Influence factors of organic compounds leaching from PE pipes and the potential toxic effects on E. coli and rat C6 glioma cell

MATERIALS

Sodium hypochlorite (analytically pure, AP) was obtained from Guangzhou Chemical Reagent Factory. R2A Agar was prepared from Sinopharm Chemical Reagent Co., Ltd. RPMI 1640 medium (without L-glutamine and phenol red) was purchased from HyClone (Logan, UT). FBS and Dulbecco’s balanced phosphate-buffered saline (PBS) without calcium and magnesium salts was obtained from Gibco BRL (Paisley, UK). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. GC-MS (TRACE GC2000/TRACE MS, ThermoQuest Co., UK), UV-Vis (Ultra-violet-visible spectroscopy) (SP-756PC, Shanghai) and Micro-plate reader (Thermo, USA) were used in this study.

Relevant parameters for GC-MS

Helium functions as the carrier gas at a constant flow of 1.2 mL/min. One microlitre of the solution was injected in the split-splitless mode (the split was opened after 30 s) into the injection port at a temperature of 280 °C. The MS scan range was from 20 m/z to 650 m/z (full scan) for 3.6 times every second under an electron impact condition of 70 EV. A capillary column (Hewlett-Packard, HP-MS5 30 m, 0.25 mm i.d., 0.25 mm film thickness) was employed and the oven temperature was held at 45 °C for 1 min, raised by 5 °C/min to 300 °C, and then held at 300 °C for 10 min.

Colony counting method

For counting the colony, R2A agar, soaked solution from the PE pipes and blank solution (ultrapure water) were sterilized in a pressure vapor sterilizer (121 °C, 100 kP, and 30 min) and Petri dishes were sterilized in a dry heat sterilization pot at 160–170 °C for 2 h as well. Under sterile conditions, a colony of E. coli was taken, diluted 1,000-fold, and then 100 μL solution was inoculated into a soaked solution of PE pipes to culture overnight in the biological culture cabinet at 37 °C. At the same time, the control samples were also
prepared. The bacterium was appropriately diluted by sterilized water and then 100 μL solution was inoculated into the plate, which was placed in the incubator for 0 h, 5 h, 10 h and 24 h, respectively. Each experiment was performed in at least triplicate. The colony count was analyzed by colony counter.

Solid phase extraction method

A soaked ultrapure water sample (2,000 ml) from the PE pipe was extracted by the SPE apparatus. Moreover, reference samples were also prepared for analysis. Finally, each SPE micro-column was dried with nitrogen, and the volume of the samples set to 1 mL.