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Production of Alkali Treated Cottonii from *Eucheuma Cottonii* on Pilot Plant Scale Using Response Surface Methodology

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ABSTRACT

Alkali Treated Cottonii (ATC) is processed from red algae (*Eucheuma cottonii*) with alkaline treatment. Important factors affecting the commercial production of ATC are KOH concentration, temperature and process time. Variation in alkaline treatment conditions process will effect the yield and gel strength of the ATC. The optimum conditions for the alkaline treatment were KOH concentration, temperature and process time from Eucheuma cottonii were determined using response surface methodology (RSM). Based on the RSM approach is known that KOH concentration, temperature and process time had a significant influence on the seaweed alkaline treatment to ATC. Optimum alkaline treatment conditions obtained from KOH concentration (7%), temperature 78.81ºC and process time 2 h. Under the optimal conditions obtained the yield of 30.62% and gel strength of 1.114.69 g.cm^{-2}.

Keywords: alkalinization, ATC,carrageenan, pilot plant, RSM
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1. INTRODUCTION

Since the declaration of seaweed in the revitalization program, that the government seeks to develop downstream industries of seaweed through the development industry of value added is ATC. These efforts in order to reduce foreign exchange, and facilitate seaweed communities generally live in coastal areas. However, these efforts still need to be improved related to the high demand for Indonesia would carrageenan, which is reflected in the volume of imports reached 1, 320, 818 tons in 2011.

ATC is processed seaweed *E. Cottonii* that have undergone a process of alkalinization. The use of alkaline the process of eliminating a role in the processing of six sulfate groups of the monomer units into 3,6-anhydro-D-galactose, there by increasing the gel strength [13,2,7]. In addition it serves to prevent alkaline hydrolysis of carrageenan [6]. KOH chosen because of the effects of the kappa carrageenan cations that produce the gel is stronger than the other alkali such as NaOH and $Ca(OH)_2$ [20,4,15]. KOH concentration, temperature and duration of the alkalinization process will determine the quality of the resulting ATC.

Some research on the treatment of ATC has been done that further development is more focused on the research on a pilot plant scale. Pilot plant scale is a scale to obtain the optimal operation and precise control before heading to the commercial production or industrialization. On a scale pilot plant is expected characteristics of the product obtained is not much different from the laboratory scale, although the equipment and conditions between the two different processes. Therefore, it is necessary to test the optimum conditions were obtained at laboratory scale to pilot plant scale, so that later can be obtained optimum processing conditions ATC with a pilot plant scale.

The purpose of this study is to obtain alkalinization process technology of ATC on a pilot plant scale in order to obtain the ATC with a value of yield and gel strength is optimal. The resulting process technology can be used as a reference for applied and developed on a scale of SMEs, with the hope of producing carrageenan can meet the quality standards required for carrageenan.

2. Methodology

Dried seaweed (*E. cottoniii*) was harvested in January 2014 in Jeneponto, Indonesia. In the laboratory, all of the seaweeds were washed with tap water to remove the salt, sand and attached epiphytes. The process was repeated twice. The 'clean seaweed' sample was processed further. Potasium hydroxide was used to process dry seaweed to be ATC was purchased from sentana store in Makassar.

A. Design of ATC on pilot plant scale

Alkaline treatment tank (200 L) used on pilot plant scale is made of a stainless steel vessel and cylindrical double jacket with a water heater that is placed between the cylinder. A temperature sensor is placed inside the vessel to control the temperature. The alkaline treatment tank was heated by burner (premium oil) and it was placed outside of the alkaline treatment.

B. ATC processing

The 'clean seaweed' sample (20 kg) were cooked in hidroxide (KOH) solution. The cooking temperature was controled to be constant, whereas the KOH concentration and cooking time were fixed to the desired value. The process condition alkaline treatment design which will be explained in Table 2. After the alkaline treatment, the seaweed were neutralized by washing under running tap water until they had a pH of about 8-9 [8]. The material was then chopped. Finally, the ATC were recovered and dried at sun for 2-3 days, weighed and milled into fine powder to pass 60 mesh.

C. Determination of yield

The ATC yield (%) was determined according to the formula:

$$Yield\ (\%) = \frac{Wc}{Wm}.100$$

where Wc is the ATC weight (g) and Wm is the dry seaweed weight (g) used for alkali treatment [16].

