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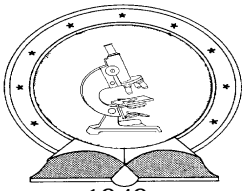

LIVING AND NON-LIVING BIOMASS AS BIOSORBENTS FOR HEAVY METAL REMOVAL FROM WASTEWATERS

Egyetemi doktori (PhD) értekezés

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Témavezető

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..……..

Debrecen, 2012.01.20. Tonk Szende-Ágnes

Tanúsítom, hogy Tonk Szende-Ágnes doktorjelölt 200.. – 200.. között a fent megnevezett Doktori Iskola *Teresztris és Kvantitatív Ökológia* programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Az értekezés elfogadását javasolom.

..…….

Dr. Tóthmérész Béla
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Debrecen, 2012.01.20.

LIVING AND NON-LIVING BIOMASS AS BIOSORBENTS FOR HEAVY METAL REMOVAL FROM WASTEWATERS

Értekezés a doktori (Ph.D.) fokozat megszerzése érdekében a Környezettudomány tudományágban

Írta: *Tonk Szende-Ágnes*

Készült a Debreceni Egyetem Juhász-Nagy Pál Doktori Iskolája (*Kvantitatív és Terresztris Ökológia* programja) keretében.

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THE SUMMARY OF THE PH.D THESIS

A PH.D. DOLGOZAT ÖSSZEFOGLALÓJA

LIVING AND NON-LIVING BIOMASS AS BIOSORBENTS FOR HEAVY METAL REMOVAL FROM WASTEWATERS

ÉLŐ ÉS ÉLETTELEN BIOMASSZA ALKALMAZÁSA BIOSZORBENSKÉNT A NEHÉZFÉMEK SZENNYVÍZBŐL VALÓ ELTÁVOLÍTÁSÁRA

A TÉMA JELENTŐSÉGE

„… Amikor a természetet szépnek látjuk, mi is ott vagyunk, tehát a szépség a természet és a mi közös alkotásunk. A szemlélt természetben benne vagyunk mi is, aminthogy a természet is bennünk van, mint szemlélőkben.” (Tavaszy Sándor)

Az elmúlt évszázadban környezetünk szennyeződése óriási mértékben megnövekedett. Az ipari fejlődés, az emberi populáció rohamos növekedése, valamint a környezetünkbe kibocsájtott toxikus vegyületek hatásai mind hozzájárultak környezetünk szennyezettségéhez. Különösen aggasztó méreteket ölt világszerte a kémiai környezetszennyezés, amely egy globális probléma forrása és a földi élet alapjait veszélyezteti.

A kémiai környezetszennyezés kérdéskörében egyre nagyobb figyelem fordul a nehézfémekkel összefüggő veszélyek felé, hiszen a mikroelemek és a toxikus nehézfémek felhalmozódása meghatározó humán-egészségügyi, ökológiai, biológiai jelentőséggel bír. A mikroelemek és a nehézfémek kibocsájtása az ipari korszak fejlődésével többszörösére emelkedett, amelyben a közlekedés és az ipari tevékenység mellett a mezőgazdasági modernizáció szintén potenciális nehézfém-szennyező forrássá vált.

Az utóbbi évtizedek kutatási eredményei, valamint a környezet állapotára vonatkozó felmérések igazolták, hogy az ipari körzetekben, városokban, közlekedési főútvonalak mentén, és sok esetben a szennyvizek, szennyvíziszapok mezőgazdasági területeken történő elhelyezésével kritikus mértékben megemelkedett a talaj nehézfémtartalma. Ezek a nehézfémmel szennyezett területek alapvető környezeti- és humán-egészségügyi problémát jelentenek. A talajok hosszú évekig képesek a nehézfémeket toxikus mennyiségben akkumulálni anélkül, hogy azok akut mérgező hatása megnyilvánulna, amelyek ha közvetve vagy közvetlenül bekerülnek a táplálékláncba -így az emberi szervezetbe is (pl. a nehézfémmel szennyezett területeken termeszett növények elfogyasztásával) ott felhalmozódhatnak és az élő szervezetek heveny vagy idült károsodását, végső esetben pusztulását idézhetik elő.

Örömmel mondhatjuk, hogy az elmúlt két évtizedben a szennyezett természeti elemek (pl. földtani közeg, felszín alatti vizek) állapotának felmérésére, megismerésére és megtisztítására irányuló tevékenység lavinaszerűen megindult. Így a környezetvédelem keretein belül a

kármentesítés fokozatosan sajátos iparággá fejlődött ki, amely iránt egyre inkább fokozódó társadalmi és gazdasági igény jelentkezik. A kármentesítési technológia a szennyezett területen a helyi adottságok alapján történik, így egyre elterjedtebbek az olyan módszerek, amelyeknél az élő rendszerek bonyolult folyamatait használják fel a végső cél eléréséhez.

A környezeti rehabilitáció (kármentesítés) egyik alternatív új lehetősége a bioremediáció, mely olyan technológiákat foglal magába, melyekben mikroorganizmusokat használnak a szennyezett közeg (talaj, talajvíz, felszíni víz vagy felszíni vízi üledék) környezeti kockázatának csökkentésére, megtisztítására. A bioremediációs technológiák lehetővé teszik, hogy bizonyos szennyezett területet olcsón, mégis hatékonyan tisztítsanak meg. Napjainkban a bioremediációs technológiák számos kutatás témáját képezik, s fontos területét jelentik a biotechnológiai eljárásoknak.

A bioremediáció egyik területe a bioszorpció. Ez egy olyan módszer, melynek során mikroorganizmusok alkalmazhatók adszorpciós folyamatokban. A leggyakrabban alkalmazható mikroorganizmusok a baktériumok, gombák és az algák. Az eljárás különböző típusú szennyeződések megkötésére, eltávolítására alkalmas, leggyakrabban a nehézfémek adszorpciós tulajdonságait vizsgálták. Az előnyös gazdasági, ökológiai és technológiai szempontok felvetik a módszer ipari alkalmazását.

Jelen dolgozat a bioszorpciós folyamaton alapuló víztisztítási módszerekkel foglalkozik. A bioszorpciós folyamatot különböző új típusú adszorbensekkel, Cd^{2+}, Zn^{2+} és Cu^{2+} ionokkal szennyezett vizes oldatokban vizsgáljuk. Bioszorbensként élesztősejteket és zöld algát használunk szuszpenzió, illetve immobilizált formában. Az immobilizáció Na-alginátttal történik. Az adszorpciós kapacitás növelése érdekében az immobilizáció során bentonitot is használtunk különböző tömegarányú keverékek formájában. Az adszorpciós folyamatot minden esetben az idő függvényében vizsgáltuk az adszorpciós egyensúly eléréséig, a folyamatot adszorpciós izotermákkal jellemeztük, egyes esetekben kinetikai vizsgálatokat is végeztünk.

A bioszorpciós mechanizmus tanulmányozása érdekében a biomasszán különböző kémiai kezeléseket végeztünk. A kémiai kezelések igazolták a sejtfal funkciós csoportjainak (-COOH, -HO, $-NH_2$, -SH) fontos szerepét az adszorpciós folyamatban.

Külön eredményként értékeljük a sörgyári fermentációs folyamatokból visszamaradt élesztősejtek alkalmazásának tanulmányozását. Eredményeink igazolták, hogy a „fáradt" élesztősejtek sikeresen alkalmazhatóak a szennyvíztisztításban, szuszpenzióban illetve immobilizált formában egyaránt.

Számos Európai Fórum és nemzetközi projektek sora bizonyítja, hogy nagy jövője van a környezeti, gazdasági és társadalmi szempontból előnyös biotechnológiáknak.

Meggyőződésem, hogy a környezetszennyezés csökkentése hosszútávon a felnövekvő generációk, környezeti károk előidézésének elkerülésére irányuló, tudatos neveléssel érhető el, míg a meglévő problémákat csakis a környezetvédelem és a környezetvédelmi biotechnológia rohamléptékű fejlődése oldhatja meg. Hiszek abban, hogy kutatómunkám sorozata valamilyen szinten hozzájárul a bioremediációs módszerek fejlődéséhez.

CÉLKITŰZÉSEK

A PhD dolgozat a környezetszennyezés általános témakörével foglalkozik, ezen belül a nehézfémek (Cd^{2+}, Zn^{2+}, Cu^{2+}) megkötési lehetőségeit vizsgálja, különböző bioszorbensek alkalmazásával. A dolgozatban új típusú bioszorbensek adszorpciós képességét tanulmányoztuk az alkalmazási lehetőségek figyelembevételével. *Scenedesmus opoliensis* zöld algát és *Saccharomyces cerevisiae* tenyésztett, kereskedelmi, valamint sörgyári-hulladék élesztősejteket tanulmányoztuk élő, élettelen és immobilizált formában. A dolgozat röviden ismerteti a témához kapcsolódó szakirodalmat, hangsúlyozva az önálló kutatás irányait.

A disszertáció célkitűzései a következők:

- A *Scenedesmus opoliensis* zöld algák bioszorbensként alkalmazása nehézfémek eltávolítására, a bioszorpciós kapacitás meghatározása és értékelése.
- Az immobilizált *Saccharomyces cerevisiae* élesztősejtek nehézfémekkel szembeni adszorpciós képességének vizsgálata.
- Immobilizált bentonit és élesztőkeverékek alkalmazása nehézfém eltávolítására adszorpciós oszlopban ipari szennyvizekből (adszorpciós kapacitás, áttörési görbék, deszorpciós folyamatok tanulmányozása).

- Az immobilizált sörgyári élesztősejtek adszorpciós kapacitásának, eltávolítási hatékonyságának vizsgálata, valamint a bioszorpciós folyamat leírása az adszorpciós egyensúlyi és kinetikai modellek segítségével.
- Az immobilizált sörgyári élesztő és az immobilizált kereskedelemben kapható friss élesztő bioszorpciós kapacitásának összehasonlítása és vizsgálata, valamint a bioszorpciós folyamat bemutatása az adszorpciós idő és a termodinamikai vizsgálatok alapján.
- Az immobilizált *Saccharomyces cerevisiae* (DSM 1333) tenyésztett élesztő bioszorpciós kapacitásának vizsgálata, valamint a bioszorpciós folyamat leírása az adszorpciós egyensúlyi és kinetikai modellek segítségével.
- Az élesztősejtek kémiai kezelésének és hatásának vizsgálata a bioszorpciós kapacitásra, valamint a bioszorpciós folyamat hatékonyságának meghatározása.
- A sejtfal felszínén levő funkciós csoportok szerepének meghatározása a bioszorpciós folyamatban Fourier Transzformációs Infravörös Spektroszkópia (FTIR) segítségével.

KÍSÉRLETI MÓDSZEREK

A dolgozat bioszorpción alapuló szennyvíztisztítási módszerekkel foglalkozik. A kísérleteinkben Cd^{2+}, Zn^{2+}, Cu^{2+} ionok adszorpciós tulajdonságait vizsgáljuk. Bioszorbensként élő, élettelen *Saccharomyces cerevisiae*, valamint *Scenedesmus opoliensis* zöld algát használtunk. A bioszorbenseket vizes szuszpenziós formában, illetve immobilizált formában alkalmaztuk. Minden esetben tanulmányoztuk az adszorpciós egyensúlyt, az adszorpciós kapacitást meghatározó paramétereket, illetve kinetikai vizsgálatokat is végeztünk. A bioszorpciós mechanizmus meghatározásának érdekében vizsgáltuk a különböző típusú kémiai kezelések hatását.

A kísérleti módszereket, valamint a kapott eredményeket az alábbi fejezetek foglalják össze.

1. A Cd^{2+} ionok eltávolítása mesterséges szennyvizekből *Scenedesmus opoliensis* zöld algák segítségével

A fémion alapú oldatot analitikai tisztaságú $Cd(NO_3)_2x4H_2O$ sóból állítottuk elő a szükséges mennyiségű desztillált vízzel. A kadmium oldat különböző koncentrációit a törzsoldat hígításából nyertük. Az oldatban levő

nehézfém ionok koncentrációját az atomabszorpciós spektrofotométerrel (SensAA Dual GBS Scientific Equipment, Australia) határoztuk meg.

A *Scenedesmus opoliensis* algákat a kolozsvári (P. Richter) Biológiai Kutató Intézet gyűjtéséből származtak, melyet Kuhl-Lorenzen (KL) táptalajon szaporítottak. A bioszorpciós folyamatot folytonos mágneses keverés mellett szobahőmérsékleten vizsgáltuk (15 ml tömény alga, 150 ml különböző koncentrációjú 4.35, 12.7, 20.49 mg Cd^{2+} /L kadmium oldat). A Cd^{2+} ionok koncentrációját az idő függvényében követtük, a mintákat 45 μm M.E. Cellulose filter segítségével átszűrtünk, majd atomabszorpciós spektrofotometriával (l=228.8 nm, 0–2.5 mg/L koncentráció tartományban, standard kadmium oldattal végzett kalibrációval) határoztuk meg. A biomassza szárazanyag tömege 0.66 g volt.

2. A Zn^{2+} ionok eltávolítása mesterséges szennyvizekből immobilizált *Saccharomyces cerevisiae* sejtek segítségével

Vizsgálatainkhoz a kereskedelemben kapható *Saccharomyces cerevisiae* (Pakmaya) élesztőt alkalmaztuk.

Immobilizálás módszere

Az élesztőt sűrű szuszpenzió formájában nátrium-alginátal elegyítettük, majd az oldatot kalcium ionokat tartalmazó pufferbe csepegtettük. Az így létrejött, élesztő tartalmú Ca-alginát golyócskákat használtuk fel a további vizsgálataink során.

Az adszorpciós kapacitás jellemzésére a maximálisan adszorbeált Cd2+ mennyiségét mg-ban, 1 g bioszorbensre vonatkoztatva a következő képlet alapján határoztuk meg:

$$q_e = [(C_0 - C_e)/m] \cdot V$$

ahol, q_e - adszorbeált Cd mennyisége mg-ban, 1 g biomasszára vonatkoztatva, C_0 - kiindulási Cd koncentráció, (mg/L), C_e - egyensúlyi Cd koncentráció, (mg/L), m - bemért biomassza mennyisége, (g), V - Cd oldat térfogata, (L).

A fémion alapú oldatot analitikai tisztaságú $ZnSO_4 \times 7H_2O$ sóból állítottuk elő desztillált vízzel. A cink oldat különböző koncentrációit a törzsoldat hígításából nyertük (129.60 mg Zn^{2+}/L, 213.41 mg Zn^{2+}/L és 304.88 mg Zn^{2+}/L). A kísérletet szakaszos körülmények között folyamatos mágneses keveréssel végeztük, állandó kinetikai paraméterek mellett. Az oldatban levő cink ionok koncentrációját spektrofotometriás módszerrel (kálium-ferrocianáttal, l =420 nm-en, STAS 6327-81 szerint) mértük.

3. Immobilizált bentonit és élesztő keverékek alkalmazása Cd^{2+} eltávolítására adszorpciós oszlopban

Vizsgálataink során a kereskedelmi forgalomban levő Fort Benton (B) nevű bentonit mintát alkalmaztuk por alakjában (d < 0.2 mm), valamint a szintén kereskedelmi forgalomban levő Pakmaya (D) sütőélesztőt. Felhasznált vegyszerek $Cd(NO_3)_2 \times 4H_2O$, nátrium-alginát és $CaCl_2$).

Az adszorbens immobilizációhoz különböző mennyiségű bentonitot (2, 4, 6, 8 g) és élesztőt (2, 4, 6, 8 g) különböző kombinációkban (8g B, 6g B + 2g D, 4g B + 4g D, 2g B + 6g D, 8g D) alkalmaztuk, melyet 50 ml desztillált vízben szuszpendáltunk. Ezt a szuszpenziót elegyítettük 1 g Na-alginátttal és 2 ml etanollal. Ezt a keveréket egy perisztaltikus pumpával 0.2 M $CaCl_2$ oldatba csepegtettük. Így jöttek létre a kálcium-alginát (4.0 ± 0.2 mm átmérőjű) gélbezárt gyöngyök, melyet további 0.2 M $CaCl_2$ oldatban tartottunk 4°C-on 1 órán keresztül, a keresztkötések kialakulása végett. Használat előtt a gyöngyöket desztillált vízzel lemostuk. A nehézfémionok koncentrációját atomabszorpciós spektrofotométerrel (SensAA Dual GBS Scientific Equipment, Australia) mértük.

A nehézfémionok bioszorpciós vizsgálata szakaszos körülmények között folytonos mágneses keverés mellett (825 rpm, 100 ml kadmium oldat, mely tartalmazta a Ca-alginát bentonit gyöngyöket), illetve adszorpciós oszlopban (32 mm) történt. Az adszorpciós oszlopon a kadmium oldat átfolyási ideje 4 ml/perc volt.

A bioszorpciós folyamatot a kadmium koncentráció meghatározásaival követtük, a mintákat 5 illetve 15 percenként mértük. Vizsgáltuk a bentonit és élesztő arányának hatását, valamint az adszorpciós folyamatot (20°C, pH = 5.4).

4. Immobilizált hulladék sörgyári élesztősejtek alkalmazása Cd^{2+} ionok eltávolítására. Egyensúly és kinetika

A bioszorbenst, mint hulladék biomasszát a Csíki sörfőzdéből (Csíkszereda, Románia) kaptuk, mely a különböző fermentációs folyamatokból visszamaradt melléktermék. A bioszorbens előkészítése a kifáradt élesztő mosásával, majd szárításával történt. Az immobilizálási folyamat, valamint a nehézfém koncentráció meghatározása a már ismert módszerekkel történt.

5. Szuszpendált és immobilizált hulladék sörgyári élesztősejtek és kereskedelmi élesztő bioszorbenskénti alkalmazása Cd^{2+} eltávolításra. Termodinamikai tanulmány

A bioszorpció vizsgálatára négyféle biszorbenst használtunk:

1. Szuszpendált sörgyári hulladék biomassza
2. Immobilizált sörgyári hulladék biomassza
3. Szuszpendált kereskedelmi (Pakmaya) friss élesztő biomassza
4. Immobilizált kereskedelmi (Pakmaya) friss élesztő biomassza

A bioszorpció tanulmányozására mesterséges kadmium ionok vizes oldatát használtuk (C=5.75 mg Cd^{2+} l^{-1}), analitikai tisztaságú $Cd(NO_3)_2x4H_2O$ sóból.

Tanulmányoztuk a érintkezési idő befolyását, jellemeztük egyensúly szempontjából a bioszorpciós folyamatot, valamint termodinamikai vizsgálatokat végeztük (295, 308 és 323 K, és állandó pH értéken, pH = 5.5). A kadmium ionok mennyiségét az oldatban pHoenix Electrode Co. ion-szelektív elektróddal a pH-t pedig Jenway 3330 pH méterrel mértük.

Meghatároztuk a termodikamikai paramétereket (Gibbs-féle szabadenergiát (ΔG°), az entalpiát (ΔH°) és az entrópiát (ΔS°).

$$\Delta G° = -RT \ln K_d$$

$$\ln K_d = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R}$$

ahol: R az univerzális gázállandó, T hőmérséklet (K), és K_d terjedési együttható (l g^{-1}).

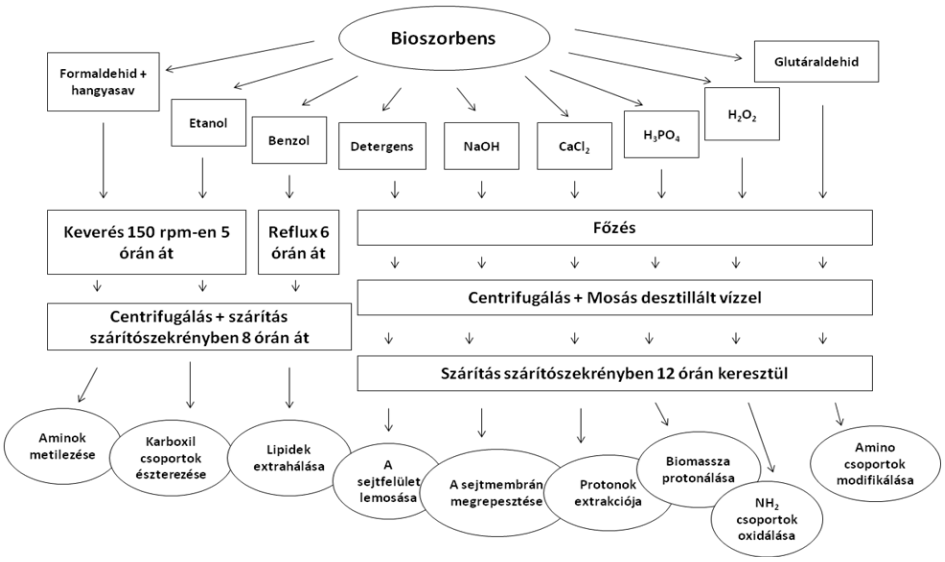
6. A Cd^{2+} ionok bioszorpciója immobilizált *Saccharomyces cerevisiae* sejtekkel. Adszorpciós egyensúly és kinetikai tanulmány.

A bioszorpciós vizsgálatainkhoz tenyésztett *Saccharomyces cerevisiae* (DSM 1333) élesztőtörzset használtunk, melyet a Pécsi Orvostudományi Egyetem, Orvosi Mikrobiológiai és és Immunitástani Intézetben tenyésztettünk, majd liofilizált formában használtuk. A tenyésztés körülményei: Müller-Hinton táptalaj (3 % glükóz, pepton, yeast-extra, NaCl, pH=7).

Az immobilizálás, valamint a bioszorpciós folyamat tanulmányozása az általunk kidolgozott módszer alapján történt.

7. A felületkezelés hatása a hulladék sörélesztő Cd^{2+}, Zn^{2+}, és a Cu^{2+} adszorpciójára.

A felületkezelés módszerét az 1. ábra foglalja össze:



1. ábra. A felületkezelés módszereinek grafikus összefoglalása.

Az infravörös spektroszkópia (FTIR) vizsgálatokat Jasco 615 típusú spektrofotométerrel végeztük, (hullámhossz tartomány 400-4000 cm^{-1}, felbontás 2 cm^{-1}).

EREDMÉNYEK

1. A Cd^{2+} ionok eltávolítása mesterséges szennyvizekből *Scenedesmus opoliensis* zöld algák segítségével

Kísérleteink igazolják, hogy a *Scenedesmus opoliensis* zöld alga alkalmazható bioszorbensként Cd^{2+} ionok eltávolítására a szennyvizekből. Kísérleteinkben a következő paramétereket vizsgáltunk:

1. A nehézfémionok koncentrációjának változását az idő függvényében
2. A bioszorpciós folyamat hozamát
3. Az alga nehézfémmegkötő képességét

A kadmium ionok koncentrációjának változását követve megállapítható, hogy két óra expoziciós idő után a nehézfém koncentráció jelentősen csökken, gyorsabban az első 10 perc alatt, majd 60 perc múlva eléri az egyensúlyi koncentrációt.

Következésképpen, mindhárom kadmium koncentráció esetében (C_1=4.36 mg Cd^{2+}/L, C_2=12.7 mg Cd^{2+}/L, C_3=20.48 mg Cd^{2+}/L) az adszorpció hozama 50-52%-os volt, a bioszorpciós kapacitás értéke 0.67 és 3.28 mg Cd^{2+}/g között változott. Az eredmények igazolják, hogy lehetséges a Cd^{2+} ionok eltávolítása szennyvizekből *Scenedesmus opoliensis* zöld algák segítségével.

2. A Zn^{2+} ionok eltávolítása mesterséges szennyvizekből immobilizált *Saccharomyces cerevisiae* sejtek segítségével

Vizsgálataink során sikeresen alkalmaztuk az immobilizált *Saccharomyces cerevisiae* sejteket Zn^{2+} ionok eltávolítására mesterséges szennyvizekből. A bioszorpciós kísérleteinkhez immobilizált biomasszát használtunk. A kezdeti koncentráció C_1 = 129.60 mg Zn^{2+}/L, körülbelül 55 – 60 perc eltelte után a vizsgált nehézfémion tartalom a 0-hoz közelít.

Meghatároztuk a maximálisan adszorbeált mennyiségeket három fémion koncentrációra nézve (C_1 = 129.60 mg Zn^{2+}/L, C_2 = 213.41 mg Zn^{2+}/L, C_3 = 304.88 mg Zn^{2+}/L). Általános következtetésként elmondható, hogy az általunk használt élesztő, valamint a kidolgozott módszer alkalmas a nehézfémionok eltávolítására a szennyvizekből.

3. A Cd^{2+} eltávolítása adszorpciós oszlopban ipari szennyvizekből immobilizált bentonit és élesztő keverékek alkalmazásával

A bentonitminta, élesztő biomassza és ezek keveréke hatékonynak bizonyult a mintaoldatokban található kadmium eltávolításánál.
A legnagyobb adszorpciós kapacitást akkor értük el, amikor a Ca-alginát gyöngyök csak bentonitot tartalmaztak. A bentonit koncentráció csökkenésével, az adszorpciós kapacitás is csökkent, az élesztőmennyiség növelése ellenére is, kivéve a tiszta élesztőminta esetét, mely köztes értéket eredményezett.

A kísérleteket szakaszos körülmények között, illetve adszorpciós oszlopokkal végeztük.

4. Immobilizált hulladék sörgyári élesztősejtek alkalmazása Cd^{2+} ionok eltávolítására. Egyensúly és kinetika

Ebben a tanulmányban a (Ca-alginát gyöngyökkel) immobilizált, csíkszeredai sörgyártásból származó hulladék biomasszát (élesztősejteket), sikeresen használtuk bioszorbensként a Cd^{2+} ionok vizes oldatból való eltávolítása céljából. A kálcium-alginát megfelelő mátrixnak bizonyult sörélesztő sejtek immobilizációjára.

A maximális bioszorpciós kapacitás számításaink szerint 5,96 mg Cd^{2+} /g^{-1} volt 169 mg Cd^{2+} /L^{-1} kezdeti koncentráció esetén.

Az egyensúlyi adszorpció jellemzésére a Langmuir és a Freundlich adszorpciós izotermákat használtuk. A korrelációs együtthatók alapján, arra a következtetésre jutottunk, hogy a Langmuir izoterma alkalmasabb a kadmium bioszorpciós egyensúlyi folyamatok leírására.

A bioszorpciós folyamat leírására az első és pszeudo-másodrendű kinetika szerinti modelleket használtuk. Az elvégzett matematikai számítások alapján úgy találtuk, hogy az adszorpciós folyamat pszeudo-másodrendű kinetikai modell szerint történik, és az ehhez a kinetikához kapcsolódó paraméterek is meghatározásra kerültek.

A tanulmányban bemutatott eredmények bebizonyították, hogy a fermentációs iparból származó bioszorbens, a sörgyártás során visszamaradt hulladék biomassza, mely egy ipari folyamat melléktermékeként olcsón és nagy mennyiségben hozzáférhető, sikeresen használható a kadmium ionok eltávolítására vizes oldatból.

5. Szuszpendált és immobilizált sörgyári biomassza és kereskedelmi élesztő bioszorbenskénti alkalmazása Cd^{2+} ionok eltávolítására. Termodinamikai tanulmány.

