

Production of N-acetylglucosamine from shrimp shells' chitin using intracellular chitinase from *Mucor circinelloides*

^{1,*}Halim, Y., ¹Fransiska, ^{1,2}Hardoko and ¹Handayani, R.

¹Food Technology Department, Universitas Pelita Harapan, Jl. M.H. Thamrin Boulevard, Lippo Karawaci, Tangerang 15811, Indonesia

²Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Jl. Veteran No. 1 Malang 65113, Indonesia

Article history:

Received: 29 March 2020

Received in revised form: 4 May 2020

Accepted: 15 May 2020

Available Online: 30 May 2020

Keywords:

Chitinase,
Chitin,
Intracellular,
Mucor circinelloides,
N-acetylglucosamine

DOI:

[https://doi.org/10.26656/fr.2017.4\(5\).135](https://doi.org/10.26656/fr.2017.4(5).135)

Abstract

Chitin is a natural biopolymer found in shrimp shells and can be processed into N-acetylglucosamine which is extensively used as a dietary supplement to treat osteoarthritis, back pain and knee pain. This research was conducted to determine the optimum pH, temperature, substrate concentration and incubation period to produce N-acetylglucosamine using crude and semi pure intracellular chitinase extracted from *Mucor circinelloides*. Chitinase activity was measured to determine optimum pH and temperature by using various pHs (3, 4, 5, 6, 7, 8 and 9) and temperatures (30°C, 40°C, 50°C, 60°C, 70°C and 80°C). Different substrate concentrations (0.5%, 1.0%, 1.5% and 2.0%) and incubation periods (2, 4, 6 and 24 hrs) were used to determine the optimum condition to produce N-acetylglucosamine. Results showed that crude intracellular chitinase had an optimum pH of 5 with chitinase activity of 4.16±0.07 U/mL and optimum temperature of 60°C with chitinase activity of 4.22±0.07 U/mL. The optimum substrate concentration obtained was 0.5% and the optimum incubation period obtained was 6 hrs with about 961.67±9.13 ppm N-acetylglucosamine produced. Semi pure intracellular chitinase had an optimum pH of 4 with chitinase activity of 4.75±0.09 U/mL and optimum temperature of 50°C with chitinase activity of 5.03±0.08 U/mL. The optimum substrate concentration obtained was 1.5% and the optimum incubation period obtained was 4 hrs with about 1150.56±12.55 ppm N-acetylglucosamine produced.

1. Introduction

Chitin is the most abundant natural amino polysaccharide that consists of N-acetylglucosamine monomers attached to each other via β (1,4) glycosidic linkages (Robiah, 2015). Chitin is also non-toxic, biodegradable and biocompatible in nature (Anitha *et al.*, 2014). Chitin is very widely distributed, especially in animals. Higher concentrations up to 85% of chitin are found in Arthropoda such as shrimps (Savitri *et al.*, 2010). The exoskeleton of *Penaeus monodon* shrimp contains up to 10% chitin, up to 40% proteins and 45-50% minerals. The extraction of chitin from shrimp shells involves two steps, including demineralization and deproteinization process, which can be conducted by chemical and biological method (Arbia *et al.*, 2012).

Glucosamine is the end hydrolytic product of chitin that can be obtained through chemical and enzymatic hydrolysis. Glucosamine, in the form of glucosamine

sulfate, glucosamine hydrochloride, or N-acetylglucosamine is extensively consumed as a dietary supplement to treat osteoarthritis, back pain and knee pain. Glucosamine sulfate and glucosamine hydrochloride pose some defects including harsh hydrolysis conditions as both are obtained through chemical hydrolysis of chitin. To overcome it, glucosamine can be obtained through enzymatic hydrolysis of chitin using chitinase (Wang *et al.*, 2008).

Even though chitinase can be obtained from many sources, chitinase of filamentous fungi have shown to have higher activity levels than those of plants and bacteria (Wang and Yang, 2007). Chitinolytic fungi include *Trichoderma*, *Mucor*, *Beauveria*, *Aspergillus* and *Penicillium* (Brzezinska *et al.*, 2014). Fungi used in this research was *Mucor circinelloides* isolated from previous research by Veronica (2018), which showed high chitinolytic activity. Fungi could produce chitinase both extracellularly and intracellularly (Yamazaki *et al.*,

*Corresponding author.

