Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 204–210
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Enzyme extraction by ultrasound from sludge flocs

YU Guanghui, HE Pinjing*, SHAO Liming, ZHU Yishu

State Key Laboratory of Pollution Control and Resource Reuse, Key Laboratory of Yangtze River Water Environment, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China. E-mail: solidwaste@mail.tongji.edu.cn

Received 01 March 2008; revised 11 July 2008; accepted 28 July 2008

Abstract

Enzymes play essential roles in the biological processes of sludge treatment. In this article, the ultrasound method to extract enzymes from sludge flocs was presented. Results showed that using ultrasound method at 20 kHz could extract more types of enzymes than that at 40 kHz and ethylenediamine tetraacetic acid (EDTA) methods. The optimum parameters of ultrasound extraction at 20 kHz were duration of 10 min and intensity of 552 W/g TSS. Under the optimum condition, ultrasound could break the cells and extract both the extracellular and a small part of intercellular enzymes. Ultrasound intensity was apparently more susceptive to enzyme extraction than duration, suggesting that the control of intensity during ultrasound extraction was more important than that of duration. The Pearson correlation analysis between enzyme activities and cation contents revealed that the different types of enzymes had distinct cation binding characteristics.

Key words: enzymes; extracellular polymeric substances; extraction method; sludge flocs; statistical analysis; ultrasound

DOI: 10.1016/S1001-0742(08)62252-4

Introduction

Proteins, polysaccharides, and lipids were the major organic matters in sewage sludge, which must be hydrolyzed to smaller units by enzymes before subsequent degradation (Frølund *et al*., 1995; Cadoret *et al*., 2002; Sheng and Yu, 2006). Therefore, enzymes played essential roles in the biological processes of sludge treatment (Tcuber and Brodisch, 1977). The concentration of enzymes, location of the enzymes, and product transport mechanisms all influenced the reaction rate in the biological processes (Morgenroth *et al*., 2002). Thus, measurement of enzyme activities was the most direct way to study mechanisms and biological reaction. Additionally, measurement of enzyme activities was an alternative method to assess microbial biomass and activity of sludge, and acted on indicators of specific processes such as COD and phosphorus removal (Richards *et al*., 1984; Nybroe *et al*., 1992).

In recent years, there had been a growing interest on the study of enzymes from sludge flocs (Teuber, and Brodisch, 1977; Richards *et al*., 1984; Frølund *et al*., 1995; Goel *et al*., 1998; Cadoret *et al*., 2002; Nielsen *et al*., 2002; Whiteley *et al*., 2002). It was found that a large proportion of enzymes were immobilized in sludge flocs by adsorption in the extracellular polymeric substances (EPS) matrix (Frølund *et al*., 1995). EPS and cells formed bioaggregates, such as biofilms and sludge flocs (Nielsen and Jahn, 1999). The researchers (Goel *et al.*, 1998; Confer and Logan, 1998; Whiteley *et al*., 2002) collected sludge samples from one sewage treatment plant, and they reported that most extracellular enzymes were bound with cell, pellet, or the organic particulate matters in sludge flocs. To date, however, no standardized methods for enzymes extraction from sludge flocs exist.

Enzymes were considered to be an integrated part of EPS matrix (Frølund *et al*., 1995). Therefore, enzymes in the sludge flocs could also be extracted by EPS extraction methods. Different methods had been developed for EPS extraction (Frølund *et al*., 1996; Liu and Fang, 2003; Comte *et al*., 2006a, 2006b), but not all of them could be used for the enzymes extraction as some of them could lead to enzymes inactivation or just partial extraction (Gessesse *et al*., 2003). For example, cation exchange resin (CER) was regarded as an efficient standard method to extract EPS (Henze, 2007). However, sole CER could not efficiently extract all the enzymes in the sludge flocs (Frølund *et al*., 1995; Gessesse *et al*., 2003). The reason was that CER was highly selective for Ca^{2+} and Mg^{2+} bound EPS (Park and Novak, 2007). Therefore, CER could only extract the enzymes bound with Ca^{2+} and Mg^{2+}.

Ultrasound could effectively disintegrate sludge flocs and released enzymes embedded in the sludge flocs (Tiehm *et al*., 2001; Whiteley *et al*., 2002). On the other hand, ultrasound could enhance the enzyme activities in the sludge flocs (Yu *et al*., 2007, 2008), suggesting that ultrasound is a good method to extract enzymes from sludge flocs. To our knowledge, no previous work has been conducted

* Corresponding author. E-mail: solidwaste@mail.tongji.edu.cn

on the enzymes extraction by ultrasound method. The objective of this article was to optimize the process parameters of ultrasonic extraction. A better knowledge on this issue could not only provide a suitable enzyme extraction method but further deepen our understanding on the roles of enzymes in wastewater treatment processes. Protease, α-amylase, and α-glucosidase had been reported to hydrolyze proteins and polysaccharides (Goel *et al*., 1998). Alkaline phosphatase hydrolyzed phosphomonoesters to provide an alternative source of phosphorus for the cells, while acid phosphatase was reported to be involved in internal cell metabolism (Kloeke and Geesey, 1999). Thus, the five enzymes were selected for this study due to their essential roles in the sludge treatment.

