Soil Sci. Plant Nutr., **23** (1), 9–19, 1977

DRYING EFFECT ON MINERALIZATIONS OF MICROBIAL CELLS AND THEIR CELL WALLS IN SOIL AND CONTRIBUTION OF MICROBIAL CELL WALLS AS A SOURCE OF DECOMPOSABLE SOIL ORGANIC MATTER DUE TO DRYING

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Received May 20, 1974

1) The mineralization of several kinds of microbial cells added to soil were accelerated considerably by the drying effect.

2) When microbial cells were roughly divided by mechanical procedure into two parts, *i.e.*, cytoplasmic and cell wall substances, and separately added to soil with or without drying previously, the former was mineralized very quickly both with and without drying previously and its mineralization was not accelerated by the drying effect. The latter without drying previously was mineralized rather slowly, and the latter with drying previously was mineralized very quickly and remarkably. Furthermore, the former with and without drying previously left hardly any residual matter in soil, but the latter without drying previously left considerable residual matter because of making a complex resistant to microbial decomposition with colloid materials such as clay minerals and humus, and mineralization of the residual matter was remarkably accelerated by the drying effect.

3) From the results mentioned above, it may be concluded that microbial cell wall substances remaining in soil clearly contribute as a source of soil organic matter becoming decomposable due to drying.

From the results of previous papers (*9*, *10*), it was assumed that microbial cells and their cell walls contributed as a source of soil organic matter becoming decomposable due to drying (hereinafter referred to as the decomposable soil organic matter). In this paper, therefore, several experiments were carried out as to the effect of drying on mineralization of microbial cells and their cell walls added to soil.

EXPERIMENT 1 MINERALIZATION OF FRESH AND DRIED MICROBIAL CELLS IN SOIL

Materials and methods

Ten g of artificial soil (8 g of sea sand, 0.25 to 0.50 mm in diameter+2 g of mont-

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Table 1. Culture media.

A.niger, P. chrysogenum, S. cerevisiae, C. lipolitica		*Ar. simplex, B. subtilis, M. ammoniaphilum*	
Glucose	20.0 g	Meat extract	10.0 g
Peptone	5.0 g	Peptone	10.0 g
Yeast extract	2.0 g	NaCl	2.0 g
KH_2PO_4	1.0 g	Distilled water	1 liter
$MgSO_4 \cdot 7H_2O$	0.5 g	pH	6.8–7.0
Distilled water	1 liter	Shaking culture, at 30°C overnight	
pH	5.6–5.8		
Shaking culture, at 30°C for 2 to 3 days			

morillonite clay prepared from paddy soil taken at the Agricultural Experiment Station in Nagano Prefecture) was weighed in a 500 ml incubation bottle (*13*). Microbial cells added to soil were *Aspergillus niger* (fresh and dried), *Saccharomyces cerevisiae* (fresh and dried), *Penicillium chrysogenum* (dried), *Candida lipolitica* (dried), and *Microbacterium ammoniaphilum* (dried). The microbial cells were cultured with shaking in each culture medium shown in Table 1. They were then collected and washed with distilled water four times. Dried cells were prepared by the drying of fresh cells at 100°C for 1 hr and at 70°C overnight, and ground in a mortar (<60 mesh). After they were added, the mineral nutrients and the inoculum were also added to soil as described in the previous paper (*10*). Soil was adjusted with distilled water to bring the moisture content to 60 per cent of the maximum water holding capacity and to pH 6.5, and incubated at 30°C.

Carbon evolved as CO_2 during the incubation period was determined by a gravimetry method (*13*). Total carbon was determined by Tyulin's method (*6*). Mineral nitrogen in soil was extracted with N KCl and determined by Conway's microdiffusion method using Devarda's alloy as a reducing agent (*5*). Total nitrogen was determined by micro-Kjeldahl method using reduced iron as a reducing agent (*12*).

Results and discussion

Mineralization of microbial cells in soil is shown in Table 2. The mineralization rates of fresh and dried cells of *A.niger* were 23.0 per cent in C and 17.2 per cent in N; and 39.9 per cent in C and 27.4 per cent in N, respectively. The mineralization rates of those of *S.cerevisiae* were 14.1 per cent in C and 11.4 per cent in N; and 59.0 per cent in C and 46.6 per cent in N, respectively. In the cases of three dried cells, the rates were from 54.5 to 64.8 per cent in C and from 40.9 to 51.6 per cent in N, respectively.

