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Degradation kinetics of chlortetracycline in wastewater using ultrasonication assisted laccase

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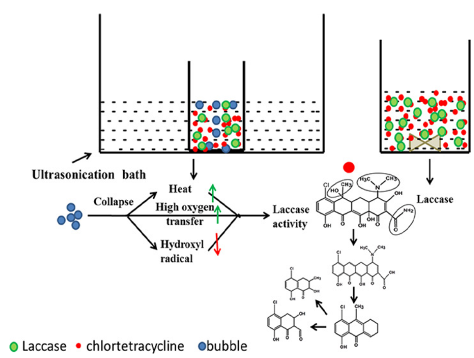
HIGHLIGHTS

- Novel use of laccase coupled UlS technique to degrade antibiotics in wastewater.
- UAL treatment achieved maximum degradation of CTC in lower time.
- Degradation rate was increased by 5.6 folds in UAL treatment.
- Laccase at 0.5 U/L removed over 80% of 2 μg/L CTC in 2 h at pH 4.5 by UlS assistance.

GRAPHICAL ABSTRACT



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ABSTRACT

Chlortetracycline (CTC) is widely used as a veterinary antibiotic and is considered as a recalcitrant pollutant. In this study, spiked CTC (2 mg L^{-1}) in wastewater was degraded using laccase from the white rot fungi, *Trametes Versicolor* combined with ultrasonication (UlS). Over 60% of CTC was removed in 2 h by UlS assisted laccase (UAL) treatment where laccase treatment alone took 2 days to degrade 87% of CTC under similar CTC concentration (2 mg L^{-1}), laccase dose (0.5 IU) and pH 6.0 conditions. UAL treatment showed 5.3 folds higher CTC degradation rate compared to laccase alone treatment at pH 6.0. Further, pH optimization of UAL treatment was done and pH 4.5 was found to be optimum wherein 80% of CTC degradation was obtained which is 2.6 folds higher compared to degradation at pH 6.0. The UAL treatment with optimized pH was not only increased CTC degradation efficiency (~80%) but also reduced the degradation time to 2 h. The obtained results highlighted the enhanced degradation rate, efficiency and unaltered stability of laccase during UAL treatment which can be used for oxidizing other tetracycline groups of antibiotics. Moreover, laccase and UAL treatments showed similar degradation products and no estrogenic activity.

1. Introduction

The rapid increase in population, income, and urbanization has driven the global consumption of milk and meat in developing countries [1]. Current industrial farming with extensive use of antibiotics is likely helping to meet the growing demand for animal products. The current intake of antibiotics in animals has exceeded the human consumption [2]. This is due to their massive usage in growth

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promotion and disease prevention purposes apart from treating infections. As a result, animal farming became a key source for continuous release of veterinary antibiotics into the environment [3,4]. Among antibiotics, tetracyclines (TCs) are traditional and extensively using broad-spectrum antibiotics in the market across the world. Chlortetracycline (CTC) is the first TC antibiotic that has been discovered and used for veterinary purposes. The low absorption (25–40%) of CTC in livestock led up to 70–90% release into the environment by animal excretions. This results in the detection of CTC in water sources across the world around 0.08–0.61 μg/L [5,6]. The continuous detection of antibiotics and their exposure to microorganisms accelerate the development and spread of antibiotic resistance [7]. Apart from continuous release and detection, CTC is able to form stable metal complexes which have more toxicity and are persistent [8,9].

Conventional biological treatment plants are not designed for effective removal of emerging and low concentration (ug/L-ng/L) contaminants including antibiotics. Studies have shown the negative effects of antibiotics on the biological treatment processes and other pollutant biotransformation processes [10,11] even at low concentrations. Advanced physical and chemical methods, such as ultraviolet treatment, ozone treatment, adsorption and membrane filtration processes have been proven to some extent inefficient for the complete degradation/removal of antibiotics [12]. Studies so far have reported using higher amounts of chemical reagents to achieve enhanced degradation efficiencies incurring costs [13]. Hence, the research is continuously growing on these technologies to make them low cost, efficient, and safe applications. Specifically, the research is exploiting hybrid technologies which have synergistic effects on degradation of a wide range of contaminants and are cost-effective [14].

