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ABSTRACT

Small secondary treatment plants have reported a variety of pH problems in the past. One such problem has been an unacceptably low pH in a plant's discharge waters. To study the possibility of this problem being biologically induced, organisms in the aeration tank of a plant reporting low pH values were identified, counted, compared to pH data from the plant's aeration tank and innoculated into sterilized wastewater. It was found that protozoans numerically dominated the eukaryotic organisms and bacteria the prokaryotic group. The most dominant protozoan, Zoothamnium sp., was found to have a + correlation with the pH values of the aeration tank. Three bacteria, Escherichia coli, Pseudomonas mallei and P. aeroginosa, lowered pH values of sterilized wastewater from a pH of 7 to as low a pH as 4.8 over a 96 hour test period. The greatest decrease in pH occurred over the first 36 hours of the test period.

INTRODUCTION

Similar to many small secondary treatment plants, Henniker Sewage Treatment Plant in Henniker, New Hampshire has had a pH problem since its opening in 1976 (Damour and Ward, 1981). The specific problem at Henniker's plant is one of low pH in the discharge water. This problem could be caused by a number of factors which we can group into two

main categories; externally induced and internally induced pH changes.

Among those externally induced changes could be included the release of large amounts of pH - influencing chemicals into the wastewater system. The following calculations illustrate this possibility. Let's assume that 100 gallons of concentrated nitric acid (15 M) was dumped into a wastewater treatment system with a holding capacity of 300,000 gallons. This amount of acid would contain the following H^+ equivalents:

$$\frac{100 \text{ gal.} \times 15 \text{ M}}{0.264 \text{ gal/L}} = 5682 \ H^+ \text{ EQUIVALENTS (or c.6E3)}$$

If the facility operator tried to compensate with 60 lbs. of soda ash, this amount of soda ash would contain the following number of base equivalents:

$$\frac{60 \text{ lbs of } Na_2CO_3}{53 \text{ g/base equivalent}} \times 453.6 \text{ g/lb.} = 514 \text{ base equivalents}$$

It can be seen that this amount of base would be inadequate to neutralize 100 gallons of HNO_3 conc. The actual ratio for neutralization is approximately 7 lbs of soda ash to 1 gal. of HNO_3 conc. Therefore 60 lbs. of soda ash would neutralize approximately 8½ gal. HNO_3 conc. All of these calculations are based on the assumption that there is no buffering activity or neutralization of the acid in the water before treatment.

Internally induced changes in pH include those that are due to biochemical activity of the organisms involved in the

biological purification of wastewater (Lijklema, 1969). These biologically induced changes in pH values are caused by many factors including carbon dioxide production, ammonia oxidation and nitrate production (Lijklema, 1969).

The degree of internally induced change depends on many environmental parameters, including the initial pH of the influent. pH levels apparently can influence the efficiency of the biological purification process (George and Gaudy, 1973). Low and high pH values generally restrict various biochemical processes involved in the purification process. High pH, for example, can restrict the nitrification process and, therefore, prevent the lowering of pH by this process.

pH exerts a strong, selective influence on the species present (George and Gaudy, 1973). A lowered diversity of species could restrict the range of adaptive responses to the great variety of environmental changes (temperature, flow rates, etc.) observed in secondary treatment plants.

Not only the degree of change in pH but the length of exposure to pH changes can disrupt these purification processes. Length of exposure to low pH was found to influence the ability of the treatment system to decover to a steady state system (Clark and Speece, 1971). The length of exposure in an anaerobic situation was found by them to be 12 hours. Within this 12 hour limit, their laboratory system could recover from a low pH of 5 to a steady-state pH of 8.

George and Gaudy (1973) found that the actual "allowable" decrease in pH could not be closely defined, although a drop of approximately one pH unit could be accommodated with a small change in species response capability. However, they did find that, in their continuous flow experiments, systems could recover biochemical efficiency even after drops in pH from 7 to 3. They were unable to observe a correlation between severity of acid shock and recovery time to a former steady-state level.

