Application of halophilic bacteria in traditional solar salt pond: a preliminary study

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Abstract. Microbial community in salt ponds have an important role in salt quality production. One of important microorganisms is halophilic bacteria and archaea which has been reported to positively correlated with salt quality. This manuscript reported a preliminary study on application of the halophilic bacteria consortia on to traditional salt pond in Pati. An amount of 0.5% (v/v) of the halophilic bacterial consortia was applied in 20 Baume (Be) salt pond sizing 20 m². After the saltwater reached 25 Be, the halophilic-treated saltwater was moved to the crystallization pond. Salt has been harvested by scrubbing cristal salt manually after 3-5 days, the time used by salt farmers in Pati. Yield, NaCl content, salt impurities, crystal whiteness, and compactness were parameters studied in this trial. Salt produced from pond interfered with halophilic bacteria consortia had higher NaCl content by 2%, lower content of impurities (Mg and Ca) and higher whiteness degree. SEM analysis showed that crystal salt produced from halophilic pond showed more compact, cubical form compared to the crystal salt from untreated pond which was more fragile. However, the yield of salt produced was not different between treated and untreated pond.

Keywords: application, halophilic consortia, traditional solar salt pond, salt quality

1. Introduction

Globally, about one-third of the sodium chloride production, i.e about 200 million tonnes per year, is produced in solar salt ponds (Davis 2000). Indonesia is an example of a country that producing salt through solar salt system, mostly conducted by traditional solar salt pond. Salt production is categorized low in quality and productivity, and up to now, salt production from salt producers in Java such as Pati and Indramayu is mostly had low NaCl (NaCl under 90%) and high content of impurities. Efforts have been done to improve salt quality by purification, namely washing and introducing some chemicals in removing impurities since in the processing ponds, one of them has been introduced as Bestekin method and application of ramsol, claimed as natural coagulant. On the other hand, the biological communities in the solar salt ponds have been neglected so far.

Biological communities in solar salt ponds have been reported for their important role and well related to salt quality (Davis 2000, Giordano et al 2014, Oren 2010). Some biological communities are
advantageous by supporting salt crystal formation and minimizing impurities, while some other are disadvantages by releasing damaging quantities of mucilage and resulting in low-quality salt. A salt field with the biological community in equilibrium will produce good-sized solid crystals with low impurities, taking advantage of natural evaporation maximally.

Predominant microorganism inhabitant in the salt environment such as a salt lake and ponds has been reported to be brine shrimp (*Artemia salina*), algae (*Dunaliella salina*), bacteria (members of the family Halobacteriaceae and Haloanaerobiaceae, methanogens, etc.), and cyanobacteria (*Oscillatoria* sp.) (Shadrin et al 2015). Amongst all of those organisms, 2 microorganisms i.e halophilic bacteria and archaea, and microalgae *Dunaliella salina*, influenced directly in the quality of salt produced. Halophilic bacteria and archaea have been reported to positively related to salt quality. Meanwhile, the presence of *Dunaliella salina* is directly connected to the low quantity and quality of contaminants in the salt by alternating NaCl crystalline structure, specifically of crystallite size (Giordano et al 2014).

An important role of halophilic bacteria in the salt production was reported since the 1970s. The bacteria was responsible for the quality and quantity of the salt produced by solar salt system (Manikandan and Senthilkumar 2017). The use of halophilic bacteria after enrichment of saltwater with *Artemia salina* was reported to improve quality and productivity of solar salt pond in Madura, which is already well known for the best salt quality producer in Indonesia. The resulting salt production was having NaCl content of 98.12% and its productivity was 84 tonnes salt Ha⁻¹ per season, while control treatment produced salt with NaCl content of 95.6% and the productivity was 51 tonnes salt Ha⁻¹ per season (Marihati et al 2014).

