Factors that influence the accumulation of fatty acids in Prorocentrum micans

Carla Aguilar[1], Hanna Hernandéz[2], Lennin Ramos[2], Anthony Soto[2], Roberta Soares[3], Carlos Yure B. Oliveira[4], Jéssika Lima de Abreu Abreu[3], and Alfredo Gálvez[5]

[1]Instituto del Mar del Perú
[2]Instituto del Mar del Peru
[3]Universidade Federal Rural de Pernambuco
[4]Universidade Federal de Santa Catarina
[5]UFRPE

April 19, 2024

Abstract

Microalgae, historically relevant to aquaculture sector, can effectively contribute to the sustainability in several production chain. The physical and chemical environmental factors play a direct or indirect role in shaping the composition of their intracellular compounds of these organisms. Among these compounds, fatty acids are particularly noteworthy, with various species harboring an extensive array of them. Notably, the marine dinoflagellate Prorocentrum micans excels in the production of essential polyunsaturated fatty acids (PUFA), including DHA and EPA. The objective was to determine which factors contribute to the accumulation of the PUFA in a P. micans strain. A factorial design of 3x3 composed by the following factors: illumination (5, 12.5, 20 µmol photons m-2 s-1), nitrate dose (0, 75, 150 mg ml-1) and inoculum volume (50, 125, 200 ml) was examined to determine the better growth conditions for P. micans. Furthermore, lipid profile and the fatty acids profile were analyzed at the end of the cultivation. The results determined that, maximum averages of DHA (20%) and EPA (1%), in the biomass, were registered in cultures without nitrate, low irradiance (5 µmol photons m-2 s-1) and high volume of inoculum (200 ml). The maximum cell concentration recorded was 3.1×104 cells ml-1, at the end of the culture. So, it can be concluded that the evaluated strain is easily adapted to the culture flow and, the culture conditions to which it was subjected allows its biomass to be considered an interesting alternative as lipid input in aquaculture activity.

Factors that contribute to the accumulation of fatty acids in*Prorocentrum micans*: a novel source of lipids for aquaculture

Carla Aguilar S[1], Hanna Hernández A[1], Lennin Flores R[2], Anthony Ruiz S[2], Roberta Borda Soares[3], Carlos Yure B. Oliveira[4], Jéssika Lima de Abreu[3*] and Alfredo Olivera Gálvez[3]

1 Germplasm Bank of Aquatic Organisms, Directorate of Aquaculture Research, Instituto del Mar del Perú, Peru

2 Instrumental Analysis Laboratory, Directorate of Aquaculture Research, Instituto del Mar del Perú, Peru

3 Fisheries and Aquaculture Department, Federal Rural University of Pernambuco, Brazil

4 Botany Department, Center for Biological Sciences, Federal University of Santa Catarina, Brazil

List of abbreviations

PUFA - polyunsaturated fatty acids

DHA - C22:6n-3 docosahexaenoic acid

EPA- C20:5n-3 eicosapentaenoic acid

FAMEs - fatty acid methyl ester

BGOA - Aquatic Organisms Germplasm Bank

mg ml^{-1} - milligram per milliliter

ng ml^{-1} - nanogram per milliliter

pg ml^{-1} - picogram per milliliter

µmol – micromole

*Corresponding author: Jéssika Lima de Abreu

Federal Rural University of Pernambuco, Department of Fisheries and Aquaculture, St. Rua Dom Manuel de Medeiros, s/n - Dois Irmãos, Recife - PE, Zip code: 52171-900

Tel.: +55 87 98581 3442

E-mail address: jessik.labreu@gmail.com

Abstract

Microalgae, historically relevant to aquaculture sector, can effectively contribute to the sustainability in several production chain. The physical and chemical environmental factors play a direct or indirect role in shaping the composition of their intracellular compounds of these organisms. Among these compounds, fatty acids are particularly noteworthy, with various species harboring an extensive array of them. Notably, the marine dinoflagellate *Prorocentrum micans* excels in the production of essential polyunsaturated fatty acids (PUFA), including DHA and EPA. The objective was to determine which factors contribute to the accumulation of the PUFA in a *P. micans* strain. A factorial design of 3x3 composed by the following factors: illumination (5, 12.5, 20 µmol photons $m^{-2}s^{-1}$), nitrate dose (0, 75, 150 mg ml^{-1}) and inoculum volume (50, 125, 200 ml) was examined to determine the better growth conditions for *P. micans* . Furthermore, lipid profile and the fatty acids profile were analyzed at the end of the cultivation. The results determined that, maximum averages of DHA (20%) and EPA (1%), in the biomass, were registered in cultures without nitrate, low irradiance (5 µmol photons m^{-2} s^{-1}) and high volume of inoculum (200 ml). The maximum cell concentration recorded was 3.1×10^4 cells ml^{-1}, at the end of the culture. So, it can be concluded that the evaluated strain is easily adapted to the culture flow and, the culture conditions to which it was subjected allows its biomass to be considered an interesting alternative as lipid input in aquaculture activity.

Keyword : *P. micans* DHA, EPA, nitrate

Introduction

Due to the global changes that humanity faces to maintain, protect and improve food security, aquaculture has several challenges to overcome, mainly sustainability, biosecurity, regulatory structures in accordance with ocean ecosystems and food supplies, since these charge greater relevance due to the nutritional level they perform, especially as unique and extremely diverse providers of omega-3 fatty acids and essential bioavailable micronutrients (MUSTAFA and SHAPAWI, 2015; FAO 2022). In the aquatic environment, microalgae are the base of the trophic chain, capable of accumulating a series of active compounds, among which fatty acids stand out, especially polyunsaturated fatty acids (PUFAs). important for the development and reproduction of zooplankton organisms, seeds and other secondary consumers in their initial levels of development (IKAWA, 2004; HERNÁNDEZ, 2016; GONÇALVEZ et al., 2017; REMMENS et al., 2018; PELTOMA et al., 2019; OLIVEIRA et al., 2022).

The importance of microalgae in the diet of the larval stages of various aquatic organisms is mainly due to the presence of polyunsaturated fatty acids such as; C22:6n-3 docosahexaenoic acid (DHA) and C20:5n-

3 eicosapentaenoic acid (EPA) that are synthesized and transferred through the food chain ingested by secondary consumers, as they play a crucial role in development neural, tissue formation, physiological functions, cell walls and energy storage of the latter, it is due to this, that various studies refer to a connection between the nutritional value of microalgae and their lipid profile (ADAME et al., 2012; GONCALVEZ et al., 2017; RAJA et al., 2018; PELTOMA et al., 2019).

With the development of the aquaculture industry and the demand that this implies, in terms of quality and quantity of organisms produced in closed or open spaces, inputs rich in lipid compounds are increasingly scarce or are replaced by alternative products that lack the necessary nutrients for a balanced feed of aquatic organisms (PELTOMA et al., 2019). In this context and given that microalgae contain interesting lipid profiles, they are positioned as a potential resource so that, through mass cultivation processes, biomass or extracts can be obtained, as raw material or biochemically adequate input for feeding cultivable organisms, also considering that microalgal productivity does not interfere with food safety and favors the environment (ATKINSON, 2013; FU et al., 2017; LORENZEN et al., 2017; REMMENS et al., 2018; PELTOMA, 2019).

