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*"Science and mathematics as proponents
for sustainable future"*

**The Fourth International Conference and
Workshops on Basic and Applied Sciences
(4th ICOWOBAS)**

&

**Regional Annual Fundamental Science
Symposium 2013 (11th RAFSS)**

**Le Grandeur Palm Resort
Johor, Malaysia
3-5 September 2013**

PROCEEDING OF THE
“4TH INTERNATIONAL CONFERENCE &
WORKSHOP ON BASIC AND APPLIED
SCIENCES”

&

“11TH REGIONAL ANNUAL FUNDAMENTAL
SCIENCE SYMPOSIUM 2013”

(ICOWOBAS-RAFSS 2013)

“Science and mathematics as proponents for sustainable future”

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Organized by

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Potency of Sargassum SP from Madura Strait as Irreversible Hydrocolloid Impression Material

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ABSTRACT

Sargassum sp is one type of brown algae which abundantly available on Madura Strait, Indonesia. Brown algae has been used is one of raw material source of natrium alginates. One type of brown algae found growing in waters of Indonesia is Sargassum sp. Sargassum sp as the member of division Phaeophyta could be found in Madura Strait. Alginate as irreversible hydrocolloid impression material is quite important in dentistry. The need of alginate is fulfilled by supply from abroad. Meanwhile, the availability of Sargassum sp is overflow. **Purpose** : This research aims are to explore the potency of brown alga Sargassum sp from its basic compound natrium alginate and to examine its mechanical characteristic and biocompatibility. **Methods** : The methods of this research including extraction natrium alginate from Sargassum sp, synthesis dental impression material and mechanical characteristic (compressive strength), and MTT Assay. **Results** : Extraction result is natrium alginate powder with cream colour, odorless, dissolved in water. The compressive strength is showed the best result 127,8 kPa in the sample with the addition of trisodium phosphate 4%, but this value was still far away from the value of control material. **Conclusion** : The dental impression material with natrium alginate from Sargassum sp from Madura strait has mechanical properties which still far away from the control but the biology characteristic of dental impression material based on natrium alginate from Sargassum sp has been showed excellent result (viability cell in the range 84,2 – 85,7 %). This means that the hydrocolloid impression material based on Natrium alginate from Sargassum sp extraction is non toxic.

| Sargassum sp | Irreversible Hydrocolloid Impression Material | Natrium Alginate | Compressive Strength | Non Toxic |

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1. INTRODUCTION

Indonesia is known as the world's second mega biodiversity country. The diversity is including flora, fauna and mine. There is the abundance of algae in the Indonesia territorial waters. One of the most important algae is Sargassum sp. Sargassum sp as the producer of natrium alginate, the main substance for dentistry irreversible hydrocolloid impression material could grow in the calm or wavy and craggy territorial. Sargassum sp. has cylindrical thallus shape or flattened, lots of branching resembles trees on land, leaves widened, tapering like a sword, has solitaire air bubble, the main stem round, somewhat harshly and the shape of holdfast is discoid. Its leaves edge is rarely serrated and wavy with tip is curved or tapered. Its colour is brown, relatively large-sized, grow and flourish on strong base substrate. The upper part resembles bilateral symmetrical shaped shrub or radial and equipped with growth part.¹

Sargassum sp. classification :

Kingdom : Plantae
 Divisio : Phaeophyta
 Class : Phaeophyceae
 Ordo : Fucales
 Family : Sargassaceae
 Genus : Sargassum
 Species : Sargassum sp.²

One of dental impression material is alginate. Alginate Impression material are widely used for a variety of applications. In prostodontics, they are used for recording

impressions of edentulous ridge. In orthodontics, they are used for recording impressions prior to appliances constructions and extensively for recording impressions for study model construction.³ Alginate has become the material of choice because the accuracy of line and shape reproduction, the comfortness of patient, and its easy mixing and modification.⁴ Alginate impression material is the irreversible hydrocolloid impression material. Irreversible hydrocolloid mean that if alginate has been mixed with other substance and the chemistry reaction has happened, then alginate could not back to its original form. Main component of hydrocolloid impression material is natrium alginate. If natrium alginate has been mixed with water then it would become sol form and as reactor could be added calcium sulphate. Diatom earth and silica gel as filler which has function to increase the strength, hardness, to influence setting time and physical properties of alginate gel. Accelerator and retarder material was needed to arrange setting time. Kalium sulphate was act as accelerator. Natrium or trisodium phosphate was act as retarder.⁵ PEG (Polyethylene Glicol) has been added to coat impression material powder so that the powder can not easily to steam like dust.

Research about the influenced of retarder which were trisodium phosphate and kalium oxalat to the alginate impression material was performed in 2008. Impression material with addition of trisodium phosphate 0,3 gram has been yield flatter surface, homogen, and the highest decomposition temperature which was 550°C. The availability of alginate before has been gained from abroad.

