BIOFOULING CONTROL WITH UV/PEROXIDE
- A Laboratory Study

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INTRODUCTION

This investigation has been conducted to evaluate the effectiveness of hydrogen peroxide followed by irradiation with ultraviolet light as a method for controlling biofouling. The work was sponsored by Photox International, Inc. The scope of this project has been limited to a preliminary analysis of the treatment effectiveness and includes only laboratory results. A more comprehensive study will include field data and more laboratory work.

Biofouling

The term biofouling refers to the undesireable formation of a microbial film (biofilm) consisting of microorganisms and their products, possibly followed by a succession of higher life forms. Biofouling is a major cause of energy losses in fluid transport systems and heat transfer systems.

Energy loss occurs in fluid transport systems due to increase in frictional resistance to flow within equipment and piping resulting in decreased capacity for gravity flow systems, or greater power consumption in pumped systems. Characklis (1) in a literature review of frictional resistance due to biofouling cited one example of a 55 percent reduction of original flow capacity in a 61 cm ID, 80 km long water supply line due to a thin slimy film approximately 650 μm thick. Laboratory experiments using a 1.27 cm ID tube have shown a frictional resistance increase of up to 5 times due to a 500 μm thick biofilm (2).

Energy losses in heat transfer systems are due to the insulating effect of biofilm on power plant and chemical plant condenser surfaces. Reduced efficiency, caused by biofouling in condensers, costs the power industry approximately $400 million a year (1976 dollars) for extra fuel which is equivalent to 25 million barrels of oil (3). Laboratory experiments using a 1.27 cm ID tube have shown heat transfer to drop as much as 30 percent due to a 150 μm thick biofilm (4).

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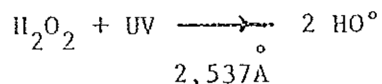
Control of biofouling is frequently accomplished by chlorine addition. However, use of chlorine has recently come under attack due to the potential health and environmental hazards of trihalomethanes (THMs) generated by chlorination of organic compounds in water.

In applications placing a cooling tower on zero discharge, increased biofouling problems may be realized due to accumulation of organics from recycling wastewater and use of rainfall runoff. The situation is countered by increased chlorine addition. The chlorine demand is high and the reacted chlorine remains in the recirculating system increasing chloride concentrations. The increased chloride concentration results in an increased corrosion rate and a natural buildup of brine which results in an increase in total dissolved solids which will make some blowdown necessary.

Economic considerations, energy conservation demands, increasingly stringent regulations on potentially toxic chlorine residuals (and their reaction products) and the demand for reuse of water require a systematic understanding of factors influencing biofouling and its control. This paper presents laboratory data evaluating one alternative biofouling control process, the Photox Process, which uses hydrogen peroxide addition prior to ultraviolet irradiation.

The Photox Process

The Photox process consists of adding hydrogen peroxide to the water followed by irradiation with ultraviolet light (UV) at a wavelength of 2,537Å. The hydrogen peroxide, as it passes through a high-intensity UV chamber, absorbs the 2,537Å radiation energy and divides into two moles of hydroxyl free radicals:



$$H_2O_2 + UV \xrightarrow[2,537\text{Å}]{} 2\ HO^\circ$$

These free radicals have an oxidation potential second only to flourine as shown in Table 1 (5).

An oxidant in combination with UV can actually sterilize water. Bayliss (6) reported that UV irradiation at 2,537Å of B. subtilis spores in the presence of hydrogen peroxide (0.150.45M) destroyed more than 99.99 percent of the spores. She reported, however, that irradiation of the spores in the presence of higher concentrations of peroxide markedly reduced the kill, possibly because at high peroxide concentrations a good deal of radiation was being absorbed by the peroxide. Bayliss' work also showed that irradiation in the presence of less than 0.6 M hydrogen peroxide produced a synergistic kill. The combination was 4,000 times as effective as UV irradiation followed by hydrogen peroxide treatment. Bayliss concluded the effect was due to the formation of hydroxyl free radicals.

A hydrogen peroxide-UV process would cost only a few cents per 3,500 ℓ. This is extremely economical* and provides total viral and bacterial inactivation, no THMs or other suspected carcinogenic compounds produc-

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* An ozone process on an equivalent oxidant basis is in excess of 5.5 times more expensive than the hydrogen peroxide-UV process.

tion, and elimination of odors.

The reaction rate constants of the hydroxyl free radicals compared with ozone's are generally one billion times faster.

Ananthaswamy (7) also has shown that hydrogen peroxide combined with near-UV irradiation enhances the killing of phage T7. The maximum effect was observed to be at 3,400Å.

Aside from the germicidal effect of the Photox Process, oxidation of organics, including "refractory" organics is possible by the hydroxyl radicals (8). The combination of killing the fouling bacteria and destroying their energy source suggests that the Photox process may offer an excellent alternative for control of biofouling.

Laboratory Test Methods

The experimental system used in this research consists of four identical annular fouling reactors (AFR), each receiving the same water but treated differently as follows:

- No treatment. This was considered the control reactor.
- Treatment with hydrogen peroxide only.
- Treatment with the Photox process (UV plus hydrogen peroxide).
- Treatment with UV only.

Annular Fouling Reactor

Figure 1 illustrates details of the AFR along with pertinent dimensions. The AFR is constructed of acrylic plastic and consists of two concentric cylinders, a stationary outer cylinder and a rotating inner cylinder. Removable acrylic slides for mass measurements are located between the two cylinders, close to the outer cylinder. Mixing within the AFR is accomplished by the pumping action of four draft tubes and an impeller mounted at the bottom of the cylinder.

As biofouling develops on the cylinder surfaces the biofilm accumulation is monitored in two ways:

- Dry biofilm mass is determined by removing biofilm from one of the removable acrylic slides using a rubber policeman and flushing with distilled water. The resulting biomass is placed on a 5.7 cm diameter, tared aluminum dish and dried at 60°C for approximately 12 hours. After drying, the mass of the dish plus dry biofilm is determined and the difference in mass from the clean dish and the dish plus biofilm gives the dry biofilm mass.

