Study of Glucosamine Production from Shrimp Shells by Fermentation Using *Trichoderma harzianum*

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Abstract

Shrimp shells are one of chitin sources in Indonesia which is potential to be converted into multifunctional glucosamine. This research was aimed to study the glucosamine production by fermentation using *Trichoderma harzianum*. Method used was experimental fermentation with pH treatment of 3-5 and fermentation duration of 10-20 days, and designed using Response Surface Methodology (RSM). Results showed that fermentation duration of 10-20 days has reached the optimum point of glucosamine production from shrimp shells using *T. harzianum*. The highest production of D-glucosamine in fermentation using *T. harzianum* occurred on initial pH of 5.41 and fermentation duration of 15 days (18,294.95 ppm), while the highest N-Acethyl-D-Glucosamine production occurred on initial pH of 3.00 and fermentation duration of 20 days (127,000.00 ppm).

Keywords: fermentation, glucosamine, shrimp shells, *T. harzianum*.

INTRODUCTION*

Shrimp is one of the important export commodities for fishing industry in Indonesia. Majority of shrimp export from Indonesia is done in form of frozen unshelled shrimp, therefore shrimp shell is considered as waste or unused by-products. The shrimp waste produced is around 30-35% of total weight of a shrimp [1]. Shrimp waste, such as shells can cause environmental problem if they are left unutilized.

Meanwhile, shrimp shells contain 25-40% of protein, 45-50% of calcium carbonate and 15-20% of chitin, depend on the types of shrimp [2]. Thus, shrimp shells can be used as a source of protein, calcium and chitin. Among those three components, the most potential component, in terms of economic value, is chitin. This is because chitin and its derivatives are very useful to be used in many industrial fields [3].

Chitin and its derivatives are commonly used in several fields, such as medical and health, i.e. they can be used as immunoadjuvant (non-specific stimulator of immune response) and basic material to make surgical suture [4]. N-acetyl glucosamine compound, the product of chitin hydrolysis, is used in treatment of osteoarthritis and used as supplement [5,6]. In aesthetic field, chitin derivatives can be used as basic ingredients for cosmetics, toothpaste, body cream and hair treatment products. In textile, they are widely used as coating material for cellulose fiber, nylon, cotton and wool [4].

Glucosamine is one of chitin derivatives that are widely used. Glucosamine production can be done physicochemically [7-11], enzymatically [12-15] and microbiologically [16]. To produce glucosamine chemically is relatively fast, but the chemical compounds used are dangerous for the environment, meanwhile to produce glucosamine enzymatically, the limitations are on the stability of enzyme and enzyme extraction cost that are quite expensive [17,18]. To produce monomer (likes glukosa, glucosamine) using microorganism is more advantageous because it is easier, more practical and the waste does not pollute the environment [19].

Glucosamine production using microorganisms is usually done by fermentation. There are many kinds of microorganisms, including bacteria and molds that can produce chitinolytic enzyme that can break down chitin into glucosamine. One of the microorganisms that can produce chitinase quite actively is *Trichoderma*. One of the strains, *T. viride*, produces chitinase enzyme with enzyme activity value of about 210.14 $U.mg^{-1}$ [20].

Trichoderma have several strains and in this research, the characteristics of *Trichoderma harzianum* in fermenting chitin into glucosamine with different pH condition and fermentation duration using RSM method, was studied.

MATERIALS AND METHODS

Method used in chitin fermentation was experimental method with design and analysis using Response Surface Methodology. Treatment

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or random variables applied in this fermentation experiment were pH (X_1 = 3, 4, 5) and fermentation duration (X_2 = 10, 15, 20 days). The determination of fermentation duration was based on report [21,22] that optimum fermentation duration of *Trichoderma* was 15 days, whereas the pH of fermentation was based on optimum pH for *T. harzianum* growth [23], i.e. pH of 5.6. The experimental matrix was derived by Software Design Expert, as can be observed on Table 1. The fixed factor was nutrition composition inside the fermentation media (KH_2PO_4 and $MgSO_4$) and fermentation temperature, i.e. about 30°C (room temperature). Therefore, the treatment applied in this research was combination from initial pH column and duration column, i.e. 21 treatments (run column).

