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Synthesis and evaluation of hydroxypropylated potato starch as polymeric support for benzo[a]pyrene degradation by Fenton reaction

Ana-Maria Rosu[a,b,c], Etienne Veignie[a,b], Gheorghe Surpateanu[a,b,c], Gheorghe Brabie[d], Doru Neculai Miron[c], Catherine Rafin[a,b,*]

[a] Univ Lille Nord de France, Lille 59000, France
[b] ULCO, Unité de Chimie Environnementale et Interactions sur le Vivant, Dunkerque 59140, France
[c] University 'Vasile Alecsandri' of Bacau, Faculty of Engineering, Department of Food Products Engineering, Bacau 600115, Romania
[d] University 'Vasile Alecsandri' of Bacau, CCIMT Centre of Research, Faculty of Engineering, Bacau 600115, Romania

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ABSTRACT

In order to perform an efficient Fenton reaction of benzo[a]pyrene (BaP) in the presence of starch as a reaction matrix, a computer modeling study conducted on amylopectin as a component of potato starch allows to identify three more stable sites, among six, available for BaP and iron complexation. For the purpose of enabling the formation of such a stable complex, starch was irreversibly modified by hydroxypropylation for favoring the accessibility of BaP to available complexation sites. The results show that such an irreversible modification significantly consequently increased starch solubilization in cold water. Hydroxypropyl starch derivative obtained in the optimized synthesis conditions (molar substitution of 0.73) increased significantly the BaP solubility and consequently influenced its degradation (38%) by Fenton oxidation. We might hypothesize that starch depolymerisation occurring through a radical chain mechanism during the Fenton reaction allows the formation of carbon centred radicals, permitting therefore an oxidation attack of BaP.

1. Introduction

Benzo[a]pyrene (BaP), a fused pentacyclic aromatic hydrocarbon, has a very low aqueous solubility (3.8 μg/L) and a high octanol/water partitioning coefficient (6.04), which suggests its preference for non-aqueous phases (Rivas, 2006). It has been classified by the US Environmental Protection Agency as a priority pollutant because of its carcinogenicity, teratogenicity and acute toxicity. Such polycyclic aromatic hydrocarbons (PAHs) are quite recalcitrant to biodegradation in soil because of their aromatic and condensed structure leading to low solubility and then high chemical stability. Among the processes whereby these compounds are removed from the environment, advanced oxidation processes are widely investigated and, in particular, the Fenton reaction which is currently one of the most powerful oxidizing reactions available. In 1894, Fenton discovered that several metals have special oxygen-transfer properties that improve the use of hydrogen peroxide (Fenton, 1894). Some metals have a strong catalytic power to generate highly reactive hydroxyl radicals. The Fenton reaction is used to treat a large variety of pollutants (e.g. phenols, formaldehyde, benzene, toluene, ethylbenzene and xylenes commonly named BTEX, pesticides and PAH) in various ecosystems (water and soil) (Kulik, Goi, Trapido, & Tuhkanen, 2006; Murray & Parsons, 2004).

In previous work (Veignie, Rafin, Landy, Fourmentin, & Surpateanu, 2009), we demonstrated the interest of using cyclodextrins which are a family of cyclic oligosaccharides that are composed of α-1,4-linked glucopyranose subunits (Bender & Komiyama, 1978; Szejtli, 1988, 1998). For most non-polar contaminants, the formation of cyclodextrin-contaminant complexes in the hydrophobic interior of the cyclodextrin molecule increases the apparent solubility of many low-solubility organic contaminants. In our study, hydroxypropyl-β-cyclodextrin (HPBCD) significantly increased the solubilization of BaP, a prerequisite for being oxidized by the hydroxyl radicals generated during the Fenton reaction (Flotron, Delteil, Padellec, & Camel, 2005). However, even if HPBCD seems to be promising for developing PAH soil polluted treatment by Fenton oxidation and also integrated treatment combining chemical and biological oxidation (Rafin, Veignie, Fayeulle, & Surpateanu, 2009), its rather high cost could limit its use for large scales applications.