D. Determination of gel strength

Gel strength was determined using a Texture analyzer (TAXT Plus, Stable Micro Systems Ltd., Surrey, England). ATC solution was prepared by dissolving 3 g of ATC powder and KCl 0.6 g to 197 ml of distilled water with continuous magnetic stirring at 80ºC for 15 min. For the gel strength analysis, this solution was placed in plastic tubes (40 mm diameter, 50 mm height, flat bottom) which were kept under refrigeration 10ºC for 16 hours before analysis. All analyses were carried out in duplicate [12].

E. Experimental design and statistical analysis

Before the development of the study through RSM, a first set of tests were performed to select the relevant factors for independent variables including KOH concentration (A), temperature (B), and time (C) as shown in Table 1. The ranges of KOH concentration (6-8 %), temperature (70-90 °C), and time (1- 3 h) were obtained based on the results of preliminary experiments. The model proposed for the response Y is given below:

Y=x0+x1A+x2B+x3C+x4A2+x5B2+x6C2+ x7AB+x8AC+x9BC

where x0 was offset term, x1, x2 and x3 were related to the linear effect terms, x4, x5 and x6 were connected to the quadratic effects and x7, x8 and x9 were associated with the interaction effects.

The ATC processing included important processes, process conditions alkaline treatment

KOH concentration (%), temperature (ºC,B) and time (h,C) were independent variables studied to optimize yield and gel strength of *E. cottonii* extracts. The extraction yield (%) and gel strength ($g.cm^{-2}$) are the most important things for ATC production. These two responses were selected together as the dependent variables for the combination of the independent variables. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. A Box Behnken design consisting seventeen experimental runs was employed including five replicates at the center point[14]. Seventeen extraction condition combinations were generated by the software program of Design Expert version 7.0® (DX 7.0®) as shown in Table 2.

Table 1 Experimental design range and values of the independent variables in the RSM with Box-Behnken design

Independent variabels	Symbol	Coded level	
		-1	1
KOH concentration (%)	A	6	8
Temperature (°C)	B	70	90
Time (h)	C	1	3

F. Verified Model

The adequacy of the polynomial model was expressed by the multiple coefficient of determination, R^2. The significance of each coefficient was determined by using F value and P value. Optimizations of alkali treatment conditions including the KOH concentration, temperature, and process duration for maximizing a quantitative yield and gel strength determination of ATC were calculated by using the predictive equation from RSM. The optimum condition was verified by conducting experiments under these conditions. The response was monitored and the results were compared with the model predictions.

Table 2 Experimental design range and values of the independent variables.

Runs	A	B	C
	KOH concentration (w:v)	Temperature (ºC)	Time (h)
1	6	70	2
2	8	70	2
3	6	90	2
4	8	90	2
5	6	80	1
6	8	80	1
7	6	80	3
8	8	80	3
9	7	70	1
10	7	90	1
11	7	70	3
12	7	90	3
13	7	80	2
14	7	80	2
15	7	80	2
16	7	80	2
17	7	80	2

3. RESULT AND DISCUSSION

The effect of alkaline treatment conditions were independent variables studied to optimize yield and gel strength of ATC on pilot plant scale. The experimental results were shown in Table 3. The yield and gel strength of ATC which was alkaline treatment with different process conditions is showed that yield of ATC are in the range 22.49-33.68% and the gel strength values were 790.60-1281.95 $gr.cm^{-2}$. The yield value obtained were within ranges reported by [18] for K. alvarezii strain brown from Sao Paulo, Brazil (25–35%); [1] for Hypnea bryoide in Oman (30.05-33.16%); [20] for Furcellaria lumbricalis and Coccotylus truncatus (27-31%); [21] for K. alvarezii (31.17%). Whereas the amount of gel strength content obtained in the present study was considerably higher than that reported by [20] (350 g.cm-2); [7] (168.86 g.cm^{-2}); [3] (1279 g.cm^{-2}).

The summary of the results obtained from the effect of independent variables; The KOH concentration, temperature and time on each independent from Box-Behnken design are shown in Table 4.

The ANOVA of the quadratic regression model indicates the model to be significant. There is only a 0.05% chance that a "Model F-Value" this large could occur due to noise. Model P value (Prob>F) is very low (<0.0005). The lack of fit is an indication of the failure for a model representing the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error14]. If there is a significant lack of fit which could be indicated by a low probability value, the response predictor is discarded. The lacks of fits model were not significant (P>0.05) meaning that these models were sufficiently accurate for predicting the relevant responses [9].