Observations by George and Gaudy (1973) further showed that a pH change from neutral to acid in their laboratory experiments was accompanied by a shift in predominating species of bacteria and protozoans to filamentous fungi. Low pH values, according to them, are deleterious to growth of protozoans but do favor fungal growth. This latter finding is especially significant for the efficiency of biological purification because it has been shown by Pillai and Subrahamanyan (1944) that protozoan activity is extremely important in the treatment processes. Their importance is often reportedly greater than that of bacterial activity.

Specific protozoans have been found to be responsible to a large degree for the success of biological purification. Vorticella is one such protozoan (Curds and Cockburn, 1970), and apparently its activity can be influenced by pH values. A 48 hour run in closed, aerated cylinders showed that Vorticella abundance decreased as pH increased.

The pH of treatment waters is of critical concern to the associated town for a number of reasons including the need to comply with E.P.A. required pH of discharge waters and that of the cost of the neutralization process. At one stage in the operation of the Henniker plant, up to 100 pounds of industrial grade baking soda was added to the effluent daily to attain the required pH of 6.5. Cost per 100 pound bag at the beginning of 1981 amounted to $19 (Damour and Ward, 1981).

Corrective measures, other than chemical neutralization, have been studied. They include the removal of chemical oxygen demand (COD) at pH values as low as 2.6 (Randall et al., 1972) and the innoculation of appropriate microbes which can operate efficiently at either low or high pH values (Heukelekian and Gellman, 1951). However both of these processes can be costly.

Much still remains to be learned about the ecology of secondary treatment waters as well as the relationship between the associated flora and fauna and the pH of the waters. The general applicability of any results are viewed with the knowledge that each treatment plant apparently has characteristic flora and fauna that set it apart from other plants (Varma et al., 1975). Therefore, as more plants are studied, solutions to the general category of plant pH problems could become more clear.

METHODOLOGY

Sample Collection

From three to four times per month during the period 9/80 to 8/81, two sterilized, five gallon jars were lowered into an actively aerating section of the aeration tank at the Henniker Sewage Treatment Plant, Henniker, New Hampshire. The jars were allowed to fill to capacity, were raised and capped.

Identification of Organisms

Immediately upon return to the laboratory, the jars were agitated and three, 9 ml. aliquots were drawn from each of the two jars. To each aliquot was added one ml. of 12% formaldehyde solution as a fixative. The aliquot sample jars were capped and agitated before a sample was drawn and placed either in the Sedgwick-Rafter Counting Chamber or the Levy Chamber for microscopic identification and counting of protozoans, algae and blue-green algae. At least six sample counts for protozoans and algae each were done per collection. The count numbers were corrected for the formaldehyde dilution, and counts were averaged for the statistical analyses. The total number of counts done during the period 9/80 to 8/81 amounted to 432 sample counts of 15 dominant microbes for a total of 6480 individual counts.

For bacterial and fungal identification three on ml. aliquots from the collecting jars were spread on previously

prepared Sabourad-Dextrose plates (fungal determination) and Bacto-agar plates (bacterial determination). The plates were marked and stored in an incubator at 25°C for 48 hours. Isolation and identification of the most frequently appearing bacteria were done using Bergey's Manual (1970) and Standard Methods (1971).

Bacterial Growth Experiments

A closed, aerated system for each organism was devised and consisted of five sterilized, cotton-plugged ehrlenmeyer flasks. Into each of these flasks was placed a sterilized aeration tube containing a 0.25 μ filter. The tubes were connected to a commercial aeration motor designed for home aquarium use and the air flow rate was adjusted equally among each flask after samples were introduced.

Once bacterial isolation was achieved, single pure cultures were innoculated into 500 mls of sterile wastewater which was adjusted to a pH of 7.0 with the addition of sodium bicarbonate. The sample was allowed to aerate for four days. pH was tested during that time with a digital pH meter attached to a previously alcohol-sterilized, distilled H_2O washed, glass electrode. Cultures were maintained at 25°C. Ten experimental runs each for E. coli, P. mallei and P. aeroginosa were accomplished for the period 9/80 to 8/81.

