On the influence of temperature on the CO_2-assimilation of Helodea canadensis

by

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INTRODUCTION.

In a former publication about the influence of temperature on physiological processes of the yeast [1]) I showed that in general the studies on this subject had not been carried out in such a manner that important conclusions could be drawn from the results obtained. It was especially demonstrated in the above mentioned study that the present data do not answer to the question whether there is a difference between the influence of a rise of temperature on a physiological and on a physical or chemical process.

This view also concerns the influence of temperature on the CO_2-assimilation, notwithstanding that Blackman [2]) studied this subject on much better principles than his predecessors.

With regard to the discussion of Blackman's experiments my above mentioned study [3]) can be referred to, and critical reference to earlier investigations [4]) will be omitted here, partly because they have already been

[1]) Dissertation, de Bussy, Amsterdam 1912.

[2]) Proceedings Roy. Soc. Vol. *83 B.*, 1911, p. 374.

[3]) pag. 74.

[4]) See: W. Pfeffer. Pflanzenphysiologie Bd. I.

criticised in that publication, partly because the results, to be mentioned further on, contain a criticism on many of these observations.

When the influence of temperature on a process is to be estimated accurately, the velocity of that process at a certain moment should be determined as accurately as possible. But this is only to be obtained when the velocity is constant. When this is not the case, one has to be contented by measuring the mean velocity during a space of time which should be taken as short possible.

Now we will describe a method which will enable us to determine the mean velocity of assimilation during a very short space of time ($\pm$ 5 minutes). This velocity was determined by measuring titrimetrically the amount of oxygen formed by the assimilation. Meanwhile care had to be taken that the amount of available carbondioxide should be constant and that this gas should be present in so great a quantity that it did not diminish too much during the assimilation, in other words that the amount of carbondioxide could not be a limiting factor in the meaning attached to it by Blackman[1]). Likewise the same care had to be taken in reference to the available energy of light. In these circumstances it might be expected that a change of the CO_2-assimilation during variation of temperature, was exclusively caused by this factor.

Shoots of *Helodea canadensis*, which had been put up in the laboratory for some time before the beginning of the experiment, were used as testing material. Water containing carbondioxyde, but no oxygen was streaming along these shoots, and the oxygen formed in the light of an electric lamp was measured.

In a treatise, entitled: „Ueber den Gasaustausch der Wasserpflanzen. Ein Beitrag zur Kritik der Blasenzähl-

[1]) Ann. of Botany. *19*, 1905. p. 281.

methode" Hans Kniep[1]) pointed out that the gaseous exchange of assimilating shoots of *Helodea canadensis* is a complicated process, especially caused by the difference between the rate of diffusion of carbondioxyde, oxygen and nitrogen. Even the velocities of the dissolving and escaping of these gasses into and from the liquid and from and into the intercellulars (invasion and evasion) seem to play a part.

In our experiments the circumstances are much more simple than in Kniep's, because no gasbubbles are evading from the cut ends of the *Helodea*-shoots in the water which is free of oxygen and streaming along these shoots. So the oxygen will come into the water only by diffusion. Moreover we had no nitrogen in our gasmixture. Yet it will be obvious that probably in our experiments the processes pointed out by Kniep also took place. It will even be proved that the results obtained, have presumably been determined by similar processes.

Thus the experiments, which are to be discussed here, will lead to the conclusion that in fact we did not measure the influence of temperature on the CO_2-assimilation itself, but that physical processes have exerted their limiting influence. In spite of this circumstance we are publishing our results now, because they may indicate to others the probable way to obtain the desired results.

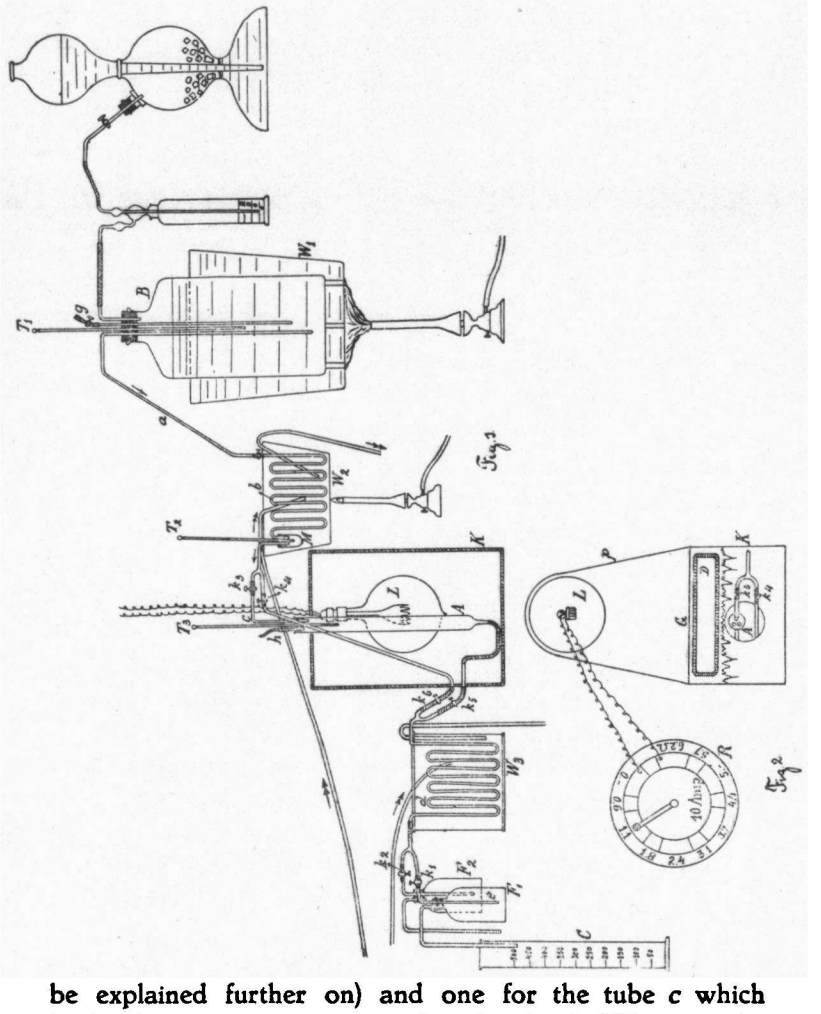
§ 1. The apparatus.

