INHIBITION OF SULPHATE REDUCTION IN WATERLOGGED SOILS

By

R. VÁMOS and I. STEFANDEL

Plant Physiology of University, Szeged
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The hidrogen sulphide formed in certain waterlogged soils means very serious problem from the point of view of rice cultivation. Namely the H_2S inhibits the uptake of water and nutrients even it may cause the destruction of the roots (4, 5, 6, 7, 8, 9, 13). The appearance of disease due to this injurious effect (brusone, Brand, aki-ochi etc.) in unfavourable weather conditions, especially with little sunshine, produced considerable damage. Similar weather conditions were in our country in 1949, 1954, 1955. The injury mainly occurs following the stem elongations, when in ALBERDA's (2) opinion amount of oxigen carried into the roots, is reduced. In cool and sunless weather the plant is unable to overcome the H_2S, so the roots die off. In the years without brusone 1950, 1951, 1952, 1956, 1957, 1958 the insolation and warmth were abundant. In these years the injurious effect of H_2S was controled by oxigen secured by vigorous photosynthesis and respiration.

Experiments carried out so far to control the disease (drainage, liming, application of »red lime« and calcium-oxide etc.) have shown favorable effect to a certain degree, the result, however was not satisfactory. Consequently it seemed necessary to find an other control method. The conclusion could be drawn from the experiences that results could be reached rather by inhibiting the formation of H_2S than the binding the liberated H_2S. The H_2S is formed by protein decomposition and chiefly by sulphate reduction (1, 3, 10, 12, 13, 14). The bacteria reducing the sulphates utilized the hydrogen as energy source formed by fermentation of cellulose and other organic matters (10, 12, 13). In acidic condition H_2S is liberated from the sulphides which injured the rice plant in different ways. The circumstances and factors of H_2S formation are shown in *Fig. 1.* (12, 13, 14).

Laboratory experiments were made on the inhibition of H_2S formation.

Material and method

Limeless meadow soil and limeless alkali soil *(szik)* »inclined« to disease, were used. The organic matters not decomposed were removed from the soils grown in aerated condition and passed throw 1 mm sieve.

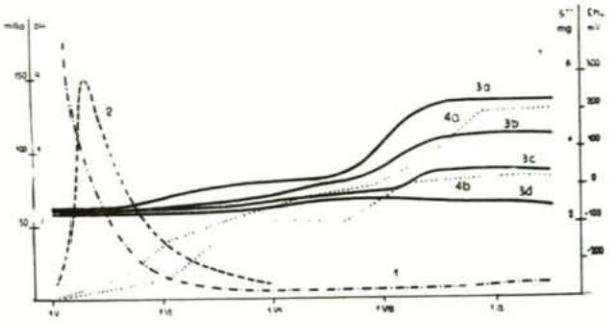

Fig. 1. Fields examinations

Curve No. 1. = Eh_7,
No. 2. = number of aerobic bacteria.
No. 3a, 3b, 3c, 3d = pH-values of flood water. 3a = lime free alkali soil in 1955. 3b = lime free meadow soil in 1955. 3c = lime free meadow soil in 1956. 3d = lime free meadow soil in 1957.
No. 4a = S−−mg/100 g in lime free alkali soil. 4b = S−− mg/100 g in lime free meadow soil.

1,5 g cotton was placed between the wet clay disks made of these soils, except the control pots. The cotton was soaked with sulphate and sulphate-nitrate solutions. Then the disks were placed in aluminium boxes lined with glass, then in thermostat at 28 C°.

Table 1 shows the results of the basal examinations of the soils used.

Table 1.

Type of soils	„inclination„	pH		alkalinity as Na_2CO_3	hidr. acid.	$CaCO_3$ in top soil %	Total salts %
		H_2O	KCl				
meadow soil	inclined	6,6	5,3	no	5,0	22	0,14
limeless alkali soil	inclined	6,4	5,3	no	6,8	no	0,11
alkali soil rich in lime	not inclined	8,2	—	0,09	—	no	0,26

Experimental

Exp. No. 1. Limeless meadow soil, limeless alkali soil and alkali soil rich in lime were put in boxes in water saturated conditions without cotton, as described above. The sulphide content of the boxes were determined on the 14. and 24. day following the start.

