Optimizing pre-cooking treatment condition for reducing lead (Pb) content in Seaweed (Gracilaria sp.)

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Abstract. Seaweeds are increasingly used in Indonesian cuisines due to their nutritional value and health benefits. In contrast to these benefits, they accumulate lead during their growth and exhibit potential health risks upon ingestion. To overcome these risks, some appropriate pre-cooking treatments are desired to reduce lead content. Sample of Gracilaria sp. will be treated by soaking before cooking. This study applied the Taguchi method to determine optimum pre-cooking treatment conditions to reduce the level of lead in Gracilaria sp. The control factors included soaking temperature, time reaction, potential of hydrogen and water volume are used for this research. The levels of lead were determined by Atomic Absorption Spectroscopy (AAS). After these processes, lead contents were found to be successfully reduced and the results show that the optimal conditions during the soaking process can reduce the levels of lead of Gracilaria sp. until 56%.

1. Introduction
Indonesia has several varieties of seaweed that are abundant, and their cultivation has the potential to improve the country's economy. Food and Agriculture Organization (FAO) shows for the year 2015, Indonesia has emerged as the second largest producer of cultured seaweed in the world following China. Of the global production of farmed aquatic plants (largely seaweeds) at around 29.4 million tons, Indonesia contributed almost 38 percent (11.3 million tons) compared to China’s 47 percent (14 million tons) [1, 2]. Nowadays, seaweed in Indonesia are not only valuable source of natural products for commercial importance but also source of food for daily consumption. Seaweeds are proved to be the rich sources of minerals elements and recommended as food supplements to help meet daily intake of essential mineral and trace elements [3]. Because of these benefits, seaweeds are increasingly used for cuisines and can eaten raw, cooked or processed.

On the other hand of the benefits, some seaweed species show a high affinity for metals and can accumulate metals during their growth. In some condition, seaweeds have been used as bioindicators of metal pollution in estuarine and coastal waters. Metal pollution can be derived from industry, agriculture, and domestic wastes that disposals into the waters without any standardized waste treatment process. One of the toxic heavy metal is lead (Pb). The impact of toxic elements like Pb on human health is dangerous enough. These elements can be very damaging even at low levels when ingested over a long period time. Gracilaria sp. is one of the most cultivated seaweed species in Indonesia which has
the ability to absorb metals and can be consumed. The level of metal contaminants is an important determinant for the safety of edible seaweeds [4, 5].

Several studies have shown that processing practices may have a positive effect on the reduction of toxic elements in foodstuffs. Processing practices such as washing, soaking, drying, grilling, boiling or cooking can effectively remove heavy metals in some foodstuffs [6]. Process to make agar and geluring from seaweed also reported to be able reduce heavy metals from seaweed [7, 8]. Washing and soaking are pre-cooking treatments that must be passed before cooking treatment on seaweed products. Control factors during soaking including temperature, time, hydrogen potential, and water volume need to be analysed to determine the role of each factor to reduce heavy metals in seaweed.

2. Methods

2.1. Material

Material that used in this study was Gracilaria sp., which was taken from Tanjungsari Hamlet, Kupang Village, Jabon District, Sidoarjo Regency, East Java. Gracilaria sp is cultivated in brackish water ponds and has been known to be contaminated with Pb due to the flow of the Porong river as a discharge from Lapindo mud [7].

![Figure 1. Source of seaweed (Gracilaria sp.) samples in this study. The specific location at the pond is shown in the red area.](image)

Sample of Gracilaria sp was taken using the quadrant transect method. The transects were randomly placed at the station that was determined, then the samples were analyzed with AAS to determine the initial lead content.

2.2. Design experiment using Taguchi methodology

Taguchi was employed as an optimization technique, with designed a system of distinct orthogonal arrays where decreases the number of experiments without negative impact on the range of operating conditions and keeping all the necessary information intact. Many design arrays can be implemented using Taguchi methodologies such as L4, L8, L9, L12, L16, L18, L27, and L64 with a central focus on the main effects on a process [9]. In the present experimental investigation, four controllable factors were considered with each factor at three levels as shown in Table 1. An L9(3^4) orthogonal array designed by Taguchi has been used to determine the optimum experimental conditions for maximum reduce of heavy metal. Nine groups combination of coded parameters has been performed at Table 2.

| Table 1. Controllable Factors and Their Levels |
|-----------------------------------------------|
| Factor | Description | Levels |
|--------|-------------|--------|
|        |             | L1     | L2     | L3     |
| A      | Temperature (°C) | 30     | 50     | 70     |
| B      | Time (minutes)    | 30     | 60     | 90     |
2.3. S/N Ratio and ANOVA

Another advantage of Taguchi method is its ability to compare and quantify the effect of factors on a process. These attributes can be explored with the aid of analysis of variance (ANOVA) or analysis of signal-to-noise ratio (S/N ratio). Using ANOVA and S/N ratio, the significant effects of key factors on the response can be determined, and the best factor levels for a given process can be identified from the selected factor levels as the optimum parameters for the process [10].

