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# Hydrogen Peroxide as a Supplemental Oxygen Source for Activated Sludge: Microbiological Investigations

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**Summary.** Parallel bench-scale activated sludge systems were operated using air or hydrogen peroxide as oxygen source. The use of $H_2O_2$ resulted in a temporary decrease of COD reduction, an increase of the catalase activity of the activated sludge, a depression of the nitrification, and a marked decrease of some filamentous organisms. Enumeration of some microbiologic groups indicated that the counts of enterobacteria, coliforms, staphylococci, and streptococci were lower in the $H_2O_2$ unit than in the parallel air unit. Also the use of $H_2O_2$ did not induce the selection of bacterial species that are more resistant to $H_2O_2$. The increase in catalase activity after $H_2O_2$ addition might be the result of a stimulation of catalase synthesis in catalase positive microorganisms.

Because of its flexibility and high removal efficiency, the activated sludge system has become very popular for the biological treatment of waste waters. With this system, it is very important to provide enough oxygen for the aerobic microorganisms. Until now, oxygen was mostly supplied from the air using compressed-air aerators or surface aerators. Because of the low driving force for the transfer of oxygen from the gas phase to the liquid phase, the oxygen transfer rate with conventional aeration equipment was limited. There has been much research during the last years on the use of pure oxygen for biological treatment processes (Chapman et al., 1976), although different opinions exist (Kalinske, 1976). Recently, some authors proposed hydrogen peroxide as a supplemental oxygen source in biological processes.

In many aerobic bacteria, hydrogen peroxide can be formed by the oxidation of some reduced flavoproteins by molecular oxygen (Stanier et al., 1971). $H_2O_2$ is a toxic substance and most aerobic organisms contain an enzyme, catalase ($H_2O_2$:$H_2O_2$ oxidoreductase, EC 1.11.1.6), which decomposes the peroxide to water and oxygen.

*List of Abbreviations:* COD: chemical oxygen demand, mg $O_2$/l; $COD_{eff}$: chemical oxygen demand of the effluent, mg $O_2$/l; DO: dissolved oxygen, mg $O_2$/l; MLSS: mixed liquor suspended solids, g dry weight/l; SVI: sludge volume index, ml settled sludge per liter/MLSS (ml/g); F:M: sludge loading factor or the Food to Microorganisms ratio, g COD/g MLSS.day

The normal catalase contains iron-porphyrin prosthetic groups and is inhibited by cyanide or azide. Other bacteria (e.g., many *Lactobacilli*) contain catalase only when grown with hematin. Sometimes they contain an unusual catalase without iron-porphyrin which is not inhibited by cyanide or azide.

Hydrogen peroxide is bactericidal in high concentrations. It has been used extensively in medicine and sanitation. $H_2O_2$ is also one of the components of the antimicrobial system of milk (Klebanoff et al., 1966; Steele and Morrison, 1969) and leucocytes (Mc Ripley and Sbarra, 1967; Selvaraj et al., 1974). The mechanism of disinfectant action is not known. Some authors found a correlation between the catalase content of the cells and their sensitivity to $H_2O_2$ (Amin and Olson, 1968; Yosphe-Purer and Henis, 1976). Others noted that a catalase-negative strain (*Lactobacillus plantarum*) was slightly sensitive to $H_2O_2$ (Gregory and Fridovich, 1974). Their results suggested that cell death was caused by the superoxide radical $O_2^-$ which is normally formed through the univalent reduction of oxygen and primarily by the hydroxyl radical OH· generated from the reaction: $O_2^- + H_2O_2 \rightarrow OH\cdot + O_2$. When more $O_2^-$ is formed in actively growing cells, this mechanism would explain the higher sensitivity of young, actively growing cells to $H_2O_2$ (Yosphe-Purer and Henis, 1967).

