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On Vitamins in Sewage Sludge

III. Vitamin B_{12} Activity of Different Types of Sewage Sludge

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The vitamin B_{12} activity of different types of sewage sludge were studied by means of microbiological estimation and bioautography.

Digested sludge from a plant where the activated sludge process is used was found to have about the same vitamin B_{12} activity per ml (and a considerably higher activity calculated on dry solids basis) but higher percentage of cyanocobalamin than digested sludge from plants where no aeration took place.

Average and limit values are given for the vitamin B_{12} activities of samples of digested sludge collected from a sewage plant during a period of 3 years.

During the bacterial decomposition of sewage sludge, considerable amounts of factors of the vitamin B_{12} group are formed as reported by several authors[1-4]. The values found by different authors for "activated sludge" and for digested sludge vary greatly. There are not only great differences between the values for activated sludge on one hand and digested sludge on the other but also between the values for each kind of sludge. In the present work an attempt was made to investigate whether the type of microbial sludge decomposition has some influence on the amount and kind of vitamin B_{12} activity formed and to follow the vitamin B_{12} activity of digested sludge from a single sewage plant over a longer period of time in order to elucidate whether there exist seasonal variations.

EXPERIMENTAL

Sludge samples from some sewage plants using different methods of sludge treatment were autoclaved at pH 5.5 in the presence of KCN, at 121° C, for 10 min. After centrifugation, a phosphate solution was added to the centrifugate in order to remove calcium ions. The vitamin B_{12}-activity of the centrifugate was then estimated with *Escherichia coli* 113-3 using the cup-plate and tube methods as well as bioautography. Estimations with *Lactobacillus leichmannii* and *Ochromonas malhamensis* have also been made in some cases.

The analyses were made in triplicate and repeated on three or more different days.

RESULTS

Table 1 gives a comparison of the vitamin B_{12} activity of different types of sewage sludge.

I. In the plants Ia, Ib and Ic, digestion of the settled sewage sludge is used but no biological treatment of the effluent. *No aeration* of the sewage takes place at any stage of the treatment. The digestion tanks are provided with stirrers and arrangements for artificial heating (33° C).

II. In plant II the sludge is digested in a large earth basin without stirring or artificial heating. The samples taken at different depths and different

Table 1. Vitamin B_{12}-content of different types of sewage sludge. (For description of the different sewage plants, See text.)

Sewage plant and type of sludge	Number of samples	Average tot. vit. B_{12} activity a)		Average distribution of vit. B_{12}-factors in % of total vit. B_{12}-activity b)			
		mg/l	mg/kg dry solids	Cyano-co-bala-min	Factors A + pseudovit. B_{12} c)	Factor B	Factors C_1 + C_2
Ia							
Fresh sludge	3	0.2	6	40	40	5	20
Digested sludge	27	1.0	14	50	25	5	20
Ib							
Digested sludge	4	0.7	14	50	20	10	20
Ic							
Digested sludge	3	0.9	13	50	20	20	30
II							
Digested sludge	14	(0.9)	(13)	(60)	(30)	(5)	(5)
III							
Digested sludge	2	2.0	25	60	15	5	20
IV							
Fresh sludge	1	0.2	6	50	40	5	5
Digested sludge	3	0.8	25	80	20	—	traces
Activated sludge	3	0.04	11	90	10	—	traces
V							
Digested sludge	1	0.7	11	70	30	—	—
"Biological sludge" (from settling tanks)	1	0.1	3	85	5	5	5
"Scrapes" from biological beds	1	0.3	6	85	5	5	5

a) estimated with *E. coli* 113-3 in the tube-assay

b) after chromatographic separation

c) or possibly also factor III (Bernhauer[4]) which has the same R_F-value as factor A in the chromatographic method applied by us.

distances from the inlet and outlet showed great variations in vitamin B_{12} and dry solids contents. In this case it is not possible to calculate an "average" witamin B_{12} content of the sludge.

III. Plant III receives its sewage from a food factory.

IV. In plant IV the sewage is treated by the activatd sludge process (step aeration).

V. Plant V is equipped with tanks for digestion of sludge and with biological filters for (aerobic) treatment of the effluent from the settling tanks.

DISCUSSION

It can be seen in Table 1 that, *inter alia*, two factors may exert special influence on the vitamin B_{12} activity of decomposed sludge:

1) the nature of the incoming fresh solids.

Thus digested sludge from sewage plant III which receives its sewage from a food factory has a much higher vitamin B_{12} activity (2 mg/l sludge corresponding to 25 mg/kg dry solids) than ordinary municipal digested sludge.

2) the way in which the sewage has been treated.
   viz. a) digestion of settled sludge
   b) digestion of settled sludge in combination with the activated sludge process.

The influence of the first factor has only been investigated in one case. Thus, it is only possible to draw attention to the high vitamin B_{12} activity of food factory sludge — no other comments can as yet be made. The influence of the second factor, however, deserves more attention.

Digested sludge from plants where no part of the sludge has been submitted to aeration before digestion (plants I a, b, c, and II) contains 0.75—1.0 mg/l sludge corresponding to 13—14 mg/kg dry solids of vitamin B_{12} activity. The percentage distribution of the different vitamin B_{12} factors in such a sludge is: cyanocobalamin, about 50 %; factors A + pseudovitamin B_{12}, 15—30 %; factors C_1 + C_2, 20—30 %; factor B, 5—20 %.

Table 2. Vitamin B_{12} activity of digested sludge from sewage plant I a. (Tube assay with E. coli 113-3.)

Total		Average values of 27 samples	Limit values of 27 samples
	mg/l sludge	1.0	0.6 — 1.8
	mg/kg dry solids	14	7 — 30
Cyanocobalamin, % of tot. vit. B_{12} activity		50	15 — 80
Factor A*	—»—	25	10 — 40
Factor B	—»—	5	0 — 30
Factors C_1+C_2	—»—	20	0 — 35

* including pseudovitamin B_{12}.

In the plant V, where the activated sludge process is applied, the sewage is mixed with part of the settled activated sludge and aerated. The excess activated sludge is removed and transferred to digestion tanks *. The digested sludge from this plant has a very high vitamin B_{12} activity when calculated on a dry solids basis: 25 mg/kg solids. Still more surprising is the fact that as much as 80 % of the vitamin B_{12} activity of the sludge is due to cyanocobalamin, factor B being absent and factors C_1 and C_2 occurring only in traces. The high proportion of cyanocobalamin is still more pronounced in the activated sludge where it reaches 90 % of the total vitamin B_{12} activity. In the "biological" sludge and "biological scrapes" from plant V (both derived from aerobic decomposition of organic solids), the corresponding figure is 85 %.

The average values and the variations of the vitamin B_{12} activity of the 27 samples of digested sludge from the sewage plant I a, collected over a period of 3 years, are given in Table 2.

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