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BIOSORPTION IN TREATMENT OF WASTE WATER

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Abstract: The imperative of environment protection brings out the biomass use in recovery of toxic or valuable metals from diluted effluents. This fact is due to biosorption, which is more efficient in retention of cations present at low concentrations in aqueous solutions, that the conventional treatment, involving reduced energetic consumptions too.

INTRODUCTION

Assurance of life quality represent an imperative for super industrialized modern society, which must to offer the biotechnological, ecological and efficient alternatives, in conditions of very high demographic explosion, the exhaustions of basic materials sources, the pollutants diversification and increase of environmental pollutions. Don't to have to ignore that the ecological risk products have the complex structures and compositions, are present in small among, of µg order, and have an accumulative toxicity during of the time [Gorduza et al, 2002; Tofan et al,2002].

In this context are imposed the strict laws for environmental protection like, for example, the normative referring to quality of drink water (table 1.) [Streat, 1995].

Table 1. The maxim admissible concentration (C) of pollutants, in drink water.

Pollutant	C, µg/ L
Cd	5
Hg	1
Pb	50
Pesticides and related compounds	0.1
Polycyclyc aromatic hydrocarbons	0.2
Insecticides, herbicides, fungicides	0.5

The chemical species presented in trace concentrations are important due their associations with discharges in environment and with health of plants, animal and peoples.

In many cases, the difference between the essential concentration and the concentration of some elements in environment, critical depends by suitable drawing and analysis of samples with attention focus by minim contamination, the utilization of some suitable reference materials and the correct selection of analytical methods. It can affirmed that interest manifested for trace elements is due their dual role in plants, animal and people health, namely their essentiality and their potential to be dangerous (figure 1) [Gorduza et al,2002;Prichard et al,1996].

The environment understanding by scientists is very important as well as their knowledge about the identity and the quality of pollutants and of another chemical species from water, air, soil and biological systems. Therefore, the actual techniques of chemical analysis, suitable utilized, are essential in environment analysis.

1. BIOSORPTION

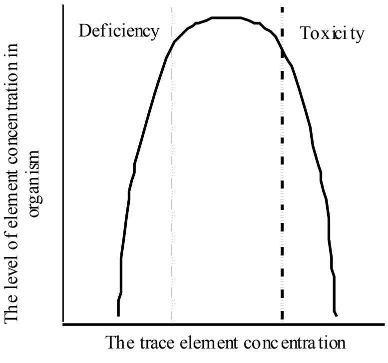

Figure 1. The efect of trace element concentration

The utilization of biological materials in technological practice for metals holding from wastes waters is relative new. The biotechnologies involves low ost price, offer the ecological solutions for toxic metallic ions recovery [Brown,1991]. The natural supports, generate by plants, mushrooms and microorganisms from soil or waters, is biodegradable, being integrals in carbon cycles and compatible with environmental autoreglation processes [Gorduza et al,2002].

The new technologies of waste waters treatment to refer to microorganisms capacity of metallic ions sorption and has imposed the elucidation of some aspects, like: the interactions between ligands from walls cell and metallic ions, the kinetics of retention process and the thermal effects of biosorption.

The frequent applied methods, in waters depolution (the precipitation, the oxidation, the reduction, the ionic exchange, the filtration, the electrochemical treatment, the reverse osmoses, the recovery by evaporation and the solvent extraction) not assure the complete remove of metallic ions, need the high consumption of reagents and energy and to endappear formation of mud and toxic secondary products, for the contaminants concentration by 10-100 ng/L [Singleton et al,1995]. In this context, the **biosorption** represent an alternative to the elimination existent technologies of compounds with ecological risk.

The thermal effect estimation of biosorption involved the activation energy determination of microorganism – metal systems, use the adsorption constant rate and the Langmuir constant. The thermal effects size of biosorption process can represent an important criterion for kinetics and thermodynamic approach of interaction from microorganism – metal system.

The imperative of environmental protection to bring in attention, the utilization of biomass in toxic or valuable recovery from dilute effluents, because the biosorption is more efficient in retention of cations existed in low concentration from aqueous solutions, than conventional treatments by ionic exchange, and has low energy consumption.

