Growth rate of *Pseudomonas fluorescens* in liquid fertilizer from brown seaweed (*Sargassum sp.*) extracts

J Basmal¹, M E Aribowo², Nurhayati¹ and R Kusumawati¹
¹ Research Center for Marine and Fisheries Product Processing and Biotechnology, Indonesia  
² University of the Brawijaya, Malang, East Java, Indonesia  
E-mail: jamalbasmal24@gmail.com

**Abstract.** The purpose of this study was to observe the growth rate of *Pseudomonas fluorescens* bacteria as biological agents to stimulate plant growth and inhibit the growth of pathogenic bacteria. The method used were aimed to extract the micro, macro and growth stimulating substances contained in the thallus of *Sargassum* seaweed which had been fermented for 5 days using 0.3% KOH solution and then heated at 70°C for 2 hours. The filtrate obtained was formulated by adding silage and molasses which were then sterilized at 121°C for 15 minutes. The *P. fluorescence* bacteria were inoculated into the formulation solution and observed for 20 days with a span of 5 days against pH values, thickness, total nitrogen, C organic, electric conductivity (EC), total dissolved of solid (TDS) and growth rate of *P. fluorescens*. The results showed that the pH value did not show significant differences but the viscosity value, total nitrogen, C organic, EC, TDS, and TPC were significantly different. The best results were found on the 10th day of inoculation of *P. fluorescence* in a non-sterile formulation solution with a pH value of 3.76, thickness of 2.95 cPs, total N-0.62%, C organic 0.1%, EC 9,814 µS / cm, TDS 4,602 ppm and Total Plate Count of 10.6 log CFU/ml.

1. **Introduction**

It is known that seaweed besides containing hydrocolloid also contains vitamins, proteins, minerals and growth substances. Basmal et al. [2] found that seaweed contained growth-promoting substances such as auxin, cytokinin and gibberellins. The mineral content and growth booster substances are mostly contained in the liquid seaweed. The mineral content and growth stimulating substances that exist in the seaweed thallus are very useful for increasing crop production. According to Serrani et al. [15] the content of growth booster substances can increase tomato production.

Although seaweed contains N-P-K but the amount does not meet the requirements set by Indonesian Minister of Agriculture which says that N-P-K in organic fertilizer must contain a minimum of 4%. To overcome this, seaweed or sap liquid can be added to other organic material such as fish meal or fish silage which is rich in minerals, vitamins and complete amino acids. Besides that, fermented manure can also be added which is also rich in organic matter. Other materials that can be used are molasses which have high C organic and are very much needed by plants.

The combination of seaweed, fish silage and molasses is not enough if applied as organic fertilizer as required by the Minister of Agriculture which states that single or multiple organic fertilizers must contain a minimum of 15% C organic and a minimum of 4% N-P-K content. This is impossible to achieve by organic fertilizers unless there are additional inorganic ingredients. Another alternative combination of sap liquid with other organic materials such as fish silage and molasses can be used as biological fertilizers by adding microbes as a N fixator from air, Other nutrients, P and K, are sufficiently available.
in the soil but are still mixed with other ingredients, therefore decomposers are needed to release P and K from other materials.

Elekhtyar [5] reported genera such as *Pseudomonas*, *Bacillus*, *Azospirillium*, *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Serratia*, *Rhizobium*, *Enterobacter*, *Burkholderia*, *Klebsiella*, *Clostridium*, *Vario–vovax*, *Xanthomonas* and *Phylobacterium* can be used to release N-P and K also release plant growth promoting Rhizobacteria (PGPR). PGPR is a secondary metabolite (regulator of plant growth, fitohormones and biologically active substances) produced by microbes which facilitates the availability and absorption of certain nutrients from the root environment and inhibits plant pathogenic organisms in the rhizosphere. The present of the PGPR will increase the root exudation component e.g. sugars, amino acids, organic acids, vitamins, enzymes and organic or inorganic ions. PGPR are secondary metabolite, isolated from the rhizosphere, which when applied to seeds or crops, enhanced the growth of the plant or reduce the damage from soilborne plant pathogens. Suyono and Salahudin [16] said that many *Pseudomonas* found on land, plants and water such as *Pseudomonas aeruginosa*, *Pseudomonas* sp, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Pseudomonas syringae*, *Pseudomonas stutzeri* dan lain-lain.

