THE OHIO JOURNAL OF SCIENCE

Vol. 70 MAY, 1970 No. 3

FUNGI ASSOCIATED WITH THE ACTIVATED-SLUDGE PROCESS OF SEWAGE TREATMENT AT THE LEBANON, OHIO, SEWAGE-TREATMENT PLANT[1]

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ABSTRACT

Samples of materials in various stages of sewage treatment were obtained at monthly intervals between October, 1967, and April, 1968, from the Lebanon, Ohio, sewage-treatment plant. From both secondary and tertiary processes, a total of 93 species or species groups of fungi, including filamentous and yeast-like types, was recovered. Methods of sampling and isolation techniques, and data for several chemical and physical measurements made on the samples are presented. Comparisons of bacterial and fungal populations tested show that, on the basis of colonies recovered per ml of sample, effluent from the activated sludge aerator yielded 41 bacteria to one fungus, in contrast to a ratio of 1430 bacterial cells to one fungus cell in raw sewage. Many of the more common species of fungi present in the Lebanon sewage-treatment plant have also been recovered from similar-process materials in sewage-treatment plants at Dayton, Ohio, a number of sewage-treatment plants in the Chicago, Illinois area, a small waste-stabilization pond system in a different watershed near Lebanon, and from other sewage-treatment plants elsewhere. Fungi, being reducer organisms, are admirably adapted to a habitat in which their assimilative cells are continually bathed in a nutrient medium such as that offered by sewage in the process of treatment by the activated-sludge process.

INTRODUCTION

In general, throughout the literature dealing with sewage treatment, when the activated-sludge process is considered, emphasis is placed on the zoogloeal bacteria as the organisms responsible for good floc formation in an efficient treatment process. Literature of this subject has been reviewed by Cooke and Pipes (1969). That fungi are also present has been recognized for some time, especially in reference to one of the several types of bulking for which filamentous organisms are considered responsible, but it is only recently that the presence of fungi has been thought of as beneficial.

The present study was initiated in order to determine the kinds of fungi present in a small activated-sludge-type sewage-treatment plant, those techniques which would develop the fungal populations adequately, and the numbers of bacteria present at the same time. At the same time, a search was also made for fungi which might be associated with tertiary-treatment processes in an adjacent wastewater-renovation pilot-plant, in which the influent was the final product of the secondary plant.

METHODS

The data presented here were collected as a result of a search for as many kinds of fungi as possible in materials collected in critical stages of a sewage-

[1]Manuscript received May 28, 1969.

treatment process. The flow chart in Figure 1 shows from where samples were obtained: 1. raw sewage, 2. primary settled sewage, 3. aeration-tank influent, 4. aeration-tank effluent, 5. secondary effluent (effluent from a secondary settling-tank), 6. return sludge, and 7. primary settled sludge. Samples were collected once a month for seven months between October, 1967, and April, 1968. Samples were obtained from each of the seven process stages listed above on each date: Oct. 12 and 30, and Dec. 14, 1967, Jan. 25, Feb. 20, March 27, and April 23, 1968.

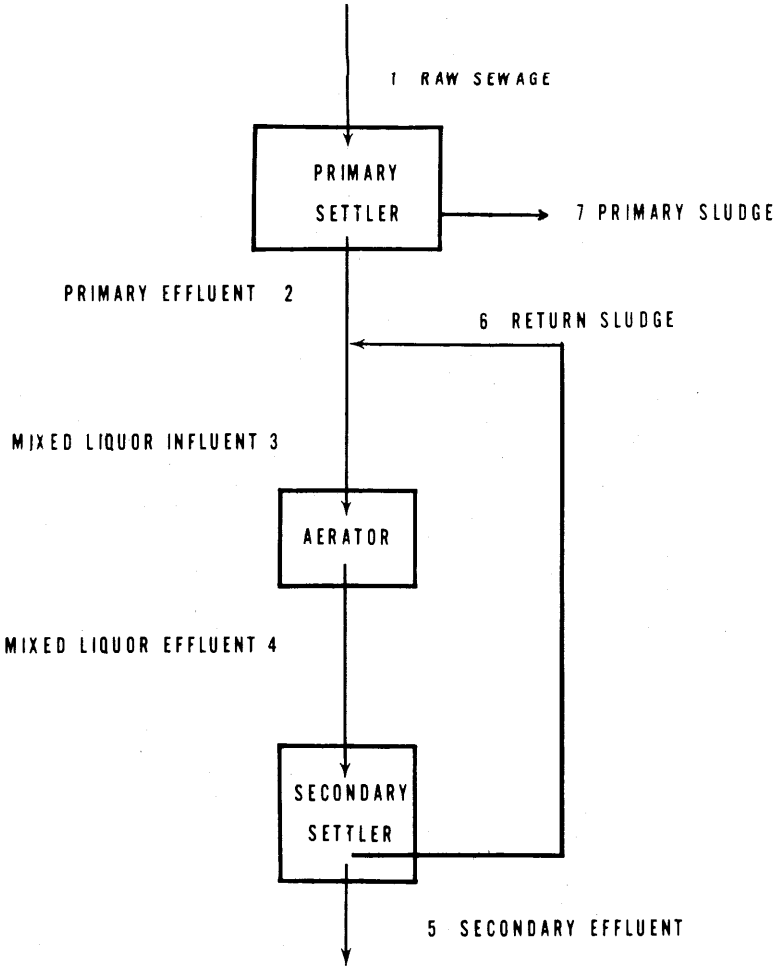

FIGURE 1. Flow chart of Lebanon, Ohio, secondary sewage-treatment plant (R. V. Villiers).

Samples were obtained in the morning, returned to the Cincinnati laboratory, and processed the same day. Samples were also taken from tertiary-treatment units which were in operation in the adjacent pilot plant on the same sampling date. Available tertiary-treatment units are indicated in the flow chart in Figure 2.

At the laboratory, portions of each sample were immediately removed for bacteriological analysis. Total count and the count of *Pseudomonas* spp. were the only bacteriological determinations made of these samples. Residues of all samples were analysed for total organic carbon and for organic nitrogen.

For development of fungus populations, samples were processed as in the Laboratory Guide (Cooke, 1963), using pour plates in replicates of five with neopeptone-dextrose agar, with and without rose bengal, but with Tetracycline, and using shaken-flask culture with YNB–1% glucose and YNB–20% glucose (Cooke, 1965). In order to determine whether or not *Candida albicans* was present, plates were prepared in which ABY and BiGGY agar (Nickerson, 1953), as well as Pagano-Levin Base agar (Difco, 1962), were used.

