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Mutagens in Wastewaters Renovated by Advanced Wastewater Treatment

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As clean water resources become scarce and the need to protect our environment becomes urgent, wastewater reclamation will be increasingly practiced. Advanced Wastewater Treatment (AWT) processes are used following conventional treatment, not only to protect certain lakes and rivers but also to produce high quality water for reuse. Reuse applications of wastewater include well established uses such as irrigation, formation of recreational lakes, industrial uses and others which are only beginning to be considered such as ground water recharge, and direct domestic reuse. Unintentional direct reuse of wastewater occurs widely at the present time via consumption of drinking waters drawn from polluted rivers and lakes.

Prior to considering reuse of renovated wastewaters for potable purposes, the potential hazards from the consumption of renovated wastewaters must be assessed. Wastewaters contain many hazardous chemicals for which currently no criteria exist. The advanced wastewater treatment technology available today leaves many potentially hazardous chemicals unchanged and in some instances introduces new and potentially toxic chemicals (SHUVAL and GRUENER 1973, GLAZE and HENDERSON 1975). Further complications arise from the fact that residual or introduced trace pollutants vary from one situation to the next depending upon the nature of the industrial waste which enters the sewage system.

In view of the growing recognition that the majority of human cancers are due to the presence of chemicals in the environment, the concern about the presence of carcinogenic compounds in renovated wastewaters is justified. The present study was undertaken to detect chemical carcinogens/mutagens in municipal wastewaters and determine their removal and/or formation during wastewater renovation by various advanced treatment processes, using histidine requiring mutants of Salmonella typhimurium (AMES 1975). The microbial assay has been demonstrated to be highly efficient in detecting potential human carcinogens as mutagens (McCANN et al. 1975, McCANN and AMES 1976).

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EXPERIMENTAL

Advanced Wastewater Treatment Plants and Sampling Details

Principal AWT processes currently in use fall into three categories: biological, physical-chemical and land applications. Three AWT plants located in the northeast representing different treatment processes were selected for this study. Samples studied from each plant included influent for AWT (secondary effluent from conventional treatment process), effluent after partial advanced treatment (in some cases) and final AWT effluent. The details of the selected treatment plants and sampling points are given in Table 1. Although the Lake George village sewage treatment plant does not receive any industrial effluents and hence the presence of mutagens in wastewaters would be unlikely, a rationale for its inclusion was to examine the possibility of microbial synthesis of mutagens during land infiltration of wastewaters. Synthesis of strong mutagens by microbial action has been noted in other environments (ALEXANDER 1974).

Twenty-four hour composite samples were collected from each plant with the help of an ISCO automatic composite sampler. The rate of sample collection was maintained at approximately 250 ml every 30 minutes. Final effluent samples from the rapid sand filtration system at Lake George were obtained from two different depths with the help of porous cup lysimeters installed at 25 ft and 60 ft depths in the sand beds and maintained under negative pressure. The samples were withdrawn from the lysimeters by application of positive pressure with a pump.

All samples were kept in wet ice during the compositing period and transportation. In view of the generally recognized quality fluctuations of the influent wastewaters, samples were collected from each plant at two dates several weeks apart. Samples were sterilized by serial filtration with 1200 nm, 450 nm and finally 200 nm membrane filters (Millipore Corp.), for use in the bioassay.

Mutagenicity Assay

Ames Salmonella typhimurium assay employing strain TA100 and 1535 as base-pair substitution mutants and strain TA98 as frame shift mutant (AMES 1975) was used in these studies. To facilitate detection of low concentrations of mutagens suspected in wastewaters, the bioassays were performed by preparing the assay media (base agar layer) in the filter sterilized wastewaters under investigation. Thus, suspected mutagens would be present in the bottom agar whereas bacteria and mammalian activation systems (where added) would be in the agar overlay. The ability of the assay to detect mutagens when present in bottom agar was confirmed using known mutagens. This modification of the assay permitted incorporation of up to 20 ml wastewater/assay (70% v/v). Metabolic

TABLE 1

Details of the Advanced Wastewater Treatment (AWT) systems studied.

