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STUDIES ON THE PHYSIOLOGY OF A LAVER, *PORPHYRA TENERA* KJELLM
III. CHEMICAL FACTORS INFLUENCING UPON THE PHOTOSYNTHESIS

By

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To bring out the factors controlling the photosynthesis of *Porphyra* will be a most effective way to produce an increased yield. In the preceding paper (1), analyses of some physical factors effecting the growth and mineral nutrition of *Porphyra* were made.

The present paper deals with the effect of chemical factors upon the photosynthesis which is considered to be necessary for the growth of *Porphyra*.

Materials and Methods

In the experiment, the laver samples which grew in different areas as Matsukawa-ura, Matsushima Bay and Onagawa Bay were used. (Fig. 1)

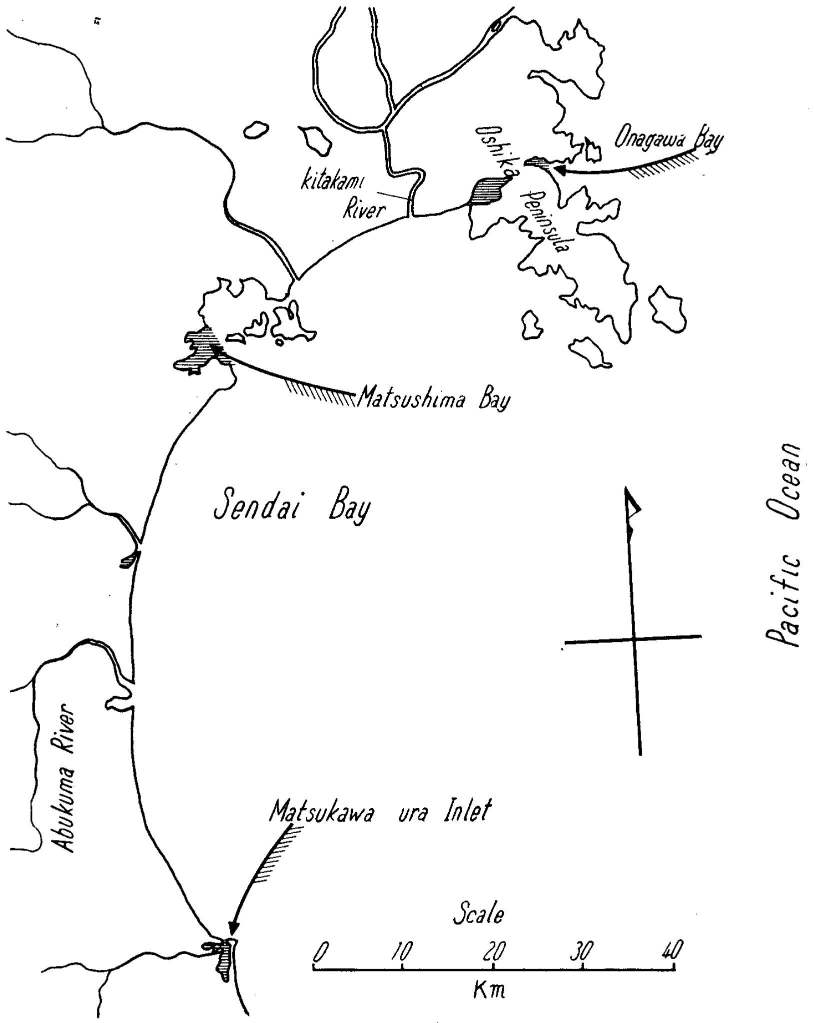
Laver samples were treated with the same way as the methods described in the previous paper and were cultured under the addition of various kinds of substances. After two days, the photosynthesis of each sample was measured by Warburg's manometric method at 15.0°C under a constant illumination of two 150 w electric lamps. The average of five series which consist of 20 pieces respectively was taken as the representative value.

The sea water used for the experiment was collected at Onagawa Bay in summer. It was filtered and heated to kill any organisms which had passed the filter.

Results and Discussion

1. Silicate

The effect of several silicates on the photosynthesis of *Porphyra* are shown in Fig. 2 (A). The photosynthesis was increased by the addition of several silicates except water glass. The increase in photosynthesis was 14



show the cultural grounds.

Fig. 1. Distribution of cultural grounds of laver in Sendai Bay and sampling stations.

per cent in glass wool and silica gel and 20 per cent in sodium silicate (anhydrous).

