Photocatalysis and catalytic wet air oxidation degradation and toxicity of bisphenol A containing wastewaters

Buddhika Rathnayake^a, Anne Heponiemi^{b*}, Marjo Huovinen^c, Satu Ojala^a, Minna Pirilä^a, Jarkko Loikkanen^c, Saïd Azalim^{b,d}, Mohammed Saouabe^a, Rachid Brahmi^e, Kirsi Vähäkangas^c, Ulla Lassi^{b,} and Riitta L. Keiski^a

^a Environmental and Chemical Engineering, University of Oulu, P.O. Box 4300, FI-90014 Oulu, Finland

^b Sustainable Chemistry, University of Oulu, P.O. Box 4300, FI-90014 Oulu, Finland

^c School of Pharmacy/Toxicology, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland

^d Department of Physics, St John's University, 8000 Utopia Parkway, Queens, NY 11439, USA

^e University of Chouaib Doukkali, Avenue Jabrane Khalil Jabrane, B.P.299, 24000 El Jadida, Morocco

* Corresponding author: email address <u>anne.heponiemi@oulu.fi</u>

ABSTRACT

Bisphenol A (BPA) is a commonly used chemical in consumer products. It is an endocrine disrupter that has potentially significant negative effects on human health. The use and chemical stability of BPA have resulted in the appearance of the chemical in wastewaters. Since the current wastewater treatment technologies are not effective enough to remove BPA, new methods to degrade BPA are required. In this paper, we report the efforts made towards developing a bi-functional catalyst for consecutive catalytic wet air oxidation-photocatalytic

water treatment. It was found that 2.5% Pt/Ti_{0.8}Ce_{0.2}O₂ is a potential bi-functional catalyst for the consecutive treatment. Concentration and toxicity of BPA were successfully reduced by catalytic wet air oxidation. Although BPA was further reduced by photocatalysis, it was not reflected in further decrease of cell toxicity. Thus wet-air oxidation combined with photocatalysis is a promising approach for the reduction of BPA.

Keywords: photocatalysis; CWAO; endocrine disruptor; cell viability; reactive oxygen species

1. Introduction

A wide variety of hazardous chemical substances, such as phenolic compounds, are found in rivers and lakes. Most of the phenolic compounds originate from the agro-industrial sector, but also other branches of industry contribute to these discharges. A well-known example is bisphenol A (BPA), which has been used in the production of plastics and in epoxy resin liners for beverage and food packaging since 1940's. The annual production of BPA in 2015 worldwide was more than 5 million tons, which makes BPA a high production volume chemical [1]. BPA was synthesized already in 1891 and it is still widely used in day-to-day products in spite of its negative effects on human health, some of which were already known before the production was commercialized. Because BPA affects reproductive tissues and the brain it is classified as a xenoestrogen [2]. The effects are wide-ranging, including changes in the structural development of the brain, disruption of estrogen regulation at different levels, and effects on social behavior. Epigenetic alterations e.g. effects on the expression of genes regulating DNA methylation, are among the potential molecular mechanisms behind these effects [3]. Due to the before mentioned reasons, carcinogenicity of BPA is also speculated on.

More importantly, exposure of people to BPA has been clearly proven by urinary samples, which justifies the importance of the removal of BPA from wastewaters [2,4-7].

The traditional wastewater treatment methods are not effective in the BPA removal. For example, biological treatment using activated sludge fails completely, because BPA stays in activated sludge [8,9]. Moreover, the phenolic group of BPA inhibits biological treatments [10,11]. One possible method to remove BPA from wastewater is the photocatalytic treatment. Metal oxide and sulfide semiconductors, such as TiO₂, ZnO, CdS and ZnS are materials typically used in photocatalytic processes. These materials are efficient due to the electronic structure of the metal atoms having a filled valence band and an empty conduction band [12]. Among these materials TiO₂ seems the most interesting, because, depending on the particle size, it has appeared fairly non-toxic (for a recent review see [13], it has a reasonable price, it is easily available, photochemically stable and it has been recognized to have very good photocatalytic activity. The photocatalytic activity of TiO₂ is also related to its crystalline structure, specific surface area, porosity and density of the surface OH groups. These parameters affect the production of electron-hole pairs, adsorption and desorption phenomena as well as redox reactions [14]. TiO₂ exists in three crystalline forms: rutile, anatase and brookite, of which anatase and rutile are used in photocatalysis. The anatase-phase has a bandgap of 3.23 eV that corresponds to the wavelength of UV-light, and it is considered more active than rutile in the degradation of organic compounds. Nevertheless, the combination of these two structures typically produces the most active catalyst. Industrially available Evonik AEROXIDE[®] TiO₂ P25 (P25; 80% anatase, 20% rutile) is so far the most active commercially available catalyst for photocatalytic processes [15].

The use of photocatalysis in the treatment of industrial wastewaters may become expensive due to the high operation costs, but may also be ineffective if organic loading is very high. Combining photocatalysis with another effective wastewater treatment method is therefore beneficial. In this study the combination of photocatalysis and catalytic wet air oxidation (CWAO) has been considered. In contrast to ambient reaction conditions of photocatalysis, in CWAO the pollutants in wastewaters are exposed to high pressure (0.5-5 MPa) and temperature (125-200 °C) in the presence of a catalyst to produce more biodegradable organic compounds, CO₂ and water. CWAO is a suitable treatment method especially for highly toxic and refractory organic compounds [16]. Several heterogeneous catalysts, such as carbon materials, transition metals and their oxides, have been used in CWAO [17]. However, noble metals (e.g. Pt, Ru, Ag, Pd) supported on metal oxides, such as CeO₂, ZrO₂ and TiO₂ and their mixed oxides, have been found to be the most efficient catalysts [18-20].

As known, similar types of catalytic materials could be used in the two processes described. Using an optimized bi-functional catalyst would enable an interesting possibility to combine photocatalysis and CWAO as a hybrid process that could be run sequentially in the same reactor construction to reach the best purification degree. Thus, the development of such a catalytic material was one of the most important aims of this study. In the current work we aimed to modify the TiO₂ catalyst so, that its operation range would be widened from photocatalysis to CWAO, which is the novelty in this work. Only a few earlier studies exist related to the combination of photocatalysis and CWAO [21,22], and they employ two separate catalysts. Since BPA was selected as a model compound, one part of the study was devoted to the toxicity of wastewater containing BPA before and after the treatments. This multidisciplinary approach is important, since the degradation of BPA may produce more toxic by-products than the original compound itself. In addition, the concentrations of these products may occur at such low levels that the chemical detection becomes very difficult even though they may still have toxic effects.