The microalgae with considerable lipid profiles are those of the group of diatoms and dinoflagellates, the first of which is characterized by accumulating EPA and the second DHA in a greater proportion compared to other fatty acids (SEXTON and LOMAS 2018; ZULU et al., 2018; PELTOMA et al., 2019), both groups are widely distributed in the aquatic environment and even when some of them face certain factors such as; temperature, irradiance, salinity tend to proliferate forming algal blooms, only dinoflagellates can adapt very well due to their mixotrophic or heterotrophic condition to sudden changes, losing their inhibitory capacity on the development of other organisms (LEE et al., 2017; ABD et al. ., 2022).

Within the group of dinoflagellates, *Prorocentrum micans*turns out to adapt quite easily to culture conditions, it stands out for its high content of DHA over other lipid components, which makes it a potential resource whose biomass would be an interesting input or raw material in the production of aquaculture products (KATTNER and BROCKMANN, 1978; SUH et al., 2015; HERNANDEZ et al., 2019).

In culture, *P. micans* is a species whose growth is mainly impacted by thermal and light variation, as well as by nutrient levels, especially nitrates (LEE et al., 2017; REMMENS et al., 2018). The variations of these factors determine the lipid profile of the species; among the fatty acids that can be recorded and are related to algal bloom events are considered; octadecapentaenoic acid C18:5n3 (OPA) and octadecatetraenoic acid C18:4n3 (ODTA), the latter quite toxic (IKAWA, 2004).

Therefore, to explore and evaluate the factors that contribute to the accumulation of fatty acids of interest in the aquaculture industry in*P. micans* and minimize or inhibit toxic substances, light intensity and nitrate content should be taken into account, given which are critical factors that affect the photosynthesis of these algal organisms, also considering that the toxic activity of PUFAs may be related to photooxidation (IKAWA, 2004; LEE et al., 2017; REMMENS et al., 2018).

In this sense, the objective of the present study was to evaluate the culture conditions in a controlled environment that contribute to the accumulation of fatty acids with emphasis on DHA and EPA in the marine dinoflagellate *P. micans* strain IMP-BG-036, with the purpose of that is considered a potential input for the preparation of enrichments or other products that are required in the aquaculture activity. It is necessary to mention that the afore mentioned strain was evaluated and selected from a set of strains of the same species isolated from different places on the Peruvian coast and was the one that best adapted to the culture flow with high levels of various fatty acids, among which stand out the DHA.

Material and methods

2.1 Culture conditions

The biological material was the IMP-BG-036 strain, corresponding to the marine dinoflagellate *P. micans* , which is part of the strain of the Aquatic Organisms Germplasm Bank (BGOA). The cultivation was of the batch type, for 11 days, enriched with medium FL, arranged in 250ml glass flaks and in triplicate, submitted

to a temperature of 18degC, with photoperiod 12 h, according to experimental design factors detailed in Table 1.

[Table 1]

The data generated was subjected to a complete factorial experiment 23, with 3 replicates and 3 central points, completely randomized, the treatments were shown in table 2.

[Table 2]

2.2 Determination of growth characteristics

The evaluation of cell density was carried out using cell counts, after a period of 11 days of triplicate culture, in a Sedgwick-Rafter chamber, extracting a 2 ml culture sample set in lugol at 4%.

2.3 Determination of lipid content

The lipid profile was determined using the Folch method (1957). 50 ml of sample was centrifuged for analysis, then 3 ml of chloroform: methanol (1:1) was added to perform lipid extraction. A Branson 2510 ultrasonic bath was used to enhance lipid extraction, the chloroform:lipid phase was separated with ultrapure water and evaporated with nitrogen gas. The result was expressed as weight percentage.

2.4 Determination of fatty acids

The derivatization of these compounds was carried out using the method of Ichihara and Fukubayashi (2010). 0.2ml toluene, 1.5ml methanol and a mixture of 1ml concentrated hydrochloric acid with 4.3ml methanol were added to the extracted lipids and the mixture was incubated at 45degC, then FAMEs were extracted with 1.5ml hexane and 1ml of water. Varian CP-3800 Gas Chromatograph conditions were as follows (Table 3).

[Table 3]

Statistical analysis

For the evaluation of the data obtained, an analysis of variance (ANOVA) and Tukey's post hoc test will be carried out to evaluate the value of significance (α=0.05). Likewise, for the relationship of the lipid profile and fatty acids against the crops, a design of significant effects and graphs (Pareto) will be carried out in the statistical program Minitab 16.

Results

3.1 Growth characteristics of the P. micans strain IMP-BG-036

Figure 1 shows that the cell concentration was affected by all the factors as a whole: the volume of the inoculum, the lighting on the culture and the dose of nitrate in the medium, without there being a single selective factor. Although the standardized effects of the Pareto Diagram show that the factor with the greatest impact was the volume of the inoculum, the interaction between it and the lighting, as well as the dose of nitrate and lighting allowed the recording of the maximum cell concentrations at the end of the culture period on day 11. This result ($3.1x10^4$ cel.ml^{-1}), was obtained with a lighting of 5 mol.m^{-2}.sec^{-1}, an inoculum volume of 200 ml and without nitrate dose. in the culture medium (Table 4).

[Figure 1]

[Table 4]

One aspect observed during the counting process and not considered in the design was the number of empty shells found; with higher incidence in crops whose inoculum was 200 mL. In those with a smaller volume of inoculum (50 and 125 ml), the medium was more diluted, and the valves found were very few.

3.2 Content and determination of fatty acids

The experimental design allowed evaluating 27 combinations with the 3 factors considered: lighting, nitrate dose and inoculum volume. The result allowed recording 12 fatty acids for this strain, among which are distinguished; 3 saturated fatty acids (C14:0 myristic, C16:0 palmitic and C18:0 stearic); 3 monounsaturated fatty acids (Palmitoleic C16:1n-7, Oleic C18:1n-9 and Vaccenic C18:1n-7) and 6 polyunsaturated fatty acids (Linoleic C18:2n-6, Linolenic C18:3n-3, Stearidonic C18:4n-3, Docosahexanoic: DHA C22:6n-3, Eicosapentaenoic: EPA C20:5n-3 and Octadecapentaenoic: OPA C18:5n-3).

The selective factor for the accumulation of DHA at the biomass level (ng ml^{-1}) was mainly the volume of the inoculum, followed by the lighting factor in interaction with the dose and the volume separately, by themselves they did not have selective representativeness (Figure 2a). On the contrary, at the cellular level (pg.cel^{-1}), the factors related to illumination and nitrate dose were selective on the accumulation of DHA, the first being the most relevant. However, the inoculum volume interacts with the other factors during the process (Figure 2b).

[Figure a, b]

Although, the interaction of the 3 factors (inoculum volume, lighting and nitrate dose) contribute to the accumulation of DHA fatty acid at the cellular level (pg.cel^{-1}). This condition is evidenced when these factors interact at their maximum levels, that is, when the cultures were exposed to a lighting of 20 µmol.m^{-2}.seg^{-1} and an inoculum volume of 200 ml respectively (Fig 3).

