Dental Journal
Majalah Kedokteran Gigi
Volume 46 Number 2 June 2013

Research Report

A study of extraction and characterization of alginates obtained from brown macroalgae *Sargassum duplicatum* and *Sargassum crassifolium* from Indonesia

Decky J. Indrani[1] and **Emil Budianto**[2]
[1]Department of Dental Materials Science, Faculty of Dentistry, Universitas Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia
Jakarta – Indonesia

ABSTRACT

Background: *Worldwide commercially available alginate have been used for tissue engineering purposes. The macroalgae Sargassum obtained from Indonesia have been used for various purposes, however, they have not been applied for tissue engineering scaffolds.* ***Purpose:*** *This study was aimed to extract alginate from the macroalgae Sargassum from Indonesia sea and to characterize in morphology, chemical element and functional groups.* ***Methods:*** *Macroalgae Sargassum duplicatum (S. Duplicatum) and Sargassum crassifolium (S. Crassifolium) were collected from Banten, Indonesia. Extraction of alginates were carried out using the alkaline extraction procedure. Scanning electron microscopy as well as X-ray Fluorescence and Fouirer Transform Infra-Red spectroscopy were used to characterize the extracted powders. Obtained data from the extracted powders were compared to those of the commercially available alginate.* ***Results:*** *Extraction using the alkaline method has resulted in S.duplicatum and S.crassifolium alginate powders. Alginate particles were suggested as irregular shapes with various dimension. Element components were mainly Na and Ca, whereas, minor elements were considered as negative impurities. COO^- and C-O-C groups were evident in the finger print regio. The characteristics of Alginates extracted from the macroalgae S.duplicatum and S.crassifolium found similar to those of the commercially available alginate.* ***Conclusion:*** *Extraction obtained from the macroalgae S.duplicatum and S.crassifolium showed the typical alginate and the morphology, chemical element and functional groups were in agreement with those of the commercially available alginate.*

Key words: *Alginate extraction, morphology, chemical element, functional groups*

ABSTRAK

Latar belakang: *Alginat dari berbagai penjuru dunia telah digunakan untuk kegunaan rekayasa jaringan. Alginat dari alga makro Sargassum yang diperoleh dari Indonesia telah digunakan untuk berbagai kegunaan, namun ini belum diterapkan untuk scaffold jaringan.* ***Tujuan:*** *Untuk mengekstrak alginat dari alga makro Sargassum perairan Indonesia dan untuk memperoleh karakteristik alginat dalam morfologi, unsur kimia dan gugus fungsi.* ***Metode:*** *Alga makro Sargassum dari spesies Sargassum duplicatum (S. Duplicatum) dan Sargassum crassifolium (S. Crassifolium) diperoleh dari Banten, Indonesia. Ekstraksi alginat dilakukan dengan menggunakan prosedur ekstraksi alkali. Scanning electrone microscope, X-ray fluorescence dan Fouirer transform infra-red spektroscope digunakan untuk mengarakterisasi bubuk alginat hasil ekstraksi. Data yang diperoleh dari serbuk laginat dibandingkan dengan yang tersedia secara komersial.* ***Hasil:*** *Ekstraksi menggunakan metode alkali telah menghasilkan serbuk alginat dari S.duplicatum dan S. crassifolium. Morfologi partikel alginat terlihat tidak teratur dengan berbagai dimensi. Elemen Na dan Ca muncul sebagai komponen utama, sedangkan, elemen minor dianggap sebagai pengotor. Gugus fungsi COO-dan COC terdeteksi di regio sidik jari. Karakteristik alginat S.duplicatum dan S.crassifolium ditemukan sesuai dengan karakteristik alginat yang tersedia secara komersial.* ***Simpulan:***

Serbuk yang diperoleh dari alga makroi S. duplicatum dan S.crassifolium menunjukkan kekhasan alginat dan morfologi, unsur kimia dan kelompok fungsional alginat sesuai dengan yang tersedia secara komersial.