A két különböző forrásból származó élesztőt (kereskedelmi élesztő és sörgyártásból származó élesztőhulladék biomassza) két formában, (szuszpendált és immobilizált) vizsgáltuk meg a kadmium ionok bioszorpciós folyamata során. Kiszámítottuk az adszorpció termodinamikai paramétereit, beleértve a Gibbs-féle szabadenergiát ($\Delta G°$), az entalpiát ($\Delta H°$) és az entrópiát ($\Delta S°$). A kapott eredmények azt mutatták, hogy a Cd^{2+} bioszorpciója a *Saccharomyces cerevisiae* által megvalósítható, spontán és endoterm folyamat. A négy tesztelt bioszorbens közül a legnagyobb hatékonyságot és adszorpciós kapacitást a sörgyártásból származó élesztőhulladék biomassza esetében kaptuk, mely bizonyítja, hogy alkalmazható, olcsó, alternatív bioszorbens lehet különböző szennyeződések eltávolítására.

6. A Cd ionok bioszorpciója immobilizált *Saccharomyces cerevisiae* sejtekkel. Adszorpciós egyensúly és kinetikai tanulmány.

A kutatás az immobilizált tenyésztett *Saccharomyces cerevisiae* sejtek (DSM 1333) vizes oldatból történő adszorpciós képességét vizsgálta a Cd^{2+} ionokra nézve. Az eredmények azt mutatták, hogy a kezdeti Cd^{2+} koncentráció nagymértékben befolyásolta a kadmium bioszorpciót. A bioszorpciós kapacitás növekedett a kezdeti kadmium koncentráció növelésével. A tanulmányozott fémionok bioszorpciója gyors folyamat, mely gyakran három órán belül eléri az egyensúlyi állapotot; a maximális bioszorpciós kapacitás 3.7825 mg Cd^{2+}/g volt 99.75 mg Cd^{2+} /L^{-1} kezdeti koncentráció esetén.

Az egyensúlyi adszorpciós folyamatok leírására a Langmuir és Freundlich adszorpciós izotermákat alkalmaztuk.

Azt tapasztaltuk, hogy a kadmium bioszorpciója a szorbensen főként a fizikai és az ioncsere kölcsönhatásokon alapszik, és ezt az adszorpciós izotermák eredményei is igazolták.

A nehézfémionok bioszorpciója immobilizált *Saccharomyces cerevisiae* sejteken a Langmuir izoterma modell szerint következik be.

Az elvégzett matematikai számítások alapján úgy találtuk, hogy az adszorpciós folyamat leírása során a kinetikai adatok jól igazodtak a pszeudo-másodrendű kinetikai modellhez. Az ehhez a kinetikához kapcsolódó paraméterek is meghatározásra kerültek. Kísérleteinkben

összehasonlítottuk a tenyésztett *Saccharomyces cerevisiae* sejtek, illetve a kereskedelmi, valamint a sörgyári élesztősejtek adszorpciós kapacitását, valamint tanulmányoztuk az adszorpciós egyensúly termodinamikáját és kinetikáját.

7. A felületkezelés hatása a hulladék sörélesztő Cd^{2+}, Zn^{2+}, és a Cu^{2+} adszorpciójára. Infravörös spektroszkópiás vizsgálatok eredményei

Az adszorpciós kapacitás növelése, valamint az adszorpciós mechanizmus felderítésének érdekében különböző típusú kezeléseket alkalmaztunk az élesztősejtek esetében.

Kísérleteink igazolják, hogy a sejtfal felületén levő karboxil csoportoknak alapvető szerepük van a bioszorpciós folyamatban, mikor ezeket a csoportokat megváltoztattuk (észterezési reakció) az adszorpciós kapacitás nagymértékben lecsökkent.

A sörélesztő felületén levő funkciós csoportokat az IR spektroszkópiával jellemeztük. A vizsgált spektrumok alátámasztják a vizsgálataink egyik alapelvét, mely szerint a NaOH-al való kezelés nem módosítja a sejtfelületi funkciós csoportokat, de igenis annál inkább megkönnyíti a fémionok adszorpcióját a sejt felületen, ezáltal elősegítve a megfelelő aktív kötőhelyek kialakulását. Feltételezzük továbbá, hogy hidrolízises reakció megy végbe, mely karboxil (-COOH), karboxilát (-COO) és alkohol (-OH) csoportok kialakulásához vezet, mely növeli a kationok bioszorpcióját.

A benzollal való kémiai kezelés során a sejtfalban levő lipidek nagyrésze szétroncsolódik, ezáltal számos kötőhely szűnik meg, ami az adszorpciós kapacitás csökkenését eredményezte.

A foszforsavval történt kezelés szintén az adszorpciós kapacitás csökkenését eredményezte, aminek feltehetően az az oka, hogy H^+ ionok kötődnek a sejtfelszín aktív kötőhelyeihez, ezáltal megakadályozzák a nehézfém ionok adszorpcióját. A sejtfal felületének polimér struktúrája negatív töltést mutat a szerves és szervetlen csoportok ionizációja miatt, ami arra utal, hogy minél nagyobb a biomassza elektronegativitása, annál nagyobb a vonzereje, így a nehézfém kationok adszorpciója is könnyebben kellene végbemenjen. Ennek ellenére a H^+ ionok a savas előkezelés hatására megváltoztatják a sejtfal elektronegativitását, ennek következtében csökken az adszorpciós kapacitás.

A metilezés adszorpciós kapacitáscsökkentő hatása azzal magyarázható, hogy a sejtfelszínen található aminosavak amino-csoportjai metileződnek, mely egy fontos adszorpciós kötőhely megszűnését eredményezi.

A FTIR vizsgálatok alapján bebizonyosodott, hogy a biomassza sejtfelületén levő funkciós csoportok fontos szerepet játszanak a bioszorpciós mechanizmusban. Igazoltuk annak létezését és módját, amelyben a kationok hozzákapcsolódnak a funkciós csoportokhoz a sejtfelületen, ezt a tényt igazolja a funkciós csoportokhoz kapcsolt csúcsok elmozdulása az IR spektrumon.

Ezen felül az IR vizsgálatok megmagyarázzák a NaOH-os kezelés hatását. Igazolják továbbá, az észterezett sörélesztő alacsonyabb adszorpciós kapacitását, ezáltal kiemelve az adszorpcióért felelős funkcionális csoportok jelentőségét.

A kísérleteink igazolják tehát, hogy a biomassza sejtfalainak kémiai szerkezete és funkciós csoportjai nagymértékben meghatározzák a bioszorpció hatékonyságát. A NaOH-al való kezelés mindhárom kation esetben növelte az élesztősejtek adszorpciós kapacitását, ami azt jelzi, hogy az alkáli vegyületek alkalmasak lehetnek a különböző víztisztítási technológiák hatékonyságának növelésében.

ÚJ TUDOMÁNYOS EREDMÉNYEK

1. A kutatási eredmények lehetővé tették új bioszorpciós módszerek kidolgozását. Kétféle mikroorganizmust vizsgáltunk, *Saccharomyces cerevisiae* gombát, valamint *Scenedesmus opoliensis* zöld algát, ezeket alkalmaztunk bioszorbensként a nehézfémek (Cd^{2+}, Zn^{2+}, Cu^{2+}) szennyvízből való eltávolítására bioszorpció útján. Az adszorpciós folyamat tanulmányozása kétféleképpen történt: (1) szakaszos körülmények között (szuszpenziós, illetve Na-alginátt*a*l immobilizált élesztősejtekkel), valamint zöld algákkal (szuszpenzió formában), és (2) adszorpciós oszlopban végzett vizsgálatokkal.

2. Kifejlesztettünk egy módszert a nehézfémek szennyvízből való eltávolítására, melynek során bioszorbensként *Scenedesmus opoliensis* zöld algát alkalmaztunk vizes szuszpenzióban. Három minőségi paraméter vizsgálatára került sor: (1) a nehézfémionok koncentrációjának meghatározására az oldatban az algával való érintkezés után; (2) a

bioszorpciós folyamat hozamának kiszámítására; (3) az algák nehézfém megkötő képességének tanulmányozására az adszorpciós folyamatban, valamint vizsgáltuk a különböző paraméterek hatását bioszorpciós folyamatra nézve.

3. Kidolgoztuk az élesztősejtek immobilizálásának módszerét Na-alginnáttal, ez a mátrix alkalmas a *Saccharomyces cerevisiae* sejtek immobilizálására. Abban az esetben, amikor immobilizált *Saccharomyces cerevisiae* sejteket alkalmazunk Zn^{2+} ionok eltávolítására mesterséges szennyvizekből, az oldatban levő nehézfém teljes mértékben megkötődött a biomasszán. Eredményeink összhangban vannak a szakirodalomban található adatokkal, melyek szerint a fémmegkötés a bioszorpciós folyamatokban két lépésben megy végbe. Az első lépés a sejtfelület irányába való diffúzió, itt megy végbe a tulajdonképpeni adszorpció, a sejtfelületet felépítő funkcionális csoportokhoz való kötődés. A második lépés a sejt belsejébe való szállítás (transzportálás), ez a fémion koncentráció lassú csökkenését jelenti. Ez a csökkenés azzal magyarázható, hogy a fém áthalad a sejtfalon és megtörténik a sejten belüli (intracelluláris) akkumuláció. Ez a folyamat addig tart, amíg be nem áll az adszorpciós egyensúly a biomassza mennyiség és a szennyező (nehézfém) mennyiség között.

4. Az immobilizálási módszert kiterjesztettük a bentonit és élesztőkeverékek Na-alginnáttal való immobilizálására. A bentonitminta, az élesztő biomassza és ezek keveréke hatékonynak bizonyult a mintaoldatokban található kadmium eltávolításánál. A legnagyobb adszorpciós kapacitást akkor értük el, amikor a Ca-alginát gyöngyök csak bentonitot tartalmaztak. A bentonit koncentráció csökkenésével az adszorpciós kapacitás is csökkent, az élesztőmennyiség növelése ellenére is, kivéve a tiszta élesztőminta esetét, mely köztes értéket eredményezett. A kísérleteket szakaszos körülmények között, illetve adszorpciós oszlopokkal végeztük.

5. Az adszorpciós vizsgálataink során, a fémmegkötésre vonatkozóan kiemelkedő eredmények születtek a csíkszeredai sörgyártásból származó hulladék élesztősejtek esetén. Kísérleteinkben az élesztősejteket sikeresen alkalmaztuk bioszorbensként úgy szuszpenzió, mint immobilizált formában, a Cd^{2+} ionok vizes oldatából való eltávolítására.

6. Az egyensúlyi adszorpció jellemzésére a Langmuir és a Freundlich adszorpciós izotermákat használtuk. A korrelációs együtthatók alapján arra a következtetésre jutottunk, hogy a Langmuir izoterma alkalmasabb a

kadmium bioszorpciós egyensúlyi folyamatok leírására. A bioszorpciós folyamat leírására az első és pszeudo-másodrendű kinetika szerinti modelleket használtuk. Az elvégzett matematikai számítások alapján úgy találtuk, hogy az adszorpciós folyamat pszeudo-másodrendű kinetikai modell szerint történik, és az ehhez a kinetikához kapcsolódó paraméterek is meghatározásra kerültek.

7. A sörgyártásból származó biomassza esetén a kapott eredmények azt mutatják, hogy a Cd^{2+} bioszorpciója *Saccharomyces cerevisiae* sejtek által megvalósítható, spontán és endoterm folyamat. Kiszámítottuk az adszorpció termodinamikai paramétereit, beleértve a Gibbs-féle szabadenergiát ($\Delta G°$), az entalpiát ($\Delta H°$) és az entrópiát ($\Delta S°$).

8. A csíkszeredai sörgyártásból származó bioszorbens esetén tanulmányoztuk a biomassza sejtfelületét alkotó funkcionális csoportok szerepét az adszorpciós folyamatban, és megállapítottuk, hogy a fermentációból visszamaradt élesztősejtek nagyobb adszorpciós kapacitással rendelkeznek.

9. Az adszorpciós kapacitás, valamint az adszorpciós mechanizmus felderítése érdekében a biomasszán különböző kémiai kezeléseket végeztünk – pl. metilezés, észterezés, extrahálás, protonálás, stb., – tanulmányozva ezáltal a sejtfal funkciós csoportjainak szerepét az adszorpciós folyamatban. Megállapítható, hogy az a szennyező mennyiség, melyet egy bioszorbens képes eltávolítani csak a kinetikai egyensúlytól, valamint a biomassza felületi összetevőjétől, funkciós csoportjaitól függ. A szorpció végbemenetele a szorbens magas affinitásától is függ, valamint a szennyező és a biomassza specifikus funkciós csoportjai között levő interakció mechanizmusától. Vizsgálataink alátámasztják, hogy a biomassza kémiai természete nagymértékben meghatározza a bioszorpció hatékonyságát. Bebizonyítottuk továbbá, hogy a sejtfal szerkezetéből mely csoportok befolyásolják döntően az adszorpciós kapacitást.

10. Kísérleteink igazolják, hogy a sejtfal felületén levő karboxil csoportoknak alapvető szerepük van a bioszorpciós folyamatban, ugyanis mikor ezeket a csoportokat megváltoztattuk (észtereztük), az adszorpciós kapacitás nagymértékben lecsökkent.

11. Javasolható a víztisztítási folyamatban a NaOH-al kezelt biomassza alkalmazása, mivel mindhárom kation esetében növekedett az élesztősejtek adszorpciós kapacitása, ami azt jelzi, hogy az alkáli vegyületek alkalmasak lehetnek a különböző víztisztítási technológiák hatékonyságának növelésében.

12. A FTIR vizsgálatok alapján bebizonyosodott, hogy a sejtfelületen levő funkciós csoportok alapvető szerepet játszanak a nehézfém megkötésben. Igazoltuk annak létezését és módját, amelyben a kationok hozzákapcsolódnak a funkciós csoportokhoz a sejtfelületen, ezt a tényt igazolja a funkciós csoportokhoz kapcsolt csúcsok elmozdulása az IR spektrumon.

13. Az általunk használt módszer ipari alkalmazása szempontjából igen lényeges kiemelni, hogy egy költséghatékony lehetőséggel állunk szemben, ugyanis a sörgyártásból származó hulladék biomassza ingyen vagy igen alacsony áron szerezhető be az egyes ipari létesítményekből. Ugyanakkor, tekintettel a sörgyártási folyamatok széleskörű elterjedtségére, a hulladék biomassza nagy mennyiségben állhat rendelkezésre, ami – a jelentős gazdasági előnyök mellett – eredményesen járulhat hozzá a szennyvíztisztítási folyamatokhoz, s ezáltal a veszélyes hulladékok mennyiségének csökkentéséhez.

THE TOPIC SIGNIFICANCE

The pollution of environment increased considerably in the past century. Industrial expansion, the rapid growth of human population, as well as the effects of released toxic compounds all had their impact on the pollution of the environment. The worldwide proportion of chemical pollution become alarming.

Dangers related to heavy metals, as the accumulation of micro-elements and toxic heavy metals can have decisive human health related, ecological and biological consequences. The emission of micro-elements and heavy metals considerably increased with industrial evolution, where beside traffic and industrial activities, the modernization of agriculture has also become a potential source of heavy metal pollution.

In the recent decades the levels of heavy metals in the soil have become critically higher as a result of the placement of sewage water and sewage sludge on agricultural fields, around industrial districts, in cities, and along main traffic roads. These contaminated areas represent basic environmental and health-related problems. Soils are able to accumulate heavy metals for long periods of time without any sign of toxic effects, yet in case these metals reach the nutrition chain (for instance by the consumption of plants grown on contaminated fields, and thus they enter the human body) they can accumulate there and cause acute or chronic damage, ultimately bringing about the destruction of living organisms.

Over the past two decades the activities to assess and recognise the status of contaminated natural elements (such as geological media, waters under surface), and to clean them from pollutants have multiplied exponentially. As a result, environmental protection has become a specific industry, for which a cumulative social and economic demand emerges. Remediation technologies depend on the local characteristics of contaminated fields; therefore, methods that rely on the highly sofisticated processes of living organisms are used more often to achieve the best results.

AIM OF THE WORK

It examines the opportunities of heavy metal (Cd^{2+}, Zn^{2+}, Cu^{2+}) binding by using various biosorbents. The adsorption capacity of new types of biosorbents was studied to explore the possibilities of industrial applications. We have studied *Scenedesmus opoliensis* green algae and cultivated commercial and brewery waste *Saccharomyces cerevisiae* yeast cells in living, non-living and immobilised form.

The dissertation has got the following objectives:

- To evaluate the biosorption capacity of the *Scenedesmus opoliensis* green algae for heavy metal removal.
- To compare the biosorption capacity of immobilized *Saccharomyces cerevisiae* cells to adsorb heavy metals.
- To compare the combined adsorptive properties of *Saccharomyces cerevisiae* cells and bentonite in immobilized form and to establish adsoption capacities towards cadmium from aqueous solution.
- To investigate the biosorption of cadmium ion by Romanian immobilized brewery waste biomass, to determine the removal efficiency and adsorption capacity and to describe the biosorption process with the adsorption equilibrium and kinetic models.
- To investigate the biosorption capacities of suspended and immobilized brewery waste biomass for cadmium removal, to compare with suspended and immobilized commercial fresh yeast and to describe the biosorption process (effect of contact time and thermodynamics).
- To determine the potential of *Saccharomyces cerevisiae* immobilized living cells (DSM 1333) to adsorb cadmium ions and to describe the adsorption isotherm and kinetic studies.
- To study the influence of the chemical treatment and to determine the efficiencies of the biosorption process.
- To use Fourier Transform Infrared Spectroscopy (FTIR) analyses to determine functional groups and their functionality on the cell wall surface.

1. INTRODUCTION

1.1. Cd^{2+} Removal from Synthetic Wastewaters Using *Scenedesmus opoliensis* Green Algae

The rapid industrial development, various wastes containing different metal ions are directly or indirectly discharged into the environment, rising serious environmental pollution problems and threatening marine life [1]. There are many processes that can be used for the removal of metals from wastewaters including chemical precipitation, coagulation, solvent extraction, electrolysis, membrane separation, ion exchange and adsorption [2]. Microorganisms can remove heavy metal ions by a large variety of processes such as cell walls biosorption, entrapment in extracellular capsules and uptake by membrane transport of the metal ions into cell cytoplasm, microprecipitation and oxidation-reduction reactions. Some or all of these processes could take place in living microorganisms [3]. Metal ions are adsorbed first on the cells surface by the interactions between the metal ions and the metal-functional groups such as carboxyl, phoshate, hydroxyl, amino, sulphur, sulphyde, thiol, etc., present in the cell wall and then they penetrate the cell membrane and enter the cells [4]. Cadmium is one of most toxic heavy metals whose toxicity is attributed in part to its ability to accumulate in tissues. There are some reports on the destruction of the chloroplast by heavy metal ions at higher concentrations [5]. In fact, cadmium ions disorganize chloroplasts and cause the damage of photosynthetic pigments [6]. As a consequence of this, the photosynthetic activity could severely be affected, causing growth inhibition or complete death of the cells [7, 8]. The purpose of this study is to evaluate the biosorption capacity of the *Scenedesmus opoliensis* algae for Cd^{2+} from aqueous solutions.

1.2. Removal of Zn^{2+} from some synthetic wastewaters by immobilized *Saccharomyces cerevisiae* cells

Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metals from industrial waste streams [1]. Most studies of biosorption for metal removal involved the use of either laboratory-grown microorganism or biomass generated by the pharmacology and food processing industries

or wastewater treatment units. The biosorption of heavy metal ions using microorganisms is affected by several factors. These factors include the specific surface properties of the organism (biosorbent) and the physicochemical parameters of the solution such as temperature, pH, initial metal ion concentration and biomass concentration [2]. Non-living biomass appears to present specific advantages in comparison to the use of living microorganisms. Killed cells may be stored or used for extended periods at room temperature, they are not subject to metal toxicity and nutrient supply is not necessary. Moreover, the pretreatment and killing of biomass either by physical or chemical treatments [3, 4] or crosslinking [5] are known to improve the biosorption capacity of biomass. For example, the immobilization of biomass has the advantages of using an adsorption column in a multi-cycle biosorption process. *Saccharomyces cerevisiae* is an inexpensive, readily available source of biomass for heavy metal removal from wastewaters and possesses good metal-binding potential [6-8]. Yeasts are a growth form of eukaryotic microorganisms classified in the kingdom Fungi, with about 1,500 species described [9]. Investigations conducted by several researchers demonstrated that *S. cerevisiae* is capable of accumulating heavy metals such as Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^{2+} and Ni^{2+} [10-14]. The main application of zinc is as a coating for the protection of steel against corrosion. Zinc itself forms an impervious coating of its oxide on exposure to the atmosphere, and, hence, the metal is more resistant to ordinary atmospheres than iron, and it corrodes at a much lower rate. The toxicity of the metals increases sharply in the order Zn < Cd < Hg. The free zinc ion in solution is highly toxic to plants, invertebrates, and even vertebrate fish. The free zinc ion is also a powerful Lewis acid up to the point of being corrosive [15]. Metal uptake by microorganisms occurs in two stages: first stage consisting in passive adsorption of metal ions to the external cell surface, and second stage in which metal ions are subsequently transported through the cell membrane into the cell itself [16, 17].

The aim of this study was to test and compare immobilized *Saccharomyces cerevisiae* cells for their capacity to adsorb Zn^{2+}, which is a widely distributed heavy metal in water. Although calcium alginate is a cheap, non-toxic, and abundantly available immobilization matrix, insufficient literature was found about Zn^{2+} removal by alginate immobilized biosorbents.

1.3. Fixed Bed Studies for Cd^{2+} Removal from Model Solutions Using Immobilized Bentonite/Yeast Mixtures

Wastewaters contaminated with heavy metals represent a serious environmental problem because they do not undergo biodegradation and are accumulated [1] into vegetal and animal cells, with different toxic effects. Heavy metals can be released into the aqueous environment from a variety of sources such as metal smelters, effluents from plastic, textile, microelectronic and wood preservative-producing industry, and even fertilizer and pesticide usage [2]. In addition, mining, mineral processing and extractive-metallurgical operations also generate toxic liquid wastes. Environmental engineers and scientists are facing with the challenging task to develop appropriate low cost technologies for effluent treatment [3]. Conventional methods for removing metals from aqueous solutions include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery. These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1-100 mg/L [4]. Another major disadvantage with conventional treatment technologies is the production of toxic chemical sludge and its disposal/treatment becomes a costly issue and is not environmental friendly. Therefore, removal of toxic heavy metals to an environmentally safe level manner became a great importance process [3].

Cadmium is one of the most toxic heavy metals, heaving half-life of 10-30 years and its accumulation in human body affect kidney, bone and also causes cancer. Cadmium enters in the environment by natural (rock weathering, forest fires, and volcanic activities) and more important anthropogenic activities. Its use is increasing in industrial applications such as electroplating, pigments and electronic (batteries) industries. Mineral fertilizers applied on soil or solid wastes deposited in inappropriate places contains also cadmium compounds, which can be adsorbed on soil particles or can be mobilized in groundwater [5,6]. Because of its mobility in soil, cadmium may be transferred to plants and accumulated in roots, stems and leaves. As a consequence, because of the high toxicity of Cd^{2+}, the crops may become unfit for animal and human consumption, and crop yields may be drastically reduced [5].

Beside conventional methods presented above, eco-friendly processes such as biosorption and phytoremediation are used more often in order to eliminate heavy metals from different environments [3, 7].

Biomaterials of microbial and plant origin interact effectively (adsorption and bioaccumulation) with heavy metals. Due to their unique chemical composition, metabolically inactive dead biomass sequesters heavy metal ions and metal complexes from solutions, which obviates the necessity to maintain special growth-supporting conditions. Metal adsorption by various types of biomaterials can find enormous applications for removing and recovery of heavy metals from their solutions [8].

For the economical reason, researchers have paid much attention to various by-products from fermentation industry, such as *Saccharomyces cerevisiae*, because they are produced in large quantities. Although *Saccharomyces cerevisiae* is a mediocre biosorbent, it is extensively examined as a biomaterial in biosorption studies for heavy metals removal [8]. Vieira et al. have shown that the questioned yeast has commercial application as biosorbent because at least four reasons. In the first place, *Saccharomyces cerevisiae* is easy to cultivate at large scale; as a result it can grow with unsophisticated fermentation techniques and inexpensive growth media. Second, the biomass of *Saccharomyces cerevisiae* can be obtained from various food and beverage industries. Third, *Saccharomyces cerevisiae* is not usually a waste, but a commercial commodity and considered safe, therefore, biosorbent made from *Saccharomyces cerevisiae* may be easily accepted by the public when applied in practice as it can be used at large scale with low cost, especially for treating of large amount of wastewater containing heavy metal in low concentration. Fourth, attempt is to use *Saccharomyces cerevisiae* as biosorbent, but not the last, is an ideal model organism to identify the kinetics of the biosorption in metal ion removal, especially to investigate the interactions at molecular level [8].

Alginate beads are often used as a support for biomaterials because of their natural origin with no toxicity towards immobilised micro-organisms or environment and because of their biodegradability after utilisation [5].

Besides biomaterials, other natural cheap materials such as bentonite can be used with success to remove heavy metals from aqueous solutions. Removal of heavy metal ions such as lead, copper, cadmium, cobalt, iron, nickel, zinc from their solutions, was studied on bentonite (suspended in aqueous solution) from deposits situated all over the world [9-14].

In order to study the combined adsorptive properties of *Saccharomyces cerevisiae* cells and bentonite, a series of mixtures consisting of bentonite and baker’s yeast, immobilized in calcium alginate

were used to establish adsorption capacities towards cadmium from aqueous solution.

1.4. Application of immobilized waste brewery yeast cells for Cd^{2+} removal. Equilibrium and kinetics

Heavy metal ions, such as cadmium, lead or mercury, are highly toxic to living organisms. Cadmium is one of the three most toxic heavy metals, its toxicity being attributed in part to its ability to accumulate in living organisms. Cadmium tends to accumulate slowly over time in bones, liver and kidneys, where it can damage normal functions.

Adsorption of metals by microbial biomass and agricultural materials is a relatively recent method for the removal and recovery of metals. This method was used to remove toxic metals from industrial liquid waste products, and due to its high efficiency, it looks more attractive by comparison to other processes [1]. Various kinds of microbial biomass (*e.g.* yeast, algae, fungi) [2-4]. and agricultural by-products (*e.g.* rice straw, soybean hull, sugarcane bagasse, peanut shell, pecan and walnut shells, almond shells, olive stones, and peach stones) [5,6] have been tested for this purpose. For example, Norris and Kelly [7] studied the adsorption of cadmium and cobalt ions by *Saccharomyces cerevisiae* yeast surface. Biosorption is considered to be a fast physical and/or chemical process depending on the yeast type and treatment. The biosorption rate depends on the type of the process. According to literature, biosorption can be divided into two main processes: adsorption of the ions on cell surface and bioaccumulation within the cell [8].