2. Materials and methods

2.1. Catalyst preparation

In this study, both simple and binary oxides were prepared by a sol-gel method. The sol-gel process involves calcination of gels obtained by controlled hydrolysis of alkoxides dissolved in the parent alcohol. The preparation can be adjusted by temperature, used solvent, pH and reaction time, and the final product is in powder form. The final product in the case of TiO_2 consists of either pure anatase or a mixture of anatase and rutile phases. Anatase appears in the temperature range of 400-650 °C, while rutile is formed only at around 650 °C [23].

The preparation of TiO₂-H₂O, TiO₂-HNO₃, CeO₂, ZrO₂, Ti_{0.8}Ce_{0.2}O₂, Ti_{0.5}Zr_{0.5}O₂ and Ti_{0.7}Zr_{0.3}O₂ supports are following the procedure described in [24]. The Ti(OC₄H₉)₄ (Fluka \geq 97%), Ce(NO₃)₃·6H₂O (Alfa Aesar 99.5%) and ZrO(NO₃)₂·5H₂O (Sigma-Aldrich 99%) or Zr(OC₄H₉)₃ (Sigma-Aldrich 80%, for mixed oxides), were first dissolved in 0.5 molL⁻¹ ethanol (VWR Int. 99.8%) and then mixed together in the desired molar ratio (Ti_{0.8}Ce_{0.2}O₂, Ti_{0.5}Zr_{0.5}O₂ and Ti_{0.7}Zr_{0.3}O₂). Further, the solutions were heated to 80 °C and an aqueous solution of ethanol (5 vol%) was added dropwise under constant stirring. After a few minutes the gel was formed and the mixture was maintained at 80 °C for 1.5 h. After overnight maturing, the excess ethanol and water were evaporated first in a sand bath at 100 °C for 5 h and then in a ventilated oven at 120 °C for 20 h. The grinded supports were calcined at 500 °C or 600 °C for 2 h. The mechanical mixture of CeO₂ and TiO₂ was prepared by mixing them together (20% CeO₂ and 80% TiO₂).

The addition of 1% silver on the Ag/TiO₂-P25 and Ag/TiO₂-HNO₃ catalysts was done via complexing. 0.1575 g of AgNO₃ was dissolved in 20 mL of water, which was then added to 9.25 mL of ammonia solution (0.2 molL⁻¹) dropwise. The obtained samples were then aged at 25 °C for 48 h. After that, 9.9 g of TiO₂-P25 or TiO₂-HNO₃ was added to the solution. The mixture was stirred for 48 h and dried at 50 °C for 24 h and at 120 °C for 48 h on a sand bath. The calcination was performed at 500 °C for 3 h with a heating rate of 5 °C min⁻¹. Finally, the

catalysts were reduced under hydrogen at 500 °C. The platinum catalyst (2.5 wt%) was prepared by wet impregnation followed by dry evaporation of support in a rotating evaporator using an aqueous solution of $Pt(NO_3)_2(NH_3)_4$ (Alfa Aesar 99.99%) as a precursor. The Pt catalyst was dried and calcined similarly to supports and finally activated under a H₂ flow (70 mLmin⁻¹) at 350 °C for 3 h in order to obtain Pt⁰. All the prepared catalysts are later indicated as described in Table 1.

Table 1. I	Denotation	of the	prepared	catalysts
------------	------------	--------	----------	-----------

Catalyst	Denotation
Aeroxide TiO ₂ -P25 (Evonik), commercial catalyst	P25
TiO ₂ -H ₂ O ^a , calcined at 600 °C	Ti-600
TiO ₂ -H ₂ O ^a , calcined at 500 °C	Ti-500
TiO_2^a with acid hydrolysis by HNO ₃ , calcined at 600 °C	Ti-N-600
TiO_2^a with acid hydrolysis by HNO ₃ , calcined at 500 °C	Ti-N-500
TiO _{0.5} ZrO _{0.5} , calcined at 500 °C	0.5Ti-0.5Zr
ZrO ₂	Zr
1% Ag/TiO ₂ -P25	Ag/P25
1% Ag/TiO ₂ with HNO ₃ used in preparation	Ag/Ti-N
CeO ₂	Ce
$Ti_{0.8}Ce_{0.2}O_2$	0.8Ti-0.2Ce
2.5% Pt/Ti _{0.8} Ce _{0.2} O ₂	Pt/0.8Ti-0.2Ce

^aTiO₂, prepared by the sol-gel method

2.2. Catalyst characterization

The Brunauer-Emmett-Teller (BET) surface area and pore sizes were determined from N₂ adsorption-desorption isotherms at -196 °C using a Micromeritics ASAP 2020 analyzer. The phase composition of the samples was determined from X-ray powder diffraction data measured by a PANalytical X-Pert Pro X-ray diffraction equipment. The diffractograms were determined at 20 range from 15° to 90° using a scan speed of 0.017° s⁻¹ at CuK α radiation at a

wavelength 0.15406 nm and the Powder Diffraction File (PDF) database supplied by the International Centre for Diffraction Data (ICDD) was used for the identification of the crystalline phases. The elemental composition of the mixed oxides were analyzed with X-ray photoelectron fluorescence (XRF) using a PANalytical Axios instrument. The noble metal content was analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Perking Elmer Optima 5300 DV instrument.

2.3. Catalytic bisphenol A degradation experiments

CWAO reactions were carried out for 3 h in a 0.3 L stainless steel batch reactor equipped with a magnetically driven stirrer. In the beginning of the reaction, 160 mL of aqueous solution of BPA (Sigma Aldrich \leq 99%) with the concentration of 60 mgL⁻¹ and 2 gL⁻¹ of a catalyst were introduced into the reactor. The reactor was purged with nitrogen, heated to the reaction temperature (160 °C) and the reaction was activated with the addition of air (2 MPa). During the reaction, pressure was kept constant and at regular intervals of time water samples (7x5 mL) were taken from the reactor.

Degradation of BPA by photocatalysis was conducted in two different vertical reactors, one made of quartz (1.0 L) and the other of Teflon (0.5 L). The quartz reactor was used in the evaluation of catalyst activities in BPA degradation. The Teflon reactor was used in connection with consecutive tests due to the smaller amount of sample obtained after the CWAO. The quartz reactor was a circulating batch reactor equipped with a peristaltic pump (60 Lh⁻¹) to maintain circulation. The circulation was used to ensure good agitation and to control the temperature with a heat exchanger. The experiments were done at room temperature and the stability of the temperature was followed by a thermocouple (Delta OHM HD2128.1). Synthetic air (6.5 cm³s⁻¹) was introduced into the reactor through a sintered glass tube to enhance the dispersion of gas to the solution. The compartment, where the reactor was placed during the

reaction, contained six UV-B lamps (Philips, 15 W) that were placed outside the reactor. The pollutant water solution (15 mgL⁻¹) together with 200 mgL⁻¹ of a catalyst were used in the experiment.

