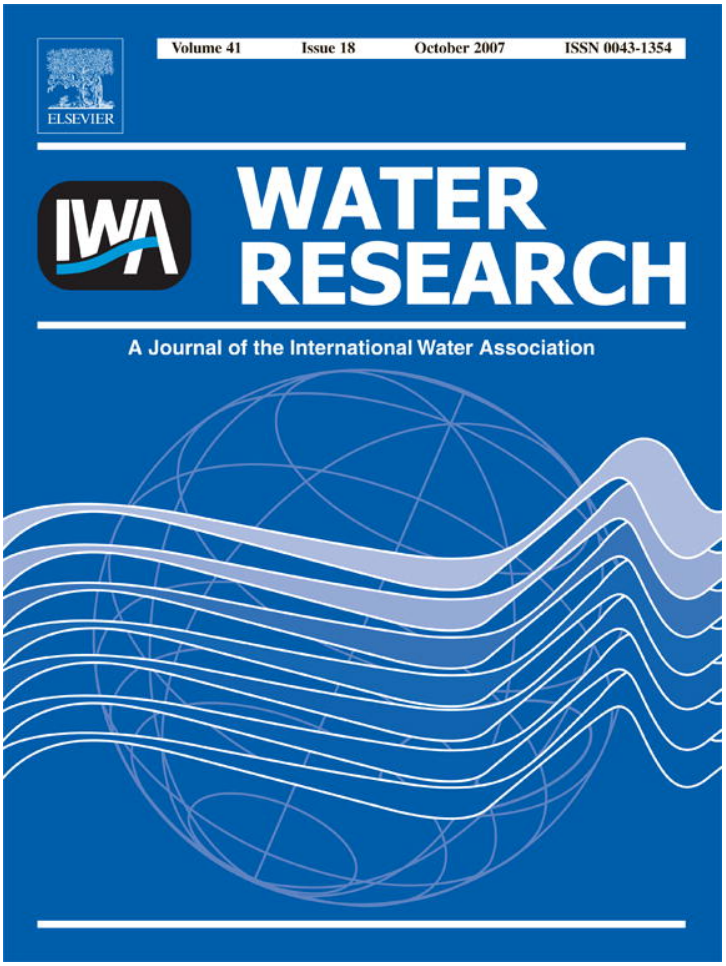

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Review

Biosorption and me

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ABSTRACT

Biosorption has been defined as the property of certain biomolecules (or types of biomass) to bind and concentrate selected ions or other molecules from aqueous solutions. As opposed to a much more complex phenomenon of *bioaccumulation* based on active metabolic transport, *biosorption* by dead biomass (or by some molecules and/or their active groups) is passive and based mainly on the "affinity" between the (bio-)sorbent and sorbate. A personal overview of the field and its origins is given here, focusing on R&D reasoning and know-how that is not normally published in the scientific literature. While biosorption of heavy metals has become a popular environmentally driven research topic, it represents only one particular type of a concentration-removal aspect of the sorption process. The methodology of studying biosorption is based on an interdisciplinary approach to it, whereby the phenomenon can be studied, examined and analyzed from different angles and perspectives—by chemists, (micro-)biologists as well as (process) engineers.

A pragmatic science approach directs us towards the ultimate application of the phenomenon when reasonably well understood. Considering the variety of parameters affecting the biosorption performance, we have to avoid the endless empirical and, indeed, alchemistic approach to elucidating and optimizing the phenomenon—and this is where the power of computers becomes most useful. This is all still in the domain of science—or "directed curiosity". When the knowledge of biosorption is adequate, it is time to use it—applications of certain types of biosorption are on the horizon, inviting the "new technology" enterprise ventures and presenting new and quite different challenges.

Contents

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1. Biosorption and the interdisciplinary challenge

"*Booohuuuuumiiiil …*"—a booming voice calling my name reverberated through the hallway of a temporary lab building at the University of Western Ontario and I jumped from whatever I was doing. I knew that this hurricane barreling down the hall could be none else but my Ph.D. supervisor Jim Zajic—in a good mood, overflowing with some idea or news, with his lab coat tails flailing behind him. He called me by my full first name when he was in a good mood and those were the days when academic supervisors wore lab coats and personally taught their students how to do things in the world of experimental science. And that's what I came back to school for, a few years after getting my engineering degree. Biochemical engineering was my choice and, as a good Czech, I had become involved in brewing a better pint of beer during my first years of "job experience" working in the Czech Food Research Institute. In my encounters with biochemists and microbiologists there, I realized that there was hardly any communication between "us" engineers and "them"—the natural sciences crowd. While my notion of the smallest living thing appeared to be the fire-ant that I could barely see, they talked about yeasts, enzymes and electron microscopy. That made me realize that I needed to learn about the "bio-things" and hope that some of the bio-people may eventually even pick up the significance of boundary conditions of the differential equation and process scale-up parameters. Then we could thus start to understand each other and do really interesting things together.

Professor James Zajic was one of those biochemists-turned-engineers—and, for a good measure, he added a law degree (!) on top of it all ("I was not going to be put down all the time by those corporate lawyers!") when he was building his long career with Kerr-McGee Corporation in the USA. He made me uphold my part of the "interdisciplinary bargain" by enrolling me in post-graduate courses in biochemistry and microbiology ("Boya, you may be a good engineer—but you have to pick up that 'bio'!"). That made for probably the toughest few years of my life, and it was all in the English language somewhat foreign to me—with all its various accents from Scottish and Welsh ones to the Texan drawl. There was a little research group of us—interdisciplinary graduate students working on different areas of what was labeled as biochemical engineering. I mention all this because that is where and how I learned also about biosorption. In our discussions surfaced the question about the fallout from the early nuclear experiments in the Pacific—the concentration of some strange nuclear elements in the plankton was so conspicuously high after them! Well, if that kind of biomass seems to effectively concentrate some of the fallout elements, could we somehow use this peculiar property?

That question was deposited in my mind during those early days. And to me these are the origins of the idea of "biosorption". It took quite a few years before there came any action on that basic "curiosity-driven" idea. Only when I became a tenured and established academic did I find enough courage, and time, to act on it. With my advancing years, I give a full credit to my students—we, as Professors, are generally only as good as our students are. And I do realize that my career advances and all that I have become is only thanks to all my students and researchers that I worked with. In this respect, the biosorption "sacrificial lamb" walked one day through my door. His name was Marios Tsezos and he was full of enthusiasm and energy—ok, let's try how, for instance, uranium is picked up by biomass, microbial biomass (I was a fermentation man). Some microbial biomass was right on my desk in front of us—it was a filamentous mold *Rhizopus arrhizus*. One microbiologist friend of mine gave me his industrial culture isolate producing interesting lipids. He will have to forgive me using it for quite a different purpose. This fungus is known as a common bread mold, easy to cultivate in my fermentors.