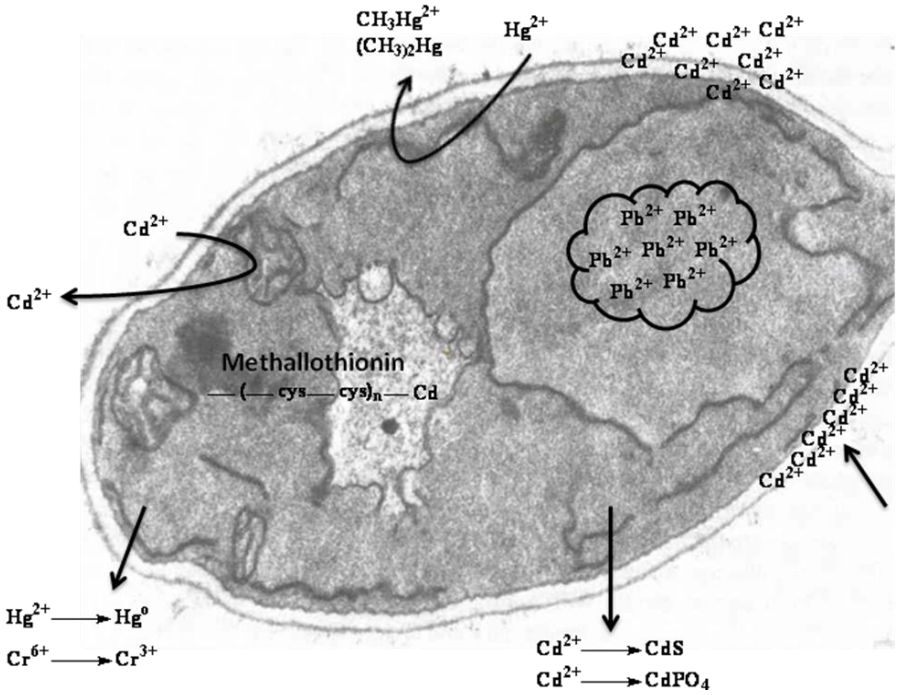


Fig. 1. The *Saccharomyces cerevisiae* yeast cell organelles and compartments (TEM) [36].

Uptake capacity of biomass is always affected by many factors like pH, temperature, initial concentration of biomass and metal ions, culture condition and some others, such as the presence of various ligands and metal ions. If all culture conditions are the same, the biosorption capacity of a biomass depends mainly on the type of biomass cells. To understand the interaction between the metal ions and the biomass, the hard and soft principle of metal ions proposed by Nieboer and Richardson has been widely used [9-12].

The commercial applications of biomass as biosorbents has been hindered by operational limitations associated with their physical characteristics, such as small particle size, low density, poor mechanical strength, low rigidity and solid/liquid separation problems [13] these difficulties can be overcome by entrapment of microbial biomass in immobilized preparations. The efficiency of these preparations as potential metal biosorbents can be further enhanced by using plant waste material as immobilizing matrix [14]. Immobilization techniques is one of the key

elements for the practical application of biosorption, especially by dead biomass [15]. The most commonly used matrix materials for the immobilization of microbial cells via entrapment has been the carbohydrate polymers such as alginate, chitosan, chitin and carboxymethyl-cellulose [16], polysulfone, polyacrylamide, polyurethane, and silica [17]. The selection of immobilization matrix is crucial in the application of immobilized biomass. The polymer matrix determines the mechanical strength, rigidity, and porosity characteristics and chemical resistance of the final biosorbent particle to be utilized for successive sorption–desorption cycles so, it is very important to choose the appropriate immobilization matrix in every case [18]. Natural polymers such as Na-alginate have been used as matrix for cell immobilization. Chang and co-workers found that the adsorption capacity of Ca-alginate immobilized cells was greater than that of polyacrylamide-entrapped cells for adsorption of Cd^{2+} [19].

Another authors studied the removal of different heavy metals (Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, etc.) onto diverse immobilized bacteria, fungi, algae, yeasts, etc. (*Spirulina platensis, Laminaria digitata, Aspergillus niger*, etc.) [20-24]. All of them proved to be very efficient in heavy metal ions removal from aqueous solutions.

The yeast biomass has been successfully used as biosorbent for removal of Ag, Au, Cd, Co, Cr, Cu, Ni, Pb, U, Th and Zn. A number of literatures have proven that *Saccharomyces cerevisiae* can remove toxic metal, recover precious metals and clean radionuclides from aqueous solutions to various extents. The advantages of *Saccharomyces cerevisiae* as biosorbents in metal biosorption, the forms of *Saccharomyces cerevisiae* in biosorption research, biosorptive capacity of *S. cerevisiae*, the selectivity and competitive biosorption by *Saccharomyces cerevisiae* were depicted in detail by Wang and Chang (2006) [25].

The same authors, in 2009, reviewed metal uptake capacity of *Saccharomyces cerevisiae*, in different forms (calculated for dry biomass), and found values ranging between 10 and 300 mg M^{n+} g^{-1}. In case of cadmium, uptake (adsorption capacity) capacity is usually above 10 but less than 100 mg Cd^{2+} g^{-1} dry mass. It should be noted that comparing results from different literatures involves in standardizing the different ways the adsorption capacity may be expressed. At same time, metal uptake, should be compared in almost the same equilibrium concentration of metals in solution for the purpose of evaluating performance of the biomaterial. In

particular, there is no standard measurement of dry weight of biomass, i.e. no standard of dry temperature and dry hours when drying biomass [18].

Various kinds of immobilized *Saccharomyces cerevisiae* have been studied with different support materials, which can be used in practical biosorption [15-26].

The objective of this work was to investigate the biosorption of Cd^{2+} ion by Romanian immobilized brewery waste biomass. Cadmium removal efficiency and adsorption capacity were determined. Adsorption equilibrium (Langmuir and Freundlich isotherms) and kinetic models (first and pseudo second order) were used to describe the biosorption process.

1.5. Suspended and immobilized brewery waste biomass and commercial yeast as biosorbents for Cd^{2+} removal. A themodynamic study

Pollution by toxic heavy metals is a global environmental problem. Heavy metals are nonbiodegradable and can be accumulated in living organisms [1]. Cadmium is a dangerous pollutant originating from metal plating, metallurgical alloying, mining, ceramics and other industrial operations.[2] According to Romanian legislation, the maximum concentration limit for Cd^{2+} discharge into surface waters is 0.2 mg l^{-1} and in potable water is 5×10^{-3} mg l^{-1}[3, 4].

Heavy metals can be removed from wastewater using conventional (ion exchange, adsorption, solvent extraction, chemical precipitation, reverse osmosis, ultrafiltration) and advanced (biosorption and phytoremediation) methods [5].

Biosorption utilizes the ability of certain materials to accumulate heavy metals from aqueous solutions by either metabolically mediated or physico-chemical pathways of uptake [6]. The major advantages of biosorption over conventional treatment methods include low cost, high efficiency of metal removal from dilute solution (e.g., less than 100 mg heavy metal l^{-1}), minimization of chemical and/or biological sludge, reusability of biomaterial, short operation time and the possibility of metal recovery [7, 8]. Various types of biomass including bacteria, [9] yeast, [10] fungi [11] and algae [12] have been investigated with the aim of finding more efficient and cost-effective metal removal biosorbent.

The word biomass usually refers to the material derived from the living beings after their death. The immobilization in alginate and the fermentation processes for brewery industry produce yeast biomass [13].

Saccharomyces cerevisiae can be used to remove toxic metals, recover precious metals and clean radio-nuclides from aqueous solutions to various extents. *S. cerevisiae* is not only a by-product of some fermentation processes, but also can be easily obtained in substantial quantities at low costs [10]. Often, the economics of the process can be improved by using waste biosorbent instead of cultured biosorbent [14]. As a biosorbent, *S. cerevisiae* not only successfully removes metals from wastewaters but also eases the burden of disposal costs associated with the waste [15].

Yeast cell walls, consisting mainly of polysaccharides, proteins and lipids, offer many functional groups that can bind ions such carboxylate, hydroxyl, sulphate, phosphate and amino groups which can act as binding sites for metals [13, 16].

The purpose of this work was to investigate the biosorption capacities of suspended (SBW) and immobilized (IBW) brewery waste biomass for cadmium removal. The results were compared with suspended (SFY) and immobilized (IFY) commercial fresh yeast. Effect of contact time and thermodinamics were used to describe the biosorption process.

1.6. Biosorption of Cd^{2+} Ions By Immobilized Cells of *Saccharomyces cerevisiae*. Adsorption Equilibrium and Kinetic Studies

The presence of industrial effluent containing heavy metals into freshwater poses serious problems to the ecological system including humans as they are toxic even at low concentrations [1,2]. One of the most common toxic metals found in industrial effluents is cadmium. It may come from various industrial sources such as electroplating, fertilizers, mineral processing and battery manufacturing [3, 4].

The removal of this metal from waters and industrial wastewaters has become a challenge for researchers. Manny studies confirm that various biological materials including fungi, algae, bacteria and yeast could be used in biosorption process to remove metal ions in wastewater [5, 6.] The biosorption process is a passive uptake that utilizes cell wall of biomass to sequester the metal ions from aqueous solutions [7, 8]. The presence of functional groups on biomass cell wall such as carboxyl, hydroxyl, ketones

and amino groups will involve a physical-chemical interaction between the metal ions during the biosorption processes [9].

Metal uptake is dependent not only on the type of species of microorganism, but also on growth conditions. Growth conditions considerably influence the composition of all yeast and thereby the binding abilities of cells for metal ions also. Since cell wall structure and the metabolic state of the cell depend on substrate composition, the growth in different media should influence the capacity and selectivity of metal uptake by creating other binding sites or diverse enzymatic system within the cell [10, 14].

Arica et all. (2001) said that the cell immobilization is one of the methods used to overcome the incorporating free suspended cell in industrial operations. It offers several advantages include minimal clogging in continuous systems, easy to separate from the reaction system and can be regenerated and reused the immobilized cells for a few cycles [3]. Natural polymers mostly used, as the matrix for the immobilization of cells is the alginate.

This study was carried out to determine the potential of immobilized living cells of *Saccharomyces cerevisiae* (DSM 1333) to adsorb cadmium (II) ions. Adsorption isotherm and kinetic are proposed.

1.7. Effect of surface modification of waste yeast from brewery onto Cd^{2+}, Zn^{2+}, and Cu^{2+} adsorption

The increase in usage of heavy metals in industrial activities has caused the existence of them in wastewater.

Microorganisms including bacteria, algae, fungi and yeast are found to be capable of efficiently accumulating heavy metals [1, 2]. Bioadsorption mechanisms involved in the process may include ion exchange, coordination, complexation, chelation, adsorption and microprecipitation [3, 4].

The term of “biosorption” is used to describe, in general, the property of microbial biomass to sequester heavy metals from aqueous systems. Earlier reserchers have used brewery waste *Saccharomyces cerevisiae* for removing silver and uranium from laboratory-prepared aqueous solution. Metal uptake by biosorption is reported to occur through interactions with functional groups native to the biosorbent cell wall [5, 6].

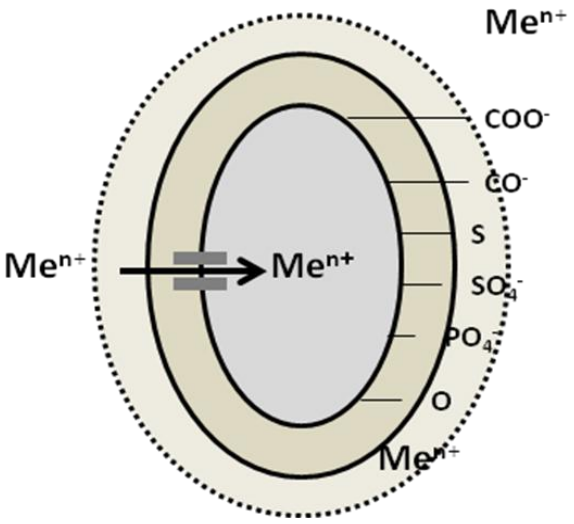


Figure 1. The mechanism of the biosorption
1. Diffusion to the surface of the cell. 2. Sorption. 3. Binding to the groups on the surface of the cell. 4. Transport into inside of the cell. 5. Binding in organo-mettalic form - metal is excluded from metabolic process [22].

Biomass cell walls, such as *Saccharomyces cerevisiae* consisting mainly of polysaccharides, proteins and lipids, offer many functional groups that can bind metal ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. In addition to these functional binding groups, polysaccharides often have ion exchange properties. Pretreatment and killing of biomass either by physical or chemical treatments or cross-linking are known to improve the biosorption capacity of biomass [7, 8].

For adsorption of heavy metals, surface chemistry of the biosorbent plays a key role since adsorption is favoured by the presence of oxygen-containing functional groups which can be very different accoring to the nature of the biosorbent: carboxylic, phosphate, sulphate, amino, amide and hydroxyl groups are the most commonly found [9,10]. These specific functional groups are essential for the adsorption of heavy metals due their chelating attributes. In particular, acidic groups (such as carboxylic) for pH values greater pKa are mainly in dissociated from and can exchange H^+ with metal in solution. At pH lower than pKa values, complexation phenomenon can also occur, especially for the carboxylic groups [11]. The reaction can occur due to ion exchange reaction of the metal cation with the hydrogen ion previously attached:

$$M^{n+} + n(\text{-COOH}) \Leftrightarrow (\text{-COOH})_n M + nH^+$$

The concentration of such acidic groups can be increased by chemical treatment involving oxidation. Depending on the chemical activation method, partial oxidation take place and the biomass surface becomes rich in a variety of functional groups whose nature and concentration depend on the method of activation, chemicals used, and temperature of preparation.

The advantages of biosorption over conventional treatment methods include, low cost, high efficiency, no additional requirement, possibility of regeneration of biosorbent and metal recovery [12].

The objective of this work was to investigate the effect of chemical treatments of brewery waste biomass on Cd^{2+}, Zn^{2+}, Cu^{2+} biosorption and to confirm by Fourier transform infrared spectroscopic analyses the groups responsible for the metal adsorption. Identification of the functional groups on the yeast cell wall would also help determine the mechanism responsible for the binding of target metals [13].

Metal ions removal efficiency and adsorption capacity were determined.

2. MATERIALS AND METHODS

2.1. Cd^{2+} removal from synthetic wastewaters using *Scenedesmus opoliensis* green algae

The cadmium solution was prepared by dissolving $Cd(NO_3)_2x4H_2O$ (analytically reagent) in deionized water. Axenic monoalgal cultures of *Scenedesmus opoliensis* P. Richter, obtained from the culture collection of Cluj Biological Research Institute [11], were grown in Kuhl-Lorenzen (KL) nutrient media supplemented [12]. The biosorption process was conducted in batch conditions in a Berzelius flask (beaker) where we poured 15 ml of concentrated algae solution over 150 ml solution of Cd^{2+} of different concentrations (4.35, 12.7 or 20.49 mg Cd^{2+}/L). The solutions thus formed were placed on a magnetic stirrer and there were continuously mixed, at 25ºC. In order to determine residual Cd^{2+} concentration in the solution, 2 ml samples were taken out at specific time intervals with a syringe and filtred using M.E. Cellulose filter the pore dimension of 0.45 μm. The analytical method employed in the Cd^{2+} concentration measurements was atomic absorption carried out with a Senso AA Spectrometer. Calibration was performed within a linear calibration range of cadmium. To determine the dry mass of algae 15 ml of initial green solution were dried to constant mass, in a drying oven, at 105ºC. The weighted dried biomass was 0.66 g.

2.2. Removal of Zn^{2+} from some synthetic wastewaters by immobilized *Saccharomyces cerevisiae* cells

Microorganism

The microorganisms were obtained from commercial type *Saccharomyces cerevisiae* cells (Pakmaya).

Biosorbent immobilization

For calcium alginate immobilization of yeast, 2.5 g biosorbent (baker yeast's) was suspended in 30 ml alginate solution (3g Na-alginate mixed with 1 ml ethanol was added to 100 ml distillated water and incubate for 30 minutes). A 100 ml aliquot of alginate - biosorbent suspension containing 2% Na-alginate was added drop by drop to 1000 ml of 2% $CaCl_2$ solution with a peristaltic pump. Alginate drops solidified upon contact with $CaCl_2$, forming beads and thus entrapping biosorbent particles. The beads

were allowed to harden for 30 min and then were washed with distillated water in order to remove excess of calcium ions.

Metal uptake

The retention capacity of biosorbent, q_e, was calculated.

Biosorption experiments

The stock solution of Zn^{2+} was prepared by dissolving a weighed quantity of $ZnSO_4 \times 7H_2O$ in deionized water. The immobilized biosorbent, 2.75 g, was added in a flask over 150 ml Zn^{2+} solution (129.60 mg Zn^{2+}/L, 213.41 mg Zn^{2+}/L and 304.88 mg Zn^{2+}/L) under continuous magnetic stirring at 200 rot/min for 2 h. The experiment was continued until a constant Zn^{2+} ion concentration was obtained. 1 ml samples were taken at different intervals of time and analyzed in order to determine Zn^{2+} concentration.

Zn determination

Zn^{2+} ions concentration was determined in the supernatant according to STAS 6327-81 using spectrophotometric method (potassium ferricyanide, l = 420 nm, UV/VIS JENWAY 6305 spectrophotometer).

2.3. Fixed Bed Studies for Cd^{2+} Removal from Model Solutions Using Immobilized Bentonite/Yeast Mixtures

We used a commercial bentonite (B) sample from Fort Benton distributed by Interker-Wein Kft., Hungary. The bentonite sample was used as powder, (d < 0.2 mm), without any chemical treatment. We used also commercial baker's yeast (D) produced by Pakmaya (wet). All chemicals used in this study were analytical reagent grade ($Cd(NO_3)_2 \times 4H_2O$, alginic acid sodium salt and $CaCl_2$).

In order to obtain the bentonite/baker's yeast mixtures (we will refer to the bentonite-yeast mixture as adsorbent along the paper) immobilized in alginate beads we used the cross-linking procedure with calcium alginate, which is an adapted version of the method for treatment of fungi biomass outlined by Schiewer et al. (1995) and Zhao and Duncan (1997) [17, 18].

For adsorbent immobilization, various quantities of bentonite (2, 4, 6, 8 g) and baker's yeast (2, 4, 6, 8 g) in different combinations (8g B, 6g B + 2g D, 4g B + 4g D, 2g B + 6g D, 8g D), were suspended in 50 ml distilled water. This suspension was next blended with a mixture formed from 1 g Na-alginate and 2 ml ethanol. The mixture was then dropped with a peristaltic pump into a 0.2 M $CaCl_2$ solution. During this process, alginate-

bentonite-yeast mixture drops were gelled into beads with a diameter of 4.0 ± 0.2 mm. The Ca-alginate immobilized adsorbent beads were stored in 0.2 M $CaCl_2$ solution at 4°C for 1 hour to cure. The beads were rinsed with distilled water for remove excess of calcium ions and stored at 4°C prior to use.

For the heavy metal ion removal study we used model monocomponent solutions containing cadmium ions of 40 and 120 mg Cd^{2+}/L. The concentration of cadmium ions in solution was determined using a flame atomic absorption spectrophotometer (SensAA Dual GBS Scientific Equipment, Australia).

The heavy metal ions removal process was realized in a batch reactor under magnetic stirring (825 rpm), using 100 ml of cadmium solution in which Ca-alginate bentonite beads obtained from the desired quantity of adsorbent were suspended. For the fixed bed column experiments we used a 32 mm diameter column, in which Ca-alginate beads obtained from the desired quantity of adsorbent were placed. Cadmium solution passes the fixed bed with a flow rate of 4 ml/min.

In order to determine the exact concentration of cadmium ions and establish the evolution of the removal process, in batch conditions, samples of 1 mL (dilution in each case was 25) from the supernatant were collected at different time intervals, every 5 minutes for the first 30 minutes and next every 15 minutes until equilibrium was reached. In case of the fixed bed experiments, 50 ml solution is collected at the outflow of the column every 12.5 minutes until the adsorbent is exhausted (exhaustion point).

We studied the influence of the bentonite and baker's yeast quantity, cadmium concentration in solution over the process efficiency in batch and fixed bed conditions. The experiments were carried out at room temperature (20°C) and without any modification of the pH value (pH 5.4 of the initial cadmium solution).

The amount of adsorbed cadmium (adsorption capacity q_e, mg/g) was calculated using equation 1 (the calculated values of removal efficiencies and adsorption capacities should be regarded according to the precision of the determination methods we used). We also calculated the removal efficiencies (E, %), equation 2, in order to establish the effectiveness of the considered adsorbent in the heavy metal ion removal process, in batch conditions.

$$q_e = \frac{(C_0 - C_t)}{w} \cdot \frac{V}{1000} \quad (1)$$

where,

C_0 is the initial cadmium concentration (mg Cd^{2+}/L),
C_t is time t cadmium concentration (mg Cd^{2+}/L),
V = 100 ml, and
w is the quantity of the adsorbent (g).

$$E = \frac{C_0 - C_t}{C_0} \cdot 100 \quad (2)$$

2.4. Application of immobilized waste brewery yeast cells for Cd^{2+} removal. Equilibrium and kinetics

Biosorbent

The biosorbent, brewery waste biomass, *Saccharomyces cerevisiae*, was collected from CIUC brewery (Miercurea-Ciuc, Romania) after used in fermentation processes and transported to the laboratory in plastic containers. The yeast was then washed with bi-distillated water, separated by vacuum filtration, and dried in a hot air oven at 80°C for 24 hours.

Biosorbent immobilization

The cross-linking procedure with calcium alginate that we used is an adapted version of the method for treatment of fungi biomass outlined by Schiewer and coworkers [27, 28].

For immobilization of yeast, 2g of biosorbent (brewery waste biomass) was suspended in 50 ml distilled water. This suspension was next blended with a mixture formed from 1g Na-alginate and 2 ml ethanol. The mixture was then dropped with a peristaltic pump into a 0.2 M $CaCl_2$ solution. During this process, the drops of alginate-biomass mixture were gelled into beads with a diameter of 4.0±0.2 mm. The Ca-alginate immobilized yeast beads were stored in 0.2 M $CaCl_2$ solution at 4°C for 1 hour to cure and to form the cross-linking bonds. The beads were rinsed with distilled water for remove excess of calcium ions and stored at 4°C prior to use.

Cadmium solution preparation

The stock metal ion solution was prepared by dissolving $Cd(NO_3)_2 \times 4H_2O$ of analytical grade reagent in an appropriate amount of

distilled water. Cadmium solutions of different concentration (10, 24, 48, 100, 169 mg L^{-1}) were obtained by diluting the stock solution. The concentration of Cd^{2+} ions in the supernatant fluids was determined using a flame atomic absorption spectrophotometer (SensAA Dual GBS Scientific Equipment, Australia).

Metal biosorption studies

Experiments were realised in batch conditions with continuously magnetic stirring (875 rpm) at room temperature (20°C), pH = 6.5, for 3 hours. Immobilized brewery yeast biomass obtained as described before, was contacted with 100 ml of initial cadmium solutions, as described. Kinetics studies were performed using different concentrations of cadmium solutions. In order to determine the exact concentration of cadmium ions and establish the evolution of the removal process, samples of 100 μL (dilution in each case was 50) from the supernatant were collected at different time intervals (see Fig. 1, in page 53).

The amount of adsorbed cadmium was calculated using the (1) equation in page 37.

2.5. Suspended and immobilized brewery waste biomass and commercial yeast as biosorbents for Cd^{2+} removal. A themodynamic study

Materials

In our experiments we used four types of biosorbents that contain the same strain, namely *Saccharomyces cerevisiae*, as described bellow: (a) suspended brewery yeast waste biomass (SBW) – the brewery waste biomass was collected from CIUC brewery (Miercurea-Ciuc, Romania) after being used in fermentation processes and transported to the laboratory in plastic containers. The yeast was then washed with bi-distilled water and separated by vacuum filtration, dried in a hot air oven at 80°C for 24 hours; (b) immobilized brewery yeast waste biomass (IBW) – the brewery waste biomass was immobilized using the cross-linking procedure with calcium alginate, an adapted version of the method for treatment of fungi biomass outlined by Schiewer and coworkers; [20, 21] (c) suspended fresh yeast (SFY) – commercial baker's yeast produced by Pakmaya (wet); (d) immobilized fresh yeast (IFY) – commercial baker's yeast immobilized using the procedure described above.

For cadmium ions biosorption study we used synthetic monocomponent solutions containing Cd^{2+} ions (5.75 mg Cd^{2+} l^{-1}), prepared from $Cd(NO_3)_2x4H_2O$ salt, analytically pure reagent. Effect of contact time and thermodynamic study of cadmium biosorption were conducted at 295, 308 and 323 K, and constant pH for the synthetic solutions (pH = 5.5). Cadmium ions in solution were determined using an ion-selective electrode pHoenix Electrode Co. and a Jenway 3330 pH meter. The cadmium ions biosorption process was carried out in a batch reactor under magnetic stirring (750 rpm), using 0.978 g *Saccharomyces cerevisae* suspension (SBW, SFY) and alginate immobilized beads (IBW, IFY) within 100 ml cadmium solution of the established concentration. In order to determine the concentration of cadmium ion, water samples were taken every 5 minutes until equilibrium was reached.

In order to establish the effectiveness of the biosorbent samples in the heavy metal ion removal process, removal efficiencies and adsorption capacities were calculated. Removal efficiencies of cadmium ions were calculated using equation (1), where V = 100 ml, while adsorption capacities were calculated with equation (2), see page 37.

The calculated values of removal efficiencies and adsorption capacities should be considered with respect to the precision of the determination method used.

Biosorption thermodynamics

The thermodynamic parameters were determined using the equilibrium constant, K_d (q_e/C_e), which depends on temperature. The modification in free energy (ΔG°), entropy (ΔS°) and enthalpy (ΔH°) associated with the adsorption process were calculated using the following equations: [17,18]

$$\Delta G° = -RT \ln K_d \quad (1)$$

$$\ln K_d = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R} \quad (2)$$

where: R is the universal gas constant, T is temperature (K), and K_d is the distribution coefficient ($l\ g^{-1}$).

According to van't Hoff equation (2), enthalpy (ΔH°) and entropy (ΔS°) parameters can be calculated from the slope and intercept of the plot $\ln K_d$ versus 1/T.

2.6. Biosorption of Cd^{2+} Ions By Immobilized Cells of *Saccharomyces cerevisiae*. Adsorption Equilibrium and Kinetic Studies

Microorganism, media and culture conditions

Saccharomyces cerevisiae (DSM 1333) yeast was used in this study. The yeast was provided from University of Pécs Medical School, Department of Medical Microbiology and Immunology (Hungary), in the lyophilized form. The composition of growth medium was Müller-Hinton substrate (3 % glucose, pepton, yeast-extra, NaCl, pH=7). The medium was sterilized by autoclaving at a pressure of 1.5 atm and temperture of 121^{o}C for 20 minutes. The pure yeast culture grown in an incubator at 30^{o}C, 200 rot/min for 48 hours (New Brunswick Scientific). During the process the growth of yeast was controlled by measuring the absorbance of the culture. After completion of the yeast production the suspension was centrifuged 4500 rpm for 30 minutes, and two times washed with steril PBS (phosphate-buffer solution). Cells were then lyophilized and used in this form for all trials.

Biosorbent immobilization

The cross-linking procedure with calcium alginate that we used, is the current method for treatment of biomass [11].

The immobilization of yeast was carried out as follows: 2 g of lyophilized biosorbent was suspended in 50 ml distilled water. This suspension was next blended with a mixture formed from 1g sodium-alginate and 2 ml ethanol. The mixture was then dropped with a peristaltic pump into a solution containing 0.2 M $CaCl_2$. During this process, the drops of alginate-biomass mixture were gelled into beads with a diameter of 4.0±0.2 mm. The Ca-alginate immobilized yeast beads were stored in 0.2 M $CaCl_2$ solution at 4°C for 1 hour to cure and to form the cross-linking bonds. The beads were rinsed with distilled water for remove excess of calcium ions and stored at 4°C prior to use.

Cadmium solution preparation

The stock cadmium solution was prepared by dissolving $Cd(NO_3)_2 \times 4H_2O$ of analytical grade reagent in an appropriate amount of distilled water. Cadmium solutions of different concentration (4.82, 9.74, 24.15, 38.5, 84.39 and 99.75 mg/L) were obtained by diluting the stock solution. The concentration of Cd^{2+} ions from different samples was determined using a flame atomic absorption spectrophotometer (SensAA Dual GBS Scientific Equipment, Australia).