Kata kunci: *Ekstraksi alginat, morfologi, elemen kimiawi, gugus fungsi*

Correspondence: Decky J. Indrani, c/o: Departemen Ilmu Material Kedokteran Gigi, Fakultas Kedokteran Gigi Universitas Indonesia. Jl. Salemba Raya No. 6 Jakarta 10430, Indonesia. E-mail: decky@ui.ac.id

INTRODUCTION

The failure or loss of an organ or tissue is one of the most numerous and costly problems in human health care. Tissue engineering, that integrates a variety of science and engineering disciplines to create functional organ or tissues for transplantation, evolved as one of the most promising therapies in regenerative medicine.[1] Scaffold-guided tissue engineering for cells growth matrix made of biopolymer were appealing due to their structural similarities to the macromolecular-based human tissues.[2] Among biopolymers, alginate enabled more efficient penetration of cells into scaffold matrices.[3,4] To enhance its biological performance, alginate has been combined with other biomaterials. Blends of alginate and chitosan have been used to regenerate cell in soft tissues.[5,6] Composites of alginate and hydroxyapatite seeded with osteoblast have also been observed for bone tissue engineering.[7,8] Bone tissue engineering may be an advantage, for instance, to increase ridge width due to resorption from tooth loss or for the treatment of edentulous patients if they lack the appropriate bone volume.

Worldwide alginates are collected from macroalgae of sub-tropical seas. For example, algae species of *Laminaria* from Norwegia, France, and Japan, as well as, species of *Macrocystis* from North America and Australia have produced alginates and made them important industries for food and nutrients,[9] and other industries, such as, cosmetics, textile protection, fertilizers, pharmaceuticals, and biotechnology.[10] In dentistry, alginates have been applied for impression materials.[11] In tropical seas of Malaysia, Phillipine and Indonesia, more than 400 species of macroalgae are widely spreaded and mainly consist of *Sargassum* and *Turbinaria*. Indonesia has a lot of islands with broad coral rock, allowing huge supply of macroalgae as raw material for alginate and is a potential renewable marine resource in great abundance.[12,13] Of the locals observation around Pamengpeuk shore, Banten, Indonesia, the macroalgae *Sargassum* are abundant and floated to the beach, at high tide. The locals had not have much information about the role of alginate in the industry, accept its downstream products, such as for food, syrup and gelling. Small industries of alginates have produced cosmetics, textile protection, fertilizers, and pharmaceuticals, limited for thickening, stabilizing and emulsifying agent.[13,14]

Sargassum, as one of the many species of macroalgae from Indonesia, are currently being researched. Characterization of alginates have been based on requirements for quality standards, i.e. water and ash content, ashing temperature, and biochemistry analysis, such as thickening and stabilizing properties, gel-forming, estimation of content, etc.[9,14,15] Although pharmaceutical uses from *Sargassum* alginates has not been evaluated fully, they have started an early stage of research as scaffold materiasl for tissue engineering scaffolds. However, neither of their characterizations were available. For tissue engineering, alginates scaffolds should be biocompatible to facilitate the process of cell regeneration.[16,17]

Biocompability of alginate scaffold including the biodegradability and pore size for growth of cells was necessary.[18] Biodegradability is an essential factor as scaffolds were absorbed by the surrounding tissues. Alginate is generally accumulated by minerals from the sea.[10] Besides, there were also fukosantin pigment and polyphenol bound in the plant that make them become dark brownish colour.[9] For this, pure alginate is required. Next, a high porosity and adequate pore size which can seed and grow cells are necessary; to secure mechanical properties of the scaffold appropriate for the cells.[8,18] For this, alginate should be able to gelled and cross-linked through ionic bonding with divalent cations that act as bridges.[19,20] Essential factor to cross-linked alginate was the presence of carboxyl and carbonyl functional groups;[17] besides, the existance of those functional groups are admired as they mimic the part of the protein group in human.[2] As an addition, algiante powder should show morphology typical alginate.

