ILMU KELAUTAN: Indonesian Journal of Marine Sciences March 2019 Vol 24(1):7-14 ISSN 0853-7291

Formation of Eco-friendly Silver Nanoparticle Microalgae using *Chlorella vulgaris*

Hermin Pancasakti Kusumaningrum[1]*, Muhammad Zainuri[2], Widianingsih[2], Wahyu Dewi Utari Haryanti[2], Indras Marhaendrajaya[3] and Robertus Triaji Mahendrajaya[2]

[1]*Biotechnology Laboratory, Biology Department, Faculty of Sciences and Mathematics, Diponegoro University*
[2]*Faculty of Fisheries and Marine Science, Diponegoro University*
[3]*Faculty Sciences and Mathematics, Diponegoro University*
Jl. Prof. Soedarto, SH Tembalang, Semarang, 50275, Indonesia
Email: herminsakti@gmail.com

Abstract

Biosynthesized silver nanoparticles (AgNPs) using organism have spurred great interest as a antimicrobial and biomedical agents. Green microalgae have advantages as they are easily available, grow rapidly and producing varieties metabolites. Synthesized of AgNPs from microalgae C. vulgaris offer environmentally antimicrobial agent. The objectives of the study is producing AgNPs microalgae using C. vulgaris as eco-friendly antimicrobial agent. The research methods was conducted by synthesizing silver nanoparticle microalgae using C. vulgaris following by characterization under UV–visible spectroscopy, transmission electron microscopy (TEM), and scanning electron microscope (SEM) and Energy-dispersive X-ray spectroscopy (EDX). The research result showed AgNPs C. vulgaris microalgae were produced with and without agitation treatment under different condition. The synthesized AgNPs C. vulgaris exhibited a maximum absorption at 312 nm and 398 nm, and EDX analysis had determined that abundance chemical elements presented in a sample were carbon and silver. The TEM analysis revealed that they are spherical form. The spot of EDX analysis showed the presence of silver atoms. The SEM analysis shows the spherical shaped with some silver particle inside of the cell. These resut indicated that formation of silver nanoparticle microalgae using C. vulgaris has been succesfully obtained under the treatment.

***Keywords**: nanoparticle, silver, microalgae, TEM, SEM, C. vulgaris*

Introduction

Recently, synthesis of silver nanoparticles using microorganisms, algae and plant extracts is already performed. The development of bionanotechnology in microalgae has shown that nano silver biosynthesis (nano silver) has increased the ability of microalgae as antimicrobial, antifungal and anticancer (Devi and Bimba, 2012; Rajesh et al., 2012; Sudha *et al*., 2013; Anuradha *et al*., 2014; Patel *et al*., 2015; Duong *et al*., 2016; El-Sheekh and El-Kassas, 2016; Rajeshkumar *et al*., 2012). Integration of microalgae with nano silver has wider the spectrum of antimicrobials capability in microalgae. This development also makes nano silver microalgae become an acceptable antimicrobial product because it is environmentally safe.

Nanobiotechnology dealing with metal nanoparticles and living organisms has spurred great interest due to its wide application range in almost every field of science and technology such as materials and manufacturing, nanoelectronics, information technology, medicine and health care, energy, biotechnology, food storage, household products, disinfectants, biomonitoring and environmental remediation (El-Nour *et al*., 2010; Balashanmugam and Kalaichelvan, 2015; Dahoumane *et al*., 2016). Eco-friendly nanoparticles have advantages especially in compatibility with pharmaceuticals over physical, chemical and microbial synthesis. High cost, inefficient treatment, contamination of toxic chemicals were leading to several effects when silver nanoparticles are being applied in the medical and pharmaceutical applications (Balashanmugam and Kalaichelvan, 2015; El-Sheekh and El-Kassas, 2016).

Chlorella is widely used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industries. *C. vulgaris* contains 17 amino acids of both essential amino acids and non essential essential amino acids. *C. vulgaris* also contains high levels of 34 lipid acid, Omega 3, Omega 6, Omega 9, AA, DHA and PUFA. Its carotenoid content is α-carotene as much as 0.24 $mg.g^{-1}$ and β-carotene as much as 0.86 $mg.g^{-1}$.

