Operation of full-scale biological phosphorus removal plant

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THE FIRST FULL-SCALE INSTALLATION of a biological process * for phosphorus removal from wastewater was placed in operation at Seneca Falls, N. Y., in July 1973. This paper reports primarily on the first 30 days of operation of the process, during which it was intensively monitored. After the demonstration period, Seneca Falls officials decided to install the process on a permanent basis. Significant aspects of the extended operation are also reported. The technical and economic findings are presented and discussed.

BACKGROUND

The process. The biological process for phosphorus removal [1] has been described through laboratory [2, 3] and pilot plant [4, 5] development. It is based on the use of activated sludge microorganisms to transfer phosphorus from inflowing wastewater to a small concentrated substream for precipitation. There are several variations of the process. All are based on subjecting the activated sludge to anaerobiosis to induce phosphorus release into the substream and to provide phosphorus uptake capacity when the sludge is returned to the aeration basin. The flow diagram seen in Figure 1 is that used at Seneca Falls.

The sludge organisms take up phosphorus in the aeration basin under normal aeration. The mixed liquor then flows to the secondary clarifier. The phosphate-rich organisms are transferred from the bottom of the secondary clarifier to a thickener-type holding tank: the phosphate stripper. The settling sludge quickly becomes anaerobic and, thereupon, the organisms surrender phosphorus, which is mixed into the supernatant. The phosphorus-rich supernatant is removed from the stripper as a small substream, which is then dosed with lime for chemical precipitation of the phosphorus. The thickened, anaerobic sludge is returned to the aeration tank.

Pilot plant tests. Extensive pilot plant tests of the method were conducted during 1970–72. The pilot plant [6] was first operated on synthetic wastewater and, subsequently, on primary effluent from the Baltimore municipal wastewater treatment plant. Pilot plants were then operated in wastewater treatment facilities in Washington, D. C., Piscataway, Md., and Chicago, Ill. In each case, primary effluent from the main treatment plant supplied the pilot plant. A total of approximately 1 yr of operating experience, under summer and winter conditions, tested the process over the parametric ranges shown in Table I. The process was shown to be stable and rugged; the results of these tests are summarized in Table II. Effluent total phosphorus levels were consistently below the generally applied standard of 1.0 mg/l.

While highly encouraging, the pilot plant data required full-scale verification. For one thing, the pilot tests had never actually precipitated the phosphate for final removal, but merely demonstrated the production of low-phosphorus effluent and of the high-phosphorus supernatant stream. This meant, of course, that the problem of sludge handling was not addressed in the pilot tests. Problems associated with diurnal variation in flow and quality were not addressed either. A full-scale demonstration was needed to determine whether the entire process could operate satisfactorily in a complete wastewater treatment system, to establish whether the data

* Phostrip® process, marketed exclusively by Union Carbide Corp.

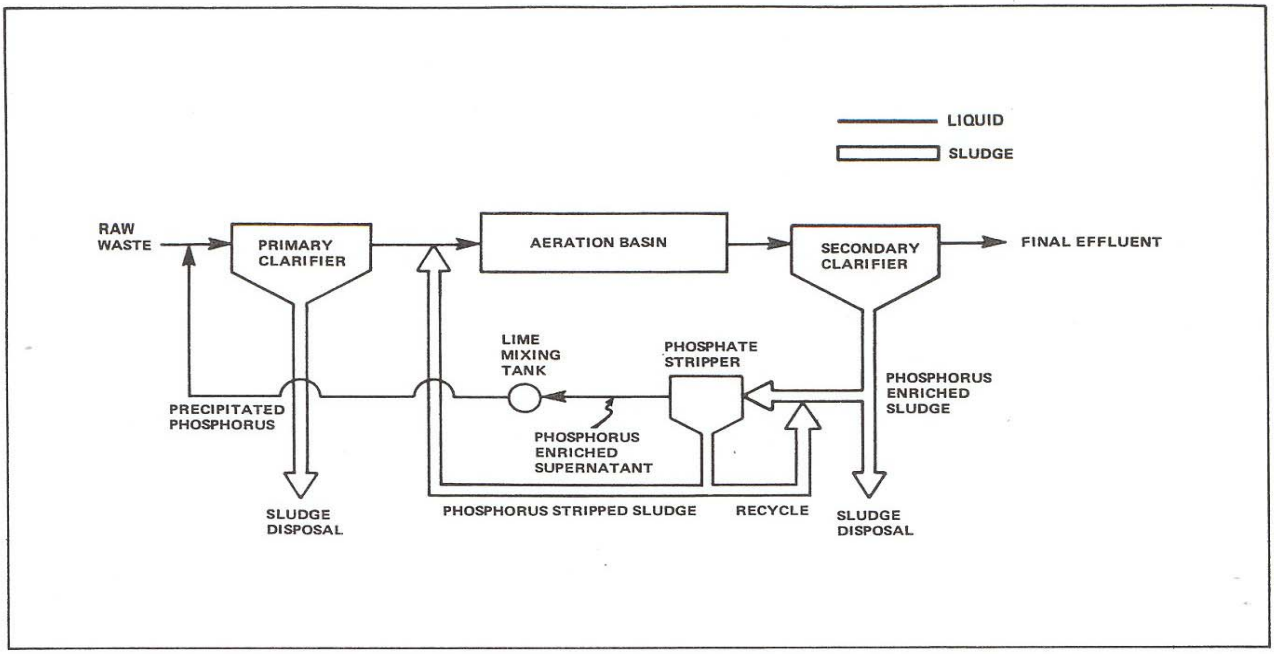

FIGURE 1.—Phosphorus removal flow pattern at Seneca Falls.

obtained from the pilot plant tests could be extrapolated to full scale, and to obtain cost data.

FULL-SCALE DEMONSTRATION

Seneca Falls. Seneca Falls, N. Y., lies within the Great Lakes drainage basin and, in keeping with the U. S.-Canadian treaty on the Great Lakes, it was ordered by the State of New York to reduce total phosphorus in its treatment plant effluent to 1 mg/l. Investigating low-cost methods for phosphorus removal, Seneca Falls officials contracted with Biospherics Inc., Rockville, Md., for a full-scale test of the process.

TABLE I.—Process Parametric Test Ranges

Parameter	Range
Total phosphorus:	
Influent	2.0–11.4 mg/l
Stripper Supernatant	14.0–84.0 mg/l
Sludge (Total phosphorus/SS)	2.1–7.1%
SS:	
Influent	24–250 mg/l
Effluent	2–51 mg/l
Mixed liquor	610–3,350 mg/l
Return anerobic sludge	9,850–36,300 mg/l
BOD:	
Influent	35–270 mg/l
Effluent	1–43 mg/l
Return sludge flow to stripper	11–37%
Stripper supernatant flow	3.3–28.3%
Anaerobic detention period	5–30 hr
Temperature of influent	11°–26°C

The Seneca Falls Paul W. Simson Wastewater Treatment Plant,[7] seen in Figure 2, is a modern, dual-train, activated sludge treatment plant with a total capacity of 3.5 mgd (13,247 cu m/day). The plant consists of a bar screen, comminutor, two circular primary clarifiers, two completely mixed aeration basins with mechanical aerators, two rectangular secondary clarifiers, and a chlorine contact chamber.

Waste activated sludge is discharged into the primary clarifier in which, together with the primary sludge, it is collected for transfer to a single sludge thickener. From there it is transferred to a two-stage, heated digester from which the sludge is drawn into tank trucks for land disposal at an offsite location.

Final effluent is chlorinated and discharged into a branch of the Erie Canal between Seneca Lake and Lake Cayuga.

TABLE II.—Pilot Plant Summary Data

Project and Time Period	Phosphorus (as P)						
	Influent	Final Effluent					
		Total				Orthophosphate	
		Unfiltered		Filtered			
	Concentration (mg/l)*	Concentration (mg/l)	Removal (%)	Concentration (mg/l)	Removal (%)	Concentration (mg/l)	Removal (%)
Synthetic waste (4/10–5/8/70)	9.6 OP	—	—	—	—	0.12	98.8
Baltimore wastewater (7/9–8/9/70)	6.1 OP	—	—	—	—	0.13	97.9
Washington, D. C. (1/12–2/29/72)	6.4 TP	0.69	89.1	0.28	95.5	0.07	—
Washington, D. C.† (2/12–2/29/72)	5.7 TP	0.92	83.7	0.45	92.2	0.05	—
Piscataway, Md. (1/12–2/29/72)	6.3 TP	0.70	89.2	0.15	97.6	0.17	—
Piscataway, Md.‡ (2/9–2/29/72)	5.5 TP	0.77	86.0	0.11	98.0	0.04	—
Chicago, Ill. (8/4–8/14/72)	3.0 TP	0.27	91.0	0.17	94.4	0.07	—
Chicago, Ill.‡ (8/15–8/25/72)	2.5 TP	0.20	92.2	0.15	94.2	0.17	—

* OP = orthophosphate phosphorus; TP = total phosphorus.
† 50% overload resulting in 4-hr aeration.
‡ "Split flow" operation routing only 50% of return sludge through stripper tank.

