 3 hours at a temperature of 100° lost 6.9 % in weight, leaving only 1.8 % not accounted for by the calculation. The combustion value of the material works out as 4400 cal. per g. or 228 cal. for the organisms in 1 litre.

The gauze-filtered sample gave as the absolutely dominating organism a species of *Chlamydomonas*, while a few individuals of *Ankistrodesmus falcatus* were present.

Dry weight 48.0 mg.

Ash, 2 determinations, 17.8 and 17.3 %, mean 17.7 %.

	cc. CO ₂ per g.	cc. O ₂ absorbed per g.	R.Q.	cc. N ₂ per g.
Combustion (a)	698	848	0.82	36
(b)	691	841	0.82	42

The average corresponds with 305 mg. of crude protein, 237 mg. of carbohydrate and 140 mg. of fat, leaving 318 mg. for ash and water. Drying at 100° gave 9.8 % extra water and we have 45 mg. per g. not accounted for. The combustion value is 4100 cal. per g. or 197 cal. for the organisms in 1 litre.

The analysis of the water gave per litre:

	CO ₂ cc.	O ₂ cc.	N ₂ cc.	R.Q.
Paper-filtered	13.17	14.47	0.65	0.91
Gauze-filtered	11.06	12.10	0*	0.915
Original water	8.29	9.12	0.44	0.91
Increase paper-filtered	4.88	5.35	0.21	0.91
Increase gauze-filtered	2.77	2.98	?*	0.93

* There must be an error in this result, but its source could not be detected. The apparatus was found to be airtight in a test performed just after the analysis.

Assuming a caloric value for the oxygen of 5.0 we have in the paper-filtered sample an increase in organic material corresponding to 27 cal. or 12 % of the combustion value of the plankton and in the gauze-filtered of 15 cal. or 8 % of the plankton.

¹ We are indebted to Mr G. Nygaard for the determination of the organisms in our samples. The variety *tenellus* of *A. Falcula* turns out to be new and will be described by him.

SUMMARY.

All the culture experiments agree in showing that the organic material synthesised by the assimilation of freshwater algae is almost quantitatively stored in the cells of the algae, while a fraction amounting at most to 10 % may possibly be lost to the surrounding water. We think it most probable that the organic substances directly lost during assimilation are wholly negligible, and that the losses observed in these experiments are mainly due to dead and decomposed organisms.

REFERENCES.

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