



废水培养微藻藻种遴选及其组分积累的研究进展

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摘要: 利用废水培养微藻能够降低微藻的培养成本, 同时削减污染。微藻的蛋白质、多糖和油脂等组分是影响其后续资源化利用的重要因素。本文重点综述了以废水为基质培养微藻的研究进展, 从组分积累的角度, 分析了微藻种类的选择依据, 探讨了影响微藻生长的因素和提高产量的方法, 并对藻体中组分的合成机制进行了讨论, 提出未来废水培养微藻技术面临的挑战和可能的解决方法。

关键词: 废水处理; 微藻培养; 筛选; 微藻组分; 强化方法

Research progress on species screening and organic component accumulation in microalgae cultivation with wastewater

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Abstract: Using wastewater to cultivate microalgae can reduce cultivation cost and pollution.

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Proteins, polysaccharides, and lipids of microalgae are major factors affecting the utilization of microalgae resources. The research progress on microalgae cultivation with wastewater as the substrate was reviewed. Centering on component accumulation, we expound the selection basis of microalgae species, the factors affecting microalgae growth, the methods to improve microalgae yield, and the synthesis mechanism of components in microalgae. Finally, we put forward the challenges and possible solutions for microalgae cultivation in the future.

Keywords: wastewater treatment; microalgae cultivation; screening; microalgae components; strengthening method

微藻是一类生长周期短的单细胞藻类, 其分布极其广泛, 具有重要的经济价值, 但其产业化过程面临微藻生长量低和培养成本高等问题。目前, 利用废水培养微藻是资源化领域的研究热点, 它不仅可以降低微藻的生产成本和土地占用, 还兼有消减污染的环境效益^[1]。微藻细胞组分是影响其资源化利用的重要因素, 通常与微藻种类有关。例如杜氏藻、四尾栅藻、布朗葡萄藻和小球藻含有较多的脂质积累^[2-5], 莱茵衣藻、伪鱼腥藻和螺旋藻中碳水化合物的积累显著^[6-8], 因此它们被广泛应用于生物燃料的开发。某些螺旋藻则含有较多的蛋白质组分, 可用于制备饲料和缓释肥料^[9]。对于同一藻种, 培养条件和废水组成亦对其生物量和组分积累产生影响。选择适合的藻种并优化培养工艺是实现微藻技术处理废水的基础与关键。

以废水为基质培养微藻具有良好的可持续性, 是国内外研究关注的焦点。在此背景下, 本文从微藻组分积累及微藻资源化利用的角度, 着重介绍了不同微藻的组分特性及其对废水的适应性, 综述了影响微藻生物量和组分积累的关键因素, 并探讨了强化培养微藻的工艺方法。最后, 总结了利用废水培养微藻技术的瓶颈和应用前景, 为微藻资源化利用技术的推广提供依据。

1 微藻种类的选择

1.1 以污染物削减为导向的微藻选择

微藻种类繁多, 应用于污水处理的藻种需满足以下标准: 首先, 微藻应有较强的污染物去除能力; 其次, 能耐受废水中的污染物(如重金属离子、高氨氮、高毒性化合物等); 最后, 采用诱变育种方式来改变微藻的遗传信息, 可获得优良性状的藻株^[10]。根据常见的污水类型, 表 1 列举了近几年废水处理中常用的微藻种类, 并归纳了微藻去除污染物的机理和应用场景。许多研究已经尝试采用微藻处理城市生活污水、畜禽养殖废水、食品工业废水和含金属离子的工业废水, 其中小球藻(*Chlorella*)和栅藻(*Scenedesmus*)由于其高生长速率、高环境耐受性而成为各类废水处理中最受欢迎的藻种。采用小球藻共培养模式处理啤酒厂废水时, 在适宜的工艺条件下, 废水中氮磷的去除率可达 90%以上^[11]。褐藻(如 *Ascophyllum nodosum*、*Fucus spiralis*、*Laminaria hyperborea* 和 *Pelvetia canaliculata*)则可以去除石化废水中的过渡金属离子^[12]。但污水中过高的金属离子也会抑制微藻生长, 且不同藻种对金属离子的耐受性也存在差异。同时微藻也可作为抗生素的非靶标生物, 对于去除农业养殖废水中的药物具有一定优势, 可降低抗性基因传播的风险。Qu 等^[13]对斜生栅藻进行离子诱变, 发现诱变后的微藻