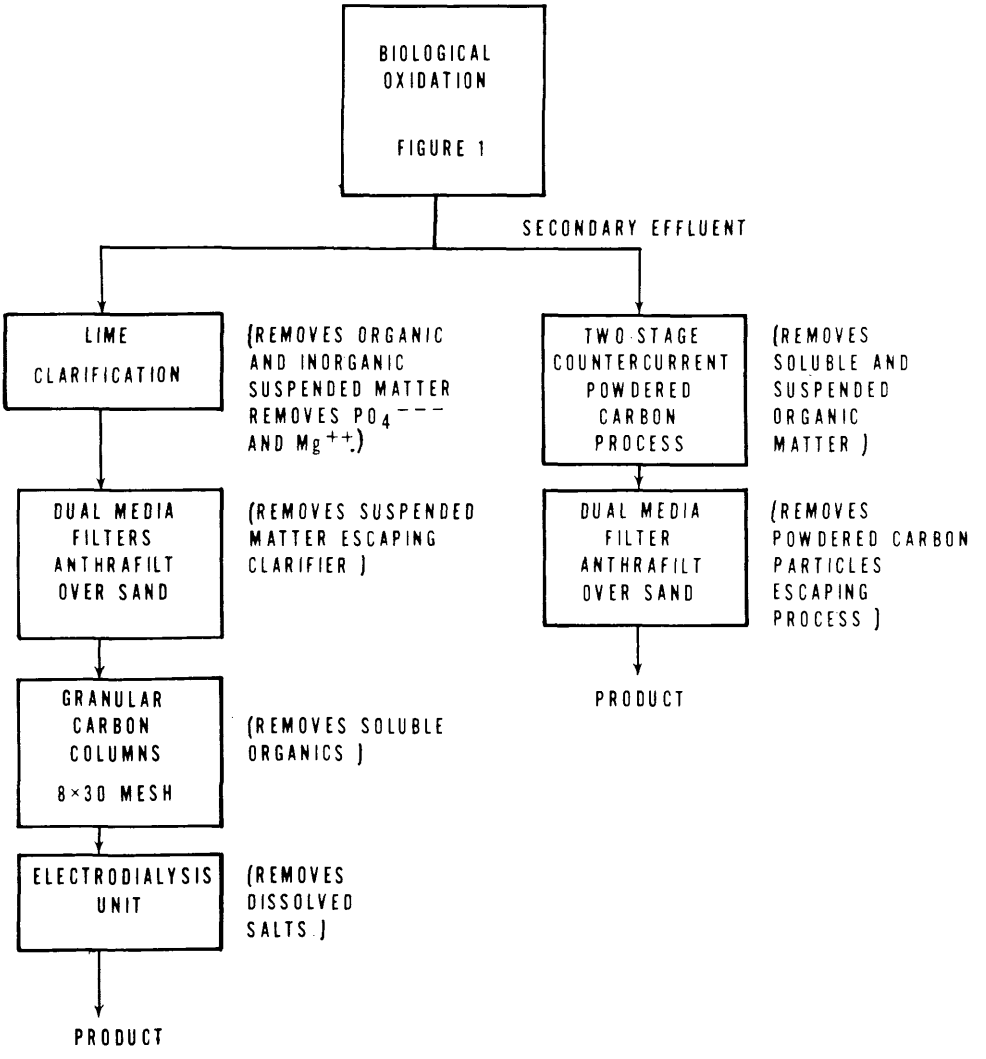

FIGURE 2. Flow chart of Lebanon, Ohio, tertiary sewage-treatment pilot plant (R. V. Villiers).

For relatively even distribution of sample and for partial break-up of floc, 1:10 dilution samples were shaken on the rotary shaker for a half hour at 130–150 oscillations per minute. Incubation of all preparations was at room conditions of light and temperature. Agar pour-plates were read after seven days, and other agar plates were read upon adequate colony development. Shaken-flask cultures were removed from the shaker after 64–98 hours. Colonies were identified according to procedures outlined in the Laboratory Guide (Cooke, 1963). It should be

noted that, for counting the fungus colonies, replicates of five plates were poured for each sample and for each medium. Counts presented in Tables 1, 2, and 3 were taken from neopeptone-dextrose-rose bengal-Tetracycline agar plates. In addition, microscopic observations showed mycelial filaments in much of the activated-sludge floc obtained on each of the seven sampling dates.

RESULTS

Numbers of bacteria and fungi recovered from samples obtained in the Lebanon sewage-treatment plant and in the tertiary-treatment pilot plant are given in Tables 1 and 2. In addition, certain pertinent data concerning the sewage are

TABLE 1

Lebanon Sewage-Treatment Plant Data

Date	Code	SVI	SS	BOD	DO	TOC	Org. N.	pH	Bacteria Total/ml $\times 10^5$	Fungi: Yeast-like IN*/ml $\times 10^3$	Fungi: Filamentous Colonies/ml $\times 10^3$	Fungi: Filamentous Colonies/gm QDW** $\times 10^6$
						1. RAW SEWAGE						
10-12-67	A	121	220	190	2.4	128	15.2	7.35	60	10	1.2	6.4
10-30-67	B	117	225	192	1.9	122	15.6	7.5	140	1	9.0	0.09
12-14-67	C	112	225	178	.9	66	8.4	NT	NT	0.2	1.9	2.1
1-25-68	D			194		125	11.2	NT	NT	3	1.8	2.0
2-20-68	E			190		86	11.2	NT	46	3	1.6	1.8
3-27-68	F			169		98	2.3	NT	3.9	10	3.7	0.61
4-23-68	G			184		65	10.4	6.8	29	0.1	8.0	1.3
						2. PRIMARY SETTLED SEWAGE						
	A		76	72		52	10.4	7.5	29	0.1	2.2	1.
	B		78	74		58	6.4	7.1	34	0.2	15.0	0.11
	C		64	71		33	0.9	NT	NT	0.2	1.4	1.03
	D			77		28	5.2	NT	NT	0.1	2.0	1.4
	E			70		43	6.8	NT	51	4.3	0.8	0.56
	F			78		40	7.6	NT	1.2	1.0	2.4	0.26
	G			75		39.5	7.0	6.2	17	0.001	7.0	0.73
						3. MIXED LIQUOR INFLUENT						
	A				(2.5)	1000	115.0	7.0	85	1.0	160.0	37.0
	B				(2.9)	1000	187.0	7.3	73	1.0	5.8	1.3
	C				(---)	324	9.5	NT	NT	20.0	12.0	1.6
	D					648	133.0	NT	NT	2.9	15.0	0.24
	E					1200	270.0	NT	2.4	30.0	42.0	9.5
	F					343	52.0	NT	5.9	100.0	12.0	0.17
	G					306	108.0	6.1	180	10.0	24.0	0.8
						4. MIXED LIQUOR EFFLUENT						
	A					680	145.0	6.9	34	1.0	220.0	76.0
	B					420	110.0	7.0	28	10.0	520.0	1.8
	C					103	4.0	NT	NT	20.0	6.4	2.3
	D					238	59.8	NT	NT	2.0	17.0	5.8
	E					1000	130.0	NT	1.3	3.0	17.0	5.4
	F					408	80.0	NT	4.9	1.0	10.0	0.52
	G					450	115.0	6.3	170.0	1.0	24.0	0.8
						5. FINAL PRODUCT						
	A		11	10	3.9	19.2	5.7	7.2	2.9	0.1	1.3	1.2
	B		9	10	3.9	119.0	12.2	7.0	2.1	0.1	23.0	0.19
	C		9	12	4.0	22.0	4.5	NT	NT	0.3	0.64	0.0054
	D			12		26.5	4.9	NT	NT	0.3	2.2	1.8
	E			15		29.5	<0.05	NT	0.68	0.001	0.9	0.0075
	F			12		12.0	2.0	NT	0.16	0.1	0.26	0.075
	G			11		11.0	1.9	6.6	4.3	0.1	0.23	0.011

