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*Low Energy Bacteria Preservation of Extremely Halophilic Archaea Haloferax Lucentense and Haloferax Chudinovii Immobilized using Natural Zeolite*

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ABSTRACT

The methods of microbial cells preservation were already known by liquid drying, freeze-drying, and freezing. Those methods could preserve bacteria cells in a long period of time but its survivability was relatively low and used relatively high energy during preservation. Immobilization was known as entrapping, attaching or encapsulating bacterial cells in a suitable matrix. This research was conducted to know the suitability of zeolite as immobilization carrier and also as preservation matrix of two halophilic archaea *Haloferax chudinovii* and *Haloferax lucentense*. Variable of this research was the type of the carrier which was raw zeolite, 110°C and 300°C heat-activated zeolite carrier, parameters measured in this study was physical and chemical of zeolite such as chemical content, Si/Al ratio, surface area and pore volume, and biochemical assay, bacterial cells numbers after immobilization and bacterial cells after preservation as bacterial response to the immobilization and preservation. Heat activation was significantly affecting the zeolite chemical composition, carrier surface area, and pore volume. Compared to other pretreated zeolite, highest quality zeolite was obtained in 110°C pretreated zeolite which has which has 65,625 m<sup>2</sup>/gr for surface area 0,071 cc/gr pore volume and 5,13 Si/Al ratio. . The bacterial cells obtained after immobilization process was 1,8x10<sup>7</sup> cfu/g, 3,0 x 10<sup>7</sup> cfu/g, and 2,1x10<sup>7</sup> for raw zeolite, 110°C pretreated zeolite and 300°C zeolite respectively. After 4 months preservation, the slight reduction of the bacterial cells was observed. Immobilization halophilic archaeae using zeolite as carrier was proven as low cost and effective preservation method due to relatively simple process and unspecific preservation temperature requirements.

1. INTRODUCTION

Halophilic bacteria or halobacteria are known as single cell microorganism inhabit saline environment. Halobacteria are considered as halophilic archae living in hypersaline environment and having pigment ranging from yellow to red. Mostly halophilic archaea are found in crystallization ponds due to high salinity requirement (250-300 g/l) of NaCl (A Oren, 2010). Halophilic archaea have ability to cope with high osmolarity pressure due to their

ability to compensate intercellular osmotic pressure using salt in mechanism and compatible solute (Das sarma, 2001). Halophilic archaea were also known to their ability to survive inside the halite crystal in long period of time (Norton et al., 1993).

Bacteria cells preservation has been known for a long time. Lyophilization or freeze-drying method was the oldest methods used to preserved bacteria cell in which

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bacteria cell was frozen and then dried using freeze drying apparatus (Heckly, 1961). Freeze drying apparatus consisting of vacuum machine, freezing apparatus and drying apparatus, this method has relatively low survival percentage due to extreme temperature applied during its processes and also the requirement of suitable protective media are strictly needed. To obtain maximum preservation time the freeze dried sample must be stored in 1-4°C storage or even lower temperature (Kupletskaia and Netrusov, 2011). The limitation of freeze-drying method were very selectively depended on the microorganism characteristic. Preservation of bacterial cell commonly done by refrigeration (storage in < 4°C), ultralow freezer (-86°C), cryofreezing (-130 to -145°C) this preservation cost high energy to operate.

Zeolite is naturally found in nature and commercially used by many human activities. Zeolite has white-greenish soft minerals, it consists commonly of a tetrahedral crystalline microstructure built from alumina (AlO<sub>4</sub>) and silica (SiO<sub>2</sub>) (Jha, B; Singh, 2016). Zeolite composed in three categories which are extraframework cations, framework and sorbed phase due to its high adsorption properties. Upon incorporation of Al with SiO<sub>2</sub> the surface of zeolite becomes negatively charged, in this condition extraframework of organic and inorganic cations is needed to made the surface positively charged (Payra and Dutta, 2003). Zeolite is also known to have molecular sieve, ion exchange and catalytic features.

Immobilization is known as a common technique to preserve and improve bacteria activity. Immobilization is microbial engineering by trapping, attaching and encapsulating the enzyme or bacterial cells into a matrix or carrier (Woodward, 1988). The suitable matrix characteristic for immobilizing bacterial cell must have high cell loading capacity, simple and nontoxic for targeted bacteria, mechanically stable, bacteria and matrices were easily separated (Abdelmajeed et al., 2012). Common carriers used for immobilizing bacterial cells are divided into inorganic materials such as zeolite, clay, porous glass, ceramics and organics materials /polymers (Suzana et al., 2015). The advantages of bacterial cell immobilization

compared to enzyme immobilization is cheaper due to reduction of the separation process, the possibility of multiple reactions occurred due to various enzymes produced by bacteria cells, and the presence of bacterial biosynthesis that supports the existence of longer or complex chain of enzyme reactions (Jack and Zajic, 1977). Increased ability and biochemical activity of immobilized bacteria are well known, research done by Shindo et al., (2001) investigated continuously ethanol production using *Saccharomyces cerevisiae* immobilized in inorganic matrices (zeolite) carriers which have twice larger fermentation activity compared to un-immobilized *Saccharomyces cerevisiae*. In other hands (Omarova et al., 2012) shown that immobilized *Rhodococcus* spp using organic polymers were used in the degradation of crude and oil products. Many methods used to immobilizing bacteria cell and the most common bacterial cell immobilization was encapsulation using alginate polymer (Zommere and Nikolajeva, 2018). Otherwise, immobilization of bacterial cells could probably has a negative impact to bacterial biochemistry activities.