*) Corresponding author

ijms.undip.ac.id
DOI: 10.14710/ik.ijms.24.1.7-14
Received : 08-06-2018
Accepted : 15-08-2018

(Merin *et al.*, 2010; Sivakumar *et al*., 2011; Kusumaningrum and Zainuri, 2015). Although synthesis and characterization of silver nanoparticles on microalgae *C. vulgaris* had been conducted by Annamalai and Nallamuthu (2016), there are no reports concerning synthesis of AgNPs on *C. vulgaris* in higher concentration of silver using agitation treatment, their effect to the cell and how much concentration of silver in cell of microalgae.

Materials and Methods

Microalgae material

C. vulgaris microalgae were obtained from Brackishwater Aquaculture Development Centre (BBPBAP) on Jepara Indonesia. They were held in seawater tanks, recirculated and aerated, with the temperature set at 25-28^{0}C and salinity at 30-32‰. The tanks were monitored daily. The microalgae were cultivated using sea water enriched with Walne media.

Microalgae Media

Walne media for microalgae *C. vulgaris* growth and cultivation consist of NaH_2PO_4 20 $g.L^{-1}$; NaNO3 10 $g.L^{-1}$; $FeCl_3$ 0.15 $g.L^{-1}$; Na_2EDTA 45 $mg.L^{-1}$; $^{1}H_3BO_3$ 3.36 $g.L^{-1}$; $MnCl_2.4H_2O$ 0.36 $g.L^{-1}$, trace metal solution 1 $mL.L^{-1}$, and 1 L distilled water. Trace metal solution was consist of $NaMoO_4.5H_2O$ 0.39 $g.L^{-1}$; $MnCl_2.4H_2O$ 1.81 $g.L^{-1}$; H_3BO_3 2.86 $g.L^{-1}$; $ZnSO_4.7H_2O$ 0.222 $g.L^{-1}$; $CuSO_4.5H_2O$ 0.079 $g.L^{-1}$; $Co(NO_3)_2.6H_2O$ 0.0494 $g.L^{-1}$; pH was adjusted 6.8. All of the trace metal ingredients were dissolved in 200 mL of distilled water. The solution was adjusting the pH to 7.6 with HCl or NaOH while boiled for 10 min, and bring it into 1 L. Solution sterilization was conducted by autoclaving at 120^{0}C and 15 $lb.in^{-2}$ (103 kPa and). The medium for microalgae cultivation was using by adding 0.1mL solution to each 10mL of seawater (McVey and Moore, 1983; Bidwell and Spotte, 1985).

Preparation of 2 mM AgNO3 solution

The solution of 2 mM $AgNO_3$ was prepared by dissolving 1000 ppm $AgNO_3$ with size about 1-10 nm in double distilled water and stored in amber coloured bottle to avoid auto oxidation of silver nitrate.

Formation of microalgae silver nanoparticles

Formation of microalgae silver nanoparticles was conducted using modification from some studies (Rajeshkumar *et al*., 2012; Sudha *et al*., 2013; Ibraheem *et al*., 2016). The 100 mL microalgae extract with total cell count 10^{-5}–$10^{-7}mL^{-1}$ was added to 250mL $AgNO_3$ 2 mM solution, then the mixing solution was stirred for 6 hours using magnetic stirrer to perform agitation treatment on the sample (CVA). The same experiment was carried out with mixing solution without agitation treatment (CVWA). As a control, fresh *C. vulgaris* microalgae in Walne media and $AgNO_3$ solution was used. The formation of silver nanoparticles was indicated by color change in solution from green to green brownish followed by brownish color.

UV –Visible spectra analysis

Synthesized silver nanoparticles were initially characterized by taking small aliquot of sample in to UV–Visible spectrophotometer absorption spectra at 300-700 nm using Shimadzu UV -1800 Spectro-photometer.

SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was carried by using Zeiss, EV-18 model. Samples of the dray material from aqueous solution of the silver nanoparticles (AgNPs) were prepared by centrifu-gation at 8,000 rpm for 5 min. The pelet was dried. The SEM micrographs have been produced with magnifications 3000, 5000, 10000 and 20000 x (diameters). SEMs are equipped with x ray analytical capabilities. Thus topographic, cristallographic, and compositional information can be obtainaed rapidly, efficiently, and simultaneously from the same area.

Energy-dispersive X-ray spectroscopy (EDS, EDX, EDXS, XEDS, EDXA or EDXMA)

The X-ray excitation technique used for the elemental analysis or chemical characterization of a sample.

