Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2008; 33 (2) ; 64–70.

Research Article [Araştırma Makalesi] Yayın tarihi 23 Temmuz, 2008 © TurkJBiochem.com

[Published online 23 July, 2008]

A Low Cost Immobilization Agent From an Invasive Marine Alga: *Caulerpa racemosa* var. *cylindracea* Biomass In Bovine Serum Albumin Immobilization

[Yayılımcı Deniz Algi *Caulerpa racemosa* var. *cylindracea* Biyokütlesinden Sığır Serum Albuminine Yönelik Düşük Maliyetli İmmobilizasyon Ajanı]

Sevilay Cengiz,
Levent Cavas,
M. Kadir Yurdakoc

Dokuz Eylül University, Faculty of Arts and Sciences, Department of Chemistry, Kaynaklar Campus, 35160 Buca, İzmir, Türkiye

Yazışma Adresi
[Correspondence Address]

Levent Cavas

Dokuz Eylül University, Faculty of Arts and Sciences, Department of Chemistry, Kaynaklar Campus, 35160 Buca, İzmir, Türkiye
Tel: +90 232 4128701
Fax: +90 232 4534188
Email: lcavas@deu.edu.tr

Kayıt tarihi: 22 Ocak 2008, Kabul tarihi: 1 Temmuz 2008

[Received: 22 January 2008, Accepted: 1 July 2008]

ABSTRACT

Objectives: *Caulerpa racemosa* var. *cylindracea* is a marine green alga which has been widely invading sublittoral ecosystem of the Mediterranean Sea since 1991. Inasmuch as there is no eradication method related to this species so far, use of the dried biomass of *C.racemosa* for immobilization of bovine serum albumin was studied in the present study.

Materials and Methods: *Caulerpa racemosa* var. *cylindracea* was collected from Seferihisar – İzmir by SCUBA diving. Immobilization studies were done by using batch technique under different conditions concerning the determination of optimum temperature, ionic strength, pH and adsorbent dosage.

Results: Optimum pH, ionic strength, temperature and amount of adsorbent dosage was found as 7 (pH), 50 mM, 25 ^{0}C and 10 mg, respectively.

Conclusion: According to results of this paper, dried and powdered form of *Caulerpa racemosa* var. *cylindracea* might be used in some biomolecule immobilization studies as a low cost immobilization agent. This paper proposes an alternative application of biomass of *Caulerpa racemosa* var. *cylindracea* after a possible eradication method which will be carried out in future.

Key Words: Bovine serum albumin, biological pollution, *Caulerpa racemosa* var. *cylindracea*, immobilization

ÖZET

Amaç: Yeşil bir deniz algi olan *Caulerpa racemosa* var. *cylindracea* 1991 yılından bu yana Akdeniz'in sublittoral ekosisteminde yayılımcı özellik göstermektedir. Günümüze kadar bu türü yok etmeye yönelik bir metod geliştirilemediğinden ötürü, sunulan bu çalışmada bu türün kurutulmuş biyokütlesinden yararlanılarak sığır serum albüminine immobilizasyonu yapılmaya çalışılmıştır.

Materyal ve Metot: *Caulerpa racemosa* var. *cylindracea* Seferihisar-İzmir'den SCUBA dalışları ile toplanmıştır. İmmobilizasyon çalışmaları; optimum sıcaklık, iyon şiddeti, pH ve adsorban dozajını belirlemeye yönelik farklı koşullar altında kesikli yöntem kullanılarak yapılmıştır.

Bulgular: Çalışmada optimum pH, iyonik şiddet, sıcaklık sırasıyla 7, 50 mM, 25 ^{0}C ve 10 mg olarak tespit edilmiştir.

Sonuçlar: Bu çalışmanın sonuçlarına göre, kurutulmuş ve toz haline getirilmiş *Caulerpa racemosa* var. *cylindracea* bazı biyomoleküllerin immobilizasyonunda ucuz immobilizasyon ajanı olarak kullanılabilir. Bu çalışma, gelecekte bu türün yok edilmesine yönelik geliştirilecek muhtemel bir metod sonucu ortaya çıkacak bu alg biyokütlesinin değerlendirilmesine alternatif bir yaklaşım sunmaktadır.