The exhaustion of metals resource to bring in actuality, the application of biological treatments for concentration and recovery of some metals with industrial utilizations, using the microorganisms obtained by techniques of genetic engineering [Brown, 1991]

The application of biological methods and for other metals, about Cu, U and Au depends by economical factors and by used strategy. Due their value and importance, is very important the recovery of metals as: Mo, Co, Pb, Zn and Ni. The success of microbiological methods in waste waters treatments depends by factors as: the in situ depolution capacity, the low energy cost, the reduced investigation expenses, the ability to transformation of residual products in resource, the favorable impact with environment and the moderate operation conditions.

2. THE SUPPORT MATERIALS USED IN BIOSORPTION

2.1 THE BIOMASS

The biomass represent on unity, potential carried of reused substances and energy, which correspond to the total quantity of carbon and nitrogen, incorporated by biological processes, in organic polymeric material. The principal, components of biomass are: cellulose, hemicelluloses, lignin and starch (figure 2) [Batt, 1991]. The biomasses have much biotechnological and ecological utilization.

The main source of biomass is represent the plants, with an annual production by about 10^{11} tons and is a result of photosynthesis. Thus, the biomass is a regenerative resource. The biomass is refer to agricultural and chemically biomass (phytomass) [Enciclipedia,1988].

The phytomass included: lignin – cellulose biomass, consist in wooden plants, starch and inulin biomass, the aquatic biomass, biomass obtained as agricultural refuse, domestic biomass, used in technologies for biogas obtain.

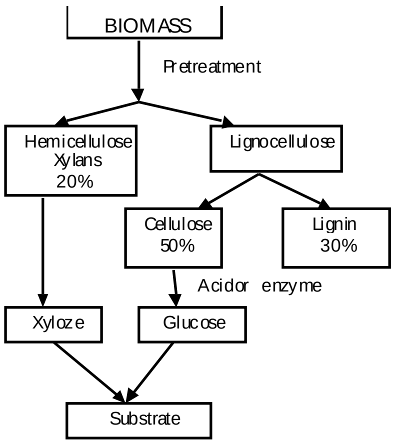

Figure 2. Component of lignocellulosic biomass

The biomass composition depends by origin, and from this reason the conversion processes in substrate with concrete utilizations, is very complicate.

The biomass is used as:

- Substrate in recovery/decontamination processes of waste waters;
- Substrate or cultural media in fermentation process.

The biomass utilization as substrate depends by: low cost price for conservation and transport from biomass source to utilization place, utilization of residual biomass as substrate in other technological process for to decrease the cost, the concentration and selective retention capacity of metallic ions from solutions to low concentrations (< 1 ng/L), ensured the recirculation potential for metal recovery [Batt, 1991].

The biomass capacity of wastes waters treatment is manifested in mechanisms as: chelatization, solubilization initiated by protons, formations of inorganic complexes, ionic exchange and dissolution of metals bond of substrate by reduction reactions, but the important contribution have the chelatization.

The biosorptive interactions between biomass and cations have applications in water decontamination from nuclear, photographic and extractive industries, the retained metal is desorbed after.

The metal complexation with extra cells polymers and with the polymers from cell walls permitted to the microorganisms to survive in aqueous media, which contain an among of metal over admits toxicity limits [Brown,1991].

Above 98% from present metal in aqueous solution can be adsorbed by biomass, and to provide the possibility to metal recovery from wastes waters.

In general, the metal adsorption systems on biomass is characterized by high value of ratio between the wet volume and dry mass, than the ionic exchange systems which is more less efficient in case of high flow of solution. The utilization of these materials permitted the diminution of metal concentration in final effluent, and is recommended in the final treatment process, in succession with the conventional treatments.

By lyophilized biomass of *Saccharomyces cerevisiae*, produced in beer industries can be retained the silver ions from aqueous solutions, by covalent bond mechanism, which involved the substitution of some protons from biomass [Singleton et al,1995].

Actinomycetes manifest biosorption affinity for metals due the present anionic groups in cell wall. The microorganisms in contact with solutions which contain mixture of metallic ions, accumulate different the cations, in variant limits (table 2). An example is represented by the utilization of refuse by micelles from antibiotic industries for rare earths separations, by sorption to pH = 2 –6 [Hancock, 1986].

Some microorganisms have de-toxicity and metals transformation effects. Thus, Hg, Pb, As, Au, Sn, Pt, Se can be mutilated, while Te, As, V, Mo, Hg can be reduced by *Micrococcus sp.* in some cases until to free metal step [Brown, 1991].