Sulphide was formed in traces or in very small amount in meadow soil and limeless alkali soils. No sulphide formed in alkali soil rich in lime.

Exp. No. 2. To demonstrate the relation of fermentation of cellulose and of sulphate reduction limeless meadow soil was placed in 4 boxes, limeless alkali soil and alkali soil rich in lime in 2—2 boxes with 1,5 g cotton between the soil disks. The 2 boxes containing meadow soil were sterilized in autoclave. After 14 days is was stated that the cotton and contiguous soil layer in the boxes containg limeless alkali soil and meadow soil, became black due to the ferrous sulphide as, H_2S and the ferrous compounds of the soil formed ferrous sulphide *(Fig. 2.)* Ferrous sulphide could be observed neither in sterilized meadow soil nor in alkali soil rich in lime.

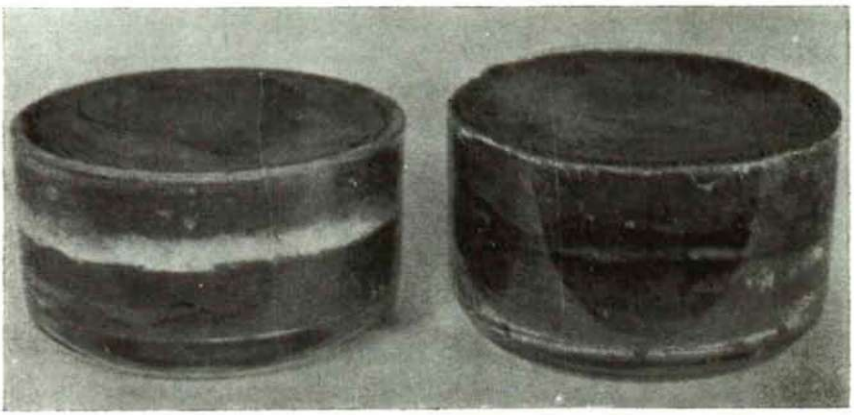

Fig. 2.

Exp. No. 3. In order to increase the sulphate reduction in limeless meadow soil and alkali soil rich in lime 30 mg $(NH_4)_2SO_4$ in 2 boxes and 30 mg Na_2SO_4, also in 2 boxes, were soaked by cotton. Here was observed that in meadow soil $(NH_4)_2SO_4$ increased the sulphide formation. The sulphide formation in the alkali soil rich in lime failed again, although the considerable number of butyric acid bacteria and vigorous gas-formation indicated, that the fermentation of cellulose was promoted by $(NH_4)_2SO_4$. *(Fig. 3).*

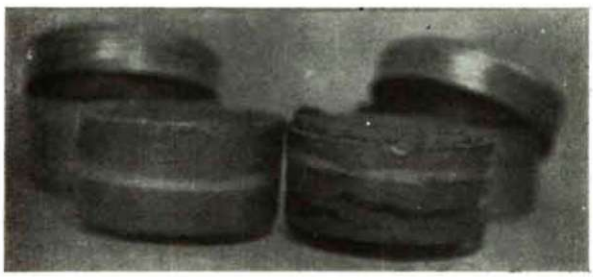

Fig. 3.

Exp. No. 4. The further experiments were carried out only with meadow soils. In these experiments 30 mg $(NH_4)_2SO_4$, 30 mg NH_4NO_3 and 30 mg KNO_3 were soaked by cotton in the separated boxes. After the incubation could be stated that merely the cotton treated with $(NH_4)_2SO_4$ become black, while the cotton treated with nitrates remained white in all the boxes and no sulphide was formed.

Exp. No. 5. To elucidate whether the nitrate treatment is able to control H_2S formation in the presence of a greater amount of $(NH_4)_2SO_4$ the following experiments were made. 80 mg $(NH_4)_2SO_4$ was soaked in 6 boxes containing limeless meadow soil, 2 of the 6 boxes were the controls, 30 mg KNO_3 and 30 mg NH_4NO_3 were added to the remaining 2—2 boxes. Following incu-

bation no change occured in the results, as the ferrous sulphide formation failed again in the boxes treated by nitrate. The results are in the *Table 2.*

