DEGRADATION OF MIX HYDROCARBONS BY IMMOBILIZED CELLS OF MIX CULTURE USING A TRICKLE FLUIDIZED BED REACTOR

Final Report

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Summary

The microorganisms, capable of degrading mix hydrocarbons were isolated from the soil samples collected from the hydrocarbon contaminated sites. The mix cultures were identified as *Pseudomonas acidovorans, Flavobacterium indoltheticum* and *Phyllobacterium rubiaceum*. The bacterial cells of mix cultures were immobilized in calcium-alginate solution in the form of beads. A trickle fluidized bed air-uplift-type reactor designed to study the degradation of mix hydrocarbons was filled with 0.85% normal saline containing the immobilized cells of mix culture. The immobilized beads were aerated with different amounts of CO_2-free air. The normal saline saturated with BTXs was circulated in the bioreactors at the rate of 2-4 ml/min. The biodegradation of BTXs by the immobilized beads of mix culture was monitored by determining the concentrations of the BTXS and the metabolites formed during their degradation in the samples at regular intervals using GC. The peaks obtained through the degradation of BTXs were not identiified and quantified in this study.

Chemicals:

All chemicals of 99% purity, such as Benzene, Xylene, Toluene, Sodium Alginate etc., were obtained from Sigma Chemical Company, St. Louis, MO.

Isolation of Mix Cultures:

Different soil samples around industrial sites contaminated with hydrocarbons were collected, stored in polyethyelene bags and transported to the laboratory on ice. The mix cultures were isolated from the contaminated soil samples by enrichment culture techniques using the phosphate buffer medium (PBM), consisting of the following (g/L): K_2HPO_4, 4.3; KH_2PO_4, 3.4; $(NH_4)_2SO_4$, 2.0; Mg $Cl_2.H_2O$, 0.3; amended with 0.5 ml of a trace element solution containing (mg/L), $MnCl_2.4H_2O$, 1.0; $FeSO_4.7H_2O$, 0.6; $CaCl_2$), 2.6; and $NaMoO_4.2H_2O$, 6.0.

A 1:10 dilution of each sample was made with sterile PBM and the suspension was incubated at room temperature for 1 h. One ml of the suspension was then transferred into tubes, containing 9 ml of sterile PBM. Different concent-rations of hydrocarbons (BTXs) ranging from 2 mM -1000 mM were then added. The tubes were then incubated at 25°C. After 7 days of incubation, the tubes were examined for turbidity. A loopful of the turbid sample was also streaked onto PBM agar plates (1.5% Difco Agar) containing BTXs for the identification of mix cultures.

Identification of Mix Cultures:

The microorganisms isolated from the contaminated sites were identified according to the method of Biolog GN Microplate. The mix cultures were identified as *Pseudomonas*

acidovorans,*Flavobacterium indoltheticum* and *Phyllobacterium rubiaceum*.

Growth of Mix Cultures:

The mix cultures isolated from the contaminated soil samples were grown in large quantities in fermentor. The optimal temperature and pH for the growth of mix cultures were found to be 7.0 and 30°C respectively. The 72 h old bacterial cells were centrifuged at 10,000 x g for 10 min at 5°C. The pellet thus obtained were suspended in 0.85% normal saline.

