Chemical Engineering Journal 163 (2010) 273–283

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Degradation of endocrine disrupting bisphenol A during pre-treatment and biotransformation of wastewater sludge

D.P. Mohapatra[a], S.K. Brar[a,*], R.D. Tyagi[a], R.Y. Surampalli[b]

[a] INRS-ETE, Université du Québec, 490, Rue de la Couronne, Québec, Canada G1K 9A9
[b] US Environmental Protection Agency, P.O. Box 17-2141, Kansas City, KS 66117, USA

ARTICLE INFO

Article history:
Received 9 June 2010
Received in revised form 23 July 2010
Accepted 25 July 2010

Keywords:
Bisphenol A
Wastewater sludge
Pre-treatment
Rheology
Zeta potential
Laccase activity

ABSTRACT

The effect of various pre-treatment methods, including alkaline hydrolysis (AH), thermal hydrolysis (TH), thermal alkaline hydrolysis (TAH), thermal oxidation (TO) and thermal alkaline oxidation (TAO), on solubilization and simultaneous degradation of bisphenol A (BPA), an endocrine disruptor, in wastewater sludge (WWS) were investigated. The results showed that among AH, TH and TAH pre-treatments, TAH significantly improved the solubilization of WWS (41.6% suspended solid (SS), 70.7% volatile suspended solid (VSS) and 48.5% chemical oxygen demand (COD)) with higher degradation of BPA (38.4%). SS, VSS and COD solubilization were observed to be lower in TO and TAO pre-treatment as compared to TAH pre-treatment. However, higher degradation of BPA (75.0% and 78.9%) was observed in TO and TAO pre-treatment due to the presence of oxidation process. The effects of rheology and zeta potential on degradation of BPA in raw sludge and different pre-treated sludges were also investigated. The results showed that decrease in viscosity and particle size and increase in zeta potential resulted in higher degradation of BPA. BPA degradation by laccases produced by *Sinorhizobium meliloti* in raw and pre-treated sludge was also determined. Higher activity of laccases (230.9 U L^{-1}) was observed in TAH pre-treated sludge resulting in high degradation of BPA (21.9%) suggesting concomitant biological degradation of BPA.

1. Introduction

Endocrine disrupting compounds (EDCs) have caused various adverse health effects which have been reported in recent years. The EDCs are defined as “exogenous substances or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations” [1]. Bisphenol A (BPA) has been identified as EDC by the U.S Environmental Protection Agency (EPA), World Wide Fund for Nature (WWF) and is declared as a social, environmental and global issue [2].

An effective treatment and disposal of sludge has been one of the major concerns in wastewater treatment processes to degrade organic pollutants including BPA. Many authors extensively investigated and reviewed sludge treatment and disposal, pointing to the challenges involved and future possibilities of reuse [3]. However, the presence of toxic organic compounds in wastewater sludge (WWS) pose problems to sludge recycling making its final disposal a challenge. Further, pre-treatment of WWS is adopted to solubilize and/or to reduce the size (in case of polymers) of organic compounds, in order to make them more easily biodegradable [4].

Numerous research findings reporting effect of WWS treatment/pre-treatment on EDCs have been compiled by Scrimshaw and Lester [5]. In fact, the pre-treatment of WWS and subsequent bioconversion by microorganisms is a very promising approach to dispose and reuse these residues. It allows sludge volume reduction, destruction of organic compounds, improvement of sludge quality and potential gains from value-added product sales. There are several kinds of pre-treatment methods studied so far which involve chemical treatment, mechanical treatment, oxidative treatment, biological hydrolysis or combination of any two of these methods [6].

Out of the existing pre-treatment methods, alkaline hydrolysis (AH), thermal hydrolysis (TH) and thermal alkaline hydrolysis (TAH) are known to increase biodegradability, convert biorecalcitrant molecules to readily biodegradable by-products and/or detoxify the medium by enhancing the mineralization of organic matter and degradation of recalcitrant compounds during biolog-

---

Abbreviations: AH, alkaline hydrolysis; BPA, bisphenol-A; D_{43}, volume mean diameter; LC–MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; $\log K_{ow}$, octanol–water partition coefficient; SS, suspended solids; SPE, solid phase extraction; SCOD, soluble chemical oxygen demand; TH, thermal hydrolysis; TAH, thermal alkaline hydrolysis; TO, thermal oxidation; TAO, thermal alkaline oxidation; TS, total solids; TCOD, total chemical oxygen demand; VSS, volatile suspended solids; WWS, wastewater sludge.

[*] Corresponding author. Tel.: +1 418 654 3116; fax: +1 418 654 2600.
E-mail address: satinder.brar@ete.inrs.ca (S.K. Brar).

1385-8947/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2010.07.062

ical treatment [7]. Sludge hydrolysis is a technique to break the major part of the sludge solid fraction into soluble and less complex molecules, and thus has effects on the viscosity and the filterability of sludge [8], which contribute significantly to removal of pathogens and organic compounds [9]. Advanced oxidation processes (AOPs) have been proposed as an attractive alternative method for the treatment of potential EDCs present in water, wastewater and WWS, among others. Thermal oxidation (TO) and thermal alkaline oxidation (TAO) are oxidative treatment processes, characterized by the generation of hydroxyl radicals which oxidize and mineralize organic molecules present in WWS. However, majority of research on EDCs, including BPA so far has covered their treatability in aqueous solutions. To the best of our knowledge, no studies have been reported on the degradation of BPA in WWS by AH, TH, TAH, TO and TAO pre-treatment processes and moreover, the effect of rheological changes of WWS during these pre-treatments on removal of BPA.

The indigenous microbial flora of WWS can produce panoply of enzymes, such as fungal or bacterial peroxidases, tyrosinases and laccases, which form a group of phenol-oxidising enzymes that degrade or detoxify organic pollutants [10]. An enzyme-catalyzed polymerization and precipitation process has been widely studied as a new method for the treatment of aqueous phenols [11], with the bulk of the work reported using laccases and peroxidases. Laccases produced by *Sinorhizobium meliloti* [12], possesses the potential to degrade or detoxify organic compounds, especially with phenolic groups present in WWS. However, no specific studies have been reported on degradation of BPA in raw sludge and different pre-treated sludges by the activity of laccase produced by *S. meliloti*.

Therefore, the present study constituted following objectives: (i) effect of different pre-treatment methods including AH, TH, TAH, TO and TAO on the solubilization of WWS and simultaneous elimination of BPA; (ii) effect of rheology and zeta potential changes on BPA removal/degradation during different pre-treatment of WWS; (iii) cell growth of *S. meliloti* in different pre-treated sludges and its correlation with BPA; (iv) effect of laccases produced by *S. meliloti* for degradation of BPA in WWS.

2. Materials and methods

2.1. Reagents

BPA (98% purity assay) was obtained from Sigma–Aldrich (Ontario, Canada). HPLC-grade methanol (MeOH), dichloromethane (DCM), acetone and chloroform, used for cleaning and extraction purposes, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). Acetic acid, hydrogen peroxide, sodium hydroxide and sulphuric acid were supplied by Fisher scientific (Ontario, Canada) and were of analytical grade. HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Sample collection and analysis

Secondary sludge used in the study was collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada) which receives wastewater originating from domestic zones, industries, commercial enterprises and institutions present in the city. Sampling was performed on September 23, 2009 between 8:30 and 10:00 a.m. There was no rain or snow precipitation on the day of sampling. Samples were collected in pre-cleaned amber glass bottles with aluminum foil-lined caps and stored under dark conditions at $4 \pm 1\,^{\circ}$C. The sludge suspended solids (SS) was concentrated from 1.5% (w/v) to higher SS concentrations by gravity settling and centrifugation of the settled sludge at $1600 \times g$ for 3 min in a Sorvall RC 5C plus Macrocentrifuge (rotor SA-600). The supernatant was discarded in order to obtain a concentration of 30 g L^{-1} SS.

2.3. Pre-treatment of wastewater sludge

Different pre-treatments, such as AH, TH, TAH, TO and TAO were conducted with optimum SS concentration (30 g L^{-1}) [13]. The concentration of BPA was measured in raw sludge (secondary sludge) before pre-treatment and after each pre-treatment. In each case, a spiked BPA sample (0.12 mg) was treated to compare the results with the unspiked sample.

2.3.1. Alkaline hydrolysis (AH)

Alkaline hydrolysis was adjusted by adjusting the pH of sludge to 10.0 ± 0.1 by using 4 N NaOH. Approximately 0.062 g of NaOH solution was added per gram of SS to obtain the required pH. Later, the sludge was placed in incubator shaker (Infors HT Multitron Standard, Switzerland) at $30 \pm 1\,^{\circ}$C and 200 rpm for 24 h.

