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Nitrogen and Phosphorus Removal from Combined Sewage Components by Microbial Activity[1]

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ABSTRACT

FINSTEIN, M. S. (Rutgers, The State University, New Brunswick, N.J.). Nitrogen and phosphorus removal from combined sewage components by microbial activity. Appl. Microbiol. **14**:679–684. 1966.—When primary domestic sewage sludge was combined with settled sewage or secondary-treatment plant effluent, synergism resulted. The activity (measured by oxygen uptake, and the removal of Kjeldahl nitrogen and orthophosphate from solution) which resulted from incubating sludge together with settled sewage exceeded the sum of the activities when these components were incubated separately. A similar synergistic effect occurred with sludge and effluent. The sewage sludges were deficient in readily available nitrogen, but no shortage of phosphorus was demonstrated. The addition of ammonium and orthophosphate salts to sludge, in concentrations equivalent to those found in settled sewage and effluent, stimulated sludge oxygen uptake at least 80% as much as settled sewage or effluent. It is suggested that the synergism reflects increased microbial activity resulting from widened carbon-nitrogen and carbon-phosphorus ratios achieved by combining sludge with nutrient-rich settled sewage or effluent.

The undesirable growth of algae in receiving waters has directed attention to the importance of removing plant nutrient elements, as well as carbonaceous pollutants, from waste waters (14). Biological treatment of domestic sewage does not effectively remove nitrogen and phosphorus, the elements of greatest concern. This difficulty has its origin in the nutrient imbalance of sewage with respect to the requirements of heterotrophic bacteria for respiration and cell synthesis. The imbalance consists of an excess of nitrogen and phosphorus relative to carbon. The portion of the nitrogen and phosphorus that is required for microbial synthesis is retained as activated sludge or trickling filter slime, whereas excess nutrient is discharged in the final effluent.

Primary sedimentation may have the effect of aggravating the nutrient imbalance by withholding from aerobic treatment the sewage fraction richest in carbonaceous constituents (Table 1). Since any decrease in the amount of microbiologically available carbon in the feed, relative to nitrogen and phosphorus, would be detrimental to nutrient removal, primary sedimentation may adversely affect the mineral quality of secondary effluent. Primary sedimentation prior to aerobic treatment is practiced at most secondary-treatment installations.

The present paper explores the possibility of utilizing primary sludge to enhance the removal of nitrogen and phosphorus from domestic sewage during a brief aerobic treatment.

MATERIALS AND METHODS

Sampling. Raw unsettled sewages, activated sludge mixed liquors, and unchlorinated final effluents were obtained from three municipal treatment plants and a state institution in New Jersey. Domestic waste comprises the bulk of the municipal sewages, and sanitary waste that of the institutional flow. Samples were obtained on weekdays between the hours of 9 and 11 AM. At most, 1.5 hr was required to transport the samples to the laboratory.

Sewage preparation. After 5 gal (18.9 liters) of sewage was settled for approximately 0.75 hr, most of the supernatant fluid was siphoned off, a portion being put aside for experimental use. The remaining material was transferred to an Imhoff cone for an additional 0.75 hr of settling; the resulting supernatant fluid was then discarded, and the sludge (concentrations ranged from 10,800 to 24,000 mg per liter) was blended for 2 min at approximately 10,000 rev/min with an Omni-Mixer (Ivan Sorvall, Inc., Norwalk,

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TABLE 1. *Relative abundance of carbonaceous, nitrogenous, and phosphorous materials in raw domestic sewage fractions*[a]

Fraction	Ratio	Value	Reference
Sludge	COD/organic N[b]	26.3	5
Supernatant fluid	COD/organic N[b]	18.3	
Sludge	COD/Kjeldahl N[c]	35.0	6
Sludge	BOD/Kjeldahl N[c]	18.5	
Sludge	BOD/P[d]	55.5	
Supernatant fluid	BOD/total N	8.3	2 and 3
Supernatant fluid	BOD/total P	45.2	
Feces	Organic C/total N	11.3	9
Whole sewage	Organic C/total N	4.4	

[a] Average data, except reference 9, where single values are given. Sludge corresponds to the fraction of sewage that settles, and supernatant fluid to the fraction that does not settle. COD = chemical oxygen demand; BOD = biochemical oxygen demand. *Note.* Other investigators found about 8% of the phosphorus in sewage to be associated with the sludge (4).

[b] Kjeldahl N with the use of NH_3-free samples.