Brown algae were contained alginate. One of brown algae which abundantly seen in Indonesia territorial and has economical value is Sargassum sp. Sargassum sp. has potency to be utilized as producer of natrium alginate which has been known as raw material the making of alginate dental impression material. Some research has been done but the result has not used and produced directly as dental impression material. This research aims are to explore the potency of brown alga Sargassum sp from its basic compound natrium alginate and to examine its mechanical property and the biocompaibility of the material.

2. EXPERIMENTAL

2.2 Materials, method and instruments

The source of Sargassum sp were collected from Madura strait by cutting the holdfast as close to the attachment of possible. The other material are aquadest, water, HCl 5%, Na₂CO₃ 4%, NaOCl 12%, NaOH 10%, isopropanol (IPA), calsium sulphate, silica gel, calium sulphat, PEG, diatom earth, and trinatrium phosphate.

The process of this research has been divided into 2 parts. The first part is the extraction of natrium alginate and second part is synthesis of irreversible hydrocolloid.^{6,7} The extraction of natrium alginate has been done by this followed processes. Dry brown algae Sargassum sp. has been immersed in HCl 1% for 1 hour. After 1 hour of immersion in acid solution, brown algae has been washed. Then Na₂CO₃ 4% has been added and the mixture has been heated in the temperature 60°C for 2 hours. Brown algae then has been diluted with aquades and has been erated for ± 30 minutes. After that, brown algae has been filtered. The result then has been bleached and stirred with NaOCl 12% solution. HCl 5% then has been added until pH value reached 2-3 (acid). The next step is to filter to gain alginate acid in the form of foam wadding. The foam wadding has been washed with water to eliminate dangerous acid sludge and the NaOH 10% has been added until pH was 9. Alginate acid which has been convert to natrium alginate then has been added Iso Prophyl Alcohol (IPA) (99%) with the ratio 1:2 (IPA : acid alginate). Separated natrium alginate then has been filtered and dried. The extraction result in the form of natrium alginate powder then ready to be composed as impression material.

The second process of irreversible hydrocolloid impression material making has been done by mixing all the composition material using mortar and pestle. The composition material were consist of natrium alginate 19%, calsium sulphate 40%, calium sulphate 15%, diatom earth 4%, silica gel 15%, and PEG 7%. There were 5 variation trinatrium phosphate percentage of impression material sample which were 0% (sample A), 1% (sample B), 2% (sample C), 3% (sample D), and 4% (sample E). Samples examinations are consist of mechanical characteristic (compressive strength) and cytotoxicity test using MTT Assay.^{6,7}

FTIR test was performed to know the purity of natrium alginate sample from the Sargassum sp. First, sample is mashed and mixed with KBr with ratio 1 : 20 gram using tiny mortar until well-mixed. Sample then constituted into thin pellet with the thickness less than by pressing equipment. Sample then is inserted to the tube inside FTIR machine and is illuminated by Jasco FTIR 4200. The data could be read as absorption FTIR graph.

The mechanical characteristic of irreversible hydrocolloid impression material is compressive strength. Compressive strength is the maximum strength of material in receiving the pressure. Compressive strength is calculated by comparing force to cross sectional area in the sample which receiving this force (stress). The compressive strength is performed by pressing the sample in the hard table until it was crushed. The difference between the lowest load until the highest load must be recorded. The highest load which could be received until it was destructed is the maximum load which could be outstayed by the sample. The equipment for this compressive strength is Autograph AG-10TE Shimadzu. The procedure of compressive strength are measuring length and width as the data of surface area (A). The test is performed until the sample is cracking. The data as force (F) with denomination kN. Data as compressive strength (σ) then is processing by the formula :

$$\sigma = \frac{F}{A}$$

One of the requirement of medical and dentistry material is the biocompatibility of the material. The material must be non toxic, non allergenic and could be accepted by the human body. The cytotoxicity test was performed using BHK-21 culture cell. Samples are dental impression material with variety of trinatrium phosphate 0%, 1%, 2%, 3%, and 4%. Media control are from the mixture of eagle and bovine serum. Cell control are the mixture of BHK-21 and eagle. BHK-21 cell was stored at 85°C in nunc bottle with eagle media and was placed in incubator at 37 °C. Then it was centrifugated with 3000 rpm for 10 minutes. Deposition which was cell culture has been token and the supernatant which was media has been discarded. Cell culture ± 20 ml has been inserted to small roux bottle and has been added eagle media and sample inside it. Then it was inserted to the CO₂ incubator with 37 °C for 24 hours. Cell culture was divided to the 96 microwell plate. 1,5 ml in each well has been added eagle media and sample, then they have been inserted to CO₂ incubator with 37 °C for s24 hours. After confluent, media was discarded and washed by PBS and MTT. Microwell plate then has been incubated for 4 hours. Cell culture then has been emitted from CO₂ incubator then DMSO (dimetil sulfoxida) has been added. DMSO's act as stopper of reaction between cell and MTT. Cell culture then to be place in shaker and the reaction has been observe using Elisa Reader Thermo Scientific-Multiskan EX. Observation result of Elisa Reader in the form of table and the cellular proliferation inhibition rate (CPIR) was calculated using the following formula: CPIR = (1 - average A value of experimental group / average A value of control group) × 100%.⁸