2.3.2. Thermal hydrolysis (TH) and thermal alkaline hydrolysis (TAH)

Thermal hydrolysis was conducted by adjusting the pH of sludge to 7.0 ± 0.1 by addition of either 4 N NaOH or 4 N H_2SO_4. The digestion process was performed by using a Multiwave-microwave sample preparation system (Paar Physica, Perkin Elmer) with infrared temperature control and hydraulic pressure control with feedback function capable of reducing microwave power near the limit of operating pressure. The sludge was transferred to PTFE digestion vessels, 100 mL capacity and the microwave digestion process was conducted at microwave power of 1000 W, operating pressure of 3000 kPa and temperature $140 \pm 1\,^{\circ}$C for 30 min.

Thermal alkaline hydrolysis was conducted by adjusting the pH of sludge to 10.0 ± 0.1 by adding 4 N NaOH solution followed by microwave digestion. The microwave digestion for TAH pre-treatment followed the same procedure as in TH pre-treatment.

2.3.3. Thermal oxidation (TO) and thermal alkaline oxidation (TAO)

Thermal oxidation was conducted by adjusting the pH of sludge to 7.0 ± 0.1 and the digestion process was carried out by microwave digestion at $140 \pm 1\,^{\circ}$C for 30 min. After digestion, pH of sludge was adjusted to 3.0 ± 0.1 by adding 2 N H_2SO_4. Later, 0.01 mL of H_2O_2 (30%, v/v) per gram of SS was added to sludge.

Thermal alkaline oxidation was carried out by adjusting the pH of sludge to 10.0 ± 0.1 by adding 4 N NaOH solution and then the same method was followed as for TO pre-treatment.

For convenience, AH, TH and TAH pre-treatments have been grouped together and the other category, based on oxidation has been enumerated for TO and TAO pre-treatments.

2.4. Analytical methods

The samples were subject to different analysis including BPA within two to three days to avoid degradation or transformation of native samples. Analysis of pH, ammonical nitrogen, phosphorus, total solids (TS), suspended solids (SS), volatile suspended solids (VSS), total and soluble chemical oxygen demand (TCOD and SCOD), total and soluble organic carbon (TOC and SOC) were carried out as per the Standard Methods [14]. SS_0, VSS_0 and $SCOD_0$ referred to as the parameters of WWS before pre-treatment, while SS_S, VSS_S and $SCOD_S$ referred to the parameters after pre-treatment. The soluble increment of SS, VSS and SCOD were calculated as follows:

$$S_{ss} = \frac{(SS_0 - SS_s)}{SS_0} \times 100\% \qquad (1)$$

$$S_{vss} = \frac{(VSS_0 - VSS_s)}{VSS_0} \times 100\% \qquad (2)$$

$$SCOD_{increment} = \frac{(SCOD_s - SCOD_0)}{TCOD} \times 100\% \qquad (3)$$

2.5. BPA analysis

2.5.1. Sample preparation

Sludge was frozen using liquid nitrogen prior to lyophilization by the freeze-dry system (Dura Freeze Dryer, Kinetics). About 0.5 g of lyophilized and homogenized sample was transferred to a glass tube and 20 mL of dichloromethane–methanol (7:3, v/v) was added to the tube. The extraction was carried out by microwave assisted extraction method (MARS microwave extractor, CEM Corporation, North Carolina, USA). This system allowed simultaneous irradiation of 14 extraction vessels. Microwave power was 1200 W (100%) and the extraction was performed in a temperature-controlled mode. The extraction temperature was 110 ± 1 °C and programmed as follows: ramp to 110 ± 1 °C for 10 min, holding at 110 ± 1 °C for 10 min. The extract was separated by centrifugation at 7650 × g for 15 min and the procedure was repeated three times. The extracts were combined, concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and redissolved in 100 mL of HPLC grade water.

2.5.2. Clean-up

Solid phase extraction (SPE) method was used for clean-up and pre-concentration of extract. Sep-Pak Plus C18 environmental cartridges were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were preconditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. Sludge extracts (100 mL) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (−15) psi.

The elution was performed by adding 2 × 4 mL of methanol/dichloromethane (3:1, v/v) mixture to the cartridge at a flow rate of 1 mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 1 mL prior to liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) analysis. The overall scheme of the analytical procedure used for the determination of BPA in WWS is presented in Fig. 1.

2.5.3. LC–MS/MS analysis

LC–MS/MS analyses were performed on a TSQ Quantum Access (Thermo Scientific, Mississauga, Ontario) with a Finnigan surveyor LC pump equipped with a 120-vial capacity sample management system. The analytes were separated on a 3 μM, 100 mm × 2.1 mm Hypersil Gold C18 reverse phase column (Thermo Scientific, Peterborough). The sample injection volume was set at 10 μL.

A binary mobile phase gradient with water [A] and methanol [B] was used for analyte separation at a flow rate of 200 μL min^{-1}. The gradient was performed as isocratic 5% A and 95% B for 6 min. The identification of BPA was done in a full-scan mode by matching the retention time and mass spectrum with true standards. Quantitative LC–MS/MS analysis was carried out in negative ionization (NI) condition and in selective reactions monitoring (SRM) mode using BPA d_{16} as internal standard.

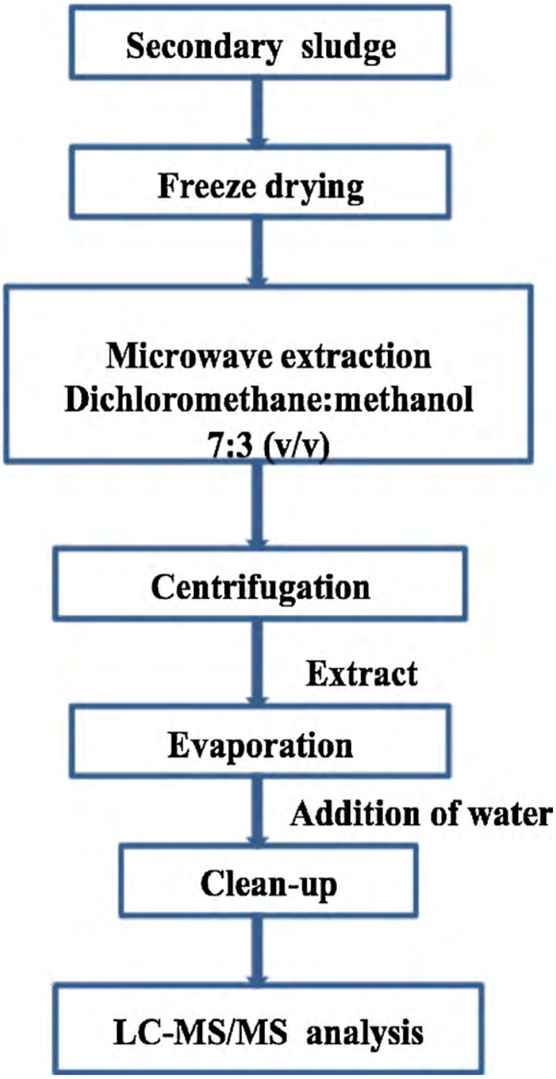

Fig. 1. Schematic diagram of analytical procedure used for analysis of BPA in wastewater sludge.

2.6. Rheological study

2.6.1. Viscosity

Viscosity of raw sludges and different pre-treated sludges were measured by using a rotational viscometer Brookefield DVII PRO+ (Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) equipped with Rheocalc32 software. The viscosity data acquisition and analysis was carried out using Rheocalc V2.6 software, (B.E.A.V.I.S.–Brookfield Engineering Advanced Viscometer Instruction Set). All measurements were performed at 25 ± 1 °C, 36.69 s^{-1} shear rate and viscosity was referred to as “apparent viscosity”.

2.6.2. Particle size analysis

Particle size analysis was carried out by using Fritsch Laser particle sizer analysette 22, which is based on LASER diffraction principles. The stirrer and recirculation pump speed were also kept moderate at 250 and 500 rpm, respectively to minimize the damage of sludge particles. For analysis, each sample was diluted approximately 400-fold in tap water and analyzed in triplicate. This method is based on the principles of Fraunhoffer diffraction and Mie scattering. The results were then averaged to produce the particle size distribution, and the readings were recorded as particle volume percent in 51 discrete particle ranges between 0.1 and 1000 μm.

Table 1
Changes in physical–chemical parameters before and after pre-treatment of WWS.

