学校编码：10384　　　　分类号______密级____
学号：32420131152307　　　　UDC____

厦门大学

硕 士 学 位 论 文

高密度培养紫球藻生产花生四烯酸的研究

Study on high density cultivation of red microalgae *Porphyridium purpureum* for Arachidonic acid production

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论文提交日期：2016 年 04 月
论文答辩日期：2016 年 05 月

答辩委员会主席：______________
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2016 年 05 月

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摘 要

非可再生化石资源的大量消耗导致了严重的环境问题和气候问题，这使得可再生且碳中性的生物质资源日益受到人们的关注。生物质可作为一种再生资源，在替代化石资源获取燃料、化学品和材料等领域具有广阔前景。其中，微藻具有将废弃物转化成生物燃料、食品、饲料原料以及其他高附加值生化产品的巨大潜能。海洋食物链中，微藻可作为多不饱和脂肪酸（PUFAs）的初级生产者，其种类繁多、含量丰富，具有诸多优点并且富产多种高附加值的生物活性物质。特别是近年来微藻已成为人们获取花生四烯酸（Arachidonic acid，ARA）非常重要的来源。ARA 的传统来源是从动物肝脏、猪肾上腺、动物血液、鱼油和蛋黄等中提取的，但因其含量极低导致 ARA 的产量低成本高；另外，微生物发酵法是目前常用的 ARA 生产方法，虽然微生物具有生物量大、油脂含量丰富和不饱和脂肪酸组成比较合理等优点，但它易受外界环境变化或其他因素的影响而退化，降低产油量和油脂产量；利用微藻制取 ARA 不仅能够降低成本，又可以提高 ARA 的产量，还能弥补动、植物资源的紧缺问题，更有利于 ARA 大规模地被使用，这样生产厂家与消费者均可从中受益。紫球藻是微藻的一种，其富含藻红蛋白，油脂，维生素，色素以及胞外多糖。特别是紫球藻富含长链不饱和脂肪酸，包括占总脂肪酸含量高达 36%的 ARA 及 17% 的 EPA。本论文以厦门大学筛选得到的富含 ARA 的紫球藻（*Porphyridium purpureum* CoE1）为研究对象，选择适合该藻种生长的培养基，通过培养条件的优化以及关键因子的调控，使细胞在较高的生长速度条件下达到高密度，进而大大提高 ARA 和总脂肪酸的产量。

首先，研究不同浓度的外源细胞分裂素 6-苄氨基嘌呤（6-BA）对紫球藻生长及合成脂肪酸含量与成分的影响。在人工海水培养基（ASW）中，添加不同浓度的 6-BA，在一定条件下进行光照培养（25 ℃，110 μmol/m^{2}s 连续光照，1 L/min 通气量），诱导藻细胞合成并积累多不饱和脂肪酸。结果表明：随着培养时间的延长，紫球藻的生物量逐渐增加；与对照相比，添加 0.6 mg/L~6.0 mg/L 6-BA 对紫球藻的生长有不同程度的促进作用，其中 0.6 mg/L 6-BA 对藻细胞生长的促进作用最显著，促进效果随 6-BA 浓度升高依次递减，当浓度增加到 9.0 mg/L 时，

6-BA 抑制了紫球藻的生长；添加 6.0 mg/L 细胞分裂素不仅促进紫球藻总油脂的积累，还有利于紫球藻合成不饱和脂肪酸。添加适当浓度的细胞分裂素可明显促进藻细胞生长，同时也增加了不饱和脂肪酸的产量。

其次，考察了通气量（0.5~3 L/min）、光照强度（110~220 μmol/m^2s）、温度（25 ℃, 30 ℃和 33 ℃）和培养时间这几个单一变量因子对紫球藻生物量积累和脂肪酸合成的情况的影响。研究结果表明，高通气量和适中温度（30 ℃）不仅可以促进紫球藻生物量的积累，还可以促进总脂肪酸和 ARA 的合成。而过高的光照强度没有促进紫球藻生物量的积累，反而抑制了脂肪酸的合成。所以，紫球藻在 ASW 培养基中，3 L/min 通气量，165 μmol/m^2s 的光照强度，30 ℃条件下生长最好，并且在这些培养条件下，培养至稳定期，藻细胞生产的总脂肪酸最多，在接近稳定期时收获的 ARA 产量最高。

最后，考察了 $NaHCO_3$（0.04~1.2 g/L）浓度和 KH_2PO_4（0.00~0.14 g/L）浓度对紫球藻生物量积累和脂肪酸合成的情况的影响。实验结果如下：提高培养基中 $NaHCO_3$ 浓度可以提高紫球藻的生物量，在 0.8 g/L $NaHCO_3$ 浓度时，紫球藻生长得最好，紫球藻合成的总脂肪酸和 ARA 产量最高，分别达 408.90 mg/L 和 104.35 mg/L。另外，培养基中 KH_2PO_4 的含量对紫球藻中总脂肪酸和 ARA 的产量影响很大，当磷酸盐浓度为 0.035 g/L 时，总脂肪酸和 ARA 的产量可以提高到 666.38 mg/L 和 159.73 mg/L；同时 KH_2PO_4 浓度对紫球藻脂肪酸组成也有明显的影响，当磷酸盐浓度降低时，UFA/TFA 和 ARA/EPA 的比例都被提高，说明在磷限制条件下有利于紫球藻中不饱和脂肪酸和 ARA 的合成。在低磷条件下，能促进 C16:0 合成 C18:2 的Δ6-去饱和酶的酶活性可能会增强，从而提高了不饱和脂肪酸的含量；也可能在此条件下，长链不饱和脂肪酸的合成更倾向于 ω6 路径，同时Δ17-去饱和酶活性被抑制所致。缺磷培养的方法提高了紫球藻不饱和脂肪和 ARA 的合成，可为 ARA 的商业规模培养作借鉴。