From these results, the mineralization rate of dried cells was clearly higher than that of fresh cells, though its values varied more or less among the kinds of microorganisms added. It is suggested that mineralization of microbial cells in soil may be considerably accelerated due to drying of soil. In such a drying effect it is considered that the change of decomposability of microbial cells due to their death by drying may be included. As described later, however, the fact that the drying significantly affected mineralization of microbial cell wall substances indicates due to the accelerating effect

Table 2. Mineralization of microbial cells.

Microbial cells	Amounts of cells added		C/N ratio	Mineralized for 2 weeks		Mineralization rate	
	C	N		C	N	C	N
	(mg/100 g soil[1])			(mg/100 g soil[1])		(%)	
A. Fresh	230	19.2	11.9	53	3.3	23.0	17.2
A. Oven-dried	178	17.5	10.2	71	4.8	39.9	27.4
S. Fresh	277	45.6	6.1	39	5.2	14.1	11.4
S. Oven-dried	404	62.0	6.5	236	28.9	59.0	46.6
P. Oven-dried	420	55.0	7.6	256	22.5	61.0	40.9
C. Oven-dried	420	89.2	4.7	272	46.0	64.8	51.6
M. Oven-dried	211	51.0	4.2	115	26.0	54.5	51.0

A: A. niger, S: S. cerevisiae, P: P. chrysogenum, C: C. lipolitica, M: M. ammoniaphilum.

[1] 8 g of sea sand+2 g of montmorillonite clay prepared from paddy soil taken of the Agricultural Experiment Station in Nagano Prefecture.

on mineralization of cells due to drying itself.

Furthermore, mineralization rates varied among the microbial species. It is considered that the variation may depend on the differences of structure and components of microbial cell walls. Several workers (*1–3, 7, 8*) reported that mineralization of fungi in soil depended on the contents of a lignin-like substance, a melanic substance, and fucose which were contained in fungi cell walls. In any case, it is assumed that mineralization rate of dried cells might be higher than that of fresh cells.

So, Experiment 2 as to the drying effect on mineralization of microbial cells and their cell wall substances in soil were carried out.

EXPERIMENT 2 DRYING EFFECT ON MINERALIZATION OF MICROBIAL CELLS AND THEIR CELL WALL SUBSTANCES IN SOIL

Materials and methods

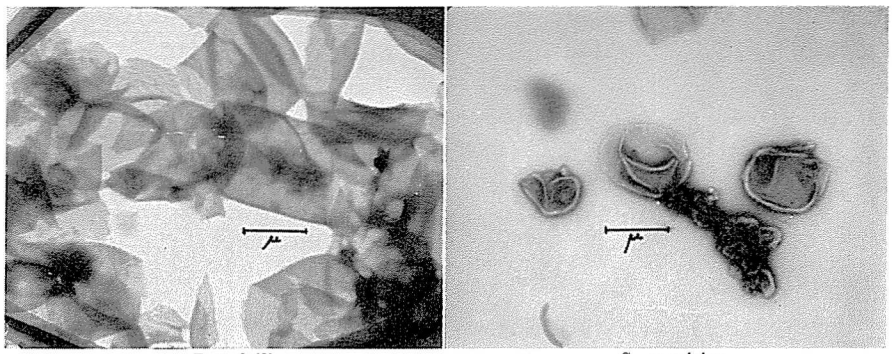
A) Arthrobacter simplex and Bacillus subtilis: Ten g of sea sand or artificial soil (8 g of sea sand+2 g montmorillonite clay prepared from paddy soil taken at Ariake, Saga Prefecture) was weighed in a 500 ml incubation bottle as in Experiment 1, respectively. Fresh cells of *Ar.simplex* or *B.subtilis* prepared by shaking culture of the medium shown in Table 1 were added to sand or soil respectively. They were incubated under the same experimental conditions as in Experiment 1. Just after the addition of fresh cells, a part of them were oven-dried at 80°C for 2 hr, moistened and incubated under the same experimental conditions. The non-heat treatment and heat treatment samples were shown as "Control" and "Oven-dried" respectively, to be described later.

Preparation of cytoplasmic and cell wall substances contained in both bacteria were made by using the ultrasonic equipment of Kaijo Denki Co. The procedure is shown in Table 3. Electron micrographs of cell wall substances after ultrasonic treat-

Table 3. Preparation of cytoplasmic and cell wall substances.