Immobilization processes are also used to preserve bacteria cells in order to keep the bacteria in perfect condition when it comes to use. Research done by Krumnow et al (2009) obtained that *E.Coli* and *Bacillus subtilis* were well preserved for 64 days in accacia gum and pullulan matrices with temperature and humidity variation, thus result are also affected by the bacteria characteristic such as avoid dehydration capability and ability to produce spore. There are many variation of immobilization carriers used for preserving bacteria cells such as sol gel matrix (Nassif et al., 2003) and PVA (Poly Vinyl Alcohol) (Efremenko and Tatarinova, 2007). The matrix selection used in immobilization process are adjusted with the bacteria characteristic and the purpose of immobilization. Bacteria cell immobilization are also has function for maintain bacteria ability to produce specific proteins. Desimone et al (2005) found that *E. Coli* producer of recombinant proteins were immobilized in silicone oxide matrix and it preserved perfectly in 4°C and 20°C storage for 60 days.

*Haloferox chudinovii* and *Haloferox lucentense* are an example of halophilic archaea which found in hypersaline environment from Sampang region in Madura Indonesia. Potential use of these haloferax species are still limited due to lack of study about this species. The aim of this research was to obtain information about possibility of low energy preservation of two halo archaetae species *Haloferox chudinovii* and *Haloferox lucentense* using immobilization method.

## 2. METHODS

### 2.1 Carrier Pretreatment, Morphological visualization and quantification Halophilic bacteria preparation

#### a. Carrier Pretreatment

The Natural zeolite used as a carrier in this research was obtained from a local chemical store in Semarang. Pretreatment of the carrier used in this research was done by activating the zeolite using heat treatment to remove organic impurities in the zeolite pore. Heat activation was done by heating the zeolite using furnace at 300°C for 4 hours (Djaeni et al., 2010) and heating using the oven at 105°C for 24 hours (West and Strohfus, 1997), treated zeolite was then cooled in a desiccator.

#### b. Morphological Visualization and Quantification

Quantification of immobilization carriers was done using Brauner-Emmet-Teller (BET) method and Barret-Joyner-Halenda (BJH) method (Quantachrome instrument), this quantification was to determine zeolite surface area and pore size for each pretreatment variations. Morphological visualization of immobilization carriers and the immobilized sample was done using SEM-EDX (Jeol JSM 6510 Analytical Scanning Electron microscope and JED 2300 EDS for EDX analysis) analysis was carried out to obtain the difference between two heat activation to zeolite physical and chemical properties.

### 2.2 Culture preparation and immobilization procedure

#### a. Culture Preparation

Extremely halophilic archaea *Haloferox chudinovii* and *Haloferox lucentense* strain used in this research was

laboratory isolate. Pre-culture was conducted by inoculating each 2 loops pure culture of *Haloferox chudinovii* and *Haloferox lucentense* into different sterile conical flask glass filled with 50ml of modification of extremely halophilic liquid medium consisting NaCl 24% (w/v), yeast extract 0,25 gr/l, tryptone water 0,5gr/l, MgSO<sub>4</sub>.7H<sub>2</sub>O 20 gr/l, KCl 2 gr/l, and trisodium citrate 3 gr/l (Nilawati et al., 2017), the cultures were incubated and shaken in shaker incubator at 39°C for 7 days. The culture Harvested after 7 days incubation and then mixed into 250ml sterile conical flask and stirred to make the mixture homogenous. The obtained mixture was stirred for 15 min and counted the cell using total plate count (TPC) method to determine the number of the viable cell, TPC was done by diluting 1 ml of harvested mixed culture and then diluted in series 11 fold in 9ml sterile distilled water, the number of bacterial were expressed as CFU g<sup>-1</sup>. Obtained initial culture was 10<sup>11</sup>

#### b. Immobilization Procedure

The immobilization was carried out by inoculating 50ml pure culture of *Haloferox lucentense* and *Haloferox chudinovii* each in sterile erlenmeyer flasks and then each 10gr natural and pretreated zeolite as immobilization carrier was added to the culture. Immobilization procedure was done under shaking condition at 200rpm for 24 hours in order to obtain maximum absorption of the cultured cell on the zeolite carrier matrices. Immobilized cells were then harvested by separating supernatant and the culture filtrate and dried in room temperature for 24 hours.

### 2.3 Analysis

#### a. Biochemical Assay

Biochemical assay of *Haloferox lucentense*, *Haloferox chudinovii*, and immobilized *Haloferox lucentense* and *Haloferox chudinovii* was determined using Vitek 2 Technology (Biomereux) by culturing 10 w/v immobilized sample in liquid modified haloferax medium and incubated in shaker incubator in 150rpm for 7 days at 39°C. The growth cultures then harvested and stored in a sterile glass tube with screw cap and then transferred for biochemical assay.