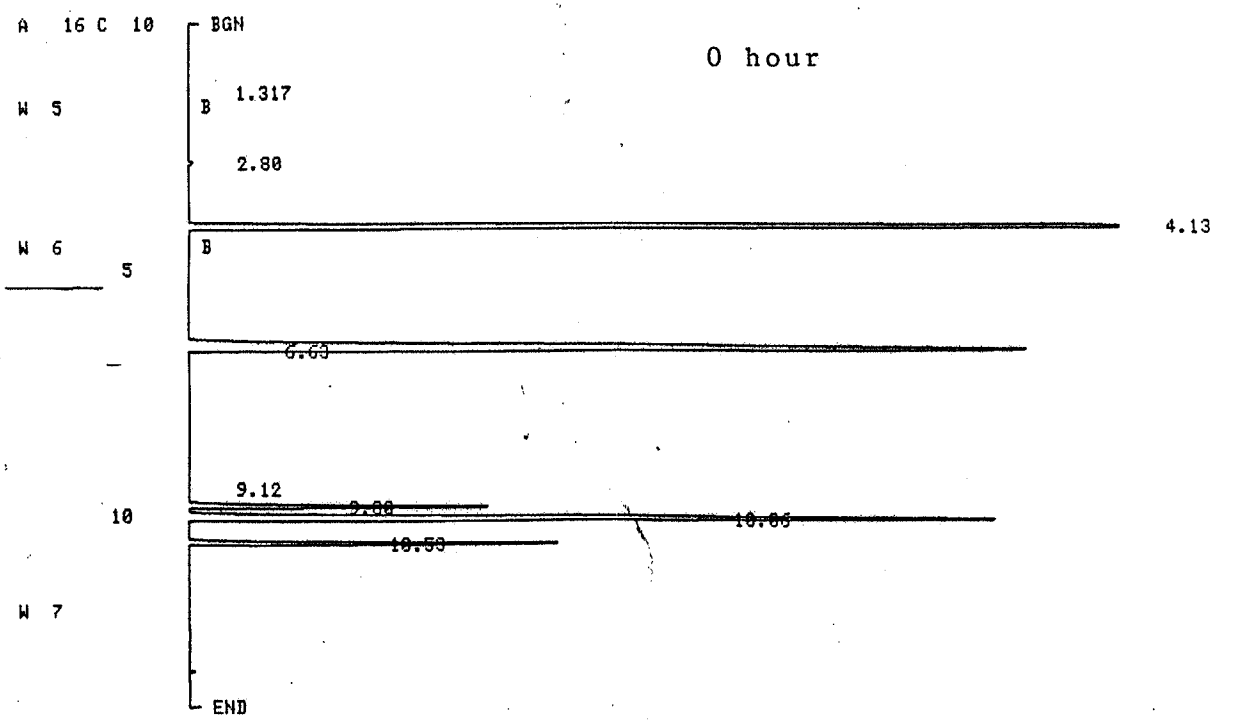
Immobilization of Mix Cultures in Sodium Alginate:

Equal volumes of cell slurry [200-400 mg/100 ml] and sterile 4% sodium alginate solution were mixed together. The alginate-cell mixture was added dropwise to cold 0.2 M $CaCl_2$ soltuion. Each drop was hardened into a bead containing entrapped cells of mix culture. The beads were allowed to harden further in $CaCl_2$ solution for 24 h at 5°C. The typical following immobilization of cells with alginate was about 0.6 - 0.8 g of beads per ml of the cell-alginate suspension. Individual beads had a diameter of 1-3 mm with an average wet and dry weight of 14 and 0.7 mg respectively. At the time of immobilization, each bead contained 1.5-2.5 x 10^8 viable cells as determined by pour plate count method of disrupted beads.

Degradation of Mix Hydrocarbons:

The degradation of mix hydrocarbons by the immobilized cells of mix cultures was monitored in the air-uplift-type reactor. The immobilized beads of mix culture present in the bioreactor were supplied with 0.85% normal saline saturated with BTXs at different flow rates of 2-4 ml per min. The immobilized cells were aerated with CO_2-free air at different