Table 2. The retention capacity of some microorganisms

Microorganisms	Metallic ion	pH	Capacity, mg/g dry material
Saccharomyces cerevisiae	Cu,Cd,Co	6,2	25,4;19,1;6,5
STREPTOMYCES RIMOUS	Zn Co,Ni	- -	2,9 -
STREPTOMYCES NOURSEI	Ag/Cd/Co/Cr/ Cu/Ni/Pb/Zn	6,0/6,5/5,8/5,5/5,5/5,9/ 6,1/5,8	38,6/3,4/1,2/10,6/9,0/0,8/36,5/1,6

The *Escherichia coli* bacterium reduced the mercury ion (Hg(II)) to metallic mercury, which them diffused from cells and are volatilized in atmosphere. The *Micrococcus luteus* immobilize Pb in his cell walls, less than 1 % from retained lead ions being accumulated in cytoplasm [Cripps et al, 1990].

The polysulphonate biomass is selective for heavy metals, thus that from waste water can be removed more than 99 % from present amounts of Zn, Cd and Mn ions, than 9 % for Ca(II) and 4 % Mg(II), respectively [Brown, 1991].

The radium adsorption by *Penicillium chrysogenum* is more 14 times high than the conventional system capacity of ionic exchange.

By dry biomass, which contain the *Pllurotus putrida* mushroom Cd(II) can be retained, to pH=5.5-6 [Cibangri et al,1999]. The biomass which contain two mushrooms from *Mucorales* class, one cultured in laboratories, or eventual immobilized with *Rhizopus arrhizus*, and other extracted from *Mucor miehe* industrial refuses retain Cu (II) from solutions [Brady et al,1999]. By a biomass obtained from bacteria's (*Buccilus subtilis*), mushrooms (*Penicillium chrysogenum*) and marine plants (*Sargassum fluitants*), the gold is extracted cyanuric solutions, to pH=2, the retention capacity being 3.2 –8 μmoll /g [Niu et al,1999]. By a biomass, which contain *Streptomyces rimous, Penicilinium chysogenum* and *Saccharromyces carlesbergensis* and *Saccharomyus cerevisiae* dregs can be realized the biosorption of Zn, Co and Ni, in presence of ca and Na ions, and the results is superior than the results obtained by filtration, centrifugation or flotation techniques [Zouboulis et al,1999].

The biomass inactivation by thermal or with solvent treatments determined the misrepresentation of basis material and has obtained the inactive biomass (for example the dead *Actinomycete* bacteria's, by *Streptomyces* type) [Batt,1991; Matis et al,1997]. The inactive biomass can have more high adsorption capacity, than no distort material, being more densely and many times recyclable. Thus, the inactive cells by *Thiobaillus ferrooxidans* can adsorb with 10 –40 % much more uranium than the live cells [Batt,1991].

The most metal remove techniques from effluents use the biomass generated as secondary products or as refuse from industrial process.

The residual biomass can be by three types [Gorduza et al,2002]:

- the liquid refuses with contained aliphatic carboxylic acids and amino-acids (effluents from time of harvests ensilage or from to horned reproduction farms);
- the solid refuses, with high contend of proteins and peptides, represented by meal from blood, refuses of carcasses from slaughter –house, etc;
- the solid refuse characterized by high among of carbohydrates – molasses from sugar fabrication.

The residual biomass can be utilized as such, chemical or physical modified, by oxidation and hydrolysis, respectively.

An accessible and cheap source of biomass for metals recovery is represented by the pharmaceutical and food industries, by microorganisms used for enzymes and antibiotics products, respectively (table 3). The biomass can be obtained from diverse microorganisms: mushrooms, algae[Simmons et al,1995; Singleton et al,1995;sag et al,1995], bacteria's [Batt,1991], microorganisms, isolated from different media: soil, waters, mud, plants, rocks [Eccles, 1997].

Utilization of industrial residual biomass as sorptiv material, increases the microbial technologies profitableness. For example: the dregs residual biomass represented an important material source with biosorptiv properties, is cheaper, easy for recuperate to end of fermentation and produced in high among.