	Type of soil	Treaments	Smg/100 g	
1	meadow soil	without cotton	0,58	0,48
	limeless alkali soil	„ „	traces	traces
	alkali soil rich in lime	„ „	no	no
2	meadow soil	cotton	11,2	13,0
	„ „	„ (sterilized)	no	no
	limeless alkali soil	cotton	7,2	5,8
	alkali soil rich in lime	„	no	no
3	meadow soil	cotton + 30 mg $(NH_4)_2SO_4$	20,2	16,7
	„ „	„ ± 30 mg Na_2SO_4	8,8	—
	alkali soil rich in lime	„ + 30 mg $(NH_4)_2SO_4$	no	no
	„ „	„ + 30 mg Na_2SO_4	no	no
4	meadow soil	cotton 30 mg $(NH_4)_2SO_4$	20,2	16,7
	„	„ 30 mg NH_4NO_3	no	no
	„	„ 30 mg KNO_3	no	no
5	meadow soil	cotton + 80 mg $(NH_4)_2SO_4$	26,4	19,8
	„	„ „ „ + 30 mg NH_4NO_3	no	no
	„	„ „ „ + 30 mg KNO_3	no	no

Fom the abowe results the conclusions can be drawn:

1. No H_2S liberated from the alkali soils rich in lime, where the brusone failed.

2. The sulphate reduction can not occur without hydrogen forming processes. SZABOLCS, MÁTÉ et al. (11) could not demonstrate sulphide, due to sulphate reduction in their investigation with S^{35} without hydrogen source. The above statement is supported by their experiments. The presence of the hydrogen forming bacteria was found an the basis of the pigment in soil samples examined.

Clostridium werneri Bergey et al	brown pigment
Cl. cellulosolvens Cowles et Rettger	no pigment
Cl. dissolvens Bergey et al.	yellow pigment
Cl. omelianskii Hennberg	winered pigment

3. The presence of nitrate ions inhibits the sulphate reduction. Probably the nitrate-ions are reduced by atomic hydrogen due to cellulose fermentation:

$$KNO_3 + 8\,H = KOH + NH_3 + 2H_2O$$

The sulphate reducers are missing the hydrogen as their energy-source,

Beside the chemical reduction also microbiological nitrate-reduction occurs in the mud. Due to these processes nitrite is also formed from the

nitrate, the presence of which has a toxic effect on the autotrophic sulphate reducers. The presence of nitrite-ions has been repeatedly demonstrated from the water pressed from the cotton.

Fig. 4. Clostridium dissolvens

Fields experiments

These experiments are under way. The have a positive effect on the basis of the observation so far made. For this purpose the ammonium nitrate mixed with powered lime (Hungaria product: »*Péti só*«) proved to be the most suitable. The roots keep their white colour on the place of the application. Lateral roots with healthy root-hair developed abundantly. Atemps are being made to determine time of application, reduction rate of nitrate i. e. duration of its favourable effect in our futher examinations.

Summary

The hydrogen sulphide formed in waterlogged soils means a serious problem in rice cultivation. It inhibits the water and nutrients uptake, even may cause root-rot. The disease due to these injurious effects (browning disease, »brusone«, Brand, aki-ochi, ect.) under unfavourable weather conditions, chiefly little insolation brought about considerable damages (i. e. in years: 1949, 1954, 1955). The injury appeared mainly following the stem-elongation, when the oxigen supply in the root is greatly reduced.

My previous experiments for controling the injuries showed that results can be obtained nor with H_2S-binding, but by inhibition of its formation. The H_2S is mainly formed by sulphate reduction, for which the celluloze (butyric acid) fermentation procides the hydrogen source.

To eliminate the hydrogen required for the reduction experiments were carried out in laboratory and in fidels. 1,5 g cotton placed between wet rice

soil disks in glasses was soaked in 30—80 mg/100 g $(NH_4)_2SO_4$ and Na_2SO_4, moreover in some glasses 30—50 mg/100 g NH_4NO_3 or KNO_3. 14 days after waterlogging and incubation 8,8—26,0 mg sulphide was formed in the controls and only in the glasses containing sulphates. While in the glasses containing nitrates beside the sulphates the sulphide formation failed.

The atomic hydrogen formed by butyric acid fermentation in the reduction of the nitrate-ions was utilized, consequently could not be utilized by the sulphate reducing bacteria, as a energy-source.

The experiments in fields yielded also positive results and for this purpose the ammonium nitrate mixed with powdered lime proved to be the most suitable.

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