Email: yuniwati.halim@uph.edu

2007), but intracellular chitinase from fungi has not been studied extensively.

Moreover, to produce N-acetylglucosamine, chitinase activity is affected by several factors such as substrate concentration, temperature, pH and fermentation time (Hao *et al.*, 2012). Therefore, the objectives of this research were to determine the optimum pH, temperature, substrate concentration and incubation period to produce N-acetylglucosamine using crude and semi pure intracellular chitinase extracted from *M. circinelloides*.

2. Materials and methods

2.1 Materials and equipment

Materials used in this research were chitin isolated from shrimp shells of *Penaeus monodon* obtained from PT. Lola Mina located at Muara Baru, Jakarta, Indonesia, *Mucor circinelloides* culture obtained from Veronica (2018) previous study, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), Bradford reagent, bovine serum albumin (BSA), Sigma-Aldrich DNS (3,5-Dinitrosalicylic acid) reagent, Sigma-Aldrich N-acetylglucosamine standard, 70% ammonium sulfate, sodium phosphate dibasic (Na_2HPO_4), magnesium sulfate (MgSO_4), 37% hydrochloric acid (HCl), sodium hydroxide (NaOH), distilled water, phosphate buffer (pH 7.4), phosphate buffer (pH 8), sodium citrate, citric acid, monosodium dihydrogen orthophosphate (NaH_2PO_4), glycine and potassium sodium tartrate.

2.2 Extraction of intracellular chitinase of *M. circinelloides*

The extraction of chitinase from *M. circinelloides* was done based on the method by Struszczyk *et al.* (2008) with modifications. Firstly, 3 mL of mold culture was added to 300 mL of PDB that have been fortified with 0.5% Na_2HPO_4 , 0.5% MgSO_4 , and 0.5% chitin and incubated at room temperature for 2 days using shaker. After incubation, media was then centrifuged at 3500 rpm for 10 mins at 4°C. The precipitate obtained was suspended in 4 mL of lysis buffer per 80 mL of culture. The mixture was then sonicated using 47 kHz for 10 mins and centrifuged at 3500 rpm for 10 mins. The supernatant produced was crude intracellular enzyme.

To purify the enzyme, the supernatant was further added with 70% ammonium sulfate with ratio 1:1 and left to settle for 18 hrs. Then it was centrifuged at 3500 rpm for 10 mins. The precipitate was then dissolved in 8 mL of 0.05 M phosphate buffer (pH 8) per 150 mL initial culture. The solution obtained was semi pure intracellular chitinase.

2.3 Determination of optimum pH and temperature of chitinase

The determination of optimum pH and temperature of chitinase was done based on Annamalai *et al.* (2011) with modifications. To determine the optimum pH, the substrate was prepared by mixing buffer solution of pH 3, 4, 5, 6, 7, 8 and 9 with 1% chitin. The substrate was then sterilized. About 1 mL of the substrate was added with 1 mL of crude or semi pure chitinase and incubated for 1 hour at room temperature. The mixture was centrifuged at 13000 rpm for 5 mins. The chitinase activity was then analyzed using the DNS method.

To determine the optimum temperature, 1 mL of the substrate with optimum pH obtained was added with 1 mL of crude or semi pure chitinase and incubated at different temperatures (30°C, 40°C, 50°C, 60°C, 70°C and 80°C) for 1 hr. It was then centrifuged at 13000 rpm for 5 mins. The chitinase activity was then analyzed using the DNS method.

2.4 Determination of optimum substrate concentration and incubation period of chitinase

Determination of optimum substrate concentration and incubation period of chitinase was done based on the method done by Annamalai *et al.* (2011) with modifications. The substrate of different concentrations (0.5%, 1%, 1.5% and 2%) with optimum pH were each added with 1 mL of crude or semi pure chitinase and incubated at optimum temperature at different incubation periods (2, 4, 6 and 24 hrs). After incubated, it was centrifuged at 13000 rpm for 5 mins. N-acetylglucosamine obtained was then analyzed using the DNS method.