The role of the silicon in red algae nutrition such as ***Porphyra*** is not yet clear, though it is considered that silicates may play some part in the uptake of cation by the cells. Harder and Witsch (2) obtained increased yields of moss cultures of diatoms by the addition of sodium "wasserglas" up to 0.16 per cent, though 0.3 per cent produced an inhibition. In this experiment, however, the photosynthesis was decreased by the addition of 0.005 per cent of water glass. The cause of this inhibition must be due to the rise of pH (8.63) by the addition of water glass.

2. ***Phosphorus***

As already reported in a previous paper (1), a deficiency of phosphorus

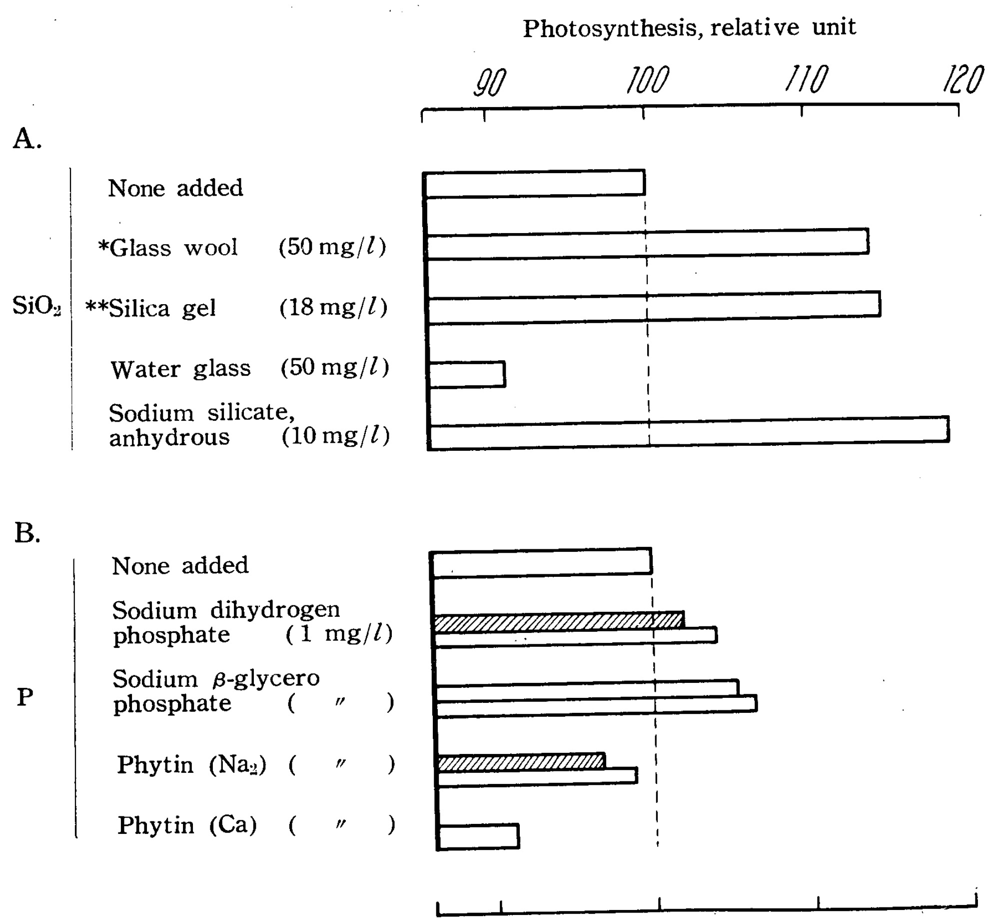


Fig. 2. Effect of adding various silicates (A) and phosphorus compounds (B) on the photosynthesis of *Porphyra*.
* Glass wools washed by a hot cleaning mixture and distilled waters then rubbed to powder after drying and suspended in distilled water. The filtrate of this suspension was used.
** The filtrate of this suspension was used.

inhibited the photosynthesis of ***Porphyra***, but it was not improved remarkably by the addition of the phosphorus.

In this experiment, the effects of various forms of phosphate upon the photosynthesis of *Porphyra* were compared with each other. The results are shown in Fig. 2 (B). These phosphates had no remarkable effect on the photosynthetic behavior in the concentration of P 1 mg per liter, though the photosynthesis was increased slightly by the addition of potassium phosphate (monobasic) and sodium β-glycerophosphate.

