Mycopathologia et Mycologia applicata, vol. 42, 1–2, pag. 89–111, 1970.

FUNGI IN THE LEBANON SEWAGE TREATMENT PLANTS AND IN TURTLE CREEK, WARREN CO., OHIO

by

WM. BRIDGE COOKE*

ABSTRACT

Using a variety of isolation techniques in agar and liquid media culture, 101 species of filamentous fungi and yeasts were isolated from samples of materials from Turtle Creek, Warren Co., Ohio, and 100 species of fungi were isolated from materials from secondary and tertiary treatment processes in the Lebanon, Warrren Co., Ohio, sewage treatment plant. The combined list of fungi from the two types of materials numbered 142 species. Based on such habitat factors as content of total organic carbon, and organic nitrogen, the distribution of these fungi was discussed especially in relation to seven sampling points in the secondary-type treatment system, eight in the tertiary-type treatment systems, and five sampling points along Turtle Creek, two above the outfall from the Lebanon sewage treatment plant, one at the outfall, and two below that outfall. It is concluded that, as in the cases of Lytle Creek, Clinton Co., Ohio.; the Bear River in the Cache Valley, Utah and Idaho; and the Cache la Poudre River, Colorado, a group of fungi made up of many of the same species is adaptable to the conditions resulting from organic enrichment of streams resulting from sewage or industrial waste additives to the streams. These organisms contribute to the removal of this polluting organic matter. At least monthly sampling from any group of stations is needed to describe the populations more accurately and more meaningfully.

INTRODUCTION

It has been shown in previous studies that fungi are present in large numbers in both sewage treatment plants and in natural streams, reaches of which receive effluents from sewage treatment plants. Thus, in a study of Lytle Creek, Clinton Co., Ohio, which receives the effluent from the Wilmington sewage treatment plant, it was shown (COOKE, 1961) that above the point at which this effluent was received larger numbers of species represented by smaller numbers of colonies were present than at or below the point at which the outfall was received. As the stream continued downward, the numbers of species increased, and the numbers of colonies decreased although in the more polluted reach, smaller numbers of

* Formerly, Mycologist, Microbiological Treatment Activities, Advanced Waste Treatment Research Laboratory, Robert A. Taft Water Research Center, Federal Water Pollution Control Administration, U.S. Dept. of the Interior. Present address: 1135 Wilshire Ct., Cincinnati, Ohio 45230.
Accepted for publication: 9.II.1970.

species represented by larger numbers of colonies were recovered.

In the case of sewage treatment plants, it has been shown that in a trickling filter-type plant (COOKE, 1958, 1959a, b, COOKE & HIRSCH, 1958) fungi are important members of the surface film of organisms effecting biological treatment on the stone in trickling filter beds as well as being present throughout the whole sytem, especially in processes directly related to the treatment of sewage. In a waste stabilization pond system, fungi (COOKE & MATSUURA, 1969), including yeasts (COOKE & MATSUURA, 1963), were shown to be abundantly present throughout the system, especially in areas which were specifically designed for the treatment of sewage or waste waters. In the type of plant using the activated sludge process, COOKE & PIPES (1968, 1970), and COOKE (1970) have shown that fungi are important members of the population involved in the biological treatment of sewage.

In developing concepts of the sources of these fungi for the production of populations in sewage treatment systems or polluted streams, it has been shown in the cited references that populations of fungi are present in the habitat in the case of streams, and in the influents to sewage treatment plants. These populations reach the sewage treatment plant through runoff from any surface, through sewage and industrial waste of most types, and through air spora fallout. They reach the stream through runoff from the soil, air spora fallout, and through natural occurrence in the stream.

In May, 1969, an opportunity was presented to demonstrate the occurrence of fungi in a small polluted stream to Dr. FAUSTA ROGLEDI, visiting microbiologist from the Italian Water Pollution Research laboratory. Samples were taken from five points on Turtle Creek, Warren Co., Ohio. These points included two above the outfall of the Lebanon sewage treatment plant, the second of which was at the point of reception of sewage bypassing the treatment plant for one of several operational reasons; the area at the outfall point; and two points downstream from the plant at about 1.6 and 2.9 miles below the outfall point. Sampling at these points was repeated in October, 1969, and a complete set of samples was obtained from points within the secondary and tertiary systems of sewage treatment operated at the Lebanon sewage treatment plant by the City of Lebanon, and by the Advanced Waste Treatment Research Laboratory of the Federal Water Pollution Control Administration as one of the facilities of the Robert A. Taft Water Research Center.

METHODS

Samples were collected in the field in plastic vials with snap-on caps. The vial was inserted in the stream; water, water and sediments from the stream bottom in pools, and in two cases in riffles, and stream bank soil at or just above the water line were scooped into the vials without touching the inside or lip of the vial or without

handling the material being sampled. Sewage treatment plant samples were poured into the vials aseptically. The vials were returned to the laboratory and refrigerated over night. In the meantime, dilution waters and media had been prepared.

Media used for demonstration of fungus populations included both liquid and agar types. Using membrane filter sterilization, liquid media based on yeast nitrogen base (YNB, Difco) using 1 % and 20 % glucose were prepared, and dispensed, using sterile glassware, at the rate of 50 ml per 250 ml Erlenmeyer flask. These flasks were cotton stoppered, numbered, and kept on the laboratory bench until inoculated. Agar media were prepared using neopeptone-dextrose agar, and neopeptone-dextrose agar with rose bengal (COOKE, 1963). These media were distributed at the rate of 10 ml per tube in culture tubes which were metal capped and stored in a hot water bath until plates were ready to be poured. In addition, hemp seed was halved and autoclaved in preparation for selective isolation of aquatic fungi, if present. For the October samples, an additional set of plates of media was prepared using several types of dehydrated pellet-type agar media.

Following preparation of the media, the dilution water, and collection of the samples, dilutions of the samples were prepared. To 135 ml of distilled water were added 15 ml of sample. At the same time, pairs of soil cans received identical 15 ml units of the sample. From the weighed sample in these cans, which were weighed prior to use, the water was evaporated in an oven at 95° C overnight, and the oven dry (OD) weight of the sample was obtained. To disperse the sample through the dilution water, the 1 : 10 dilution flasks were placed on a rotary shaker at slow speed (about 150 oscillations per minute) for a half hour. At the end of this time the flasks were removed and from those from which greater than 1 : 10 dilutions were required 5 ml were removed and added to 45 ml distilled water. This process was repeated for 1 : 1000 and 1 : 10,000 dilutions in case of samples rich in organic matter such as stream bottom sediments, bank soils and sewage sludges.