The plant has a smallcraft wastewater pumpout station to service pleasure craft and barges, which must store wastes for shore discharge according to New York State law. The boat wastes and the supernatants from the thickener and digester are returned to the primary clarifier.

The plant, capable of any mode of activated sludge operation, is normally used as a standard activated sludge plant.

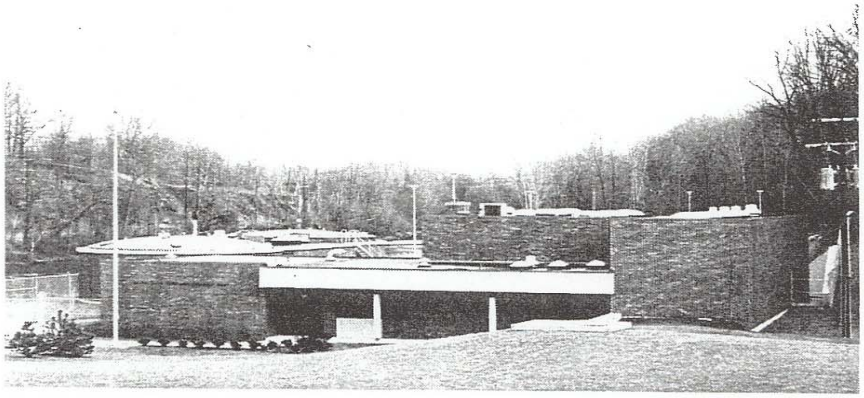

FIGURE 2.—Paul W. Simson Wastewater Treatment Plant, Seneca Falls, N. Y.

TABLE III.—Detailed Analysis of Primary Effluent from Seneca Falls Before Phosphorus Removal Process Installation (February 13, 1973)

Parameter	Concentration (mg/l)
BOD	110.0
Total dissolved solids	680.0
Total phosphorus	5.6
Orthophosphate (PO_4-P)	3.3
Total Kjeldahl nitrogen	25.0
Ca	220.0
Mg	33.5
K	5.0
Na	67.0
Cl	115.0
SO_4	126.0
Fe	0.72
Cd	<0.01
Cr	<0.01
Cu	0.09
Pb	<0.1
Hg	0.0023
Ni	<0.01
Ag	<0.01
Zn	0.12
CN	<0.1
Emission spectrographic analysis of primary effluent:	Concentration*
Al	trace–low
Ag	trace
B	trace
Ba	trace
Ca	medium–high
Cu	trace
Fe	trace–low
Mg	medium
Mn	trace
Na	medium–high
Ni	trace
Pb	trace
Si	low–medium
Sn	trace
Sr	trace–low
Ti	trace
P	low–medium
K	low–medium

* High = > 100 mg/l; medium = 10 to 100 mg/l; low = 1 to 10 mg/l; trace = 0 to 1 mg/l.

Conversion of plant. An analysis of the Seneca Falls primary effluent that was made approximately 4 months before the demonstration operation is presented in Table III.

An inspection of the plant revealed that its conversion to the phosphorus removal process would be relatively easy. The plant had been built in anticipation of receiving wastewater from an adjacent jurisdiction. The hookup had not yet occurred. Therefore, if the entire flow of approximately 1 mgd (3,785 cu m/day) were processed through only one train of the system, the primary clarifier of the second train could be used as the phosphate stripper tank. The clarifier was two to three times larger than desired for this purpose, but its use permitted quick conversion to the new process. The use of a single train to treat the entire flow caused that train to operate closer to its design loading.

The piping additions required to convert the plant (Figure 2) to the flow pattern shown in Figure 1 were made with 4-in. (10.16-cm) inside diam, aluminum irrigation tubing and 3-in. (7.62-cm) inside diam, flexible hose, all laid above ground (Figure 3). Pumping was provided by three submersible pumps † driven by 1-hp motors and rated at 100 gpm (0.378 cu m/min).

One submersible pump was placed in the sludge distribution box to intercept the return sludge from the secondary clarifier and force it through approximately 150 ft (45.7 m) of the irrigation tubing into the primary clarifier serving as the stripper tank. The thickened sludge was conveyed to the center well by the existing rotary sludge collector. Existing piping and valving permitted the sludge to flow from the center well by gravity to a scum pit adjacent to the tank (Figure 4). The second Goulds submersible pump was placed in the scum pit to force the now anaerobic sludge back to the downstream compartment of the sludge distribution box supplying the aeration tank.

The sludge collector motion was too slight to infuse the phosphate secreted by the sludge layer into the supernatant for removal. Elutriation of the dissolved phosphate from the sludge into the supernatant was accomplished by recycling return anaerobic sludge back to the stripper tank in the same line conveying secondary

† Model 3882, 1 hp, Goulds Pumps, Inc., Seneca Falls, N. Y.

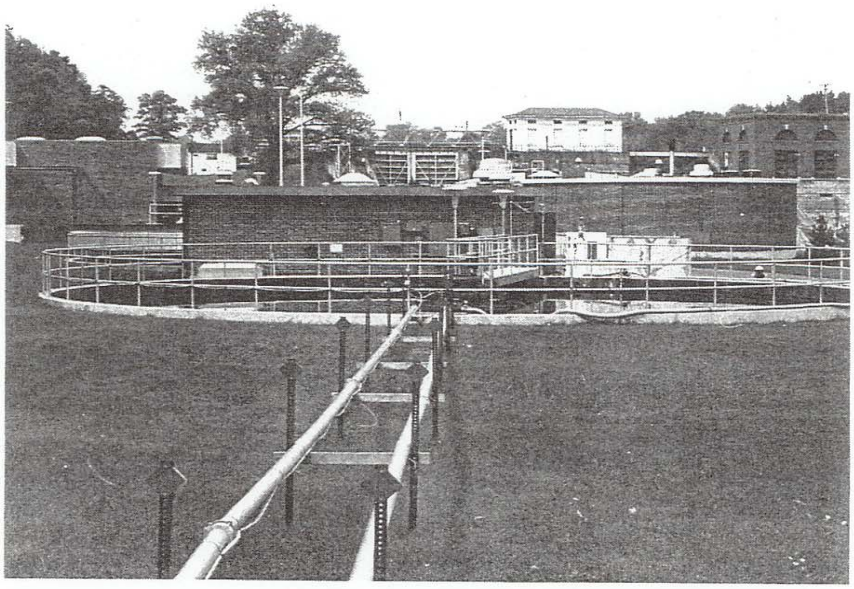

FIGURE 3.—Tubing installed to convert Seneca Falls plant. Secondary sludge is delivered (left tube) from sludge distribution box to primary clarifier converted into phosphate stripper tank shown in background and anaerobic sludge from stripper is returned (right tube and hose) to aeration tank. Lime slurry tank with mixer is on far side of stripper beside 1,000-gal (3,785-l) concrete mixing tanks, only one of which was used.

sludge to the stripper. This recycle provided a return sludge flow to the stripper tank consisting of approximately two parts of secondary clarifier underflow and one part anaerobic sludge. The mixing obtained was adequate to distribute the phosphorus into stripper tank supernatant, while still yielding a sharp anaerobic sludge blanket boundary.

Phosphate-rich supernatant generated in the stripper tank overflowed its peripheral weir into the launder. The launder drop pipe was sealed with a concrete plug fitted with an emergency, raised intake pipe to convey any possible tank overflow to the aeration basin. The supernatant was removed from the launder by the third Goulds submersible pump for discharge into a 1,000-gal (3.8-cu m) concrete tank in which the phosphorus was reacted with lime. Lime slurry was prepared daily in a 400-gal (1.5-cu m) storage tank. A variable-speed Moyno pump metered the slurry through a 1-in. (2.5-cm) garden hose discharging near the suction intake of the pump in the stripper tank launder. Thus, the lime was sucked in with the phosphate-rich supernatant, and the two were mixed in the approximately 20 ft (6.1 m) of 2-in. (5.1-cm) inside diam, galvanized pipe discharging into the 1,000-gal (3.7-cu m) tank, which provided 10 min additional reaction time. The mixture was then discharged by gravity to the center of the primary clarifier via an approximately 40-ft (12.2-m) long section of the aluminum irrigation tubing (Figure 5). All necessary valving was installed in the tubing, and mechanical joints were used throughout. In order to control the mixed liquor suspended solids (MLSS) level, a suspended solids (SS) meter [8] was installed

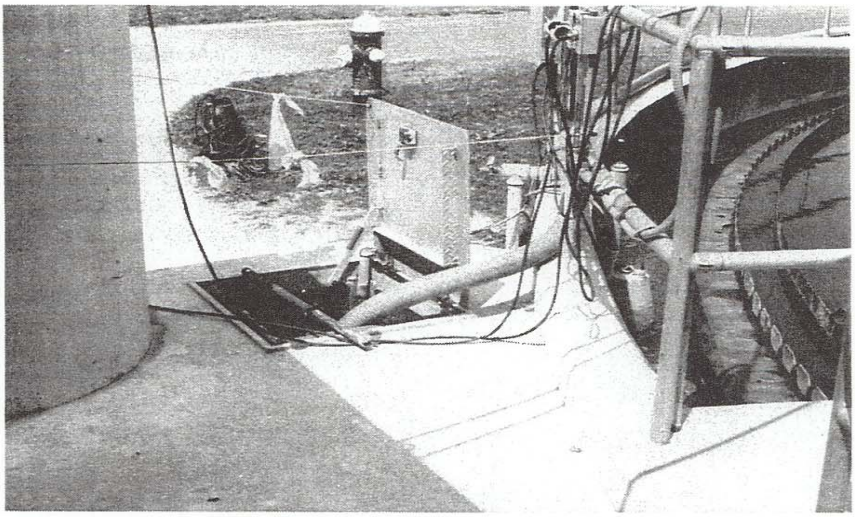

FIGURE 4.—Scum pit valved to center well of adjacent stripper tank, showing anaerobic return sludge line supplied by submersible pump within pit. Pump obtaining stripper tank supernatant from launder is seen at right. Tank at left delivers lime slurry to intake of this pump.

at the discharge end of the aeration basin.