表 1 常用于废水处理的微藻种类

Table 1 Microalgae species used in wastewater treatment

Microalgae species	Wastewater types	Compounds	Pollutant removal	Mechanisms	References	
<i>Chlorella</i>	<i>Chlorella vulgaris</i>	Domestic secondary effluent	COD, N, P, heavy metal	TN 87.7% TP 76.7%	Assimilation/ adsorption	[14]
		Brewery wastewater	COD, N, P, trace heavy metal	TN 90.6% TP 97.4%	NA	[11]
	<i>Chlorella pyrenoidosa</i>	Synthetic wastewater	COD, N, P	COD 83.6% TN 89.4% TP 91.4%	Assimilation/ precipitation	[15]
		Dairy wastewater	N, P, trace iron ions	TN 87.0% TP 3.0%	Assimilation	[16]
<i>Chlorella sorokiniana</i>	Heavy metal wastewater	Cu ²⁺ , Cd ²⁺ , As(III), As(V)	Cd ²⁺ 65.0%	Surface adsorption/ bioadsorption	[17]	
<i>Scenedesmus</i>	<i>Scenedesmus obliquus</i>	Pharmaceutical wastewater	Carbamazepine	28.0%	Biodegradation/ photolysis	[18]
		Brewery wastewater	COD, N, P	COD 61.9% TN 88.5% TP 40.8%	Assimilation	[19]
	<i>Scenedesmus quadricauda</i>	High saline piggery wastewater	COD, N, P, high salinity	COD 89.0% TN 88.0% TP 60.0%	Assimilation	[20]
	<i>Scenedesmus</i> LX1	The effluent of an electronic device factory	COD, N, P, trace heavy metal	TN 46.0% TP 100.0%	Bioaccumulation/ adsorption	[21]
<i>Chlamydomonas</i>	<i>Chlamydomonas mexicana</i>	Municipal wastewater	COD, N, P	Inorganic nutrients 91.8%	Assimilation	[22]
		Pharmaceutical wastewater	Carbamazepine	35.0%	Biodegradation/ photolysis	[18]
<i>Chlamydomonas debaryana</i>	Domestic secondary effluent	COD, N, P	NA	Assimilation	[23]	

NA: not available.

对城市生活污水中 TP、TN、NH₄⁺-N 和 COD 的去除率分别达到 95.72%、80.30%、87.25% 和 85.43%。

微藻可以通过同化、吸附、生物降解和光解等途径去除污染物,图 1 总结了微藻对废水中主要污染物的去除机理。废水中有机物、氮磷等营养物质在同化作用下被微藻吸收利用。有机物可参与藻细胞碳骨架的合成,微藻可利用的碳源种类主要包括有机碳源和无机碳源,分别对应微藻的自养培养模式和异养培养模式。氮是其蛋白质、叶绿素、遗传物质和能量

转移的主要来源,废水中铵氮、尿素、硝酸盐氮和亚硝酸盐氮均可被微藻吸收利用,而磷则主要以无机磷的形态被微藻同化利用,用于合成 ATP、磷脂、核苷酸和核酸等^[24]。微藻对污水中较低浓度的抗生素、农药和重金属等有毒物质的去除机制主要包括:①吸附作用。微藻可以吸附多数带正电的重金属离子^[25];②生物富集。活体微藻可将金属离子、抗生素等有毒物质吸收、储存在液泡中;③生物降解和光解;④活体微藻在细胞外将重金属积累或沉淀^[26]。