When the final dilutions had been prepared, five tubes of agar were removed from the water bath for each type of agar and each level of dilution in which the test was to be made. In some cases it was thought desirable to test two different dilution levels, the higher one chosen for use and the next higher one. For pour places, both neopeptone-dextrose and neopeptone-dextrose-rose bengal agars were used. To both types of media was added 0.05 ml of a 1 g/150 ml solution of tetracycline per tube containing 10 ml of agar. Following this, 1 ml of the dilution of the sample to be used was added to each tube, and the contents of the tube were promptly poured into a 9 cm plastic petri dish. The dishes were labeled and stacked on the laboratory bench for incubation at room conditions of light (daylight and fluorescent light during working hours) and temperature (approx. 27° C, or varying between 25°—30° C) for seven

days. At the end of that time the plates were read and inventoried. The total number of colonies was counted, those colonies which could readily be identified were listed, and in the May series were counted. Those colonies not readily identified were picked and inoculated on neopeptone-dextrose agar slants for future study, or mounted for direct microscopic examination. The plates were then discarded by autoclaving. Critical identification of some isolates was completed by specialists to whom the cultures were submitted.

Portions of the samples were submitted to personnel in the Analytical Applications Laboratory for determination of total dissolved solids (TDS), total organic carbon (TOC), chemical oxygen demand (COD), and a number of inorganic ions. The determination of pH was made on portions of the 1 : 10 dilution of the samples the same day or shortly after plating. Within limits of time, Mr. B. A. KENNER tested samples for bacterial content.

To each of a pair of flasks of YNB, one flask having 1 % glucose, the other 20 %, was added 1 ml of the 1 : 10 dilution of each sample. The flasks were then placed on the rotary shaker at low speed (approx. 150 oscillations per min) for 88 hours. Following their removal they were placed on the laboratory bench for approximately four hours during which time yeast cells settled to the bottom. Balls, chunks, or floc of filamentous fungus tissue settled or remained in suspension, and bacterial cells, if present, remained in suspension. Using a loop, a quantity of yeast cells were then picked from the interface between the liquid and the bottom of the tilted flask and streaked on Diamalt agar plates. These plates were incubated at room conditions of light and temperature (see above) for two or three days after which colonies were picked for study or for purification. Purified yeast colonies were identified to species using a series of morphological and physiological tests, or in the October samples, were identified only to genus by morphological appearance. Those filamentous fungi appearing in this test were placed on Diamalt agar and tentatively identified following the development of conidia and conidiophores.

A portion of the 1 : 10 diluted sample, or of the original sample, if sufficient material was available, was placed in a 9 cm plastic petri dish, an autoclaved halved hemp seed was added, and the plate incubated at room conditions of light and temperature on the laboratory bench. At the end of 1—2 weeks seeds on which aquatic fungi developed were transferred to petri dishes containing distilled water in which activated carbon had been boiled and filtered off and to which fresh halved autoclaved hemp seeds were added. Identification of resulting aquatic fungus growth was completed by Dr. ROLAND SEYMOUD, University of Pittsburgh.

To each of several plates of different agar media was added 1 ml of 1:10 diluted sample. This was spread over the surface of the agar. Fungus colonies growing on these plates were added, when identifiable, to the list of species obtained from each of the stations sampled.

RESULTS

A list of the locations sampled with a few notes about each habitat is presented in Table I. Station numbers used in this table will be used in other tables and in the discussion which follows.

TABLE I. Locations and Habitats Sampled

Station Number	Sample Number	Location and Habitat
		Turtle Creek, Warren Co., Ohio
1		Above County Road bridge just east of the sewage treatment plant.
	1	Water
	2	Water and Sediment in Pool
	3	Bank Soil
2		At Bypass Outfall, Lebanon Sewage Treatment Plant
	4	Water
	5	Water and Sediment in Pool
	6	Bank Soil
3		At the Outfall, Lebanon Sewage Treatment Plant.
	7	Water
	8	Water and Sediment in Pool
	9	Bank Soil
4		1.6 mi. below the Sewage Treatment Plant, at the US 42 bridge.
	10	Water
	11	Water and Sediment in Pool
	12	Bank soil
5		2.9 mi. below the Sewage Treatment Plant, along Columbia Road.
	13	Water
	14	Water and Sediment in Pool
	15	Bank Soil
		Lebanon Sewage Treatment Plant
	16	Raw sewage
	17	Primary Settled Sewage
	18	Mixed Liquor Influent to Activated Sludge Tanks
	19	Mixed Liquor Effuent from Activated Sludge Tanks
	20	Final Product – Sewage Treatment Plant Effluent
	21	Return Sludge
	22	Primary Settled Sludge
		Advanced Waste Treatment Research Laboratory Tertiary Treatment Processes
	23	Primary Settled Sewage (Process Influent) (17)
	24	Lime Clarifier Effluent
	25	Lime Clarifier Filter Effluent
	26	Effluent from Carbon Column 1
	27	Effluent from Carbon Column 2
	28	Effluent from Carbon Column 3
	29	Influent to the Villiers Activated Sludge Unit (17)
	30	Effluent from the Villiers Activated Sludge Unit

Results of tests of samples for TDS, COD, TOC, organic nitrogen, and pH (delayed), are given in Table II. These results are based

Table II. Physical and Chemical Characteristics of Samples

Sample Number	TDS	COD	TOC	Org. N		C/N Ratio	pH (delayed)
1	mg/l*	477	11.1	6.4	0.5	13	7.9
2	%	–	.25	.08	.01	8	8.2
3	%	–	.66	.13	0.014	9	8.2
4	mg/l	428	12.5	5.4	0.7	8	7.4
5	%	–	.84	.45	0.982	0.46	7.9
6	%	–	.34	.27	0.004	67.5	8.2
7	mg/l	765	1,130.0	255.0	41.5	6.2	7.8
8	%	–	.43	.01	0.014	0.7	8.0
9	%	–	2.37	1.29	3.36	0.38	9.0
10	mg/l	605	20.6	8.2	0.88	10.00	8.1
11	%	–	2.87	.38	0.102	3.7	8.9
12	%	–	1.98	.65	0.08	8.0	8.5
13	mg/l	614	27.6	9.4	2.4	4	8.3
14	%	–	.21	.10	0.007	14	8.4
15	%	–	.13	.04	0.005	8	8.3
16	mg/l	1370	477.	133.	11.1	12	8.0
17	mg/l	1072	230.	47.	7.0	6.7	8.0
18	mg/l	955	6,170	1,520	201.0	7.5	7.5
19	mg/l	907	2,910	672	121.0	5.5	7.6
20	mg/l	839	172	44	2.0	22.0	7.5
21	mg/l	961	13,180	2,500	393.0	6.4	7.6
22	mg/l	4008	58,360	19,680	1,070.0	18.4	6.7
23	mg/l	1057	127	50.5	8.3	6.1	8.0
24	mg/l	1068	40.3	11.6	2.3	1.5	7.6
25	mg/l	1168	42.0	10.6	1.6	6.6	7.6
26	mg/l	966	40.9	10.6	1.4	7.6	7.6
27	mg/l	963	23.5	6.6	1.5	4.4	7.6
28	mg/l	924	23.8	4.6	1.6	2.9	7.6
29	mg/l	852	2,910.	312.0	145.0	2.2	7.4
30	mg/l	854	23.2	6.4	1.3	5.0	7.5

* Mg/l – milligrams per liter for liquid samples
% or milligrams per gram for solid samples.

on samples collected Oct. 9, 1969. A carbon/nitrogen ratio is given based on the ratio to total organic carbon (TOC) to organic nitrogen.