[Figure 3]

Figure 4a shows that the selective factor in the accumulation of EPA at the biomass level (ng ml^{-1}) was the volume of the inoculum, followed by the interaction of the lighting factor with the volume and dose of nitrate separately, realizing that, the selectivity in this case, is evidenced through the interaction of these factors. While, at the cellular level (pg ml^{-1}) there is not a single selective value that contributes to the accumulation of EPA, but all in parallel, only a joint interaction between the nitrate dose and the volume of the inoculum is evidenced (Figure 4b).

[Figure 4]

The principal components graph showed that, at the cellular level (pg cel^{-1}), the accumulation of EPA fatty acid requires a minimum inoculum volume, without nitrate doses and lighting of 20 µmol photons m^{-2} s^{-1}, (Figure 5a). The interaction of factors that was recorded for the accumulation of EPA fatty acid at the cellular level was between the volume of the inoculum (50 ml) and the dose of nitrate (without nitrate), even though the average values were of the order of 2.5 pg cel^{-1}(Figure 5b).

[Figure 5]

A fatty acid whose presence called attention because it is related to HABs was OPA and for this reason the Pareto Diagram is presented for the factors involved in its accumulation. At the biomass level (mg ml^{-1}) two selective factors are evident, lighting and the volume of the inoculum, the other two factors are an interaction of the three: Lighting and inoculum volume and lighting and nitrate dose. At the cellular level (pg cel^{-1}) the selective factor was lighting, no factor interaction was recorded (Figure 6). Both the principal component plot and the interaction plot were not determinative for this fatty acid.

[Figure 6]

During the cell count, a high incidence of empty valves was recorded in the cultures whose inoculum was 200 ml. This condition may have been related to the low levels of oil concentration at the cell level and high concentrations at the biomass level. On the other hand, the cultures with 50 ml inoculum did not present the presence of empty valves, they were more diluted and exposed to a maximum illumination of 20 µmol photons m^{-2} s^{-1} and a double dose of nitrate.

Of the 12 fatty acids registered for the IMP-BG-036 strain of *P. micans* , 11 reached their maximum average values, in the nitrate-free medium, independent of lighting and inoculum volume, these were in descending

order; OPA (1783 ng ml^{-1}), Palmitic (1367 ng ml^{-1}), DHA (1162 ng ml^{-1}), Steridonic (647 ng ml^{-1}), Vaccenic (472 ng ml^{-1}), Myristic (133 ng ml^{-1}), Linolenic (95 ng ml^{-1}), Stearic (84 mg ml^{-1}), Palmitoleic (73 ng ml^{-1}), EPA (71 ng ml^{-1}) and Linoleic (70 ng ml^{-1}). The only one that registered its highest average value in the medium with double dose of nitrate, minimum lighting factor and inoculum volume was the Oleic (103 ng ml^{-1}).

Given the interest in relevant fatty acids in aquaculture products, the biomass level is compared with the content at the cellular level only of DHA and EPA; where it is observed that, in the medium lacking nitrate, the maximum average values at the biomass level are recorded when; lighting was minimal and the inoculum volume was maximum, while the maximum mean values at the level of cell content were recorded when the lighting factor was maximum independent of the inoculum volume factor (Table 5).

[Table 5]

The lipid variety for the *P. micans* strain IMP-BG-036 recorded in table 6 shows that, when the nitrate dose was doubled, it did not exceed the average concentrations recorded in Table 5, in neither of the two levels evaluated. biomass and cell. However, the pattern that the highest values are shown in low lighting conditions is maintained, despite the fact that the nitrate dose was double, taking the L1 culture medium as reference.

[Table 6]

The results recorded in Table 7, where the factors were intermediate, did not exceed the values recorded in Table 6.

[Table 7]

Discussion

The dinoflagellate *P. micans* is one of the many microalgae that, due to its vertical movement in the water column, can adapt to fairly wide light intensities and thus experience the absorption of a variation of nutrients in that path (JEON et al., 2010). Also, during the photosynthetic process two important factors are the light intensity and the nutrients available in the medium, which are related to the growth of all photosynthetic organisms (LEE et al., 2017). In this sense, the present study considered three factors at three levels each for the cultivation of the strain; illumination of 5, 12.5 and 20 $\mu mol.m^{-2}.seg^{-1}$; the dose of nitrate as part of the nutrients of the culture medium of 0, 75 and 150 $mg.ml^{-1}$ and the volume of the inoculum (50, 125 and 200 ml), so that some of these combinations of variables allow cells carry out the synthesis of active substances such as fatty acids more easily.

The studies by Lee et al. (2017) also recorded that, with the increase in light intensity from 10 to 200 $\mu E.m^{-2}.seg^{-1}$, in Guillard medium with silicate, the cell concentration of *P. micans* increases between 10,000 to 12,000 $cel.ml^{-1}$ and the nitrate concentration decreases from 100 uM to 1.5uM over time (between 6 to 20 days). In the present study, cell concentrations greater than 30,000 $cel.ml^{-1}$ were recorded in the medium without nitrate dose and with a light intensity of 5 $\mu mol.m^{-2}.seg^{-1}$, after 11 days of culture. Even when the light intensity was 20 $\mu mol.m^{-2}.seg^{-1}$, the average cell concentration was higher than 12,000 $cel.ml^{-1}$. From these results it can be concluded that it is necessary to evaluate the components of the medium in order to strengthen cell growth.

A relevant aspect of microalgal diversity, including dinoflagellates, is the fatty acid content that characterizes them, since they play an important role in their nutritional quality and growth, as well as in the organisms that consume them; Thus, a correlation of the lipid profile of microalgae with its nutritional value is established and, from this, its usefulness as food can be determined (LEE et al., 2017), indirectly, its use as an input for various products. During the present study, the IMP-BG-036 strain of *P. micans* recorded the presence of 12 types of fatty acids, including saturated, monounsaturated and polyunsaturated, of the latter the presence of DHA and EPA becomes important due to their relevance in aquaculture activity.

An important aspect for the accumulation of fatty acids is the lack of nitrate in the medium (REMMENS et al., 2018). Of the 12 fatty acids registered for the strain IMP-BG-036 of *P. micans* , 11 reached their maximum average values, in the nitrate-free medium, independent of lighting and inoculum volume, these were in descending order; OPA (1783 $mg.ml^{-1}$), Palmitic (1367 $mg.ml^{-1}$), DHA (1162 $ng.ml^{-1}$), Stearidonic (647 $mg.ml^{-1}$), Vaccenic (472 $mg.ml^{-1}$), Myristic (133 mg.ml-1), Linolenic (95 $mg.ml^{-1}$), Stearic (84 $mg.ml^{-1}$), Palmitoleic (73 $mg.ml^{-1}$), EPA (71 $mg.ml^{-1}$) and Linoleic (70 $mg.ml^{-1}$), the only fatty acid that was recorded in the medium with a double dose of nitrate was Oleic (103 $mg.ml^{-1}$).