Parameters	Raw sludge	Alkaline hydrolyzed sludge	Thermal hydrolyzed sludge	Thermal alkaline hydrolyzed sludge	Thermal oxidative sludge	Thermal alkaline oxidative sludge
pH	6.5	9.8	7.3	10.0	2.6	3.4
TS ($g L^{-1}$)	33.1 ± 0.6	33.0 ± 0.5	32.8 ± 0.6	31.7 ± 1.2	32.5 ± 0.9	32.5 ± 0.7
SS ($g L^{-1}$)	30 ± 0.31	23.3 ± 0.8	20.0 ± 0.7	17.5 ± 0.5	19.3 ± 0.7	17.8 ± 1.0
VSS ($g L^{-1}$)	14.9 ± 0.08	10.8 ± 0.1	7.3 ± 0.2	4.4 ± 0.5	5.6 ± 0.08	4.6 ± 0.09
TCOD ($g L^{-1}$)	17.3 ± 1.5	17.2 ± 3.2	17.1 ± 1.8	16.8 ± 2.8	16.9 ± 3.3	16.6 ± 4.0
SCOD ($g L^{-1}$)	3.0 ± 0.5	7.0 ± 0.9	9.4 ± 2.8	11.4 ± 1.2	10.2 ± 3.0	11.0 ± 2.6
Ammonical nitrogen ($g L^{-1}$)	0.1 ± 0.03	0.2 ± 0.01	0.4 ± 0.06	0.7 ± 0.02	0.3 ± 0.07	0.4 ± 0.05
Phosphorus ($g L^{-1}$)	0.3 ± 0.07	0.3 ± 0.04	0.3 ± 0.03	0.4 ± 0.07	0.2 ± 0.02	0.3 ± 0.06
TOC ($g L^{-1}$)	282 ± 10	282 ± 7	283 ± 11	282 ± 14	278 ± 4	282 ± 6
SOC ($g L^{-1}$)	0.15 ± 0.08	0.2 ± 0.01	0.23 ± 0.01	0.32 ± 0.08	0.26 ± 0.07	0.29 ± 0.05

±refers to standard error.

Floc disruption at D_{43} (volume mean diameter) analogous to the particle size expressed as diameter 43% size distribution cutoff points was chosen as average particle size as it showed the volume mean diameter.

2.7. Zeta potential

Surface charge (zeta potential) measurement of flocs formed before and after pre-treatment of sludge was carried out by using a zetaphoremeter (Zetaphoremeter IV and Zetacompact Z8000, CAD Instrumentation, Les Essarts le Roi, France) and Smoluckowski equation [15]. Raw and pre-treated sludge sample of 10 μL was diluted 100 times with distilled water before passing through the zetaphoremeter.

2.8. Rhizobial growth

The fast-growing *S. meliloti* strain A_2 (Agriculture and Agri-food Canada, Sainte-Foy, Quebec, Canada) was used throughout this study. Cultures were maintained at 4 ± 1 °C on yeast mannitol agar (YMA) slants [16]. Cell production was carried out in different liquid media: the standard medium yeast mannitol broth (YMB), raw sludge, alkaline hydrolysis sludge, thermal hydrolysis sludge, thermal alkaline hydrolysis sludge, thermal oxidation sludge, thermal alkaline oxidation sludge and each sludge sample with spiked BPA. All sludge samples were sterilized at 121 ± 1 °C for 30 min prior to inoculation. The YMB medium contained the following constituents in grams per liter: K_2HPO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2; NaCl, 0.1; yeast extract, 1; and mannitol, 10.

The strain A_2 was grown at 30 °C for 72 h on a rotary shaker at 200 rpm and the cell counts were determined on YMA plate supplemented with Congo red (0.25%) after a serial dilution on saline solution (NaCl, 0.85% (w/v)). The samples were plated in triplicates. For enumeration, 30–300 colonies were counted per plate. The results were expressed in colony forming units per mL (CFU/mL). Colonies were counted after incubation of plates for 0, 6, 9, 12, 24, 36 and 48 h at 30 °C.

2.9. Laccase activity

Laccase activity was measured with 2,2-azino bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in 0.1 M phosphate–citrate buffer (pH 4). Oxidation of ABTS was determined by the increase in A420 ($\varepsilon 420 = 36\ (\mathrm{mM\ cm})^{-1}$) [17]. One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per min.

2.10. Statistical analysis

All data presented in Table 1 and Figs. 2, 4, 6–8 are representative of at least three independent experiments with all samples measured in triplicates within each experiment. Repetition of statistical results was carried out using Statistical 6.0 software for Windows by employing the Student's *t*-test.

3. Results and discussion

3.1. Effects of pre-treatment on solubilization of WWS and BPA removal

Table 1 shows the characteristics of raw sludge (non pre-treated) and pre-treated sludge by AH, TH, TAH, TO and TAO methods. The SOC value observed in raw sludge was 0.15 g L^{-1} and a higher value of 0.32 g L^{-1} was observed in TAH pre-treatment process. The lower organic carbon solubilization observed in TO and TAO as compared to TAH pre-treatment process was due to formation of aliphatic acids in the medium during reaction of hydroxyl radical with BPA [18].

The pre-treatment efficiency was analyzed in terms of SS, VSS and organic matter (COD) solubilization and simultaneous BPA concentration reduction. Pre-treatment of WWS was adopted to improve the solubilization of sludge and to reduce the organic compounds [19]. The solubilization of SS, VSS and COD in WWS and simultaneous BPA degradation during different types of pre-treatment methods is presented in Fig. 2. As seen in Fig. 2, higher SS, VSS and COD solubilization was observed during thermal pre-treatment processes as compared to alkaline pre-treatment process. The concentration of BPA observed in raw sludge was 5.2 μg g^{-1} and the concentration decreased to 4.3, 3.8, 3.2, 1.3 and 1.1 μg g^{-1} in AH, TH, TAH, TO and TAO pre-treated sludge, respectively. LC–MS/MS chromatogram of BPA degradation during

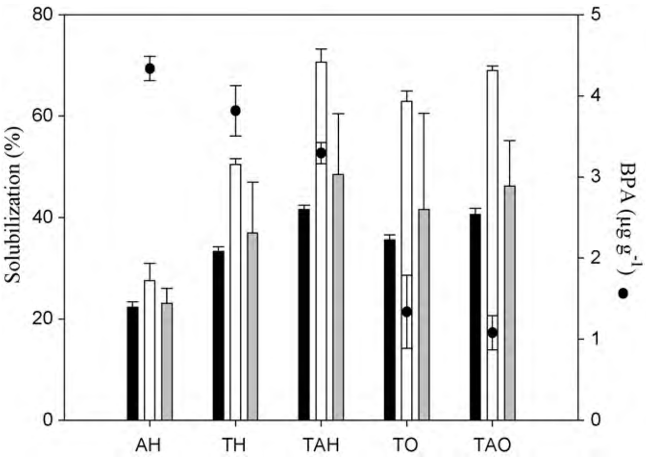

Fig. 2. The solubilization of SS, VSS and COD in raw sludge after pre-treatment and simultaneous BPA degradation. ■ SS solubilization, □ VSS solubilization, ▨ COD solubilization.

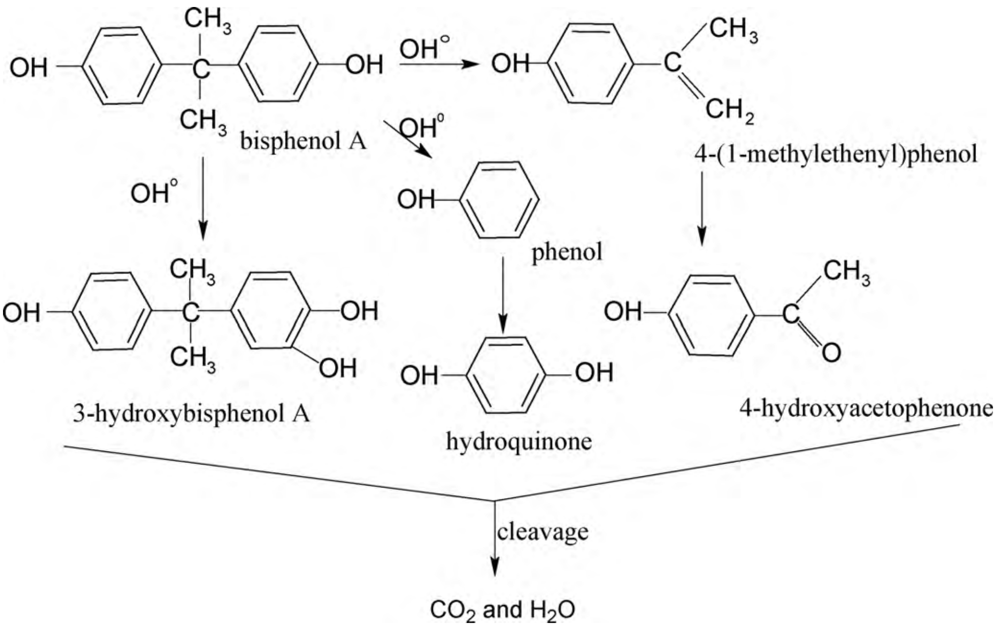

Fig. 3. Different possible degradation pathways of BPA during thermal oxidation and thermal alkaline oxidation treatment of wastewater sludge.

different pre-treatment processes is presented in Fig. 1s (supplementary material). Higher degradation of BPA was observed in thermal pre-treated sludge as compared to alkaline pre-treated sludge. Higher temperature (60–180 °C) during thermal pre-treatment processes leads to more lysis or disintegration of sludge cells [3]. Heating of sludge causes hydration of proteins, polysaccharides, lipids and other intracellular macromolecules secreted from disrupted sludge cells [20]. With the improvement of sludge's solubilization during thermal pre-treatment, organic substances will be transferred from solid to aqueous phase, which resulted in the increase of soluble protein and soluble carbohydrate concentration in aqueous phase and decrease of SS, VSS and COD in solid phase.

