Chapter 4

CHITOSAN FOR PESTICIDE CONTROL ON ENVIRONMENTAL PROTECTION AND WATER PURIFICATION

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ABSTRACT

The present review is focused on the diverse approaches employing chitin and/or chitosan as either active or passive components which have been employed to pesticide control in environmental protection and water purification. In some cases, ideas are presented in order to contribute in the solution of particular problems associated with this topic. The first section is dedicated to introduce the most important basic elements in the work as for example classification of the pesticides (according to their toxicological effects, chemical similarities and type of plague to control), description of the chitin and chitosan and some general applications of these biomaterials on removal of pollutants. Second section briefly analyzes some strategies employed in order to minimize the effects of the indiscriminate application of pesticide, through previous actions, as controlled release and protected dosing system. Third part is dedicated to discuss the diverse action modes which chitin or chitosan can be used for pesticide removal, including their uses in coagulation/flocculation process, filtration membranes and as adsorbents. Some applications of these materials as supports of pesticide-degradating agents are presented in the section four including enzymes micro-organisms and catalysts. Finally, a section dedicated to trends in this topic is presented. In general, the review shows that these materials posses high potentialities for use in pollutants removal. Applications as adsorbent appear to be the most promising at short term, especially to either anionic or acid pesticides, considering that perhaps chitosan is the unique natural polycation. Similarly, some proposal to produce new composite materials with

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applications on pesticide removal, using processes involving chitin and chitosan are presented.

1. INTRODUCTION

Daily significant volumes of pesticides are discharged from countless sources creating a serious environmental problem, especially when these substances are poured into rivers and lakes and other water reservoirs. Pesticides accumulation in water sources used for human consumption is also highly worrying for human health, because, in general, conventional water treatment methods (i.e., coagulation-flocculation, sedimentation, and conventional filtration) do not seem to facilitate either removal or transformation of pesticides in drinking water (U.S. Environmental Protection Agency, 2000).

From the environmental and human health point of view, discovery of new methods and materials that allow improving level of control, removal or transformation of these pollutants has cardinal importance. As in other areas, a lot of effort has been paid to increasingly use environmental friendly materials. Chitosan, a biopolymer coming from diverse natural sources but mainly obtained by chemical transformations of chitin (one of the renewable materials more abundant in nature) has been extensively studied during the last twenty years by its high potential in water treatment.

This review focuses on the principal systems based in chitin and/or chitosan (and their derivatives) that have been reported for the controlled and/or protected release, removal or biodegradation of pesticides. Due to the present relevance of this topic, which no doubt will continue in the near future, some ideas on the production of either materials or methods for removal of pesticide based on these polymeric materials are briefly presented.

Table 1. Recommended WHO pesticide classification according to their toxicological effects

Class		LD_{50} for the rat (mg/Kg weigth)			
		Oral		Dermal	
		Solids	Liquids	Solids	Liquids
Ia	Extremely hazardous	5 or less	20 or less	10 or less	40 or less
Ib	Highly hazardous	5 − 50	20 − 200	10 − 100	40 − 400
II	Moderate hazardous	50 − 500	200 − 2000	100 − 1000	400 − 4000
III	Slightly hazardous	Over 500	Over 2000	Over 1000	Over 4000

The LD_{50} value is a statistical estimate of the number of mg of toxicant per kg of bodyweight required to kill 50% of a large population of test animals.

Table 2. Pesticide classification according to their chemical similarities

Code	Type of compound	Code	Type of compound
AS	Arsenic compounds	OP	Organophosphorus compounds
BP	Bipyridylium derivatives	OT	Organotin compounds
C	Carbamates	PAA	Phenoxyacetic acid derivatives
CO	Coumarin derivatives	PZ	Pyrazoles
CU	Copper compounds	PY	Pyrethroids
HG	Mercury compounds	T	Triazine derivatives
NP	Nitrophenol derivatives	TC	Thiocarbamates
OC	Organochlorine compounds		

Table 3. Pesticide classification according to type of controlled plague

Acaricides	Algicides	Antifeedants
Avicides	Bactericides	Bird repellents
Herbicides	Fungicides	Herbicide safeners
Insecticides	Insect attractants	Insect repellents
Molluscicides	Mammal repellents	Nematicides
Rodenticides	Virucides	

1.1. Classification of Pesticides

Pesticides can be organized in groups according to diverse considerations, ranging from their toxicological effects to chemical properties, including type of plagues to be controlled. Thus, considering their toxic effects in the human beings, the pesticides have been classified by the World Health Organization (WHO, 2004) in three main groups (Table 1). Table 2 shows pesticide classification according to their similar chemical properties; use of the same antidote is among the properties more important to consider in this classification. Table 3 shows pesticide classification considering the type of controlled plague.

1.2. Chitin and Chitosan

Chitosan is a well studied linear polysaccharide which can be considered, from the point of view of their repetitive units, a copolymer: poly-(β-1,4-glucosamine-co-N-acetyl- β-1,4-glucosamine). It occurs naturally in several fungi, especially *Mucor* species. However, commercial chitosan is usually prepared by a chemical chitin N-deacetylation reaction. It has been demonstrated that acetylated (GlcNAc; β (1$\rightarrow$4) 2- acetamido – 2 – deoxy – b – D 4 - glucopyranose) and deacetylated units (Glc; β (1$\rightarrow$4) 2-amino-2-deoxy-b-D-glucopyranose) are randomly distributed along the chains, in proportions defined by a parameter known as deacetylation degree (DD) (i.e., DD is the fraction of deacetyladed units in the polymer chain) as it is illustrates in Figure 1.
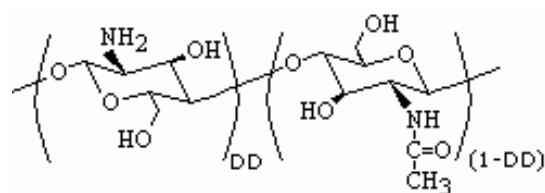
Figure 1. Repetitive structural chemical units of chitosan. Monomer units are randomly distributed in the chain.

Chitosan physicochemical properties are markedly depending on intrinsic characteristics of the polymer (molar mass, DD, distribution of repetitive units) and the experimental conditions in which the material is studied (temperature, pH, ionic strength, solvent, associated counterions, etc.). Thus, chitosan is insoluble in neutral and basic aqueous medium but it is very soluble in acidic aqueous solutions. Similarly, materials with $DD < 0.50$ are insoluble even in aqueous acid solutions but solubility increases when DD get higher values. Protonation of amine moieties on the deacetylated units is responsible for solubilization of chitosan in acidic aqueous medium because quaternary amine salt formation (Figure 2) destroys the intra and intermolecular hydrogen bonds in which these participate.

Due to its natural origin, chitosan possesses intrinsic advantages such as low cost, biodegradability, biocompatibility, non-toxicity, good sorption properties and film forming capacity. In particular, protonated amino groups enable properties such as antibacterial, protein affinity and water solubility. On the other hand, non-protonated amine groups and hydroxyl groups favor heavy metal chelation and facilitate some polymer modification reactions.