There are several biomolecules that various groups of microalgae accumulate in response to the environment where they develop and the strategies to which they can be exposed (FU et al., 2017; SUN et al., 2019). After an exhaustive study on the importance of fatty acids extracted from microalgae strains for aquaculture purposes, one of the interesting species due to its lipid profiles, especially its DHA and EPA content, was *P. micans* (HERNÁNDEZ et al., 2019). Although it is an organism related to the proliferation of Harmful Algal Blooms (HABs), it is necessary to mention that its inhibitory capacity for the development of other organisms can be affected by the variation of certain factors such as lighting or some nutritional components in the environment. medium as the source of nitrates and phosphates mainly (IKAWA, 2004; LEE et al., 2017; ABD et al., 2022).

On the other hand, the studies by Peltoma et al., (2019), determined that dinoflagellates, regardless of their habitat, register concentrations between 10 to 15% of DHA and less than 5% of EPA. In the present study, even when the results did not show statistically significant differences, the average concentrations of EPA did not exceed 3% and for DHA they did not exceed 30%.

When evaluating the influence of luminosity, nitrate doses and initial concentration of the inoculum, it can be observed that the fatty acid profiles of the produced biomass did not show differences among themselves. The results indicate that the species has great potential for its use in obtaining fatty acids, regardless of the culture conditions that are subjected to it.

Acknowledgments

The authors would like to thank the National Council for Scientific and Technological Development - CNPq for the financial support (Process nº 167938/2022-3, PQ 309669/2021-9 and PQ 308063/2019-8), the Fundação de Amparo a Ciência e Tecnologia of PE - FACEPE (Process nº BFP - 0131 - 5.06/22) and finally the Foundation Coordination for the Improvement of Higher Education Personnel - Capes (Process nº 88882.436231/2019-01).

Authors' contributions

CAS: Investigation, Conceptualization, Data curation, Formal analysis, Visualization, Writing - original draft, Writing- review & editing. HHA: Investigation, Data curation, Formal analysis, Writing- review & editing. LFR: Investigation, Data curation, Formal analysis, Visualization, Writing - original draft. ARS: Formal analysis, Data Curation, Writing- review & editing; RBS: Formal analysis, Writing- review & editing. CYBO: Conceptualization, Data curation, Writing- review & editing. JLA: Data curation, Formal analysis, Writing- review & editing. AOG: Supervision, Investigation, Project administration, Visualization, Writing -review & editing.

Conflict of interest

Authors declare that there is no conflict of interest regarding the publication of this article.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

ABD, H. M. EL FATAH; DINA M. ALI; MOHAMED IBRAHIM. Seasonal dynamics and ecological drivers of *Prorocentrum Micans* Ehrenberg dinoflagellate blooms in Qarum Lake Egypt. Egyptian Journal of Aquatic Research, 2022. https://doi.org/10.1016/j.ejar.2022.07.001

ADARME VEGA T. C.; DAVID K Y LIM; MATTHEW TIMMINS; FELICITAS VERNEN; YAN LI and PEER M SCHENK. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production, Review. Microbial Cell Factories 2012, 11 – 96. http://www.microbialcellfactories.com/content/11/1/96

ATKINSON N. The potential of microalgae meals in compound feeds for aquaculture. International Aquafeed, 16 (5) 14 – 17, 2013.

FAO. 2022. El Estado Mundial de la Pesca y la Acuicultura 2022. Hacia la Transformacion Azul. https://doi.org/10.4060/cc0461es

FOLCH, J.; LEES, M; SLOANE STANLEY, G.H. A simple method for the isolation and purification of total lipides from animal tissues. The Journal of biological chemistry. 1957. 226, 497-509.

FU, W.; D.R. NELSON; Z. YI; M. XU; B. KHRAIWESH; K. JIJAKLI; A. CHAIBOONCHOE; A. ALZAHMI; D. AL-KHAIRY; S. BRYNJOLFSSON; K. SALEHI-ASHTIANI. In Studies in Natural Products Chemistry. Chapter 6: Compuestos Bioactivos de Microalgas: Bioactive Compounds From Microalgae: Current Development and Prospects, 2017. Vol. 54. page 199 – 217. http://dx.doi.org/10.1016/B978-0-444-63929-5.00006-1

GONCALVES, ANA M.M.; JOAO C. MARQUES; FERNANDO GONCALVES. Fatty Acids' Profiles of Aquatic Organisms: Revealing the Impacts of Environmental and Anthropogenic Stressors. Intech Open, Chapter 6, 2017

HERNANDEZ ACEVEDO, H.; LEENIN FLORES RAMOS; ANTHONY RUIZ SOTO. Acidos grasos en cepas de microalgas del Banco de Germoplasma de Organismos Acuaticos del Instituto del Mar del Peru (IMARPE). Revista peruana de biologia 26(3): 369 - 378 (2019) doi: http://dx.doi.org/10.15381/rpb.v26i3.15356

HERNÁNDEZ A, I. Efecto de la dieta en la composición de ácidos grasos del alimento vivo utilizado en la crianza larvaria de peces marinos. Tesis de Maestría, Centro Interdisciplinario de Ciencias Marinas - Instituto Politécnico Nacional: CICIMAR-IPN, La Paz, B.C.S, 2016.

ICHIHARA K. and FUKUBAYASHI Y. Preparation of fatty acid methyl esters for gas-liquid chromatography. J Lipid Res. 2010 Mar; 51(3):635-40.

IKAWA, MIYOSHI. Algal polyunsaturated fatty acids and effects on plankton ecology and other organisms. UNH Center for Freshwater Biology Research, 6(2): 17-44 (2004).

JEONG, HAE JIN; YEONG DU YOO; JAE SEONG KIM; KYEONG AH SEONG; NAM SEON KANG; TAE HOON KIM. Growth, Feeding and Ecological Roles of the Mixotrophic and Heterotrophic Dinoflagellates in Marine Planktonic Food Webs. Ocean Science Journal (2010) 45(2):65-91. DOI 10.1007/s12601-010-0007-2

KATTNER, G. G., and U. H. BROCKMANN. Fatty-Acid Composition Of Dissolved And Particulate Matter In Surface Films. Marine Chemistry, 6(1978) 233-241

LEE, KYUNG HA; HAE JIN JEONG; HYE JEONG KIM; AN SUK LIM. Nitrate uptake of the red tide dinoflagellate *Prorocentrum micans* measured using a nutrient repletion method: effect of light intensity. Algae 2017, 32(2): 139-153. https://doi.org/10.4490/algae.2017.32.5.20

LORENZEN J.; NADINE, I.G.L; MARLENE TIPPELT; ANDREA STEGE; FARAH QOURA; ULRICH SOHLING; THOMAS BRUCK. Extraction of microalgae derived lipids with supercritical carbon dioxide

in an industrial relevant pilot plant. Bioprocess Biosyst Eng (2017) 40:911–918. DOI 10.1007/s00449-017-1755-5

MUSTAFA S. and ROSSITA SHAPAWI. Preface In Aquaculture Ecosystems: Adaptability and Sustainability, first edition, 2015.