A side-view of the apparatus used for the experiments is schematically represented in Fig. 1; Fig. 2 schematically represents a view from the upperside.

The cylindrical glassvessel A (35 c.M. long and 2.5 c.M. in diameter) is used for holding the *Helodea*-shoots. The

[1]) Jahrb. f. Wiss. Bot. 56 Pfefferband. 1915, S. 460.

top-end is closed with a rubber-stopper with three holes: one for a thermometer T_3, one for a tube *k*, which can be closed by a valve (and the use of which will



be explained further on) and one for the tube *c* which leads the water containing the dissolved CO_2 into the vessel A along the shoots. This water has beforehand

been warmed to the desired temperature, and it leaves the vessel A through a tube, fixed to the narrow bottom-end.

Reservoir *B* contains the water that is prepared in the way, which will be discussed further on. Moreover the reservoir B is connected with a CO_2-apparatus in order that the outflowing water may be replaced by gaseous CO_2.

The water in the reservoir *B* is kept at a temperature of about 65° C., the thermometer T_1 indicating the temperature of this water.

The rubber-stopper of the flask *B* is provided with a siphon *a*, leading off the water and with a tube *g*, which can be closed by a valve. It reaches to the bottom of the flask and its use will be explained while discussing the preparation of the water. Before flowing along the *Helodea*-shoots the water passes through a glass-tube *b* that has been bent several times. This tube is placed in a waterbath W_2 which is warmed at a lower temperature than W_1. The temperature of W_2 is to be regulated by a current of tapwater and if necessary, also by a gasburner. In connection with the velocity of the watercurrent through the tube *b* the temperature of W_2 is chosen so that the water reaches the *Helodea*-shoots at the desired temperature. This temperature is controlled at the end of the tube *b* by leading the water through a short, wide tube M, containing a thermometer T_2.

Here it should be noted that this way of regulating the temperature of the overflowing water has been chosen to exclude saturation and over-saturation of this water by CO_2. In the case of experiments at a high temperature a casual saturation and over-saturation might cause a development of gas-bubbles in the cylindrical vessel A, the result of which might be a loss of oxygen, the bubbles carrying away this gas. This saturation and over-saturation of the water, possible in the reservoir B, will be prevented by cooling the water in the bath

W_2 to a lower temperature than it had in the bath W_1.

The water after having been raised to the desired temperature and having passed the shoots, is again conducted through a glasstube *d* which has been bent several times and which is also placed in a waterbath: here it is cooled to a low temperature (that of the tapwater) by means of a water current. After this the water is caught in one of the small flasks F_1 and F_2, different precautions being taken, which will be described further on. The liquid, displaced from the flasks by the overflowing water, is caught in a measuring cylinder, in order to determine the velocity of the watercurrent.

It will be evident that the current is brought about by siphonic action and that the velocity can be regulated by valves.

Figure 1 shows that it is also possible to conduct the water immediately to the flasks, without flowing along the *Helodea*-shoots. Before and behind the cylindrical vessel **A** a T-piece is put in the water-pipe and by opening and shutting the valves k_3, k_4, k_5 and k_6 the course of the water may be regulated.

Lastly it should be mentioned that a half-watt lamp **L**, with a maximum power of 4000 candles was used as the source of light. By linking in a rheostat **R** (see Fig. 2) the light-intensity could be regulated. The daylight was excluded entirely and at the same time care had been taken to reflect the light of the lamp as much as possible on the shoots by enveloping the lamp with white asbestus, covered outside with a black cloth and by placing the vessel **A** in a small wooden box **K**. This box is furnished at back and front with a pane of glass, the front one to arrest the heat of the lamp, the back one for observation of the shoots during the experiment.

In the upper side of the box is an opening, to allow the passage of the tube **A**. This box **K** is also covered with a black cloth.

Finally between the lamp and the cylindrical vessel is a bowl D, containing a solution of alum to arrest the heat-radiation of the lamp.

§ 2. Preparation of the water and determination of the dissolved CO_2.