amounts of 50 and 100 mlml/min through the top of the reactor. The degradation of the mix hydrocarbons was monitored in the samples (5 ml) collected from aqueous sample port at regular intervals starting from 0 h. The samples were extracted with 5 ml of hexane and the concentrations of BTXS and their metabolites formed during their degradation by the immobilized beads of mix culture were determined by Gas Chromatography. The detection and injection temperatures were 275°C and 225°C. Hydrogen pressure was 14.0 PSIG. The oven temperature was 35°C and increased to 220°C at 15°C/min. The peaks thus obtained were not identified and quantified in the present study.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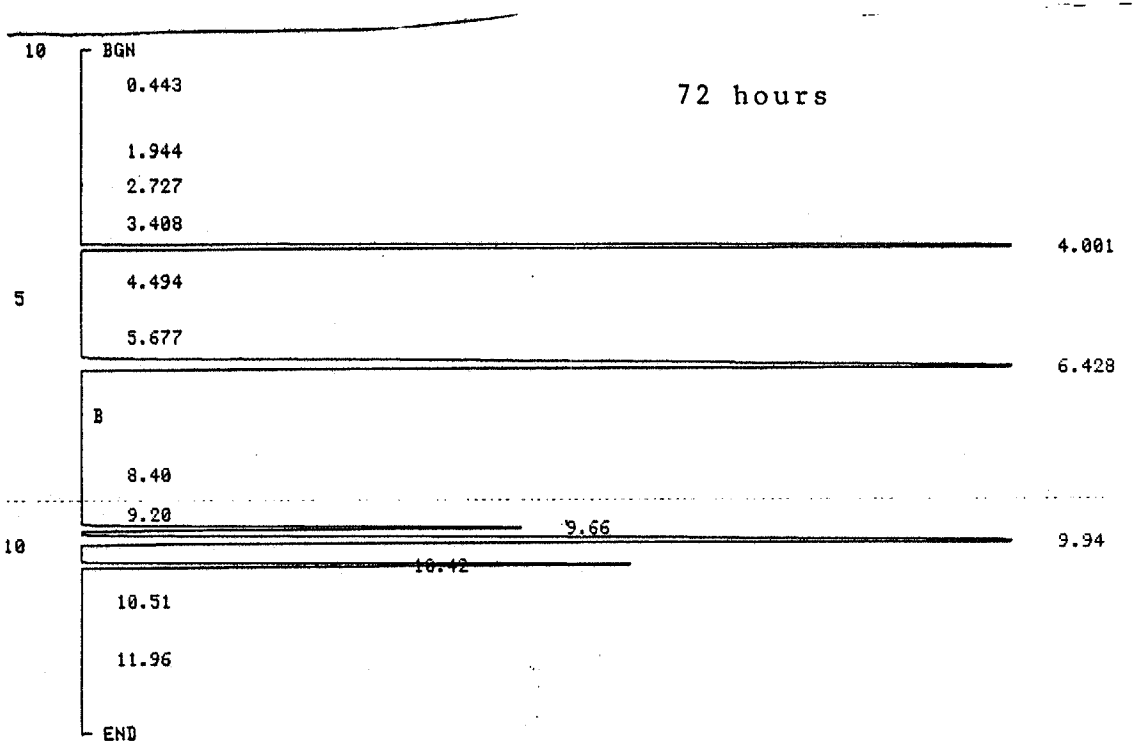
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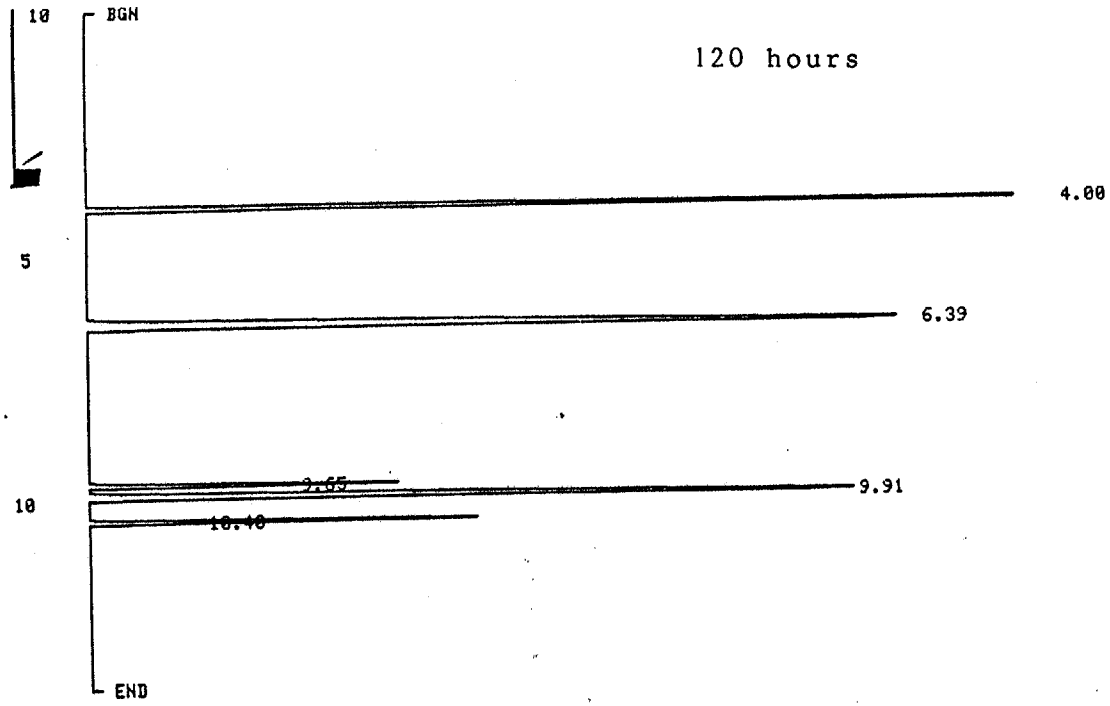