Anahtar Kelimeler: Sığır serum albümini, biyolojik kirlilik, *Caulerpa racemosa* var. *cylindracea,* immobilizasyon

Introduction

Caulerpa racemosa var. *cylindracea* is a marine green alga which has been widely invading sublittoral ecosystem of the Mediterranean since 1991. Eleven Mediterranean countries (Albania, Algeria, Crotia, Cyprus, France, Greece, Italy, Libya, Malta, Spain, Tunusia and Turkey) are under thread of this highly invasive marine alga (1,2). No valid eradication method has been proposed in literature for *Caulerpa racemosa* invasion so far. Inasmuch as *Caulerpa racemosa* var. *cylindracea* shows high regeneration capacity in a large range of both temperature and ionic strength and easiness in cultivation, the dried biomass of this species might be used as an alternative solution for removal of unwanted material from waste water or as a carrier in immobilization studies. The some usage of *Caulerpa* species has been proposed in the recent literature. Pavasant et al. (3) also showed the biosorption characteristics of some heavy metals such as Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} by using dried *Caulerpa lentillifera.* According to their research, they have concluded that *Caulerpa lentillifera* could be used for removal of heavy metals from low strength waste water. Pavasant and Marungrueng (4) have investigated the adsorption of a dye, Astrazon Blue FGRL onto *Caulerpa lentillifera* from aqueous solution. It was found that the adsorption capacity of *Caulerpa lentillifera w*as 49.26 $mg.g^{-1}$. In another study, Misheer et al. (5) have showed that *Caulerpa racemosa* has high arsenic, boron and titanium bioaccumulating capacity. In a very recent study, Aravindhan et al. (6) have demonstrated that beach dried *Caulerpa scalpelliformis* adsorbs 27 mg dye per gram of seaweed. They have mentioned that *Caulerpa scalpelliformis* could be a promising material for the development of a low cost biosorption technology for the removal dye effluents. Marungrueng and Pavasant (7) reported that *Caulerpa lentillifera* exhibited greater sorption capacities than activated carbon for some basic dyes such as Astrazon Blue FGRL, Astrazon Red GTLN and methylene blue in their new paper. There are large numbers of studies in literature on protein adsorption onto different materials (8). Protein adsorption onto immobilization agent is of importance for some application fields such as biosensor development (9), drug delivery (10), immunoassay (11) and artificial tissues and organs (12) and isolation and purification of some biomolecules (13). Of about 60 % of blood plasma is composed by albumins and they provide 80 % osmotic pressure of blood (14). Because of bovine serum albumin (BSA) can be obtained commercially and inexpensively with high purity and with slow denaturation characteristics, it has been widely using as a model protein in immobilization researches (8). Inasmuch as Turkish coastlines are invaded by *Caulerpa racemosa* var. *cylindracea*, this species as a raw material for immobilization research could be obtained easily underwater after a possible eradication method this would be based on manual uprooting. Therefore, immobilization characteristics of *Caulerpa racemosa* var. *cylindracea* for BSA are aimed to investigate in the present study.

Materials and Methods

Caulerpa racemosa var. *cylindracea* was collected from Seferihisar – İzmir by SCUBA diving. Underwater view of *Caulerpa racemosa* var.*cylindracea* was shown in Picture 1. The location of study area where algae material collected was shown in Figure 1. The deep of sampling area was 3-5 meters and the sampling area was away from any industrial factories. The algal material was transported to laboratory immediately in wet conditions and then the material was washed with deionised water. The rizoids and other epiphytes were removed carefully. After that material was dried in oven at 80 °C for 16 hours. Dried materials were grinded with mortar and pestle. Grinding material was boiled in water to remove possible proteins from materials then dried as before mentioned (Picture 2). SEM pictures have been taken in Marseille University in France (Figure 2). Material dehydrated was coated with gold palladium and examined through a Hitachi S570 Scanning electron microscope.