Depending on the desired output quality characteristics, three types of Signal-to-Noise (S/N) ratios are available: (1) smaller is better, (2) nominal is best, and (3) larger is better [11]. Since the present study involves maximizing the reduction of heavy metal, the S/N ratio for the case of ‘larger is better’ was evaluated as per the formula is given below:

\[
\frac{S}{NR} = -10 \log_{10}(\frac{1}{n} \sum_{i=1}^{n} \frac{1}{y_i^2})
\]

where ‘n’ represents total number of replications of each test run and \(y_i\) represents the percentage removal of heavy metal realized in replication experiment ‘i’ carried out under the same experimental conditions of each test run.

### Table 2. Test Runs

| Run | Temperature (°C) (A) | Time (minutes) (B) | pH (C) | Water Volume (D) |
|-----|----------------------|--------------------|--------|------------------|
| 1   | 30                   | 30                 | 5      | 1:10             |
| 2   | 30                   | 60                 | 7      | 1:15             |
| 3   | 30                   | 90                 | 9      | 1:20             |
| 4   | 50                   | 30                 | 7      | 1:20             |
| 5   | 50                   | 60                 | 9      | 1:10             |
| 6   | 50                   | 90                 | 5      | 1:15             |
| 7   | 70                   | 30                 | 9      | 1:15             |
| 8   | 70                   | 60                 | 5      | 1:20             |
| 9   | 70                   | 90                 | 7      | 1:10             |

However, identification of the most influenced factor and contribution of each of the factors on the yield is not possible using S/N ratio analysis. The limitations of S/N ratio analysis were achieved by carrying out ANOVA of the data according to:

\[
\text{Contribution of factor, } \% = \frac{SS_f}{SS_T} \times 100
\]

where \(SS_f\) is the sum of squares of factors, and \(SS_T\) is the total sum of squares of all the parameters.

A validation test with five trials was carried out with the set of optimum parameters. The variation was checked between predicted and experimental response.

2.4. Lead Determination

Response of this experiment is the level of lead that was measured by using Atomic Absorption Spectroscopy (AAS). Flame atomic absorption spectroscopy, FAAS, is a well-known quantitative elemental analysis method for a wide range of samples. It is simple, inexpensive, rapid, and applicable to wide range of samples [12].

3. Results and Discussion
The percentage reduction of lead was determined by taking the average of the lead reduction realized in experimental runs replicated thrice under the same experimental conditions in the order shown in Table 3.

### Table 3. Percentage reduction of lead with conditions determined by orthogonal array

| Experiment | Parameter Code | Experiment | R1 | R2 | R3 |
|------------|----------------|------------|----|----|----|
| 1          | 1 1 1 1        | 39,584     | 39,824 |
| 2          | 1 2 2 2        | 54,572     | 53,929 |
| 3          | 1 3 3 3        | 51,738     | 50,252 | 50,605 |
| 4          | 2 1 2 3        | 43,942     | 43,589 | 43,199 |
| 5          | 2 2 3 1        | 47,254     | 48,199 | 48,048 |
| 6          | 2 3 1 2        | 43,589     | 43,602 | 44,610 |
| 7          | 3 1 3 2        | 45,567     | 43,589 | 44,610 |
| 8          | 3 2 1 3        | 49,219     | 48,287 | 48,514 |
| 9          | 3 3 2 1        | 50,038     | 50,151 | 49,861 |

The average of percentage reduction in lead from nine group combination is around 39.80% to 54.42% (Table 4). Based on significant difference test, the highest average of reduction is group combination 2 which has 54.42% differentiation compared to the lowest average of percentage reduction in group combination 1 with 39.80%.

### Table 4. Mean ($\bar{y}$) and S/N Ratio

| Exp | R1   | R2   | R3   | $\bar{y}_{Exp} = \frac{\sum_{i=1}^{3}y_1}{3}$ | $S_{N/R} = -10 \log_{10}\left(\frac{\sum_{i=1}^{3}1}{\sum_{i=1}^{3}y_i^2}\right)$ |
|-----|------|------|------|---------------------------------------------|--------------------------------------------------|
| 1   | 40,013| 39,584| 39,824| 39,807                                      | 31,999                                            |
| 2   | 54,572| 54,761| 53,929| 54,421                                      | 34,126                                            |
| 3   | 51,738| 50,252| 50,605| 50,865                                      | 34,126                                            |
| 4   | 43,942| 43,589| 43,199| 43,577                                      | 32,784                                            |
| 5   | 47,254| 48,199| 48,048| 47,834                                      | 33,594                                            |
| 6   | 43,589| 43,602| 43,955| 43,715                                      | 32,812                                            |
| 7   | 45,567| 43,589| 44,610| 44,589                                      | 32,980                                            |
| 8   | 49,219| 48,287| 48,514| 48,673                                      | 33,745                                            |
| 9   | 50,038| 50,151| 49,861| 50,017                                      | 33,982                                            |

S/N Ratio graphic response of percentage reduction in lead with ‘larger the better’ quality characteristic from nine group combination ranged from 31.99 – 34.71. The S/N Ratio highest value of ‘larger the better’ percentage reduction is 2, and the lowest is 1. This proves that the combination factor level of concept 2 has the biggest effect on the variety of reduction response and must be controlled to meet the expected quality. Group combination 2 consists of temperature 30ºC, with time 60 minutes, in pH 7 and ratio seaweed : water volume is 1:15.