Coefficient of determination R^2 is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for a good fitted model [11,9]. The value of determination coefficient R^2 = 0.8861 (yield) and 0.9033 (gel strength) implies that the sample variations of 88.61% for the yield and 90.33% for the gel strength of carrageenan are attributable to the independent variables, namely the KOH concentration, temperature and time. Thus, the analysis of variance showed that the predicted 2nd order models were statistically suitable.

A. Effect of alkaline treatment conditions on yield of ATC

The parity plot (Fig. 2 and Fig 5) showed a satisfactory correlation between the experimental and predicted values of ATC yield and gel strength wherein the points cluster around the diagonal line indicated the good fit the model, since the deviation between the experimental and predicted values minimal [11]. Fig. 3 shows the combinations of components that affects each of the yield values through the different colors.

Table 3 Results of experiment

Formula	KOH (%)	Temperature (°C)	Time (h)	Yield (%)	Gel strength (g/cm^2)
1	6	70	2	24.38	1257.40
2	8	70	2	31.49	1062.60
3	6	90	2	26.77	1281.95
4	8	90	2	28.81	1036.10
5	6	80	1	23.51	891.45
6	8	80	1	23.96	820.85
7	6	80	3	27.42	1019.30
8	8	80	3	30.88	805.75
9	7	70	1	22.49	957.25
10	7	90	1	23.69	976.40
11	7	70	3	30.49	790.60
12	7	90	3	28.29	902.40
13	7	80	2	30.99	1190.35
14	7	80	2	27.86	1134.85
15	7	80	2	32.22	1085.75
16	7	80	2	32.36	1053.60
17	7	80	2	33.68	1061.95

Table 4 ANOVA for surface response model

Response	Model	Equation	Significant Model (P<0.05)	*Lack of fit* (p>0.05)	R^2
Yield	*Quadratic model*	Yield = $1.63A - 0.16B + 2.93C - 1.27AB + 0.75AC - 0.85BC - 1.68A^2 - 1.88B^2 - 3.30C^2$	Significant (0.013)	Not significant (0.8474)	0.8861
Gel strength	*Quadratic Model*	Gel strength = $-90.60A + 16.13B - 15.99C - 12.76AB - 35.74AC + 23.16BC + 15.94A^2 + 38.27B^2 - 236.91C^2$	Significant (0.008)	Not significant (0.2386)	0.9033

The blue color indicates the values of lowest yield, whereas the red color indicates the values of highest yield. Line consisting of the points on the contour plot is a combination of three components of formula with different proportions that generates a response value of the same yield. Response surface for the effect of KOH concentration and temperature on the yield of ATC is presented in Fig.4.

