Gmail **ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id>**

Re: AACL Bioflux - manuscript reception confirmation

7 messages

Tudor Papuc <ptudor2008@yahoo.com> Mon, May 17, 2021 at 8:56 PM
Reply-To: Tudor Papuc <ptudor2008@yahoo.com>
To: ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id>

I am back with the final version. There are only some minor comments about the references and citations. Please correct based on them, like you did before, with track changes.
After you make the corrections, please read again carefully all the manuscript. If you wish to add or change anything, please do so, but mark the changes, so I can check them. This would be the last time you can make any changes to this manuscript. After publication, we cannot change anything in the paper.

After you do all the above, send me back the corrected manuscript, and I will publish it in 1-2 days.
Thank you,

Best Regards,
Tudor Păpuc
Editor, Bioflux

On Wednesday, April 21, 2021, 05:12:37 PM GMT+3, ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:

Thank you very much for your help and kindness

Best Regards
Andi Setiawan.

On Tue, Apr 20, 2021 at 10:15 PM Tudor Papuc <ptudor2008@yahoo.com> wrote:

> Thank you, I received the document and will soon send you a final version for a last check (or maybe for some more minor changes).
>
> Best Regards,
> Tudor Păpuc
> Editor, Bioflux
>
> On Monday, April 19, 2021, 09:09:38 PM GMT+3, ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:
>
> Dear Mr. Tudor Papuc
> How are you, I hope you are in good health.
> Sorry if I can't complete the repair
> manuscript on time.
> I've tried to make corrections to the manuscript
> according to your suggestion using track changes.
> Hopefully in accordance with your expectations.
> Thank you for your help and kindness.
>
> Best Regards
> Andi Setiawan

On Tue, Mar 23, 2021 at 11:09 PM ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:

Dear Mr. Tudor Pupac
Thank you for your information and I am very happy to hear from you.
I can understand that you must be very busy. I will read the paper
more carefully and improve according to your suggestions

Best regards
Andi Setiawan

On Tue, Mar 23, 2021 at 9:16 PM Tudor Papuc <ptudor2008@yahoo.com> wrote:

Hello, I am finally back with the paper with comments. Sorry for the long wait. One reviewer gave a negative review, because there are too few references. I managed to find another 2 reviewers to give positive evaluations. What you need to do is this:

1. Read all the paper carefully, because the English was corrected and the text was formatted.

2. Read carefully all the comments first (before starting the corrections) and try to correct as best as you can. **Please work on this version of the manuscript. Please mark your changes (highlight with yellow, or use track changes; you can also leave the comments), so I can check them**. If you cannot correct, do not wish to do so, or have your own explanations, please write the reason as a reply to the comment or as a new comment.

3. If you have anything to add/change to the text not based on comments, please do so, but mark the changes like in point 2.

4. Try to respect the formatting when making changes.

5. After you make the corrections, please check again, to make sure everything is in order.

6. Send me back the corrected version of the manuscript.

The references will be checked after you make the changes.
I will check it, give it a final form, and send you the final version for a last check before publication.

We will try to publish it in April, if you manage to make to corrections in 2-3 weeks.

Thank you,

Best Regards,

Tudor Păpuc

Editor, Bioflux

On Saturday, August 15, 2020, 03:17:53 AM GMT+3, ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:

Thank you very much for your information.

Best regards,
Andi

On Fri, Aug 14, 2020 at 1:54 PM Tudor Papuc <ptudor2008@yahoo.com> wrote:

> There is no mistake. The payment was confirmed, and I sent you the invoice for the payment you made.
> As previously mentioned, the invoice is sent immediately after payment confirmation.
>
> There is no mistake and no need to make another payment.
>
> Best Regards,
> Tudor Păpuc
> Editor, Bioflux
>
> On Friday, August 14, 2020, 09:18:59 AM GMT+3, ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:
>
> Dear Tudor Papuc
>
> I am really sorry to make a mistake for a payment.
> My student will post the underpayment on Tuesday next week.
> Thank you very much for your understanding
>
> Best regards,
> Andi
>
> On Fri, Aug 14, 2020 at 12:30 AM Tudor Papuc <ptudor2008@yahoo.com> wrote:
>
> > Please find attached the invoice for the payment you made.
> > Thank you,
> >
> > Best Regards,
> > Tudor Păpuc
> > Editor, Bioflux
> >
> > On Monday, August 10, 2020, 08:48:20 PM GMT+3, ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:
> >
> > Thank you very much for your information and support.
> > Take care and I will look forward to hearing from you!
> >
> > Sincerely yours,
> >
> > Andi
> >
> > On Mon, Aug 10, 2020 at 4:21 PM Tudor Papuc <ptudor2008@yahoo.com> wrote:
> >
> > > Thank you, we are confirming the payment, but everything should be ok.
> > > I have contacted the reviewer and expect a reply.
> > >
> > > Best Regards,
> > > Tudor Păpuc
> > > Editor, Bioflux

On Friday, August 7, 2020, 12:47:59 PM GMT+3, ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:

Dear Tudor Papuc,
I hope you are always healthy.
In this email I attached scan receipt for payment
And for reviewer the manuscript, I recommend
Mr. Novriyandi Hanif Ph.D
novriyandi@gmail.com
or
nhanif@apps.ipb.ac.id
Thank you very much for your kindness

Sincerely yours,

Andi

On Wed, Aug 5, 2020 at 10:31 PM ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:

> Dear Tudor Papuc,
> I am very happy to hear your good news.
> Thank you very much for your kindness.
> Tomorrow, My student will arrange for payment.
> And I will send a scanned receipt as soon as possible.
>
> Best regards
> Andi

On Wed, Aug 5, 2020 at 2:06 AM Tudor Papuc <ptudor2008@yahoo.com> wrote:

> Hello,
> I am back with some good news.
>
> Your paper is preliminary accepted for publication. Please find attached the preliminary acceptance letter. The final acceptance or rejection will be confirmed after receiving the double-blind peer reviews. If you can, please recommend 1 reviewer for your paper. I found 1, but it seems difficult to find the second reviewer.
>
> After this acceptance you should pay 250 USD for publication (open access).
>
> As the payment and bank transfer may be time consuming you may start the process of payment right now; we will send you the invoice immediately after the payment.
>
> Please let us know when the payment is done (scanned receipt) in order to start the publishing process.
>
> In case the peer-reviews suggest rejection, we will return the payment. The payment will not be returned in case of withdrawal request by authors or simply lack of feedback.