A comparison between five pre-treatment processes, such as AH, TH, TAH, TO and TAO, were carried out to observe the significance of alkaline conditions in thermal process (TAH and TAO pre-treatment) for SS, VSS and COD solubilization and simultaneous degradation of BPA. It was observed that TAH pre-treatment was more efficient (41.6% SS, 70.7% VSS and 48.5% COD) for solubilization of sludge and simultaneous degradation of BPA (38.4%) as compared to AH and TH pre-treatment process. The SS, VSS and COD solubilization observed in AH and TH pre-treatment was 22.3%, 27.6% and 23.1% and 33.3%, 50.5% and 37%, resulting in 16.6% and 26.7% of BPA degradation from raw sludge, respectively. TAO pre-treatment process also showed higher SS, VSS and COD (40.6%, 69.2% and 46.2%, respectively) solubilization and higher degradation of BPA (78.9%) as compared to AH, TH and TO pre-treatment processes. The SS, VSS and COD solubilization observed in TO pre-treatment was 35.6%, 62.9% and 41.6%, resulting 75% of BPA degradation from raw sludge. However, when a comparison was done in between TAH and TO pre-treatment, higher SS, VSS and COD solubilization was observed in TAH pre-treatment with lower degradation of BPA. The higher degradation of BPA during TO pre-treatment process as compared to TAH pre-treatment process was due to formation of hydroxyl radicals (discussed later). Tanaka et al. [21] compared three different methods of pre-treatment, NaOH addition (chemical), heating (thermal) and heating with NaOH addition (thermochemical) in terms of VSS solubilization of waste activated sludge from domestic, commercial and industrial wastewater. They observed that the thermochemical pre-treatment gave the best result in terms of VSS solubilization (40–50%) when sludge was heated at 130 °C for 5 min with the dose of 0.3 g NaOH/g VSS. Sodium hydroxide at relatively low dosage level is effective in solubilizing WWS at ambient temperature [22]. There are two distinct phases in hydrolysis under temperature and alkaline condition, initial rapid hydrolysis phase where solubilization started with the hydrolysis of carbohydrate followed by aminoacids and proteins and finally by fats and lipids and the subsequent phase where most of the organic matter are solubilized. Alkaline treatment becomes especially effective in terms of sludge solubilization and organic matter removal when combined with thermal hydrolysis [23].

Among AH, TH, TAH, TO and TAO pre-treatment process, the order of efficiency of pre-treatment method for solubilization and BPA degradation was TAH > TAO > TO > TH > AH and TAO > TO > TAH > TH > AH, respectively. Higher sludge solids and organic matter solubilization in TAH pre-treatment process leads to higher BPA degradation followed by TH and AH pre-treatment process. However, no significant difference of SS, VSS and COD solubilization between TAH and TAO pre-treatment process was observed. It was assumed that in TAO pre-treatment process, higher COD solubilization will be observed as compared to TAH pre-treatment. However, the formation of some aliphatic acids, such as oxalic, formic and acetic acid during reaction of hydroxyl radical with BPA (discussed later) act as recalcitrant towards the oxidation action leading to poor COD solubilization [18]. Valo et al. [24] studied TAH pre-treatment effect on the solubilization of WWS under the following conditions: 17.1 g TS L^{-1}, 3.65 g L^{-1} KOH, 170 °C for 60 min and observed that the pre-treatment process can solubilize upto 83% of COD. Similarly, Barnabe [13] has also confirmed the 70–80% solubilization of COD on TAH pre-treatment of WWS. Chiu et al. [25] studied the solubilization of WWS (1%, w/v) pre-treated with the combination of ultrasonic and alkaline (NaOH, 40 meq L^{-1}) and observed 50–60% of SS solubilization.

However, despite higher solubilization of solids and organic matter, TAH pre-treatment process was found to be less efficient for degradation of BPA as compared to TO and TAO pre-treatment process. This change may be attributed to, the possibility as to why advanced oxidation processes such as ultrasonication, ozonation, Fenton's oxidation and photocatalysis were preferred for degradation of potential EDCs including BPA, rather than the hydrolysis process [26]. In these advanced oxidation processes, chemical reaction plays an important role rather than physical process. These processes require the addition of catalysts and oxidants to the solution which lead to the formation of free hydroxyl radicals responsible for the degradation of EDCs [27].

Higher removal of BPA in TO and TAO pre-treatment was due to the presence of hydrogen peroxide oxidant. Thus, TO and TAO pre-treatment processes were characterized by the generation of hydroxyl radicals, which is the strongest known oxidant [13]. The hydroxyl radical oxidized and mineralized BPA into CO_2 and inorganic ions during TO and TAO pre-treatment of WWS. The possible pathway of degradation of BPA during TO and TAO pre-treatment processes is presented in Fig. 3 based on m/z ratios obtained during LC–MS/MS analysis. It was found that during TO and TAO pre-treatment, three intermediates, such as hydroquinone (6.4 min retention time), 4-hydroxyacetophenone (11.9 min retention time) and 3-hydroxybisphenol A (13 min retention time) were formed when hydroxyl radical reacted with BPA. Aromatic ring cleavage takes place due to continuous reaction of these intermediates with hydroxyl radicals leading to formation of light-weight hydrocarbons, such as formic acid, propionic acid and formaldehyde among others [18]. Further, mineralization of the solution gives rise to the formation of CO_2 and H_2O. Torres et al. [18] studied the effect of ultrasonic pre-treatment process on degradation of BPA in aqueous solution and observed 80% of removal after 30 min of reaction.

3.2. *Effects of rheology on BPA degradation during different pre-treatments of WWS*

The pre-treatment processes will typically influence the rheological behavior by modifying overall sludge properties, including structure, strength and size of sludge flocs and sludge composition. Fig. 4 presents the effect of viscosity and particle size on removal of BPA from raw sludge during different pre-treatment processes. The viscosity observed in raw sludge was 123.8 mPa s and the value decreased to 56.4, 42.8, 26.3, 69.4 and 58.1 mPa s after AH, TH, TAH, TO and TAO pre-treatments, respectively. The decrease in viscosity from raw sludge to pre-treated sludge was mainly due to increase in percentage of solubilization of SS, VSS and COD. WWS pre-treatments rupture suspended solids (microbial cells), liberate the nutrients, partially solubilize the suspended solids, increase the soluble chemical oxygen demand, decrease viscosity and improve the overall WWS biodegradability [13]. However, higher viscosity was observed in TO and TAO pre-treated sludge as compared to AH, TH and TAH pre-treated sludge. The increase in viscosity during TO and TAO pre-treatment process was mainly attributed to decrease in pH (discussed later).

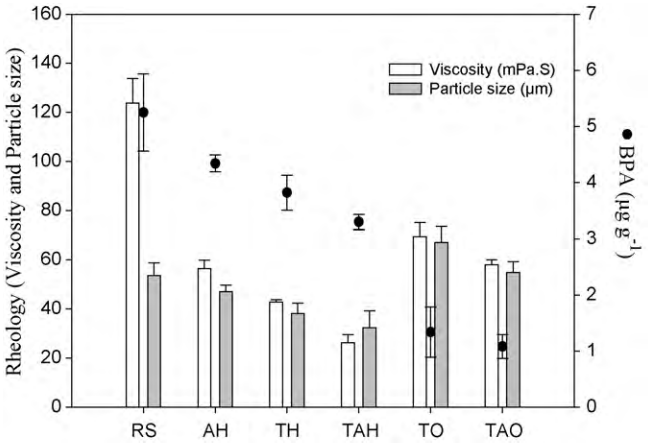

Fig. 4. Effect of viscosity and particle size on removal of BPA from raw sludge during different pre-treatment processes.