"Putting together" the *R. arrhizus* biomass with uranium in solution was easy. Uranium served as a good and unpopular enough nuclear fuel element of interest from several angles—concentrating it and removing it from solution sounded like a good proposition. Already early on we had to learn something about sorption equilibrium and corresponding isotherms to quantify the sorption behavior. I don't know if "luck" is the basis of all discoveries as often said, but there was certainly some serendipity in discovering that our microbial biomass was accumulating good quantities of uranium from the surrounding solution. The high atomic weight of uranium made our results sound even more impressive—when expressing its uptake, as we inadvertently did it, in mg U/g biomass. Our enthusiasm was certainly aided by quite an accidental public exposure. Our first uranium biosorption results were presented at the ACS Annual Conference—exactly at the time of the infamous Three Mile Island nuclear incident in the USA (Pennsylvania, 1979), where one of the reactors almost melted down leaving behind thousands of gallons of highly radioactive water sitting in the basement of the containment building. "Prof, could you remove the elements from the solution—could we use biosorption to assist with the clean-up there?" was one interesting question from the press people at the Conference. Needless to say, those serendipitous circumstances generated some waves and quite a bit of enthusiasm.

Please do not consider it as immodest, as it is for the convenience's sake that I will use examples of our own work

and references in this personal review. Every one of them, in turn, contains a wealth of references that will initiate a chain reaction of information on each specific topic addressed. In addition, during more than two decades of our research on biosorption, we accumulated almost 3000 most relevant references in that field—they are all now available in the EndNote format (www.biosorption.com/order).

2. The threat of metals in the environment

The toxicity and health hazards associated with heavy metals have been established beyond any doubts. What kinds of metals should we be interested in examining—in terms of removing their threat from the environment by biosorption? Realize that eventually quite a bit of time and energy is spent on the metal(s) that we choose for biosorption studies. However, more toxicology of heavy metals will not be discussed here—there are volumes of it, and while some metals are clearly toxic toxicology and classification of others is still subject of extensive research. There are at least three major points to consider when choosing the metal for biosorption studies to focus on:

(1) metal toxicity (direct health threat);
(2) metal costs (recovery interests);
(3) how representative the metal may be in terms of its behavior (scientific studies).

The "Big Three" metals are known for their high toxicity and impact: lead, mercury and cadmium. All have had their share of negative publicity even in the media as they have been connected to major poisoning or health hazards. Thee Big Three are closely followed by others, perhaps not as ubiquitous, such as arsenic, that has been a poison of choice for centuries, and chromium, used in large quantities industrially for quite some time. Note that the most toxic forms of these two appear as anionic species (–) in solution (arsenate and chromate).

Arsenic literally "surfaced" relatively recently in what has been reported in the media as "the largest poisoning in the history of mankind"—affecting close to 70 million people (!) in Bangladesh and Eastern India. In these regions, the (organic) contamination of surface waters forced the population to drill wells for supposedly safer drinking and irrigation water supply. However, as it was established only recently, the underground well water contains enough arsenic, naturally leaching from the local geological formations, to cause severe toxicity effects when used. This is a typical case of a not unusual non-anthropogenic pollution that threatens the entire population segments. This type of arsenic-related problem is not limited only to the above-mentioned areas, it occurs locally in many others in China, South America and even the USA. While there are indications that biosorption could be used to economically treat the arsenic-contaminated water (Niu and Volesky, 2007b), there is no immediate remedy for this particular catastrophe that is currently still in progress. Similarly for mercury, both anthropogenic (gold mining in Amazonia) and natural (sub-arctic lakes), that has been recognized as the cause of health problems in local populations. However, for all practical priority reasons, the metal biosorption studies are focusing on mainly anthropogenic point sources of metal releases into the environment. Among these, the following four appear as the main priority targets, particularly in the industrialized world:

(1) acid mine drainage (AMD)—associated with mining operations;
(2) electroplating industry waste solutions (growth industry);
(3) coal-based power generation (throughput of enormous quantities of coal);
(4) nuclear power generation (uranium mining/processing and special waste generation).

Most of the metals originating from the above sources occur in simple cationic (+) forms. While the toxicity of lead has been well known for some time, the toxicology and, correspondingly, the limits to presence in surface waters of copper, zinc, nickel and some others are still being debated in many areas of the world.

It is important to note that just plain high uranium toxicity to humans, quite apart from its perhaps higher radioactivity "fame", prompted a special interest. Similarly for thorium, another nuclear-cycle element, also briefly examined for biosorption in our laboratory earlier (Tsezos, 1980). Metal biosorption studies rapidly focused on toxic heavy metals such as lead and cadmium, leaving somewhat behind mercury and chromium (Kratochvil et al., 1998), which are of a "different kind" and much more difficult to study. What makes some metallic elements easier or more difficult to study is their solution chemistry and, correspondingly, ionic state(s). We purposely shunned lead and chromium in our earlier work because of their more complex solution chemistry. Even uranium prepared some surprises in terms of its ionic states containing either one or two uranium atoms—that obviously affected the overall uptake of this metal (Yang and Volesky, 1999b).

Anionic metal species or complexes offer a specific challenge in both their toxicity and sequestration. Some notorious toxic species of arsenic and chromate (Cr(VI), Cr(III) is a cation) are in this group. The major arsenic species found in environmental samples are anionic complexes of arsenite As(III), arsenate As(V), arsenious acids (H_3AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$), arsenic acids ($HAsO_4$, $H_2AsO_4^-$, $HAsO_4^{2-}$), dimethylarsinate (DMA), monomethylarsenate (MMA), arsenobetaine (AB) and arsenocholine (AC). Among the arsenic compounds in the environment, of particular interest is arsenite, which is 10 times more toxic than arsenate and 70 times more toxic than the methylated species, DMA and MMA. DMA and MMA are moderately toxic, whereas AB and AC are virtually non-toxic. These facts indicate why it would be of a priority interest to develop methods for the selective removal of anionic As(III) complex. Among other anionic metal complexes are more rare but also toxic selenate (usually existing as two anionic species in solution: $HSeO_4^-$ and SeO_4^{2-}) and vanadate (usually existing as an anionic V(V) oxy-ion complex). Corresponding to their anionic state, all these compounds require different types of biosorbents.

Last but not least, two aspects affecting the choice of metal to study in conjunction with biosorption are the recovery aspect for metals with high enough price tags and the consideration of how typical a metal could be, representing perhaps a whole group, enabling thus to extend the findings to that group. A typical example for the latter aspect would be, for instance, the valence of the metal in its ionic state: while potassium could represent monovalent cations, copper or cadmium could serve as simple representatives of divalent cations, aluminum could well represent trivalent cations.

For a long time, people were asking me about gold : "—well, if you can concentrate and recover heavy metals, why not do it with gold?". Following some initial trepidation on our part—gold is so inert!—I eventually broke down and asked an interesting person to look at biosorption of gold. That person was Nural Kuyucak, who walked into my university office barely speaking any English—Turkish only. Following our mostly signing "conversation", I understood that while her husband studies in the neighboring Department of Metallurgy, she has a suitable background and driving interest to do something on biosorption with my research group. Gold jumped into my mind—"Nural, why don't you test some biomass for the uptake of gold ?". Her eyes shone with excitement and Nural disappeared. For some time, she kept quiet but I saw her keeping busy in the lab and waving her hands as she "talked" to other students. A few months later, Nural came back and in much improved English reported her gold uptake results. Just too good to be true—almost one half of the biomass weight in gold uptake! Go back, my friend, check your procedures, check your analytics, check everything, this could hardly be so. Ok, she shrugged and disappeared again only to show up some time later, firm and confident with her results.