Caulerpa racemosa powder with particle size of 100 µm diameters was used for this study. Elemental analysis is carried out in Vario EL III CHN Elemental Analyzer in Organische Chemie Abteilung, TU-Darmstadt, Germany. The results of elemental analysis were shown in Table 1. The functional groups of *Caulerpa racemosa* var.*cylindracea* were interpreted using Fourier Transform Infrared (FT-IR) spectroscopy technique (Perkin Elmer, Spectrum BX) and the results are shown in Table 2. BET specific surface area of *Caulerpa racemosa* was

Picture 1. Underwater view of Caulerpa racemosa var.cylindracea. Photo was taken in Gümüldür-İzmir (Depth -15 m., ©Levent Cavas)

Figure 1. Location of study area where alga material collected

Picture 2. Grinding -crude- biomass of *Caulerpa racemosa* var. *cylindracea*

determined as 7 m^2/g by adsorption of N_2 at 77K with Quantachrome Autosorb Automated Gas Sorption System. Total pore volume for pores with diameter less than 5479.1 A^0 at P/Po =0.99650 was 2.385 x 10^{-2} cm^3 g^{-1} and average pore diameter is 136 A^0.

Determination of protein concentration

Protein concentration was determined by Bradford method (15). Bovine serum albumin (BSA) was used as standard.

Immobilization of BSA

In the experimental studies, the effects of initial BSA concentration, pH, temperature, ionic strength, adsorbent dosage and contact time on the immobilization were studied. The initial concentration of BSA in the immobilization medium was varied between 0.03 and 0.8 mg mL^{-1}. The pH of the immobilization medium was varied from 4 to 12 by using different buffer systems

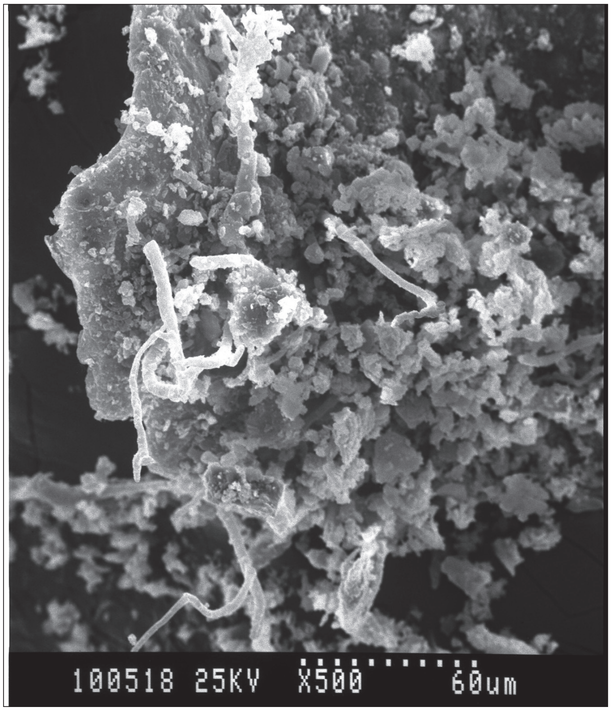

Figure 2. SEM picture of the dried Caulerpa racemosa var. cylindracea

(0.05 M CH_3COOH for pH 4; 0.05 M KH_2PO_4 - K_2HPO_4 for pH 7-9 and 0.05 M NaOH – KCl for pH 12). The temperature, ionic strength and the adsorbent dosage of the immobilization medium were varied from 15 - 65 °C, 0.01- 0.5 M and from 2 - 50 mg, respectively.

For immobilization of BSA onto *Caulerpa racemosa,* dried and grinded material (10 mg) were incubated with 1 mL buffer solutions (at selected pH) containing different amounts of BSA at 35 °C for 1 hour, which is proven to reach equilibrium. At the end of this period, the immobilized protein was separated from the residual aliquot by centrifuging (10000 rpm for 5 min at + 4 ˚C) and the amount of immobilized BSA was determined by measuring the concentration of the free protein in the supernatant. The concentration of BSA was determined spectrophotometrically at 595 nm with Bradford method using a calibration curve prepared previously. The amount of BSA immobilized by *Caulerpa racemosa* powder was calculated by measuring the initial and final concentrations of BSA in the medium. After these experiments 100 µg mL^{-1} BSA concentration was

Table 1. The results of elementel analysis of Caulerpa racemosa powder with and without BSA

Sample	C/N Ratio	N,C,H (%)
Caulerpa racemosa + BSA	13.085 ± 0.332	N 2.540 ± 0.004 C 33.235 ± 0.799 H 5.327 ± 0.078
Caulerpa racemosa	12.095 ± 0.134	N 2.440 ± 0.065 C 29.500 ± 0.481 H 4.799 ± 0.034