### b. Viability cell during preservation

The number of viable cell were determined as viable counts (cfu) per gram immobilized sample. After dried in room temperature for 24 hours immobilized *Haloférx lucentense* and *Haloférx chudinovii* sample were transferred into a sterile plastic clip and then stored in room temperature for 4 months (Alvarez et al., 2007). Viability cell was done after immobilization process and after 4 months preservation using TPC method with triple replicates for each variation, 1 gram of each immobilized sample were serially diluted  $10^{-9}$  fold dilution and each dilution was mixed using vortex to make homogeneous suspension, then 1ml aliquot of  $10^{-4}$ - $10^{-9}$  dilution were plated in solid modified haloferax medium which was then incubated for 21 days at 39°C.

## 3. RESULT AND DISCUSSION

### 3.1 Physical and chemical characteristic of carriers

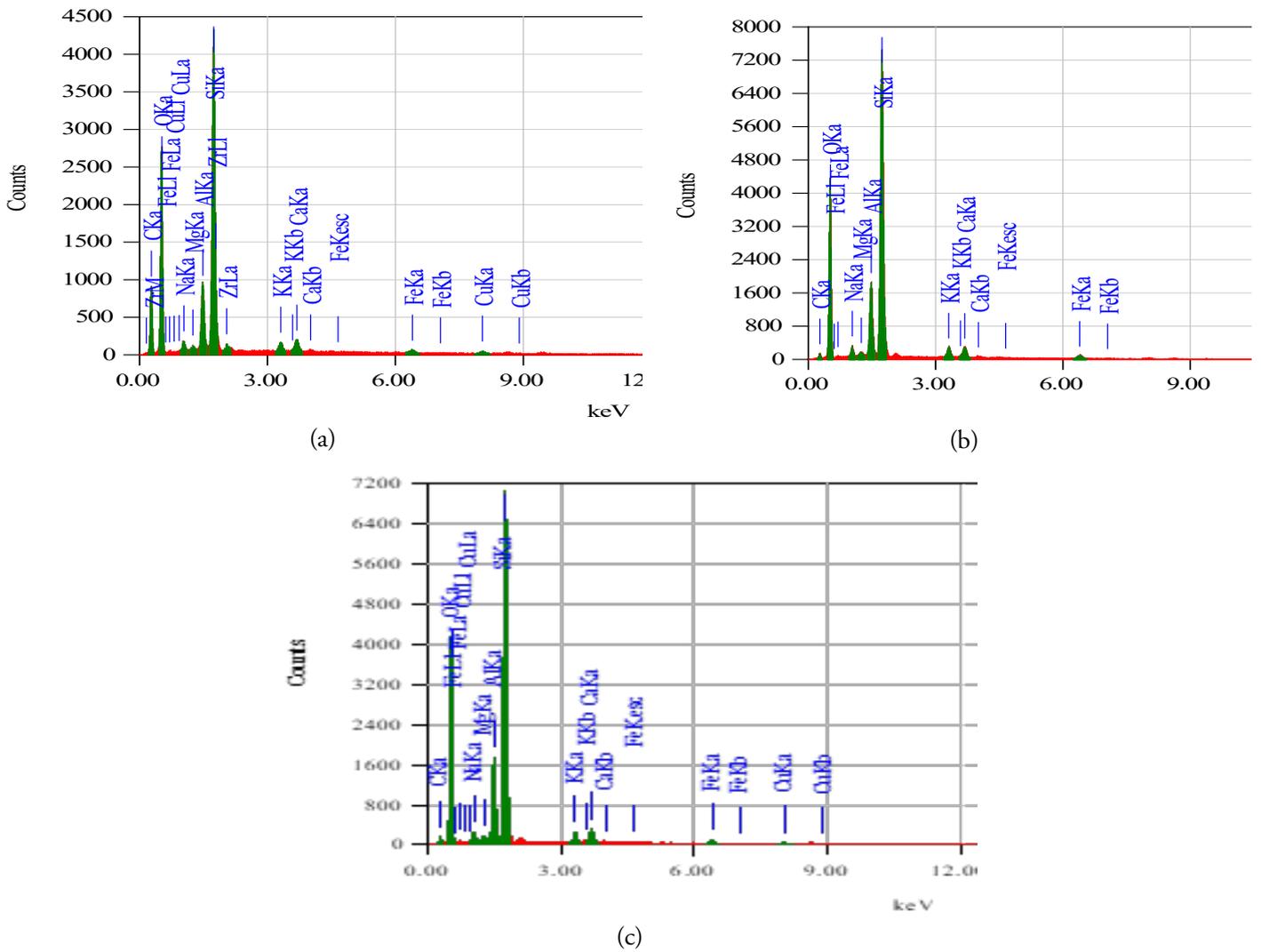
Zeolite is a porous material which consists of  $Al_2SO_3$  and  $SiO_2$  bonded together in such way formed tetrahedral structure (Mery et al., 2012).  $CaCO_3$ , organic and water vapor are the most common impurities found in zeolites that could cover the porous that led to decreasing the zeolites ability to adsorb targeted substances (Fuoco, 2012). Heat treatment and chemical addition are the most common pretreatment done to activate and or enhance zeolite ability to adsorb liquid and gasses. Chemically activated zeolite has several disadvantages when used as bacteria cell immobilization carrier which was longer activation process, the possibility of hazardous chemical left in the carrier matrices that has a negative effect on immobilized bacteria.