- Torque on the rotating shaft of the inner cylinder is monitored. As the biofilm develops, an increase in torque results. Previous work has shown good correlation between biofilm thickness and torque measurements (9).

Water Supply

A schematic of the water supply to each AFR is given by Figure 2. Dilution water consists of domestic tap water (Bozeman, Montana) passed through a carbon column for chlorine removal followed by 5 μm filtration. Temperature is controlled in the dilution water storage tank and aeration is provided. A mixed microbial culture is supplied to the AFR from

a chemostat which was initially inoculated by activated sludge from the Bozeman Municipal Sewage Treatment Plant. The chemostat receives a continuous feed of nutrients (glucose, trypticase soy broth and micronutrients). Glucose is added directly to the AFR as an energy source for biofilm growth.

Cleaning Procedure

Cleaning procedures between experiments consist of dismantling the AFR, removing the impellers and washing all parts in warm water and mild detergent.

Experimental Design

Figure 3 presents a schematic of the experimental design. Experiments were conducted to compare the following conditions:

° No Treatment. This is considered the control reactor and is used to ensure proper growth conditions are present. The control reactor also allows comparison between experiments since the mixed microbial populations may vary from one experiment to another.
° Treatment with UV only. Water supply to this AFR first passed through the UV chamber to determine the effectiveness of UV treatment alone.
° Treatment with H_2O_2. This AFR receives H_2O_2 to determine if the Photox Process is any more effective than H_2O_2 without UV.
° Treatment with the Photox Process. H_2O_2 is added to the AFR water supply followed by irradiation of the water with UV at 2,537Å.

Operating Conditions

Operating conditions are given in Table 2. All parameters remain constant for each experiment except for variation of H_2O_2 concentration.

Analytical Techniques

H_2O_2 concentrations are checked periodically by a method developed by Graf and Penniston (10).

Viable microbial concentrations are determined by the Standard Methods Spread Plate Technique (11). This method is employed in determining the density of aerobic microbes present in the four individual AFR's. The microbial cells are isolated by delivering 0.1 ml. of the diluted samples onto solid TLY agar plates (Trypticase Soy + 10% Lactos and 3% yeast extract). The inoculum is spread uniformly over the agar by use of a sterile glass rod bent to 90°.

After 36-48 hours incubation at 28°C plates containing 20-200 colonies are chosen and the colonies counted by normal techniques and reported as colony forming units per milliliter.

A direct count of microbes (both dead and viable cells) is determined by the epi-illuminated fluorescent microscope technique developed by Hobbie, et al. (12).

RESULTS

Experiments are conducted with either one or two AFR's operated as

a control (no treatment) while the remaining AFR's are operated with treatment (Photox Process, UV alone, or H_2O_2 alone). The only variation in operating conditions is a change in H_2O_2 concentration (1 to 5 mg/ℓ).

Torque Measurements

Torque (N-cm) measured on the rotating AFR shaft is used to indicate change in frictional resistance caused by the formation of biofilm. Figure 4 shows the increase in torque from an experiment using a H_2O_2 concentration of 5 mg/ℓ. In this experiment, one control is used and the remaining AFR's are treated with UV alone, H_2O_2 alone, and the Photox Process. The torque increase follows a typical pattern seen in biofouling studies. Initially an induction period occurs where little or no growth is present, followed by a growth phase where logarithmic increase is evident. After the growth phase, a plateau is reached where no more growth occurs and, in fact, some sloughing may occur (9).

Results show both H_2O_2 alone and the Photox Process to be effective in minimizing biofouling as expressed by change in torque. The Photox Process shows less fouling than H_2O_2 alone, however, the difference is not dramatic. UV alone has little effect on preventing biofouling as expressed by torque change. In this case, the induction period for the AFR with UV alone is less than the control AFR.

Figure 5 compares time-smoothed data from control experiments to time-smoothed data of the H_2O_2 alone and Photox Process when H_2O_2 concentration is 5 mg/ℓ. Data is time-smoothed and then averaged for each 10 hour time interval. The Photox Process is shown to be effective in preventing biofouling as indicated by torque increase of less than 0.08 N-cm. H_2O_2 alone is also effective in reducing biofouling but not to the extent of the Photox Process. H_2O_2 alone results in torque readings of up to 0.40 N-cm. The control AFR shows a plateau torque of 2.3 N-cm.

Figure 6 compares data of the control AFR to data of the H_2O_2 alone and Photox Process when 1 mg/ℓ H_2O_2 is used. At this H_2O_2 concentration, treatment effectiveness is reduced. Plateau torque for the H_2O_2 alone is 2.2 N-cm which is close to the plateau of 2.3 N-cm for the control. The Photox Process shows a small effect at reducing biofouling with a plateau torque of 1.7 N-cm.

Figure 7 compares time-smoothed data for control experiments to time-smoothed data for treatment with UV alone. Results show almost no significant difference betweeen treatment with UV alone and no treatment.

Mass Measurements

Mass on the removable slides is measured at the end of each experiment. Figure 8 summarizes these measurements for experiments where a plateau torque is reached. Mass is averaged for each experimental condition. The following observations are noted:

- The Photox Process with 5 mg/ℓ H_2O_2 is the most effective treatment. Little or no mass is measured at the end of an experiment.
- H_2O_2 alone at 5 mg/ℓ is less effective than the Photox Process

but still shows some activity.

° At H_2O_2 levels of 1 mg/ℓ treatment effectiveness is greatly reduced. H_2O_2 alone at 1 mg/ℓ is completely ineffective.

° UV treatment alone reduces production of biomass even though torque measurements shows no difference between the control and UV.