2.5 Chitinase activity and N-acetylglucosamine concentration analyses

The chitinase activity was analyzed using the DNS method (Jha and Modi, 2017). An aliquot (1 mL) of the sample was then added with 1 mL of DNS reagent and 1 mL of 4% potassium sodium tartrate. The mixture was then heated in a boiling water bath for 15 mins to stop the reaction in the mixture. About 1 mL of the heated mixture was then diluted with 4 mL of water. The diluted mixture was then measured at 540 nm using UV-Vis spectrophotometer to determine its N-acetylglucosamine concentration. A standard curve was prepared using N-acetylglucosamine standard with concentrations of 200, 400, 600, 800 and 1000 ppm. Furthermore, 1 μmol of N-acetylglucosamine produced by the chitinase is defined as one unit (U) of chitinase activity.

2.6 Data analysis

All data obtained from the experiment were analyzed using SPSS version 22.

3. Results and discussion

3.1 Characteristics of isolated chitin

The isolated chitin used in this research was obtained through demineralization and deproteinization process of shrimp shells. The yield of isolated chitin was $6.42 \pm 0.07\%$. According to Arbia *et al.* (2012), shrimp shells contain up to 10% of chitin depending on the species of the shrimps. Moreover, the ash content of isolated chitin $0.23 \pm 0.02\%$, lower than previous research done by Poeloengasih *et al.* (2008), which was about 2.34%. It is because the demineralization process in this research was about 2 hrs, longer than research by Poeloengasih *et al.* (2008), which was 15 mins. The protein content of isolated chitin was $0.8 \pm 0.02\%$, lower when compared to those obtained by Islam *et al.* (2016) which was 1.8%. The difference in chitin's protein content could be caused by the difference in temperature and time used in the deproteinization process. In this research, the temperature used was 80°C for 2 hrs, whereas in research by Islam *et al.* (2016), the temperature used was $70\text{--}75^\circ\text{C}$ for 1 hr. Temperature is an important parameter that affects chitin's purity as the reaction rate in the deproteinization process increases with increasing temperature (Percot *et al.*, 2003). Lower ash content and protein content of isolated chitin in this research showed that deproteinization and demineralization process was done effectively. Furthermore, to ensure that chitin was obtained from these processes, measurement of the degree of deacetylation (DD) was also done. The degree of deacetylation (DD) of chitin obtained in this research was 23.06%. According to Arbia *et al.* (2012), chitin had a DD value of less than 50%, while chitosan had a DD value of higher than 50%.

3.2 Optimum pH and temperature of chitinase

Effect of pH on crude and semi pure intracellular chitinase obtained on this research could be seen in Figure 1 (a) and (b), respectively. The statistical results using ANOVA showed that there was a significant effect of pH on chitinase activity ($p \leq 0.05$).

Crude intracellular chitinase activity in this research showed to be at its optimum at pH 5 with the enzyme activity of 4.16 ± 0.07 U/mL. Sharaf (2005) reported that fungi mostly secrete its chitinase in acidic conditions. Brzezinska *et al.* (2014) mentioned that optimum pH for chitinase activity was around 5-8. Priya *et al.* (2016) also found that the chitinase activity of *Beauveria bassiana* and *Trichoderma harzianum* were shown to be optimum at pH 5.5 and 6.5, with the chitinase activity of 5.08 U/mL and 6.4 U/mL, respectively. However, crude intracellular chitinase activity in this research was comparable its crude extracellular chitinase activity which was 4.38 ± 0.06 U/mL (Halim *et al.*, 2019).

On the other hand, the enzyme activity of semi pure chitinase obtained in this research was optimum at pH 4 with chitinase activity of 4.75 ± 0.09 U/mL. This indicates that the enzyme activity of semi pure chitinase obtained was 1.14 times higher than that of crude chitinase obtained. Kim *et al.* (2003) and Pratiwi *et al.* (2015) found that the chitinase purified using 75% and 80% ammonium sulfate had an activity up to 1.90 and 2.21 times higher than before it was purified, respectively.