For some preliminary experiments tapwater was used, which after having been thoroughly boiled (to expell the dissolved oxygen) was cooled in an atmosphere of CO_2 and then poured into the reservoir B. In fact an active absorption of CO_2 took place in water prepared in this way.

Yet, in such water the determination of the free and the half-free CO_2, both a source of the CO_2-assimilation (as contrasted with the CO_2 from the carbonate) is rather a protracted process because of the simultaneous presence of magnesium-carbonate and calcium-carbonate. In order to save time some experiments were made with distilled water similarly prepared. However it was obvious, that by using this water the *Helodea*-shoots did not assimilate nearly as well as in the prepared tapwater, notwithstanding the distilled water also contained a great quantity of dissolved CO_2. Most probably this decrease is to be attributed to the absence of half free CO_2 in the distilled water; for, when a quantity of potash, almost equivalent to the carbonate in the tapwater, was added before bubbling the CO_2 through the distilled water, the CO_2-assimilation took place with the same intensity as in the tapwater.

Afterwards I found that Angelstein [1]) had also observed that in distilled water the assimilation-process of waterplants is nearly stopped and that it begins when $KHCO_3$ is supplied.

[1]) Cohn's Beitr. z. Biologie d. Pfl. 9, 1911, S. 93.

However, though the use of distilled water to which potash had been added, caused a simplification of the measuring of the free and half free CO_2, which will be discussed further on, yet the boiling and cooling of large quantities of water still took much time.

Now I succeeded in driving the oxygen out of the water in a simpler way, viz. by bubbling through hydrogen, followed by CO_2. The hydrogen pushed away the oxygen and afterwards was pushed away by the CO_2, which partially bound to the potash as $KHCO_3$, partially dissolved in a free state.

The preparation as it was at last applied was carried out in this way: To 10 L. distilled water in the reservoir **B** (fig. 1) 750 mgr. potash was added. After this the water was heated up to $\pm$ 65° C., during this heating, and also when that temperature was reached, hydrogen was kept bubbling through the water. This all happened within one hour, a hydrogen apparatus being connected with the above mentioned tube *g*.

Further CO_2 was kept bubbling through the water by means of the same tube *g*, during 2 hours, whilst the water was kept permanently at $\pm$ 65° C.

Only after this was the tube *g* shut and the CO_2-apparatus connected with the reservoir **B** in the way shown in the figure.

In order to determine the quantity of free and half free CO_2 in this water, a flask of 100 cM^3. was filled (in a way to be discussed later on) with this water, which as will be clearly seen, had not been flowing along the *Helodea*-shoots. Thereupon this water was poured over as quickly as possible into another bottle, the contents of which were a little more than 160 cM^3. Immediately 50 cM^3. of a baryta-solution of $\pm$ 0.1 normal and 10 cM^3. of a $BaCl_2$-solution of $\pm$ 0.5 % were added to it, and so the flask was nearly filled. After closing, the flask was shaken

thoroughly, and was left to stand overnight. The next day 50 cM^3. of the clear liquid, which was above the precipitate was carefully pipetted and titrated with a solution of HCl of about o.1 N. From these data the total quantity of CO_2 (free and half free) could be calculated [1]).

From this titration which was repeated at every experiment, it appeared that the water, prepared in the way above described, contained in minimum 200 mgr. CO_2 pro Liter, generally the quantity was somewhat larger.

Now, 109 mgr. $KHCO_3$ can be obtained from the 75 mgr. potash which were added to the water before bubbling through the CO_2, which $KHCO_3$ is equivalent to 48 mgr. CO_2 per Liter. So the excess of 152 mgr. CO_2 must have been dissolved in the water.

From this we see firstly, that there is an excess of free CO_2 (as was to be expected), which is of importance, because it gives the certainty that all the K_2CO_3 has in fact been transposed into $KHCO_3$, which is harmless to the assimilation, unlike carbonate, the harmful influence of which has been shown by Nathanson.

But secondly we wish to draw attention to the fact that in our experiments the total quantity of CO_2 meant a large excess. Presently we will see that in these experiments quantities of oxygen were formed, which (in maximum) are equivalent to only 4.7 mgr. CO_2 pro Liter water. This means a very small decrease of the quantity of CO_2, which therefore remains practically constant during the experiment.

§ 3. Description of the method of the experiments and calculation of the results.

a. Putting in the Helodea-shoots. After the water has

[1]) This method has been derived from the: Codex Alimentarius, No. 3 „Het water."

been prepared in the manner described above, the shoots can be placed in the cylindrical vessel **A** for the assimilation-experiments.

Shoots having 700 à 750 leaflets (not counting the buds) were always chosen for the experiments.

The shoots were fastened with a piece of raffia to a glass rod, which, after removing the rubber stopper, was put in the vessel **A**, which was closed again afterwards. By means of the CO_2-apparatus the water was now passed from the reservoir **B** through the apparatus, but first the valve k_5 was shut, whilst the valve in tube *h* was open. In this way the air could escape from the tubes *a* and *b* and also from the reservoir **A**. After this the tube *h* was shut and the valve k_5 was opened.

