Int Aquat Res (2022) 14:127-137
https://doi.org/10.22034/IAR.2022.1950929.1234

ORIGINAL RESEARCH

Effect of ferric chloride (FeCl3) concentration on pigment *production of Porphyridium Cruentum*

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Received: 27 January 2022 / Accepted: 27 May 2022 / Published online: 02 June 2022

Abstract Microalgae are unicellular organisms with different shapes and sizes. Wide range of biomolecules (protein, fatty acids, carotenoids, phycobiliproteins, etc.) could be produced and accumulated by these organisms. *P. cruentum* is known as a source of high-value biomolecules named phycobiliproteins. These pigments used in various areas such as food, cosmetics, nutraceutical and pharmaceutical industries. In this study, the effect of ferric chloride ($FeCl_3$) concentration ($0.234 \times 10^{-5} – 1.17 \times 10^{-5}$ M) in F/2 medium on growth and pigment composition of *P. cruentum* was investigated. Using the 0.585×10^{-5} M of $FeCl_3$ (group C) provides higher cell number than other concentrations on *P. cruentum* culture ($P<0.05$). The 0.234×10^{-5} $FeCl_3$ was provided the highest phycoerythrin production while the highest cellular phycoerythrin accumulation was obtained with 5.85×10^{-5} $FeCl_3$ concentration. It was found that pigment accumulation was induced by low and high concentrations of ferric chloride. As a result of this study, it was concluded that the ferric chloride could be used as a promoter for pigment accumulation in *P. cruentum* biomass.

Keywords Microalgae . *Porphyridium cruentum* . Pigments . Ferric chloride . Phycobiliproteins

Introduction

Microalgae are unicellular organisms, which are able to accumulate high amounts of poly-unsaturated fatty acids (PUFA), pigments, sterols and protein (Volkman, 2016; Niccolai et al. 2019). Microalgae species contain economically valuable pigments such as chlorophylls, carotenoids and phycobiliproteins. Pigments have become more important after increase of awareness on healthy food of the consumers (Shakeri et al. 2018). Instead of synthetic equivalents, natural pigments are non-toxic and non-carcinogic (Begum et al. 2016). Microalgae biomasses and their pigments are used as colorant and source of bioactive compounds in foods such as chocolates, chewing gums, pasta and yoghurt (Fradique et al. 2013; Robertson et al. 2016; Palabıyık et al. 2018; Genç Polat et al. 2020). These compounds have various bioactive properties as vitamin precursors, antioxidants, anti-inflammatory and anti-carcinogenesis activites (Sousa et al. 2008; Katiyar and Arora 2020). Algae are classified into different divisions or phyla based on the pigmentation and specific biochemical properties and cell wall components in each species. Pigmentation determines the habitats for each genera or species that are found apart from the varying adaptability potential (Richmond 2004).

Porphyridium cruentum is a red microalga which contains chlorophylls, carotenoids and high amounts of phycobiliproteins. High value pigments phycobiliproteins are already used in food, cosmetic, nutraceutical and pharmaceutical industries (Manirafasha et al. 2016; Khatoon et al. 2018).

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The requirement of nutrients on algae growth can be divided into macro-elements, i.e. nitrogen and phosphorus and micro-elements, i.e. ferric compounds, manganese, copper, zinc, etc., (Ushizaka et al. 2011; Agrawal 2012). Ferric compounds are essential micronutrients for the growth of phytoplankton due to their critical role in various metabolic processes including chlorophyll synthesis, photosynthetic electron transport, respiration, nitrate reduction, nitrogen fixation processes, etc., (Fujii et al. 2010; Alexova et al. 2011). Ferric chloride is indicated as the most appropriate iron source for the rapid growth of *Dunaliella tertiolecta* but, it is stated that higher dry weights were obtained with ferric EDTA and ferric ammonium sulfate (Rizwan et al. 2017). Also, the results of the same study showed that the use of both ferric EDTA and ferric chloride provided higher lipid content in *Dunaliella tertiolecta* biomass than ferric ammonium sulfate (Rizwan et al. 2017). Therefore, it can be stated that different ferric compounds are effective on the growth parameters, and even the biochemical composition of microalgae.

The accumulation of phycobiliproteins at low concentrations in cells, is one of the obstacles against their use more commonly. It has already proven that the biochemical composition of microalgae can be altered by the modification of environmental conditions and culture medium substances (Renaud et al. 2002; Procházková et al. 2014; Fernandes et al. 2020). By this study, it was aimed to determine the effects of ferric chloride ($FeCl_3$) concentration on growth and pigment accumulation of *Porphyridium cruentum*.

Materials and methods

Porphyridium cruentum (Ben Gurion University, Israel) stock was cultured in Aquaculture Department, Faculty of Fisheries, Kastamonu University.