Table 1. Experimental Design

Std	Run	Block	Initial pH	Duration (days)
11	1	Block 1	5.41	15.00
21	2	Block 1	4.00	15.00
12	3	Block 1	5.41	15.00
3	4	Block 1	5.00	10.00
16	5	Block 1	4.00	22.07
2	6	Block 1	3.00	10.00
15	7	Block 1	4.00	22.07
4	8	Block 1	5.00	10.00
5	9	Block 1	3.00	20.00
20	10	Block 1	4.00	15.00
17	11	Block 1	4.00	15.00
14	12	Block 1	4.00	7.93
19	13	Block 1	4.00	15.00
9	14	Block 1	2.59	15.00
13	15	Block 1	4.00	7.93
6	16	Block 1	3.00	20.00
10	17	Block 1	2.59	15.00
8	18	Block 1	5.00	20.00
18	19	Block 1	4.00	15.00
7	20	Block 1	5.00	20.00
1	21	Block 1	3.00	10.00

Note: Std = Standard

Preparation of Chitin from Shrimp Shells

Chitin isolation from *Vannamei* shrimp shells was performed by demineralization step using HCl and deproteination using NaOH [24]. First, *Vannamei* shrimp shells were washed and dried under sunlight until dry and then milled using Buhrmill and sieved using 60 mesh sieve to obtain shrimp shells powder.

Shrimp shells powder was then added with 1.5 M HCl with ratio of 1:15 (w/v) between sample and solvent. The mixture was then heated at temperature of 70-80°C for 4 hours under agitation of 50 rpm, and was filtered afterwards. The solid obtained was washed with aquadest to remove the remaining HCl. Then, the solid was dried in the oven with temperature of 70°C for 24 hours and cooled inside the desiccator, to obtain low mineral shrimp shells powder.

Demineralized shrimp shells powder was put into beaker glass and added with 3.5% NaOH solution with ratio of 1:10 (w/v). The mixture was then heated at temperature of 65-70°C for 4 hours under agitation of 50 rpm. The mixture was filtered to obtain chitin solids and washed with aquadest until neutral pH is obtained.

Fermentation of Chitin from Shrimp Shells using *T. harzianum*

Fermentation of chitin using *T. harzianum* was performed using semi solid fermentation system. The substrate consists of 5 gram of chitin, 0.05 gram of KH_2PO_4, 0.25 gram of $MgSO_4$, 0.05 gram of urea, 1 gram of glucose, and 25 ml of aquadest [25].

The substrate was then sterilized using autoclave with temperature of 121°C for 15 minutes. When the substrate has cooled down, pH was set according to the treatment using 0.1 M NaOH and/or 0.1 M HCl. Each substrate was added with 1 mL of *T. harzianum* spore and fermented at room temperature using shaker fermenter.

Chitinolytic Activity Test by Clear Zone Measurement

Chitinolytic activity test was done by observing the clear zone formed on agar media enriched with chitin, surrounding the growth of molds. Agar-chitin media was made from PDA added with 0.5% colloidal chitin (w/v media) [26, 27] and acidified using 50% lactic acid for about 0.14% from total volume of media [28]. This media was then sterilized using autoclave with temperature of 121°C, pressure of 1 atm for 15 minutes. The media was then poured into Petri dish and let solidify. After the media has solidified, a well with 6 mm diameter was made, aseptically. Then, 20 µL of *T. harzianum* spores with density of $20.48\times10^7.mL^{-1}$, $38.48\times10^7.mL^{-1}$, $63.52\times10^7.mL^{-1}$, $158.72\times10^7.mL^{-1}$, were respectively added into the well (6.0 mm diameter), and incubated at temperature of 35°C for 48 hours. Chitinolytic activity was determined by measuring the clear zone formed, i.e. by subtracting diameter of clear zone formed from diameter of well.

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Glucosamine Analysis using Spectrophotometry Method [29]

About 0.01 gram of fermented sample was dissolved uisng 1 mL of 0.25 M CH_3COONa to obtain concentration of 10,000 $mg.L^{-1}$. This solution was then diluted gradually until concentration of 12 ppm and 100 ppm were obtained. Each sample (concentration of 12 ppm and 100 ppm) was reacted with same concentration of PITC. The absorbance of sample solution of 12 ppm concentration that has been added with PITC was determined using UV-Vis spectrophotometer at wavelength of 273 nm to obtain absorbance of D-Glucosamine compound. Meanwhile, the absorbance of sample solution of 100 ppm concentration that has been added with PITC was determined using UV-Vis spectrophotometer at wavelength of 584 nm to obtain absorbance of N-Acethyl-D-Glucosamine compound. These absorbance results were then plotted on standard curve of D-Glucosamine and N-Acethyl-D-Glucosamine.