"Would you bet you shoe on the gold uptake that we see here?"—I jokingly asked. Without further ado, she took off her shoe and put it on my desk. And this is how we came to a patent on biosorption of gold (Volesky and Kuyucak, 1988) and Nural eventually earned her "gold" Ph.D. Biosorption of trivalent cationic gold Au^{+3}, to be more exact—note the valence, it is of importance. Because in practice, gold is most often extracted from ores with a cyanide solution, forming an anionic gold–cyanide complex that is very effectively sorbed by activated charcoal—and that process is hard to beat, as we learned somewhat later (Niu and Volesky, 1999). Needless to say, the gold case is a typical example of metal sorption for the purpose of not only metal removal from solution but also its recovery—the driving force being the high metal value. There are quite a few other metals of interest in this category. Of some interest for recovery are the rare earth elements such as lanthanum, europium and ytterbium (Diniz and Volesky, 2005), all forming mainly trivalent cations in solution.

3. The mystery of biomass–metal interaction

It is rather a tedious job to examine one by one all kinds of biomaterials for their capacity to bind and concentrate various metal species. There are biomass types of many various kinds (e.g. leaves, wood or agricultural residues, waste crustacean biomass—anything renewable that grows, including microbes), and while some of them may not sequester metals to any appreciable degree others show a promising potential. One only wishes that there were some kind of a preliminary sign of that capacity. For practical and eventual biosorption process scale-up reasons, we are looking for abundant biomass, easily available in large quantities and as cheap as possible, as waste types are actually very desirable. A high-sorbing but rare biomass type may be interesting to study—but what if we eventually wanted to have tons of it? I remember the uproar I caused at one large world congress of seaweed scientists by asking their plenary gathering where we could obtain several thousand tons of *Sargassum* seaweed, our best metal biosorbent, required for decontamination of one huge poisonous mining-site lake. My question really fired their imagination because collecting such amounts of wild *Sargassum* would possibly wipe out the entire world stocks of it. And nobody cultivated *Sargassum* so far—but it is possible …

Similarly to special anionic exchange resins, biosorbents that are capable of sequestering anions contain appropriate chemical active groups within their structures. It is often the NH_2 group that is active and ubiquitous in fungal cell walls as well as in the chitin/chitosan components of crustacean exoskeletons (e.g. crab shells) (Niu and Volesky, 2006, 2001, 2003).

We know that there are chemical active sites in the biomass that are responsible for sequestering metals from the surrounding solution. Now that we understand more just what kinds of sites these are, we can focus and conduct our explorations more intelligently, saving ourselves a lot of time, energy and disappointment. Obviously, we can start manipulating the natural biosorbent by chemically introducing the binding sites (Holan and Volesky, 1995). However, that invariably increases the cost of the biosorbent, bringing it closer to the domain of man-made ion exchange resins—and those are what "environmental biosorption" has to compete with as the eventual process cost-effectiveness is of a major concern.

The early strong indications of ion exchange being at the root of biosorption metal uptake (Treen-Sears et al., 1984) led us to examining the active chemical groups involved in the metal binding. The most important of them are summarized in Table 1. The knowledge of the cell wall composition and its differences among microbial types came handy (Fig. 1a–d). While our *Rhizopus* and other molds have prominent chitin layers in their cell walls, bacteria have quantities of peptidoglycan (gram+) and teichoic acid (gram−) in their walls—all of these featuring important ion-exchange active groups in their structures. In these cursory examples, one can easily see the importance of the biosorbent structure knowledge that originates from different science disciplines such as biochemistry and microbiology. The addition of seaweeds as a source of biosorbent materials (Davis et al., 2003c; Figueira et al., 2000) only underlines the need for interdisciplinary expertise. Their cell wall biopolymers such as alginate feature carboxylic groups active in metal sequestering (Davis et al., 2003a, b). Just why some carboxylates bind metal ions and others don't remains a challenging question to be answered—perhaps with the aid of contemporary thermodynamically based computer molecular modeling, a chemistry domain.

Table 1 – Major binding groups for biosorption

Binding group	Structural formula	pK_a	HSAB classif.	Ligand atom	Occurrence in selected biomolecules
Hydroxyl	–OH	9.5–13	Hard	O	PS, UA, SPS, AA
Carbonyl (ketone)	>C=O	–	Hard	O	Peptide bond
Carboxyl	–C=O | OH	1.7–4.7	Hard	O	UA, AA
Sulfhydryl (thiol)	–SH	8.3-10.8	Soft	S	AA
Sulfonate	O || –S=O || O	1.3	Hard	O	SPS
Thioether	>S	–	Soft	S	AA
Amine	$–NH_2$	8–11	Int.	N	Cto, AA
Secondary amine	>NH	13	Int	N	Cti, PG, peptide bond
Amide	–C=O | NH_2	–	Int	N	AA
Imine	= NH	11.6–12.6	Int	N	AA
Imidazole	–C-N-H || >CH H-C-N	6.0	Soft	N	AA
Phosphonate	OH | –P=O | OH	0.9–2.1 6.1–6.8	Hard	O	PL
Phosphodiester	>P=O | OH	1.5	Hard	O	TA, LPS

PS = polysaccharides; UA = uronic acids; SPS = sulfated PS; Cto = chitosan; PG = peptidoglycan; AA = amino acids; TA = teichoic acid; PL = phospholipids; LPS = lipoPS.

Chemistry background and knowledge are extremely useful in exploring biosorption. Information on the active sites involved in the sequestration of sorbates is derived by using either simpler techniques such as titration (Fourest et al., 1996; Fourest and Volesky, 1996; Naja et al., 2005) or more sophisticated instrumental analyses (Figueira et al., 1999a) including spectroscopy (e.g. infra-red and Raman spectroscopy, electron dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS)), electron microscopy (scanning and/or transmission), nuclear magnetic resonance (NMR), X-ray diffraction analysis, etc. Each of these techniques is capable of revealing certain aspects of the state of sorbate and its interaction with the binding site. This type of information on the binding mechanism is important, for instance, in composing the mathematical model of the sorption phenomenon that is eventually used for computer sorption process simulations guiding further experimental work and for predicting sorption performance under different operating conditions. However, it is important to realize that all those sophisticated analytical techniques may be very expensive and the type of information they yield may not always be so crucial to understanding and evaluating the phenomenon.

