·综 述·

外源添加剂对微藻生长和高价值生物产物积累的影响

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吴亚伟, 王方忠, 陈磊, 张卫文. 外源添加剂对微藻生长和高价值生物产物积累的影响[J]. 生物工程学报, 2025, 41(1): 173-198.
WU Yawei, WANG Fangzhong, CHEN Lei, ZHANG Weiwen. Effects of exogenous additives on growth and high-value bioproducts accumulation of microalgae[J]. Chinese Journal of Biotechnology, 2025, 41(1): 173-198.

摘 要：近年来，随着合成生物学的飞速发展，基因工程和分子操作手段日益完善。微藻作为微生物细胞工厂的代表性宿主菌株之一，在生产油脂、色素、蛋白质和生物燃料等高附加值生物产品方面得到了广泛的应用，并在生化能源、食品药品以及环境保护等领域展现出广阔的应用前景。然而，目前基于微藻工艺的生产效率仍然很低，限制了其大规模的工业应用。除了改良菌种和优化培养外，基于外源化学添加剂进行调控也是一种有益的优化策略。这种方法依赖于直接的表型筛选，不需要深入解析生物产品合成过程中涉及的代谢和分解代谢途径中的分子靶点，就可以快速提高微藻高附加值生物产品的产量，获得所需的表型。虽然已有大量关于使用外源添加剂等替代手段来提高微藻生长和高附加值生物产品的产量的研究，但关于添加剂的种类、用途和所针对的菌株以及相关的分子作用机制的分类总结还不够系统和全面。本综述对近年来应用化学诱导剂或增强剂来改善微藻培养中细胞生长和高附加值生物产物积累的实例进行总结，重点介绍了用于微藻培养的外源添加剂的种类、外源添加剂及其组合对微藻生长和高价值生物产品积累的影响以及相关作用的分子机制，为研究人员利用合成生物学的方法来开发合适的细胞底盘以及利用微藻来进行大规模的工业化生产提供了有用的信息。
关键词：添加剂；微藻；生长；生物产物

资助项目：国家重点研发计划(2020YFA0908703)；国家自然科学基金(32101172)
This work was supported by the National Key Research and Development Program of China (2020YFA0908703) and the National Natural Science Foundation of China (32101172).
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Received: 2024-04-19; Accepted: 2024-07-04; Published online: 2024-07-05

Effects of exogenous additives on growth and high-value bioproducts accumulation of microalgae

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Abstract: With the rapid development of synthetic biology, genetic engineering, and molecular manipulation methods in recent years, microalgae, as representatives of microbial cell factories, have been widely used as hosts in the production of high-value bioproducts, such as oils, pigments, proteins, and biofuels, demonstrating promising prospects of application in biochemical energy, food and drugs, and environmental protection. Despite these advancements, the low production efficiency of microalgae limits their industrial application. In addition to strain improvement and culture condition optimization, the regulation by exogenous chemical additives serves as a promising optimization strategy. This method relies on straightforward phenotypic screening and circumvents the necessity for intricate understanding of molecular targets in the metabolic and catabolic pathways involved in the synthesis of bioproducts. It enables rapid yield increasing of high-value bioproducts from microalgae and obtaining the required phenotypes. Although studies have reported the use of alternatives means such as exogenous additives to improve the growth of microalgae and the yield of high-value bioproducts, the classification and summarization of the types, applications, targeted strains, and molecular mechanisms of these additives are not comprehensive. Here, we review the studies using chemical inducers or enhancers to improve cell growth and high-value bioproduct accumulation in microalgae in recent years. This paper focuses on the types of exogenous additives, the effects of exogenous additives and their combinations on microalgae growth and high-value bioproduct accumulation, and the molecular mechanisms of related effects. We aim to provide information for researchers to use methods of synthetic biology to develop suitable cell chassis and harness microalgae for industrial production.

Keywords: additives; microalgae; growth; bioproducts

近年来，对于微藻的研究日益受到重视，使得藻类在各领域的研究工作蓬勃发展，特别是在高价值生物产品的生产方面。相对于传统经济作物，以微藻作为高附加值生物产品的原料是一个有吸引力的选择，因为它们生长周期短、生长速度快，可以积累大量的脂质、碳水化合物、蛋白质以及对人类健康十分有益的类胡萝卜素、角鲨烯、虾青素和二十二碳六烯酸(docosahexaenoic acid, DHA)等。同时，微藻的营养物质丰富、生产成本较低、经济效益高，可以在水质较差的

水或海水中生长，可以利用工业烟气等来源的二氧化碳，并且还可以去除工业、农业和城市废水中的污染物以及氮和磷等营养物质来提供额外的收益，净化节约了水资源，保护了环境[1]。并且，微藻在生产高附加值生物产品时不受季节变化、场地和气候的影响，被认为是一类可用于工业化大规模生产的微生物之一。

微藻也是蛋白质、脂质、糖类和色素的重要来源，其在化妆品、食品、药品、工业材料甚至生物燃料等领域具有巨大的应用潜力[2]。其中，微藻的大规模培养是藻类生物产品放大生产应用中最具挑战性的步骤，因此提高微藻的生物量(dry cell weight, DCW)和/或高价值生物产物的含量可以提高藻类培养的经济可行性，从而有利于微藻的大规模工业化生产除了菌种改良和培养优化之外，目前提高微藻生长和促进高附加值生物产品积累的方法主要包括实验室适应性驯化[3]、诱变[4]、基因工程[5]和基于外源性化学添加剂调控。适应性驯化依赖于自然突变和外部压力来筛选高产菌株，这需要相对较长的时间[3]。诱变是随机的，获得所需表型的突变体需要进行高通量的筛选，这无疑会导致成本较高、时间较长、工作量较大等问题[4]。基因工程方法往往操作复杂、成本较高，并只适用于遗传操作体系成熟、分子生物学操作手段完善的微藻。同时，基因水平的操作会导致物种发生可遗传的变化，并且公众对转基因菌株的安全问题的担忧限制了此类策略在环境保护、食品生产和农业中的应用。相反，利用外源化学添加剂进行调控是一种有益的替代策略，通过在培养基中添加简单易获取、价格低廉的化学诱导剂，进行简单的表型筛选，确定可以提高微藻生物量和/或高附加值生物产品含量的潜在添加剂及其组合，就可以快速获得所需表型，从而能够在较短时间内有效提高微藻的生长和高价值生物产品的积累，节约时间的同时降低了生产成本，具有较高的经济效益。

自 20 世纪 30 年代以来，已有大量的研究报道了使用外源添加剂等替代手段来提高微藻生长和高附加值生物产品的积累，但关于这些已报道过的添加剂的种类、作用和所针对的物种以及相关的分子作用机制的分类总结还鲜有报道。为此，本文主要对近年来应用外源添加剂来改善微藻细胞生长和生物产物积累的研究进行了全面总结，旨在确定可以提高微藻生物量和/或高附加值生物产品产量的外源添加剂以及相关的分子作用机制。最后，总结了对外源添加剂在微藻中的应用现状，并对外源调节剂高维度的组合添加做出展望，以期为相关的研究人员提供有价值的参考信息。

1 添加剂分类

近年来，已经有大量的研究发现使用外源添加剂可以影响微藻生长和生物产品的积累，增强藻类产物的产量和细胞干重[6]。其中涉及的添加剂种类高达数十种，按其结构、性质、作用大致可分为抗氧化剂、氧化剂、信号转导剂、植物激素、胺类、维生素和微量元素以及其他化学物质等。在微藻的大规模培养中，使用外源添加剂来促进细胞生长和高附加值生物产品的积累是一种非常经济有效的方法[6]。

1.1 抗氧化剂

已知抗氧化剂对微藻生产高附加值产物具有积极的影响，因为它可以增强总抗氧化能力(total antioxidant capacity, T-AOC)并减少细胞中活性氧(reactive oxygen species, ROS)如 H_2O_2 和 O_2^-的产生，从而可以减缓 ROS 引起的氧化应激[7]。在食品工业中，由于形成不良的次生脂质过氧化产物会导致食品变质(例如营养价

值、安全性和外观)，因此，许多合成的商业抗氧化剂，如芝麻酚[7-9]、槲皮素[10]和丁基羟基茴香醚[11]，被用于延缓氧化和过氧化过程。同时，大量的研究表明，抗氧化剂具有促进微藻细胞生长、脂质积累和色素合成等高价值生物产物合成的能力(表 1)，这可能是因为抗氧化剂具有清除细胞内 ROS 的能力。

1.2 氧化剂

活性氧通常被认为是氧化应激的细胞毒性诱导剂[16]，ROS 可与脱氧核糖核酸(deoxyribonucleic acid, DNA)、脂质和蛋白质等生物大分子发生反应并造成损伤，进而导致蛋白质功能丧失甚至细胞死亡。但最近的证据表明，某些 ROS 还具有信号转导功能，因此通过作为信号分子可以参与微藻的细胞增殖、分化、凋亡和细胞衰老，从而改善细胞生长和生理反应[16]。外源添加合适类型和剂量的 ROS 已被证实可诱导微藻虾青素的积累，且对虾青素的大规模生产具有潜在的价值(表 2)。