AWT Plant	Treatment Processes	Sampling Points	Dates of Sampling
Model Advanced Wastewater Treatment Plant, Piscataway, MD	Lime addition, Recarbonation, Duel media filtration, Breakpoint chlorination, Activated Carbon Adsorption	1. Secondary effluent from conventional treatment process (AWT influent) 2 Before breakpoint chlorination (AWT Midpoint) 3. After carbon adsorption (AWT final)	4-24-77 and 7-6-77
Bay Park Wastewater Renovation Plant, East Rockaway, LI	Biological denitrification column*; Alum addition, Sand filter, Activated carbon adsorption, Chlorination.	1. Secondary effluent from conventional treatment process (AWT influent) 2. After chlorination (AWT effluent)	8-31-77 and 10-12-77
Lake George Village Sewage Treatment Plant Lake George, NY	Filtration through natural delta sand beds**	1. Unchlorinated secondary effluent from conventional treatment process (AWT effluent) 2. At two depths in the sand bed: 25 ft and 60 ft.	10-9-77 and 1-3-78

* JERIS and OWENS (1975)

** AULENBACH et al. (1975, 1976)

activation of the mutagens was achieved by incorporating rat liver homogenate in the assay. The procedure used for preparation of the liver homogenate was the same as described by AMES et al. (1975).

Mutagenicity assays on various samples collected from one AWT plant at a particular date were carried out at the same time together with distilled-demineralized water control for spontaneous reversion rate and positive mutagenesis controls. The results expressed are averages of triplicate determinations.

Wastewaters were assayed for the presence of histidine using an enzymatic assay which involved conversion of histidine to uraconic acid by ℓ-histidine ammonia lyase (E.C. 4.3.1.3) and its measurement by increase in absorbance at 277 nm (HASSALL 1971).

RESULTS AND DISCUSSION

Secondary as well as AWT effluents from Bay Park and Piscataway AWT Plants showed significant mutagenesis in the base pair substitution mutants TA100 and 1535 of S. typhimurium (Fig. 1,2,3,4). The mutagens responsible for the effect were detoxified to a large extent by the presence of mammalian liver enzymes in the assay. These wastewaters failed to revert the frame shift mutant TA98 but exhibited strong toxicity towards this strain, decreasing the spontaneous reversion rate as much as three-fold with some samples. Our inability to detect frame shift mutagens in the wastewaters may be due to high sensitivity of this strain towards non-mutagenic toxicants in wastewaters. Decreasing the volume of wastewaters in the assay relieved the inhibition without inducing mutagenic response. Influent and AWT effluent samples from the rapid sand filtration process at Lake George failed to cause mutagenesis in the Salmonella strains tested but showed an inhibitory effect on the spontaneous reversion rate of TA98. Enzymatic hydrolysis by β-glucuronidase of mutagens possibly present in wastewaters as conjugates (COMMONER et al. 1974), did not affect the level of mutagenesis observed in any of the samples studied.

In view of the low level of mutagenic activity, it became important to determine if histidine may be present in wastewaters and responsible for the increased number of revertants. Studies carried out in our laboratory showed that the presence of histidine beyond the trace levels required in the assay (AMES 1975) causes increase in the number of spontaneous revertants which may be confused with a mutagenic response. Assay of the wastewaters for histidine by specific enzymatic method having a detection limit of 1 μg/ml revealed that histidine was not present in measurable amounts. In addition, wastewaters spiked with $\leq$ 1 μg/ml histidine failed to exhibit any significant influence on the spontaneous reversion rate. Thus wastewater-induced increase in the reversion rate was true mutagenesis and not due to histidine.