In order to explore the potential of carbohydrate substitutions by hydroxypropyl groups for enhancing PAH solubilization, we chose to modify by hydroxypropylation more economical car-

* Corresponding author at: ULCO, Unité de Chimie Environnementale et Interactions sur le Vivant, 145, avenue Maurice Schumann, Dunkerque 59140, France. Tel.: +33 (0)3 28 65 82 78.
E-mail address: rafin@univ-littoral.fr (C. Rafin).

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bohydrates such as starch. Starch is a natural, renewable, and biodegradable polymer produced by many plants as a source of stored energy. Starches are composed of unbranched and slightly branched amylose molecules (BeMiller, 1997) and highly branched amylopectin (Manners, 1989). Such chemical modifications of starches with monofunctional reagents, such as alkylene oxides, have been used to introduce new properties of starch and extending the applications of starch for instance in food, paper and textile fields (Kshirsagar & Singhal, 2008). In this work, the chemical modifications of starch were undertaken to investigate the potential use of renewable resources for soil rehabilitation applications.

The scope of the research described here includes the following aims: firstly, a computer modeling study on the fragment of starch and both components (amylose and amylopectin) to evaluate the potential sites of inclusion of BaP and iron (Fe); secondly, investigations of optimal conditions to synthesize hydroxypropylated potato starch monitored by nuclear magnetic resonance (NMR), and finally, the efficiency of obtained hydroxypropylated starch ethers was studied on BaP solubilization and BaP degradation by Fenton reaction.

2. Materials and methods

2.1. Chemicals

BaP at 96% HPLC purity was purchased from Fluka (St. Quentin Fallaviers, France). Propylene oxide (PO) at 99% purity, sodium hydroxide (NaOH) micropearls for analysis, $FeSO_4{\cdot}7H_2SO_4$ at 99.5% purity and standard reagent hydrogen peroxide (H_2O_2) 30% were purchased from Acros Organics (Noisy-Le-Grand, France). Potato starch and dimethyl sulphoxide (DMSO) at 99.5% purity were provided by Panreac Quimica SA (Barcelona, Espana). Methanol (MeOH), dichloromethane (DCM), acetone and other chemicals, except when specified otherwise, were obtained in the highest purity available from Fisher Scientific (Illkirch, France). Hydroxypropyl-β-cyclodextrin (HPBCD) was kindly donated by Roquette Frères (Lestrem, France). Distilled deionized water was used throughout this work.

Table 1
Computed energies of complexes.

Site[a]	$\Delta E^{b}_{\text{amylopectin–BaP}}$ (kcal/mol)	$\Delta E_{\text{amylopectin–Fe}}$ (kcal/mol)
1	−43.3	−2337.2
2	−40.4	−2258.7
3	−137.5	−2297.6
4	525.9	2298.4
5	66.8	2307.7
6	503.1	2267.4

[a] Site number where host molecule can be included with the formation of complexes.
[b] Computed complexation energy calculated by the MM3 method as described in Section 2.

2.2. Molecular modeling

Starch fragment was acquired from data provided by the Cambridge Structural Data Base Center. The structural manipulations on amylose and amylopectin were made using the CAChe library on PC. The study of complex formed was performed by applying a general procedure of multiconformational search with the MM3 force field. The potential energy variation (ΔE) depending on the variation of the dihedral angles (defined between BaP, Fe and starch fragment) is recorded with rotational increments. Thus, using a dummy atom placed in different positions, the docking of the inclusions of BaP has been performed. Results obtained with amylopectin (sites available for BaP complexation and computed energies of complexes) are shown, respectively, in Fig. 1 and Table 1.