1.3 信号转导剂

细胞信号转导剂不仅可以影响植物的某些生长发育过程，还可以作为重要的信号分子出现在生物胁迫和非生物胁迫中，提高植物的抗逆性[22]。近来，有大量的研究表明植物信号转导剂也是一类重要的添加剂，其不仅可以调节微藻的细胞生长、脂质积累、蛋白质合成以及虾青素积累等一系列重要的生物合成过程[16,23-25]，而且还可以诱导这些合成过程的显著增强，是促进微藻高价值生物产物合成的潜在有价值的培养添加剂(表 3)。

表 1 抗氧化剂对微藻生长和高价值生物产物积累的影响

Table 1 Effects of antioxidants on growth and accumulation of high-value bioproducts in microalgae

Additive names	Microbial species	Products	Mechanism analysis	References
Sesamol	*Crypthecodinium cohnii*	DHA (11.3%), biomass (44.2%)	\	[7]
Quercetin	*Chlorella vulgaris*	Biomass (2.5 times), lipid (1.8 times), chlorophyll a (+)	Transcriptional regulation/ stimulate metabolism	[10]
Butyl hydroxyanisole (BHA)	*Crypthecodinium cohnii*	Lipid (8.8%)	Stimulate metabolism	[11]
	Heveochlorella sp.	Lipid (25.0%)	Transcriptional regulation	[12]
Propyl gallate (PG)	*Crypthecodinium cohnii*	\	\	[11,13]
Melatonin (MLT)	*Heveochlorella* sp.	Lipid (36.6%)	Transcriptional regulation	[12]
Fulvic acid (FA)	*Heveochlorella* sp.	Biomass (+), lipid (+)	Transcriptional regulation	[12]
	Monoraphidium sp. FXY-10	Lipid (21.8%)	Transcriptional regulation	[14]
	Haematococcus pluvialis	Biomass (10.6%), astaxanthin (86.9%)	Transcriptional regulation	[15]
Butylhydroxytoluene (BHT)	*Heveochlorella* sp.	Lipid (+)	Transcriptional regulation	[12]

"+" represents that exogenous additives have a promoting effect on the accumulation of microalgae high-value bioproducts; "\" represents no positive effect, and the specific number represents the percentage increase in the accumulation of microalgae high-value bioproducts compared with the control.

表 2 氧化剂对微藻生长和高价值生物产物积累的影响

Table 2 Effects of oxidants on growth and accumulation of high-value bioproducts in microalgae

Additive names	Microbial species	Products	Mechanism analysis	References
Methylviologen (MV)	*Haematococcus pluvialis*	Astaxanthin (32.9%), carotenoid (+)	Stimulate metabolism	[16-17]
	Chlorococcum sp.	Astaxanthin (+)	\	[18]
Methylene blue (MB)	*Haematococcus pluvialis*	Astaxanthin (41.9%), carotenoid (+)	Stimulate metabolism	[16-17]
2,2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH)	*Haematococcus pluvialis*	Astaxanthin (29.2%), carotenoid (+)		
Hydrogen peroxide	*Haematococcus pluvialis*	Astaxanthin (20.6%), carotenoid (+)	Stimulate metabolism	[16-17]
	Chlorococcum sp.	Astaxanthin (72.3%)	\	[18-19]
	Chlorella vulgaris	Astaxanthin (53.2%), carotenoid (38.9%), chlorophyll (29.8%)	\	[20]
	Spirulina platensis	Carotenoid (6.7 times)	\	[21]
Sodium hypochlorite	*Chlorella vulgaris*	Astaxanthin (32.4%), carotenoid (67.7%), chlorophyll (46.8%)	\	[20]

"+" represents that exogenous additives have a promoting effect on the accumulation of microalgae high-value bioproducts; "\" represents no positive effect, and the specific number represents the percentage increase in the accumulation of microalgae high-value bioproducts compared with the control.

1.4 植物激素

植物激素是天然或人工合成的化学信使，可以调节植物和微藻生长、发育的各个方面，通常极低浓度即可发挥活性[22]。已有多篇报道称植物激素也可以刺激非植物微生物的生长，包括细菌和真菌[34-36]。同时，有充分的证据表明，植物激素或类似物已被证明为促进非植物微生物高价值生物产物合成的潜在有价值的培养添加剂。植物激素主要包括生长素、细胞分裂素、赤霉素、乙烯和脱落酸这 5 类(表 4)。

1.4.1 生长素及其化学类似物

生长素是一类数量众多、研究广泛的植物激素，在调控植物生长发育方面起着至关重要的作用[30]。生长素除了对植物细胞分裂和分化起作用外，还在细胞、组织和器官之间起信号传递作用[37]。生长素通过这种方式协调和整合植物生长发育以及植物对环境胁迫的生理反应。

近年来，已有大量研究证实生长素对藻类生理生化过程的影响，生长素不仅能够刺激植物的生长和发育，而且还能刺激细菌、某些蓝藻和真菌的生长和发育[34,62,79]。生长素在微藻的生长和代谢中发挥着多种作用，即使极低浓度的生长素也可以诱导生物量的产生，并促进高价值生物产物的积累[52]。同时，生长素及其化学类似物早已作为外源添加剂被大量地添加到微藻培养基中用于调节微藻高价值生物产物的合成(表 4)。

表 3 信号转导剂对微藻生长和高价值生物产物积累的影响

Table 3 Effects of signal transducers on growth and accumulation of high-value bioproducts in microalgae

Additive names	Microbial species	Products	Mechanism analysis	References
Salicylic acid (SA)	*Crypthecodinium cohnii*	Lipid (13.5%)	Stimulate metabolism	[22]
	Haematococcus pluvialis	Astaxanthin (30.6%), biomass (+)	Stimulate metabolism/ transcriptional regulation	[16,23-24]
	Chlorella vulgaris	Biomass (40.0%), nucleic acid (60.0%), protein (60.0%), chlorophyll (70.0%), carotenoid (57.0%), saccharide (41.0%)	\	[25]
Jasmonic acid (JA)	*Crypthecodinium cohnii*	\	\	[22]
	Haematococcus pluvialis	Astaxanthin (50.6%)	Stimulate metabolism	[16]
	Chlorella vulgaris	Cell numbers (42.2%), chlorophyll (71.8%), fucoxanthin (80.8%), carotenoid (59.8%), saccharide (33.8%), protein (71.3%)	\	[26]
Methyl jasmonate (MJ)	*Haematococcus pluvialis*	Biomass (+), carotenoid (+), chlorophyll (+), astaxanthin (+)	\	[24]
	Fucus vesiculosus	Fucoidan (58.0%)	\	[27]
Brassinolides (BRs)	*Crypthecodinium cohnii*	\	\	[22]
	Spirulina platensis	Biomass (26.0%)	\	[28]
	Haematococcus pluvialis	Astaxanthin (25.9 times)	Transcriptional regulation	[29]
	Chlorella vulgaris	Biomass (237.0%), protein (296.0%), chlorophyll (228.4%), saccharide (2.0 times), nucleic acid (3.0 times)	\	[30-33]

"+" represents that exogenous additives have a promoting effect on the accumulation of microalgae high-value bioproducts; "\" represents no positive effect, and the specific number represents the percentage increase in the accumulation of microalgae high-value bioproducts compared with the control.

表 4 植物激素对微藻生长和高价值生物产物积累的影响

Table 4 Effects of phytohormones on growth and accumulation of high-value bioproducts in microalgae

Additive types	Additive names	Microbial species	Products	Mechanism analysis	References
Auxins and its chemical analogues	Iodoacetic acid	*Crypthecodinium cohnii*	Lipid (4.6%)	Stimulate metabolism	[22]
	1-naphthylacetic acid (NAA)	*Crypthecodinium cohnii*	\	\	[22]
		Haematococcus pluvialis	Biomass (117.0%)	\	[35]
		Chlorella vulgaris	Biomass (133.0%), carotenoid (131.6%), chlorophyll (170.2%), protein (92.7%), saccharide (91.0%), lipid (137.3%)	Transcriptional regulation	[35,37-41]

(待续)

(续表 4)