1 Materials and methods

1.1 Sludge samples

Activated sludge samples were collected from the aerated basin and returned pump house of a municipal wastewater treatment plant (WWTP) in Shanghai, China. The plant treated 75000 m^3/d of wastewater (93% domestic and 7% industrial sewage) using anaerobic-anoxic-oxic process. The collected samples were transported to laboratory within 30 min after sampling.

1.2 Ultrasound equipments

Two types (20 and 40 kHz) of ultrasound equipments were applied to extract enzymes from sludge flocs in this study. Table 1 lists the parameters of the ultrasound equipments.

Table 1 Parameters of the two ultrasound equipments

Parameter	Equipment I	Equipment II
Producer	Shanghai Sonxi Co., Ltd., China	Kunshan Ultrasonic Equipment Co., Ltd., China
Model	FS-600	KQ-300 DE
Type	Probe	Water-bath
Frequency (kHz)	20	40
Electricity supply (V, kHz)	220, 50	220, 50
Maximum energy output (W)	600	300
Treated volume (mL)	10000	0.5–500

1.3 Enzymes extraction protocol

Ultrasound protocol was selected to extract enzymes from sludge flocs. Since the ethylenediamine tetraacetic acid (EDTA) method had high EPS extraction efficiency, it was selected as a counterpart (Yu *et al*., 2007). Figure 1 illustrates the extraction protocol applied in this work. In brief, the sludge flocs were settled for 1.5 h at 4°C with supernatant decanted carefully by a siphon. The decanted fraction of sludge flocs was taken as slime that contained few enzymes (Frølund *et al*., 1995; Nielsen and Jahn, 1999). The sludge sediment was collection, whose characteristics are listed in Table 2. The sediment was then centrifuged at 2000 ×*g* for 15 min. The collected bottom sediment was re-suspended to its original volume using a pH 7 buffer solution consisting of Na_3PO_4, NaH_2PO_4, NaCl, and KCl. The molar ratio of these components was 2:4:9:1. The conductivities of the buffers were adjusted with distilled water to match those of the sludge sediment samples listed in Table 2. The suspension was centrifuged

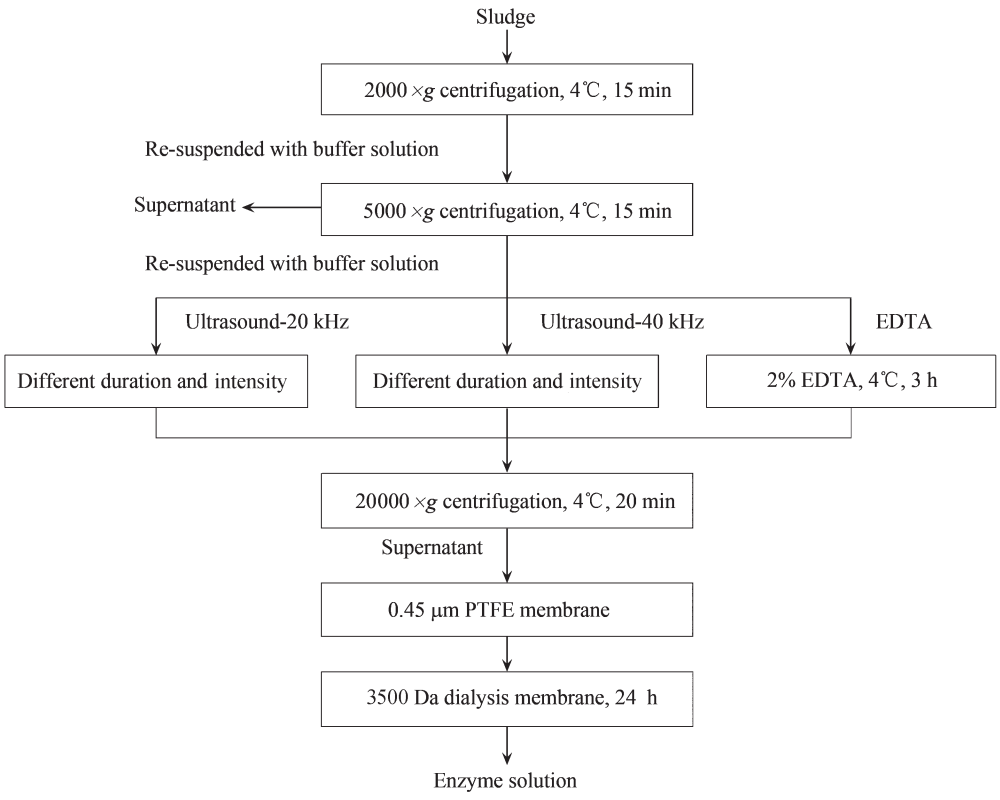

Fig. 1 Enzymes extraction protocol from sludge flocs.