Results and Discussion

UV–Visible spectra analysis

Marine microalgae like *C. vulgaris* contain a number of biodynamic compounds of therapeutic value. These compounds provide valuable ideas for the development of new drugs against microbial infections and contamination (Kusumaningrum and Zainuri, 2014). The present study was conducted to use the marine microalgae, *C. vulgaris* for the synthesis of nanosilver microalgae. The microalgae extract may act as reducing and capping agents in silver nanoparticles biosynthesis. Reduction of silver ions into nanosilver microalgae during exposure of the *C. vulgaris* extracts could be followed by color change. Studies have indicated that biomolecules like protein, carbohydrates, lipids and phenols not only play a role in the capping of the nanoparticles, but also play an important role in reducing the ions to the nano size. The biomolecules found in these extracts like enzymes, vitamins, proteins, amino acids, and polysaccharides play a vital role in the reduction of Ag+ ions. The addition of silver nitrate solution into microalgae solution will change the reaction mixture turning brown,due to the excitation of the surface plasma vibrations, indicated of the formation of silver nanoparticles.

Characterization of nanosilver microalgae were primarily performed by UV-Visible spectroscopy, which is proved to be a very useful technique for the analysis of these nanoparticles.

A single broad peak was observed for microalgae, control and treatment that was correlated to plasmon excitation of the nanosilver microalgae as illustrated on Figure 1. Another researcher was in agreement with this result, in founding that absorption of silver nanoparticle of *C. vulgaris* was about 400 nm (Annamalai and Nallamuthu, 2016); and also 400-450 nm for *Chlorella* and *Scenedesmus* (Patel *et al*., 2015)

The research based on the absorbance and wavelength values also show the synthesis of silver nanoparticles with agitation provide stability silver nanopartice of microalgae. The agitation accelerates the process of forming silver nanoparticles. The absorbance value increases with the increasing contact reaction time. As the microalgae suspension was mixed with the aqueous solution of the silver ion complex it was changed from green to brown colour. This is due to the excitation of the surface plasma vibrations, which indicates the formation of the nanosilver microalgae. UV-Visible Spectrograph of nanosilver microalgae has been recorded as a function of time by using quartz cuvette with distilled water as the reference.

Formation of the nanosilver microalgae of *C. vulgaris* monitored by UV–Vis spectroscopy exhibits a strong absorption due to the collective oscillation of the conduction electrons, after appropriate excitation by suitable radiation. This phenomenon is adressed as localized surface plasmon resonance, which is highly dependent on the size and shape of the nanoparticles.

SEM analysis

The SEM analysis showed morphological, cellular ultrastructural changes of *C. vulgaris* cells after 160 hours of exposure with AgNPs which also accomplished by the differences in surface topography as the electron beam sweeps across the specimen. As showed in Figure 2., the morphology of *C. vulgaris* cell with silver addition maintained a smooth exterior, a round and spherical shape with size 0,4–0,8 μm (CVA), 0,4-0,9 (CVWA), 0.91–1,43 μm (CV). Its also showed that agitation treatment did not caused greater effect on cell structure and morphologycaused by intense contact among AgNP particles and cells surface. This result was in contrast with another researcher which was proven that nanoparticles can caused change in morphology and dimensions of green algae *Chlamydomonas reinhardtii* and *Dunaliella salina* (Dahoumane *et al*., 2017). The AgNP microalgae also revealed spherical and cuboidal nanoparticles. Similar phenomenon was reported by Annamalai and Nallamuthu (2016) and Balashanmugam and Kalaichelvan (2015).

EDX analysis

Quantitative determination of bulk elemental composition and the location of AgNPs on cell surface for *C. vulgaris* as a control, AgNPs of *C. vulgaris* with agitation treatment and AgNPs of *C. vulgaris* without agitation treatment, were analyzed using the combination of both X-ray (EDX) and ZAF factor. Result shows that *C. vulgaris* and AgNPs microalgae contains four major basic elements of microalgae consist of Carbon (C), Oxygen(O), sodium (Na), calsium (Ca). EDX analysis for AgNPs of *C. vulgaris* shows the addition of two different elements Cu (Cuprum) and silver (Ag) as illustrated in Figure 3, 4, 5. Some other chemical compounds are also found in $AgNO_3$ solution in very small quantities. Although C, O. Na, kalium (K), magnesium (Mg) and chloride (Cl) was used as a standard concerning their based on their high concentration in the microalgae cell (Mandalam and Palsson, 1998). The EDX analysis also showed the high concentration of Ag indicating the formation of microalgae nano particle. Characteristic peaks was supported the