Table 2. Functional groups in the dried Caulerpa racemosa var.cylindracea

Functional Group	Standard Wavenumber (Skoog and Leary, 1992) (cm^{1}) (21)	Wavenumber from the results (cm^{-1})
Hydroxyl; O-H	3250-3700	3200-3600
Carboxyl; COOH	2400-3300	2800-3000
Amine; NH_2	3300-3500	3200-3600
C-O	1050-1300	1050-1150
Sulfonyl; S=O	1040-1200	1050-1150
Carbonyl; C=O	1670-1780	1600-1700
S-O	550-650	560

chosen for further experiments. In these experiments, ionic strength, pH and temperature were changed and the effects of these conditions on immobilization were determined in the optimized conditions.

Results

Optimum BSA immobilization conditions onto low cost powder obtained from an invasive marine alga: *Caulerpa racemosa* var. *cylindracea* are aimed to investigate in the present study.

According to Figure 3, optimum immobilization pH (18 % remaining BSA) was determined at 7 with one hour shaking time. There is no statistical difference among shaking times at pH 7. On the other hand, pH 10 and 12 showed ignorantly lower immobilization ratio compared to pH 7. Immobilization efficacy at pH 4 is very close to pH 7.

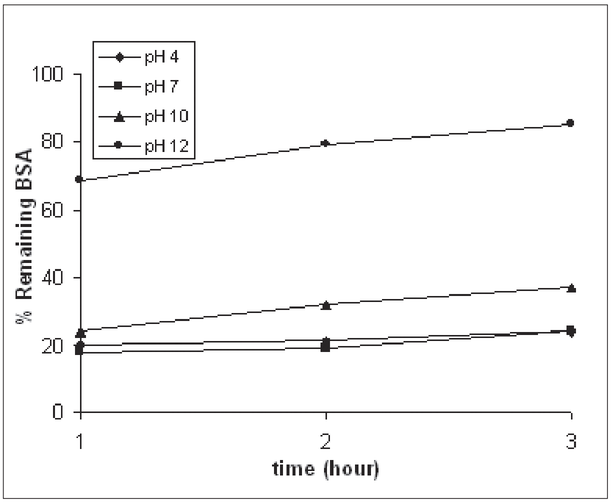

Figure 3. Effect of pH on BSA immobilization onto *Caulerpa racemosa* powder. Reactions were performed at 35 °C in buffers (50 mM) of various pH values. (BSA)= 100 µg/mL. Adsorbent dose=10 mg.

Figure 4 showed the effect of temperature on BSA immobilization onto *Caulerpa racemosa*. Maximum immobilization was observed at 25 ˚C with 1 hour shaking time. The immobilization results at 15, 25 and 35 ˚C were very similar, whereas the result at 65 ˚C significantly different compared to other results.

The effect of ionic strength on BSA immobilization onto *Caulerpa racemosa* was showed in Figure 5. Maximum immobilization condition was observed at 50 mM. Lower ionic strength decreased immobilization efficacy.

Figure 6 shows adsorption kinetics of BSA onto *Caulerpa racemosa* at different initial BSA concentrations. Increasing BSA concentrations were well in line with the increased amount of adsorbed BSA.

The effect of adsorbent dosage on BSA immobilization onto *Caulerpa racemosa* was showed in Figure 7. According to results, the best adsorbent dosage was observed for 10 mg. Fluctuations were observed for other adsorbent dosages.

Figure 8 shows the effect of initial BSA concentration on its immobilization onto *Caulerpa racemosa* at different

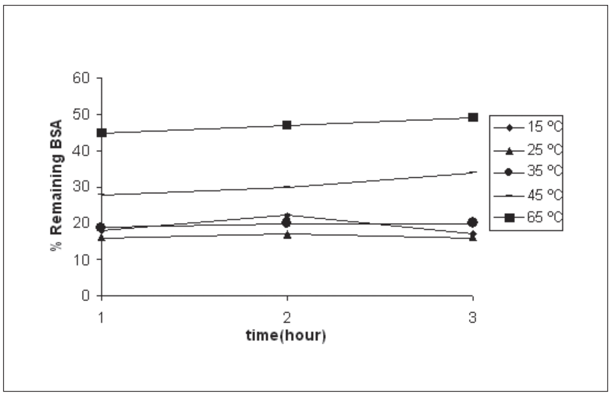

Figure 4. Effect of temperature on BSA immobilization onto *Caulerpa racemosa* powder. Reactions were performed at pH=7 in phosphate buffer (50 mM) of various temperature values. (BSA)=100 µg/mL. Adsorbent dose=10 mg.