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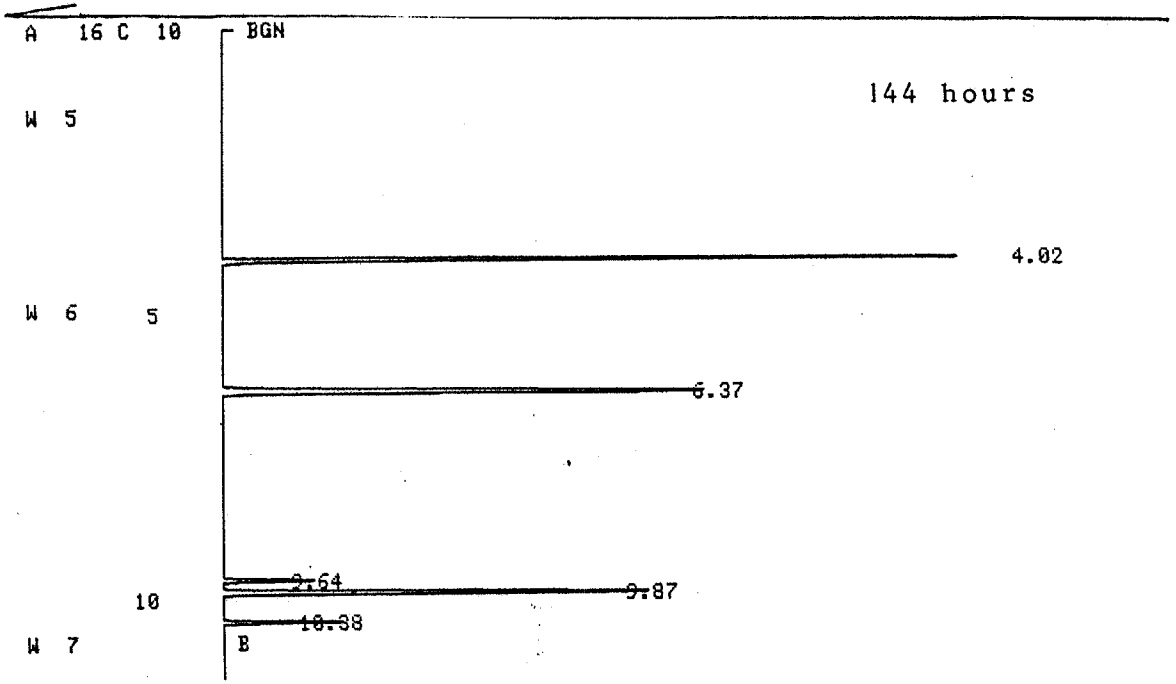
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10