TABLE 1—*Continued*

Date	Code	SVI	SS	BOD	DO	TOC	Org. N.	pH	Bacteria Total/ml ×10⁵	Fungi: Yeast-like IN*/ml ×10³	Fungi: Filamentous Colonies/ml ×10³	Fungi: Filamentous Colonies/gm ODW** ×10⁶
						6. RETURN SLUDGE						
	A					2000.0	332.0	6.7	35.0	1.0	3.7	5.8
	B					1670.0	308.0	7.1	78.0	2.0	500.0	0.78
	C					925.0	119.0	NT	NT	300.0	23.0	3.6
	D					1950.0	401.0	NT	NT	300.0	22.0	3.4
	E					3250.0	440.0	NT	4.3	0.2	55.0	8.6
	F					19.0	104.0	NT	5.9	10.0	20.0	0.076
	G					964.0	275.0	6.3	380.0	1.0	47.0	0.18
						7. PRIMARY SETTLED SLUDGE						
	A					10,500.0	386.0	5.8	230.0	100.0	8300.0	21.0
	B					12,300.0	1155.0	6.1	1600.0	300.0	660.0	0.17
	C					16,000.0	lost	NT	NT	20.0	200.0	5.0
	D					15,900.0	1255.0	NT	NT	20.0	130.0	3.3
	E					NS	NS	NS	NS	NS	NS	NS
	F					18,000.0	930.0	NT	490.0	100.0	250.0	5.8
	G					1,400.0	120.0	6.8	600.0	10.0	41.0	0.96

*IN—Indicated Number.
**ODW—Oven Dry Weight.
SVI—Sludge Volume Index.
SS—Suspended Solids.
NT or NS—Not sampled.

TABLE 2

Lebanon Tertiary Processes

Date	Code	Total Organic Carbon	Organic Nitrogen	Bacteria: Total Count/ml ×10³	Bacteria: *Pseudomonas* per ml ×10³	Number of Species	Fungi: *Colonies per ml* Filamentous	Fungi: *Colonies per ml* Yeast-like
				Lime Clarifier Effluent				
10–12–67	A	9.0	1.6	8.0	5.2	7	10	
10–30–67	B	NT	NT	NT	NT	2	5	20
12–14–67	C	NT	NT	NT	NT			
1–25–68	D	15.2	2.3	NT	NT	5	17	1
2–20–68	E	16.6	2.5	51.0	49.0	18	22	3500
3–27–68	F	12.0	1.1	1.0	0.7	4	50	
				Lime-Clarifier Filtered Effluent				
	E	15.8	1.8	180.0	170.0	8	22	870
4–22–68	G	7.0	0.9	4.8	2.7	6	10	
				Granular-Carbon-Column Feed				
	G	6.6	0.95	5.0	4.4	5	2564	

TABLE 2—*Continued*

Date	Code	Total *Organic* Carbon	Organic Nitrogen	Bacteria: Total Count/ml ×10³	Bacteria: *Pseudomonas* per ml ×10³	Fungi: Number of Species	Fungi: *Colonies per ml* Filamentous	Fungi: *Colonies per ml* Yeast-like
				Granular-Carbon-Column Effluent				
	A	3.0	0.7	1.7	1.6	2	30	
	B	NT	NT	NT	NT	4	4	
	E	<0.6	0.1	70.0	40.0	4	40	
	G	2.0	0.4	0.69	0.39	7	14	
				Powdered-Carbon-Column Effluent				
	A	9.0	3.9	3.7	28.0	8	240	
	D	8.0	0.15	NT	NT	11	27	20
	G	2.8	0.35	2.0	1.5	7	32	
				Electrodialysis-Unit Product				
	A	1.2	0.9	3.0	2.7	9	150	
	B	NT	NT	NT	NT	2	3	
	F	5.0	0.9	1.3	0.4	6	44	
	G	2.6	0.25	1.0	0.7	3	18	
				Electrodialysis-Unit Filter Surface				
4- 3-68	Washing	NT	NT	280.0	190.0	<8	<50,000	
	Scraping	NT	NT	3300.0	3200.0	<8	<50,000	

TABLE 3

Averages of Bacterial and Fungal Populations in Lebanon Samples Based on 7 sets of samples, Oct.-Apr.

Sewage Treatment Stage	Average Numbers of Bacteria per ml sample ×10⁶	Average Numbers of Fungi: per ml sample ×10³	Average Numbers of Fungi: per Gram oven dry weight ×10⁶	Ratios Bacteria : Fungi: per ml sample	Ratios Bacteria : Fungi: per Gram oven dry weight
Raw sewage	5.6	3.9	2.0	1430 : 1	2.7 : 1
Primary Settled Sewage	2.6	4.4	0.93	600 : 1	2.9 : 1
Aerator Influent	6.9	38.7	7.2	179 : 1	.95 : 1
Aerator effluent	4.8	116.0	13.2	41 : 1	.4 : 1
Final Product	0.5	4.1	0.47	132 : 1	1.2 : 1
Return Sludge	10.1	96.0	3.2	105 : 1	3.1 : 1
Primary Settled Sewage	73.0	1,083.0	6.0	67 : 1	12.1 : 1

also given. From available plant records, data were obtained on sludge-volume index (SVI), suspended solids (SS), biochemical oxygen demand (BOD), and dissolved oxygen (DO) for the dates on which the samples were collected; these are included in Table 1. Total organic carbon and organic nitrogen are given for all samples in parts per million. Total aerobic bacteria, and total numbers of *Pseudomonas* observed on membrane filters, have also been listed. While total bacterial counts were originally reported in terms of numbers per 100 ml, these have been corrected to numbers of bacteria per ml in Tables 1 and 2, since parallel records of fungi are based on numbers per ml.