Installation. Components for the conversion of the plant began arriving on June 1, 1973, and the installation was complete within 1 wk. Sludge wasting was stopped, and sludge return flow was directed through the phosphate stripper tank and back to the aeration basin. In approximately 10 days, an adequate amount of sludge accumulated in the stripper tank to begin the new mode of operation. The disadvantage of the oversized stripper tank became apparent when the desired volume of anaerobic sludge made a layer only several inches deep at the bottom of the tank. Because of the danger that this layer would be overpumped, thereby interrupting the return of sludge to the aeration basin, it was necessary to carry a deeper blanket of sludge. This imposed a longer anaerobic detention time than the 10 hr planned, but no adverse effects resulted.

Start-up. After about 2 wk of building up and cycling through the aerobic and anaerobic conditions imposed on it, the sludge, to which no seed had been added, acclimated as was predicted from the pilot plant studies performed elsewhere. This was announced by a rapid rise in the phosphorus level of the supernatant and a marked decline in that of the final effluent.

Demonstration period. When the total phosphorus content of the final effluent decreasd below 1 mg/l, an intensive sampling and monitoring program was begun. On-site analyses were carried out by the laboratory staff of the Seneca Falls treatment plant. Split samples and samples for additional analyses were air freighted to a Rockville, Md., laboratory. The ss meter in the aeration basin, the dissolved oxygen (DO) probe monitoring and controlling DO in the aeration basin, and the influent flow meter were calibrated and continuous records maintained. Return sludge and anaerobic return sludge flows were controlled by valve settings daily calibrated by precision timing of the displacement of large measured volumes in the sludge distribution box and the phosphate stripper tank launder. Pumps were stopped or quick-acting valves were closed for several minutes to make each of the measurements.

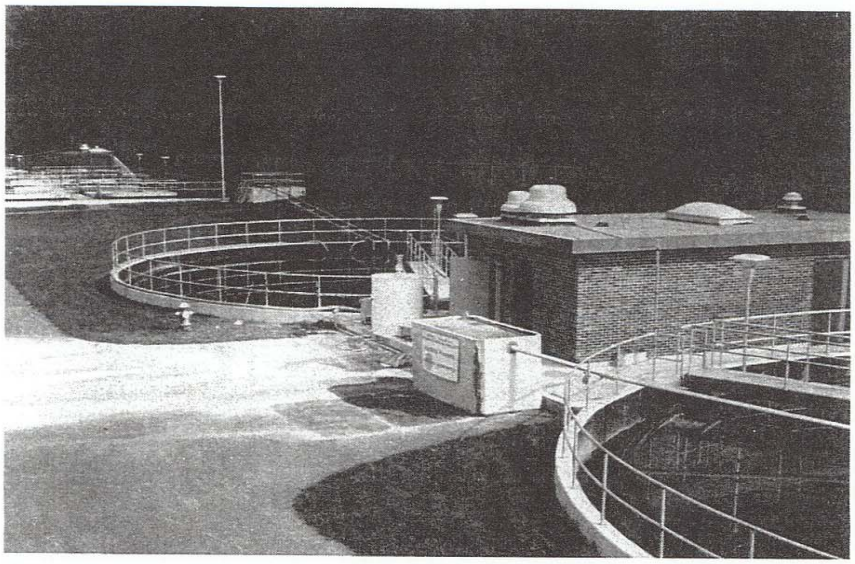

FIGURE 5.—Primary clarifier (foreground) receives lime-dosed supernatant from mixing tank. Lime slurry tank, stripper tank sludge distribution box, and aeration basin are in background.

Flow rates throughout the plant were properly controlled, and no serious imbalances developed. A daily check or adjustment of the discharge valve returning the anaerobic sludge to the aeration tank maintained the desired concentration of MLSS. The adjustment was automatically accommodated by changes of the detention period of the anaerobic sludge in the phosphate stripper and by changes in the overflow rate of the supernatant. When the supernatant flooded the launder to a depth of several inches, a pressure switch activated the supernatant discharge pump. A clock switched with the pump motor was read daily to determine the duty cycle. Sludge blanket levels in the phosphate stripper, secondary clarifier, and sludge thickener were monitored with a portable detector.

In accordance with the experimental design, the intensive sampling and monitoring program was conducted for 30 days.

RESULTS

During the demonstration period, the total phosphorus content of the raw wastewater averaged 6.3 mg/l.‡ The average total phosphorus of the effluent was 0.55 mg/l, approximately 50 percent less than the 30-day average limit of 1 mg/l imposed by the State of New York. The daily phosphorus data are presented in Table IV, along with data on biochemical oxygen demand (BOD) and SS.

Basic design and operating data for the demonstration are shown in Table V. Of particular interest is the return sludge flow to the phosphate stripper, which averaged 24 percent of the raw wastewater flow. Before conversion, the plant had been returning sludge at the rate of approximately 100 percent of the raw wastewater flow because of the poor settleability of the secondary sludge. The sludge volume index (SVI) of the mixed liquor was approximately 200, and the plant was

‡ This was despite the fact that a phosphate laundry detergent ban was started on June 1, 1973, just before the test. The ban had little effect on the influent phosphorus levels during the demonstration program, as is indicated by the sample of primary effluent taken on February 13, 1973, before the ban, which contained 5.6-mg/l total phosphorus (Table III).

TABLE IV.—Seneca Falls Daily Results*

Date: 1973	Phosphorus				BOD		SS				
	Total			Ortho							
	Raw Waste (mg/l)	Effluent (mg/l)	Sludge (%)	Stripper Supernatant (mg/l)	Raw Waste (mg/l)	Effluent (mg/l)	Effluent (mg/l)	Mixed Liquor (mg/l)	SVI	Return Activated Sludge (mg/l)	Return Anaerobic Sludge (mg/l)
7/19	5.4	0.74	—	26.0	—	—	6.0	1,540	110	7,480	15,000
20	6.5	0.87	2.09	26.0	186.0	3.9	8.0	1,410	106	5,980	16,700
21	6.7	0.68	2.75	22.0	—	—	3.0	1,580	101	8,380	15,600
22	6.0	0.52	2.87	30.0	—	—	2.0	1,810	99	7,900	14,350
23	8.6	—	2.85	26.0	—	—	—	1,760	85	6,810	15,300
24	7.1	1.0	2.64	30.0	186.0	4.4	2.0	1,240	113	8,000	15,600
25	6.5	0.72	2.66	35.0	—	—	3.0	1,200	108	7,840	15,500
26	7.5	0.62	3.89	28.0	—	—	2.0	1,400	107	7,520	16,350
27	6.8	0.68	2.70	43.0	140.0	5.7	2.0	1,110	117	7,520	16,200
28	6.1	0.58	3.08	35.0	—	—	1.0	1,630	104	8,180	15,800
29	7.5	0.40	3.75	47.0	—	—	1.0	1,460	109	8,880	15,000
30	6.4	0.72	3.80	47.0	—	—	2.0	1,650	97	10,320	15,100
31	6.4	0.57	2.88	47.0	—	—	7.0	1,070	140	8,440	16,000
8/1	4.9	0.53	2.84	55.0	—	—	2.5	1,160	120	10,320	16,250
2	7.2	0.40	3.08	60.0	—	—	2.0	1,290	116	9,060	16,200
3	6.5	0.36	3.57	43.0	105.0	2.7	2.0	1,300	107	9,780	16,400
4	7.0	0.55	3.38	46.0	—	—	6.0	1,220	139	8,018	17,350
5	7.5	0.37	3.00	47.0	—	—	1.5	1,610	118	7,500	16,200
6	7.8	0.68	3.32	41.5	—	—	4.5	1,300	146	7,800	15,800
7	4.5	0.46	—	45.0	135.0	1.2	4.0	—	—	—	—
8	4.0	0.58	3.60	47.0	—	—	1.5	1,490	114	7,800	16,300
9	5.0	0.46	3.29	60.0	—	—	3.5	1,450	104	8,700	16,680
10	5.0	0.29	3.76	57.0	188.0	5.5	1.0	1,870	107	9,760	14,950
11	4.5	0.54	3.63	52.0	—	—	2.5	1,630	123	8,350	15,700
12	5.7	0.54	2.92	60.0	—	—	—	1,550	130	6,180	15,700
13	6.8	0.69	3.06	89.0	—	—	—	1,510	112	8,260	15,500
14	6.1	0.3	3.17	48.0	165.0	1.3	—	1,520	138	9,680	15,800
15	3.3	4.0	2.7	50.0	—	—	—	880	195	1,320	18,650
16	6.0	0.49	2.79	63.0	—	—	—	1,300	138	—	15,000
17	5.9	0.20	2.47	55.0	—	—	—	1,750	120	9,900	16,950
Max	8.6	1.0†	3.89	89.0	188.0	5.7	8.0	1,870	146	10,320	18,650
Min	4.0	0.20	2.09	22.0	105.0	1.2	1.0	1,070	85	5,980	14,350
Avg	6.3	0.55†	3.10	45.3	158.0	3.5	3.0	1,457	115.3	8,309	15,910