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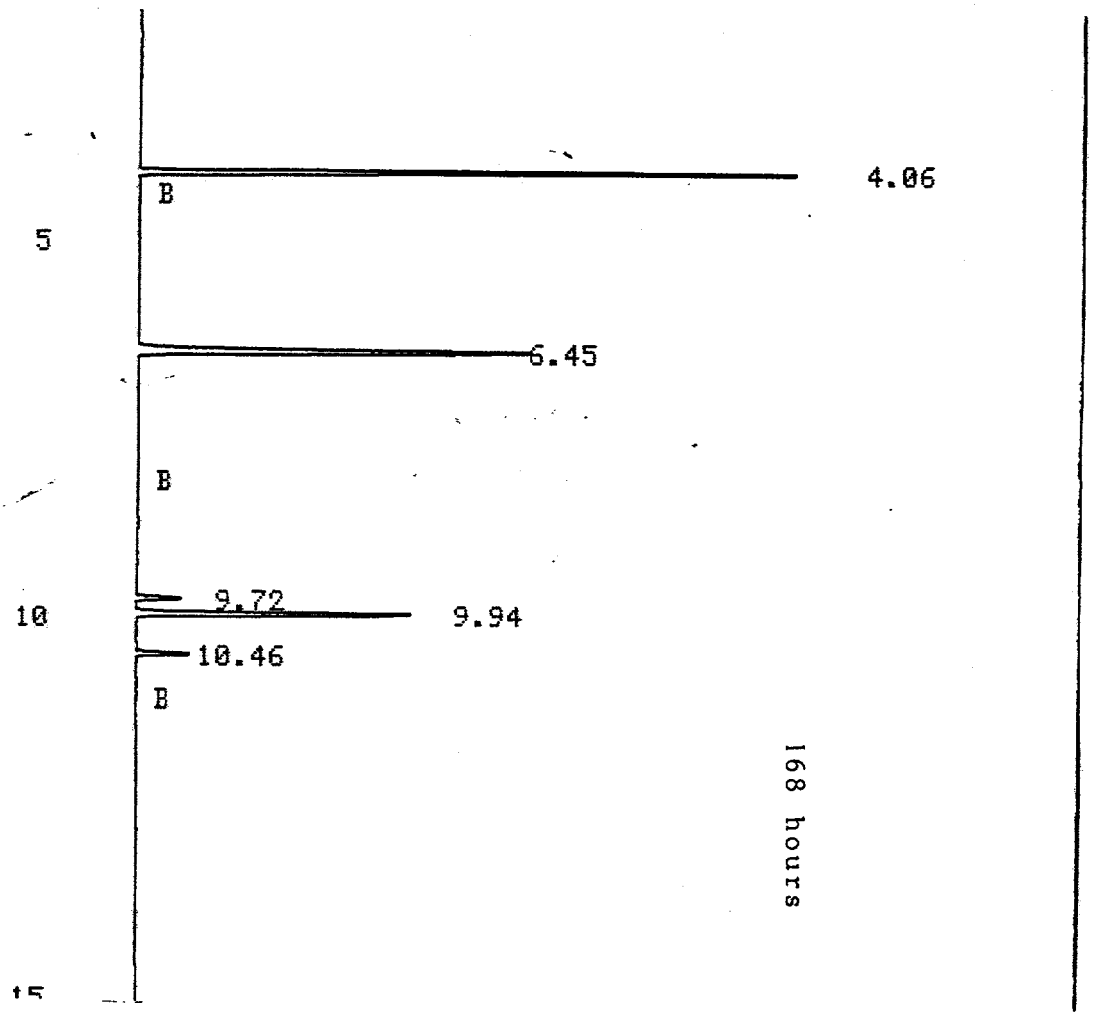