Despite its antimicrobial activity, $H_2O_2$ can be used as an oxygen source for activated sludge. Chin et al. (1973) noted that a large percentage of waste water treatment plant difficulties are caused by a lack of oxygen and that $H_2O_2$ can efficiently solve this problem. Cole et al. (1974) also concluded that good COD reductions can be obtained using $H_2O_2$ as a supplemental source of oxygen. These authors thought that the high cost of $H_2O_2$ might limit its generalized use as an oxygen source, but that it could be a viable solution for sporadic problems occurring with temporarily overloaded systems and for preventing anaerobic conditions in secondary sedimentation tanks.

Although it was demonstrated that $H_2O_2$ can be used as an oxygen source for activated sludge, there are indications that some bacteria of activated sludge are more sensitive to $H_2O_2$ than others. It was found that the use of $H_2O_2$ depressed the nitrification (Cole et al., 1974) and that $H_2O_2$ could be used to prevent filamentous bulking (Cole et al., 1973). This work investigates the influence of $H_2O_2$ as oxygen source on the microbiological composition of activated sludge and examines the possibility of some adaptation or selection of the activated sludge bacteria in the presence of $H_2O_2$.

## Materials and Methods

Laboratory-scale completely-mixed activated sludge systems (Fig.1) consisted of an aeration volume (2.5 or 5.0 l) and a separate sedimentation cylinder. Settled sludge was recirculated continuously by means of a peristaltic pump. Except for one experiment, two parallel activated sludge systems were run. One of them was aerated with 40 NL air/l·h to supply enough dissolved oxygen and to obtain homogeneous mixing. A second system was stirred magnetically and dissolved oxygen concentration was maintained at a fixed level (4 ± 0.5 mg/l) by the addition of $H_2O_2$ (3%, wt/vol) with a peristaltic pump, which was monitored by a dissolved oxygen controller (Wiss.-Tech. Werkstätten, Weilheim, Germany, Oxygen Controller OXI 139). Synthetic wastes were added continuously as influents to the activated sludge units with a gravity flow system

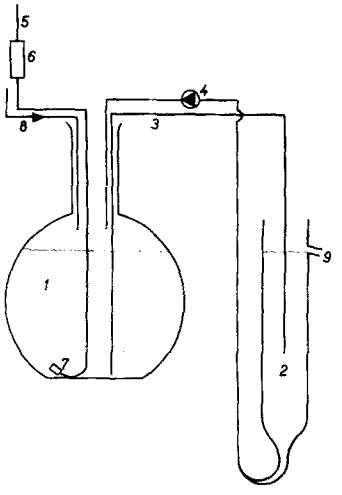


**Fig. 1.** Schematic diagram of the experimental apparatus 1. aeration unit (capacity of mixed liquor: 2.5 l or 5 l) 2. sedimentation unit (diameter, 3 cm, capacity, 175 ml) 3. siphon 4. peristaltic pump 5. compressed air 6. air flowmeters 7. ceramic sparger 8. feed supply line 9. effluent discharge

(Parker, 1967). The synthetic wastes contained different carbon sources, such as terephtalic acid (obtained from Amoco Chemicals Belgium), glucose + nutrient broth, or glucose + starch + nutrient broth. Details are given later. The influent flow rate was adjusted to obtain a sludge loading (F:M ratio) of 0.4—0.5 g COD/g MLSS.day. The sludge retention time and MLSS were controlled by the daily wastage of a certain amount of mixed liquor.

Chemical oxygen demand (COD) and mixed liquor-suspended solids (MLSS) were determined by Standard Methods (1971). A modified sludge volume index (SVI) was determined by allowing the mixed liquor to settle for 30 min in a graduated cylinder of 100 ml. Ammonium nitrogen was determined colorimetrically after nesslerization of the samples (Strauch, 1965). Nitrite was analyzed by the method of Montgomery and Dymock (1961) and nitrate by a 2,4-xylenol method (Department of the Environment, 1972). The protein content of activated sludge was analyzed by the Lowry method (Herbert et al., 1971). The catalase activity of activated sludge was measured following a permanganate titration method (Sridhar and Pillai, 1974), except that the enzyme activity was expressed as mmoles $H_2O_2$ decomposed/g MLSS.min. Catalase-positive or negative microorganisms were determined by mixing a loopful of cells in a drop of 3% $H_2O_2$ solution on a clear slide and inspection for the production of oxygen bubbles within one minute (Sirockin and Cullimore, 1969).