4. The sorption equilibrium—and where the mistakes are made

In studying biosorption behavior, we always endeavored to avoid micro-precipitation phenomena and their contribution to the uptake by maintaining a lower pH around pH 4.5–5 in the sorption system. The pH control in the system is important because it affects both the configuration of the active ion-exchange sites as well as the ionic state of the sorbate in the solution. At low pH the concentration of protons is high and the ion-exchange sites become solidly protonated. This, on the other hand, indicates the possibility of displacing the metals sequestered on the biosorbent by a

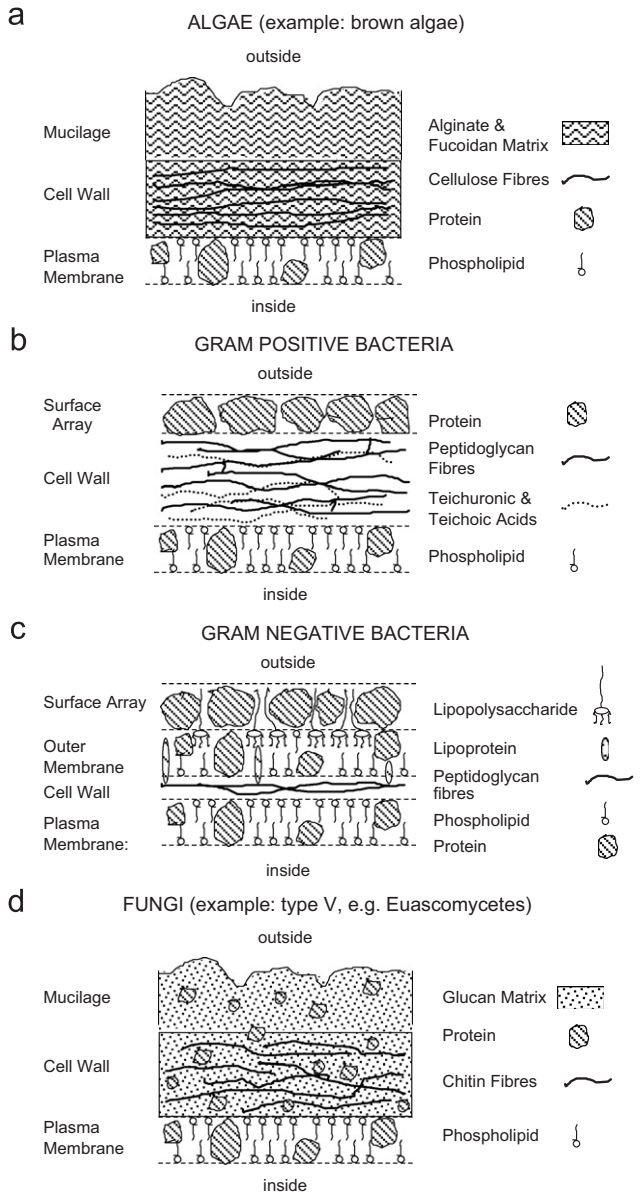

Fig. 1 – Schematic outline of the cell wall structures of (A) seaweeds; (B) gram+ bacteria; (C) gram− bacteria; (D) fungi.

simple acidic wash. The regeneration of the biosorbent material enables its multiple reuse, further increasing the economy of its use.

The most preliminary information on the performance of any given sorption system comes from the equilibrium sorption studies. These simple solid–liquid contact tests represent thus possibly the most important aspect. By definition, "enough time" has to be afforded for the contact before sorption equilibrium is reached between the sorbate sequestered on the solid sorbent and the sorbate concentration in the liquid phase. Only when there is no more change in that concentration can we consider that equilibrium has been reached. The summary of the sorption system performance is reflected in the sorption isotherm whereby the equilibrium (final or residual) sorbate concentration (C_f) is plotted against the sorbate uptake by the sorbent solids (q)

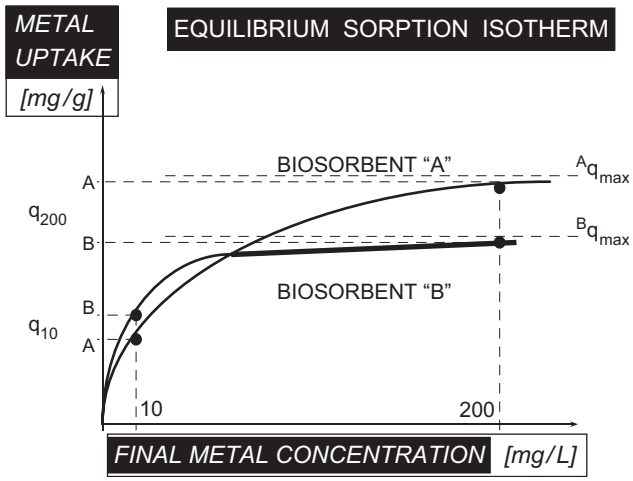

Fig. 2 – Comparative example of sorption isotherm curves: Sorbent B performs better (higher q at q_{10}) than Sorbent A at lower equilibrium concentrations (e.g. $C_f = 10$ mg/L).

(Fig. 2). The degree of the sorbent "affinity" for the sorbate determines its distribution between the solid and liquid phases.

That somewhat mysterious word "affinity" appears so as to explain the basis of the sorption behavior. However, its own substance is rather elusive—what is it really that provides for two moieties to be attracted to each other and to eventually lock up together. At the bottom of the answer lies another single word for a simplistic answer pointing to the swirl of electrons—thermodynamics. Yes, the basis of sorption—and most of other types of behavior based on different types of reactions, including the basis of life itself—seems to be in thermodynamics. The thermodynamic explanation of the sorption behavior, of the affinity that we observe, with appropriate equations and all, is the elusive one that science is striving for. Correspondingly, the thermodynamically based all-encompassing models of the sorption phenomenon, while attempted (Jossens et al., 1978; Najm et al., 1991; Radke and Prausnitz, 1972), remain an outstanding scientific challenge. There is still a long way to go …

The quality of the sorbent material is judged according to how much sorbate it can attract and retain in an "immobilized" form. For this purpose, it is customary to determine the metal uptake (q) by the biosorbent as the amount of sorbate bound by the unit of solid phase (by weight, volume, etc.). The calculation of the metal uptake [mg Metal/g (dry) sorbent] is based on the material balance of the sorption system: sorbate which "disappeared" from the solution must be in the solid:

$$q = V\,(\mathrm{L})\,(C_i - C_f)\,(\mathrm{mg/L})/S\,(\mathrm{g})\,(\text{in weight units mg/g}),$$

$$V\,(\mathrm{L})\,C_i\,(\mathrm{mg/L}) = \text{all the sorbate in the system (mg)},$$

$$V\,(\mathrm{L})\,(C_f)\,(\mathrm{mg/L}) = \text{the sorbate left over in the solution (mg)}.$$

V is the volume of the metal-bearing solution contacted (batch) with the sorbent (L); C_i and C_f are the initial and equilibrium (residual) concentrations of the metal in

the solution, respectively. They have to be analytically determined (mg/L); S is the amount of the added (bio)sorbent on the *dry* basis (g).