Microbial Concentrations

Microbial concentrations are measured to determine the effectiveness of the Photox Process as a biocide. Table 3 presents data comparing viable and direct counts in water before and after treatment by the Photox Process. The results show viable counts are reduced three orders of magnitude from 2.2×10^5 organisms per ml to 1.5×10^2 organisms per ml. Direct counts show the actual number of organisms (both dead and viable cells) to be the same (approximately 4×10^6 organisms/ml before and after Photox Process treatment.

Visual Observations

During experiments conducted with H_2O_2 concentrations of 5 mg/ℓ the AFR with the Photox Process treatment maintains clarity and is "squeaky" clean when dismantled. Furthermore, there is no long filaments attached to the brackets holding the removable slides. Conversely, the AFR treated with H_2O_2 alone appears cloudy and has a "slimy" surface when dismantled. Long filaments are observed to attach to the slide brackets. The control and UV treatments are observed to have more attached film than either the Photox Process treatment or treatment with H_2O_2 alone.

DISCUSSION

These experiments have been conducted as a preliminary analysis of the effectiveness of the Photox Process as a method for controlling biofouling.

Limitations of Reported Results

Several limitations must be considered when attempting to apply these results to biofouling in the field:

° The microbial inoculum for all laboratory experiments is composed of a variety of microbial species. Experimental conditions can favor the dominance of one or a few species and, thus, microbial population diversity could differ between experiments. Furthermore, the treatment may be effective for one type of microorganism and not another. Microbial population diversity in the field will be site-specific.

° Oxidant demand (organics or reduced inorganics) in the process water influence the effectiveness of the Photox Process. Therefore, concentration of hydrogen peroxide required may be site-specific.

° The UV chambers used in this project result in an increased retention time for the water supply entering the AFR's. The two reactors receiving UV treatment had a water supply which was held in the UV chambers for approximately 10 minutes. This means the water supply treated with the Photox Process had 10 minutes greater exposure to H_2O_2 than the reactor treated with

H_2O_2 alone.

Effectiveness of the Photox Process as a Biocide

All methods of observing biofouling in the AFR's (torque, mass and visual observations) are in agreement and indicate the following:

- ° Treatment with the Photox Process at 5 mg/ℓ is effective in preventing biofouling in this system. At 1 mg/ℓ the effectiveness is substantially reduced. A "breakpoint" concentration for maximum efficiency of H_2O_2 application should be somewhere between 1 and 5 mg/ℓ H_2O_2.
- ° Treatment with H_2O_2 alone at 5 mg/ℓ is effective in reducing biofouling but not to the extent of the Photox Process. The difference between H_2O_2 alone and the Photox Process is not dramatic in these experiments. A more in-depth study might show a greater difference in effectiveness between the Photox Process and H_2O_2 alone near the Photox Process "breakpoint" concentration of H_2O_2.
- ° Treatment with 1 mg/ℓ H_2O_2 alone shows little or no effectiveness in reducing biofouling.

Microbial cell enumeration is limited to only one experiment and compares both viable and direct counts of organisms before entering the Photox Process (control) to organism counts after the Photox Process. The results show the effectiveness of the Photox Process as a biocide by reducing the viable count by three orders of magnitude. The direct count shows the concentration of organisms whether viable or dead and these results show no difference in direct counts after Photox Process treatment; this indicates no substantial oxidation of the organisms is occuring but they are being killed. Further work is necessary to determine the effectiveness of UV alone or H_2O_2 alone as biocides.

Effectiveness of Treatment With UV Alone

Results show little or no effectiveness in reducing biofouling as indicated by torque measurements. Conversely, mass measurements show UV is effective in reducing the plateau mass to approximately half of the control value. A plausible explanation for the apparent contradiction might be that UV is effective in reducing only microorganisms that do not cause frictional resistance (torque increase). Observations have shown filamentious microorganisms result in increased frictional resistance whereas non-filamentious microorganisms do not (9). Therefore, the effectiveness of UV in reducing plateau mass may be due to the elimination of non-filamentious microorganisms.

Comparison of the Photox Process to Chlorine Treatment

Previous work using the AFR system to evaluate the effectiveness of chlorine treatment allows a comparison with the Photox Process. As indicated by Table 4, experimental conditions varied . The chlorine experiments were conducted with 16 mg/ℓ trypticase soy broth and 4 mg/ℓ glucose which provided a richer environment for biofilm growth. Furthermore, the input microbial concentration differed. All other conditions were alike (i.e. AFR residence time, rotational speed and temperature). These results indicate the Photox Process at 5 mg/ℓ H_2O_2 is as effective in preventing biomass accumulation as chlorine at 2.4 mg/ℓ or lower

("breakpoint" for chlorine dosage has not been determined).

Clearly, further work is necessary in comparing the effectiveness of chlorine and the Photox Process as treatments for biofouling. An important criteria is the degree to which the Photox Process can oxidize organics, particularly "refractory" organics without creating hazardous compounds. Another consideration in such a comparison is the cost of one treatment versus another.

CONCLUSIONS

The work presented is preliminary and the number of experiments are few. However, for this experimental system, the study shows the Photox Process to be a promising treatment for the control of biofouling.

The following can be concluded from this investigation for the experimental system used:

1. The Photox Process, which uses hydrogen peroxide addition prior to ultraviolet irradiation, can be an effective treatment for the prevention of biofouling.

2. The Photox Process is more effective as a biofouling treatment than hydrogen peroxide alone at the same concentration as used in the Photox Process.

3. The Photox Process is effective in preventing biofouling and the effectiveness is dependent on the hydrogen peroxide concentration. Further work is expected to show a "breakpoint" peroxide concentration between 1 and 5 mg/ℓ.

4. Ultraviolet irradiation alone at 2,537Å has no effect on reducing the effects of frictional resistance caused by biofouling (torque increasing on the AFR rotating shaft). However, the UV is effective in reducing plateau biomass accumulation.

5. The Photox Process is effective in killing substantial numbers of microorganisms. However, the process does not oxidize the dead organisms.