To compare the microbiological composition of parallel activated sludge systems, several dilutions of homogenized activated sludge samples were plated on different selective and nonselective media. Homogenization occurrred with a Waring Blendor during 3 min at maximum speed. According to methods described earlier (Poffé et al., 1973), we determined: total counts on tryptone soya agar (Oxoid) and on the medium of Jones (1970), *Pseudomonas* and other nonfastidious gram-negative bacteria, *Arthrobacter* and other gram-positive bacteria, enterobacteria, staphylococci, and fecal streptococci. Coliforms were counted on violet red bile agar (Oxoid) after anaerobic incubation at 37°C for 24 h. To determine the amount of aerobic spores, several dilutions of activated sludge samples were heated for 10 min at 80°C and seeded on

tryptone soya agar (Oxoid); colonies were counted after incubation for 48 h at 25°C. Autotrophic nitrifying bacteria were enumerated by a MPN procedure using 10-fold dilutions with 5 tubes per dilution. Ammonia oxidizers were grown in a medium described by Höfflich (1969). After three weeks of incubation, the tubes were examined for nitrite with sulphanilic acid and α-naphtylamine and for nitrate with diphenylamine reagent (Boltz, 1958). Nitrite oxidizers were grown in a medium as used by Soriano and Walker (1973). After three weeks of incubation, the tubes were examined for nitrate with diphenylamine reagent (Boltz, 1958). Interfering nitrite was reduced with ureum. Numbers of nitrifying organisms per ml were obtained from McCrady's table.

## Results and Discussion

In a first experiment, two parallel bench-scale activated sludge systems were fed with a solution containing: terephtalic acid, 1800 mg/l; $NH_3$, 135 mg $NH_3$-N/l; $H_3PO_4$, 27 mg $H_3PO_4$-P/l; $CaCl_2.2H_2O$, 50 mg/l; $MgSO_4.7H_2O$, 50 mg/l; $FeCl_3.6H_2O$, 10 mg/l. To prepare 10 l influent solution, 18 g terephtalic acid were dissolved in 1 l distilled water containing 8 g NaOH. When all terephtalic acid was dissolved, ammonia (1.35 g $NH_3$-N, 12.3 ml of $NH_3$ solution containing 110 g N/l) and phosphoric acid (270 mg $H_3PO_4$-P, 1 ml $H_3PO_4$ 85%) were added, and the pH was adjusted at 7.5 with HCl. This solution was diluted with distilled water while the other salts were added. Activated sludge from a municipal treatment plant was adapted to this peculiar influent in a bench-scale activated sludge system with normal aeration at a gradual increasing sludge loading. After 10 days of adaptation, the mixed liquor was spread over two parallel activated sludge systems and to one of these systems (B) $H_2O_2$ addition started. The mixed liquor vol was 2.5 l and, with a daily wastage of 125 ml, the solids retention time was 20 days. The influent flow rate was adjusted to obtain a sludge loading of 0.4 g COD/g MLSS.day. The pH of the mixed liquor remained constant between 7.5 and 8.0.

**Table 1.** Characteristics of mixed liquor and effluents of parallel bench-scale activated sludge systems when air (A) or peroxide (B) was used as oxygen source; $H_2O_2$ addition to B begun at day 1. The carbon source was terephtalic acid, the sludge loading was 0.4 g COD/g MLSS. day and the sludge retention time was 20 days (first experiment)