Standard curve of D-Glucosamine and N-Acethyl-D-Glucosamine were prepared by dissolving 0.01 gram of standard D-Glucosamine or N-Acethyl-D-Glucosamine in 10 mL of 0.25 M CH_3COONa and allowed to stand for 24 hours (to stabilize the solution). After that, standard D-Glucosamine or N-Acethyl-D-Glucosamine solution with concentration of 1,000 ppm as a stock solution was obtained. The stock solution was then diluted to obtain concentration of 3, 6, 9 and 12 ppm. About 10 mL of each solution was added with 10 mL of PITC solution, homogenized for 5 minutes and derivatives compound, i.e. phenylthiourea (PTH), was obtained. The absorbance of this compound can be determined according to the wavelength used. In this case, PITC solution was prepared by dissolving PITC using methanol that has the same concentration with glucosamine standard solution.

RESULT AND DISCUSSION

Characteristics of Chitin from Shrimp Shells

Table 2 shows that chitin used has not fulfilled the commercial chitin standard in terms of protein content, although its moisture content, mineral content and deacetylation degree have fulfilled the commercial chitin standard. High protein content in chitin indicates that deproteination process was not enough. This might be caused by insufficient NaOH concentration and/or insufficient deproteination process. Effectivity of deproteination process depends on the concentration of basic solution and temperature used [30]. Besides, protein content of chitin is related to its moisture content [30]. Lots of protein matrix that have been degraded cause hydrophilic properties of protein to bind water molecule becomes weaker[33]. As a result, the moisture content will become less [31]. It is shown by the moisture content of shrimp shells which is about 60-65% [32].

Table 2. Characteristics of Chitin and Dried Shrimp Shells

Parameter (%)	Dried Shrimp Shells	Chitin	Commercial Chitin
Moisture	10.07± 0.26	6.69±0.21	<10*
Protein	42.41±4.21	13.2 ±1.42	<1*
Mineral	19.75±0.39	1.42±0.09	<2.5*
Deacetylation degree	-	35.16	15-70**

Note : *Commercial food grade chitin[33];
**Chitin standard Protan Laboratories[34]

Chitinolytic Activity of *T. harzianum* Molds

Chitinolytic activity of molds is defined as the ability of molds to degrade chitin into other simpler compounds, e.g. glucosamine by chitinase enzyme. This activity can be measured by clear zone formation around the wells, paper disc or colony [20,35,36]. In this research, chitinolytic activity obtained based on clear zone formation was used to determine the amount of spores added during chitin fermentation. The results of clear zone based on density of *T. harzianum* spores can be observed on Table 3.

Table 3. Clear zone formation based on density of *Trichoderma harzianum* spores

Density (spore.mL^{-1})	Clear zone (mm)*
20.48×10^7	6.52 ± 0.73
38.48×10^7	5.93 ± 0.91
63.52×10^7	4.67 ± 1.28
158.72×10^7	2.95 ± 0.17

***Note**: clear zone = diameter of clear zone – diameter of well

The size of clear zone formed shows the amount of N-acetylglucosamine formed from chitin breakdown by chitinase enzyme. The larger the clear zone, the higher amount of chitinase formed, which means the higher the chitinolytic activity [36]. Based on Table 3, it can be observed that the largest clear zone diameter for *T. harzianum* molds was obtained from spores with density of 20.48 x 10^7 spores.mL^{-1}, with clear zone of 6.52 ± 0.73 mm, and then decreased as the spore density was increased. The smallest clear zone occurred at density of 63.52×10^7 spores.mL^{-1} with clear zone of 2.95 ± 0.17 mm. This might happen because the spore density of 20.48 x 10^7 spores.mL^{-1} was the most effective;

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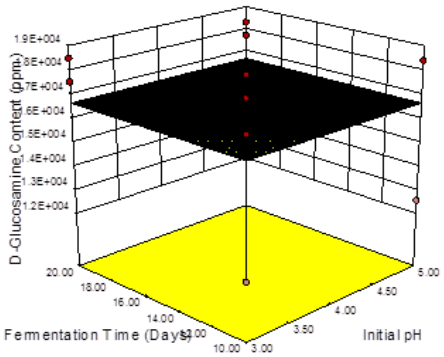
therefore there was no growth competition among the molds. Thus, *T. harzianum* can work optimally to degrade chitin during fermentation. Chitin degradation is done by chitinase enzyme that has different activity based on the types and stage of growth of microorganisms, and factors which influence the growth of microorganisms, such as pH, temperature and fermentation period [12,37,38]. Chitinase activity from *Trichoderma* sp. and *T. viridae* was 33.19 IU.mL^{-1} and 18.77 IU.mL^{-1}, respectively [20].