Additive types	Additive names	Microbial species	Products	Mechanism analysis	References
		Spirulina platensis	Cell numbers (+)	\	[42]
		Scenedesmus quadricauda	\	\	[43]
		Cladophora glomerata	Chlorophyll (+)	\	[44]
		Thraustochytrium roseum	Biomass (41.7%)	\	[36]
	Naphthoxyacetic acid (BNOA)	*Crypthecodinium cohnii*	Lipid (10.7%)	Stimulate metabolism	[22]
	2,4-dichlorophenoxyacetic acid (2,4-D)	*Crypthecodinium cohnii*	\	\	[22]
		Chlorella vulgaris	Biomass (84.7%), protein (96.1%), chlorophyll (134.8%), saccharide (+), lipid (96.8%), carotenoid (90.8%)	Transcriptional regulation	[37,39,41,45]
		Spirulina platensis	Cell numbers (24.6%), protein (+), chlorophyll a (12.9%)	\	[42,45]
		Haematococcus pluvialis	Biomass (+)	\	[46]
		Dunaliella salina	Biomass (+)	\	[46]
		Scenedesmus quadricauda	Biomass (+), chlorophyll a (14.3%)	\	[43,47]
		Pavlova viridis	Biomass (+)	\	[48]
		Chlamydomonas reinhardtii	Biomass (28.2%), chlorophyll a (25.7%)	\	[49]
		Gracilaria vermiculophylla	Biomass (+)	\	[50]
	3-indoleacetic acid (IAA)	*Scenedesmus obliquus*	Biomass (86.3%), fatty acid (23.1%)	\	[51]
		Chlamydomonas reinhardtii	Biomass (61.0%), protein (35.0%), chlorophyll (81.0%)	\	[52]
		Chlorella vulgaris	Biomass (83.0%), carotenoid (158.2%), protein (170.9%), saccharide (83.7%), chlorophyll (173.5%), lipid (20.0%)	Transcriptional regulation	[30,37,39,40,53-57]
		Amphora coffeaeformis	Biomass (41.7%)	\	[58]
		Navichla corymbosa	Biomass (17.9%)		
		Spirulina platensis	\	\	[42]
		Pavlova viridis	\	\	[48]
		Scenedesmus quadricauda	Biomass (15.0%), chlorophyll (25.8%)	\	[43,59-60]

(待续)

(续表 4)

Additive types	Additive names	Microbial species	Products	Mechanism analysis	References
		Gracilaria vermiculophylla	Biomass (+)	\	[50]
		Cladophora glomerata	Chlorophyll (3.0 times)	\	[44]
		Codium fragile subsp. *tomentosoides*	Biomass (+)	\	[61]
		Euglena sp.	Biomass (+),carbohydrate (9.2%), protein (148.5%), lipid (40.3%), chlorophyll (+), carotenoid (+)	\	[62]
		Thraustochytrium roseum	Biomass (19.4%)	\	[36]
	Diethyl aminoethyl hexanoate (DAH)	*Scenedesmus obliquus*	Biomass (147.4%), fatty acid (25.8%)	\	[51]
	1-triacontanol (TRIA)	*Chlamydomonas reinhardtii*	Biomass (54.0%), protein (44.0%), chlorophyll (43.0%)	\	[52]
		Amphora coffeaeformis	Biomass (42.0%)	\	[58]
		Navichla corymbosa	Biomass (40.4%)		
		Pavlova viridis	Biomass (11.1%)	\	[48]
	Indole-3-butyric acid (IBA)	*Crypthecodinium cohnii*	\	\	[22]
		Chlorella vulgaris	Carotenoid (3.1%), protein (13.0%), saccharide (24.0%), biomass (83.0%), chlorophyll (240.0%)	\	[30,37-38, 40,55]
		Spirulina platensis	\	\	[42]
		Pavlova viridis	Biomass (+)	\	[48]
		Cladophora glomerata	Chlorophyll (1.5 times)	\	[44]
		Nostoc linckia	Biomass (64.0%), carotenoid (+), chlorophyll a (~20.0%)	\	[63]
		Thraustochytrium roseum	Biomass (30.6%)	\	[36]
	Indole-3-lactic acid (ILA)	*Chlorella vulgaris*	Biomass(+), carotenoid (10.0%)	\	[37]
	Tryptamine (Trp-NH_2)	*Chlorella vulgaris*	Biomass (114.2%), carotenoid (+), chlorophyll (140.3%), protein (100.0%)	\	[37,39,64]

(待续)

(续表 4)

Additive types	Additive names	Microbial species	Products	Mechanism analysis	References
	Indolyl-3-propionic acid (IPA)	*Chlorella vulgaris*	Cell numbers (83.0%), protein (38.0%), chlorophyll (57.0%), saccharide (51.0%)	\	[30,40,55]
		Scenedesmus quadricauda	\	\	[43]
	Anthranilic acid (AA)	*Chlorella pyrenoidosa*	Biomass (110.2%), protein (91.3%), chlorophyll (112.9%)	\	[39]
	Naphthyl-3-sulphonic acid (NSA)	*Chlorella pyrenoidosa*	Biomass (130.7%), protein (81.9%), carotenoid (95.9%), chlorophyll (148.0%)		
	Phenylacetic acid (PAA)	*Chlorella pyrenoidosa*	Chlorophyll (162.8%), biomass (65.6%), protein (111.2%), carotenoid (147.9%), saccharide (96.3%)	\	[38-40]
		Nostoc muscorum	Biomass (59.0%)	\	[64]
		Tolypothrix tenuis	Biomass (48.0%)		
	2-methyl-4-chlorophenoxyacetic acid (MCPA)	*Scenedesmus quadricauda*	Cell numbers (+)	\	[43]
	Tryptophol	*Chlorogloea fritschii*	Biomass (24.0%)	\	[64]
	Isatin	*Anacystis nidulans*	Biomass (19.0%)		
		Chlorogloea fritschii	Biomass (21.0%)		
Gibberellin	Gibberellin (GA)/ gibberellic acid (GA_3)	*Crypthecodinium cohnii*	\	\	[22]
		Thraustochytrium roseum	DHA (79.1%), lipid (43.6%), biomass (14.4%)	Stimulate metabolism	[36,65]
		Chlorella vulgaris	Protein (51.0%), saccharide (57.0%), chlorophyll (203.0%), carotenoid (29.0%), biomass (61.0%), lipid (12.0%)	Transcriptional regulation	[38,56-57, 66-69]
		Chlamydomonas reinhardtii	Biomass (68.0%), protein (26.0%), chlorophyll (68.0%)	\	[52]
		Microcystis aeruginosa	Biomass (+), protein (+), chlorophyll a (+)	\	[70]
		Scenedesmus quadricauda	Biomass (7.0 times), protein (3.5%), chlorophyll (46.1%)	\	[59,67,71]
		Dictyosphaerium pulchellum	Biomass (11.0 times)	\	[67]
		Amphora coffeaeformis	Biomass (19.9%)	\	[58]
		Navichla corymbosa	Biomass (26.7%)		
		Cyclotella cryptica	Biomass (+)	\	[72]

(待续)

(续表 4)

Additive types	Additive names	Microbial species	Products	Mechanism analysis	References
		Haematococcus pluvialis	Astaxanthin (30.8%)	Stimulate metabolism	[16]
		Spirulina platensis	Cell numbers (+)	\	[42]
Cytokinin	Kinetin (KT)	*Crypthecodinium cohnii*	\	\	[22]
		Chlamydomonas reinhardtii	Biomass (69.0%), protein (25.0%), chlorophyll (59.0%)	\	[52]
		Haematococcus pluvialis	Biomass (+)	\	[46]
		Dunaliella salina	Biomass (+)		
		Chlorella vulgaris	RNA (110.0%), biomass (71.2%), carotenoid (85.2%), protein (161.0%), saccharide (43.0%), chlorophyll (226.0%)	\	[37,56,67,73]
		Scenedesmus quadricauda	Biomass (71.7%), protein (12.2%)	\	[59,67,71]
		Dictyosphaerium pulchellum	Biomass (61.5%)	\	[67]
		Thraustochytrium roseum	DHA (57.3%), biomass (105.6%)	\	[36]
	2-chloroacrylicacid	*Crypthecodinium cohnii*	Lipid (10.0%)	Stimulate metabolism	[22]
	6-benzylaminopurine (6-BAP)	*Aurantiochytrium* sp.	DHA (52.8%), lipid (25.9%)	Stimulate metabolism	[74]
		Chlorella vulgaris	RNA (110.0%), biomass (+), carotenoid (94.3%), protein (102.0%), saccharide (43.0%), chlorophyll (226.0%)	\	[37,73]
		Spirulina platensis	Cell numbers (+)	\	[42]
		Gracilaria vermiculophylla	Biomass(+)	\	[50]
	Allantoin (AT)	*Chlorella vulgaris*	Carotenoid (24.0%)	\	[37]
	Thidiazuron (TDZ)	*Chlorella vulgaris*	Biomass (83.0%), chlorophyll a (160.0%)	\	[38]
	Zeatin (ZT)	*Crypthecodinium cohnii*	\	\	[22]
		Chlorella vulgaris	RNA (110.0%), biomass (67.0%), protein (185.0%), chlorophyll (226.0%), carotenoid (89.0%), saccharide (43.0%), lipid(+)	Transcriptional regulation	[38,57,73]
	N,*N'*-diphenylurea (DPU)	*Chlorella vulgaris*	RNA (125.0%), cell numbers (130.0%), protein (3.0 times), chlorophyll (226.0%), carotenoid (89.0%), saccharide (57.0%)	\	[73]

(待续)

(续表 4)

Additive types	Additive names	Microbial species	Products	Mechanism analysis	References
Ethylene	Ethrel	*Scenedesmus quadricauda*	Biomass (27.4%), protein (+), nucleic acid (35.2%)	\	[75]
		Haematococcus pluvialis	Astaxanthin (92.1%)	\	[76]
		Chlorella vulgaris	Fatty acid (+)	Stimulate metabolism	[77]
Abscisic acid	Abscisic acid (ABA)	*Crypthecodinium cohnii*	Lipid (11.1%)	Stimulate metabolism	[22]
		Chlamydomonas reinhardtii	Fatty acid (13.0%), protein (32.0%), saccharide (172.7%), biomass (100.0%), chlorophyll (39.0%)	\	[52,78]
		Chlorella vulgaris	Biomass (1.3 times), lipid (148.0%), protein (+), carotenoid (+), chlorophyll a (+)	Transcriptional regulation	[41,57]
		Haematococcus pluvialis	Astaxanthin (64.2%)	Stimulate metabolism	[16]

"+" represents that exogenous additives have a promoting effect on the accumulation of microalgae high-value bioproducts; "\" represents no positive effect, and the specific number represents the percentage increase in the accumulation of microalgae high-value bioproducts compared with the control.