Please note that the Publishing House Policy does not allow to start the manuscript editing prior payment.

Here are the info for payment;
take care to write correct the details:

Beneficiary: Bioflux SRL
City: Cluj-Napoca
Country: Romania, European Union

SWIFT CODE of the bank: BTRLRO22

Account USD: RO68BTRL01302202L28614XX

Bank: BANCA TRANSILVANIA

Important! When bank transfer is used to pay a publication fee, please pay attention to which modality of payment you chose! There are three options: Ben, Our, Us.

Use always "Us" option (meaning that all the bank transfer costs are in your concern/ authors support them and not the publisher). If you forgot to mention that and you let the bank to set "Ben" or "Our" options, we receive about 225 usd instead of 250 usd. Such payments are not valid and you need to pay again for the rest of the sum and one more transfer charge. This situation is not desirable.

There is a second method of payment, by Paypal, with credit card. Please let me know if you choose this method and I will redirect you to our colleague which is in charge of this procedure.

Let me know if you need more details.

Thank you for publishing with us!

Best regards,

Tudor Papuc

Editor, Bioflux

Visit our journals:

Aquaculture, Aquarium, Conservation & Legislation www.bioflux.com.ro/aacl

Advances in Environmental Sciences www.aes.bioflux.com.ro

Human & Veterinary Medicine www.hvm.bioflux.com.ro

Advances in Agriculture & Botanics www.aab.bioflux.com.ro

Animal Biology & Animal Husbandry www.abah.bioflux.com.ro

Extreme Life, Biospeology & Astrobiology www.elba.bioflux.com.ro

Porcine Research www.porc.bioflux.com.ro

Rabbit Genetics www.rg.bioflux.com.ro

ProEnvironment Promediu http://journals.usamvcluj.ro/index.php/promediu

Poeciliid Research www.pr.bioflux.com.ro

On Friday, July 17, 2020, 02:10:20 PM GMT+3, ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:

Dear Tudor Papuc
I am very happy to hear this information from you.
Thank you very much for your kindness.

Best regards.
Andi Setiawan

On Fri, Jul 17, 2020 at 3:21 PM Tudor Papuc <ptudor2008@yahoo.com> wrote:

> Yes, the paper will be preliminary accepted.
> I will send you the preliminary acceptance letter and payment details in 7-10 days.
>
> Best Regards,
> Tudor Păpuc
> Editor, Bioflux

On Wednesday, July 15, 2020, 08:07:09 AM GMT+3, ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:

Dear Tudor Papuc,
How are you ?
I hope your work is going well.
In this change,I would like to trace the status of my manuscript.
I and my students would be really happy if the manuscript can be published at AACL Bioflux.
Thank you very much for your consideration.
I will look forward to hearing from you.

Best regards
Andi Setiawan.

On Fri, Jun 19, 2020 at 8:21 PM ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> wrote:

> Dear Tudor Papuc
> Thank you very much for information
> I will look forward to hearing from you
>
> Best regards
> Andi
>
> On Fri, Jun 19, 2020 at 7:41 PM Tudor Papuc <ptudor2008@yahoo.com> wrote:
>
> > Dear Dr. Andi Setiawan,

This is a message of confirmation regarding the reception of your manuscript **Chromatographic isolation of ß-Phycoerythrin from Porphyridium cruentum using ceramic hydroxyapatite**, submitted to our journal AACL Bioflux. I will soon contact you with further information regarding your manuscript.

For any questions, please contact me, on this email address.

Please try to keep one message thread (respond/ask with “Reply”) for an easier message management.

Thank you for considering our journal.

Best regards,

Tudor Papuc

Editor, Bioflux SRL

Visit our journals:

Aquaculture, Aquarium, Conservation & Legislation www.bioflux.com.ro/aacl

Advances in Environmental Sciences www.aes.bioflux.com.ro

Human & Veterinary Medicine www.hvm.bioflux.com.ro

Advances in Agriculture & Botanics www.aab.bioflux.com.ro

Animal Biology & Animal Husbandry www.abah.bioflux.com.ro

Extreme Life, Biospeology & Astrobiology www.elba.bioflux.com.ro

Porcine Research www.porc.bioflux.com.ro

Rabbit Genetics www.rg.bioflux.com.ro

ProEnvironment Promediu http://journals.usamvcluj.ro/index.php/promediu

Poeciliid Research www.pr.bioflux.com.ro

Manuxcript Alkausar final version .doc
1079K

ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> Thu, May 20, 2021 at 7:52 PM
To: Tudor Papuc <ptudor2008@yahoo.com>

Thank you very much for your support.
In this email, I have attached the final revised manuscript accordingly
with your suggestions.
Your referrals are very helpful for us in improving the writing of manuscripts.
We hope that you are always in good health.

Best regards,
Andi Setiawan

[Quoted text hidden]

Ed. Manuxcript Alkausar final version.doc
1082K

Tudor Papuc <ptudor2008@yahoo.com> Sun, May 23, 2021 at 2:43 PM
Reply-To: Tudor Papuc <ptudor2008@yahoo.com>
To: ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id>

Congratulations! The manuscript has been published!
You can find it in the attachment, or on our site: http://www.bioflux.com.ro/docs/2021.1351-1358.pdf.

Thank you for your hard work, cooperation, and patience!
Thank you for publishing with us!

Best Regards,
Tudor Păpuc
Editor, Bioflux

[Quoted text hidden]

2021.1351-1358.pdf
664K

ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> Sun, May 23, 2021 at 4:29 PM
To: Tudor Papuc <ptudor2008@yahoo.com>

I am very happy to hear from you.
Thank you very much for your support and assistance.