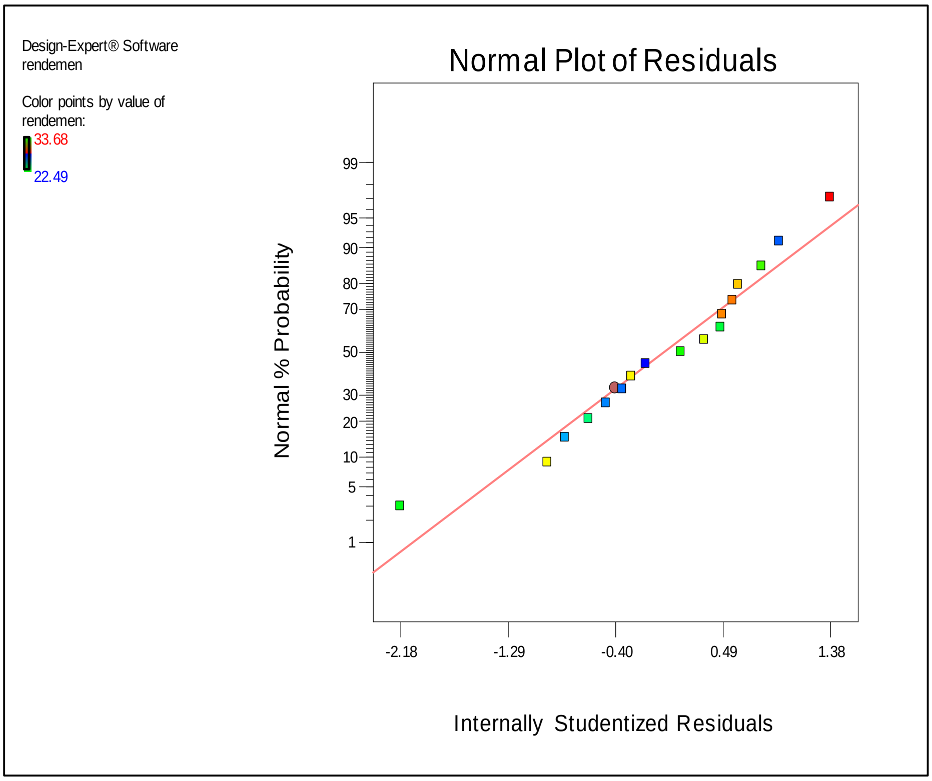

Fig. 1. Normality of internally studentized residuals of ATC yield

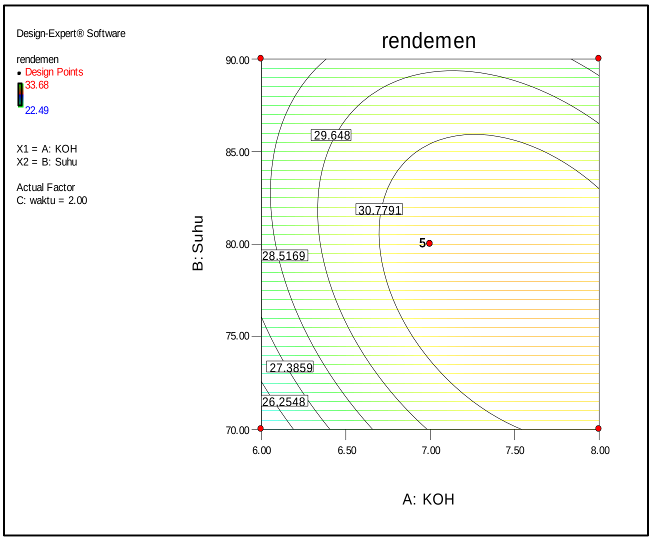

Fig. 3 Countour plot for the effect of KOH concentration and temperature on the yield of ATC.

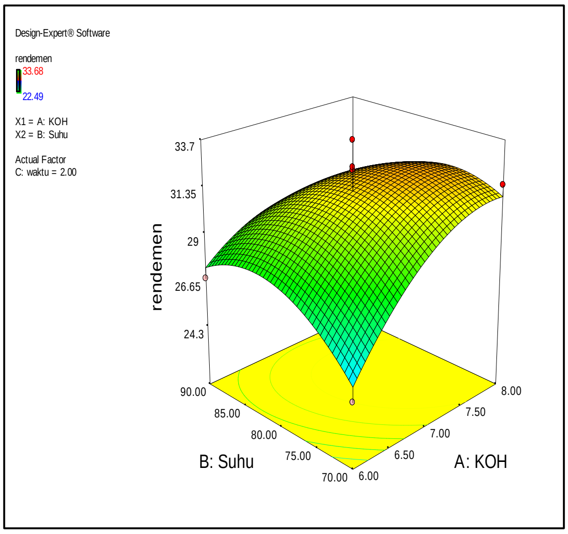

Fig. 4. 3D graphic surface optimization of ATC yield versus KOH concentration and temperature.

The effect of alkaline treatment conditions on ATC yield is shown in Fig. 3 and 4. The analysis of the results showed that the KOH concentration was the only highly significant factor affecting the yield of ATC. ATC yield showed strong positive correlation with KOH concentration. It was found that with the increasing KOH concentration the yield will be increasing. It has also been cited that increase in polysaccharide yield is due to the strong effect of extraction time–temperature on the mass transfer rate of the water soluble polysaccharides in the cell wall [22]. Further increase of temperature leaded to the decreasing the yield of ATC. This was due to higher temperature could have resulted in some degradation of the polysaccharide [21. The decrease of yield of carrageenan products has been reported to be a result of heating temperature higher gave high solution concentration and it was difficult to separate between filtrate and residue [19].

B. Effect of process conditions on gel strength of ATC

Fig. 6 shows the combinations of components that affect each of the gel strength values through the different colors. The blue color indicates the values of lowest gel strength whereas the red color indicates the values of highest gel strength. Line consisting of the points on the contour plot is a combination of three components of formula with different proportions that generates a response value of the same gel strength. Response surface for the effect of KOH concentration and temperature on gel strength of ATC is presented in Fig. 7.