Numbers of yeasts and of filamentous fungi were obtained in the Fungus Studies Laboratory, as were pH levels when opportunity permitted. Numbers of fungi are reported both as total colonies observed per ml of sample and as total colonies per gram dry weight of sample. In obtaining the latter value, the amount of oven-dry matter (at 100°C) was determined for the first set of samples only. It was assumed that samples obtained from comparable stages in the treatment process on later dates did not differ greatly from the first samples collected in the series. Averages of the values for the total count of bacteria per ml, and of the corrected total number of fungus colonies per ml of sample and per gram dry weight for each stage in the treatment process sampled are presented in Table 3. Ratios given here are for bacteria in relation to fungi present, based both on values per ml and values per gram oven-dry-weight.

An alphabetical list of species of fungi, regardless of systematic position, isolated from all secondary and tertiary treatment samples, is given in Table 4. Without considering incompletely identified yeasts, 93 species of fungi are listed. Of the yeasts, 32 species of white yeasts and two of red yeasts were identified from

TABLE 4

Alphabetical list of species of fungi isolated from various sewage-treatment processes at Lebanon, Warren Co., Ohio

Allescheria boydii Shear
Alternaria alternata (Fries) Keissler
Aspergillus spp.
flavipes (Bainier and Sartory) Thom and Church
flavus Link ex Link
fumigatus Fresenius
niger van Tieghem
cf. *puniceus* Kwon and Fennell
sydowii (Bainier and Sartory) Thom and Church
ustus (Bainier) Thom and Church var. *ustus*
versicolor (Vuillemin) Tiroboshi
Aureobasidium pullulans (de Bary) Arnaud
Botrytis cinerea Persoon
Candida brumptii Langeron and Guerra
catenulata Diddens and Lodder
curvata (Diddens and Lodder) Lodder and Kreger-van Rij
guilliermondii (Castellani) Langeron and Guerra
humicola (Daszewski) Diddens and Lodder
intermedia (Ciferri and Ashford) Langeron and Guerra
krusei (Castellani) Berkhout
mycoderma (Reess) Lodder and Kreger-van Rij
parapsilosis (Ashford) Langeron and Talice
pelliculosa Redaelli
scottii Diddens and Lodder
tenuis Diddens and Lodder
tropicalis (Castellani) Berkhout
utilis (Henneberg) Lodder and Kreger-van Rij
?*zeylanoides* (Castellani) Langeron and Guerra

TABLE 4. (*Continued*)

Cephalosporium spp.
Chaetomium funicolum M. C. Cooke
Cladosporium cladosporioides (Fresenius) de Vries
Coniothyrium fuckellii Saccardo
Cryptococcus spp.
diffluens (Zack) Lodder and Kreger-van Rij
laurentii (Keufferath Skinner
luteolus (Saito) Skinner
Epicoccum purpurascens Ehrenberg ex Schlechtendahl
Fusarium spp.
acuminatum Link
aquaeductuum (Radelmacher and Rabenhorst) Saccardo
oxysporum Schlechtendahl
roseum Link ex Fries
solani (Martius) Appel and Wollenweber
Geotrichum candidum Link ex Persoon
Gliocladium spp.
roseum (Link) Bainier
Gliomastix murorum (Corda) Hughes var. *felina* (March) Hughes
Moniliales spp.
Mucor spp.
hiemalis Wehmer
plumbeus Bonorden
Paecilomyces marquandii (Mason) Hughes
varioti Bainier
Penicillium spp.
chrysogenum Thom
funiculosum Thom
janthinellum Biourge
lilacinum Thom
martensii Biourge
ochro-chloron Biourge
variabile Sopp
vermiculatum Dangeard
Phoma spp.
herbarum Westendorp
Prototheca stagnora W. B. Cooke
Pyrenochaeta sp.
Rhinocladiella mansonii (Castellani) Schol-Schwarz
Rhizopus spp.
Rhodotorula spp.
glutinis (Fresenius) Harrison var. *glutinis*
mucilaginosa (Jörgensen) Harrison
Septoria sp.
Torulopsis aeria (Saito) Lodder
candida (Saito) Lodder
colliculosa (Hartmann) Saccardo
famata (Harrison) Lodder and Kreger-van Rij
glabrata (Anderson) Lodder and de Vries
holmii (Jörgensen) Lodder
inconspicua Lodder and Kreger-van Rij
?saki (Saito and Ota) Lodder and Kreger-van Rij
versatilis (Etchells and Bill) Lodder and Kreger-van Rij
Trichoderma viride Persoon ex S. F. Gray *sensu latissimo*
Trichosporon spp.
capitatum Diddens and Lodder
cutaneum (de Buermann, Gougerot and Vaucher) Ota var. *cutaneum*
fermentans Diddens and Lodder
margaritiferum (Stautz) Buchwald
Verticillium sp.
lateritium Berkeley
White yeast spp.

secondary-process materials, while eight of these were also recovered from tertiary-treatment processes.

Of the 93 species of fungi listed for the samples studied, 90 species were recovered from processes in the secondary-treatment plant, and 37 in the tertiary processes. Of those in the secondary plant, 13 filamentous fungi were found at least once in each of the seven processes sampled on each of the seven dates on which samples were taken. Two of these fungi were found in all 49 possible combinations of circumstances. Six species were found in six of the processes on at least one of the seven sampling days, two were found in five, five in four, ten in three, ten in two, and ten in only one process on only one sampling date. The data for distribution of the 37 species in the tertiary systems sampled are presented in Table 5.