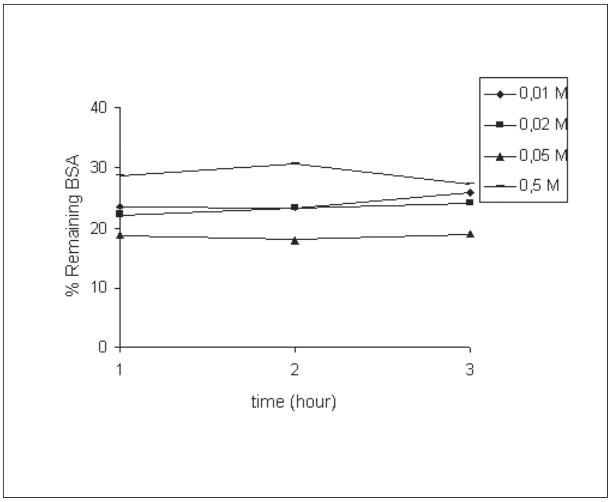

Figure 5. Effect of ionic strength on BSA immobilization onto *Caulerpa racemosa* powder. Reactions were performed at 35 °C in phosphate buffer pH=7.0 of various ionic strength values. (BSA)=100µg/mL. Adsorbent dose=10 mg.

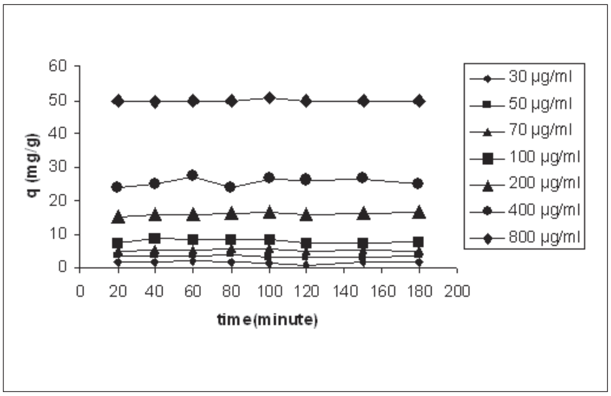

Figure 6. Adsorption kinetics of BSA onto *Caulerpa racemosa* at different initial BSA concentrations. Adsorbent dose=10 mg, T=30 °C, pH=7.0.

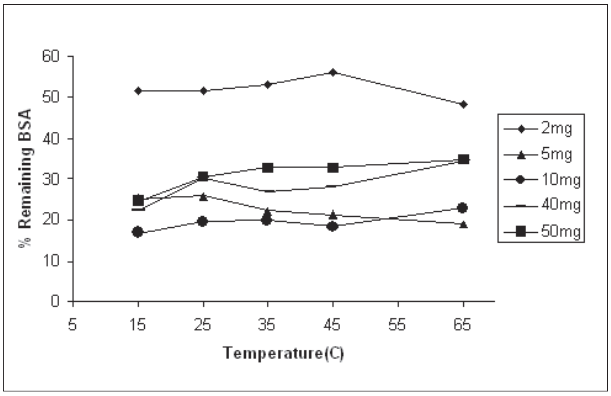

Figure 7. Effect of adsorbent dosage on BSA immobilization onto *Caulerpa racemosa* powder. Reactions were performed in phosphate buffer (pH=7.0, 50 mM) at various temperature values. (BSA)=100 µg/mL (45 min).