#### 3.1. S/N Ration Approach

Besides the average calculation and S/N Ratio of percentage reduction in lead, the calculation of factor effect and S/N Ratio were also conducted to identify the effect level from factor towards the average of observed quality. The average effect of every level from each factor has resulted from the calculation of the average quality value from the contained product concept. The average effect of level from each factor then subtracted with the highest score with the lowest score to find the deviation as the average effect value from the factor level [13].
As mentioned before, the character of S/N Ratio used in this paper is the ‘larger the better’. Therefore, the greater delta value of a parameter is the better. The optimum conditions on this approach obtained by sorting the delta in an order of significantly affecting the process. The higher the delta value signifies the more effect its parameter contributes.

### Table 5. Optimum Conditions by Utilizing S/N Ratio

|     | A   | B   | C   | D   |
|-----|-----|-----|-----|-----|
| Level 1 | 33.61 | 32.59 | 32.85 | 33.19 |
| Level 2 | 33.06 | 34.02 | 33.83 | 33.50 |
| Level 3 | 33.57 | 33.64 | 33.57 | 33.55 |
| Delta  | 0.55  | 1.43  | 0.98  | 0.36  |
| Rank   | 3     | 1     | 2     | 4     |

The highest plots of each parameter were chosen as could be seen in Figure 2, the optimum conditions obtained by this approach are as follows: A1, B2, C2 and D3, with the reason that the ratio set was the larger the better [10]. The same optimum conditions could be seen in Table 5 by the delta values therefore, it shows that the order of factors ranking which has the highest number of deviation in a row is factor B, C, A, D.

![Main Effects Plot for S/N Ratio](image)

**Figure 2. Main Effects Plot for S/N Ratio**

### 3.2. ANOVA Approach

In this part, the aim of using the Analysis of Variance (ANOVA) is also to determine the significance of parameters on reduction lead in *Gracilaria* sp. ANOVA is a statistically decision-making tool to detect differences in the average performance and helps testing the significance of all main factors. ANOVA method was utilized to understand the percentage of contribution of each parameter [10]. Based on the calculation of S/N ratio, ANOVA value can be obtained. The most significant parameter was determined by calculating the percentages contribution. Based on the data in Table 6, the ANOVA method was computed with some quantities such as degrees of freedom, squares sums, F-ratio, variance, square pure sum and contribution of percentage.

### Table 6. ANOVA Approach
These results show that the precooking time contributes the most by 56.92% and this is followed by the pH of water contributes 26.63%, the temperature of 11.32%, and ratio seaweed:water volume of 3.64 % as the influence factor for reduction of lead. This proves that precooking time and the pH of water are the most significant parameters contribute to reduction lead content in the precooking process while temperature and water volume only have small effects towards the reduction in lead.

3.3. Verification

A verification test with five trials was carried out with the set of optimum parameters. The variation was checked between predicted and experimental responses. Five experiments under the optimum parameters to verify the predicted value that could be seen in Table 7.

| Experiment | R |
|------------|---|
| 1.         | 55,872 |
| 2.         | 56,575 |
| 3.         | 56,348 |
| 4.         | 55,984 |
| 5.         | 56,893 |
| Mean       | 56,334 |
| Standard Deviation | 0,420 |

The verification test is based on reassessing the percentage reduction in lead. The purpose of the verification test was to confirm the validity of the predicted value. From the verification experiment, it is known that the optimum combination selected has been in the confidence interval and has confirmed its value.

4. Conclusion

Taguchi experimental design with L9 (3^4) orthogonal array was used to optimize the process parameters for maximum percentage removal of heavy metals Pb in Gracilaria sp. The optimized experimental parameters were soaking temperature 30°C, time 60 minutes, pH 7 and water volume 1:20. The contribution of each parameter towards the percentage removal of lead is of the following order: time (56.92%) > pH (26.63%) > temperature (11.32%) > water volume (3.64%). Then, percentage reduction of heavy metal realized in the confirmation experiment is higher than all the test runs. The optimization process that performed by Taguchi method brings out the best conditions to be used in precooking treatment that could actualize to reduction the lead content in Gracilaria sp. In addition to the above, optimization using a different set of process parameters may further help in the enhancement of process efficacy.

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