THE EFFECT OF ITS NITROGEN CONTENT ON THE DECOMPOSITION OF THE POLYSACCHARIDE EXTRACT OF CHONDRUS CRISPUS

MARGARET RUTH BUTLER

(From the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts[1]*)*

It has been frequently pointed out (Keys, Christensen and Krogh; Johnson; Waksman and Carey, et al.), that the bacterial decomposition of organic matter in stored sea water is limited by the supply of available nitrogen. A rather striking illustration of this arose from an experiment in which the organic material used was the purified polysaccharide extract of the marine alga, *Chondrus crispus,* which provides as well a natural source of nitrogen.

Method

Oxygen consumption was used as the index of decomposition. The customary procedure of storing glass-stoppered bottles (of approximately 200 ml. capacity) under water, at room temperature (20–25° C.), in the dark, was followed. The oxygen remaining was determined by the Winkler titration method, at various intervals. The results given are averages of at least two bottles, more often of three.

Experimental

In a preliminary experiment, the rate of decomposition of the extract was compared with that of the plant itself. The results, given in Table I, show the whole plant to be more readily decomposed than the extract. This fact was attributed to the lower quantity of nitrogen in the latter. For instance, plants containing 1.20 per cent nitrogen, on a dry weight basis, might yield an extract having only 0.30 per cent of nitrogen (Butler, 1936). The method of preparing the extract was described in a previous paper (Butler, 1934).

Table II shows the effect of various amounts of nitrogen, added as nitrate, on the decomposition of the extract.

It is evident from this table that the decomposition was increased in proportion to the amount of nitrogen added, up to a certain point

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only; other factors then apparently become limiting. In seven days the decomposition occasioned with the addition of 0.042 mgm. of nitrate nitrogen was as great as with 0.140 mgm., at least as measured by oxygen consumption. The fact that, with the lower quantity of nitrate, seven days were required to bring about the same amount of decomposition that was accomplished in three days with the larger quantity, suggests a possibility of the nitrogen being utilized more than once. This, however, is not likely, for von Brand, Rakestraw and Renn (1937) have shown that decomposition stops only after an interval varying

TABLE I

Decomposition of Chondrus crispus and its Polysaccharide Extract

Material	Mg. added per bottle	Oxygen consumed (ml. per liter)				
		1 day	2 days	3 days	5 days	6 days
Sea water only.....	—	0.09	0.24	0.36	—	0.57
Chondrus.....	3	1.11	1.71	1.89	—	4.32
"	5	—	1.26	—	3.48	—
"	10	1.38	3.60	4.32	—	5.46*
"	20	—	3.18	—	5.88*	—
Extract.....	25	0.18	0.54	0.81	—	1.47
"	5	—	0.81	—	1.92	—
"	10	0.66	1.17	1.68	—	3.36
"	20	—	1.68	—	5.31	—

* All oxygen consumed.

from 8–20 days. A more probable explanation is that the bacterial population supported by the smaller quantity of nitrogen has a much lower total metabolism than that of the abundant population when nitrogen is more plentiful.

The bacterial numbers given in Table II were furnished by Dr. Margaret Hotchkiss at the Woods Hole Oceanographic Institution, and are simply included here as a matter of record, but will not be discussed.

Having seen the effect on the breakdown of the *Chondrus* extract of adding various quantities of inorganic nitrogen, the effect of different quantities of organic nitrogen became of interest. Since extracts from different collections of *Chondrus* contain varying amounts of nitrogen (Butler, 1936), it was a relatively simple matter to study the effect of this naturally occurring organic nitrogen on the decomposition of the polysaccharide complex. For the purpose, a series of bottles was prepared in which each group contained the extract from a different monthly collection of *Chondrus* plants. As each extract from the plants col-

lected at monthly intervals contained a different quantity of nitrogen, a natural series was provided, in which each member had a different nitrogen content. Furthermore, this nitrogen is in that form in which it most probably occurs under natural conditions, in the plant itself.

Great care was taken to make up each extract in exactly the same concentration, 25 mgm. of extract per liter of sea water. This insured that any individual sample was directly comparable with all the others in the series. Oxygen determinations were made after one day of storage. The results are given in Table III along with the percentage

TABLE II

Effect of Added Nitrate on Decomposition of Chondrus Extract

Extract added (mg. per bottle)	Nitrate nitrogen (mg. per bottle)	Oxygen consumed (ml. per liter)		No. bacteria per ml.
		3 days	7 days	3 days
0	0	0.53	0.74	—
0	0.014	0.55	0.80	90,000
0	0.042	0.55	1.24	—
5	0	1.32	4.07	560,000
5	0.014	3.27	5.02	980,000
5	0.042	4.28	5.28*	—
5	0.140	5.28*	5.28*	3,200,000

* All oxygen consumed.

In this experiment the sea water was enriched by the addition of 1 mg. K_2HPO_4 per liter.

of nitrogen in each sample. It is readily seen that a correlation exists between the two and is most direct where nitrogen is low. This seems, therefore, to furnish definite evidence of the limiting effect of nitrogen on the decomposition of, at least one type of, organic material such as occurs in the sea. It suggests also that the low level of nitrogen during the summer may be partially responsible for the slow rate of decomposition.

While in the present discussion it is assumed that the decomposition is brought about by bacteria, the possibility that the oxygen is consumed by other organisms is not excluded. The water used may, undoubtedly, have contained larger organisms. It was surface water collected in Vineyard Sound and filtered through a No. 25 net of bolting silk. The bacterial considerations cannot be discussed here although evidence was obtained for the bacterial nature of the decomposition. However, the important fact remains that the consumption of oxygen on storing the

polysaccharide extract of *Chondrus* in sea water is correlated with the amount of nitrogen which it contains naturally.

TABLE III

Oxygen Consumption of Various Extracts of Chondrus crispus *

Extract	Nitrogen (per cent dry weight)	Oxygen consumption occasioned in one day by addition of extract (ml. per liter)
January	1.92	0.92
February	2.30	1.05
March	2.40	1.21
April	2.18	1.12
May	1.40	1.09
June	0.82	—
July	0.34	0.23
August	0.37	0.27
September	0.32	0.20
October	0.39	0.24
November	1.28	0.65
November	0.80	0.51
January	2.34	0.80

* The sea water used in this experiment showed an oxygen consumption of 0.55 ml. per liter in one day.

SUMMARY

The decomposition of *Chondrus crispus,* as measured by oxygen consumption, has been shown to be more readily accomplished on storing in sea water than that of its polysaccharide extract. This has been attributed to the higher nitrogen content of the former.

Inorganic nitrogen added to the polysaccharide extract of *Chondrus* increased its decomposition.

Samples of the extract containing different quantities of nitrogen have been found to decompose in direct proportion to the amount of nitrogen which they contain.

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