PROJECT OBJECTIVES

The overall objective of this research project was to study the influence of organisms appearing in wastewaters of sewage treatment plants on observed pH changes in the sewage effluent.

The specific objectives were two.

1. to identify major bacteriological, fungal, algal and protozoan complements appearing in the secondary stage of domestic sewage treatment in selected New Hampshire plants and

2. to study the influence of these organisms on pH changes occurring during the domestic sewage treatment process.

Tables 2 and 3 list not only the major protozoan, algal and blue-green algal components in the Henniker Sewage Treatment Plant for the period 9/80 to 8/81 but also the frequency of appearance of these same components. It can be seen from these tables that Aspidisca sp., Machrotrochela sp., Vorticella sp. and Zoothamnium sp. were the protozoans appearing in the largest numbers consistently through the test period. Mougetia sp. was the most frequently appearing alga and Spirulina sp. and Oscillatoria sp. the most frequently appearing blue-green algae. The total numbers of protozoans were far greater than the total numbers of algae and blue-green algae.

Table 1. Weekly Averages of Effluent pH, Temperature (°C) and Sewage Flow (millions of gallons per day) for the Period July 1980 to September 1981 at Henniker Sewage Treatment Plant, Henniker, New Hampshire. Data Provided by Plant Superintendent and Operator.

Week of	$\bar{x}$ Daily pH	$\bar{x}$ Daily Temperature (°C)	$\bar{x}$ Daily Flow (m.g.d.)
7/1/80	5.2	Data not available	0.079
7/7/80	5.1	July 1, 1980 to	0.094
7/14/80	5.0	January 5, 1981	0.087
7/21/80	4.7		0.088
7/28/80	4.6		0.088
8/4/80	5.0		0.098
8/11/80	4.3		0.089
8/18/80	4.3		0.092
8/25/80	4.4		0.095
9/1/80	5.1		0.113
9/8/80	4.7		0.136
9/15/80	4.4		0.132
9/22/80	5.6		0.127
9/29/80	4.3		0.128
10/6/80	4.2		0.128
10/13/80	4.3		0.124
10/20/80	4.2		0.122
10/27/80	4.1		0.129
11/3/80	4.3		0.138
11/10/80	4.6		0.122
November 17, 1980 to January 4, 1981 - Data not Available			
1/5/81	7.2	2.3	0.123
1/12/81	7.2	2.9	0.145
1/19/81	7.2	5.5	0.127
1/26/81	7.2	6.4	0.122
2/2/81	7.1	4.0	0.096
2/9/81	7.2	5.9	0.131
2/16/81	7.2	9.2	0.135
2/23/81	7.2	9.2	0.149
3/2/81	6.8	7.9	0.154
3/9/81	6.1	8.9	0.147
3/16/81	6.0	7.8	0.144
3/23/81	5.7	9.0	0.127
3/30/81	5.4	10.4	0.085
4/6/81	6.2	11.1	0.130
4/13/81	6.0	10.4	0.138
4/20/81	5.6	11.4	0.137
4/27/81	5.2	13.7	0.130
5/4/81	4.9	14.1	0.133
5/11/81	5.3	15.0	0.133
5/18/81	7.2	13.8	0.127
5/25/81	7.0	16.6	0.094

Table 1. (Continued)

Week of	$\bar{x}$ Daily pH	$\bar{x}$ Daily Temperature (^{o}C)	$\bar{x}$ Daily Flow (m.g.d.)
6/1/81	6.5	16.4	0.080
6/8/81	6.5	16.8	0.079
6/15/81	6.6	18.8	0.074
6/22/81	6.9	18.0	0.081
6/29/81	6.7	18.2	0.081
7/6/81	6.7	21.4	0.081
7/13/81	6.7	20.2	0.079
7/20/81	6.8	19.7	0.085
7/27/81	6.7	18.5	0.080
8/3/81	6.6	20.8	0.080
8/10/81	6.7	21.0	0.080
8/17/81	6.7	18.5	0.083
8/24/81	6.8	18.6	0.075
8/31/81	6.9	18.9	0.087
9/7/81	6.8	18.5	0.124
9/14/81	7.3	18.7	0.128

Table 2. Average Frequency (# per cm^3) of Selected, Dominant Phytoplankton in Sewage Effluent from Henniker Sewage Treatment Plant, Henniker, New Hampshire for the period 9/80 to 8/81.