TABLE 3. THE MICROORGANISMS USED IN METALS BIOACCUMULATION

Element	Microorganisms capable for bioaccumulation	Element	Microorganisms capable for bioaccumulation
Aluminium	*Aspergillus niger*	Lead, molybdenum	*Spirogyra sp.*
Arseniu	*Dumiella sp.*	Magnesium	*Gallionella sp.*
Cadmium	*Zoogloea ramigera*	Nickel	*Chlamydomonas sp.*
Cesium	*Saccharomyces cerevisiae*	Palladium	*Thiobacillus ferrooxidans*
Chrome, vanadium	*PSEUDOMONAS*	Radium	*Penicillium chrysogenum*
Cobalt	*AERUGINOSA*	Selenium	*Pseudomonas sp.*
Cooper	*Chlorella regularis*	Silver	*Anaerobic sludge*
Gold, platinum	*Cladosporium resinae*	Strontium	*Citrobacter sp.*
Hafnium, zirconium	*Chlorella vulgaris*	Thorium, uranium	*Rhizopus arrhizus*
Indium, mercury	*Klebsiella aerogenes* *Escherichia coli*	Iron	*Leptothrix sp.*

2.2 THE ALGAE

The algae, representative of marine flora, is characterized by high polysaccharides contained, beside of proteins, minerals, etc. The algae, represented a source for human and animal food, agriculture fertilizer, basis material for the food, pharmaceutical and cosmetics industries. Some marine algae can be utilized in depolution of contaminable waters with toxic organic and inorganic substances [Simionescu et al,1974].

The marine algae chemization processes depend by the following factors: alga specie, zone and season of harvest depositing conditions. By organic compounds from algae is remark [Gorduza et al,2002]:

- Polysaccharides;
- Cellulose, manna, xylan, in proportion of 2.5-95%, similarly with land plants;
- Alginic acid, laminarin, fucoidin, agar, carrageen, in proportion of 7-34 %, specific the algae and with structural and physiological functions;
- Cyclic-polyoli (D-manitol), in proportion of 6.86- 24.33%;
- Vitamins: B_2, B_{12}, A, D, C, PP, H;
- Proteins, sterides, flavonoides, polyphenols and ureides, in proportion of 12.3-35.6 %;
- Pigments in form of chromoproteides (chlorophyll's, carotenoides).

The algae cells can adsorb metals from natural aqueous media or industrial effluents. Some types of algae manifested affinity for Au, Hg, Ag, U, Zn, Cd and Cu. The retention capacity of metals is attributed to ionic exchange mechanism, but are not excused and the covalent binding [Christ et al,1990].

The RMN spectroscopy applications for retention mechanism elucidation for Cd, adsorbed by on algae, indicated the covalent binding of constitutive carboxylic groups [Majidi et al,1990].

To cation adsorption by algae has observed that the constitutive carboxylic groups are implied in Cu and Al binding, but these not interfere in Au retention [Gardea-Torresdey et al,1990].

To gold retention by *Chorella vulgaris*, Au(III) is bond to S atom and/or N, [Pittet et al,1999] being observed and the competitive biosorption of Cr(VI) and Fe(III) ions [Aksu et al,1997].

3. THE BIOSORBENTS

The biomass utilization in depolution technologies, in chromatographic column respectively, has determined the immobilization of these by different types of supports, with variable forms and porosities, that guide to a new class of sorbents – **biosorbents** [Matis et al,1997;Jeffres et al,1997]. The biosorbents can be use in complete treatment of refuels, or in finishing treatment, associated with another materials, like active coal.

For to remove the cadmium ions, for example, from aqueous media are need the following steps [Matis et al,1997]:

- he biosorption of metallic ions by direct contact between wastes water and biosorbent;
- the separation by filtration, sedimentation, centrifugation or flotation of the biomass;
- the elution from biomass of retained cadmium ions;
- the reutilization of biomass and of eluent.

Such process not have an suitable efficiency, being recommended the immobilization technologies, where the biomass is converted in other form which can be utilizable, similar with the ionic exchangers. For example, to biomass inclusion in chitosan granules, the magnetite added are indicated, for to separation facility [Rorrer et al,1993].

The biosorbents is efficient for to metallic ions remove from aqueous media, in presence of organic compounds or dissolved salts, which is considered poisons for to ionic exchange resins and have the sorption propriety superiors in comparison with conventional materials from final treatment of effluents. The biosorbents remove selectively, heavy metals ions from waters, which have high concentration of K(I), Na(I), Ca(II), Mg(II) and of Cl^-, SO_4^{2-} anions [Gorduza et al,2002].