6. H_2O_2 alone is more effective at controlling biofouling than UV alone.

RECOMMENDATIONS

The following is recommended for the results of this study:

1. Examine the influence of H_2O_2 concentration on the effectiveness of the Photox Process.

2. Examine the influence of UV intensity on the effectiveness of the Photox Process.

3. Perform experiments with more defined, relevant mixed microbial populations.

4. Compare the effectiveness of H_2O_2 addition before UV irradiation to H_2O_2 addition after UV irradiation.

5. Compare the effectiveness of the Photox Process to other biofouling control methods.

6. Determine the effectiveness of the Photox Process in oxidizing organics, particularly the refractory organics.

7. Further testing should include a more intense analytical effort on terms of microbial and chemical parameters.

ACKNOWLEDGEMENTS

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Table 1
Oxidation Potentials

Species	Potential
Fluorine	3.06
Hydroxyl radical	2.80
Ozone	2.07
Hydrogen peroxide	1.77
Permangante	1.67
Chlorine dioxide	1.50
Hypochlorous acid	1.49
Chlorine	1.36

TABLE 2

Operating Parameters

AFR Rotational Speed	200 rpm
AFR volume	660 ml
Residence Time in AFR	10 min
Dilution Water Temperature	25°C
Nutrient Feed to Chemostat	200 mg/ℓ glucose
	25 mg/ℓ trypticase soy broth
	plus micronutrients
Residence Time in Chemostat	8 hours
Microbial Cell Concentration Entering AFR	Approximately 10^5 viable cells per ml after dilution
Glucose Concentration Entering AFR	5 mg/ℓ after dilution
Hydrogen Peroxide	1 to 5 mg/ℓ after dilution

TABLE 3

Microbial Cell Concentrations Before and After Treatment by the Photox Process (H_2O_2 at 5 mg/ℓ)

	Viable Plate Counts (CFU/ml)	Direct Counts (cells/ml)
Before Treatment	2.2×10^5	4.0×10^6
After Treatment	1.5×10^2	4.4×10^6

TABLE 4

Comparison of the Photox Process to Other Biofouling Treatments

Treatment	AFR Input Nutrient Concentration	Treatment Concentration	Plateau Mass
	(mg/ℓ)	(mg/ℓ)	(g/m^2)
Photox Process	5 glucose	5 (H_2O_2)	$\leq$ 0.05
Chlorine	16 trypticase Soy Broth plus 4 glucose	5	0
	16 trypticase Soy Broth plus 4 glucose	2.5	0