In Table III are presented the results of colony counts of fungal plates poured from the samples and of bacterial tests made on the samples. Only three samples were checked for bacterial counts. These included primary settled sewage in the secondary treatment plant (sample 17), carbon column 2 effluent in the tertiary treatment plant (27), and stream water in Turlle Creek just below the point of receiving the outfall from the sewage treatment plant.

TABLE III. Results of 1969 Sampling: Turtle Creek and Lebanon Sewage Treatment Plant

Sample Number	Combined Numbers of Fungus Colonies	Bacterial Populations (October) Fecal Coliforms	*Salmonella* spp.	*Pseudomonas aeruginosa*	Fungus Colonies per gram oven dry weight of sample June x10⁴	Fungus Colonies per gram oven dry weight of sample October x10⁴	Yeast Cells per ml sample by inspection June x10⁴	Yeast Cells per ml sample by inspection October x10⁴
1	23				0.28	0.14	0.001	10.0
2	23				33.0	6.0	0.1	1.0
3	23				6.5	45.0	—	1.0
Sta. 1	49							
4	20				0.04	0.06	0.004	20.0
5	16				350.0	30.0	0.3	20.0
6	18				45.0	50.0	0.002	20.0
Sta. 2	50							
7	25	16.6×10^4	1,100	210	0.009	0.9	0.002	10.0
8	18	(42,000/gal.)	(7,900/gal.)		260.0	30.0	1.0	20.0
9	28				14.0	60.0	—	10.0
Sta. 3	52							
10	16				0.006	5.0	0.002	0.2
11	26				32.0	27.0	1.0	10.0
12	30				37.0	55.0	—	10.0
Sta. 4	57							
13	17				0.007	0.65	0.002	0.001
14	21				41.0	60.0	0.1	10.0
15	33				6.7	50.0	—	1.0
Sta. 5	54							
16	20					0.9		1.0
17	18	8×10^6	34	1,100		1.3		10.0
18	21	(1,300/gal.)	(42,000/gal.)			7.0		10.0
19	23					6.0		10.0
20	18					0.5		10.0
21	24					7.0		10.0
22	18					32.0		10.0
Secondary	37							
23	13					1.0		10.0
24	11					0.06		0.001
25	7					0.09		—
26	5					0.076		—
27	7	14.4×10^4	210	240		0.066		—
28	5	(7,900/gal.)	(9,000/gal.)			0.1		—
29	18					20.0		10.0
30	8					0.86		0.2
Tertiary Sewage Tr.	29 45							

TABLE IV Species of Fungi Isolated from Turtle Creek Samples (Including Species from the Lebanon Sewage Treatment Plant.)

Times Appearing Turtle Creek (of 10)	Species	Turtle Creek 1		Turtle Creek 2		Lebanon STP Secondary Processes * in effluent	Lebanon STP Tertiary Processes	Turtle Creek 3		Turtle Creek 4		Turtle Creek 5	
		June	Oct	June	Oct			June	Oct	June	Oct	June	Oct
10	*Aspergillus niger*	×	×	×	×	×*	×	×	×	×	×	×	×
10	*Geotrichum candidum*	×	×	×	×	×*	×	×	×	×	×	×	×
10	*Mucor hiemalis*	×	×	×	×	×	×	×	×	×	×	×	×
10	*Penicillium* spp.	×	×	×	×	×*	×	×	×	×	×	×	×
10	*Penicillium lilacinum*	×	×	×	×	×	×	×	×	×	×	×	×
10	*Rhinocladiella mansonii*	×	×	×	×	×*	×	×	×	×	×	×	×
10	*Trichoderma harzianum*	×	×	×	×	×	×	×	×	×	×	×	×
10	White yeasts	×	×	×	×	×*	×	×	×	×	×	×	×
9	*Cephalosporium* spp.	×	×	×		×	×	×	×	×	×	×	×
9	*Cladosporium cladosporioides*	×	×	×	×	×*	×		×	×	×	×	×
9	*Fusarium aquaeductuum*	×	×	×	×	×*	×		×	×	×	×	×
9	Moniliales spp.	×	×	×	×	×	×	×		×	×	×	×
8	*Aspergillus fumigatus*	×	×	×		×		×	×	×	×	×	×
8	*Fusarium oxysporum*	×	×		×	×*	×	×	×		×	×	×
8	*Penicillium funiculosum*	×	×	×	×	×*			×	×	×	×	×
8	*Penicillium rubrum*	×	×		×	×*	×	×	×	×	×		×
8	*Phoma* spp.	×	×		×	×	×	×	×		×	×	×
8	*Zygorhynchus moelleri*	×	×	×				×	×	×		×	×
7	*Rhodotorula* spp.		×	×	×	×*	×	×	×		×		×
6	*Alternaria alternata*	×	×	×		×	×		×		×		×
6	*Phoma herbarum*	×		×	×	×	×	×		×		×	
6	*Gliocladium catenulatum*		×		×	×				×	×	×	×
6	*Trichosporon cutaneum*			×	×	×*	×	×	×		×		×
5	*Aspergillus ochraceus*	×			×			×			×		×
5	*Fusarium roseum*	×	×		×	×*						×	×
5	*Gliocladium roseum*	×		×		×		×		×		×	
5	*Candida* spp.		×		×	×*	×		×		×		×
5	*Torulopsis* spp.		×		×	×*	×		×		×		×
4	*Aspergillis flavus*	×		×		×	×	×			×		
4	*Cephalosporium* sp. (P)	×	×		×						×		
4	*Coniothyrium* spp.	×		×						×		×	
4	*Curvularia lunata*	×			×		×				×		×
4	*Dictyochus monosporus*	×			×				×			×	
4	*Scopulariopsis brevicaulis*	×						×	×				×
4	*Rhizopus nigricans*		×		×	×*	×				×		×
4	*Cryptococcus* spp.		×			×*	×		×		×	×	×
4	*Epicoccum purpurascens*		×		×	×	×				×		×

TABLE IV. (Continued)