Not before the air had been driven out of the other tubes and the water had flowed through the apparatus for some time (to take up all the air still attached to the sprigs and the glasswalls) was the water caught in the flasks.

b. Collecting the water. We have already told, that the water is caught in one of the flasks F_1 and F_2. The contents of these flasks being about 300 cM^3. was accurately measured. Before the rubber stoppers were put on, the flasks were filled up to the brim with an alcohol-solution of 7 %, tinged with methylenblue. The current of water was lead to the bottom of the flask by the tube *e* and displaced the blue alcohol which was floating on the surface. In this way, mentioned by Blackman[1]), I succeeded in collecting the water whilst avoiding its contact with the air. The disappearance of the blue colour told the moment when the whole was driven out of the flask and its contents consisted only of the ordinary effluent water.

By opening first the valve k_1 and shutting k_2 and by reversing the manipulation, F_1 will be filled first and then

[1]) Proc. of the Roy Soc. of London. Vol. 83 B. 1911. p. 379.

F_2. Moreover, when F_2 is still being filled, F_1 can be replaced by a third flask F_3. In this way the water may be caught in a consecutive series of flasks, or, if necessary, also with regular intervals. After loosening the flasks they were immediately supplied with the reagents and then shut air-tight with a glass stopper.

c. Determination of the velocity of the watercurrent.

We showed already in § 1, that overflow of fluid, from the flasks, can be collected in the measuring cylinder **C**, which enables us to measure the velocity of the watercurrent. By opening the valves entirely, about 70 à 80 cM^3. water per minute streamed through our apparatus. Generally we measured the mean assimilation-rate during a period of $4^1/_2$ à 5 minutes. In this space of time about 350 cM^3. streamed through the apparatus, when the valves were opened entirely. Because of this we have ever since expressed the velocity of the watercurrent by measuring the time necessary for collecting 350 cM^3. water, also when the valves were not openend entirely.

d. Determination of the amount of oxygen.

After having fixed the amount of CO_2 in a sample of the water from the reservoir **B**, which had not flowed along the shoots, the (very small) amount of oxygen in a similar sample had to be determined. After this the water was guided along the *Helodea*-shoots by turning the valves, and then the amount of dissolved oxygen had to be determined from the water samples successively collected.

The amount of oxygen was determined according to the method of Winkler[1]), titrating with a N/100 solution of $Na_2S_2O_3$ the quantity of jodium equivalent to the oxygen dissolved in the water. Therefore in our

[1]) See: Tiemann—Gärtner: Handb. d. Unters u. Beurt. d. Wässer. S. 308. Braunschweig 1895.

tables, we will also mention the number of cM^3. N/100 $Na_2S_2O_3$ necessary for the titration.

It may incidentally be remarked here that the method of Winkler is also applied by Kniep[1]) for the investigation of the CO_2-assimilation. This investigator covered the water, in which the assimilating *Helodea*-shoots were put, with a layer of olive-oil. Apart from the circumstance that this method of arranging the experiments did not suit our purpose, it may be noticed here that some experiments done in the „Laboratory for microscopical anatomy" showed that this way of shutting off the oxygen is very imperfect. In fact in this way the oxygen of the air permeates as quickly into the water as when the water is directly in connection with the air.

e. Calculation of the average velocity of assimilation.

The quantity of oxygen present in the flasks having once been fixed, by means of these data and by taking into account the capacity of the flasks, the quantity of oxygen which would be present in 350 cM^3. water of the same concentration of O_2 can be immediately calculated.

We expressed the amount of oxygen by the equivalent quantities of N/100 $Na_2S_2O_3$; 1 cM^3. N/100 $Na_2S_2O_3$ being equivalent to 0.08 mgr. O_2.

Taking this quantity of $Na_2S_2O_3$-solution necessary for the original water to be: a_1, and that for the water after having flowed along the shootss: a_2 and indicating the velocity of the watercurrent by t, viz. the time in minutes necessary for collecting 350 cM^3. when the velocity was constant, then the average velocity of assimilation during the period of filling the flasks expressed in mgr. oxygen per minute is given by the following formula:

$$v = (a_2 - a_1) \frac{60}{t} \times 0{,}08 \text{ mgr.}$$

[1]) Jahrb. f. Wiss. Bot. *56*, Pfefferband. 1915, S. 460. and Handwörterb d. Naturw. Bd. 7 (1912). S. 701.

f. Determination of the intensity of the light.

In the experiments with varying quantities of light the light of the lamp was measured by Weber's photometer, different resistances having been put in. As the intensity of the electric current could be considered to be constant, the intensity of the light could be deduced from the resistance that was put in during the experiment. In all our experiments the lamp was placed at the same distance from the *Helodea*-shoots, so that at that point the intensity of the light was changing proportionally to that of the lamp.

§ 4. Intensity of light and velocity of assimilation.

According to what has already been pointed out in the Introduction it is necessary to work with an excess of light-energy when studying the influence of temperature on the velocity of the assimilation. At least this is the view that is most accepted since Blackman's theory about limiting factors.

Therefore when presently discussing some experiments about the connection between the intensity of the light and the velocity of assimilation, we do not in the least mean to give an explanation of this interesting problem, which would require much more accurate investigations. We have only tried to find out which intensity of light was required in our way of arranging the experiments, to be sure of an excess of light-energy.