Seed germination was significantly improved with inoculated rice seeds by PGPR *Pseudomonas fluorescens* (PGPRPf) as inoculation [5] while leaf mustard plants inoculated with *Pseudomonas fluorescens* and *Bacillus subtilis* had longer roots than those without the inoculation of *Pseudomonas fluorescens* and *Bacillus subtilis* [4]. Research [1,6] showed that PGPR (Plant Growth Promoting Rhizobacteria) could be used as a biological control agent by inducing resistance in plants. PGPR also serves to increase nitrogen capture, synthesis of phytohormones, dissolving minerals such as phosphorus and siderophores to availability of iron in plant roots. Luttege et al. [8] stated that phytochrome and phytohormon are signal system defense of plants. This can be seen with the increasing of resistance compound such as peroxide that occurs in the plant tissues. Park et al. [20] said that *Pseudomonas fluorescens* SS101 can produce volatile organic compounds (VOC) as a driver of plant growth and play an important role in modulating plant growth and inducing systemic resistance (ISR) to pathogens.

By paying attention to the amount of environmental damage due to the use of inorganic fertilizers, the modern agricultural system of crop fertilization will shift from the use of non-environmentally friendly inorganic fertilizers to organic fertilizers or biological fertilizers.

The purpose of this study was to look at the growth rate of *Pseudomonas fluorescens* in biological fertilizer formulations.

2. Materials and Methods

The type of seaweed used was *Sargassum* seaweed obtained from Biniuangeun beach - Banten. Seaweed was collected from the collector washed with seawater, dried and then taken to the Center for Marine and Fisheries Product and Biotechnology Research for further treatment. Fish to be produced into fish silage was obtained from Kronjo-Tenggerang Fish Landing Place while molasses was obtained from sugar factories in the Bantul area of Central Java. The species of microbes used was *Pseudomonas fluorescens* obtained from the collection of the Research Center for Marine and Fisheries Biotechnology and Product Processing. The chemicals used were technical grade such as phosphoric acid and potassium hydroxide.

*Sargassum* washed and then soaked in a solution of 0.3% (w/v) KOH with a ratio of 1 part *Sargassum*: 10 for well water for 5 days. Then cooked at 70°C for 2 hours. The filtrate then separated from the seaweed. The resulting seaweed liquid was then separated from thallus. The filtrate obtained was then mixed with fish silage and molasses with a ratio: 72%: 21%: 7%. Then divided into two to get sterilization treatment at 121°C for 15 minutes and non-sterilization. After reached room temperature, the mixed medium was then inoculated with *P. fluorescens* with a ratio of 1: 10. The process of observing the growth rate of *P. fluorescens* was carried out for 20 days from a range of 0, 5, 10, 15 and 20 days. Observations made were the growth rate of *P. fluorescens*, N (Metode Kjeldal Foss), viscosity using Brookfield, P, K, & C organic used method from Balai Penelitian Tanah, EC & TDS used EC-Meter.
3. Results and discussion