The sorption uptake q can be expressed in different units depending on the purpose of the exercise:

(1) For practical and engineering process evaluation purposes, which are eventually concerned with process mass balances, it is customary to use *weight per (dry) weight* [e.g. mg of metal sorbed per g of the (dry) sorbent material].
(2) Ultimately, mainly because of the reactor volume considerations (e.g. a packed-bed column), the uptake may also be expressed on a *per volume* basis (e.g. mg/L). However, the volume porosity (voids) may present a complication in quantitative comparison of biosorption performance.
(3) Only when working on the stoichiometry of the process and when studying the functional groups and metal-binding mechanisms, it may be useful to express q on a *molar* or *charge equivalent* basis—again, per unit weight or volume of the sorbent (e.g. mmol/g or meq/g).

All these units are relatively easily inter-convertible. The only problem may arise with the sorbent weight–volume conversions. For scientific interpretations, the sorbent material dry weight basis is thus preferred. It entailed quite some work to convert various quantifications of biosorption to a common basis as done in one of the few comprehensive earlier reviews (Volesky and Holan, 1995) . Needless to say, many new results appeared in the literature since then.

The use of "wet biomass weight", unless the (wet weight/dry weight) conversion is well specified, should be discouraged. Different biomass types are likely to retain different moisture contents, intracellular as well as that trapped in the interstitial space between the cells or tissue particles (e.g. seaweed particles). Different types of biomass obviously compact in a different way.

4.1. *Experimental sorption isotherm*

It is relatively simple and easy to obtain laboratory equilibrium sorption data for a single sorbate. A small amount of the sorbent tested is brought into contact with solution containing the given sorbate. However, the "environmental" parameters in the sorption system (particularly pH) have to be carefully controlled at the given value over the entire period of contact until the sorption equilibrium is reached. It may take a few hours or much longer depending on the size of sorption particles and the time it takes until they attain sorption equilibrium. A simple preliminary sorption dynamics test will establish the exposure time necessary for the given sorption system to equilibrate. That is determined by time-based analyses.

Safely "enough" time will then have to be allowed for the sorption system to reach equilibrium. Still quite frequently appearing wrong reporting of "isotherms" makes it useful to include here the following outline of the general experimental procedure to obtain data points for the sorption isotherm (Volesky, 2003) (Fig. 3):

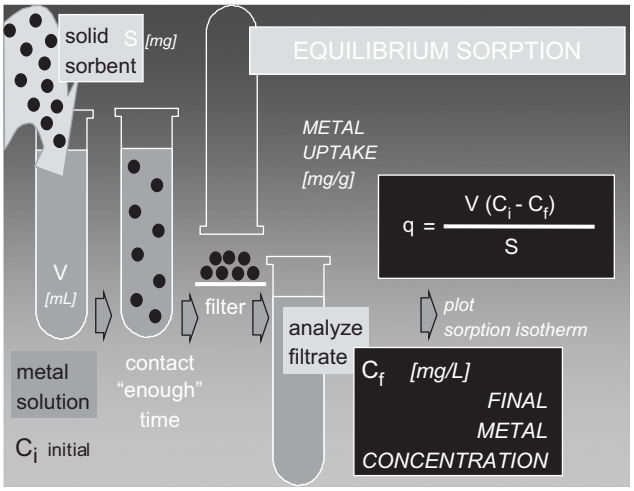

Fig. 3 – Outline of the experimental procedure to obtain data points for the sorption isotherm.

(1) Prepare the sorbate in solution at the highest concentration of interest.
(2) Make dilutions to cover the entire concentration range (from 0-blank, to the max.).
(3) Adjust the "environmental parameters" (e.g. pH, ionic strength, etc.).
(4) Determine analytically the sorbate initial concentrations (C_i) in all the liquid samples.
(5) Distribute the samples into appropriate-volume containers (record V = 30–150 mL of liquid) such as flasks or test tubes (in duplicate, triplicate or as required).
(6) Weigh accurately each (approximate) amount of the (bio)sorbent solids to be used in each contact test and record each amount (S mg).
It may help to be able to roughly estimate the anticipated sorption uptake so that there is a well-detectable sorbate final concentration left in the solution at equilibrium in each sample. If there is too much of solids added, there may be virtually no sorbate left in the solution for a reliable analysis.
(7) Add the sorbent solids into each sample solution and provide for rather gentle mixing over the contact period ("enough" time).
(8) Make sure the "environmental" parameters (pH) are controlled at a constant value during the contact period (use appropriate acid or base for the purpose; do not "dilute" the sorption system by adding excessive volume).
(9) At the end of the contact period, separate the solids from the liquid (decantation, filtration, centrifugation, etc.)
(10) Analyze the liquid portion for the residual, final, equilibrium sorbate concentration (C_f).
(11) Calculate the sorbate uptake: $q = V\ (L)\ (C_i - C_f)\ (mg/L)/S\ (g)$
Note that q could also be determined directly by analyzing the separated solids and thus closing the

material balance on the sorbate in the system. However, this usually presents analytical difficulties (digestion–liquefaction of solids and/or very sophisticated analytical methods may be required).

A variation of this approach is the "tea-bag experiment", whereby a "small" amount of sorbent is physically separated (contained in a permeable "bag") in a large volume of solution that would not change its sorbate concentration, thus $C_f = C_i$ and only the solids are analyzed.

In either case, the C_f in the liquid must be known for the sorption isotherm plotting:

(12) Plot the sorption isotherm q vs C_f.

Note that for all practical purposes the choice of experimental variables narrows usually down to two: concentration C_i and the amount of sorbent solids S contacted. One or the other or both can be varied. In the above procedure, it was the concentration of sorbate dilutions C_i.

The key point is to obtain measurable and different values of C_f at the end of the contact experiment.

From the equilibrium principles, it is easily seen that the initial concentration of sorbate (C_i) is of little relevance in these kinds of sorption tests. It can assist in identifying the final concentration range, which, of course, depends on the amount of sorbent solids (S) in the system. Also note that one has no control over the value of C_f, it sort of "happens" during the experiment. This must not happen, for instance, to pH that has to remain constant. It is important to emphasize that the sorption isotherm procedure assumes that all the external sorption system parameters are not changing (constant pH, ionic strength, another sorbate concentration, etc.).

5. The process considerations and sorbent regeneration

Due to the concentration difference driving force for sorption (between the sorbate in the solution and that already sequestered), generally the most effective configuration of the sorption system is that based on a flow-through fixed-bed type of a reactor/contactor (Fig. 4). The sorption column would generally not exceed approximately 1.8 m in diameter and 4–5 m in height. The scaling up of the process to accommodate larger flows is attained by simply multiplying the number of columns that would operate in parallel. Rarely, usually because of special process requirements, a mixed contactor could also be used. Different, usually multi-stage, countercurrent configurations could then be arranged.

