MethodsX 4 (2017) 214–217

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Method Article

Purely ultrasonic enzyme extraction from activated sludge in an ultrasonic cleaning bath

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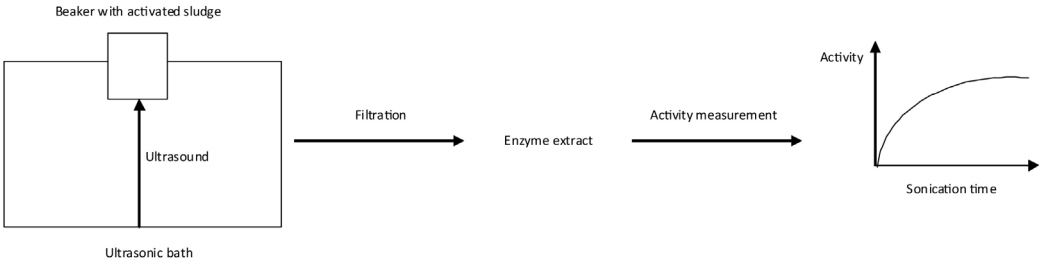
GRAPHICAL ABSTRACT



ABSTRACT

Enzymes are important in biological wastewater treatment systems, since they are responsible for breakdown of macro- and micropollutants, thereby making the pollutants available for metabolism. Enzyme activity has been investigated in particular in activated sludge processes, since the activated sludge technology is the most important and widely spread wastewater treatment technology used today. Various methods have been used to extract enzymes from activated sludge in order to measure their activity, these include stirring with additives like detergents and cation exchange resins, ultrasonication (with probes) and combinations of the three [1–3]. In this article we describe a method for purely ultrasonic enzyme extraction from activated sludge using low power ultrasound generated by an ultrasonic bath and no additives. The method essentially consists of:

- Sonication of the sludge sample using a glass beaker and an ultrasonic bath.
- Filtration of the sample in order to obtain the enzyme extract.
- Measurement of enzyme activity by fluorescence spectrometry using a substrate that yields a fluorescent product.

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http://dx.doi.org/10.1016/j.mex.2017.07.003

ARTICLE INFO
Method name: Purely ultrasonic enzyme extraction from activated sludge
Keywords: Enzymes, Extraction, Ultrasound
Article history: Received 27 September 2016; Accepted 11 July 2017; Available online 18 July 2017

Methods detail

Activated sludge sampling and TSS/VSS measurement

An activated sludge sample (1 l) was taken from the aeration tank of the municipal wastewater treatment plant Petange (50 000 PE) in Luxemburg and transported to the laboratory within 30 min. Half of the sample was directly subjected to enzyme extraction. The other half was aerated over night using an aeration pump (SCHEGO M2K3) and subsequently subjected to enzyme extraction. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined. In order to do so the sludge sample was filtered using glass fiber filters (1 μm pore size, PALL). The residue was dried over night at 105 °C in a drying oven (Memmert UN 110) and subsequently incinerated at 550 °C for 1.5 hours in a muffle furnace (Nabertherm). The weights were recorded.

Ultrasonic enzyme extraction

An activated sludge sample (50 ml) was centrifuged for 5 min at 3000 rpm (Thermo Scientific SL 16 Centrifuge). The sediment was resuspended to original volume (50 ml) with sodium chloride solution (0.14 mol/l, Merck). Part of the sample (40 ml) was than sonicated in an ultrasonic bath (VWR Ultrasonic Cleaner USC-TH, 45 kHz, 180 W) using a glass beaker (100 ml) and various sonication times (0, 2.5, 5, 10, 15, and 20 min). The ultrasonic power in the beaker was determined by disequilibrium calorimetry described elsewhere (submitted). The ultrasonic power in the beaker was found to be 5.71 W. After sonication, the sludge was filtered using syringes (TERUMO) and membrane filters (0.45 μm, VWR). This gave the enzyme extracts.

Enzyme assay

Esterase activity was measured by fuorescence spectroscopy using fluoresceine diacetate (Sigma-Aldrich) as substrate according to the method described by Obst [4]: The substrate solution was prepared by dissolving 10 mg fluoresceine diacetate in 5 ml ethylenglycol-monomethylether (SERVA). This solution was then diluted (1:2) with deionized water, which gave the final substrate solution. For the essay 200 μl of enzyme extract were pipetted into 3 cavities of a microtiter plate (Perkin Elmer) and 10 μl of substrate solution were added. For the blank 10 μl of substrate solution were added to 200 μl sodium chloride solution (0.14 mol/l, Merck). For the photometric blank 10 μl of substrate solution were added to 200 μl sodium chloride solution. A standard solution was prepared by dissolving 10 mg fluorescein sodium (Sigma-Aldrich) in 100 ml deionized water. An aliquot of 0.1 ml was diluted to 100 ml with deionized water (final concentration 0.1 mg/l). For the standards 10, 40, 70, 100, 130, 160, and 200 μl of standard solution were filled up to 200 μl with deionized water and 10 μl of substrate solution were added. The pH of all cavities was adjusted to 7.5 by addition of 30 μl HEPES-buffer (CALBIOCHEM). An overview of the microtiter plates preparation is given in Table 1. The excitation wavelength of the fluorescence spectrometer (Perkin Elmer LS55) was set to 485 nm and the emission wavelength was set 538 nm respectively. The resulting progress curves and enzyme activities were evaluated in Excel.