Cells
↓
→ Ultrasonic treatment
19.5 kHz
Ar. simplex, *B. subtilis* : 20 min
S. cerevisiae : 25 min
↓
Centrifuge
3,000–4,000 rpm, 20 min
↓ ↓
—ppt. sp.
(Undestroyed cells)

Centrifuge
10,000 rpm, 10 min
↓ ↓
ppt. sp.
(Cell wall substances) (Cytoplasmic substances)



B. subtilis *S. cerevisiae*

Fig. 1. Electron micrographs of cell wall substances after ultrasonic treatment (×10,000).

ment are shown in Fig. 1. These substances were added to soil respectively and incubated as mentioned above.

Regarding cell wall substances from *B.subtilis*, the air-drying effect (air-dried at room temperature for 1 week) on their mineralization in three soils (Table 4) was examined. Total carbon evolved in the experiment was calculated as follows: carbon evolved as CO_2 during the air-drying treatment was determined and added to carbon mineralized during the incubation period. Carbon and nitrogen mineralized from cell wall substances were calculated as follows: carbon and nitrogen mineralized from soil without addition of cell wall substances was determined, and these values were subtracted from those of soil with such additions. Though a little carbon and nitrogen were mineralized from the soil without such additions during the incubation period,

Table 4. Properties of soils.

Soil	Texture	Major clay mineral	Clay content	Total C	Total N	Mineral N	CEC
				(%)		(mg/100 g soil)	(me/100 g soil)
Ushiro-kawachi	Lic	Kaolin	40.2	0.23	0.04	8.2	11.8
Isahaya	Lic	Mont-morillonite	44.2	0.40	0.06	12.0	31.9
Choyo	L	Allophane	14.5	0.47	0.01	6.2	11.5

Soils were treated with H_2O_2 solution and soil organic matter was removed. These treatments were repeated four times. After washing with distilled water, soils were air-dried and ground in a mortar.

Table 5. Drying effect on the mineralization of cells (*Ar. simplex, B. subtilis*).

		Amounts of cells added	Treatment	Mineralization of cells			
				for 2 weeks		for 4 weeks	
				Amounts mineralized	Drying effect	Amounts mineralized	Drying effect
Carbon				(mg C/100 g sand[1] or soil[2])			
Ar.	Sand	50	Control	28	2	39	1
			Oven-dried	30	(4.0)	40	(2.0)
		100	Control	52	4	64	4
			Oven-dried	56	(4.0)	68	(4.0)
	Soil	100	Control	52	3	60	4
			Oven-dried	55	(3.0)	64	(4.0)
B.	Soil	71	Control	41	2	51	2
			Oven-dried	43	(2.8)	53	(2.8)
		142	Control	72	4	85	4
			Oven-dried	76	(2.8)	89	(2.8)
Nitrogen				(mg N/100 g sand[1] or soil[2])			
Ar.	Sand	16.5	Control	9.8	0.4	14.2	0.4
			Oven-dried	10.2	(2.4)	14.6	(2.4)
		33.0	Control	18.0	1.3	18.4	1.0
			Oven-dried	19.3	(3.9)	19.4	(3.0)
	Soil	33.0	Control	15.9	0.6	17.2	1.0
			Oven-dried	16.5	(1.8)	18.2	(3.0)
B.	Soil	23.6	Control	11.7	0.9	12.5	0.7
			Oven-dried	12.6	(3.8)	13.2	(3.0)
		47.2	Control	22.6	1.7	25.3	1.3
			Oven-dried	24.3	(3.6)	26.6	(2.8)

Ar: *Ar. simplex,* *B:* *B. subtilis.*

Figures in parenthesis show the rate of drying effect: (Drying effect)/(Amounts of cells added) × 100 (%).

[1] Sea sand (diameter: 0.25 to 0.50 mm). [2] 8 g sea sand+2 g of montmorillonite clay prepared from paddy soil taken at Ariake, Saga Prefecture.

Table 6. Drying effect on the mineralization of cytoplasmic substances (*Ar. simplex*, *B. subtilis*).