| day | MLSS g/l | | SVI ml/g | | $COD_{eff}$ mg/l | | catalase activity mmol $H_2O_2$/g MLSS.min | |
|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B |
| 1 | 2.17 | 2.07 | 460 | 480 | 20 | 20 | 14 | 14 |
| 2 | 2.05 | 2.0 | 490 | 480 | 30 | 370 | – | 36 |
| 3 | 2.26 | 2.24 | 440 | 490 | 60 | 80 | 9 | 44 |
| 4 | 2.37 | 2.23 | 440 | 340 | 40 | 50 | – | 80 |
| 5 | – | – | – | – | – | – | – | – |
| 6 | 2.50 | 2.07 | 410 | 340 | – | – | – | – |
| 7 | 2.55 | 2.06 | 390 | 215 | 40 | 70 | 10 | 72 |
| 8 | 2.92 | 2.07 | 340 | 180 | – | – | – | 89 |
| 9 | 2.90 | 2.10 | 345 | 170 | 40 | 60 | – | 105 |
| 10 | – | – | – | – | – | – | – | – |
| 11 | 2.93 | 2.15 | 340 | 210 | – | – | – | 98 |
| 12 | 2.85 | 2.20 | 345 | 180 | 50 | 50 | 12 | 96 |

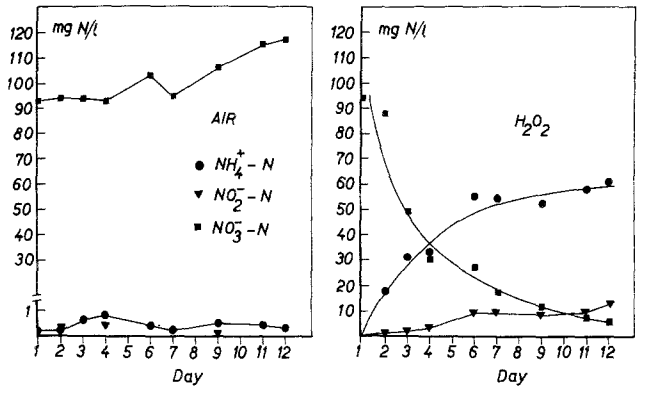


**Fig. 2.** Evolution of nitrogen compounds in the effluents of parallel bench-scale activated sludge systems when air (A) or hydrogen peroxide (B) was used as oxygen source. After a previous adaptation period with air, hydrogen peroxide addition to system B started at day 1 (first experiment)

Results of different analyses are given in Table 1. As shown, the initial modified sludge volume index is very high for both sludges. Microscopic examination of the sludges indicated the presence of a vast amount of filamentous organisms embedded in a sheath. Switching from air to $H_2O_2$ resulted in a rapid decrease of the number of filaments in the mixed liquor. Seven days after the start of $H_2O_2$ addition, almost all trichomes disappeared from the system. At that moment the SVI decreased.

The values of the effluent COD for both systems show that high COD reductions were obtained even when $H_2O_2$ was used as an oxygen source. However, during the first day of $H_2O_2$ addition, the COD of the effluent is higher. Table 1 also includes values about the catalase activity of both sludges. It indicates that in the system with $H_2O_2$ this activity was 7 to 10 times as high as in the parallel system. The protein content of the activated sludges was the same for both systems (50% of the dry weight). Results on the nitrification during the first experiment are given in Figure 2, which shows that in the aerated system almost all excess of ammonium was oxidized to nitrate. However, in the other system, the addition of $H_2O_2$ caused a thorough disturbance of the nitrification process indicated by high concentrations of ammonium-N and low levels of nitrate. Enumeration of nitrifying microorganisms revealed a decrease in the number of ammonia oxidizers from 150 germs/ml to 20 germs/ml 10 days after the start of $H_2O_2$ addition, while the number of nitrite oxidizers remained at about 80 germs/ml during the whole experiment.

In a second experiment, two bench-scale activated systems were fed with the same influent and under the same conditions as during the first experiment. The results of the first experiment were confirmed. The use of $H_2O_2$ caused an inhibition of the nitrification, a temporary decrease of the COD reduction and an increase of the catalase activity of the sludge. During this experiment, filamentous growth was observed in none of the systems.