The experiments were performed at two temperatures, namely 24° C. and 36° C. The latter temperature, as will be evident later on, was not noxious to the assimilation function, though it was very near the injurious temperature.

It might be noted here that it was not sufficient to establish the required excess of light at a low temperature. It is possible that a quantity of light- energy which means

an excess at a low temperature, becomes a limiting factor at high temperature in consequence of the increase of velocity.

The results of the experiments with various intensities of light are given in the tables I and II. The period of exposition of the shoots to the different intensities of light is mentioned in the 3rd column. At each of the applied intensities two or more flasks were filled with water, which had been conducted along the *Helodea*-shoots. The indication and the contents of these flasks are given in the 4th column. The first flask of each series was filled with water, which had not flowed along the shoots, but had been collected directly.

Notwithstanding the inevitable errors in the observations, the results show that at both temperatures the rate of assimilation becomes independent of the light-intensity when this is more than about 2000 Hefner-candles.

From these results it seems right to suppose that the intensity of light might be a limiting factor. Further on it will appear whether this conclusion is to be considered correct.

At our further experiments an intensity of 2482 candles was applied, which was obtained by putting in a resistance of 1.1 Ω.

When the ligth is less than 2000 candles the velocity of assimilation seems to depend very much on the light-intensity. Even from our (rather rough) data one would draw the conclusion of a proportionality between these values. It should be noticed that the results of both tables are not to be compared, as they were obtained with different shoots.

§ 5. The velocity of the assimilation when the circumstances are constant and when the velocity of the watercurrent is changed.

It seemed not improbable that noxious influence would be effected by an action of the light during a longer

Table I.

Relation between the velocity of assimilation and the intensity of light at 24° C.

Resistance put in.	Intensity of the light of the lamp.	Begin and end of the exposition to the light.	Indication and contents of the flasks.	cM^3. N/100 $Na_2S_2O_3$ per flask.	cM^3. N 100 $Na_2S_2O_3$ per 350 cM^3. water.	Time for collecting 350 cM^3. water.	Rate of assimilation in mgr. O_2 per minute.
Water collected directly.		11h.22′—11.35′	A 277.7 cM^3.	0.7	0.9	—	—
0 Ω	3377 candl.	11.50′—12.8′	C 316.9 cM^3.	9.6	10.6	4′30″	0.169
			D 317.8 „	9.8	10.8	4′30″	0.174
			E 265.3 „	8.7	11.5	4′30″	0.193
1.1 Ω	2482 candl.	12.13′—12.31′	F 289.6 cM^3.	9.4	11.3	4′35″	0.213
			G 291.9 „	9.4	11.2	4′50″	0.173
			H 314.6 „	10.2	11.3	4′50″	0.174
1.8 Ω	1816 candl.	12.38′—12.50′	I 297.5 cM^3.	9.5	11.2	5′2″	0.154
			K 281.2 „	9.4	11.3	5′5″	0.167
3.1 Ω	1133 candl.	12.55′—1.7′	L 313.9 cM^3.	9.9	11.0	5′15″	0.155
			M 303.8 „	8.6	9.9	5′15″	0.137
6.2 Ω	612 candl.	1.12′—1,22′	N 300.3 cM^3.	6.2	7.2	7′16″	0.066

Table II.

Relation between the velocity of assimilation and the intensity of light at 36° C.

Resistance put in.	Intensity of the light of the lamp.	Begin and end of the exposition to the light.	Indication and contents of the flasks.	cM^3. N/100 $Na_2S_2O_3$ per flask.	cM^3. N/100 $Na_2S_2O_3$ per 350 cM^3. water.	Time for collecting 350 cM^3. water.	Rate of assimilation in mgr. O_2 per minute.
Water collected directly		11.45′—11.51′	A 277.7 cM^3.	0.9	1.1	—	—
1.1 Ω	2482 candl.	12.11′—12.23′	E 265.3 cM^3.	7.—	9.2	4′20″	0.147
			F 289.6 „	7.8	9.8	4′30″	0.150
0 Ω	3377 candl.	12.28′—12.40′	G 291.9 cM^3.	8.2	9.8	4′31″	0.152
			H 314.6 „	8.4	9.3	4′37″	0.144

period than that mentioned in the preceding section. Such a detrimental influence would render the results of our further experiments somewhat uncertain.

Anyhow in the first place constancy of velocity of assimilation under unaltered circumstances at a harmless temperature should be required, else the experiments would not be reliable. In order to establish this, some *Helodea*-shoots were exposed during 1 hour at 24° C. to the light of 2482 candles, the water being collected in a continuous series of flasks. The filling of one flask took about $4^1/_2$ à 5 minutes.

On the whole 10 flasks were filled, the first one with water that had not streamed along the shoots. Table III shows the results of this experiment.

Table III.

The rate of assimilation when the circumstances are constant (24° C., 2482 candles).