1.4.2 赤霉素

赤霉素是除生长素外的植物调节物质中的主要类群之一[42]。其与生长素在生理活性和结构上都不同[57]，最早鉴定出的赤霉素是赤霉酸[80]。赤霉素广泛参与植物生长发育的各个阶段，在种子萌发到衰老过程中，赤霉素打破种子休眠，促进种子发芽、细胞伸长、叶片增大以及开花结果，使作物提早成熟[52,70]。此外，许多研究集中在赤霉素对微藻生长和代谢产物的影响上，有大量的研究表明赤霉素能够促进微藻的生长，并对蛋白质、脂质、糖类、叶绿素、总类胡萝卜素等必需代谢产物的产生有积极影响(表 4)。

1.4.3 细胞分裂素

细胞分裂素在植物体内具有多种生理作用，包括刺激细胞分裂、分化、生长和发育，调节种子休眠和萌发，促进侧芽发育、延缓叶片衰老，防止植物早衰及花果脱落等[37,73]。然而，细胞分裂素不仅刺激植物的生长和发育，而且还会影响微藻的生长与代谢，对初级及次级代谢产物(如蛋白质、脂质、糖类以及核酸等)的产生有积极作用(表 4)，因此，细胞分裂素也是调节微藻细胞生长和生物产物积累的一类重要的添加剂。

1.4.4 乙烯

乙烯是一种气态植物激素，参与调节植物的一系列生理过程，尤其是对植物的生长发育、成熟、衰老以及果实成熟、脱落和对生物(病原体入侵)和非生物胁迫(包括干旱、高盐和寒冷)等环境因素的耐受方面均起重要的生理作用[75-76]。长期以来，乙烯一直被认为是一种生长抑制剂，抑制许多植物开花结果，但越来越多的证据表明，乙烯也可以促进生长和生物合成，这主要是由剂量决定的[75]。据报道，乙烯作为添加剂对微藻生长也具有显著的刺激作

用，可以诱导其产生有价值的化学物质，如色素、蛋白质和脂肪酸等(表 4)，长期以来，通过外源添加乙烯来正向调节微藻细胞生长和高价值生物产物积累一直是大规模工业应用提高生产力的一种替代策略。

1.4.5 脱落酸

脱落酸是植物五大天然生长调节剂之一，参与调节了植物的一系列生理过程，包括抑制细胞分裂、分化、生长和发育，引起叶、花和果实的脱落，刺激芽进入休眠状态，增加植物对高温、干旱、严寒、盐胁迫和水涝等环境胁迫的耐受性，是一种抑制生长的植物激素[41,78]。然而，脱落酸的添加却能显著刺激包括莱茵衣藻和小球藻在内的多种微藻的生长与代谢[52,57]，使其生物量、蛋白质、脂质、糖类和色素的产量显著提高(表 4)。

1.5 胺类

胺类是一类具有生物活性的低分子量脂肪族含氮碱，参与调节了植物的一系列生理过程，包括植物的生长发育和代谢以及以信号分子的形式调节植物对严寒、干旱、水涝和盐胁迫等环境胁迫的耐受性，是细胞生长和代谢的重要调节物质[81-82]。同时，细胞内的蛋白质、核酸、许多激素和生物碱等都是胺的衍生物，在细胞生命活动中扮演着重要的角色[83]。基于此，胺类作为重要的外源添加剂在藻类细胞中的作用受到越来越多的关注和研究，有大量的研究表明胺类可以调节微藻的生长与代谢以及初级和次级代谢产物的产量(表 5)。

1.6 微量元素

微量元素是一类含量极其微小但却具有强大的生物学作用，是机体正常生命活动所不可或缺的一类物质。在海洋藻类的生长过程中，微量元素是藻类细胞本身和藻类细胞酶活性中心不可或缺的组成部分[66,84]；同时，微量元素对微藻的细胞密度、脂肪酸含量以及脂质积累也有一定的影响[85]，是微藻脂质形成过程中不可缺少的一类物质，适当浓度的微量元素可促进细胞生长、脂质形成以及色素合成，但浓度过高会对微藻产生毒害作用(表 6)。

表 5 胺类对微藻生长和高价值生物产物积累的影响

Table 5 Effects of amine on growth and accumulation of high-value bioproducts in microalgae

Additive names	Microbial species	Products	Mechanism analysis	References
Ethanolamine (ETA)	*Scenedesmus obliquus*	Lipid(83.3%)	Stimulate metabolism	[81]
	Crypthecodinium cohnii	Lipid(18.8%)	Stimulate metabolism	[22]
Putrescine Spermidine	*Chlorella vulgaris*	Biomass(+), protein(+), saccharide(+), chlorophyll(+)	\	[38,82]
Spermine Agmatine	*Chlorella vulgaris*	Cell numbers(+), chlorophyll(+), protein(+), saccharide(+)	\	[82]

"+" represents that exogenous additives have a promoting effect on the accumulation of microalgae high-value bioproducts; "\" represents no positive effect, and the specific number represents the percentage increase in the accumulation of microalgae high-value bioproducts compared with the control.

表 6　微量元素对微藻生长和高价值生物产物积累的影响
Table 6　Effects of trace elements on growth and accumulation of high-value bioproducts in microalgae

Additive names	Microbial species	Products	Mechanism analysis	References
Ferrum (Fe^{2+}/Fe^{3+})	*Nannochloropis oculata*	Lipid (48.4%)	\	[86]
	Chlorella vulgaris	Biomass (+), lipid (+), chlorophyll (+)	\	[38,87]
	Thraustochytrids	Biomass (63.3%)	\	[84]
	Microcystis aeruginosa	Biomass (+)	\	[88]
	Haematococcus pluvialis	Astaxanthin (+), carotenoid (+)	\	[17]
	Chlorococcum sp.	Astaxanthin (+)	\	[18]
Cuprum (Cu^{2+})	*Nannochloropis oculata*	\	\	[86]
	Thraustochytrids	Biomass (69.1%)	\	[84]
Cobalt (Co^{2+})	*Nannochloropis oculata*	\	\	[86]
	Thraustochytrids	Biomass (24.4%)	\	[84]
Molybdenum (Mo^{6+})	*Nannochloropis oculata*	Lipid (46.1%)	\	[86]
	Thraustochytrids	Biomass (24.4%)	\	[84]
Zinc (Zn^{2+})	*Nannochloropis oculata*	Lipid (121.7%)	\	[86]
	Thraustochytrids	Biomass (49.6%)	\	[84]
	Microcystis aeruginosa	Biomass(+)	\	[88]
Manganese (Mn^{2+})	*Nannochloropis oculata*	Lipid (8.4%)	\	[86]
	Thraustochytrids	Biomass (69.1%)	\	[84]
Plumbum (Pb) Cadmium (Cd)	*Chlorella vulgaris*	\	\	[66]
Nickel (Ni)	*Thraustochytrids*	Biomass (17.4%)	\	[84]
Magnesium (Mg^{2+})	*Haematococcus pluvialis*	Astaxanthin (29.9%)	Stimulate metabolism	[16]
	Thraustochytrids	DHA (+), biomass (+), lipid (+)	\	[89]
Calcium (Ca^{2+})	*Thraustochytrids*	DHA (+), biomass (+), lipid (+)	\	[89]

"+" represents that exogenous additives have a promoting effect on the accumulation of microalgae high-value bioproducts; "\" represents no positive effect, and the specific number represents the percentage increase in the accumulation of microalgae high-value bioproducts compared with the control.

