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Reduced Toxicity of an Aqueous Coal-Conversion Effluent Following Waste Disposal Treatment

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Recent concern over the depletion of natural gas and oil has brought into focus other forms of energy, especially coal. In most cases, however, the direct burning of coal is not environmentally suitable; therefore, a significant interest in coal-conversion technology exists within both private industry and government. Since the aqueous effluent from a single commercial conversion plant may range from 0.4 to 1.2 million gallons per day and contain hundreds or thousands of different compounds (HERBES et al. 1976), it is essential to examine such effluents as potential environmental hazards.

One of the more promising coal-conversion schemes is the Solvent Refined Coal (SRC) process which can produce fuel low in sulfur and ash from coals high in these substances. The SRC process is outlined in detail in reports by PERRUSSEL et al. (1975), SCHMID (1974), PETERSEN (1975), PETERSEN et al. (1976), and HUFFMAN (1976).

Chemical analyses of the untreated waste process-liquid stream from the SRC plant (Fort Lewis, Washington) show that it has a high pH and contains high concentrations of sulfur, phenol, and cresols in addition to process "naphtha" (PETERSEN 1975). This waste process-liquid stream, however, is fed through a waste-water-oil disposal system which includes a bio-reactor unit and sand and carbon filters. The liquid waste effluent from this water-oil disposal system has a nearly neutral pH and a greatly reduced sulfur and phenol content (PETERSEN et al. 1976).

The purpose of the present report is to examine the biological effects of the liquid effluent stream from the "oxycontact" bio-reactor unit on a model

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system — the ciliate Tetrahymena pyriformis. This model system has been used to examine the toxicity of untreated process streams and pure compounds from a variety of coal-conversion processes (SCHULTZ et al. 1977, 1978; SCHULTZ and DUMONT 1977).

MATERIALS AND METHODS

General

The organism used in these studies, the ciliate Tetrahymena pyriformis, was selected because of its wide distribution in freshwater ecosystems and because it is representative of ciliate populations in general. It is very well characterized and possesses a metabolism very similar to that of higher eukaryotes (ELLIOTT 1973); in addition, it provides a rapid and inexpensive bioassay system. Tetrahymena pyriformis, strain GL-C, were reared in axenic cultures in a semidefined proteose peptone medium (SCHULTZ and DUMONT 1977). Cultures were grown without shaking in a 28°C water bath. Stationary-growth-phase cultures (3 to 4 days old, 90,000 cells/ml) were used throughout the investigation. The treated process-water tested (provided by the Pittsburg and Midway Coal Mining Co., Fort Lewis, Washington) was collected following chemical and biological treatment and sand filtration in the waste-water—oil disposal system of the SRC pilot plant. A small sample from the carbon filtration unit was also examined. For more details on the treatment process see PERRUSSEL et al. (1975). Before they were tested, the process-water samples [the sand-filter stream (SFS) and the carbon-filter stream (CFS)] were subjected to Millipore filtration to remove any organisms which may have been present.

Behavior

The behavior of test cells was observed with a phase-contrast microscope. Specimen samples were obtained following the procedure described by TINGLE et al. (1973) [i.e., centrifugation, washing, and suspension in distilled water containing 20 mg/liter $CaCO_3$ (pH 7.4), which approximates soft water]. Cells were exposed to concentrations of 25, 50, 75, and 100% of the SFS or CFS, and their behavior was monitored for 24 hr. Cells suspended in 20 mg/liter $CaCO_3$ served as controls. In each case the general reaction and the appearance of the organisms were recorded, with particular attention being paid to changes in shape and motility at various times, and the percent of cells altered was estimated.

Oxygen Uptake

The effect of the SFS on the respiration rate of Tetrahymena was examined following the procedures of SCHULTZ et al. (1977) by use of a Gilson Respirometer (model GR-20) with the temperature stabilized at 28°C.

Reaction vessels were employed, each containing 4 ml of cell suspension. SFS (1 ml at a concentration 10 times that desired for final testing) was placed in the side arm of the reaction flask and later mixed with the sample. Experiments were performed with final SFS concentrations of 5, 10, 15, and 20%. Due to the small quantity of the CFS sample, respiratory measurements could not be made.