The effect of KOH concentration indicated that decrease in gel strength up to certain values. However, further increase in temperature and extraction time showed a increase in gel strength of ATC. This was suspected due to higher temperature causing carrageenan degradation, thereby producing carrageenan fragments and lowering gelling ability. Similar results have been reported for other Gracilaria cliftonii and different samples [10].

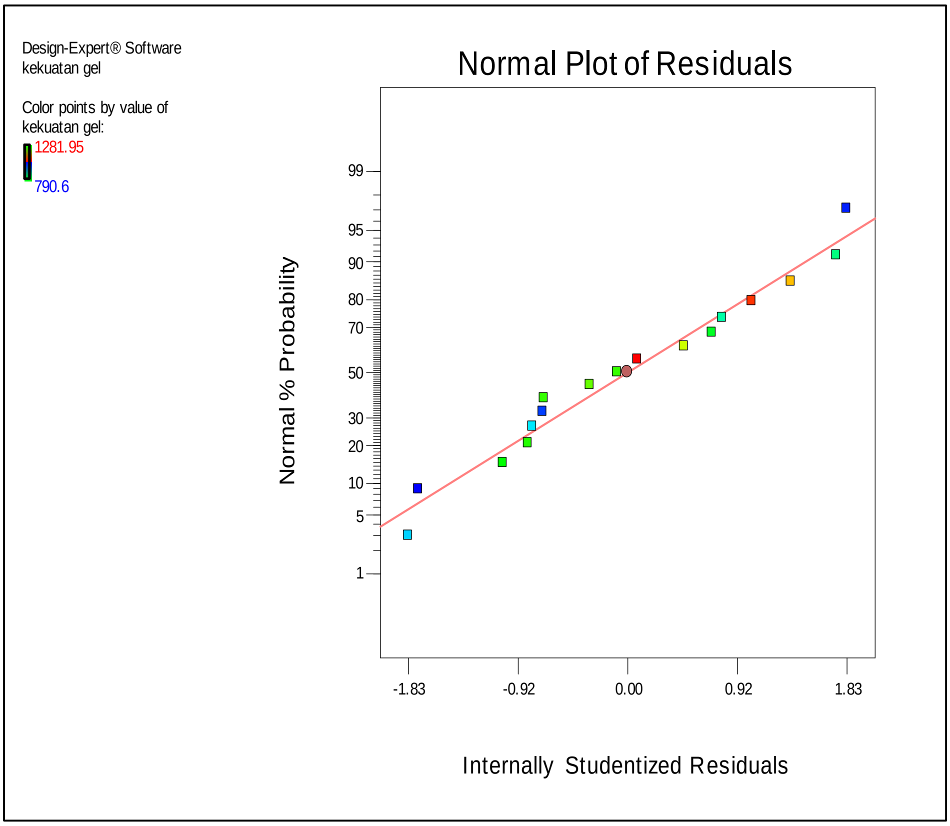

Fig. 5 Normality of internally studentized residuals of ATC gel strength

C. Optimization of process conditions pilot plant scale

The optimization of multiple responses was developed using desirability functions with the responses to be maximized [23]. Table 5 shows the components and optimized a response, the target value, the minimum and maximum limits, as well as the importance of the optimization phase of the formula with program of DX 7.0®. The final results of the optimization phase in the form of a new extraction conditions is determined based on predetermined targets. The program will build solutions to desire a different value. The higher a desirability value (approaching 1) means that the optimal conditions for the process to produce an optimal response.

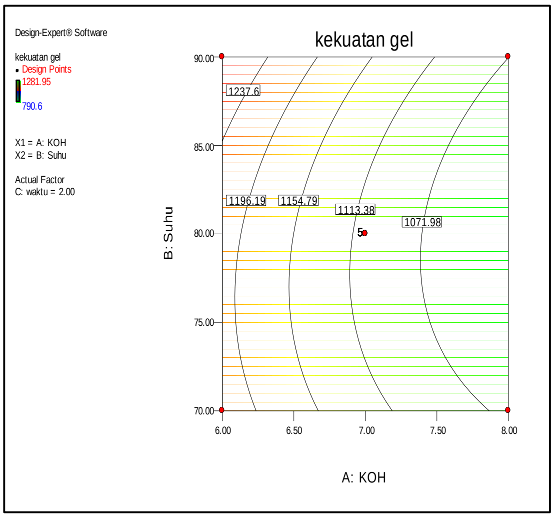

Fig. 6 Contour plot for the effect of alkali treat ment the KOH con centration to tempe rature on gel strength of ATC.