Organism $\left(\frac{\text{\# filaments or cells}}{cm^3}\right)$

Date	Oscillatoria sp.	Spirulina sp.	Mougeotia sp.	Cladophora sp.	Dinobryon sp.	pH
9/3/80	4	73	5	6	0	4.3
9/17/80	35	73	12	0	1	4.4
9/22/80	6	39	2	0	0	5.9
9/28/80	5	25	0	1	0	4.5
10/6/80	4	50	0	0	2	4.0
10/15/80	0	41	0	4	0	4.3
11/19/80	1	10	0	104	0	6.3
12/2/80	2	18	0	2	0	7.2
12/10/80	9	30	6	0	1	6.9
12/17/80	2	28	2	0	5	6.9
1/5/81	0	27	6	0	2	7.2
1/12/81	0	24	5	0	2	7.2
1/19/81	0	40	6	0	6	7.4
1/27/81	1	32	22	2	4	7.1
2/10/81	0	31	16	0	4	7.4
3/2/81	0	49	196	0	0	7.0
3/17/81	0	40	85	0	1	6.2
4/6/81	2	27	40	0	0	5.9
4/21/81	2	21	5	0	0	5.6
5/12/81	7	18	8	1	0	5.3
5/26/81	1	26	16	0	2	7.1
6/9/81	2	21	37	0	6	6.5
6/23/81	0	19	126	1	7	7.0
7/8/81	1	23	45	1	5	6.7
7/22/81	0	24	67	7	2	6.7
8/5/81	2	20	51	0	3	6.7
8/19/81	1	29	33	0	5	6.6

Table 3. Average Frequency (# per cm^3) of Selected, Dominant Zooplankton in Sewage Effluent from Henniker Sewage Treatment Plant, Henniker, New Hampshire for the period 9/80 to 8/81

Organism (# per cm^3)

Date	Vorticella sp.	Zoothamnium sp.	Machrotrochelia sp.	Aspidisca sp.	Centropysis sp.	Euchlanis sp.	Amphisiolla sp.	Uronema sp.	Opisthenecia sp.	Malacophrys sp.	pH
9/3/80	1035	440	2220	400	296	592	0	0	0	0	4.3
9/17/80	1130	1035	590	1330	0	290	0	0	0	0	4.4
9/22/80	740	1300	148	2663	148	0	0	0	0	0	5.9
9/23/80	400	3402	0	5909	0	0	0	0	0	0	5.7
9/28/80	440	740	2200	1775	140	0	0	0	0	0	4.5
9/30/80	296	1479	0	4290	150	0	0	0	0	0	4.4
10/6/80	300	590	0	1923	145	0	0	0	0	0	4.0
10/10/80	290	300	296	8728	100	0	0	0	0	0	4.3
10/15/80	2515	3994	880	6805	1480	1330	0	0	0	0	4.3
11/5/80	2960	3254	148	4882	1301	890	0	0	0	0	4.3
11/12/80	300	870	0	1780	145	0	0	0	0	0	4.6
11/19/80	147	2219	0	2071	0	296	0	0	0	0	6.3
12/2/80	0	0	0	404	0	0	0	0	0	0	7.2
12/10/80	150	1035	0	444	0	135	0	0	0	0	6.9
12/17/80	5620	4885	0	1183	296	410	4150	0	0	0	6.9
1/5/81	1183	3846	0	1627	0	130	3254	0	0	0	7.2
1/12/81	592	5621	0	2510	100	0	4738	148	0	0	7.2
1/19/81	740	6657	0	1600	0	0	9320	0	0	0	7.4
1/27/81	700	5917	0	740	0	0	138	886	880	296	7.1
2/10/81	450	4439	0	454	0	0	148	3027	5178	1627	7.4
2/17/81	1485	4330	0	110	0	0	2810	1010	1445	5759	7.2
2/23/81	1035	3846	0	296	0	0	740	900	0	3240	7.3
3/2/81	730	3400	0	2220	0	0	150	425	0	1470	7.0
3/17/81	700	459	0	108	0	0	296	0	112	0	6.2
3/24/81	0	592	0	0	0	0	580	0	400	0	5.9
4/6/81	0	0	0	0	0	0	0	286	0	0	5.9
4/13/81	1020	885	0	0	0	0	0	0	98	0	5.7
4/21/81	570	856	0	0	0	0	778	0	568	0	5.6
4/28/81	1188	0	0	0	0	0	1154	0	868	0	5.3
5/5/81	1630	244	0	0	0	0	2810	0	1332	0	4.9
5/12/81	668	195	0	555	0	0	1010	0	1440	0	5.3