Microalgae cultivation

Flat bottom flasks (1 L) were used in the study. According to Guillard, (1975) F/2 medium was prepared without any iron source. $FeCl_3$ (Fisher Scientific UK, Ferric chloride hexahydrate) was added into the flasks as a source of iron at different concentrations as given in Table 1. Inoculation of the experimental groups was arranged at an initial density of 0.4 x 10^6 cells/mL. All trials were done in triplicate. Experimental groups were cultivated at 21±1ºC. Philips Master TL-D 865 36W fluorescent lamps were used (45 µmol/m^{-2}/s^{-1}) as the light source with 24:0 L:D cycle. Cultures were aerated with air without CO_2 addition and was filtered passing through 0.2-micron syringe filters to avoid of contamination.

Study model

Cell numbers were counted daily under the light microscope with using the Neubauer chamber. Pigments were determined spectrophotometrically. Chlorophyll *a,* total carotenoids, phycobiliproteins (Phycoerythrin, R-phycocyanin, Allo-phycocyanin and total phycobiliproteins) amounts were calculated by using Equations 1-6 (Macias-Sánchez et al. 2005; Zou and Richmond 2000; Gantt and Lipschultz 1974);

Chlorophyll *a* (µg/ml) = 13,9 A_{666} (1)

Total carotenoids (µg/ml) = 4,5 A_{475} (2)

Phycoerythrin (PE)= (A_{545nm}0.572(A_{620nm})+0.246(A_{650nm}))/5.26 (3)

R-phycocyanin (R-PC)= (A_{620nm}0.666(A_{650nm}))/3.86 (4)

Allo-phycocyanin (A-PC)= (A_{650nm}0.105(A_{620nm}))/4.65 (5)

Total phycobiliprotein (Total PBP) = (PE)+(R-PC)+(A-PC) (6)

Statistical analysis

All cultivation experiments and pigment analysis were performed in triplicate and all data are expressed as the mean with standard deviation. Results were analyzed by one-way ANOVA with significance level at $P \leq 0.05$ and Tukey's multiple comparison test was performed by using SPSS Statistics 23 (IBM, US).

Table 1. Experimental groups and $FeCl_3$ concentrations

Group	A	B	C	D	E	F	G
$FeCl_3$ (molar concentration)	0.00	0.234×10^{-5}	0.585×10^{-5}	1.17×10^{-5}	2.34×10^{-5}	5.85×10^{-5}	11.7×10^{-5}
Per/Con	0% (N.C.)	20%	50%	Control	200%	500%	1000%

*Per/Con; indicates the ratios of experiment groups to standard concentration of $FeCl_3$ in f/2 medium (Guillard, 1975). N.C. indicates Negative Control.

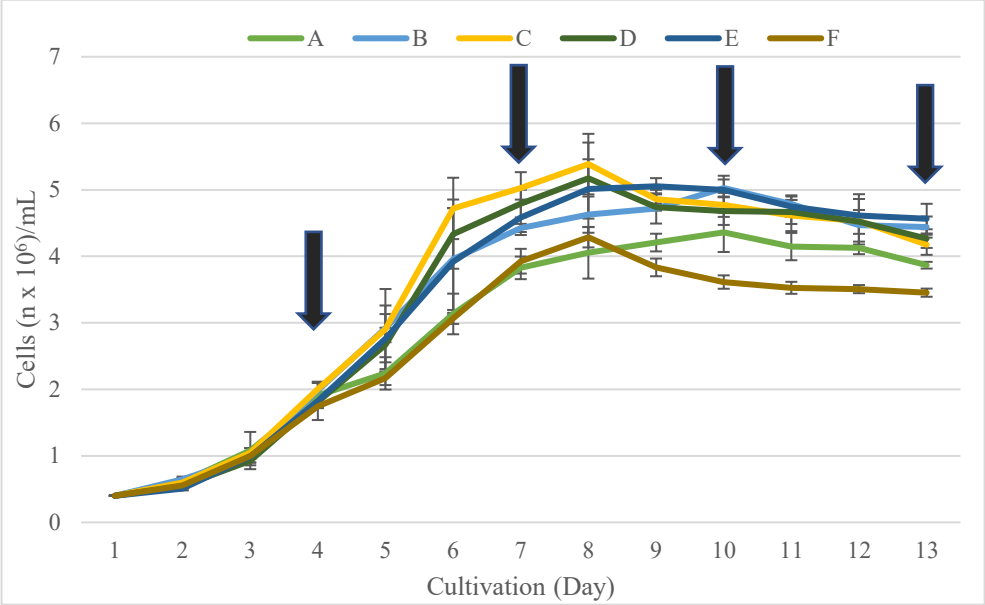

Fig. 1 Cell numbers of *P. cruentum* at different $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234×10^{-5} $FeCl_3$, Group C; 0.585×10^{-5} $FeCl_3$, Group D; 1.17×10^{-5} $FeCl_3$, Group E; 2.34×10^{-5} $FeCl_3$, Group F; 11.7×10^{-5} $FeCl_3$. (Arrows point the harvest times).