Table 1. UV-Visible absorption spectrum of microalgae and control

Microalgae	UV-Visible absorption spectrum
$AgNO_3$	343 nm
C. vulgaris	410 nm
Nanosilver *C. vulgaris* with agitation	312 nm
Nanosilver *C. vulgaris* without agitation	398 nm

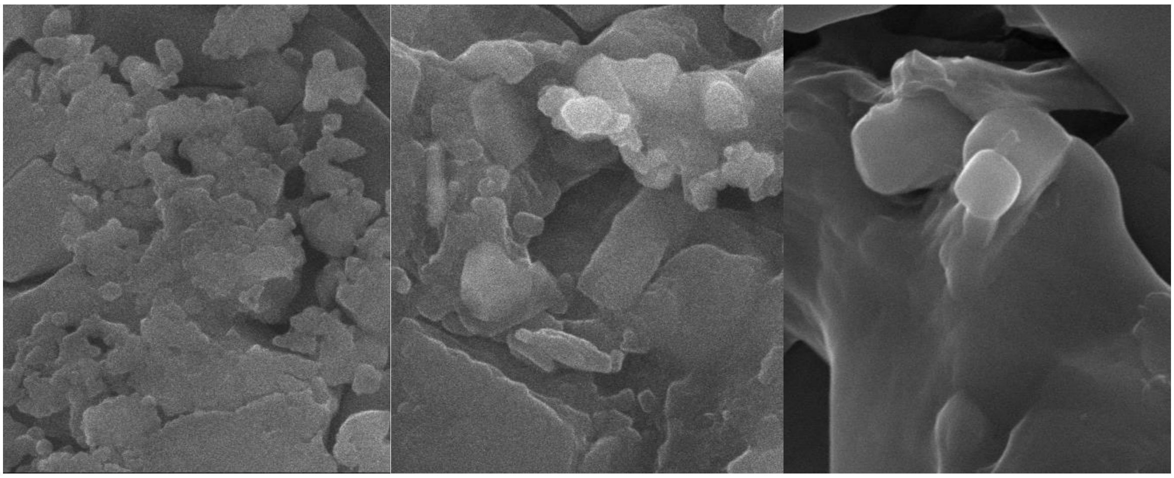
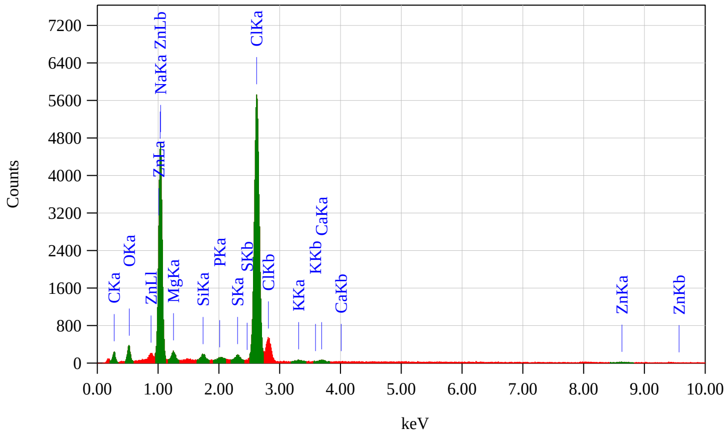


Figure 2. SEM image of nanosilver microalgae formed by *C. vulgaris* (left to right= nanosilver microalgae *C.vulgaris* without agitation CVA, nanosilver microalgae *C.vulgaris* with agitation, *C.vulgaris*) (x20.000μm)