The overall performance of the processes based on the sorption column mode of operation is judged by the column operating time (until the column sorption capacity is exhausted), characterized by the sorbate break-through point as determined at the column exit (Fig. 5). Since the fixed-bed column does get eventually saturated, a process arrangement is often used whereby one column is in active sorption operation while another one is being prepared (regenerated and cleaned) during its stand-by period. That column

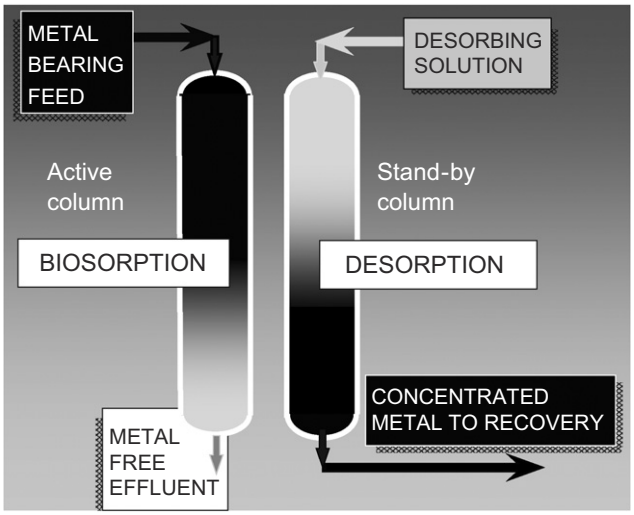

Fig. 4 – The flow-through fixed-bed type of a reactor/contactor sorption column.

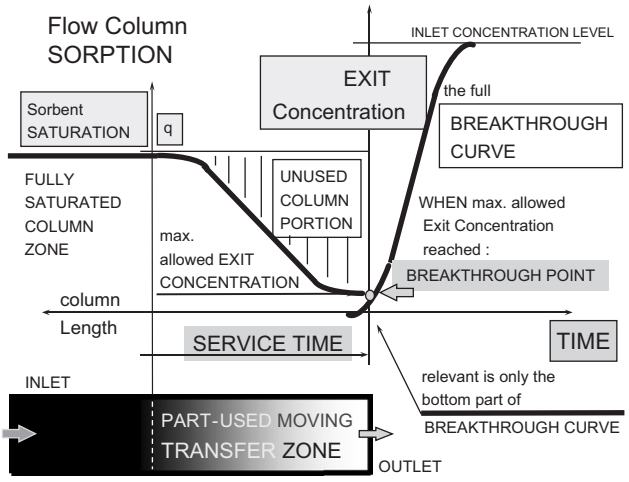

Fig. 5 – The sorbate break-through point as determined by its concentration detected at the column exit. Note the very important "unused column portion" determined by the length of the "transfer zone" formed inside the column.

preparation usually consists of elution of the sequestered sorbate with simultaneous or sequential sorbent regeneration, clean-up and washing. Sorbent regeneration capacity is an important characteristic that results in a better overall process economy.

Cationic biosorbents, for example, could be regenerated by a simple acidic wash (Aldor et al., 1995) (e.g. HCl) that quickly releases the deposited metal, making way for its very high concentration in the desorbing solution (Yang and Volesky, 1999a) and making it suited for economic recovery. The acid-based metal desorption may be followed by further steps to prepare the biosorbent for the most efficient subsequent uptake cycle. This may entail pre-saturating the active sites of the biomaterial with more pH-neutral calcium or potassium cations in order to avoid an uncontrollable acidic wave inside the column caused by the release of

ion-exchanged protons. Some overall process optimization may be useful in this respect.

6. Use models and optimize biosorption performance

Because they yield the most important information, it is mandatory to do all the equilibrium sorption studies prior to the continuous-flow sorption column tests. It is quite useless to do the latter with inadequate attention to the former and also without applying the appropriate methodology for evaluating and generalizing the column sorption experimental results. Such methodology is usually based on computer modeling of the process that must go hand in hand with the experimental column work. Otherwise, literally hundreds of experimental break-through curves could be generated from the column by varying values of selected process parameters (e.g. flow rate, column length, packing density, etc.)—with no useful interpretation possible or with trivial and obvious conclusions. Instead, these results could be generated in a very short time by computer simulations that need not even be so perfect (Figueira et al., 1999b; Naja and Volesky, 2005, 2006). However, there seems to be a professional barrier in the computer work to be undertaken in this direction. There is an example of a simple test question to be asked prior to column studies: "What flow rate is to be applied?" The answer is far from obvious and it could easily be seen that it must usually be generated from somewhere—perhaps best from computer simulations of the

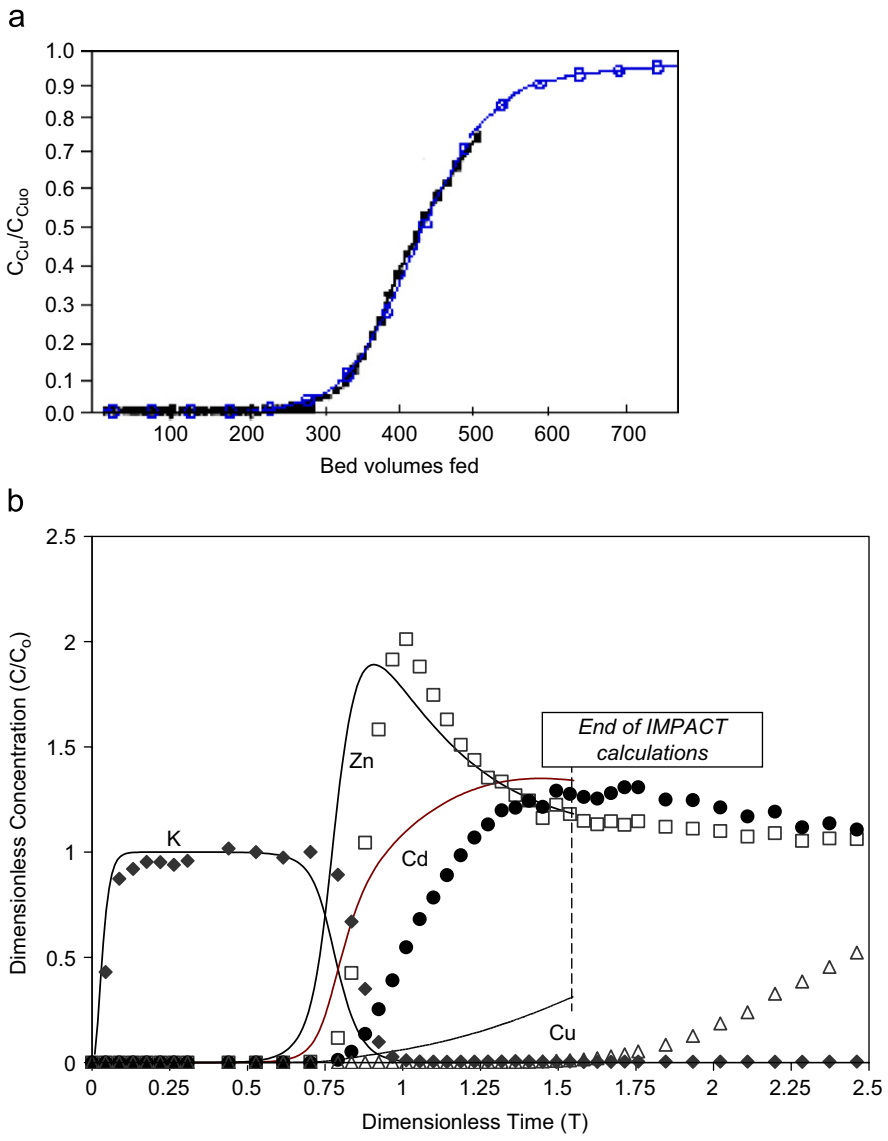

Fig. 6 – An example of a sorption column performance computer simulation as it closely reflected the experimental points: (A) comparison of experimental (■) and model (□) breakthrough curves for Ca-Sargassum and feed containing 1 meq/L Cu^{2+}; (B) comparison of experimental and model breakthrough curves for K-Sargassum and feed containing equimolar amounts of Cd, Cu and Zn. The modeling was not very successful and the computer program "Impact" used crashed at the the point indicated.

process. To just adopt some flow rate based on "experience" ("others used it") is usually not adequate, particularly in the case of biosorption, where there is virtually no experience available and the nature and configuration of biosorbent materials vary extremely broadly.