Results

Growth

Experimental cultures were started with the cell concentration at 0.4±0.00 x 10^6 cells/mL. Since the beginning of the experiment, it was found that the extreme $FeCl_3$ concentration inhibits the growth of *P. cruentum* (group G). The highest cell concentration was determined for Group C at 8th day as 5.39±0.46 x 10^6 cells/mL whereas group F had the lowest cell concentration as 4.29±0.15 x 10^6 cells/mL (Fig. 1). At the exponential phase (day 4), there were no significant differences between any of experiment groups ($P<0.05$). However, cell number of the experimental groups were differed by the beginning of decreasing growth phase (day 7) ($P<0.05$). Cell number of Group F was significantly lowest group at the stationary (day 10) and death (day 13) phases ($P<0.05$). Group B and E were the groups reached to the highest cell numbers among the all groups in both culture phases (stationary and death) and were significantly different than groups A and F ($P<0.05$).

Pigments

Chlorophyll *a* values were significantly different for each growth phases ($P<0.05$). At the exponential phase, the significant difference was identified between groups A and C ($P<0.05$). Groups E and F were accumulated the highest amount of chlorophyll *a* at the decreasing growth phase. At the death phase, negative control group (A) was reached to the highest chlorophyll *a* amount among all groups as 2.93±0.06 µg/mL, and it was followed by group B (2.84±0.05 µg/mL) (Fig. 2).

Group A was contained the lowest chlorophyll *a* amount per cell at exponential phase as 0.68±0.05 while group F was contains the highest amount as 0.80±0.08 pg/cell. However the effect of using $FeCl_3$

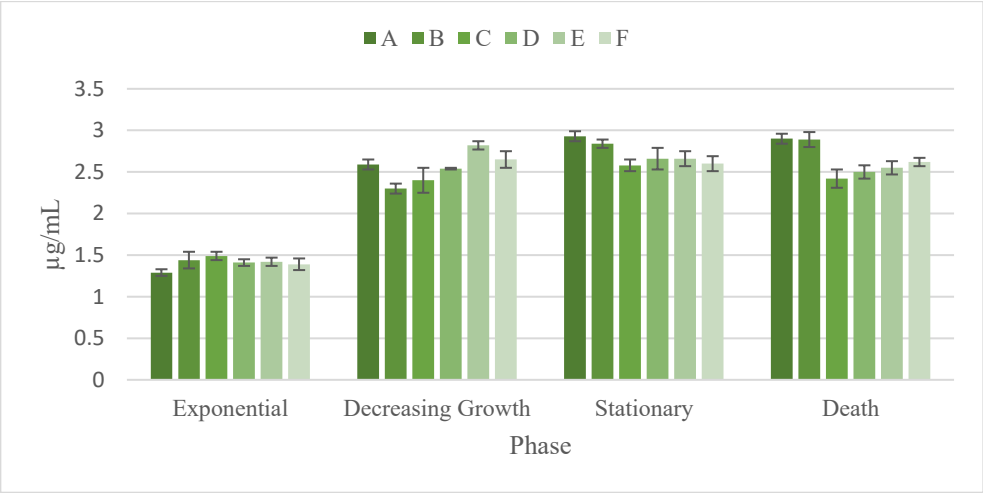

Fig. 2 Chlorophyll *a* amounts of *P. cruentum* cultivated by using various $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234 × 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$.

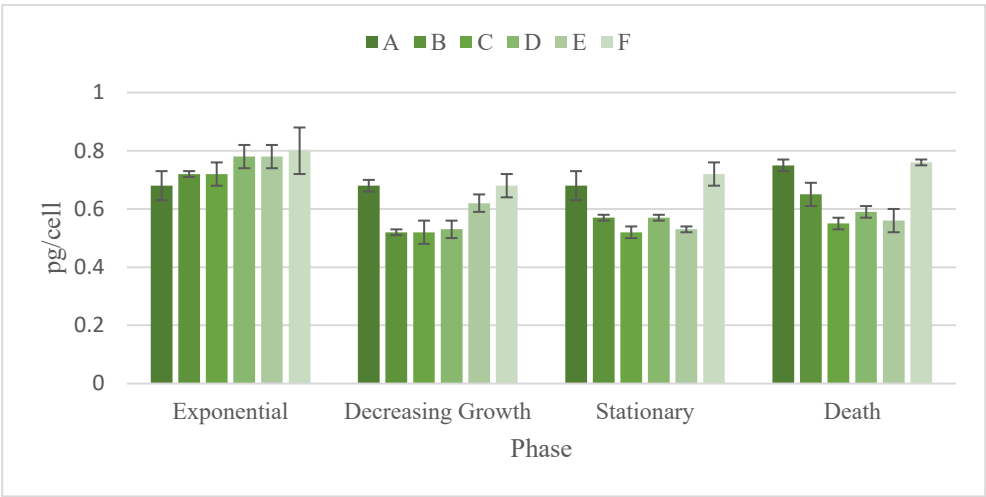

Fig. 3 Chlorophyll *a* amounts per cell of *P. cruentum* at different $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234 × 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$.

was not significant. Decrease in cellular chlorophyll *a* accumulation were found at the decreasing growth phase for all groups except group A. However, chlorophyll *a* per cell of group B, C and D were significantly lower than group A, E and F at that phase ($P<0.05$). At stationary and death phases, group A and F samples were containing significantly higher chlorophyll *a* than the other groups ($P<0.05$). At the death phase, chlorophyll *a* per cell amounts of group A and F were found as 0.75±0.02 and 0.76±0.01 pg/cell, respectively (Fig. 3).