[c] Essentially equivalent to total N.

[d] Essentially equivalent to total P.

Conn.). Settling and blending was carried out in the cold.

Oxygen uptake studies. Blended primary sewage sludge (5.0 ml), supernatant fluid (15.0 ml), and effluent (15.0 ml) were transferred to 125-ml Warburg flasks of the BOD type (Aminco Corp., Silver Springs, Md.) and, where appropriate, combined therein. When needed, the volume was adjusted to 20.0 ml with distilled water. The flasks were seeded with 0.1 ml of concentrated, blended, activated sludge. Final sewage sludge concentrations in the Warburg flasks ranged from 2,700 to 6,000 mg per liter. Incubations were for 6 hr, during which the flasks were shaken in water (20 C) at 100 strokes per min through an arc of approximately 14 cm. Oxygen uptake was measured by routine procedures (13). When used as a nutrient supplement, phosphate was adjusted to *p*H 7. Acid-washed glassware was used throughout.

Filtration. After completing the O_2-uptake measurements, the contents of replicate Warburg flasks were combined and transferred to a filtering apparatus. This consisted of a funnel holding a no. 42 Whatman filter paper which drained into a Millipore filter holder (Millipore Filter Corp., Bedford, Mass.) fitted with two membranes, RA and HA, in that order (porosities, 1.2 and 0.45 μ, respectively). The final filtrate was collected in a conical flask. Filtration was carried out overnight in the cold without application of suction, a procedure designed to minimize the risk of losing ammonia. Most of the liquid passed through the filter paper within 1 hr. Passage through the membranes was slower and occasionally was not completed overnight, necessitating the application of positive air pressure to complete the filtration. Zero-time samples were similarly filtered.

Assays of filtrates. A qualitative test for nitrite was carried out with the reagents of Rider and Mellon (10). The other determinations were performed quantitatively: total nitrogen by a Kjeldahl procedure (8), and orthophosphate by the molybdophosphoric acid method, with the use of the organic reductant (1). For the latter determination, color development was measured with a Beckman spectrophotometer, model DB (Beckman Instruments, Inc., Fullerton, Calif.) with the use of 4-cm cells. Zero-time values of combined treatments (e.g., sludge plus supernatant fluid) were calculated as the sum of the zero-time values of the individual components.

RESULTS

Synergism is used herein to describe the heightened activity which resulted from incubating sewage sludge together with homologous supernatant (settled sewage) or treatment-plant effluent, compared with the sum of the activities under separate incubation. Measured by O_2 uptake and the removal of nitrogen and phosphorus from the solution, synergism was demonstrated with waste and effluent from all of the locations sampled (Table 2); sources 1, 2, and 3 are different municipal treatment plants, and source 4 is the plant at a state institution. The *p*H of the sludges ranged from 6.7 to 7.0, and remained essentially unchanged during incubation; supernatant fluid and effluent *p*H generally rose from neutrality to about 8.0. In combined treatments, the *p*H was that of the sludge. Most of the O_2 uptake originated with the sludge, which consumed on the average five times more O_2 than the supernatant fluid alone, and an average of 21 times more than effluent alone. Nitrite was not detected in the filtrates, indicating that nitrification did not contribute to the O_2 uptake.

In all trials, the synergistic effect was greater when measured by nutrient removal than by O_2 uptake; the mean values of the ratios (combined/summed) are: O_2 uptake, 1.3; nitrogen removal, 3.0; and PO_4-P removal 4.0 (source 1 values omitted).

Because each component contributed soluble nutrients, before incubation the combined treatments had higher concentrations of nitrogen and phosphorus than did the corresponding individual treatments (Table 3). Although synergism occurred in all trials, the enhancement of phosphorus removal in combined treatments was not always sufficient to reduce this nutrient below the level of the corresponding supernatant fluid-alone or effluent-alone treatments. For example, with materials from source 1, phosphate in the combined sludge and supernatant fluid treatment declined during incubation from 9.9 to 5.8 mg per liter, whereas, in the supernatant fluid-alone treatment, the value was stable at approximately 5.5 mg per liter. The components from source 3 gave similar results. In the other five comparisons, after incubation the combined treatments had less residual phosphate than the corresponding supernatant fluid-alone or effluent-alone treatments. Nitrogen removal was more vigorous; in seven of eight comparisons, after incubation the combined treatment had less residual nitrogen in solution than supernatant fluid or effluent alone. In the eighth comparison (source 4), residual nitrogen in the sludge plus effluent treatment was equal to that of the effluent alone.

