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Removal of Butylated Hydroxyanisole with Enzyme Based Polymerization Using Organo-Clays

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Abstract

3,5-di-tert-butyl-4-hydroxytoluene (BHT) and 2-tert-butyl-4-methoxyphenol (BHA) are used as antioxidants in food. However, considerable concern has arisen from experiments showing that BHA and BHT are potent enhancers of chemically-induced mutagenesis and carcinogenesis in laboratory animals. This work describes a method for the removal of buytlated hydroxyanisole (BHA) compound with an enzyme based polymerization onto organo-clay from aqueous solution. BHA bound organo-clay was prepared from horseradish peroxidase (HRP)-catalyzed polymerization reaction onto nanolayer of organosimectite. The approach described the enzyme catalyzed oxidation of BHA to quartamin modified smectite.

Key Words: BHA, BHT, smectit, organoclay nanocomposites.

INTRODUCTION

Phenolic compounds have been interested by different researchers because of their physiological and physical-chemical properties as well as their anticarcinogenic and high antioxidant capacity. 3,5-di-tert-butyl-4-hydroxytoluene (BHT) and 2-tert-butyl-4-methoxyphenol (BHA) are used as antioxidants in food. However, experiments have arisen considerable concern, showing that BHA and BHT are potent enhancers of chemically-induced mutagenesis and carcinogenesis in laboratory animals [1-3]. Furthermore, there is evidence that in rodents BHA is carcinogenic in the forestomach [4] and BHT in the liver [5-7]. Because of their carcinogenic properties there is a great need for a new and selective technique to be used in BHA separation while treating wastewater.

Adsorption is a well-known technique to separate organic compounds from water. The use of activated carbon as adsorbent for the removal of phenolic compounds is a very common practise [8-10]. The adsorption behavior of various phenols by polymeric adsorbents and ion exchange resin [11-13], the use of clay and orgonoclay as an adsorbent [14,15], molecularly imprinted adsorbents [16-18] and the use of enzymatic polymerization [19-21] have been studied for the purpose of removing phenolics from aqueous solution.

In enzymatic polymerization, horseradish peroxidase (HRP) catalyzes the oxidation of phenolics with hydrogen peroxide, which generates

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phenoxyl radicals. These radicals couple to form larger oligomers which are insoluble in water and can be easily separated by filtration or sedimentation. The enzymatic method had one significant disadvantage: the relatively short catalytic lifetime of the enzyme, which was attributed to the inactivation of the peroxidase [22].

The objective of this study was to extend the catalytic lifetime of this enzyme to improve an economical method for the selective removal of the chosen BHA phenolic and to optimize the reaction parameters in a quartamin modified smectite dispersion medium. The polymer which was produced during horseradish peroxidase (HRP) catalyze oxidation was crosslinked to the organosmectite.

MATERIALS AND METHODS

Materials

HRP and hydrogen peroxide were purchased from Sigma Chemical Company. BHA, BHT and 4-aminoantipyrine were supplied by Aldrich and used as received. All other chemicals were of reagent grade and were purchased from Merck AG. All the water used in the experiments was purified by using a Barnstead (Dubuque, IA) ROpure LP reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANO pure organic/colloid removal and ion exchange packed-bed system.

The smectite (S) used throughout the study was obtained from Northern Anatolia, Turkey and crushed and sieved to have particles of size 200 μm. Some chemical and physical properties of simectite are given in the study of Genç et al.[23].

Instrumentation

High performance liquid chromatography (HPLC) experiments were carried out with Dionex Bio-LC model HPLC system, equipped with a photodiode array dedector. BHA concentrations were determined by measuring the absorbance at 293 nm at 1 $mLmin^{-1}$ and 70/30 Acetonitrile/ultra pure water. Enzyme activity measurement was carried out by the colorimetric monitoring of the oxidative coupling of 4-aminoantipyrine and phenolics in the presence of hydrogenperoxide at 510 nm according to Metelitza et al. [24] with 1601 model Shimadzu UV-spectrometry system. A Fisher Scientific, Accumet® Basic AB15 pH-meter was used to measure pH values.

The experiments were performed in replicates of three and the samples were analyzed in replicates of three as well. For each set of presented data, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin error.

Preparation of Quartamin Modified Smectite

The organoclay was prepared in the way described by Tabtiang et al. and Say et al [25,26]. The simectite (20 g) was dispersed in deionized water (500 mL) at 80ºC. A solution of quartamin [dimethyl(dihydrogenated tallow) ammonium chloride] (0.05 mol) and concentrated HCl (5 mL) in deionized water (100 mL) was added, and the solution was heated and stirred for 3 h. The suspension was filtered, and the solid residue was washed with hot distilled water until no chloride was left. The product was dried at 55 ºC for several days in a fan oven, then dried under vacum for 24 h, yielding the quartamin modified simectite (QS).