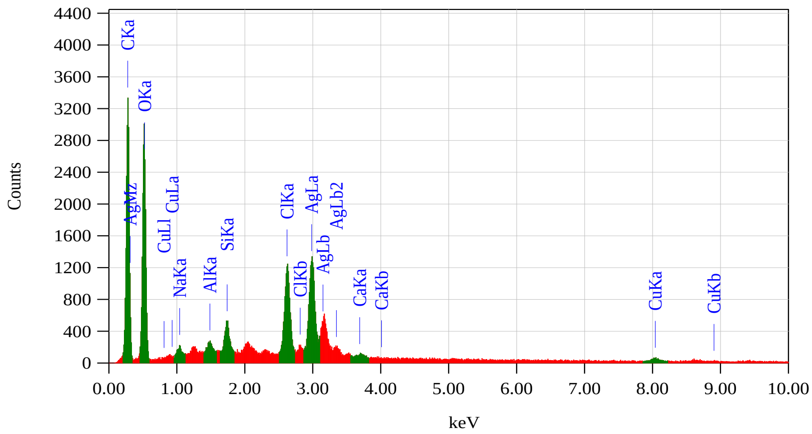
ZAF Method Standardless Quantitative Analysis(Oxide)
Fitting Coefficient : 0.0181
Total Oxide : 24.0

Element	(keV)	Mass%	Sigma	Mol%	Compound	Mass%	Cation	K
C K	0.277	37.78	0.14	70.05	C	37.78	0.00	4.6763
O		9.82						
Na K	1.041	21.39	0.12	10.36	Na2O	28.83	36.36	35.4530
Mg K	1.253	0.90	0.04	0.82	MgO	1.49	1.45	0.8598
Si K	1.739	0.53	0.04	0.42	SiO2	1.13	0.74	0.7708
P K	2.013	0.17	0.04	0.06	P2O5	0.39	0.22	0.2717
S K	2.307	0.48	0.03	0.33	SO3	1.19	0.58	0.8480
Cl K	2.621	28.03	0.07	17.61	Cl	28.03	0.00	55.6671
K K	3.312	0.19	0.02	0.05	K2O	0.23	0.19	0.2929
Ca K	3.690	0.24	0.02	0.14	CaO	0.34	0.24	0.4062
Zn K	8.630	0.47	0.06	0.16	ZnO	0.59	0.28	0.7542
Total		100.00		100.00		100.00	40.05	

Figure 3. The quantitative determination of bulk elemental composition of C. vulgaris

result in representing the dominant material in the cell of microalgae. These result were in agreement with Bhowmick *et al*. (2009). The use of EDX and ZAF Factor for AgNPs of *C. vulgaris* analysis is proven to be representative method for showing the elemental composition as supporting by previous result (Newbury and Ritchie, 2013; Trincavelli *et al*., 2014; Wassilkowska *et al*., 2015)

The EDX analysis was performed for the confirmation of *C. vulgaris* silver nanoparticles. Figure 3 shows the evidence of EDX analysis in the



ZAF Method Standardless Quantitative Analysis(Oxide)
Fitting Coefficient : 0.0444
Total Oxide : 24.0

Element	(keV)	Mass%	Sigma	Mol%	Compound	Mass%	Cation	K
C K	0.277	75.39	0.05	96.07	C	75.39	0.00	61.7291
O		3.26						
Na K	1.041	0.54	0.03	0.18	Na2O	0.73	2.76	0.7218
Al K	1.486	0.40	0.03	0.11	Al2O3	0.75	1.73	0.5568
Si K	1.739	1.14	0.05	0.62	SiO2	2.45	4.80	1.9172
Cl K	2.621	3.84	0.03	1.66	Cl	3.84	0.00	8.4684
Ca K	3.690	0.21	0.02	0.08	CaO	0.29	0.60	0.3692
Cu K	8.040	1.17	0.06	0.28	CuO	1.46	2.16	1.9397
Ag L	2.983	14.05	0.09	1.00	Ag2O	15.10	15.34	24.2979
Total		100.00		100.00		100.00	27.39	

Figure 4. The quantitative determination of bulk elemental composition on AgNPs of *C. vulgaris* with agitation treatment