We have studied the culturable and unculturable inhabitant of solar salt ponds microorganism, and have isolated culturable halophilic microorganisms from solar salt pond from Sampang Indonesia. The previous study on application of the halophilic consortia in the laboratory (using 3 L saltwater) showed that addition of 0.5% and 1% halophilic bacteria improved salt content (NaCl) by 2% and 4%, respectively, compared to the untreated sample. Therefore, the objective of this study was to apply the bacteria consortia on to traditional salt pond in Pati to improve their salt quality. Pati is one of important solar salt ponds in Indonesia, located in north coast of Java island. Pati is also center of iodine salt production, that also role as home of 100 small scale industries of iodine table salt. Therefore, improving salt quality of solar salt pons in Pati will be worthed and helped the industries to obtain good quality of raw material for iodine table salt. This study was a preliminary study where the pond used was very limited, as part of salt farmer ponds sizing of 180 m³ (figure 1), in 1 cycle of production.

2. Materials and methods

2.1 Materials
Halobacteria consortia were refreshed and cultured in the laboratory following Oren and Litchfield (1999) with modification, i.e. the medium composition used was NaCl (20%), KCl (5%), MgCl₂·H₂O (5%), NH₄Cl (5%), dan yeast extract (10%). NaCl and medium for bacteria cultivation in the laboratory were in p. a grade while for field application, the medium used was technical grade, locally produced according to Marihati et al (2014). Media for propagation: Sugar (1 kg) was diluted with warm water and added on to cooked fish meal. Then, the media was filtered and used to culture the halophilic consortia from the laboratory. The cultivation of the bacteria was using 10% starter, using saline water Baume (Be) 20, at room temperature, 24 hours.

2.2. Field trial.
We used part of farmer salt ponds in Pati with layout and size of each pan as figure 1 below.
Figure 1. Salt ponds used for the trial. The solar salt pond used for halophilic application.

The seawater as raw material was in the Boezem pond, and first, the water flowed to the spray ponds (right) which will increase the Baume (Be) of the water to Be 11 about 5-7 days by evaporation combined with sprayer. The water from this spray pond flow to seawater pond II to reach Be 12-17 about 2-3 other days, and continue to pond III for another 2-3 days to obtain water Be 17-20. This water (Be20) was ready to be enriched with the halophilic consortia in the enrichment pond. And, when the Baume of enriched pond water was 25 which usually took about 3 days, the water then flowed to the
crystallization pan. Bittern was excluded from the crystallization pan after the salt pan reached 28-28.5 Be which could be reached for 7-10 days, depending on the weather and wind.

2.3. Application. 0.5% cultivated halophilic bacteria consortia (10% of the total volume) was added into treatment pan having saline water of 20 Be. The treated water, without separating the halophilic consortia) was moved to crystallization pan after the 25 Be was achieved.

2.4. Analysis. NaCl was analyzed based on titrimetry 18-11/MU/SMM-SIG, while Ca and Mg were using ICP OES 18-13-1/MU/SMM-SIG. The analysis was conducted in PT Saraswanti Indo Genetech, Bogor Indonesia. Whiteneeds degree of salt measured using Whiteness Meter Kett Electric Laboratory C-100-3. Meanwhile, crystal salt profile was analyzed using SEM. Both whiteness and crystal profile were conducted at Physic Laboratory of R and D Marine and Fisheries Product Processing and Biotechnology.

3. Results and discussion

Addition of halophilic bacteria on to solar salt ponds before crystallization step was indicated by the development of pinky color in the pan containing 20 Be seawater after the treatment. Change in colour of the water was slightly noticed after 2 days of adding 0.5% of the halophilic bacteria treatment on to the 20 Be seawater pan, however the color was not developed until the water reach 25Be, and moved onto the crystallization pan. Halophilic microorganisms, i.e salt-loving microorganism and one of them are halophilic bacteria, were reported to have high carotenoid pigment (Oren and Rodriguez-Valera 2001, Oren 2002), Therefore the growth can be detected by the development of pink color. The red extremely halophilic bacteria and Archaea was reported to be found abundantly in the crystallizer ponds, so are the halophilic microalgae such as *Dunaliela salina*. Many halophilic bacteria and Archaea use glycerol, the metabolite produced by the halophilic microalgae as carbon and energy source (Oren 2010). In this preliminary trial, we did not analyze the biodiversity of microorganism in the crystallization pan during applications.