TABLE 5
Distribution of Fungi in Tertiary-Treatment Processes

Species	Lime Clarifier		Granular Carbon Column		Powdered Carbon Column	Electrodialysis Unit		
						Filter		
	Product	Filtered	Feed	Effluent	Effluent	Effluent	Washings	Scrapings
Moniliales spp.	x	x	x	x	x	x	x	x
Phoma spp.	x	x	x	x	x	x	x	x
Penicillium lilacinum	x	x	x	x	x	x		
Rhinocladiella mansonii	x	x	x	x	x	x		
Rhodotorula spp.	x	x	x	x	x	x		
White yeasts spp.	x	x	x	x	x	x		
Geotrichum candidum	x	x	x	x*		x		
Aspergillus versicolor	x	x		x	x			
Rhodotorula mucilaginosa	x	x		x	x			
Cephalosporium spp.	x			x	x	x		
Trichoderma viride	x	x					x	x
Fusarium oxysporum	x	x			x			
Candida krusei	x	x			x			
Penicillium ochrochloron	x	x				x		
Fusarium aquaeductuum	x		x	x				
Cladosporium cladosporioides	x			x		x		
Cryptococcus sp.	x	x						
Rhodotorula glutinis	x	x						
Aureobasidium pullulans	x			x				
Mucor hiemalis	x			x				
Aspergillus flavus	x							
Aspergillus ustus	x							
Trichosporon sp.	x							
Candida intermedia	x							
Torulopsis inconspicua	x							
Penicillium spp.		x		x	x	x	x	x
Penicillium janthinellum				x			x	x
Trichosporon cutaneum	x			x	x			
Torulopsis glabrata			x					
Rhizopus sp.				x				
Septoria sp.				x				
Candida utilis				x				
Cryptococcus luteolus				x				
Talaromyces vermiculatum				x*				
Phoma herbarum					x			
Aspergillus niger						x		
Fusarium spp.							x	x
Mucor spp.							x	x
Species per habitat	26	16	9	21	14	12	7	7
Number of Samples	4	2	1	5	6	4	1	1

*—Slime from carbon granules yielded these species.

The species of filamentous fungi are listed in Table 6 by their occurrences on the seven sampling dates. The numbers given under each date identify the sewage-treatment process from which, in isolations that were made, the named fungus was found. In the last column of Table 6, the total number of times the fungus was observed is listed. In Table 7, letters representing the collecting dates of each fungus are given for each process. Again, in the last column of Table 7, the total number of times the fungus was observed is listed.

In addition to total numbers of colonies observed on the pour-plates, which were prepared, and which are reported above, two other types of observations were made on fungus colonies. In the first of these, a portion of the dilute sample was placed in yeast nitrogen base with either 1% or 20% glucose and shaken for three or four days. At the end of this time, the growth which appeared could be recorded as an indicated number, and cells could be streaked onto Diamalt agar plates from which, after purification, identifyable colonies could be recovered and studied. Results of identification of these colonies are given in Table 8.

TABLE 6

Distribution of Fungi in Lebanon Secondary-Sewage-Treatment Plant by Date and Process (Numerals represent process stages indicated below)

Species	10–12–67 A	10–30–67 B	12–14–67 C	1–25–68 D	2–20–68 E	3–27–68 F	4–23–68 G	Number of times appearing
Rhinocladiella mansonii	1234567	1234567	1234567	1234567	1234567	1234567	1234567	49
White Yeasts spp.	1234567	1234567	1234567	1234567	1234567	1234567	1234567	49
Geotrichum candidum	1234567	1234567	1234567	1 34567	1234567	1234 67	1234567	47
Moniliales spp.	1234567	1234567	1234567	3 56	1234 67	123 567	1234567	43
Penicillium spp.	1234567	12 567	23 56	1 34 67	1234 67	12 4 67	1234 67	37
Rhodotorula spp.	234567	12 7	123 5 7	3 5	1234567	123 567	1234567	35
Penicillium lilacinum	1234 67	1234567		123 567	3 567	123456	123456	34
Mucor hiemalis	34 67	234 67	1 4 67	34 67	6	3	234 67	24
Fusarium oxysporum	1234567		67	1 5	1234 67	1	567	21
Fusarium aquaeductuum	2 4 7		1234		1234 6	12 7	123 567	21
Trichosporon spp.	12 567	12 5 7	12 567	7	1 5	2 456	67	21
Trichosporon cutaneum	1 34 67	123456	12	1	1 345 7	4	1	21
Cephalosporium spp.	1234567	1234567	2 7	3				17
Phoma spp.	2 4		1	3 6	1 3 56	2 4	1 34567	17
Cladosporium cladosporioides	2345	1 3	12 45		1	3	1 3 5	15
Trichoderma viride	1 567	12 6		67	3 6	6		12
Aspergillus flavus	4	1 7	3		4 6	1 4 6	23	11
Phoma herbarum	234567	7	2 5			6		10
Aspergillus niger		123456	1		3 6			9
Cryptococcus spp.			3	12 6	123 5			8
Penicillium ochrochloron	3 567	567						7

Process stages:

1—Raw sewage
2—Primary settled sewage
3—Aerator influent
4—Aerator effluent
5—Final product
6—Return sludge
7—Primary settled sludge

Species present in 5 or fewer samples include:

5: *Aureobasidium pullulans, Penicillium janthinellum, Pyrenochaeta* sp., and *Verticillium lateritium;*

4: *Fusarium* spp., *Mucor* spp., *Rhizopus* spp., *Verticillium* sp.;

3: *Aspergillus* spp., *A. flavipes, A.* cf. *puniceus, A. versicolor, Coniothyrium fuckelli, Epicoccum purpurascens, Gliocladium* spp., *Gliomastix murorum* var. *felinum, Mucor plumbeus, Penicillium funiculosum;*

2: *Aspergillus fumigatus, Fusarium roseum, Gliocladium roseum, Paecilomyces varioti, Penicillium chrysogenum, Trichosporon margaritiferum;*

1: *Alternaria alternata, Aspergillus sydowii, A. ustus, Botrytis cinerea, Chaetomium funicolum, Fusarium acuminatum, Paecilomyces marquandii,* and *Penicillium martensii.*

TABLE 7

Distribution of Fungi in Lebanon Secondary-Sewage-Treatment Plant by Process and Date
(Letters represent dates indicated below)