Table 1 shows the quantification of zeolite composition in all pretreatment variable used in this research. Carbon content considered as impurities in zeolite and it has significantly decreased due to heat treatment applied, it decreased from 54,41% to 13,07% and 15,45% in 110°C pretreatment and 300°C pretreatment respectively. In other hands physical heat treatment increased the percentage of  $SiO_2$  and  $Al_2O_3$ , initial

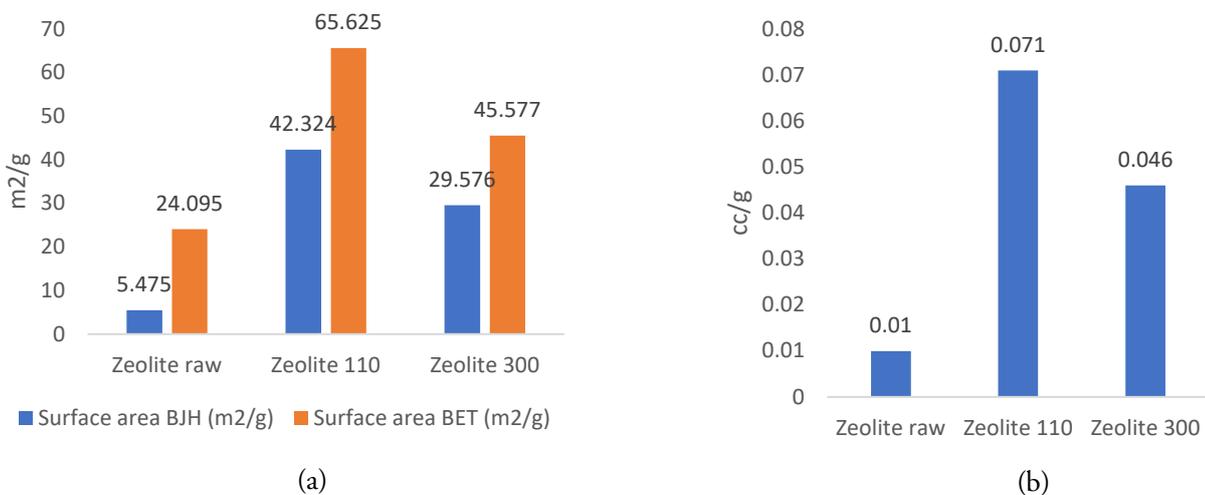
percentage of raw zeolite was 32,48% and 5,88% while the 110°C and 300°C pretreated zeolite has increased twice which was 64,89/12,47% and 62,79%/11,82% respectively. The increasing of  $SiO_2/Al_2O_3$  percentage occurred because of elimination or reduction of organic impurities and evaporated water vapor which trapped in zeolite pore by heat treatment. Heat treatment applied in this experiment was not impacting other impurities such as  $CaO$  and  $K_2O$  minerals this phenomenon occurred because the heat applied was not high enough to decompose the substances due to zeolite calcination temperature was ranging between 1200-1300°C (Shindo et al., 2001). The importance of heat treatment was to enhance the  $Ca^+$ ,  $K^+$ ,  $Na^+$  ions in zeolite, thus needed for making the zeolite surface become more positively charged to accommodate adhesion or adsorption of negatively charged bacteria cell wall. Calcination was not needed in this experiment because of the physical and chemical properties needs for immobilizing bacteria cell was not specifically required, the importance of heat treatment was reduction of organic and vapor impurities that could caused blocking or inhibition of bacterial adsorption or adhesion.

**Table 1.** Chemical Composition of Carriers

Element	Mass %		
	Raw Zeolite	110°C Pretreated zeolite	300°C Pretreated zeolite
C	54,41	13,07	15,45
Na <sub>2</sub> O	0,96	2,02	1,64
MgO	0,29	0,57	0,75
Al <sub>2</sub> O <sub>3</sub>	5,88	12,67	11,82
SiO <sub>2</sub>	32,48	64,89	62,79
K <sub>2</sub> O	0,89	2,13	1,84
CaO	1,38	2,57	2,44
FeO	0,9	2,08	2,13
CuO	1,53	-	1,13
ZrO <sub>2</sub>	1,29	-	-
Si/Al ratio	5,52	5,12	5,31



Figures 1. EDX visualization Raw Zeolite (a), Pretreated 110°C zeolite (b) and 300°C (c)



Figures 2. Physical properties of carrier, Carrier surface area (a) and Carrier pore volume (b)