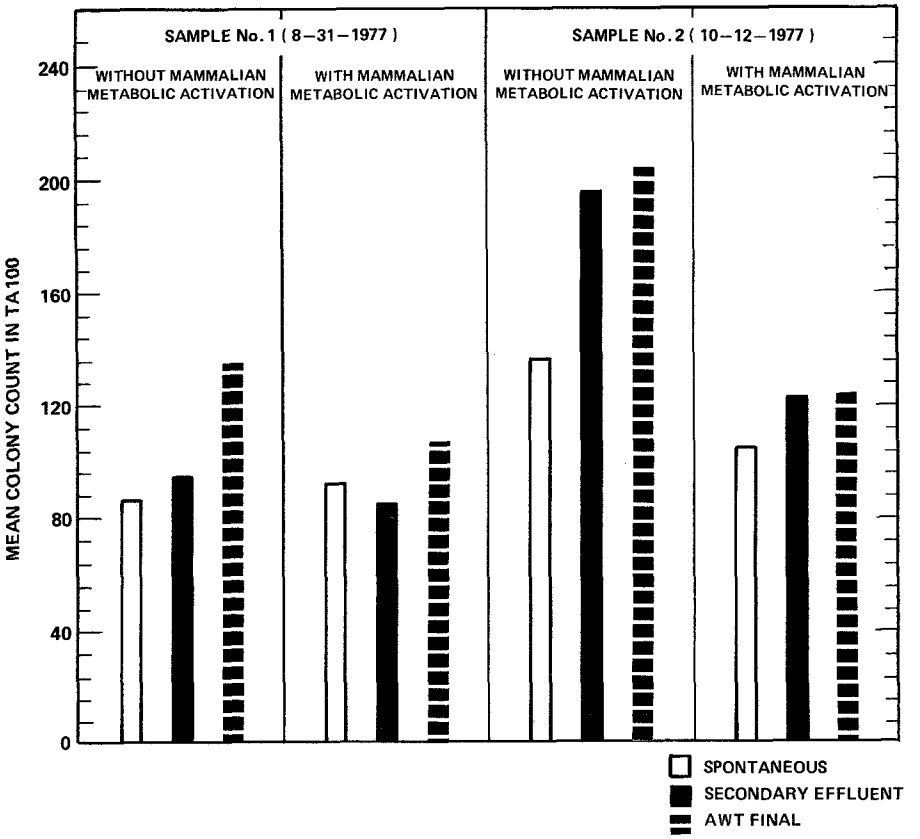

Figure 1. Mutagenic response of the Wastewaters from Bay Park Wastewater Renovation Plant, in Salmonella Strain TA100.

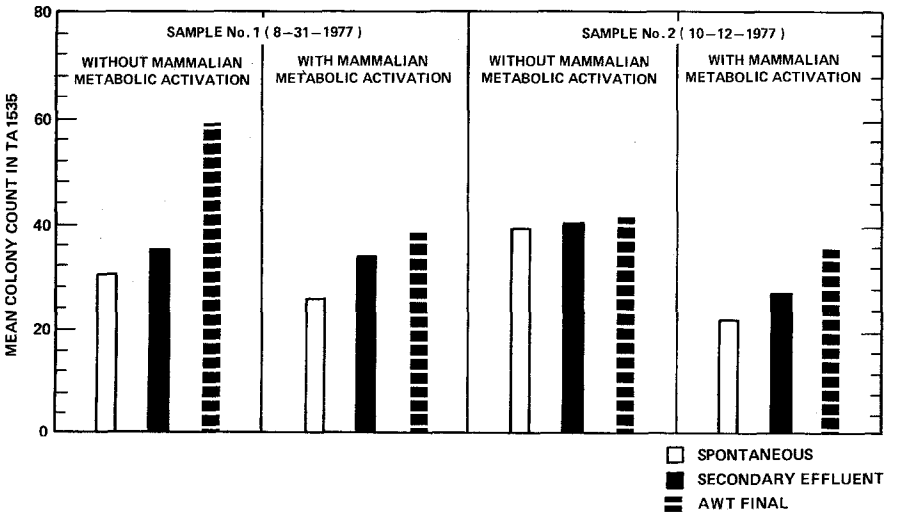

Figure 2. Mutagenic response of the Wastewaters from Bay Park Wastewater Renovation Plant, in Salmonella Strain TA1535.