Biosorption studies

The immobilized pure yeast biomass was contacted with 100 ml of initial cadmium solution. The reaction mixture was agitated at 875 rpm on a rotary shaker at room temperature (20°C), pH 6.8-7.2, for 3 hours.

Kinetics studies were performed using different concentrations of cadmium solutions (4.82, 9.74, 24.15, 38.5, 84.39, 99.75 mg/L).

In order to determine the exact concentration of cadmium ions and establish the evolution of the removal process, samples of 100 μL from the supernatant were collected at different time intervals.

The amount of cadmium (II) ions bound by biosorbent was calculated using the equation (1) in page 37.

2.7. Effect of surface modification of waste yeast from brewery onto Cd^{2+}, Zn^{2+}, and Cu^{2+} adsorption

Biosorbent

The biosorbent, brewery waste biomass, *Saccharomyces cerevisiae*, was collected from CIUC brewery (Miercurea-Ciuc, Romania) after used in fermentation processes and transported to the laboratory in plastic containers. The yeast was then washed with bi-distillated water and separated by vacuum filtration and dried in a hot air oven at 80°C for 24 hours.

Chemical treatment of biosorbents

The yeast was chemically treated to study the influence of various functional groups on biosorption of Cd^{2+}, Cu^{2+} and Zn^{2+}. 2 grams of biomass was pretreated in 10 different ways as described below:

- 20 ml formaldehyde + 40 ml formylic acid, agitation on rotary shaker at 150 rpm for 5 hours, centrifugated in 5000 rpm after dry hot air oven 70°C for one night → this way obtained the amine-methylated biosorbent
- 65 ml ethanol + 0.6 cc hydrogen cloride, agitation on rotary shaker at 150 rpm for 5 hours, centrifugated in 5000 rpm after dry hot air oven 70°C for one night → this way obtained the carboxyl-esterified biosorbent
- 50 ml 40 % (vol/vol) NaOH solution boiled for 15 min, centrifugated in 5000 rpm after dry hot air oven 70°C for one night

- 10 ml 10 % (vol/vol) H_3PO_4 solution boiled for 15 min, centrifugated in 5000 rpm after dry hot air oven 70°C for one night
- 10 ml 10 % (vol/vol) H_2O_2 solution boiled for 15 min, centrifugated in 5000 rpm after dry hot air oven 70°C for one night
- 10 ml 0.5 % (vol/vol) commercial laundry detergent solution, boiled for 15 min, centrifugated in 5000 rpm after dry hot air oven 70°C for one night
- 75 ml benzene heat under reflux conditions for 6 hours, centrifugated in 5000 rpm after dry hot air oven 70°C for one night $\rightarrow$ this way obtained the lipide-extracted biosorbent
- 10 ml of 2 % (vol/vol) gluteraldehyde solution boiled for 15 min, centrifugated in 5000 rpm after dry hot air oven 70°C for one night
- 50 ml 1% (vol/vol) $CaCl_2$ solution autoclaving for 30 min, centrifugated in 5000 rpm after dry hot air oven 70°C for one night

After each pretreatment with chemicals the biomasses were washed with generous amounts of deionized water. The NaOH pretreated biomass was washed with deionized water until the pH of the solution was in a near neutral range (pH 6.8-7.2). The dried sample was then ground, using a blender and sieved to pass through a 100-mesh sieve to obtain uniform particle size.

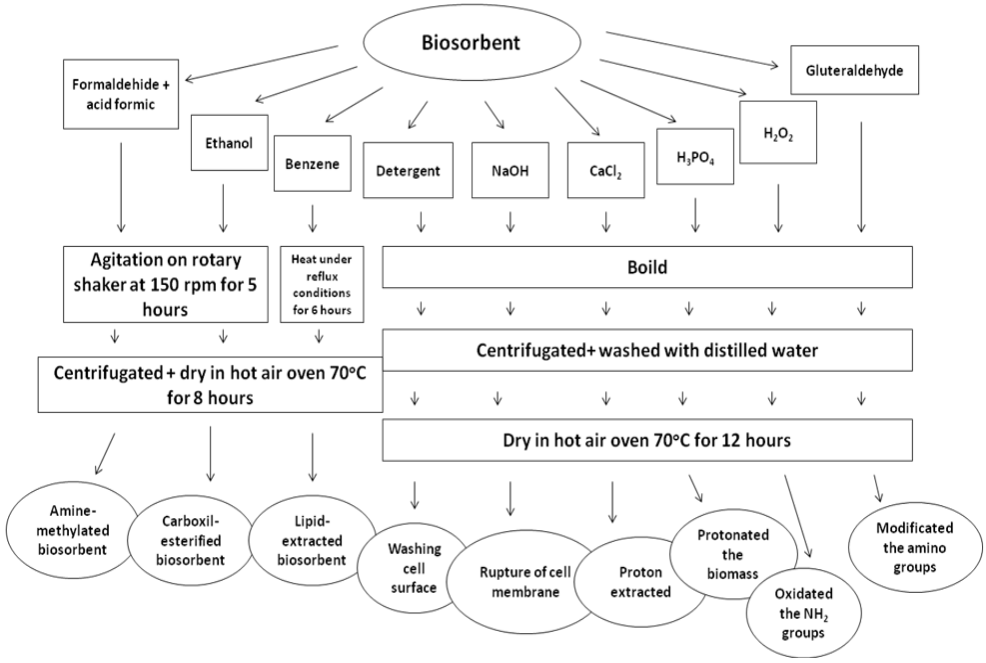


Figure 1. Schematic of the chemical treatments performed on brewery yeast

Metal biosorption studies

The batch equilibrium method was used to determine sorption of heavy metals by the tested yeasts. The initial heavy metal concentrations was 45.25 mg Cd^{2+}/L, 49.9 mg Zn^{2+}/L, mg Cu^{2+}/L. All biosorption experiments were carried out in 250 ml Erlenmeyer flasks containing 0.1 g dried biosorbent in 50 ml of the tested metal ion solution. This suspension was agitated for 3 hours in room temperature on a rotary shaker 150 rpm. The biomass was separated by centrifugation at 12000 rpm for 5 min and residual metal ion concentration was measured in the supernatant.

In order to determine the exact concentration of cadmium ions and establish the evolution of the removal process, samples of 100 μL from the supernatant were collected at different time intervals (5, 10, 15, 20, 25, 30, 40, 50, 50, 60, 75, 90, 105, 120, 135, 150, 165 and 180 min).

The amount of adsorbed cadmium was calculated using the equation (1) in page 37. [14].

3. RESULTS AND DISCUSSION

3.1. Cd^{2+} removal from synthetic wastewaters using *Scenedesmus opoliensis* green algae

As biosorbent we used *Scenedesmus opoliensis* green algae. Heavy metal ions removal was tested using synthetic Cd^{2+} solutions (C_1=4.36 mg Cd^{2+}/L, C_2=12.70 mg Cd^{2+}/L and C_3=20.49 mg Cd^{2+}/L), in dynamic regime (under magnetic, continuous stirring at 200 rot/min for 2 h), at room temperature and pH=5.2. The biomass of live algae *Scenedesmus op.* was added to the synthetic solutions of heavy metal ions. In order to determine residual Cd^{2+} concentration in the solution, 2 ml samples were taken out at specific time intervals.

The difference between the initial and remaining metal concentration was assumed to be taken up by the biosorbent. Evolution of Cd^{2+} concentration in time as a function of initial concentration is presented in figure 2. We found that in all three cases (three solution containing different quantities of cadmium ions) the concentration of Cd^{2+} significantly decreases, faster in the first 10 minutes from the beginning of the experiment, while the equilibrium was reached after 60 minutes for solution with initial concentration C_1, 70-80 minutes for C_2 and approximately 120 minutes for the most concentrated initial solution C_3 (figure 1). For adsorption yields, calculated values were placed around 50%, with small differences between the three initial concentrations, increasing from 50.90% for C_1=4.36 mg Cd^{2+}/L to 52.84% for C_3=20.49 mg Cd^{2+}/L (figure 2).

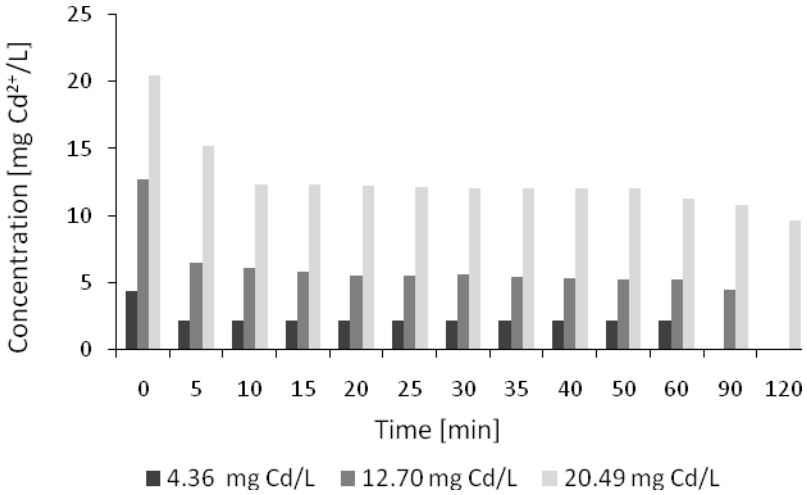


Figure 1. Evolution of Cd^{2+} concentrations in time for initial solutions containing different quantities of cadmium ions; 0.66 g dry mass biosorbent/15 ml solution.

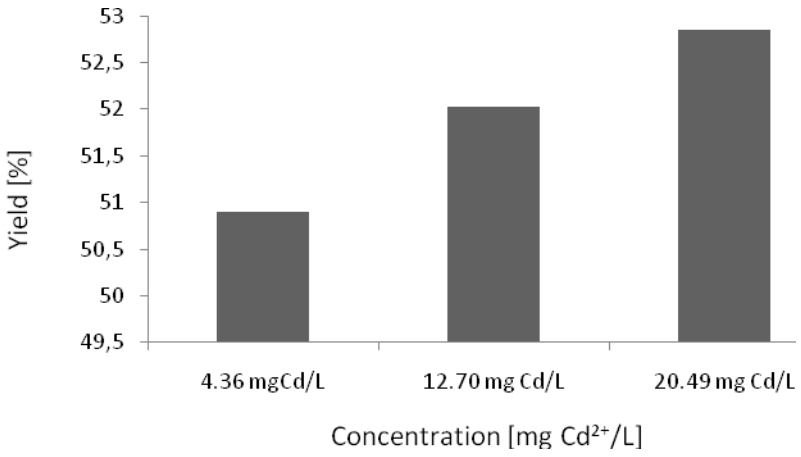


Figure 2. Maximum yield (%) values for Cd^{2+} adsorption on algae biosorbent; influence of the initial concentration of cadmium ions.

The retention capacity, q_e of algal material adsorbent increases from 0.67 mg Cd^{2+}/g adsorbent for C_1=4.36 mg Cd^{2+}/L to 3.28 mg Cd^{2+}/g adsorbent for C_3=20.49 mg Cd^{2+}/L (figure 3). Some of our previous studies [9] using dead algae as biosorbents (destroyed through thermal exposure) showed that the biosorption results were comparable with those from present paper (with live algae), or are even better. One possible explanation could be the metabolic extracellular products, which may form complexes with metals to retain them in solution [10].

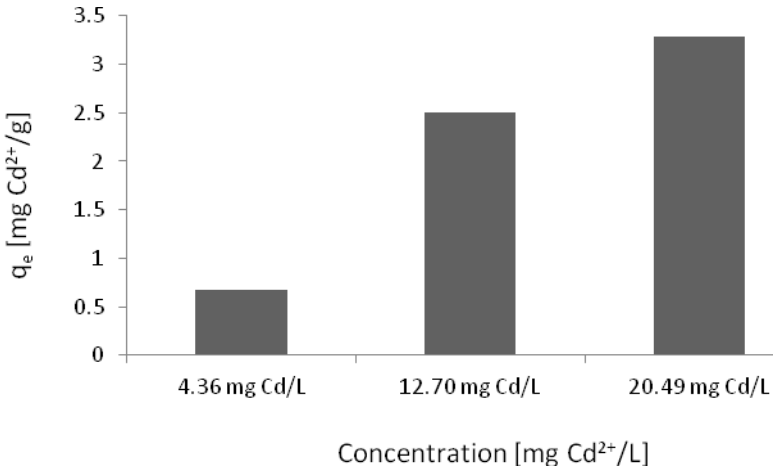


Figure 3. Maximum absorption capacity values for algae biosorbent; influence of the initial concentration of cadmium ions.

3.2. Removal of Zn^{2+} from some synthetic wastewaters by immobilized *Saccharomyces cerevisiae* cells

In order to investigate the effects of different Zn^{2+} concentration on metal uptake of immobilized *S. cerevisiae* cells, the biosorption experiments were conducted increasing the heavy metal ion initial concentration (129.60 mg/L; 213.41 mg/L; 304.88 mg/L). Figure 1 shows the variation of Zn^{2+} concentrations during the biosorption process. As it can be seen in figure 1, the initial Zn^{2+} concentration decreases in every biosorption experiment. In the first 25 minutes from the beginning of the experiment, Zn^{2+} concentration drops significantly (exponential decrease). This trend is followed, in all cases, by a slowly decrease until a constant value

(equilibrium concentration) was reached. For the initial concentration C_1=129.60 mg Zn^{2+}/L, after approximately 55 – 60 minutes the heavy metal ion content from analyzed samples goes to zero, which means that Zn^{2+} ions are totally retained on the immobilized *S. cerevisiae.*

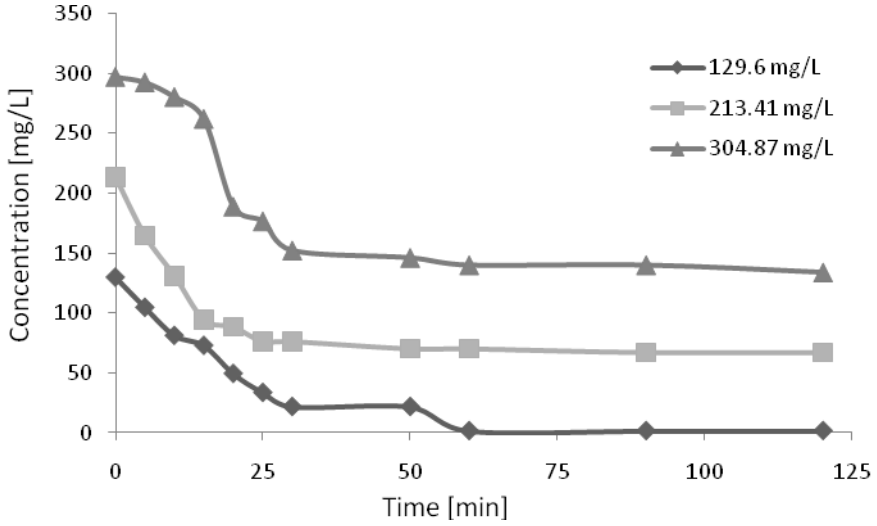


Figure 1. The biosorption of Zn^{2+} dependence of time for immobilized *S. cerevisiae* cells, at different initial Zn^{2+} concentrations. The biosorbent concentration was 2.75 g/150 ml (dry mass/volume).

Our results are in agreement with literature data regarding biosorption mechanism that is considered to take place in two stages [18]. In a first stage (dynamic regime) a pseudo-equilibrium is reached, while in a second stage (that takes place in some cases in static regime, on longer time intervals) a slowly decrease of metal concentration takes place. This decrease may be explained as a metal crossing through the cell wall, when intracellular accumulation takes place [18].

The adsorption yield, η [%], was calculated as follows:

$$\eta = \frac{C_i - C_f}{C_i} \cdot 100$$

where,

C_i is the initial concentration of solution (C_1, C_2, C_3), mg/L

C_f is the final concentration of Zn^{2+} in solution, mg/L.

For this parameter, the calculated values are ranging between 98.80% (for C_1 = 129.60 mg Zn^{2+}/L) and 56% (for C_3 = 304.88 mg Zn^{2+}/L).

The metal uptake is influenced by the initial Zn^{2+} concentration. The retention capacity, q_e, of yeast adsorbent increases from 5.1 mg Zn^{2+}/g adsorbent for C_1=129.60 mg Zn^{2+}/L to 7.1 mg Zn^{2+}/g adsorbent for C_3=304.88 mg Zn^{2+}/L (figure 2).

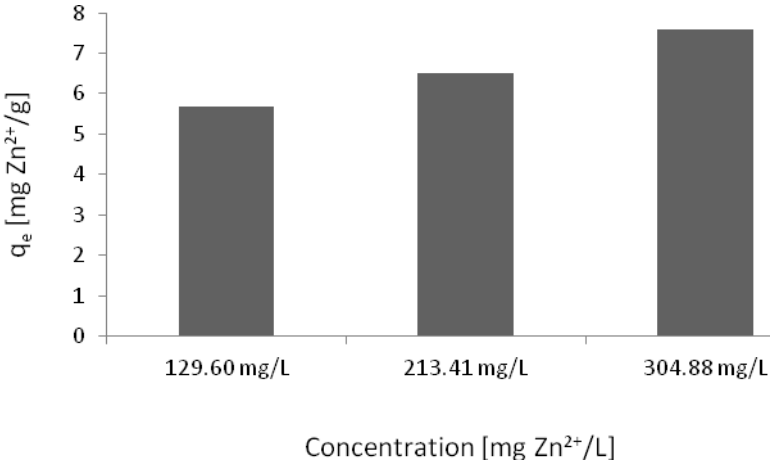


Figure 2. The influence of the initial concentration over the Zn^{2+} uptake, q_e, by the immobilized *S. cerevisiae*. The biosorbent concentration was 2.75 g/150 ml (dry mass/volume).

3.3. Fixed Bed Studies for Cd^{2+} Removal from Model Solutions Using Immobilized Bentonite/Yeast Mixtures

Batch study

The influence of the adsorbent type and ratio over the time evolution of cadmium concentration in batch conditions is presented in figure 1. Analyzing the decrease of concentration in time for all samples we observed some differences in the evolution of the adsorption process (cadmium concentration trends). In case of samples containing a higher quantity of baker's yeast (8g D, 6g D + 2g B) it is easy to observe that adsorption takes place in three distinct regions corresponding to cell surface adsorption, membrane diffusion and adsorption equilibrium, [15]. These regions are evidenced on the graph by the presence of two steps. As the baker's yeast

quantity decreases, these specific regions are disappearing being replaced by a continuous decrease, evolution specific to inorganic adsorbents [14,16].

Adsorption equilibrium was reached in 90 minutes for betonite increasing to 150 minutes for the baker's yeast. This decrease of the adsorption rate in case of baker's yeast could be associated with the diffusion limitation of the heavy metal ions through the cellular membrane.

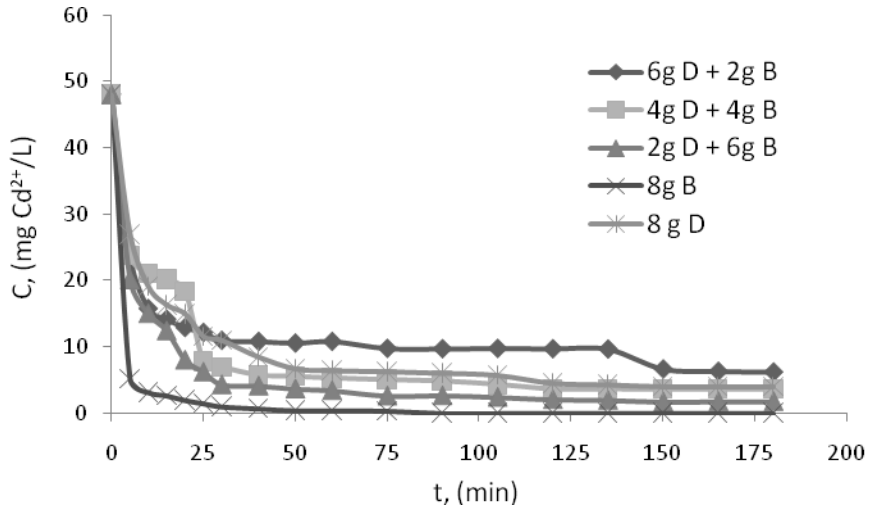


Figure 1. Influence of the adsorbent type and ratio over the time evolution of cadmium concentration in batch conditions (C_i = 40 mg Cd^{2+}/L, T = 20°C, pH = 5.4, 8g D, 6g D + 2g B, 4g D + 4g B, 2g D + 6g B, 8g B).

The influence of the adsorbent type and bentonite/baker's yeast ratio over the cadmium removal process, in terms of maximum removal efficiencies and adsorption capacities are presented in figures 2 and 3. The highest value of the removal efficiency was obtained in case of bentonite (100%). As the bentonite quantity decreases in the adsorbent mixture, figure 2, removal efficiency decreases to 87.08 % for the 6g D + 2g B mixture. When only the baker's yeast is present in the alginate beads, an increase of the removal efficiency to a value close to that corresponding to the 1:1 mixture ratio, 4g B + 4g D, (92.50%), 91.56% was calculated. Accordingly, the adsorption capacities (maximum values, calculated at equilibrium) will follow the same trend. The highest value was obtained when 8g of bentonite were immobilized, 0.6 mg Cd^{2+}/g adsorbent.

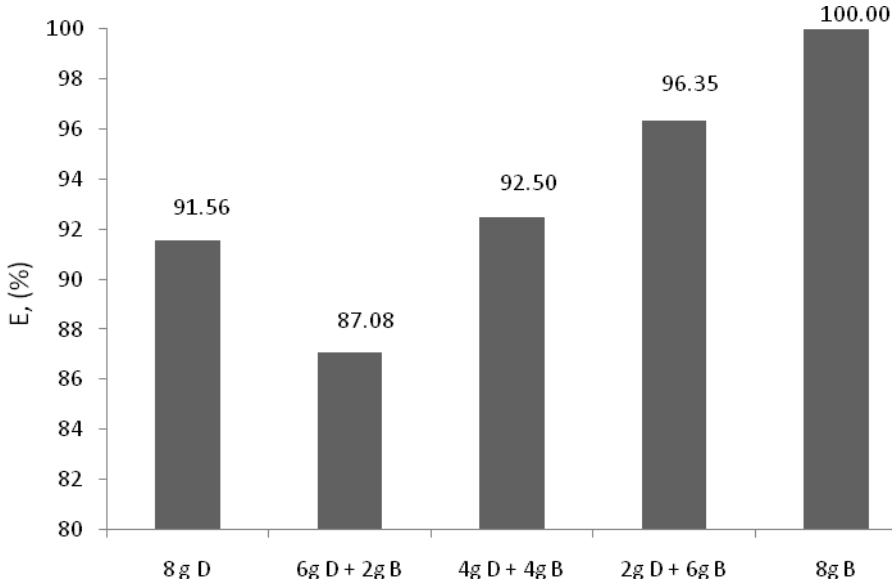


Figure 2. Maximum removal efficiency values for cadmium removal in batch conditions – influence of the adsorbent type and ratio (C_i = 40 mg Cd^{2+}/L, T = 20°C, pH = 5.4, 8g D, 6g D + 2g B, 4g D + 4g B, 2g D + 6g B, 8g B.

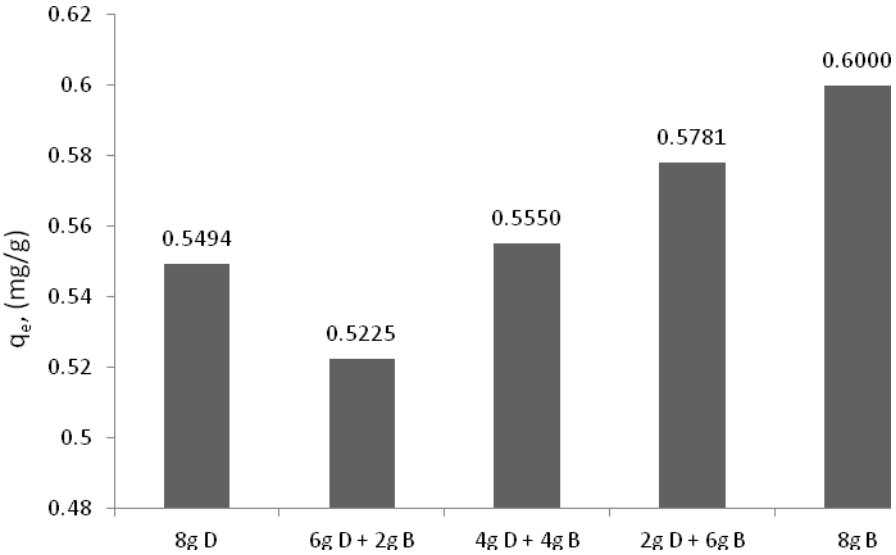


Figure 3. Adsorption capacity values for cadmium removal in batch conditions – influence of the adsorbent type and ratio (C_i = 40 mg Cd^{2+}/L, T = 20°C, pH = 5.4, 8g D, 6g D + 2g B, 4g D + 4g B, 2g D + 6g B, 8g B.

Fixed bed column study

Results obtained when the cadmium removal experiments were carried out in fixed bed column are presented in terms of cadmium concentration time evolution (breakthrough curves) and adsorption capacities at exhaustion point. As can be observed from figures 4 and 5, when only baker's yeast is present in the alginate beads, the breakthrough curve is steeper, concentration of cadmium at the outflow of the column increases faster, indicating a favourable kinetic in this case. We can concluded that using this flow rate, the adsorption in case of the baker's yeast takes place rapidly but only on the membrane cell, the diffusion through the membrane being restricted, therefore the adsorption capacity will be smaller. In all cases the breakthrough point is around 75 minutes, while the exhaustion point was reached after approximately 250 minutes.

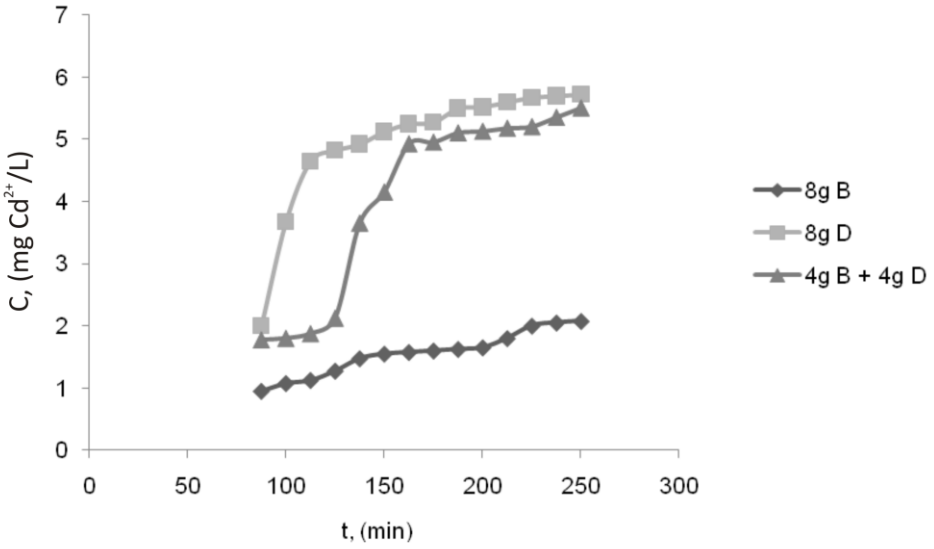


Figure 4. Influence of the adsorbent type and ratio over the cadmium concentration evolution in time in fixed bed studies – breakthrough region (C_i = 40 mg Cd^{2+}/L, T = 20°C, pH = 5.4).