	Amounts of cytoplasmic substances added	Treatment	Mineralization of cytoplasmic substances			
			for 2 weeks		for 4 weeks	
			Amounts mineralized	Drying effect	Amounts mineralized	Drying effect
Carbon			(mg C/100 g soil[1])			
Ar.	53	Control	31	− 1	40	0
		Oven-dried	30		40	
	106	Control	57	− 6	65	− 6
		Oven-dried	51		59	
B.	69	Control	43	− 2	52	− 2
		Oven-dried	41		50	
	138	Control	75	−11	88	−11
		Oven-dried	64		77	
Nitrogen			(mg N/100 g soil[1])			
Ar.	21.1	Control	11.2	−0.4	15.4	−0.1
		Oven-dried	10.8		15.3	
	42.2	Control	23.8	−0.6	26.2	−0.1
		Oven-dried	23.2		26.1	
B.	28.2	Control	14.8	−1.8	16.0	−0.2
		Oven-dried	13.0		15.8	
	56.4	Control	31.0	−1.9	34.1	0.5
		Oven-dried	29.1		34.6	

Ar: Ar. simplex, *B: B. subtilis*.

[1] 8 g of sea sand+2 g of montmorillonite clay prepared from paddy soil taken at Ariake, Saga Prefecture.

Table 7. Drying effect on the mineralization of oven-dried cell wall substances (*B. subtilis*).

Amounts of cell wall substances added (1)	N mineralized for 2 weeks		Drying effect	Rate of drying effect
	Control (2)	Oven-dried (3)	(3)−(2)	$\frac{(3)-(2)}{(1)}\times 100$
	(mg N/100 g sand[1])			(%)
22.3	7.2	8.5	1.3	5.8
65.3	24.6	28.7	4.1	6.3

[1] Sea sand (diameter: 0.25 to 0.50 mm).

the drying effect was not recognized. Furthermore, the fixation of ammonium during drying treatment and the loss of nitrogen during the incubation period did not occur under these experimental conditions.

Analytical procedures were made according to Experiment 1.

B) S.cerevisiae: Preparation of cytoplasmic and cell wall substances of *S.cerevisiae*, experimental conditions and analytical procedures were all made as described in A) above.

Table 8. Drying effect on the mineralization of air-dried cell wall substances (*B. subtilis*).

Soil	Amounts of cell wall substances added (1)	Mineralization of cell wall substances for 2 weeks		Drying effect (3)−(2)	Rate of drying effect $\frac{(3)-(2)}{(1)}\times 100$
		Control (2)	Air-dried (3)		
Carbon			(mg C/100 g soil)		(%)
U	229	148	162	14	6.1
I	229	107	128	21	9.2
Nitrogen			(mg N/100 g soil)		(%)
U	75.3	35.1	38.5	3.4	4.5
I	75.3	26.0	30.6	4.6	6.1
C	75.3	25.8	30.5	4.7	6.2

U: Ushirokawachi, I: Isahaya, C: Choyo.

Results and discussion

A) Ar.simplex and B.subtilis: The drying effect on mineralization of cells is shown in Table 5. An acceleration effect on mineralization of cells due to drying was recognized and the rates of the drying effect in *Ar.simplex* and *B.subtilis* were 2.0 to 4.0 per cent in C and 1.8 to 3.9 per cent in N; and 2.8 per cent in C and 2.8 to 3.8 per cent in N, respectively. As to the results at 2 to 4 weeks of incubation, the mineralization of cells increased as time went by, but the rates of the drying effect were almost equal in both microbial cells and about 2 to 4 per cent.

From the results obtained, it was indicated that mineralization of microbial cells was accelerated by oven-drying. So, after separating roughly two parts, cytoplasmic and cell wall substances, by mechanical procedure, experiments as to the drying effect on mineralization of their substances were carried out.

The drying effect on mineralization of cytoplasmic substances is shown in Table 6. They were mineralized very quickly and their mineralization was not accelerated by drying. The drying effect on mineralization of protein (gelatin) in soil was also examined, and it was not recognized.

The drying effects on mineralization of cell wall substances of *B.subtilis*, *i.e.*, the oven-drying and the air-drying effects, are shown in Table 7 and Table 8, respectively. As seen in Table 7, mineralization was clearly accelerated by oven-drying. Rates of the drying effect were higher on cell wall substances (5.8 to 6.3 per cent) than on cells (2.8 to 3.8 per cent, Table 5). As seen in Table 8, the air-drying effect was clearly recognized and the rates were higher in cell wall substances than in cells. The rates of mineralization in three soils became lower in the following order: Ushirokawachi > Choyo ≒ Isahaya, but the rates of air-drying effect became lower in the following order: Choyo ≒ Isahaya > Ushirokawachi. These differences among the soils may be caused by the kinds of clay, clay content, free iron content, *etc.* The cause of the differences is a subject for future study.