Among the three pre-treatment processes, AH, TH and TAH, lower viscosity was observed in TAH pre-treatment. The lower viscosity during TAH pre-treatment was mainly due to higher solid solubilization. Structural units of suspension in WWS are typically larger in size and knitted close to each other at higher concentration of solids. As particle concentration increased, a network of particles is formed with the number of interactions increasing rapidly with the increase in volume concentration of particles leading to increase in resistance to flow of sludge [28]. Therefore, higher solid solubilization resulted in lower resistance to flow leading to decrease in viscosity.

The order of viscosity and BPA degradation for AH, TH and TAH pre-treatment processes were AH > TH > TAH and TAH > TH > AH, respectively. These results indicated that increase in solubilization of WWS during pre-treatment resulted in decrease in viscosity and hence higher degradation of BPA. Decrease in viscosity improved mass transfer rate leading to higher reaction rate and easy transportation of BPA mainly its partitioning causing higher degradation of BPA within the medium.

The increase in viscosity during TO and TAO pre-treatment as compared to AH, TH and TAH pre-treatment process was due to operating conditions, i.e. acidification (lower pH) and oxidation by

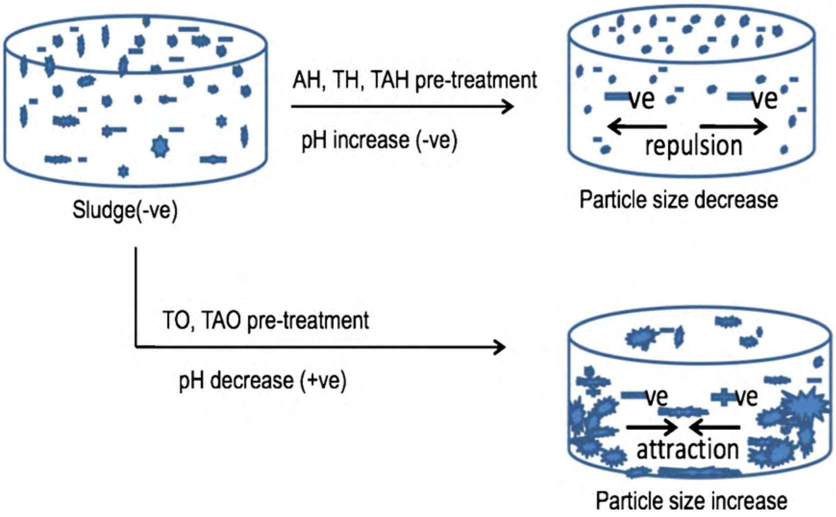

Fig. 5. Schematic representation of change in particle size during different pre-treatment processes.

free hydroxyl radicals. The reaction conditions will lead to changes in inter-particle interactions between sludge flocs and their components. The decrease in pH will result in a decrease in electrostatic repulsions between flocs (which are mostly negatively charged) causing higher resistance to flow leading to higher viscosity [29]. In between TO and TAO pre-treatment, the order of viscosity and BPA degradation were TO > TAO and TAO > TO, respectively. Higher viscosity observed in TO pre-treated sludge as compared to TAO pre-treated sludge was due to lower pH. However, higher removal of BPA was observed in TO and TAO pre-treated sludge at higher viscosity compared to AH, TH and TAH pre-treated sludge, was mainly attributed to the presence of oxidation process.

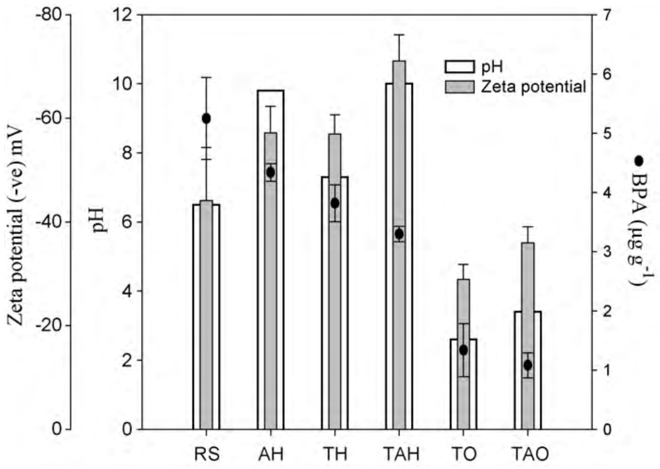

Fig. 6. Correlation of zeta potential values of raw and pre-treated sludges with degradation of BPA.

As seen in Fig. 4, the particle size of raw sludge decreased after AH, TH and TAH pre-treatment and increased after TO and TAO pre-treatment. The schematic presentation of increase and decrease in particle size during different pre-treatment process due to change in pH is presented in Fig. 5. Our previous studies have demonstrated that pre-treatment can result in a change in particle size (larger volume formation of fine particles) [30]. Among the AH, TH and TAH pre-treatment, the order of larger formation of fine particles was TAH > TH > AH. The lower particle size (26.3 and 42.7 μm) observed in TAH and TH pre-treated sludge, respectively was due to the cell wall degradation. Thermal pre-treatment at 60–180 °C disintegrated the cells and liberated intracellular substances [3]. During experimentation, it was observed that in TH and TAH pre-treated sludge, many minute cavities appeared on the surface of sludge particle (picture not shown), caused by the disruption of microbial cells leading to liberation of intracellular substances into the bulk liquid. Higher temperature used in both the pre-treatment processes breaks flocs and releases intracellular material. This results in increase of soluble protein concentration in aqueous phase leading to lower particle size. As discussed earlier, alkaline condition in thermal process (TAH pre-treatment) was more efficient in sludge cell lysis or disintegration as compared to TH pre-treatment. This might be the reason for lower particle size observed in TAH pre-treated sludge. The scavenging of organic compounds is largely influenced by the particle size distribution [31]. The decrease in particle size in TAH pre-treated sludge caused higher interaction (due to more fine particles, increasing the reaction rate) of BPA with OH^- ions, converting it to corresponding salt and other forms. Thus, lower concentration of BPA was observed in TAH pre-treated sludge as compared to AH and TH pre-treated sludge. While, AH pre-treated sludge comprised OH^- ions, but the reaction rate was not thermally enhanced and in TH, there was no reacting hydroxyl species which can react with BPA. In order to verify this hypothesis, we used the Arrhenius equation (Eqs. (4–5)).

$$K = Ae^{E_A/RT} \tag{4}$$

$$\ln K = \ln A - \frac{E_A}{RT} \tag{5}$$

where, K is the rate constant, E_A is the activation energy, R is the gas constant, T is the temperature. Higher temperature and pressure used during TH and TAH pre-treatment process lead to generation of some radical species. With higher temperature, activation energy decreases resulting in higher rate of reaction between BPA and these radical species causing higher degradation of BPA in TH and TAH pre-treated sludge as compared to AH pre-treated sludge. Further, higher degradation of BPA in TAH pre-treated sludge as compared to TH pre-treated sludge was due to further reaction of OH^- ions with BPA.

The increase in particle size after TO and TAO pre-treatment was due to decrease in pH (pH 3). Neyens et al. [23] observed that particle size can be modified by the presence of acids, i.e. supracolloidal particles (1–100 μm range) decrease with acidic pH. Since repulsive electrostatic interactions created from the surface charge of sludge particles are minimized at pH in the range of 2.6–3.6, leading to proximity of small particles as also seen in Fig. 6. In between TO and TAO pre-treatment, lower particle size was observed in TAO pre-treatment resulting in higher removal of BPA. Thus, decrease in particle size increased the reduction of BPA by enhancing the reaction kinetics between the substrate and reactant (depending on the pre-treatment). However, higher removal of BPA was observed in TO and TAO pretreated sludge at higher particle size as compared to AH, TH and TAH pre-treated sludge. This ambiguity was mainly due to the oxidation of BPA by hydroxyl radicals in TO and TAO pre-treatment.

3.3. Effect of zeta potential on BPA degradation during different pre-treatments of WWS

The zeta potential obtained in raw and pre-treated sludges at different pH is presented in Fig. 6. The zeta potential values of −44.1, −57.2, −56.8, −71.1, −28.9 and −35.98 mV were reported for raw, AH, TH, TAH, TO and TAO pre-treated sludge, respectively. Among the five pre-treatment processes, lower zeta potential was observed in TO pre-treatment with pH 2.6 and highest in TAH pre-treatment with pH 10.09. Higher zeta potential was observed in AH, TH and TAH pretreatment as compared to TO and TAO pre-treatment. Lower zeta potential in TO and TAO pre-treatment was due to addition of acid (lower pH) which resulted in neutralization of charge within the medium. Meanwhile, higher zeta potential in AH and TAH was due to addition of alkali (higher pH) that results in net increase in negative charge.