Table 5. Components and optimized response, targets, limits, and importance in the optimization phase formula.

Components & response	Goal	Lower limits	Upper limits	Impor-tance
KOH concentration (%)	In Range	6	8	3 (+++)
Temperature (°C)	In Range	70	90	3 (+++)
Time (h)	In Range	1	3	3 (+++)
Yield (%)	Maximize	22.49	33.68	5 (+++++)
Gel strength (g.cm-2)	Maximize	790.6	1281.95	5 (+++++)

On the optimization process, the recommended process conditions are KOH concentration 7%, temperature 78.81 °C and times of 2.06 h. Under the optimal conditions obtained the yield of 31.57% and gel strength of 1102.23 g.cm^{-2}.

D. Verified of Model

In the verified stage, the actual response values obtained were compared with the predicted value of the response generated by the program. Table 6 shows that the suitability of the model equation for predicting the optimum response values was tested using the selected optimal conditions. Results of verified solutions are not exactly the same, but all the values were within ranges between 95% prediction interval low and 95% prediction interval high. Thus the that the empirical models developed were reasonably accurate. The 95% prediction interval is the range in which we can expect any individual value to fall into 95% of the time [17].

4. CONSLUSION

The Response Surface Methodology (RSM) can effectively be used to determine the optimum process conditions for ATC production on pilot plant scale from *E. cottonii*. Process condition with alkaline treatment was optimized with maximize the

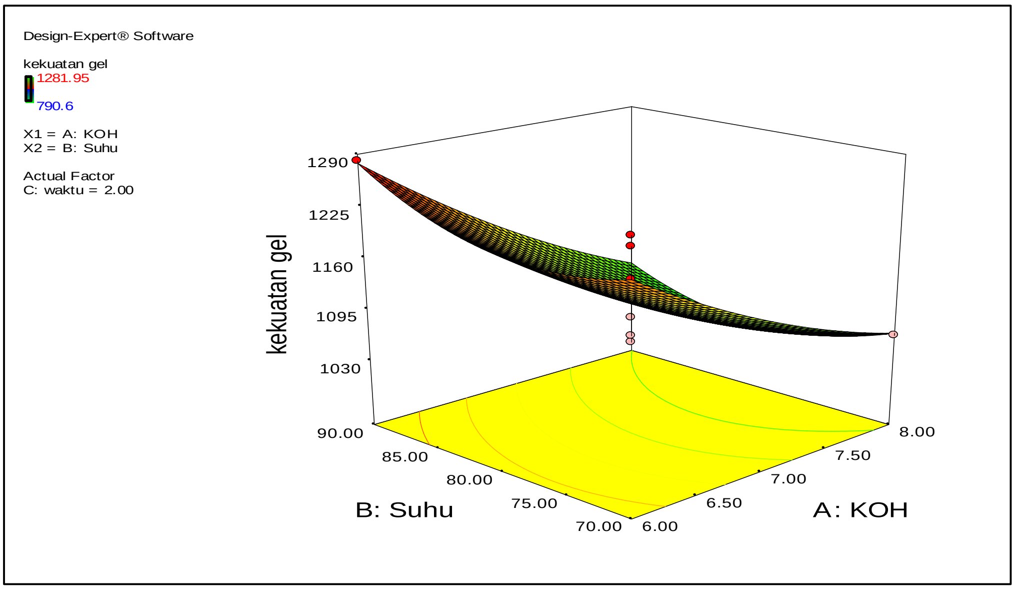

Fig. 7. 3D graphic surface optimization of ATC gel strength versus ratio of KOH concentration and temperature

yield and gel strength content. Second-order polynomial models were obtained for predicting extraction yield and gel strength. Using the contour plots, the optimum set of the operating variables are obtained graphically in order to obtain the desired levels of the carrageenan properties which is suitable for the further development and aplicated for the construction industry in the form of Small Medium Business. Optimum process condition for maximizing yield, and gel strength were the KOH concentration (7%), temperature (78.81°C) and process time (2 h). In these conditions the ATC product obtained with the yield 30.62% and gel strength 1,114, 69 g.cm^{-2}.

Table 6. Comparison of predicted and actual response value verified optimum process conditions with Box-Behnken design

Response	Formula			
	Predicted	Verified	95%PI Low	95%PI High
Yield(%)	31.57	30.62	29.65	33.50
Gel strength (g.cm^{-2})	1102.23	1.114.69	1028.85	1175.61

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