Measured as O_2 uptake, the synergism resulted largely from the stimulatory effect of supernatant fluid or effluent on the uptake originating in the sludge component, and inorganic nutrients partially substituted for supernatant fluid or effluent (Table 4). The estimate (Table 4) of the extent to which ammonium and orthophosphate salts substituted for supernatant fluid or effluent is conservative; the *true* value would be given by the expression:

$$\text{substitution} = \frac{\text{excess sludge uptake (nutrient-supplemented)} \times 100}{\text{excess sludge uptake (combined with supernatant fluid or effluent)}}$$

where excess uptake is the uptake of sludge when supplemented or combined minus the uptake of sludge alone. The numerator of the approximation (footnote, Table 4) corresponds to the numerator of the *true* expression; the denominator of the approximation, however, is larger to the extent to which enhanced uptake of supernatant fluid or effluent contributed to the synergism. Thus, the inorganic nutrients probably substituted somewhat more effectively for supernatant fluid

TABLE 2. *Synergism resulting from combining domestic waste components*[a]

Source	Components incubated	O_2 uptake (μliters/flask)[b]		Net decrease of nutrients in solution (μg/flask)[c]			
				Kjeldahl N		PO_4-P	
		Summed	Combined	Summed	Combined	Summed	Combined
1	Sludge-supernatant fluid	2,270 ± 60	2,930 ± 120	220	620	4	85
	Sludge-effluent	1,890 ± 70	2,870 ± 160	130	530	−11[d]	91
2	Sludge-supernatant fluid	5,770 ± 50	7,240 ± 270	570	1460	117	234
	Sludge-effluent	5,340 ± 60	6,290 ± 280	570	810	126	252
3	Sludge-supernatant fluid	3,810 ± 30	4,900 ± 140	400	890	41	159
	Sludge-effluent	3,390 ± 20	4,300 ± 130	300	640	43	204
4	Sludge-supernatant fluid	2,970 ± 20	4,210 ± 40	—	—	30	73
	Sludge-effluent	2,530 ± 20	3,460 ± 30	—	—	20	178

[a] Warburg flasks contained primary sludge alone, supernatant fluid (settled sewage) alone, effluent alone, or combined sludge and supernatant fluid, or sludge and effluent. Where needed, the volume was adjusted with distilled water. Incubation time, 6 hr. Summed = components incubated separately and values summed. Combined = components incubated in a common flask.

[b] Mean of three replicates ±2 standard deviations.

[c] Values derived from single determinations of pooled replicates.

[d] Net increase during incubation.

TABLE 3. *Nitrogen and phosphorus concentrations before and after incubation of domestic sewage components*

Source	Components incubated	Incubation[a]	Concn of nutrients in solution[b]	
			Kjeldahl N	PO_4-P
			mg/liter	*mg/liter*
1	Sludge	B	12	4.3
		A	6	4.4
	Supernatant fluid	B	44	5.6
		A	39	5.4
	Effluent	B	20	7.2
		A	21	7.7
	Sludge + supernatant fluid	B	56	9.9
		A	25	5.8
	Sludge + effluent	B	32	11.5
		A	6	7.0
2	Sludge	B	32	7.7
		A	9	1.2
	Supernatant fluid	B	41	4.6
		A	35	5.3
	Effluent	B	16	6.7
		A	11	7.0
	Sludge + supernatant fluid	B	73	12.3
		A	9	0.6
	Sludge + effluent	B	48	14.4
		A	8	1.8
3	Sludge	B	20	10.5
		A	5	8.0
	Supernatant fluid	B	41	6.3
		A	36	6.7
	Effluent	B	18	8.2
		A	18	8.0
	Sludge + supernatant fluid	B	61	16.8
		A	17	8.8
	Sludge + effluent	B	38	18.7
		A	6	8.5
4	Sludge	B	—	2.8
		A	7	1.8
	Supernatant fluid	B	19	1.6
		A	10	1.1
	Effluent	B	1	2.8
		A	1	2.8
	Sludge + supernatant fluid	B	—	4.4
		A	8	0.8
	Sludge + effluent	B	—	5.6
		A	1	1.2

[a] B = before incubation; A = after incubation.

[b] Data from same experiments reported in Table 2.

and effluent than is indicated by the estimated values.