1.3. Recovery of Pesticides Using Chitin and Chitosan

Applications of chitin and chitosan (and their derivatives) in water treatments have extended rapidly due to the competitive advantages of these materials (Kawuamura, 1991; Majeti & Ravi, 2000), i.e., their natural origin, biodegradability and low cost. Similarly, facility to chemical modification makes them extremely attractive materials for development of friendly environmentally systems for water purification. In that sense, these biopolymers have been used in different activities related with water remediation such as: (a) removal of diverse pollutants including heavy metals (Varma *et al.*, 2004) (considering its high selectivity to transition metallic ions of the group III - but not for metallic ions of the groups I and II - at low concentrations (Muzzarelli, 1973)), dyes (Cestari *et al.*, 2004), oils (Ahmad *et al.*, 2004); (b) coagulant agent (Bratskaya *et al.*, 2002); (c) flocculant agent (Divakaran & Pillai, 2001); (d) adsorbent (Crini, 2005); (e) ultra-filtration membranes (Verbych *et al.*, 2005); etc.

Chitin and chitosan possesses important features which enable them to be used in agriculture as biocide agent (Devlieghere *et al.*, 2004), growth stimulant (Barka *et al.*, 2004), elicitor (Prapagdee *et al.*, 2007), etc. Also, they have been employed as matrix in controlled pesticide release (Hirano, 1978; McCormik *et al.*, 1982; Texeira *et al.*, 1990; Larez, 2008). Nevertheless, information about their utilization in systems for pesticide removal is scarce, in

spite of this type of pollutants has been included in the list of hazardous compounds for the environment and human health (Häggblom & Valo, 1995).

Table 4 shows some group-representative pesticides on the WHO classification, where chitosan has been employed as a component of the formulation for release (controlled and/or protected), removal and/or biodegradation.
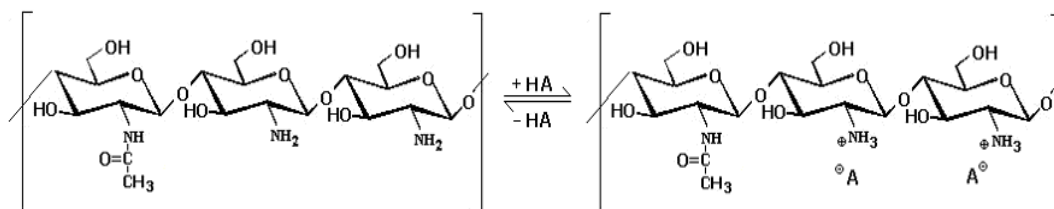

Figure 2. Protonation reaction of chitosan in acidic aqueous medium.

Table 4. Some representative-group pesticides on recommended WHO classification related to chitosan studies

Group IA	Group IB	Group II	Group III
Brodifacoum	**Dichlorvos**	**Carbaryl**	**Isoproturon**
Rodenticide	Insecticide	Insecticide	Herbicide
Parathion	**Warfarin**	**Diquat**	**Malathion**
Insecticide	Rodenticide	Herbicide	Insecticide
Ethoprophos	**Nicotine**	**Paraquat**	**MCPA**
Insecticide (soil)		Herbicide	Herbicide
Hexachlorobenzene		**Lindane**	**Dicamba**
Fungicide (seed)		Insecticide	Herbicide
Mercuric chloride		**7Imidacloprid**	**Oxadixyl**
Fungicide (soil)		Insecticide	Fungicide

2. PESTICIDES CONTROLLED DOSING EMPLOYING CHITIN AND CHITOSAN

2.1. Controlled Release of Pesticides

Substitution of traditional agrochemical formulations by controlled release systems helps, among other benefits, to avoid the employment of excessive quantities of active substances, constituting this action by itself an environmental protection strategy. The principal aims pursued with the use of these systems are:

- Protection of active agents
- To allow the automatic release of the proper active agent at the selected place and at appropriate rate.
- Ensure concentration level at optimal limits and time providing higher specificity and persistence.

- Sustained release in time is one of the goals more pursued in the agriculture because this allows assuming the control of diverse problems as:
- The effects of the released substances are spread in time, which produces substantial economic savings due to it is possible to exercise a better control of the employed quantities.
- The release happens when the plant needs it, generally in minor doses that those obtained when the active agent is added alone.
- Reduction of the number of applications, diminishing the contact of the workers with agrochemicals and the hours dedicated to this labor, as well as stress in the plants.
- Diminish the risk of human beings and animals by toxic contamination since localized release control is assured.
- Use of right agrochemical doses, which obviously carries minors economic costs.
- More friendly environmental systems, especially when degradation of the biomaterial used as support not affect the quality of the soil.
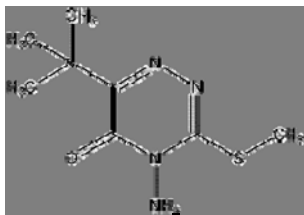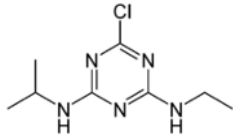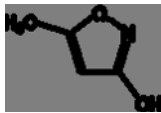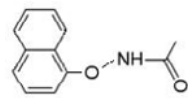
One of the firsts reports proposing the use of chitosan and chitosan derivatives (as membranes) for controlled release of agrochemicals was carried out by Hirano (1978). Likewise, chitin was proven as matrix for release of an agrochemical in the beginnings of the eighties, when the chemical union of the pesticide Metribuzin, and its subsequent release, to this biopolymer was reported (McCormik et al., 1982). Later, Texeira et al., (1990) reported the controlled release of Atrazine (a common herbicide used in corn fields) using films and pearls made with chitosan (and derivatives) hydrogels. These studies showed that herbicide covered with chitosan presents a rapid initial release, followed for a rate constant stage; similarly, chitosan hydrogels achieved to extend release period up to 7 months, compared with only 4 days obtained with the herbicide without covering.

More recently, the chemical coupling of the insecticide Carbaryl (1-naphthyl methylcarbamate) onto chitosan has been reported either by direct conjugation on the glucosamine ring or through a spacer between this and the insecticide moiety (Chirachanchai et al., 2001). Table 5 shows some chitin/chitosan-based systems related to controlled release of agrochemical.

2.2. Protected Dosing of Pesticides

Some very ingenious systems that allow a relatively safe application – or protected dosing – of a chemical agent to a specific plague have been designed, as for example the "anti-escape traps" used to apply localized pesticides without affecting other susceptible species by lethal effects of the active agent. The rodenticide Brodifacoum has been employed in this type of application (Creeger & Fakiro, 2007). Chitosan has been used in these systems, taking advantage of the adhesive properties of their solutions, as one of the carrier polymers for the pesticide. Thus, it adheres to the rodent and is then eaten during the animal's grooming.

**Table 5. Some chitin/chitosan-based systems related to controlled release
of agrochemical**

Matrix	Pesticida	References
Chemically substituted chitin	Metribuzin	McCormick *et al*., 1982
Films and pearls of chitosan	Atrazine	Texeira *et al*., 1990
Microcapsules of chitosan prepared by interfacial reaction	3-methyl-5-hydroxyisoxazole	Yeom *et al., 2000*
Pesticide chemically modified chitosans	Carbaryl	Chirachanchai *et al*., 2001
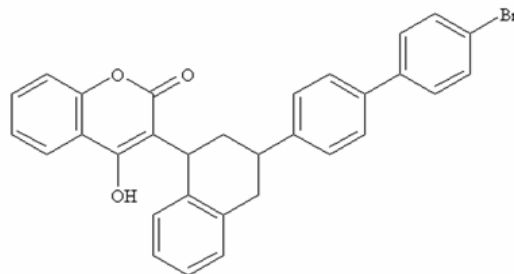
Brodifacoum

On the other hand, very recent studies have shown that these materials can also be microencapsulated by the formation of polymer surface layers around pesticide microparticles, in order to protect other susceptible species from their action. In that sense, the insecticide Imidacloprid has been protected with alternating layers of chitosan and alginate, using the technique known as layer by layer auto-assembling (Guan *et al*., 2008). Additionally, photocatalysts that promote the degradation of the micro-encapsulated pesticide were studied.