The equations, calculations and contribution of the computer in modeling biosorption in the quest for prediction of its performance start already during the equilibrium studies where stoichiometric relationships provide the basis for formulation of equilibrium equations leading to expressing the sorption equilibrium constants. The foundations for this line of work were laid almost a hundred years ago by Langmuir and Freundlich and many more who derived the various early sorption equilibrium models. While these proved most useful in our biosorption work (Chong and Volesky, 1995, 1996; Niu and Volesky, 2007a; Schiewer and Volesky, 1995a, b; Schiewer and Volesky, 1996, 1997a, b, 2000; Yang and Volesky, 2000), it is essential to realize that most of these models do not reflect the actual phenomena taking place on the ionic or molecular level. Whether they fit the experimental data does not prove much in particular; indeed, most often this is an exercise in curve fitting to experimental data sets. However, the quantitative mathematical equilibrium representation eventually provides the necessary input in the form of an equation in a more complex equation set that describes dynamic sorption, e.g. in the column. Together with equations characterizing the mass transfer and fluid flow in the sorption system, the equilibrium relationship becomes part of the sorption system model. Solving all those model equations simultaneously allows us to describe the sorption system behavior and gives us the tool for predicting its performance.

The current confluence of computer power, numerical software methodology and accumulated knowledge in sorption and biosorption fields offers an enormous opportunity to examine processes and their performance "virtually"—in their computer simulation. Not that I would be a computer maniac, but my experience keeps bringing me to it in a full experimental cycle. Nowadays, we don't have to do any difficult programming ourselves as the case used to be; for solving our mathematical model equations of the processes that we study, we have now commercial solver programs such as COMSOL (FEMLAB, 2004) that are user-friendly and so powerful. They allow us to solve complex sorption models sensitive to the more important external sorption system parameters such as pH, ionic strength and even ionic speciation (Naja and Volesky, 2006; Niu and Volesky, 2007a; Yang and Volesky, 2000). Fig. 6 shows an example of a sorption column performance computer simulation as it closely reflected the experimental points.

The purpose of computer process simulations is actually dual: (1) to guide the experimental work (by assisting in the selection of the most important and informative experiments), and (2) to optimize the column sorption performance together with, eventually, also the overall performance of the entire sorption process to be operated (multiple columns, including the regeneration operation(s) and stand-by timing). Every new experiment generates new data that, in turn, could subsequently also serve for improving the mathematical process model (Fig. 7).

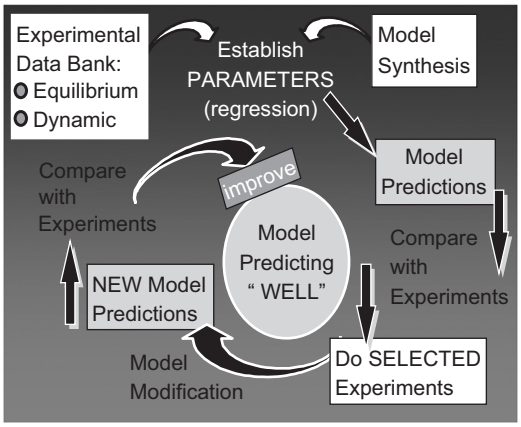

Fig. 7 – Mathematical modeling and computer simulation guides the next round of experimental work, which, in turn, serves as a basis for further model improvements to make it better reflect the reality.

The final optimization of the process is then performed based on the costs—"optimization by the dollar"—because that is eventually the ultimate criterion. In this area, the "good science" must provide the basis for (cost-)effective process engineering.

6.1. *From the lab to applications—a new technology venture*

A considerable amount of research on biosorbent materials has developed a solid basis of knowledge and indicated their enormous potential. At the early stage of considering biosorption as a useful technology, the highest priority is at least some preliminary and approximate assessment of its commercial potential and application feasibility. Correspondingly, these early indications should be based on some basic studies that need to be completed:

A. Assessment of the competing technologies
For cases where metal removal from contaminated industrial effluents is considered, the following process alternatives can be considered for a more detailed evaluation and feasibility assessment:

Precipitation	Bio-reduction
Reverse osmosis	Ion exchange

The overall performance and process application modes of biosorption justify a comparison with the ion exchange technology. In the comparison of ion exchange and biosorption processes, the following hold:

- The same equipment (i.e. pipes, columns, etc.) can be used with both (a given treatment installation can be interchangeably used with both types of sorbents).
- According to all estimates, biosorbents can be at least an order of magnitude cheaper (1/10).
- Only a shorter life cycle can be assumed for biosorbents. The limits of ion exchange resins have, to a large degree, been reached and these products are considered a

Fig. 8 – The main steps required prior to the actual launching of the biosorption technology venture.

chemical commodity now. The growth rate of the ionex technology appears to have been a "flat" one already for quite some time. The price of ion exchange resins, that are hydrocarbon derivatives, is invariably linked to that of crude oil. Needless to say, crude oil is a finite resource and, in addition to that disadvantage, its price is also very much subject to the world trading stability. The most compelling reasons for using biosorption technology, based on renewable or waste raw materials, are that it is effective and inexpensive. That certainly guarantees the possibility of easily opening new markets. There is also an extremely high development potential associated with the new concept of biosorption. The main steps required prior to the actual launching of the biosorption technology venture are shown in Fig. 8.

B. Assessment of costs of new biosorbents
Estimation of the costs of preprocessing and drying the raw biomass to prevent its degradation has to be carried out for selected representative types of biomass available in large quantities. Preliminary technical work needs to be carried out on the processing necessary for biomass formulation into a biosorbent product suitable for process uses. It is anticipated that different raw biomass materials (algae, fungi, bacteria) will require different and specific treatment for their optimal formulation into finished ready-to-use products. This part would entail specifically planned small-scale laboratory work and preliminary optimization of the procedures involved in obtaining an efficient and cost-effective biosorbent material.

C. Assessment of the market size
The potential application for biosorption appears to be enormous as huge markets already exist for cheap biosorbents. Electroplating and metal finishing operations, mining and ore processing operations, smelters, tanneries and printed circuit board manufacturers are a few of the industries in which metal bearing effluents pose a problem. All together, more than a thousand tons of heavy metal is released into Canadian waters by polluting industries in the area of fabricated metal products industry alone.