Amounts of the carotenoids were varied between 0.35±0.01-0.41±0.02 µg/mL at the exponential phase. Group B and C samples were contained significantly higher amounts of carotenoids than groups A, D and E at this phase ($P<0.05$). During decreasing growth and stationary phases, carotenoid concentrations were increased for all groups. Group A was the highest carotenoids containing group at the stationary and the death phases. (Fig. 4). Negative control group was accumulated significantly higher value of carotenoids only than group F at the stationary phase, however was accumulated higher amount of carotenoids than groups C, D, E and F at the death phase, statistically ($P<0.05$).

Carotenoids per cell amounts were not significantly differed at the exponential phase between the groups. Cellular carotenoids accumulation was decreased during the decreasing growth phase for all groups (Fig. 5). At this phase, cells of group F were containing the highest carotenoids ($P<0.05$). Also, group A and F samples were accumulated higher amounts of carotenoids per cell than other groups at the stationary

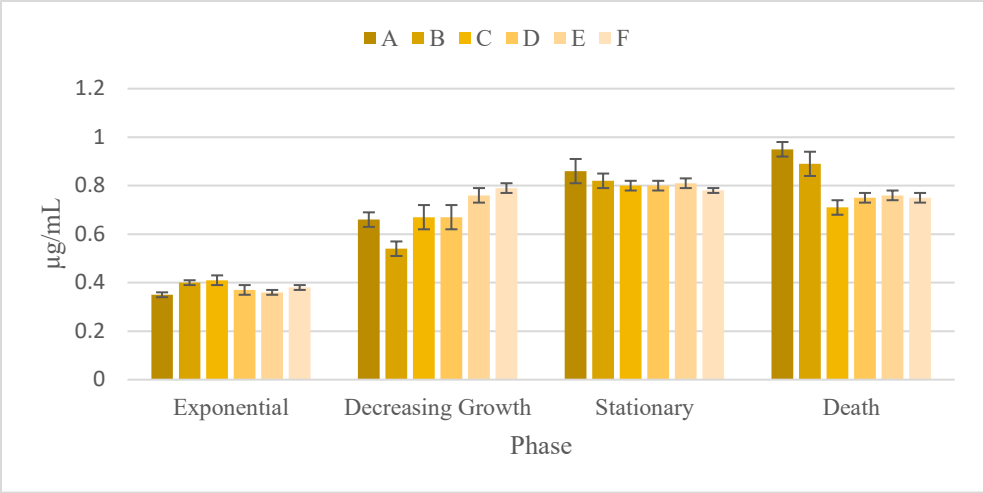

Fig. 4 Carotenoids amounts of *P. cruentum* at different $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234 x 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$.

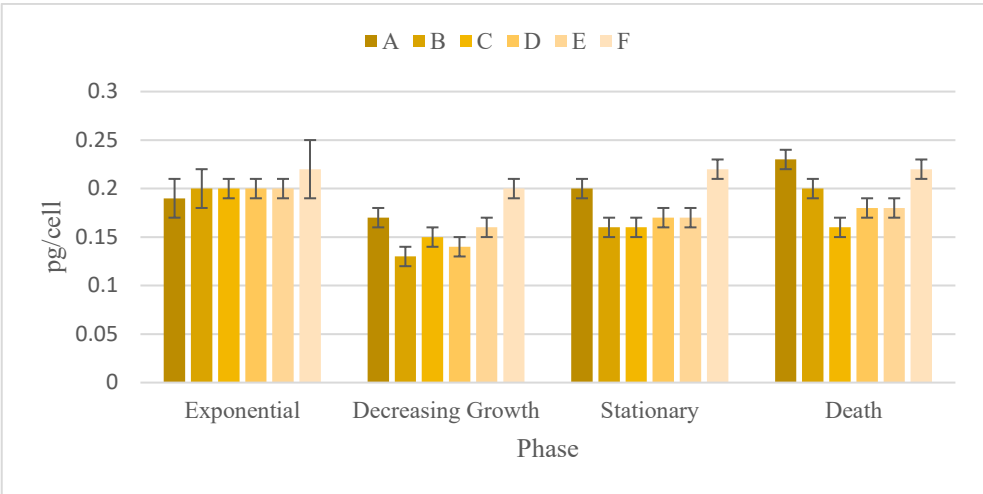

Fig. 5 Carotenoids amounts per cell of *P. cruentum* at different $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234 x 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$.

and the death phases ($P<0.05$).

Phycoerythrin contents of all groups were at the highest level at decreasing growth phase. Decreasing trend of phycoerythrin content was started after the decreasing growth phase for all groups. The lowest phycoerythrin amounts were accumulated by Group D at all growth phases (Fig. 6). Higher phycoerythrin contents were found in group A, B and F at all growth phases except exponential phase.