3. RESULTS & DISCUSSION

Extraction result is sodium alginate powder with cream colour, odorless, dissolved in water. Fourier Transform Infra Red (FTIR) result is showed that the extraction of *Sargassum* sp succeed to form sodium alginate (Figure 2). Extraction of sodium alginate from *Sargassum* sp. refers to modified extraction method.^{6,7} Drying process was using freeze drying. Sodium alginate powder was cream in colour, odorless, dissolved in water. This result was matched with Farmakope requirement 1974.⁹ Sodium alginate powder has been tested using FTIR to confirm extraction result of sodium alginate. FTIR spectrum (Figure 2) of sodium alginate ($C_6H_7O_6Na$)_n was showed by absorption peak in the frequency 3465,4, 1658,48, 1413,57, and 1026,91 cm^{-1} . According to the PAVIA¹⁰ absorption peak 3.500 cm^{-1} - 3200 cm^{-1} is specific for hydroxyl group (O-H), absorption peak 1600 cm^{-1} – 1680 cm^{-1} for carbonil (C=O) group and absorption peak between 1000 – 1300 cm^{-1} for carboxyl group (CO). Sodium in the isomer alginate was located in absorption peak 1614 cm^{-1} and 1431 cm^{-1} .¹¹ Based on the peak, it could be confirmed that the powder from synthesis result of brown algae *Sargassum* sp was sodium alginate.

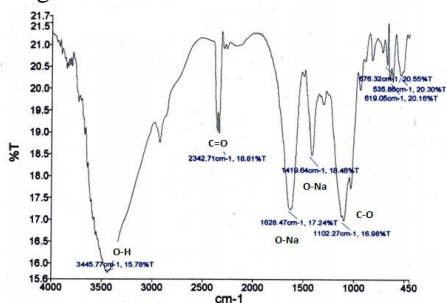


Figure 1. Control sample (Sigma Aldrich)

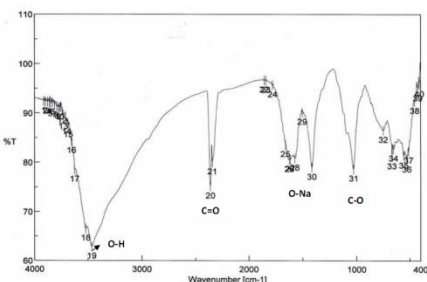


Figure 2. Sample with Na alginate from *Sargassum* sp

Compressive strength is the capacity of a material or structure to withstand loads tending to reduce size. It can be measured by plotting applied force against deformation in a testing machine. Some material fracture at their compressive strength limit; others deform irreversibly, so a given amount of deformation may be considered as the limit for compressive load. Compressive strength is a key value for design of structures. Table 1 is presenting the compressive strength value of samples. According to this table, the

compressive strength value of samples were lower than control material.

Table 1 Table compressive strength of impression material

Sample	A (m^2)	F (N)	δ (kPa)
Control	0,00009	23,5	261,1
A+0	0,00012	5,5	45,8
B+1	0,000096	2	20,8
C+2	0,000091	4,5	49,5
D+3	0,000091	6	65,9
E+4	0,00009	11,5	127,8

A = commercial product of alginate (Sigma Aldrich)

B = 1% trisodium phosphate

C = 2% trisodium phosphate

D = 3% trisodium phosphate

E = 4% trisodium phosphate

Compressive strength of impression material were 45,8-127,8 kPa.¹² This value is far away from the control value which was 261,1 kPa. This is because the sample was brittle compared with control. Compressive modulus and strength increased with polymer concentration. Improvement in mechanical properties with increasing alginate concentration was attributed to the increase in polymer chain density and entanglement.¹³

Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT Reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation. MTT is yellow soluble molecules, which could be used to measure cellular enzymatic activity, based on the ability of viable cell to reduce MTT salt. The mechanism is that the yellow tetrazolium salt would be reduce in the cell which has metabolic activity. Mitochondria of viable cells play important role in this process to yield dehydrogenase. If dehydrogenase is not active because of cytotoxicity effect, then formazan would not be formed. The level of formazan

is in line with the enzymatic activity of viable cell. Table 2 is presenting the percentage of viability cell of samples.

Table 2 The percentage of viability cell between control material and samples based on Natrium alginate from *Sargassum* sp

Sample	Percentage of viability cell (%)
Control	65
A+0	84,2
B+1	83,9
C+2	85
D+3	85,7
E+4	85

According to MTT test, we can conclude that all sample is non-toxic because the percentage of viable cell were above 50%.¹⁴

4. CONCLUSION

It is concluded, therefore, that this research has been succed to extract natrium alginat from *Sargassum* sp, and showed the mechanical property which still far away from the control but the biology characteristic of dental impression material based on natrium alginate from *Sargassum* sp has been showed excellent result. This material is non toxic related on the viability cell value has mechanical properties which almost fulfilled requirements eventhough still need further research to optimize the mechanical property and biocompatibility.

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