Studies on the Scheme of Decomposition of Oxalic Acid by Some Wood-rotting Fungi, and its Effect on the Change of pH in a Glucose Malt Extract Medium

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Introduction

According to the previous work,[1] three following types of change of pH in the medium were recognized; (cf. Fig. I)

(1) The acidity of the medium increased in about ten days till its pH value reached a min. of ca. 4.0, and then reversed direction of the pH change and the reaction of the medium turned remarkably to alkaline side.

(represented by *Schizophillum commune* FR.)

(2) The acidity of the medium increased in about 15~30 days till its pH value reached a min. of ca, 4.0, and then the pH value remained almost constant, approaching slightly to alkaline side.

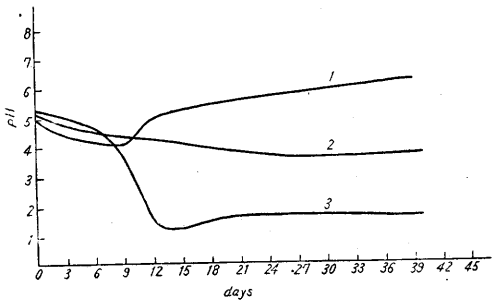
(represented by *Cortinellus edodes* (BERK.) SAWADA)

(3) The acidity of the medium increased in about twelve days till the pH value reached a min. of ca, 1.8, and stayed on the same pH.

(represented by *Poria vaporaria* FR.)

MONTOGOMERY[2] (1936) stated his view that the reversal in the direction of the pH change was caused by the free ammonium ion released from a mycelium which was autolysed after the growth

Fig. 1. Change of pH in glucose malt extract medium.
1 ·····*Schizophillum commune* FR.
2 ·····*Cortinellus edodes* (BERK.) SAWADA
3 ·····*Poria vaporaria* FR.



reached the max.. BIRKINSCHAW[3] (1940) made a similar experiment on the change of pH in the solution of malt in which various fungi were growing, and in relation to the reversal in the direction of the pH change, it was concluded that the acids first formed was later oxidised, being used after the depletion of the sugars as a source of energy, and white rot fungi gave a positive test for oxidase. As the result of previous authors' work,[1] it was recognized that CO_2 output was remarkably larger amount than O_2 uptake in the respiration of *Schizophillum commune* FR. in a glucose malt extract solution. This excess CO_2 output for ordinary respiration was supposed to have been caused by the decomposition of oxalic acid which the fungus produced.

Experiments

Warburg apparatus was available to measure the volume of O_2 uptake and CO_2 output with fungous mats in a solution of oxalic acid at 30° C, and an increase or decrease of oxalic acid during this experiment was titrated by 1/100 N $KMnO_4$ solution.

Table. 1

oxalic acid in 10 ml distil. water g	pH	consumed volume of 1/100 N $KMnO_4$ (F=0.817) in 10 ml of the solution ml
0.01	1.8	19.80
0.0091	4.4	2.30
0.00083	5.4	0.45
0	7.0	0

Discs were cut from mycelial mats with a cork borer 11 mm in diameter, and were washed in distilled water for one hour. Oxalic acid solutions were prepared in various concent-

Fig. 2. *Schizophillum commune* FR.

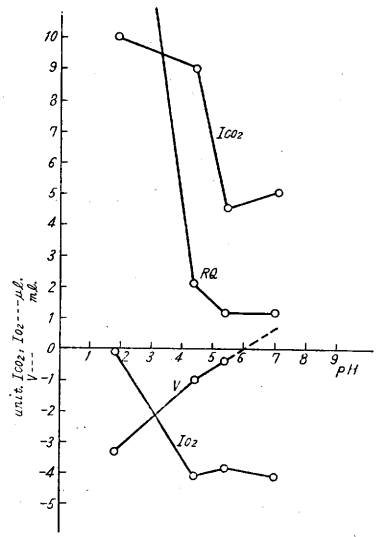
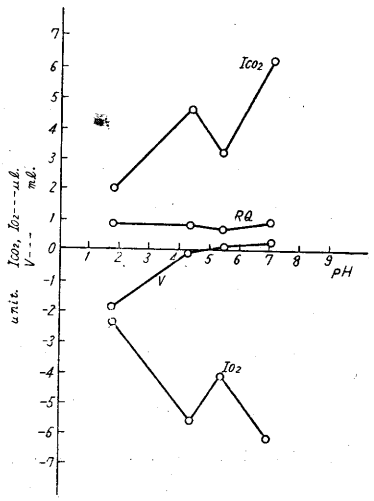