In a third experiment, the use of $H_2O_2$ as an oxygen source for activated sludge was investigated using an influent solution containing: glucose, 2500 mg/l; nutrient broth, 500 mg/l; $(NH_4)_2$ $HPO_4$, 500 mg/l; NaCl, 50 mg/l; $CaCl_2 \cdot 2H_2O$, 50 mg/l; $MgSO_4 \cdot 7H_2O$, 50 mg/l; $FeCl_3 \cdot 6H_2O$, 10 mg/l. The bench-scale activated sludge unit had an aeration vol of 5.0 l. By daily wastage of 500 ml mixed liquor, the solids retention time was 10 days. The sludge loading was 0.5 g COD/g MLSS.day. After 7 days of aeration, $H_2O_2$ addition was started. As shown in Table 2, the SVI increased sharply during the first days. This was caused by an explosive growth of filamentous organisms. These again were sheathed bacteria, probably *Sphaerotilus* species. After 3 days of $H_2O_2$ addition, many empty sheaths were observed and after 6 days almost all filaments disappeared.
This resulted in a decreasing SVI. During this experiment, the COD reduction was always good, even just after the start of $H_2O_2$ addition. As in the previous experiments, the use of $H_2O_2$ induced an increase of the catalase activity of the sludge. The evolution of the respiration rate of the sludge indicates that the use of $H_2O_2$ resulted in a decrease of the substrate respiration from about 0.70 mg $O_2$/g MLSS.min to 0.32 mg $O_2$/g MLSS.min, although the endogenous respiration is not affected. A few days after the start of $H_2O_2$ addition, the substrate respiration again reached the initial level.

In the following experiments, the influence of the use of $H_2O_2$ on the microbiological composition of activated sludge was studied more closely. To this end, during a fourth experiment two parallel activated sludge systems were fed with a solution containing: glucose, 2500 mg/l; nutrient broth, 1000 mg/l; $(NH_4)_2$ $HPO_4$, 150 mg/l; NaCl, 50 mg/l; $CaCl_2$, $2H_2O$, 50 mg/l; $MgSO_4 \cdot 7H_2O$, 50 mg/l; $FeCl_3 \cdot 6H_2O$, 10 mg/l. The influent flow rate was adjusted to obtain a sludge loading of 0.4 g COD/g MLSS. day

**Table 2.** Characteristics of mixed liquor and effluent of a bench-scale activated sludge system during experiment 3. The mixed liquor was aerated until day 7 and from that day $H_2O_2$ was added to maintain a constant DO level. The carbon source was glucose + nutrient broth, the sludge loading was 0.5 g COD/g MLSS. day and the sludge retention time was 10 days

| day | MLSS g/l | SVI ml/g | $COD_{eff}$ mg/l | catalase activity mmol $H_2O_2$/g MLSS. min | $O_2$ uptake rate[a] $mgO_2$/g MLSS.min endogenous substrate | |
|---|---|---|---|---|---|---|
| 1 | 1.49 | 104 | – | – | 0.08 | 0.77 |
| 2 | 1.67 | 180 | 64 | 9 | 0.09 | 0.85 |
| 3 | 1.76 | 290 | – | – | 0.06 | 0.69 |
| 4 | – | – | – | – | – | – |
| 5 | – | – | – | – | – | – |
| 6 | 2.31 | 416 | 50 | 10 | 0.07 | 0.68 |
| 7 | 2.25 | 440 | – | – | 0.08 | 0.72 |
| 8 | 2.02 | 480 | 55 | 34 | 0.12 | 0.32 |
| 9 | 1.77 | 525 | – | – | 0.11 | 0.46 |
| 10 | 1.67 | 539 | 50 | 67 | 0.07 | 0.64 |
| 11 | – | – | – | – | – | – |
| 12 | 1.86 | 414 | – | – | – | – |
| 13 | 2.27 | 300 | 45 | 79 | 0.08 | 0.68 |

[a]100 ml of mixed liquor were aerated in an 100 ml erlenmeyer flask and the decrease of DO was recorded with an oxygen electrode. As substrate 0.05% glucose + 0.05% nutrient broth were used

and the sludge retention time was 7 days. After an adaptation period with aeration for both systems, $H_2O_2$ was added to one system (B) to maintain the DO level at $4 \pm 0.5$ mg/l. In a fifth experiment, parallel activated sludge systems were operated in the same way as during the fourth experiment, but only the influent solution was different; glucose, 2500 mg/l and nutrient broth, 1000 mg/l were replaced by glucose, 1000 mg/l, soluble starch, 1000 mg/l and nutrient broth, 2000 mg/l. The results on the microbiological composition of both sludges together with the catalase activity are given in Table 3 for experiment 4 and in Table 4 for experiment 5. The percentage of catalase-positive colonies is indicated in parentheses.