In comparison with some commercial ionic exchanger resins, the polisulfone granules which contain a biomass based by green –blue alga can be of 4 times more efficient in Zn and Mn adsorption, to a initial concentration of 5 mg/ L and 1 mg /L respectively, in time what the ionic exchanger resins are applicable to cations concentration more than 18 mg/L. The granules with immobilized alga can extract 90% of cadmium quantity from waste water, which contain 45 µg Cd/L, in time what the ionic exchange resins adsorb only 15 – 45 % [Batt,1991].

The biosorbents efficiency in effluents depollution process from uranium extractive industry was estimated, comparatively with IRA –400 resin ethalon. Thus, the *A. niger* tablets adsorb, to pH= 4, 14 times more uranium, and the *Rhizopus arrhizus* biomass retain uranium by 4 times more then the resin. By *Rhizopus arrhizus* was obtained and the following values of retention capacity: 0.089 g Pb/g; 0.056 g Ni/ g; 0.054 g Fe/g; 0.026 g Cu/g [Gorduza, et al,2002].

Are to interest the biosorbents obtained by preparative methods associated with sequential modification, by genetic engineering technologies. Thus, by microbian synthesis can be introduced in Streptomyces cerevisiae and in Escherichia coli, clones of natural and synthetic genes [Gorduza, et al,2002].

3.1 THE BIOSORBENTS FROM BIOMASS AND ALGAE

The biomass immobilized in pearls or granules can represented the filling material for hydrometalurgical prelucration equipment, reactors with mixer in fix and fluidized strate, or can replace the ionic exchanger resins. As immobilization supports can be use: chitosan [Rorrer et al,1993], chitosan – carboxymethil –cellulose [Peter et al,1997], polyacrylamide gel [Maecaski et al,1985], polyacrylamid – hydrazine [Peter et al,1997], polysulfone [Awadalla et al,1994], Karrageenane [Peter et al,1997], calcium alginate [peter et al,1997;Kierstan et al,2000;Jin et al,1999] and silica [Mahan et al,1992].

By treated of *Bacillus subtilius* biomass with alkali can be obtain an granule biosorbent, with an increase retention capacity of metals cations: 0.8 mmol Ag/ g; 1.9 mmol Cd/ g; 2.1 mmol Zn/ g; 2.4 mmol Cu/ g; 2.9 mmol Pb/ g; respectively, the retention capacity being more than 99 % [Broon et al,1994].

The *Actinomycet* bacterian cells can be encapsulated in different matrices and permit to obtain an biosorbent which can be utilizable as biological component in tests for 1, 3 – diclorpropene and 1,2 – dibrommethane from water [Broon et al, 1994].

The immobilization of *Zoogloea ramigera* in calcium algilate and the reticulation of resulted material, by treated with 1% triethilentetraamine and 1% glutaric dialdehide leaded to on biosorbent with superior properties than the inreticulated biosorbent, with a high mechanical resistance and cadmium adsorption capacity, even after 30 adsorption – desorptin cycles [jin et al,1999].

An biomaterial, recent tested is the membrane from hen egg shell (MCOG), which manifest affinity for some metals (table 4). The retention of metallic ions with toxic potential is make by thiol and disulfure groups of this biosorbent [Eccles, 1997].

Table 4. The retention capacity of (MCOG) for some metals (mg/ g)

Metals	Fe(II)	Fe(III)	Cu	Zn	Ca	Pd	Au	Co	Cd	Ag	Pt
pH	2,0	4,0	6,0	6,0	6,0	4,0	4,0	6,0	6,0	6,0	4,0
Capacity	12,5	4,0	9,5	6,5	2,5	250	550	8,0	15,0	15,0	280

The algae immobilization by supports lead to efficient biosorbents in heavy metals remove from waters with organic refuse, which poison the ionic exchanger resins [Brown, 1991]. The obtain of biosorbents by algae basis is an application interest problem. The algae immobilization in polymer matrix determined an increase of 10^2 times of concentration capacity for heavy metals ions from waste waters, with a g/ L order cation concentration. As support for algae immobilization are use: polyacrylamide gel, polyuretanic foam and calcium algilate reticulate gel, which lead to obtain of some biosorbents applicants as filters and efficient in toxic metallic ions (Hg) recovery from aqueous media [Gorduza et al,2002].