Table 1 presents characters and yield of salt production after 3 days process in the crystallization pan, shorter than it should be (5 days, time traditionally used in salt production) due to the weather. Halophytic treatment did not show better productivity compared to the traditional one. However, halophilic consortia treatment produced salt with higher NaCl, from 84.9% to 91.4%. One reason for might be because the halophilic consortia were not developed well during enrichment. Addition of 0.5% consortia without addition of nutrition might not sufficient to develop the growth and number of the bacteria. Marihati *et al* (2014) reported that enrichment treatment sizing 100 m² (contained seawater 20 Be of 15-20 m³; depth of water was 15 cm) has been added with 200 L halophilic as starter and 400 L halophilic nutrition. Using this composition, the treatment was capable to increase NaCl content from 95.6% to 98.12% and productivity from 51 tonnes salt Ha⁻¹ per season to 84 tonnes salt Ha⁻¹ per season. The nutrition used was *Artemia salina* powder which is rich in protein as N sources for the halophilic consortia, while in this study, the addition of nutrition after application, was not conducted. The survival and growing of the halophilic applied then depending on the microflora and availability of simple compound in the enrichment pond that can be served as nutrition for halophilic bacteria. Unfortunately, counting of the halophilic microorganism, i.e microalgae dan bacteria, was not conducted. The other reason was the crystallization process was not optimal due to the weather so the process was cut only 3 days, instead of 5 days, the time needs for crystallization process of the traditional solar salt production by most salt farmer in Pati. However, in Madura, the final process, i.e crystallization pond was 10 days (Marihati *et al* 2014). Solar salt ponds are series of evaporating seawater ponds by wind and solar energy, therefore, contact time of saltwater with wind and heat from solar is very important during all the successive process of solar salt production. Impurities started to precipitate and sink since the
beginning, in the downstream process salt ponds, and by this system, salt with purity 99.7% or more can be achieved (Davis 2000, Davis 2006).

The impurities such as magnesium and calcium can be reduced using marine cyanobacteria. Reduction of magnesium content from 831.88 mg.100g$^{-1}$ to 502.26 mg.100g$^{-1}$ and calcium content from 288.26 mg.100g$^{-1}$ to 182.09 mg.100g$^{-1}$ from solar salt ponds has been achieved using marine cyanobacteria during a single trial. The experiment was in Gujarat coast of India for the production of industrial grade salt (Mishra et al 2000). The role of halophilic consortia which excrete metabolite might be by reducing co-precipitation between salt crystal and organic material such as Mg and Ca in the Be28 - Be 28.5 in the crystallization pan (Marihati et al 2014). Therefore, the impurities in the crystal salt can be reduced.

| Harvest condition                         | Conventional (without treatment) | Halophylic treatment |
|-------------------------------------------|----------------------------------|----------------------|
| Height of water and time of harvest (4 cm, 3 days) | 1.785 kg                        |                      |
| Height of water and time of harvest (6 cm, 3 days) | 1.728 kg                        |                      |
| NaCl                                      | 89.4%                            | 91.48%               |
| Magnesium                                 | 0.831 mg.g$^{-1}$                | 0.502 mg.g$^{-1}$    |
| Calcium                                   | 0.288 mg.g$^{-1}$                | 0.182 mg.g$^{-1}$    |

The treated salt product was whiter in color compared to the untreated one. This is because the treated experiment was using geomembrane but not the untreated one, consequently the untreated process of a conventional solar salt system produced darker salt. Whiteness degree of salt products measured using Whiteness Meter Kett Electric Laboratory C-100-3 showed untreated salt was 60% whiteness degree while the treated one was about 75%.

Figure 2. (A): treatment halophilic bacteria and (B): control, without halophilic; magnificent 100x.

Figure 2 showed the profile of salt crystal production which was analyzed using SEM. Halophylic treatment produce solid, more cubical form of crystal (A), while (B) showed the control that is more
fragile, not solid and less cubical form of crystal. The crystals with hollow, contain brine inside, and also have high concentrations of organic and inorganic contaminants is a crystal from low-quality salt (Davis 2000). While Rahaman and Jeyalakshmi (2009) reported that good quality of solar salt ponds was large in salt crystal size, which can be obtained after 25 days observation of an application of Halobacterium culture as one of implementing biological management.

4. Conclusion

A single trial of salt processing in solar salt pond treated with 0.5% of halophilic bacteria produced salt with higher NaCl content, fewer impurities namely Mg and Ca, showing solid and more cubical form of crystal. However, there was no difference in yield of salt harvested between halophilic treated and untreated processes.

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