The optimum pH of semi pure chitinase obtained in this research showed a slight difference when compared to the optimum pH of crude chitinase (pH=5). Pandey *et al.* (2017) reported that optimum pH depends on the degree of purity of the enzyme. Martinez *et al.* (2007) also mentioned that purified chitinase from *Trichoderma atroviride* had an optimum pH of 4.0.

Another factor that contributes to enzyme activity is temperature. Effect of temperature on crude and semi

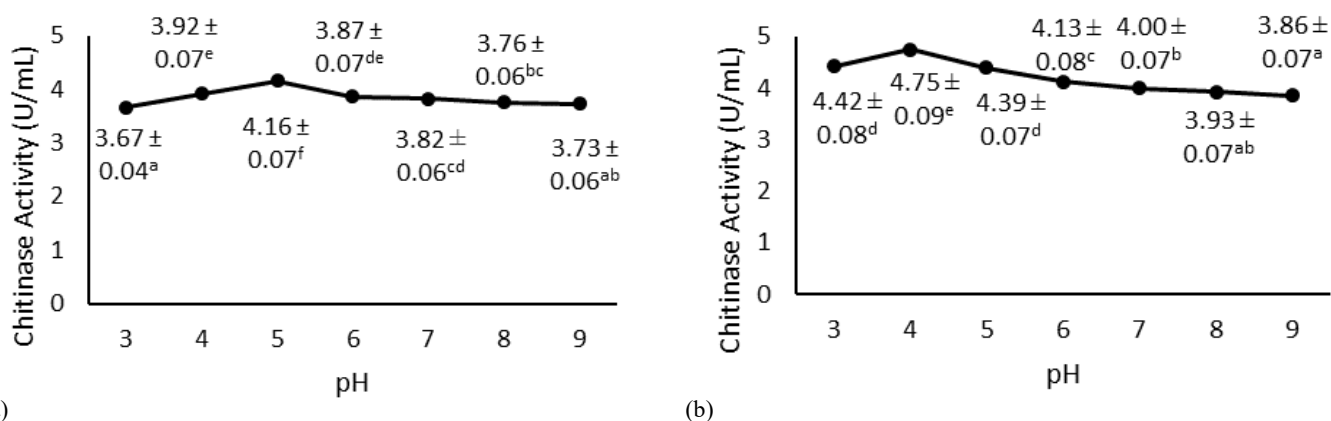


Figure 1. Effect of pH on (a) crude intracellular chitinase and (b) semi pure intracellular chitinase activity. Values are expressed as mean \pm standard deviation. Values with different superscript letters indicate significant difference ($p \leq 0.05$).

pure intracellular chitinase obtained on this research could be seen on Figure 2 (a) and (b), respectively. The statistical results using ANOVA showed that there was a significant effect of temperature on chitinase activity ($p \leq 0.05$).

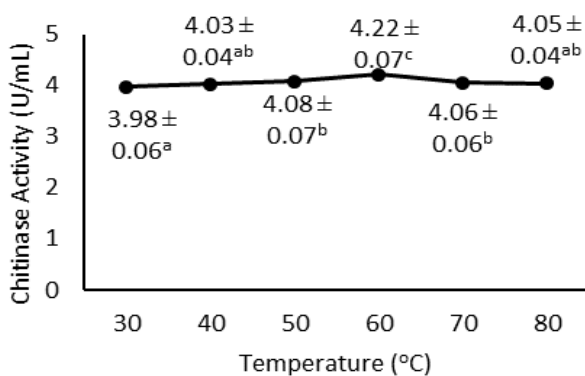
Figure 2 (a) shows that the optimum temperature for crude chitinase activity obtained in this research was at 60°C with chitinase activity of 4.22 ± 0.07 U/mL. This is in accordance with the theory by Pandey *et al.* (2017) in which the optimum temperature for most fungal chitinase activity is within 40 to 60°C. Moreover, De Marco *et al.* (2004) and Chen *et al.* (2013) found that chitinases obtained from *Trichoderma harzianum* and *Rhizopus oryzae* had an optimum temperature of 60°C.