Among AH, TH and TAH pre-treatment, TAH revealed higher zeta potential as compared to AH and TH pre-treatment. However, no significant difference of zeta potential was observed in between AH and TH pre-treated sludge even though the pH is higher in AH pre-treatment. The results may be due to smaller floc size during TH pre-treatment (Fig. 5). The particles come together and flocculation takes place (formation of large particle size) at low zeta potential and vice versa. Based on DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory, a higher (negative) value of zeta potential is expected to result in larger electrostatic repulsion within the flocs, leading to a higher sludge-shear sensitivity and, consequently, smaller floc sizes [32]. The higher zeta potential in TAH pre-treatment resulted in formation of larger number of sludge flocs per unit volume of WWS. As the size of the flocs decreased with increasing zeta potential, overall availability of sorbed BPA molecules to react with OH^- ion increased resulting in higher degradation of BPA during TAH pre-treatment.

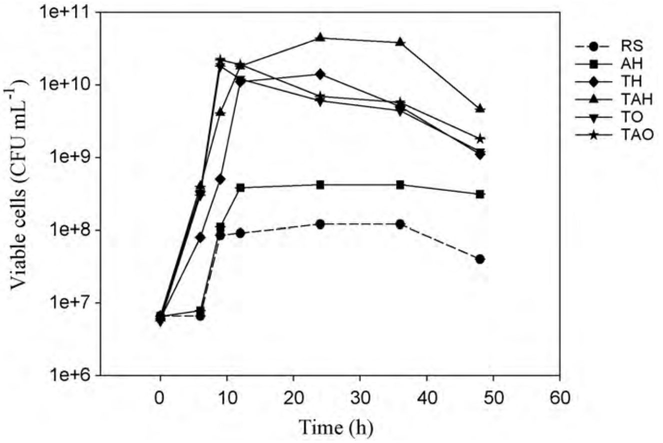

Fig. 7. *S. meliloti* growth curves in raw sludge and different pre-treated sludges.

Increase in zeta potential leads to decrease in viscosity as the particle size decreases. It can be explained well by using Henry equation which established a relationship between zeta potential, electrophoretic mobility and viscosity.

$$U_E = \frac{2\,\varepsilon\, z f(Ka)}{3\eta} \qquad (6)$$

where, z is the zeta potential, η is the viscosity, U_E is the electrophoretic mobility, ε is the dielectic constant and $f(Ka)$ is the Henry's function. However, all the three phenomenon, increase in zeta potential, decrease in viscosity and particle size occur simultaneously within the media leading to increased reaction rate and partitioning of BPA causing higher removal in TAH pre-treated sludge followed by TH and AH. In between TO and TAO pre-treatment, higher zeta potential was observed in TAO pre-treatment resulting in higher removal of BPA as per the phenomenon discussed earlier.

3.4. *S. meliloti* growth profile

The cell growth of *S. meliloti* in raw sludge, different pre-treated sludges and each sludge with spiked BPA was observed. Fig. 7 presents the viable cell counts of *S. meliloti* growth in raw sludge and different pre-treated sludges (unspiked). Spiked BPA sample (0.12 mg) was taken in order to compare the cell growth of *S. meliloti* with unspiked sample (data not reported). No difference was observed in cell growth of *S. meliloti* in spiked and unspiked samples. However, the generation time of viable cells in spiked sample was observed 12 h later than the unspiked ones.

It was observed that in AH, TH, TAH, TO and TAO pre-treated sludge, the growth of *S. meliloti* in terms of viable cells enhanced. The increase in viable cell count in pre-treated sludge as compared to raw sludge was probably due to higher SS, VSS and COD solubilization of raw sludge. As *S. meliloti* is an aerobic bacteria, the increase in solubilization during different pre-treatment processes leads to higher oxygen diffusion and consequently increased cell count. The concentration of suspended solids in sludge affected the growth of *S. meliloti* in secondary sludge from municipal wastewater treatment plant (Quebec, Canada) [33]. Other factors, such as decrease in viscosity and particle size also increased the oxygen transfer rate (OTR) within the medium leading to higher cell multiplication.

Among the three types of pre-treated sludges, AH, TH and TAH, high count of viable cells (4.4×10^{10} CFU mL^{-1}) was observed in TAH pre-treated sludge at 24 h. The higher cell count of *S. meliloti* in TAH pre-treated sludge was due to higher SS, VSS and COD solubilization as compared to other pre-treated sludges. The order of sludge solids solubilization and cell count of *S. meliloti* was TAH > TH > AH and TAH > TH > AH, respectively. Possible reason of higher cell count in TAH pre-treated sludge was due to lower viscosity as compared to AH and TH pre-treated sludge. Higher solids solubilization and decreased viscosity during TAH pre-treatment resulted in higher oxygen or substrate mass transfer within the medium leading to higher cell count [34]. In between AH and TH pre-treated sludge, higher solids solubilization and lower viscosity was observed in TH pre-treated sludge causing higher cell count. Higher cell count (1.4×10^{10} CFU mL^{-1}) in TH pre-treated sludge was observed in 24 h. In between TO and TAO pre-treated sludge, higher solids solubilization and lower viscosity was observed in TAO pre-treated sludge again leading to higher cell count.

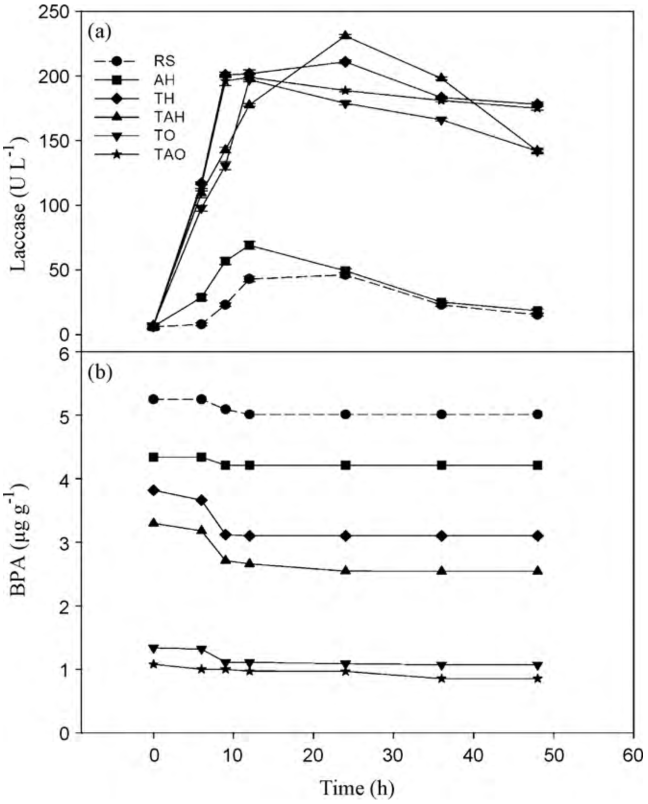

Fig. 8. BPA degradation in raw sludge and different pre-treated sludge due to laccase activity: (a) laccase activity and (b) BPA degradation.

3.5. Laccase activity and BPA degradation

Fig. 8a and b, presents the laccase activity profile and simultaneous BPA degradation in raw and different pre-treated sludges. It was evident that *S. meliloti* was capable of producing laccase enzyme in raw and pre-treated sludge. In AH pre-treated sludge, lower activity of laccase was observed as compared to other pre-treated sludges. The results were in concordance with less viable cell count of *S. meliloti* in AH pre-treated sludge. Rosconi et al. [12] carried out a comparative study between *Rhizobium* sp. to identify the laccase strain and they observed higher identity (99%) was established with *S. meliloti*. They also observed the higher production of laccase with higher cell count of *S. meliloti*.