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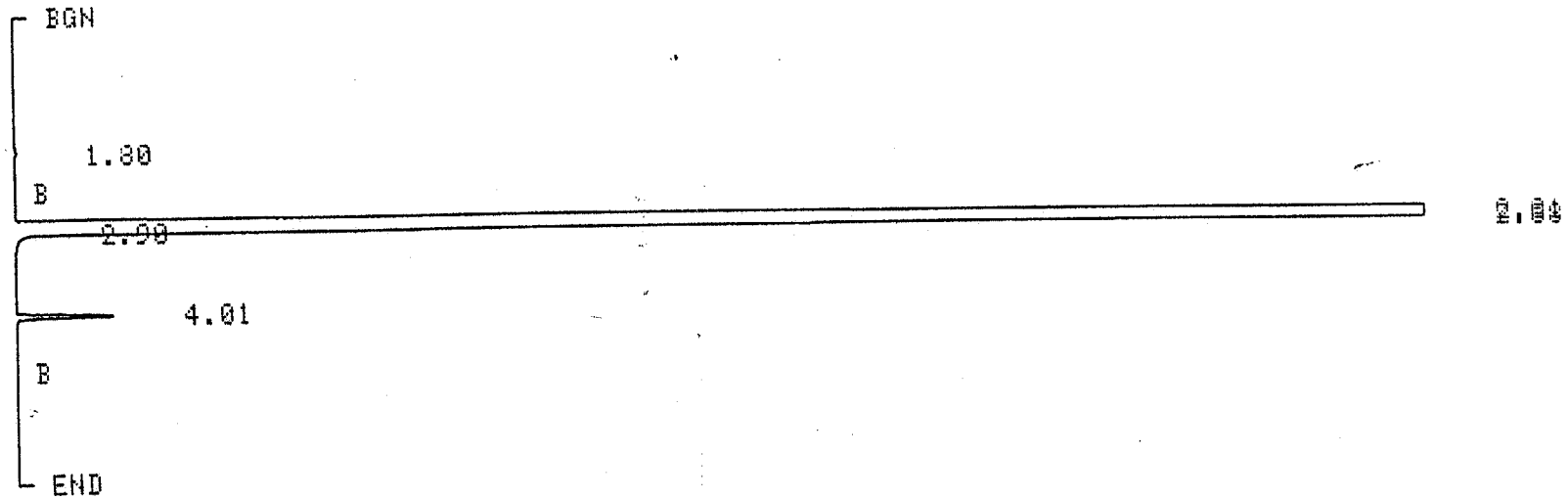
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