3. *Heavy Metals*

Manganese, Cobalt and Copper ions:

The relationships between the photosynthesis and the concentrations of Mn as sulphate, Co as sulphate and Cu as sulphate are shown in Table 1.

The addition of cobalt sulphate up to Co 50 μg per liter accelerated the photosynthesis, though 100 μg per liter produced an inhibition. In the copper ion, it inhibited the photosynthesis even in the concentration of 15 μg per liter.

Table 1 Effect of metal-ions upon the photosynthesis of *Porphyra*

Metals	Concentration (μg/l)	O_2 produced (μl/h/mg)	Photosynthesis relative unit	Catalytic Activity K_{30}
Manganese. (20 h at 13.0°C)	0	7.12	100	
	0.5	7.70	108	
	1.0	6.22	87.4	
	2.5	7.06	99.1	
	5	6.93	97.3	
	10	6.70	94.1	
	20	6.62	93.0	
	50	7.74	?	
Cobalt. (66 h at 13.0°C)	0	7.25	100	(K^{20h}_{30}) 5
	20	7.74	107	12
	50	7.47	103	21
	100	7.13	98.4	49
	200	6.82	94.1	77
	500	6.48	89.4	143
Copper. (120 h at 17.2°C)	0	17.75	100	(K^{5h}_{30}) 5
	10	17.95	101	44
	20	12.30	69.3	60
	40	12.42	70.0	94
	60	13.27	74.8	148
	80	10.95	61.7	298
	100	10.30	58.0	338
	150	7.82	44.1	544
	300	3.57	20.1	564

Note: These photosynthesis were measured by the oxygen bottle under a illumination of 100 w electric lamp (35 cm)

Iron :

The requirement of iron for the growth of algae has been well substantiated (3~9), though the way in which the iron is provided is a subject of controversy. The solubility of iron in alkaline solutions is very low and Cooper (10) concluded that less than 10^{-7}mg per liter of ionic iron can exist in the sea water in equilibrium with ferric hydroxide.

It is known that one molecule of EDTA (ethylene dia nine tetra-acetic acid) makes a metal chelate compound with one molecule of metals in the solution above pH 6.0. Therefore, in the present experiment, Fe-EDTA was made, and then the effect of the addition of Fe-EDTA on the photosynthesis was examined comparing with the addition of other several ferric compounds. The photosynthetic activity of ***Porphyra*** was activated remarkablly as shown in Fig. 3 rather more than the other forms of ferric compounds by the addition of Fe-EDTA, though there was a little difference in the effect by the environments where the laver had grown.

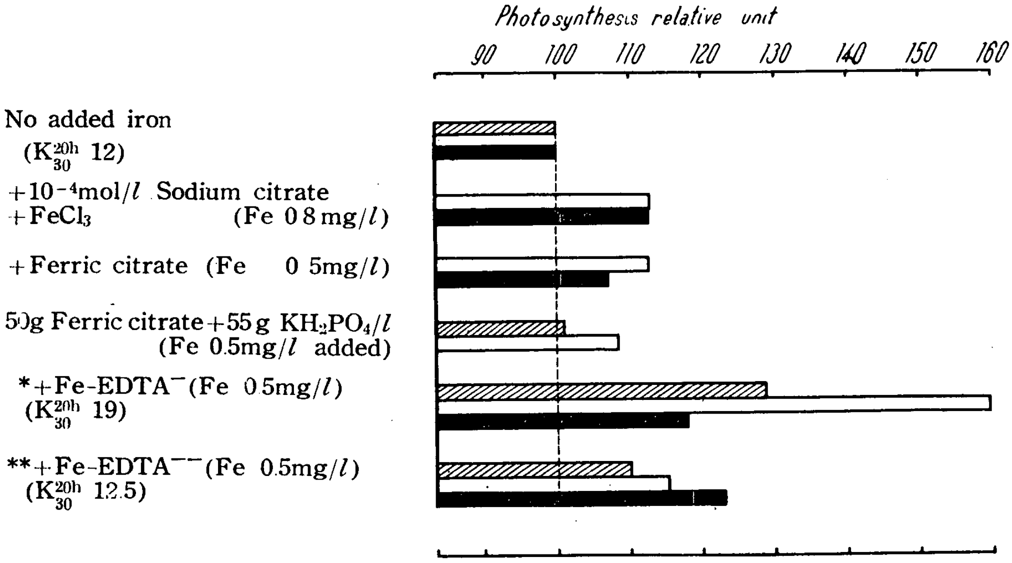


Fig. 3. Effect of adding various ferric compounds on the photosynthesis.
* Chelation from $Fe_2(SO_4)_3(NH_4)_2SO_4 \cdot 24H_2O$.
** Chelation from $FeSO_4 \cdot 7H_2O$.
▨ *Porphyra* collected from Matsukawa-ura, □ from Onagawa Bay,
■ from Matsushima Bay. (Signs are the same for following figures.)