Best Regards,
Andi Setiawan
[Quoted text hidden]

ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> Sun, May 23, 2021 at 4:31 PM
To: radhoalkausar@gmail.com

[Quoted text hidden]

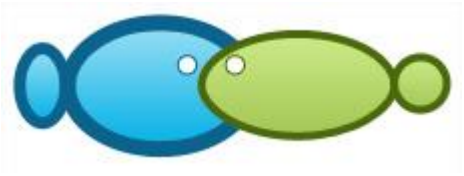
2021.1351-1358.pdf
664K

Radho Alkausar <radhoalkausar@gmail.com> Sun, May 23, 2021 at 4:37 PM
To: ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id>

Baik pak terima kasih informasinya
[Quoted text hidden]

ANDI SETIAWAN <andi.setiawan@fmipa.unila.ac.id> Mon, May 31, 2021 at 9:11 AM
To: osy.datul@gmail.com

---------- Forwarded message ---------
From: **Tudor Papuc** <ptudor2008@yahoo.com>
Date: Sun, May 23, 2021 at 2:43 PM
[Quoted text hidden]
[Quoted text hidden]



Chromatographic isolation of ß-phycoerythrin from *Porphyridium cruentum* using ceramic hydroxyapatite

[1]Radho Alkausar, [1]Ni L. G. R. Juliasih, [1]Buhani, [2]Syafei, [3]Zeily Nurachman, [1]Andi Setiawan

[1] Department of Chemistry, Faculty of Science, Lampung University, Bandar Lampung, Indonesia; [2] Center for Marine Cultivation Fisheries, Bandar Lampung, Indonesia; [3] Biochemistry Division, Faculty of Mathematics and Natural Sciences, Institute of Technology Bandung, Bandung, Indonesia. Corresponding author: A. Setiawan, andi.setiawan@fmipa.unila.ac.id

Abstract. The pigment ß-phycoerythrin is useful as a natural pigment in food and cosmetics, and also serves as a fluoroprobe for laboratory analyses. This study evaluated the effectiveness of ceramic hydroxyapatite as a stationary phase to purify ß-phycoerythrin using medium-pressure liquid chromatography. The microalga, *Porphyridium cruentum*, was cultivated in artificial sea water consisting of 20% effluent biogas of tapioca waste water, as well as in modified F2 nutrient medium consisting of NaH_2PO_4 $2H_2O$ and $NaNO_3$, stock solution of trace elements (Na_2EDTA, $NaMoO_4$ $2H_2O$, $CoCl_2$ $6H_2O$, $FeCl_3$ $6H_2O$, $CuSO_4$ $5H_2O$, $ZnSO_4$ $7H_2O$, $MnCl_2$ $4H_2O$), and stock solution of vitamins (biotin, cyanocobalamin, and thiamine HCl). *P. cruentum* biomass was harvested after 8 days by centrifuging at 8000 g for 10 min at 4°C. Fresh biomass was resuspended in buffer solution and sonicated for 30 min. Then the slurry was centrifuged at 12000 g for 4 min. ß-phycoerythrin-rich supernatants were pooled and treated as a crude extract. The crude extract was then passed over a ceramic hydroxyapatite column at medium pressure. Ceramic hydroxyapatite was prepared using a precipitation method and sintering at 1200°C. The composition and microstructures of the ceramic were characterized with a scanning electron microscope equipped with an energy dispersive X-ray spectrometer. The purity of ß-phycoerythrin was confirmed using UV-Vis spectroscopy. Ceramic hydroxyapetite offers a scalable method for isolation of ß-phycoerythrin. Moreover, effluent biogas of tapioca waste water should be considered as an alternative cultivation medium for sustainable production of microalgae. This study succeeded in cultivating *P. cruentum* in modified F2 media with 20% biogas waste and utilizing ceramic hydroxyapatite to recover 60% of ß-phycoerythrin with an A546/A280 ratio of 3.995.
Key Words: ion chromatography, microalgae, phycobiliprotein, waste water.

Introduction. *Porphyridium cruentum*, a unicellular, marine red alga, belongs to the phylum *Rhodophyta*. This microalga has received considerable attention in recent years because of its production of ß-phycoerythrin (BPE) which is widely used as a colorant in food, nutraceuticals, and pharmaceuticals (Gujar et al 2019). However, to date, commercial cultivation of *Porphyridium* spp. has not been achieved due to high production costs. Many factors can influence production costs, such as the microalga species, cultivation condition, and isolation techniques. Due to its diverse applications in various fields, it seemed worthwhile to develop a simple, cost effective method to recover BPE from *P. cruentum* to meet the increasing demand.

Researchers have made many attempts to cultivate *Porphyridium* species and to devise simple methods to extract and purify BPE from them. Several studies showed that waste water can be used as media to cultivate *Porphyridium* sp. (Widiyaningsih et al 2013; Ulusoy Erol et al 2020). Application of agricultural waste for mass cultivation of *Porphyridium* sp. is a first step to reduce cost and to ensure sustainability of biomass production. *Porphyridium* sp. productivity depends on various parameters, such as temperature, pH, and carbon and nitrogen sources (Su et al 2016). However, information about using effluent biogas from tapioca waste water as a carbon source is still limited.

The second crucial step concerns optimized recovery of BPE from microalgal biomass. Some methods have been reported for extracting and purifying BPE from *Porphyridium* biomass (Gallego et al 2019; Ardiles et al 2020). Extraction and purification are essential steps to optimize BPE recovery. Microwave-assisted extraction, ammonium sulfate precipitation (Cai et al 2012), coupled with ion-exchange chromatography (Bermejo Roman et al 2002) have been employed to purify BPE from *Porphyridium* sp.

In the present study, we report a new method for efficient BPE production from *P. cruentum* cultivated using effluent biogas derived from industrial tapioca waste water. This microalgal strain grows normally in a 20% mixture of the effluent from biogas process and artificial sea water. BPE was recovered from biomass *P. cruentum* extract using a single medium-pressure ion-exchange chromatography step with ceramic hydroxyapatite (HAp) as the stationary phase.