Phycoerythrin per cell amounts were varied depending on the sample group for each culture phase. At the exponential phase, cells of group F were accumulated highest amount of phycoerythrin (8.46±0.07 pg/cell). Cellular phycoerythrin accumulation was decreased for all groups after the exponential phase. Group A and F were accumulated highest amount of phycoerythrin per cell at the decreasing growth phase, while group C, D and E had lower phycoerythrin per cell amounts than the other groups at the decreasing growth and stationary phase ($P<0.05$). Cellular phycoerythrin contents of group F and A samples were higher than the others at the death phase and were found as 5.99±0.17 pg/cell and 5.45±0.14 pg/cell, respectively (Fig. 7).

In this study, phycobiliproteins are expressed as the sum of PE (phycoerythrin), R-PC (R-phycocyanin) and A-PC (allo-phycocyanin). Highest phycobiliprotein accumulation was determined at the decreasing growth phase. In this phase, phycobiliprotein contents of samples of group C, E, B and D

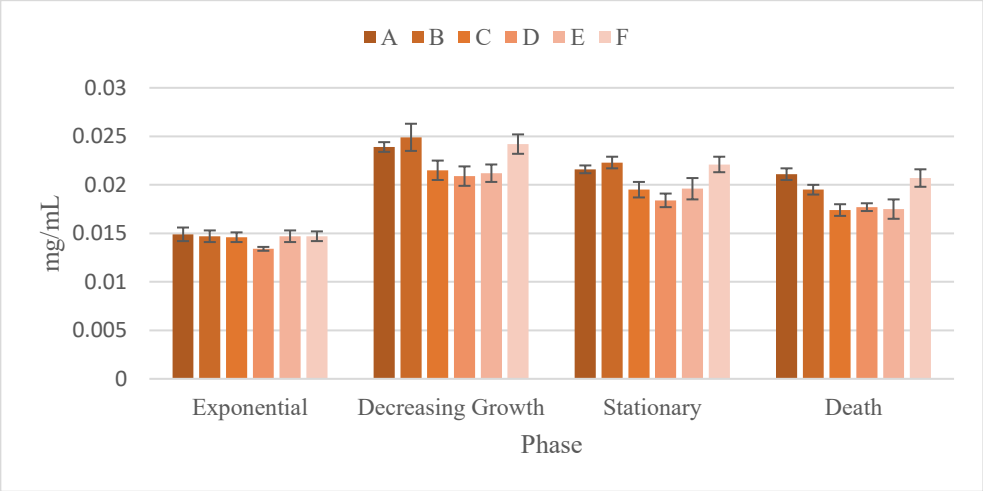

Fig. 6 Phycoerythrin amounts of *P. cruentum* at different $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234 x 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$.

were 0.0319±0.0006 mg/mL, 0.0311±0.0017 mg/mL, 0.0310±0.0015 mg/mL and 0.0310±0.0015 mg/mL, respectively (Fig. 8). However, phycobiliprotein amounts of samples were not significantly different except exponential phase ($P<0.05$).

Cellular phycobiliprotein accumulation of group F was determined as 10.68±0.92 pg/cell which was the highest value for the exponential phase, whereas Group C samples (9.07±0.38 pg/cell) had the lowest phycobiliprotein per cell content for this phase. For each sample of groups, the lowest phycobiliprotein values were determined at the stationary phase. Phycobiliprotein per cell values of group C, B and E were 5.53±0.23 pg/cell, 5.61±0.22 pg/cell and 5.71±0.19 pg/cell at that phase, respectively (Fig. 9). Group F was accumulated highest phycobiliprotein per cell among all groups at both the stationary and death phases, significantly. ($P<0.05$).

PE/PB (phycoerythrin/phycobiliproteins) ratios of P. cruentum biomass were affected by different $FeCl_3$ concentrations in the culture medium. Highest PE/PB ratio at the exponential phase was 0.807±0.020 (group A) and it was followed by group F with 0.792±0.016. However, there was no significant difference at this phase ($P>0.05$). At the decreasing growth phase, group C and D samples had the lowest PE/PB ratio values. Also, PE/PB ratios of group A, B and F samples were significantly higher at the decreasing growth and stationary phases. PE/PB values of Group F was the highest at the decreasing growth (0.750±0.005) and stationary phases (0.821±0.014) (Table 2). However, PE/PB ratio of group A was significantly higher than the other experiment groups at the death phase ($P<0.05$).