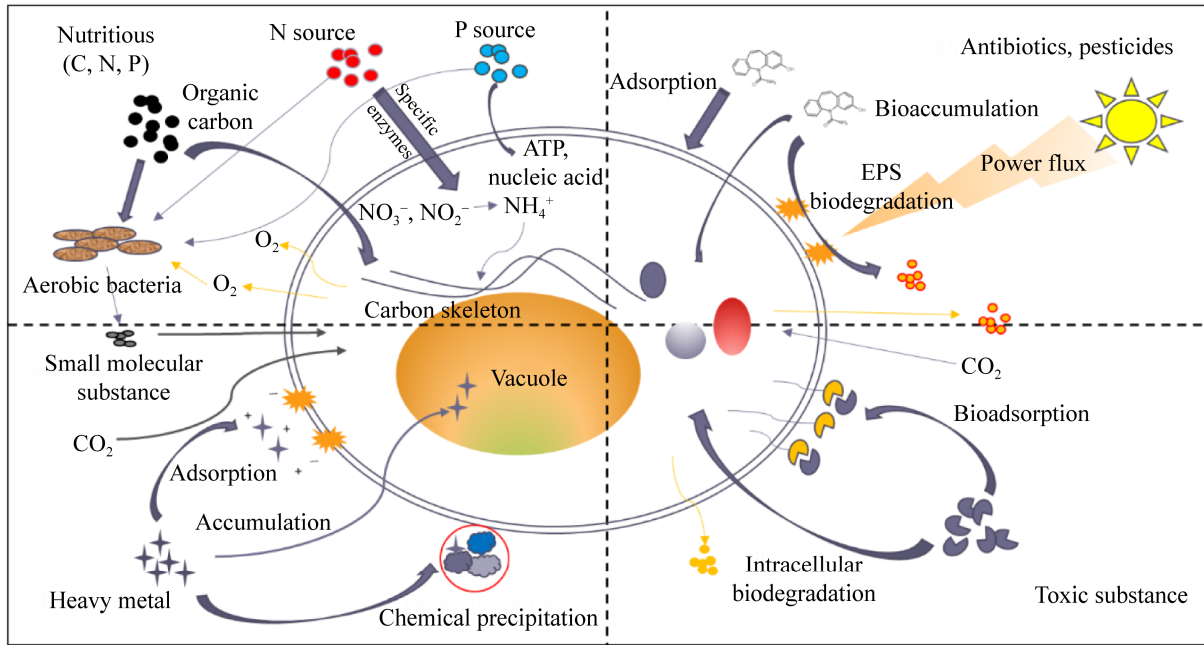


图 1 微藻去除各类污染物的机理

Figure 1 Mechanism of removal of various pollutants by microalgae.

1.2 以收获藻类生物质为导向的微藻选择

1.2.1 可用于回收生物质能的微藻

微藻细胞中可用于生物质能开发的组分主要是脂质和碳水化合物。脂质可作为生物燃料的原料，碳水化合物可作为发酵工业中的碳源^[27]。表 2 列举了不同培养条件下脂质含量较高的微藻，目前脂质含量较高的藻种包括四尾栅藻(*Scenedesmus quadricauda*)、布朗葡萄

藻(*Botryococcus braunii*)、杜氏藻(*Dunaliella tertiolecta*)及普通小球藻(*Chlorella vulgaris*)等，其单细胞脂质含量均超其干重的 55%。Zhan 等^[5]的研究发现 *Scenedesmus quadricauda* 细胞中脂质含量高达 66.1%。研究表明，添加赤霉酸可以引发藻细胞内脂质合成基因的显著上调，使得 *Botryococcus braunii* 中脂质含量增加^[3]。部分藻种如 *Cryptocodinium cohnii* 中脂质含量

表 2 富含脂质的微藻汇总

Table 2 Summary of lipid-rich microalgae

Microalgae species	Lipid content/%	Cultivated in	Inoculation amount	Period/d	Max biomass concentration	References
<i>Scenedesmus quadricauda</i>	66.1	mBG11	2×10^6 cells/mL	20	51.7 mg/L	[5]
<i>Botryococcus braunii</i>	60.3	BG11+GA3	1×10^5 cells/mL	15	0.5 g/L	[3]
<i>Dunaliella tertiolecta</i>	57.0	Two-stage cultivation with sodium azide intervention and salinity stress	3×10^7 cells/mL	12	7.5×10^7 cells/mL	[2]
<i>Cryptocodinium cohnii</i>	54.0	50% carbon source is acetic acid	NA	9	51.0 g/L	[28]
<i>Chlorella vulgaris</i>	55.3	N11	NA	NA	1.0 g/L	[29]

mBG11, BG11 and N11 are medium types; GA3: gibberellic acid; NA: not available.