Table 9. Ultrasonicating effect on the mineralization of cell wall substances (*B. subtilis*).

Amounts of cell wall substances added	N mineralized for 2 weeks		Ultrasonicating effect	Rate of ultrasonicating effect	N mineralized for 2 weeks	Drying effect	Rate of drying effect
	Control	Ultrasonicated			Oven-dried		
(1)	(2)	(3)	(3)−(2)	$\frac{(3)-(2)}{(1)}\times 100$	(4)	(4)−(2)	$\frac{(4)-(2)}{(1)}\times 100$
	(mg N/100 g sand[1])			(%)	(mg N/100 g sand[1])		(%)
29.4	10.7	11.6	0.9	3.1	11.5	0.8	2.7

[1] Sea sand (diameter 0.25 to 0.50 mm).

Table 10. Drying effect on the mineralization of cell wall substances (*S. cerevisiae*).

Soil	Amounts of cell wall substances added	Mineralization of cell wall substances for 2 weeks			Drying effect	Rate of drying effect
		Control	Treatment			
	(1)	(2)	(3)		(3)−(2)	$\frac{(3)-(2)}{(1)}\times 100$
Carbon		(mg C/100 g soil)				(%)
U	747	116	Air-dried	231	115	15.4
			Oven-dried	270	154	20.6
I	747	148	Air-dried	262	114	15.2
			Oven-dried	313	165	22.1
C	747	184	Air-dried	326	142	19.0
			Oven-dried	330	146	19.5
U[1]	49	42	Oven-dried	40	−2	—
Nitrogen		(mg N/100 g soil)				(%)
U	113.1	37.2	Air-dried	42.5	5.3	4.7
			Oven-dried	45.0	7.8	7.0
I	113.1	23.6	Air-dried	29.7	6.1	5.9
			Oven-dried	37.5	13.9	12.5
C	113.1	28.8	Air-dried	32.7	3.9	3.5
			Oven-dried	35.5	6.7	5.9
U[1]	9.2	8.0	Oven-dried	7.9	−0.1	—

U: Ushirokawachi, I: Isahaya, C: Choyo.

[1] Cytoplasmic substances of *S. cerevisiae* were added in order to compare them with cell wall substances of *S. cerevisiae*.

Further, ultrasonic treatment employed in the preparation of cell wall substances may have an acceleration effect on mineralization of cell wall substances in soil. Using cell wall substances of *B.subtilis*, therefore, the effect of ultrasonicating on mineralization of cell wall substances was examined complementarily. The oven-drying effect (80°C for 2 hr) was compared with the ultrasonicating effect (19.5 kHz for 20 min). Experimental conditions were the same as mentioned above. The result obtained is shown in Table 9. Mineralization of cell wall substances was clearly accelerated by ultrasonic treatment and its effect almost equalled the oven-drying effect under this experimental condition. From the result mentioned above, it can be assumed that mineralization of cell wall substances prepared by ultrasonic treatment was

accelerated more or less as compared with the oven-drying effect. Besides the ultrasonic treatment, however, no other method preparing microbial cell wall substances without alteration of their chemical composition for a short time has been published to date.

B) S.cerevisiae: The drying effect on mineralization of cell wall substances is shown in Table 10. The drying effect on cell wall substances of *S.cerevisiae* was clearly recognized in three soils, but the drying effect on cytoplasmic substances of them (see Ushirokawachi) was not recognized as same as those of *Ar.simplex* and *B.subtilis*. As to the drying effects, the oven-drying effect was higher than the air-drying effect. And both effects in three soils decreased in the following order: Isahaya<Ushirokawachi<Choyo.

As described in A) and B), the drying effect was clearly shown with microbial cells and their cell wall substances, but not with their cytoplasmic substances. From these results, the following assumption may be given as to the reason why microbial cells applied to soil showed the drying effect. When microbial cells are applied to soil and decomposed in it, their cytoplasmic substances may be decomposed very quickly as compared with their cell wall substances. The cell wall substances may be left in portion for a longer time because of their resistant nature to microbial decomposition. On that occasion, they may be held in the form of a complex with organic and/or mineral colloids in soil which are much more resistant to microbial decomposition. If once dried, however, mineralization of cell wall substances held as such a complex in soil may be accelerated. Thus, cell wall substances held in soil will contribute as a source of decomposable soil organic matter due to drying.

