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Effect of Simultaneous Ozone and Activated Carbon Treatment on 1,2-Dihydroxybenzene Genotoxic Effects

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Ozone is one of the most powerful oxidants and could readily destroy highly toxic aromatic organic compounds, usually found in industrial wastewaters (Rice 1997). However, toxic by-products may be generated as a result of incomplete oxidation (Bourbigot et al. 1986; Adams and Randtke 1992). Recently, ozone oxidation combined with activated carbon adsorption has been identified as a suitable treatment option (McKay and McLeavey 1988; Zaror 1997). Toxic chemicals may be effectively removed by adsorption on activated carbon. Moreover, dissolved ozone may react with both dissolved organic contaminants and adsorbed species, increasing the extent of oxidation, and eventually, reducing genotoxic activity (Bourbigot et al. 1986).

Unfortunately, there is little data on the effectiveness of mutagenic activity removal by these treatment systems, which provides the main incentive for this work. The objective of this study was to assess the effect of ozone oxidation and activated carbon adsorption on the toxicity of 1,2-dihydroxybenzene in aqueous solutions. 1,2-dihydrohybenzene (CAS number 120-80-9) has been identified as a by-product of ozonation from a wide range of toxic phenolic compounds, and is said to be responsible for the mutagenic activity found in many effluents from chemical plants. Aromatic hydrocarbons present in the environment may be metabolised to quinones which are thought to be cytotoxic and carcinogenic to man, due to direct interaction with DNA. Bioreactivity of polyhydroxylated benzenes and their molecular mechanisms of toxic action are widely reported in the literature (O'Brien 1991). Those compounds may be toxic to cells by a number of mechanisms including redox cycling, arylation, intercelation, induction of DNA strand breaks, generation of site-specific free radicals, and interference with mitochondrial respiration (Smith et al. 1985; Kari 1989). In cultured cells, quinone induce DNA damage, such as the induction of single strand breaks, by several mechanisms, possibly involving active oxygen species (Coleman et al. 1989). It is important to mention that those events leading to DNA damage or some other perturbation of cellular function may have major deleterious consequences for cells and organisms (Terrence et al. 1992).

The potential presence of genotoxic activity was evaluated using samples taken during the course of treatment with dissolved ozone and combined ozone/activated carbon by the Ames test using *Salmonella Typhimurium* TA-98 and TA- 100.

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Induction of mutation in bacteria is a very sensitive indirect indicator of DNA damage. The theory that carcinogenesis involves a somatic mutation has led to the use of mutagenicity test as a prescreen for the detection of potential chemical carcinogens.

MATERIALS AND METHODS

Ozonation experiments were carried out in a 1.5 L Pyrex reactor, connected to an Ozocav OEC240X ozone generator, with rated capacity 6 (mmol $O_3 min^{-1}$) from pure oxygen. Mixing was provided by mechanical stirring at 240 rpm. The ozone gas mixture was fed into the reactor through a sintered glass sparger, located 3 cm beneath the stirring blades. Exhaust residual ozone gas was bubbled through an iodine solution, 1 (mol dm^{-3}), at pH 12 to secure total ozone destruction before final release to the environment. A HCl/KCl buffer was used to keep pH 2 during ozonation. Test chemicals were synthesis grade, purchased from Merck Ltd.

Bituminous-based Filtrasorb 400 (Calgon Carbon Corp., Pittsburgh, PA) was used in this study. This carbon featured a specific BET surface area and a micropore volume of 845 (m^2/g), 0.394 (cm^3/g), respectively. Ozone concentration in aqueous solution was determined by the Indigo method proposed by Bader and Hoigne (1981).

Typically, 1 L aqueous buffered solution was fed into the reactor, together with 0.02 kg activated carbon, a 20°C and pH 2. Then, 6 (L min^{-1}) O_3/O_2 gas mixture was bubbled into the reactor at a constant rate, and 20 mL liquid samples were taken at 6 and 20 min. This experiment was conducted in the absence of activated carbon in the reactor. Each run was conducted in triplicate and composite samples were prepared for mutagenicity analyses.

Mutagenicity analyses were conducted using *Salmonella typhimurium* microsomal test, according to the method described by Ames et al. (1975), and modified by Maron and Ames *(1983).* *Salmonella typhimurium* TA-98 and TA-100 strains were employed; these were kindly supplied by Dr. P. Sanchez, (CETEBS, Sao Paulo, Brazil). All tester strains were routinely checked for confirmation of genotypes. Concurrent tests for spontaneous reversion, positive control reversion, and tester strain viability were performed with each experiment. Viability counts averaged 9.0 x 10^8 colony forming units (CFU)/ mL.