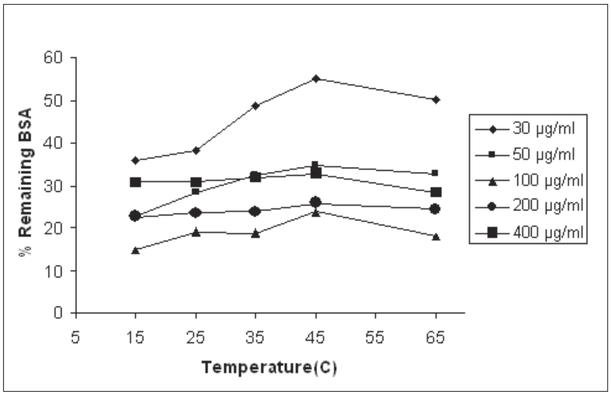

Figure 8. Effect of initial BSA concentration on BSA immobilization onto *Caulerpa racemosa* powder. Reactions were performed in phosphate buffer (pH=7.0, 50 mM) at various temperature values. Adsorbent dose=10 mg. (45 minutes).

temperatures. The best immobilization was observed for 100 µg/mL at 35 °C.

Elemental analysis showed that percentages of N, C and H in the raw *Caulerpa racemosa* and BSA immobilized *Caulerpa racemosa* were 2.44 ± 0.065 %, 29.5 ± 0.481 %, 4.79 ± 0.034 % and 2.54 ± 0.004 %, 33.24 ± 0.799 %, 5.33 ± 0.078 %, respectively. The C/N ratio in the raw *Caulerpa racemosa* and BSA immobilized *Caulerpa racemosa* were 12.095 ± 0.134 and 13.085 ± 0.332, respectively (Table 1). These results may confirm the immobilization of BSA onto *Caulerpa racemosa*. The functional groups are used to understand the mechanism of binding on the algal surface (3,4). In order to find which functional groups have roles on binding, FT-IR analyzes were performed. The results are shown in Table 2. Several functional groups such as hydroxyl, amine, amino, carboxylic acid, sulfonyl which affect the immobilization efficacy were determined. It was found a existence of FT-IR spectrum difference between *Caulerpa racemosa* and BSA immobilized *Caulerpa racemosa*. The new peaks occurred at a wave number 1368 and 1157 cm^{-1} for BSA immobilized *Caulerpa racemosa*. These peaks might be associated with binding of BSA on the algal surface with the related functional groups.

Discussion

Invasive form of *Caulerpa racemosa* var. *cylindracea* invades Mediterranean Sea since 1991. Eleven Mediterranean countries are now suffering from this invasive seaweed. Because of its secondary metabolite called caulerpenyne herbivorous fishes can not consume this species. Some sea slugs (*Lobiger serradifalci* and *Oxynoe olivaceae*) which are considered as potential consumers of Caulerpales member can not limit this invasion because these slugs can provide a contribution to invasion of *Caulerpa racemosa var. cylindracea* through punctuating and grazing of Caulerpales member (16,17). There are many published papers on the fast regeneration property of invasive *Caulerpa* species (1,18,19). Since there is no valid eradication method for *Caulerpa racemosa* var. *cylindracea,* biomass of this invasive species could be considered as a low cost material for immobilization studies.

BSA is a model protein in protein immobilization researches since its cheapness and commercially availability. Therefore, immobilization of BSA onto *Caulerpa racemosa* powder was aimed to investigate in the preliminary study.

Isoelectric point of the BSA studied is 4.7. Therefore, BSA is positively charged at pH 4. On the other hand, it is negatively charged at pH 7, 10 and 12 (20). Observation of optimum immobilization pH value at 7 could have been caused by the different charges of BSA and surface of *Caulerpa racemosa* powder. Lower immobilization of BSA at pH 4 can support previous comment since BSA is positively charged at this pH values. The immobilization decreased at pH=4 due to the electrostatic repulsions between the species. The optimum temperature for the best immobilization was found as 25 ºC. However, some enzymes such as catalase which is widely used for industrial purposes has high Q_{10} value and when the environmental conditions are considered, 35 °C was chosen as an optimum temperature value. In the literature, similar temperature values can be found. Hu et al. (20) and Salgin et al.. (14) found optimum temperature values for BSA immobilization as 37 °C and 30 °C, respectively. The high protein levels at 65 ˚C could have been caused by the release of protein from support material. Because our material includes membrane proteins however it does not release at moderate temperatures up to 40 ˚C. Optimum ionic strength was found as 50 mM. Increased ionic strength caused decreases in the BSA immobilization. This might have been caused by interaction of negatively charged BSA molecule with Na^+ ions in immobilization medium. Interactions of BSA with Na^+ ions could have changed the ionic structure of BSA. As the concentration of BSA increase on the constant adsorbent dosage, the amount of adsorbed BSA is increased. Optimum adsorbent dosage was chosen as 10 mg because we observed lower immobilization of BSA under 10 mg adsorbent dosage. 100 µg/mL BSA concentrations were found for maximum immobilization. Various adsorbent types and their adsorption capacities were summarized in Table 3.