3.1. pH value

The pH value of the liquid biofertilizer before the sterilization process was 3.52 and after the sterilization process the pH value dropped to 3.28. During the process of fermentation the pH value in the sterilization treatment in ranged from 3.28 to 3.41 while the treatment of non-sterile pH values during the fermentation process ranged from 3.52 to 3.33 (figure 1). In the treatment of the sterilization process there was a decrease in pH value from 3.52 to 3.28. This decrease is the possibility of a reaction between the sap liquid and silage during the sterilization process. The observation of the pH value of fish silage was 2.7. While the pH value of sap liquid was 9.64, the mixing of sap liquid with fish silage and molasses had resulted in a pH mixture of 3.52. The sterilization process had caused a reaction between fish silage and sap liquid to reduce the pH value. Differences in pH values between sterilization and non-sterilization treatments have different pH values up to the tenth day of the fermentation process, then in the fermentation process from day 15 to 20 days no significant differences occur. The highest pH value for the first 10 days of fermentation was found in non-sterilizing treatments. The pH value has an effect on microbial growth. Suyono and Salahudin [16] said that Pseudomonas spp will grow at pH 5-9 and aerobic condition. Based on the pH value produced in sterile and non-sterile treatments, the highest change in pH value was found in the non-sterile treatment, this may be in the non-sterile treatment there were other microbes that develop and most likely come from fish silage. Handajani [7] reported that lactic acid bacteri (LAB) was found in fish silage to produced acidic pH conditions.

![Figure 1](image_url)

**Figure 1.** pH value on sterile and non-sterile treatments during fermentation.

3.2. Viscosity

The viscosity value in the sterilization treatment of *P. fluorescens* growing medium ranged from 2.12 - 2.75 cPs during the 20 days fermentation process, whereas in the non-sterilization treatment the medium of *P. fluorescens* ranged between 2.275 - 2.835. The pH value tended to increase in the sterilization treatment while the non-sterilization tended to decrease. At the end of the fermentation process the highest viscosity was found in the sterilization treatment. Viscosity values could be derived from sap liquid, fish silage and molasses, but increased viscosity values during the microbial inoculation process might be caused by microbial activity during the fermentation process. Based on figure 2 changes in viscosity values have no significant effect and tended to return to the initial pH value like mentioned by Patricio [11] the sudden slope change occurs during fermentation, and the viscosity value will return to a value close to the initial value.

Basmal *et al.* [2] found that viscosity value in the sap liquid of *Sargassum* in range of 2.7 - 3.1 cPs while the results of this research on the treatment of sterilized and non-sterilized liquid fertilizers ranged from 2.12 - 2.96 cPS. The low viscosity value of sterilized and non-sterilized liquid fertilizer treatment after *P. fluorescens* inoculation might probably caused by the presence of microbial activity.
of *P. fluorescens* which makes the resulting low viscosity of biological fertilizers. The results also found the pH value of sterilization and non-sterilization treatments ranged from 2.93 - 3.76. The decrease in pH value had caused alginate in the biological fertilizer solution to be converted to alginic acid. As a result, the thickness of the resulting biological fertilizer was low.

**Figure 2.** Viscosity value on sterile and non-sterile treatments during fermentation.

### 3.3. Total Nitrogen

The total N value in the sterilization treatment ranged between 0.66 - 0.65%, whereas in the non-sterile treatment between 0.60 - 0.61% during the inoculation process 20 days at room temperature. In figure 3, the fluctuating total N curve shows that in the 10th day of inoculation there was a decrease and then there was an increase in the 20th day inoculation. In the sterilization treatment, the total N reduction was 0.01% while the sterilization treatment took a 0.01% increase at the end of the 20th day inoculation. The total N in the sterilization treatment was higher than the non-sterilization treatment during the 20th day inoculation process (figure 4). Overall, the total N sterilization process on the growth medium of *P. fluorescens* was higher than the non-sterilization treatment.

It is well known that *P. fluorescens* is a group of N fixation bacteria that can take N from the air. In the sterilization treatment, the growth medium of *P. fluorescens* had no direct contact with the outside air while in the non-sterilization treatment the medium grew *P. fluorescens* was left open so that the air could be in contact with the growing medium. The total N content in the *P. fluorescens* growing medium non-sterilization could attract N from the air. In the sterilization process, there is no contact with air, as a result, *P. fluorescens* utilizes the total N present in the growing medium *P. fluorescens* as a result of the total N decreasing (figure 3). Mantelin, and Touraine [9] said that relatively large amounts of atmospheric N reach the plant as ammonia released by the bacteroids.