相对稍低，然而采用批次补料异养培养方式高密度培养，当乙酸占碳源 50%时，可以获得高达 51 g/L 的微藻生物量^[28]。以废水作为底物培养上述微藻时，微藻中脂质积累受废水组分和工艺条件的影响，还需进一步的研究讨论。

微藻中碳水化合物主要包括葡萄糖、淀粉、纤维素以及各种多糖类物质，葡萄糖和淀粉可用于生产生物乙醇、氢气^[30]。如表 3 所示，微藻细胞中碳水化合物含量高的藻种主要包括莱茵衣藻 (*Chlamydomonas reinhardtii*)、斜栅藻 (*Scenedesmas obliquus*)、伪鱼腥藻 (*Pseudanabaena* sp.)、螺旋藻 (*Spirulina* sp.) 以及小球藻 (*Chlorella vulgaris*) 等，其单细胞中碳水化合物含量达 50%–70%，其中，*Chlamydomonas reinhardtii*、*Chlorella vulgaris* 等被认为具有生物乙醇生产的潜力。研究表明，通过 CO₂ 浓缩机制和氮饥饿处理，可以进一步提高 *Chlamydomonas reinhardtii* 的碳水化合物含量(达 71%)^[8]。

1.2.2 可用于回收蛋白质的微藻

微藻中蛋白质可以用于生物医药或饲料开发^[27]。目前，相比利用微藻处理废水同步回收脂质和碳水化合物，回收蛋白质的研究较少。用于回收蛋白质的藻种研究主要集中于螺旋藻 (*Spirulina*)，其蛋白质含量高达 60.0%–70.0%，此外还富含维生素，特别是维生素 B₁₂、矿物质等^[9]。Pereira 采用 Zarrouk 培养基培养 *Spirulina*，最终藻细胞内蛋白质含量达到 65.5%^[35]。通常认为，微藻中大分子物质的积累取决于细胞生物量及胞内大分子物质含量^[36]。微藻在生长前期，主要进行生物量积累，进入生长平衡期后开始积累非生长相关的大分子物质，如蛋白质、脂质和碳水化合物等^[37]。微藻在生长过程中，藻细胞会根据营养素的浓度比例，对光合作用的碳分配进行程序化改变，进而影响其组成成分含量^[38]，在以废水为基质的异养培养模式中，当微藻处于氮限制条件时，其代谢途径会从蛋

表 3 富含碳水化合物的微藻汇总

Table 3 Summary of carbohydrate-rich microalgae

Microalgae species	Carbohydrates content/%	Cultivation	Inoculation amount	Period/d	Max biomass concentration/(g/L)	References
<i>Chlamydomonas reinhardtii</i>	71.0	Two-stage cultivation (HS, HSN)	3.0×10 ⁵ cells/cm ³	4	0.7	[8]
<i>Scenedesmus obliquus</i>	68.8	Freshwater after filtration and sterilization	0.3 g/L	10	4.2	[31]
<i>Pseudanabaena</i> sp.	61.0	Improved WC, adjusted nitrogen source concentration	NA	6	0.4	[6]
<i>Spirulina platensis</i>	58.0	20% Zarrouk	0.2 g/L	14	3.2	[7]
<i>Chlorella vulgaris</i> FSP-E	54.4	Improved Basal, nitrogen starvation	0.1 g/L	5	7.2	[32]
<i>Spirulina</i> sp. LEB 18	52.3	Brackish groundwater with 25% Zarrouk addition	0.5 g/L	30	1.1	[33]
<i>Neochloris aquatica</i> CL-MI	50.5	Improved swine wastewater, N/P=5/1	3.6 g/L	7	6.1	[34]

HS and HSN are high salt and high salt nitrogen deficient medium respectively; WC: Zarrouk and Basal are medium types; NA: not available.

白质合成转向脂质和碳水化合物合成^[36]。而常见的废水组分往往以碳为主, 脂质和碳水都是碳氢化合物, 蛋白质的合成则需要 N、P、S 等元素, 这可能是回收微藻蛋白质的限制因素之一。因此, 改变废水中营养素的比例为微藻积累蛋白质提供了一种研究思路。

微藻用于废水处理时, 特定藻种处理某一类型废水, 如氮磷浓度较高的农业养殖废水培养微藻时, 其生物量和脂质产率会相应增加^[39]。废水用于微藻培养时, 微藻中大分子物质积累只能在有限范围(10%–40%)内发生变化, 此变化与废水中营养物浓度水平、培养方式和环境因子调节等有关^[40]。在微藻大分子物质积累方面, 除上述调节方式外, 还可通过过表达或抑制胞内大分子物质合成路径及其竞争途径中的关键酶基因来提高相应含量。如通过过表达叶绿体中脂肪酸合成路径中的酶基因、过表达内质网中三酰甘油的合成路径酶基因、抑制油脂合成的竞争途径酶基因以及抑制油脂分解的途径酶基因^[41]等方式可提高藻细胞中脂质含量。因此, 以导向性为目的选择微藻是合理应用微藻技术的重点。