Glucosamine Production

Glucosamine that is obtained from chitin fermentation can be in form of D-glucosamine and N-Acethyl-D-Glucosamine. The correlation between pH and fermentation duration of shrimp shells chitin for glucosamine production according to RSM program can be observed on Figure 1.

A) D-Glucosamine



B) N-Acethyl-D-Glucosamine

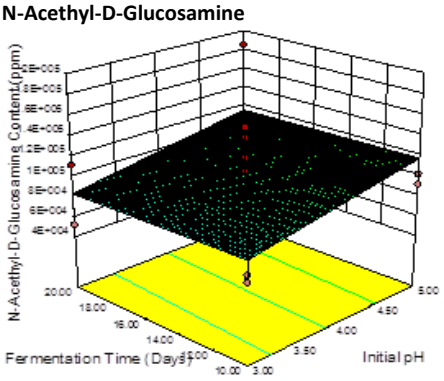


Figure 1. Three-dimensional RSM graph of correlation between pH and fermentation duration of shrimp shells chitin for glucosamine production using *T. harzianum*

Three-dimensional RSM graph on Figure 1 shows the flat shape of correlation between pH and fermentation duration of shrimp shells chitin for glucosamine production, which means there was no optimum point. This phenomenon shows there was no significant effect of fermentation duration and pH on D-Glucosamine and N-acetyl-D-Glucosamine obtained. This might happen because the fermentation duration was too long; therefore the production of D-Glucosamine is already maximum since the first observation (day 10) and there was only slight difference in glucosamine content on the following observations. It is based on a previous research that stated the production of chitinase by fermentation using *T. harzianum* reached its highest amount after 72 hours (day 3) when *T. harzianum* mycelia starter was used and after 96 hours (day 4) when *T. harzianum* spore starter was used [39].

This statement is also supported by another research that stated chitinolytic activity of *T. harzianum* reached its optimum amount in between day 2 and day 3 [40]. No significant change of glucosamine production after it has reached its optimum point is also in accordance with a research [41] which stated after 172 hours, glucosamine fermentation using *T. harzianum* mold starter did not undergo significant increase. Because there was no optimum point for glucosamine production, the glucosamine production can be observed based on interaction between pH and fermentation duration, as can be seen on Figure 2.

Figure 2 shows that the highest D-glucosamine amount obtained from pH treatment of 5.41 and fermentation duration of 15 days, which produces D-glucosamine content of 18,294.95 ppm. This amount of glucosamine is still higher compared to other research reports. Fermentation using chitinase from *Pseudomonas* sp. was optimum after 35 hours, which produces 0.164 mg.mL^{-1} 164 ppm of glucosamine [14], using *Actinomycetes* ANL-4 starter produces highest glucosamine about 9,784.25 ppm on day 5 [29], using *Aspergillus* sp. BCRC 31742 produces highest glucosamine of 7,480 ppm [42].

The highest N-acetyl-D-Glucosamine production in fermentation of shrimp shells chitin using *T. harzianum* obtained from pH treatment of 5 and 10 days of fermentation, which produces N-acetyl-D-Glucosamine of 127,000.00 ppm. This amount of glucosamine is still higher compared to other research reports. Fermenta-tion using *Aspergillus rogulosus* 501 on day 10 obtained N-acetyl-D-Glucosamine of 2,228 ppm [43], fermentation using enzyme combination from *T. harzianum* and *T. reesei* was highest after 72 and 96 hours of incubation, which was about 4,040 ppm [39], fermentation using *T. harzianum* was

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highest after 212 hours, which was about 190 ppm [40].