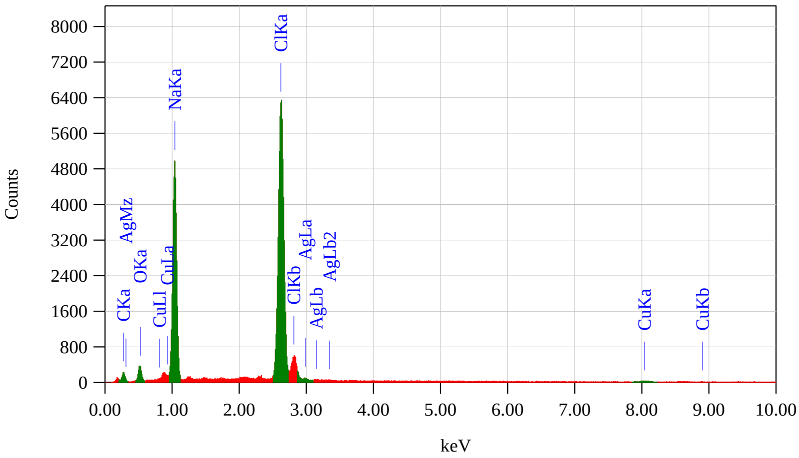
spot profile mode for each treatment. The EDX analysis combined with SEM was used also to characterize the chemical composition and the location of AgNPs on cell surface (Anuradha *et al*., 2014). The chemical composition of $AgNO_3$ as illustrated the EDX analysis on Figure 3 was containing 31,9 % concentration of Ag characterized by the high peak appearance in the XRD image as marked by green colour. The sharp diffraction patterns of the XRD spectra indicates a pure crystalline silver structure. The X-rays of *C. vulgaris* are scattered by diffraction owing to the unique crystalline structure of the material analyzed. This result was in agreement with previous study that the silver metals attached to the cell wall (Patel *et al*., 2015). Energy Dispersive Analysis of X-ray (EDAX) gives qualitative as well as quantitative status of elements that may be involved in the formation of AgNPs.

TEM analysis

The TEM analysis had showed the detailed information of the three-dimensional structure of silver nanopartice of microalgae from planar serial sections. Figure 6 showed the construction of the microalgae cell image from a through-focal series of images formed in a scanning confocal optical microscop. Its also exhibited the silver crystal that entering into the cell that was not cause the disruption of cell wall or making lysis of the cell. This result also exhibited that the silver metals not only attached to the cell wall but also embedded in the cell. This analytical method also proven the characterization of the particle structure and confirm the presence of the nanoparticles. Silver ions released from AgNPs may penetrate inside the cell membranes interacting with sulfur and phosphorus containing compounds such as proteins and DNA (Jyoti *et al*., 2016).

The study showed that production of microalgae silver nanoparticle using *C. vulgaris* can be performed under the concentration of 2 mM of silver under agitation treatment. This result was in contrast with other researcher in having the lysis of the cell in 1mM of silver concentration Annamalai and Nallamuthu (2016). This study also exhibited that the cell still maintaining their structure stability while containing about 32% of the silver. This result showing the indication that agitation treatment was supporting the stability of the cell in dispersing the silver material around the cell.

These data showed implication that silver nanoparticle microalgae can be potentially saved for



ZAF Method Standardless Quantitative Analysis(Oxide)
Fitting Coefficient : 0.0194
Total Oxide : 24.0

Element	(keV)	Mass%	Sigma	Mol%	Compound	Mass%	Cation	K
C K	0.277	35.43	0.14	67.80	C	35.43	0.00	3.8099
O		8.49						
Na K	1.041	23.67	0.10	11.83	Na2O	31.91	46.54	36.5197
Cl K	2.621	30.84	0.07	19.99	Cl	30.84	0.00	57.5180
Cu K	8.040	0.80	0.06	0.29	CuO	1.00	0.57	1.1952
Ag L	2.983	0.77	0.05	0.08	Ag2O	0.82	0.32	0.9571
Total		100.00		100.00		100.00	47.43	

Figure 5. The quantitative determination of bulk elemental composition on *C. vulgaris* without agitation treatment