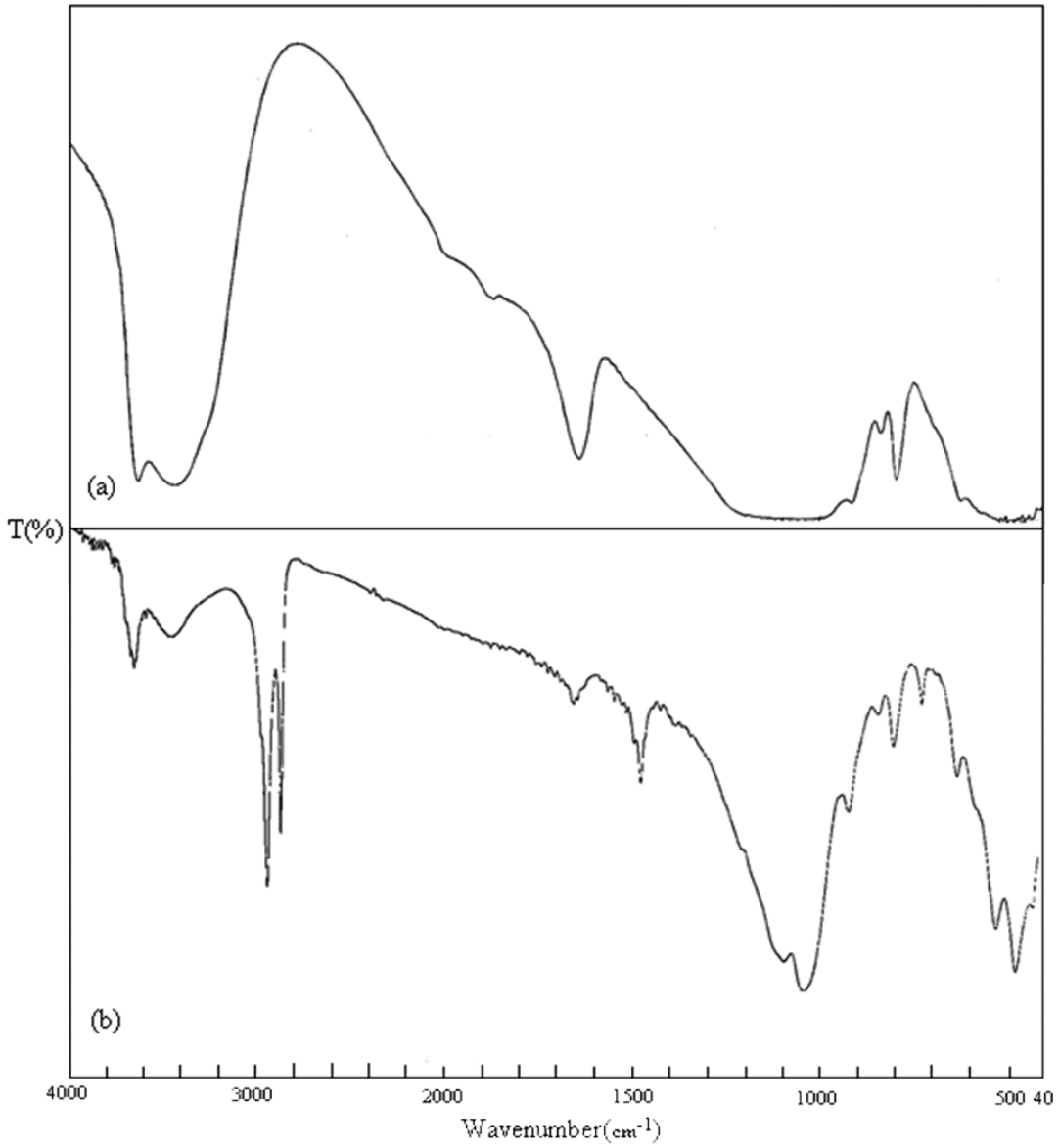

Figure 1. FTIR spectra of a. Smectite b. Organosmectite.

FTIR spectra of smectite and organosmectite (Figure 1) were obtained using FTIR spectrophotometer (Jasco Corparation, Made in Japan; FT/IR-300 E). FTIR spectra of organosmectit is given below: FT-IR (KBr, cm^{-1}): N-H stretching in the 3400 cm^{-1}, C-H stretching in the 2920 cm^{-1} and C-H (concerning CH_2 group) stretching in the 2840 cm^{-1}.