PELTOMA E.; HEIDI HALLFORS; SAMI J. TAIPALE. Comparison of Diatoms and Dinoflagellates from Different Habitats as Sources of PUFAs. Marine drugs 2019, 17, 23; doi:10.3390/md17040233

REMMERS I. M.; RENE H. WIJFFELS; MARIA J. BARBOSA; PACKO P. LAMERS. Review: Can We Approach Theoretical Lipid Yields in Microalgae?. Trends in Biotechnology, March 2018, Vol. 36, No. 3. https://doi.org/10.1016/j.tibtech.2017.10.020

RAJA, R.; ANA COELHO; SHANMUGAM HEMAISWARYA; PARKAVI KUMAR; ISABEL S. CARVALHO; ARUN ALAGARSAMY. Review Article: Applications of microalgal paste and powder as food and feed: An update T using text mining tool. Beni-Suef University Journal of Basic and Applied Sciences 7 (2018) 740–747. https://doi.org/10.1016/j.bjbas.2018.10.004

SEXTON JULIANNE P. and MICHAEL W. LOMAS. In Microalgae in Health and Disease Prevention, Chapter 4: Microalgal Systematics, page 73-107, 2018. https://doi.org/10.1016/B978-0-12-811405-6.00004-9.

SUN, X. M; LU-JING REN; QUAN-YU ZHAO; LI-HUI ZHANG; HE HUANG. Application of chemicals for enhancing lipid production in microalgae-a short review. Bioresource Technology 293 (2019) 122135. https://doi.org/10.1016/j.biortech.2019.122135

SUH, SUNG-SUK; SO JUNG KIM; JINIK HWANG; MIRYE PARK; TAEK-KYUN LEE; EUI-JOON KIL; SUKCHAN LEE. 2016. Fatty acid methyl ester profiles and nutritive values of 20 marine microalgae in Korea. Asian Pacific Journal of Tropical Medicine (2016) 191-196. http://dx.doi.org/10.1016/S1995-7645(14)60313-8.

ZULU N. N.; KRZYSZTOF ZIENKIEWICZ; KATHARINA VOLLHEYDE; IVO FEUSSNER. Current trends to comprehend lipid metabolism in diatoms, Review. Progress in Lipid Research 70 (2018) 1–16. https://doi.org/10.1016/j.plipres.2018.03.001

Tables

Table 1: Factors and levels of factorial design

Factors	**Units**	**Levels**	**Levels**	**Levels**
		-1	0	+1
Inoculum volume	ml	50	125	200
Luminous intensity		5	12.5	20
Nitrate dosage(*)	$g.ml^{-1}$	0	1	2

(*) Level 0 = no dose, level 1 = 75mg.ml-1, level 2 = 150mg.ml-1.

Table 2: Experimental design with the strain selected to enhance its lipid profile, subjected to nitrogen stress, according to possible combinations at chance between levels and variables.

N° experiments	Inoculum volume (ml)	Luminous intensity ($umol.m^2.seg^{-1}$)	Nitrate dosage ($g.ml^{-1}$)	Fatty acid ($pg.cel^{-1}$)	Final cell conc ($cel.ml^{-1}$)
1	50	5	0	average	
2	200	5	0	–	

N° experiments	Inoculum volume (ml)	Luminous intensity (umol.m^2.seg^{-1})	Nitrate dosage (g.ml^{-1})	Fatty acid (pg.cel^{-1})	Final cell conc (cel.ml^{-1})
3	50	5	0.2		
4	200	5	0.2	–	
5	50	20	0	–	
6	200	20	0		
7	50	20	0.2	–	
8	200	20	0.2	–	
9	125	12.5	0.1		

Table 3 . Instrumental parameters and conditions of the chromatograph used (Varian CP-3800)

Instrumental parameters	Conditions
Injection volume	1 µL
Injection mode	spitless
Injection time	0.5 min
Injector temperature	250 ºC
Capillary column	Restek, FAMEWAS ® WCOT fused silica 30m x 0.25 mm x 0.25 µm
Carrier gas	Helium
Gas flow	1 mL min^{-1}
Temperature program	120 ºC for 1 min, 30 ºC min^{-1} up to 160 ºC, 160 ºC for 1 min, 4 ºC min^{-1} up to 240 º
Total time of temperature program	30.33 min
Detector temperature	260 ºC

Table 4: Cellular concentration of *P. micans* , strain IMP-BG-036 at the end of culture

N° experiments	Vol., inoculum (ml)	Vol., inoculum (ml)	Luminous intensity (µmol/m^2/s^1)	Nitrato dosage (g/ml)	Cell Concentration (cel/mL)	Cell Concentration (cel/mL)
1	1	50	5	0	1.5E+04	(±334.33)
2	2	200	5	0	2.91E+04	(±216.76)
3	3	50	5	0.2	1.29E+04	(±242.53)
4	4	200	5	0.2	2.77E+04	(±257.26)
5	5	50	20	0	1.26E+04	(±161.11)
6	6	200	20	0	1.52E+04	(±1346.01)
7	7	50	20	0.2	1.71E+04	(±525.73)
8	8	200	20	0.2	1.96E+04	(±300.04)
9	9	125	12.5	0.1	2.62E+04	(±391.64)

Table 5: Variety of the lipid content of the strain IMP-BG-036 of *P. micans* , under controlled culture conditions, induced by three abiotic factors; Inoculum volume (ml), Illumination (µmol.m^{-2}.sec^{-1}) and nitrate dose.

Factores			Biomasa		células	
			mg/mL		pg/cel	
Vol (inóculo)	IL	Dosis	C 20:5n-3 (EPA)	C 22:6n-3 (DHA)	C 20:5n-3 (EPA)	C 22:6n-3 (DHA)
50	5	0	45.762	546.770	3.355	40.086
50	5	0	47.221	576.197	3.090	37.709
50	5	0	54.753	710.548	3.596	46.665
		Prom	**49.245**	**611.172**	**3.347**	**41.487**
200	5	0	76.861	1190.331	2.445	37.860
200	5	0	61.709	1091.423	2.389	42.260
200	5	0	75.390	1205.509	2.517	40.255
		Prom	**71.320**	**1162.421**	**2.450**	**40.125**
50	20	0	51.821	670.027	3.781	48.883
50	20	0	50.687	687.513	4.128	55.986
50	20	0	45.103	608.412	3.853	51.971
		Prom	**49.204**	**655.317**	**3.920**	**52.280**
200	20	0	42.936	453.998	2.976	31.469
200	20	0	53.487	713.799	3.388	45.215
200	20	0	52.459	755.053	3.401	48.945
		Prom	**49.628**	**640.950**	**3.255**	**41.876**

Table 6: Variety of the lipid content of the strain IMP-BG-036 of*P. micans* , under controlled culture conditions, induced by three abiotic factors; Inoculum volume (ml), Illumination ($\mu mol.m^{-2}.sec^{-1}$), with a double dose of nitrate.