2 促进微藻生物量和组分积累的途径

2.1 水样预处理方法

污水中所含的悬浮颗粒、高浓度有毒物质和有害微生物等严重抑制微藻的生长繁殖。因此, 在使用微藻处理废水之前, 须采用合理的方法对污水进行预处理。常用的预处理方法包括固液分离、废水组分调节和去除有毒有害物质等。

悬浮物会影响污水的透光率, 并且容易诱导微藻的沉降, 使微藻过度聚集, 影响微藻的生长^[42]。在实验室条件下多采用物理法(沉降、过滤等)去除悬浮物后用于微藻的培养^[43]。然而对大部分有机废水仅去除悬浮物仍达不到微藻

正常生长的要求。首先, 高浓度的有机废水通常色度较高, 会抑制微藻的光合作用。其次, 废水中碳、氮和磷源的形式会影响微藻的吸收效率。铵态氮一般作为微藻的首选氮源^[44], 但高浓度的铵态氮($\geq 280\text{--}300\text{ mg/L}$)同样会抑制微藻的生长^[45–46]。废水稀释是常用的调节组分的方法, 但过高或过低的稀释倍数均不利于微藻的生长^[47]。Deng 等^[48]研究发现, 微藻很容易利用乙酸、丙酸、异丁酸、丁酸、戊酸、异戊酸等小分子有机酸, 因此可采取厌氧消化将污水中的有机质转化为微藻易利用的状态。最后, 废水中过量的重金属^[49]、高浓度的抗生素可以通过抑制叶绿体、核糖体来影响微藻的光合作用^[50]。所以, 需要通过各种物理、化学等方法去除这些有害物质, 以满足培养微藻的最佳条件。

2.2 使用添加物

近年来, 植物生长调节剂已开始被应用于微藻的生长领域。它通过调节微藻内部生化途径来促进微藻的生长, 甚至可以诱导微藻产生油脂或色素^[51]。生长素、吲哚乙酸(IAA)、水杨酸(SA)、茉莉酸(JA)、赤霉素(GA3)和黄腐酸(FA)等植物激素促进微藻生长的研究均有报道^[52]。在 IAA 以及类似物的影响下, 能促进微藻的生长和脂质积累^[53]。对植物激素进行综合利用可显著提高微藻细胞生长和代谢产物积累的效率^[52]。Hunt 等^[54]通过联合使用萘乙酸、赤霉素和玉米素发现, 微藻的生产率比对照增加了 170%。另外, Chen 等^[1]采用固定化微藻模式, 发现以海绵为细胞固定载体时进一步提高了 *Chlorella sorokiniana* AK-1 最大生物量和蛋白质生产率, 分别达到 8.080 g/L 和 0.272 g/(L·d)。

2.3 工艺条件优化

微藻培养工艺中主要分为光能自养、化能异养和混合培养 3 种基本模式, 其中以废水为底物的微藻培养技术通常为混养模式, 微藻生

长和组分积累受培养参数的影响,如环境光照和温度、pH值、藻菌系统和营养胁迫等。

光照强度和光照周期(光暗比)可直接影响微藻的光合作用,从而改变微藻的组分和生物量^[55]。培养初期宜选用低光强以防止出现藻细胞光漂白现象,随着生物量的升高光强也应逐渐升高^[56],以最大限度地增加CO₂同化,同时尽可能减少光抑制^[57]。光强满足后,光周期也会影响微藻生长。何振平等^[58]研究发现,初期塔胞藻的生物量随光照周期的延长而增加,当达到一定光照时间后其生长反而受到抑制。显然,光强和光周期并非单独作用于微藻,如果光强弱,则要延长光照时间以保证微藻生长,相反则需减少光照时间^[59]。通常微藻光饱和点的光照强度范围为200–400 μmol/(m²·s)^[60]。总之,光照会直接影响微藻生物量及其细胞中碳水化合物的合成和积累。