Table 3. (continued)

Date	Organism (# per cm^3)										
	Vorticella sp.	Zoothamnium sp.	Machrotrochelia sp.	Aspidisca sp.	Centropysis sp.	Euchlanis sp.	Amphisiolla sp.	Uronema sp.	Opisthenecia sp.	Malacophrys sp.	pH
5/19/81	115	260	0	1131	0	0	330	0	780	0	7.2
5/26/81	380	1380	0	76	0	0	0	0	440	0	7.1
6/2/81	1375	1821	95	0	0	0	0	78	488	0	6.7
6/9/81	3047	2254	188	0	0	0	3168	0	110	0	6.5
6/16/81	2116	3009	50	0	0	0	3370	990	80	0	6.6
6/23/81	4940	1802	111	0	0	0	842	1106	0	0	7.0
7/1/81	894	3327	767	0	0	0	4802	886	0	0	6.7
7/8/81	1791	2108	921	0	0	0	0	3020	0	0	6.7
7/15/81	3300	1990	519	0	0	0	0	808	0	0	6.9
7/22/81	1140	2200	381	0	0	0	0	66	0	0	6.7
7/29/81	1485	1364	1297	0	0	0	0	0	0	0	6.7
8/5/81	980	660	571	76	0	0	0	0	0	0	6.7
8/12/81	1250	2139	1144	260	0	0	0	0	0	0	6.7
8/19/81	1581	710	2010	109	0	0	0	0	0	0	6.6

Because of the lack of appearance of fungi on the Sabourad-Dextrose plates during the first three weeks of September and because of an increasing awareness of the size of our Task, we elected to postpone the fungal identification component.

The major bacteria isolated were, in decreasing order of frequency, as follows:

Escherichia coli

Streptococcus fecalis

Pseudomonas mallei

Pseudomonas aeroginosa

Staphlococcus aurosa

In order to study the influence of these organisms on pH changes in the secondary treatment process, it was decided to add a statistical approach to the experimental approach outlined in the "Methodology" section. Table 1 is a summary of pertinent data collected by Damour and Ward (1981) during our testing period, 9/80 - 8/81. These dates include pH in the aeration tank. We then noted population counts on dates common to both protozoan counts and algal counts and ran correlation statistics with these and the pH data. Table 4 lists the calculated correlation coefficients (r) for the most frequently appearing plankton.

Those organisms showing a positive ($\geq$ 0.50) correlation between numbers and pH were:

Dinobryon sp.

Zoothamnium sp.

Those showing a negative correlation were:

Spirulina sp.

Centropyxis sp.

All other correlations were below $\pm$ 0.50. This included the lowest correlation value of 0.00 for *Vorticella* sp.

For our experimental approach, we chose to use three bacteria which were in abundance and which we isolated in pure culture from our aeration tank samples. The results of this experiment are listed in Table 5.

It can be seen that in each case the pH of the contained amount of medium was lowered from neutral to the acidic range.

Finally, our objective of studying more than one treatment plant within the constraints of our test time and budget was totally unrealistic.

Table 4. Correlation coefficients (r) for frequency of individual species and pH at Henniker Sewage Treatment Plant for the period 9/80 to 8/81.