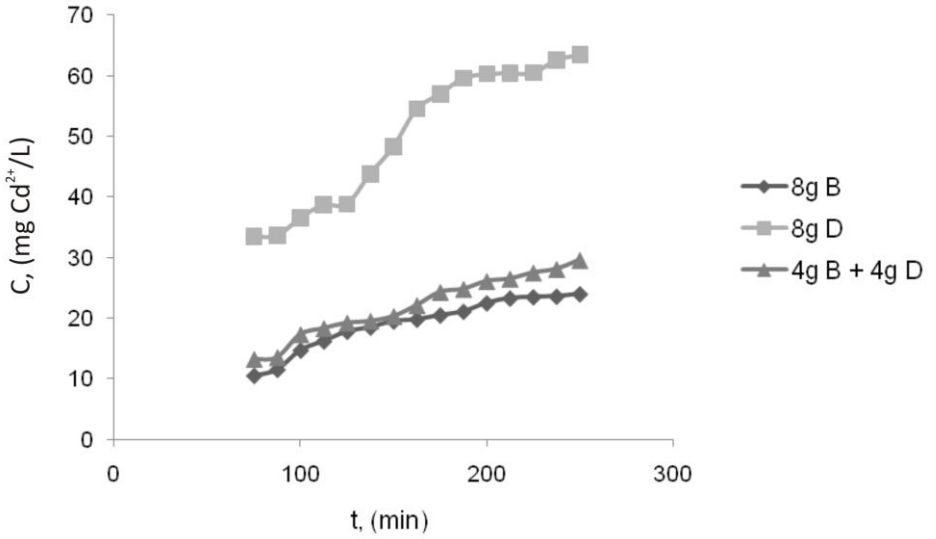


Figure 5. Influence of the adsorbent type and ratio over the cadmium concentration evolution in time in fixed bed studies – breakthrough region (C_i = 120 mg Cd^{2+}/L, T = 20°C, pH = 5.4).

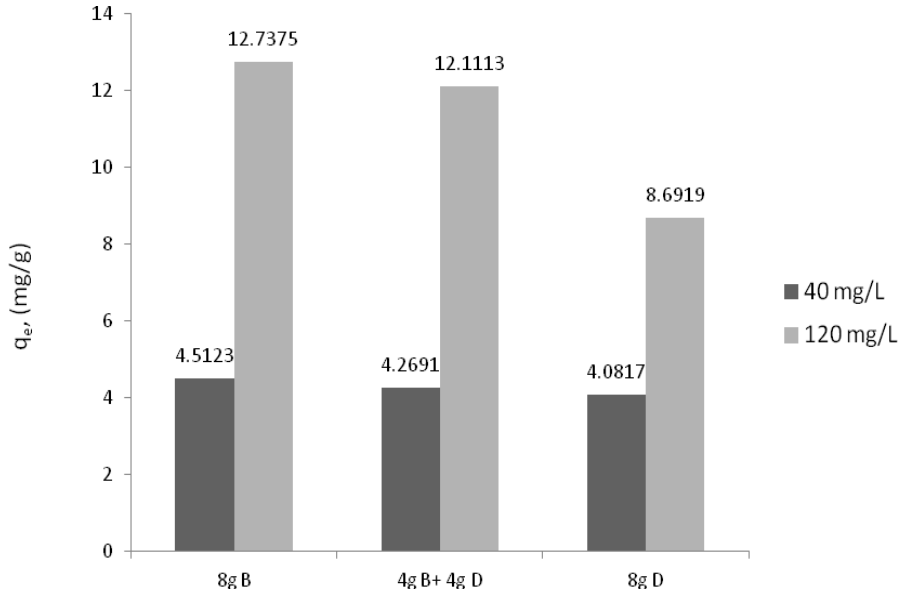


Figure 6. Maximum adsorption capacity values for cadmium removal in fixed bed – influence of the initial cadmium solution concentration, and adsorbent type and ratio (C_i = 40, 120 mg $Cd^{2+/}$L, T = 20°C, pH = 5.4, 8g D, 4g D + 4g B, 8g B).

3.4. Application of immobilized waste brewery yeast cells for Cd^{2+} removal. Equilibrium and kinetics

Cadmium biosorption

The dynamics of cadmium uptake until equilibrium by waste brewery biomass for various initial cadmium concentrations is represented in Fig. 1.

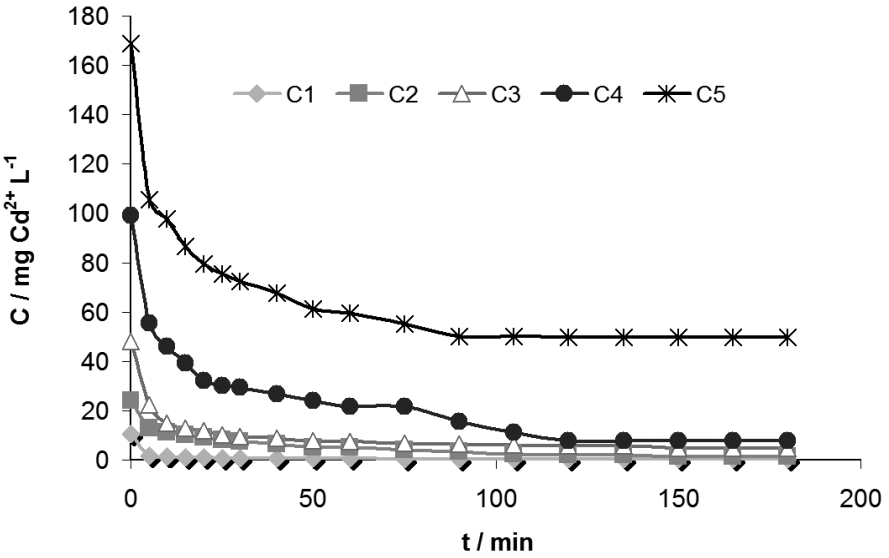


Figure 1. Dynamics of cadmium uptake by waste brewery biomass for various initial cadmium concentrations; C_1 = 10 mg Cd^{2+} L^{-1}, C_2 = 24 mg Cd^{2+} L^{-1}, C_3 = 48 mg Cd^{2+} L^{-1}, C_4 = 100 mg Cd^{2+} L^{-1}, C_5 = 169 mg Cd^{2+} L^{-1}.

Following the decrease of concentrations in time, we can observe three distinct zones, which represent:

(a). one rapid decrease in the first *5* minutes, corresponding to an adsorption of cell surface by the interactions between the metal ions and the metal-functional groups such as carboxyl, phoshate, hydroxyl, amino, sulphur, sulphyde, thiol, etc., present in the cell walls;

(b). a slow decrease of cadmium concentration corresponding to metal ions that penetrate the cell membrane and enter inside the cells; this decrease of the adsorption rate could be associates with a diffusion limitation of the heavy metal ions transport through the cell wall.

(c). the attainment of adsorption equilibrium between metal ions from solution and the immobilized cell surface. Biosorption equilibrium was reached in 150 minutes for all considered cadmium initial concentrations.

Our results are in good agreement with those from literature [29-32].

Maximum adsorption capacities (Fig. 2) increase from 0.5003 mg Cd^{2+} g^{-1} for the initial 10 mg Cd^{2+} L^{-1} to 5.9600 mg Cd^{2+} g^{-1} for the initial 169 mg Cd^{2+} L^{-1}.

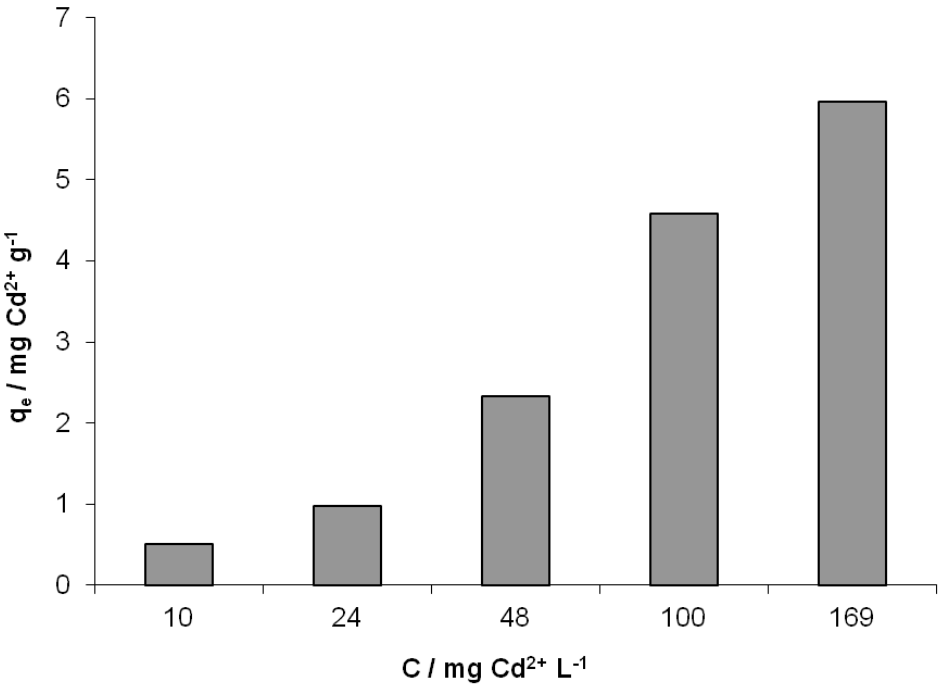


Figure 2. Maximum adsorption capacities obtained during cadmium adsorption experiments – initial concentration influence.

Adsorption equilibrium models

Cadmium biosorption equilibrium was described using Langmuir and Freundlich models, which were widely used to fit biosorption data [26, 33]. Langmuir model suggests a monolayer adsorption, with no lateral interaction between the adsorbed molecules. Freundlich model assumes heterogeneous adsorption due to the diversity of adsorption sites or diverse nature of the adsorbed metal ions, free or hydrolyzed species [33].

The Langmuir isotherm can be expressed as follows:

$$q_e = \frac{q_{max} \cdot b \cdot C_e}{1 + b \cdot C_e} \quad (2)$$

where, q_e is the solid-phase adsorbate concentration at equilibrium (mg g^{-1}),

q_{max} is the maximum adsorption capacity corresponding to the monolayer adsorption capacity (mg g^{-1}),

C_e is the concentration of Cd^{2+} solution at equilibrium (mg L^{-1}), and

b is related to the strenght of adsorbent-adsorbate affinity.

A linear expression of the Langmuir isotherm, eq. (2), is expressed as:

$$\frac{1}{q_e} = \frac{1}{q_{max} \cdot b} \cdot \frac{1}{C_e} + \frac{1}{q_{max}} \quad (3)$$

From the $1/q_e$ to $1/C_e$ linear plot – Fig. 3 –, q_{max} and b values were calculated to be 17.4825 mg Cd^{2+} g^{-1} and 0.0660 (L mg^{-1}). Experimental values (q_e and C_e), represented in Fig. 4, fitted well on a Langmuir isotherm type.

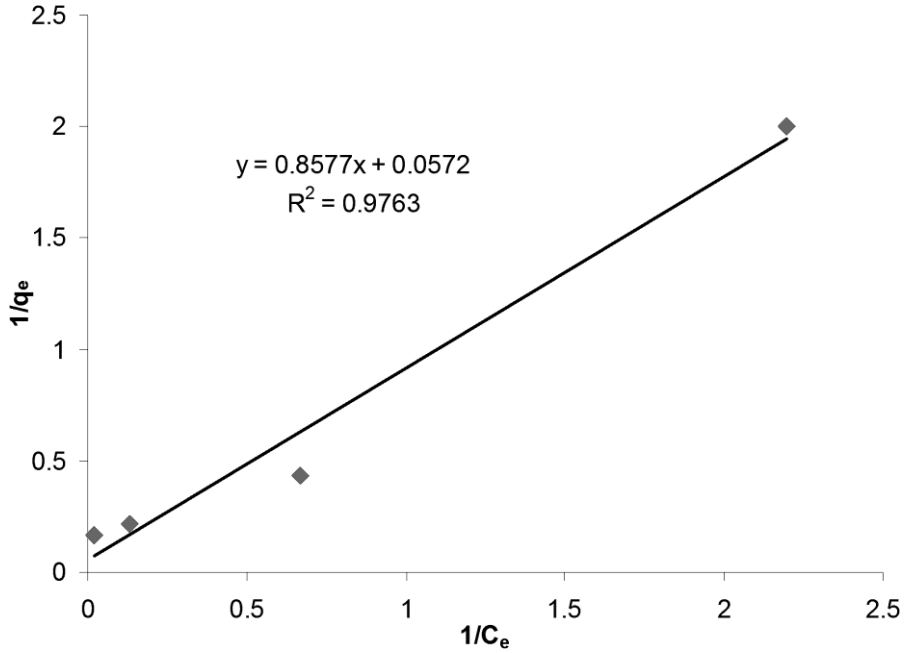


Figure 3. Langmuir adsorption model of cadmium biosorption on immobilized brewery waste biomass.

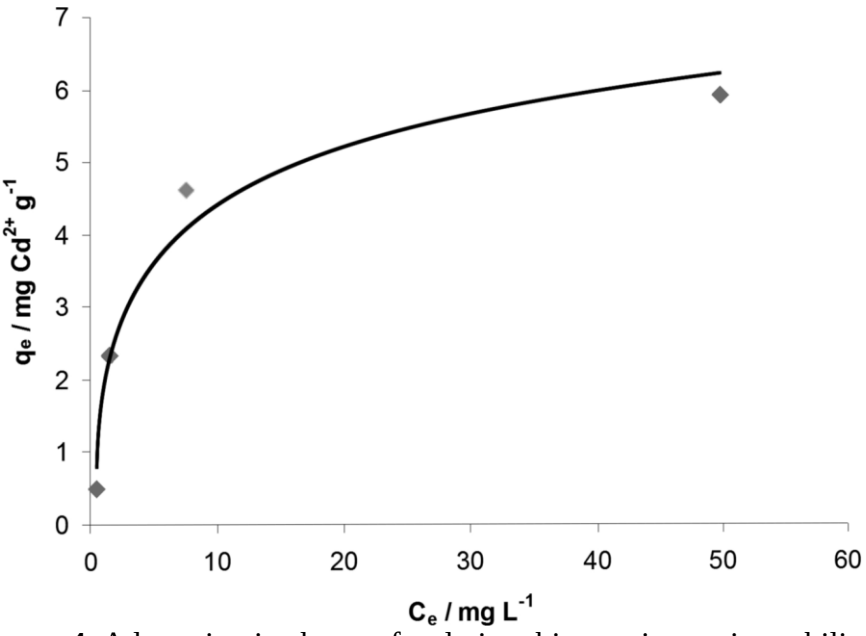


Figure 4. Adsorption isotherm of cadmium biosorption on immobilized brewery waste biomass.

The Freundlich isotherm can be expressed as:

$$q_e = k \cdot C_e^{(1/n)} \tag{4}$$

in logarithmic form (linear)

$$\log q_e = \log k + \frac{1}{n} \cdot \log C_e \tag{5}$$

where, k is related to adsorption capacity, and n is related to intensity of adsorption.

From the $\log q_e$ to $\log C_e$ linear plot – Fig. 5 –, we determined a correlation coefficient of 0.9183, which is smaller than that obtained for the Langmuir model, 0.9763. Therefore we concluded that cadmium biosorption on immobilized brewery waste biomass takes place on a Langmuir isotherm.

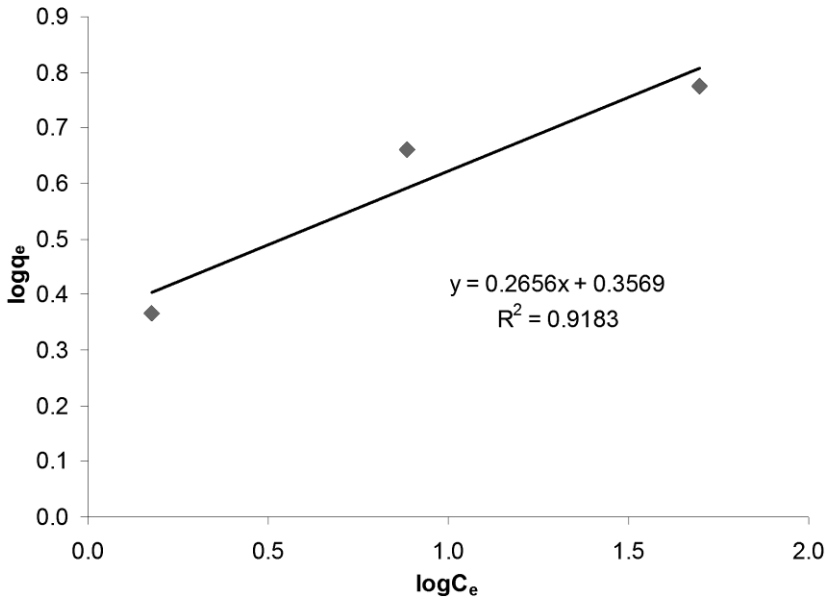


Figure 5. Freundlich adsorption model of cadmium biosorption on immobilized brewery waste biomass.

Kinetic models

Kinetic data were analyzed using first and pseudo-second order models. Using these models it is possible to investigate the mechanism of adsorption and rate controlling steps [19,33-35].

First order equation for the adsorption of liquid/solid system based on solid capacity, can be expressed as follows:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (6)$$

Integrating eq. (2) from the boundary conditions t = *0* to t = t and q_t = *0* to q_t = q_t, gives:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (7)$$

where,

q_e and q_t are the amounts of cadmium adsorbed (mg g^{-1}) at equilibrium and time t, respectively, and

k_1 is the rate constant of first order adsorption (min^{-1}).

In order to determine the rate constant and equilibrium cadmium uptake, the straight line plots of $\ln(q_e-q_t)$ against t, eq. (7), were made at five different initial cadmium concentrations. Correlation coefficients between 0.8126 and 0.9513 were obtained (figure not shown).

The pseudo second order kinetic model is derived on the basis of the adsorption capacity of the solid phase, which assumes that measured heavy metal ion concentrations are equal to cell surface concentration, expresses as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \tag{8}$$

Integrating eq. (8) from the boundary conditions t = 0 to t = t and q_t = 0 to $q_t = q_t$, gives:

$$\frac{1}{(q_e - q_t)} = \frac{1}{q_e} + k_2 t \tag{9}$$

where,

q_e and q_t are the amounts of cadmium adsorbed (mg g^{-1}) at equilibrium and time t, respectively, and

k_2 is the rate constant of first order adsorption (g $mg^{-1} \cdot min^{-1}$).

Equation (9) can be rearranged in linear form, as follows:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{10}$$

In order to determine the rate constant and equilibrium cadmium uptake, the straight line plots of t/q_t against t, eq. (10), were made at five different initial cadmium concentrations. Correlation coefficients between 0.9966 and 1.0000 were obtained (Fig. 6 and Tab. 1).

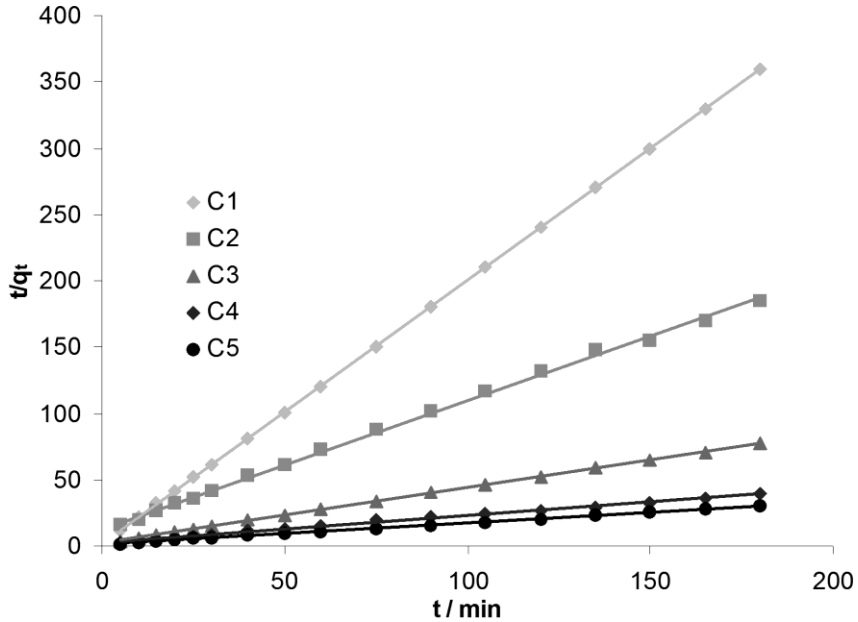


Figure 6. Correlation of the experimental data using the pseudo second order model for cadmium biosorption on immobilized brewery waste biomass; C_1 = 10 mg Cd^{2+} L^{-1}, C_2 = 24 mg Cd^{2+} L^{-1}, C_3 = 48 mg Cd^{2+} L^{-1}, C_4 = 100 mg Cd^{2+} L^{-1}, C_5 = 169 mg Cd^{2+} L^{-1}.

C / mg Cd^{2+} L^{-1}	q_e (exp) / mg Cd^{2+} g^{-1}	q_e (calc) / mg Cd^{2+} g^{-1}	k_2 / g $(mg \cdot min)^{-1}$	R^2
10	0.5003	0.5033	2.2189	1.0000
24	0.9715	1.0331	0.0727	0.9986
48	2.3250	2.3958	0.0754	0.9998
100	4.5825	4.8614	0.0186	0.9966
169	5.9600	6.2657	0.0211	0.9994

Table 1. Second order adsorption kinetic parameters.

If we compare correlation coefficients for the first and pseudo second order models, we can conclude that cadmium biosorption on immobilized brewery waste biomass can be classified as pseudo second order.

3.5. Suspended and immobilized brewery waste biomass and commercial yeast as biosorbents for Cd2+ removal. A themodynamic study

Effects of contact time and temperature

Effect of contact time on Cd^{2+} concentration during the biosorption process for all four considered biosorbents, SBW, IBW, SFY, and IFY at constant temperature (295 K) is presented in figure 1. Following the evolution in time of cadmium ions concentration it was observed that in the first 5 minutes from the beginning of the experiment there is a significant decrease, while the equilibrium was reached after another 35 minutes. For all considered biosorbents, equilibrium was reached in approximately 40 minutes. Values of cadmium ions concentration determined at equilibrium were smaller in case of SBW and IBW (0.71 and 0.91 mg Cd^{2+} l^{-1}, respectively) by comparison with SFY and IFY (4.16 and 2.33 mg Cd^{2+} l^{-1}, respectively). The difference observed between SBW and IBW, could be attributed to the internal diffusion limitation that occur when adsorption takes place on immobilized form (beads). Regarding to the temperature effect over the biosorption process, presented in figure 2 for IFY biosorbent, an important decrease of cadmium ions concentration was observed with an increase in temperature from 295 to 323 K. Equilibrium concentrations decreased from 2.33 mg Cd^{2+} l^{-1} at 295 K to 0.93 mg Cd^{2+} l^{-1} at 323 K. This effect could be attributed to the intensification of the diffusion processes and to the fact that the biosorption process was endothermic. It can be seen that the adsorbed amount of Cd^{2+} increased with contact time up to 40 min, and on this point, it attained the maximum removal. Therefore, 40 minutes was selected as the optimum contact time for all further experiments.

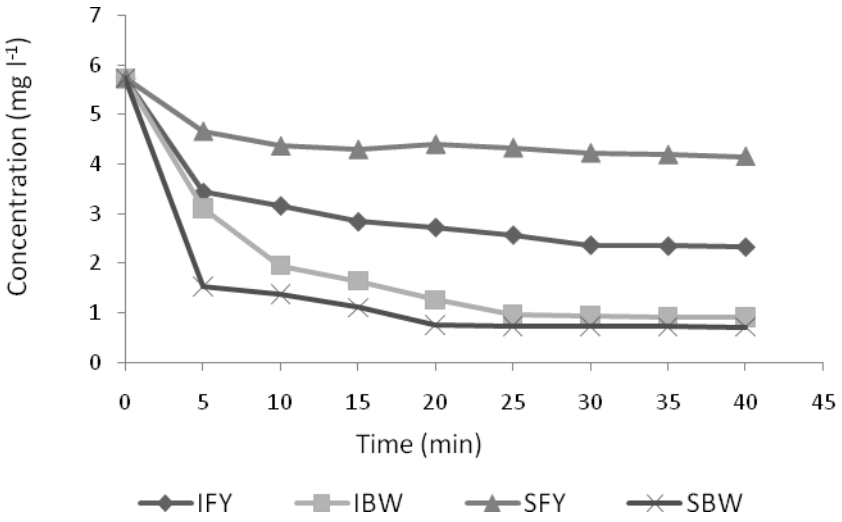


Figure 1. Effect of contact time on Cd^{2+} concentration during the biosorption process for SBW, IBW, SFY and IFY biosorbents at constant temperature (295 K).

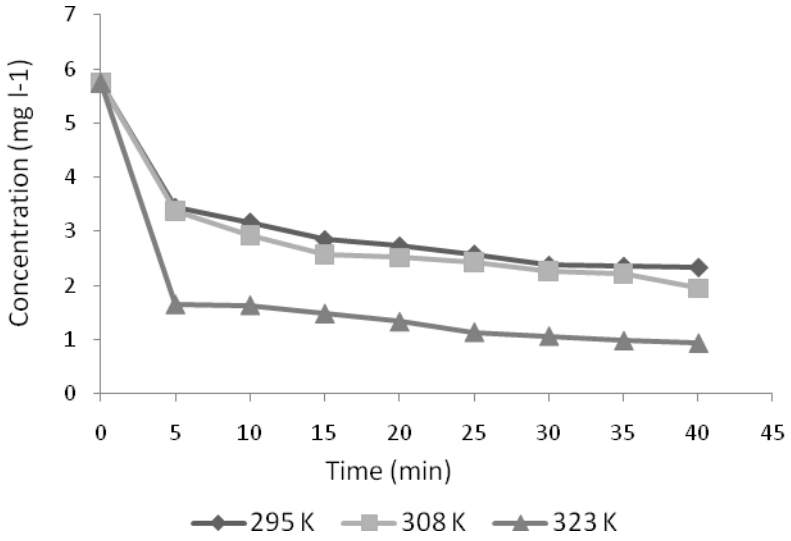


Figure 2. Effect of contact time and temperature (295, 308, 323 K) on Cd^{2+} concentration during the biosorption process for IFY biosorbent.

Previous conclusions are also reflected by the maximum biosorption efficiency values and adsorption capacities at equilibrium, figures 3 and 4. Highest biosorption efficiencies were found for SBW, with the maximum value of 99.83% calculated at 323 K. This result could indicate also that cadmium biosorption process onto considered biosorbents was endothermic. Also, adsorption capacities have maximum values for SBW and IBW biosorbents, 0.5841 and 0.5531 mg Cd^{2+} l^{-1}, respectively (figure 4). In all cases when brewery waste biomass was used for biosorption, higher values for adsorption capacities were calculated, fact that can be attributed to the destruction of the yeast cell walls, which led to an increase of the surface available for biosorption. In order to exemplify the influence of the temperature over the adsorption capacity, in figure 5, we presented values obtained in case of SBW biosorbent. Adsorption capacities increase with temperature from 0.5133 mg Cd^{2+} l^{-1} at 295 K to 0.5852 mg Cd^{2+} l^{-1} at 323 K.