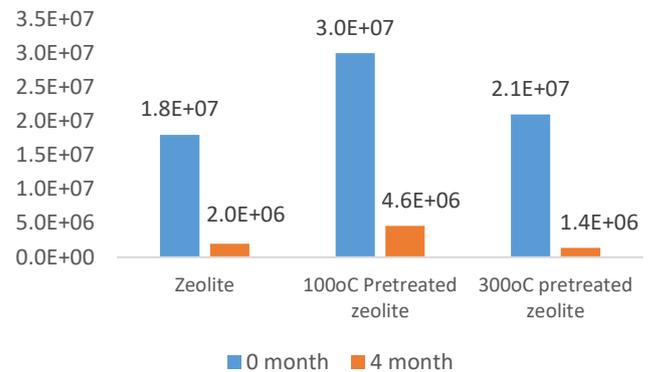
Another impact affected by heat treatment was Si/Al ratio, untreated zeolite has 5,52 Si/Al ratio, 110°C and 300°C heat treated zeolite has Si/Al ratio 5,12 and 5,52 respectively, the lower ratio of Si/Al and higher SiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> content means higher quality of the zeolite (Mustain *et al.*, 2014). Hydrophobicity and ion exchange capacity of zeolite could be known from its Si/Al ratio, higher ratio means higher hydrophobicity and lower ion exchange capacity (Kubota *et al.*, 2008). Si/Al ratio are also affect zeolite pore shape and its distribution. Zeolite modification could be done by physical or chemical treatment or both depend on needs, especially Modification were needed to obtain specific shape and size of zeolite pore. For example, zeolite modified to become nano-pore zeolite was used as immobilization carrier of  $\alpha$ -amylase enzyme (Talebi *et al.*, 2016). Another physical properties affected by heat activation was zeolite surface area and pore volume.

Figures 2 shows the BET and BJH surface area and pore volume analysis of untreated and treated zeolite carrier. Since the organic impurities were reduced and the water evaporated due to heat application the surface area of the carrier was increased it was confirmed by the BET surface area in mother carrier (raw zeolite) was 24,095 m<sup>2</sup>/g and the highest surface area was 110°C pretreated zeolite which has 65,625 m<sup>2</sup>/g and followed by 300°C pretreated zeolite which has surface area 45,577 m<sup>2</sup>/g. Surface area analysis using the BJH method has the same trends with BET method. Highest BJH surface area was occurred in 110°C treated zeolite which has 42,324 m<sup>2</sup>/g, followed by 300°C treated zeolite and zeolite raw which has 29,576 m<sup>2</sup>/g and 5,475 m<sup>2</sup>/g respectively. Increased pore volume has same trend with surface area, pretreated 110°C zeolite has 0,071 cc/g it was the highest volume compared to two others carriers.

### 3.2 Immobilization

Immobilization bacterial cells onto zeolites matrix has been investigated by many reseracher, Weiß *et al.*, (2013) investigated that anaerobic bacteria grows and colonized in activated zeolites, another work done by Hrenovic *et al.*, (2011) has investigated phosphate-

accumulating bacterium *A. Junii* could immobilized in zeolitized tuff.



**Figure 3.** Number of immobilized bacteria cells (Cfu) in different zeolite carriers

After 24 H of bacterial immobilization process and then dried achieved natural zeolite has the lowest number of bacteria which was 1,8x10<sup>7</sup> cfu/g and followed by 300oC pretreated zeolite with 2,1x10<sup>7</sup> cfu/g. The highest immobilized bacteria cell was achieved using 110oC pretreated zeolite as immobilization carrier which has the number of bacteria 3,0 x 10<sup>7</sup> cfu/g. The result After 4 months preservation observed that reduction of bacterial cell in immobilized carrier were occurred in all carrier variations, raw zeolite carrier was reduced to 2x10<sup>6</sup> cfu/gr, 110oC pretreated carrier was 4,5x10<sup>6</sup> cfu/gr and 300oC pretreated zeolite was 1,4x10<sup>6</sup> cfu/gr. Reduction of viable cells number was normally happened during initial preservation. Previous research done by (Sakane *et al.*, 1992) found that several halophilic archaeobacteria preserved using liquid drying method could survive approximately to more than 3 years at 5oC storage but reduced survival rates was still occurred and cell viability was reduced until 2 weeks preservation but relatively stable after 2 weeks. Preservation using liquid drying method has several disadvantages such as obligation to use protective media as immobilization carrier, the number of viable cells after immobilization process was regarded low due to extreme heat exposure during liquid drying process, and the immobilized cell must be stored at 5oC to gain maximum preservation condition. Compared to another research, Alvarez *et al* (2007) obtained the number of viability cell of

*E. Coli* immobilized in silica oxide and its derivate which stored at 20°C has a reduction varying from 102 to 103 cfu/ml in 60 days preservation.