Characterizations of alginate scaffolds for tissue engineering have not been well addressed. To achieve the goal, therefore, characterization of *Sargassum* powder, i.e. morphology of alginate, chemical element and functional groups, should be carried out procede the characterization of alginate scaffold. Furthermore, extraction of alginate from the the macroalgae *Sargassum* was needed. Extracted and named by a Scottish scientist in 1881, alginic acid was realized as a kind of polysaccharide consisting of copolymers D-mannuronic and L-guluronic acids. They were found in cell walls of macroalgae and composed of 3 kinds of polymers: i.e. alginates, cellulose, and complex heteroglycans. Alginates were presented mainly as the Ca salt of alginic acid, although Mg, K and Na salts may also be present; they do not dissolve in water.[21] This structural integrity of alginate can be broken down with the use of extraction, to allow the direct transformation of the mixed salts (Na^{+}, Mg^{+2+}, and Ca^{2+}) of alginate into

sodium alginate, in order to obtain alginate that dissolves in water.[15,22] The purpose of the present study, therefore, was to extract alginate obtained from the macroalgae *Sargassum* from Indonesia and to characterize the morphology, chemical element and functional groups. The information obtainded from the present study can be used partly to consider the use of the alginate extracted from the macroalgae in preparing scaffold of hydroxyapatite and alginate composite for scaffoled material for tissue engineering.

MATERIALS AND METHODS

All chemicals used for alginate extraction process were analyst grade, obtained from Merck (USA). Two species of the macroalgae, i.e. *Sargassum duplicatum* JG Agardh (*S. duplicatum*) and *Sargassum crassifolium* JG Agardh (*S. crassifolium*), collected in November 2009 from Pamengpeuk shore of Banten, Indonesia, were obtained from the Biotechnology Laboratory, Ministry of Marine Affairs and Fisheries–RI. A commercially available alginate powder of alginic acid sodium salt from brown algae, purchased from Sigma (Germany), was used routinely for a comparison.

Macroalgae were collected by cutting the thallus about 40 cm from the top of the plant and were washed with water to remove impurities, such as sand, etc, and were sun-dried for at least three days. All alginate extraction experiment were conducted on fronds of *S.duplicatum* and *S. crassifolium* macroalgae. Leaves of the plant were cut into pieces, stored in aerated bags and kept in shaded and ventilated site, until they were taken for extraction.

Alginate extraction using alkaline protocol used in the present study was a laboratory adaptation of the industrial process and was conducted with a serial step, as described earlier.[15–23] First of all, pieces of algae leaves were rinsed and immersed in water until they were expanded and then immersed in a 0.3% HCl solution for one hour. For each extraction experiment, 50 g of algae pieces were rinsed with distilled water and soaked in 1 L of a 4% (w/w) Na_2CO_3 solution under continuous stirring a least two hours. At the end of the extraction, supernatant were separated from the solution by means of a vibrating screen and then acidified using HCl 10%. The resulting alginic acid, in the form of fiber foam, were rinsed with water on a filter screen. Solution of 10% NaOH was added to produce sodium alginate (Na-alginate). No decoloration procedure was included to the extraction process. Extracted Na-alginate fibers were recovered from solution by oven drying. Finally, dried Na-alginate fibers were milled to obtain smooth powders. Samples were powders of the Na-alginate, extracted from the macroalgae *S. duplicatum* (*S. duplicatum* alginate) and from the macroalgae *S. crassifolium* (*S. crassifolium* alginate), and, the commercially available alginate. All samples were then characterized.

Scanning electron microscopy (SEM) was conducted to study the morphology of the alginate samples. Alginate powder samples were mounted on a sample holder. The convert into electrically conductive samples, the samples were coated with an ultrathin gold coating deposited on the surface of the samples by low-vacuum ion sputter apparatus. Coated samples were moved to scanning electron microscope (Zeus, Germany) and were then scanned and convert to an image through a monitor to visualize particle shapes.

X-ray fluoroscence (XRF) spectroscopy was used to quantify chemical elements of the alginate samples. Each sample was prepared in a plastic ring mold (2.5 cm in diameter and 0.5 mm in thickness) and was compressed under a hydraulic press. The Na-alginate powder together with the mold was then loaded in XRF spectrometer (JEOL JSX-3211, Japan). The samples were scanned by XRD spectrometer and X-ray beam would interact with the samples. Data collected from the measurements were recorded using a software programme in the XRF spectrometer. Measurements were conducted three times.