In conclusion, *Caulerpa racemosa* var. *cylindracea* is going on its wide invasion in Mediterranean Sea and is negatively affecting sublittoral ecosystem of Mediterranean. There is still no valid eradication method for this invasive species. According to results of this paper, dried and powdered form of *Caulerpa racemosa* var. *cylindra-*

Table 3. The adsorption capacity of different adsorbents for BSA under optimum conditions

Reference	Kind of adsorbent	Initial BSA concentration	Maximum adsorption capacity for BSA
Tanyolaç and Özdural (22)	Magnetic polyvinyl butyral microbeads	-	17.7 mg BSA/g adsorbent
Tanyolaç et al (23)	Polyvinyl butyral membrane	10 mg/mL	427 µg/cm^2 (35.44 mg/mL membrane)
Katiyar et al (24)	MCM-41	10 mg/mL	104 mg/g
Zhang et al (22)	Chitosan microspheres	2.5 mg/mL	12.7 mg/g dry bead
Zhang et al (25)	Cibacron Blue F3GA attached chitosan microspheres	2.5 mg/mL	95.2 mg/g
Ma et al (26)	Magnetic poly (styrene–divinylbenzene–glycidyl methacrylate) microspheres	3 mg/mL	<10 mg/g
Ma et al (26)	Modified magnetic poly microspheres	3 mg/mL	80.2 mg/g
Putman et al (8)	Zirconium oxide powder	-	> 15 mg/g (increases with equilibrium concentration)
Putman et al (8)	Phosphated zirconium oxide powder		> 3 mg/g (increases with equilibrium concentration)
Avramescu et al (27)	Functionalised ethylene vinyl alcohol copolymer (EVAL) membranes	1 mg/mL	0.5 – 0.55 µg/cm^2 20 – 22 mg/g per membrane mass
The present study	*Caulerpa racemosa* powder	0.1 mg/mL	8.2 mg/g dry weight

cea could be used in some biomolecule immobilization studies as a low cost immobilization agent. This paper proposes an alternative application of biomass of *Caulerpa racemosa* var. *cylindracea* after a possible eradication method which will be developed in future.

Acknowledgement

The authors would like to thank Prof. Dr. Peter Claus and Prof.Dr.Marc Verlaque from TU-Darmstadt/Germany and from Université de la Méditerranée for elemental analysis and SEM pictures, respectively.

References

[1] Verlaque M, Durand C, Huisman JM, Boudouresque CF, Parco Y. (2003) On the identity and origin of the Mediterranean invasive *Caulerpa racemosa* (Caulerpales Chlorophyta), European Journal of Phycology. 38: 325– 339.

[2] Ould-Ahmed N, Meinesz A. (2007) First record of the invasive alga Caulerpa racemosa (Caulerpales, Chlorophyta) on the coast of Algeria. Cryptogamie Algologie. 28: 303-305

[3] Pavasant P, Apiratikul R, Sungkhum V, Suthiparinyanont P, Wattanachira S, Marhaba TF. (2006) Biosorption of Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} using dried marine green macroalga *Caulerpa lentillifera*. Bioresource Technology. 97: 2321-2329.

[4] Pavasant P, Marungrueng K. (2006) Removal of basic dye (Astrazon Blue FGRL) using macroalga *Caulerpa lentillifera*. Journal of Environmental Management. 78: 268-274.

[5] Misheer N, Kindness A, Jonnalagadda SB. (2006) Seaweeds Along KwaZulu-Natal Coast of South Africa-4: Elemental Uptake by Edible Seaweed *Caulerpa racemosa* (Sea grapes) and the Arsenic Speciation. Journal of Environmental Science and Health. A 41: 1217-1233.

[6] Aravindhan R, Rao JR, Nair BU. (2007). Removal of basic yellow dye from aqueous solution by sorption on green alga *Caulerpa scalpelliformis*. J Hazard Mater. 142: 68-76.