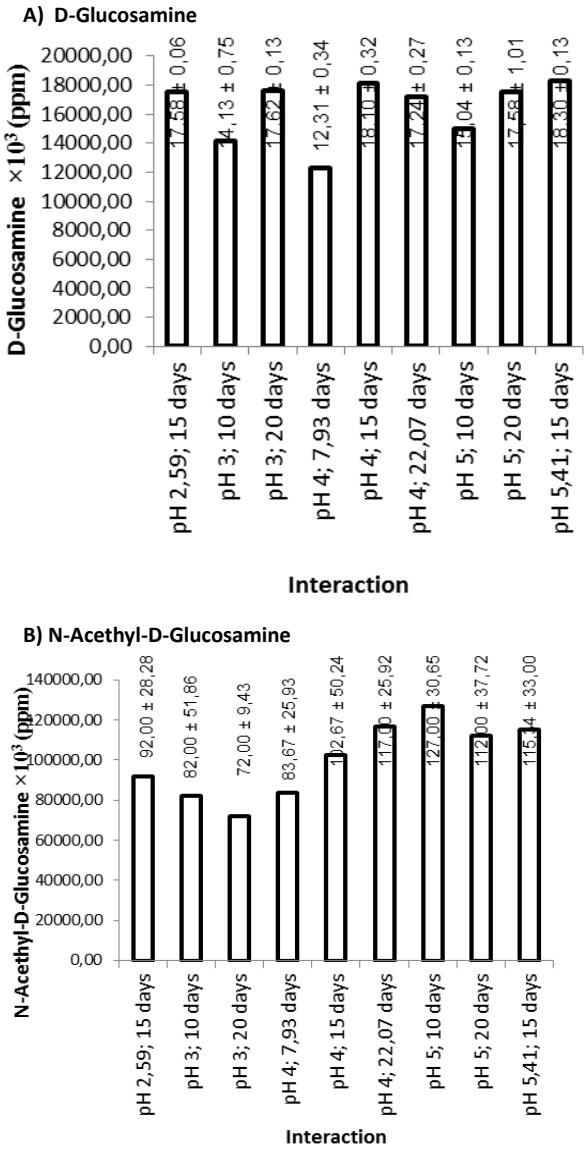

Figure 2. Graph of interaction between fermentation factors and their influence on glucosamine content

CONCLUSION

The fermentation duration applied on optimization of fermentation of shrimp shells chitin for glucosamine production using *T. harzianum* was too long. The highest production of D-Glucosamine from fermentation using *T. harzianum* occurred on initial pH of 5.41 and fermentation duration of 15 days, which reached about 18,294.95 ppm, whereas the highest production of N-Acethyl-D-Glucosamine occurred on initial pH of 3.00 and fermentation duration of 20 days, which reached about 127,000.00 ppm.

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REFERENCES

[1] Darmawan, E., S. Mulyaningsih, F. Firdaus. 2007. Karakteristik khitosan yang dihasilkan dari limbah kulit udang dan daya hambatnya terhadap pertumbuhan *Candida albicans*. *Logika.* 4. 207-213.

[2] Foucher, J.P., G.K. Westbrook, A. Boetius, S. Ceramicola, S. Dupre, J. Mascle, J. Mienert, O. Pfannkuche, C. Pierre, D. Praeg. 2009. Structure and drivers of cold seep ecosystems. *Oceanography.* 22. 92-109.

[3] Hargono, Abdullah, I. Sumantri. 2008. Pembuatan kitosan dari limbah cangkang udang serta aplikasinya dalam mereduksi kolesterol lemak kambing. *Reaktor.* 12(1). 53-57.

[4] Kaban, J. 2009. Modifikasi kimia dari kitosan dan aplikasi produk yang dihasilkan. Master Thesis. Department of Chemistry. University of North Sumatera. Medan.

[5] Sashiwa , H., S. Fujishima, N. Yamano, N. Kawasaki, A. Nakayama, E. Muraki, K. Hiraga, K. Oda, S. Aiba. 2002. Production of N-Acetyl-D-Glucosamine from chitin by crude enzymes from *Aeromonas hydrophila* H-2330. *Carbohyd.Res.* 337. 761-763.

[6] Kardiman, C. 2013. Manfaat glukosamin, kondroitin, dan metilsulfonilmetana pada osteoartritis. *CDK-211.* 40 (12). 936-939.

[7] Suptijah, P., B. Ibrahim, Ernawati. 2014. Pemanfaatan limbah krustasea dalam pembuatan glukosamin hidroklorida dengan metose autoklaf. *Jurnal Teknologi Perikanan dan Kelautan.* 5(2). 173-181.