* All samples except mixed liquor and return sludges were composited hourly: 8:00 AM to 4:00 PM daily.
† Deleting Aug. 15, 1973, value resulting from 8 mgd (30,280 cu m/day) + flow.

threatened with bulking sludge. Within several days after initiation of anaerobic detention of the return sludge, the SVI improved dramatically. For the 30-day demonstration period, the SVI averaged 115, ranging from 85 to 146. Settling in the stripper was also very good, and the solids concentrated to the point at which the return flow from the stripper to the aeration basin was about 10 percent of the raw wastewater flow.

Aeration is provided by three mechanical rotors located at approximate third points in the basin. The first two aerators, driven by two-speed 10-hp (7.5-kw) electric motors, are top rated at 40 lb (18.14 kg) of oxygen/hr at 20°C and zero DO. The low-speed setting drops the oxygen rating to 16 lb (7.25 kg)/hr. The third aerator, at the discharge end of the basin, is driven by a 15-hp (11.2-kw) electric motor and is rated at 49 and 20 lb (22.2 and 9.07 kg) of oxygen/hr at high and low speed, respectively. The first two aerators were operated by DO feedback control to maintain an average of 1.5 mg/l DO at the two-thirds

TABLE V.—Design and Operating Data, Seneca Falls Demonstration

Parameter	Quantity
Plant design flow (for the 0.5 plant used) (mgd)	1.75
Average plant flow during 30-day demonstration (mgd)	0.90
Range in plant flow (mgd)	0.2–8.0
Dry weather normal diurnal flow range (mgd)	0.2–1.0
Detention periods (based on average flow) (hr):	
Primary clarifier	3.9
Aerator	5.5
Secondary clarifier	3.2
Phosphate stripper (sludge)	
Max	26
Min	8
Avg	18
Lime mixing tank	0.166
Return sludge flow to phosphate stripper (% of raw flow)	24
Return sludge flow from stripper to aerator (% of raw flow)	10
Phosphate-enriched supernatant flow (% of raw flow)	14

Note: Gal × 3.785 = l.

point of the basin, with limits set at 0.5 and 2.5 mg/l. These aerators operated at high speed only 20 percent of the time and maintained an average DO at the control point of 2.2 mg/l. The third aerator was kept at low speed at all times. The aeration rate was consistent with the plant's normal rate, with no significant increase in oxygen demand being required for the phosphorus removal process.

The BOD determinations were made by the Seneca Falls plant staff. Samples were taken at 4-day intervals, with the results presented in Table IV. The final effluent samples were obtained before chlorination and contact detention. Nonetheless, the results were as good as those previously obtained for chlorinated effluent at this highly effective treatment plant.

The orthophosphate content of the stripper tank supernatant is shown in Table IV. The lime slurry used to precipitate the phosphorus was made with agricultural lime labeled as 70 to 75 percent CaO. Five 50-lb (22.68-kg) bags were used each day. Thus, the average dose as CaO was 250 × 0.725 = 181 lb (82.10 kg)/day for the average 0.9-mgd (3,406-cu m/day) total flow of the plant. This is a dosage of only 24.1 mg/l as CaO based on total plant flow. Stripper supernatant was returned to the primary clarifier with less than 1 mg/l dissolved phosphorus after the precipitation. Probably less lime could have been used, because the supernatant was diluted approximately sevenfold by the incoming wastewater and could have carried more phosphorus without an adverse effect. Further savings in lime over the normally required dose of 300 to 600 mg/l for post-precipitation treatment would soon approach marginal utility, however.

No other chemicals were applied. The pH of the system was monitored; results are shown in Table VI. Except for the lime-dosed supernatant, the range was near neutral, quite narrow, and consistent. Most significant, however, is the fact that the supernatant phosphorus was effectively removed at an average pH of only 9.2, as opposed to the pH of 11 required to achieve similar phosphorus reductions by treatment of the entire wastewater flow. The small volume into which the phosphorus was concentrated and the high phosphorus content of the supernatant are the key factors producing the large chemical savings inherent in the process.[3]

At the beginning of the demonstration program, the lime-dosed supernatant was

TABLE VI.—Seneca Falls Treatment Plant pH Profile during Demonstration

Value	Raw Waste	Primary Effluent	Aerator	Final Effluent	Return Activated Sludge	Return Anaerobic Sludge	Stripper Supernatant	Lime-Dosed Supernatant	Thickener Overflow	Digester Supernatant
Max	7.6	7.9	7.6	7.8	7.6	7.3	7.5	9.7	7.9	7.3
Min	6.9	7.1	7.0	7.0	7.0	6.8	6.8	8.8	7.2	7.1
Avg	7.3	7.6	7.4	7.5	7.3	7.0	7.2	9.2	7.5	7.2

TABLE VII.—Waste Sludge Characteristics during Demonstration at Seneca Falls

Characteristic	Raw Waste Sludge	Digested Waste Sludge
Total solids (%)	3.2	5.4
Specific resistance (M kg^{-1})	1.39×10^{14}	1.02×10^{14}
Filter yield (psf/hr)	1.28	3.23

discharged into three 1,000-gal (3.80-cu m) tanks connected in parallel for final settling and separate sludge removal. The settled chemical sludge was hauled to the drying beds used by the plant. Several such applications showed that the lime sludge spread and dried well, leaving a whitish, cracked surface within several days. After about 1 wk of operation in this fashion, however, the sludge handling was simplified by returning the lime-dosed supernatant from the mixing tank directly to the primary clarifier for settling and eventual introduction into the digester. No adverse consequences to digester operation occurred during the course of the demonstration or in the subsequent 9 months of operation.

Based on lime dosage, it is estimated that sludge production will be increased by 20 to 30 percent by the biological phosphorus removal process. This compares with increases of approximately 100 and 50 percent, respectively, for the lime addition to the primary and the mineral addition methods. Total solids, specific resistance, and filter yields of the unconditioned raw and digested sludges are presented in Table VII. The values are within the normal range for unconditioned, combined sludges.

Because of the varying and uncertain detentions of return flows from the thickener and digester, correlating these flows with the influent raw wastewater originally producing them is extremely difficult. Accordingly, a mass balance of total phosphorus entering the plant through the raw waste and leaving the plant via the effluent and the digested sludge was made with the last value obtained by difference. This balance, presented in Table VIII, shows that 91.8 percent of the total phosphorus entering the plant was ultimately removed in the digested sludge.

Storm overload. One of the advantages postulated [4] for the new process was that the stripper tank provides a valuable sludge reservoir for the activated sludge process. Solids may be stored when influent wastewater strength is low, or they may be added to the mixed liquor when the strength of the influent wastewater increases. Thus, better maintenance of the food:microorganism ratio is possible than in the conventional activated sludge plant. This hypothetical benefit of the sludge reservoir was dramatically proven on August 15, 1973. Infiltration from an intense summer rainstorm suddenly produced a flow exceeding 8.0 mgd (30,280 cu m/day) (offscale) at the treatment plant influent meter. Most of the MLSS and the sludge in the secondary clarifier were quickly lost over the final weir because of the turbulence induced by the high flow. The MLSS declined to 350 mg/l, and clarity in the secondary clarifier, as measured by the depth of water through which a target could be seen, was reduced to 2 in. (5.1 cm) [down from dry weather values of approximately 24 in. (61 cm) before installation of the new process and 36 to 54 in. (91.4 to 137.1 cm) during its operation]. The treatment plant operators stated that the normal recovery time for such episodes was 2 to 3 days. It was decided to test the ability of the stripper tank sludge reservoir to restore the plant to normal operation. At 8:00 AM, when

TABLE VIII.— Phosphorus Mass Balance at Seneca Falls

Point of Process	Average Daily Total Phosphorus	
	(lb/day)	(%)
Plant influent	47.2	100
Plant effluent	3.9	8.2
Digested sludge*	43.3	91.8

* By difference.
Note: Lb × 0.454 = kg.

the storm began, all wasting of sludge was stopped. At 10:00 AM, the decision to attempt the rescue operation was made, and the valve returning sludge from the stripper tank to the aeration basin was opened wide to build up MLSS. Table IX is a chronology of events with respect to key parameters.