Imidacloprid

3. REMOVAL OF PESTICIDES USING CHITIN AND CHITOSAN

The search to low-cost systems for contaminants removal in water purification processes is a very active and extensive scientific research area because of the complexity involved in the diversity of pollutants that can water possess, considering that most of the times coming from dissimilar sources. Additionally, when considering the situation of water contaminated with pesticides, the picture becomes more worrying, especially in relation to the human health because, as it has been mentioned at the beginning, methods generally used in conventional water treatments seem to have little or no effect on the successful removal of them (U.S. Environmental Protection Agency, 2001).

On the other hand, although in certain cases some additional purification processes, including disinfection, irradiation and/or softening of the water, could promote the conversion of pesticides in less toxic products, there will always be the possibility that this procedures could also induce the formation of more toxic sub-products, whose effects are unknown because of the scarce information on them.

3.1. Coagulation/flocculation

Chitosan has been studied as a coagulant or flocculant for a wide variety of aqueous suspensions (Table 6) (Pan *et al*., 1999; Huang & Chen, 1996) In many of these studies the mechanism responsible for separation have not been clearly established and the terms coagulation and flocculation can be used indistinctly (Roussy *et al*., 2005), with some results pointing towards mechanisms of charge neutralization (Ashmore & Hearn, 2000) whereas others indicate that chitosan can operate by means of the bridging mechanism (Chen *et al*., 2003; Guibal *et al*., 2006). These discrepancies have been explained, in general terms, considering the effective density of charge of the polyelectrolyte employed (Strand *et al*., 2003).

The ability of chitosan for coagulation and flocculation has been related to: a high content of –OH groups, which makes the polymer hydrophilic and contributes to chelating effects; cationic charge derived from protonation of the amine groups at acidic pH; an electron pair for each amine groups (more available at pH close to or greater than the pKa =6.5). In addition, chitosan with moderate to high molecular weight can provides bridging mechanisms for coagulation/flocculation process.

Table 6. Some systems in which chitosan has been studied as coagulant/flocculant agent

Suspension material	References
Yeast	Weir *et al.*, 1993
Proteins	Savant & Torres, 2000
Humic susbtances	Bratskaya *et al.*, 2002
'model colloid' polymer latices	Ashmore *et al.*, 2001
Mammalian cells	Riske *et al.*, 2007
Bacteria	Hughes *et al.*, 1990; Strand *et al.*, 2003
Oil/water emulsions	Bratskaya *et al.*, 2006
Latex particles	Ashmore & Hearn, 2000
Silt	Divakaran & Pillai, 2002

The use of chitosan as clarifying agent for water purification, regarding to the removal of turbidity, has been well documented for several years. Pioneering studies (Penistone & Johnson, 1970) rapidly associated a major efficiency in the clarification of suspensions of montmorillonite with a higher content of deacetylated groups on chitosan. Afterwards, studies showed that this biomaterial was also effective to reduce turbidity in suspensions of organic material proceeding from vegetable sources (Bough, 1975a) as well as of proteins-containing suspensions (Bough, 1975b).

Chitosan has been studied as flocculant either alone (Divakaran and Pillai, 2001), or in combination with other cationic flocculants (Pinotti *et al.*, 2001) or modified to increase its content of cationic groups (Jian-Ping *et al.*, 2007) or to increase the ionic character of these groups (Lárez *et al.*, 2003). Table 6 shows some of the systems for which coagulation/flocculation has been studied using chitosan as clarifying agent.

In spite of chitosan had been reported as an effective coagulant in surface water treatment, there is little information on its employment as coagulant agent for pesticides. Nevertheless, it would be expected that solutions of chitosan in acidic aqueous medium could work as coagulant for water-insoluble pesticide applied as a very fine dust (but that can later form colloidal suspensions in water) as for example the Methyl-parathion (solubility in water 50 ppm).
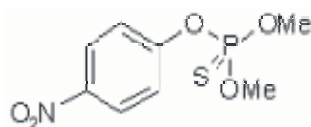
Methyl parathion

Some of these systems are really interesting considering that they might generate new materials with high potential to be used as adsorbents in processes of water purification, according to recent trends in the employment of immobilized biomass for these purposes (Aksu, 2005), including pesticide removal as discussed later on. Figure 3 shows a basic

scheme for generation of new adsorbents, employing chitosan in coagulation/flocculation processes of aqueous biomass suspensions.

3.2. Filtration

Membrane separation technology has gained increasing interest due to new developments of highly selective materials which possess also good chemical and mechanical stabilities. Some of the more interesting applications of membranes related with separation of azeotropic and gaseous mixtures, and proteins, have been recently review by Xu *et al*. (2008).

The use of membranes for filtration processes generally result effective on organic pollutants removal when are applied after a coagulation (Leiknes *et al*., 2004) or adsorption (Ericsson & Trägardh, 1996) previous step. Although both processes (adsorption or coagulation) by themselves are little effectives to remove this type of pollutants, they are generally necessary to avoid the rapid plugging of the membrane by removing larger particles. Some similar systems that substantially improve the level of pesticide rejection have been reported (Van der Bruggen *et al*., 1998; Kosutic *et al*., 2005).

Chitosan has been studied in filtration systems for purifying of contaminated water with persistent organic pollutants (POP´s). In 1986, Thomé & van Daele reported the use of a chitosan filter as a complement to traditional activated carbon filter, which completely eliminated the components of the mixture known as Aroclor 1260, a polychlorinated biphenyl (PCB) mixture containing approximately 38% $C_{12}H_4Cl_6$, 41% $C_{12}H_3Cl_7$, 8% $C_{12}H_2Cl_8$, and 12% $C_{12}H_5Cl_5$ with an average chlorine content of 60%.
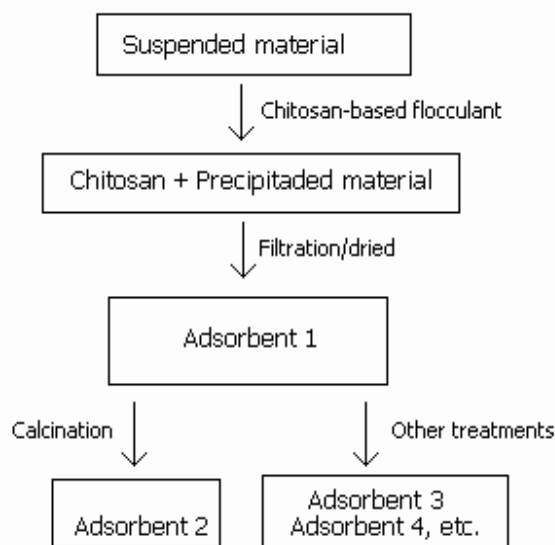

Figure 3. A basic scheme for generation of new adsorbents employing chitosan for precipitation of suspended biomass.