Another reactor used was an annular type batch reactor having a UV-B lamp (Philips Cleo PL-L 36W) covered with a quartz tube fixed in the middle of the reactor. Synthetic air feed at 2.5 cm^3s^{-1} was supplied into the reactor during the experiments. The temperature was manually controlled by adjusting the cooling water circulation in a steel coil placed inside the reactor. The test solution was constantly agitated by a magnetic stirrer with 250 rpm. The experiments were done with the BPA solutions taken after the CWAO experiments together with the recovered catalysts from the same CWAO experiments with the catalyst dose of 200 mgL⁻¹. All the experiments were done without adjusting the pH. In order to establish the adsorptiondesorption equilibrium between the catalyst and the BPA solution, a 30 min dark period was used prior to starting the photocatalytic experiments. Then, the UV-B lamps were switched on and the photocatalytic experiment was continued for 3 h. The samples were drawn for analysis at the pre-defined sampling times and they were filtered with a 0.2 µm filter (Millipore) to remove suspended catalyst particles.

2.4. Water sample analysis

The concentrations of BPA and the possible oxidation by-products were analyzed by HPLC using Shimadzu SCL 10A at a wavelength of 226 nm. Waters SunFire C 18, 5 μ m particle column (2.1 mm x 100 mm) at 40 °C was used for the separation with 45% acetonitrile and 0.1% formic acid solution. The flow rate was set to 0.5 mLmin⁻¹ after which a 20 μ L or 30 μ L (BPA concentration < 10 mgL⁻¹) sample was injected. The TOC content was analyzed by a Skalar Formacs^{HT} TOC/TN analyzer (samples from CWAO) and GE Sievers 900 (samples from photocatalysis). For the Skalar Formacs TOC analyzer the sample was first acidified, stirred

and purged in order to remove the inorganic carbon. The organic carbon was oxidized at temperature of 750-950 °C in the presence of a catalyst to CO_2 , which was measured at the wavelength of 4.2 µm by NDIR detection. For the analysis by the GE Sievers 900 the sample was first acidified and the organic matter was oxidized by the combination of a liquid oxidant and UV-light to CO_2 , which was measured by an electrical conductivity measurement. In both systems the TOC was calculated automatically. Moreover, the possible leaching of the active metals (Ag, Pt) during the experiments was determined by ICP-OES using a Perking Elmer Optima 5300 DV ICP-OES instrument.

2.5. Cell culture and experiments to study toxicity of bisphenol A

Human MCF-7 breast adenocarcinoma cells were cultured in a Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 gL⁻¹ glucose (BioWhittaker, Belgium) and supplemented with 4.4 mgmL⁻¹ insulin (Sigma), 10µgmL⁻¹ gentamicin (Gibco, UK), and 9% heat inactivated fetal bovine serum (FBS, Sigma, USA). Cells were cultured at 37 °C in an incubator with a humidified atmosphere containing 95% air and 5% CO₂. Three types of experiments were done with MCF-7 cells.

Firstly, MCF-7 cells were seeded in 48-well plates (30 000 cell/well) and exposed to 50 μ M or 200 μ M BPA (Sigma, dissolved in dimethyl sulfoxide, DMSO, Sigma) for 24, 48 or 72 h. Control cells were exposed to 0.1% DMSO. The viability with MTT-test and reactive oxygen species (ROS) were analyzed. Secondly, MCF-7 cells were exposed to the BPA-model water (60 mgL⁻¹), CWAO-treated, and CWAO + photocatalysis treated waters for 48 h. Shortly, 10x DMEM culture medium (Sigma, with 4.5 gL⁻¹ glucose, L-glutamine and sodium pyruvate) was supplemented with 0.4% NaHCO₃ (Sigma, USA) and 9% FBS (Sigma, USA). Medium was diluted to 1x with control water, or with the BPA-waters (final concentration of the waters are lower than the original ones because of the medium preparation, see Table 2). Before pipetting

to the cells, waters were filtered with 0.45 μ m filter (Acrodisc® Syringe Filter, Pall Life Sciences) and warmed in 37 °C water bath. Viability was tested with MTT-test, and ROS were analyzed by a fluorescence method [25]. Thirdly, MCF-7 cells were exposed to BPA-water diluted from the BPA model water (60 mgL⁻¹) to give 45, 30, 20, 10 or 5 mgL⁻¹ in the culture to study viability with MTT-test.

Table 2. BPA concentrations in water samples and in cell culture. For cell culture the purified water samples were further diluted due to the necessary addition of cell culture medium components.

Purification method	Concentration of BPA	Concentration in
	in purified water	MCF-7 cell culture
	(mgL ⁻¹)	(mgL ⁻¹)
No purification (original)	60	45
CWAO	16.1	12.1
CWAO	21.9	16.4
CWAO + photocatalysis	9.48	7.11
CWAO + photocatalysis	10.3	7.73

2.6. Measurement of cell viability

MCF-7 cell viability was determined by the MTT-reduction assay [26] where an exogenously administered MTT solution [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] is converted into colored formazan by functionally normal mitochondria inside the cells. After the exposure, the medium was discarded and new MTT-containing (0.5 mgmL⁻¹) medium was pipetted to every well and incubated in 37 °C for 2 h. Afterwards, MTT-medium was replaced with SDS-DMF buffer, pH 4.7 for 40 min to solubilize the formazan crystals. Absorbance was measured at 570 nm using ELx 800 plater reader (Biotek Instruments, Inc.). Results were expressed as a percentage of the controls exposed to 0.1% DMSO or MilliQ-H₂O. Four parallel wells per exposure in a 48-well plate were used in viability experiments. The mean of these values were calculated and handled as a single independent experiment. Cell viability results

are expressed as the mean \pm SD. Statistical significance was analyzed with one-way ANOVA followed by Tukey's multiple comparison test in all comparisons.

2.7. Analysis of reactive oxygen species

The production of ROS was measured with a method that uses 2'7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) as the substrate (described earlier in [25]. Cells were exposed to BPA-waters on 48-well plates in the same way as in viability experiments. After 48 h exposure, medium was removed and cells were loaded with 5 μ M of H₂DCFDA in HEPES buffered Hanks balanced salt solution (HBSS; pH 7.4) for 30 min in the dark at room temperature. Afterwards, fluorescence of DCF was measured with VictorTM Plate Reader at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. DCF fluorescence values were normalized to total cell number based on the maximum propidiumiodide (50 μ M) fluorescence measured at an excitation wavelength of 531 nm and an emission wavelength of 615 nm after digitonin (160 μ M) treatment as described earlier [25]. Production of ROS is expressed as percentage of control. Four parallel wells per exposure in a 48-well plate were used in ROS experiments. The mean of these values were calculated and handled as a single independent experiment. ROS results are expressed as the mean \pm SD. Statistical significance was analyzed with one-way ANOVA followed by Tukey's multiple comparison test in all comparisons.