However, the actual environmental figures do not appear to be well consolidated as the environmental politics dialogue evolves. As biosorbent technology may prove cheaper and more competitive with time, it is anticipated that its new applications, otherwise perhaps not feasible, will significantly increase together with the scope of potential clients.

While the high cost of the ion exchange process limits its application (as demonstrated by the huge amount of untreated effluents still released), the cost advantage of biosorption technology would guarantee a strong penetration of the large market of heavy-metal polluting industries. It can easily be envisaged that cheaper biosorbents would open up new, particularly environmental, markets so far non-accessible to ion exchange resins because of their excessive costs, which make them prohibitive for clean-up operation applications.

The ion exchange market is as well established as the technology itself. The manufacture and supply of ion exchange hydrocarbon-derived polymer-based resins is concentrated in the hands of a very few transnational giant chemical companies. Rohm & Haas, Dow Chemicals, Bayer and only a few more are the ones that have monopolized the ion exchange market. It is worth mentioning that the exact figures of the ion-exchanger sales volume and value are rather difficult to get from usual information sources. These figures appear to be a key to assessing the potential market for biosorbents.

6.2. *Identification of potential synergies and partners*

Naturally, ion exchange *manufacturers* should be watching the developing field of biosorption particularly closely as the new biosorbents could extend their own product lines. Biosorption, as a direct competitor of ion exchange, is a tool that engineering *consulting companies* could use when designing wastewater treatment systems for their polluting clients. Biosorption would allow them to gain competitiveness by having a wider palette of remedial processes—if they acquire the appropriate know-how. Their virtually "possessive" clients, interested mainly in having their problem solved, are usually not keen on spreading the experience, mainly because it is not along their lines of business. The *supply* side for suitable raw biomass represents a large new business opportunity and a good partnership chance.

Continuing, strong and quite diversified R&D work in the field of biosorption cannot be overestimated. Within this framework, not only can more effective engineering process design/optimization tools be developed but also a contribution from the marketing and biomass supply sides would be most useful and very desirable for the start-up of a viable commercial enterprise.

7. Biosorption future—metals are only the "tip of the iceberg" (Volesky, 2003)

There is a completely different type of biosorption then discussed so far here—focused on the purification and recovery of high-value proteins, steroids, pharmaceuticals

and drugs like digoxin or vinblastin, with costs in thousands of dollars per gram. And this may be possibly biosorption at its best—and most challenging. Recovery of the high-priced pharmaceuticals—or any pharmaceuticals for that matter—is actually using this "other side of the spectrum" in biosorption: not environmentally oriented low-cost biosorption but the process of biosorption at its very sophisticated and very demanding form, biosorption targeted at product recovery; biosorption that is at the very cutting edge of the newest area of human endeavor, that explores the very foundations of living nature and life itself—*biotechnology*.

Every engineer dreams about the "silver bullet" process that would enable the recovery of one type of a molecule from a mixture of many—in one step. A process that would take the "pea-soup" pouring from some kind of a special molecule-making process and pick that one target molecule from the process in one simple and short step of an operation. That step could be, and very often already is, a sorption process—due to its efficacy in an aqueous environment that happens to be the medium of all biosystems. Recovery and purification of a precious bio-molecule from the mixture is often so complicated, difficult and costly that the eventual price for this compound becomes as high as to put it out of the realistic reach. Many sequential procedures of extraction, repeated fractionations, precipitations, re-dissolutions, distillations and such are just so involved and inefficient that the yields of these purification process sequences become miniscule and drive the costs of the recovery quite impractical.

Chromatographic procedures, based on sorption, are currently already quite widely used for separating, recovering and purifying all kinds of compounds. When bio-material is used as a sorbent, we certainly have biosorption whereby one special biosorbent can aim for and lock, very specifically, that one particular molecular compound out of a mixture of perhaps several hundreds of them—sometimes in only one step (Fig. 9).

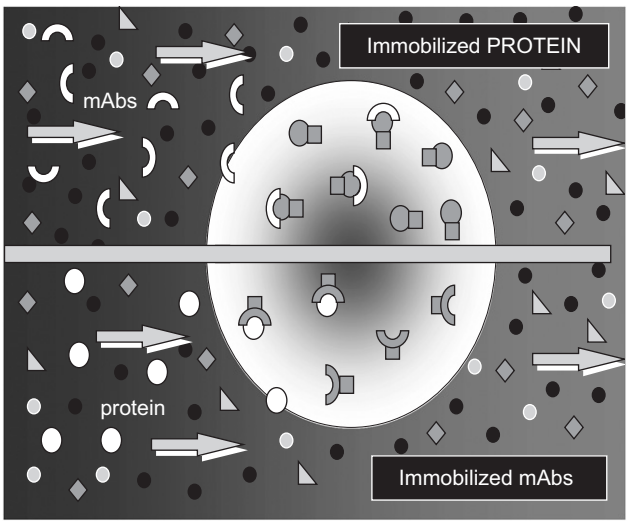

Fig. 9 – Selective sorption of a protein molecule by monoclonal antibodies and a vice-versa sorption.

7.1. *Antibodies as a biosorbent example*

Antibodies can serve as an example of an effective and very specific bio-compound manufactured by the living system. Produced by immune system, these compounds are made so as to recognize and lock one particular type of a target molecule. We understand the function of some of these compounds and we even know how to obtain them. When we "immobilize" them on a suitable solid support and fix them in a suitable environment such as a chromatographic column, we can have that "silver bullet" process for locking and thus extracting, recovering and purifying the one desirable target molecule out of the mixture.

And also the other way around: we can recover precious antibodies from a mixture by a procedure that uses the immobilized target (e.g. a protein molecule) for the antibody in order to lock the antibody itself. Usually, chromatographic procedures are used for these purposes. And thus, chromatography and sorption that provides its foundations have become the operations of choice for the recovery and purification of precious biochemical products.

In essence, this could be an example of the dream "silver bullet", a one-step recovery process. Naturally that bio-recovery is not as simple as introduced here, but the principle and the outline of new biosorption processes are here: a very powerful technology—a whole new direction in the product recovery that could assist in making applications of a number of other biotechnological advances (such as gene manipulation) feasible and practical. This could be the basis for developing biosorption into a technique for recovering and purifying, for example, at least some of those highly desirable high-value pharmaceutical compounds hard to recover through conventional multi-step procedures. This aspect could add an entirely different dimension to the research on biosorption (Volesky, 2003).

And if I had another 10 years to tear into an interdisciplinary research challenge, this is what I would turn my attention to—"*Booohuuuuumiiiil …*"; isn't this another exciting area of *biosorption*—and biochemical engineering?

Acknowledgements

I meant what I wrote about my indebtedness to my students, on all levels, and to all the research collaborators that I worked with over the years. There is not enough space to list them all here, but the names of quite a few of them appear in the Reference section below. Again, thank you all, my friends, it has been a privilege to work with you!

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