More recently, Assis & de Britto (2008) have reported the modification of glass porous membranes with complex polyelectrolytes formed by alternating deposition of chitosan and carboxymethylcellullose for removal of the herbicide Atrazine. These authors have proposed a tentative model for the binding of the herbicide to the polyelectrolyte complex implying the formation of hydrogen bridge links and/or complex charge transfer to non-specific sites, which are dynamically available as a function of conformational changes of the deposited polymers.
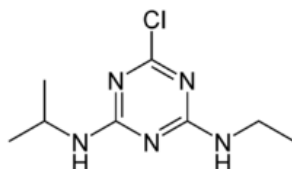
Atrazine

A potential application of chitosan based membranes for selective removal of chemical substances, including pesticides, is related to the use of molecular recognition sites in the DNA. These sites can generally bind some specific substances in a preferential form and may even discern between optical isomers. It is known that chitosan forms polyelectrolyte complexes with the DNA (Mao *et al.*, 2001) and, in general, the properties of such polycomplexes can be controlled by means of the selection of appropriated conditions for their formation (Higuchi *et al.*, 1997). A very elegant application of membranes formed with this type of complexes, which could serve as model for application in the removal of pesticides, is the chiral separation of phenylalanine with the chitosan/DNA polycomplex (DNA proceeding from testicles of salmon) (Matsuoka *et al.*, 2006).

Another possibility for pesticide removal using chitosan based membranes is related to current trend towards the formation of the so called molecularly imprinted membranes. During the manufacture of this type of membranes the molecule to retain is employed as a mold inside the polymer; later on it is taken out by repeated rinse with solvent and then an imprinted polymeric matrix is obtained, which preserves memory on the shape, size and interactions of the "printed" molecule (Andersson *et al.*, 1996; Silvestri *et al.*, 2004).

A similar system to the previously described can be formed by placing the molecule to retain between layers of polyelectrolytes of opposite charges, deposited layer by layer, as already it has been done in systems of liberation of drugs (Manna & Patil, 2008). Posterior removal of the mold molecule leaves available the sites for molecular recognition

3.3. Adsorption

Methods based on the adsorption of POP´s have probably been the most effective route for removal them, having been tried a great variety of material adsorbents. Among the numerous studied adsorbents activated coal has been catalogued as the most effective due to its great superficial area, though also it has more elevated costs (Aksu, 2005). Table 7 presents a list of other materials that have been tested as adsorbents for pesticides.

Table 7. Several materials studied as adsorbents for pesticides removal

| Adsorbent	Adsorbed pesticide	Reference
Zeolites	Atrazine, lindane and diazinone	**Lemić et al. (2006).**
Clays	Penconazole, linuron, alachlor, atrazine and metalaxyl	Sanchez-Martin et al. (2006)
Peat	Paraquat, Diquat, and Amitrole	Mac Carthy & Djebbar (1986)
Ocean peat moss	Azinphosmethyl	Aroguz (2006)
Fly ash bagasse	Lindane and malathion	Gupta et al. (2002)
Cork	α-Cypermethrin	Domingues et al. (2007)
Rice husk	Methyl parathion	Akhtar et al. (2007)
Diatomaceous earth	Atrazine and organo-phosphorus pesticides	Agdi et al., (2000)
Iron oxides	Atrazine, isoproturon, mecoprop, 2,4-D, and bentazone	Clausen & Fabricius (2001)
Pine bark	Lindane and heptachlor	Ratola et al. (2003)
Almond shell residues	Pentachlorophenol	Estevinho et al. (2006)
Rubber granules	2,4-D and Atrazine	Alam et al. (2007)
Pyrolized crab shells	Azinphosmethyl	Gulen et al. (2005)

Studies on Adsorption of Pesticides Using Chitin and Chitosan

Chitosan has received considerable attention as a possible adsorbent regarding metal removal due to its recognized chelating activity and, specially, because it can be obtained to low cost from a diversity of natural sources (Babel & Kurniawan, 2003). Similarly, it has also been extensively studied as adsorbent for dyes (Wu et al., 2001). Nevertheless, in spite of the increasing interest in these applications, there are a few references directly related to the adsorption of pesticides and mechanisms related to these processess.

Probably the first report on the capacity of these materials for pesticide adsorption was the work of Richards and Cutkomp (1946). These authors, associating the major sensibility for DDT poisoning to the possession of a chitinous cuticle and considering the capacity of chitin to adsorb DDT from colloidal suspensions, proposed that chitinous cuticles could concentrate DDT selectively to produce a higher dose inside the animals. Two years later Lord (1948) demonstrated that DDT − and some structurally related compounds (showed below) − can be adsorbed on this biomaterial in quantities approximately equal and with similar rates.
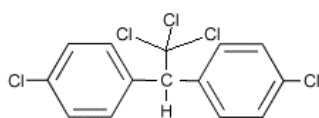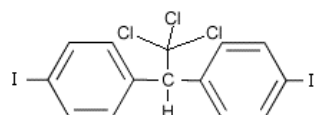

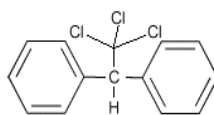DDT (1:l:l-Trichloro-2:2-bis (4'-chlorophenyl)ethane
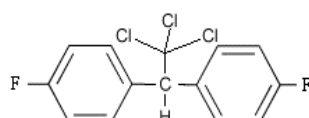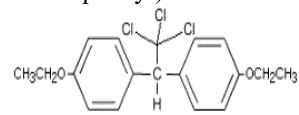
1:l:l-Trichloro-2:2-bis (4'-fluorophenyl)ethane
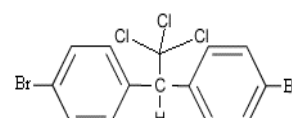
1:1:1-Trichloro-2:2-bis (4'-bromophenyl)ethane

1:1:1-Trichloro-2:2-bis (4'-iodophenyl)ethane

1:1:1-Trichloro-2:2-diphenyl)ethane

1:1:1-Trichloro-2:2-bis (4'-ethoxyphenyl)ethane

At 1972, Masri & Friedman compared the capacity of some materials, natural and synthetic (like chitosan, polymanines, cellulose derivatives, starch derived polyamines, and copolymers of styrene/amine-styren), to bind the fungicide mercuric chloride ($HgCl_2$), obtaining promising results with all of them. Their studies pointed towards the free amine groups in the chitosan and the polyamines as the binding sites for Hg^{+2} ions and, more importantly, towards the possible role of the natural polyamines on distribution of the mercury in the environment.

Later, Davar & Wightman (1981) showed that as it should be expected because the cationic nature of the protonated amino groups, chitosan can strongly adsorb some chlorinated herbicides derived from the fenoxi-acetic acid, as for example MCPA; dicamba; 2,4-D; 2,4,5-T; whose structures are shown down below. The driven force for the fast adsorption of these substances on chitosan is related to the strong electrostatic attraction between the positively charged amine groups on the biopolymer ($-NH_3^+$) and the negatively charged carboxylic groups ($-COO^-$) from the acids. The occurrence of these interactions have been shown for several systems including: (a) linear chitosan/long hydrophobic chain carboxylic acid, with precipitation of the simplex formed (Wei & Hudson, 1993); (b) crosslinked chitosan/long hydrophobic chain, with the occurrence of the phenomenon denominated gel collapse (Lárez et al., 1995; Barreiro-Iglesias et al., 2005); (c) linear chitosan/linear negatively charged polyelectrolyte, with formation of the so called polyelectrolyte complexes (Lárez et al., 2002; Peniche and Arguelles, 2001); etc.