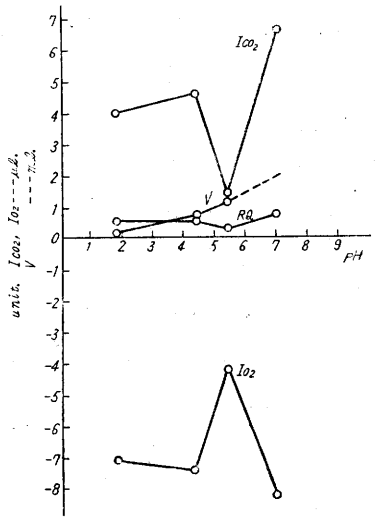
Fig. 3 *Cortinellus edodes* (BERK.) SAWADA



rations as in table 1.

Two pieces of discs were placed in vessels containing 10 ml oxalic acid solution, and KOH plus filter paper wicks were used for determination of O_2 uptake. Results are showed in Fig. 2, Fig. 3 and Fig. 4.

Fig. 4. *Poria vaporaria* FR.



Io_2 = μl O_2 taken up per hour

Ico_2 = μl CO_2 given off per hour

$RQ = Ico_2/Io_2$

$V = b - a$ ml

a……value titrated in initial 10 ml substrate by 1/100 N $KMnO_4$

b……value titrated in 10 ml substrate by 1/100 N $KMnO_4$ after measuring the gas exchange

V shows the volume corresponding to oxalic acid increased during the measurement of the gas exchange.

Schizophillum commune FR. (Fig. 2)

Io_2 decreased but Ico_2 remarkably increased and V decreased with increasing concentration of oxalic acid in substrate. At pH 1.8 substrate, the large amount of CO_2 was liberated but O_2 uptake was approximately zero.

Cortinellus edodes (BERK.) SAWADA (Fig. 3)

Io_2 and Ico_2 decreased with increasing concentratin of oxalic acid in substrate. V increased a little within the range of pH 4.0~7.0, but decreased within the range of pH 4.0~1.8 of the substrate.

Poria vaporaria FR. (Fig. 4)

Io_2 and Ico_2 decreased with increasing concentration of oxalic acid in substrate, but V increased with decreasing concentration of oxalic acid in substrate.

The result of *Schizophillum commune* is noticeable in respect to the fact that a great deal of CO_2 was put out, though O_2 uptake was little.

The conceivable schemes to decompose oxalic acid are as follows;

1. $(COOH)_2$ + hydrogen acceptor $\rightarrow CO_2$
2. $(COOH)_2 + O_2 \rightarrow H_2O_2 + 2CO_2$, $H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2$
3. $(COOH)_2 \rightarrow HCOOH + CO_2$

1. FODOR[4] (1930) observed the hydrogen donator function of oxalates in the seed of corn using the Thunberg method. OGURA and NAGAHISA[5] (1937) investigated the

hydrogen donator function of various substrate with *Aspergillus oryzae*, and oxalate showed rather negative hydrogen donator function. With *Schizophillum commune* Fr., it was determined by the time needed for reduction of methylene blue by Thunberg method.

The composition of reaction mixture was as follows : mycelium 1.0 ml, 1/5 M phosphate buffer 0.3 ml, 1/10 M oxalate or distil. water 0.3 ml, 0.02 % methylen blue 0.2 ml. Test tube was evacuated to 10 mm Hg after the mixture was added, and then placed in a water bath at 30° C. Result obtained is shown in table 2, which reveals that oxalate have a weak hydrogen donator function within the range of pH 6~8, but rather negative within the range of pH 3~5.

Table. 2.

pH	3	4	5	6	7	8
add. oxalate	95	74	75	48	25	30 min.
add. distil. water	85	62	73	60	32	35 min.