1.7　其他化学物质

除了以上具有鲜明结构、功能特征的抗氧化剂、氧化剂、信号转导剂、胺类以及植物激素和类似物外，包括化学药物、醇类、酸类、无机盐类、烷烃类、多环芳烃类、有机磷类和油脂类物质等在内的其他化学物质作为重要的一类外源添加剂也能够诱导微藻的生长和高价值生物产品的积累(表 7)。

2　外源添加剂对微藻产物的影响

微藻的细胞化学成分包括碳水化合物、脂质、蛋白质、类胡萝卜素和核酸，此外，它还含有多种有机成分，如维生素、矿物质和其他次生代谢产物化合物等。如今，微藻被认为是生产许多生物质产品的细胞工厂，如脂质、碳水化合物、蛋白质和色素等，更重要的是外源添加剂的添加可以显著地改变微藻细胞生长和/或各种高附加值生物产品的产量，对微藻的影响是广泛而深远的。因此，本文系统总结了外源添加剂对微藻生物量、脂质积累、蛋白质含量、色素含量、碳水化合物含量以及核酸含量的影响。

表 7 其他化学物质对微藻生长和高价值生物产物积累的影响

Table 7 Effects of other chemicals on growth and accumulation of high-value bioproducts in microalgae

Additive types	Additive names	Microbial species	Products	Mechanism analysis	References
Drugs	Calliterpenone	*Cyanobacterium synechocystis*	Biomass (316.1%), lipid (130.8%), carbohydrate (140.3%)	Transcriptional regulation	[80,90]
	Indomethacin (IM)	*Chlorella vulgaris*	Biomass (70.0%), nucleic acid (60.0%), protein (43.0%), chlorophyll (>90.0%), carotenoid (70.0%), saccharide (100.0%)	\	[91]
	Vinblastine	*Haematococcus pluvialis*	Astaxanthin (+)	\	[92]
	Ethyl 2-methylacetoacetate (EMA)	*Selenastrum capricornutum*	Biomass (+)	\	[93]
Alcohols	Methanol	*Chlorella vulgaris*	Biomass (69.0%), chlorophyll a (160.0%)	\	[38]
	Ethanol	*Crypthecodinium cohnii*	DHA (+), biomass (+), lipid (+)	\	[94]
Acids	Acetic acid	*Crypthecodinium cohnii*	Biomass (134.6%), lipid (260.0%)	\	[95]
		Aurantiochytrium sp.	\	\	[96]
	Propanoic acid	*Aurantiochytrium* sp.	\	\	[96]
	Isobutyric acid	*Aurantiochytrium* sp.	\		
	Butyric acid	*Aurantiochytrium* sp.	\		
	Isovaleric acid	*Aurantiochytrium* sp.	DHA (46.7%)		
	Valeric acid	*Aurantiochytrium* sp.	DHA (74.0%)		
	Humic acid (HA)	*Chlorella vulgaris*	Biomass (72.0%), chlorophyll a (160.0%)	\	[38]
	Acrylic acid	*Crypthecodinium cohnii*	\	\	[13]
	Oleic acid	*Thraustochytrium aureum*	Biomass (~9.2%), lipid (~9.5%)	\	[97]
	Palmitic acid	*Thraustochytrium aureum*	DHA (36.8%), lipid (24.1%)		
	Stearic acid	*Thraustochytrium aureum*	Lipid (12.5%)		
	Ethylene diamine tetraacetic acid (EDTA)	*Nannochloropis oculata*	Biomass (+), lipid (49.7%)	\	[86]
	Glutamate	*Aurantiochytrium* sp.	Lipid (90.9%)	\	[98]
Inorganic salts	Sodium chloride	*Spirulina platensis*	\	\	[28]
		Haematococcus pluvialis	Astaxanthin (2.2 times), lipid (24.1%), carbohydrate (23.1%)	\	[92]
	Citrate	*Chlorella vulgaris*	Biomass (5.6 times), lipid (+), chlorophyll a (+)	\	[99]
		Scenedesmus sp.	Biomass (+)	\	[100]
	Acetate	*Chlorella vulgaris*	Lipid (+), biomass (7.0 times)	\	[99,101]

(待续)

(续表 7)

Additive types	Additive names	Microbial species	Products	Mechanism analysis	References
		Haematococcus pluvialis	Biomass (25.5%), carotenoid (100.0%), astaxanthin (110.5%)	\	[17,102-103]
		Monodus subterraneus	Fatty acid (100.0%), lipid (5.0 times)	\	[104]
		Aurantiochytrium sp.	DHA (33.3%)	\	[98]
		Haematococcus pluvialis	Chlorophyll (66.7%), protein (32.0%)	\	[92]
	Nitrate	*Haematococcus pluvialis*	Carotenoid (118.9%), carbohydrate (+), protein (59.0%), lipid (39.2%), astaxanthin (21.4%), chlorophyll (42.9%), biomass (3.0 times)	\	[92,102]
	Malonate	*Haematococcus pluvialis*	Biomass (27.3%), carotenoid (13.0 times), astaxanthin (2.0 times)	\	[102]
Alkanes	Dodecane	*Crypthecodinium cohnii*	DHA (8.3%)	\	[105]
Polycyclic aromatic hydrocarbons	Paraquat	*Scenedesmus quadricauda*	\	\	[47]
	Norflurazon	*Crypthecodinium cohnii*	\	\	[13]
Organic phosphorus	Glyphosate	*Scenedesmus quadricauda*	Biomass (+), chlorophyll a (10.7%)	\	[47]
	Fenitrothion	*Chlamydomonas reinhardtii*	Biomass (+), chlorophyll a (+)	\	[49]
	Diazinon Dimethoate Malathion Phenthoate Quinalphos	*Chlamydomonas reinhardtii*	\		
Oil class	Linseed oil	*Thraustochytrium aureum*	Biomass (44.7%), lipid (52.7%)	\	[106]
	Glycerol	*Chlorella vulgaris*	Biomass (+), lipid (+)	\	[101]
	Polysorbate 80	*Thraustochytrium aureum*	DHA (107.6%), biomass (88.9%), lipid (15.2%), fatty acid (25.3%)	\	[107]
	Vegetable oil	*Thraustochytrium aureum*	Biomass (12.3%), lipid (72.6%)	\	[97]

"+" represents that exogenous additives have a promoting effect on the accumulation of microalgae high-value bioproducts; "\" represents no positive effect, and the specific number represents the percentage increase in the accumulation of microalgae high-value bioproducts compared with the control.

2.1 外源添加剂对微藻生物量的影响

生物量是评价利用微藻生产高价值生物产物以及进行大规模扩大生产的一个最基础也是最重要的指标，丰富的生物量可以弥补微藻中高价值产物含量低的不足，并且生物量的高低直接决定了高价值生物产物产量的高低。更重

要的是，外源添加剂对微藻的生长具有显著的影响，比如，Ma 等[108]发现随着奥利司他添加浓度的增加，宿主菌株的 DCW 显著增加，在 1 000 mg/L 奥利司他的作用下，其 DCW 是对照的 1.25 倍。同样，在培养基中添加 0.5 mmol/L 芝麻酚显著促进了隐甲藻的生长，使其生物量浓度比对照提高了 44.2%[7]。更重要的是，Park 等[52]研究发现除了脱落酸外，其他 4 种植物激素，吲哚-3-乙酸、赤霉素、激动素和 1-三十烷醇，均显著促进了莱茵衣藻的生长，在其最佳浓度下，分别使生物量增加了 61.0%、68.0%、69.0%和 54.0%。综上，外源添加剂能够显著促进微藻生长。

2.2 外源添加剂对微藻脂质积累的影响

由于化石燃料的日益枯竭、不可再生，全球对可再生和可持续能源的需求日益增加，生物燃料以其可再生、无毒、可生物降解等优势被公认为是一种环境友好的燃料替代来源，而微藻脂质作为生物燃料的重要原料，其产量的提升就显得尤为重要。有大量的研究表明外源添加剂对藻类脂质积累有积极的影响，据报道，大叶紫珠萜酮的添加能够显著促进蓝藻中脂质的积累，在其最佳添加浓度下，脂质产量相对于对照提高了 130.76%[90]。在 Ma 等[10]研究中也出现了类似的现象，即槲皮素的使用提高了总脂质含量，与对照相比，在 15 μg/L 槲皮素处理下小球藻的脂质含量增加了 1.8 倍。同样，Cheng 等[81]用 2 mmol/L 外源乙醇胺处理斜生栅藻，发现斜生栅藻的总脂质含量从对照组的 12.0%提高到 22.0%。而脂肪酸作为微藻脂质的主要成分，其组成和产率已被用作生产高价值化合物的潜在指标。目前的研究表明，外源添加剂可以诱导微藻高价值脂肪酸 DHA 的产生和积累。Yu 等[65]研究表明，在 4 mg/mL 赤霉素处理下，破囊壶菌 YLH 70 的二十二碳六烯酸产量显著增加，与对照相比，提高了 79.1%。同样，吐温 80 的使用对脂肪酸组成也有显著影响，在 1.0%吐温 80 的处理下，破囊壶菌的总脂肪酸含量从 177.6 mg/g 显著增加到 222.5 mg/g，二十二碳六烯酸产量从 118 mg/L 显著增加到 245 mg/L[107]。综上，基于外源添加剂进行调控是提高微藻脂质积累和高价值脂肪酸产量的一种有益的替代策略。