3. Results and discussion

BPA is a much used plasticizer which, however, is a known reproductive toxicant with endocrine disrupting properties and potentially damaging human health (for recent reviews, see e.g. [1,27]. Because BPA is a common contaminant in community water, it is essential to create approaches to purify the water and ensure the results of purification. In this study, we report

that CWAO and photocatalysis clearly reduced the concentration of BPA in water, which reflects in the toxicity of the water in studies using cells of human origin. Further studies would be needed to get full idea of the potential beneficial effects of these water purification procedures on the reproductive toxicity of BPA.

3.1. Toxicity of bisphenol A

BPA as a pure compound is clearly dose-dependently toxic to human MCF-7 breast cancer cells as analyzed by the MTT-test (Fig. 1). At all studied time points (24, 48 and 72 h) BPA decreased the viability of the cells statistically significantly when added to give 200 μ M (equals to 46 mgL⁻¹) concentration in the cell cultures (Fig. 1 d)-e)). A decrease in cell number and changes in cell morphology were seen (Fig. 1 a)-c)). Recently, BPA has shown toxicity also in other types of cells relevant for reproduction. Toxicity was indicated by the reduction of aromatase activity in human JEG-3 placental trophoblastic cancer cells [28]. In mouse GC-2 spermatocyte-derived cells BPA inhibited proliferation and increased apoptosis [29]. The significance of these findings lie in the fact that disturbance in the balance of cell proliferation and apoptosis reduce capacity of tissue renewal and is a mechanism of organ toxicity.

Another measure of potential toxicity is the increase in reactive oxygen species or ROS (for a review, see [30]). ROS can damage DNA and are also involved in other mechanisms of cellular toxicity, such as lipid peroxidation [31]. The same concentration (200 μ M which equals 46 mgL⁻¹), which decreased viability, also increased ROS formation statistically significantly at all studied time points (Fig. 2). Our results and those from the literature support the involvement of ROS in BPA toxicity. BPA-induced ROS increase was also seen in human JEG-3 cells [28] and in mouse spermatocyte-derived GC-2 cells [29]. In vivo work with male rats has shown that BPA leads to an increase in ROS production and is one of the causes of liver fibrosis [32]. Furthermore, in pregnant women the two biomarkers of oxidative stress, 8-hydroxy-

deoxyguanine and 8-isoprostane were significantly associated with BPA concentration in urine [33].



Figure 1. Light microscopy of human MCF-7 breast cancer cells in culture. A) Control (0.1% DMSO). B) 50 μ M BPA. C) 200 μ M BPA. Cells were exposed for 48 h. Magnification 10X. Viability of MCF-7 cells after treatment with 50 μ M and 200 μ M BPA for D) 24 h, E) 48 h and F) 72 h was analyzed by MTT test. MTT test reflects mitochondrial function of the cells. In controls only the solvent, DMSO in similar concentration was added. For comparison with control One-way ANOVA and Tukey's multiple comparison test were used. The columns represent mean \pm SD (n=4), *P < 0.05, **P< 0.01, ***P< 0.001.



Figure 2. The production of reactive oxygen species (ROS) as analyzed by the H₂DCFDA method [25] in human MCF-7 breast cancer cells exposed to 50 μ M or 200 μ M BPA in DMSO for 24, 48 or 72 h. In controls only the solvent, DMSO in similar concentration was added. For comparison with control, One-way ANOVA and Tukey's multiple comparison test were used. The columns represent mean ± SD (n=4), *P < 0.05, **P< 0.01, ***P< 0.001.

3.2. Hybrid catalyst development

The hybrid catalyst development route is described in Figs. 3 and 4. The different steps in the preparation procedure aim finally towards a catalyst that could be used in both photocatalytic and CWAO processes. The first set of the catalysts was selected mainly due to the photocatalytic activity of the materials. In addition, CeO₂ was selected due to its good activity in CWAO. A selected part from the first set of catalysts was tested in both processes.

P25	Commercial reference catalyst
Ti-600 Ti-500	 'Base' catalyst, to observe the effect of calcination temperature on the phase composition of TiO₂ Calcination at 500 °C and 600 °C
Ti-N-600 Ti-N-500	 pH adjustment by HNO₃ Calcination at 500 °C and 600 °C
Zr	 Another photocatalytically active material that also works in CWAO
Ce	• Known to work in CWAO

Figure 3. The first set of catalysts and why they were selected.

3.3. Testing and characterization of TiO₂, ZrO₂ and CeO₂ catalysts

Table 3 shows the characterization results for the first set of catalysts and Table 4 shows BPA and TOC removal percentages by photocatalysis and CWAO for the corresponding catalysts. The BET surface area and pore volume of the reference catalyst P25 were significantly higher than the corresponding values for the other catalysts. This is important, since the higher BPA and TOC removal percentages were achieved by photocatalysis with P25. However, P25 did not show adequate activity towards removing BPA by CWAO. Ti-600 and Ti-500 were used as base catalysts to observe the effect of calcination temperature on the phase composition of TiO₂. The contents of anatase and rutile were 31% and 69% for Ti-600 and 94% and 6% for Ti-500, respectively, but a considerable difference in photocatalytic degradation was not observed. Then, the effect of pH during catalyst preparation was studied with Ti-N-600 and Ti-N-500.

These samples showed similar BPA removal percentages as the base catalysts. Furthermore, a significant decrease in TOC removal was observed, which was probably due to the increased acidity of these catalysts [34]. Thereafter, the experiments were performed with Zr, another photocatalytically active material that is known to be active also in CWAO. It was observed that Zr led to markedly lower BPA conversion and TOC removal by photocatalysis compared to titania catalysts. Moreover, the BPA reduction by CWAO was not sufficient. The CeO₂ catalyst is known to be active in CWAO with several compounds and as it can be seen from Table 4, a high conversion of BPA was achieved with Ce. Unfortunately, CeO₂ is not photocatalytically active material i.e. the removal of BPA by photocatalysis was less than 60%.

Table 3. Specific surface areas (BET), pore volumes, pore sizes and phase compositions of the first set of catalysts.