Enzymatic Polymerization of BHA

The effect of enzymatic polymerization of BHA in aqueous solution using S and QS, respectively, was performed as follows. S and QS (0.2 g) were added into a mixture of 20 mL of BHA (100 mgL^{-1}) in borate-phosphate buffer (pH 7.0) and HRP (0.025-0.25 UmL^{-1}). Then, hydrogen peroxide (30 %, 0.2 mL) was added to the dispersion medium. Reaction mixture was carried out at 25ºC with continuos stirring using magnetic stirrers. All reactions were run for 5 h. Polymerization efficiencies were measured by the determination of BHA by HPLC.

RESULTS AND DISCUSSION

Comparison of BHA Transformation for Smectite and Organosmectite

Figure 2 illustrates the application of enzyme

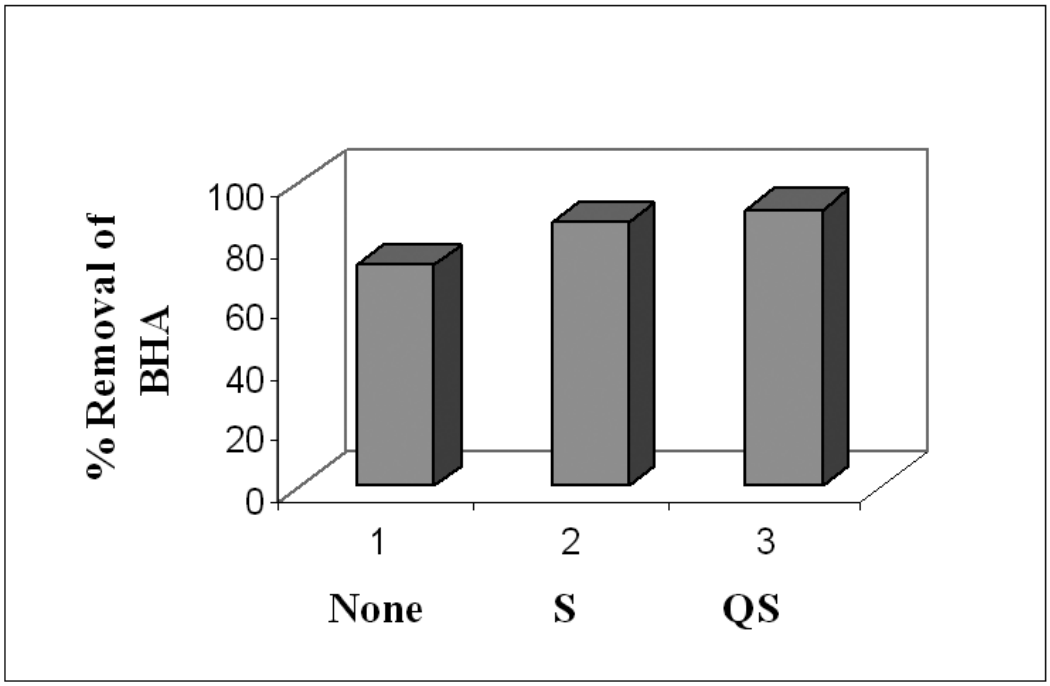

Figure 2. Comparison of Polymerization Efficiency of BHA for S and QS.

mediated polymerization processes to immobilize BHA. The enzyme immobilized S and QS can retain its ability to mediate oxidative coupling processes over long periods and BHA polymers (poly(BHA)) which were produced during the HRP catalysed oxidation are fixed to the S and QS. The addition of HRP may be accomplished through a nanolayer of polymer organosmectit (PQS) or by constructing a permeable reactive barrier with immobilized HRP. In this section, the experiments were designed to achieve a removal of at least 86.5 and 91.0 % of the initial BHA which present in the beginning solution using S and QS, respectively. The results in Figure 2 showed that the removal efficiency of BHA increased when enzyme immobilized S and QS was used.