Among TH, TAH, TO and TAO pre-treated sludge, higher activity of laccase was observed in TAH pre-treated sludge. This trend might be due to higher viable cell count of *S. meliloti* in TAH pre-treated sludge. The laccase activity maximum was observed at 24 h in TAH and TH pre-treated sludge and 12 h of incubation in TO and TAO

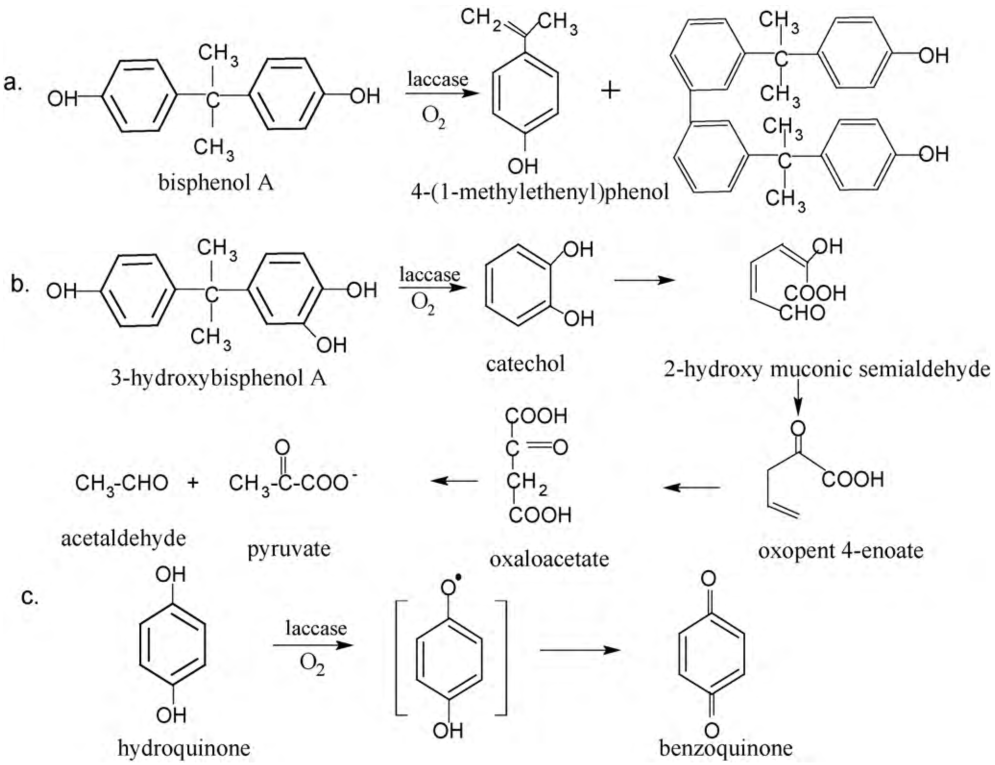

Fig. 9. Proposed pathways of BPA and its by-products degradation formed during TO and TAO pre-treatment by laccase activity; (a) BPA degradation, (b) 3-hydroxybisphenol A degradation and (c) hydroquinone degradation [37,38].

pre-treated sludge, respectively. As seen in Fig. 6a, laccase activity started increasing from 6 to 12 h of fermentation time in raw sludge and TH, TAH, TO and TAO pre-treated sludge. However, in between 12 and 24 h, increase in activity of laccase was observed in TAH and TH pre-treated sludge and decreased activity was observed in TO and TAO pre-treated sludge. The results were in agreement with the increase in cell count of *S. meliloti* upto 24 h in TAH and TH pre-treated sludge and decrease after 12 h in TO and TAO pre-treated sludge.

As seen in Fig. 8a and b, degradation of BPA was observed in raw sludge and TH, TAH, TO and TAO pre-treated sludge with increasing laccase activity except AH pre-treated sludge where laccase activity was lower. Thus, laccase was capable of degrading BPA in these media. Laccases have multiple copper atoms in their active sites and utilize molecular oxygen as the oxidant for a variety of phenols, to form corresponding reactive quinines [35]. Laccases produced by *S. meliloti* [12] possess the potential to degrade or detoxify organic compounds, especially with phenolic groups present in WWS simultaneously during bioconversion of WWS into value added products. A proposed pathway of degradation of BPA and its by-products, formed during TO and TAO pre-treatment of WWS by laccase activity is presented in Fig. 9a–c. As seen in the figures, oxidation by $OH^{\bullet}$ radicals during TO and TAO pre-treatment degraded BPA in raw sludge, characterized by production of by-products which are further degraded by activity of laccase. Hence, the contribution of two mechanisms such as oxidation degradation by radicals followed by biodegradation by laccase enzyme enhanced the degradation of BPA from raw sludge.

Degradation of BPA with increasing laccase activity during TH, TAH, TO and TAO might be due to solubilization of organic compounds which resulted in higher activity of laccase to degrade BPA. Alkaline and oxidative treatments of lignocellulosic materials can induce swelling in particulate organics, making the cellular substances more susceptible to enzymatic attack during saccharification [36].

The concentration of BPA observed at 0 h of incubation in raw, AH, TH, TAH, TO and TAO pre-treated sludge were 5.2, 4.3, 3.8, 3.2, 1.3 and 1.1 $\mu g\,g^{-1}$, respectively. The concentration of BPA observed after 48 h of laccase activity in raw sludge, AH, TH, TAH, TO and TAO pre-treated sludge were 5.1, 4.2, 3.3, 2.5, 1.0 and 0.7 $\mu g\,g^{-1}$, respectively. Significant concentration of BPA (0.7 $\mu g\,g^{-1}$) was degraded due to laccase activity in TAH pre-treated sludge.

Among AH, TH, TAH, TO and TAO pre-treatment, it was observed that TAH pre-treatment was more efficient (21.9%) in degradation of BPA in raw sludge due to relatively higher laccase activity induced by high viable cell count. Another reason might be lower particle size (larger formation of fine particles) and higher zeta potential observed in TAH pre-treated sludge. The larger formation of fine particles in TAH pre-treatment resulted in larger number of small particles per unit volume of the medium so that BPA was adsorbed on these large surface flocs. Further, laccase produced by *S. meliloti* have enhanced access to BPA and finally favor biodegradation of the compound.

The concentration of BPA observed in raw sludge was 5.2 $\mu g\,g^{-1}$ and the concentration reduced to 4.2, 3.3, 2.5, 1.0 and 0.7 $\mu g\,g^{-1}$ in AH, TH, TAH, TO and TAO pre-treated sludge, respectively after combined application of pre-treatment process and laccase activity. The pre-treatment processes AH, TH, TAH, TO and TAO for BPA degradation became more efficient (19.2%, 40.3%, 51.9%, 80.8% and 86.7%, respectively) when a combined application of different pre-treatment processes and laccase activity were taken into account.

Further investigation is required to identify the different by-products formation during these pre-treatment processes and classify these by-products on the basis of toxicity as compared to the parent compound. Thus, future work is under progress in our laboratory to identify these by-products, their toxic effects and to understand whether there is any residual H_2O_2 still present in the pre-treated sludge during TO and TAO pre-treatment process where higher degradation of BPA was observed.

4. Conclusions

The degradation of BPA in raw sludge by AH, TH, TAH, TO and TAO pre-treatment and laccase activity were encouraging and led to the following conclusions:

1. Among AH, TH, TAH, TO and TAO pre-treatment, the higher SS, VSS and COD solubilization was observed in TAH pre-treatment, however, BPA degradation was higher in TAO pre-treatment due to the contribution of oxidation by $OH^\bullet$ radicals and thermal alkaline treatment.
2. AH, TH, TAH, TO and TAO pre-treatment caused a decrease in apparent viscosity of sludge. Among AH, TH and TAH pre-treatment, decreased viscosity and particle size and increased zeta potential was observed in TAH pre-treatment causing higher removal (38.4%) of BPA from raw sludge.
3. Among TO and TAO pre-treatment, decreased viscosity and particle size and increased zeta potential was observed in TAO pre-treatment causing higher removal (78.9%) of BPA from raw sludge.
4. The viable cell count of *S. meliloti* increased in TH, TAH, TO and TAO pre-treated sludge and higher growth (4.4×10^{10} CFU mL^{-1}) was observed in TAH pre-treatment due to higher percentage of SS, VSS and COD solubilization as compared to other pre-treatment process.
5. Higher laccase activity was observed in TAH pre-treated sludge leading to higher biodegradation of BPA (22.9%) as compared to TH, TO and TAO pre-treated sludge.
6. During AH, TH and TAH pre-treatment processes, BPA removal may be attributed to four different parameters, namely solubilization, lower viscosity, lower particle size and higher zeta potential. In TO and TAO pre-treatment process, BPA removal was also attributed to these four parameters along with intervention by $OH^\bullet$ radicals.
7. During further biotransformation of the pre-treated sludges, BPA removal was enhanced by the action of laccase enzymes.

Acknowledgements

The authors are sincerely thankful to the Natural Sciences and Engineering Research Council of Canada (Discovery Grants A4984 and 355254, STP235071, Canada Research Chair) and INRS-ETE for financial support. The views or opinions expressed in this article are those of the authors and should not be construed as opinions of the U.S. Environmental Protection Agency.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cej.2010.07.062.