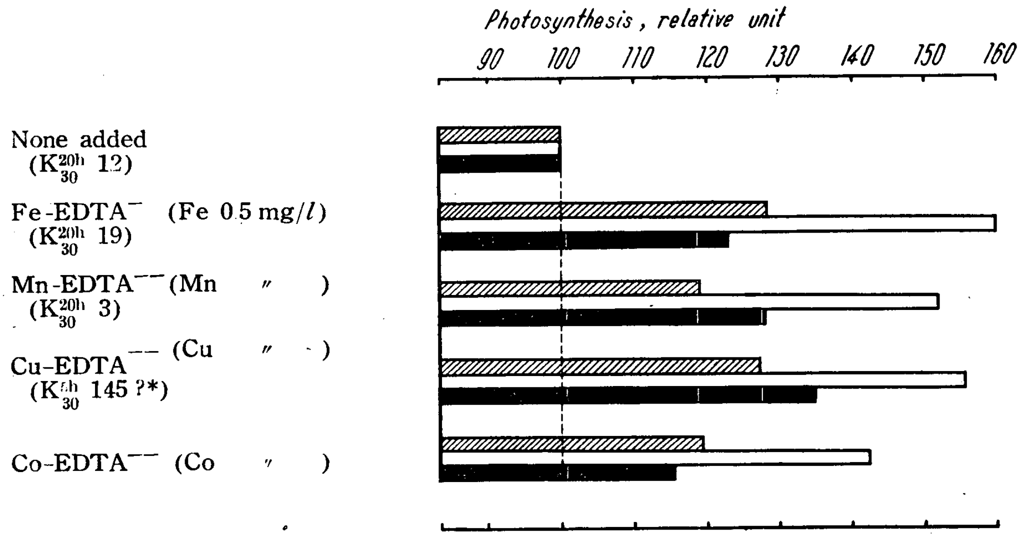


Fig. 4. Effect of several metal chelate compounds on the photosynthesis.
* It may contain free Cu ions.

Metal Chelate Compounds:

The effect of the addition of metal chelate compounds on the photosynthesis is shown in Fig. 4. From this results, it is apparent that additions of Fe-EDTA, Mn-EDTA, Cu-EDTA and Co-EDTA have more or less significant effects on the photosynthesis of *Porphyra*. On the other hand, the additions of the chelate compounds of Zn, Mo and B inhibited the photosynthesis.

Effect of several Metal Chelate Compounds:

The three kinds of medium; i) filtered sea waters, ii) miquel's sea waters and iii) filtered sea waters enriched with 5 mg N (as NH_4NO_3), and 0.5 mg P (as KH_2PO_4), 10 mg SiO_2 (as $Na_2Si_2O_5$) and 0. 5 mg of Fe, Mn, Cu and Co (as Metal-EDTA) were made and used. The photosynthesis was measured after being cultured in each medium for two days. As shown in Fig. 5, the photosynthetic activity of *Porphyra* which was cultured in (iii) medium was nearly the same as the one cultured in miquel's sea water.

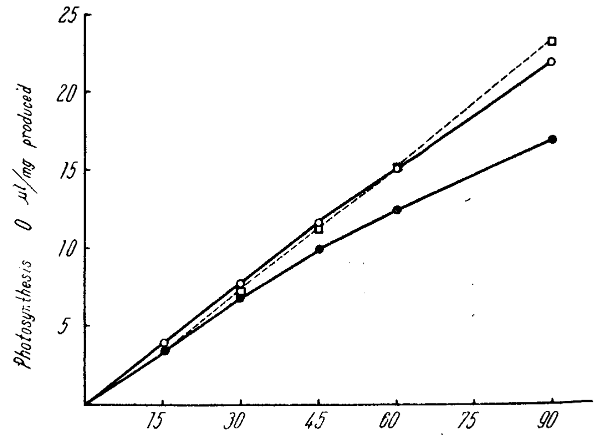


Fig. 5. Photosynthesis of *Porphyra* in filtered inshore sea water (solid circles), miquel's sea water (Squares) and filtered inshore water enriched with 5 mg N(as NH_4NO_3), 0 5 mg P (as KH_2PO_4), 10 mg SiO_2 (as $Na_2Si_2O_5$) and 0 5 mg Fe, Mn, Cu and Co (as Metal-EDTA) (circles).