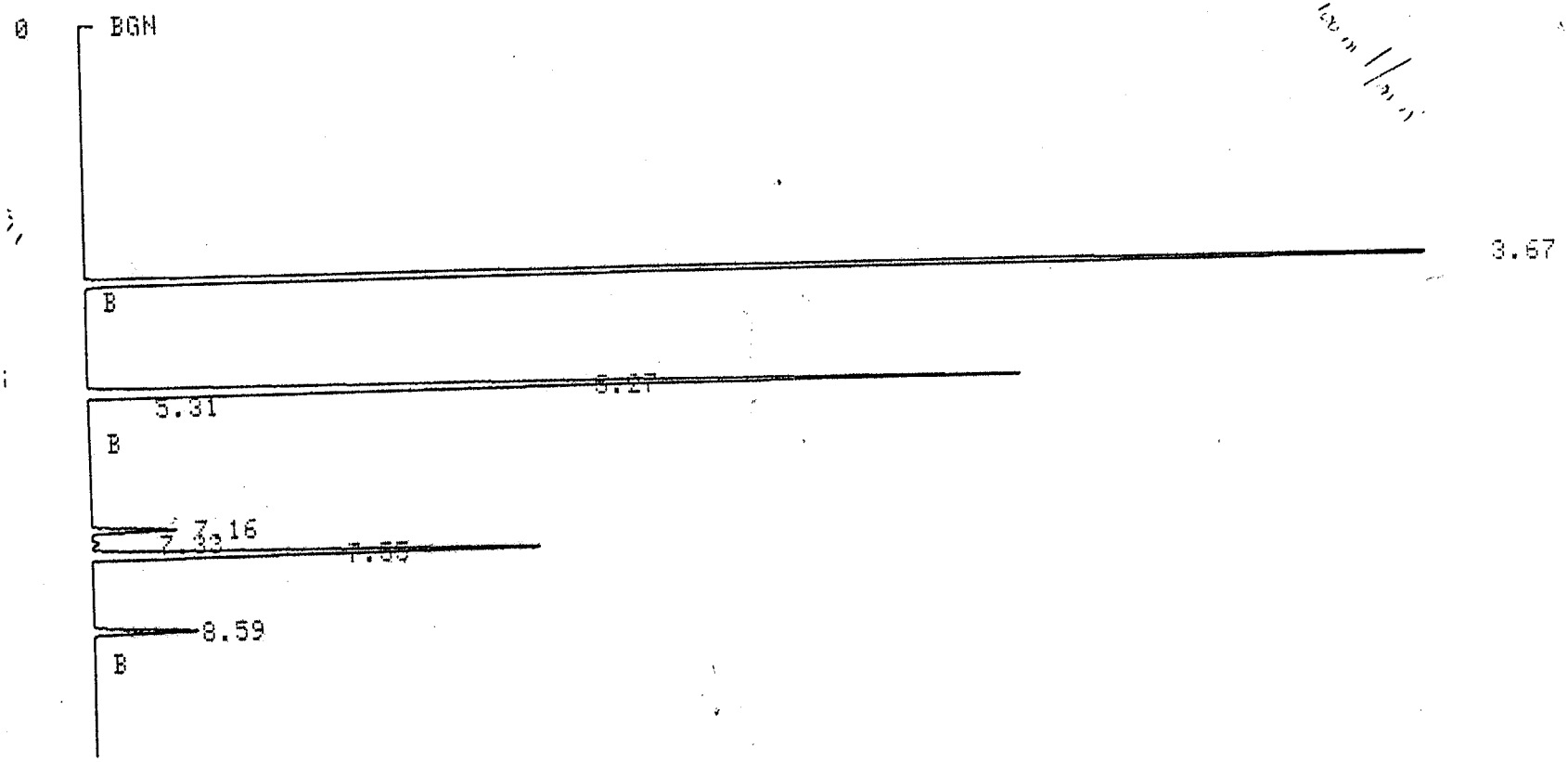
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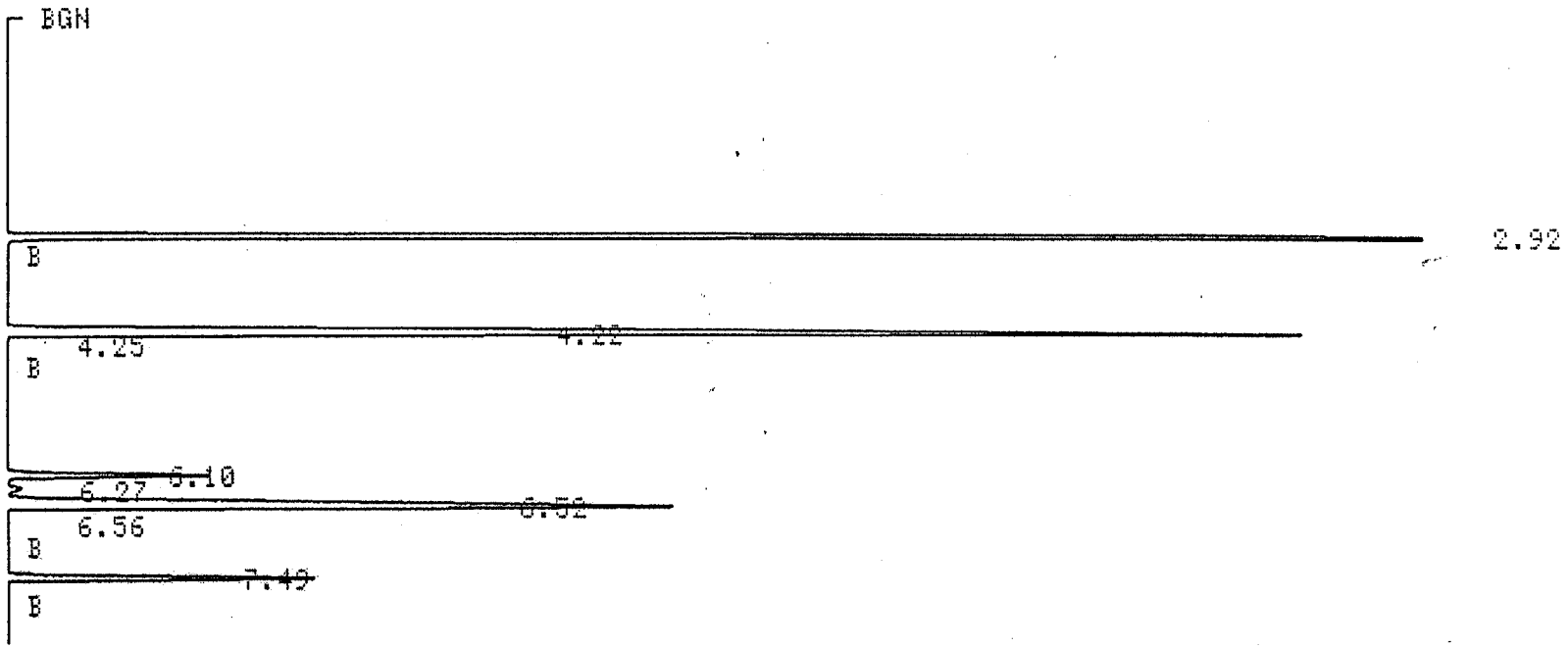