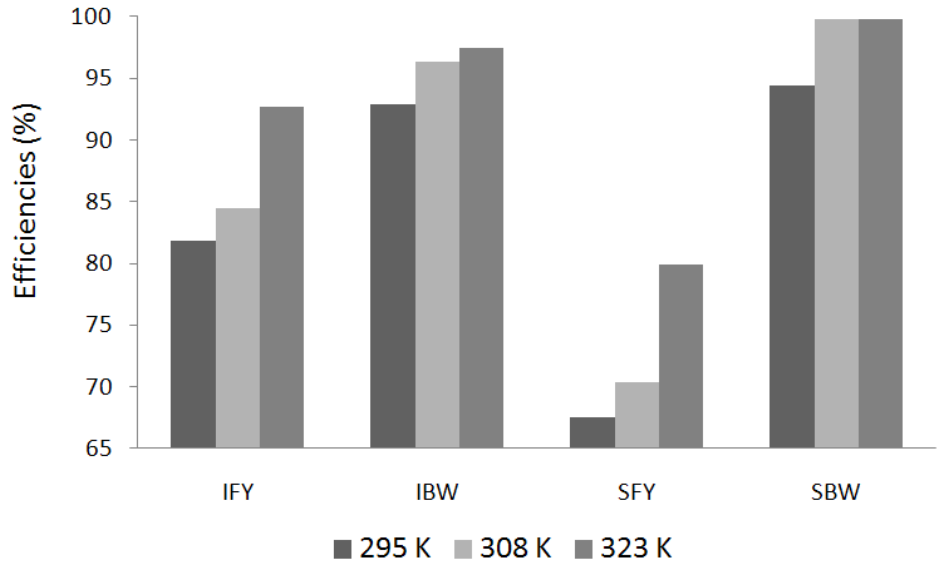


Figure 3. Effect of temperature on the maximum removal efficiencies (E,%) of Cd^{2+} onto SBW, IBW, SFY, and IFY biosorbents.

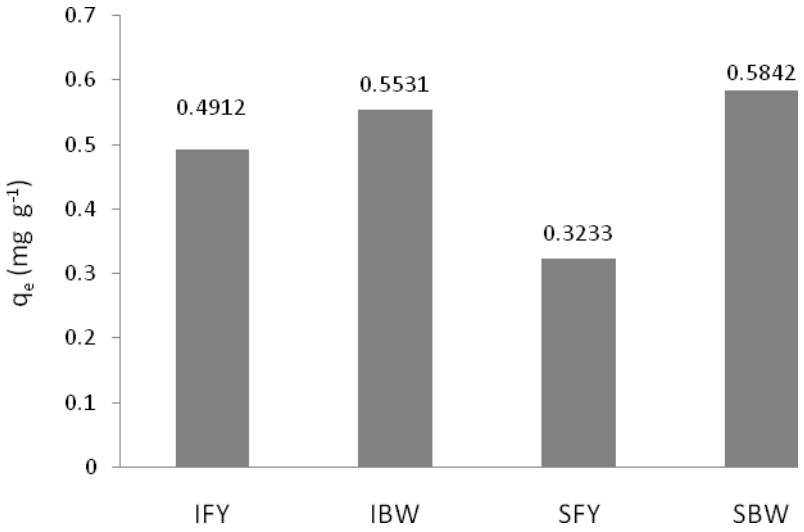


Figure 4. Effect of biosorbent type over the adsorption capacity values (q_e, mg Cd^{2+} g^{-1}) at constant temperature (323 K).

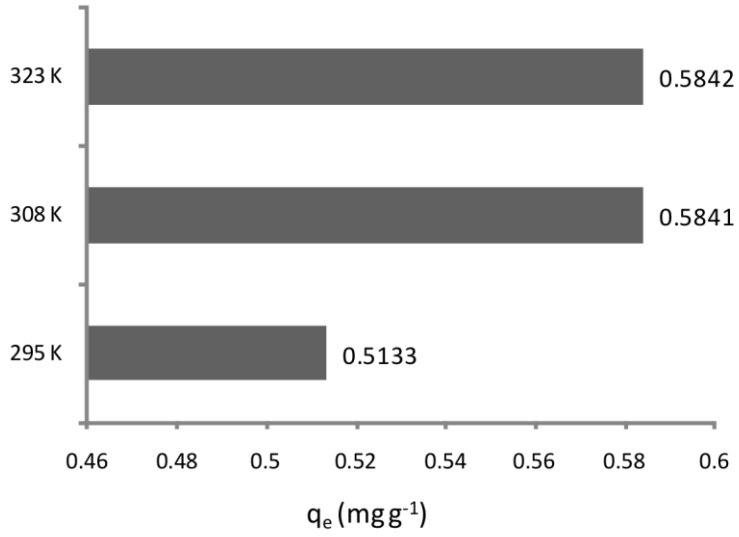


Figure 5. Effect of temperature on the adsorption capacity values (q_e, mg Cd^{2+} g^{-1}) for SBW biosorbent.

Biosorption thermodynamic parameters

The thermodynamic parameters for the biosorption process were computed from the $\ln K_d$ vs 1/T plots, figure 6, for a constant yeast quantity (0.978 g and 100 ml cadmium solution) in batch conditions under magnetic stirring. K_d values calculated as (q_e/C_e) are presented in table 1. These plots have good linearity with high values for regression coefficient (0.919-0.932).

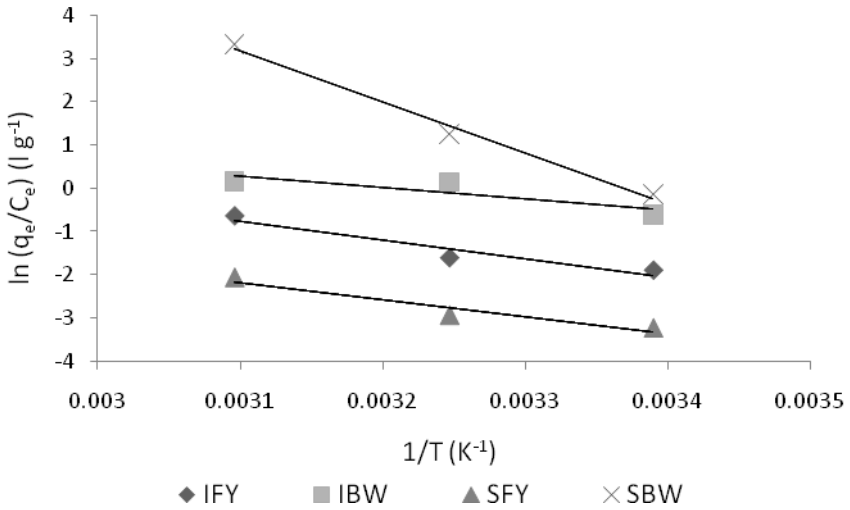


Figure 6. Plot of $\ln K_d$ vs 1/T for the estimation of the thermodynamic parameters for Cd^{2+} biosorption onto SBW, IBW, SFY, and IFY.

T, [K]	K_d, (l g^{-1})			
	IFY	**IBW**	**SFY**	**SBW**
295	0.1494	0.5434	0.0388	0.7184
308	0.1995	1.1487	0.0522	25.8998
323	0.5276	1.1799	0.1256	27.9084

Table 1. Distribution coefficient, K_d (l g^{-1}), values calculated at different temperatures (295, 308 and 323 K) for SBW, IBW, SFY, and IFY biosorbents.

Cd^{2+} –biosorbent interactions, at 295-323 K, took place through sufficiently strong endothermic interactions accompanied by thermo-dynamically favorable entropy and Gibbs energy changes ($\Delta G°$) [17, 18].

The enthalpy values for IFY–Cd, IBW–Cd, SFY–Cd, and SBW–Cd interactions vary from 21.8200 to 102.9863 kJ mol^{-1} (table 2). The positive ΔH° values indicate the endothermic nature of the biosorption processes at temperatures between 295 and 323 K.

The corresponding adsorption entropy values are ranging between 0.0697 for IBW and 0.3514 kJ K^{-1} mol^{-1} for SBW, positive values suggesting a certain degree of randomness at the solid–solution interface during the biosorption process [17]. In case of SBW biosorbent the highest value of ΔS° confirms the best results discussed previously in terms of efficiency and adsorption capacity, by a highest randomness degree.

The negative values of Gibbs free energy calculated from experimental results, indicated the fact that the cadmium biosorption process is feasible and spontaneous for IBW at 323 K and for SBW at temperatures varying from 295 to 323 K (table 2). In case of all studied biosorbents the decrease in ΔG° values with increase in temperature shows that the biosorption process is endothermic and it is favored by an increase in temperature [19].

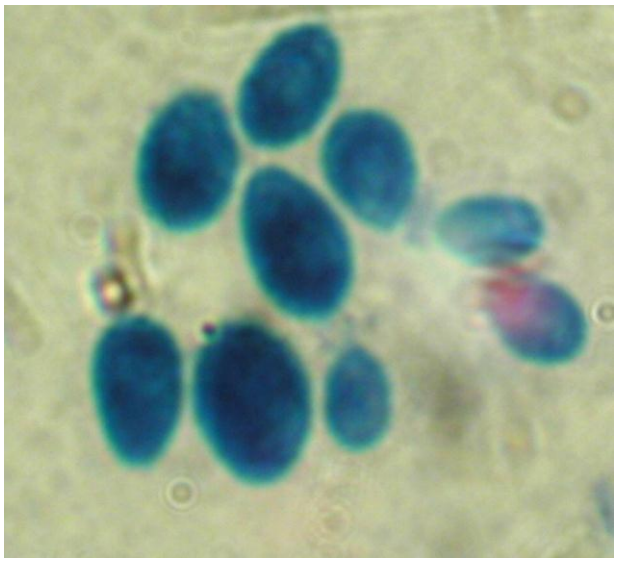
Biosorbent material	**ΔS°, (kJ mol^{-1} K^{-1})**	**ΔH°, (kJ mol^{-1})**	**ΔG°, (kJ mol^{-1})**		
			295 K	**308 K**	**323 K**
IFY	0.1047	35.9747	5.0715	3.7097	2.1384
IBW	0.0697	21.8200	1.2547	0.3484	-0.6972
SFY	0.0853	33.4307	8.2422	7.1322	5.8514
SBW	0.3514	102.9863	-0.6785	-5.2468	-10.5179

Table 2. Thermodynamic parameters for Cd^{2+} biosorption onto SBW, IBW, SFY, and IFY biosorbents (0.978 g yeast, 100 ml cadmium solution, 40 minutes)

3.6. Biosorption of Cd^{2+} Ions By Immobilized Cells of *Saccharomyces cerevisiae*. Adsorption Equilibrium and Kinetic Studies

Cadmium biosorption

The biosorption of cadmium ions on the pure *Saccharomyces cerevisiae* strain (DSM 1333) in immobilized form was investigated in biosorption equilibrium experiments. The effects of initial cadmium ion concentration on the biosorption capacity of cadmium onto immobilized cells were studied.



Saccharomyces cerevisiae (DSM 1333) (own photo)

The dynamics of cadmium uptake until equilibrium by biomass for various initial cadmium concentrations is represented in Figure 1.

Maximum adsorption capacities increase from 0.2197 mg Cd^{2+}/g for the initial 4.82 mg Cd^{2+}/L to 3.7825 mg Cd^{2+}/g for the initial 99.75 mg Cd^{2+}/L.

Equilibrium isotherm models

Langmuir and Freundlich models were used to determine the sorption equilibrium between the biosorbent and metal ions.

The Langmuir model assumes that a monomolecular layer is formed when biosorption takes place without any interaction between the adsorbed molecules [12]. Freundlich isotherm is an empirical equation based on a heterogeneous adsorption due to the diversity of adsorption sites or diverse nature of the adsorbed metal ions, free or hydrolyzed species [13].

The Langmuir isotherm equation has a hyperbolic form:

$$q_e = \frac{q_{max} \cdot b \cdot C_e}{1 + b \cdot C_e} \qquad (2)$$

where q_e is the solid-phase adsorbate concentration at equilibrium (mg/g),

q_{max} is the maximum adsorption capacity corresponding to the monolayer adsorption capacity (mg/g),

C_e is the concentration of Cd^{2+} solution at equilibrium (mg/L), and

b is related to the strenght of adsorbent-adsorbate affinity.

A linear expression of the Langmuir isotherm, eq. (2), is expressed as:

$$\frac{1}{q_e} = \frac{1}{q_{max} \cdot b} \cdot \frac{1}{C_e} + \frac{1}{q_{max}} \tag{3}$$

We calculated q_{max}=2.7129 mg Cd^{2+}/g and b=1.9359 (L/mg) (see Figure 2). Experimental values (q_e and C_e), represented in Figure 3, fitted well on a Langmuir isotherm type.

The empirical Freundlich isotherm equation is:

$$q_e = k \cdot C_e^{(1/n)} \tag{4}$$

in logarithmic form (linear)

$$\log q_e = \log k + \frac{1}{n} \cdot \log C_e \tag{5}$$

where k is related to adsorption capacity, and

n is related to intensity of adsorption.

From the $\log q_e$ to $\log C_e$ linear plot – Figure 4 –, we determined a correlation coefficient of 0.96, which is smaller then that obtained for the Langmuir model, 0.9804. Therefore we concluded that cadmium biosorption on immobilized biomass takes place on a Langmuir isotherm.

Kinetic models

The kinetic of cadmium adsorption on biosorbent was determined with two different kinetic models, i.e. the first- and second order.

The first-order rate equation may be represented as follows:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \tag{6}$$

Integrating eq. (6) from the boundary conditions t = 0 to t = t and q_t = 0 to $q_t = q_t$, gives:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{7}$$

where q_e and q_t are the amounts of cadmium adsorbed (mg/g) at equilibrium and

time t, respectively, and

k_1 is the rate constant of first order adsorption (1/min).

In order to determine the rate constant and equilibrium cadmium uptake, the straight line plots of $\ln(q_e-q_t)$ against t, eq. (7), were made at five different initial cadmium concentrations.

The pseudo second order kinetic model is derived on the basis of the adsorption capacity of the solid phase, which assumes that measured heavy

metal ion concentrations are equal to cell surface concentration, expresses as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (8)$$

Integrating eq. (8) from the boundary conditions t = 0 to t = t and $q_t = 0$ to $q_t = q_t$, gives:

$$\frac{1}{(q_e - q_t)} = \frac{1}{q_e} + k_2 t \quad (9)$$

where q_e and q_t are the amounts of cadmium adsorbed (mg/g) at equilibrium and

time t, respectively, and

k_2 is the rate constant of first order adsorption (g/mg·min).

Equation (9) can be rearranged in linear form, as follows:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (10)$$

In order to determine the rate constant and equilibrium cadmium uptake, the straight line plots of t/q_t against t, eq. (10), were made at five different initial cadmium concentrations. Correlation coefficients between 0.9941 and 0.9998 were obtained (Figure 5 and Table 1).

If we compare correlation coefficient for the first and pseudo second order models, we can conclude that cadmium biosorption on immobilized biomass can be classified as pseudo second order, fact confirmed by the literature scientific results [15]. The mathematical method for determination of kinetic parameters was the same utilized by Tonk and al. [16].

Figures and Tables

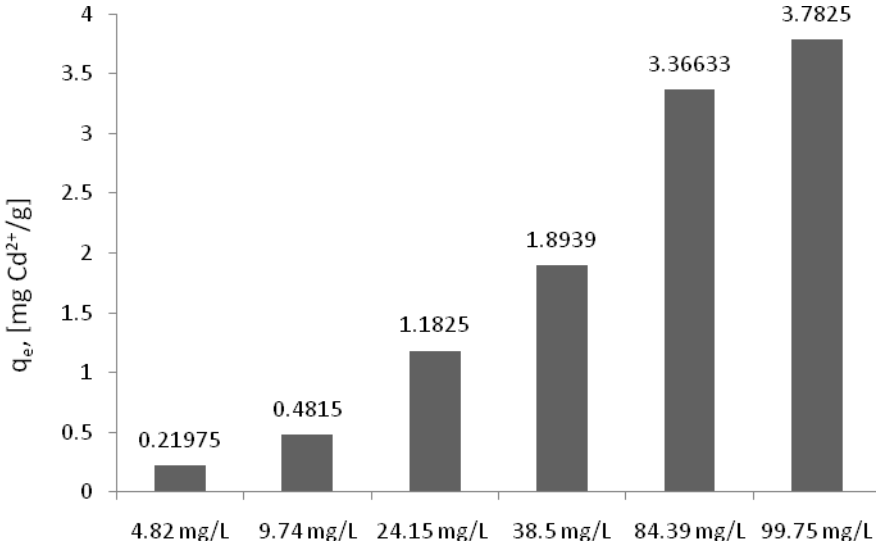


Figure 1. Maximum adsorption capacities obtained during cadmium adsorption experiments – initial concentration influence.

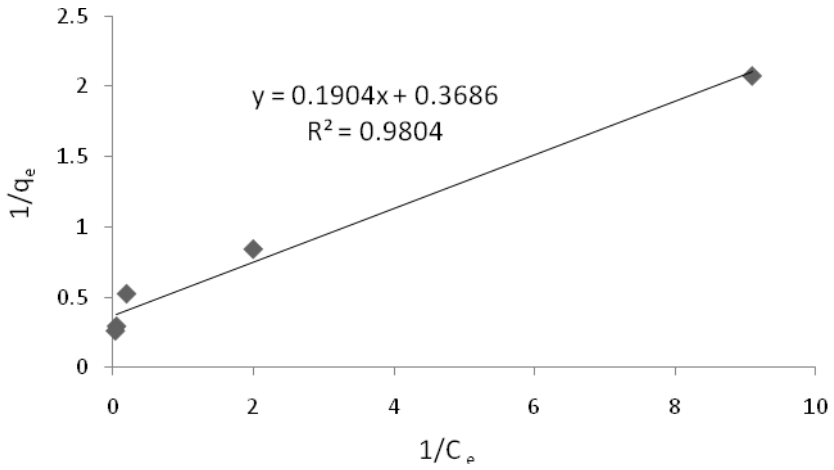


Figure 2. Langmuir adsorption model of cadmium biosorption on immobilized biomass.

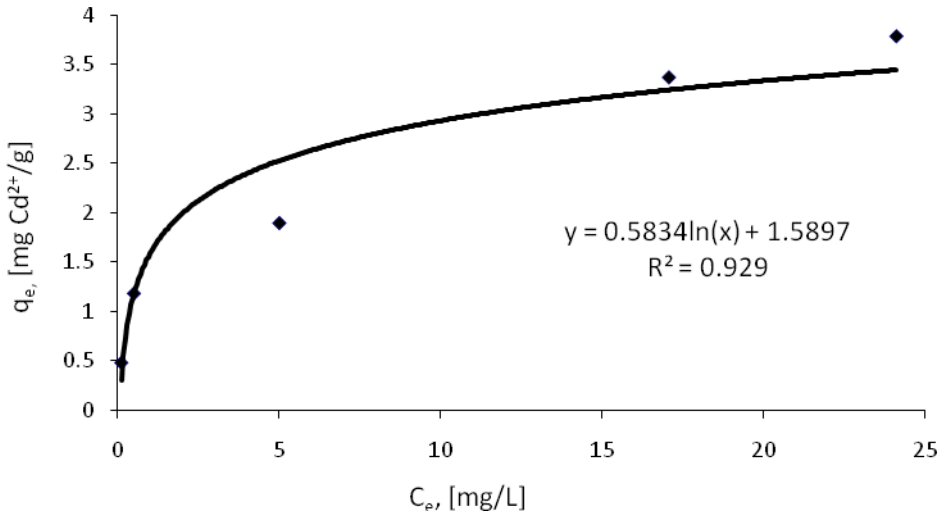


Figure 3. Adsorption isotherm of cadmium biosorption on immobilized biomass.

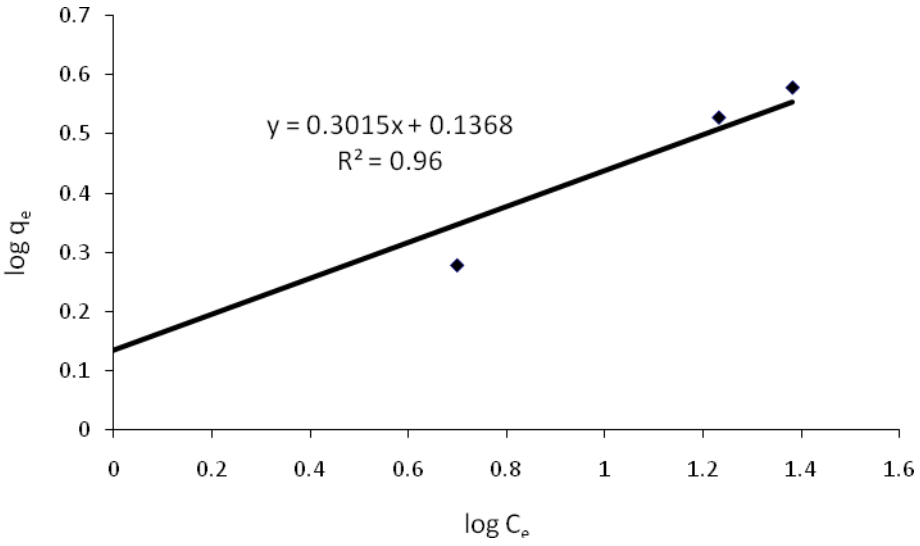


Figure 4. Freundlich adsorption model of cadmium biosorption on immobilized biomass.

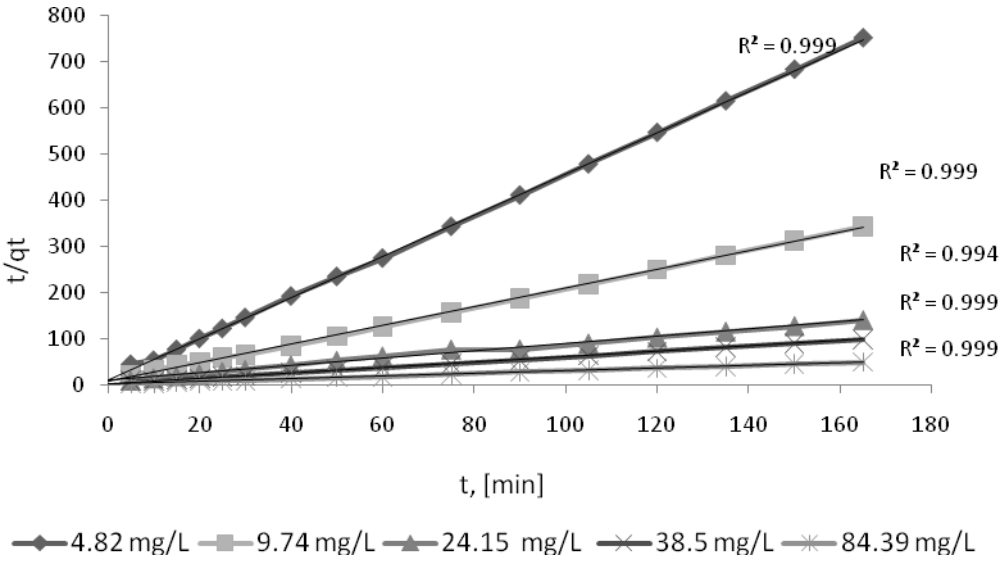


Figure 5. Correlation of the experimental data using the pseudo second order model for cadmium biosorption on immobilized biomass

Table 1. Second order adsorption kinetic parameters.

C, (mg Cd^{2+}/L)	q_e (exp), (mg Cd^{2+}/g)	q_e (calc), (mg Cd^{2+}/g)	k_2, (g/mg·min)	R^2
4.82	0.2197	0.2240	1.7967	0.9998
9.74	0.4815	0.4960	0.5860	0.9992
24.15	1.1825	1.2631	0.0706	0.9941
38.5	1.6750	1.7199	0.1719	0.9991
84.39	3.3663	3.4328	0.1129	0.9998

3.7. Effect of surface modification of waste yeast from brewery onto Cd^{2+}, Zn^{2+}, and Cu^{2+} adsorption

1. NaOH

Removal of surface impurities, rupture of cell membrane and exposure of available binding sites for metal bioadsorption after pretreatment may be the reason for the increase in metal bioadsorption.

Some researchers showed that alkali treatment of biomass may destroy autolytic enzymes that cause putrefication of biomass and remove

lipids and proteins that mask reactive sites. The researchers found that cell walls could be ruptured using NaOH treatment. Besides, the pretreatment could release polymers such as polysaccharides that have a high affinity towards certain metal ions [1, 15].

The explanation they offered is that the increase in the metal uptake after the protein removal steps is brought about by the unmasking of some of the cellular groups, which cannot participate in the sorption process without treatment with alkali [16].

In the case of pretreatment with NaOH, hydrolysis reactions can occur, causing high dissolution of organic substances from the biomass. The hydrolysis reactions can lead to the formation of more carboxylic (-COOH), carboxylate (-COO), and alcohol (-OH), groups in the pretreated biomass, which enhances the cationic biosorption [17].

2. Detergent

Most of the commercial detergents also contain a main alkalis as one of their ingredients, so that the treatment with detergents the biosorption effectiveness of metal ions would result in the removal process [16].

On the other species the detergent treatment reduces the biosorption capacity.

3. Metilation of amine – formaldehide and acid formic treatment

The treatment of biosorbent with HCHO-HCOOH causes methylation of amines present on the cell wall of biosorbent. The reaction occurs as follows:

$$RCH_2NH_2 + HCHO + HCOOH \rightarrow RCH_2N(CH_3)_2 + CO_2 + H_2O$$

Methylation of amine groups prevents their participation in metal biosorption and hence reduces the biosorption capacity [13, 5].

4. Esterification of carboxylic groups – ethanol treatment

According to literature Drake (1996), treatment of biosorbent with methanol results in esterification of carboxylic acids present on the cell wall of biosorbent and the reaction occurs as follows:

$$RCOOH + CH_3OH \rightarrow RCOOCH_3 + H_2O$$

In our case was used ethanol (which is chemically similar) in the presence of concentrated HCl, the carboxylic acids present on the cell wall may be esterified as follows:

$$RCOOH + CH_3CH_2OH \rightarrow RCOOCH_2CH_3 + H_2O$$

This treatment gives information about the significant role of carboxyl groups in the cell wall, which contributes to heavy metals biosorption [5].

5. Benzene

The treatment of biosorbent with benzene extracts the lipid fraction of biosorbent. [5].

6. Gluteraldehyde

Gluteraldehyde is a cross-linking reagent with multifunctional groups. According to Jialong, gluteraldehyde pretreated *Saccharomyces cerevisiae* biomass retains almost all its original biosorption capacity.

Some studies reported an increase in the adsorption capacity, which is a different cell wall structure due.

While the vast majority of fungi have a chitin-glucan cell wall, *S. cerevisiae* possesses a mannan-glucan cell wall, which contains only 1 % chitin [16].

In yeast surface in gluteraldehyde reaction with amino groups, resulting in a Schiff base, which according to the following reaction take place [18]:

$$CHO(CH_2)_3CHO + H_2N{-}\sim\sim \rightarrow CHO(CH_2)_3\text{-}CH{=}N{-}\sim.$$

7. $CaCl_2$

That pretreatment were performed for the conversation of the active binding sites from the H^+ to the Ca^{2+} form. This substitution may favor the biosorption of metals, because, due to the size of the ions, it should be easier to exchange metal for calcium than for H^+ [17].