Times Appearing Turtle Creek (of 10)	Species	Turtle Creek 1		Turtle Creek 2		Lebanon STP Secondary Processes * in effluent	Lebanon STP Tertiary Processes	Turtle Creek 3		Turtle Creek 4		Turtle Creek 5	
		June	Oct	June	Oct			June	Oct	June	Oct	June	Oct
4	*Trichoderma hamatum*				×	×			×		×		×
3	*Penicillium expansum*	×		×				×					
3	*Rhizopus* sp.	×								×		×	
3	*Verticillium lateritium*	×		×		×						×	
3	*Torulopsis famata*	×		×		×		×					
3	*Fusarium 'dimerum'*		×			×*					×		×
3	*Gliocladium fimbriatum*		×			×			×		×		
3	*Myrothecium verrucaria*		×						×			×	
3	*Fusarium equiseti*				×					×			×
3	*Fusarium* spp.			×		×	×	×				×	
3	*Paecilomyces farinosus*			×				×		×			
3	*Trichosporon* spp.			×		×	×	×		×			
3	*Candida krusei*			×		×	×	×				×	
3	*Penicillium velutinum*				×	×*					×		×
3	*Penicillium martensii*					×	×	×			×		×
2	*Gliomastix murorum* var. *felina*	×				×				×			
2	*Penicillium variabile*	×		×									
2	*Cephalosporium acremonium*		×		×	×*							
2	*Trichosporon capitatum*		×			×	×		×				
2	*Sphaeropsis* sp.			×									×
2	*Fusarium oxysporum* var. *redolens*				×				×				
2	*Cylindrocarpon* sp.		×								×		
2	*Pyrenochaeta terrestris*					×		×		×			
2	*Penicillium janthinellum*					×	×		×		×		
2	*Penicillium ochrochloron*					×	×				×		×
1	*Chaetomium funicolum*	×				×							
1	*Penicillium oxalicum*	×											
1	*Penicillium ?tardum*	×											
1	*Penicillium verruculosum*?	×											
1	*Fusarium solani*		×										
1	*Helminthosporium*? vagans		×										
1	*Actinomucor elegans*		×										
1	*Peyronellaea glomerata*		×										
1	*Candida curvate*			×		×							
1	*Candida ?parapsilosis*			×		×							
1	*Candida pelliculosa*			×		×							

TABLE IV. (Continued)

Times Appearing Turtle Creek (of 10)	Species	Turtle Creek 1		Turtle Creek 2		Lebanon STP Secondary Processes * in effluent	Lebanon STP Tertiary Processes	Turtle Creek 3		Turtle Creek 4		Turtle Creek 5	
		June	Oct	June	Oct			June	Oct	June	Oct	June	Oct
1	*Candida ?scottii*			×		×							
1	*Penicillium frequentans*			×									
1	*Thraustotheca clavata*			×									
1	*Aspergillus ustus*				×	×	×						
1	*Mucor* sp.					×	×	×					
1	*Pyrenochaeta* sp.					×		×					
1	*Rhodotorula mucilaginosa*					×	×	×					
1	*Penicillium chrysogenum*					×		×					
1	*Penicillium jiczynskii*								×				
1	*Colletotrichum atramentarium*								×				
1	*Chaetomium globosum*								×				
1	*Achlya* sp.								×				
1	*Phytophtora* sp.								×				
1	*Trichoderma polysporum*									×			
1	*Penicillium fellutanum*									×			
1	*Penicillium imbricatum*									×			
1	*Verticicladiella penicillata*									×			
1	*Candida melinii*									×			
1	*Calcarisporium arbusculum*										×		
1	*Scopulariopsis* sp.										×		
1	*Humicola grisea*										×		
1	*Penicillium thomii*												×
1	*Aureobasidium pullulans*					×	×						×
1	*Monochaetia* sp.												×
1	*Ulocladium botrys*												×
1	*Fusarium* L3H15-24												×
1	*Papulaspora* sp.												×
1	*Nigrospora sphaerica*												×
	Candida intermedia					×	×						
	Cryptococcus luteolus					×	×						
	Rhodotorula glutinis					×	×						
	Torulopsis glabrata					×	×						
	Torulopsis inconspicua					×	×						
	Aspergillus versicolor					×	×						
	Aspergillus spp.					×							
	Aspergillus flavipess					×							

TABEL IV. (Continued)

	Lebanon STP Secondary Processes * in effluent	Lebanen STP Tertiary Processes
Aspergillus cf. *puniceus*	×	
Aspergillus sydowii	×	
Botrytis cinerea	×	
Candida brumptii	×	
Candida catenulata	×	
Candida guilliermondii	×	
Candida humicola	×	
Candida mycoderma	×	
Candida tenuis	×	
Candida tropicalis	×	
Candida utilis	×	
Candida ?zeylanoides	×	
Coniothyrium fuckelii	×	
Cryptococcus diffluens	×	
Cryptococcus lauventii	×	
Fusarium acuminatum	×	
Gliocladium sp.	×	
Mucor plumbeus	×	
Paecilomyces marquandii	×	
Paecilomyces varioti	×	
Prototheca stagnora	×	
Scedosporium apiospermum	×	
Torulopsis aeria	×	
Torulopsis candida	×	
Torulopsis colliculosa	×	
Torulopsis holmii	×	
Torulopsis ?saki	×	
Torulopsis versatilis	×	
Trichosporon fermentans	×	
Trichosporon margaritiferum	×	
Verticillium sp.	×	
Septoria sp.		×
Talaromyces vermiculatus		×

TABLE V. Species of Fungi Isolated from the Sewage Treatment Plants at Lebanon, Ohio

Species	Number of Stations in which appearing Systems: Secondary		Tertiary	
	1967–8	1969	1967-68	1969
	ABCDEFG	H	ABCDEFG	H
Moniliales spp.	7	–	8	5
Fusarium aquaeductuum	7	4	3	3
Fusarium oxysporum	7	7	3	3
Geotrichum candidum	7	7	5	3
Mucor hiemalis	7	5	2	1
Penicillium spp.	7	7	6	6
Penicillium lilacinum	7	5	6	8
Rhinocladiella mansonii	7	7	6	8
Rhodotorula spp.	7	7	6	2
Trichosporon spp.	7	1	1	1
Trichosporon cutaneum	7	7	3	2
White Yeast spp.	7	7	6	3
Cephalosporium spp.	7	7	4	1
Phoma spp.	7		8	
Candida spp.				5
Torulopsis spp.		7		1
Aspergillus niger	6	6	1	1
Trichoderma harzianum	6	6	4	2
Aspergillus flavus	6		1	
Phoma herbarum	6		1	
Rhodotorula mucilaginosa	6		4	
Cladosporium cladosporioides	5	5	3	7
Cryptococcus spp.	5	2	2	1
Aureobasidium pullulans	5		2	1
Penicillium janthinellum	5		3	
Candida krusei	5		3	
Pyrenochaeta sp.	5			
Torulopsis holmii	5			
Torulopsis versatilis	5			
Verticillium lateritium	5			
Penicillium rubrum		5		1
Penicillium velutinum		5		
Penicillium ochrochloron	4		3	
Candida intermedia	4		1	
Rhizopus nigricans	4	3	1	
Fusarium spp.	4		1	
Mucor spp.	4		1	
Candida parapsilosis	4			
Cryptococcus laurentii	4			
Torulopsis candida	4			
Torulopsis famata	4			
Rhodotorula glutinis	4		2	
Trichosporon capitatum	4			1
Verticillium spp.	4			
Cephalosporium acremonium		4		
Aspergillus spp.	3			
Aspergillus flavipes	3			
Aspergillus cf. *puniceus*	3			
Aspergillus versicolor	3		4	2
Penicillium funiculosum	3	5		
Gliomastix murorum var. *felina*	3	1		

