VITAMIN B₁₂ CONTENT IN ANAEROBIC DIGESTION PRODUCTS OF ACTIVATED SLUDGE¹

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As a means of the treatments of activated sludge produced in the municipal sewage treatment plants in Japan, the majority of the by-product is sold as fertilizer after being dried in Nagoya City, while the substance is not necessarily reasonably treated in the other cities, *e.g.*, in Osaka City it is abandoned in the sea. On the other hand, methane fermentation tanks are established for the purpose of decreasing the amount of activated sludge produced in most of the sewage treatment plants. In the earlier days of the study on the preparation of crystalline vitamin B₁₂ from activated sludge, digested sludge was also supposed to contain a larger amount of the vitamin, if little or no destruction of the vitamin occurred during digestion. Contrary to the expectation, the results obtained with the samples of both Kyoto and Gifu Cities showed that there was little or no difference in vitamin B₁₂ content between digested and activated sludge.

The recent discovery in the authors' laboratory that a considerably large amount of vitamin B₁₂ was contained in the bacterial cell fraction obtained from methane fermentation of night soil (1) and distillers' wastes (2) led to the reinvestigation on the vitamin content of anaerobic fermentation products in municipal sewage treatment plants. In the previous experiment, investigation was made on the sludge sedimenting at the bottom of digester, while in this paper the supernatant in digester was dealt with. Sjöström (3) and Bernhauer (4) reported the discovery and isolation of cyanocobalamin and other related factors in digested sludge, but it is not well known what part of fermentation products was the object of their investigation.

THE STATE OF DIGESTING ACTIVATED SLUDGE IN MUNICIPAL SEWAGE TREATMENT PLANTS

The structure of digester and digesting system are more or less different with each municipal sewage treatment plant. In Gifu City, 130 m³ per day of activated sludge produced in the plant of the city are fermented for about 20 days in a closed type of digester followed by digesting for 40 days in an open system of tank.

¹ Researches on Vitamin B₁₂ Resources. III.

The supernatant is discharged, and digested sludge is sold as fertilizer after drying on sand bed. In Nagoya City, activated sludge produced in three plants at Horidome, Tsuyuhashi and Atsuta is transported to Tenpaku treatment plant by pump, dried on sand bed, and sold as "dried sludge fertilizer". An excess of activated sludge, however, in rainy season is subjected to digestion. In Toyohashi City, sewage sludge which sediments in a primary settling pond is digested after mixing up with activated sludge, while in Kyoto City the majority of sludge fed into digesting tank is discharged before being well digested due to the much larger amount of sludge charged than the capacity of tank. In most cases, fermentation is promoted by warming the contents of tanks at 30–35°, but spontaneous digestion process without warming is employed in Gifu City.

In this paper, investigations on vitamin B₁₂ were presented with the digested sludge samples of Gifu (Fig. 1), Toyohashi and Nagoya Cities.

FIG. 1 *Digester for Activated Sludge of Gifu City*

EXPERIMENTAL AND RESULTS

The supernatant in digesters was centrifuged for 1 hr at 3,000 rpm, and the moisture and vitamin B₁₂ content of the supernatant liquor prior to and after centrifugation, and of the precipitates were measured. Microbiological assay methods with *Lactobacillus leichmannii* ATCC 4797 and *Escherichia coli* 215 were employed for the estimation of vitamin B₁₂. Alkali-stable factors tested with several of the samples proved to be negligible.

The results obtained from the duplicate investigations in Gifu City are shown in Tables I and II, and these with the samples of Nagoya and Gifu are in Table III.

TABLE I
The Change in Vitamin B₁₂ Content in the Process of Digesting
Activated Sludge in Gifu City

Sample	Moisture in %	Vitamin B ₁₂ content in γ/g			
		<i>L. leich.</i> method		<i>E. coli</i> method	
		On a wet basis	On a dry basis	On a wet basis	On a dry basis
Activated sludge	98.44	0.10	6.4	0.096	6.2
Supernatant	99.93	0.006	8.6	0.005	7.1
Centrifuged supernatant ^a	99.94	0.001	1.7	0.001	1.7
Precipitate ^a	95.89	1.3	31.6	1.2	29.2
Digested sludge	96.8	0.36	11.1	0.33	10.2

^a The samples obtained by centrifuging the supernatant. Also the same in the following Tables.