Catalyst	BET	Pore volume	Pore size	Anatase	Rutile
	(m^2g^{-1})	(\mathbf{mLg}^{-1})	(nm)	(%)	(%)
P25	53	0.096	7	82	18
Ti-600	3.6	0.010	11	31	69
Ti-500	7.8	0.022	11	94	6
Ti-N-600	1.6	0.006	15	13	87
Ti-N-500	1.2	0.003	9	94	6
Zr	12	0.014	5		
Ce	98	0.359	15		

	Removal (%)							
Catalyst	Photocatalysis,	UV-B, after 3h	CW	AO				
	BPA	тос	BPA	ТОС				
P25	100	79	37	11				
Ti-600	99	27						
Ti-500	96	39	27	20				
Ti-N-600	97	16						
Ti-N-500	96	10						
Zr	67	8	33	15				
Ce	56	11	91	85				

Table 4. BPA and TOC removal percentages by photocatalysis and CWAO for the first set of catalysts.

The results confirmed that the catalysts used typically in photocatalytic treatments were not directly effective in CWAO and further development of these catalysts is required. This was expected, since in CWAO, the materials having high oxygen storage capacity and representing low temperature for activation of molecular oxygen are generally more active, such as CeO₂. In photocatalysis, semiconducting materials (e.g. TiO₂) are used that are able to generate highly active hydroxyl radicals that are able to oxidize organic pollutants in water. Even though both the materials are metal oxides, combining these required features in the same material is a challenging task. The experiments showed us clearly the starting point for the catalyst development and the next step in our study was to modify the catalyst composition in such a way that the efficiency in both the processes could be achieved. A natural continuation is the preparation of mixed oxides.

3.4. Testing and characterization of the improved catalysts

Fig. 4 shows the development route of the second set of the catalysts.



Figure 4. Development route of the second set of catalysts.

In order to enhance the performance of the photocatalyst in CWAO, Zr and Ce were added into TiO₂, and further, Ag and Pt were introduced as additives. The characterization results and BPA and TOC removals are presented in Tables 5 and 6, respectively. The addition of Zr and Ce increased the surface area of the catalyst significantly compared to pure oxides. According to the XRD analysis both mixed oxides have an amorphous structure while Ce and Ti catalysts were crystalline. The amorphous structure of the mixed oxides and smaller crystallite sizes of CeO₂ and ZrO₂ explain the higher surface area of 0.5Ti-05.Zr and 0.8Ti-0.2Ce compared to single oxides [35]. However, no increase in the removal of BPA by CWAO was observed in the 0.5Ti-0.5Zr catalyzed reaction, but in photocatalysis the result was slightly better than with Zr. The abatement of BPA by CWAO over the 0.8Ti-0.2Ce catalyst was still high, similar to

Ce (Table 4) and also a slight improvement in the photocatalytic activity was observed (Table 6). Further, with the Ag/P25 catalyst the total removal of BPA was achieved in the photocatalytic reaction, but unfortunately leaching of Ag occurred during the experiment and thus no CWAO of BPA was observed over this material (50% of Ag leached compared to the Ag content of the fresh catalyst). Due to the leaching of Ag, Pt was added as an active metal on the 0.8Ti-0.2Ce support. The addition of Pt decreased the surface area slightly, but the removal of BPA by photocatalysis increased significantly compared to the pure support. Furthermore, no leaching of Pt was observed neither in the photocatalysis nor the CWAO experiment. The removal of TOC in photocatalysis was very low with all the catalysts except Ag/P25. However, it should be noted that TOC was measured by two different methods, which could explain the TOC results.

Based on the results, currently the best choice as a bi-functional catalyst is Pt/0.8Ti-0.2Ce. To understand better the importance of the interaction of compounds in the mixed oxide a test with a mechanical mixture was done. It was observed that some benefit in photocatalysis is gained when TiO_2 and CeO_2 are in separate phases. In contrast, CWAO seems to work better with the mixed oxide.

Table 5. Specific surface areas (BET), pore volumes, pore sizes and phase compositions of the second set of catalysts.

Catalyst	BET	Pore volume	Pore size	Anatase	Rutile
	(m^2g^{-1})	(mLg ⁻¹)	(nm)	(%)	(%)
0.5Ti-0.5Zr	159	0.088	2.2		
0.8Ti-0.2Ce	123	0.127	4.1		
Ag/P25	51	0.365	28	78	22
Pt/0.8Ti-0.2Ce	104	0.116	4.5		
CeO ₂ +TiO ₂					
mech. mixt.	23	0.083	14		

	Removal (%)						
Catalyst	Photocatalysis	s, UV-B, after 3h	CW	AO			
	BPA	ТОС	BPA	ТОС			
0.5Ti-0.5Zr	85	7	33	20			
0.8Ti-0.2Ce	64	5	77	80			
Ag/P25	100	61					
Pt/0.8Ti-0.2Ce	89	6	72	48			
CeO ₂ +TiO ₂ mech.	100	9	63	52			

Table 6. BPA and TOC removal percentages by photocatalysis and CWAO for the second set of catalysts.

3.5. Understanding of the hybrid process concept

To take one step closer to the final hybrid process the most potential catalyst (Pt/0.8Ti-0.2Ce) was tested in sequential treatment of BPA. The experiments were carried out first with CWAO and the resulting effluent was collected for the further treatment with photocatalysis. During the sequential treatment, the samples were collected for toxicity analysis from the original solution, after CWAO and after photocatalysis.

The results of the two consecutive tests are presented in Table 7, both for the developed hybrid catalyst and for the mechanical mixture of CeO₂ and TiO₂. During the CWAO treatment BPA degraded to about half of its initial concentration after which in photocatalysis, 84% removal of BPA was achieved. In terms of TOC the total degradation seems to stay a bit over 50%, and it seems that the photocatalytic treatment after CWAO is not able to improve the TOC removal significantly. When comparing the results of consecutive tests to earlier results, it can be seen that the BPA removal is higher than in CWAO alone and the TOC removal is higher than in photocatalysis or CWAO alone. A significant benefit from separate phases of CeO₂ and TiO₂ was not observed in these experiments. This result shows the viability of the consecutive

processing by using a bi-functional catalyst. Furthermore, the analysis of a spent hybrid catalyst after the consecutive test did not show any Pt leaching.

Table 7. Measured BPA concentrations, TOC and total removal percentages related to the consecutive experiments.