TABLE V. (Continued)

Species	Number of Stations in which appearing Systems: Secondary			Tertiary		
	1967–8 ABCDEFG		1969 H	1967-68 ABCDEFG		1969 H
Coniothyrium fuckelii	3					
Epicoccum purpurascens	3					
Gliocladium sp.	3					
Mucor plumbeus	3					
Candida guilliermondii	3					
Candida pelliculosa	3					
Candida curvata	3					
Torulopsis aeria	3					
Fusarium roseum	2		1			
Paecilomyces varioti	2		1			
Candida utilis	2		1			
Aspergillus fumigatus	2					
Candida catenulata	2					
Candida tenuis	2					
Candida ?zeylanoides	2					
Cryptococcus diffluens	2					
Gliocladium roseum	2					
Penicillium chrysogenum	2					
Torulopsis colliculosa	2					
Trichosporon margaritiferum	2					
Gliocladium fimbriatum			2			
Alternaria alternata	1		4			1
Aspergillus sydowii	1		1			
Aspergillus ustus	1		2	1		
Penicillium martensii	1		1			2
Cryptococcus luteolus	1			1		
Torulopsis glabrata	1			1		
Torulopsis inconspicua	1			1		
Candida brumptii	1					
Candida humicola	1					
Candida mycoderma	1					
Candida scottii	1					
Candida tropicalis	1					
Botrytis cinerea	1					
Chaetomium funicolum	1					
Fusarium acuminatum	1					
Paecilomyces marquandii	1					
Prototheca stagnora	1					
Torulopsis ?saki	1					
Trichosporon fermentans	1					
Fusarium 'dimerum'			1			1
Gliocladium catenulatum			1			
Scedosporium apiospermum			1			
Pyrenochaeta terrestris			1			
Trichoderma hamatum			1			
Septoria sp.				1		
Talaromyces vermiculatum				1		1
Curvularia lunata						1
Total species 100	85	97	37	37	45	28

In parallel with other sets of samples being tested at the same time, tests were run on fecal coliforms, *Salmonella* spp. and *Pseudomonas aeruginosa.*

Colony counts of all types of fungi appearing on pour plates are given in Table III, as are cell counts for yeasts in shaken-flask culture. Colonies on agar were counted, this count was corrected for the dilution used, and for the oven dry weight of the sample used. Cell counts for yeasts are given by inspection of the amount and type of growth occurring in the flasks in which 1 : 10 dilutions were used. This technique follows the outline given by COOKE (1965a). The numbers of species found at each station during all sampling in the Lebanon sewage treatment plant (COOKE, 1970), and at each sampling on Turtle Creek, are given as totals for each sample, and for each station.

The list of species recovered from all samples obtained from Turtle Creek and from the Lebanon sewage treatment plants is presented in Table IV. Species isolated from each habitat are composited for each station. These habitats included water, water and sediments in the stream bottom, and soil from the creek shore at or near the interface of the water and the soil. In addition to data for the two dates on which samples were collected, May and October, at each of the five stations, a composited list of the species for the secondary and tertiary treatment systems at the Lebanon sewage treatment plant is inserted at the point at which the outfall from the plant reaches the creek below station 2 and just above station 3. An asterisk following the listing for the secondary treatment plant indicates that the species concerned was represented among the eight sets of samples obtained at that plant in the final product flowing from the final settling tank to the creek. It will be noted in all cases that these species were present in materials collected in Turtle Creek itself at stations 1 and 2.

Within the secondary sewage treatment plant at Lebanon, raw sewage, primary settled sewage, mixed liquor influent to the aerator, effluent from the aerator, final product from the secondary settling basin, return sludge, and primary settled sludge were sampled on each of eight occasions. The results of sampling of the first seven sets have been described (COOKE, 1970). These results are summarized in the species list for the Lebanon sewage treatment plant in Table V. In column 1 the species are listed. In column 2 the number of habitats from which the species obtained in the first seven sets of samples are given. In column 3 the number of habitats from which these species were recovered in the October samples are indicated. All but three of these species also occurred in the tertiary treatment systems as indicated in the report for the first seven sets of samples (COOKE, 1970); these are indicated in column 4. The fifth and final column indicates those species recovered from sample in the tertiary system in October, 1969. Locations samples included: primary settled sewage (influent from the secondary treatment plant), ef-

fluent from the lime clarifier, effluent from the lime clarifier filter, effluents from the three carbon columns the influent to which was the effluent from the lime clarifier, and two samples from the activated sludge unit designed by R. V. VILLIERS: the influent which is from the effluent from the primary settling tank in the secondary treatment plant, and the effluent from the unit. The numbers in these columns refer to the number of process samples from which the species was recovered.

DISCUSSION

Turtle Creek

As is true of other streams studied, Turtle Creek contains an excellent population of fungi. Colony counts (Table III) are high in the bank samples collected at all stations. They are almost as high in all but one of the stream bottom samples, and in this one, station 1, in a relatively clean portion of the stream, colony numbers are only a tenth of those at lower stations. On the other hand, stream water carries the largest number of disseminules (resulting in colonies on the plates) at station 4. Water from stations 1 and 2 has low numbers of fungus colonies, but that at station 3, carrying outfall from the sewage treatment plant, and at station 5, has an intermediate number of colonies.

According to Table II, total organic carbon and organic nitrogen are highest at station 3 which receives outfall from the sewage treatment plant. This becomes diluted or dispersed as the water flows down the stream 1.6 miles to station 4, but there is a slight build-up in the next 1.3 miles resulting in higher values at station 5. The source of this added organic carbon and organic nitrogenis at present unknown; the stream flows through farmland and might accept runoff from septic tanks, barns, or other sources of heavy loads of organic matter.

COOKE (1968a) has shown that fungi grow well under conditions in which the carbon: nitrogen ratio is 9 to 12 : 1, the C : N ratio of natural soil. At C : N ratios which are lower, such as rich culture media, heavier growth of fungi of loss than typical nature can be expected. It is not known to what extent lower C : N ratios depress fungal growth. However, under conditions such as found in Turtle Creek, where organic matter is added to the stream indiscriminately, as at station 2 when sewage in excess of that which can be treated adequately or even minimally in the sewage treatment plant is added to the stream through a bypass, the C : N ratio can be increased, or when treatment plant effluent is added regularly as at station 3, it can be decreased. As long as the supplies of carbon and nitrogen, in the presence of other nutrients which may be required, are available, the fungus population responds by the production of increased amounts of cells. In doing this the fungi aid in removal of polluting organic substances and contine the treatment process.