Factores			Biomasa		Células	
			mg/mL		pg/cel	
Vol (inóculo)	IL	Dosis	C 20:5n-3 (EPA)	C 22:6n-3 (DHA)	C 20:5n-3 (EPA)	C 22:6n-3 (DHA)
50	5	2	29.148	468.506	2.504	40.250
50	5	2	34.672	557.878	2.251	36.226
50	5	2	24.024	418.571	2.062	35.919
		Prom	**29.281**	**481.652**	**2.272**	**37.465**
200	5	2	47.190		1.843	
200	5	2	61.906	1098.480	2.219	39.381
200	5	2	62.044	1050.256	2.097	35.498
		Prom	**57.047**	**1074.368**	**2.053**	**37.440**
50	20	2	64.981	1077.017	3.921	64.985
50	20	2	54.342	793.994	2.958	43.214
50	20	2	47.646	629.289	2.915	38.496
		Prom	**55.656**	**833.433**	**3.264**	**48.899**
200	20	2	50.913	839.848	2.520	41.577
200	20	2	58.784	1059.592	3.064	55.225
200	20	2	63.696	1011.543	3.263	51.821
		Prom	**57.798**	**970.328**	**2.949**	**49.541**

Table 7: Variety of the lipid content of the strain IMP-BG-036 of*P. micans* , under controlled culture conditions, induced by three abiotic factors; Inoculum volume (ml), Illumination ($\mu mol.m^{-2}.seg^{-1}$), with a dose of nitrate (According to culture medium L1)

Factores			Biomasa		células	
			mg/mL		pg/cel	
Vol (inóculo)	IL	Dosis	C 20:5n-3 (EPA)	C 22:6n-3 (DHA)	C 20:5n-3 (EPA)	C 22:6n-3 (DHA)
125	12.5	1	69.186	1138.509	2.675	44.015
125	12.5	1	71.093	1134.641	2.704	43.153
125	12.5	1	72.416	1204.989	2.749	45.736
		Prom	**70.898**	**1159.380**	**2.709**	**44.301**

Figure capitions

Figure 1: Factors involved in cell concentration during the cultivation of the *P. micans* strain IMP-BG-036 under laboratory conditions, using the Pareto Diagram

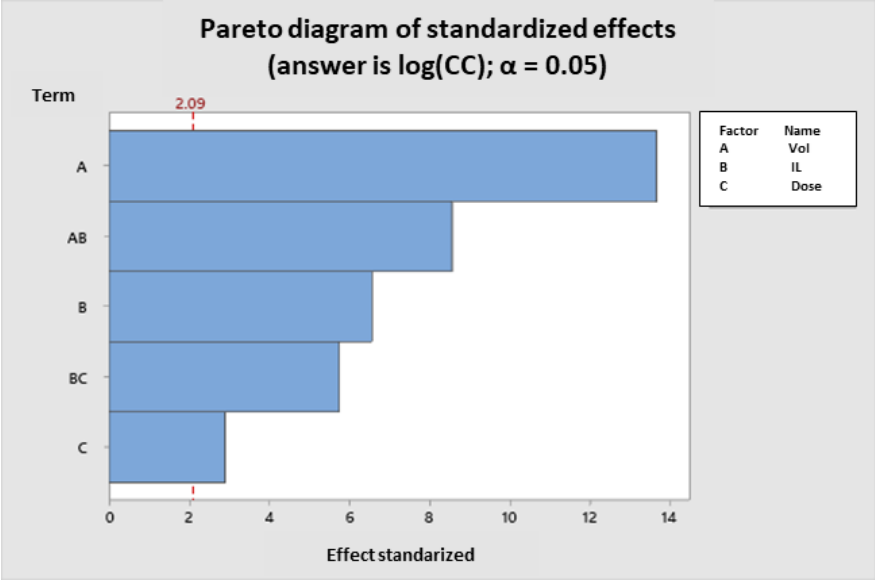
Figure 2: Factors involved in the accumulation of DHA polyunsaturated fatty acid, a) biomass level ($ng.ml^{-1}$), b) cellular level ($pg.cel^{-1}$) of the IMP-BG-036 strain of *P. micans* under laboratory conditions, using the Pareto Diagram.

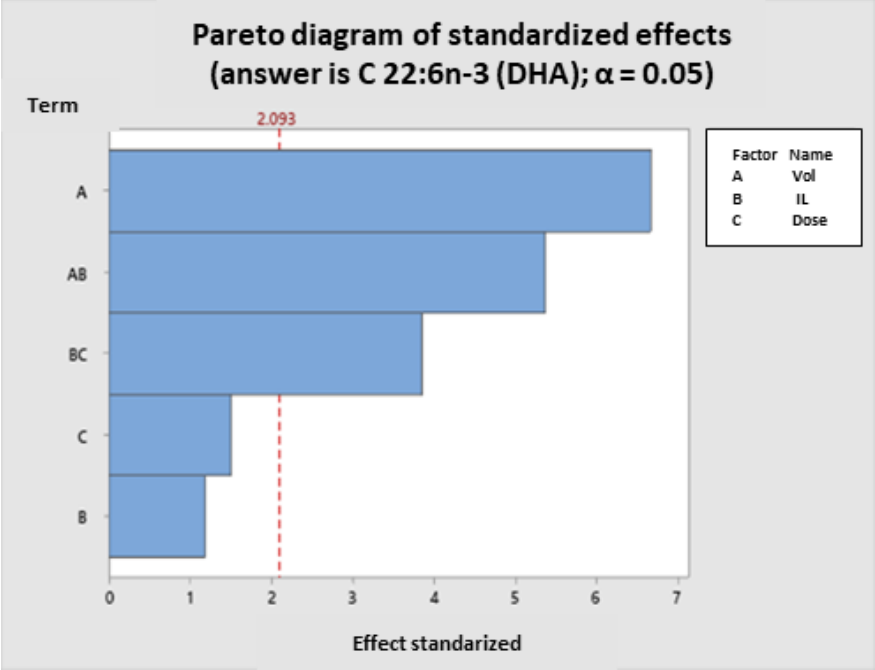
Figure 3: Graph of interaction of factors involved in the accumulation of the fatty acid DHA of the strain IMP-Bg-036 of *P. micans* , in culture.

Figure 4: Factors involved in the accumulation of EPA polyunsaturated fatty acid, a) biomass level ($ng.ml^{-1}$), b) cellular level ($pg.cel^{-1}$) of the IMP-BG-036 strain of *P. micans* under laboratory conditions, using the Pareto Diagram.