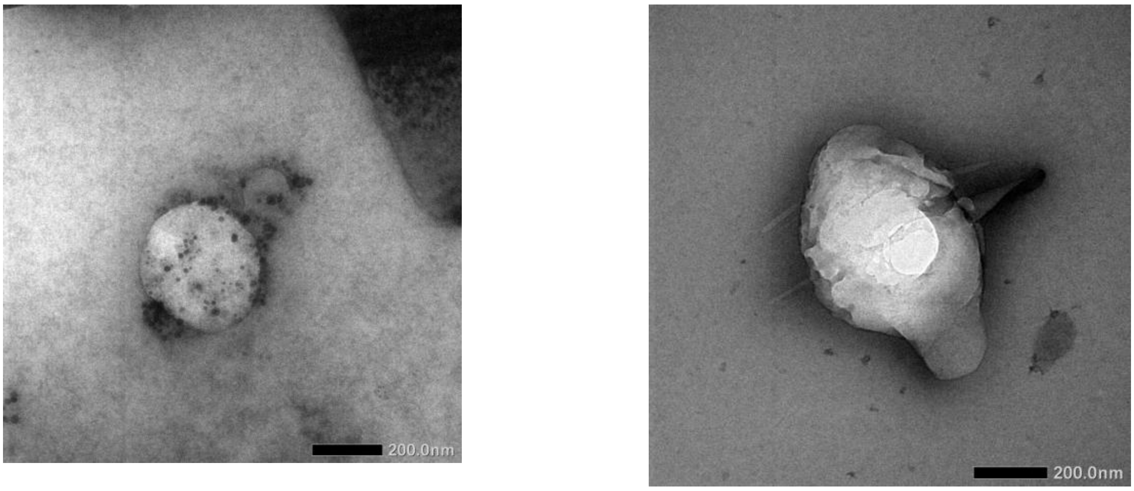


Figure 6. TEM analysis of AgNPs of *C. vulgaris* cell with agitation (left) and without agitation (right) (200 nm)

application on human body at low concentrations, capable for large scale production because of fast multiplication of cells, and environmentally safe, comparing with chemical and physical methods as in agreement with previous research (El-Sheek and El-Kassas, 2016, Ibraheem *et al*., 2016). Interestingly, the use of microalgae as organic coating material shown by this study has opened up opportunities for the development of this potency in future studies especially for biomedical applications.

Conclusion

The present study reveals that the microalga *C. vulgaris* is good source for the synthesis of silver nanoparticles at a high silver concentration. The formation of silver nanoparticles was confirmed by characterization using UV-Vis, SEM, EDX and TEM techniques. The microalgae silver nanoparticles formed were quite stable in the solution. The agitation treatment act as the surface active stabilizing molecules and cell structure for the synthesis of silver nanoparticles.

Acknowledgments

The authors gratefully thank to Directorate of Research and Public Services (Dirlitabmas), Indonesian Ministry of Research, technology and Higher Education according to Letter of Assigment of Hibah PUPT, Number: 344-43/UN7.5.1/PP/2017 date 5 May 2017 in funding this research.

References

Annamalai, J. & Nallamuthu, T. 2016. Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency. *Appl. Nanosci*. 6(2): 259–265

Anuradha, G., Syama Sundar B., Sreekanth kumar J., & Ramana, M.V. 2014. Synthesis and Characterization of Silver Nanoparticles from *Ocimum basilicum L. var.thyrsiflorum*. *Eur. J Acad. Essays* 1(5): 5-9

Balashanmugam, P. & Kalaichelvan, P.T. 2015. Biosynthesis characterization of silver nanoparticles using *Cassia roxburghii* DC. aqueous extract, and coated on cotton cloth for effective antibacterial activity. *Int. J. Nanomedicine.* (Suppl 1: Challenges in biomaterials research) 10: 87–97

Bidwell, J.P. & Spotte, S. 1985. Artificial Sea Water: Formulas and Methods. Boston, Massachusetts: Jones and Bartlett Publisher. 349 p.

Bhowmick, T.K., Suresh, A.K., Kane, S.G., Joshi, A.C., & Bellare, J.R. 2009. Physicochemical characterization of an Indian traditional medicine, *Jasada Bhasma*: detection of nanoparti cles containing non-stoichiometric zinc oxide. *J. Nanopart. Res.* 11: 655–664. doi: 10.1007/s11051-008-9414-z

Dahoumane, S.A., Mechouet, M., Alvarez, F.J., Agathos, S.N. & Jeffryes, C., 2016. Microalgae: An outstanding tool in nanotechnology. *Bionatura.* 1(4): 196-201. doi: 10.21931/RB/ 2016.01.04.7

Devi, J.S. & Bhimba, B.V. 2012. Anticancer Activity of Silver Nanoparticles Synthesized by the Seaweed *Ulva lactuca* Invitro. 1: 242. doi:10.4172/scientificreports.242

Duong, T.T., Le, T.S., Tran, T.T.H., Nguyen, T.K., Ho, C.T., Dao, T.H., Le, T.P.Q., Nguyen, H.C., Dang, D.K., Le, T.T.H. & Ha, P.T. 2016. Inhibition effect of engineered silver nanoparticles to bloom forming cyanobacteria. *Adv. in Nat. Sci. Nanosci. Nanotechnol. 7*(3): 035018.