Species	Raw Sewage 1	Primary Settled Sewage 2	Acrator Influent 3	Acrator Effluent 4	Final Product 5	Return Sludge 6	Primary Settled Sludge 7	Number of Times Appearing
Rhinocladiella mansonii	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	49
White yeasts spp.	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	49
Geotrichum candidum	ABCDEFG	ABC EFG	ABCDEFG	ABCDEFG	ABCDE G	ABCDEFG	ABCDEFG	47
Moniliales spp.	ABC EFG	ABC EFG	ABCDEFG	ABC E G	ABCD FG	ABCDEFG	ABC EFG	43
Penicillium spp.	AB DEFG	ABC FG	A CDE G	AB DEFG	ABC	ABCDEFG	AB D FG	37
Rhodotorula spp.	BC EFG	ABC EFG	A CDEFG	A E G	A CDEFG	A EFG	ABC EFG	37
Penicillium lilacinum	AB D FG	AB D FG	B DEFG	AB FG	B DEFG	AB DEFG	AB DE	34
Mucor hiemalis	C	B G	AB D FG	BCD FG		ABCDE G	ABCD G	23
Trichosporon spp.	ABC E	ABC F		F	ABC F	A C FG	ABCD G	23
Fusarium oxysporum	A DEF	A E	A E	A E	A DE G	A C E G	A C E G	23
Fusarium aquaeductuum	C EFG	A C EFG	C E G	A C E	G	E G	A FG	21
Trichosporon cutaneum	ABCDE G	BC	AB E	B EF	B E	AB	A E	20
Cephalosporium spp.	AB	ABC	AB D	AB	AB	AB	ABC	17
Phoma spp.	C E G	A F	DE G	FG	E G	DE G	G	16
Cladosporium cladosporioides	BC E G	A C	AB FG	A C	A C G			15
Trichoderma viride	AB	B	E		A	AB DEF	A D	12
Aspergillus flavus	B F	G	C G	EF	EF	EF	B	10
Phoma herbarum	A C		A	A	A C	A F	AB	10
Aspergillus niger	BC	B	B E	B	B	B E		9
Cryptococcus spp.	DE	DE	C E		E	D		8
Penicillium ochrochloron			A	E	AB	AB	AB	8

Dates of sampling:
A—10-12-67 B—10-30-67 C—12-14-67 D—1-25-68 E—2-20-68 F—3-27-68 G—4-23-68

Species appearing in 5 samples or fewer are listed under table 6.

TABLE 8

Distribution of Yeasts in Lebanon Secondary-Sewage-Treatment Plant by Process and Date
(Letters represent dates indicated below)

Species	Raw Sewage 1	Primary Settled Sewage 2	Aerator Influent 3	Aerator Effluent 4	Final Product 5	Return Sludge 6	Primary Settled Sludge 7	Number of Times Appearing
Unidentified White Yeasts	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	ABCDEFG	ABCDE G	ABCDEFG	48
Rhodotorula spp.	BC EFG	ABC EFG	A CDEFG	A EFG	A CDEFG	AB EFG	A C EFG	37
Trichosporon spp.	ABC E	ABC EF	C	E	ABC F	ABCD FG	A C	23
Trichosporon cutaneum	ABCDE G	BC	AB E	B DEF	A DE	A E	AB	22
Candida intermedia	F	FG	C E G	G		FG	E	10
Rhodotorula mucilaginosa	F	AB G	E G	E G	C	G	A F	12
Candida parapsiolosis	DEFG	D	E	EF	G	E G		11
Torulopsis versatilis	E	A E	B EF			E	C E	9
Torulopsis aeria	E	A	A E	D	D	A	A D	9
Torulopsis holmii	CD	CD F	DE	A D				9
Candida krusei	A E	E	C	C		G	C	7
Cryptococcus spp.	E	DE	F		E	E	D	7
Torulopsis famata	A F	A EF		G	E			7
Torulopsis candida	D F		G	G	A D			6
Candida guilliermondii		F	DE	D	D	D		6
Candida pelliculosa			A			AB D	A D	6
Rhodotorula glutinis	F				CDE	F		5

Dates of sampling:

A—10-12-67 B—10-30-67 C—12-14-67 D—1-25-68 E—2-20-68 F—3-27-68 G—4-23-68

Species appearing in four or fewer samples include:

4: *Trichosporon capitatum, Cryptococcus laurentii;*
3: *Candida curvata;*
2: *Candida utilis, C. ?zeylanoides, C. tenuis, C. catenulata, Torulopsis colliculosa, Cryptococcus diffluens, Trichosporon margaritiferum;*
1: *Torulopsis inconspicua, T. glabrata, T. ?saki, Candida scottii, C. tropicalis, C. brumptii, C. mycoderma, C. humicola, Trichosporon fermentans, Cryptococcus luteolus, Prototheca stagnora.*

The categories "*Rhodotorula* spp." (or red yeasts) and "white yeasts" represent unidentified colonies which were observed. Where white yeasts appear on all seven dates in all seven processes, the individual species making up the complex may have been isolated only once, or may have appeared on all seven dates in all seven processes.

Because *Candida albicans* is an organism which could be present in sewage and sewage-polluted water, and because it had been isolated previously only two or three times from such waters, two different types of media used to screen cultures of this fungus from similar-appearing fungi in the laboratory were tried in an attempt to recover this yeast from samples in this series. The first of these were two bismuth-sulphite media, in which *C. albicans* colonies should appear black with or without a darkened halo. The second of these was Pagano-Levin Base agar, on which *C. albicans* colonies should appear cream to bright pink. Various species of yeast, yeast-like, and filamentous fungi appeared on these media, but in no case could a definite assignment to *C. albicans* be made. Colonies suspected of being that species were picked for future study, but growth patterns in each case were sufficiently different to rule out the recognition of this species in the series of samples from which isolations were made.

DISCUSSION

The Nature of Sludge

The composition of sewage is unknown, varying from place to place and from time to time. The elemental composition probably can be determined, but not the structure, if one should want to consider sewage as a chemical entity. Any listing of elemental components would produce an inaccurate picture. One-third to one-half of the contents of the solid fraction of sewage is cellulosic in nature (Maki, 1954) and comes from a variety of sources. The BOD of sewage is thought to be about 300 ppm in "average" untreated sanitary sewage. The DO of this sewage approaches zero, according to the Winckler test. The glucose content cannot be determined easily and is thought to approach zero. Parenthetically, it may be noted that most common fungi of sewage grow well on hexose sugars, while a few grow on pentose sugars, in contrast to the waste stream in the paper-pulp industry where pentose-using yeasts are common. Depending on the conditions of the use of the environment, the nature of the wastes discharged to the trunk sewer, and other factors, traces of a variety of substances may be present in waste waters, such as odor- and taste-producing compounds, pesticides, stereoids, toxins, antibiotics, and a variety of other compounds.

How these elements, ions, compounds, substances, etc., are put together, and their relation to each other, is unknown. Mingling, mixing, blending, and other phenomena occur in the waste streams as they flow from their sources, merge, and increase in volume. Oxygen in these waters, and in the air through which they pass, is quickly depleted as the requirements of oxygen-demanding fractions of these waste components are satisfied. This means that there is a minimum of oxygen for the microorganisms living in these waters which require this essential element.