Fouirer transform infra-red (FTIR) spectroscopy was utilized to confirm the existance of functional groups in the alginate samples. A total of 5% (w/w) sample was mixed with dry potassium bromide (KBr), with respect to the pellet technique. The mixture was ground into a fine powder using an agate mortar and was compressed into a disc under a hydraulic press. Each sample was then mounted in and scanned by FTIR Spectroscopy (SpectrumOne, Perkin Elmer, Japan) at 4 mm/s over a wave number region of 4000-450cm^{-1}. FTIR transmission spectra obtained from the measurement were recorded using a software programme for FTIR. Measurements were conducted three times in the alginate samples.

RESULTS

Alkali extraction used in the extraction of the macroalgae *S. duplicatum* and *S. crassifolium* has resulted solid powder of alginates (Figure 1). *S. duplicatum* and *S. crassifolium* alginates were pale yellow and dark brown, respectively. In contrast, and the commercially available alginate were in white. Results from the characterizations showed comparison of *S. duplicatum*, *S. crassifolium* and the commercially available alginates in the morphology, chemical elemental and functional groups.

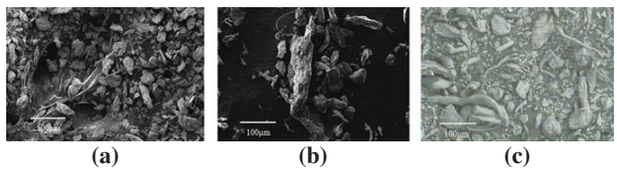
Morphology of *S. duplicatum*, *S. crassifolium* and the commercially available alginates revealed from SEM were presented in Figure 2. *S. duplicatum* and *S. crassifolium* alginates displayed shapes of irregular particles in varied size. Some were long, whereas, others were round. Some of which form the helical structure extending up to 200 µm and 20 µm in diameter. The variation in morphology and dimension coincided with those seen in the commercially available alginate.

Quantitative chemical elements detected in *S. duplicatum*, *S. crassifolium* and the commercially available alginates yielded by XRF spectroscopy were listed in Table 1. It can be seen from Table 1 that S and Fe elements were seen in low amount in all alginate powders. Surprisingly, the quantitative of Si (16%) in *S. duplicatum* alginate and Cl (30%) in *S. crassifolium* alginate were considerable. All chemical elemental were not detected in the commercially available alginate. As expected, the major chemical element components were Na and then Ca.

FTIR spectra of the *S. duplicatum*, *S.c rassifolium* and the commercially available alginates in the band range of 4000–450 cm^{-1} were illustrated in Figure 3. A broad envelop occured between the band of 3500-3000 cm^{-1} was assigned to stretching vibration of OH (hydroxyl) group. It is obvious that the presence of OH^- ions around 3000 cm^{-1} correspond to adsorbed H_2O, derived from moist of the samples. In the fingerprint region, the band around 1626-1623 cm^{-1} were correspond to asymmetrical and symmetrical stretching modes of COO (carboxyl) group. Another COO with similar modes also appeared at the band at 1421 cm^{-1}. Other charateristic peaks around the band at 1027 cm^{-1} derived from C-O-C (ether) group, as aliphatic alcohol with C-O (carbonyl) substituent. The functional groups obtained from both extracted alginate were in agreement with those appeared in the commercially available alginate. As an addition, the extraction has resulted Na-alginate as ini Figure 4. A possible mechanism of the addition of NaOH into alginic acid producing Na-alginate was because of the weak bond between H^+ and COO^- was broken causing Na^+ ions, possibly, forming COO-Na.

(a) (b) (c)

Figure 1. Alginate powders from the macroalgae a) *S. duplicatum*, b) *S. crassifolium* and the c) commercially available alginate.



(a) (b) (c)

Figure 2. SEM micrographs of the Na-alginates obtained from the macroalgae a) *S. duplicatum*, b) *S. crassifolium* and c) commercially available.

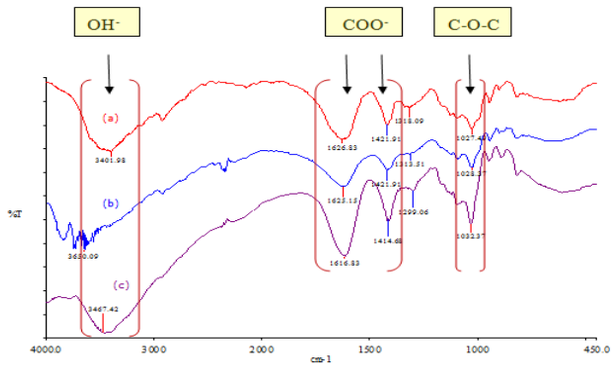


Figure 3. FTIR spectra of Na-alginates obtained from the macroalgae a) *S. duplicatum*, b) *S.crassifolium* and c) available commercially.