This is reflected in Table III by the demonstration of relatively heavy growths of filamentous fungi in the presence of added organic matter. It was hoped that within the distance sampled on Turtle Creek at least one of the stations would demonstrate lower populations of fungi. However, COD values (Table II) for water in all stations are relatively high indicating that all stations chosen have probably become polluted to some extent, in one case to a great extent.

Yeast populations along Turtle Creek show that in the three stations upstream there are large numbers of yeast cells. These numbers decline rapidly below the treatment plant. In the benthic and shore samples, however, yeast populations remain high in the October set. The numbers reported in Table III reflect only the results obtained by inspection of flasks following primary incubation (COOKE, 1965a). Where only white or red yeasts appeared in the primary flasks, the reporting value of '1' was used; where red yeasts could be demonstrated in addition to white in a flask, the reporting value of '2' was used. These values were then corrected through the dilution factor. From growth in the flasks it could not be detected how many species of yeasts were involved. In flasks in which 1: 10, 1:100 and 1:1000 dilutions were used most stations yielded *Geotrichum candidum*. In addition to this, and in the two higher dilutions, species of *Candida*, *Torulopsis*, and *Trichosporon* appeared. In general, without developing a series of morphological and physiological tests, species in these genera were not identified. Among the red yeasts, at least two, possibly more, species of *Rhodotorula* could be expected, but without using a series of nutritional tests these could not be determined. In the first series of isolations from Turtle Creek samples in May there was an opportunity to partially process isolates of yeasts from these samples. Some of the results appear on the list of species presented in Table IV, and in the column listing numbers of yeast cells recovered in May when fewer were found than in October. The value 40 for water at station 2 indicates that four species were isolated from a 1:10 dilution of the sample. The value 3,000 for stream bottom sediment at station 2 indicates that three species were recognized and that the highest dilution from which yeasts were recovered was 1:1000. Additional species were found but identifications for those not listed were incomplete and the identification of those species listed should have been rechecked.

It will be noted that there are considerable differences between colony counts for some stations between May and October samples. Had sampling taken place at more frequent intervals, lower values might have been found in some instances, and a smoother curve might be developed for populations recoverable using the techniques described above.

The list of species of fungi recovered from Turtle Creek in Table IV is presented according to their occurrence in the five sampled stations. Inserted between stations 2 and 3, at approximately the

point of outfall, are those species isolated from the secondary and tertiary treatment systems at the Lebanon sewage treatment plant. These records are not included in the presented values given in column 1. It will be noted that 20 of the species listed for the secondary treatment system are marked with an asterisk (*). These species were recovered from the effluent from that treatment plant in the October study. Of those species isolated from sewage treatment plant samples, 52 were not isolated from stream samples obtained upstream from the outfall of the sewage treatment plant at station 3. In addition to the 41 species found in the treatment plant but not recovered from Turtle Creek, 15 were not recovered from station 3 although they appeared in stations 4 or 5 or both. Based on only two sets of isolations from the stream, in contrast with eight from the sewage treatment plant, a complete and valid comparison of these data cannot be made.

Both the stream and the sewage treatment plant receive inoculum for fungal populations from the same or similar sources. The stream flows through the city of Lebanon after draining a large agricultural area east, north , and northeast of Lebanon. It picks up runoff from fields, woodlands, farms, farm buildings, and overflows or outfalls from septic tank systems in suburban developments. The sewage treatment plants receive runoff from houses, lots, out-buildings and streets, as well as municipal sewage and wastes from small businesses and industries. It is possible that the sanitary sewage could carry organisms not received from other sources, but sampling has not been critical enough to sort out species which might be restricted to one or another of the habitat-types listed above.

Within the series of species isolated from Turtle Creek are three groups of the four based on apparent habitat preferences among fungi (COOKE, 1957a). Of the 101 species isolated from the stream, none can be considered restricted to pollution habitats, lymabionts. However, 28 species are thought to be lymaphiles. These include species isolated from all habitats at least once or on both sampling occasions. Assigned to the lymaxenes are 33 species isolated from only 2, 3, or 4 of the sampled stations. Usually these were obtained only once, sometimes they were recovered from both sets of samples obtained from each station. While it is possible that in reality some of the 39 species considered lymaphobes are either lymaxenes or lymaphiles, in this pair of isolation studies these species appeared only once in each station or only once in the two studies. In other streams, or in other studies involving populations of sewage treatment plants, some of these species have necessarily been considered lymaxenes or lymaphiles. Assignment to these categories is strictly subjective and subject to reinterpretation as more studies become available, or as more critical studies are made.

Lebanon Sewage Treatment Plant

Table V presents a composite list of the species of fungi isolated from all samples obtained in the secondary and tertiary treatment processes at the Lebanon sewage treatment plant. Since 13 of the 100 species listed were obtained only in the 8th or October set of isolations, it may be assumed that the law of diminishing returns is showing its effect and that fewer species will be recovered in each of any later samplings. However, it cannot be assumed as yet that the total distribution of species recoverable is known since 14 of the 37 species recovered in October 1969 have less or more complete patterns of distribution than those shown in the 1967 and 1968 series.

Of the 100 species of fungi recovered from the secondary and tertiary treatment systems, 97 were obtained from the secondary and only 45 from the tertiary system. Of these 45 species, a number were present only in the influent to the processes sampled, that is, in the primary settled sewage of the secondary system. Values obtained for total organic carbon and organic nitrogen are sufficiently high in all treatment stages to permit growth of fungi, and the C : N ratio of each stage sampled is adequate to support growth of fungi in each of the locations sampled. In some cases, this ratio would indicate that a larger than normal amount of growth could be expected. However, as a result of lack of sufficient nutrients, or of satisfactory environmental conditions, yeast cells were not recovered to a great extent from most tertiary treatment samples. If present, these can be considered to be attached to substrata such as particulate carbon granules or lime slurry particles and thus would not necessarily be recovered from effluents from these processes.

Within this particular series of isolations, it can be considered that no lymabionts are present, that 45 of the species listed are lymaphiles, that 31 of the species are lymaxenes, and only 24 of the species are lymaphobes. However, this analysis is not quite accurate when species within each category are compared on the basis of their occurrence in other series of samplings. Some of the species listed among the lymaxenes and lymaphobes have been reported elsewhere as lymaphiles. However, no species reported in earlier studies as lymabionts have been recovered from the Lebanon sewage treatment plant processes.