At 2:45 PM, MLSS, as measured by the ss meter, attained 1,200 mg/l, whereupon the return flow from the stripper tank was reduced to normal. At 4:00 PM, the MLSS gave an indication of falling again. Accordingly, the return of sludge from the stripper tank was increased until 5:00 PM, when it was again returned to normal. In all, the sludge inventory in the stripper tank was reduced by 9 in. (22.8 cm), equal to 15,789 gal (59.76 cu m). If necessary, the tank could have provided sufficient solids for several additional such storms. As seen in Table IX, by 2:00 PM, orthophosphorus effluent levels had decreased below 1.0 mg/l. The plant stabilized and was back in normal operation within about 12 hr after the storm began. On the following day, an intensive sampling was conducted, and an hourly composited 24-hr sample showed a total phosphorus of 0.2 mg/l in the effluent (as opposed to the 0.49 mg/l shown for the August 16, 1973, hourly 8:00 AM to 4:00 PM, composited sample in Table IV).

Continued operation. On conclusion of the demonstration, village officials decided to keep the new process operating. Biospherics Inc. personnel instructed the Seneca Falls treatment plant staff in the daily routine. Except for minor mechanical difficulties, primarily because of leaves clogging the plant's return sludge pump, the process was operated throughout the winter. Except for periods of such difficulties, analyses, although not as detailed or frequent as those obtained during the demonstration period, showed the same high levels of performance as reported herein. The very cold winter temperatures of upper New York State had no adverse effect on the efficiency of phosphorus removal or on other aspects of the process. The process did not produce any odor or other nuisance problems. The process was operated until the following May, when it was felt that sufficient experience had been obtained to warrant a permanent installation at Seneca Falls.

TABLE IX.—Storm Overload Events, Seneca Falls Demonstration August 15, 1973

Time	Effluent PO_4-P (mg/l)	MLSS (mg/l)	Clarity* (in.)
8:00 AM	1.1	450	—
9:00 AM	—	—	2
10:00 AM	—	350	2
11:00 AM	2.5	450	—
12:00 PM	—	650	12
1:00 PM	1.3	850	14
2:00 PM	0.7	1,050	20
3:00 PM	0.4	1,250	—
4:00 PM	0.2	1,250	19
7:00 PM	0.2	1,350	24

* Clarity = Visual extinction depth in final clarifier.

Note: In. × 2.54 = cm.

COSTS

The biological process for phosphorus removal offers a dramatic saving in operating costs as a result of the reduced chemical requirement. Capital costs comparisons between it and competing methods will vary depending on the installation. Even in those instances in which the capital costs for the new process are higher, however, the greatly reduced operating costs quickly give it the advantage in total cost per million gallons treated.

Capital costs. The principal item of capital cost for the new method is the stripper tank. The tankage required is approximately 5 to 10 percent of average daily flow. In one instance, for a 5-mgd (18,925-cu m/day) plant (which would serve a population of approximately 50,000), the stripper tank was sized at 300,000 gal (1,162.5 cu m). The cost for this tank is $80,000, as reported [9] for the design of the process into the 5-mgd (18,925-cu m/day), Carpentersville, Ill., plant. The sludge collector, chemical contact tank, and mixer cost an additional $28,000, for a total capital cost of $108,000. In cases in which mineral addition can meet the stan-

TABLE X.—Estimated Operating, Capital, and Total Costs Per Million Gallons for Biological Phosphorus Removal Process, Two-Stage Lime, and Mineral Addition at 10 and 50 MGD

Item	Biological Process		Two-Stage Lime		Mineral Addition	
	10 mgd	50 mgd	10 mgd	50 mgd	10 mgd	50 mgd
Electricity	0.75	0.25	6.50	4.25	0.50	0.10
Natural gas	—	—	6.00	5.50	—	—
Alum	—	—	—	—	35.00	35.00
Polymer	—	—	—	—	3.50	3.50
Lime or makeup lime	2.50	2.50	11.50*	11.50*	—	—
Operating labor	5.00	3.00	30.00	9.00	3.00	1.50
Equipment maintenance	1.25	0.50	5.75	2.75	0.75	0.25
Solids disposal	4.50	4.50	20.00*	20.00*	10.00	10.00
Total operating cost, $/mil gal	14.00	10.75	79.75	53.00	52.75	50.35
Capital cost, $/mil gal	15.40†	10.40†	50.00	30.00	2.00	2.00
Total cost, $/mil gal	29.40	21.15	129.75	83.00	54.75	52.35
$/cap/yr	1.34	0.96	5.92	3.79	2.50	2.39

* Using lime recovery.
† Includes system cost and fees.
Notes: Capital amortized at 6%, 25 yr; no flow equalization or filtration facilities for any of the three processes (1973 dollars).
Gal × 3.785 = l.

dard without requiring equalization tanks or filtration, the corresponding capital costs (for chemical storage, chemical metering, and polymer feeding) are approximately $50,000 according to Environmental Protection Agency (EPA) data.[10] In this instance, the capital cost for the biological process would exceed that of mineral addition by $58,000, which difference, at 6 percent interest over 25 yr, would cost $2.46/mil gal ($0.65/1,000 cu m) treated, an amount small in comparison with the savings in chemical costs. Actually, Carpentersville officials found that the mineral addition method was not adequate. The conventional phosphorus removal method they would have had to use is "lime addition before the primary settler." Capital costs for this process would have been $165,000 according to the Carpentersville consulting engineer, $57,000 more than for the biological process.

Chemical costs. Bulk chemical costs for the new method are about $2.00 to $2.50/mil gal ($0.53 to $0.66/1,000 cu m) treated compared with $30 to $50 ($7.95 to $13.21/1,000 cu m)[10] for the cheapest other process capable of substantially the same phosphorus removal. Several of the communities that have tried the latter method report[11] actual chemical costs closer to $50 to $60/mil gal ($13.21 to $15.85/1,000 cu m) treated, however.

Operation and maintenance costs. Costs for operation and maintenance are only slightly more than for any of the other phosphorus removal processes and are considerably less than for some (those in which lime is recalcined, for example). At Seneca Falls, no additional staff was required, and operation and maintenance were handled within the normal operating day.

Sludge disposal costs. Sludge disposal for chemical methods to remove phosphorus is estimated[12] at about $10/mil gal ($2.64/1,000 cu m) of wastewater treated. The biological process of phosphorus removal produces only about 20 to 30 percent as much chemical sludge as lime precipitation and about 50 percent as much as mineral addition. Hence, sludge disposal costs for the new process offer an advantage of $5 to $8/mil gal ($1.32 to $2.11/1,000 cu m) of wastewater treated.

Detailed cost breakdown. An itemized

cost comparison for the new method and two principal alternative methods for phosphorus removal, two-stage lime addition and mineral addition to the aerator, are presented in Table X. They are based on independent engineering studies, the literature, EPA published data, and direct experience at Seneca Falls. Figures are presented for two plant sizes, 10 and 50 mgd (37,850 and 189,250 cu m/day). The costs are for "typical plants" and, of course, will vary somewhat from installation to installation, but they represent an effort to achieve an equitable comparison for the general cases. The savings in total costs with this process over the next best are $25.35/mil gal ($6.70/mil l) for a 10-mgd (37,850-cu m/day) plant and $31.20/mil gal ($8.25/mil l) for a 50-mgd (189,250-cu m/day) plant; corresponding savings in operating costs are $38.75 and $39.60/mil gal ($10.25 and $10.45/mil l).

Overall Economic Aspects

The new process offers economic advantages at two levels: at that of the plant itself and at the level of current national resource problems. In terms of the operating plant, the cost saving per million gallons of wastewater treated is dramatic. The reliability and resilience of the method make it an easy process to operate. This is in contrast to chemical dosing of the entire wastewater flow, in which variations in influent must be carefully monitored and chemical dosages adjusted accordingly for economic considerations. The new method permits the use of lime, which is the generally preferred phosphorus-precipitating agent from the standpoints of availability, ease of handling, and noncorrosive characteristics. Feed equipment is readily available and, because of the low dose rate, the equipment is small and easily managed. No polymer or other coagulant aid is required. The process provides important protection against plant washout, as demonstrated at Seneca Falls. In the many plants subject to frequent summer rains producing such washouts, average BOD removals may be improved significantly by the new process. The process is completely compatible with activated sludge, as was illustrated by the high BOD and SS removals and the improved sludge settling.

Summary

A new process for the biological concentration of wastewater phosphorus into a small substream from which it is precipitated chemically has been demonstrated at full scale. The New York State phosphorus effluent limit of 1 mg/l was easily met, and indications were given that even more stringent standards can be met. Total phosphorus effluents as low as 0.2 mg/l were obtained. The total cost of phosphorus removal and, particularly, the daily operating costs are drastically reduced over those for other methods. The method displayed technical advantages and was demonstrated to be completely compatible with the activated sludge process. It offers a means for significantly assisting the nation in meeting its phosphorus removal goals at less expense in dollars, chemicals, and energy, and in a manner that does not produce substitute pollution by large quantities of anions liberated from precipitating agents.