温度作为重要的生态因子,能够直接影响微藻细胞的生命活动。在高温或低温等逆境环境中,藻细胞内各种酶的活性会受到抑制,影响其代谢产物的合成^[58]。低温通过降低碳同化反应的活性而影响光合作用,而高温会使光合作用相关蛋白酶失活并扰乱细胞内的能量平衡降低光合作用^[61]。研究表明大部分藻类的最佳温度范围是20–30 °C^[62]。同时,温度还可以作为一种控制手段,来诱导微藻产生高价值代谢物,Converti等^[63]发现相比于30 °C,在25 °C下培养*Chlorella vulgaris*会产生更多碳水化合物和脂质。

pH值也会对微藻生长及产物合成产生多方面的影响。首先,温度和pH值均能影响各种酶的活性,进而影响藻细胞的新陈代谢;其次,pH值会影响细胞膜所带电荷的状态,从而改变细胞膜的通透性,影响微藻对营养物质的

吸收利用;另外,pH值还会改变底物中某些组分的分子状态(如HCO₃⁻),进而影响微藻对营养物的吸收利用^[56]。微藻最适的pH值因藻种而异,也因培养环境而异,一般来说微藻生长的最佳pH值为6.0–8.0^[61]。

废水中的微生物菌群亦会影响微藻生长。研究表明微藻和细菌、真菌等微生物形成的藻菌共生体系可改善水质^[64]。藻类通过光合作用释放氧气供给好氧异养型微生物进行代谢活动,而好氧微生物对有机污染物进行氧化分解,代谢产物CO₂和氮、磷又供给藻类进行光合作用,如此循环形成藻菌之间互生的关系^[15]。目前研究发现,微藻与细菌共培养对废水的处理具有较高的成本效益,且共培养后微藻易于收获^[65]。细菌除了对微藻有促进作用外,同时也对藻类有抑制和拮抗作用,这主要体现在营养供应不足时出现营养竞争^[66]。

不同微藻对营养的需求有所差异,但所有藻类对N、P、C、O等基本营养素的需求是相同的,用以构成微藻细胞的基本骨架(CH_{1.7}O_{0.4}N_{0.15}P_{0.0094})^[67]。除此之外,微量元素Mo、K、Co、Fe、Mg、Mn、B、Zn等则参与藻类细胞中的多种酶促反应^[68]。通常,无机氮和磷以铵盐和磷酸盐的形式被微藻吸收,碳源则以有机和无机态的形式被微藻利用^[61]。另外,C、N和P的浓度水平也影响微藻生物量及组分。微藻对碳源的需求量很大,对氮源的需求仅次于碳源^[56]。Chen等^[2]在两步培养条件下,结合50 μmol/L叠氮化钠和2.5 mol/L盐胁迫处理培养*Dunaliella tertiolecta*,总脂质生产力和单细胞脂质含量比对照组高出10.0%和70.5%。Cuellar-Bermudez^[6]在3种初始氮浓度(56、42、14 mg N/L)下培养*Pseudanabaena* sp.,发现14 mg N/L组生物量最低但碳水化合物含量最

高达 61%。所以, 当微藻暴露在某些环境压力下, 特别是营养胁迫时, 藻细胞生物量及其大分子化合物代谢途径会随之发生改变。

3 总结与展望

微藻作为“小型工厂”, 能够合成高附加值产物。以废水为底物培养微藻具有降低微藻培养成本的潜力, 同时能够削减环境污染。尽管目前微藻技术处理废水取得了进展, 但仍需要改进, 例如废水处理不充分; 微藻对有机物和氮磷等组分的转化途径和机理的研究仍不够彻底以及藻胞内活性物质积累较低的问题; 经废水培养后的微藻回收成本较高, 提取和利用胞内活性物质工艺不够成熟。为了优化废水处理效果, 实现微藻生物质资源化和产业化, 我们应进一步分离性能优异的微藻物种, 并证明其能够对大规模废水内的生物抑制因子保持稳定的生物量和胞内活性物质生产的能力; 然后, 还应考虑到在废水中培养对藻生物量转化过程可能产生的风险和不利影响。另外, 在高价值产物生产过程中, 提高藻胞内活性物质产量而不影响其生长仍然是一个障碍。因此, 未来可以从分子水平研究胞内活性物质积累、代谢和应激之间的相互作用。通过将各种“组学”技术与传统方法相结合, 可实现微藻高附加值产物的高生产率, 以建立一种经济有效的微藻生产, 收获并进一步转化为燃料、饲料添加剂及食品等的产能体系。只有通过这种方式, 才能充分利用废水作为资源, 实现可再生和可持续的微藻生物质生产。

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