Material and Method

Synthesis and characterization of HAp. HAp powder was prepared using a wet chemical precipitation method from $CaCl_2$ $2H_2O$ and Na_2HPO_4 (Ramesh et al 2013). An aqueous solution of 0.3 M Na_2HPO_4 was added dropwise to a 1.66 M solution of $CaCl_2$ at a rate of 1 mL min^{-1}. During this process, the pH of the solution was adjusted to 10 using NaOH solution. The reaction was carried out at 32°C with continuous stirring (800 rpm). The resulting precipitate was aged for 15 h and then repeatedly centrifuge-washed with distilled water until the pH became neutral. Then it was dried at 80°C for 24 h. For sintering, HAp was heated to 1200°C for 1 h using a furnace Lenton type UAF 16/10. The HAp surface was characterized using scanning electron microscopy/dispersive X-ray spectroscopy (SEM-EDX) Zeiss EVO MA 10 at Technical Service Unit, Integrated Laboratory of Innovation and Technology Center Lampung University, Indonesia.

Algal strain and cultivation conditions. This study was performed using the microalga *P. cruentum*. Microalgae were obtained from the culture collection of the Biochemistry Laboratory Institute of Technology at Bandung, Indonesia. A stock culture was maintained in a 250-mL flask containing modified artificial sea water (ASW). The starter culture was grown in a 6×60 cm glass column containing ASW (g L^{-1}) composed of: 27 g NaCl, 5.6 g $MgCl_2.6H_2O$, 1.5 g $CaCl_2.2H_2O$, 1 g KNO_3, 0.07 g K_2HPO_4, 6.6 g $MgSO_4.7H_2O$, and 0.04 g $NaHCO_3$ while media were adjusted to a pH between 7.4-8.2. Illumination was provided by fluorescent lamps producing 350 µmol photons m^{-2} s^{-1}. The photoperiod was 12:12 h (light to dark), and the growth temperature was maintained at 28±2°C. An additional carbon source of media culture and agitation were supplied with an aerator. Microalgal cells were harvested via centrifugation (8000 g for 10 min at 4°C).

Preparation of a crude extract. *P. cruentum* was cultivated in modified F2 mixed with 20% effluent biogas. The modified F2 nutrient medium consisted of the following (pH 8): NaH_2PO_4 $2H_2O$ 0.0056 g; $NaNO_3$ 0.0752 g; stock solution of trace elements - 1 mL L^{-1} (Na_2EDTA 4.15 g, $NaMoO_4$ $2H_2O$ 0.20 g, $CoCl_2$ $6H_2O$ 0.011 g, $FeCl_3$ $6H_2O$ 3.17 g, $CuSO_4$ $5H_2O$ 0.012 g, ZnSO4 $7H_2O$ 0.024 g, $MnCl_2$ $4H_2O$ 0.19 g); and stock solution of vitamin mix - 1 mL L^{-1} (cyanocobalamin 0.0004 g, thiamine HCl 0.13 g, and biotin 0.0005 g) (Hawrot-Paw et al 2020). Microalgae were harvested after eight days of cultivation, and biomass was collected using a HITACHI CF 16 RXII centrifuge at 8000 g for 10 min at 4°C. After that, pelleted *P. cruentum* were mixed with 0.1 M buffer phosphate (pH 6.7, 0.05 M K_2HPO_4, 0.05 M KH_2PO_4) and sonicated to homogeneity. Then the cell lysate was centrifuged again. The supernatant was gradually saturated with 60% ammonium sulfate. The resulting solution was kept for 2 h at 4°C and centrifuged at 12000 g for 15 min at 4°C, using a TOMY CAX-370 centrifuge. The precipitate was kept at 4°C until use.

Purification and characterization. Purification of a crude extract of BPE was accomplished using medium-pressure liquid chromatography (MPLC). Chromatography was performed on a Buchi type Sepacore X50 system connected with an inner diameter 2x12 cm plastic column of ceramic HAp (20 g). The mobile phase consisted of sodium

phosphate buffer (pH 6.8, 1 M NaH_2PO_4, 1 M Na_2HPO_4) with 0.1% NaCl using a 10 mL sample loop at a flow rate of 20 mL min. Samples (2 mL) were loaded onto the HAp column and eluted with a linear gradient of 0.1% NaCl (from 20% to 90%). The resulting eluent was monitored by UV absorbance at 220, 254, and 364 nm and 5 mL fractions were collected. The purity of isolated BPE was determined from the A_{565}/A_{280} ratio using a Cary 50 spectrophotometer (Bennet & Bogorad 1973).

Results and Discussion

Synthesis and characterization. The reaction of Na_2HPO_4 with $CaCl_2$ yielded 16.9 g of white, crystalline HAp. Crystalline HAp was sintered at 1200°C to obtain ceramic HAp (15.8 g). HAp synthesis was performed several times to obtain 60 g of ceramic HAp. Surface morphology of ceramic HAp was investigated with SEM, showing that HAp had formed agglomerated, rod-shaped structures (Figure 1).