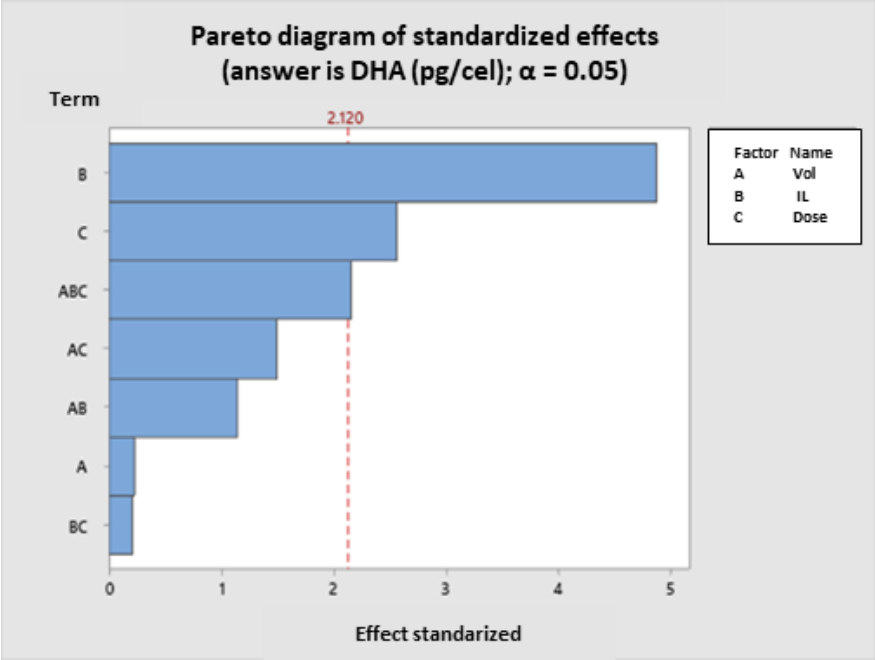
Figure 5: a) Graph of principal components that determines the impact of the factors volume of the inoculum, illumination and dose of nitrate on the accumulation of EPA at the cellular level, b) Graph of interaction of factors that intervene in the accumulation of the fatty acid EPA of the strain IMP-Bg-036 of *P. micans* , in culture under controlled conditions.

Figure 6: Factors involved in the accumulation of the polyunsaturated fatty acid OPA, a) biomass level ($ng.ml^{-1}$), b) cellular level ($pg.cel^{-1}$) in the culture of the strain IMP-BG-036 of *P. micans* under laboratory conditions, using the Pareto Diagram.