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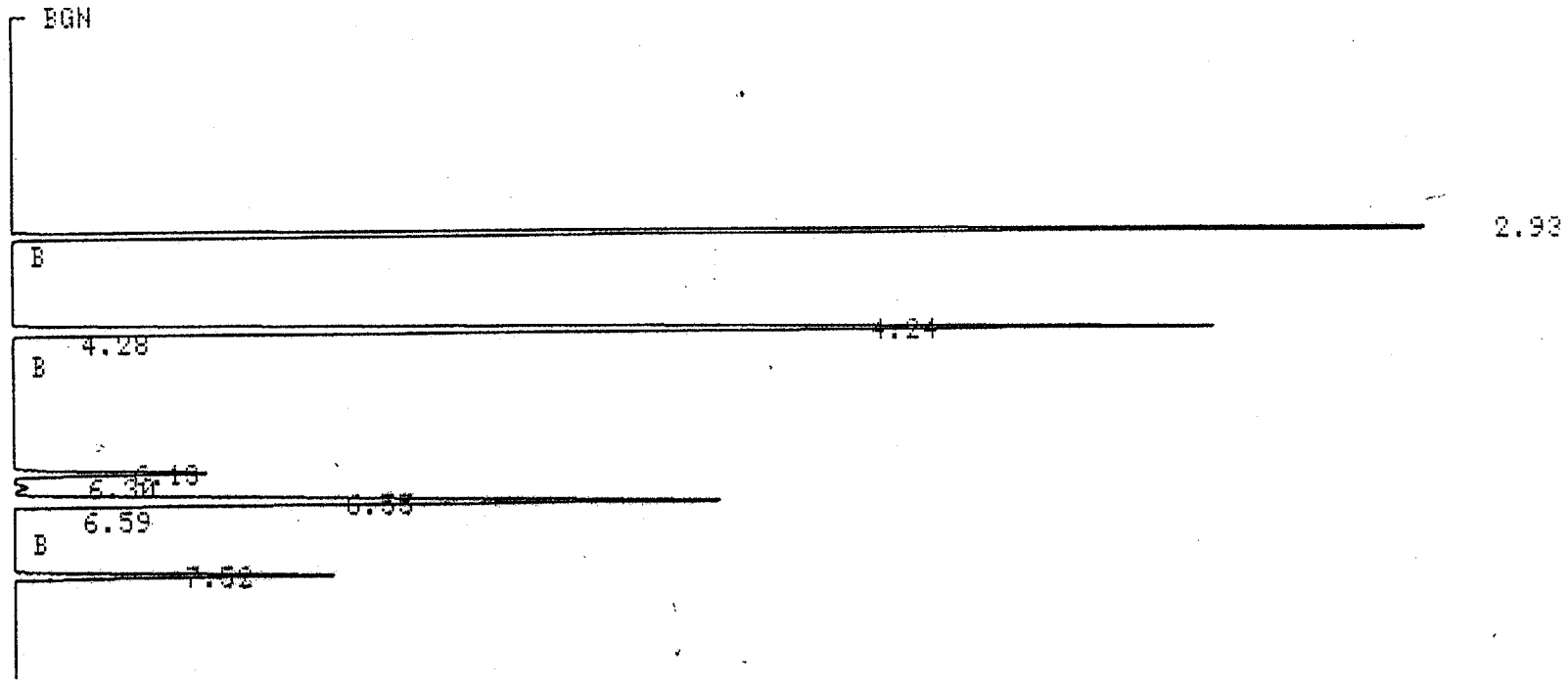
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