All assays were performed with and without metabolic activation, using an exogenous metabolic system consisting of a tissue homogenate. A 9000 x g rat liver supernatant (S9), provided by Molecular Toxicology Inc (Moltox, Boone, NC), was used as the tissue homogenate. All assays were conducted in duplicate plates per dose level. A minimum of five doses per sample were tested.

Mutagenicity was expressed in terms of the number of revertants per litre. The sample Mutagenicity Ratio (MR) is estimated as:

$$MR = \frac{\text{Number of revertants in the sample}}{\text{Number of spontaneous natural revertants}}$$

A positive response was considered when MR≥ 2, and a clear dose-response trend over three or more doses. A sample was classified as negative if neither criterion was satisfied, and marginal if only one criterion was met.

RESULTS AND DISCUSSION

Results from Ames tests are shown in Tables l-5. These tables show that all samples, including the untreated 20 mM 1,2-dihydroxybenzene aqueous solution, presented a negative mutagenic response when TA98 strain was used. This strain respond to frameshift mutation.

When TA98 strain was assayed with metabolic activation (ie., in the presence of S9 liver homogenate), no mutagenic response was observed. The S9 extract is an exogenous metabolic system of mammalian cells. Certain chemicals (pro-carcinogens/pro-mutagens) are not biologically effective unless they are converted to their active form by mammalian metabolic enzymes. Addition of mammalian microsomal enzymes (S9 fraction) in the bacterial assay, increases its scope for the detection of such pro-carcinogens/pro-mutagens.

Table 1. Ames test results. Initial untreated 1,2-hydroxybenzene aqueous solution, 2,2000 (mg/l), pH 2, 20°C

	Mutagenicity Ratio(*)			
Dose (mL)	TA-98	TA-98 + S9	TA-100	TA-100 + S9
0.2	1.1 (34)	0.3 (8)	2.4 (236)	1.9 (194)
0.5	1.1 (32)	0.1 (4)	2.4 (240)	2.1 (206)
1.0	0.8 (24)	0.2 (7)	2.8 (284)	2.1 (208)
1.5	1.1 (32)	0.3 (10)	2.1 (210)	2.4 (240)
2.0	3.1 (93)	0.3 (10)	1.9 (189)	2.8 (278)
Control	(30)	(30)	(100)	(100)

(*) Number of revertants /plate shown in parenthesis

However, as seen in Table 1, the untreated 1,2-dihydroxybenzene sample shows a clear mutagenic response when TA100 strain was used. This strain detects base pair substitution. This is a clear indication that 1,2-dihydroxybenzene mutagenic action takes place by a base pair substitution mechanism. Similar results were obtained when TA100 was cultured in the presence of S9 rat liver extract, confirming the presence of a direct-acting mutagen. This is in agreement with literature reports which show that quinonic and other related aromatic compounds are mutagenic to Salmonella TA104 strain (Chesis et al. 1984).

Table 2. Ames test results. 1,2-hydroxybenzene aqueous solution, treated with Ozone for 6 min, at pH 2 and 20°C.

	Mutagenicity Ratio(*)			
Dose (mL)	TA-98	TA-98 + S9	TA-100	TA-100 + S9
0.2	0.5 (15)	0.5 (15)	1.8 (176)	1.8 (182)
0.5	0.8 (24)	0.7 (21)	1.1 (110)	1.5 (154)
1.0	0.2 (5)	0.7 (20)	1.9 (188)	1.6 (156)
1.5	0.0 (0)	0.0 (0)	1.4 (140)	1.2 (196)
2.0	0.0 (0)	0.0 (0)	1.4 (140)	1.3 (103)
Control	(30)	(30)	(100)	100

Table 3. Ames test results. 12-dihydroxybenzene aqueous solution, treated with Ozone in the Presence of Activated Carbon, for 6 min, at pH 2 and 20°C.