References

[1] International Program on Chemical Safety (IPCS), Establish the scientific basis for safe use of chemicals, and to strengthen national capabilities and capacities for chemical safety. http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors.en/, 2005 (accessed 02.11.05).

[2] D.P. Mohapatra, S.K. Brar, R.D. Tyagi, R.Y. Surampalli, Physico-chemical pre-treatment and biotransformation of wastewater and wastewater sludge-fate of bisphenol A, Chemosphere 78 (2010) 923–941.

[3] E. Neyens, J. Baeyens, A review of thermal sludge pre-treatment processes to improve dewaterbility, J. Hazard. Mater. 98 (2003) 51–67.

[4] G. Lehne, A. Muller, J. Schwedes, Mechanical disintegration of sewage sludge, Water Sci. Technol. 43 (1) (2001) 19–26.

[5] M.D. Scrimshaw, J.N. Lester, Fate and behavior of endocrine disruptors in sludge treatment and disposal, in: J.W. Birkett, J.N. Lester (Eds.), Endocrine Disruptors in Wastewater and Sludge Treatment Processes, CRC Press LLC and IWA Publishing, London, 2003, pp. 145–176.

[6] X. Liu, H. Liu, J. Chen, G. Du, J. Chen, Enhancement of solubilization and acidification of waste activated sludge by pretreatment, Waste Manage. 28 (2008) 2614–2622.

[7] S.M. Contreras, M.F. Rodriguez, F.C. Monami, C. Sans, S. Espulgas, Contributation of the ozonation pre-treatment to the biodegradation of aqueous solutions of 2,4-dichlorophenol, Water Res. 37 (2003) 3164–3171.

[8] N.J. Anderson, D.R. Dixon, P.J. Harbour, P.J. Scales, Complete characterization of thermally treated sludges, Water Sci. Technol. 46 (10) (2002) 51–54.

[9] U. Kepp, I. Machenbach, N. Weisz, O.E. Solheim, Enhanced stabilization of sewage sludge through thermal hydrolysis—3 years of experience with full-scale plant, Water Sci. Technol. 42 (9) (2000) 89–96.

[10] S.B. Subramanian, S. Yan, R.D. Tyagi, R.Y. Surampalli, A new, pellet-forming fungal strain: its isolation, molecular identification and performance for simultaneous sludge-solid reduction, flocculation and dewatering, Water Environ. Res. 80 (9) (2008) 840–852.

[11] J. Wu, J.K. Bewtra, N. Biswas, K.E. Taylor, Effect of H_2O_2 addition mode on enzymatic removal of phenol from wastewater in the presence of polyethylene glycol, Can. J. Chem. Eng. 72 (1994) 881–886.

[12] F. Rosconi, L.F. Fraguas, G. Martinez-Drets, S. Castro-Sowinski, Purification and characterization of a periplasimic laccase produced by *Sinorhizobium meliloti*, Enzyme Microb. Technol. 36 (2005) 800–807.

[13] S. Barnabe, Hydrolyse et oxidation partielle des boues d'epuration pour la production de *Bacillus thuringiensis* HD-1. Ph.D. thesis, INRS-ÉTÉ, Universite de Quebec, Quebec, Canada, 2004, p. 233 pp.

[14] APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 21st edition, 2005, American Public Health Association, Washington, DC.

[15] B.Q. Liao, D.G. Allen, I.G. Droppo, G.G. Leppard, S.N. Liss, Surface properties of sludge and their role in bioflocculation and biosettleability, Water Res. 35 (2) (2001) 339–350.

[16] J.M. Vincent, A manual for the practical study of root-nodule bacteria, in: International Biological Programme Handbook No. 15, Blackwell, Oxford, 1970.

[17] P.J. Collins, A.D.W. Dobson, Regulation of laccase gene transcription in *Trametes versicolor*, Appl. Environ. Microbiol. 63 (1997) 3444–4345.

[18] R.A. Torres, F. Abdelmalek, E. Combet, C. Petrier, C. Pulgarin, A comparative study of ultrasonic cavitation and Fenton's reagent for bisphenol A degradation in deionised and natural waters, J. Hazard. Mater. 146 (2007) 546–551.

[19] M. Weemaes, W. Verstraete, Evaluation of current wet sludge disintegration techniques, J. Chem. Technol. Biotechnol. 73 (1998) 83–92.

[20] A. Erdincler, P.A. Vesilind, Effect of sludge cell disruption on compactibility of biological sludges, Water Sci. Technol. 42 (9) (2000) 119–126.

[21] S. Tanaka, T. Kobayashi, K. Kamiyama, M.L. Bildan, Effects of thermo chemical pre-treatment on the anaerobic digestion of waste activated sludge, Water Sci. Technol. 8 (1997) 209–215.

[22] J.G. Lin, C.N. Chang, S.C. Chang, Enhancement of anaerobic digestion of waste activated sludge by alkaline solubilization, Bioresour. Technol. 62 (1997) 85–90.

[23] E. Neyens, J. Baeyens, R. Dewil, B. De heyder, Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering, J. Hazard. Mater. 106B (2004) 83–92.

[24] A. Valo, H. Carrere, J. Philippe, Thermal, chemical and thermo-chemical pre-treatment of waste activated sludge for anaerobic digestion, J. Chem. Tech. Biotech. 79 (11) (2004) 1197–1203.

[25] Y.C. Chiu, C.N. Chang, J.G. Lin, S.J. Huang, Alkaline and ultrasonic pre-treatment of sludge before anaerobic digestion, Water Sci. Technol. 36 (1997) 155–162.

[26] S. Esplugas, D.M. Bila, L.G.T. Krause, M. Dezotti, Ozonation and advanced oxidation technologies to remove EDCs and pharmaceuticals and personal care products in water effluents, J. Hazard. Mater. 149 (2007) 631–642.

[27] G.V. Korshin, J. Kim, L. Gan, Comparative study of reactions of endocrine disruptors bisphenol A and diethylstilbestrol in electrochemical treatment and chlorination, Water Res. 40 (2006) 1070–1078.

[28] L.H. Mikkelsen, The shear sensitivity of activated sludge. Relations to filterability, rheology and surface chemistry, Coll. Surf. A: Physicochem. Eng. Aspects 182 (2001) 1–14.

[29] N. Tixier, G. Guibaud, M. Baudu, Effect of pH and ionic environment changes on interaparticle interactions affecting activated sludge flocs: A rheological approach, Environ. Technol. 24 (2003) 971–978.

[30] T.T.H. Pham, S.K. Brar, R.D. Tyagi, R.Y. Surampalli, Ultrasonication of WWS-Consequences on biodegradability and flowbility, J. Hazard. Mater. 163 (2009) 2–13.

[31] S.K. Marttinen, R.H. Kettunen, J.A. Rintala, Occurrence and removal of organic pollutants in sewages and landfill leachates, Sci. Total Environ. 301 (2003) 1–12.

[32] J.C.T. Vogelaar, A.D. Keizer, S. Spijker, G. Lettinga, Bioflocculation of mesophilic and thermophilic activated sludge, Water Res. 39 (1) (2005) 37–46.

[33] F. Ben Rebah, R.D. Tyagi, D. Prévost, R.Y. Surampalli, Wastewater sludge as a new medium for rhizobial growth, Water Qual. Res. J. Can. 37 (2002) 353–370.

[34] M. Verma, S.K. Brar, R.D. Tyagi, J.R. Valero, R.Y. Surampalli, Wastewater sludge as a potential raw material for antagonistic fungus (*Trichoderma* sp.): Role of pre-treatment and solids concentration, Water Res. 39 (2005) 3587–3596.

[35] N. Tanaka, T. Tonosaki, M. Nose, N. Tomidokora, N. Kadomura, T. Fujii, M. Tniguchi, Treatment of model soils contaminated with phenolic endocrine dis-

rupting chemicals with laccase from *Trametes* sp. in a rotating reactor, J. Biosci. Bioeng. 92 (4) (2001) 312–316.
[36] M. Tagaki, Pretreatment of lignocellulosic materials with hydrogen peroxyde in the presence of manganese compounds, Biotechnol. Bioeng. 29 (1987) 165–170.
[37] G. Hublik, F. Schinner, Characterization and immobilization of laccase from *Pleurotus ostreatus* and its use for the continuous elimination of phenolic pollutants, Enzyme Micro. Technol. 27 (2000) 330–336.
[38] C.I. Nair, K. Jayachandran, S. Shashidhar, Biodegradation of phenol, Afr. J. Biotechnol. 7 (2008) 4951–4958.