Are to interest the biosorbents obtaining by immobilization of algae cells to macromolecular supports, like: copolymer (ethyl acrylate –ethylene glycol dimetacrylate), polyacrylamide gel, polyuretanic foam and reticulate gel of calcium algilate [Harris et al,1990;Fry et al,1993].

As inert support for biopelicles can be use and the granulate active coal. Thus, a *Pseudomonas sp.* biofilm by granulate active coal have the capacity to adsorb 0.85 mg Cu/ g; 0.75 mg Zn/ g; 0.4 mg Ni/ g and the adsorption capacity for herbicides (antrazine) [Mahan et al,1992].

The maxim retention capacity of Cu from aqueous media, in concentration of 0.53 g/L is 0.323 g Cu/ g of *Z. ramigera* dry cell, in static conditions. In continue process, the retained quantity decrease to half, due biomass flocculation, before as Cu ions to occupied the centers with adsorption potential. Was obtained the following values for retained quantity per g of *Z. ramigera* dry biosorbent: 0.085 g Pb; 0.067 g Fe(III); 0.054 g Ni; 0.035 g Cu [Sağ et al,1995].

4. THE ENZYMES IN WASTE WATERS TREATMENT

The physical (adsorption) and chemical (oxidative methods) treatment processes have the limited selectivity and depollution efficiency, being conveyable for treatment of waste waters, with a low contends of chemical pollutants compounds.

The choice of some refuse treatment process suppose the evaluation of some factors, such: technical and economical feasibility, but and the absence of ecological risks [Aitken,1993].

In place of methods which transform the compounds with toxic potential from a phase in other, like the transfer of volatile organic compounds from waste waters in atmosphere, are preferred the destruction proceedings of pollutants materials the proceedings of selective removing of pollutants, the nontoxic material mass being biological treated.

The enzymes utilization offer alternatives for refuse treatments by which can be realized on selective remove of pollutants [Grumman,1970]. These depollution preceding is based by chemical reagents specificity for certain substrate or for appropriate compounds, what permit utilization of chemical reagents which assure the stoichiometrical efficiency and eliminated the secondary reaction risk, in reproducibility and economical conditions.

The enzymes actuated on "recalcitrant" pollutants for their removed by precipitation or by transformation in outer products, which reduce pollution action, or which can remove by classical proceedings [Nicell, 1997;Aitken et al,1987].

The enzymes utilization are indicated in the following cases [Bollag, 1992;Bollag,1992]:

- remove of specific chemical products from complex mixture of refuse, before these to be subdue of biological treatment;
- remove the chemical compounds hared in low concentration in mixtures, for which cannot be applied the biological treatment by mixt culture;
- the final treatment of waste waters, after pretreatment;
- the treatment of occasional refuse , after deversation in isolate places;
- the treatment of small volume of waste waters, but with high concentration in deversation point, for to avoided some interaction with pollutants materials from other installations;
- the "in situ" transformations with enzymes of pollutants from soil or subteran contaminated waters.

The advantages of enzymes utilization in treatments of pollutants can be considered realizable from technical and economical point of view, if the reaction products have a decrease toxicity degree and a biodegradability more advanced than the products resulted by other treatments. Than, the enzymes utilization objectives is the diminution of pollutants toxicity, but are showed and the increase of toxicity cases [Aitke et al,1987; Bollag,1992].

The enzymes attack selective the chemical compounds by refuse mixture. The enzymes specificity was verified by laboratory studies referring to selective oxidation of 2,4-diclorphenol, in low concentrations, in presence of other organic species, follow of extension of method to treatment of waste waters [Gorduza et al,2002].

For treatment efficientization process must assure the control of reaction conditions. The refuse composition and volume can varied in high limits, in short time period, being the risk formation of some inhibitors for enzymes activity.

The most industrial or domestic refuse is not sterile, pH and temperature being outside of optima interval, which make necessary anticipation of some microbian degradation of enzymes. The aqueous refuse treatments with enzymes are impose the following conditions: to homogenize the flow and refuse composition, the pH control, respectively.

The reactor for enzymatic depollution are the conventional, homogenate, mixt (with discontinue, semicontinue or continue working). The selection of reactor type implied: economical analysis, the establish of reaction kinetics and to knowledge of enzyme inactivation mechanism.