Indication and contents of the flasks.	cM³. N/100 $Na_2S_2O_3$ per flask.	cM³. N/100 $Na_2S_2O_3$ per 350 cM³. water.	Time for collecting 350 cM³. water.	Rate of assimilation in mgr. O_2 per min.
A[1]) 277.7 cM³.	0.8	1.00	—	—
C 316.9 „	7.4	8.2	4'40"	0.124
D 317.8 „	8.9	9.8	4'40"	0.152
E 265.3 „	7.2	9.5	4'30"	0.152
F 289.6 „	8.3	10.—	4'37"	0.158
G 291.9 „	8.2	9.8	4'35"	0.155
H 314.6 „	8.6	9.5	4'40"	0.148
I 297.5 „	8.4	9.9	4'43"	0.152
K 281.2 „	8.—	9.9	4'45"	0.152
L 313.9 „	9.1	10.1	4'45"	0.151
M 303.8 „	8.6	9.9	4'55"	0.146

[1]) Water collected directly.

The first observation excepted, the differences between the numbers obtained were not larger than could be expected as a result of the many errors in the observation. Indeed the rate of assimilation seemed to be constant under unaltered circumstances.

The fact that at the first observation the numbers were lower than at the following ones, we have observed several times, and will be discussed further on.

In the previous experiment and the experiments about the influence of temperature, the velocity of the watercurrent was kept as constant as possible. However this did not succeed entirely, so it was necessary to examine the casual influence of the velocity of the watercurrent on the results. Table IV gives the results of a series of experiments, carried out on that purpose. The maximum velocity of the watercurrent, which could be obtained with our apparatus, was gradually diminished to the half. The first flask was as usual filled with water, that had not passed along the shoots.

Table IV.

The rate of assimilation when changing the velocity of the watercurrent (24° C., 2482 candles).

Indication and contents of the flasks.	cM³. N/100 $Na_2S_2O_3$ per flasks.	cM³. N/100 $Na_2S_2O_3$ per 350 cM³. water.	Time for collecting 350 cM³. water.	Rate of assimilation in mgr. O_2 per min.
A[1]) 277.7 cM³.	2.6	3.3	—	—
C 316.9 „	11.3	12.5	4′30″	0.164
D 317.8 „	11.6	12.8	4′30″	0.169
E 265.3 „	12.7	16.1	6′40″	0.162
F 289.6 „	14.3	17.3	7′5″	0.160
G 291.9 „	19.—	22.9	9′25″	0.164

[1]) Water collected directly.

From these results it is evident that the rate of assimilation is independent of the velocity of the watercurrent, therefore it was of no consequence that this velocity was not entirely constant in our experiments.

Still more important is a second conclusion which can be drawn from this result, viz. that the CO_2 was available in such an excess that the amount of CO_2 was not a limiting factor. For, decrease of the velocity of the watercurrent means a slower replacing of the water which is streaming along the shoots and which is loosing its CO_2. When this does not influence the rate of assimilation we may conclude that the assimilation has reached its maximum under the circumstances of our experiments.

§ 6. The assimilation and the temperature.

The way of arranging the experiments for different harmless temperatures needs no interpretation after the previous remarks. As to the experiments at injurious temperatures it is quite different, as then the velocity of assimilation is decreasing during the experiment. The only thing to do here is to determine a mean velocity during the period of filling the flask, which in our experiments was 4.5 à 5 minutes, the flasks being changed every 6 minutes. Supposing however the change of the velocity to be regular, then we might consider this mean velocity to be the real velocity 3 minutes after putting on the flask. So when changing the flasks every 6 minutes we thus establish the velocity of the assimilation 3, 9, 15 and 21 minutes after putting on the first flask.

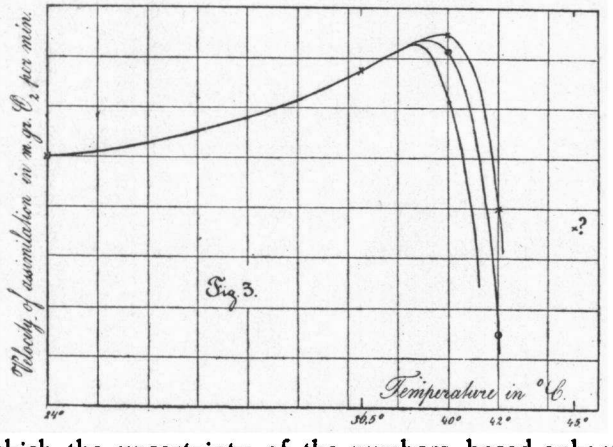
Moreover these experiments present the difficulties that fresh *Helodea*-shoots had to be used for every injurious temperature, so the results could not be compared with one another. This difficulty can be removed by determining the rate of assimilation of the shoots at a same harmless temperature before the beginning of the actual experiment

at the higher temperatures. As one may assume approximately that at other temperatures the velocities of assimilation are related in the same way as at this low temperature, all the results obtained can be reduced to a standard-value and so become comparable.

Now at the beginning of every experiment we always fixed the velocity of assimilation at 24° C. and finally we have calculated all values supposing that at 24° C. the shoots assimilated at a rate of 0.200 mgr. O_2 per minute.

The results are given in the Tables V and VI, the first of which is divided in 4 superdivisions according to the separate series of experiments. The last column of Table V shows the average of the 3 observations about the rate of assimilation at 24° C. In Table VI the recalculated velocities of assimilation are given.