Meanwhile, the optimum temperature for the activity of semi pure chitinase was at 50°C with chitinase activity of 5.03 ± 0.08 U/mL. Binod *et al.* (2005) and Koppurapu *et al.* (2012) found that the activity of purified fungal chitinases from *Penicillium aculeatum* and *Paecilomyces thermophila* were optimum at 50°C. The optimum temperature obtained for the semi-pure chitinase was different from that obtained for the crude chitinase because the optimum temperature of chitinase is dependent on the degree of purity of enzyme (Pandey *et al.*, 2017).

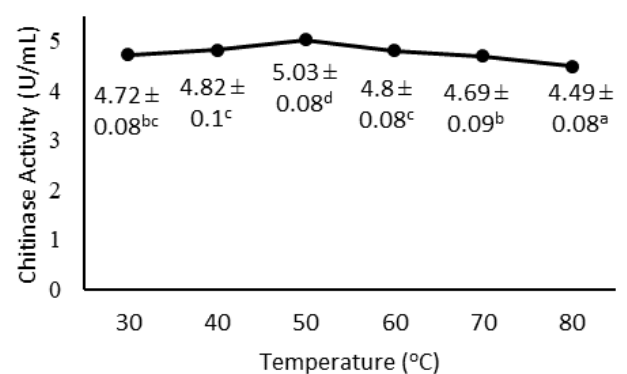
3.3 Optimum substrate concentration and incubation period on N-acetylglucosamine production

To determine the optimum substrate concentration and incubation period in producing N-acetylglucosamine, both the optimum pH and temperature obtained for crude and semi pure intracellular chitinase were used. Figures 3 and 4 show the effect of substrate concentration and incubation period on N-acetylglucosamine concentration using crude intracellular chitinase and semi pure intracellular chitinase, respectively.

Statistical results showed that both substrate concentration and incubation period and their interaction



(a)



(b)

Figure 2. Effect of temperature on (a) crude intracellular chitinase and (b) semi pure intracellular chitinase activity. Values are expressed as mean±standard deviation. Values with different superscript letters indicate significant difference ($p \leq 0.05$).

gave significant effect on N-acetylglucosamine produced ($p \leq 0.05$). Using crude intracellular chitinase, the highest N-acetylglucosamine concentration was obtained at 0.5% substrate concentration-6 hrs incubation period with the concentration of 961.67 ± 9.13 ppm. Moreover, in the reaction using semi pure intracellular chitinase the highest N-acetylglucosamine concentration was obtained at 1.5% substrate concentration-4 hrs incubation period with a concentration of 1150.56 ± 12.55 ppm.

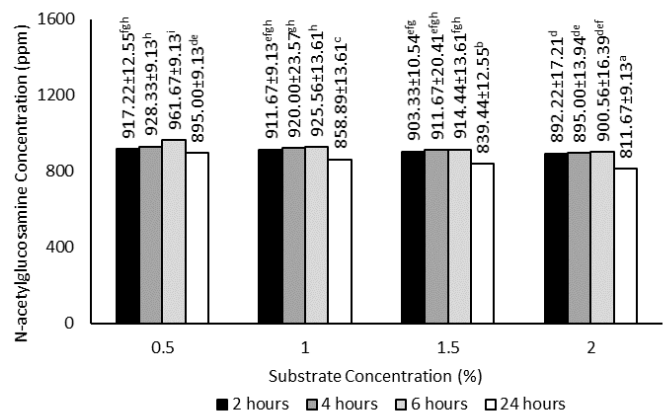


Figure 3. Effect of substrate concentration and incubation period on N-acetylglucosamine concentration using crude intracellular chitinase. Values are expressed as mean±standard deviation. Values with different superscript letters indicate significant difference ($p \leq 0.05$).