Acknowledgments

Credits. The authors wish to express appreciation to the village of Seneca Falls, N. Y., for undertaking the demonstration, to the Goulds Pump Co., Seneca Falls, N. Y., for the loan of its pumps, and to the Procter & Gamble Co., Cincinnati, Ohio, for funding the demonstration. We also wish to thank the following persons for their valuable personal cooperation: Patrick F. Cammuso, superintendent, Dept. of Water & Sewer, Village of Seneca Falls; Mike Capparelli and Al Hawker, chief operator and operator, Paul W. Simson Wastewater Treatment Plant; R. S. Bowles, Procter & Gamble Co.; and Barry Forster, Faculty Research Engineer, University of Maryland (formerly with Biospherics).

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APPLIED MICROBIOLOGY, Dec. 1973, p. 867-873

Vol. 26, No. 6
Printed in U.S.A.

Method for Radiorespirometric Detection of Bacteria in Pure Culture and in Blood

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Received for publication 5 July 1973

Methods are described for the detection of low numbers of bacteria by monitoring $^{14}CO_2$ evolved from ^{14}C-labeled substrates. Cell suspensions are filtered with membrane filters, and the filter is then moistened with 0.1 ml of labeled medium in a small, closed apparatus. Evolved $^{14}CO_2$ is collected with $Ba(OH)_2$-moistened filter pads and assayed with conventional radioactivity counting equipment. The kinetics of $^{14}CO_2$ evolution are shown for several species of bacteria. Fewer than 100 colony-forming units of most species tested were detected in 2 h or less. Bacteria were inoculated into blood and the mixture was treated to lyse the blood cells. The suspension was filtered and the filter was placed in a small volume of labeled medium. The evolved $^{14}CO_2$ was trapped and counted. A key development in the methodology was finding that an aqueous solution of Rhozyme and Triton X-100 produced lysis of blood but was not detrimental to bacteria.

The use of radioisotopes to detect the presence of microorganisms of medical significance was first reported by Levin et al. (7). The basic technique involved collection of bacteria on a membrane filter, immersion of the filter in a medium containing ^{14}C-labeled substrates, and collection of metabolically produced $^{14}CO_2$. Subsequent publications described the use of [*1*-^{14}C]lactose for a one-step, presumptive coliform test (8, 9) and the use of ^{14}C-formate in an inhibitory broth for a one-step, confirmatory fecal coliform test (10, 15). A quantitative relationship between evolved radioactivity and numbers of organisms was found.

Scott et al. (18, 19) confirmed the findings of the Levin group by using *m*-Endo broth containing ^{14}C-formate. Levin et al. have continued to develop the basic test as a means of life detection on other planets (5, 6, 11-14).

DeLand and Wagner (3) reported a radiometric method for the detection of bacterial growth in blood cultures. Their procedure involved the monitoring of $^{14}CO_2$ gas which was flushed from liquid culture bottles containing ^{14}C-labeled D-glucose. Washington and Yu (20) tested the method of DeLand and Wagner on simulated blood cultures and on a limited number of patient blood cultures. They reported that the radiometric method did not provide earlier evidence of bacteremia than did routine procedures and that it was impossible to detect 4 to 4,250 colony-forming units (CFU) within 6 h. Deblanc et al. (1) compared 2,967 blood cultures by conventional techniques and found that bacteria were detected more rapidly 70% of the time by the radiometric method. Waters (21) and Previte (16), by using an automated radiometric method, reported that detection times for various bacteria decreased with increased cell numbers. Depending upon species, inocula of 100 cells were detected in 6 to 14 h.

This publication describes modifications of the above-referenced coliform procedures for the detection of low numbers of various pathogenic bacteria in pure cultures and also in blood after lysis and filtration. Lysis of blood and filtration prior to radiorespirometric detection of bacteria is an important aspect of the procedure. Antibacterial agents present in blood are eliminated, evolution of $^{14}CO_2$ by blood cells which can mask detection of low numbers of bacteria is greatly decreased, low liquid volume and high specific activity of ^{14}C-labeled substrates promote a rapid response, and isolated colonies, which provide confirmation and material for isolation and sensitivity determinations, appear on the filter subsequent to positive detection.

Although the method has been developed specifically for detection of bacteremia, the

procedure is adaptable for spinal fluid and other filterable fluids for which a rapid sterility determination is needed.

MATERIALS AND METHODS

Cultures. Cultures of *Escherichia coli*, *Staphylococcus aureus*, hemolytic *Streptococcus*, *Salmonella paratyphi* B, *Haemophilus aphrophilus*, and *Cardiobacterium* sp. were supplied by James D. MacLowry of the National Institutes of Health. Cultures of *Pseudomonas aeruginosa*, *Klebsiella* sp., *Salmonella typhi*, *Shigella dysenteriae*, *Enterobacter aerogenes*, *Serratia marcescens*, and *Proteus vulgaris* were supplied by the Center for Disease Control, Atlanta, Ga. Cultures were maintained on Trypticase soy agar (TSA) with the exception of *H. aphrophilus* and *Cardiobacterium* which were maintained on TSA agar plus dextrose which had been enriched with 2 g of yeast extract, 20 mg of hemin, and 2 mg of nicotinamide adenine dinucleotide per liter. Eighteen to 24 h prior to an experiment, cultures were inoculated in broth medium and incubated at 37 C. Each culture was then serially diluted in Trypticase soy broth (TSB) medium before inoculation into the test system. Cell numbers were determined immediately before each experiment by spread plate techniques.

Lysis of blood. Normal blood specimens were supplied by the National Institutes of Health Clinical Center. They were drawn in 8-ml (yellow-cap) vacutainer tubes containing 0.05% sodium polyanathol sulfonate (Becton Dickinson).

Two techniques for lysing blood were principally used throughout the study. They were the modified technique of Rose and Bradley (17) and a Rhozyme procedure developed during this study. The former method was performed as follows. Blood (1 ml) was added to 19 ml of autoclaved, sterilized lysing solution (0.5 g of Triton X-100 [Sigma], 8 g of Na_2CO_3 per liter of water). The solution was allowed to stand for 3 or 4 min at room temperature and was then filtered through a 0.45-μm pore size membrane filter (Millipore Corp.). The filter was washed with 15 ml of 0.85% saline.

The Rhozyme method was performed as follows. Blood (3 ml) was added to 37.5 ml of lysing solution consisting of 4 ml of a stock solution (20 mg/ml, filtered and sterilized by membrane filtration) of Rhozyme 41 concentrate (Rohm and Haas), 1.5 ml of autoclaved 0.1% Triton X-100, and 32 ml of water. The mixture was incubated in a 37 C water bath for 30 min and then filtered through a 0.65-μm pore size 25-mm membrane filter. The filter was washed with 10 ml of sterile TSB. The concentrations of Triton X-100 and Rhozyme in the total volume of lysed blood were 0.04 mg/ml and 12.13 mg/ml, respectively.

Aliquots of the Rhozyme and Triton X-100 stock solutions were aseptically pipetted into screw-capped bottles of dilution water. These bottles of lysing solution were refrigerated and could be stored for at least 3 weeks without noticeable loss in lysing activity.

^{14}C-labeled medium. Radioactive medium was prepared in 10-ml batches as follows. To a sterile 25-ml vial were added: D-[*UL*-^{14}C]glucose, 60 μCi; [*1*-^{14}C]glucose, 20 μCi; [*UL*-^{14}C]glycine, 20 μCi; [^{14}C]formate, 20 μCi; and 0.1 ml of supplement B (Difco). Concentrated solutions of unlabeled substrates were added to bring the final concentration of each to 10^{-3} M. (Substrate additions accounted for less than 5% of the final volume.) The volume was brought to 8 ml with TSB and was filter sterilized by passage through a 0.22-μm pore size membrane filter in a microsyringe filter holder. After filtration, 2.0 ml of sterile horse serum was added. The vial was loosely capped and placed on a reciprocating shaker at room temperature overnight. This latter procedure was necessary to reduce levels of dissolved $^{14}CO_2$ in the medium. Sterile medium was stored at −5 C.

Apparatus. A radiorespirometer was constructed (Fig. 1). The design was such that: (i) membrane filters (25-mm diameter) would lie flat in a 0.1-ml volume of medium; (ii) $^{14}CO_2$-collecting pads containing aqueous $Ba(OH)_2$ could be changed at intervals without jeopardizing the sterility of the system; (iii) a tight-fitting, closed system prevented evaporation of the medium and would provide for the possible future assay of anaerobes; (iv) the entire unit was autoclavable; (v) inside heat space volume was small to promote rapid diffusion of evolved $^{14}CO_2$ and minimize the loss of $^{14}CO_2$ by adsorption on the walls of the apparatus.

The culture cups were separated from the connector and autoclaved in petri dishes. The connectors were wrapped in foil, autoclaved, and attached to a ring stand by clamps just prior to the start of an experiment.