Fig. 3 gives a diagram of the numbers of this last table,



in which the uncertainty of the numbers, based only upon a few experiments, must be taken into consideration.

From Table VI and from our diagram it follows firstly that damage of the assimilation-function only becomes perceptible at about 38° C. after the maximum time of heating which was used, viz about 40′.

Table V.

Relation between rate of assimilation and temperature (2482 candles).

1st Series of experiments.

Temperature.	Begin and end of the collecting of the water.	Indication and contents of the flasks.	cM³. N/100 $Na_2S_2O_3$ per flask.	cM³. N/100 $Na_2S_2O_3$ per 350 cM³. water.	Time for collecting 350 cM³. water.	Rate of assimilation in mgr. O_2 per min.
Water collected directly		A 277.7 cM³.	0.65	0.82	—	—
24° C.	6.6′—6.24′	C 316.9 cM³.	10.4	11.5	4′40″	0.184
		D 317.8 „	10.6	11.6	4′44″	0.185
		E 265.3 „	9.6	12.6	4′50″	0.198
36.5° C.	6.30′—7.0′	F 289.6 cM³.	13.3	16.1	4′40″	0.263
		G 291.9 „	14.3	17.2	4′55″	0.267
		H 314.6 „	14.9	16.6	5′5″	0.250
		I 297.5 „	14.8	17.5	5′10″	0.258
		K 281.2 „	14.1	17.5	5′25″	0.249

2nd Series of experiments.

Temperature.	Begin and end of the collecting of the water.	Indication and contents of the flasks.	cM³. N/100 $Na_2S_2O_3$ per flask.	cM³. N/100 $Na_2S_2O_3$ per 350 cM³. water.	Time for collecting 350 cM³. water.	Rate of assimilation in mgr. O_2 per min.
Water collected directly		A 277.7 cM³.	0.8	1.—	—	—
24° C.	12.20′—12.38′	C 316.9 cM³.	9.3	10.3	4′25″	0.169
		D 317.8 „	9.5	10.5	4′28″	0.169
		E 265.3 „	7.8	10.3	4′30″	0.166
40° C.	11.51′—1.21′.	F 289.6 cM³.	12.8	15.5	4′40″	0.250
		G 291.9 „	12.8	15.4	4′30″	0.257
		H 314.6 „	13.9	15.5	4′30″	0.258
		I 297.5 „	12.2	14.4	4′30″	0.239
		K 281.2 „	10.4	12.6	4′40″	0.206

3rd Series of experiments.

Temperature.	Begin and end of the collecting of the water.	Indication and contents of the flasks.	cM³. N/100 $Na_2S_2O_3$ per flasks.	cM³. N/100 $Na_2S_2O_3$ per 350 cM³. water.	Time for collecting 350 cM³. water.	Rate of assimilation in mgr. O_2 per min.
Water collected directly		A 277.7 cM³.	1.6	2.—	—	—
24° C.	11.30′—11.48′	D 317.8 cM³.	11.9	13.1	4′32″	0.197
		E 265.3 „	10.2	13.5	4′32″	0.204
		F 289.6 „	11.4	13.8	4′45″	0.200
42° C.	11.58′—12.22′	G 291.9 cM³.	9.0	10.8	4′30″	0.158
		H 314.6 „	4.8	5.3	4′32″	0.059
		I 297.5 „	1.4	1.7	4′32″	0
		K 281.2 „	1.4	1.7	4′37″	0

4th Series of experiments.

Temperature.	Begin and end of the collecting of the water.	Indication and contents of the flasks.	cM³. N/100 $Na_2S_2O_3$ per flask.	cM³. N/100 $Na_2S_2O_3$ per 350 cM³. water.	Time for collecting 350 cM³. water.	Rate of assimilation in mgr. O_2 per min.
Water collected directly		A 277.7 cM³.	2.3	2	—	—
24° C.	11.7′—16.25′	C 316.9 cM³.	9.85	10.9	4′50″	0.134
		D 317.8 „	10.30	11.3	4′45″	0.142
		E 265.3 „	8.80	11.6	4′50″	0.144
45° C.	11.35′—11.53′	F 289.6 cM³.	7.40	8.9	4′30″	0.103
		G 291.9 „	2.40	2.9	4′30″	0.0
		H 314.6 „	2.30	2.6	4′35″	0.0

One could try to find through extrapolation the form of the curve, that would represent the relation between temperature and rate of assimilation at higher temperatures when noxious influences could be avoided. However we have given up this extrapolation, partly because we

Table VI.

Velocities of assimilation at different temperatures after different times of heating, recalculated on a velocity at 24° C. of 0.200 m.gr. O_2 per minute.

Time of heating.	Temperature.			
	36.5° C.	40° C.	42° C.	45° C.
3 min.	0.278	0,293	0.157	0.147
9 „	0.282	0.306	0.059	0.000
15 „	0.265	0.303	0.000	—
21 „	0.274	0.285	—	—
27 „	0.264	0.246	—	—

think the numbers obtained somewhat uncertain (a greater quantity of numbers, from which an average could be taken would be necessary), partly because we are convinced that in our experiments we did not determine the relation between the actual velocity of assimilation and the temperature.