Discussion

Growth

The highest cell number of the experiment groups was determined as 5.39±0.46 cells/mL (group C). According to results of this study, 28% increase in cell number was obtained by using 0.585 x 10^{-5} M $FeCl_3$ in growth medium. Negative control (group A) and the highest concentration of $FeCl_3$ used group (F) had lower cell densities at the decreasing growth, stationary and death phases. Furhermore, our results show that, 50% $FeCl_3$ concentration (group C) in *P. cruentum* cultivation provides higher cell density than other concentrations. The effects of ferric compounds in growth medium for various microalgae have been studied for several years. In a previous study conducted for 12 years based on fluorescence measurements, the regulative role on phytoplankton biomass in both HNLC (high-nitrate low-chlorophyll) and oligotrophic water near the equator and further south of iron was determined (Behrenfeld et al. 2006). Effects of using $FeCl_3$ and ferric EDTA as iron sources on lipid accumulation and biomass productivity was investigated on marine *Chlorella* strain under laboratory conditions. Final cell density

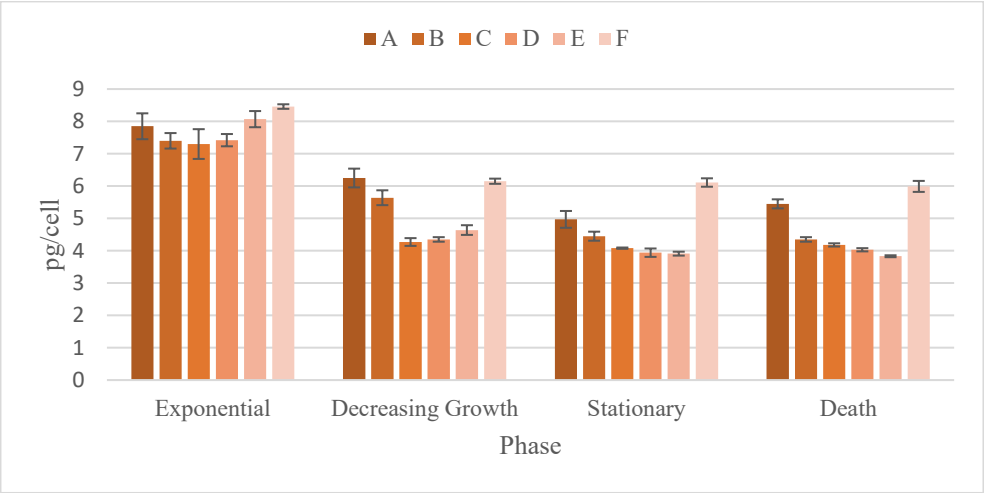

Fig. 7 Phycoerythrin amounts per cell of *P. cruentum* at different $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234 × 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$.

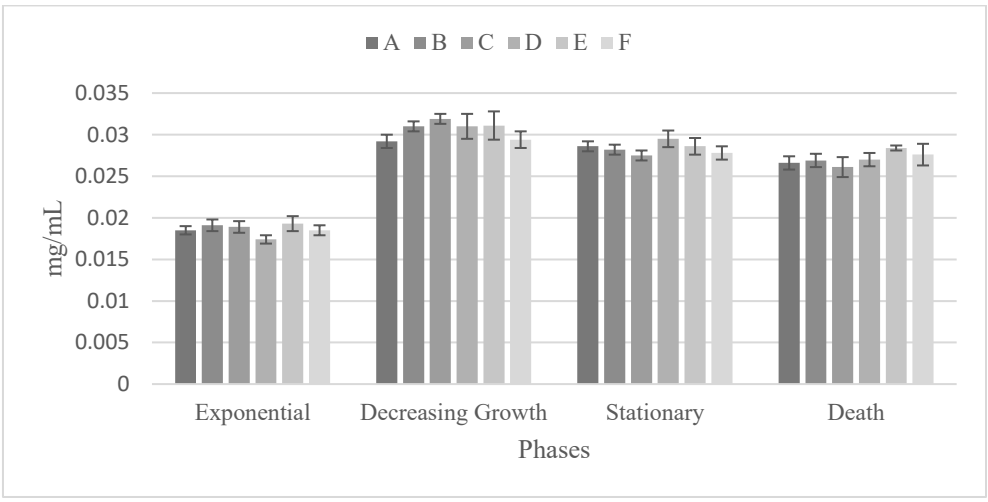

Fig. 8 Phycobiliprotein amounts of *P. cruentum* at different $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234 × 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$.