According to Poedjiadi and Supriyanti (2006), in a low concentration, the substrate is only partially bound to the active site of the chitinase. If the concentration is increased, the amount of substrate bound to the active site of the chitinase increases. However, the addition of a high concentration of substrate in the enzymatic reaction can lead to a saturated condition (Hendri *et al.*, 2007). Longer incubation period could also decrease the N-acetylglucosamine production due to the denaturation of enzymes during the reaction or inhibition from N-acetylglucosamine as the product (Sukprasirt and Wititsuwannakul, 2014).

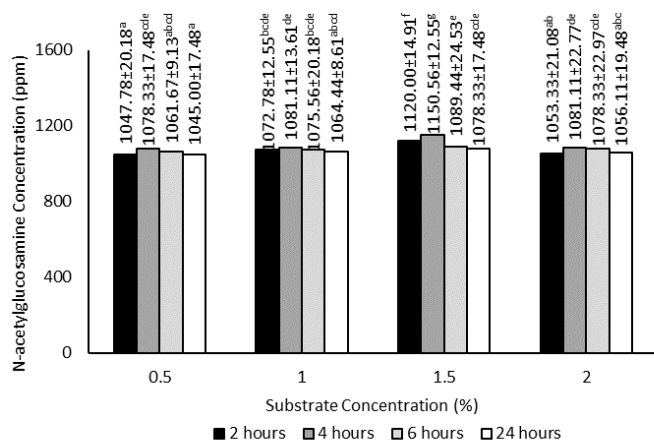


Figure 4. Effect of substrate concentration and incubation period on N-acetylglucosamine concentration using semi pure intracellular chitinase. Values are expressed as mean±standard deviation. Values with different superscript letters indicate significant difference ($p \leq 0.05$).

Results in this research show that semi pure intracellular chitinase could produce higher N-acetylglucosamine concentration in a shorter incubation period but it required a higher concentration of substrate. However, the concentration of N-acetylglucosamine produced using intracellular chitinase from *M. circinelloides* is also lower compared to its extracellular chitinase, which can produce about 2195.83 ± 15.14 ppm of N-acetylglucosamine after 2 hrs of incubation period in 1.5% substrate concentration (Halim et al., 2019). Another research by Herdyastuti and Cahyaningrum (2017) using chitinase from *Pseudomonas* sp. with amorphous modified chitin as a substrate, showed higher concentration of N-acetylglucosamine produced, i.e. about 10950 ppm, at incubation period of 8 hrs and about 22500 ppm, at 1.2% substrate concentration.

4. Conclusion

Crude intracellular chitinase of *M. circinelloides* had an optimum pH of 5 with chitinase activity of 4.16 ± 0.07 U/mL and optimum temperature of 60°C with chitinase activity of 4.22 ± 0.07 U/mL. The highest N-acetylglucosamine concentration was obtained at 0.5% substrate concentration with 6 hrs of incubation period with the concentration of 961.67 ± 9.13 ppm. Semi pure intracellular chitinase of *M. circinelloides* had an optimum pH of 4 with chitinase activity of 4.75 ± 0.09 U/mL and optimum temperature of 50°C with chitinase activity of 5.03 ± 0.08 U/mL. The highest N-acetylglucosamine concentration was obtained at 1.5% substrate concentration with 4 hrs of incubation period with the concentration of 1150.56 ± 12.55 ppm.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

This research was financially supported by the Center of Research and Community Development, Universitas Pelita Harapan through Research Project no: P-0004/FaST/I/2018.