Of the 97 species of fungi recovered from the secondary treatment processes, many, if not most, species have been recovered in earlier studies with materials from other activated sludge-type sewage treatment plants (COOKE & PIPES, 1968, 1970), trickling filter-type sewage treatment plants (COOKE, 1959a, b), and from waste stabilization ponds (COOKE & MATSUURA, 1963, 1969). This shows that there is a group of species of fungi which is readily adaptable to the habitat of the sewage treatment plant, that these fungi can live in reduced conditions of oxygen supply (TABAK & COOKE, 1968), can aid in the reduction of the biochemical oxygen demand of the habi-

tat (COOKE, et al., 1957), and can derive nutrients from this habitat (COOKE, 1957b; COOKE & BUSCH, 1958). Within the group of species are several yeasts, strains of which can be assumed to grow well at 37° C on the basis of earlier studies (COOKE, 1965b). These strains can be assumed to be of human origin, although this may not be true in all cases. Filamentous fungi which could have been derived from human sources, or which are potential human pathogens (COOKE & KABLER, 1955) include *Geotrichum candidum, Rhinocladiella mansonii, Aspergillus fumigavus,* and *Scedosporium apiospermum.* While these species are not presently the source of mild or important human disease, their occurrence in the environment, and their potential build-up as members of a population of organisms in polluted streams is of interest. *Fusarium oxyporum, F. roseum, F. solani, Geotrichum candidum, Phoma* spp., *P. herbarum, Pyrenochaeta* sp., *P. terrestris, Coniothyrium fuckelii, Alternaria alternata, Botrytis cinerea, Curvularia lunata, Septoria* sp., and possibly species of *Penicillium,* include strains which are pathogenic for vascular plants, especially those planted for crops (COOKE, 1956). Other species are known to produce antibiotics, some species are known to produce toxins, and several species cause decay in fruits and other farm products during storage, transit and marketing processes. In general, most species listed are so-called sugar fungi, fungi capable of using simple substances such as monosaccharides, including pentoses and hexoses, and disaccharides, as carbon sources. However, a few of those listed, including *Gliomastix murorum* var. *felina,* strains of *Trichoderma* species, species of *Chaetomium, Fusarium,* and possibly others, are known to be cellulolytic (SIU, 1951) and may be involved in the gradual reduction of this substance of which sewage solids include between one-third and one-half (MAKI, 1954).

In earlier studies it has been shown that there are fewer colonies recoverable from effluents from various stages in treatment processes (COOKE, 1954, 1959a). BECKER & SHAW (1955) found very poor recovery from process effluents in a pair of trickling filter-type sewage treatment plants at Pullman, Washington, and Moscow, Idaho. In activated sludge-type sewage treatment plants in the Chicago area, COOKE & PIPES (1968, 1970) found that in effluents from settling basins there were relatively fewer colonies than in sludges or other settled materials in the processes sampled. On these bases it would seem that effluents from processes at Lebanon are not carrying unusually low numbers of fungus disseminules. The nature of these habitats appears to indicate that fungus growth can be expected on solid surfaces such as walls of tanks, pipes, or any exposed surface, as well as growth on any surface such as lime particles in slurries, granular carbon particles, electrodialysis membranes, and other surfaces. To a more limited extent, except in the activated sludge floc itself, fungus growth can occur, or fungus disseminules dislodged from the parental mycelium can be carried in any of the

liquors flowing through the plant. The activated sludge floc is composed of bacterial, fungal, or protozoan growth, or any combination of these, which develops in association with a solid surface, a particle of organic matter acting as an anchoring point and as a food source, or a particle of inorganic matter bathed in a nutrient medium acting an anchoring point, no matter how small these particles may be. To this extent the activated sludge organism may be considered a member of a benthic, at at least an Aufwuchs, community (COOKE, 1956). The solid 'bottom' particle to which the organism is attached is kept in almost perpetual motion through the introduction of the forced air of the activated sludge process.

Turtle Creek and the Lebanon Sewage Treatment Plant

In the preceding section of this discussion, something has been indicated of the occurrence of certain species, strains, or groups of fungi and their activities in the Lebanon sewage treatment plant. Perusal of Table IV will show that these same groups of fungi occur with considerable frequency and regularity in materials collected in and along Turtle Creek, both above and below the outfall of the sewage treatment plant. The regular occurrence of these fungi in Turtle Creek, then, indicates that they are members of a natural population, or a population which has become adapted to the habitats presented to them in Turtle Creek through accumulation on the bottom and along the shore of enrichment materials received by the stream from the community through which it passes. Before the sewage treatment plant was built these materials accumulated in the stream through runoff from the community, from septic tanks, privies, and normal environmental washing processes induced by every rain which fell on the community, the water from which drained into the stream.

The occurrence of *Achlya* spp., *Saprolegnia* spp., *Thraustotheca clavata*, and *Phytophthora* sp. in the waters from stations above and below that into which the sewage outfall empties would seem to indicate that water passing through these stations is not completely devoid of oxygen since these fungi require well oxygenated water in which to live. Sampling has not been sufficient to determine the seasonal growth patterns of these fungi.

From the populations recovered from Turtle Creek, augmented by the populations added from the Lebanon sewage treatment plant, it appears that this stream is little different from others which have been sampled in the Fungus Studies Laboratory. The distribution of fungi in Turtle Creek does not differ markedly from that in Lytle Creek (COOKE, 1961). Lytle Creek differs from Turtle Creek in that in the drier parts of the year the effluent from the Wilmington sewage treatment plant is its only source of flow. Whether the depression of numbers of species at the outfall of that sewage treatment

plant was real or an effect of the sampling technique cannot be known since after sampling was completed, the sewage treatment plant was converted from primary-type to secondary-type using activated sludge.

Two other streams have also been described mycologically based on two sets of samples. Here only points of outfall of effluents from sewage treatment plants were sampled as stream stations so that the nature of the populations in the treatment plants themselves is not known. A series of stations on the Bear River (COOKE, 1967) in the Cache Valley of Utah and Idaho was sampled. It was found that fewer species were present in the highly organically enriched outfalls of certain food processing industries than in the cleaner water upstream, or in the more heavily organically enriched waters downstream which carried large populations of fungi. In the Cache la Poudre River (COOKE, 1968b) in northeastern Colorado, many of the same group of fungi were recovered from samples of water, bottom sediments, and shore muds. The stream drains a mountain wilderness, a recreation area, and an agricultural area in which fields are irrigated. In the latter reach it received the outfall from an overloaded sewage treatment plant, untreated wastes from a butchery, and wastes from a sugar mill. These sources of enrichment are probably responsible for the increase in numbers of fungus colonies recovered from samples from the stream, as well as an increase in numbers of species recovered along the lower reach of the river.

Sewage pollution of Turtle Creek, Lytle Creek, the Bear River, and the Cache la Poudre River results in similar phenomena. This is manifest by the increase of fungus colonies at the area of receipt of the sewage treatment plant outfall, and an increase in numbers of species of fungi in lower regions of the stream which, in the case of Lytle Creek, have been designated as the 'recovery zone'. On the basis of samples from Turtle Creek which were tested for organic carbon and nitrogen levels, it would appear that quantities of these elements are present to a great enough extent to support the growth of fungi without regard to the ratios between these elements.