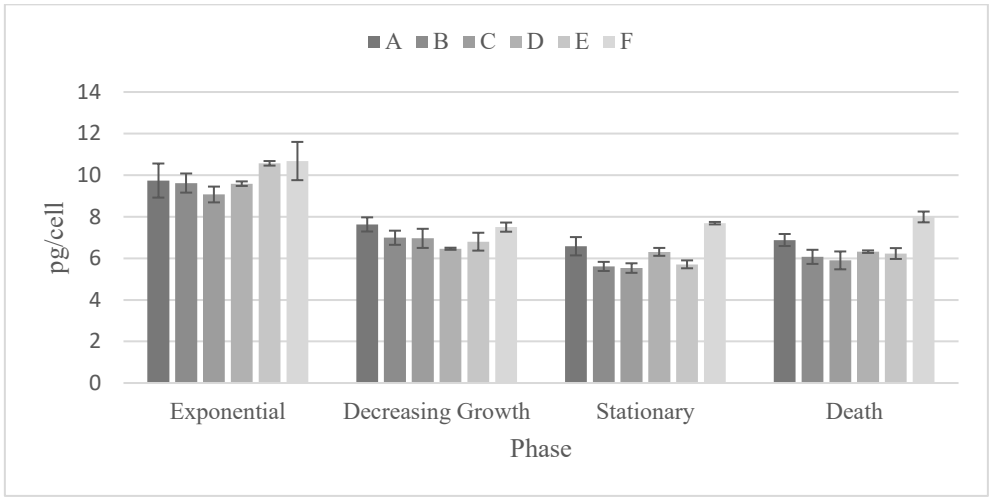

Fig. 9 Phycobiliprotein amounts per cell of *P. cruentum* at different $FeCl_3$ concentrations. Group A; 0.00 $FeCl_3$, Group B; 0.234 × 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$.

Table 2. PE/PB ratios of *P. cruentum* at different $FeCl_3$ concentrations

Group/Phase	A	B	C	D	E	F
Exponential	0.807±0.020[a]	0.771±0.018[a]	0.775±0.017[a]	0.774±0.02[a]	0.764±0.018[a]	0.792±0.016[a]
Decreasing growth	0.819±0.009[a]	0.806±0.005[a]	0.673±0.019[b]	0.674±0.009[b]	0.682±0.028[b]	0.821±0.014[a]
Stationary	0.755±0.007[a]	0.793±0.011[a]	0.709±0.028[b]	0.624±0.004[c]	0.684±0.020[b]	0.795±0.010[a]
Death	0.793±0.005[a]	0.718±0.015[b]	0.668±0.008[c]	0.638±0.005[cd]	0.615±0.029[d]	0.750±0.005[b]

*Molar $FeCl_3$ concentrations of Group A; 0.00 $FeCl_3$, Group B; 0.234 × 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$. (Letters show the statistical difference between groups, P<0.05).

Table 3. Total pigment accumulation amounts (µg/mL) of *P. cruentum* at different $FeCl_3$ concentrations

Group/Phase	A	B	C	D	E	F
Exponential	20.12±0.46	20.90±0.46	20.76±0.68	19.17±0.53	21.07±0.90	20.30±0.49
Decreasing growth	32.39±0.86	33.83±1.52	34.97±0.48	34.21±1.52	34.70±0.70	32.87±1.06
Stationary	32.41±0.48	31.84±0.36	30.87±0.70	32.97±1.09	32.06±1.01	31.16±0.81
Death	30.39±0.73	30.69±0.71	29.23±1.12	30.20±0.78	31.78±0.20	30.97±1.29

*Group A; 0.00 $FeCl_3$, Group B; 0.234 × 10^{-5} $FeCl_3$, Group C; 0.585 × 10^{-5} $FeCl_3$, Group D; 1.17 ×10^{-5} $FeCl_3$, Group E; 2.34 × 10^{-5} $FeCl_3$, Group F; 11.7 × 10^{-5} $FeCl_3$. (Letters show the statistical difference between groups, P<0.05).

was increased when chelated Fe^{3+} was added to the culture medium at late exponential growth phase, total lipid content was also increased, when cells were re-inoculated into a new medium containing high level of iron concentration (Liu et al. 2008). The key role of iron in electron transport in photosynthesis is already indicated (Peltier et al. 2010; Wan et al. 2014). However, it is also known that cyanobacteria and some algae species are able to substitute flavodoxin for ferredoxin under stress conditions such as iron limitation (Erdner et al. 1999; Tognetti et al. 2006; Karlusich and Carillo 2017). Ferredoxin replacement ability may help to explain growth of *P. cruentum* under ferric chloride deficiency. It was also stated that ferredoxin is more efficient than flavodoxin in reactions such as $NADP^+$ photoreduction (Tognetti et al. 2006). However, significant difference of cell numbers between low concentration and negative control group could be explained with this phenomenon.

Pigments

In this study, it was shown that higher cell numbers were not an indicator for pigment accumulation. Group A was the 2nd richest group of chlorophyll *a* per cell after group F. That might be the result of stress caused by absence and high concentration of $FeCl_3$. In metabolic processes such as chlorophyll synthesis, photosynthetic electron transport, respiration, nitrate reduction, nitrogen fixation processes etc., iron is needed an essential micronutrient for the growth of phytoplankton (Alexova et al. 2011; Encarnação et al. 2012; Wang et al. 2013). Also, decrease of chlorophyll *a* amount under iron deficiency had been observed in various studies (Vassiliev 1995; Henley and Yin, 1998; Desquilbet et al. 2003). Fan et al. (2014) reported that iron deficiency in *Chlorella pyrenoidosa* caused a decrease in chlorophyll content from 27 to 21.8 mg/g. In another study, the chlorophyll *a* content of *Rhodella violacea* at the exponential phase was decreased from 6.6±0.08 pg/cell to 0.74 pg/cell as a result of using lower $FeCl_3$ concentration for cultivation (Desquilbet et al. 2003). However, this effect was not observed in the present study. Chlorophyll *a* per cell amounts were varied from 0.68±0.05 pg/cell to 0.80±0.08 pg/cell between sample groups at the exponential phase. In our study, higher $FeCl_3$ group (F) was accumulated higher chlorophyll *a* but there was no dramatic decrease under iron deficiency as highlighted by Desquilbet et al. (2003). According to these findings, microalgae species should be considered as factor for each cultivation condition.