8. H_3PO_4

The acid treatment results in a reduction in the adsorption capacity, which is also supported by several studyes the researchers [1, 17].

The H^+ ions binding to the biomass after acid treatment may be responsible for the reduction in adsorption of heavy metals. Tha polymer structure of biomass surface axhibits a negative charge due to the ionisation of organic groups and inorganic groups suggested that the higher the biomass electronegativity the greater the attraction and adsorption of heavy metal cations. Thus, the remaining H^+ ions on the acidic pretreated that biomass may change the biomass electronegativity, resulting in a reduction in bioadsorption capacity 16, 1].

When acids are implicated on treatment of biomass, the H^+ ions can change the cross-linking binds, which lead to the dissolution of organic matter [17].

In addition, phosphorylation reaction can take place [19]:

$$\sim\sim\sim CH = O + H_3PO_4 \rightarrow \sim\sim\sim CH = O - PO_3H_2$$

9. H_2O_2

The H_2O_2 treatment causes modification of amino groups on the cell wall structure [19].

$$R{-}NH_2 + H_2O_2 \rightarrow R{-}H_2N{\rightarrow}O$$

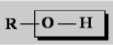
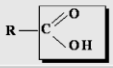
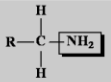
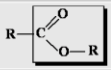
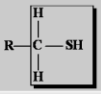
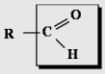
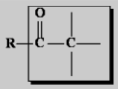
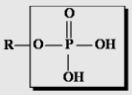
Formula of functional group	Name	Class of Compounds
	Hydroxyl	Alcohols, carbohydrates
	Carboxyl	Fatty acids, proteins, organic acids
	Amino	Proteins, nucleic acids
	Ester	Lipids
	Sulfohydryl	Cystein (amino-acid), proteins
	Carbonil, terminal end	Aldehydes, polisaccharydes
	Carbonil, internal	Ketones, polisaccharides
	Phosphate	DNA, RNA, ATP

Figure 1. Functional groups involved in the biosorption process [20].

The infrared spectroscopy (FTIR) analysis was performed with a Jasco 615-type spectrophotometer (wavelenght range of 400-4000 cm^{-1}, resolution 2 cm^{-1}).

3.7.1. The graphical interpretation

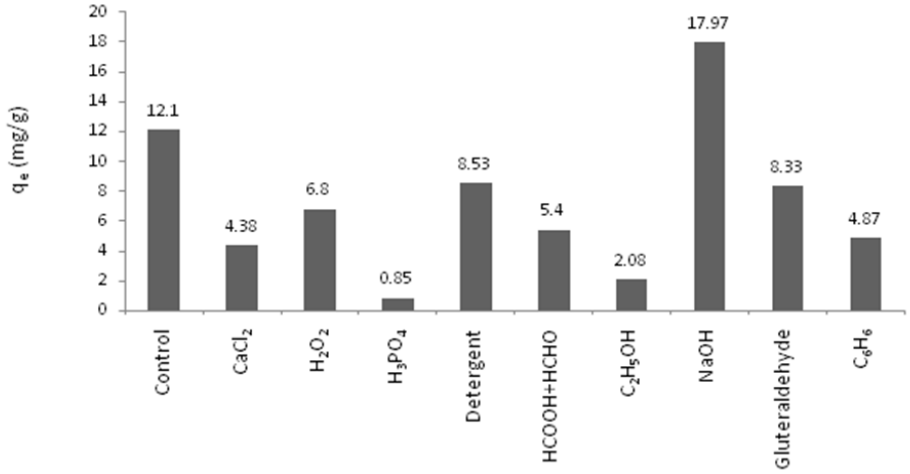


Figure 1. Maximum adsoption capacity (q_e) of cadmium for waste brewery yeast treated in different ways in comparison with the blank test (control) – untreated yeast.

Figure 1 shows the effect of chemical treatments on brewery yeast involved in cadmium adsorption. It can be seen that the treatment with NaOH is the only one, which increases metal adsorption in comparison with untreated biomass, the maximum value of adsorption increasing from 12.1 mg/g to 17.97 mg/g.

In case of cadmium biosorption on yeast biomass it can be noticed that the treatment with H_3PO_4 is the one that dimishes adsorption capacity the most, as the q_e value decreases from 12.1 mg/g to 0.85 mg/g, a fact that leads us to the conclusion that the polymeric structure of biomass surface is deeply affected by the decrease of its electronegativity.

For the rest of the treatments the decreasing of q_e is varies between 2.08 mg/g (esterification) and 8.53 mg/g (detergent).

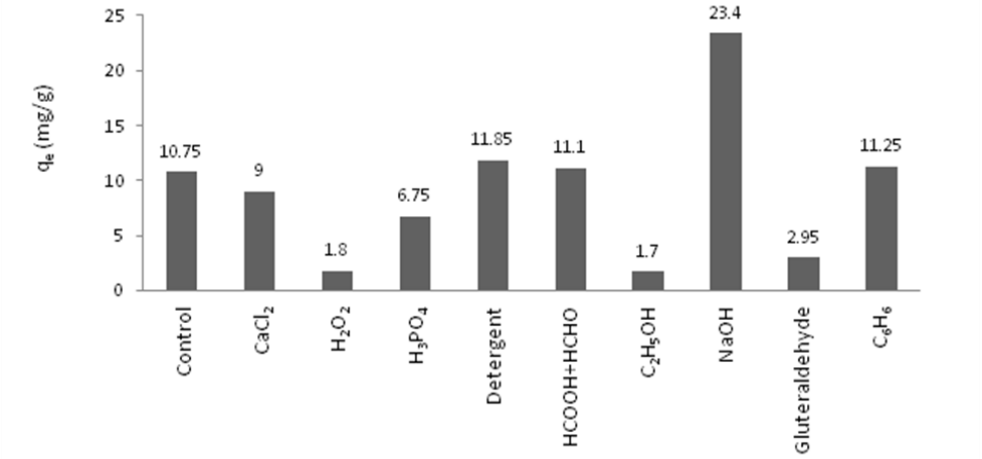


Figure 2. Maximum adsorption capacity (q_e) of zinc for waste brewery yeast treated in different ways in comparison with blank test (control) – untreated yeast

On figure 2 the influence of chemically modified yeast on Zn^{2+} adsorption can be observed.

The treatment that affects biosorption process most is the one with NaOH, when biosorption capacity goes up until 23.4 mg/g from 10.75 mg/g in the case of untreated yeast.

The largest diminishing of adsorption capacity can be observed in the case of treatment with formaldehydes and formic acid, when q_e decreases from 10.75 mg/g obtained for untreated yeast to 1.7 mg/g. This aspect is the result of the modification of carboxyl groups, which have a significant role in biosorption mechanism.

In addition to the growth mentioned for the treatment with NaOH there are three other treatments, which potentially increase adsorption capacity from 10.75 mg/g to 11.85 mg/g – detergent, to 11.25 mg/g – benzene, respectively 11.1 – mg/g formic acid and formaldehyde. In case of zinc other positive effects appear, probably as a result of the fact that zinc is a metabolic element.

The rest of the treatments decrease adsorption capacity, which can vary between 1.8 mg/g for H_2O_2 and 9 mg/g $CaCl_2$.

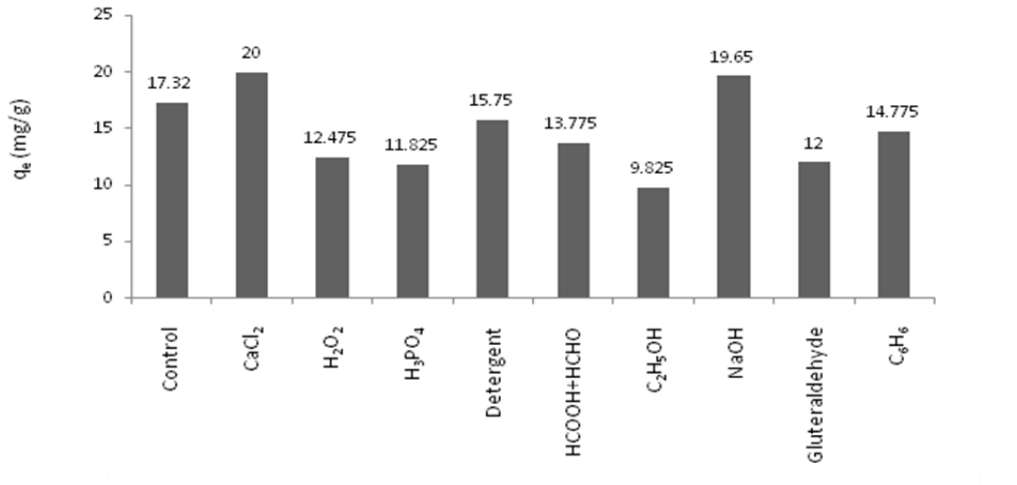


Figure 3. Maximum adsorption capacity (q_e) of copper for waste brewery yeast treated in different ways in comparison with blank test (control) – untreated yeast.

In figure 3 the influence of chemical treatments on Cu^{2+} adsorption capacity are shown.

In this case there are two treatments that improve adsorption capacity, namely with NaOH and $CaCl_2$, resulting an increase from 17.32 mg/g up to 19.65 mg/g, respectively 20 mg/g. As it can also be learnt from specialist literature, in the case of treatment with $CaCl_2$, copper adsorption capacity is improved as a result of the conversion of active binding sites from H^+ to Ca^{2+} (page 73.)

The lowest adsorption capacity can be observed in the case of treatment with ethanol, when q_e is decreasing from 17.32 mg/g to 9.825 mg/g.

The rest of the treatments lead us also to diminishing adsorption, q_e reaching values between 11.825 mg/g (H_3PO_4) and 15.75 mg/g (detergent).

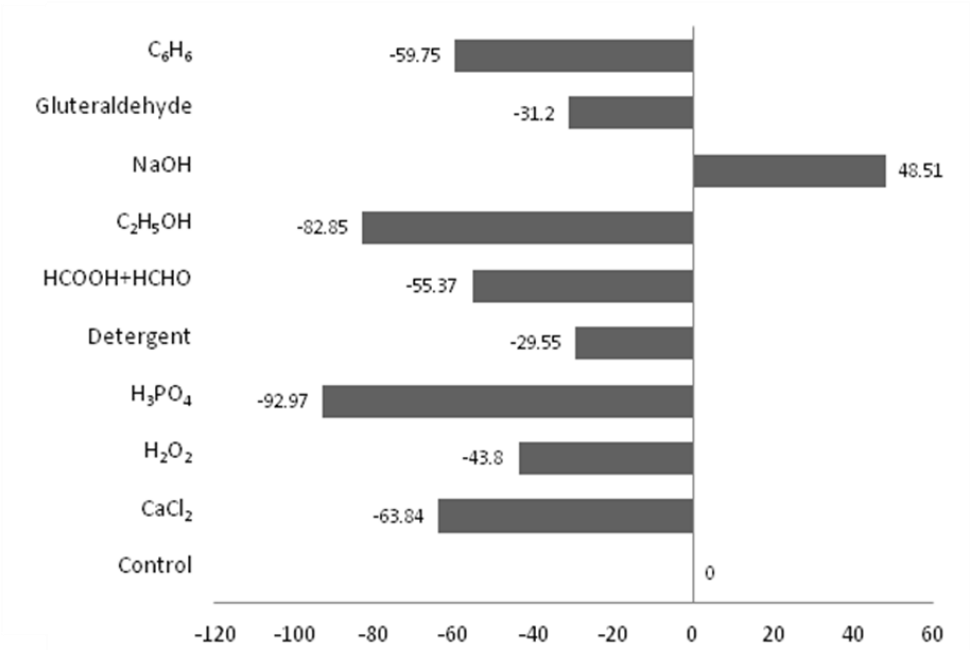


Figure 4. Increase/decrease of adsorption capacity (percentage difference, %) for different treatments related to blank test in case of cadmium adsorption.

Figure 4 shows the way how the adsorption capacity of waste brewery yeast is increased or decreased by the various treatments.

Thus, in comparison with chemically unmodified yeast, the treatment with NaOH is the only one, which increases the adsorption capacity by 48.51%, therefore we can affirm that by the elimination of uncleanliness from the cell walls, the biosorption process improves, active binding sites being more available for connecting metal ions.

The most drastic decrease of 92.97% in the case of cadmium biosorption appears when biomass treated with ethanol is used, which proves that the carboxyl groups from *S. cerevisiae* cells wall strongly contribute to metal adsorption. The rest of the treatments also lead to a decreasing adsorption capacity, with values between 82.85%, respectively 29.55%, which gives evidence that the rest of chemically modified groups have got a certain role in the adsorption process.

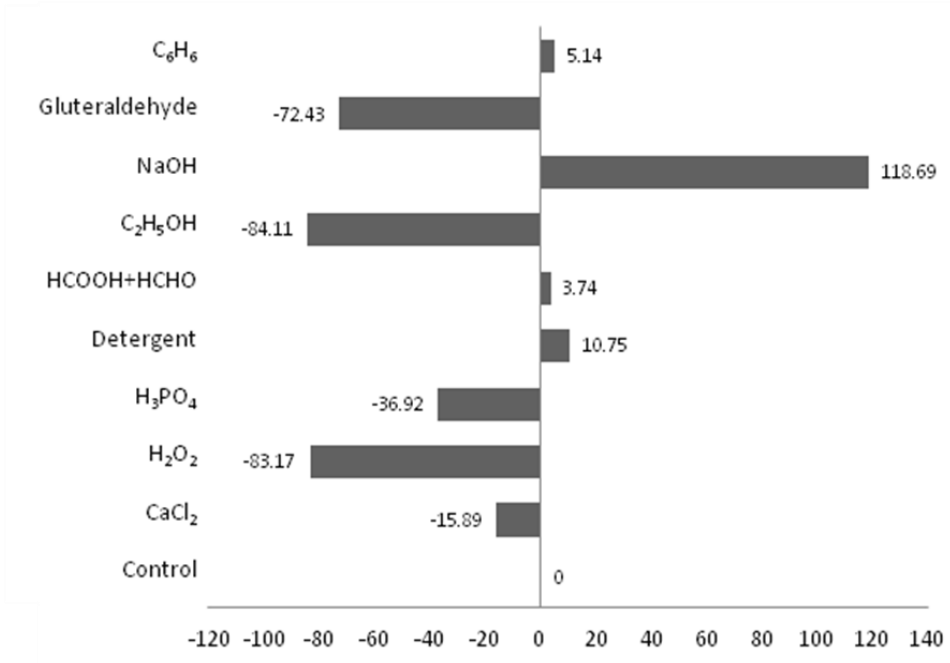


Figure 5. Increasing/decreasing of adsorption capacity (percentage difference, %) for different treatments related to blank test in case of zinc adsorption.

From figure 5 one can observe that in the case of zinc there are more chemical treatments with a positive influence on biosorption.

Just like in the case of Cd^{2+}, the most efficient treatment is the one with NaOH, which leads to increased adsorption capacity by 118.69%. Furthermore, q_e grows by values between 10.75% and 3.74%, also in the event of other treatments.

At the other hand, the highest decrease is 84.11%, at the event of using ethanol treated biomass, which underlines once again the significance of carboxyl groups regarding biosorption.

The rest of the treatments leading to decrease also produce these negative effects as a result of the modification of important sites in the process of biosorption; percentage regarding diminishing is varying between 83.17% and 15.89%.

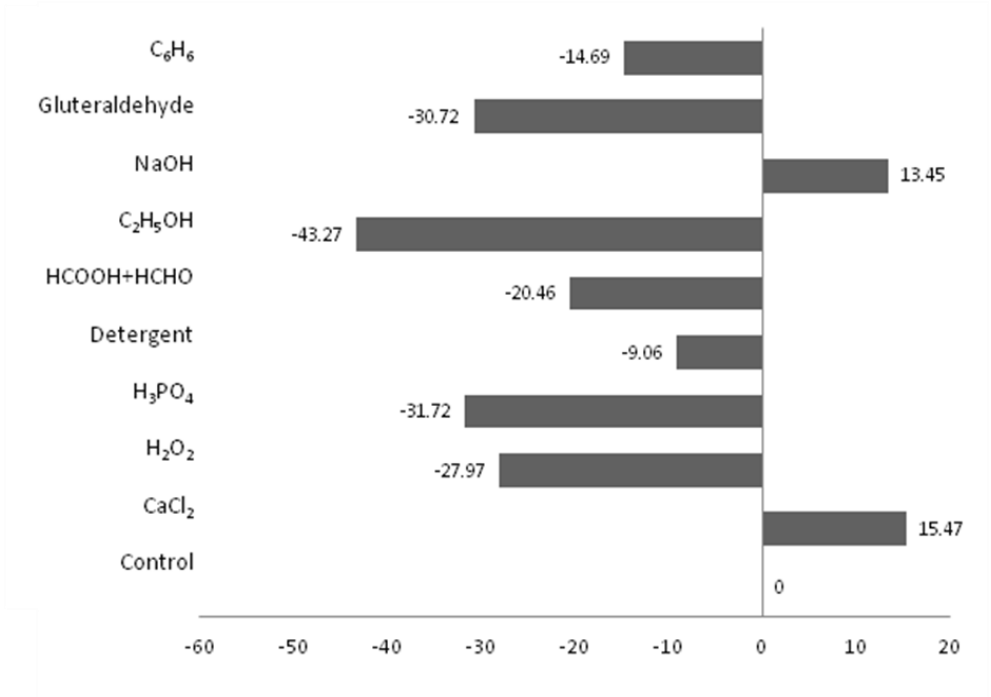


Figure 6. Increasing/decreasing of adsorption capacity (percentage difference, %) for different treatments related to blank test in case of copper adsorption.

In figure 6, differently from the two previous metals, the highest positive influence is shown in case of treatment with $CaCl_2$, treatment producing an increase of 15.47% in the case of Cu^{2+}. This aspect is confirming the surveys made in specialist literature on biomass treated with $CaCl_2$.

As in the previous cases, NaOH is produces an intensification of adsorption capacity with 13.45%.

Also here, the most obvious decrease, 43.27%, is caused by the treatment with ethanol, which again underlines the major role of carboxyl groups. The rest of the treatments produce a decreas of q_e, values situated between 31.75% and 9.06%.

On figure 7 one can observe the way how chemically unmodified yeast displays its adsorption capacity for cadmium, zinc and copper.

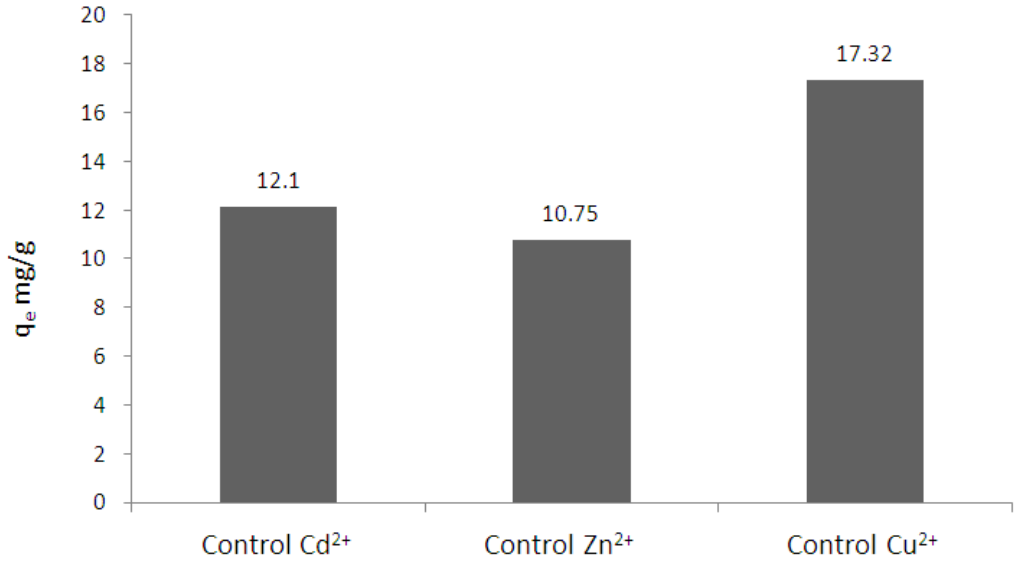


Figure 7. Maximum adsorption capacity of waste brewery yeast for three metals (control).

Analysing figure 7 one can reach the conclusion that the most efficiently adsorbed metals are copper ions, q_e value is 17.32 mg/g. One reduced adsorption capacity can be noticed in the case of zinc, with a q_e at 10.75 mg/g, a drop by 37.93% from the maximum remarked in the case of copper. In the case of cadmium, the maximum of adsorption is at 12.1 mg/g, 30.14% less than in the case of copper.

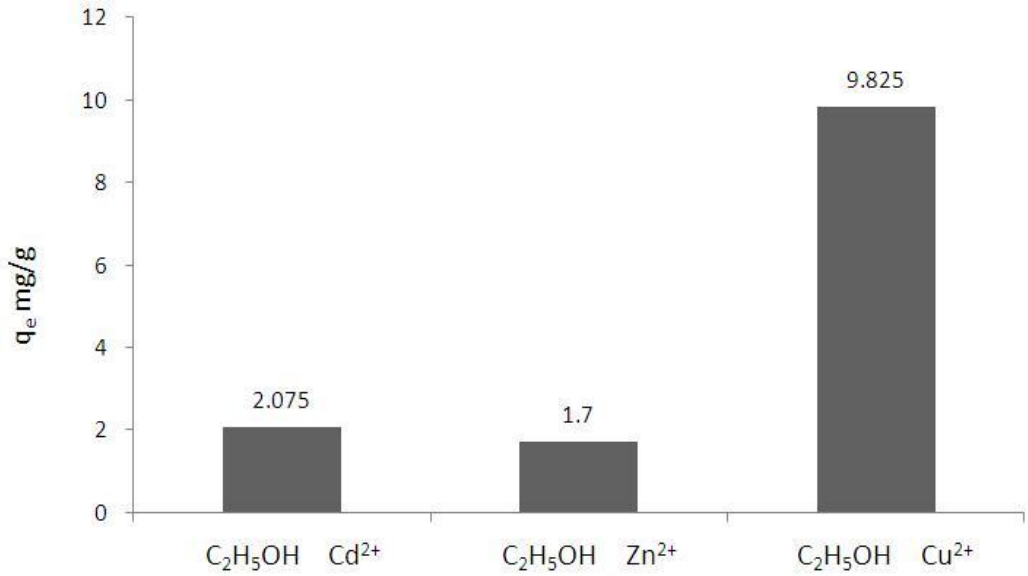


Figure 8. Maximum adsorption capacity of ethanol treated brewery yeast for three metals.

Through treatment with ethanol, namely through the esterification of carboxyl group, as presented in figure 8, the most affected adsorption capacity comes out for zinc, for which q_e reaches the value of 1.7 mg/g. Also for cadmium a pretty low maximum value of adsorption can be observed, effectively 2.075 mg/g, which is only 22.05% less than for zinc.

Differently than in the case of the two other metals, copper represents a much higher maximum, namely 9.825 mg/g, which can be the result of the fact that in the case of this metal, the alteration of carboxyl groups does not have such a negative effect on biosorption.

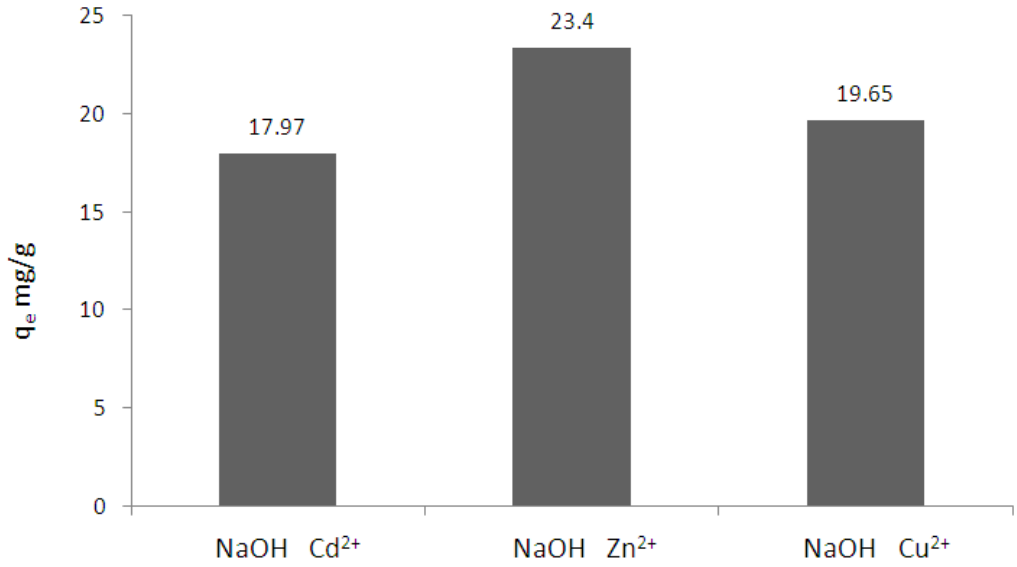


Figure 9. Maximum adsorption capacity of NaOH treated brewery yeast for three metals.

In figure 9 the adsorption maximums in the case of treatment with NaOH are presented.

Following the treatment, the strongest influence appears for zinc, which presents a maximum of 23.4 mg/g. In the case of cadmium and copper, the results are lower capacities, respectively 17.97 mg/g and 19.65, these falls to 23.21%, respectively 16.03% in comparison with adsorption capacity of zinc.

3.7.2. FTIR analyses

The functional groups on yeast surface can also be identified from an infrared spectra, which is a complex one exactly due to the several existing functional groups.

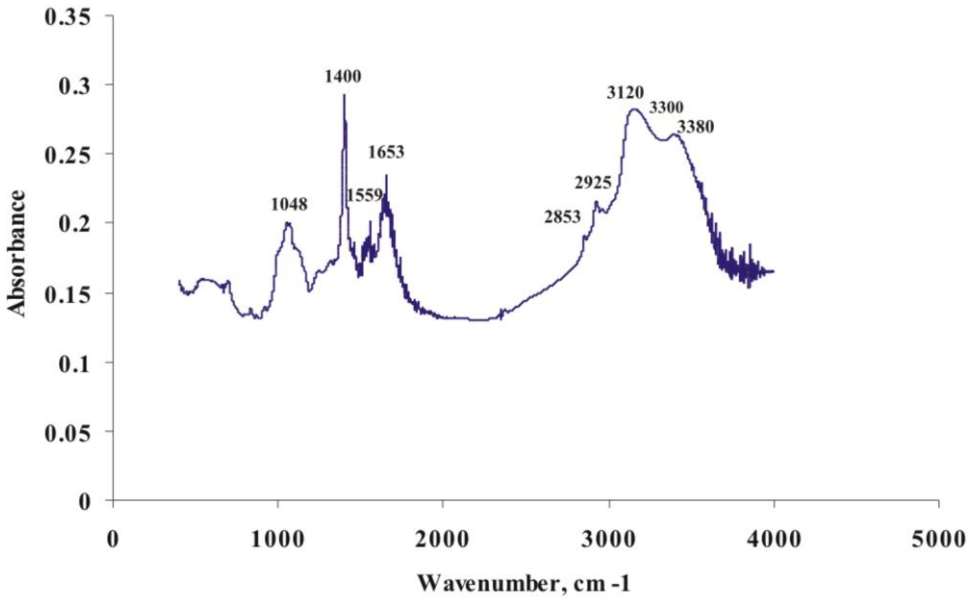


Figure 10. IR spectra for untreated waste brewery yeast.