2.3. Synthesis of hydroxypropylated starch

The reaction was carried out in a round-bottomed flask in continued high agitation under either 21 °C or 50 °C. Starch, propylene oxide and sodium hydroxide were mixed in an aprotic polar solvent quantity having like effect, firstly, the solubilization of starch and, secondly, the reaction between starch and propylene oxide. During the reaction, the following parameters varied: the solvent (DMSO or acetone), the propylene oxide concentration used (26, 32 and 64% based on starch) and the sodium hydroxide concentra-

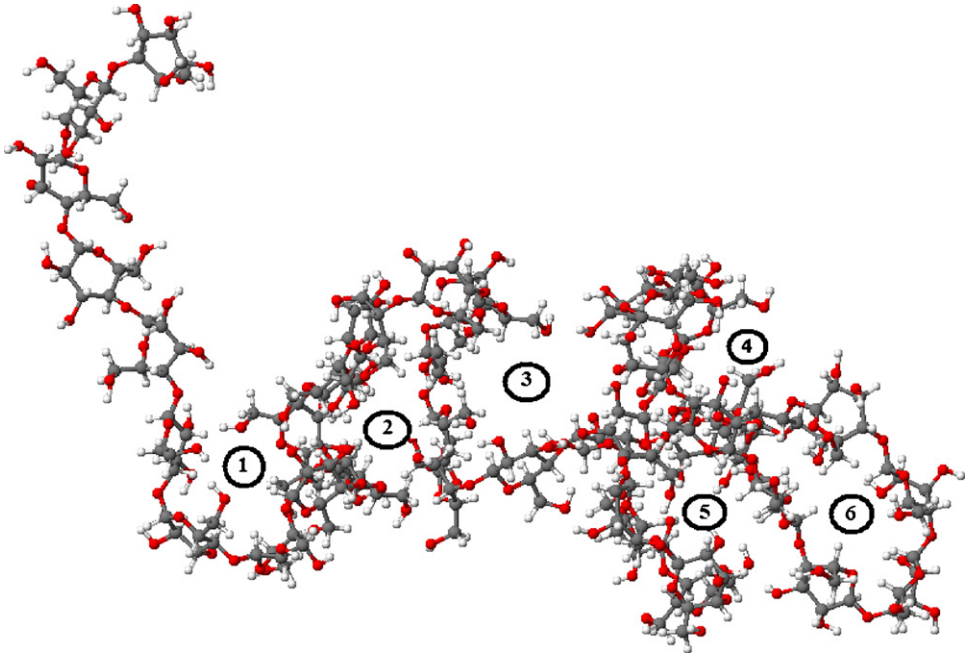

Fig. 1. Amylopectin structure with six sites available for BaP complexation.

tion (0.66 and 7.4% based on starch). On completion of the reaction, hydroxypropylated starch was neutralized with hydrochloric acid and solvent was removed by evaporation. Modified starch was dialyzed during 4 days with a cellulose membrane from Medicell International (MWCO of 12–14,000 Da). Process parameters such as temperature, solvent, starch/propylene oxide ratio, and NaOH concentration were studied with respect to the molar substitution (MS). MS represents the average number of substituents per mole of hydroxyl groups in an anhydroglucose unit of starch. The MS of hydroxypropyled starch was determined according to the method of De Graaf, Lammers, Janssen, and Beenackers (1995). Analysis of the modified starch was carried out using a 250 MHz Spectrospin NMR spectrometer (Bruker, France). ^{1}H NMR spectrum of gelatinised starch is presented in Fig. 2, as a reference, and the NMR spectra of hydroxypropylated starch ethers obtained under various synthesis conditions are related in Fig. 3.