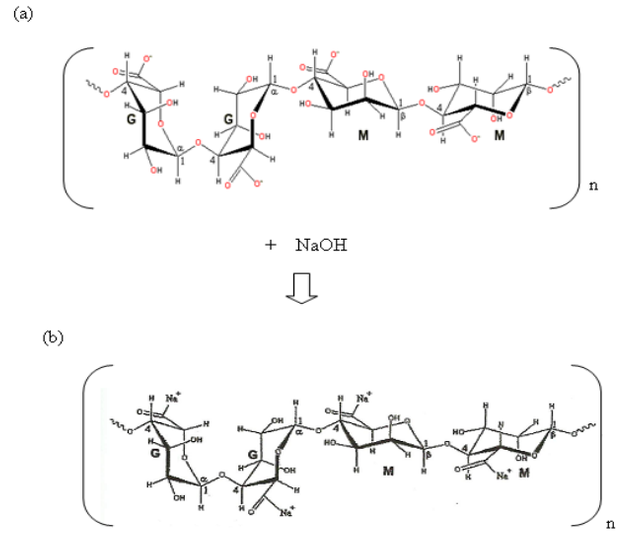


Figure 4. Schematic diagram of the incorporation of NaOH into (a) alginic acid, consisting the M and G groups resulting the (b) Na-alginate.[9]

Table 1. Chemical elements contained in the macroalgae a) *S. duplicatum*, b) *S. crassifolium* and c) available commercially

Source of alginate	Chemical elemental	wt (%)
S.duplicatum	Na	50,5
	Ca	12,1
	S	3,5
	Fe	6,9
	Si	16,4
S.crassifolium	Na	49,3
	Ca	10,2
	S	2,6
	Fe	3,2
	Cl	30,8
Commercially available	Na	73,2
	Ca	6,7
	S	8,2
	Fe	5,0

DISCUSSION

Macroalgae *Sargassum* were used in the present study because they grew abundant in areas near the shore; the collection was far easier compared with other macroalgae, and was routinely accomplished by the Ministry of Marine Affairs and Fisheries–RI. The *Sargassum* were identified as *S.duplicatum* and *S. crassifolium* by the Research Centre of Oceanography-Indonesian Institute of Sciences.

The alkaline extraction procedure used in the present study was possible to to produce *S.duplicatum* and *S. crassifolium* alginates. As mentioned previously, that alginic acid in macroalgae were presented mainly as the Ca-alginat, a salt of alginic acid which were insoluble in water. The extraction process has converted the insoluble Ca salts into *S.duplicatum* and *S. crassifolium* alginates which were soluble in water. Alkali treatment was necessary for an ion exchange process. The process was carried out by 2 steps; the first was a more efficient extraction by treating the seaweed with dilute mineral acid, as in reaction (1):
Pre-extraction:

$$Ca(Alg)2 + 2H^{+} \rightarrow 2HAlg + Ca^{2+} \quad (1)$$

According to several authors,[15, 24, 25] when Ca-alginate was converted to alginic acid, it was more readily to be extracted with alkali than the original calcium alginate. Reaction (1) was continued by extraction using alkali as shown in reaction (2).
Extraction:

$$2Halg + Na^{+} \rightarrow NaAlg + Ca^{2+} \quad (2)$$

No coloring agent was given in the extraction process. Both *S. duplicatum* and *S. crassifolium* alginates showed a distinctive brownish (Figure 1). The extremely dark brown colour demonstrated in *S. crassifolium* alginate powder was probably a reflection of fucosantin pigment, that often contained in certain algae, and probably covered other pigments. White color reflected from the commercially available alginate was ascertained as a result of color treatment at the time of the extraction process; this was always did as an effort to make alginate interesting. During alginate extraction, following the acid pre-treatment, a brown discoloration develops and this carries through the rest of the process resulting in a dark sodium alginate powder.[10] Previously, it was demonstrated that phenolic compounds are responsible for the discoloration.