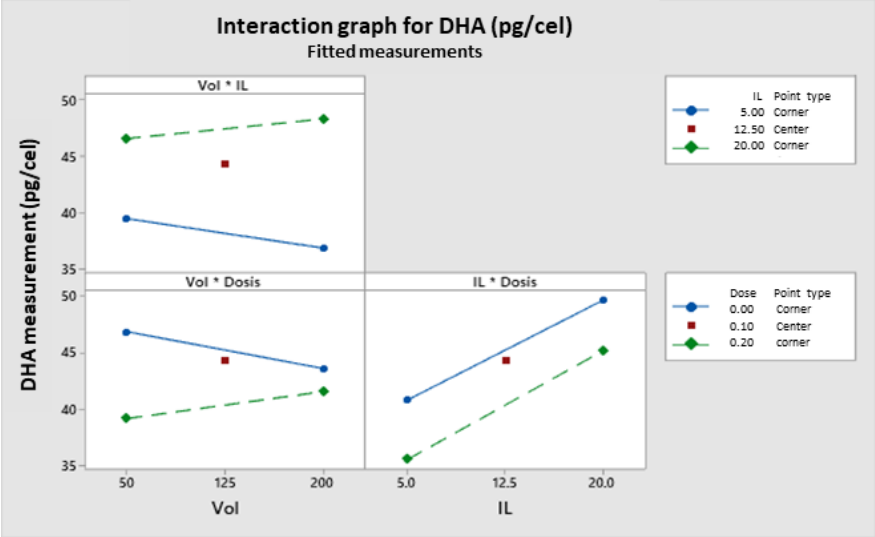


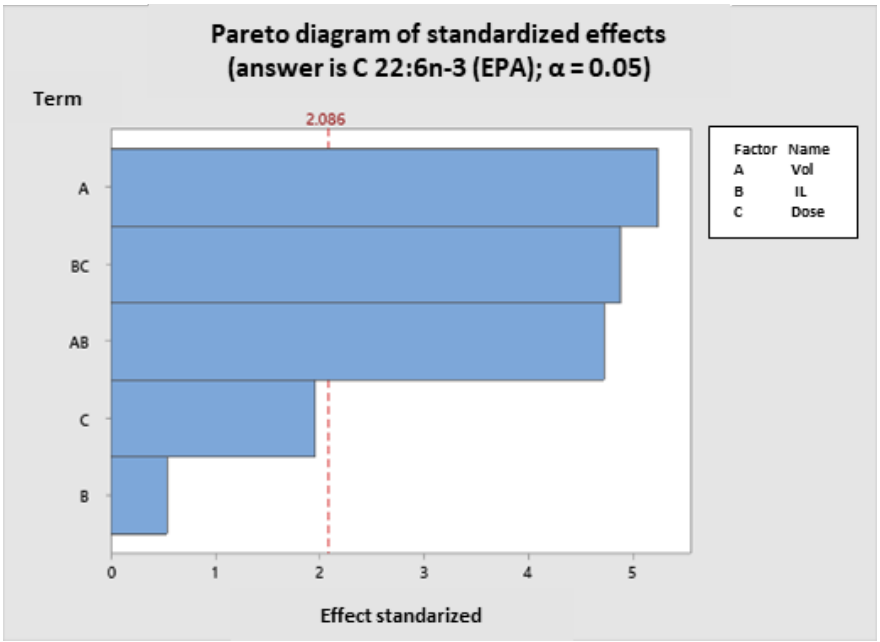


A

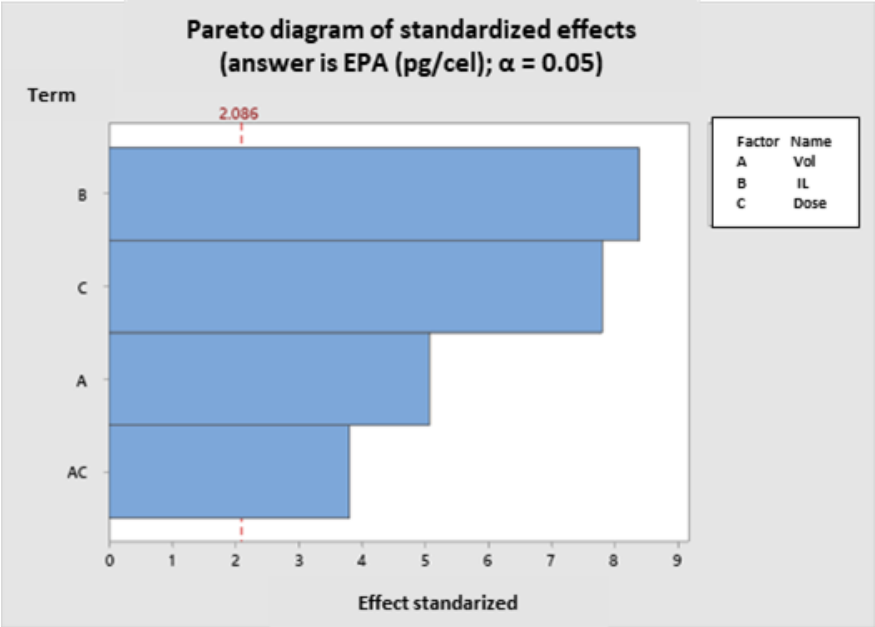


B

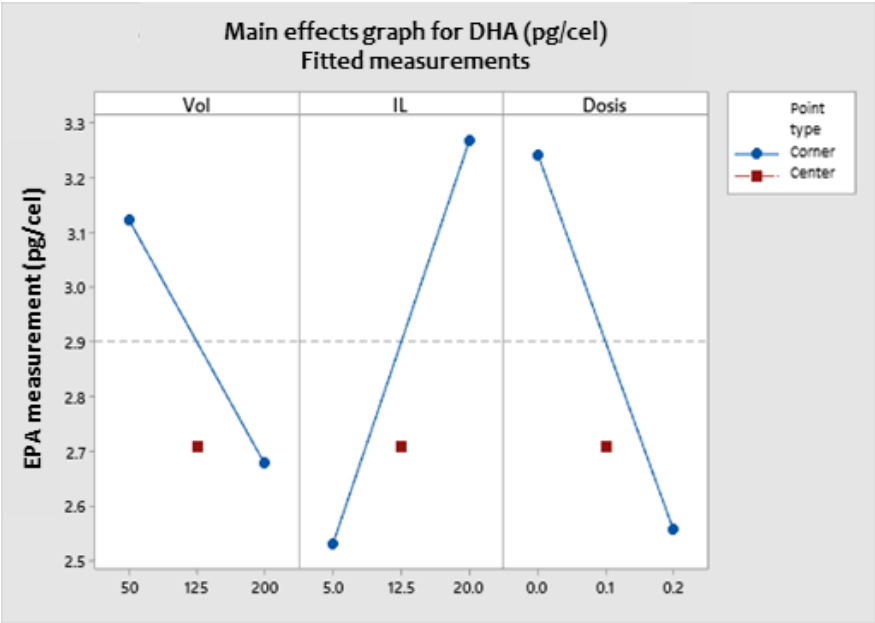




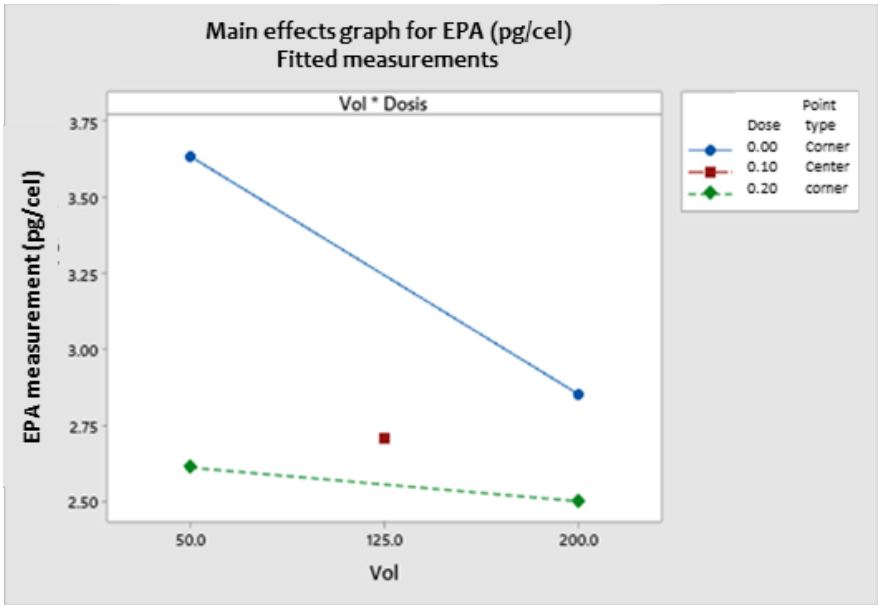
A



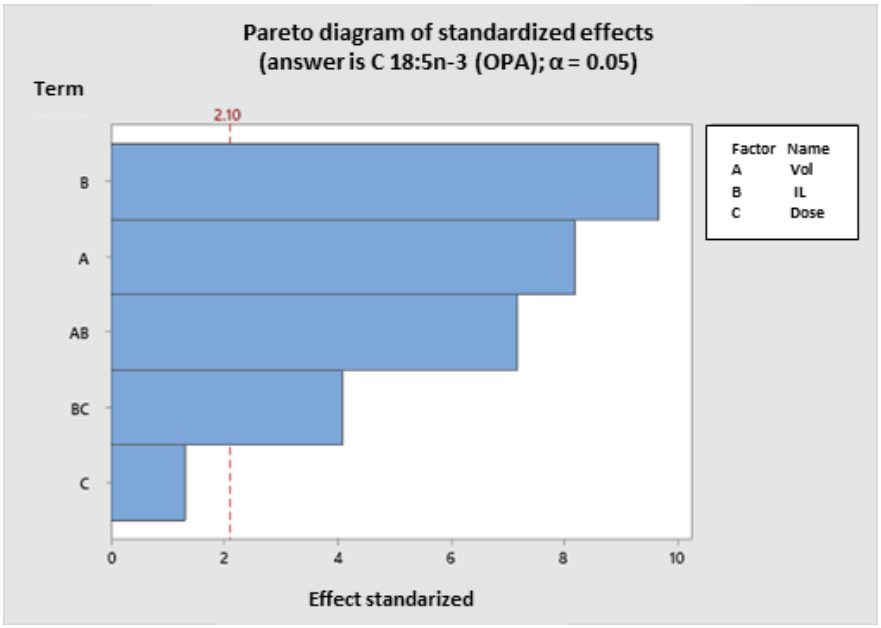
B



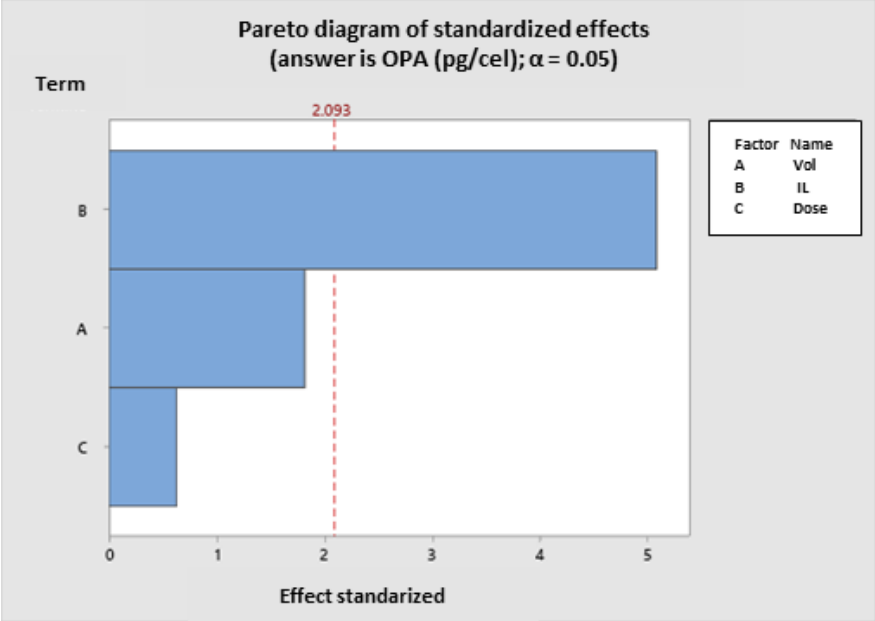
A



B



A



B