An important consideration for application of chitosan as adsorbent on the removal of this type of pesticides should have present that binding of the adsorbate can become very strong, specially in systems where may occur hydrophobic interactions through hydrophobic zones in the polymer (i.e. introduced by means of chemical modification of the original material (Jiang et al., 2006) or the hydrophobic tail of the adsorbates. These interactions favor the formation of hydrophobic domains, after of the initial ionic interaction (Lárez et al., 1995), which can to avoid the reutilization of the material.

The adsorption-desorption processes of insecticide Lindane to chitin have been studied as a model organic phase for simulating pesticide adsorption-desorption in marine systems (Gonzalez et al., 1992). These processes were studied as a function of chitin concentration, temperature, pH and salinity. Results have shown the existence of different classes of sites, with different accessibility, which at higher lindane concentration could be well described with a two sites Langmuir isotherm. At low lindane concentrations a single adsorption isotherm can be used properly. Increasing temperature and decreasing salinity resulted in both low lindane adsorption and in a more reversible process. On the other hand, an increase of pH resulted in lower adsorption of insecticide.
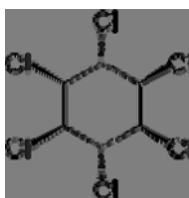

Lindane

Chemical modification of chitosan with substances that confer it a major hydrophobicity looks like a strategy to consider in the adsorption of hydrophobic pesticides. Chemical modification of chitosan to obtain more hydrophobic matrix has already been studied in other areas, as for example in systems for drug release, formation of complexes with surfactants, etc. In this sense, Martin *et al*. (2003) have studied the controlled release of the hydrophobic drug denbufylline with a glycol-chitosan (soluble in water) modified with palmitoyl chloride. Glycol-modified chitosan showed higher affinity to the drug than unmodified glycol-chitosan, and it can additionally to form physical hydrogels due to hydrophobic interactions generated through the hydrocarbonated tails of the palmitoyl residues. Similar studies for the chemical modification of chitosan with oleoyl chloride have showed that the quantity of modifier introduced in these systems can be well controlled (Lárez *et al*., 2007). Another very important advantage of these systems is that this type of modifier agents is obtained of natural sources.

MCPA   Dicamba   2,4-D   2,4,5-T
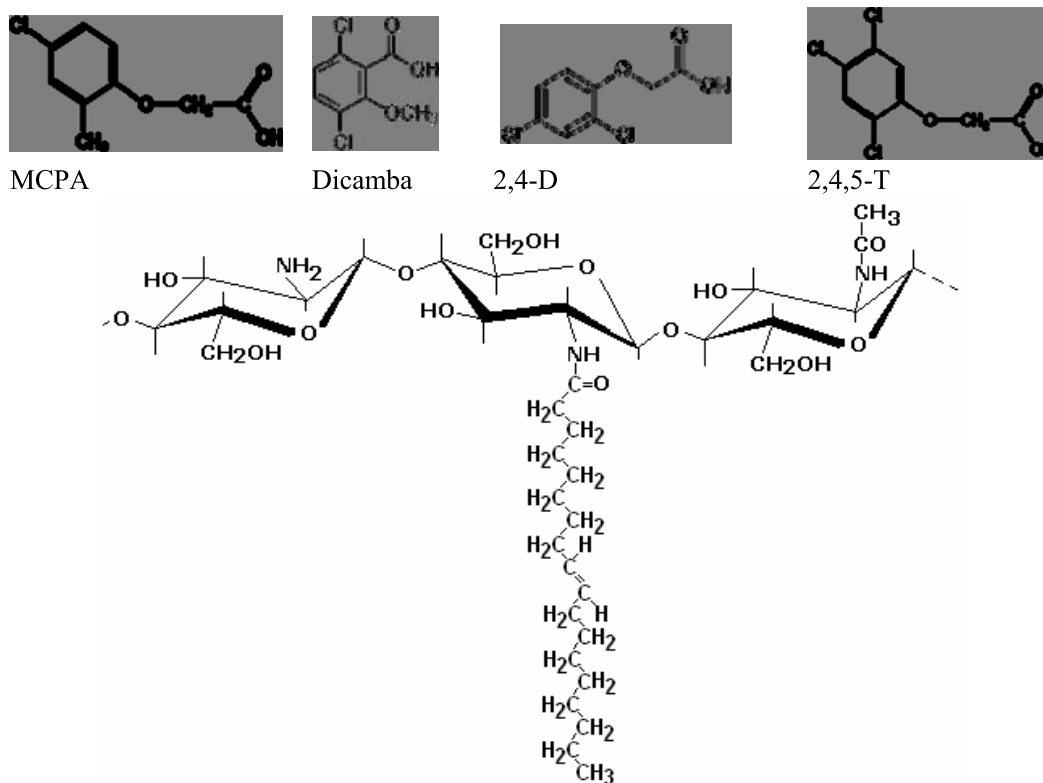
Oleoylated chitosan

On the other hand, Tanada *et al*. (1993) realized studies on the *in vitro* removal of the extremely toxic herbicide known as Paraquat (Gramoxone) using granulate chitosan. The aim of the study was obviously to use it as primary treatment for acute poisonings with this substance, which is generally mortal after being consumed by human beings. Results showed that the quantity of Paraquat adsorbed on chitosan, as well as its rate of adsorption, are higher in a saline normal medium that in either gastric artificial solution or in pure water. The superficial area of the chitosan results to be a determinant factor in all the studied solutions.

Similarly, when the hydroxyl groups in the C-6 of the chitosan is replaced by carboxymethyl or sulfonic groups, obtained materials showed a better capacity to paraquat adsorption in pure water but an inhibition of the adsorption was observed when the studies were carried out in aqueous NaCl solutions (Nakamura *et al*., 1993). Interestingly, opposite results were obtained when these modified chitosans were crosslinked, being observed an important increase in the removal of the paraquat when NaCl concentration in the external solution is smaller than 1.8%.
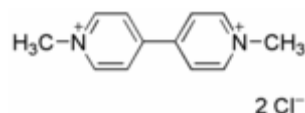

Paraquat dichloride
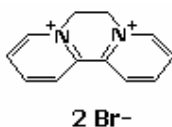
Diquat dibromide

Nakamura *et al*. (1993) also showed that polarity of these substances is very important to their adsorption on chitosan, being observed a bigger adsorption for diquat that for paraquat, due to lower polarity of paraquat. In this work was also shown the inhibitory effect of NaCl on the adsorption of the herbicides on modified chitosan with carboxylic and sulfonic pendant groups. Chitin has also been employed as adsorbent in aqueous medium for these herbicides (Bakasse *et al*. (2005). An additional study relative to pesticide adsorption on chitin is related to removal of the fungicide 4,4-*iso*-propylidene diphenol (Bisphenol A, BPA) and its derivate diphenylolpropane 4,4-dioxyaceticacid (BPAc) (Sismanoglu , 2007)
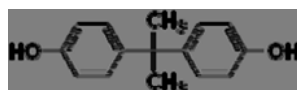
Biphenol A

Some studies have also been reported on the removal of the insecticide Methyl Parathion (Lou *et al*., 1998; Yoshizuka *et al*., 2000) employing glutaraldehyde and epichlorhydrine crosslinked chitosan based micro-particles (CMP´s), as well as the silver complexes of them (CMP-S´s). Results showed that epichlorhydrine- crosslinked CMP´s were superior for Ag^+ ions adsorption that those crosslinked with glutaraldehyde but the glutaraldehyde-crosslinked CMP-S´s were more effectives for adsorption of methyl parathion. Latter MPC-S´s showed better capacity to be re-used.