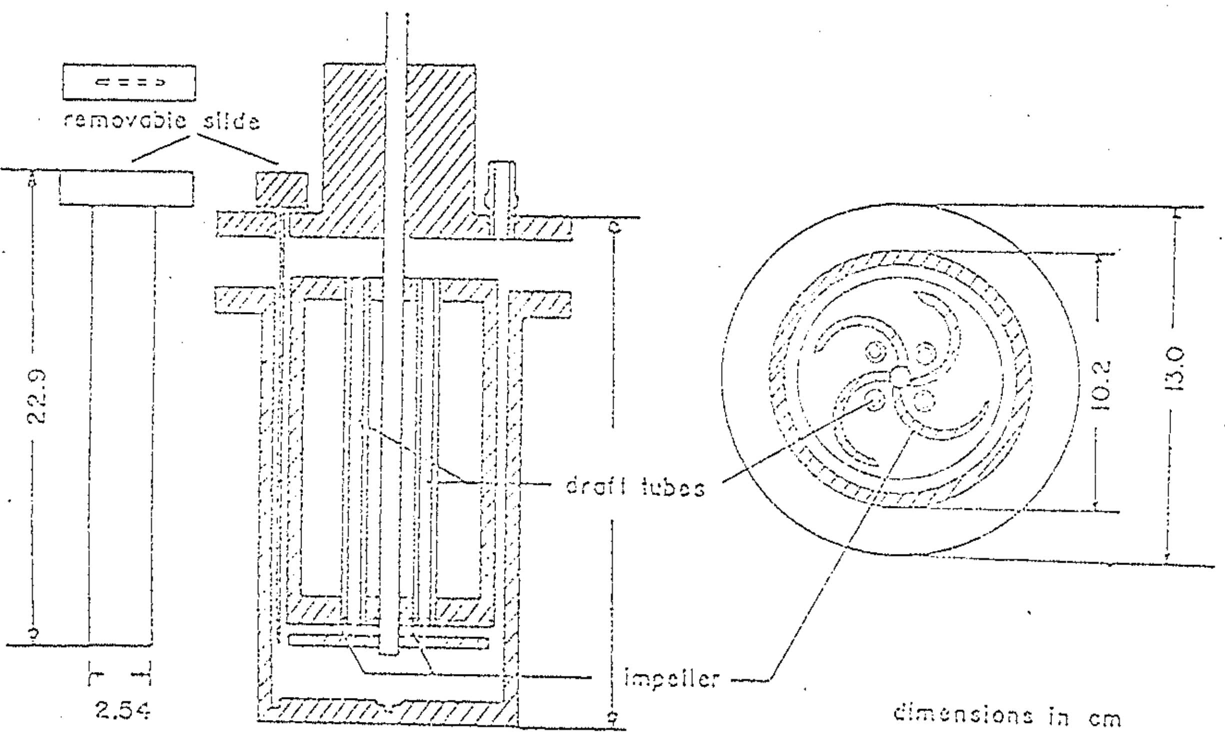

Figure 1. Annular Fouling Reactor

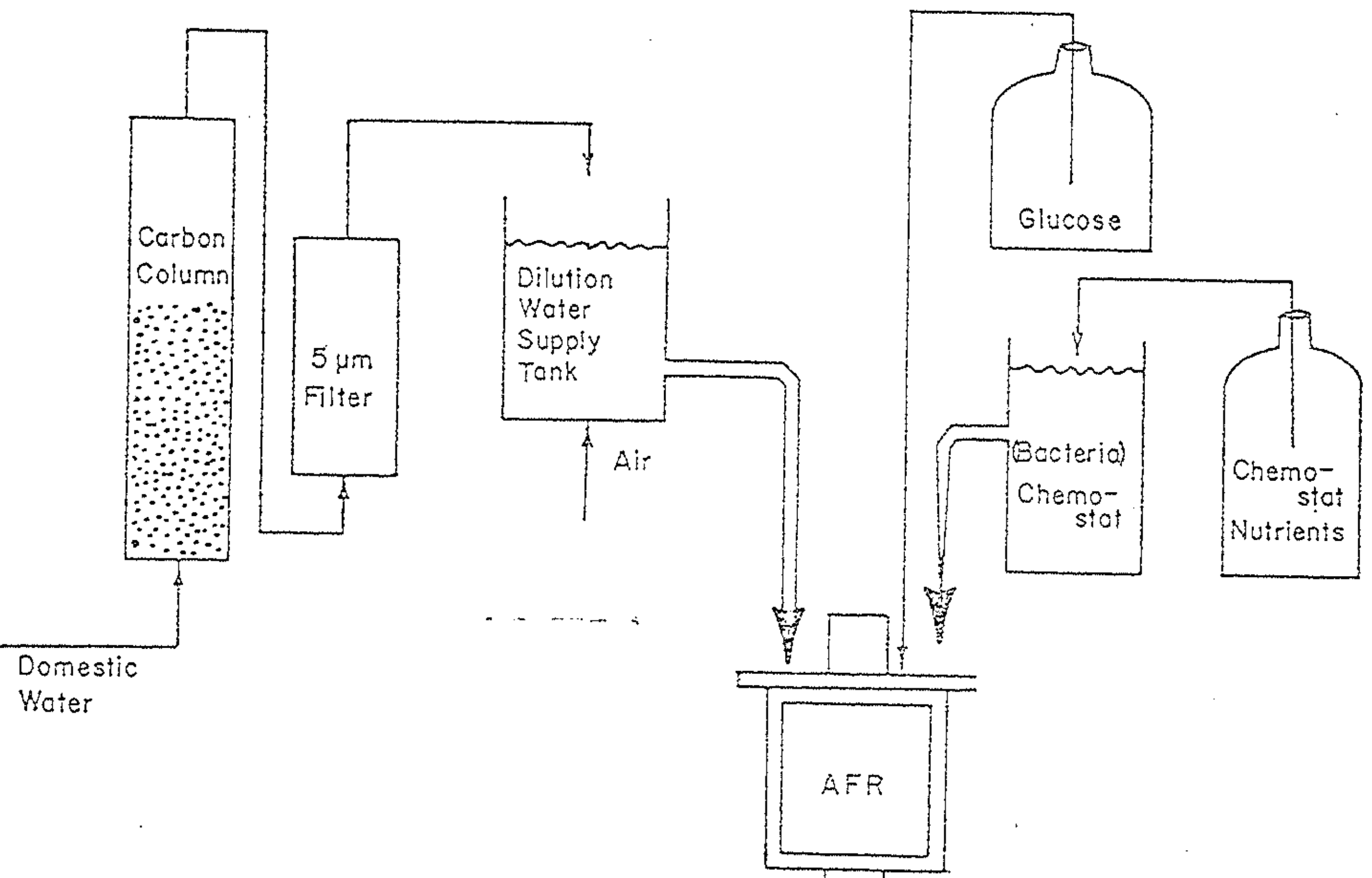

Figure 2. Schematic of Water Supply System

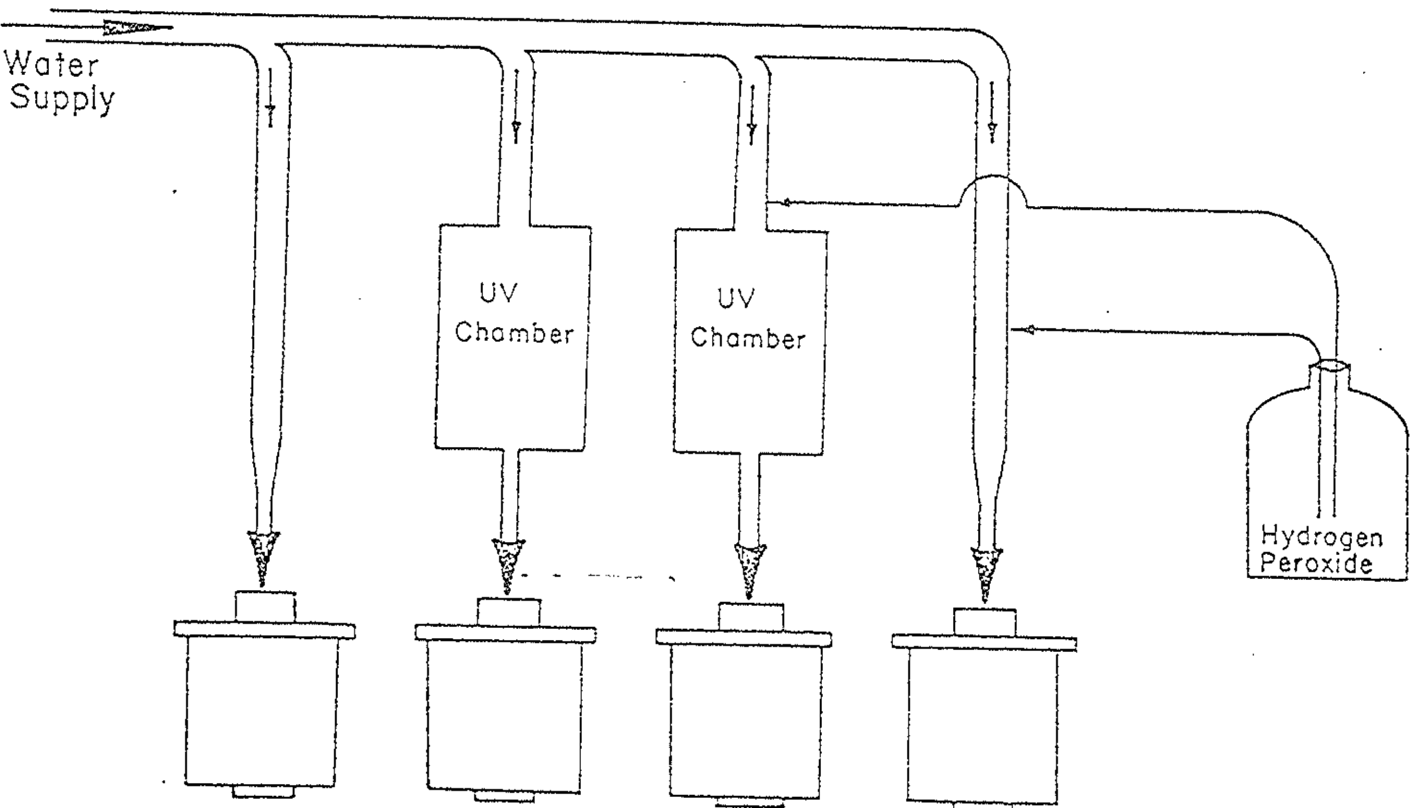