Enzyme based degradation of contaminants in wastewater is now emerging. Enzymatic treatment has benefits such as low energy requirement, easy process, no toxic effects and no adoption to conditions compare to conventional and/or chemical oxidation techniques [15,16]. These advantages make enzymatic treatment as alternatives to conventional wastewater treatment [17]. It is well known that the extracellular ligninolytic enzymes (laccase, lignin peroxidase, and manganese peroxidase) of white rot fungi have great potential in degrading environmental pollutants [18]. Laccase belongs to the oxidases family of enzymes which oxidizes many organic contaminants having phenolic and non-phenolic aromatic structures. Literature reported the wide range of compounds that are susceptible to laccase. Main classes include antibiotics (tetracyclines, sulfonamides, quinolones, penicillins, fluoroquinolones), textile dyes, Mycotoxins, endocrine disruptors, polycyclic aromatic hydrocarbons, petroleum hydrocarbons [19–22]. However, laccase oxidation alone showed lower degradation efficiency and slow degradation rate [23]. These drawbacks can be overcome by combing with other degradation methods, specially simultaneous application of ultrasonication (UlS) with enzymes that showed higher degradation especially [24,25].

Increase in enzymatic activity by UlS has been exploited for many enzyme families. In ultrasonic phenomenon, generation and growth of cavities and their subsequent collapse cause turbulence in the media generate heat and hydroxyl radicals [26]. This process enhances oxygen mass transfer owing to the enhanced interaction between enzyme and substrate molecules which is essential to speed up the degradation rate of contaminants. Apart from this, a portion of ultrasonic energy absorbed by the medium is converted into heat which increases media temperature intern increases the enzymatic activity in case of laccase. Studies have reported the enhancement and longevity of catalytic activity with the combined use of ultrasonication and enzyme [27].

Herein, crude laccase produced by the fungus, *Trametes versicolor* was used to degrade CTC which was selected as a typical veterinary antibiotic having wider application and less biodegradable nature. Several studies have reported the chemical oxidation methods to degrade CTC in wastewater. Chemical methods are non-selective, need excess reagents and harsh conditions (high temperature and pH) for efficient degradation [13]. Meanwhile, laccase treatment has a high degree of specificity towards contaminants, needs mild conditions and low energy requirements, albeit lower rates of degradation [28]. The mentioned advantages make the laccase treatment as a green technique over chemical methods. So far, very few studies were conducted on the application of ultrasonication assisted laccase (UAL) catalyzed the degradation of persistent pharmaceuticals [24,29]. In this study, the authors combined the UlS with laccase treatment to overcome the slow degradation rate by enzyme treatment alone. Current study highlighted the efficient degradation of CTC without temperature control and also pH is optimized in the UAL process to study the synergistic effect of OH radicals produced by UlS. In this context, the ultrasonic enhanced catalytic activity of laccase to degrade CTC was evaluated in wastewater. In addition, different operating parameters, such as pH, enzyme stability and kinetics during UlS were studied to determine optimum operating conditions for CTC degradation. Further, transformation products were identified by liquid chromatography-mass spectrometry (LC-MS/MS) and estrogenic activity of resulting treated streams was also determined in WW.

2. Materials and methods

2.1. Chemicals

Chlortetracycline hydrochloride was purchased from Toronto Research Chemicals (Toronto, Canada). Methanol (HPLC grade), Tween 80, disodium hydrogen phosphate, citric acid, sodium azide were purchased from Fisher Scientific (Ontario, Canada). 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) N, N-Dimethyl-4-nitrosoaniline (RNO, 97%) were purchased from Sigma-Aldrich (Mississauga, Ontario, Canada). Millipore system (Milford, MA, USA) Milli-Q/Milli-RO was used for HPLC grade water preparation.

2.2. Wastewater sample

Wastewater samples were collected from wastewater treatment plant (WWTP) of Quebec Urban Community (CUQ) (Sainte-Foy, Quebec City, QC, Canada), with a sewage treatment capacity of 13,140 m^3/h. The plant treats the sewage using primary and physical–chemical treatments and further discharges the treated effluent into the Saint-Lawrence River. WW samples used in this study were collected before UV treatment and stored under dark conditions at 4 ± 1 °C to prevent CTC degradation. The basic characteristics of WW used in this study are presented in Table 1.

2.3. Solid state fermentation

2.3.1. Microorganism and culture conditions

White-rot fungus *T. Versicolor* (ATCC 20869) was selected for solid state fermentation (SSF) to produce laccase. Potato dextrose agar Petri plates were used to inoculate the fungus and incubated for 7 days at 30 ± 1 °C. The culture plates were stored at 4 ± 1 °C and sub-cultured

Table 1
Characterization of wastewater used in this study.

Parameter	Value
pH	7.5 ± 0.3
Total solids (g/L)	0.57 ± 0.01
Suspended solids (g/L)	0.32 ± 0.08
Volatile solids (g/L)	0.17 ± 0.12
Volatile suspended solids (g/L)	0.11 ± 0.05
Total organic carbon (g/L)	0.005 ± 0.08
Alkalinity (g/L)	0.15 ± 0.09
Ammoniacal nitrogen (g/L)	0.04 ± 0.38
Chlortetracycline (μg/L)	8.54 ± 0.20