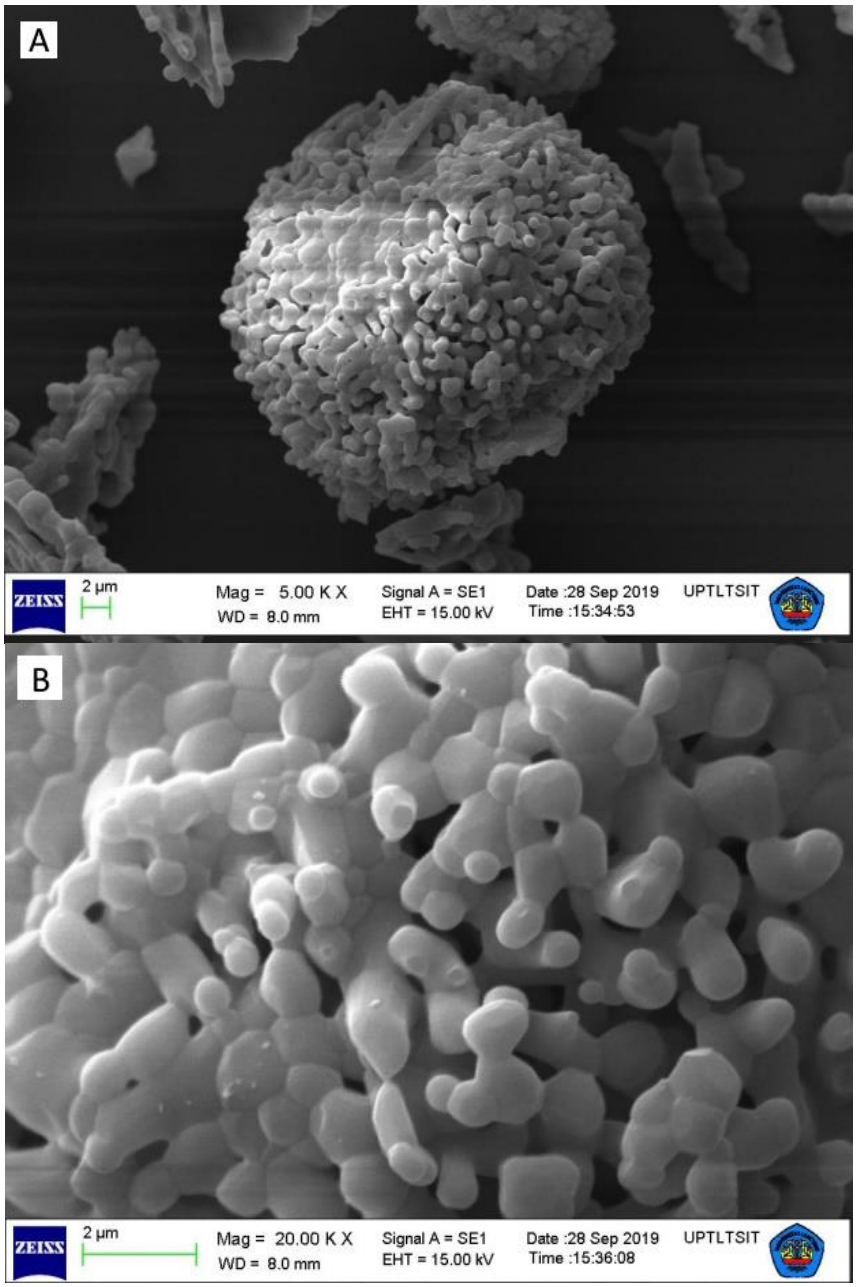


Figure 1. Visualization of sintered hydroxyapatite using a scanning electron microscope; A - magnification 500x; B - magnification 2000x.

As depicted in Figure 2, EDX spectra confirmed that ceramic HAp consists mainly of calcium, phosphorus, and oxygen, which together form calcium hydroxyl phosphate. Chemical analysis by EDX reveals a Ca/P ratio of 0.7, for the sample after heat-treatment at 1200°C (Table 1). Based on a chromatographic point of view, HAp with Ca/P ratio of 1.67 consists of positively charged pairs of calcium ions (C sites) and six negatively charged oxygen atoms associated with triplets of phosphates (P-sites). Functional groups of C-sites, P-sites, and hydroxyl groups are connected in a fixed pattern on the crystal surface (Kawasaki et al 1985). For a Ca/P ratio less than 1.67, this leaves phosphate residues as the dominant surface feature. HAp tends to repel negatively charged residues like carboxyl and phosphoryl groups (Jungbauer et al 2004).

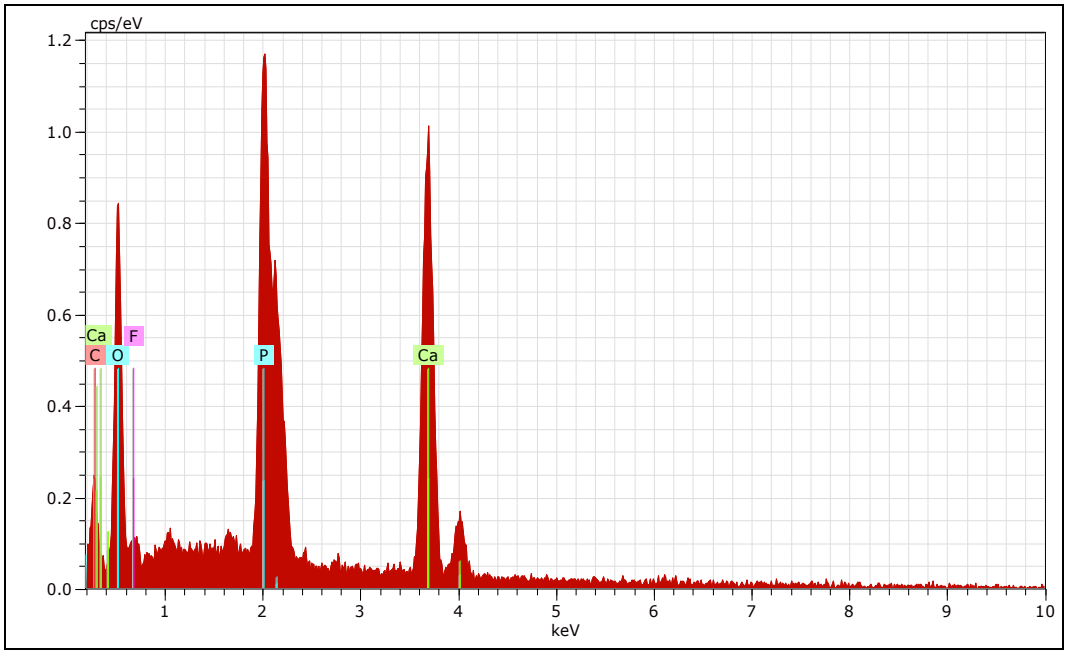


Figure 2. Energy-dispersive X-ray spectroscopy (EDX) characterization of ceramic hydroxyapatite used as a stationary phase in medium pressure liquid chromatography.