[8] Dewi, N.L., S. Bahri, H. Jaya. 2016. Penggunaan berbagai tekanan dan waktu hidrolisis pada produksi glukosamin hidroklorida dari kitosan cangkang Bekicot (*Achatina fulica*). *Kovalen*. 2(1). 22-32.

[9] Nurjannah, A., Darmanto, I. Wijayanti. 2016. Optimasi pembuatan glukosamin hidroklorida (GLcN HCl) dari limbah cangkang udang rajungan melalui hidrolisis kimiawi. *JPHPI.* 19(1). 26-35.

[10] Mukti, M.I., N. Herdyastuti. 2016. Karakterisasi N-asetilglukosamin hasil hidrolisis kitin secara kimiawi. Proceeding of

ISSN. 2087-2852
E-ISSN. 2338-1655

National Seminar on Chemistry and Its Learning. 61-64.

[11] Zaeni, M., E. Safitri, I.N. Sudiana. 2017. Pembuatan glukosamin hidroklorida dari cangkang udang dengan energi microwave *Jurnal Aplikasi Fisika.* 13(1). 22-26.

[12] Widhyastuti, N. 2007. Produksi kitinase ekstraseluler *Aspergillus rugulosus* 501 secara optimal pada media cair. *Berita Biologi.* 8(6). 547-553.

[13] Widhyastuti, N., M Ilyas. 2012. Aktivitas dan karakter kitinase isolat *Trichoderma* sp. W34 A4 asal Kepulauan Raja Ampat Papua Barat. *Biosfera.* 29(1). 1-7.

[14] Wirawan, A., N. Herdyastuti. 2013. Penentuan waktu inklubasi pada pembentukan senyawa N-asetilglukosamin yang didegradasi secara enzimatis dari kitin. *J. Chem.* 2(3): 11-13.

[15] Purnomo, E.H., A.B. Sitanggang, D. Indrasti. 2012. Studi kinetika produksi glukosamin dalam Watermiscible Solvent dan proses separasinya. Proceeding of Seminar on Research Results. Bogor Agricultural University. Bogor. 24-25.

[16] Sharaf, E.F., A.E.Q. El-Sarrany, M. El-Deeb. 2012. Biorecycling of shrimp shell by *Trichoderma viride* for production of antifungal chitinase. *Af. J. Microbiol. Res.* 6 (21). 4538-4545.

[17] Palonen, H. 2004. Role of lignin in the enzymatic hydrolysis of lignocellulose. VTT Biotechnology. Helsinki University of Technology, Espoo, Finland.

[18] Taherzadeh, M.J., K. Karimi. 2008. Pretreatment of lignocellulosic waste to improve bioethanol and biogas production. *Int. J. Mol. Sci.* 9. 1621-1651.

[19] Suhartati, F.M., W. Suryapratama, N. Iriyanti. 2003. Sintesis asam amino metionin pada Trichoderma reseii dan pengaruhnya terhadap sintesisis protein mikroba rumen. *Jurnal Peternakan dan Lingkungan.* 9. 12-16.

[20] Khikmah, N., S. Margino, R.S. Kasiamdari. 2016. Isolasi, seleksi, dan identifikasi kapang kitinolitik yang diisolasi dari tanah pembuangan limbah udang dan Rizosfer Solanaceae. *Biota.* 1(1). 1-8.

[21] Saparianti, E., T. Dewanti, S.K Dhoni. 2012. Hidrolisis ampas tebu menjadi glukosa cair oleh kapang *Trichoderma viride*. *Jurnal Teknologi Pertanian*. 5(1). 1-10.

[22] Montesqrit. 2007. Isolasi dan karakterisasi selulase dari *Trichoderma viride* dan *Rhizopus* spp dengan substrat jerami padi. *Jurnal Peternakan Indonesia.* 12(2).112-123.

[23] Kapat, A., S.K. Rakshit, T. Panda. 1996. Optimization of carbon and nitrogen sources in the medium and environmental factors for enhanced production of chitinase by *Trichoderma harzianum*. *Bioprocess Eng.* 15. 13-20.

[24] Puspawati, N.M., I.N. Simpen. 2010. Optimasi deasetilasi khitin dari kulit udang dan cangkang kepiting limbah restoran seafood menjadi khitosan melalui variasi konsentrasi NaOH. *Jurnal Kimia.* 4(1). 79-90.

[25] Saskiawan, I., R. Handayani. 2011. Hidrolisis kitin melalui fermentasi semi padat untuk produksi N-Asetilgukosamin. *Berita Biologi.* 10(6). 121-127.