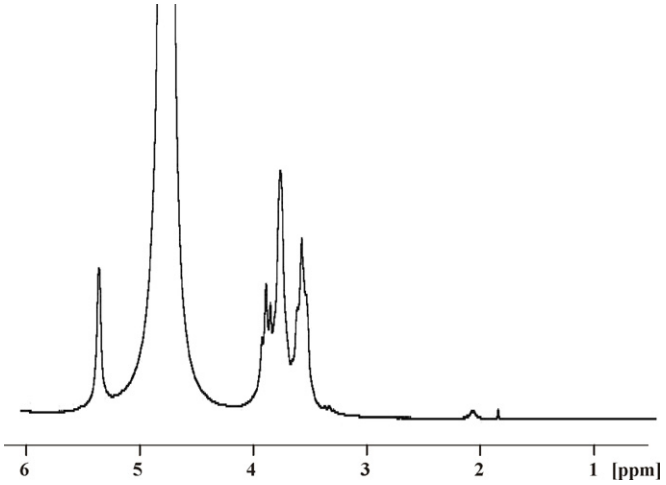

Fig. 2. ^{1}H NMR spectrum of gelatinised starch.

2.4. Modified starch solubility

Chemical modification of starch has like effect the solubilization of starch in inorganic solvent. In order to assess this parameter, a saturated solution of modified starch (either I or II) in distilled water was done at ambient temperature (21–22 °C). After 24 released hours, 10 mL of the solubilized starch was taken, lyophilized and weighed. The same procedure was performed with native starch as a control. The solubility was evaluated in three replicates for each native or modified starch and the solubility mean is expressed in Table 2.

2.5. BaP solubilization in the presence of carbohydrate polymers

BaP was initially dissolved in DCM (0.26 mg/L) and then deposited into a haemolysis tube by the addition of 375 μL of BaP solution and allowing DCM solvent to evaporate. 3 mL of a solution of HPBCD as a reference or one tested potato starch (native or modified one, I or II) was added into the haemolysis tube. All concentrations of carbohydrate polymers were tested at 70 mM equivalent glucose. The tubes were incubated in the dark for 4 days.