References

- Anitha, A., Sowmya, S., Kumar, P.T.S. and Deepthi, S. (2014). Chitin and chitosan in selected biomedical applications. *Progress in Polymer Science*, 39(9), 1644-1667. <https://doi.org/10.1016/j.progpolymsci.2014.02.008>
- Annamalai, N., Rajeswari, M.V., Vijayalakshmi, S. and Balasubramanian, T. (2011). Purification and characterization of chitinase from *Alcaligenes faecalis* AU02 by utilizing marine wastes and its antioxidant activity. *Annals of Microbiology*, 61(4), 801-807. <https://doi.org/10.1007/s13213-011-0198-5>
- Arbia, W., Arbia, L., Adour, L. and Amrane, A. (2012). Chitin extraction from crustacean shells using biological methods – a review. *Food Technology and Biotechnology*, 51(1), 12-25.
- Binod, P., Pusztahelyi, T., Nagy, V., Sandhya, C., Szakacs, G. and Pocs, I. (2005). Production and purification of extracellular chitinases from *Penicillium aculeatum* NRRL 2129 under solid-state fermentation. *Enzyme and Microbial Technology*, 36 (8), 880-887. <https://doi.org/10.1016/j.enzymictec.2004.12.031>
- Brzezinska, M.S., Jankiewicz, U., Butkowska, A. and Walczak, M. (2014). Chitinolytic microorganisms and their possible application in environmental protection. *Current Microbiology*, 68(1), 71-81. <https://doi.org/10.1007/s00284-013-0440-4>
- Chen, W.M., Chen, C.S. and Jiang, S.T. (2013). Purification and characterization of an extracellular chitinase from *Rhizopus oryzae*. *Journal of Marine Science and Technology*, 21(3), 361-366.
- De Marco, J.L., Valadares-Inglis, M.C. and Felix, C.R. (2004). Purification and characterization of an n-acetylglucosaminidase produced by a *Trichoderma harzianum* strain which controls *Crinipellis perniciosus*. *Applied Microbiology and Biotechnology*, 64(1), 70-75. <https://doi.org/10.1007/s00253-003-1490-5>
- Halim, Y., Hardoko and Pengalila, R.F. (2019). Determination of optimal fermentation condition for N-acetylglucosamine production using *Mucor circinelloides* Extracellular Chitinase. *Journal of Fisheries Sciences*, 21(2), 105-110. <https://doi.org/10.22146/jfs.45859>
- Hao, Z., Yujie, C., Xiangru, L., Zhang, X., Fang, Z. and Zhang, D. (2012). Optimization of nutrition factors on chitinase production from a newly isolated