Sewage-Treatment-Plant Populations

A variety of types of filamentous fungi and yeasts was obtained from the seven monthly samples of sewages in various stages of treatment in the Lebanon, Ohio, sewage-treatment plant, a small activated-sludge plant, and in auxiliary tertiary-treatment processes using effluent from the secondary plant. The same kinds of fungi have been found in the secondary-treatment plant at Dayton, Ohio, a trickling-filter-type plant (Cooke, 1959), and in waste-stabilization ponds used briefly several years ago at a dormitory on a penal honor farm not far from Lebanon (Cooke and Matsuura, 1963, 1969). The list of species is not greatly

different from that obtained in a study of 19 activated-sludge plants in the vicinity of Chicago, Illinois (Cooke and Pipes, 1969).

All of these plants were operated independently of each other. The sampling at Dayton and at each of the two Lebanon installations was separated by several years; an increased sophistication in sampling and isolation techniques characterized the later samplings.

Bacteria and fungi are reducing organisms and as such form the most important members of the populations in biological waste-water sewage treatment. They absorb nutrients which are either readily available, or made available through the action on waste components of exoenzymes they secrete into the habitat. The majority of bacteria and all the fungi are aerobic organisms. While oxygen is readily and always available to them in normal habitats, in sewage and in digesting sludges, free-oxygen supplies are not usually measurable by chemical means. Where free oxygen is not readily demonstrated by chemical techniques, survival and growth depend on recovery, by the organism, of trace amounts of oxygen present as dissolved oxygen, or resulting from decomposition processes (Tabak and Cooke, 1968b).

All the common reducers of sewage and sewage-treatment systems may be considered facultative anaerobes, on the basis of studies by Tabak and Cooke (1968a, 1968b). They may survive, metabolize, and grow in the presence of dissolved oxygen at levels as low as −40 Eh' (a measure of the oxidation-reduction potential). During the processes of metabolism and growth, these organisms, added in the waste-water stream or air-spore fallout, or present as natural members of populations in systems of conduction and treatment of sewage, reduce readily available compounds which thus serve as nutrients. While at their death they may add to BOD through dead cells and cytoplasm, in life they may add to BOD only through release of excess exoenzymes. During life they reduce BOD abundantly through metabolic processes, contributing to the biological treatment of waste water. On the other hand, these organisms add to the complexity of the populations present in sewage-treatment systems. While they include disease producers in man and animals, potential disease producers in man, animals, and vascular crop plants, and producers of toxins of a variety of types, their activity within the treatment system, in the secondary-treatment process, is of greater importance than their potential nuisance outside the system, where they can be eliminated by disinfection processes.

It is known that at least one fungus, one streptomycete, and several species of bacteria, which are true or facultative filament formers, cause bulking in activated sludge (an undesirable condition). It is also known that one or more filamentous fungi cause clogging of trickling filters when rocks forming the filter bed are small enough to produce only small-sized spaces between each other. It is assumed that bulking of activated sludge, or clogging of trickling filters, is triggered by an excess of wastes, including nutrients which are capable of allowing rapid growth of bulking organisms. On the basis of experiments in pure culture with presumed causal organisms in the presence of presumed nutrient compounds as sole sources of nutrients, bulking or clogging can be demonstrated. In mixed culture, however, the trigger effect of small amounts or even traces of certain types of degradable domestic or industrial wastes in a waste stream is still unknown.

As producers, algae added to this system, inadvertently or through deliberate processes, as in oxidation ponds and channels, add oxygen to the system through metabolic processes which include both normal photosynthesis and certain heterotrophic phenomena comparable to those of bacteria and fungi. Protozoans, as consumers, occur in the system as a result of additions with sewage input and as natural inhabitants. These organisms may act as reducers through accidental ingestion of whatever compounds may be passing through the system. More importantly, they have been shown to exert a type of natural control over the

bacterial population. When in balance, bacteria are ingested by protozoans and metazoans. This results in maintenance of the bacterial population in a continuous logarithmic phase of growth; if the plateau stage, at approximately a steady state, were reached, less activity could be expected from them.

Fungi in Sewage-Treatment Plants

The Lebanon sewage treatment plant is operated on the activated-sludge principle. Within the series of habitats found in this plant, the species of fungi, or decomposer organisms, which are present may be considered to be accidental inhabitants. They reach the plant in the raw sewage, or as fall-out precipitated from the air, or washed out of the air by rain. They reach the sewer from household plumbing from the bathroom, the kitchen, the laundry, or the basement drain. They include organisms which have been a part of the natural populations in man's respiratory and digestive tracts, as well as organisms from the soil washed from muddy hands or clothing, organisms washed from the surface of the body, man's clothing, the vegetables he eats, the walls and woodwork of his house, soil tracked into the basement or utility room, and other sources.

Fifty-six of the categories of fungi listed in Table 4 represent that portion of those colonies observed on isolation plates which could be relatively readily identified to species, or to genus in some cases. One of the more interesting of these was *Flammulina velutipes*, a mushroom-producing fungus usually associated with standing dead elms and other deciduous trees. In the "Moniliales" have been placed all unidentifiable molds, in the "white yeasts" all unidentifiable white yeasts. Completed identifications among the white yeasts have yielded 32 species, none of which can be distinguished by relatively simple techniques of observation.

These fungal and bacterial reducer organisms form most of the living material in the larger particulate matter and solids settled out of the raw sewage. Even in the soluble impurities, colloidal materials, and small suspended particles in the liquid which is channeled to an aerator after a brief period in the primary settling tank, a quantity of bacterial and fungal cells is found. Just before entering the aerator, return sludge is mixed with primary settled sewage, thus adding a population of microorganisms already acclimated to a habitat of this type and developed in such a habitat over a period of time. Air, yielding a quantity of oxygen to the mixed liquor, is pumped through a series of diffuser tubes into the mixed liquor as the sewage travels through the aerator tank. As the mixed liquor leaves the aerator tank, it still contains a large amount of organic matter, but this is now largely in the form of activated-sludge floc, a loose mass of microorganisms including bacteria, fungi, and protozoans.

In order to remove this mass of organic matter, the mixed liquor is piped to a final, or secondary settling tank. Here the microbial floc is settled out, becoming return sludge, and the partially clarified supernatant becomes the effluent which is discharged into the receiving stream, as the final product of the sewage-treatment plant. At Lebanon, a portion of the final product is allowed to run into adjacent Turtle Creek, while the remainder, at the time of this study, was used in several tertiary-treatment processes by a pilot-plant of the Advanced Waste Treatment Research Laboratory, Federal Water Pollution Control Administration, U. S. Department of the Interior.