**Table 3.** Microbiological composition of activated sludge of two parallel bench-scale activated sludge systems using air (A) or hydrogen peroxide (B) as oxygen source, at different days after start of the experiment (counts = number. $10^6$/mg MLSS). The percentages of catalase-positive colonies are given in parentheses. The catalase activity of the mixed liquor is expressed as mmol $H_2O_2$/g MLSS.min. The influent contained glucose + nutrient broth

| number of days after $H_2O_2$ addition | 8 | | 14 | |
|---|---|---|---|---|
| system | A | B | A | B |
| catalase activity | 7 | 36 | 12 | 45 |
| total counts on TSA[a] | 1820 (50) | 2360 (60) | 3000 (50) | 2100 (50) |
| *Pseudomonas* | 161 (50) | 56 (50) | 270 (40) | 56 (50) |
| *Arthrobacter* | 6.2 | 0.44 | 7 | 0.04 |
| enterobacteria | 58 | 0.12 | 2.7 | 0.13 |
| coliforms | 34 | 0.13 | 38 | 0.08 |
| staphylococci | 0.18 | <0.001 | 0.34 | 0.002 |
| streptococci | 5.5 | 0.015 | 2.8 | 0.01 |

[a]Total counts were determined on tryptone soya agar (TSA) (and on the medium of Jones (1970)); *Pseudomonas* and other gram-negative bacteria on sugar free agar + cristal violet; *Arthrobacter* and other gram-positive bacteria on blood agar + nalidixic acid; enterobacteria on violet red bile agar + glucose; coliforms on violet red bile agar; staphylococci on mannitol salt agar; streptococci on streptosel; See Poffé et al. (1973)

**Table 4.** Data of an experiment similar to that described in Table 3 but for an influent containing glucose + nutrient broth + starch

| Number of days after $H_2O_2$ addition | 1 | | 3 | | 8 | |
|---|---|---|---|---|---|---|
| system | A | B | A | B | A | B |
| Catalase activity | 41 | 202 | 40 | 158 | 18 | 87 |
| Total counts on TSA[a] | 160 (60) | 95 (60) | 2000 (40) | 2360 (50) | 980 (40) | 1990 (50) |
| Total counts on medium of Jones | 460 (40) | 410 (50) | 2700 (40) | 2980 (50) | 1310 (30) | 3880 (40) |
| *Pseudomonas* | 65 | <4 (40) | 86 (60) | 160 (80) | 44 (60) | 580 (70) |
| *Arthrobacter* | 68 | 21 (60) | 87 (40) | 58 (50) | 98 (40) | 140 (50) |
| Enterobacteria | 0.09 | 0.03 | 8 | 20 | 3 | 0.4 |
| Coliforms | 0.03 | 0.04 | 7 | 5 | 3 | 0.2 |
| Staphylococci | 0.001 | 0.002 | 0.0009 | 0.0008 | 0.9 | 0.2 |
| Streptococci | 0.05 | <0.001 | 0.5 | 0.07 | 1 | 0.6 |
| Aerobic spores | 0.007 | 0.003 | 0.002 | 0.002 | 0.002 | 0.0008 |

[a]Details are given in Table 3

Tables 3 and 4 show that the catalase activity of the activated sludge is higher for the systems to which $H_2O_2$ is added. They also indicate that 8 days after the start of the $H_2O_2$ addition, the number of *enterobacteria*, coliforms, *staphylococci*, and *streptococci* are lower in the system with $H_2O_2$ than in the parallel aerated system. However, the counts of *Pseudomonas* and *Arthrobacter* give different results for the two experiments. Tables 3 and 4 indicate also that the percentage of catalase-positive microorganisms does not increase markedly after addition of $H_2O_2$.