X-ray fluoroscence results showed chemical elemental contained in *S. duplicatum* and *S. crassifolium* alginates (Table 1). Observing reaction (1), the incorporation of Na^{+} ions from NaOH into Ca^{2+} ions from Ca-alginate had made them rich with both Na^{+} and Ca^{2+} ions. Therefore, the quantitative chemical elemental of Ca and Na yielded by XRF was high. As Na and Ca elements were also in a considerable amount found in the commercially available alginate, it implied that the material experienced similar method of alginate extraction and NaOH was used in the extraction process.

Chemical elemental occcured in the sample alginates were impurities. The minor amount of Fe and S elements in *S. duplicatum* and *S. crassifolium* alginates, as well as, in the commercially available alginate (Table 1) were impurities which likely to occure from the XRF instrument at the time of measurement. This possibilitiy was supported by the occurance Fe and S elements in the commercialy available alginates, as well. Whereas, the existance of the considerable amount of Si and Cl in *S. duplicatum* and *S. crassifolium* alginates, respectively, may due to mineral contaminations that originated from sea environment. It was presumed that they accumulated inside the macroalgae when producing M and G acids. This idea were supported by the absent of either Si or Cl in the commercialy available alginates. A purification process, actually, may omit impurities in *S. duplicatum* and *S. crassifolium* alginates. Nevertheless, impurities in alginate were presumed not to cause negative reaction during the overall biocompatibility of the material to human. In fact, bone mineral was known to compose of hydroxyapatite with substitutes, such as Ca Na, K, Cl, Fe, F^{-} etc, originated from the welknown biomineralization in human.[24]

FTIR spectra showed the occurance of carboxyl and carbonyl groups in *S.duplicatum* and *S.crassifolium* alginates implied the typical alginate (Figure 3). These functional groups showed the similarity to human protein, as in most organisms, polysaccharides and amino acids contained carboxylic acid (-COOH). The presence of carboxyl and carbonyl groups in *S.duplicatum* and *S. crassifolium* alginates coincided with those revield in the commercial available alginate, as well as, in a commercial available alginate analyzed studied.[17]

The present study was an initial report describing both *S. duplictum* and *S. crassifolium* alginates powders, obtained from macroalgae of Banten, Indonesia. The use of SEM, XRF and FTIR have helped in identifying and verifying the material to describe the characteristic of alginate powder. The morphology of alginate, the chemical elemental, as well as the functional groups contained in the material were found similar with those of the commercially available alginate. The commercially available alginate use in the present study has been used as scaffold material for research. It was a great expectation of both *S. duplictum* and *S. crassifolium* alginates to be used as alginate for scaffold material. Further studies, therefore, need to characterized both *S. duplictum* and *S. crassifolium* alginates to explore the posibility of the material to be used as material for scaffolds, procede to further research in application of alginate scaffolds for tissue engineering applications.

It is concluded that extraction obtained from the macroalgae *S. duplicatum* and *S. crassifolium* showed the typical alginate; the morphology, chemical element and functional groups were in agreement with those of the commercially available alginate.

ACKNOWLEDGEMENT

The financial support rendered by the Ministry of Education through the Universitas Indonesia is greatly acknowledged.