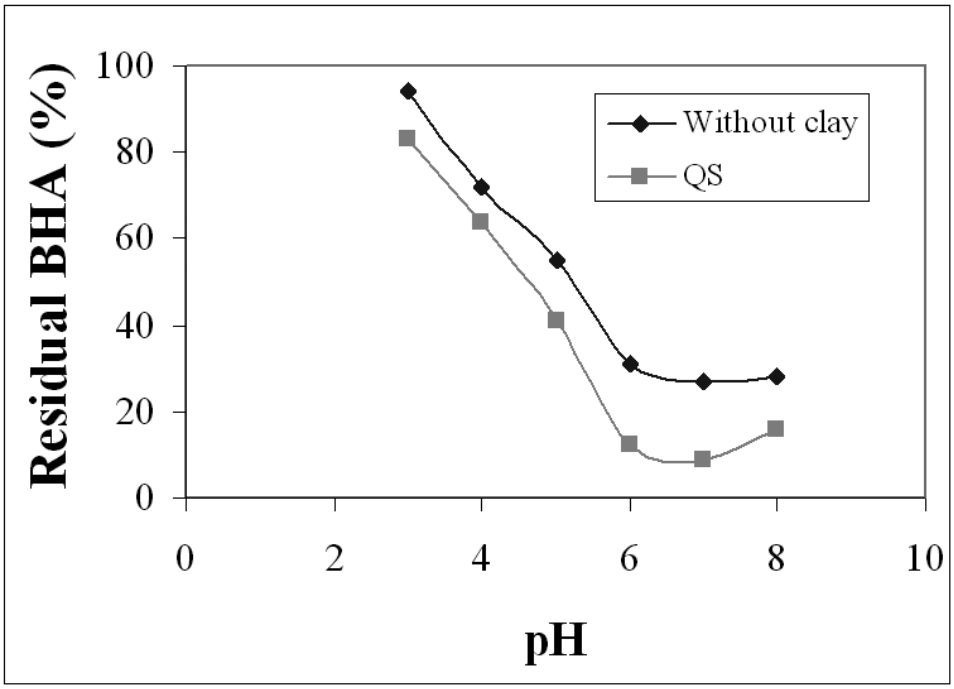

Figure 3. The Effect of pH on BHA transformation.

Removal of BHA using HRP

The effect of pH on the removal of BHA by HRP was examined in the pH range 3.0-8.0, using 100 mgL^{-1} BHA. Figure 3 demonstrates that BHA can be polymerized to a high content over a pH range 6-7. Specifically, up to 91 % transformation of BHA was achieved at pH 7.0, while nonclay polymerization was 73 % at pH 7.0. Based on these results, all further experiments were conducted at pH 7.0.

Figure 4 shows the effect of polymerization time on BHA removal. These experiments were carried out by varying the polymerization time between 15 min. and 240 min. The experiments which have been done both without clay and QS have executed with 0.2 UmL^{-1} HRP and at pH 7.0. It has been observed that the reaction which was applied using QS has reached saturation within 210 min. If clay is not applied during the reaction, the polymerization takes place in a shorter time which is 150 min. The effect of HRP which was adsorbed into organo smectite nanolayers with the help of hydrophobic effect has been continued through 210 min. That is to say the life time and the function of HRP which was adsorbed into nanolayers has been increased by this way.

In the study of Liu et al. [27], removal efficiency of modified HRP has been kept constant after 10 min. Zhang and Nicel [20] have reached a pentachlorophenol (PCP) transformation of 80 % within about 15 min. with 0.1 UmL^{-1} and the same transformation was obtained within about 40 min. with 0.05 U mL^{-1} HRP. From this point it has seen that, the life time based on the removal of different phenolics using HRP are shorter compared to the life time of HRP which was adsorbed to QS modified clays and given in this study.

The effect of the amount of HRP on the transformation of BHA was investigated at pH 7.0. The results (Figure 5) showed that the HRP dose requirements for 97 % removal of BHA decreased from 0.2 UmL^{-1} (in the absence of organoclay) to 0.15 UmL^{-1} (in the presence of organoclay) because of organosmectit immobilization. So, a smaller amount of HRP is needed for the removal of phenolic compound if immobilized organosmectite is used. This may be explained as the getting the