[26] Hsu, S.C., J.L. Lockwood. 1975. Powdered chitin agar as a selective medium for enumeration of Actinomycetes in water and soil. *Appl. Microbiol.* 29(3). 422-426.

[27] Cahyani, L. 2013. Pemanfaatan tepung cangkang udang sebagai media produksi kitinase oleh bakteri kitinolitik Isolat 26. Bachelor Thesis. Department of Biology, University of Jember. Jember.

[28] El-Gholl N.E., A.C. Alfenas, P.W. Crous, T.S. Schubert. 1993. Description and pathogenicity of *Cylindrocladium ovatum* s. nov. *Can. J. Bot.* 71. 466-470.

[29] Utami, P.H. 2014. Penetapan waktu inkubasi optimum degradasi kitin oleh kitinase dari *Actinomycetes* ANL-4 secara spektrofotometri UV-Vis. Bachelor Thesis. Department of Chemistry. University of Lampung. Lampung. 45-46.

[30] Karmas, E. 1982. Poultry and seafood technology. Noyes Data Corporation. USA.

[31] Knorr, D. 1991. Recovery and utilization of chitin and chitosan in food processing waste management. *Food Technol.* 45(1). 114-122.

[32] Cha, D.S., M.S. Chinnan. 2004. Biopolymer-based antimikrobial packaging: a review. *Critical Rev. Food Sci. Nutr.* 44. 223-237.

[33] Subasinghe, S. 1999. Chitin from shellfish waste health benefits over shadowing industrial uses. *Infofish. Int.* 3. 58-65.

[34] Bastaman, S., N. Aprinita, Hendarti. 1990. Research shrimp waste as an industrial material chitin and chitosan. Indonesian Research and Development Center of Agricultural Industrial Crops. Bogor.

ISSN. 2087-2852
E-ISSN. 2338-1655

[35] Herdyastuti, N., T.J. Raharjo, Mudasir, S. Matsjeh. 2009. Chitinase and chitinolytic microorganism: isolation, charactirization and potential. *Indones. J. Chem.* 9(1). 37-47.
[36] Pratiwi, R.S., T.E. Susanto, Y.A.K. Wardani, A. Sutrisno. 2014. Kajian pustaka: enzim kitinase dan aplikasi dibidang industri. *Jurnal Pangan dan Agroindustri.* 3. 878-887.
[37] Purkan, B., A. Azizah, Baktir, S. Sumarsih. 2014. Eksplorasi bakteri kitinolitik dari sampah organik : Isolasi dan karakterisasi enzim kitinase. *Jurnal Molekul.* 9(2). 128-135.
[38] Rachmawaty, Madihah. 2013. Potensi perlakuan awal limbah kulit udang untuk produksi enzim kitinase oleh *Trichoderma virens* pada fermentasi substrat padat. *Jurnal Bionature.* 14(1). 33-37.
[39] Sandhya, C., L.K. Adapa, K.M. Nampoothiri, P. Binod, G. Szakacs, A. Pandey. 2003. Extracellular chitinase production by Trichoderma harzianum in submerged fermentation. *J. Basic Microbiol.* 44(1). 49-58.
[40] Nampoothiri, K.M., T.V. Baiju, C. Sandhya, A. Sabu, G. Szakacs, A. Pandey. 2003. Process optimization for antifungal chitinase production by Trichoderma harzianum. *Process Biochem.* 39. 1583-1590.
[41] Bohlmann, J.A., D.O. Schisler, K. Hwang, J.P. Henning, J.R. Trinkle, T.B. Anderson, J. Donald, A. Vanderhoff. 2004. N-Acetyl-D-Glucosamine and process for producing N-Acetyl-D-Glucosamine. United States Patent US 6,693,188 B2.
[42] Sitanggang, A.B., H.S. Wu, S.S. Wang, Y.C. Ho. 2010. Effect of pellet size and stimulating factor on the glucosamine production using *Aspergillus* sp. BCRC 31742. *Biores. Technol.* 101(10). 3595-3601.
[43] Wulandari, F. 2009. Optimization of production of N-acetylglucosamine from chitin through fermentation by Aspergillus rugulosus 501. Master Thesis. Department of Biochemistry. Bogor Agricultural University. Bogor.

ISSN. 2087-2852
E-ISSN. 2338-1655