	Mutagenicity Ratio (*)			
Dose (mL)	TA-98	TA-98 + S9	TA-100	TA-100 + S9
0.2	0.5 (14)	0. 8 (24)	1.9 (185)	1.6 (164)
0.5	1.5 (46)	0.6 (19)	1.7 (168)	1.7 (165)
1.0	0.5 (15)	0.8 (23)	1.1 (111)	1.8 (182)
1.5	0.1 (2)	0.5 (15)	0.8 (78)	1.9 (190)
2.0	0.1 (2)	0.9 (28)	1.1 (106)	2.0 (198)
Control	(30)	(30)	(100)	(100)

(*) Number of revertants /plate shown in parenthesis

Tables 2 and 3 show that both ozone and combined ozone/activated carbon treatment, effectively eliminate the mutagenic activity presented by hydroxybenzene. Indeed, in all cases, the number of mutagen revertants was less than twofold the number of natural revertants. A 6 min exposure period enables total destruction of hydroxybenzene, leading to the destruction of the aromatic ring, and formation of C_6- C_4 oxidised species, mostly carboxylic, aldehydes and ketones. Moreover, residual dissolved ozone in treated samples was negligible, since all absorbed ozone was readily consumed in those oxidation reactions. However, when treatment is extended for longer periods, viz. 20 min, most organic compounds are in the form of more stable oxidised C_3 and C_2 molecules. Reactions between ozone and these species are very slow, and residual dissolved ozone rises to about 0.06 mM, increasing the solution toxicity. This effect is illustrated by results shown in Tables 4 and 5, where positive mutagenic response is presented by both T100 and T100/S9. When ozone was allowed to decompose to negligible levels, these samples showed no mutagenic activity. Ozone self-decomposition in aqueous solution generates oxygen free radicals which may produce mutagenic activity (Chesis et al. 1984).

Table 4. Ames test results. 1,2-diydroxybenzene aqueous solution, treated with Ozone for 20 min, at pH 2 and 20°C.

	Mutagenicity Ratio (*)			
Dose (mL)	TA-98	TA-98 + S9	TA-100	TA-100 + S9
0.2	0.9 (27)	1.0 (30)	2.1 (212)	1.9 (187)
0.5	0.3 (10)	0.8 (25)	1.0 (110)	2.1 (214)
1.0	0.3 (8)	0.8 (24)	3.6 (362)	1.6 (156)
1.5	0.6 (18)	0.5 (15)	1.7 (174)	2.5 (252)
2.0	0.1 (2)	0.1 (3)	0.9 (850)	3.0 (304)
Control	(30)	(30)	(100)	(100)

Table 5. Ames test results. 1,2-dihydroxybenzene aqueous solution treated with Ozone in the Presence of Activated Carbon, for 20 min, at pH 2 and 20°C.

	Mutagenicity Ratio (*)			
Dose (mL)	TA-98	TA-98 + S9	TA-100	TA-100 + S9
0.2	0.5 (14)	0.8 (24)	2.0 (196)	2.0 (204)
0.5	0.1 (3)	0.9 (28)	1.8 (178)	2.1 (210)
1.0	0.1 (3)	0.7 (22)	2.6 (262)	1.8 (177)
1.5	0.2 (7)	1.0 (30)	2.1 (205)	1.7 (171)
2.0	0.1 (3)	0.2 (6)	2.2 (223)	1.8 (178)
Control	(30)	(30)	(100)	(100)

(*) Number of revertants /plate shown in parenthesis

It is interesting to note that, in most cases, the number of TA98 revertants cultured in samples treated with ozone, and ozone combined with activated carbon, was much lower than the number of spontaneous natural revertants. This would demonstrate an acute toxic effect on *Salmonella typhimurium* TA98. However, TA100 strain viability was not affected by the treated samples.

Table 6. Ames test summary results. Mutagenic response of 1,2-dihydroxybenzene samples treated with Ozone (O_3) and Activated Carbon (AC). pH 2, 20°C

Treatment	TA-98	TA-98 + S9	TA-100	TA-100 + S9
Untreated	–	–	+	+
6 min. O_3	–	–	–	–
6 min. O_3/AC	–	–	–	–
20 min. O_3	–	–	+	+
20 min. O_3/AC	–	–	+	+/–

Table 6 summarises qualitative mutagenicity results. Both untreated and treated samples show negative genotoxic effect when assayed with TA98 strain. On the other hand, positive effects were found when untreated and treated over 20 min. were assayed with TA100 strain. Similar results were found in the presence of S9 fraction. These results show that 1,2-dihydroxybenzene is a direct acting mutagen, which in turn show its direct interaction with DNA. This is in agreement with reports that quinone cytotoxicity is attributed to DNA modification (O'Brien 1991).

Results reported herein show that ozone treatment, even in the presence of activated carbon, may not be effective in removing mutagenic activity. Ozone rapidly destroys aromatic rings, and further oxidises the reactive organic by-products; however, at higher ozone doses, dissolved ozone concentration may rise to toxic levels. Therefore, the ozone treatment time is a key design parameter and should be carefully selected to secure toxicity removal from contaminated wastewaters.

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