Table 1

Percent weight and atomic composition of ceramic hydroxyapatite

	Ceramic hydroxyapatite		
Element	*Weight %*	*Atomic %*	*Ca/P (atomic)*
O	47.85±3.05	57.45±3.05	
P	20.54±0.23	12.74±0.23	0.7±0.01
Ca	18.53±0.16	8.88±0.16	

Cultivation. Overall, *P. cruentum* required four days for adaptation in effluent biogas before entering an exponential growth phase (Figure 3a). The growth peak occurred on the 8th day of cultivation, for both growth media. After 10 days, the increase in biomass concentration in media F2 and effluent biogas was reduced. On the final day of culture, SEM was used to visualize the morphology of single cells of *P. cruentum* in effluent biogas (Figure 3b).

Single cells of *P. cruentum* grew rapidly in effluent biogas media, and displayed a characteristic red color during cultivation. The composition and physico-chemical properties of effluent biogas had no adverse effects on the morphology. After the 8th day of cultivation, the culture achieved 1.5 g of wet biomass per L, which compares favorably with cultures of other microalgae (Li et al 2019a).

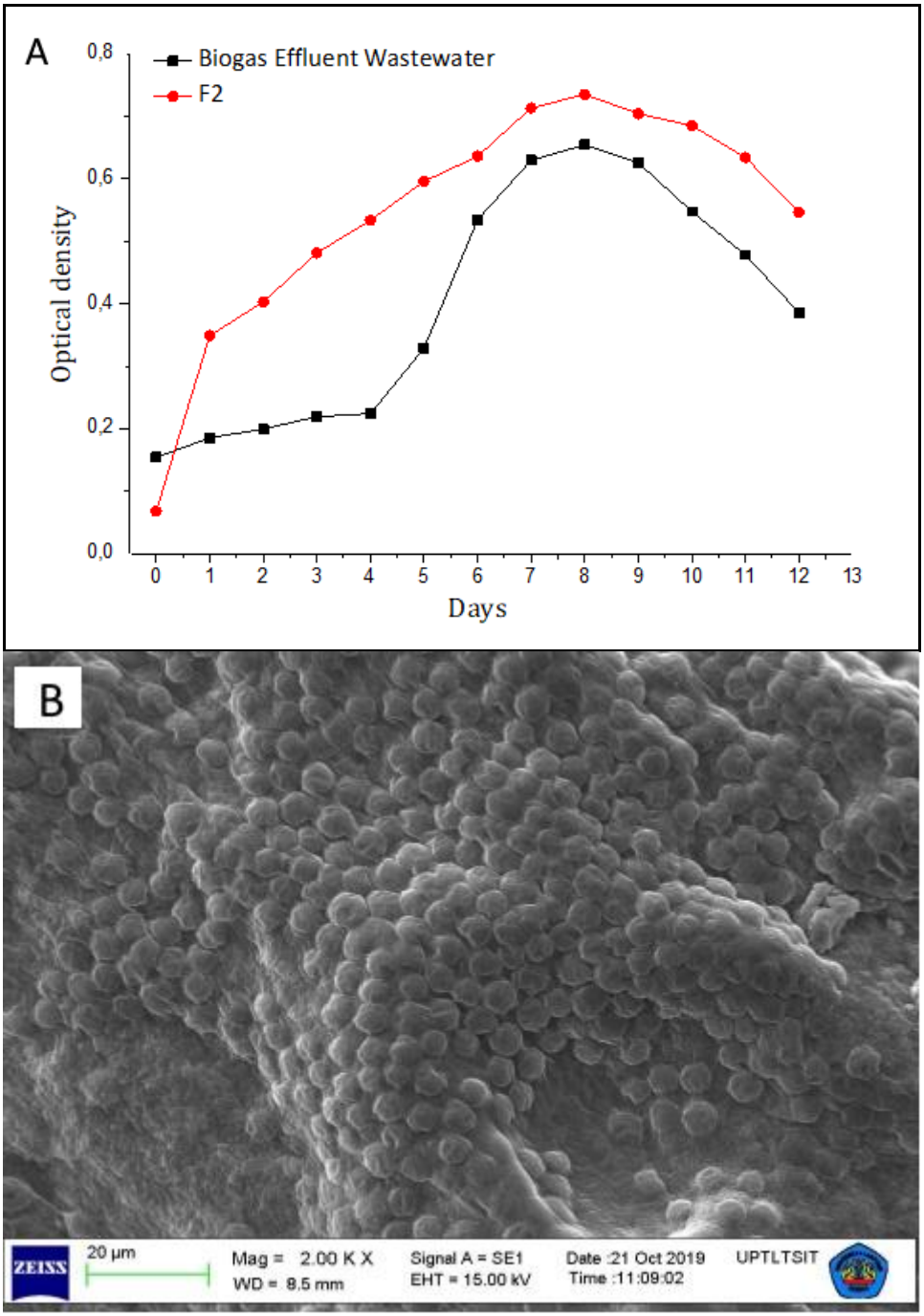


Figure 3. A - optical density growth curve of *Porphyridium cruentum* in F2 media (red) and media supplemented with tapioca waste water effluent biogas (black); B - morphology of *P. cruentum* under scanning electron microscopy (2000x magnification).

In general, microalgae can be grown in media mixed with 20% effluent biogas (Ichsan et al 2014). The BPE content in microalgal biomass depends on cultivation conditions and species. A study on *Porphyridium purpureum* (syn *P. cruentum*) showed that cells grown with sufficient nitrogen, were red, while nitrogen-limited cells were green (Li et al 2019b). The phycoerythrin content in the red microalga *P. purpureum* depends not only on nitrogen, but also on various macronutrients (Lu et al 2020).

Purification and characterization. The chromatogram showed a pronounced peak at a retention time of about 3 min after a delay of 1 min (Figure 4). Elution was accomplished at 20 mL min^{-1} with linear NaCl gradient. UV absorbance at 360 nm showed broad peak,

compared with those at 220 nm and 254 nm. Fraction 8 was collected and analyzed using a spectrophotometer.