El-Nour, K.M.A., Eftaiha, A.A., Al-Warthan, A. and Ammar, R.A. 2010. Synthesis and applications of silver nanoparticles. *Arabian J. Chem. 3*(3):135-140.

El-Sheekh, M.M. & El-Kassas, H.Y. 2016. Algal production of nano-silver and gold: Their antimicrobial and cytotoxic activities: A review. *J. Gen. Eng. Biotechnol*. 14(2): 299-310

Ibraheem, I.B.M., Abd-Elaziz, B.E.E., Saad, W.F., & Fathy, W.A., 2016. Green biosynthesis of silver nanoparticles using marine Red Algae *Acanthophora specifera* and its antimicrobial activity. *J. Nanomed. Nanotechnol*. 7: 409. doi: 10.4172/2157-7439.1000409

Jyoti, K., Baunthiyal, M. & Singh, A. 2016. Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics. *J. Radiation Res. Appl. Sci.* 9(1): 217-227. doi: 10.1016/j.jrras.2015.10.002

Kusumaningrum, H.P. & Zainuri, M. 2014. Optimization and stability of total pigments production of fusan from protoplast fusion of microalgae *Dunaliella* and *Chlorella in vivo*: Attempts on production of sustainable aquaculture natural food. *Int. J. Mar. Aqua. Res. Conserv. Co-existence*. 1(1): 1–5.

Kusumaningrum H.P. & Zainuri M. 2015. Detection of bacteria and fungi associated with *Penaeus monodon* postlarvae mortality. In: International Conference on Tropical and Coastal Region Eco-Development 2014 (ICTCRED). Hadiyanto H, Heru S & Ocky KR. eds *Proc. Env. Sc.* 23: 329–337

Mandalam, R.K. & Palsson, B.O. 1998. Elemental Balancing of Biomass and Medium Composition Enhances Growth Capacity in High-Density *Chlorella vulgaris* Cultures *Biotechnol. Bioeng.* 59(5):605-611

McVey, J.P. & Moore, J.R. 1983. Algal food cultures at the centre oceanologique du pacifique. In

Handbook of Mariculture: Crustacean Aquaculture, 2nd ed., Vol. 1 McVey, JP. ed. CRC Press, Boca Raton, p: 43–69

Merin, D.D., Prakash, S. & Bhimba, B.V. 2010. Antibacterial screening of silver nanoparticles synthesized by marine micro algae. *Asian Pacific J. Trop. Med.* 3(10): 797-799. doi: 10.1016/ S1995-7645(10)60191-5

Newbury, D.E. & Ritchie, N.W.M. 2013. Is Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM/EDS) Quantitative ?. *Scanning,* 35: 141–168

Patel, V., Berthold, D., Puranik, P. & Gantar, M. 2015. Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity. *Biotech Report.* 5: 112-119. doi : 10.1016/j.btre.2014. 12.001

Rajesh, S., Raja, D.P., Rathi, J.M. & Sahayaraj, K. 2012. Biosynthesis of Ag nanoparticles using *Ulva fasciata* (Delile) ethyl acetate extract and its activity against *Xanthomonas campestris* pv. Malvacearum. *J. Biopest*, 5: 119-128

Rajeshkumar, S., Kannan, C. & Annadurai, G., 2012. Green synthesis of silver nanoparticles using marine brown algae *Turbinaria conoides* and its antibacterial activity. *Int. J. Pharma and Bio Sci.*, 3(4): 502-510.

Sivakumar, N., Sundararaman, M. & Selvakumar, G. 2011. Efficacy of micro algae and cyanobacteria as a live feed for juveniles of shrimp *Penaeus monodon*. *Afr. J. Biotechnol*. 10(55): 11594-11599

Sudha, S.S., Rajamanickam, K. & Rengaramanujam J., 2013. Microalgae mediated synthesis of silver nanoparticles and their antibacterial activity against pathogenic bacteria. *Indian J. Exp. Bio*. 52: 393-399

Trincavelli J., Limandri S. & Bonetto R. 2014. Standardless quantification methods in electron probe microanalysis, Review. *Spectrochim. Acta* Part B 101: 76–85

Wassilkowska A., Czaplicka-Kotas A., Zielina M. & Bielski A. 2015. An analysis of the elemental composition of micro-samples using eds technique. *Technical Transactions Chem.* 133-18