Figure 3. Schematic of Experimental Design

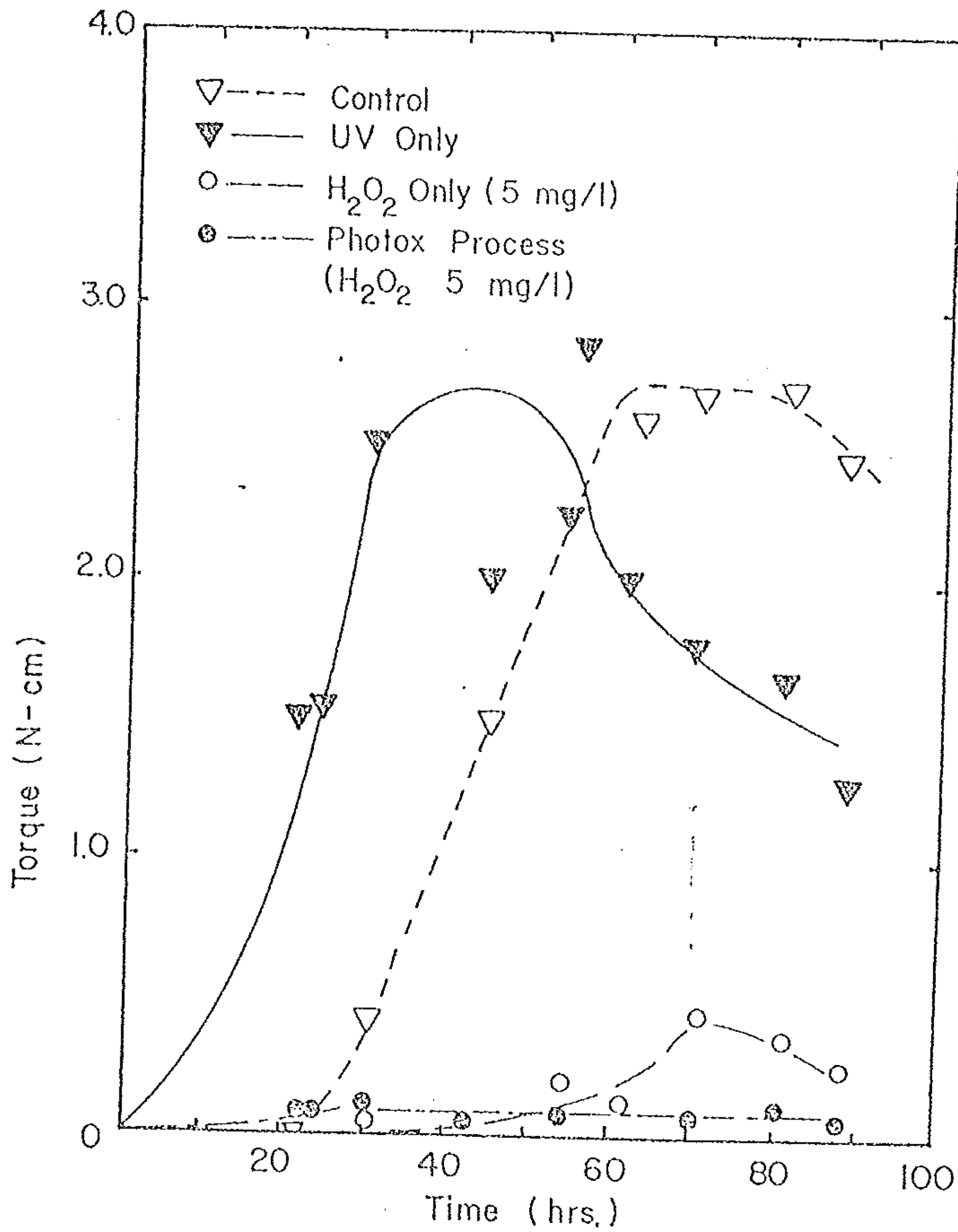

Figure 4. Torque measurements in an experiment using H_2O_2 concentration of 5 mg/ℓ in the AFR's treated by the Photox Process and by H_2O_2 alone.