TABLE II
Vitamin B₁₂ Content in the Digested Effluent in
Gifu Sewage Treatment Plants

Sample	Moisture in %	Vitamin B ₁₂ content in γ/g			
		<i>L. leich.</i> method		<i>E. coli</i> method	
		On a wet basis	On a dry basis	On a wet basis	On a dry basis
Supernatant	99.90	0.01	10.0	0.009	9.0
Upper layer of settled supernatant	99.91	0.006	6.7	0.006	6.7
Centrifuged supernatant ^a	99.93	0.002	2.8	0.003	4.2
Precipitate ^a	95.0	1.1	22.0	0.98	19.6
Digested liquor in the middle of digester	99.89	0.01	9.1	0.01	9.1
Upper layer of settled supernatant	99.92	0.006	7.5	0.006	7.5
Centrifuged supernatant ^a	99.93	0.002	2.8	0.002	2.8
Precipitate ^a	95.4	1.2	26.1	1.1	23.9

From the results given in Table I, a marked increase is recognized in vitamin B₁₂ content, since the vitamin content of the supernatant and digested sludge is 8.6 and 11.1 γ/g , respectively, as compared with that of the activated sludge of 6.2 γ/g . A precipitate fraction containing vitamin B₁₂ as much as 30 γ/g is obtained by centrifuging the supernatant, while the vitamin content of the centrifuged supernatant liquor is lower than that of the supernatant before centrifugation. Investigation with the digested fluid in the middle part of the tank showed little or no difference in the vitamin content.

TABLE III
*Vitamin B₁₂ in the Supernatant Liquor in Nagoya and
 Toyohashi Sewage Treatment Plants*

Sample	Moisture in %	Vitamin B ₁₂ content in γ/g			
		<i>L. leich.</i> method		<i>E. coli</i> method	
		On a wet basis	On a dry basis	On a wet basis	On a dry basis
N a g o y a					
Supernatant	99.87	0.006	4.6	0.005	3.8
Centrifuged supernatant ^a	99.88	0.002	1.3	0.002	1.3
Digested sludge	95.2	0.17	3.6	0.17	3.6
T o y o h a s h i					
Supernatant	99.93	0.001	1.3	0.001	1.3
Centrifuged supernatant ^a	99.93	0.0003	0.43	0.0004	0.58

No precipitates were obtained with the samples of Nagoya and Toyohashi due to too small amount of the supernatant collected. The similar result is, however, expected in the vitamin content of the precipitates of both cities since the vitamin content of the centrifuged supernatant is much lower than that of the supernatant.

A good agreement in vitamin B₁₂ values by *L. leichmannii* and *E. coli* methods seems to show that vitamin B₁₂ factors other than cyanocobalamin, if any, might be small in amount, although a quantitative estimation should be made by other suitable means such as *Ochromonas malhamensis*.

DISCUSSION

No significant difference is observed in vitamin B₁₂ content in the precipitate obtained by centrifuging the supernatant between activated sludge and night soil, while the vitamin concentration in the supernatant of the former is much lower than that of the latter. Microscopic examination of the precipitates showed that the substances mainly consisted of bacterial cells as in the case of night soil. In other words, vitamin B₁₂ seems to be accumulated in the cells of methane fermentation bacteria.

About 130 tons of activated sludge is charged and about 100 tons of supernatant is discharged daily in Gifu City. Vitamin B₁₂ contained in the supernatant is about 1 g, while the vitamin recovered in the cell fraction of high vitamin B₁₂ content by centrifugation seems to be about 0.5 g.

The bacterial cell fraction of high vitamin B₁₂ content obtained by centrifuging the supernatant is worthy of attention, being not a very great resource, since about 130 tons of activated sludge is charged and about 100 tons of supernatant is discharged.

SUMMARY

The investigation on the change of vitamin B₁₂ in the treatment of activated sludge showed that bacterial cell fraction obtained by the centrifugation of the supernatant contained vitamin B₁₂ as much as 30 γ /g.

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