During experiment 4 and 5, bacteria were isolated from nonselective media and examined for their sensitivity to $H_2O_2$. Only colonies which, on morphological grounds, seemed to dominate, were selected for these studies. Test tubes containing 5 ml plate count broth (tryptone, 5 g/l; yeast extract, 2.5 g/l; glucose, 1 g/l) with different $H_2O_2$ concentrations were inoculated with 0.1 ml of a culture of each selected strain previously grown in plate count medium at 28°C for 24 h. The tubes were incubated at 28°C on a reciprocal shaker. After 5 days, the tubes were examined and growth was established by turbidity of the culture media.

The results of this investigation are given in Table 5. Clearly, many catalase-positive organisms are much more resistant to $H_2O_2$ than catalase-negative organisms. Different catalase-positive strains, from the air system as well as from the $H_2O_2$ system, could grow with initial $H_2O_2$ concentrations of 1000 mg/l. The highest tolerated $H_2O_2$ concentration for catalase-negative bacteria was 50 mg/l. On the other hand, the highest limit for a few catalase-positive strains was 2.5 mg/l $H_2O_2$, while for some catalase-negative this was 10 mg/l. In addition to the isolated bacteria, some reference strains were tested. Thus, *Serratia marcescens* and *Pseudomonas fluorescens* could tolerate more than 1000 mg $H_2O_2$/l while *Bacillus subtilis*, *Escherichia coli*, and *Micrococcus lysodeikticus* could tolerate 500 mg/l, 300 mg/l, and 100 mg/l respectively. For the catalase-negative strains, *Streptococcus faecalis* and *Lactobacillus arabinosus* 25 mg/l was the highest limit.

**Table 5.** Influence of the $H_2O_2$ concentration on the growth of several strains isolated from bench-scale activated sludge systems when air (strains A) or $H_2O_2$ (strains B) was used as source of oxygen. Numbers indicate the number of strains that can grow at the given $H_2O_2$ concentration in the growth medium.

| $H_2O_2$ conc. mg/l | Catalase-pos. strains | | Catalase-neg. strains | |
|---|---|---|---|---|
| | A | B | A | B |
| 0 | 11[a] | 7[a] | 8[a] | 6[a] |
| 2.5 | 10 | 7 | 8 | 6 |
| 5 | 10 | 6 | 8 | 6 |
| 10 | 9 | 6 | 7 | 4 |
| 25 | 9 | 4 | 3 | 3 |
| 50 | 9 | 3 | 1 | 2 |
| 100 | 8 | 3 | 0 | 0 |
| 200 | 7 | 3 | 0 | 0 |
| 300 | 5 | 3 | 0 | 0 |
| 400 | 5 | 3 | 0 | 0 |
| 500 | 5 | 3 | 0 | 0 |
| 1000 | 3 | 2 | 0 | 0 |

[a]These figures indicate the total numbers of specific strains that were tested

## Discussion

Our experiments showed that $H_2O_2$ can be used as source of oxygen for activated sludge. However, it appeared that some microorganisms, e.g., some sheathed bacteria and some nitrifying bacteria were very sensitive to $H_2O_2$. There were indications that the dominant activated sludge microorganisms adapt themselves to $H_2O_2$.

Thus, as reported already by Cole et al. (1973), it was shown that some filamentous bacteria were destroyed by $H_2O_2$ (experiment 1,3,4, and 5). This conclusion may not be generalized to include all filamentous organisms, because we have observed during the course of other work that some of them do not respond to $H_2O_2$.