CONCLUSIONS

Certain species of soil fungi are adaptable to a habitat in water in which continual supplies of organic matter including relatively high loads of available organic carbon and organic nitrogen are present. These fungi are able to grow and produce disseminules which are carried in water throughout the sewage treatment plant, and in its outfall to the adjacent stream. Here these fungi join others which have become adapted to life in a stream which carries smaller amounts of organic matter. Depending on various factors, the newly arrived fungus may pass on through the system, or it may become adapted to life in the stream and become established

together with, or at the expense of, members of the population already present. These fungi are useful in the treatment of sewage, and in the removal of excess organic matter or polluting substances from the stream in which they produce their growth.

In order to describe the population of fungi, including yeast-like as well as filamentous fungi, more meaningfully, it is suggested that samples of stream materials from a wide range of sampling points, as well as materials from sewage treatment plants whose outfalls are contributing to the organic enrichment of the stream, and even of untreated wastes being discharged into the stream, including agricultural runoff, be studied carefully from as many points of view as possible. Chemical and physical characteristics of the samples should be determined as well as the populations of fungi. Parallel information on bacteria and other organisms present at each station would increase the usefulness of the data obtained. Not only yeast-like fungi should be studied through shaken-flask culture, but filamentous and yeastlike fungi should be determined from agar plates, since neither technique is mutually exclusive. Aquatic fungi should be determined for each sample using standard baiting techniques. For more complete data, the relation between the organisms observed in relation to food sources should be considered. Ecosystem data and potential productivity of reducer organism populations will increase the value of these data.

Zusammenfassung

Mittels verschiedener Isolierungsmethoden in festen und flüssigen Kulturnährboden sind 101 Arten von Fadenpilzen und Hefen isoliert worden vom Materialproben von Turtle Creek Warren Co., Ohio und 100 Arten von Pilzen vom Material des sekundären und tertiären Behandlungsprozesses des Abwasserbehandlungswerkes von Lebanon, Warren Co., Ohio. Die vereinigte Liste von beiden Arten des Materials umfaßte 142 Arten. Mindestens sind monatliche Proben notwendig, um die Pilzarten genauer und bedeutungsvoller zu beschreiben.

References

BECKER, J. G. & C. G. SHAW (1955) Fungi in domestic sewage treatment plants. *Appl. Microbiol.* 3: *173—180.*

COOKE, W. B. (1954) Fungi in polluted water and sewage. II. Isolation technique. *Sewage Ind. Wastes.* 26: *661—674.*

COOKE, W. B. (1956a) Colonization of artificial bare areas by microorganisms. *Bot. Rev.* 22: *613—638.*

COOKE, W. B. (1956b) Potential plant pathogenic fungi in sewage and polluted water. *Plant Dis. Rep.* 40: *681—687.*

COOKE, W. B. (1957a) Check list of fungi isolated from sewage and polluted water. *Sydowia, Ann. Mycol. II. Beih.* 1: *146—175.*

COOKE, W. B. (1957b) Nutritional requirements of nine common sewage fungi. *Sewage Ind. Wastes* 29: *1243—1251.*

COOKE, W. B. (1958) Continuous sampling of trickling filter populations. *Sewage Ind. Wastes* 30: *21—27.*

COOKE, W. B. (1959a) Fungi in sewage and polluted water. IV. The occurrence of fungi in a trickling filter-type sewage treatment plant. Proc. 13th. *Ind. Waste Conf., Purdue Univ., Engineering Bull.* 43: *26—45.*

COOKE, W. B. (1959b) Trickling filter ecology. *Ecology* 40: *273—291.*
COOKE, W. B. (1961) Pollution effects on the fungus population of a stream. *Ecology* 42: *1—18.*
COOKE, W. B. (1963) A Laboratory Guide to Fungi in Polluted Waters, Sewage, and Sewage Treatment Systems. Cincinnati. *Public Health Serv.* Publ. No. 999-WP-1.
COOKE, W. B. (1965a) The enumeration of yeast populations in a sewage treatment plant. *Mycologia* 57: *696—703.*
COOKE, W. B. (1965b) Growth of yeasts at 37°C. *Mycopath. Mycol. Appl.* 25: *195—208.*
COOKE, W. B. (1967) Fungal populations in relation to pollution of the Bear River, Idaho-Utah. *Proc. Utah Acad. Sci.* 44: *298—315.*
COOKE, W. B. (1968a) Carbon/nitrogen relationships of fungus culture media. *Mycopath. Mycol. Appl.* 34: *305—316.*
COOKE, W. B. (1968b) Some fungi of the Cache la Poudre River. Colorado. *Mycopath. Mycol. Appl.* 35: *361—372.*
COOKE, W. B. (1970) Fungi associated with the activated sludge process of sewage treatment at the Lebanon, Ohio, sewage treatment plant. *Ohio J. Sci.* 70: *129—146.*
COOKE, W. B. & K. A. BUSCH, (1957) Activity of cellulose decomposing fungi isolated from sewage polluted water. *Sewage Ind. Wastes* 29: *210—217.*
COOKE, W. B. & A. HIRSCH, (1958) Continuous sampling of trickling filter populations. II. Populations. *Sewage Ind. Wastes* 30: *138—156.*
COOKE, W. B. & P. W. KABLER (1955) Isolation of potentially pathogenic fungi from folluted water and sewage. *Public Health Rep.* 70: *689—694.*
COOKE, W. B. & G. MATSUURA (1962) A study of yeast populations in a waste stabilization pond system. *Protoplasma* 57: *163—187.*
COOKE, W. B. & G. MATSUURA (1969) The distribution of fungi in a waste stabilization pond system. *Ecology.* 50: *689—694.*
COOKE, W. B. W. A. MOORE & P. W. KABLER (1956) B. O. D. satisfaction by fungi. *Sewage Ind. Wastes* 28: *1075—1086.*
COOKE, W. B., & W. O. PIPES (1969) The occurrence of fungi in activated sludge. *Proc. 23rd. Ind. Waste Conf., Purdue Univ., Engineering Bull.* 53(2): *170—182.*
COOKE, W. B. & W. O. PIPES (1970) The occurrence of fungi in activated sludge. *Mycopath. Mycol. Appl.* 40: *249—270.*
MAKI, L. R. (1954) Experiments on the microbiology of cellulose decomposition in a municipal sewage treatment plant. Antonie van Leeuwenhoek, *J. Microbiol. Serol.* 20: *185.*
SIU, R. G. H. (1951) Microbial Decomposition of Cellulose. Reinhold Publ. Co., New York. pp.
TABAK, H. & W. B. COOKE (1968) Growth and metabolism of fungi in an atmosphere of nitrogen. *Mycologia* 60: *115—140.*