2.3 外源添加剂对微藻蛋白质含量的影响

蛋白质是组成细胞的主要有机物，具有催化、免疫和信息传递等功能，是生命活动的主要承担者，因此其含量的高低对于细胞的正常生命活动至关重要。而基于外源添加剂进行调控这一方法也早已应用于微藻的蛋白质生产之中，如在培养基中单独添加外源性油菜素甾醇 (brassinosteroids, BRs)可使小球藻细胞的蛋白质水平提高 33.0%–133.0%，而在 50 μmol/L 吲哚-3-乙酸、吲哚-3-丙酸和吲哚-3-丁酸的处理下小球藻细胞的蛋白质含量分别提高了 57.0%、38.0%和 13.0%，更重要的是，吲哚-3-乙酸和油菜素甾醇的组合添加使藻类细胞中蛋白质含量增加了 3.0 倍[30]。同样，与对照相比，在 0.1 mg/L 吲哚-3-乙酸、1 mg/L 赤霉酸、0.1 mg/L 激动素、0.1 mg/L 1-三十烷醇和 3 mg/L 脱落酸的处理下，莱茵衣藻的蛋白质含量分别增加了 35.0%、26.0%、25.0%、44.0%和 32.0%[52]。较低浓度的吲哚美辛对蛋白质的积累也有显著的促进作用，在 10^{-7} mol/L 吲哚美辛的处理下，小球藻细胞中的水溶性蛋白质含量比对照组提高了 43.0%[90-91]。因此，基于外源添加剂进行调控也是提高微藻蛋白质积累的一种有前景的替代策略。

2.4 外源添加剂对微藻色素含量的影响

虾青素是类胡萝卜素中的一种，是一类重

要的天然色素，具有许多重要的代谢功能，包括通过清除氧自由基增强免疫反应和预防癌症等疾病，而角鲨烯是一类具有高价值的萜类化合物，二者均具有抗氧化、免疫调节、抗癌、延缓衰老等功效，由于其极高的营养价值、药用价值和良好的生物活性而广泛应用于食品药品及化妆品等行业领域。因此，类胡萝卜素和角鲨烯的产量直接关系到其经济价值。更重要的是，外源添加剂策略对微藻高价值色素的积累具有显著的促进作用，据报道，黄腐酸的添加诱导了雨生红球藻虾青素的积累，与对照组相比，在 5 mg/L 和 10 mg/L 黄腐酸的处理下，虾青素产量分别增加了 86.89%和 9.78%[15]。而用活性最高的吲哚美辛浓度处理藻类可使小球藻细胞中的类胡萝卜素含量增加 70.0%，褐藻素含量增加 70.0%–140.0%[91]。氧化剂过氧化氢、2,2′-偶氮二(2-甲基丙基咪)二盐酸盐、亚甲基蓝和甲基紫精在其最佳浓度下，可使雨生红球藻中虾青素的产量分别提高 20.60%、29.17%、41.91%和 32.87%，信号转导剂水杨酸、茉莉酸和脱落酸在其最佳浓度下，也可使虾青素积累分别提高 30.55%、50.56%和 64.21%，此外，赤霉酸和金属离子 Mg^{2+}在各自的最优有效浓度下，使虾青素积累也分别提高了 30.82%和 29.87%[16]。因此，外源添加剂对于提高微藻高价值色素的产生和积累具有显著的促进作用。

2.5 外源添加剂对微藻叶绿素含量的影响

叶绿素是绿色植物和其他能进行光合作用的生物体进行光合作用时所必不可少的一类重要的天然色素。此外，它还是叶绿体的主要组成部分，主要包括叶绿素 a 和叶绿素 b，其含量的高低直接关系到光合作用效率的高低。因此，尽可能地提高叶绿素的含量就显得尤为重要。Falkowska 等[66]研究发现外源添加赤霉酸可使小球藻的叶绿素 a 和叶绿素 b 含量分别增加 64.0%和 42.0%。而 Park 等[52]研究表明所有 5 种测试激素均能提高叶绿素(a+b)的浓度，与对照相比，在吲哚-3-乙酸、赤霉酸、激动素、1-三十烷醇和脱落酸的处理下，莱茵衣藻的叶绿素(a+b)的浓度分别增加了 81.0%、68.0%、59.0%、43.0%和 39.0%。同样，生长素前体或类似物邻氨基苯甲酸、色胺、2,4-二氯苯氧乙酸、苯乙酸、萘-3-乙酸、萘-3-磺酸和吲哚乙酸的添加使用均能显著提高蛋白核小球藻的叶绿素(a+b)含量，使其含量比对照增加了 113.0%–173.0%[39]。因此，基于外源添加剂进行调控微藻叶绿素的积累无疑是一种有益的替代策略，可以改善叶绿素的积累，从而提高基于微藻生产高价值生物产物的能力。

2.6 外源添加剂对微藻碳水化合物含量的影响

碳水化合物在生命活动过程中起着重要的作用，是生命活动所需能量的主要来源，可分为糖、寡糖和多糖 3 大类，其中糖又分为单糖、双糖和糖醇。因此，碳水化合物含量的高低直接关系到生物体能否进行正常的生命活动。而如何提高碳水化合物的含量就显得尤为重要。Patel 等[90]研究发现大叶紫珠萜酮的添加能够显著促进蓝藻中碳水化合物的产生，在其最佳添加浓度下，其碳水化合物产量相对于对照提高了 140.3%。而在 10^{-8}–10^{-6} mol/L 较低浓度的茉莉酸处理下，小球藻细胞的单糖含量比对照增加了 5%–33.8%，其中，在 10^{-6} mol/L 茉莉酸的影响下，单糖含量增加最多，增加了 33.8%[26]。同样，在 Park 等[52]的研究中也观察到了类似的现象，即脱落酸处理也使莱茵衣藻的淀粉含量增加了 2 倍左右(从 2.2%增加到 6.0%)。综上，基于外源添加剂进行调控对于微藻碳水化合物的积累表现出显著的促进作用。

2.7 外源添加剂对微藻核酸含量的影响

核酸是遗传信息的携带者，在生物体的遗传、变异和蛋白质的生物合成中具有极其重要的作用，主要包括脱氧核糖核酸(deoxyribonucleic acid, DNA)和核糖核酸(ribonucleic acid, RNA)，是生物体进行正常生命活动所不可或缺的物质。更重要的是，基于外源添加剂进行调控对微藻核酸含量具有积极的影响，例如，水杨酸处理可诱导小球藻细胞中 DNA 和 RNA 含量的增加，在 10^{-4} mol/L 水杨酸的处理下，与对照相比，小球藻的 DNA 和 RNA 含量增加了 160.0%[25]。同样，吲哚美辛处理也可诱导了 DNA 和 RNA 含量增加，在 10^{-7} mol/L 吲哚美辛的处理下，与对照相比，小球藻的 DNA 含量增加了 44.0%–48.0%，RNA 含量增加了 52.0%–60.0%[91]。而 Piotrowska 等[73]研究发现外源添加 N^6-苄基腺嘌呤、激动素、反式玉米素和 *N,N'*-二苯基脲等细胞分裂素可使小球藻的 RNA 水平大幅提高，在 *N,N'*-二苯基脲处理下，RNA 含量达到最高值，比对照增加了 125.0%，相反，添加反式玉米素、激动素或 N^6-苄基腺嘌呤对 RNA 积累的影响稍小，可使 RNA 积累增加 80.0%–110.0%。因此，基于外源添加剂来改善微藻核酸的积累是一种很好的选择。

综上所述，微藻对外源添加剂的添加产生不同的反应，包括同一菌株对不同的添加剂反应不同，相同的添加剂对不同的菌株影响也不同，这就导致了外源添加剂对微藻生物产物影响的多样性。更重要的是，外源添加剂为微藻的定制高价值产物的生产提供了一种有益的替代策略，即根据生产需求，针对特定的菌株，通过加入特定的添加剂来提高特定产物的产量。

3 作用机制

近年来，外源添加剂包括植物激素和类似物以及抗氧化剂、氧化剂、信号转导剂、胺类、维生素和微量元素已被证实是促进海洋微藻生长和高价值生物产品积累的有效刺激因子。但关于外源添加剂相关作用的分子机制研究甚少，只有少数的几篇报道涉及其可能的促进机制，且并没有通过基因工程来敲除或过表达相关差异表达基因来加以验证。而目前用于探究微藻分子作用机制的手段主要包括基因组学、转录组学、蛋白质组学以及代谢组学。通过组学技术能够进一步认识微藻的生理过程、基因功能和代谢机制及其在合成高价值生物产物方面的应用，为定向改造微藻提供理论基础。而不同的添加剂根据其化学结构以及种类的不同可能直接作为合成高附加值生物产物的前体，参与转录调控或刺激代谢途径，显著影响微藻高价值生物产物的积累(表 1–表 7)。根据已有研究及其促进机制，可将其概括为 4 类：提高乙酰辅酶 A 和/或还原型辅酶II (nicotinamide adenine dinucleotide phosphate, NADPH)的供应、诱导氧化应激反应、对中心碳代谢的影响以及对相关代谢途径的调控(图 1)。