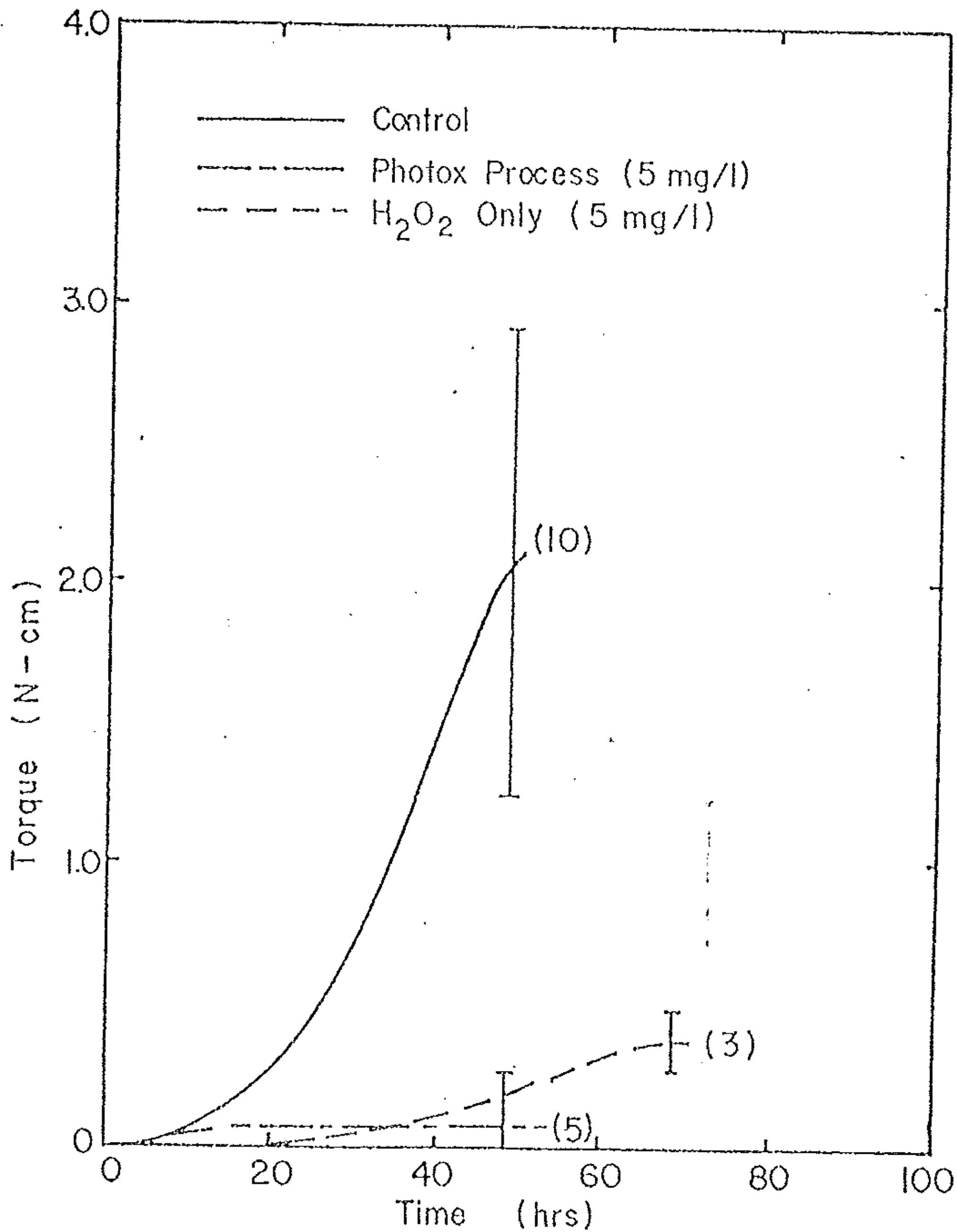

Figure 5. Torque measurements for a series of experiments comparing the control AFR to treatment by the Photox Process and treatment by H_2O_2 alone when H_2O_2 concentration is 5 mg/ℓ. The curves represent data averaged from time-smoothed plots. The numbers in parentheses refer to the number of experiments averaged. The confidence bars indicate one standard deviation.