Organism	r
PHYTOPLANKTON:	
Cladophora sp.	+0.05
Dinobryon sp.	+0.53
Mougeotis sp.	+0.27
Oscillatoria sp.	-0.46
Spirulina sp.	-0.59
ZOOPLANKTON:	
Amphisiella sp.	+0.39
Aspidisca sp.	-0.41
Centropyxis sp.	-0.51
Euchlanis sp.	-0.45
Machrotrochela sp.	-0.46
Malacophrys sp.	+0.30
Opisthonecta sp.	+0.19
Uronema sp.	+0.00
Vorticella sp.	+0.17
Zoothamnium sp.	+0.52

Table 5. Average pH of Innoculated, Previously Sterilized Wastewater from the Aeration Tank at the Henniker Sewage Treatment Plant.

TIME AFTER INNOCULATION (Hours)	E.coli	P.mallei	P.aeroginosa
0	7	7	7
6	7	7	7
12	6.8	6.9	6.8
18	6.2	6.7	6.3
24	5.7	6.3	6.0
30	5.0	6.0	6.0
36	4.8	5.7	5.9
42	4.8	5.5	5.6
48	4.8	5.3	5.5
54	4.8	5.2	5.3
60	4.8	5.3	5.5
66	4.8	5.3	5.4
72	5.0	5.4	5.4
78	5.1	5.3	5.4
84	5.6	5.5	5.3
90	5.1	5.5	5.2
96	5.0	5.5	5.4

DISCUSSION

The most frequent and most consistent organisms which appeared in the aeration tank of Henniker Sewage Treatment Tank illustrate the conclusions of Varma et al. (1975) that each treatment plant apparently has its characteristic array and/or frequency of flora and fauna. Our results did show a dominance of protozoans over algae and possibly fungi.

Vorticella was one of our predominant protozoans and this organism has been identified by Curds & Cockburn (1970) as one responsible to a large degree for the success of biological purification. However we did show that the frequency of this organism seems to have no correlation with pH values in the aeration tank. This conflicts with the laboratory experiments of Curds & Cockburn.

The alga, Dinobryon and the protozoan Zoothamnium showed a positive correlation with pH values. Correlation statistics do not necessarily show a cause and effect relationship. In order to show cause and effect it would be necessary to conduct further testing to see if low pH enhances growth of these two organisms or if these two organisms are responsible for lowering pH values.

Finally, in our closed-system analysis of bacteria and pH, all three bacteria grew in increasingly lower pH values.

The obvious conclusion is one of cause and effect. Bacteria were responsible for the lowering of pH in the test systems. Our control (without bacteria) remained at pH 7.

PRINCIPAL FINDINGS

In conclusion we have found the following relationships:

1. The dominant eukaryotic organisms at Henniker's Sewage Treatment Plant are protozoans. They include the organism Vorticella which has been shown to be important to the success of biological purification of wastewater.
2. The dominant prokaryotic organisms are coliform bacteria. This is an expected result.
3. There is a strong positive correlation between Zoothamnium and Dinobryon and pH. Whether these two organisms were actually responsible for the lowering of the pH at the treatment plant cannot be shown by these results. Further testing would be required. However the large numbers of Zoothamnium and the positive correlation make it a likely candidate.
4. Three bacteria, E. coli, P. aeroginosa, P. mallei, were shown to lower the pH of their media from 7 to as low as 4.8. pH of 4.8 is well below acceptable E.P.A. standards of discharged effluent.

Standardly, we feel that we have raised more questions than we have answered. The biological ecology of a secondary treatment system is incredibly complex, perhaps more complex than a "natural" aquatic system. Along with variables similar to those in natural systems, secondary treatment plants experience man-induced variables (foreign chemicals, widely fluctuating flow rates, changing aeration conditions, etc.)

The strongest recommendation I can make is to begin isolation of these variables for testing purposes because the maintenance of effluent pH values at and around 6.5 is required. When a plant experiences either low or high pH values, the explanation for this and the subsequent solution to the problem becomes simplified if the cause for the changing pH is specifically isolated.

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