3.1 提高乙酰辅酶 A 和/或 NADPH 的供应

乙酰辅酶 A 是脂肪酸合成的前体物质，NADPH 为脂肪酸合成提高还原力，腺嘌呤核苷三磷酸(adenosine triphosphate, ATP)为脂肪酸合成提供能量，因此充足的乙酰辅酶 A、ATP 和 NADPH 分别作为底物、能量来源和还原剂，是产生脂质积累的 3 个关键因素[96]。乙酰辅酶 A 羧化酶是脂肪酸合成的限速酶，催化乙酰辅酶 A 转化为丙二酰辅酶 A，丙二酰辅酶 A 是合成脂肪酸的底物，而苹果酸酶和葡萄糖-6-磷酸脱氢酶是脂肪酸合成所需 NADPH 的主要来源，磷酸烯醇丙酮酸羧化酶负责转运乙酰辅酶 A 用于合成脂肪酸(图 1)。因此，其活性的高低直接影响了脂肪酸产量的高低。更重要的是，许多

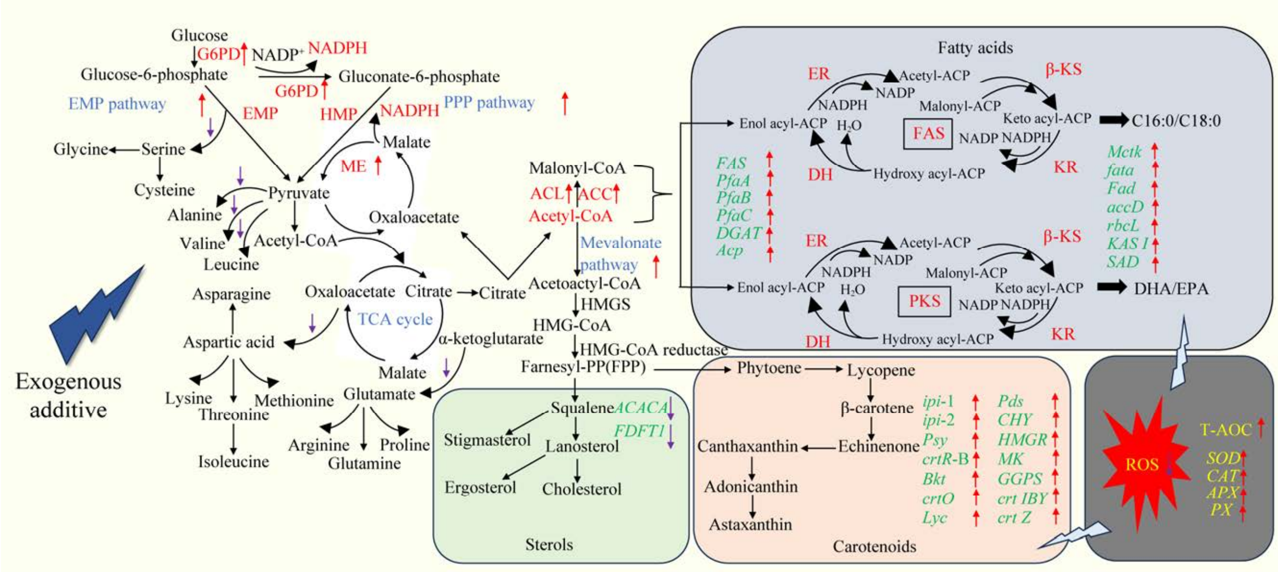

图 1　外源添加剂对微藻高价值生物产物积累影响的分子机制示意图　G6PD：葡萄糖-6-磷酸脱氢酶；ACC：乙酰辅酶 A 羧化酶；ACL：ATP-柠檬酸裂解酶；ME：苹果酸酶；EMP pathway：糖酵解途径；TCA cycle：三羧酸循环；PPP pathway：磷酸戊糖途径；ER：烯酰基 ACP 还原酶；β-KS：酮酰基 ACP 合成酶；KR：酮酰基 ACP 还原酶；DH：脱水酶；FAS：脂肪酸合成酶途径；PKS：聚酮合酶途径；MVA pathway：甲羟戊酸途径。

Figure 1　The molecular mechanism diagram of the effect of exogenous additives on the accumulation of high-value biological products in microalgae. G6PD: Glucose-6-phosphate dehydrogenase; ACC: Acetyl-CoA carboxylase; ACL: ATP-citrate lyase; ME: Malic enzyme; EMP pathway: Embden-meyerhof-parnas pathway; TCA cycle: Tricarboxylic acid cycle; PPP pathway: Pentose phosphate pathway; ER: Enoyl reductase; β-KS: β-ketoacyl synthase; KR: β-ketoacyl reductase; DH: Dehydratase; FAS: Fatty acid synthetase; PKS: Polyketide synthase; MVA pathway: Mevalonate pathway.

外源添加剂通过影响相关关键酶的活性来加强乙酰辅酶 A 和/或 NADPH 的供应来增加微藻的脂质积累。例如，有研究表明黄腐酸通过改变单针藻 FXY-10 细胞内乙酰辅酶 A 羧化酶、苹果酸酶和磷酸烯醇丙酮酸羧化酶的酶活性，显著增加了单针藻 FXY-10 的脂质积累[14]，而 Sivaramakrishnan 等[57]研究发现 4 种植物激素(玉米素、吲哚乙酸、赤霉酸和脱落酸)处理均提高了小球藻乙酰辅酶 A 羧化酶的活性，其中吲哚乙酸和脱落酸处理的乙酰辅酶 A 羧化酶活性最高，从而导致脂质积累和脂肪酸产量的显著增加。因此，在整个发酵过程中，由外源添加剂引起的这些变化说明了由组合添加策略引起的相关的脂质过量生产的潜在分子变化，为通过利用分子生物学方法和手段提高微藻的脂质产量提供了合适的靶点。

3.2　诱导氧化应激反应

ROS 通常被认为是氧化应激的细胞毒性诱导剂，但最近的研究表明 ROS 也可作为信号分子去启动许多生物产物的生产和积累，从而改善细胞生长和生理反应[16]。而与 ROS 氧化应激相关的抗氧化酶分别为超氧化物歧化酶、过氧化氢酶和抗坏血酸过氧化物酶等，外源添加剂通过改善相关抗氧化酶的活性来增强氧化应激

能力，从而显著促进微藻生物产物的积累。例如，Sivaramakrishnan 等[57]研究发现 ROS 的产生会对小球藻细胞造成氧化应激，然而在添加 4 种植物激素(玉米素、吲哚乙酸、赤霉酸和脱落酸)之后，氧化应激反过来又提高了抗氧化酶超氧化物歧化酶和过氧化氢酶的水平，这表明植物激素处理的小球藻通过提高抗氧化酶活性来应对 ROS，从而提高了生物量和脂质积累。同样，也有研究表明添加芝麻酚的隐甲藻在培养 24 h 时细胞内 ROS 水平降低了 63.5%，在 48 h 时后仍显著降低，从而促进了细胞的生长[7]。类似地，水杨酸和茉莉酸甲酯的添加均提高了雨生红球藻中抗氧化酶超氧化物歧化酶和过氧化物酶的活性，表明水杨酸和茉莉酸甲酯可以大大提高细胞抗氧化能力以抵抗氧化损伤，从而使类胡萝卜素含量显著增加[24]。而 Ma 等[10]研究指出槲皮素可以显著降低细胞中 ROS 的积累，这对于小球藻的脂质积累增加是有利的。同时，在脱落酸的处理下，莱茵衣藻细胞中的过氧化氢酶和抗坏血酸过氧化物酶这两种抗氧化酶的活性显著高于未处理的对照，从而增强对氧化胁迫的耐受性以及有助于清除 ROS，从而使生物量显著增加[78]。同样，有研究指出过氧化氢的添加使钝顶螺旋藻的抗氧化酶(即过氧化氢酶、过氧化物酶、抗坏血酸过氧化物酶和超氧化物歧化酶)的活性显著增加，从而提高了细胞的抗氧化能力，明显地保护了类胡萝卜素不被氧化，从而使细胞中类胡萝卜素含量显著增加[21]。综上，外源添加剂通过改善细胞的氧化应激能力，从而显著促进了微藻生物产物的积累。