In recent comparative studies related with the capacity of some drugs to bind to natural polymers it has been demonstrated that the rodenticide Warfarin (Coumadin) bind strongly to chitosan (Hiroki *et a*l., 2005). These results are interesting because later on Shao-Sung *et al*.

(2007) reported that ingestion of chitosan (as dietary complement) by people subjected to medicals treatments with this substance (used as anticoagulant) seems to promote its effect.
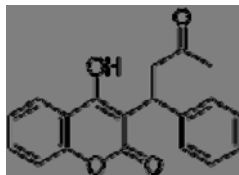
Warfarin

Adsorption of algicide Isoproturon has been studied as an intermediate step in a multiple water treatment study that included initial coagulation with poly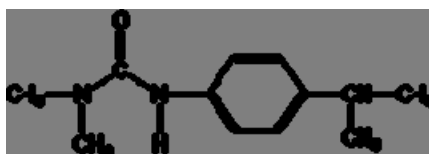(aluminum chloride) (Sarkar *et al.*, 2007), in an attempt to diminish the levels of pesticides, which, as mentioned previously, are little affected by conventional processes. Although treatment with chitosan produces better results than when using bentonite as adsorbent, the results obtained with activated carbon are significantly better.

Isoproturon

More recently, in a very interesting study dos Santos *et al.* (2008) have shown that adsorption of the herbicide Trifluralin perfectly fits the Lagmuir isotherm model. Authors, based on the experimental values obtained for adsorption enthalpy (ΔH_{ads}^{o} = -10 kJ.mol^{-1}), have suggested that process involves the electrostatic attraction of the negative dipole in the nitro group of Trifluralin and protonated amino group of chitosan, although they do not completely rule out interaction of these groups with the tertiary aromatic amine of Trifluralin. Adsorption is less affected by the addition of NaCl to the aqueous medium when the herbicide concentration is low but it is inhibited to greater extent at higher concentrations, which may also indicate that there could be some effect associated to cooperative interaction. The results were elegantly complemented with the development of an electrochemical method for analysis of Trifluralin using a glassy carbon electrode modified with chitosan.
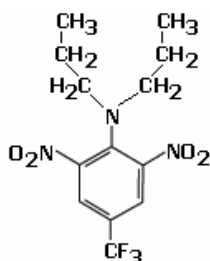
Trifluralin

Identification of electroactive species using the ability of chitosan to generate an electrode/solution interface with a greater partition coefficient for the analyte (Cruz *et al.*, 2000), has become an important tool in quantification of biomedical, such as dopamine (Wang *et al.*, 2006)and environmental related substances, such as pesticides. Some works of this type could provide valuable initial information on the removal of pesticides by using chitosan as an adsorbent although in some cases the ability of chitosan as adsorbent is not considered the important factor (Dua *et al.*, 2008).

Biosorption Using Absorbents Containing-Chitin/Chitosan Biomass

"Biosorption" is defined as the uptake of pollutants from aqueous solutions by non-growing or non-living microbial mass. The term has been related to diverse metabolism-independent processes such as physical and chemical adsorption, electrostatic interaction, ion exchange, complexation, chelation, and microprecipitation, which occur essentially in the cell wall rather than oxidation through anaerobic or aerobic biodegradation (Aksu, 2005). Although the potential of biomass related to pesticide adsorption has been known for several years (Voerman & Taemmen, 1969), we have considered adequate including in this review a small section related to the adsorption studies on these materials because its actual interest. Biomass can be usually obtained, at low cost, as wastes from a number of biotechnological processes, especially those implying inactivate biomaterials (heat killed, dried, acid and/or otherwise chemically treated). Table 8 shows some biomass-based system studied for pollutant removal. Although most of the studies on biomass-based adsorbents have been directed toward the removal of metallic ions (Mcafee *et al.*, 2001; Yan & Viraraghavan, 2008) there are also some reports on the adsorptive capacity of these materials for organic pollutants, which has been attributed, on part, to the presence of chitin and chitosan (Banks & Parkinson, 1992; Zhou & Banks, 1993; Gallagher *et al.*, 1997).

An interesting work in this direction, related with pesticide removal, is reported by Saiano & Ciofalo (2007). They have obtained an effective absorbent for removal of the fungicide Oxadixyl in aqueous solutions, which was prepared by alkaline hydrolysis of the *Phomopsis helianthi* mycelium. Chemical treatment originates a phase consisting of insoluble fractions of chitosan and glucans which may adsorb up to 6 mg of pesticide/gram of adsorbent. An excellent fitting to Langmuir isotherm model was obtained for the adsorption process.

Table 8. Some biomass-based systems studied for pollutant removal

| Biomass source	Pollutant	References
Baker´s yeast	Lindane, Dieldrin	Voerman & Taemmen, 1969
White rot fungi	Pentachlorophenol	Logan *et al.*, 1994
Rhizopus oryzae	Bromophenol blue	Gallagher *et al.*, 1997
Bacillus subtilis	2,4,6-Trichlorohenol	Daughney & Fein, 1998
Cladosporium sp	DDT, DDD and DDE	Juhasz & Naidu, 2000.
Sargassum muticum	Phenol; 2-chloro-phenol (2-CP), and 4-chlorophenol (4-CP)	Rubín *et al.*, 2006
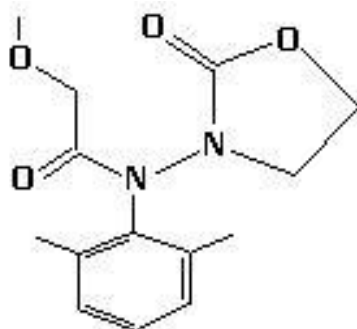
Oxadixyl

To conclude this section it is important to indicate that the uses of chitin/chitosan-containing biomass as pollutant adsorbent surely will grow in the next years due to the lower costs of these materials and, especially, because information on recycling of chitin/chitosan based adsorbents is even scarce. Thus, studies on process related to this topic, such as desorption, ionic exchange, etc., for these materials will also be necessary in the immediate future.

## Adsorption Of by-products Derived of Pesticide Transformations

In order to finalize this section related to adsorption of pesticides on chitin/chitosan is important to mention some works relatives to the adsorption of by-products coming of pesticide-degradation reactions, which have also been studied as a controlling mechanism of these pollutants. As it has been previously mentioned, information about pesticide degradation by-products, and their effects on environment and human health, is even insufficient. However, there are a lot of works related to the adsorption of phenol-related compounds (some of which are by-products coming from degradation reactions of certain pesticides) that could be considered as initial model systems to learn on control of these substances. Some few ones will be mentioned and briefly discussed.