The results on the nitrification in both systems during the first experiment revealed that the use of $H_2O_2$ induced an immediate disturbance of the nitrification process. Other investigators (Cole et al., 1974) also found a depression of the nitrification in activated sludge with $H_2O_2$ as source of oxygen. On the other hand, the results of Müller and Sekoulov (1975) indicate that $H_2O_2$ can be used as an oxygen source for the biological nitrification of secondary effluents in sand bed filters. Sekoulov (1976) assumed that the negative effect of the use of $H_2O_2$ on the nitrification, reported by Cole et al. (1974), would be the result of uncontrolled supply of high $H_2O_2$ concentrations. In our experiments, however, $H_2O_2$ is added via an oxygen controller and the DO level in the mixed liquor was held constant at $4 \pm 0.5$ mg/l. The $H_2O_2$, which was added dropwise, was decomposed immediately so that the $H_2O_2$ concentration in the mixed liquor was always very low (less than 0.5 mg/l). Enumeration of nitrifying bacteria indicated that ammonia oxidizers would be more sensitive to $H_2O_2$ than nitrite oxidizers. Other authors noted that ammonia oxidizers were also more sensitive to chlorination (Strom et al., 1976), while nitrite oxidizers were more sensitive to free ammonia (Anthonisen et al., 1976).

With respect to a possible adaptation of activated sludge to $H_2O_2$, it was shown that, while the effluent COD after the start of $H_2O_2$ addition during experiment 1 and 2 was increased, after 2 days of $H_2O_2$ addition the effluent COD returned to normal values. During the third experiment, it was shown further that the substrate respiration was affected by the use of $H_2O_2$. $H_2O_2$ induced an immediate decrease of the respiration, but after a few days of operation the initial level was reached, indicating that a possible adaptation of the sludge to $H_2O_2$ was achieved in a short time.

The higher catalase activity of the sludge after $H_2O_2$ addition might be some indication of the adaptation to $H_2O_2$. There are two possibilities for the sludge to adapt. First, the number of catalase-negative microorganisms might decrease resulting in a relative increase of catalase-positive microorganisms and/or some bacterial strains with high catalase activity might be selected. Second, the overall composition of the activated sludge might not change, but $H_2O_2$ could induce an increase of the catalase content of some strains. The results in Table 3 and 4 indicate that the percentage of catalase-positive colonies did not increase markedly. There was a distinct decrease of the number of *enterobacteria, staphylococci,* and *streptococci*, but these organisms were only a small fraction of the total microbial flora. When the sensitivity of several isolated strains to $H_2O_2$ was determined, (Table 5), it became clear that this sensitivity is very different for most strains. Several catalase-positive strains could resist up to

1000 mg $H_2O_2$/l, while others could not tolerate 2.5 or 10 mg/l. Some catalase-negative strains could survive 50 mg $H_2O_2$/l, while others did not resist 10 mg/l. Whatever the difference in sensitivity to $H_2O_2$ of the examined strains, the results indicate that strains isolated from the system with $H_2O_2$ were not more resistant than strains isolated from the aerated system. These results suggest that the use of $H_2O_2$ as oxygen source for activated sludge does not induce the selection of bacterial strains which are more resistant to $H_2O_2$ than others. Together with the fact that the use of $H_2O_2$ does not result in an increasing amount of catalase-positive microorganisms, these results suggest that the higher catalase activity of the activated sludge in the $H_2O_2$ system was not the result of a microbial shift but very probably the result of higher catalase synthesis in catalase-positive organisms in the presence of $H_2O_2$. In relation to this, Finn and Condon (1975) also showed that addition of $H_2O_2$ to *Salmonella typhimurium* cultures during the exponential growth phase stimulated catalase synthesis.

However, if not $H_2O_2$ but the hydroxyl radical ($OH^{\cdot}$) is the actual lethal agent, as is suggested by Gregory and Fridovich (1974), the concentrations of the superoxide radical ($O_2^-$) and of $H_2O_2$ are important. So, not only the content of catalase but also that of superoxide dismutase (Fridovich, 1974) must be considered, and further mechanisms which lead to the formation of $O_2^-$ and $H_2O_2$ are important.

The main differences between air systems and $H_2O_2$ systems thus remain the higher catalase activity of the $H_2O_2$ systems, the reduction of nitrification, and the decrease of some microbial populations. The disappearance of some filamentous microorganisms must certainly be mentioned. When $H_2O_2$ is used occasionally as a supplemental source of oxygen, the same effects will probably become apparent and they should be taken into consideration.

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