2. Wilhelm Franke[6] (1937) investigated the oxaloxidase of mosse and decided its aerobic decomposition scheme.

3. According to the above results by the authors, anerobic decomposition to formic acid and CO_2 may be conceivable in connection wood-rotting fungi. For the purpose of confirming this scheme, next experiment was carried out. Mycelium of wood-rotting fungi (*Schizophillnm commuue* Fr. dry weight 90 mg) was put in 50 ml of 50 % oxalic acid solution, and the air in vessel was changed with N_2 gas. After keeping at 25° C constant, the mycelium was removed and $Ca(OH)_2$ solution was added to make it neutral, a small quantity of the produced precipitation was filtered off, and the filtrate was used for determination of formic acid :

(a) After adding 1 cc aq. $HgCl_2$ to 2 cc of the filtrate, the mixture was heated to boiling and the boiling was continued for 1/2 min. A white ppt. (Hg_2Cl_2) indicated the existence of formic acid in the filtrate.

(b) After adding 2 cc of aq. $AgNO_3$ to the equal volume of the filtrate, the mixture was boiled till liquid became brown. Then a grey ppt. of silver appeared.

(c) After adding 2 cc of aq. resorcin, conc. H_2SO_4 to 2 cc of the filtrate, reddish orange colour of the solution did not change by heating.

As the result of the above three tests, it was confirmed that oxalic acid was decomposed to formic acid and CO_2 anerobically by fungous mycelium (*Schizophillum commune* Fr.).

Discussion

According to the above result, the reversal in the direction of the pH change is not only due to the NH_4 ion or oxidase, but also due to the decomposition of oxalic acid. It is conceivable that the increasing of alkali over the range of pH 5.0 is caused by NH_4 ion released from the mycelium. (cf. Fig. 2, *Schizophillum commune* Fr.)

NH_4 ion released per day may be caluculated :

$$[OH]^- = 1.0/18 \text{ pH/day} \fallingdotseq 5.5\times10^{-7}[OH]^-/\text{day}$$

Its concentration is too small to give the effect of reducing the stronger acidity in the range of pH 4.4~5.0. In this range, the change of acidity caused by decomposition of oxalic acid must play the important part of the reversal. (cf. Table. 3)

When the oxalic acid changes into the formic acid, the effect of its decomposition on pH of the solution increases with the concentration of oxalic acid.

Table. 3.

concentration pH	10^{-2}	10^{-3}	10^{-4}	10^{-5}
pH of oxalic acid solu.	0.90	3.04	3,90	4.89
pH of formic acid solu.	2.89	3.78	4.13	4.91

The growth of *Cortinellus edodes* (Berk.) Sawada is very slow compared with the other wood-rotting fungi, and if $CaCO_3$ is given in the medium, the amount of oxalic acid obtained is as large as 29 % of sugar (Nakao, Sakaguchi[7] (1942)), therefore the reason why the pH of a medium remains almost the same value after reaching the max. of 4.0 may be attributed to the balance between the production of oxalic acid, and the decomposition of it and the release of NH_4 ion.

Poria vaporaria Fr. may have weak ability to decompose oxalic acid, and produses more acid, therefore acidity in its medium increases to the max. acidity of 1.8 and then NH_4 ion released by mycelium is too weak to neutralize the strong acidity.

Summary

(1) O_2 uptake and CO_2 output in the oxalic acid solution was measured with the three wood-rotting fungi; *Schizophillum commune* Fr., *Cortinellus edodes* (Berk.) Sawada, *Poria vaporaria* Fr.

(2) It was revealed that *Schizophillum commune* Fr. decomposed the oxalic acid to formic acid and CO_2.

(3) Relationship between the reversal in the direction of pH change in the medium and the decomposition of oxalic acid was explained.

Species of the wood-rotting fungi used in this experiment originated from our laboratory.

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木材腐朽菌による蓚酸の分解機構と其の培養基 pH に及ぼす影響（摘要）

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文部教官　福　住　俊　郎
　　柳　川　林　八

(1) 木材腐朽菌（スエヒロタケ，シイタケ．ワタグサレタケ）菌蓋の蓚酸溶液に於ける酸素吸収量及び炭酸ガス発生量を Warbrug 氏検圧装置を用いて測定した．

(2) 特にスエヒロタケは顯著に蓚酸を分解し，蟻酸と炭酸ガスにすることが認められた．

(3) 木材腐朽菌培養液の pH の変化と蓚酸の分解との関係について考察した．