SEM visualization of un-immobilized and immobilized carriers used in this research were presented in Figure 4. Under microscopic visualization, halophilic archae used in this research has irregular shape and attached each others with slime-like substances (fig 4.g), those slime-like covering haloarchaea microorganism was exopolymer secreted by halophilic archaea microorganism as compatible-solute mechanism to adapt with hypersaline and other environmental stresses (Dassarma and Dassarma, 2016). Halophilic archaea also has “salt in” and “salt out” mechanism for osmoregulation or osmo-protection in order to balance the osmotic pressure with the extracellular environment (Vreeland, 2012). Salt-in or salt-out mechanism is a strategy to accumulating inorganic ions especially K<sup>+</sup> in their cytoplasm or secreting inorganic ions to their environment in order to maintain their ion gradient equilibrium (Kunte, 2009). Salt-in and salt-out mechanism works not only for osmoregulation purpose but also for intracellular enzymatic activity (Empadinhas and Costa, 2008).

Figure 4a is SEM visualization of natural zeolite before immobilized with halophilic archaea, compared to other carriers, natural zeolite surface and pores covered by impurities. Pretreated 110°C and 300°C zeolite has more clearer surface and pore (figure 4 c and 4 e). Immobilized bacteria was colonizing mostly in the surface area of the carrier, some colony in pore was observed but not fully covering or plugging the pores. The presence of *Haloférx chudinovii* and *Haloférx lucentense* were marked with cubic shape or cubic like crystal which specifically produced by extreme halophilic archaea due to salt in and salt out mechanism (Castanier et al., 1992; Malik et al., 2019). In the hypersaline and dry environment the presence of halophilic archaea and formation of NaCl crystal or ooids were inevitable due to osmoregulation mechanism described below, this phenomenon happened due to drying in immobilization process. When the formation of NaCl crystal some fluid brine would form fluid inclusion, in the

presence of halophilic archaea when the fluid inclusion was formed the halophilic archaea would use it as a shelter to survive in unfavorable condition (Lowenstein et al., 2011).

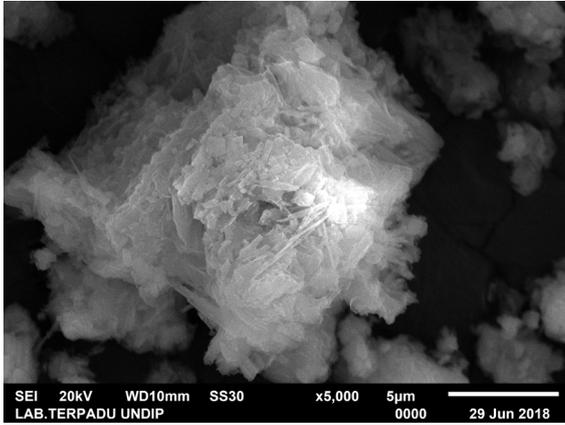
Figure 4 shown that immobilization was done mainly by adhesion mechanism, adsorption only happened due to the presence of pores in zeolite that utilize capillary pressure so it pull the culture (liquid phase) near onto zeolite surface. Most of gram-negative bacteria has negative charge while the zeolite it self has the same ionic charge, but negatively charged bacteria could also adhere on negatively charged materials (Klein and Ziehr, 1990), another possibility for bacteria to attached in zeolite surface was heat treatment that significantly turn the ionic charge of zeolite surface from negatively charged to positively charged. Adhesion or attachment of Halophilic archaea on the surface of zeolite carrier are also supported by the presence of S-layer proteins on haloarchaea cell wall that help the bacteria to attach on solid surface (Fendrihan et al., 2011). Another mechanism of halophilic archaea adhesion was exopolymers secreted by halophilic microorganism which surrounds the bacteria cells or colony that makes the bacteria attached to the zeolite surface.

Physiological and biochemical changes during immobilization may occurred due to unfavorable condition (Chauhan and Singh, 1999). The changes of Halophilic archaea biochemical pathways was investigated when trapped in halite fluid inclusion (Zerulla et al., 2014). Biochemical activity before and after immobilization process was obtained by biochemical assay and shown in table 2.

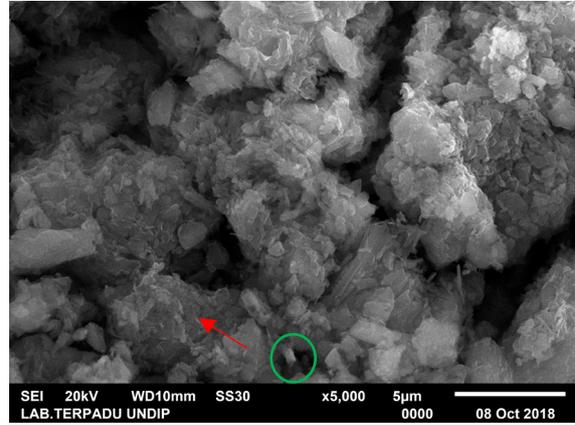
Biochemical assay shown that immobilization of halophilic archaea *Haloférx chudinovii* and *Haloférx lucentense* was not changing halophilic archaea metabolism ability (Table 2). halophilic archaeae enzymatic activity such as Lactate alkalization, Lipase enzyme, Tyrosine arylamidase, and ellman test was present in all immobilized carriers, only succinate activity who not present. From the data above shown that immobilization carriers and immobilization process were not giving a negative impact to halophilic archaea biochemical activity. Otherwise after immobilization another enzymatic activity

who were not present in single culture enzymatic assay was present in immobilized culture assay such as Phospatase, Fermentation procces, adonitol, A-glukosidase enzyme activity. Thus data confirmed that immobilization process