The cost is a decisive element in enzymatic depollution process, but exist and enzymes for which the economical factor is not priority. Thus, the enzymes which required cofactors as: adenosine – phosphates (ATP; ADP), pyridine –nucleotides (NAD; NADP) or their reduced forms (NADH, NADPH) is not indicated for practical applications, without to be elaborated the retention and regeneration methods for cofactors. The enzymes cost must be low, because these is destroyed after a suitable time of utilization. Can be utilized the enzymes immobilized by a cheaper support, which can be recovered after on some operation period. The enzymes, commercial disponible, must be stable in time and in reaction conditions, the inactivation price have influence on the process cost [Wandrey et al,1985; Ikemiand et al,1990].

The industrial interest enzymes are the extracells, which required the simple proceeding of prelucration for can be commercialized and which is produced by inactive cells or by organisms obtained by DNA recombination techniques [Aitken,1993]. Exist a series of microbian enzymes for pollutants transformation by "*in vivo*" catalytic process, but which cannot be extended to industrial scale.

The enzymes used "*in vivo*" proceedings for treatment of pollutants organic compounds (azoic colorants, phenols, clor-, brom-, methyl-, ethyl-, clormethyl-, methoxy- carboxy- phenols, insecticides, amines, fertilizers) are azoreductaze [Rafii et al,1990], cellulase [Gusakov et al,1992], cyanamidase [Ingvorsen et al,1991], depolymerase, laccase [Dec et al,1990;Claus et al,1990; Bollag, 1983], nitric hydratase [Nawaz et al,1991], peroxydases [Dec et al,1990;Claus et al,1990;Nakamoto et al,1992;Valli et al,1992], phosphoterase [Coppella et al,1990;Cajdwell et al,1991;Rowland et al,1991], polyphenol-oxydase [Wada et al,1992;Sun et al,1992], nonspecific oxygenase [Khan et al,1990].

The most studied, due the manifested specificity in refuse treatment are hydrolases for organo-phosphoric pesticides and oxidant enzymes for phenols (laccase, peroxydase and polyphenol- oxydase).

The proctease and conversion enzymes of amidon have the commercial disponibility, but the data referring to the ecological applications is not concluding. These enzymes are used in bettering of refuse biological treatments which proceeded from food industry or for solide substrate conversion (sludges) in soluble and easy biodegradable organic compounds. The *Trispine* solubilized solide material proceeded from waste waters treatment installations, and extra cells enzymes, proceeded by *Streptomyces* species, transform the polyethylene fraction of degradable plastics. The bacteria exopolyshacarides can be degraded by a depolymerase to improved the deshydratation capacity of residual biomass from biological treatment processes [Gorduza et al,2002].

The enzymes inactivation is a important element in feasibility appreciation of refuse treatment. The enzymatic inactivation involve: thermal denaturation, the lost of prosthetic group, the phases transfer, the mechanism by inactivation basis (suicide) and reverse inhibition. In aqueous solution, the proteins are susceptible to thermal degradation, which is solded with reversible or irreversible lost of enzymatic activity, sometimes with formation of some partial active intermediaries. The denaturation process is controlled by increase of enzymes concentration or by added in reaction system of some species with high molecular mass: seric albumin, gelatin, polyalchools or polyethylenglicol [Gorduza et al,2002].

In cases of enzymes produced by genetic modified organisms, the thermostability improved involve the substitution of specific sequent of aminoacids. The thermostability can be improved by utilization of enzymes in organic solvents, the method being applied for peroxydase by horse radish, polyphenol-oxydase, some immobilized enzymes as hydrolase immobilized for organo-pesticides.

Than, exist some difficulties to enzymes applications in refuse treatment, has been evidenced situation in which the enzymatic depollution is a selective solution.

CONCLUSIONS

The biological processes can replace the conventional depollution technologies which are ecological risk, very much as can be applied in finishing treatments in traditional processes, for alignments to wants of new field legislation.

The biological recovery advantages of metals offer premises for extension to applied the biological treatments processes in metal extraction and to prelucration industry, for recovery of metals which is lost in effluents and in sterile, for to reconsideration of metals reserves.

The exhaustion of metal resources uptades the biological treatment application to concentration and recovery of some metals with industrial uses, using microorganisms generating by genetic engineering techniques.

The succes of microbiological methods in wastewaters treatment depends on factors such as: the capacity of *in situ* depollution, low energetic costs, reduced investment expenses, ability to convert a waste into a resource, favourable impact with environment and moderate operating conditions.

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