- Chitolyticbacter meiyuanensis* SYBC-H1. *Brazilian Journal of Microbiology*, 43(1), 177-186. <https://doi.org/10.1590/S1517-83822012000100019>
- Hendri, J., Wardana, Suka, I.G. and Laila, A. (2007). Penentuan kadar Ca dan Mg pada hasil demineralisasi optimum kulit udang windu secara gravimetri dan spektroskopi serapan atom dimuat. *Jurnal Sains MIPA*, 13(2), 93-99. [In Bahasa Indonesia].
- Herdyastuti, N. and Cahyaningrum, S.E. (2017). Analysis of N-acetylglucosamine from enzymatic degradation of amorphous chitin. *Rasayan Journal Chemistry*, 10(1), 226-233.
- Islam, S., Khan, M. and Alam, A. (2016). Production of chitin and chitosan from shrimp shell wastes. *Journal of the Bangladesh Agricultural University*, 14(2), 253-259. <https://doi.org/10.3329/jbau.v14i2.32701>
- Jha, S.C. and Modi, H.A. (2017). Comparative analysis of chitinase activity by four different assays from soil born *Actinomycetes*, presented at the 4th International Conference on Multidisciplinary Research and Practice, Ahmedabad, 2017. Gurgaon, India: Research and Scientific Innovation Society.
- Kim, K., Yang, Y. and Kim, J. (2003). Purification and characterization of chitinase from *Streptomyces* sp. M-20. *Journal of Biochemistry and Molecular Biology*, 36(2), 185-189. <https://doi.org/10.5483/BMBRep.2003.36.2.185>
- Kopparapu, N.K., Zhou, P., Zhang, S., Yan, Q., Liu, Z. and Jiang, Z. (2012). Purification and characterization of a novel chitinase gene from *Paecilomyces thermophila* expressed in *Escherichia coli*. *Carbohydrate Research*, 347(1), 155-160. <https://doi.org/10.1016/j.carres.2011.11.002>
- Martinez, J.P., Falomir, M.P. and Gozalbo, D. (2014). Chitin: A Structural Biopolysaccharide with Multiple Applications. New Jersey: John Wiley and Sons Ltd. <https://doi.org/10.1002/9780470015902.a0000694.pub3>
- Pandey, A., Negi, S. and Soccol, C.R. (2017). Production, Isolation and Purification of Industrial Products. The Netherlands: Elsevier.
- Percot, A., Viton, C. and Domard, A. (2003). Optimization of chitin extraction from shrimp shells. *Biomacromolecules*, 4(1), 12-18. <https://doi.org/10.1021/bm025602k>
- Poedjiadi, A. and Supriyanti, T. (2006). Dasar-dasar Biokimia Edisi Revisi. Jakarta: UI-Press.
- Poeloengasih, C.D., Hernawan and Angwar, M. (2008). Isolation and characterization of chitin and chitosan prepared under various processing times. *Indonesian Journal of Chemistry*, 8(2), 189-192. <https://doi.org/10.22146/ijc.21635>
- Pratiwi, R.S., Susanto, T.E., Wardani, Y.A.K. and Sutrisno, A. (2015). Enzim kitinase dan aplikasi di bidang industri: kajian pustaka. *Jurnal Pangan dan Agroindustri*, 3(3), 878-887.
- Priya, D., Ramesh, B.N.G, Sneka, S. and Karthikeyan, S. (2016). Enhancement and detection of chitinase enzyme of fungus for the eradication of pests. *International Journal of Science Technology and Engineering*, 3(2), 169-172.
- Robiah, N. (2015). Mapping the activity of chitinase enzymes and deacetylase chitin of isolating *Actinomycetes* ANL-4 in chitin degradation during incubation time for 24 hrs. Lampung: Lampung University, BSc. Thesis.
- Savitri, E., Soeseno, N. and Adiarto, T. (2010). Sintesis kitosan, poli(2-amino-2-deoksi-D-Glukosa), skala Pilot Project dari limbah kulit udang sebagai bahan baku alternatif pembuatan biopolymer, presented Seminar Nasional Teknik Kimia, Yogyakarta, 2010. Yogyakarta: UPN "Veteran" Yogyakarta. [In Bahasa Indonesia].
- Sharaf, E.F. (2005). A potent chitinolytic activity of *Alternaria alternate* isolated from Egyptian black sand. *Polish Journal of Microbiology*, 54(2), 145-151.
- Struszczyk, K., Szczesna-Antczak, M., Walczak, M. and Antczak, T. (2008). Isolation and purification of intracellular chitosanolytic enzymes of *Mucor circinelloides*. *Progress on Chemistry and Application of Chitin*, 78(1), 107-117. <https://doi.org/10.1016/j.carbpol.2009.04.010>
- Sukprasirt, P. and Wititsuwannakul, R. (2014). A chitinolytic endochitinase and β -N-acetylglucosaminidase-based system from hevea latex in generating N-acetylglucosamine from chitin. *Phytochemistry*, 104, 5-11. <https://doi.org/10.1016/j.phytochem.2014.04.001>
- Veronica. (2018). Uji aktivitas kitinolitik kapang yang diisolasi dari kulit udang windu (*Penaeus monodon*). Tangerang: Universitas Pelita Harapan, BSc. Thesis. [In Bahasa Indonesia].
- Wang, J., Zhang, J., Song, F., Gui, T. and Xiang, J. (2015). Purification and characterization of chitinases from ridgetail white prawn *Exopalaemon carinicauda*. *Molecules*, 20(2), 1955-1967. <https://doi.org/10.3390/molecules20021955>
- Wang, L. and Yang, S. (2007). Bioprocessing for Value-Added Products from Renewable Resources. New York: Elsevier.
- Yamazaki, H., Yamazaki, D., Takaya, N., Takagi, M., Ohta, A. and Horiuchi, H. (2007). A chitinase gene, chiB, involved in the autolytic process of *Aspergillus nidulans*. *Current Genetics*, 51(2), 89-98. <https://doi.org/10.1007/s00294-006-0109-7>