In one process, the secondary effluent from the Lebanon treatment plant is sent through a powdered activated-carbon system. Here organic matter and microorganisms are removed by the activated carbon by adsorption and sedimentation methods, much as taste- and odor-causing chemicals are removed in a water-treatment plant from raw waters in a purification process that utilizes activated carbon.

In another instance, the secondary effluent is run through a lime-clarifier, which removes virtually all of the suspended organic matter and some of the micro-

organisms. The product is then processed through a granular-activated-carbon column, which removes soluble organics. The product of this process is then demineralized by an electrodialysis unit. The final product of this process should need only chlorination to make it near-potable drinking water.

Samples were obtained for testing for the presence of disseminules—spores or pieces of mycelium—from each of these processes. In all cases, fungus colonies were obtained on agar plates. The numbers were small, but the colonies were present. Granular-carbon particles and electrodialysis membranes were tested for the presence of fungus cells and these were found sometimes in larger numbers than expected, therefore too numerous to count. Effluents from the granular-carbon column, powdered-carbon process, and lime-clarifier contained fungus cells in low numbers, as did the effluent from the electrodialysis unit. In the first of these processes, the effluents contained viable cells, whether they were passed through sand filters or not. The effluent from the electrodialysis unit could have contained spores as a result of picking these up from growths on the outer surface of the filter membrane.

There are problems in the interpretation of the meaning of the numbers of fungal colonies given in Tables 1, 2, and 3. Bacteria, filamentous fungi, and yeasts are all saprobic organisms requiring preformed organic matter. At least, no photosynthetic or chemosynthetic bacteria are known to be operative in the system being considered here. Bacteria and yeasts usually form colonies which can be broken up readily into single cells, although some bacteria produce regular or irregular clumps of cells which are not readily broken up, and some yeasts produce a true mycelium which usually remains in filaments. On the other hand, filamentous fungi usually produce growths which are made up of highly branched filaments which are not readily broken up into the individual cells of which they are formed. Of the filamentous fungi, only *Geotrichum candidum* breaks up readily in such a way that each cell presumably becomes a spore or a disseminule. Because of these growth characteristics, the interpretation of the meaning of a colony count is difficult. Does a colony arise from a single cell, from a one-celled spore, from a two-to-many-celled spore, from a piece of mycelium two to ten or more cells long, or from a clump of mycelium which may include one or more complex branching systems? Time has not been available for developing data on which to base answers to these questions. In any one sample, all these types of starting points for colonies are possible.

In the method used in this study for counting colonies, no consideration was paid to the source of the colony; thus, regardless of whether it arose from one cell or from many cells, the individual colony is valued at unity, the same as each bacterial colony counted in the bacteriology laboratory, or the yeast colony in the Fungus Studies Laboratory. Yeast colonies, too, could have originated from more than one cell in some instances; the parental disseminule could have been a clump of cells from a pseudomycelium, or an elongated piece of mycelium composed of several cells. On the basis of the life form of the fungi listed in Tables 4–8, it is therefore very possible that the estimated numbers of fungal colonies listed in Tables 1–3 are low.

The volumes of fungal cells, compared with those of bacterial cells, are much greater, so that in many cases the space occupied by one fungus cell could have been occupied by 100 bacterial cells. On this basis, the ratio of bacterial cells per ml of sample to fungal cells per ml of sample yield interesting comparisons, even though these are based on colony counts which may be biased against the fungi. Here, on the average, there are many more bacteria than fungi in the raw-sewage influent to the plant. Again, on the average, in primary settled sewage entering the aerators, there is still a higher number of bacteria in relation to fungi, even allowing for the difference in size. Within the aerator influent channel, where return sludge has been mixed with primary settled sewage, the ratio decreases;

in the aerator effluent, there are relatively fewer bacteria, while in the final product, in the return sludge, there is nearly a 1:1 ratio of bacteria to fungi.

Yeasts were present in all samples taken in the secondary plant, and in many of those from the tertiary processes. On the basis of indicated numbers (Table 4), values for yeasts or yeast-like fungi reached as high as 200,000 cells per ml of sample in primary settled sludge, or as low as 10 to 200 cells per ml of sample in the final product. Thus yeast cells were available to various tertiary-treatment processes where, in one instance (on February 20, 1968), 3000 cells per ml were recovered from effluent from the lime clarifyer. In Table 1 the indicated values are based on inspection of shaken primary isolation flasks, rather than on actually identified species present in the flask. That is, when 200 cells were reported, the dilution from which they were recovered was 1:100, and two observably different types of yeast species were present.

It was of interest that, in trying to isolate the pathogenic yeast, *Candida albicans*, on each of two types of media presumably selective for it, it did not appear. In the medical mycology laboratory, in which it is important to have a rapid test for the presence of this species among a number of other yeasts and yeast-like fungi in pathological specimens, the use of acid bismuth yeast medium (ABY), or bismuth-GGY medium (BiGGY), and the use of Pagano-Levin Base agar (PLB) can aid in the determination of the presence of this yeast. Since in this study, few such colonies appeared and of these none developed other growth patterns of *C. albicans*, it is concluded that in samples collected between October 12, 1967, and April 22, 1968, *Candida albicans* was not present.

In those tertiary processes available for sampling in the period covered by this report, filamentous fungi and yeasts were present (Table 2). Neither bacteria nor fungi were as abundant as in the secondary processes, but their presence was certain, as a result of plating techniques using samples which appeared clear. Some effluents carried more fungus cells than others, but within the processes themselves, especially on the granular carbon in the carbon columns (or tubes), and on the membranes in the electrodialysis unit, growth was good to abundant and cumulative until the unit was taken down for repair or cleaning.

CONCLUSION

The growth of fungi, filamentous and yeast-like, in those structures devoted to the secondary and tertiary treatment of sewage, indicates that the liquors being treated contain substances available to these fungi as food and that the fungi are able to utilize such substances as food. As living organisms, these fungi form part of the biological population the treatment plant was designed to attract and put in use as the basic unit of the biological treatment system. These fungi are, therefore, performing a useful and necessary function in the biological treatment of sewage being presented to them as a substrate on or in which to grow.

ACKNOWLEDGEMENTS

Bacteriological counts were made by B. A. Kenner. Total organic carbon and organic nitrogen determinations were made by R. T. Williams and his staff. R. V. Villiers, Advanced Waste Treatment Research Laboratory, in charge of the tertiary-treatment pilot-plant at the Lebanon waste-water treatment plant, was most helpful in aiding in sample collection and in explaining various parts of the tertiary-treatment process. He prepared the flow charts in Figures 1 and 2.

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