[7] Marungrueng K, Pavasant P. (2007) High performance biosorbent *(Caulerpa lentillifera)* for basic dye removal. Bioresource Technol. 98: 1567-1572.

[8] Putman B, Meeren PV, Thierens D. (1997) Reduced bovine serum albumin adsorption by prephosphatation of powdered zirconium oxide. Colloid Surface. A. 121: 81-88.

[9] Lu HB, Homola JI, Campbell CT, Nenninger GG, Yee SS, Ratner BD. (2001) Protein contact printing for a surface plasmon resonance biosensor with on-chip referencing. Sens Actuat B-Chem. 74: 91-99.

[10] Glen SK, You HB, Harry C, Jan FJ, Sung WK. (1992) Release of proteins via ion exchange from albumin-heparin microspheres. J Control Release. 22: 83-93.

[11] Butler JE. (2000) Solid supports in enzyme-linked immunosorbent assay and other solid-phase immunoassays. Methods. 22: 4-23.

[12] Norman E, Williams P, Illum L. (1993) Influence of block copolymers on the adsorption of plasma proteins to microspheres. Biomaterials. 14: 193-202.

[13] Wang L, Kanani DM, Ghosh R. (2006) Purification of humanized monoclonal antibodies by membrane-based hybrid bioseparation technique. J Immunol Methods. 314: 1-8.

[14] Salgın S, Takaç S, Özdamar TH. (2006) Adsorption of bovine serum albumin on polyether sulfone ultrafiltration membranes: Determination of interfacial interaction energy and effective diffusion coefficient. J Membrane Sci. 278: 251–260.

[15] Bradford MM. (1976) A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem. 72: 248–254.

[16] Žuljevic A, Thibaut T, Elloukal H, Meinesz A. (2001) Sea slug disperses the invasive *Caulerpa taxifolia*. J Mar Biol Assoc UK. 81: 343–344.

[17] Cavas L, Yurdakoc K, Yokes B. (2005) Antioxidant status of *Lobiger serradifalci* and *Oxynoe olivacea* (Opisthobranchia, Mollusca). J Exp Mar Biol Eco. 314: 227-235.

[18] Klein J, Verlaque M. (in press) The Caulerpa racemosa invasion: A critical review. Marine Pollution Bulletin. doi:10.1016/j.marpolbul. 2007.09.043.

[19] Verlaque M, Afonso-Carrillo J, Gil-Rodríguez MC, Durand C, Boudouresque CF, Parco YL. (2004) Blitzkrieg in a marine invasion: *Caulerpa racemosa* var. *cylindracea* (Bryopsidales, Chlorophyta) reaches the Canary Islands (North-east Atlantic). Biol Invasions. 6: 269-281.

[20] Hu J, Li S, Liu B. (2005) Adsorption of BSA onto sulfonated microspheres. Biochem Eng J. 23: 259–263.

[21] Skoog D, Leary J. (1992) Principles of Instrumental Analysis, fourth ed. Saunders College Publishing, Forth Worth.

[22] Tanyolaç D, Özdural AR. (2001) BSA adsorbtion onto magnetic polyvinylbutyral microbeads. J Appl Polym Sci. 80: 707-715.

[23] Tanyolaç D, Sönmezışık H, Özdural AR. (2005) A low cost porous polyvinylbutyral membrane for BSA adsorption. Biochem Eng J. 22: 221–228.

[24] Katiyar A, Ji L, Smirniotis PG, Pinto NG. (2005) Adsorption of Bovine Serum Albumin and lysozyme on siliceous MCM-41. Micropor Mesopor Mat. 80: 311–320.

[25] Zhang J, Zhang Z, Song Y, Cai H. (2006) Bovine serum albumin (BSA) adsorption with Cibacron Blue F3GA attached chitosan microspheres. React Funct Polym. 66: 916-923.

[26] Ma ZY, Guan YP, Liu HZ. (2006) Affinity adsorption of albumin on Cibacron Blue F3GA-coupled non-porous micrometer-sized magnetic polymer microspheres. React Funct Polym. 66: 618–624.

[27] Avramescu ME, Sager WFC, Wessling M. (2003) Functionalised ethylene vinyl alcohol copolymer (EVAL) membranes for affinity protein separation. J Membrane Sci. 216: 177–193.