### 3.4. Organic carbon value

Organic C (organic material) is a part of the land which is a complex and dynamic system, which is sourced from plants and/or animals remaining in the soil that is constantly changing shape because it is influenced by biological, physical and chemical factors. As a source of nutrients for organic C plants, it can improve environmental characteristics, sustainable highland productivity, improve soil structure, while improving the consistency and stability of soil aggregates, improving soil quality, energy sources and C sources for microbodies (prokaryotes), can reduce micronutrient poisoning (eg Al on acid soils), increases the ability of the soil to hold water available to plants and improves the living system of soil bodies, especially bacteria so that all microbiological processes in the soil run more perfectly.

The results showed that organic C in the treatment of sterilized and tightly closed *P. fluorescens* growth medium without contact with outside air ranged from 0.09 - 0.10%, while the treatment of non-
sterilizing growth medium and left contact with air was found to be constant at 0.1% for 20 days \( P. \) fluorescens inoculation. Clark and Burki [3] said that \textit{Pseudomonas} and \textit{Achromobacter} required oxygen in the growth.

C organic is obtained from the process of composting or weathering organic matter both by the influence of the changing environment (temperature, pressure, humidity, open air or anaerobic conditions), enzyme and microbial activity. In the digestive process by microorganisms, a combustion reaction occurs between elements of carbon and oxygen into calories and carbon dioxide (\( \text{CO}_2 \)). This carbon dioxide is released into gas, then the decomposed nitrogen element is captured by microorganisms to build up its body. When these microorganisms die, the nitrogen element will stay with compost and become a source of nutrition for plants. Carbon is the main food source for anaerobic bacteria so that the optimum growth of bacteria is strongly influenced by this element, where carbon is needed to supply energy. Total carbon measures all inorganic and organic carbon.

\[ \text{Figure 3. Total nitrogen value on sterile and non-sterile treatments during fermentation.} \]

\[ \text{Figure 4. } C_{\text{organic}} \text{ value on sterile and non-sterile treatments during fermentation} \]
3.5. Electrical conductivity (EC)

The value of Electrical Conductivity (EC) and pH of the solution have been used to determine the quality of liquid fertilizer. The higher the concentration of the solution means the more concentrated the salt content in the solution, so the ability of the solution to deliver electric current is higher which is indicated by high EC values as well. The density of the nutrient solution is influenced by the total salt content and the accumulation of ions present in nuts. Electrical conductivity in solutions affects plant metabolism, namely in terms of the speed of photosynthesis, enzyme activity and the potential for absorption of ions by roots. The concentration of nutrient solutions will also determine the duration of the use of nutrient solutions in hydroponic systems.

![EC value on sterile and non-sterile treatments during fermentation.](image)

The results showed that the liquid fertilizer sterilization inoculated with *P. fluorescens* had a smaller value compared to the liquid fertilizer which was sterilized during the inoculation process. In the sterilization treatment the EC value was between 8872 - 9244 µS / cm (micro Siemens / cm) while the EC sterilization value was between 9184 - 9452 µS / cm. Overall the EC value during the 20-day inoculation process showed a tendency to rise both in sterile treatment and in non-sterile medium growing *P. fluorescens*. The high value of EC in the non-sterile treatment was probably not only by *P. fluorescens* which works to decipher mineral materials in liquid fertilizer medium but also assisted by other microbes. The presence of air contact between liquid fertilizer and air medium was also one of the causes of high EC because *P. fluorescens* in multiplying itself requires oxygen. Basmal *et al.* [2] reported that the EC value in liquid *Sargassum* sap ranged from 2.230 to 3.520 micro Siemens/cm, while the results of the study found that the EC value of sterilization and non-sterilization treatments ranged from 8,871 - 9,452 micro Siemens/cm which meant inoculation into liquid fertilizer can increase the EC value of biological fertilizers.