The physiological and biochemical actions of the metal chlate compounds on *Porphyra* are not yet clear. However, it is certain that Metal-EDTA is nontoxic, and allows considerable concentration of heavy metals to remain in sea water. One of the authors showed that organic matters decrease the catalytic activity of the sea water (11), and EDTA is most effective as a negative catalyst (12). The photosynthetic activity of *Porphyra* showed higher values in sea water of the lower catalytic activity (Table 1). From these facts, it is assumed that the cell of lavers uptakes the metalic ions as their stable chelate compounds with chelating agents such as EDTA and enhances its photosynthetic activity.

4. *Sodium sulphide, Gultamate and Vitamins*

The effects of Na_2S, gultamate and vitamins on the photosynthesis of *Porphyra* are shown in Fig. 6. The addition of vitamin•B_2, B_{12}, gultamate and Na_2S had an effect on the photosynthesis, especially the effect on the laver collected in early spring was remarkable.

Numerous observations (13, 14, 15) have been made in which the addition of organic divalent sulphur compounds, in the form of cystine, methionine, glutathione, thiamine or biotin, or of inorganic sulphide markedly increased the growth of diatoms. From these results, Harvey (15) pointed out that the deposition of amorphous silica from silicate in solution to form the frustules of diatoms was in some way linked with their sulphur metabolisms. Thiamine which contains sulphur has been found to be necessary for the phototrophic growth of ***Euglena gracilis*** but not when glutamate is supplied either in the light or the dark. (16)

However, the role of these sulphurs for the growth of laver is not yet clear since the requirement of silicate for the growth of ***Porphyra*** is unknown.

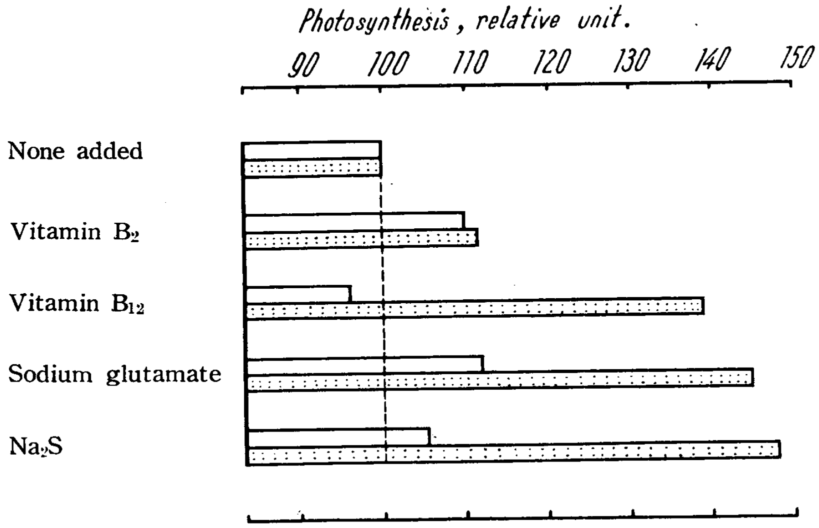


Fig. 6. Effect of Vitamin B_2, B_{12}, glutamate and Na_2S on the photosynthesis of *Porphyra*.
▦ show laver collected at Onagawa in March.
□ at Onagawa in November.

Summary

Studies were made to find the effect of chemical factors on the photosynthesis of ***Porphyra***. The obtained results are here summarized.

1. Silicates had an effect on the photosynthesis of ***Porphyra***. The photosynthesis was increased about 14 to 20 per cent by the addition of silicates, in the form of glass wool, silica gel and sodium silicate (anhydrous).

2. Iron which was supplied as Fe-EDTA remains in the sea water, and activated considerably the photosynthetic activity.

3. Mn, Cu and Co which were supplied as Metal-EDTA were nontoxic even in the their concentrations to be toxic if they were not chelated. The photosynthesis is increased remarkablly by the addition of these Metal-EDTA.

Zn, Mo and B as Metal EDTA showed negative effect for the photosynthesis.

4. The additions of vitamin B_2, B_{12}, gultamate and Na_2S accelerated the photosynthesis, especially the effect on the laver collected in early spring was remarkable.

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