Further trials with sludges derived from the four sewages revealed that nitrogen caused most of the response to inorganic nutrient supplementation. Oxygen uptake on the average was 22% (range, 14 to 40%) greater in the presence of supplemental nitrate than in its absence. Orthophosphate alone had little effect, a combination of nitrogen and phosphorus was slightly superior to nitrogen alone.

Inorganic supplementation did not appreciably effect O_2 uptake by the supernatant fractions derived from the domestic sewages. The response of supernatant fluid from the state institution (source 4) was slight. It is noteworthy that this sewage had unusually low concentrations of nitrogen and phosphorus.

DISCUSSION

The synergism appears to have resulted, in large part, from an improved nutrient balance in the combined treatments, which was conducive to microbial activity. As judged by the stimulatory effect of inorganic nitrogen on O_2 uptake, the sludges were deficient in readily available nitrogen. This deficiency could be overcome by combining sludge with nutrient-rich supernatant fluid or effluent. Although no shortage of phosphorus was demonstrated, the removal from solution of orthophosphate, as well as of nitrogen, was enhanced by incubating the components in a common flask. This is to be expected, since the increased microbial activity arising from a sufficiency of nitrogen should create a demand for any nutrient required for cell synthesis.

The magnitude of the synergistic effect was greater when measured by nutrient removal than by O_2 uptake. The reasons for this are obscure. In the presence of relatively high concentrations of nitrogen and phosphorus, luxury consumption may have occurred. Luxury consumption of phosphorus by microorganisms has been documented (11).

Because of the abundance of nitrogen and phosphorus in present-day domestic sewage, it is unlikely that biological treatment can remove these elements as completely as it removes carbonaceous pollutants (12). Recent work, however, indicates that substantial improvement is attainable (7). The data presented here suggest that the nutrient imbalance of sewage is aggravated by the common practice of primary sedimentation, and that aerobic treatment of whole sewage would more fully take advantage of the potential for biological extraction of plant nutrients. This practice is followed at a few installations, notably the activated sludge plant at Milwaukee, Wis., but the effect on nutrient removals is not known. The potential for nutrient removal

TABLE 4. *Substitution of inorganic nitrogen and phosphorus for supernatant fluid and effluent*[a]

Treatment	Run 1			Run 2		
	Components incubated	O_2 uptake	Substitution[b]	Components incubated	O_2 uptake	Substitution[b]
		μliters/flask	%		*μliters/flask*	%
a	Sludge	3,210 ± 80	—	Sludge	3,650 ± 40	—
b	Supernatant fluid	590 ± 20	—	Effluent	170 ± 30	—
c	Sludge + supernatant fluid	4,760 ± 160	—	Sludge + effluent	4,680 ± 160	—
d	Sludge + NH_4 + PO_4[c]	4,040 ± 140	87	Sludge + NH_4 + PO_4[d]	4,330 ± 90	79

[a] Experimental material from source 1. Incubation time, 6 hr. Mean of four replicates ±2 standard deviations.

[b] Substitution (%) $= \frac{(d - a) \times 100}{(c - b) - a}$.

[c] Added as $(NH_4)_2SO_4$ and Na_2HPO_4 to give final concentrations equivalent to supernatant fluid (NH_4-N, 26 mg/liter; PO_4-P, 3.3 mg/liter).

[d] Added as $(NH_4)_2SO_4$ and Na_2HPO_4 to give final concentrations equivalent to effluent (NH_4-N, 23 mg/liter; PO_4-P, 9.8 mg/liter).

may increase in the future should the disposal of garbage and other refuse through sewers become more widespread. These solid wastes increase the amount of settleable solids in sewage, and also widen the carbon-nitrogen and carbon-phosphorus ratios of this fraction.

In the present study, sludges were employed in concentrations 10 to 20 times greater than that ordinarily present in whole domestic sewage. Because the experimental conditions differed considerably from waste-treatment processes, the effect of treating whole as compared with settled sewage under operational conditions cannot be predicted. The demonstration that primary sludge effected the removal of nutrient elements from solution during a brief incubation indicates, however, that the advantages and disadvantages of primary settling should be thoroughly explored.

Another possible means of utilizing sludge is suggested by its ability to effect the removal of nutrients from treatment-plant effluent. A biological tertiary phase of treatment could be developed whereby secondary effluent could be combined with primary sludge for an additional treatment of the activated-sludge type designed to remove nitrogen and phosphorus. Exploration of this possibility would require studies with model systems simulating waste-treatment processes.

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