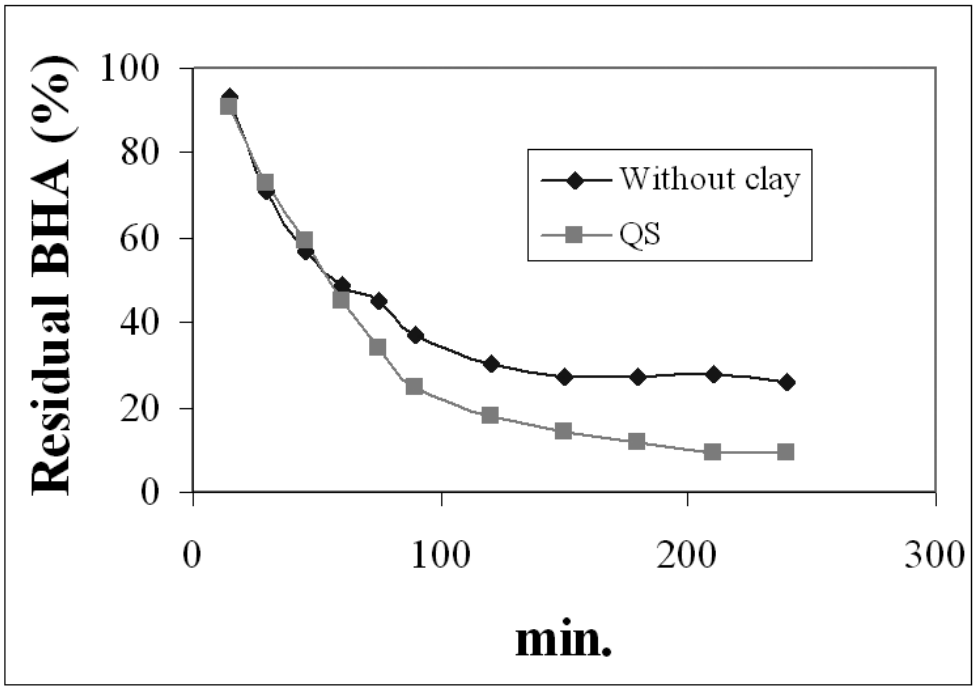

Figure 4. Time Effect on BHA Transformation (100 mgL^{-1}, 0.2 UmL^{-1} HRP, 0.1 mM H_2O_2).

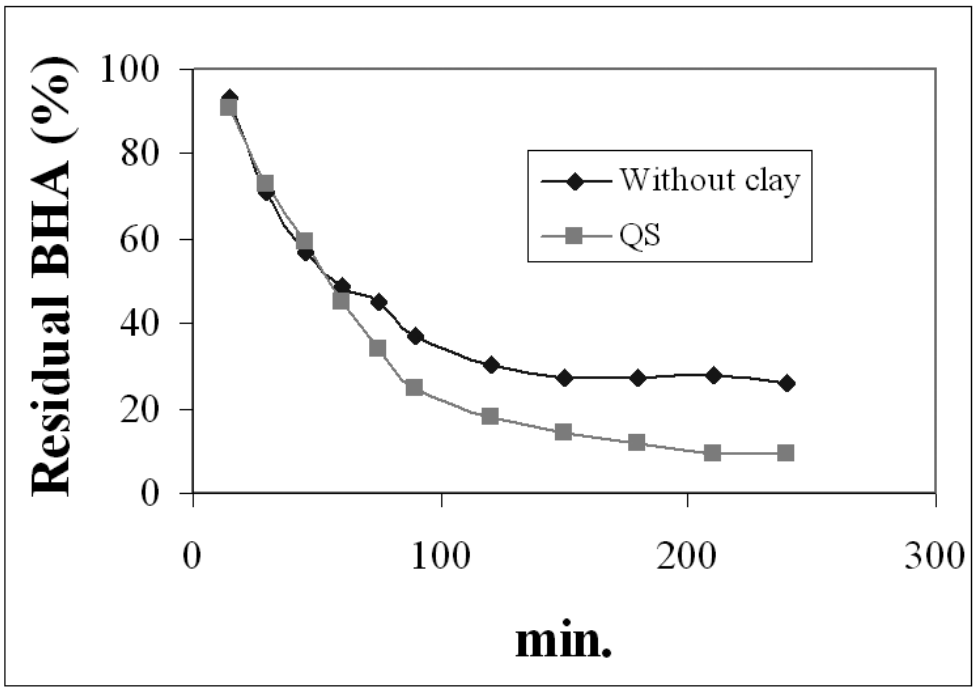

Figure 5. BHA Transformation Achieved by Different HRP (UmL^{-1}) Concentration (4 h at pH 7.0 with 100 mgL^{-1} BHA and 0.1 mL H_2O_2).

longer enzyme life because of organoclay immobilization.

CONCLUSION

The results of this study have demonstrated the applicability of using smectit for the removal of BHA. The modification of smectit by quartamin (dimethyl (dihydrogenated tallow) ammonium chloride) increased the removal efficiency of phenolics. When the smectit was used for the removal of BHA, the system reached saturation within 210 min. which is longer than without clay. In the presence of immobilized organoclay, HRP requirement for 91 % removal of BHA decreased from 0.2 UmL^{-1} to 0.15 UmL^{-1}. From here an important results can be concluded that because of organoclay immobilization, the time of enzyme life gets longer and required HRP concentration decreases for the removal of BHA.

ACKNOWLEDGEMENTS

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