Some interesting results have been reported by Ngah & Fatinathan (2006) in their studies on the capacity of chitosan flakes and chitosan pearls (glutaraldehyde-crosslinked chitosan) to adsorb p-nitrophenol (PNP), a by-product from the enzymatic degradation of the well know pesticides methyl parathion and parathion. According these authors, both systems were well described by the Freundlich isotherm model and results showed that crosslinked hydrogels have a greater capacity for adsorption (2.48 mg.g^{-1}) that the chitosan flakes (0.63 mg.g^{-1}) while adsorbate binds more rapidly to chitosan flakes (3.82×10^{-1} g.mg^{-1}.min^{-1}) that chitosan pearls (3.34×10^{-1} g.mg^{-1}.min^{-1}). Results were explained considering that chitosan pearls show higher BET surface area (0.69 m^2/g) than flakes chitosan (0.42 m^2/g), which facilitates the adsorption of p-nitrophenol due to its more loose pore structure.

Another study related to PNP adsorption on chitosan-based adsorbents has been reported by Uzun & Guzel (2004). They prepared a monocarboxymethyl-ated chitosan derivative (MCM-chitosan) by a reaction similar to:

$$QNH_2 + Cl\text{-}CH_2\text{-}COOH \rightarrow QNH\text{-}CH_2\text{-}COOH + HCl$$
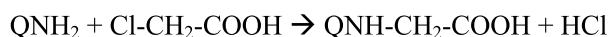
and then compared its adsorption capacity with unmodified chitosan. Results demonstrated that chitosan is more effective to remove PNP due to the MCM-chitosan lacks on promoting PNP ionization reaction:

$$NO_2\text{-}Bz\text{-}OH \leftrightarrow NO_2\text{-}Bz\text{-}O^- + H^+$$
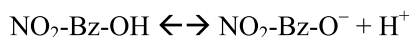
According these authors, MCM-chitosan behaves as typical zwitterions,

$$QNH\text{-}CH_2\text{-}COOH \leftrightarrow QNH_2^+\text{-}CH_2\text{-}COO^-$$
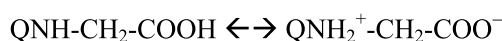
and the amino moieties exist mainly in the aminium form, which has a less proton-attracting character.

4. CHITIN AND CHITOSAN AS SUPPORT TO PESTICIDE DEGRADATION AGENTS

Degradation of organic pollutants in water has been carried out by chemical, biological and/or biochemcal processes. Chemical methods generally involve the use of strong oxidant compounds, ozonation, fenton reaction or heterogeneous photocatalysis, while biological degradation has been achieved by employing various enzymes or microorganisms (biofilters or activated sludge plants). Some approaches have involved (a) capturing of the pollutant present in the wastewater with an organic matrix (ranging from sugar cane bagasse (Crisafully *et al.*, 2008) to biopolymers) followed by disposal and/or transformation of the used matrix; (b) continuous adsorption and later hydrolysis of the pollutant until decay.

The following sections intend to present a view of the use of chitin and/or chitosan as supports of biological active materials (i.e., enzymes, microorganisms) and catalysts for pesticide degradation with the intention that these can be considered as models of similar systems.

4.1. Enzymes Supported on Chitin and Chitosan

Kim *et al.* (2007) have reported the use of various enzymes to treat waste waters. Enzymes like peroxidase (in the presence of hydrogen peroxide) and laccases (in the presence of oxygen) catalyze the oxidation of a wide variety of pollutants compounds like phenol, biphenols, anilines, benzidines, and other aromatic compound (Durante *et al.*, 2004). In particular, laccases have been used for the oxidation of phenolic dyes, phenols and chlorophenols, lignin-related diphenylmethanes, and organophosphorus compounds.

A number of oxidative enzymes from bacteria, fungi and plants have also been reported to play an important role in numerous waste treatment applications (Durán & Esposito, 2000). Peroxidases and phenoloxidases (horseradish peroxidase (HRP), lignin peroxidase and manganese peroxidase) can act on specific pollutants, transforming them into by-products

easier to treat and disposal. Degradations of 2,4-dichlorophenol, 4-chlorophenol and 2-chlorophenol catalyzed by laccase (Zhanga *et al.,*2008) is a rgood example of an enzyme pesticide removing system. Despite of the advantage of these systems, enzyme recycling remains as a problem to solve. A partial solution commonly considered involves the enzyme immobilization on biopolymer matrix which usually improves its useful life and thereby a reduction in treatment cost is achieved.

Chitin and chitosan have been used as support (entrapment or immobilization) for enzyme, cells and microorganisms (Krajewska, 2004), in order to eliminate pesticides or toxicants in water. A wide variety of enzymes have been reported to be immobilized on/in chitin- and chitosan-based gels which can be incorporated by physical and chemical ways. For example, phenol-related contaminants in water can be chemiadsorbed on chitosan and then oxidize to quinone by means of an enzymatic process employing mushroom tyrosinase immobilized on this biopolymer (Dursun & Kalayci., 2005; Sun *et al.*, 1992).

Peroxidases can also be used to remove polychlorinated phenols (PCP′s) from polluted wastewater (Quintanilla-Guerrero *et al.*, 2008) trough a process based on the redox reaction involving hydrogen peroxide. These enzymes are able to catalyze the oxidative polymerization of PCP's to form insoluble polymers (Ward *et al.*, 2001). This enzymatic treatment offers some advantages as a high degree of specificity, operation under mild conditions, high reaction rate, and, very important, low concentrations of soluble phenols are reached (Karam & Nicell, 1997). Nevertheless, during the removal process, a decrease in peroxidase activity has been observed, a common problem which in some cases has been solved by an adequate immobilization system with chitosan (Girelli *et al.*, 2006). Additionally, peroxidases from other sources such as soybean, turnip roots and bitter gourd, have been proposed as alternative to HRP, opening news an exciting opportunities in this field.

4.2. Microorganisms Supported on Chitin and Chitosan

Immobilization of cells and microorganisms on polymeric membranes and other materials seems to be an appropriated strategy to pollutant degradation. Some examples found in the recent literature including microalgae immobilization on diverse polymeric materials as polyurethane, polyacrylamide, polysaccharides, chitosan, etc., (Moreno-Garrido, 2008) This could be achieved by bead entrapment, carrier binding, adsorption techniques, encapsulation, cell coating, and film attachment (Chena *et al.*, 2007). Immobilization techniques may increase stability of biological organism to toxic environments, induce the retention of a higher concentration of microorganisms within the reactor media and help the separation of suspended biomass from waste effluents (dos Santos *et al.*, 2009). Interestingly, chitin and chitosan have been proposed as a convenient material to keep microbial cells alive (Odaci *et al.*, 2008). Some systems that have been already studied are briefly presented.

R. corynebacteriorides QBTo cells were immobilized in chitin and chitosan flakes by cultured together in both biopolymers to be employed to treat crude oil-contaminated seawater. The results showed that bioremediation was just significant when the strain immobilized on chitin and chitosan flakes were employed. Authors explained results considering the protective effect of the biopolymers which promoted biofilm formation and allowed the strain to survive (Gentili *et al.*, 2006). Similarly, algae *Scenedesmus sp. and*

Scenedesmus obliquus cells immobilized on chitosan beads have shown promising results on viability, growth and nutrients uptake and they were was efficient in removing phosphate and nitrate (Fierro *et al.*, 2008). However, authors have indicated that further studies are needed to prevent nitrite build up in water containing chitosan beads before its use for water quality management.

Another related work has reported that *Acidithiobacillus ferrooxidans*, a acidophilic bacterium capable of oxidizing ferrous sulfate, was immobilized on chitosan and crosslinked chitosan beads as a system for bioproduction of ferric iron, which could then be employed to desulfurize gases or in the treatment of acid mine drainages (Giaveno *et al.*, 2008).