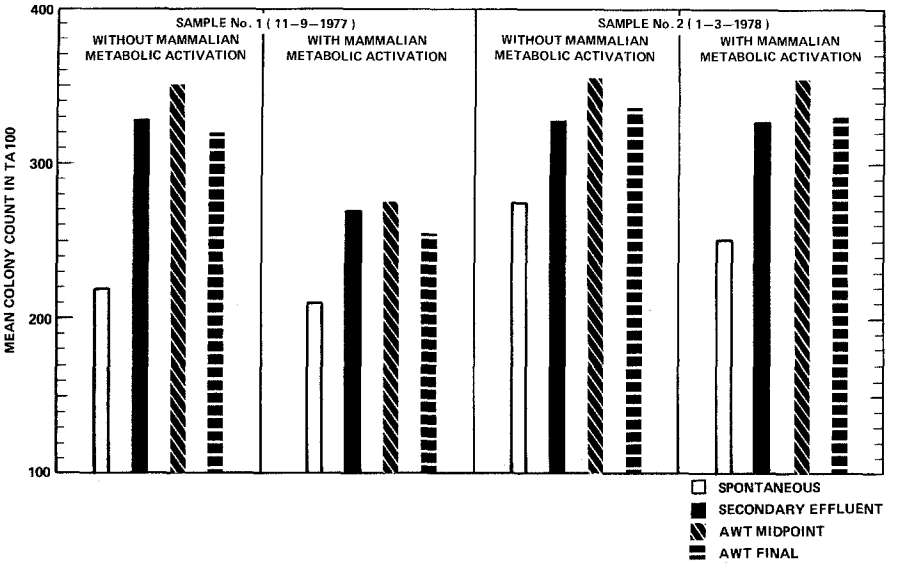

Figure 3. Mutagenic response of the Wastewaters from Piscataway Model AWT Plant, in Salmonella Strain TA100.

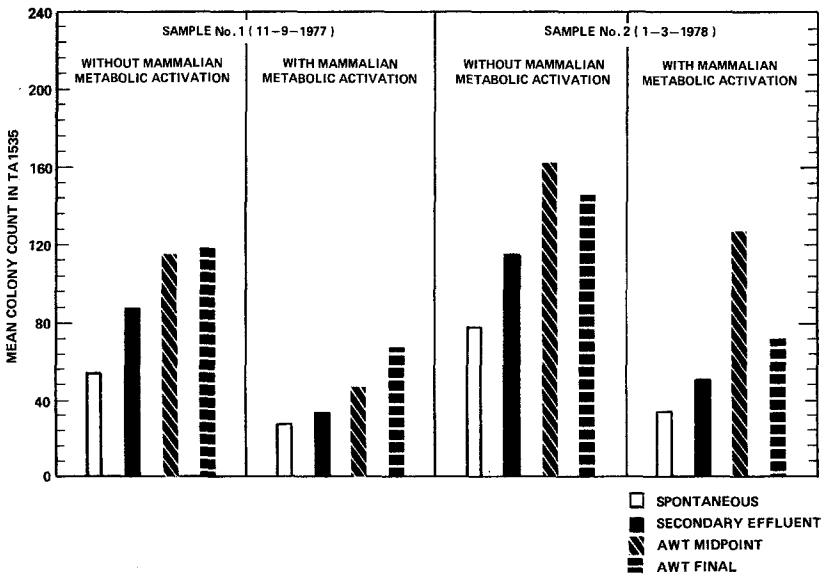

Figure 4. Mutagenic response of the Wastewaters from Piscataway Model AWT Plant, in Salmonella Strain TA1535.

Comparison of the mutagenic response of AWT influent and effluent samples revealed that the AWT methods employed at Bay Park and Piscataway not only failed to remove certain mutagenic substances which are present in the influents, but were also capable of introducing new mutagens. The formation of mutagens during treatment was particularly clear from the mutagenicity data obtained on Bay Park samples collected on 8-31-77. On the other hand, due to the relatively high mutagenic response of the influent, the data obtained on 10-21-77 samples depicts better the inability of the treatment process to remove mutagens. The inability to detect increased mutagenesis in AWT effluents over influent wastewaters with some samples may reflect the absence of mutagen precursors in those influents.

The mutagenicity data on the Piscataway AWT Plant was especially interesting because of the inclusion of an additional sampling point at mid point in AWT. The data from these studies showed that lime addition or recarbonation was capable of promoting synthesis of mutagens some of which were direct acting whereas others required metabolic activation. The mutagens formed in these treatment processes were partially removed during breakpoint chlorination and/or activated carbon adsorption. Overall, however, the mutagen concentration in the final effluent was no less than that present in the influent wastewaters and may represent the threshold mutagen concentration for these treatment methods.

The absence of mutagenic response in the Lake George secondary effluents following prolonged contact with the natural delta sand beds could be interpreted to mean that microbial synthesis of mutagens does not occur during wastewater renovation by this process. Alternatively, suitable mutagen precursors and/or cofactors needed for the synthesis of mutagens may be absent from the secondary effluents. The data does not reveal anything concerning the efficiency of the process in removing carcinogenic/mutagenic chemicals because of the absence of such compounds in the wastewaters subjected to sand filteration.

The work presented here shows that the physico-chemical and biological AWT methods studied are unable to adequately remove potentially hazardous chemicals from wastewaters and that reuse of renovated wastewaters for potable purposes may present a hazard to health.

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