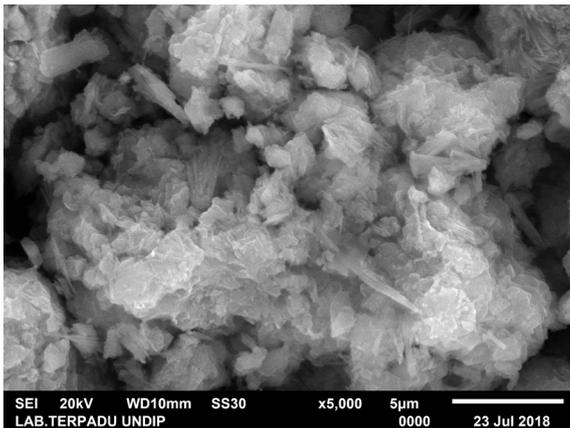
was enhancing bacteria ezymatic activity. Advantage of bacterial cells immobilization is the stability of protein and enzyme and this is not limiting their industrial utilization (Alfani et al., 1994).



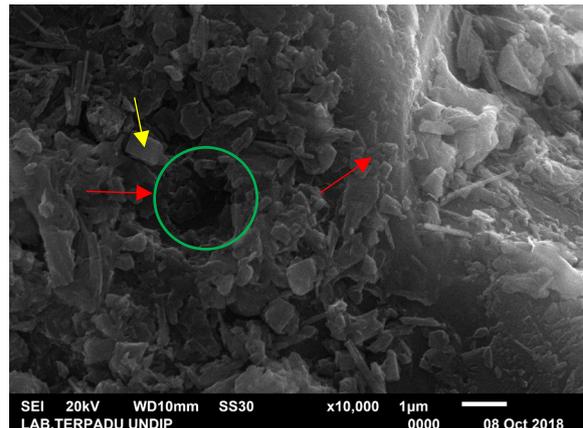
(a)



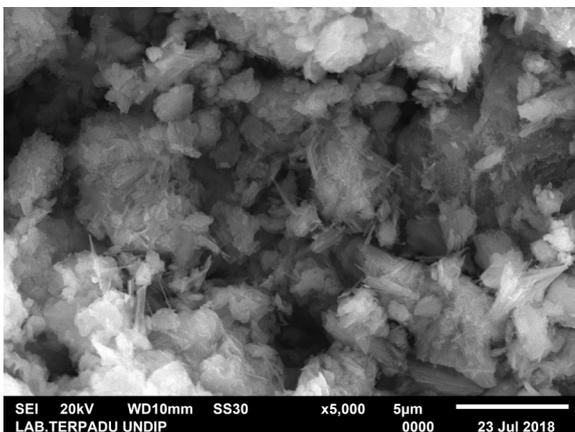
(b)



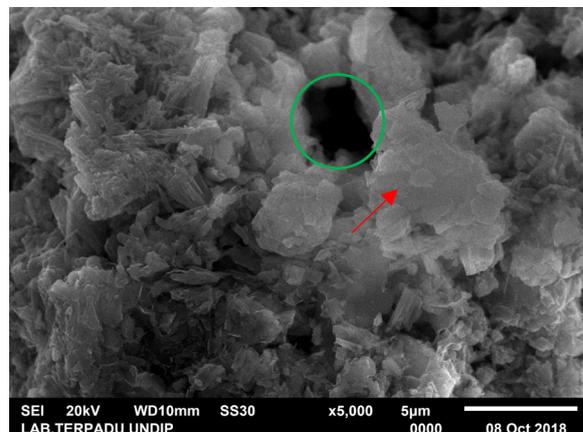
(c)



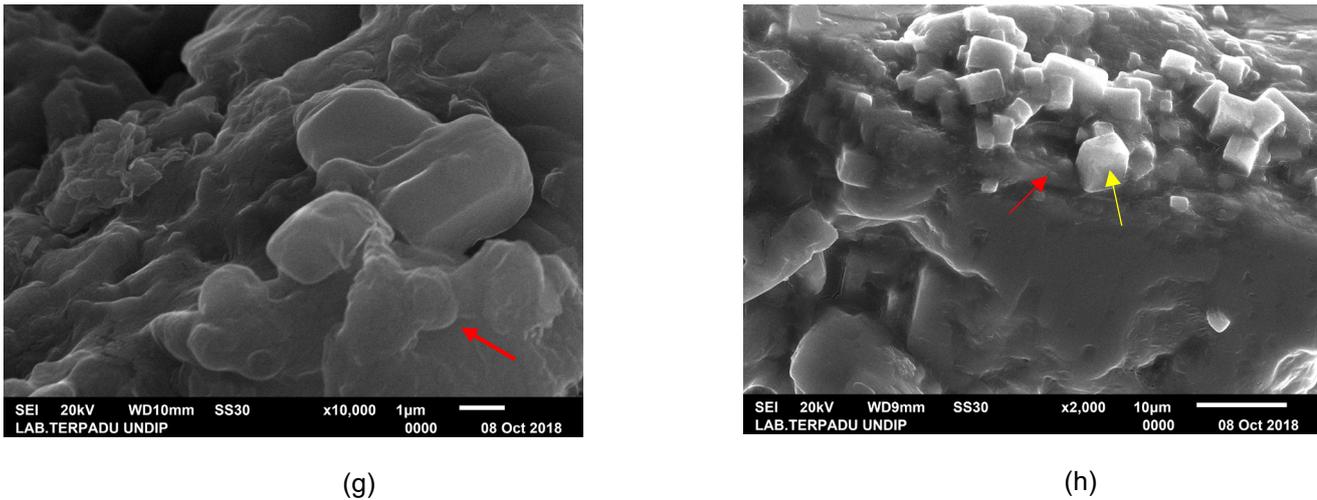
(d)