Catalyst	BPA (mgL ⁻¹)				TOC (mgL ⁻¹)			O+Pho alysis
	Initial	After CWAO	After Photo- catalysis	Initial	After CWAO	After Photo- catalysis	BPA rem (%)	TOC rem (%)
Pt/0.8Ti-0.2Ce CeO ₂ +TiO ₂	56	16	9	46	24	21	84	54
Mech. Mixture	57	21	10	46	22	21	82	54

3.6. Analysis of by-products

According to the TOC measurements of treated samples the removal of organic carbon was not as high as the degradation of BPA (Tables 4, 6 and 7). The lower TOC conversions are related to the by-products formed during the degradation and several of those have already been identified. In the study of Mezohegyi *et al.* [36] only p-hydroxyacetophenone, acetic and formic acids were identified in the final effluent after the treatment of BPA by CWAO while in the ozonation of BPA [37] almost ten compounds such as catechol and orthoquinone were detected. Further, in the photocatalytic degradation of BPA [6] three intermediates, of which one was phydroxyacetophenone, were observed. Therefore, the qualitative analysis of by-products was performed by liquid chromatography-mass spectrometry (LC-MS) after the Pt/0.8Ti-0.2Ce catalyzed consecutive test (Fig. 5).



Figure 5. The LC-MS chromatogram of treated water after consecutive test with Pt/0.8Ti-0.2Ce. In addition to BPA at 3.19 min, seven other compounds (A-G) were identified. The peaks named "UK" are unknown and the peaks marked by * are impurities.

In addition to BPA at the retention time of 3.19 min, seven compounds (A-G) were identified from the sample. Two peaks were named "UK" due to the unknown structure of these products while peaks marked by * were impurities containing e.g. Fe which was probably leached out from the CWAO reactor. In the LC-MS chromatogram the lower retention times of compounds A-F refer to the formation of more polar products than BPA during the consecutive test. Table 8 presents [M-1] ions in the negative mode of LC-MS, detected molecular formulas and tentative structures of the compounds A-G. The compounds B and C are isomers with the tentative structures of p- and o-hydroxyphenones from which 4-hydroxyacetophenone was detected earlier after CWAO [36] and photocatalytic treatment of BPA [6]. The compound A is probably formed after cleaving of methyl group from the structures B and C. Furthermore, compounds D-F with molecular weights smaller than BPA are similar with those observed also by [37-39]. However, it should be noted that the relative abundance of the compounds A and C-G are much lower than BPA, and also lower than the compound B, which creates inaccuracy to the results. Therefore, the proposed structures of these by-products are only tentative.

Table 8. LC-MS data detected from the oxidized BPA solution after the consecutive test(CWAO + photocatalysis) catalyzed by Pt/0.8Ti-0.2Ce.

Compound	Retention time (min)	[M-H]	Formula	Tentative structure
Α	1.94	121	C7H6O2	НО
В	2.03	135	$C_8H_8O_2$	но
С	2.15	135	$C_8H_8O_2$	но
D	2.38	189	$C_{12} H_{14} O_2$	HO
E	2.50	163	C9 H8 O3	НООН НО
F	2.60	209	C ₁₅ H ₁₄ O	но
BPA	3.19	227	$C_{15}H_{16}O_2$	но-Он
G	3.28	271	$C_{16}H_{16}O_4$	ноОн

3.7. The effects of wastewater treatment on cell toxicity

Treatment of BPA-water with CWAO alone or with photocatalysis decreased the toxicity of the water. Viability of the cells decreased about 60% when cultured in medium prepared with untreated 60 mgL⁻¹ BPA-water (final concentration in culture 45 mgL⁻¹ due to dilution during the preparation of the culture medium, see Table 2). When the cells were exposed to water treated by CWAO alone, or by the combination of CWAO and photocatalysis, the viability of the cells was at the control level (Fig. 6). Although photocatalysis further reduced the concentration of BPA in the purified water, no further increase in viability was seen because already CWAO alone increased the viability to control level. To make sure that the effect behind this phenomenon is really the decrease in the BPA concentration, the original BPA-water (60 mgL⁻¹) was diluted and the effect of the dilution on cell viability was studied. As shown in Fig. 7, cell viability increased with decreasing BPA concentration so that with 10 mgL⁻¹ viability was at control level.



Figure 6. The effect of purification by catalytic wet air oxidation (CWAO) alone or in combination with photocatalysis (PC) on the viability analyzed by MTT test of human MCF-7 breast cancer cells. MTT test reflects the function of mitochondria. Cells were cultured for 48 h in medium prepared in water originally containing 60 mgL⁻¹ BPA, with or without purification. Medium for control was prepared with MilliQ purified water. BPA in purified water was analyzed and the final calculated concentration in culture is given (for more details see Table 2). For comparison with control, One-way ANOVA and Tukey's multiple comparison test were used. The columns represent mean \pm SD (n=4), ***P< 0.001.



Figure 7. The effect of bisphenol A (BPA) concentration on the viability analyzed by MTT test in human MCF-7 breast cancer cells. MTT test reflects the function of mitochondria. Cells were cultured for 48 h in medium prepared in water originally containing 60 mgL⁻¹ BPA from which dilutions were made. Both of the experiments carried out are shown.

With BPA-water (final concentration of BPA in cell culture 45 mgL⁻¹) ROS increased, but not statistically significantly (Fig. 8). This is somewhat discrepant with the findings with pure BPA (Fig. 2) where the higher concentration of BPA (200 μ M or 46 mgL⁻¹) increased ROS production statistically significantly and the lower concentration (50 μ M or 11.5 mgL⁻¹) did not increase ROS at all. More importantly, CWAO increased ROS production statistically significantly. Because ROS increased, although viability was not affected, this implicates that purification creates some by-products which may be toxic to the cells.



Figure 8. The effect of bisphenol A (BPA) on the formation of reactive oxygen species (ROS) as analyzed by the H₂DCFDA -method [25] in human MCF-7 breast cancer cells. Cells were cultured for 48 h in medium prepared in water originally containing 60 mgL⁻¹ BPA, with or without purification by catalytic wet air oxidation (CWAO) alone or in combination with photocatalysis (PC). Medium for control was prepared with MilliQ purified water. BPA in purified water was analyzed and the final calculated concentration in culture is given (for more details see Table 2). For comparison with control, One-way ANOVA and Tukey's multiple comparison test were used. The columns represent mean \pm SD (n=4), *P < 0.05.

The importance of purifying wastewater from BPA stems from the fact that it is widely distributed, a potentially toxic compound, especially for reproduction. In this study it was clear that BPA can reduce the viability of MCF-7 cells which represent one toxicologically relevant tissue as a target of endocrine disruptors, hormonally regulated breast tissue. Breast cancer incidence is increasing worldwide and endocrine disruptors are putative participants in the increase (for a recent review see e.g. [40]). Probable developmental fetal origin of breast cancer and other adult onset diseases (reviewed in [41]) emphasize the importance of water purity

especially for pregnant women. When compared to the concentrations described in the literature, the concentrations used in this study are higher than typical environmental concentrations to which people are exposed [40]. On the other hand, if the wastewater purification methods are not improved and updated, concentrations in community water will inevitably increase by time.