§ 7. Final conclusions. By means of the values, to be read in fig. 3, we can calculate the temperature-coefficient $Q_{10} = \frac{V_{t+10}}{V_t}$ for the interval 24° — 34°, the latter temperature being certainly still harmless. When doing this we find a value of 1.26. Now, at such temperatures for most of the physiological processes a higher temperature-

coefficient is found. As a rule this even amounts to a value between 2 and 3, as in most chemical processes. By this circumstance it becomes very improbable that we really did determine the velocity of the assimilation-process itself. This is almost certain when we see nearer at the processes taking place in the *Helodea*-shoots during the experiments.

When those shoots are washed for some time with the water prepared in the way mentioned above, which contains no oxygen nor nitrogen and which is not saturated with CO_2, then all the gas in the intercellular spaces will be replaced by liquid. When light is thrown on it the protoplasm surrounding the grains of chlorophyl will first be saturated with oxygen and after that the cell-walls will be entirely or partly imbued with it. Through these walls the oxygen diffuses, partly directly to the outside, where it is carried off by the streaming water, partly to the water in the intercellular spaces, which is at rest. Now at a somewhat active assimilation this water may be saturated with oxygen.

If the surrounding water was not moved and if the water that is used had not been as free of oxygen as it is now, the diffusion of the CO_2 into the inside would be much quicker than the diffusion of the oxygen to the outside into the surrounding water (the diffusion-velocities in water being in a proportion of 24 : 1, according to Exner). This quicker diffusion would soon cause a saturation of the water in the intercellular spaces (connected with each other) followed by a liberation of gaseous oxygen which at last would escape through the cut end of the shoots.

In other words: here we would come again to the method of counting the liberated gas-bubbles, for the appreciation of which Kniep[1]) gave such correct views.

[1]) l. c.

From the fact, that no gas-bubbles escaped, follows that the produced oxygen was released so quickly to the outside, that either the over-saturation was not attained or not enough gaseous oxygen was produced to fill the intercellular spaces entirely. However in any case the liquid in the intercellular spaces will contain a good quantity of dissolved oxygen.

If the rate of assimilation increases by a rise of temperature it will cause but a small increase of the concentration of the oxygen in the water in the intercellular spaces. So the difference between the concentration of the oxygen inside the *Helodea*-shoots and the oxygen of the surrounding liquid can increase but a little or not at all. It is true, that the velocity of diffusion of the oxygen through the cell-walls to the outside will increase, but the temperature-coefficient of this physical process is small. It is not to be ascertained, whether the release of oxygen through the wall into the surrounding water will be influenced by raising the temperature. Here, indeed an evasion of the CO_2 from the wall and an invasion into the moving water takes place. Now Bohr [1]) demonstrated that the temperature-coefficient of the invasion of gaseous CO_2 in flowing water or saline solutions is small and negative, but that one of the evasion of CO_2 from a solution into the air is small and positive. So it seems very probable that the rate of the release of oxygen through the cell-walls into the surrounding water is modified but little by rise of temperature.

Yet this view cannot stand for the whole explanation. For, if a larger quantity of oxygen was not removed in some way into the surrounding water, when raising the temperature, the intercellular spaces would be entirely

[1]) Wiedemann's Ann. d. Physik u. Chemie. Bd. *62*, 1897. S. 644. Bd. *68*, 1899, S. 500.

filled with oxygen at the end. This gas would be given off in bubbles at the cut end. However it should be noticed that as soon as gaseous oxygen is emitted in these spaces, CO_2-laden water is pushed away from the interior cell-walls. In this way it is made comprehensible that an auto-regulation of the CO_2-assimilation takes place and that this is but little influenced by a rise of temperature. This view also explains the slower assimilation stated at the beginning of the experiments, because some time will have to elapse before the protoplasm, the cell-walls and the water in the intercellulars are saturated with oxygen. Moreover the proportionality between velocity of assimilation and intensity of light stated by us for light of less than 2000 candles comes in another view. For in light of less than 2000 candles we must consider that the auto-regulating action, referred to, will not occur. It is therefore very probable, that this auto-regulation is caused by the fact that the velocity of assimilation at a higher intensity of light has increased at such a rate that the deposit of gaseous oxygen into the intercellulars takes place, whilst this did not occur at a lower intensity. However this consideration renders doubtful the opinion, that the intensity of light could play the part of a limiting factor, which one would feel inclined to draw from our results in § 4.

Though the explanation mentioned above is to a certain degree hypothetical, yet it follows, that physical factors play a part in the experiments with *Helodea*-shoots. So it is very improbable, that the real assimilation-rate was measured. A similar conclusion may be drawn from many experiments of other investigators. Therefore one has to conclude, that only assimilating organisms of a very simple structure without intercellular spaces are fit for these kind of experiments.

This investigation has been carried out in the „Laboratory for Microscopical Anatomy" of the Technical Highschool. Herewith I wish to thank Prof. Dr. G. van Iterson Jr. for all the help he gave me and for enabling me to do these experiments.

Delft, October 1915.