![Figure 5. EC value on sterile and non-sterile treatments during fermentation.](image)

3.6. Total dissolved solute (TDS)

The TDS value of the sterilized liquid fertilizer treatment ranged from 4427 - 4636 ppm, while the treatment of non-sterilized liquid fertilizer ranged from 4600 - 4757 ppm for 20 days in the *P. fluorescens* inoculation process (figure 6). In the treatment of TDS, value sterilization tended to increase while the treatment of non-sterile TDS values tended to decrease. TDS values indicate the number of minerals dissolved in the liquid fertilizer due to *P. fluorescens* activity during the 20-day inoculation process. The more dissolved minerals, the higher the TDS value. The decrease and increase in TDS value was likely due to microbial activity in the fertilizer medium. In the treatment of medium that had been sterilized the TDS value was lower than the treatment of non-sterilized medium. The high TDS value in the treatment of non-sterilized medium due to large amount of fertilizer composting...
process by various types of microbes present in the medium of fertilizer that is not sterilized, while the fertilizer medium which is sterilized is only carried out by *P. fluorescens* bacteria.

![Figure 6. Total dissolved solute value on sterile and non-sterile treatments during fermentation.](image)

### 3.7. Total Plate Count (TPC)

The experimental results showed that the sterilization and non-sterilization treatment had an effect on the growth rate of *P. fluorescens*. The highest growth rate was found on the 10th day of inoculation of *P. fluorescens* bacteria with the highest value added to the treatment of non-sterilizing growth medium of 10.6 log CFU / ml in the log phase while the sterilization treatment of the medium was only reached 4.7 log CFU / ml in the log phase. After the tenth day, on the 15th and 20th day, the treatment of growth medium both sterilized and non-sterilized had decreased but the highest value was still in the treatment of non-sterilizing growth medium. The occurrence of differences in the amount of CFU / ml was possible in the sterilization process because there are some nutrients lost. While the non-sterilized did not occur loss of nutrients, for example, some vitamins needed by bacteria would be lost due to heat treatment.

Several factors that influence the rate of microbial growth are the availability of nutrients, conditions of growing medium and environmental conditions which include: temperature, oxygen, pH and humidity levels. There are several treatments that can damage the nutrition of growing medium because some of the vitamins and enzymes that are present in the medium grow if given high heat treatment and high pressure it is likely that some of the nutrients given will disappear. Missing some nutrients given will have an impact on the rate of microbial growth.

*P. fluorescens* is a bacterium that belongs to the rhizosphere group which can produce plant growth promoting rhizobacteria (PGPR) which is very influential to increases root surface area, increases nutrient uptake and improve plant production, inhibits the growth of other bacteria [9]. These bacteria can dominate the rhizosphere and develop rapidly, are gram-negative, motile, aerobic/facultative anaerobes [13] and are able to degrade and use large amounts of organic and inorganic compounds, interact with plants and associate in the rhizosphere that benefits agriculture [12]. *Pseudomonas* groups can produce large amounts of phytohormones especially IAA to stimulate growth [19] besides that it also produces cytokines, isopentenyl adenosine, and ribose zeatin [14]. IAA is a growth hormone auxin group which is useful for stimulating plant growth. Auxin is useful for increasing stem cell growth, inhibits the process of leaf abortion, stimulates fruit formation, and stimulates cambium growth, and inhibits the growth of armpit shoots[17]. *Pseudomonas* sp. also known to produce silicic acid which is capable of controlling tobacco necrosis virus in tobacco [10] and able to enhance crop yield by direct and indirect mechanisms [18].
Figure 7. TPC value P. flourescen on sterile and non-sterile treatments during fermentation.

4. Conclusion
The growth rate of P. flourescens has been influenced by the physical properties of growing medium and its environmental factors. In this experiment which uses two different growing medium, it is proven that there was a real effect on P. flourescens growth rate, total N, organic C, EC, TDS and viscosity value of the resulting biological fertilizer. The highest P. flourescens growth rate was found in the treatment of non-sterile growing medium namely: the highest stationer phase was achieved on the 10th day of P. flourescens inoculation which was 10.6 log CFU/ml, with a pH value of 3.76, thickness of 2.95 cPs, total N 0.62%, Corganic 0.1%, EC 9,814 μS / cm, TDS 4,602 ppm.

5. References
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