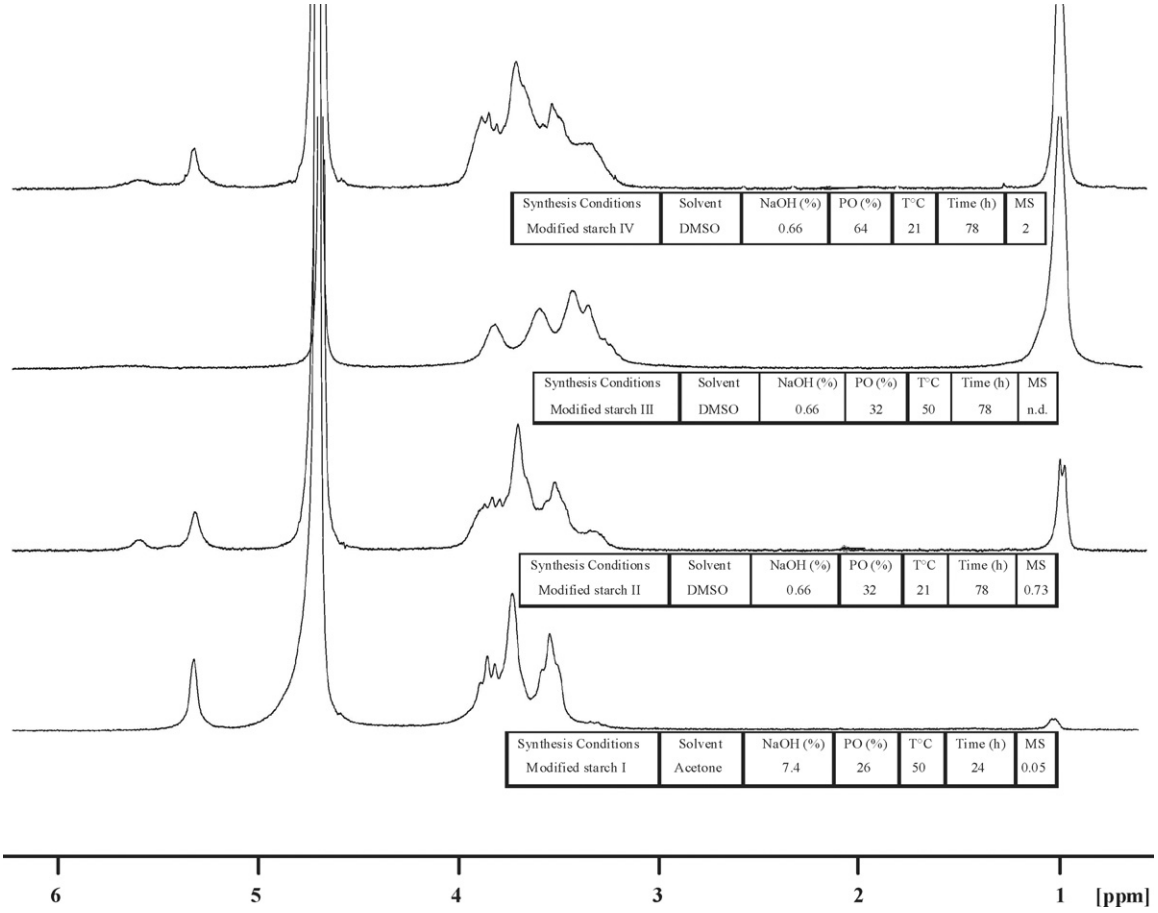

Fig. 3. ^{1}H NMR spectra of hydroxypropylated starch ethers under various synthesis conditions.

Table 2
Solubilization of benzo[a]pyrene in the presence of carbohydrate polymers and Fenton oxidation of benzo[a]pyrene.

Carbohydrate polymer			BaP solubilization		BaP Fenton oxidation		
Compound	MS[a]	Solubility (g/L)	F.I. (a.u.)[b] [BaP, μg/L]	% BaP degradation	Quantity of BaP (μg)		Ratio D/S
					Degraded (D)	Solubilized (S)	
Water	–	–	32.55 ± 0.62 [2.69]	0.00 (NS[c])	0.00	0.00	0.00
HPBCD	0.74	36.00	5282.66 ± 325.71 [131.14]	29.00 (S)	290.00	0.65	446.15
Starch	–	0.56	93.69 ± 0.59 [3.74]	0.00 (NS)	0.00	0.02	0.00
Modified starch I	0.05	6.02	118.34 ± 5.06 [10.83]	4.01 (NS)	40.10	0.05	802.00
Modified starch II	0.73	22.64	153.00 ± 13.10 [11.45]	38.38 (S)	383.80	0.06	6396.67

S, significantly different; NS, not significantly different.
[a] MS, molar substitution (calculated according to details precised in Section 2).
[b] F.I., fluorescence intensity (a.u. arbitrary unit, excitation 295 nm, emission 406 nm) of solubilized BaP in carbohydrate polymer solution (70 mM equivalent glucose).
[c] Statistical analysis performed by two-sample *t*-test comparing treatment with water control ($P = 0.01$).

The sample concentration used in this experiment was selected for two reasons. The concentration of carbohydrates polymers used for BaP solubilization should be low enough to avoid any precipitation of the formed complexes. Moreover, we used carbohydrate polymer concentration as high as possible in order to obtain a significant solubility enhancement after 4 days of incubation. Three separate experiments were conducted under the same conditions and the mean and standard errors were calculated. BaP fluorescence in carbohydrate polymer solutions was analyzed on a Perkin Elmer LS B50 spectrofluorimeter (excitation 295 nm, emission 406 nm, time integration 10 s according to Veignie et al., 2009). Results are expressed as fluorescence intensity mean value ± standard error for triplicates (Table 2).