4. Conclusions

BPA is toxic to cells and the toxicity is related to its concentration in wastewater. Moreover, the amount of BPA in wastewater is not adequately decreased by the current wastewater treatment processes. Potential technologies for more efficient BPA treatment are CWAO and photocatalysis. Especially, the combination of these methods which was more efficient in the reduction of BPA than either alone, and might bring up savings in costs and further improvements in the efficiency of the process. The results of this study show that Pt/0.8Ti-0.2Ce is a potential material to be used as a bi-functional catalyst for the consecutive photocatalytic-CWAO treatment of BPA. In the consecutive treatment of BPA (photocatalysis after CWAO) over 80% BPA and 50% TOC removal were achieved with Pt catalyst. However, several by-products were observed after consecutive treatment that may increase ROS indicating toxicity to the cells and justifying the need for the further development of the process.

Acknowledgements

The authors gratefully acknowledge the Academy of Finland for providing research funding, AOPI project (263397) within the research program for Sustainable Governance of Aquatic Resources (AKVA). Authors would like to thank PhD Tao Hu for the XRD and M.Sc. Markus Riihimäki for XRF analyses (University of Oulu). Senior technicians Virpi Koponen, Pirjo Hänninen (University of Eastern Finland), Tuomo Vähätiitto, Lotta Hekkala and Aleksanteri Nikula (University of Oulu) are acknowledged for excellent technical assistance.

Disclosure statement

No conflict of interest was reported by the authors.

References

[1] Seachrist DD, Bonk KW, Ho SM, et al. A review of the carcinogenic potential of bisphenol
A. Reprod Toxicol. 2016;59:167–182. <u>http://doi.org/10.1016/j.reprotox.2015.09.006</u>

[2] Wolstenholme JT, Rissman EF, Connelly JJ. The role of Bisphenol A in shaping the brain, epigenome and behavior. Hormones and Behavior 2011;59:296–305. http://doi.org/10.1016/j.yhbeh.2010.10.001.

[3] Ferreira LL, Couto R, Oliveira PJ. Bisphenol A as epigenetic modulator: setting the stage for carcinogenesis. Eur J Clin Invest. 2015;45:32–36. <u>http://doi.org/10.1111/eci.12362</u>.

[4] Hong CS, Wang Y, Buch B. Kinetics and products of the TiO₂, photocatalytic degradation
 of 2-chlorobiphenhyl in water. Chemosphere 1998;36:1653–1667.
 http://doi.org/10.1016/S0045-6535(97)10040-6.

[5] Staples CA, Dome PB, Klecka GM, et al. A review of the environmental fate, effects, and exposures of Bisphenol A. Chemosphere 1998;36:2149–2173. <u>http://doi.org/10.1016/S0045-6535(97)10133-3</u>.

[6] Ohko Y, Ando I, Niwa C et al. Degradation of Bisphenol A in water by TiO₂ photocatalyst.
 Environ Sci Technol. 2001;35:2365–2368. <u>http://doi.org/10.1021/es001757t</u>.

[7] Erler C, Novak J, Bisphenol A exposure: Human risk and health policy. J Pediatr Nurs.
2010; 25:400–407. <u>http://doi.org/10.1016/j.pedn.2009.05.006</u>.

[8] Marttinen SK, Kettunen RH, Rintala JA, Occurrence and removal of organic pollutants in sewages and landfill leachates. Sci Total Environ. 2003;301:1–12. http://doi.org/10.1016/S0048-9697(02)00302-9.

[9] M. Clara, B. Strenn, E. Saracevic, N. Kreuzinger, Adsorption of bisphenol-A, 17β -estradiole and 17α -ethinylestradiole to sewage sludge Chemosphere 56 (9) (2004) 843-851. <u>http://doi.org/10.1016/j.chemosphere.2004.04.048</u>.

[10] J.H.W.R.J. Hoigné, H. Bader, W.R. Haag, J. Staehelin, Rate constants of reactions of ozone with organic and inorganic compounds in water—III. Inorganic compounds and radicals, Water Res. 19 (8) (1985) 993-1004. <u>http://doi.org/10.1016/0043-1354(85)90368-9</u>.

[11] J. Spivack, T. K. Leib, J. H. Lobos, Novel pathway for bacterial metabolism of bisphenolA, J. Biol. Chem. 269 (10) (1994) 7323-7329.

[12] R. Zouaghi, Study of the photocatalytic conversion of two herbicides of the family of phenylureas in aqueous-solution method of coupling with the ultrasonic, PhD Thesis, N° 89/T.E/2006. Univ. Mentouri-Constantine, 2006.

[13] X. Zhang, W. Li, Z. Yang, Toxicology of nanosized titanium dioxide: an update, Arch Toxicol. Dec. 89(12) (2015) 2207-17. https://doi.org/10.1007/s00204-015-1594-6

[14] U. Lafont, Mesoporous titanium oxides: synthesis, characterization and surface modification, PhD Thesis, University of Montpellier, 2003.

[15] M.R. Hoffmann, S.T. Martin, W. Choi, D.W. Bahnemannt, Environmental applications of semiconductor photocatalysis, Chem. Rev. 95 (1995) 69-96.

[16] J. Levec, A. Pintar, Catalytic wet-air oxidation processes: A review, Catal. Today 124 (3-4) (2007) 172-184. <u>http://doi.org/10.1016/j.cattod.2007.03.035</u>.

[17] A. Cybulski, Catalytic wet air oxidation: are monolithic catalysts and reactors feasible?
Ind. Eng. Chem. Res. 46 (12) (2007) 4007-4033. <u>http://doi.org/10.1021/ie060906z</u>.

[18] J. Mikulová, S. Rossignol, J. Barbier, D. Duprez, C. Kappenstein, Characterizations of platinum catalysts supported on Ce, Zr, Pr-oxides and formation of carbonate species in catalytic wet air oxidation of acetic acid, Catal. Today 124 (3-4) (2007) 185-190. http://doi.org/10.1016/j.cattod.2007.03.036.

[19] J. Mikulová, S. Rossignol, J. Barbier, D. Mesnard, C. Kappenstein, D. Duprez, Ruthenium and platinum catalysts supported on Ce, Zr, Pr-O mixed oxides prepared by soft chemistry for acetic acid wet air oxidation, Appl. Catal. B 72 (1-2) (2007) 1-10. http://doi.org/10.1016/j.apcatb.2006.10.002.

[20] J. Wang, W. Zhu, X. He, S. Yang, Catalytic wet air oxidation of acetic acid over different ruthenium catalysts, Catal. Commun. 9 (13) (2008) 2163-2167.
<u>http://doi.org/10.1016/j.catcom.2008.04.019</u>.