On figure 10 the existence of peaks (maximum) can be observed at 1048, 1400, 1559, 1653, 2853, 2925 and in the domain between 3100-3400 cm^{-1}. The large and intense band appearing between 3400-3100 cm^{-1} does not permit a precise indication of functional group positions on the curve; although it can be admitted that this includes the contribution of alongation by vibration of -OH groups for carbohydrants around the value of 3380 cm^{-1}, the groups of NH proteines and peptides around the value of 3300 cm^{-1} and the band of amids II at approximately 3120 cm^{-1} [21]. At 2925 cm^{-1} and 2853 cm^{-1} two low intensity bands appear, characteristic for groups –CH, $–CH_2$, $-CH_3$ characteristic for lipids. Peaks at 1653 cm^{-1} şi 1559 cm^{-1} can be assigned to alongation vibrations of groups C=O from different proteins, respectively N-H and C-N specific peptidic connections from different

proteins. The band at 1400 cm^{-1} can be atribuated to the vibration of $–CH_2$ group from lipids and the band from 1070 cm^{-1} is specific for alcoholic group C-O. The region under 1000 cm^{-1} is the field of digital impress and the adsorption maximums can be clearly attributed to a specific vibration, as these meet the case of complex vibrational interplays.

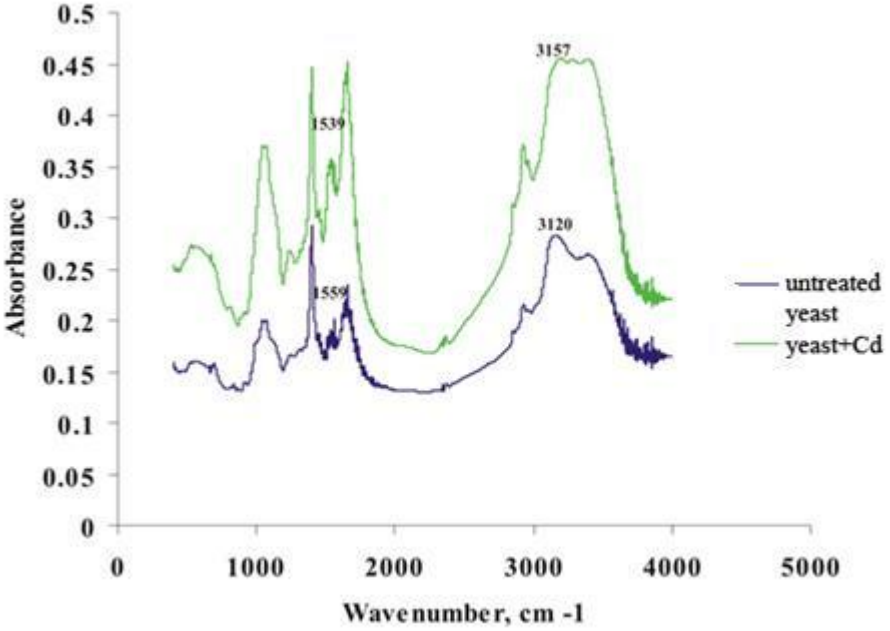


Figure 11. IR spectra for untreated yeast and yeast, on which cadmium has been adsorbed.

The peak shifts indicates the groups involved in the adsorption process. Thus, after Cd^{2+} adsorption (fig. 11) the valency vibration of N-H groups has been shifted from 1559 cm^{-1} to 1539 cm^{-1}. The shift to a lower wavelength after the adsorption of metallic ion suggests that chemical interplays have taken place between metals and amide groups.

Also, a modification of the large band can be observed, shifting to higher wavelengths from 3120 cm^{-1} to 3157 cm^{-1}, indicating the involvement of hydroxyl, carboxyl and amide groups in the adsorption process [18].

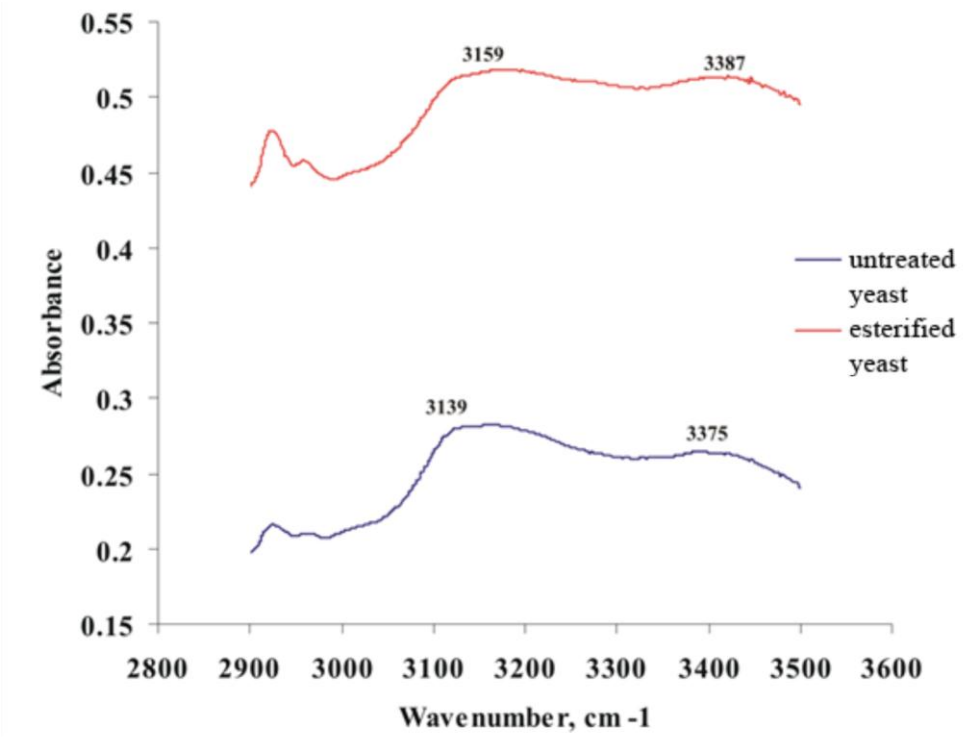


Figure 12. IR spectra for untreated yeast and esterified brewery yeast.

The existence of a chemical treatment effect is proven by the shift of peaks in the IR spectra.

Thus on figure 12, in the case of yeast treated with ethanol a shift from 3375 cm^{-1} to 3387 cm^{-1}, respectively from 3139 cm^{-1} to 3159 cm^{-1} can be observed, which suggests that interplay take part between the ethanol and the functional groups, namely the carboxyl and the amide.

On figure 13, in the case of yeast treated with NaOH, it can be remarked that the spectra obtained has the same shape as the one obtained for untreated yeast, the peaks are situated at the same values, fact marked out by the overlapping of the spectrums. This aspect thus sustains the survey hypothesis, namely the fact that NaOH treatment does not affect the group on the cells surface, on the contrary, this facilitates the adsorption of metallic ions by the elimination of impurities from cell wall surface and the exposure of active binding sites.

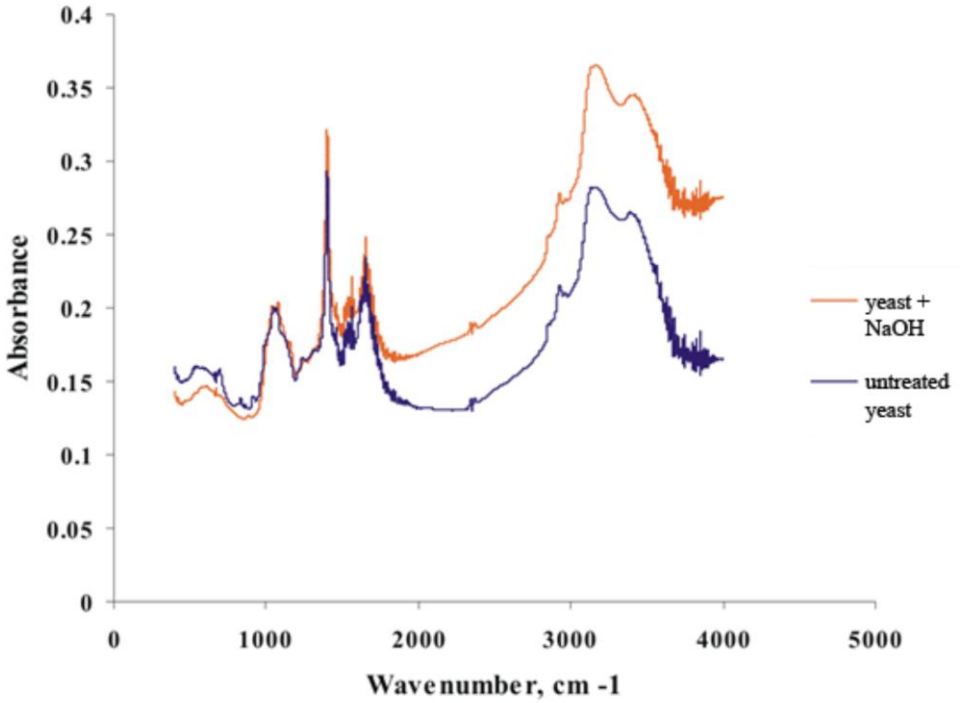


Figure 13. IR spectra for untreated yeast and yeast treated with NaOH.

Through FTIR analyses the existent functional groups on the biomass surface were primarly marked out, as well as the way how these are implicated in the biosorption mechanism. The way how cations attack functional groups, tighting them on the cells surface has been observed, a fact provent by the shift of important peaks on the IR spectra.

Similarly, yeast treated with NaOH (which lead to the biggest maximums of adsorption) was surveyed by IR, observing that this treatment does not destroy important groups in the biosorption process and esterified yeast, (lead to the lowest maximums of adsorption) was analized, pointing out the modifications in case of groups responsible for adsorption.

4. CONCLUSIONS

4.1. Cd^{2+} removal from synthetic wastewaters using *Scenedesmus opoliensis* green algae

The present study proved that *Scenedesmus opoliensis* is an effective biosorbent for the removal Cd^{2+} from wastewaters. In this respect, three quality parameters were investigated: (1) heavy metal ions concentration in solution after contact with algae, (2) the yield calculated for the biosorption process, and (3) heavy metal ions retention capacity of algae. Residual cadmium in the aqueous solutions, measured after 2 hours exposure periods, showed that the concentrations of Cd^{2+} significantly decrease, faster in initial 10 minutes, achieving the equilibrium after 60 minutes for solution with initial concentration C_1=4.36 mg Cd^{2+}/L, 70-80 minutes for C_2=12.7 mg Cd^{2+}/L and approximately 120 minutes for the most concentrated initial solution, C_3=20.48 mg Cd^{2+}/L.
In conclusion, for the three initial concentrations used in our experiment, cadmium was adsorbed up to yields of 50-52%, with retention capacity values comprised between 0.67 and 3.28 mg Cd^{2+}/g biosorbent. Hence, it is possible to remove cadmium in a simple treatment using *Scenedesmus opoliensis* green algae.

4.2. Removal of Zn^{2+} from some synthetic wastewaters by immobilized *Saccharomyces cerevisiae* cells

In this study, immobilized *S. cerevisiae* cells have been successfully used as a biosorbent for the removal of Zn^{2+} ions from synthetic wastewaters. For the successful application of biosorption, biomass needs to be immobilized to increase its mechanical strength, density, reusability and resistance to mechanical environments. In this study, Calcium alginate gel was chosen for the immobilization experiments as it is cheaply and abundantly available, nontoxic and highly selective for certain ion species. Calcium alginate proved to be a suitable material for immobilization of *Saccharomyces cerevisiae* cells. The initial Zn^{2+} concentration decreases in every biosorption experiment. For the initial concentration C_1 = 129.60 mg Zn^{2+}/L, after approximately 55–60 minutes the heavy metal ion content from analyzed samples goes to zero, which means that Zn^{2+} ions are totally removed from the water sample. The maximum retention capacity, q_e, was

calculated to be 7.1 mg Zn^{2+}/ g adsorbent for the biggest initial concentration (C_3=304.88 mg Zn^{2+}/L).

As a general conclusion we can say that using yeast as a biological filter it's a good method in reducing the concentration of heavy metals ions from wastewaters.

4.3. Fixed Bed Studies for Cd^{2+} Removal from Model Solutions Using Immobilized Bentonite/Yeast Mixtures

The bentonite sample, yeast biomass and their mixtures, proved to be efficient for the removal of cadmium from model solutions.

The highest adsorption capacity was obtained when only bentonite was included in the Ca-alginate beads in batch conditions. As the bentonite concentration decreases, the adsorption capacity decreases as well, even if the amount of yeast increases in batch conditions, excepting the case of the pure yeast sample, which has an intermediate value.

When the removal process was realized in fixed bed conditions, also the most efficient sample was the immobilized bentonite. The mixture containing the same quantity of bentonite and yeast (4 g) has intermediate values for both cadmium initial concentrations. The breakthrough point for fixed bed experiments was observed at 75 minutes in all cases.

4.4. Application of immobilized waste brewery yeast cells for Cd^{2+} removal. Equilibrium and kinetics

In this study, immobilized (Ca-alginate beads) a waste brewery biomass (yeast cells) from Miercurea-Ciuc, Romania, was successfully used as biosorbent for removal of Cd^{2+} ions from aqueous solutions. Calcium alginate proved to be a suitable matrix for immobilization of bakers' yeast cells.

The maximum biosorption capacity was calculated to be 5.9600 mg Cd^{2+} g^{-1} yeast for 169 mg Cd^{2+} L^{-1} initial concentration.

Langmuir and Freundlich adsorption isotherms were used to correlate the equilibrium adsorption data. Based on correlation coefficients, we concluded that Langmuir isotherm is more suitable to describe the cadmium biosorption equilibrium data.

Also first and pseudo second order kinetic models were applied to describe cadmium biosorption process. Based on mathematical calculations

carried out we concluded that this process has a kinetic of pseudo second order and the parameters for this kinetic were determined.

The results presented in this paper proved that a biosorbent from fermentation industry, brewery waste biomass, which is as a by-product (inexpensive and available in high quantities) of an industrial process can be successfully used to remove cadmium ions from aqueous solutions. Further investigations will be conducted in order to explain the adsorption mechanism and to establish the optimum parameters for the biosorption process.

4.5. Suspended and immobilized brewery waste biomass and commercial yeast as biosorbents for Cd^{2+} removal. A themodynamic study

Bioremediation of heavy metals pollution remains a major challenge in environmental biotechnology. Biosorption of heavy metals is one of the most promising technologies involved in the removal of heavy metals from wastewaters. *Saccharomyces cerevisiae* was selected for studying biosorption in order to assess the possibility of utilizing a brewery yeast waste biomass (CIUC brewery) for Cd^{2+} removal from monocomponent synthetic solution.

Yeast collected from two sources, used in two forms (commercial yeast and brewery waste biomass, in suspended and immobilized forms) was investigated in cadmium ions biosorption process. Thermodynamic parameters, including Gibbs free energy ($\Delta G°$), enthalpy ($\Delta H°$) and entropy ($\Delta S°$) of adsorption were calculated. The obtained results showed that the biosorption of Cd^{2+} onto *Saccharomyces cerevisiae* strain was a feasible, spontaneous and endothermic process in nature. Between the four tested biosorbents, the best efficiency and adsorption capacity was determined for the brewery waste biomass, which could be successfully used as an alternative low-cost biosorbent.

4.6. Biosorption of Cd^{2+} Ions By Immobilized Cells of *Saccharomyces cerevisiae*. Adsorption Equilibrium and Kinetic Studies

The ability of immobilized cells (DSM 1333) to adsorb cadmium ions from aqueous solution was investigated. Results showed that the initial cadmium concentration highly affected the cadmium biosorption. The

biosorption capacity increased with the initial cadmium ion concentration. The biosorption of metal ions studied is a rapid process and often reaches equilibrium within three hours; the maximum biosorption capacity was 3.7825 mg Cd^{2+}/g yeast.

Langmuir and Freudlich adsorption isotherms were used to correlate the equilibrium adsorption data.

The biosorption of cadmium on the sorbent was found to be mainly based on physical and ion-exchange interactions, and these were confirmed by the results of adsorption isotherms.

The heavy metal ions biosorption by immobilized *Saccharomyces cerevisiae* cells takes place after Langmuir isotherm model.

Based on mathematical calculations carried out, it was found that the kinetics data fitted well the pseudo-second order model to describe the adsorption process. The parameters for this kinetic were determined.

4.7. Effect of surface modification of waste yeast from brewery onto Cd^{2+}, Zn^{2+}, and Cu^{2+} adsorption

The experimental study demonstrated that yeast resulting from beer production process waste had the capacity of eliminating metallic ions from wastewaters.

At the same time, the present research points out the importance of different functional groups on the biomass cell walls and the way how these are affected by the application of several treatments.

As a cost approach, it is important to notice, that for the potential application on an industrial scale, it is extremely important to remark that yeast can be obtained free of charge or at least at a very low cost from the different industries where it is used.

Analyzing the results of experiments we can say that yeast biomass is effective to remove metal ions from aqueous solutions.

It would be convenient to use NaOH treated biomass, because in all three cases (Cd^{2+}, Zn^{2+}, Cu^{2+}) the adsorption capacities increase compared to the biomass untreated adsorption experiments. In this way it was demonstrated the efficiencies of NaOH treatment.

An other important aspect that can be sustained by the resulting data is that carboxyl groups play a fundamental role in biosorption process, and when these were modified, the adsorption capacity decrease dramatically.

From the FTIR analyses the functional groups were shown, and also the way how these are influenced as a result of metal adsorption, treatment with ethanol and treatment with NaOH, affirming once more the initial presumptions.

In conclusion, the results biosorption is a promising technology and the chemical nature of biomass is extremely important for the efficiency of the process.

5. SUMMARY OF SCIENTIFIC RESULTS

1. The research findings enabled us to develop new biosorption methods. Two types of microorganism were used, *Saccharomyces cerevisiae* and *Scenedesmus opoliensis* green algae for heavy metals (Cd^{2+}, Zn^{2+}, Cu^{2+}) removal from wastewater based on biosorption. The adsorption process was studied in two ways: (1) batch conditions (suspension and Na-alginate immobilized form of yeast) and green algae (suspension), (2) fixed bed column experiments.

2. We developed a method for the removal of heavy metals from synthetic wastewater using *Scenedesmus opoliensis* green algae in aqueous suspension. Three quality parameters were investigated: (1) heavy metal ions concentration in solution after contact with algae, (2) the yield calculated for the biosorption process, and (3) heavy metal ions biosorption capacity of algae and was studied the effect of different parameters of the biosorption.

3. We developed the method of immobilized yeast cells with Na-alginate, this matrix as a suitable material for immobilization of *Saccharomyces cerevisiae* cells. In the case of Zn^{2+} uptake with immobilized yeast cells with Na-alginate, the heavy metal (Zn^{2+}) was totally removed from the water samples. Our results are in agreement with literature data regarding biosorption mechanism that is considered to take place in two stages. In a first stage (dynamic regime) a pseudo-equilibrium is reached, while in a second stage (that takes place in some cases in static regime, on longer time intervals) a slow decrease of metal concentration takes place. This decrease may be explained by metal crossing through the cell wall, when intracellular accumulation takes place.

4. The bentonite sample, yeast biomass and their mixtures, proved to be efficient for the removal of cadmium from model solutions. The highest adsorption capacity was obtained when only bentonite was included in the

Ca-alginate beads in batch conditions. As the bentonite concentration decreases, the adsorption capacity decreases as well, even if the amount of yeast increases in batch conditions, excepting the case of the pure yeast sample, which has an intermediate value. When the removal process was realized in fixed bed conditions, the most efficient sample was also the immobilized bentonite.

5. The best results were obtained in case of waste brewery biomass from Miercurea-Ciuc, Romania for removal of Cd^{2+} from aqueous solutions. Calcium-alginate provided to be a suitable matrix for the immobilization of bakers' yeast cells.

6. Langmuir and Freundlich adsorption isotherms were used to correlate the equilibrium adsorption data. Based on the correlation coefficients, it was concluded that the Langmuir isotherm was more suitable for describing the equilibrium data of cadmium adsorption. In addition, first and pseudo-second order kinetic models were applied to describe the biosorption process. The kinetic parameters for the pseudo-second order kinetics were determined.

7. In the case of brewery yeast biomass the obtained results showed that the biosorption of Cd^{2+} onto *Saccharomyces cerevisiae* strain was a feasible, spontaneous and endothermic process in nature. Thermodynamic parameters, including Gibbs free energy (ΔG^o), enthalpy (ΔH^o) and entropy (ΔS^o) of adsorption were calculated.

8. In the case of waste yeast from brewery (Miercurea-Ciuc, Romania) a study was developed on the importance of different functional groups on the biomass cell walls and the way how these are affected by the application of several treatments. The role of functional groups in adsorption process was determined.

9. The adsorption capacity of waste yeast was affected by different chemical treatments studied, contributing thereby to the study of adsorption mechanism. Different chemical treatments were used such as: NaOH, detergent, metylation, esterification, benzene, gluteraldehyde, $CaCl_2$, phosphorylation, oxidation.

10. The treatment with ethanol (esterification of carboxylic groups) gives information about the significant role of carboxyl groups in the cell wall, which contributes to heavy metals biosorption. Another important aspect that can be sustained by the resulting data is that carboxyl groups play a fundamental role in biosorption process, and when these were modified, the adsorption capacity decreased dramatically.

11. It would be convenient to use NaOH treated biomass, because in all three cases (Cd^{2+}, Zn^{2+}, Cu^{2+}) the adsorption capacities increase compared to the biomass untreated adsorption experiments. In this way the efficiencies of NaOH treatment was demonstrated.

12. From the FTIR analyses the functional groups were shown, and also the way how these are influenced as a result of metal adsorption, treatment with ethanol and treatment with NaOH, affirming once more the initial presumptions.

13. In conclusion it can be stated that according to the results biosorption is a promising technology and the chemical nature of biomass is extremely important for the efficiency of the process.

14. Analyzing the results of experiments we can say that yeast biomass is effective to remove metal ions from aqueous solutions.

15. As a cost approach, it is important to note, that for the potential application on an industrial scale, it is extremely important to remark that yeast can be obtained free of charge or at least at a very low cost from the different industries where it is used.

16. The results proved that the biosorbent from fermentation industry, i.e., waste brewery biomass, which, as a by-product of an industrial process is inexpensive and available in large quantities, could be successfully used to remove cadmium ions from aqueous solutions.

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7. APPENDICES

Annexes for chapter 3.7.1.

Table 1. Experimental data obtained for cadmium:

Chemical treatment	Absorbance	Concentration Initial (mg/L)	Concentration Final (mg/L)	q_e (mg/g)	Efficiencies (%)
Control	0.421	45.25	21.05	12.1	0
$CaCl_2$	0.734	45.25	36.5	4.38	-63.84
H_2O_2	0.66	45.25	31.65	6.8	-43.8
H_3PO_4	0.633	45.25	43.55	0.85	-92.97
Detergent	0.871	45.25	28.2	8.53	-29.55
HCOOH+HCHO	0.564	45.25	34.45	5.4	-55.37
C_2H_5OH	0.698	45.25	41.1	2.08	-82.85
NaOH	0.822	45.25	3.05	17.97	48.51
Gluteraldehyde	0.572	45.25	28.6	8.33	-31.2
C_6H_6	0.71	45.25	35.51	4.87	-59.75

Table 2. Experimental data obtained for zinc:

Chemical treatment	Absorbance	Concentration Initial (mg/L)	Concentration Final (mg/L)	q_e (mg/g)	Efficiencies (%)
Control	0.284	49.9	28.4	10.75	0
$CaCl_2$	0.319	49.9	31.9	9	-15.89
H_2O_2	0.463	49.9	46.3	1.8	-83.17
H_3PO_4	0.365	49.9	36.5	6.75	-36.92
Detergent	0.266	49.9	26.6	11.85	10.75
HCOOH+HCHO	0.277	49.9	27.7	11.1	3.74
C_2H_5OH	0.465	49.9	46.5	1.7	-84.11
NaOH	0.031	49.9	3.1	23.4	118.69
Gluteraldehyde	0.44	49.9	44	2.95	-72.43
C_6H_6	0.274	49.9	27.4	11.25	5.14

Table 3. Experimental data obtained for copper:

Chemical treatment	Absorbance	Concentration Initial (mg/L)	Concentration Final (mg/L)	q_e (mg/g)	Efficiencies (%)
Control	0.107	40	5.35	17.32	0
$CaCl_2$	0	40	0	20	15.47
H_2O_2	0.31	40	15.05	12.475	-27.97
H_3PO_4	0.327	40	16.35	11.825	-31.72
Detergent	0.17	40	8.5	15.75	-9.06
HCOOH+HCHO	0.249	40	12.45	13.775	-20.46
C_2H_5OH	0.407	40	20.35	9.825	-43.27
NaOH	0.014	40	0.7	19.65	13.45
Gluteraldehyde	0.32	40	16	12	-30.72
C_6H_6	0.209	40	10.45	14.775	-14.69

Annex for chapter 3.7.2.

Tabel 4. The positions of the main bands of the FTIR adsorption spectrum of *Saccharomyces cerevisiae* [21].

Absorption band (cm^{-1})	Main assignment
~2960	ν_{asym} CH_3 lipids [27]
~2925	ν_{asym} CH_2 lipids [27]
~2890	CH deformation of CH_3 [12] lipids, proteins and peptides
~2875	ν_{sym} CH_3 lipids [27]
~2855	ν_{sym} CH_2 lipids [27]
~1740	C=O stretching in lipid esters [4,5,28]
~1670	Amide I: C=O vibrations of different protein structures [4,5,12,27]
~1622	β form C=O stretching in polypeptides, amide I band [27]
~1550	Amide II: N–H and C–N vibrations of the peptide bond in different protein conformations [27]
~1470	CH_2 scissoring in lipids [27–29]
~1455	Various CH_2/CH_3 bending vibrations in lipids and proteins [27,29]
~1440	CH_2 deformation mainly in proteins and peptides [28,30]
~1415	C–O–H in plane bending in proteins [4,31]
~1405	$C(CH_3)_2$ stretching mainly in proteins [4]
~1390	C=O of COO^- symmetric stretching in proteins [5,27–29]
~1370	CH_2 wagging vibrations in lipids [32], and β1,3 glucans [33]
~1350	CH_2 wagging vibrations in lipids [32]
~1340	CH_2 wagging vibrations in lipids [32]
~1300	Amide III: C–N and C–O stretching, N–H and O=C–N bending [27]
~1240	ν_{asym} PO_2^- in DNA, RNA [5,27,29] and phospholipids [4]
~1215	C–O stretching free nucleotides [27]
~1200	C–O–C carbohydrates [4]
~1156	C–O, C–OH carbohydrates [4], various contributions
~1135	Mannans [32] and β1,3 glucans [34]
~1080	ν_{sym} PO_2^- mainly from RNA [4,12,27]
~1050	Mannans [12,33,34]
~972	Mannans [12,33,34]
~1108	β1,3 Glucans [12,34]
~1025	β1,4 Glucans [12,34]
~998	β1,6 Glucans [12,34]
~915	Pyranose ring asymmetric vibrations [31,35]
~905	Mannans [35]
~880	β-Glycosidic linkage vibrations [36]
~860	α-Glycosidic linkage vibrations [37]
~822	Mannans [38]
~808	Mannans [33,34]
~780	Pyranose ring symmetric vibrations [31,35], GMP ring stretching [39]

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10. 2011-2012 Sapientia-Foundation Institute for Research Programs, Kolozsvár, Székelyföldi posztvulkanikus jelenségek tanulmányozása (10 months) – member

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