Detection procedures. Bacterial suspensions were filtered directly or added to human blood and lysing solution and filtered through a membrane filter. The filter was then transferred to the incubation cup containing 0.1 ml of radioisotopically labeled medium. The cup was immediately attached to the radiorespirometer, and $^{14}CO_2$ collection was initiated by placing an adsorbant pad in the collection cup and moistening it with one drop of saturated $Ba(OH)_2$ solution. The $Ba(OH)_2$ pads were changed at intervals, the exposed pads were dried, and the radioactivity was determined in a gas flow counter (Nuclear-Chicago Corp., model 1040).

Aliquots of sterile TSB were also filtered, the membrane filters were placed in the radiorespirometers with ^{14}C-labeled medium, and the evolved $^{14}CO_2$ was collected. Results from this sterile medium con-

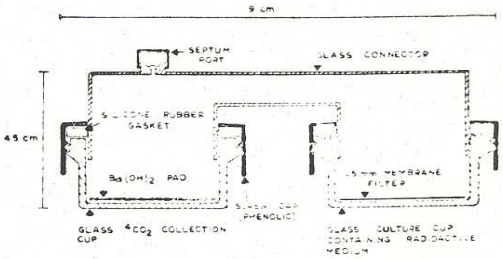

FIG. 1. *Diagram of radiorespirometer.*

stituted the control. A series of controls was conducted on each medium batch. Averages and standard deviations (σ) were determined for the controls for each time interval of incubation. To be classified as positive, a test level exceeding the average control by three σ or more was required. Different batches of ^{14}C-labeled medium displayed slightly different control levels; therefore, it was necessary to prepare a three σ curve for each batch.

RESULTS

Detection of bacteria. Incubation times producing positive signals for dilutions of various bacteria are presented in Table 1. Ten of 13 organisms tested gave a positive signal from approximately 10 CFU in less than 2 h, a significant improvement in rapidity and sensitivity of response over results reported heretofore. Higher cell concentrations were detected sooner than lower cell concentrations. Some organisms were positively detected by early readings, but produced signals below the positive level during continued incubation. Generally these organisms reverted to positive responses again later during the incubation. As

TABLE 1. *Detection times for various bacteria*

Organisms and CFU added/filter	Detection time (h)[a]												CFU recovered[b]				
	1	2	3	4	5	6	7	8	9	10	11	12	22	10	100	1,000	10,000
E. coli																	
10		−		−		−		+		+		+	+	6			
100		−		+		+		+		+		+	+		32		
1,000		−		+		+		+		+		+	+			TNTC[c]	TNTC
10,000		+		+		+		+		+		+	+				
P. aeruginosa																	
10		−		−		−		+		+		+	+	11			
100–10,000		+		+		+		+		+		+	+		TNTC	TNTC	TNTC
Klebsiella sp.																	
10	+		−		−		+		+				+	10			
100–10,000	+		+		+		+		+				+		44	TNTC	TNTC
S. aureus																	
10–10,000		+		+		+		+		+	+	+	+	15	52	TNTC	TNTC
Streptococcus (α hemolytic)																	
10	−	+		−		+		+		+		+	+	3			
100	−	+		+		+		+		+		+	+		5	9	45
1,000–10,000	+	+		+		+		+		+		+	+				
S. paratyphi B																	
10–10,000	+	+		+		+		+		+			+	7	52	TNTC	TNTC
S. typhi																	
10–10,000	+	−		+		+		−		+			+	—	—	—	—
S. dysenterae																	
10–10,000	+	+	+		+		−		−		+		−	2	2	13	TNTC
H. aphrophilus																	
10		−	−		−								−	9			
100–10,000	+	+	+		+		−		−				−		100	TNTC	TNTC
E. aerogenes																	
10–10,000	+	+	+		+		−		−				−	10	TNTC	TNTC	TNTC
S. marcescens																	
10	−	−	−		−				−		+		+	0			
100	−	−	+		+		−		+		+		+		100		
1,000–10,000	+	+	+		+		−		+		+		+			TNTC	TNTC
P. vulgaris																	
10–10,000	+	+		+		+		−					+	0	0	3	TNTC
Cardiobacterium sp.																	
10	+	+	+		+		−				+		+	0			
100	+	+	+		+		+				−		−		0		
1,000	−	+	+		+		−				+		+			0	
10,000	+	+	+		+		−				+		+				3

[a] +, 3 σ above average control; −, less than 3 σ above average control.

[b] After 24-h incubation in radiorespirometer, filters were aseptically transferred to an agar plate and incubated an additional 24 h.

[c] TNTC, Too numerous to count.

seen in Table 1, hemolytic *Streptococcus*, *H. aphrophilus*, *Klebsiella* sp., and *Cardiobacterium* showed this phenomenon. However, *Cardiobacterium* (100 CFU) which was positive at early times fell below the three σ level during continued incubation. Figures 2 and 3 show the kinetics of $^{14}CO_2$ evolution by approximately 100 CFU of bacteria in Table 1. Bacteria produce an initial curve which is parallel to, but higher than, the control curve. For most organisms, the curve breaks away from the control curve and rises sharply. In some cases, however, the bacterial curve remains at a low (even though positive) level throughout the 22-h incubation period. It appears that cells produce a small but detectable "early burst" of $^{14}CO_2$ initially. Most organisms then undergo a period of adaptation, of varying duration, and finally growth which results in the generation of considerable $^{14}CO_2$. Some organisms such as *Cardiobacterium* and *S. paratyphi* B apparently did not adapt and grow within the 22-h incubation period.

Some organisms, on the other hand, produced much $^{14}CO_2$ but were not recovered or showed poor recovery. This phenomenon appears to be due to inhibition of growth by membrane filters.

Detection of bacteria in blood. The detection of bacteria in blood by the respirometric method requires that the blood be sufficiently lysed to allow filtration through a membrane filter and to preclude a false-positive response generated by intact blood cells. However, the technique must not be damaging to bacteria. A lysing method based upon that reported by Rose and Bradley (17) was used during initial studies. Bacteria were inoculated into blood, which was then lysed, filtered, and monitored for evolution of $^{14}CO_2$. Controls were blood alone. A ratio of counts per minute evolved by the inoculated blood to the counts per minute evolved by uninoculated blood was obtained at various time intervals. Results (Fig. 4) show individual curves obtained in a number of experiments by using different media batches and blood samples. Although a threshold control level for normal blood cannot be represented, $^{14}CO_2$ evolution from low numbers of bacteria added to blood can be clearly distinguished from $^{14}CO_2$ evolution by blood cells. The method allowed low numbers of bacteria in blood to be detected within a few hours; however, adverse effects of the lysing solution on bacteria were observed. Bacterial numbers in the inoculum were verified by plate counts, and the recovery of inoculated bacteria was checked by counting colonies which occurred on the test filters. Recovery of gram-positive bacteria was generally

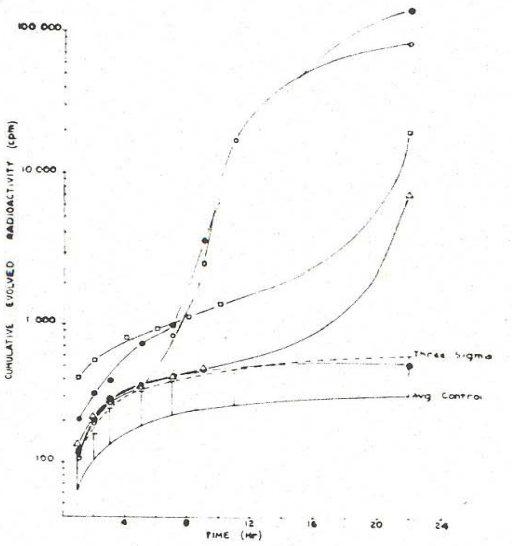

FIG. 3. *Evolution of $^{14}CO_2$ by approximately 100 CFU of: ●, Enterobacter sp; ○, S. marcescens; □, P. vulgaris; △, H. aphrophilus; and ◆, Cardiobacterium sp. The average control level and 3 σ confidence limit are given for medium alone.*

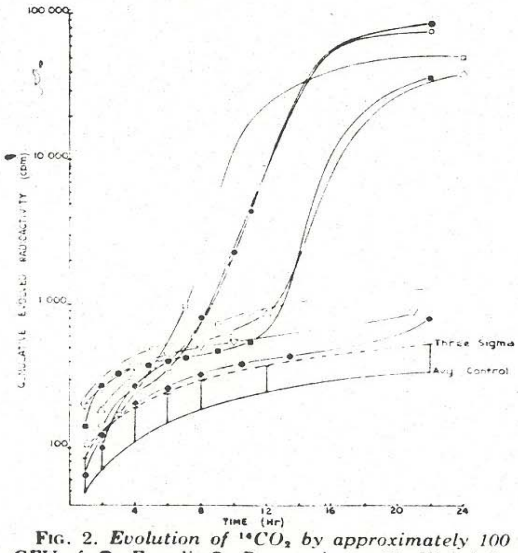

FIG. 2. *Evolution of $^{14}CO_2$ by approximately 100 CFU of: ●, E. coli; ○, P. aeruginosa; □, Klebsiella sp.; ■, S. typhi; △, S. aureus; ◇, S. dysenteriae; ▽, S. paratyphi B; ◆, alpha hemolytic Streptococcus. The average control level and 3 σ confidence limit are given for medium alone.*