3.3 对中心碳代谢的影响

一切生物的正常生命活动都靠代谢的正常运转来维持，微藻的代谢途径异常复杂，其中，中心碳代谢途径是生物体中最核心、最重要的代谢系统之一，包括糖酵解途径、三羧酸循环途径和磷酸戊糖途径，并且它涉及生物有机物的合成和降解，因此代谢途径的轻微变化会引起代谢产物产量的显著变化(图 1)。例如，Liu 等[7]研究表明在芝麻酚存在的情况下，由于苹果酸酶的抑制，NADPH 的产生减少，导致这些从头合成的饱和和单不饱和脂肪酸的积累减少，从而引起隐甲藻中脂肪酸前体的积累，另一方面，前体物质通过非依赖性去饱和酶的聚酮合成酶途径重定向到 DHA 的生物合成，这需要更少的 NADPH，从而导致 DHA 含量的显著增加。Li 等[22]基于靶向液相色谱-质谱联用仪(liquid chromatograph mass spectrometer, LC-MS)代谢组学分析指出隐甲藻中糖酵解和三羧酸循环代谢的增强以及磷酸戊糖途径代谢的减弱可能是隐甲藻脂质生物合成增加的重要原因。Yu 等[65]报道了赤霉素处理加快了破囊壶菌 YLH70 对葡萄糖的利用速率，并利用碳源生产其他代谢产物，使脂肪酸生物合成和甲羟戊酸途径中代谢物增加，而糖酵解和三羧酸循环中的代谢物减少，从而使脂质积累增加。同样，添加 6-苄氨基嘌呤能加快破囊壶菌 YLH70 对葡萄糖的利用速率，促进从糖酵解、三羧酸循环和甲羟戊酸途径到脂肪酸生物合成的代谢通量，从而促进脂质的积累[74]。综上，外源添加剂通过对中心碳代谢途径的调控可以显著影响微藻代谢产物形成过程中碳代谢通量的流向，从而可以显著改变代谢产物的产量。

3.4 对相关代谢路径的调控

代谢途径的调控是细胞内复杂的网络调控系统，关系着代谢产物的生成和降解，通过调节酶的活性、基因表达以及信号传导途径，实现对代谢途径的调控。而基因是遗传物质的基础，控制着遗传信息的传递和表达，通过操纵基因的表达水平和蛋白质合成，可以直接实现

对生物体性状和表型的精确控制。例如，Gao 等[23]研究发现 25 mg/L 和 50 mg/L 水杨酸均能提高雨生红球藻中 8 个类胡萝卜素基因的转录表达，在水杨酸处理条件下，虾青素的生物合成在转录水平上主要受 *ipi*-1、*ipi*-2、*psy*、*crt*R-B、*bkt*、*crt*O、*lyc* 和 *pds* 基因上调的调控，虾青素的高水平积累很可能是由于这 8 个类胡萝卜素基因的上调表达。同样，在黄腐酸的诱导下，雨生红球藻中 2 个虾青素生物合成基因 *PDS* 和 *CHY* 的转录水平也有所提高，表明高水平的虾青素积累可能与黄腐酸诱导的虾青素生物合成基因的上调相关[15]。而 Sivaramakrishnan 等[57]报道了小球藻脂肪酸生物合成基因 *acp*、*mctk* 和 *fata* 在 4 种植物激素(玉米素、吲哚乙酸、赤霉酸和脱落酸)处理后均上调表达，此外，*fad* 被吲哚-3-乙酸高度上调，对应于 ω-3 脂肪酸产量的显著增加。此外，Cui 等[12]研究发现在褪黑素的诱导下，与胶树小球藻脂质生物合成相关的关键基因(*accD*、*ME* 和 *rbcL*)的表达水平均上调，分别是对照组的 1.8 倍、1.6 倍和 1.9 倍，这对于脂质积累的显著增加是有利的。类似地，也有研究指出萘乙酸处理能有效诱导与脂肪酸合成相关基因(*KAS I* 和 *SAD*)的表达，其表达水平分别是对照的 2.0 倍和 1.6 倍，这与脂肪酸积累增加相一致[41]。因此，通过调控相关基因的转录表达水平来直接控制微藻的产物和产量无疑是一种很好的选择。

综上所述，不同的外源添加剂主要通过提高乙酰辅酶 A 羧化酶、苹果酸酶和葡萄糖-6-磷酸脱氢酶的活性从而增强乙酰辅酶 A 和/或 NADPH 的供应或者通过改善相关抗氧化酶(超氧化物歧化酶、过氧化氢酶和抗坏血酸过氧化物酶等)的活性来增强氧化应激能力，从而使 ROS 降低和 T-AOC 增加或者通过强化糖酵解途径、三羧酸循环途径和磷酸戊糖途径从而使微藻代谢产物形成过程中碳代谢通量导向目标产物的合成路径或者通过上调与脂肪酸合成相关的基因(*fas*、*pfaA*、*pfaB*、*pfaC*、*DGAT*、*Acp*、*Mctk*、*fata*、*Fad*、*accD*、*rbcL*、*KAS I* 和 *SAD*)或与虾青素合成相关基因(*ipi*-1、*ipi*-2、*Psy*、*crtR*-B、*Bkt*、*crt*O、*Lyc*、*Pds*、*CHY*、*HMGR*、*MK*、*GGPS*、*crt IBY* 和 *crt Z*)的表达从而显著提高微藻高价值生物产物的积累。尽管关于外源添加剂相关作用分子机制的研究还不够系统和完善，但根据上述特定的作用机制，选择合适的靶点，利用分子生物学方法和手段就可以选择性地提高微藻特定产物的产量，有利于开发出合适的细胞底盘。

4 经济可行性评价

虽然外源添加剂的使用能带来不同程度的促进效应，但是也会相应增加生产成本。因此，在进行工业化生产之前，必须从商业角度考虑添加外源添加剂对于利用微藻生产高附加值产品的生产成本。对表 1–7 中涉及的外源添加剂进行了初步的成本核算，但由于这类物质目前市场用量较小，很多甚至属于科研试剂类，价格普遍较高。例如，甲基紫精、茉莉酸、油菜素内酯、1-三十烷醇、吲哚-3-乳酸、邻氨基苯甲酸、噻苯隆、玉米素、脱落酸、胍丁胺和长春碱等物质的使用虽然会给微藻的生长和高价值生物产物的积累带来不同程度的促进效应，但由于它们的价格昂贵，在使用过程中会大幅增加生产成本，因此，未来进行大规模的工业化生产中需要慎重考虑生产成本问题。还有部分外源添加剂的使用会对环境造成污染，例如微量元素(铅、镉和镍等)、酸类物质(乙酸、丙酸和丁酸等)、多环芳烃类农药(百草枯和哒草伏)和有机磷类农药(草甘膦、杀螟硫磷、二嗪农、乐果、马拉硫磷、稻丰散和喹硫磷)由于在添加

使用过程中未被微藻充分利用，随着污水排放而残留在土壤或水体中，可能会对人类和动物造成伤害，为此在未来进行大规模的工业化生产中需要重点考虑后续污水处理成本问题。此外，部分外源添加剂(例如无机盐类物质，如氯化钠和氯化铵；氧化剂类物质，如过氧化氢和次氯酸钠)的使用还会对发酵设备——生物反应器造成腐蚀，这也无形中增加了设备的使用成本。最后，只有价格低廉、使用安全、经济可行和环境友好的外源添加剂才有可能在未来大规模的工业化生产中得到广泛应用，例如抗氧化剂(芝麻酚和槲皮素)、信号转导剂(水杨酸)、植物激素(1-萘乙酸、吲哚-3-乙酸、尿囊素和赤霉素等)、胺类(乙醇胺)和油脂类(甘油和吐温 80)物质等都是未来大规模工业化生产中极具潜力的外源性添加剂。而对于其他的化合物，还有待于从合成工艺和产品规模等方面进一步优化，来提高其使用的经济性，这也是未来希望得到加强的研究领域。

5 结语

近年来，研究人员已经对外源添加剂对微藻生长和高价值生物产品积累的影响进行了广泛的研究，尽管外源调节剂的单独添加可以显著促进微藻的生长和高价值生物产品的积累，但对外源调节剂的组合添加研究甚少，只有少数研究涉及简单的二维添加，但却取得了更大程度的提升效果。相信更高维度的组合添加(包括三维、四维等)一定可以取得更为显著的提升效果，但通过人工实验进行高维度的组合添加以及组合优化实验工作量巨大。同时，关于外源添加剂相关作用的分子机制报道甚少，只有少数的几篇文献涉及其可能的促进机制，且并没有通过基因工程来敲除或过表达相关差异表达基因来加以验证。

基于此，本课题组的研究重点集中在基于机器学习的外源化学调节剂组合添加实验(未发表数据)，即将单独添加获得的数据作为初始数据集代入模型，模型会反馈出推荐的三维、四维以及五维的添加剂组合，然后再通过将上述推荐的组合进行实际添加实验，获得具体的数据，然后再将这些数据代入模型，如此不断迭代重复，从而使模型不断优化，最终模型收敛于某一点，反馈出最优的添加剂组合。目前研究已经取得了四维组合添加的结果，可以使脂肪酸含量提高 50.0%左右。同时，将最优的三维、四维添加剂组合进行转录组分析，以寻求差异表达途径和差异表达基因，并通过基因工程敲除或过表达相关差异表达基因来加以验证，形成生物学闭环，并构建高产细胞底盘。

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