2.6. Fenton degradation of BaP

All Fenton experiments were conducted in pure distilled deionized water at pH 5.5. 500 μL of BaP–MeOH stock solution (2 g/L) are introduced into 22 mL penicillin flasks so as to give a final BaP quantity of 1 mg per flask. After solvent evaporation, carbohydrate polymers (either HPBCD, native or modified starch I or II) were added as solid and solubilized into 4.9 mL water (working concentration 70 mM equivalent glucose). Similar reaction was carried out with water control. In order to reach the equilibration of inclusion complex, Fenton reactor penicillin flasks were agitated on an orbital shaker (200 rpm) for 12 h at room temperature (22 ± 2 °C). The Fenton reaction was performed, under vigorous vortex shaking, by adding 50 μL of 0.2 M $FeSO_4$ solution and 50 μL of 1 M H_2O_2 in order to obtain a final concentration of 2×10^{-3} M $FeSO_4$ and 10^{-2} M H_2O_2.The Fenton reaction experiments were conducted overnight in the dark at room temperature. At the end of incubation, penicillin flasks were lyophilized for 3 days.

Lyophilized flasks were introduced into a Soxhlet apparatus and extracted for 16 h with DCM. Organic fractions were concentrated in 20 mL DCM/MeOH (50/50, v/v). BaP concentrations were determined using HPLC Waters 600 control system fitted with a Waters XTerra®, RP18, 5 μm, and a Waters 996 Photo Diode Array Detector. The separation was achieved with a 10 min isocatric condition of acetonitrile/water (90/10, v/v) at a solvent flow rate of 1 mL/min. Concentrations were determined by UV absorbance at 254 nm. The percentage of BaP degradation was given by the formula: $[(m_{Ec} - m_T)/m_{Ec}] \times 100$, in which m_{Ec} was the quantity of BaP recovered in extraction controls and m_T was the quantity of BaP obtained in each treatment. In extraction controls, 98% of initial BaP was recovered. Results were expressed as mean value ± standard error for three replicates (Table 2). Statistical analysis was performed by a two-sample *t*-test comparing treatments with water control.

3. Results and discussion

3.1. Molecular modeling

In the modeling study on fragment of starch and amylose, our docking strategy did not allow us to find potential inclusion complex for BaP (data not showed). Concerning the importance of Van der Waals interactions, the filling of the amylopectin cavity should be a crucial factor, ordering the stability of the amylopectin-guest complexes.

Fig. 1 showing the amylopectin structure allows us to identify six sites available for BaP complexation. By our docking strategy, we found the most stable conformer for each inclusion site. In Table 1, ΔE values represent the nature of the computed complexation energies calculated by MM3 method. Among the six sites available in amylopectin, only three are favorable based on potential energy of the inclusion complex. For the complexation of BaP with amylopectin, the highest values of ΔE (−43.3, −40.4 and −137.5 kcal/mol) have been calculated, respectively for sites 1, 2 and 3. The last three sites (4, 5 and 6) with positive values of ΔE are therefore unfavorable inclusion sites for BaP. Concerning the complexation of Fe with studied amylopectin, the highest values of ΔE (−2337.2, −2258.7 and −2297.6 kcal/mol) have been calculated for sites 1, 2 and 3 respectively, whereas the lowest values of ΔE (2298.4, 2307.7 and 2267.4 kcal/mol) have been obtained for sites 4, 5 and 6 respectively.

All these data presented in Table 1 underlined interesting results for our research strategy. Indeed, in order to perform Fenton reaction, both composites (BaP and Fe) need to be in proximity, conditions which could be realized in sites 1, 2 and 3. In summary, this theoretical study shows that amylopectin presents sites for BaP complexation. For the purpose of enabling the formation of such a stable complex, we chose to modify irreversibly starch by hydroxypropylation for favoring the accessibility of BaP to available complexation sites. Such an irreversible modification by propylene oxide will also consequently enhance starch solubilization in cold water.