[21] J.E. Duffy, M.A. Anderson, C.G. Hill, W.A. Zeltner, Photocatalytic oxidation as a secondary treatment method following wet air oxidation, Ind. Eng. Chem. Res. 39 (10) (2000) 3698-3706. <u>http://doi.org/10.1021/ie9909410</u>.

[22] A. Katsoni, H.T Gomes, L.M. Pastrana-MartÍnez, J.L Faria, J.L. Figueiredo, D. Mantzavinos, A.M.T. Silva, Degradation of trinitrophenol by sequential catalytic wet air oxidation and solar TiO₂ photocatalysis, Chem. Eng. J. 172 (2) (2011) 634-640. http://doi.org/10.1016/j.cej.2011.06.022.

[23] J. Blanchard, F. Ribot, C. Sanchez, P.V. Bellot, A. Trokiner, Structural characterization of titanium-oxo-polymers synthesized in the presence of protons or complexing ligands as inhibitors, J. Non-Cryst. Solids, 265 (1-2) (2000) 83-97. <u>http://doi.org/10.1016/S0022-3093(99)00885-6</u>.

[24] S. Azalim, M. Franco, R. Brahmi, J.M. Giraudon, J.F. Lamonier, Removal of oxygenated volatile organic compounds by catalytic oxidation over Zr-Ce-Mn catalysts, J. Hazard. Mater.
188 (1-3) (2011) 422-427. <u>http://doi.org/10.1016/j.jhazmat.2011.01.135</u>.

[25] J. Loikkanen, J. Naarala, K.H. Vahakangas, K.M. Savolainen, Glutamate increases toxicity of inorganic lead in GT1-7 neurons: partial protection induced by flunarizine, Arch. Toxicol. 77 (12) (2003) 663-671. <u>http://doi.org/10.1007/s00204-003-0498-z</u>.

[26] J. Hansen, P. Bross, A cellular viability assay to monitor drug toxicity, Methods Mol. Biol.
648 (2010) 303-311. <u>http://doi.org/10.1007/978-1-60761-756-3_21</u>.

[27] J.R. Rochester, Bisphenol A and human health: A review of the literature, Reproductive Toxicology 42 (2013) 132-155. <u>http://doi.org/10.1016/j.reprotox.2013.08.008</u>.

[28] E. Pérez-Albaladejo, D. Fernandes, S. Lacorte, C. Porte, Comparative toxicity, oxidative stress and endocrine disruption potential of plasticizers in JEG-3 human placental cells, Toxicol. In Vitro 38 (2017) 41-48. <u>http://doi.org/10.1016/j.tiv.2016.11.003</u>.

[29] L. Yin, Y. Dai, Z. Cui, X. Jiang, W. Liu, F. Han, A. Lin, J. Cao, J. Liu, The regulation of cellular apoptosis by the ROS-triggered PERK/EIF2α/chop pathway plays a vital role in bisphenol A-induced male reproductive toxicity, Toxicol. Appl. Pharmacol. 314 (2017) 98-108. http://doi.org/10.1016/j.taap.2016.11.013.

[30] X. Gu, J.E. Manautou, Molecular mechanisms underlying chemical liver injury, Expert Rev. Mol. Med. 14 (2012) e4. <u>http://doi.org/10.1017/S1462399411002110</u>.

[31] M.E. Goetz, & A. Luch, Reactive species: a cell damaging rout assisting to chemical carcinogens, Cancer Lett. 266 (1) (2008) 73-83. <u>http://doi.org/10.1016/j.canlet.2008.02.035</u>.

[32] S.E. Elswefy, F.R. Abdallah, H.H. Atteia, A.S. Wahba, R.A. Hasan, Inflammation, oxidative stress and apoptosis cascade implications in bisphenol A-induced liver fibrosis in male rats, Int. J. Exp. Pathol. 97 (5) (2016) 369-379. <u>http://doi.org/10.1111/iep.12207</u>.

[33] K.K. Ferguson, D.E. Cantonwine, T.F. McElrath, B. Mukherjee, J.D. Meeker, Repeated measures analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation and oxidative stress in pregnancy, Reprod. Toxicol. 66 (2016) 93-98. http://doi.org/10.1016/j.reprotox.2016.10.002.

[34] A.R. Ribeiro, O.C. Nunes, M.F.R. Pereira, A.M.T. Silva, An overview on the advanced oxidation processes applied for the treatment of water pollutants defined in the recently launched Directive 2013/39/EU, Environ. Int. 75 (2015) 33-51. http://doi.org/10.1016/j.envint.2014.10.027.

[35] M.A.L. Rocha, G. Del Ángel, G. Torres-Torres, A. Cervantes, A. Vázquez, A. Arrieta, J.N. Beltramini, Effect of the Pt oxidation state and Ce³⁺/Ce⁴⁺ ratio on the Pt/TiO₂-CeO₂ catalysts in the phenol degradation by catalytic wet air oxidation (CWAO), Catal. Today 250, (2015) 145-154. http://doi.org/10.1016/j.cattod.2014.09.016.

[36] G. Mezohegyi, B. Erjavec, R. Kaplan, A. Pintar, Removal of bisphenol A and its oxidation products from aqueous solutions by sequential catalytic wet air oxidation and biodegradation,
Ind. Eng. Chem. Res. 52 (26) (2013) 9301-9307. <u>http://doi.org/10.1021/ie400998t</u>.

[37] M. Deborde, S. Rabouan, P. Mazellier, J.P Duguet, B. Legube, Oxidation of bisphenol A by ozone in aqueous solution, Water Res. 42 (16) (2008) 4299-4308. http://doi.org/10.1016/j.watres.2008.07.015.

[38] K. Lin, W. Liu, J. Gan, Oxidative removal of Bisphenol A by manganese dioxide: Efficacy, products, and pathways, Environ. Sci. Technol. 43 (10) (2009) 3860-3864. http://doi.org/10.1021/es900235f.

33

[39] A.O Kondrakov, A.N. Ignatev, F.H. Frimmel, S. Bräse, H. Horn, A.I. Revelsky, Formation of genotoxic quinones during Bisphenol A degradation by TiO₂ photocatalysis and UV photolysis: A comparative study. Appl. Catal. B 160-161 (2014) 106-114. http://doi.org/10.1016/j.apcatb.2014.05.007.

[40] M. Giulivo, M. Lopez de Alda, E. Capri, D. Barcelo, Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environ. Res. 151 (2016) 251-264.
http://doi.org/10.1016/j.envres.2016.07.011.

[41] K. Vähäkangas, Chemical exposure as etiology in developmental origin of adult onset human cancer, Front. Pharmacol. 2 (2011) 62. <u>http://doi.org/10.3389/fphar.2011.00062</u>.