REFERENCES

1. Vacanti CA, Bonnassar LJ, Vacanti JP. Structural tissue engineering. In: Lanza RP, Langer R, Vacanti JP, editors. Principles of tissue engineering. San Diego: Academic Press; 2000. p. 671–82.
2. James BT, David J. Timson§, Richard J. Reece, Hazel M. Holden. Molecular structure of human galactokinase. The Journal of Biological Chemistry 2005; 280(10): 9662–70.
3. Nicola H. An alginate hydrogel matrix for the localised delivery of a fibroblast/keratinocyte co-culture to expedite wound healing. Thesis. UK: Bioengineering, School of Chemical Engineering, University of Birmingham; 2009. p. 101–4.
4. Garagorri N, Fermaniana S, Thibault R, Ambrose WM, Scheinb OD, Chakravarti S, Elisseeffa. Keratocyte behavior in three-dimensional photopolymerizable poly (ethylene glycol) hydrogels. Acta Biomater 2008; 4(5): 1139–47.
5. Majima T, Funakosi T, Iwasaki N, Yamane S, Harada K. Alginate and chitosan polyion complex hybrid fibers for scaffolds in ligament and tendon tissue engineering. J Orthop Sci 2005; 10(3): 302–7.
6. Li Z, Zhang M. Chitosan-alginate as scaffolding material for cartilage tissue engineering. J Biomed Mater Res A 2005; 75(2): 485–93.
7. Turco G, Marsich E, Bellomo F, Semeraro S, Donati I, Brun F, Grandolfo M, Accardo A, Paoletti S. Alginate/Hydroxyapatite biocomposite for bone ingrowth: a trabecular structure with high and isotropic connectivity. Biomacromolecules 2009; 10(6): 1575–83.
8. Bernhardt A, Despang, Lode A, Demmler A, Hanke T, Gelinsky M. Proliferation and osteogenic differentiation of human bone marrow stromal cells on alginat–gelatine– hydroxyapatit scaffolds with anisotropic pore struture. J Tissue Engineering Regen Med 2009; 3(1): 54–62.
9. Draget KL, Smidsroed O, Skjak-Braek G. Alginates from Algae. In: Steinbuchel A, Rhoe SK. Polysaccharides, polyamides, in the food industry, properties, production and patents. KgoA Weinbeim: Wiley-VCH Verslag GmBH & Co; 2006. p. 30.
10. McHugh DJ. A guide to the seaweed industry. FAO Fisheries Technical Paper. 2003; 441: 105.
11. Ashley M, McCullagh A, Sweet C. Making a good impression: a 'how to' paper on dental alginate. Dent Update 2005; 32(3): 169–70.
12. Yulianto K, Setiapermana F, Pettipeilohy, Mansur A. Pengembangan pengolahan rumput laut. Laporan Triwulan II. Pusat Penelitian Oseanografi – LIPI; 2002. p. 9–12.
13. Sulistijo. Penelitian rumput Laut (Algae Makro/Seaweed) di Indonesia. Pidato Pengukuhan Ahli Peneliti Utama Bidang Marikultur. Jakarta: Pusat Penelitian Oseanografi – LIPI; 2006. p. 18–21.
14. Zatnika A. Proses ekstraksi dan manfaat alginat di bidang farmasi. Jurnal Sains dan Teknologi Indonesia 2003; 5: 143–50.
15. Parthiban C, Parameswari K, Saranya C, Hemalatha A, Anantharaman P. Production of sodium alginate from selected seaweeds and their physiochemical and biochemical properties. Asian Pacific J Tropical Biomedicine 2012; 1(4): 1–4.
16. Andersen T, Strand BL, Formo K, Alsberg E, Christensen BE. Alginates as biomaterials in tissue engineering. Carbohydr Chem 2012; 37: 227–58.
17. Mohan N, Nair PD. Novel porous, polysaccharide scaffolds for tissue engineering applications. Trends Biomater Artif Organs 2005; 18: 219–24.
18. Khalil S, Sun W. Bioprinting endothelial cells with alginate for 3D tissue constructs. J Biomech Eng 2009; 131(11): 111002.
19. Kakita H, Kamishima H. Some properties of alginate gels derived from algal sodium alginates. J Applied Phycology 2008; 20(5): 93–9.
20. Kuo C, Ma PX. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. Biomaterials 2001; 22(6): 511–21.
21. Stanford ECC. New substance obtained from some of the comonomer species of marine algae. Algin Chem News 1881; 47: 254–7.
22. Yulianto K. Pengaruh konsentrasi NaOH terhadap viskositas Natrium Alginat yang Diekstraksi dari Sargassum duplicatum JG Agardh (Phaeophyta). Oseanologi dan Limnologi di Indonesia 2007; 33: 295–306.
23. Hernandez CG, McHugh DJ, Arvizu-Higuera DL, Rodriguez-Montesinos YE. Pilot plant scale extraction of alginate from macrocystis pyrifera. Conversion of alginic acid to sodium alginate. Drying and milling. J Appl Phycol 2002; 14: 445–51.
24. Weiner S, Wolfie T, Wagner HD. Lamellar bone: structure-function relations. J Structural Biology 1999; 126: 241-55.