Chena *et al* (2007) reported degradation of phenols on suspended and chitosan immobilized *Pseudomonas putida* cells forms. They observed that on the immobilized culture cell pH plays and important role for phenol degradation efficiency which may be attributed to surface properties of chitosan bed.

To conclude this section it is important to mention that studies related to the fabrication of biosensors for pesticide detection could be excellent sources of information to design biodegradation systems for these pollutants. A good example to take advantage of this type of information could be the recent paper published by Odaci *et al* (2008), where *Pseudomonas fluorescens* and *P. putida* cells immobilized on chitosan were placed at carbon and carbon nanotubes electrodes, obtaining good analytical responses when galactose, mannose and xylose were analysed (demonstrating that cells keep its activity even under the operation conditions of the biosensor). Thus, the employment of a system using *Pseudomonas fluorescens* cells immobilized with chitosan for some pesticide should be the following expected step considering that immobilized *P. putida* has already been reported to phenol removal using different chitosans (Chena *et al.,* 2007; Hsieh *et al.*, 2008).

4.3. Catalysts Supported on Chitin and Chitosan

Chitosan is characterized by a strong affinity for transition metals. The polymer can be used as a support for the preparation of heterogeneous catalysts in the form of colloids, flakes, gel beads, fibers (including hollow fibers), or immobilized on inorganic supports (alumina, silica, or other metal oxides). It has been valued as a suitable support because of its high sorption capacities for the catalytic metals, stability of metal ions (such as Pt and Pd), and physical (and chemical) versatility (Guibal *et al.*, 2005). It is a material with small specific surface area and low porosity. Additionally, the conformation of the polymer (together with its flexibility) is an important advantage for this kind of application. Despite of the work done for catalysts deposition on chitosan surfaces, at laboratory scale, water treatment information with this system is scarce.

On the other hand, in order to solve some disadvantages associated with the difficulty to separate chitosan particles when it is employed in powdered form, attempt to synthesize magnetic particles have been carried out. In fact, magnetic-modified particles of chitin, chitosan and alginate are materials suitable for PCP′s removal (Qu *et al.*, 2008). Also chitosan-bound Fe_3O_4 magnetic adsorbent has been used for acid dyes - crocein orange G (AO12) and acid green 25 (AG25)- adsorption in water (Qu *et al.*, 2008; Chang & Chen, 2005).

Some similar systems, containing an adequate catalyst, have been prepared for pollutant degradation applications. Thus, nanoscale Pd–Fe/chitosan and Pd–Fe/silica particles has been prepared and successfully employed, reaching the complete dechlorination of 1,2,4-trichlorobenzene. Also, TiO$_2$–chitosan/glass system has been proposed as a promising photodegradation–adsorption system to dye and monoazo wastewater treatment (Zainal *et al.*, 2008). Finally, a cross-linked chitosan-supported palladium catalyst (Guibal *et al.*, 2005) is claimed to be successfully used to degrade nitrophenol in aqueous dilute solutions using sodium formate as the hydrogen donor.

Other practical applications of these systems include electrochemical sensors based in similar systems have been reported to detect organophosphates insecticides such as parathion at nanogram scale (Qu *et al*, 2008).

5. FUTURE

The trend towards the increasing use of friendly environmentally substance seems to be an irreversible necessity in order to attempt preserving our planet. The consequences of the use of indiscriminate quantities of deleterious substances by mankind appear every time with major intensity (as it is being associated to the recent increment of natural disasters), forcing more drastic official restraints in many countries. Thus, chitin and chitosan seemed to have assured in the future an important position among the materials to be considered for environmental and human health applications owing to the numerous advantages that they posses, as it has previously been discussed.

Relative to chitin, its future use in this type of applications will largely depend on the discovery of more practical solvent systems than the known nowadays (i.e., 5% LiCl/N,N-dimehtyformamide), which only permit to dissolve small quantities of biopolymer and leave this contaminated with salts after a difficult solvent evaporation process. One of the major limitations of this abundant and cheap material has just been the lack of solvent systems that allow its processing, in major scale, for many other applications, including the formulation of systems related to the removal of pesticides such as adsorbents and filtration membranes. Thus, discovery of new solvent systems would facilitate preparation of chitin-based nanosystems containing immobilized either enzymes or microorganisms or catalysts, etc., which could be highly efficient in processes like biodegradation or monitoring of pollutants.

On the other hand, the processing of chitosan has less solvent limitations because its water solubility in acid medium. This fact provides to chitosan with a bigger number of applications that those of chitin. Thus, it has been used as a soluble coagulant/flocculant in water purification treatments. Limitations related to precipitation, observed when it is employed in basic medium, can be solved by an adequate chemical modification (i.e., N,N,N-trimethylated chitosan is soluble in a wide pH range). The use of a more hydrophobic or higher molecular weight chitosan can be a solution for systems requiring flocculants that work by bridging mechanism.

Flocculation of inactive biomass with chitosan can also generate some methods to produce a numerous variety of new adsorbents, as it has been schematically proposed in Figure 3. It is quite interesting that chitosan, besides acting as flocculant in these systems, can protect the co-precipitated biomass due to their biocide properties.

Regards to membrane filtration, chitosan appears as one of the best options to be considered due to the unlimited preparation possibilities that it can offer. Thus, chitosan could be used to construct membranes by diverse approaches, including the use of different chemical modifiers, physical and chemical crosslinking, molecularly imprinted membranes, polyelectrolyte complexes formation, etc., all of which can be applied to pesticide removal.

We also believe that studies in sorption/desorption process of pesticides on chitosan should be increased in the next years because nowadays it is necessary more information on these systems. This information may offer new insights on the reuse of these materials, diminishing costs. In the same direction, more research surely will be made on use of biomass-based adsorbents, including the search of new methods originating materials with higher chitin and/or chitosan proportions.

Perhaps the more exciting field on the use of chitosan for pesticide removal is that related to its use for immobilization. Certainly, it is a very active area with more and more investigations trying to build systems that imitate the nature. All of these developments are largely supported on the fast growth of biotechnology and genetic engineering, which has allowed create new cell-biosensors with more specific biorecognition elements. Thus, it is possible to obtain a biosensor that self-identify the type of pesticide present and, through electrochemical methods, liberates the appropriated microorganism or specific segment enzyme to the pesticide target.

Another possibility related to these intelligent systems should consider the use of model-based computer-aided (bioinformatic) designs for controlled pesticide release , which may become a tool to orientate the synthesis of intelligent and biomimetic systems (Muro-Suñe *et al.*, 2005). Thus, synthesis of new chitosan derivatives involving chelating and grafting groups will reinforce the fabrication of intelligent hydrogels where could coexist, covalently immobilized in different segments of the polymer, redox catalytic centers (HRP, lactase) and complexes molecular structures, like cyclodextrines (with adequate cavities to support metal ions and microorganism) (El-Tahlawy *et al.*, 2006).

Finally, bi and tri-metallic catalysts nanoparticles could be dispersed in the biopolymer matrix or biopolymers membranes in order to ensure pesticide catalytic conversion to non-toxic by-products (Ghauch & Tuqan, 2008). Thus, similar new nanoparticle systems, via encapsulation or electrostatic/ magnetic interaction with the biopolymer, should be developed in the next years.

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