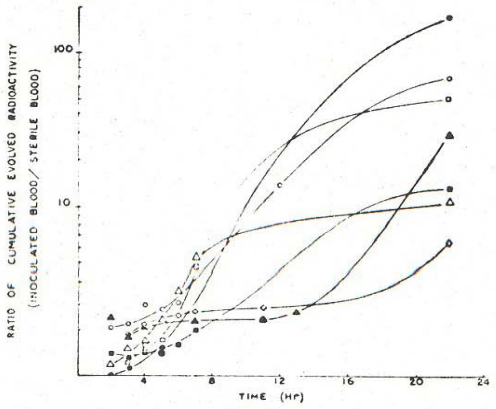

FIG. 4. *Detection of:* ●, *P. vulgaris (7 CFU);* ○, *pneumococcus (26 CFU);* □, *E. coli (10 CFU);* ▲, *Candida albicans (10 CFU);* ■, *P. aeruginosa (45 CFU);* △, *S. aureus (120 CFU); and* ◇, *enterococcus (15 CFU) in blood. Inocula were added to 1 ml of blood which was then lysed by the method of Rose and Bradley (17) and filtered.*

better than gram-negative bacteria; however, the evolution of $^{14}CO_2$ from both types was found to be decreased by the lysing agent. Farmer and Komorowski (4) also reported poor recoveries of some bacteria. Most bacteria which showed poor recovery also showed a decreased 22-h cumulative $^{14}CO_2$ evolution. However, the $^{14}CO_2$ evolution during the first 7 h was generally slightly greater for the bacteria which were inoculated into blood and then lysed than for bacteria alone.

Attempts were made to use a lysing solution less detrimental to bacterial cells than the highly alkaline 0.05% Triton X solution. The protease Rhozyme in conjunction with an aqueous solution of Triton-100 was found to be an effective, noninhibitory lysing solution. Table 2 shows the results of pure culture bacteria recovery after treatment with Rhozyme and Triton X-100. Recovery was approximately 100% with all organisms tested even in the presence of 10-fold higher concentrations of the lysing agents. Some organisms actually proliferated in the lysing solution during the 30-min incubation period.

Various final concentrations of Rhozyme (1.93 to 10 mg/liter) and Triton X-100 (0.036 to 0.3 mg/ml) were tested to determine the filterability of 4 ml of blood so treated. Filterability through a 0.65-μm pore size, 25-mm diameter membrane filter was measured. The less filterable mixtures filtered rapidly initially; but, as the filter clogged, the rate of filtration markedly decreased. Increased concentrations of either Rhozyme or Triton X-100 improved filtration. However, the Triton X-100 appeared to be a far more critical reagent. The highest concentrations of Rhozyme and Triton X-100 investigated (see Table 2) produced a filterable mixture after less than 15 min of incubation.

Several experiments were conducted to determine if bacteria may pass through the 0.65-μm pore size filter. Although very small organisms were not investigated, there was no significant difference in the number of CFU occurring on 0.45- and 0.65-μm filters used to filter bacterial suspensions of *E. coli*, *P. aeruginosa*, *S. marcescens*, and *S. aureus*.

Figure 5 shows typical results which were obtained for low numbers of *E. coli* in blood. Note that blood plus inoculum produced a

TABLE 2. *Recovery of bacteria treated with Rhozyme and Triton X-100*

Bacterium	Total vol (ml)[a]	Rhozyme (mg/ml)	Triton X-100 (mg/ml)	% Recovery
E. coli	37.5	2.13	0.040	116
E. coli	39.0	2.05	0.077	89
S. aureus	39.0	2.05	0.077	154
P. vulgaris	39.0	2.05	0.077	88
P. aeruginosa	39.0	2.05	0.077	95
E. coli	42.5	2.75	0.103	100
S. aureus	42.5	2.75	0.103	120
P. vulgaris	11.0	10.90	0.409	150
P. aeruginosa	11.0	10.90	0.409	93

[a] Volume of lysing solution only. No blood was used in these experiments.

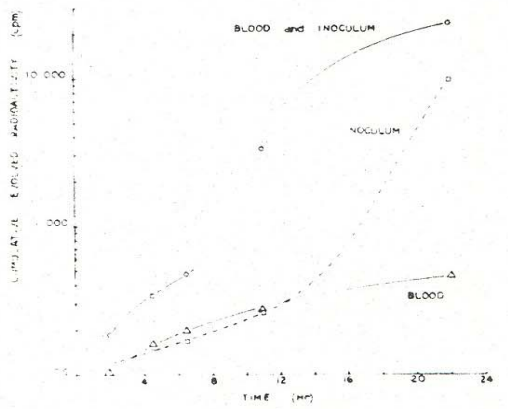

FIG. 5. *Detection of approximately 13 CFU of E. coli which were added to 4 ml of normal blood and lysed with Rhozyme and Triton X-100. Data shown are the average of duplicate determinations.*

greater and more rapid evolution than the same amount of inoculum alone. These results suggest that material from the blood which remains on the filter in some way enhances growth.

DISCUSSION

The procedure for the rapid radiorespirometric detection of bacteria in blood is shown schematically in Fig. 6.

The evolution of radioactivity from a medium containing ^{14}C-labeled compounds, by low numbers of bacteria, appears to follow a pattern which consists of an early evolution of a small but detectable amount of $^{14}CO_2$ evolution followed by a lag phase of several hours. Then a rapid evolution of $^{14}CO_2$ begins and produces a high cumulative level of evolved radioactivity. To detect the presence of microorganisms rapidly, it is necessary either to use a system which is sensitive enough to distinguish the early burst from control levels or to establish conditions which decrease the lag phase. The former approach saves valuable time and also permits detection of some organisms which do not adapt to the medium and fail to produce the characteristic rapid evolution of radioactivity after the early burst.

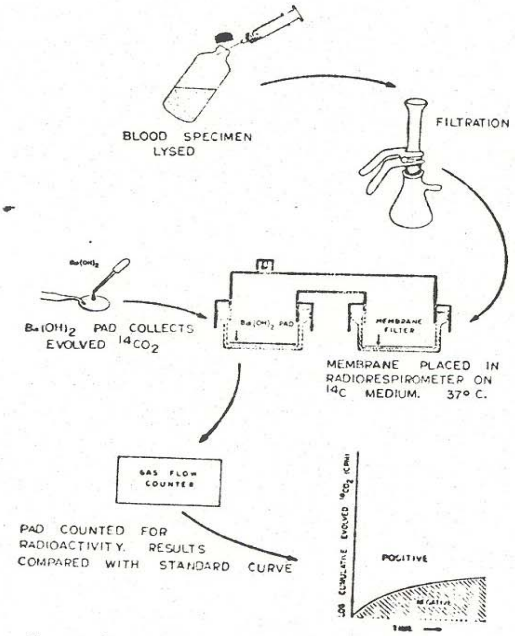

FIG. 6. *Procedure for rapid radiorespirometric detection of bacteria in blood.*

Of the two methods for lysing blood cells, 0.05% Triton X-100 plus 0.8% Na_2CO_3 and Rhozyme plus 0.005 Triton X-100, the latter appeared to be far superior. The Rhozyme-Triton X-100 method was not toxic to the bacteria tested; and, in some cases, growth actually occurred in the lysing solution.

The method herein described includes the advantage of both the membrane filter culture technique and rapid detection by radiorespiration. Lysis and filtration of blood as described concentrate bacteria and presumably wash them free of inhibitory blood constituents and antibiotics. In some cases, filtration may also provide a larger microbial sample, e.g., sterility testing of air of filterable liquids.

The use of a small amount of ^{14}C-labeled medium produces an earlier response than methods involving larger volumes of medium (1, 2, 3, 16, 20, 21). This large inoculum rapidly poises the medium and initiates growth. Lowering the pH by the bacteria as well as the large surface-volume ratio also facilitates rapid evolution of $^{14}CO_2$.

The use of a small volume (0.1 ml) of ^{14}C-labeled medium allows for the economical use of relatively high levels of radioactivity and expensive nonradioactive enrichments. The high level of radioactivity greatly increases the sentivity of the assay. Lysis and filtration eliminate much of the radiorespirometric response caused by blood cells, thereby increasing the sensitivity of the radiorespirometric detection.

Extended incubation of filters which show positive radiorespirometric results provides isolated colonies in the shortest possible time and with no additional manipulation, which may be used for sensitivity and identification determinations. The number of colonies on a filter also provides a quantitative estimate of bacterial numbers in the sample, and, in some cases, may assist in differentiating contamination from bacteremia.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health contract no. NIH-CC-72-2091.

The helpful suggestions of Robert Rose of the Millipore Corp. concerning blood lysis, the capable technical assistance of Mary Flynn and Margaret Federline, photography of figures by Patricia A. Straat, and fabrication of test apparatus by Chad Trent are gratefully acknowledged.

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