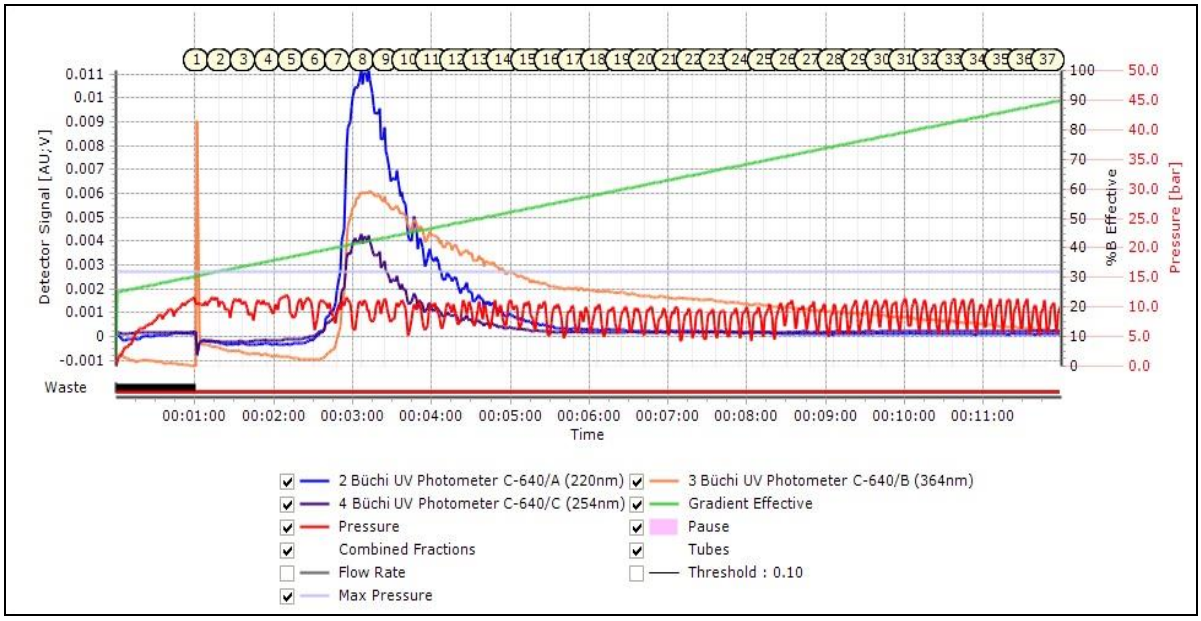


Figure 4. Elution of ß-phycoerythrin from a ceramic hydroxyapatite column (in a diameter of 2 cm x L 12 cm) at 20 mL min^{-1}, 0.1% NaCl (λ= 220, 254, and 360 nm).

The absorbance spectrum of crude *Porphyridium* extract showed absorbance peaks at 280, 545, 565, 620, and 652 nm, indicating a mixture of protein and phycobiliprotein. After separation using MPLC, the absorption spectrum of purified BPE showed absorption peaks at 545 and 565 nm. No absorbance peak was found at 620 or 652 nm, indicating the absence of phycourobilin, phycocyanin, and allo-phycocyanin in the purified BPE sample, while low absorption in the region of 280 nm suggested high purity of the BPE in fraction 8 (Figure 5). The A_{546}/A_{280} ratio of the purified BPE was 3.995, which was considered sufficient for food grade preparations (0.4-3.8) (Walter et al 2011).

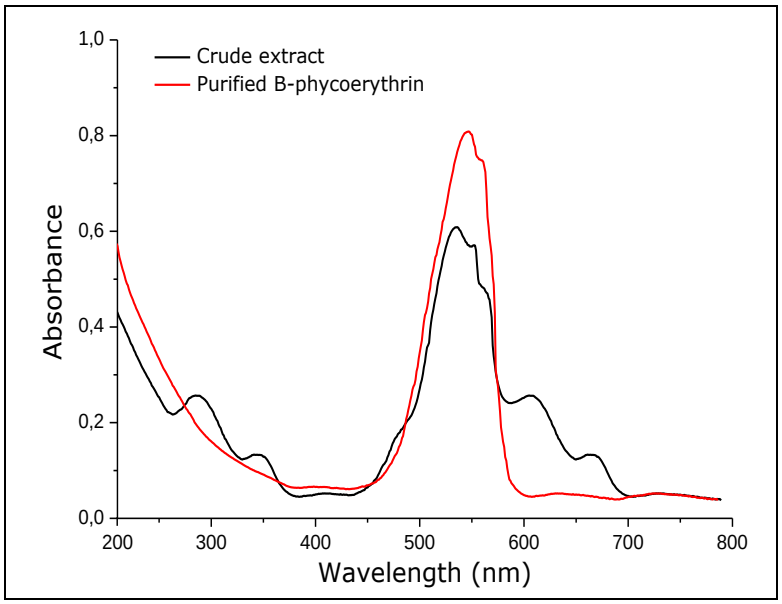


Figure 5. Absorbance spectrum of crude extract and purified ß-phycoerythrin.

Table 2

Purity ratio of ß-phycoerythrin

Sample	*A_{280}*	*A_{546}*	*Purity (A_{546}/A_{280})*	*% Recovery of BPE*
Crude Extract	0.237	0.608	2.565	60
Recover PE	0.202	0.807	3.995	

Note: BPE - ß-phycoerythrin.

Conclusions. This study successfully cultivated *P. cruentum* in F2 media with 20% effluent biogas and employed ceramic HAp to recover 60% of the BPE with an A546/A280 ratio of 3.995. We conclude that the above procedure has potential to improve the commercial production of BPE.

Acknowledgements. The authors are grateful to Lampung University for a research grant No. 1953/UN26.21/PN/2019 and thank all laboratory staff at Technical Service Unit, Integrated Laboratory of Innovation and Technology Center, Lampung University, for support of its laboratory facilities.

Conflict of Interest. The authors declare that there is on conflict of interest.