The experiment on the contribution of cell wall substances as a source of decomposable soil organic matter due to drying was carried out by using *S.cerevisiae* to make clear the assumption mentioned above. Experimental conditions were the same as described above. The amounts of cell wall substances added were 51.9 mg in C and 7.80 mg in N per 10 g of dry Isahaya soil. Tendency of the mineralization rate of cell wall substances is shown in Fig. 2. The drying effect at the beginning was recognized

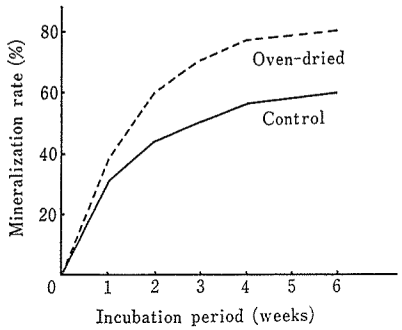


Fig. 2. Tendency of the mineralization rate of cell wall substances (*S. cerevisiae*, Isahaya soil).

as described above. Mineralization rates were considerably high until 3 weeks of incubation both in "Control" and "Oven-dried" groups, but became gradually lower from 4 to 6 weeks of incubation, and their mineralization reached a stable level at about 6 weeks of incubation. The rates at 6 weeks of incubation were 59.7 per cent in "Control" and 79.3 per cent in "Oven-dried" respectively; namely, organic matter of 40.3 per cent and 20.7 per cent remained in "Control" and "Oven-dried" respectively. Mineralization amount of decomposable organic nitrogen was larger in "Oven-dried" than in "Control" during 6 weeks of incubation. Considering the difference of mineralization rates between the two groups, it may be quite all right to consider that the amounts of residues of cell wall substances added was larger in "Control" than in "Oven-dried."

Table 11. Drying effect on the mineralization of soil organic matter remaining at 6 weeks of incubation.

Treatment at beginning	Total C Remaining at 6 weeks of incubation (1)	Treatment at 6 weeks of incubation	C mineralized for 2 weeks after treatment		Drying effect (3)−(2)	Rate of drying effect $\frac{(3)-(2)}{(1)}\times 100$	Mineral N[1] at 2 weeks after treatment	Drying effect (b)−(a)
	(mg C/100 g soil)					(%)	(mg N/100 g soil)	
Control	209	Control	(2)	9	10	4.8	(a) 42.9	3.8
	209	Oven-dried	(3)	19			(b) 46.7	
Oven-dried	124	Control	(2)	10	2	1.6	(a) 50.6	0.8
	124	Oven-dried	(3)	12			(b) 51.4	

[1] These figures include nitrogen mineralized before 6 weeks of incubation. Application amount of cell wall substances at beginning, C: 519 mg, N: 78.0 mg/100 g soil.

The drying effect on mineralization of soil organic matter remaining at 6 weeks of incubation is shown in Table 11. The drying effect in "Oven-dried" was clearly recognized, but it was not high as compared with that in "Control." Namely, the drying effect in "Control" was considerably high and the rate of the effect was significantly higher in "Control" than that in "Oven-dried." This result shows that decomposable soil organic matter due to drying was also newly accumulated during the decomposition process of cell wall substances. It was also indicated, however, that the drying effect of soil organic matter newly accumulated, *i.e.*, microbial cells newly accumulated during the decomposition process and their residues, *etc.* was not higher than that of the residues of cell wall substances added. From these results, it may be considered that the contribution of residues of cell wall substances added as a source of decomposable soil organic matter was much larger than that of newly formed microbial cells and their residues, *etc.* during the decomposition process of 6 weeks. Afterwards, however, cell wall substances and their residues newly accumulated in soil become the origin of decomposable soil organic matter. Therefore, it is likely to consider that the assumption described above is assured.

It is reported by several workers that considerable parts of soil organic matter accumulated in soil are originated from microbial cells and their residues (*4*, *11*, *14*). If we consider these reports in relation to the results described in Experiment 1 and Experiment 2, it may be concluded that microbial cells, especially their cell wall substances considerably contribute as a source of decomposable soil organic matter.

Acknowledgements. We wish to thank Mr. N. Nagao of the Laboratory of Plant Pathology for his help in the photographing of electron micrographs of cell wall substances.

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