Population Growth and Density

The effects of SFS on growth rates and population densities of *Tetrahymena* were measured spectrophotometrically following the procedures of SCHULTZ et al. (1977) with optically matched glass culture tubes. Cells were reared at 28°C, and experiments were performed with final concentrations of 5, 10, 15, 20, and 25% SFS. Absorbance at 540 nm was used to estimate population density. The lack of an adequate quantity of the CFS sample precluded measurements of its effects on population growth.

RESULTS

Behavior

Few, if any, behavioral modifications are caused by concentrations of 20% or less of the SFS or CFS. However, gradual loss of motility, change in cell shape, and lysis are directly related to concentration over the range from 25 to 100%.

Although the general scheme of cell modification is a continuum, it may be subdivided into three phases: (1) the normal pear-shaped cells become posteriorly rounded; (2) the posteriorly rounded cells become spherical; (3) the spherical cells lyse (see SCHULTZ and DUMONT 1977). The scheme of morphological and motility alterations of test cell populations is presented in Table 1.

Oxygen Uptake

The oxygen uptake of *Tetrahymena* cultures exposed to SFS in concentrations as high as 20% is not significantly different from that of controls (Fig. 1). When cultures are exposed to higher concentrations, reduction in oxygen consumption is nonlinear and dose-dependent; the reduction in oxygen uptake correlates well with the percent of cell lysis observed in the behavioral studies.

Population Growth and Density

Population growth curves of axenic cultures of *Tetrahymena* exposed to SFS concentrations from 5 to 25% for 72 hr are presented in Fig. 2. The period of exponential growth was determined for each test concentration;

TABLE 1

Summary of Morphological and Motility Changes in *Tetrahymena* Exposed to SFS or CFS Effluent for 24 hr

Effluent concentration (%)	General appearance	Motility
25	occasional lysis 25% rounded or spherical 75% normal	slightly reduced
50	10% lysis 40% rounded or spherical 50% normal	moderately reduced
75	25% lysis 50% rounded or spherical 25% normal	moderately reduced
100	50% lysis 25% rounded or spherical 25% normal	markedly reduced

replicates were pooled, and the best line for the data, where Y is absorbance and X is time, was fitted by the least-squares method of linear regression. The mean growth rate (slope of the regression line) is not significantly affected ($P > 0.05$) by the concentrations of SFS tested.

DISCUSSION

Chemical constituents of the waste stream from the SRC process have been reasonably well defined (see PERRUSSEL et al. 1975; PETERSEN et al. 1976; PETERSEN 1975). Unfortunately, similar in-depth characterizations of SFS or CFS have not been made. It is known, however, that waste-water disposal treatment removes essentially all phenols and much of the sulfur and returns the pH to nearly neutral. Thus the SFS and CFS represent comparatively clean samples. Nevertheless, the question remains as to what compounds are responsible for the biological effects on the test organisms used in this study. Based on the report of SCHULTZ and DUMONT (1977), who showed that phenol is highly toxic to *Tetrahymena*, it is likely that the reduced toxicity of the samples can be attributed to the efficient removal of most phenols.

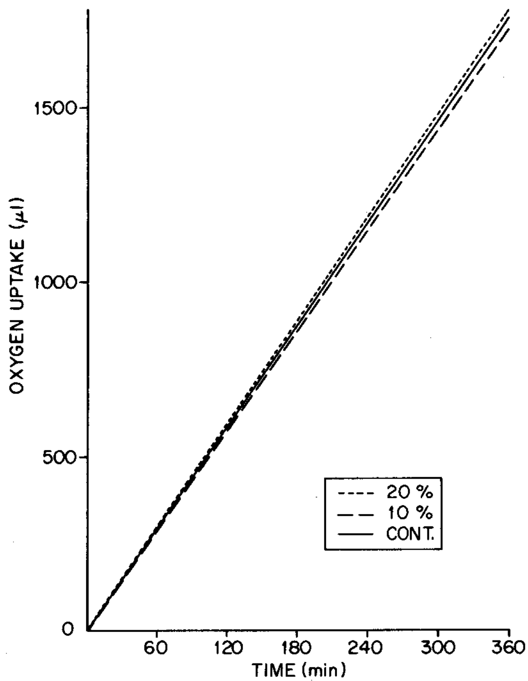

Figure 1. Effects of SFS on respiration of *Tetrahymena* at 28°C.