References

Ardiles P., Cerezal-Mezquita P., Salinas-Fuentes F., Órdenes D., Renato G., Ruiz-Domínguez M. C., 2020 Biochemical composition and phycoerythrin extraction from red microalgae: A comparative study using green extraction technologies. Processes 8(12):1628, 16 p.

Bennet A., Bogorad L., 1973 Complementary chromatic adaptation in a filamentous blue-green algae. The Journal of Cell Biology 58:419-435.

Bermejo Román R., Alvárez-Pez J. M., Acién Fernández F. G., Molina Grima E., 2002 Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*. Journal of Biotechnology 93(1):73-85.

Cai C., Li C., Wu S., Wang, Q., Guo Z., He P., 2012 Large scale preparation of phycobiliproteins from Porphyra yezoensis using co-precipitation with ammonium sulfate. Natural Science 4(8):536-543.

Gallego R., Martínez M., Cifuentes A., Ibáñez E., Herrero M., 2019 Development of a green downstream process for the valorization of *Porphyridium cruentum* biomass. Molecules 24(8):1564, 13 p.

Gujar A., Cui H., Ji C., Kubar S., Li R., 2019 Development, production and market value of microalgae products. Applied Microbiology: Open Access 5:162, 9 p.

Hawrot-Paw M., Koniuszy A., Gałczyńska M., Zając G., Szyszlak-Bargłowicz J., 2020 Production of microalgal biomass using aquaculture wastewater as growth medium. Water 12(1):106, 11 p.

Ichsan, Hadiyanto H., Hendroko R., 2014 Integrated biogas-microalgae from waste waters as the potential biorefinery sources in Indonesia. Energy Procedia 47:143-148.

Jungbauer A., Hahn R., Deinhofer K., Luo P., 2004 Performance and characterization of a nanophased porous hydroxyapatite for protein chromatography. Biotechnology & Bioengineering 87(3):364-375.

Kawasaki T., Takahashi S., Ikeda K., 1985 Hydroxyapatite high-performance liquid chromatography: column performance for protein. European Journal of Biochemistry 152(2):361-371.

Li G., Bai X., Li H., Lu Z., Zhou Y., Wang Y., Cao J., Huang Z., 2019a Nutrients removal and biomass production from anaerobic digested effluent by microalgae: A review. International Journal of Agricultural and Biological Engineering 12(5):8-13.

Li T., Xu J., Wu H., Jiang P., Chen Z., Xiang W., 2019b Growth and biochemical composition of *Porphyridium purpureum* SCS-02 under different nitrogen concentrations. Marine Drugs 17:124, 16 p.
Lu X., Nan F., Feng J., Lv J., Liu Q., Liu X., Xie S., 2020 Effects of different environmental factors on the growth and bioactive substance accumulation of *Porphyridium purpureum*. International Journal of Environmental Research and Public Health 17(7):2221, 14 p.
Ramesh S., Adzila S., Jeffrey C. K. L., Tan C. Y., Purbolaksono J., Noor A. M., Hassan M. A., Sopyan I., Teng W. D., 2013 Properties of hydroxyapatite synthesize by wet chemical method. Journal of Ceramic Processing Research 14(4):448-452.
Su G., Jiao K., Chang J., Li Z., Guo X., Sun Y., Zeng X., Lu Y., Lin L., 2016 Enhancing total fatty acids and arachidonic acid production by the red microalgae *Porphyridium purpureum*. Bioresources and Bioprocessing 3:33, 9 p.
Ulusoy Erol H. B., Menegazzo M. L., Sandefur H., Gottberg E., Vaden J., Asgharpour M., Hestekin C. N., Hestekin J. A., 2020 Porphyridium cruentum grown in ultra-filtered swine wastewater and its effects on microalgae growth productivity and fatty acid composition. Energies 13(12):3194, 9 p.
Walter A., de Carvalho J. C., Soccol V. T., de Faria A. B. B., Ghiggi V., Soccol C. R., 2011 Study of phycocyanin production from *Spirulina platensis* under different light spectra. Brazilian Archives of Biology and Technology 54(4):675-682.
Widianingsih, Hartati R., Endrawati H., Mamuaja J., 2013 Fatty acid composition of marine microalgae in Indonesia. Journal of Tropical Biology and Conservation 10:75-82.

Received: 16 June 2020. Accepted: 04 August 2020. Published online: 23 May 2021.
Authors:
Radho Alkausar, Department of Chemistry, Faculty of Science, Lampung University, 1 Sumantri Brojonegoro St., 35145 Bandar Lampung, Indonesia, e-mail: radhoalkausar@gmail.com
Ni Luh Gede Ratna Juliasih, Department of Chemistry, Faculty of Science, Lampung University, 1 Sumantri Brojonegoro St., 35145 Bandar Lampung, Indonesia, e-mail: niluhratna.juliasih@fmipa.unila.ac.id.
Buhani, Department of Chemistry, Faculty of Science, Lampung University, 1 Sumantri Brojonegoro St., 35145 Bandar Lampung, Indonesia, e-mail: buhani@fmipa.unila.ac.id
Syafei, Centre for Marine Cultivation Fisheries, Yos Sudarso St., 35454 Desa Hanura, Lampung, Indonesia, e-mail: bbpbl.lampung@gmail.com
Zeily Nurachman, Biochemistry Division, Faculty of Mathematics and Natural Sciences, Institute Technology Bandung, 10 Ganesha St., Bandung, 40132 Jawa Barat, Indonesia, e-mail: zeily@chem.itb.ac.id
Andi Setiawan, Department of Chemistry, Faculty of Science, Lampung University, 1 Sumantri Brojonegoro St., 35145 Bandar Lampung, Indonesia, e-mail: andi.setiawan@fmipa.unila.ac.id

How to cite this article:
Alkausar R., Juliasih N. L. G. R., Buhani, Syafei, Nurachman Z., Setiawan A., 2021 Chromatographic isolation of ß-phycoerythrin from *Porphyridium cruentum* using ceramic hydroxyapatite. AACL Bioflux 14(3):1351-1358.

2021.1351-1358.pdf
664K