(e)



(f)



**Figure 4.** (a) Un immobilized Raw zeolite (b) immobilized Halophilic bacteria in raw zeolite matrices (c) Un immobilized 110°C pretreated zeolite (d) immobilized 110°C pretreated zeolite (e) Un immobilized 300°C pretreated zeolite (f) immobilized 300°C pretreated zeolite (g) SEM visualization of *Haloférx chudinovii* and *Haloférx lucentense* used in this work (h) NaCl crystal in Halophilic archaea colony; red arrow: Halophilic arcaea; Yellow arrow: NaCl crystal (cubic shape); green circle: pores

**Table 2.** Biochemical assay

No.	Test Parameter		Isolate / sample variation				
	Code	Measured parameter/activity	<i>Haloférx lucentense</i>	<i>Haloférx chudinovii</i>	Immobilized in carrier	Immobilized zeolit cell in 110°C pretreated Zeolit	Immobilized cell in 300°C pretreated Zeolit
1.	ILATk	L-Lactate alkalinization	+	+	(-)	+	+
2.	BGLU	B-Glukosidase	(-)	-	-	-	(-)
3.	Lip	Lipase	+	+	+	+	+
4.	ADO	Adonitol	-	-	+	-	+
5.	AGLU	A-Glukosidase	(-)	-	+	+	+
6.	SUCT	Succinate	-	+	-	-	-
7.	dMAN	D-Mannitol	-	-	(-)	-	-
8.	TyrA	Tyrosine Arylamidase	+	+	+	+	+
9.	ELLM	Ellman test	+	+	+	+	+
10.	CMT	Courmarate	-	-	+	-	-
11.	OFF	Fermentation/glucos e	-	-	(-)	-	+
12.	PHOS	Phospatase	-	-	-	-	+

### 3.3 Survivalability of Halophilic archaea *Haloferax chudinovii* and *Haloferax lucentense*

The aim of Immobilization procedure is to preserve targeted bacteria into selected carriers. Almost all bacteria known could be preserved when it become dormant. Dormant bacteria has very slow metabolic and other biochemistry activity which impact on decreased cell division rates. Survivalability of immobilized *Haloferax lucentense* and *Haloferax chudinovii* in zeolite carrier happend due to the ability of halophilic archaea to survive in unvaforable or starvation condition (Winters et al., 2015). Exopolymer secreted by halophilic archaea were also affect the survivalability of halophilic archaea during preservation due to formation of halophilic archaea biofilm and keeping the cell together in a three dimensional structure (Toyofuku et al., 2016). Biofilm formation are considered as strategy for bacterial survivalability, since biofilms protect microorganism from variable environmental conditions (Zur et al., 2016). The preservation condition in this research were placed under room temperature in closed pastic clip which immobilized halophilic archaea is on dry state. Halophilic archaea survival in dry environment was already known (Aharon Oren, 1994), also halophilic archaea enzymatic proteins were adapt to heat and dry environment and this phenomenon made halophilic archaea could survive in dry environment for long period of time (Tehei et al., 2002).

Due to the presence of exopolymer that bind and covering the bacteria together, thus promote reduction of dehydration in bacteria micro envirnoment which avoiding bacteria to environmental stress so the survivalability of *Haloferax lucentense* and *Haloferax chudinovii* were remain high. Another mechanism of survivalability of *Haloferax chudinovii* and *Haloferax lucentense* were the formation of crystal inclusion in conjunction with saline water that trapped the bacteria inside and survive in prolonged preservation. The survivalability of immobilized bacteria was depend on the method, carriers or immobilization matrices used and targeted bacteria. All of the three points mention before have mutual functions in order to keep bacteria survivalability during post immobilization.

## 4. CONCLUSION

The numbers of viable cell was relatively high during 4 months preservation in room temperature. Heat activation of the carrier was changing its chemical and physical properties but it seems to be uneffecting bacterial survivalbility or the numbers of attached bacteria. Immobilization and preservation prcedure done in this reserach was not affecting bacterial biochemistry ability. Immobilization method using zeolite as carrier was proven to be used as suitable low cost preservation method for halophilic archaea *Haloferax lucentense* and *Haloferax chudinovii* since the immobilization method was simple and preservation condition do not need special (temperature) condition.

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