3.2. Synthesis of hydroxypropylated starch

Fig. 3 summarized the synthesis conditions used in this experiment and the characterization by NMR spectra of the modified starch obtained, in comparison with NMR spectrum of gelatinised starch (Fig. 2). Firstly, using acetone as a reaction solvent during hydroxypropylation led to a low degree of substitution (MS = 0.05) of starch (condition I). Therefore, we employed afterwards DMSO as a reaction solvent, which was chosen due to its ability to solubilize starch at room temperature (Kavitha & BeMiller, 1998). The optimization of starch hydroxypropylation in DMSO was started

by varying only one parameter at a time, keeping the others constant, as summarized in Fig. 3. The substituted starch so obtained was analyzed for MS by NMR spectra. In order to provide a method for producing superior hydroxypropylated starch ether, the first parameter studied was the reaction temperature. Therefore the temperature was increased from 21 °C to 50 °C (condition III). The NMR spectrum of the modified starch obtained in these conditions showed the loss of the equatorial proton signal (5.4 ppm) of the anhydroglucose unit of starch (De Graaf et al., 1995), indicating probably a high degree of starch depolymerisation. The temperature was fixed to a lower value of 21 °C. In the last two conditions (II and IV), the concentration of propylene oxide (either 32 or 64% based on starch) was studied. Even if this last concentration (i.e. 64%) gave a higher degree of substitution (MS = 2) in comparison with the one (MS = 0.73) obtained with only 32% PO, the enhancement of the signal intensity of hydroxypropyl group (1.1–1.2 ppm) indicated probably a polymeric substitution of PO on starch instead of a monomeric one (Helwig & Wilhelm, 1984). Moreover, such a high value (MS = 2) has been seldom reported in data reported in the literature (Biswas et al., 2008). Accordingly, the optimized conditions for preparing hydroxypropylated starch ethers chosen in this study were DMSO as a reaction solvent, 0.66% NaOH (based on starch) and 32% PO (based on starch), reaction temperature of 21 °C and reaction time of 78 h (condition II). In these soft conditions, the NMR spectrum of the modified starch was close to that of the gelatinised starch (Fig. 2), indicating a quite preservation of polymeric structure of the starch. Moreover, the MS obtained was 0.73, a value comparable to the values reported in the literature (De Graaf et al., 1995).

3.3. Solubilization of benzo[a]pyrene in the presence of carbohydrate polymers and Fenton oxidation of benzo[a]pyrene

This optimization of preparing hydroxypropylated starch ethers was conducted with the following objectives: firstly to enhance BaP solubilization, a prerequisite to stimulate the first steps of oxidation (Flotron et al., 2005) which is one of the most limiting factors for BaP degradation in both chemical and biological processes; and secondly, to evaluate the efficiency of Fenton's reagent on BaP degradation. The second experiment was conducted in the presence of each of the selected modified starches obtained in conditions I and II in comparison with the native starch and a well known cyclic oligosaccharide HPBCD (Table 2). Concerning the BaP solubilization (measured by relative fluorescence intensity and expressed by calculus in μg/L), the results indicated that, in our experimental conditions, both the modified starches significantly increased the solubility of BaP. Indeed, solubility of BaP in 70 mM equivalent glucose solution of hydroxypropylated starch derivatives was 10.83 and 11.45 μg/L respectively with modified starch I and II, a 4-fold increase compared to the BaP aqueous solubility of 2.69 μg/L (Table 2). This solubility enhancement was comparable to the one obtained with randomly methylated-β-cyclodextrin (Veignie et al., 2009). Surprisingly, the capacity of the hydroxypropylated starch derivatives to solubilize BaP did not seem to be correlated to their hydroxypropylation degree. Nevertheless, the solubility of the carbohydrate polymers obtained is strongly MS dependent. The enhancement of MS, from 0.05 to 0.73, is linked to an enhancement of water solubility from 6.02 g/L (modified starch I) to 22.64 g/L (modified starch II). This last value is in the same order of magnitude of HPBCD solubility (36.00 g/L).