There was not significant effect of using $FeCl_3$ on carotenoids content of samples at the exponential phase. After the decreasing growth phase, carotenoids of group F was started to decrease while there was an increase in same parameter for sample groups. At the death phase, negative control and low $FeCl_3$ concentration groups (A and B) were reached to the highest amounts of carotenoids. However, highest carotenoids per cell numbers were obtained at the death phase from group A and F. Cellular carotenoids amount of group F was not changed between culture phases significantly (P<0.05). $FeCl_3$ stress might be possible reason of this result. It is stated in various studies that nutrient stress may induce accumulation of pigments

of microalgae at higher amounts (Ravi et al. 2012; Encarnação et al. 2012; Saha et al. 2013).

Phycoerythrin is the most valuable and major pigment of *P. cruentum*. Highest amount of phycoerythrin was determined in groups A, B and F. All experiment groups were containing higher amount of phycoerythrin at the decreasing growth phase, while cellular phycoerythrin amount of groups was higher at the exponential phase. Highest phycoerythrin accumulation per cell was obtained from group F, A and B, respectively. It can be assumed that lack of $FeCl_3$ or its use at high concentrations induces the accumulation of phycoerythrin. Variations on culture medium and environmental conditions affect the growth and the biochemical composition of microalgae.

Opposite results were taken from total phycobiliprotein amounts. Group C, D and E were the richest groups in terms of total phycobiliprotein amounts. The higher amounts of R-PC and A-PC accumulation might be the reason for these results. Phycobiliprotein amount per cell was at the highest level for all groups at the exponential phase. Moreover the lowest level of cellular phycobiliprotein amounts were found in the stationary phase for all groups except group F. Cellular phycobiliprotein accumulation was not changed significantly at different growth phases for group F. The difference of phycobiliprotein amount per cell was not statistically significant for the rest of the experiment groups, and cellular amounts were decreased after the exponential phase. That decrease could be the result of reproduction, which may prevent the accumulation of phycobiliproteins. However, cellular phycobiliprotein accumulation was increased at the death phase. According to the results, lack of $FeCl_3$ and its excessive concentration was increased PE/PB ratio.

In a previous study, Velea et al. (2011) determined that 2g/L $NaHCO_3$ addition to culture medium under low light intensity on *Porphyridium purpureum* culture was increased growth, biomass and phycoerythrin accumulation. It is indicated that the nitrate concentration directly affects phycoerythrin accumulation of *Porphyridium purpureum* under the high light intensities (Sosa-Hernández et al. 2019). Moreover, the deficiency causes decrease in cell number of *Porphyridium marinum*, however, the higher amounts of phycoerythrin accumulation was being induced (Gargouch et al. 2018). Effects of sodium chloride, sodium nitrate, magnesium sulfate and dipotassium hydrogen phosphate amounts in culture medium on growth rate and phycoerythrin accumulation of *Porphyridium purpureum* were also reported (Kathiresan et al. 2007). Clearly, the culture medium substances and their concentrations affect the growth and biochemical composition of *Porphyridium* species.

P. cruentum biomass contains high amounts of phycobiliproteins but also chlorophyll *a* and carotenoids. At the decreasing growth phase, all experiment groups were accumulated highest amount of total pigments. Samples of group C, E and D accumulated 34.97±0.48 µg/mL, 34.70±0.70 µg/mL and 34.21±1.52 µg/mL total pigment in this phase, respectively. A decrease trend of total pigment accumulation was determined for all experiment groups at the stationary and death phases (Table 3).

Conclusions

According to the results, total pigment accumulations of experiment groups were similar, but were showed diversity by ratios of pigments. Lack of iron source and high $FeCl_3$ concentration in growth medium were induced *P. cruentum* cells to accumulate higher amounts of pigments according to results of this study. However, cell numbers were significantly lower at these conditions. It is found that concentration of ferric chloride was directly affected the cell numbers of the experimental groups.

This and previous studies show that the growth of *P. cruentum* and the accumulation of phycoerythrin are affected by the presence and concentrations of substances in the culture medium. Use of such stresses can help produce more pigments, particularly phycoerythrin, once the cultures have reached the desired density. As a result, $FeCl_3$ can be used as a potential induction parameter for *P. cruentum* pigment accumulation.

Declaration of author's contributions We hereby declare that all authors worked together in this work and participated in data analyses, discussion of results, writing and revising the manuscript. All authors have agreed to the authorship and submission of the manuscript to the journal for peer review.

Conflict of interest The authors declare no conflict of interest. This manuscript which study microalgae culture does not need an ethical approval.

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