关键词：紫球藻 生物量 总脂肪酸 多不饱和脂肪酸 花生四烯酸

Abstract

As the rapid expansion of non-renewable fossil resources in recent years, a series of serious environmental and climate problems were breaken out, which make renewable and carbon neutral biomass resources increase people’s attention more and more. In the field of substituting the fossil resources for the production of fuels, chemicals and materials, biomass has broad prospects. Microalgae，which has the great potential of converting microbial wastes into biofuels, and other high value products efficiently with simultaneous environmental bioremediation benefits, has gained more and more attention. Furthermore, owing to its rich contents and types, especially it contents valuable bioactivators, microalgae was recognized as the primary producers of PUFAs of the marine food chain, and it has becoming an important source of ARA. The traditional ARA source comes from animals including animal liver, pig adrenals, animal blood, fish oil and egg yolk and so on. Due to the low contention of ARA in these organizations, the ARA yield was quite low and it cost very high. Nowadays, ARA was mainly producted by microorganism because of its high biomass, rich content of lipid and reasonable composition of unsaturated fatty acid. However, it is sensitive to environment, which makes its features deteriorate as time go on and companied by low lipid production. Additionally, product ARA by microalgae can not only cut costs, but also can improve ARA production to make up for lack of animals and plant resources, which in favor of a large amount of ARA used, so that producers and consumers can both benefit. The red microalgae *Porphyridium purpureum* is recognized to produce a series of valuable nutrients, such as polysaccharides, phycoerythrin, lipids, vitamins and pigments. Also *P. purpurem* is known to be rich in long-chain PUFAs, which contains up to 36% of arachidonic acid (ARA) and 17% of eicosapentaenoic acid (EPA) of total fatty acids (TFA). In this study, the microalgae, *P. purpureum* CoE1, screened and maintained by the authors’ research group, was used. We chose a medium that suitable for the algae growth and fatty acid biosynthesis. Then, optimization of culture conditions and some key factors were conducted to enhance the biomass and TFA/ARA contents and yields.

Firstly, the effects of 6-Benzylaminopurine (6-BA) on the growth and fatty acid biosynthesis of *P. purpureum* were studied. During the cultivation of *P. purpureum*, different concentrations of 6-BA were introduced in ASW medium and the cultivation conditions were controlled at 25 °C, 110 μmol/m^2s continuous light illumination and supplying with 1L/min of sterile air constantly, for enhancing the production of polyunsaturated fatty acids. The results showed that the microalgae biomass increases with the cultivation time. Compared to the control trail, 6-BA of 0.6 mg/L~6.0 mg/L promotes the cell growth of *P. purpureum*. 6-BA of 6.0 mg/L enhanced the biosynthesis total lipids and unsaturated fatty acids most significantly. However, 6-BA of other concentrations conducted performed their inhibition of the biosynthesis of total fatty acids by *P. purpureum*. When 6-BA of 9.0 mg/L was introduced, the cell growth was suppressed. Addition of 6-BA at an appropriate concentration could promote the growth of *P. purpureum* cells as well as the production of unsaturated fatty acids.

Secondly, the effects of aeration rate, light intensity, culture time and temperature on biomass, total fatty acids (TFA) content/composition were studied in *P. purpureum*. Light intensities in the range of 110~220 μmol/m^2s, aeration rates ranges of 0.5~3 L/min and temperature ranges of 25 °C, 30 °C and 33 °C were tested. It was found higher aeration rate and moderate temperature of 30 °C led to more biomass and TFA/ARA production. However, higher light intensity tested did not contribute to *P. purpureum* biomass accumulation but was adverse for TFA and ARA biosynthesis. Thus, in artificial seawater medium, the highest yield of TFA was obtained with the aeration rate of 3 L/min, light intensity of 165 μmol/m^2s and temperature of 30 °C. In addition, the optimal harvest time of TFA was in stationary phase and that of ARA was approximately to the stationary phase.

Finally, effects of sodium bicarbonate (0.04~1.2 g/L) and phosphate (0.00~0.14 g/L) on biomass yield, total fatty acids (TFA) and arachidonic acid (ARA) accumulation were investigated systemically. $NaHCO_3$ dose of 0.8 g/L was preferred. The highest TFA and ARA yields were obtained， which were 408.90 mg/L and 104.35 mg/L respectively. In addition, TFA and ARA production were significantly enhanced by an appropriate concentration of phosphate, and the highest TFA yield of 666.38 mg/L and ARA yield of 159.73 mg/L were obtained at a phosphate concentration of 0.035 g/L. Interestingly, with phosphate concentration continuing to fall, UFA/TFA and ARA/EPA ratios were increased accordingly, suggesting that phosphate limitation promoted unsaturated fatty acids and arachidonic acid biosynthesis. Low concentration of phosphate may be favored to increase the enzymatic activities of Δ6-desaturase, which played a key role in catalyzing the conversion of C16:0 to C18:2, and thus the selectivity of UFA increased. Meanwhile, the increase of ARA selectivity could be attributed to ω6 pathway promotion and Δ17-desaturase activity inhibition with phosphate limitation. Phosphate limitation strategy enhanced unsaturated fatty acids (UFAs) and ARA biosynthesis in *P. purpureum*, and can be applied in commercial-scale manufacturing and commercialization of ARA.

Keywords:： *Porphyridium purpureum*; biomass; total fatty acids; polyunsaturated fatty acids; arachidonic acid

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