As illustrated in Table 2, BaP solubility seemed clearly to influence the efficiency of Fenton oxidation. The degradation efficiency increases in the following order: modified starch I (4.01%), HPBCD (29.00%) and modified starch II (38.38%). Such an efficient BaP oxidation using Fenton's reaction has already been reported (Flotron et al., 2005; Kawahara et al., 1995; Nam, Rodriguez, & Kukor, 2001). In control with water or native starch, no degradation of BaP was observed. In such conditions, the low aqueous solubility of BaP limits the quantity of soluble BaP and therefore the Fenton efficiency. These results indicate that the efficiency of Fenton treatment is strongly dependent on the capacity of HPBCD to solubilize BaP, as underlined in previous studies (Veignie et al., 2009). This result is in accordance with the results obtained by Flotron et al. (2005) who assumed that only the solubilized BaP could be oxidized by the hydroxyl radicals generated in the solution. Nevertheless, concerning the modified starch II, the high BaP degradation rate was not correlated to its capacity to solubilize BaP. The BaP fraction degraded (383.80 μg) was greatly superior to the quantity solubilized (0.06 μg). The comparison of the ratio of BaP degraded versus BaP solubilized between HPBCD (446.15) and modified starch II (6396.67), which represents almost a 10-fold enhancement, suggests the involvement of a quite different mechanism during the Fenton reaction. Indeed, during the Fenton reaction, hydrogen peroxide, when catalyzed by ferrous ions, generates strong non-specific oxidant hydroxyl radicals $OH^{\bullet}$ that react with most organic compounds. Nevertheless, due to their high reactivity, hydroxyl radicals have a very short life, limiting therefore the efficiency of Fenton reaction. Consequently, as soon as hydroxyl radicals $OH^{\bullet}$ are formed, they react with molecules in their immediate vicinity with high rate constants (on average 10^9 L/mol/s) according to Halliwell and Gutteridge (2004). In the presence of starch, we might hypothesize that H_2O_2 and $FeSO_4$ (Fenton reagents) could perform starch depolymerisation following a radical chain mechanism (Wu, Xu, Zhao, Kang, & Ding, 2010). Hydroxyl radicals, which are initially generated from H_2O_2 reduction by transition metal ions, abstract hydrogen atoms on the starch chain, providing therefore carbon centred radicals. Their decay could lead to the starch depolymerisation process, permitting probably a radical attack of BaP. Such degradation of starch through the formation of free radicals have been already described on cassava starch degraded by UV and gamma-irradiated (Bertolini, Mestres, Colonna, & Raffi, 2001), during thermolysis of plain starch (Ciesielski & Tomasik, 1996) or also for depolymerisation of polysaccharides with metallic catalysts (Wu et al., 2010). These carbon centred radicals generated during such processes are likely to be more stable due to the high molecular weight of the starch.

This hypothesis is reinforced by our previously conducted modeling studies. Indeed, the high values of ΔE calculated for sites 1, 2 and 3 underlined the complexation of Fe with studied amylopectin, favoring firstly the production of hydroxyl radicals $OH^{\bullet}$ near carbohydrates giving therefore carbohydrate-derived radicals. Secondly, the complexation of Fe by starch could block available sites on the iron (Fig. 1), on which H_2O_2 might attack. Such geometry of the iron–starch complex could lead to a progressive production of hydroxyl radicals $OH^{\bullet}$, favoring therefore a higher efficiency of Fenton treatment with the same H_2O_2 quantity. The supposed longer life of carbohydrate reactive species formed permits them to reach adsorbed BaP, allowing the Fenton reaction to take place at the solid–liquid interface and increasing consequently the efficiency of Fenton degradation. In such conditions, degradation of BaP might be due to a chain reaction occurring in starch from initiation to propagation stage, similar to lipid peroxidation mechanism (Farmer, Koch, & Sutton, 1943). This chain reaction mechanism might explain the high ration BaP degraded versus BaP solubility in the presence of modified starch II.

4. Conclusions

The modification of starch by hydroxypropylation lends to functional properties that could be very promising for our research strategy. Hydroxypropyl starch derivatives permit PAH solubiliza-

tion and were also employed as carrier matrix for complexing iron and BaP in close vicinity suitable for the Fenton reaction. These two main results are the prerequisite in order to develop a environmentally friendly treatment of PAHs for soil remediation approaches.

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