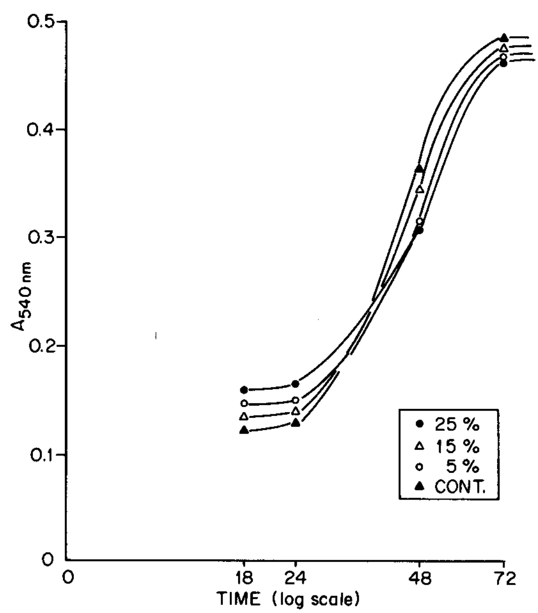

Figure 2. Effects of SFS on population growth of *Tetrahymena* at 28°C.

The effective removal of other compounds by the waste-water–oil disposal units depends, among other things, upon their molecular size, their degree of alkylation, and their substitution by heteroatoms, especially N and S, into multiring molecules (HERBES et al. 1976). The multiring compounds, in fact, appear to pass rather readily through the treatment process (HERBES et al. 1976; COOPER and CATCHPOLE 1973). Disregarding the phenols and monoaromatic hydrocarbons which are removed by waste treatment, the remaining major constituents in the effluent streams are the aromatic amines, thiophenes, and polyaromatic hydrocarbons (HERBES et al. 1976). On the basis of a comparative study of the toxicity of several classes of compounds (parent compounds as well as alkylated derivatives), it is likely that of these three major components, only the aromatic amines would be present in large enough concentrations (100–1000 mg/liter) to cause the biological effects on Tetrahymena reported here. The data of SCHULTZ et al. (1978) show that both analine and quinoline and their methyl- and dimethyl-substituted forms have toxicity ranges (500–2000 and 225–800 mg/liter, respectively) which are comparable to concentrations anticipated in treated effluent streams (HERBES et al. 1976). Whether or not these or other compounds are responsible for the toxicity of SFS and CFS must await a more detailed chemical analysis of these sample streams.

Another possibility is that the adverse effects of SFS or CFS are related to high concentrations of trace elements in the samples. Many trace elements are removed from the feed coal during the solvent-refining process, but it is not clear which of these (and what concentrations) are likely to be present in waste process water or in treated effluent (PETERSEN et al. 1976). Unfortunately trace-element analysis of treated waste water is unavailable. It is known that Tetrahymena are much more sensitive to lower concentrations of trace elements than to organic compounds (CARTER and CAMERON 1973).

Finally, although trace-element data are not available and direct comparison of untreated waste process water and SFS or CFS is not possible, it is clear that waste-water–oil disposal treatment is effective in removing many potentially toxic materials. Indirect comparison can be made, however, with gasifier condensates, which contain constituents similar to those present in SRC waste process-liquid streams (FORNEY et al. 1974; HO et al. 1976). For example, these condensates dramatically reduce respiration of test orgaisms at concentrations of 3% and significantly reduce population growth at concentrations as low as 0.4% (SCHULTZ et al. 1977). Yet SFS and CFS from the SRC process in concentrations as high as 20–25% have little effect on respiration or growth. Thus this report demonstrates that appropriate treatment of SRC waste process streams is capable of dramatically reducing toxicity, at least to one test organism.

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