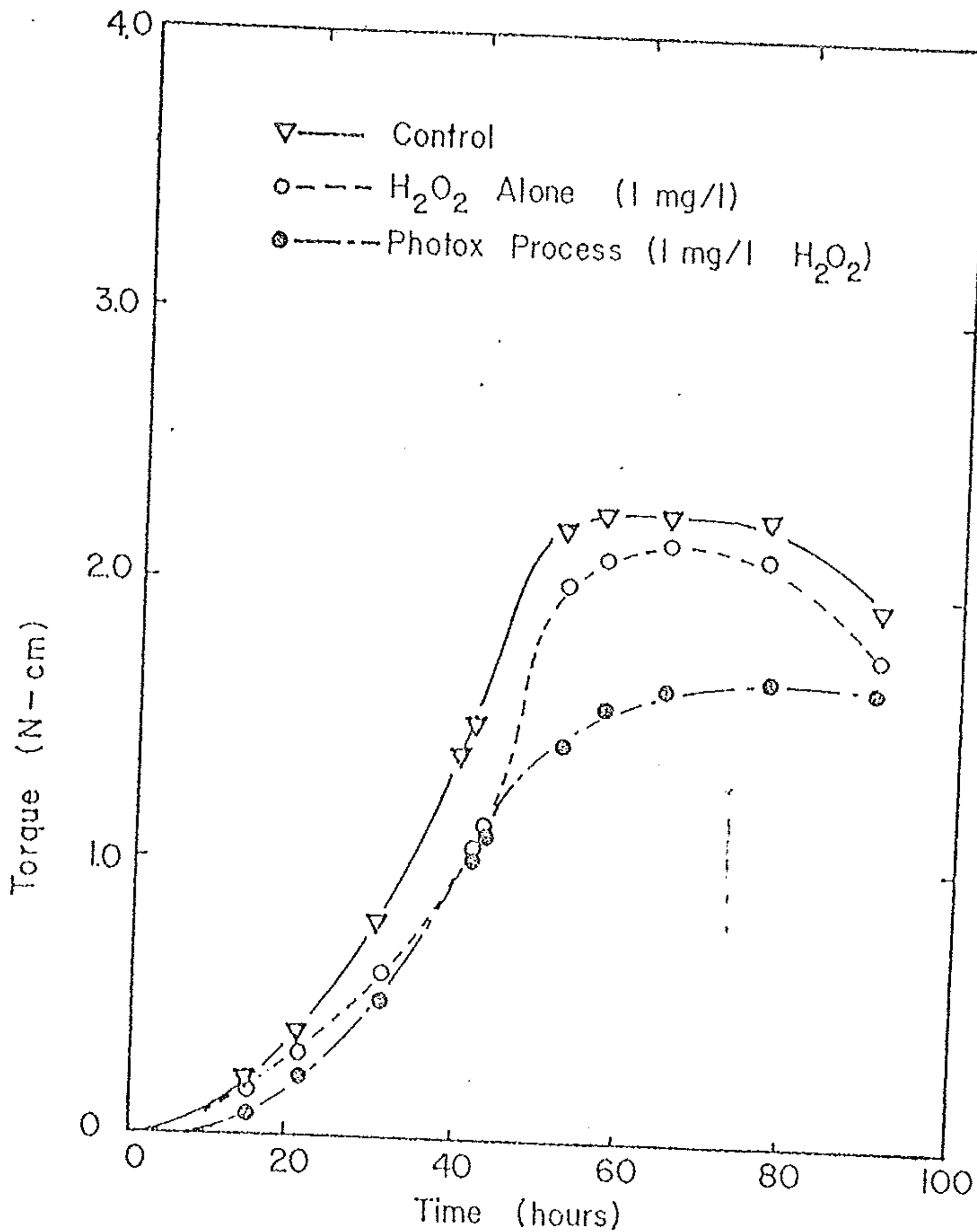

Figure 8. Torque measurements in an experiment using H_2O_2 concentration of 1 mg/ℓ in the AFR's treated by the Photox Process and by H_2O_2 alone.

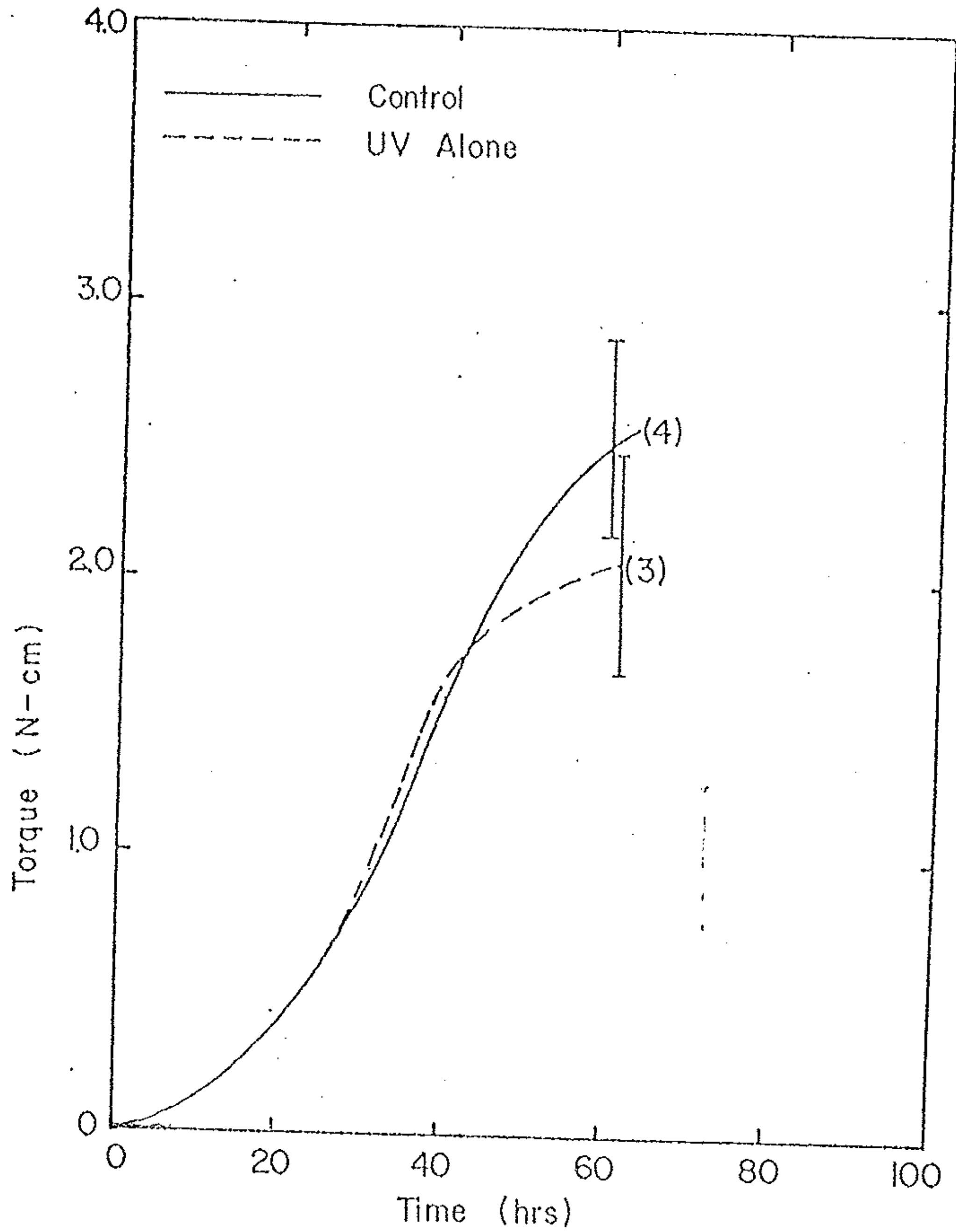

Figure 7. Torque measurements for a series of experiments comparing the control AFR to treatment by UV alone. The curves represent data averaged from time-smoothed plots. The numbers in parantheses refer to the number of experiments averaged. The confidence bars indicate one standard deviation.