Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation

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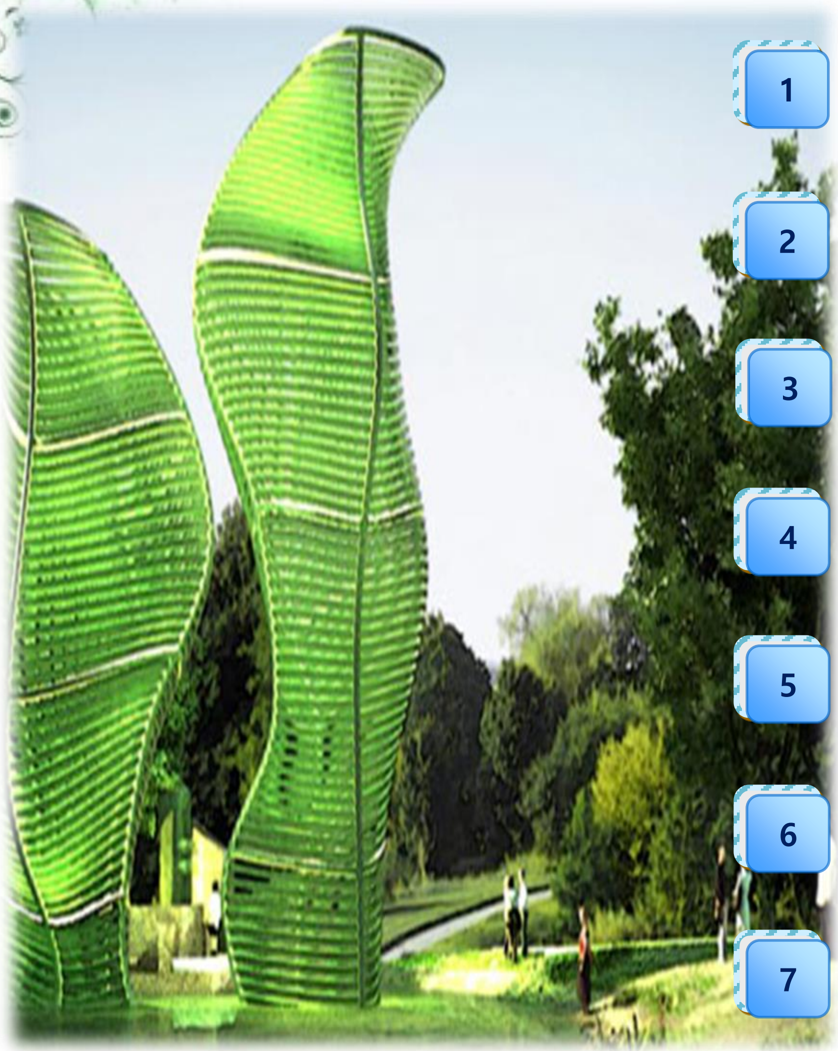
Content



nature energy ARTICLES
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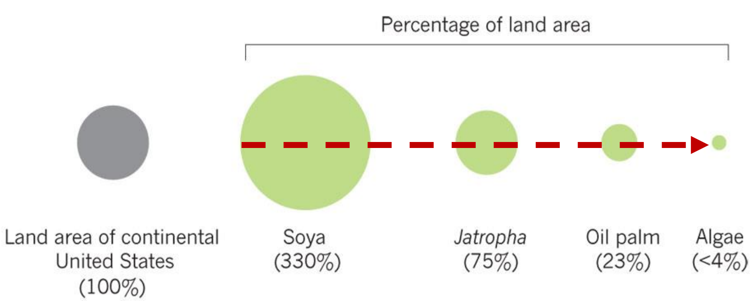
Impacts of a 32-billion-gallon bioenergy landscape on land and fossil fuel use in the US

Tara W. Hudiburg[1], WeiWei Wang[2], Madhu Khanna[2], Stephen P. Long[3], Puneet Dwivedi[4], William J. Parton[5], Melannie Hartman[5] and Evan H. DeLucia[3]*

Sustainable transportation biofuels may require considerable changes in land use to meet mandated targets. Understanding the possible impact of different policies on land use and greenhouse gas emissions has typically proceeded by exploring either ecosystem or economic modelling. Here we integrate such models to assess the potential for the US Renewable Fuel Standard to reduce greenhouse gas emissions from the transportation sector through the use of cellulosic biofuels. We find that 2022 US emissions are decreased by 7.0 ± 2.5% largely through gasoline displacement and soil carbon storage by perennial grasses. If the Renewable Fuel Standard is accompanied by a cellulosic biofuel tax credit, these emissions could be reduced by 12.3 ± 3.4%. Our integrated approach indicates that transitioning to cellulosic biofuels can meet a 32-billion-gallon Renewable Fuel Standard target with negligible effects on food crop production, while reducing fossil fuel use and greenhouse gas emissions. However, emissions savings are lower than previous estimates that did not account for economic constraints.



- The **area** required for **algae** cultivation is estimated to be significantly **less than** that for any other **biomass source**.

REVIEW

doi:10.1038/nature11479

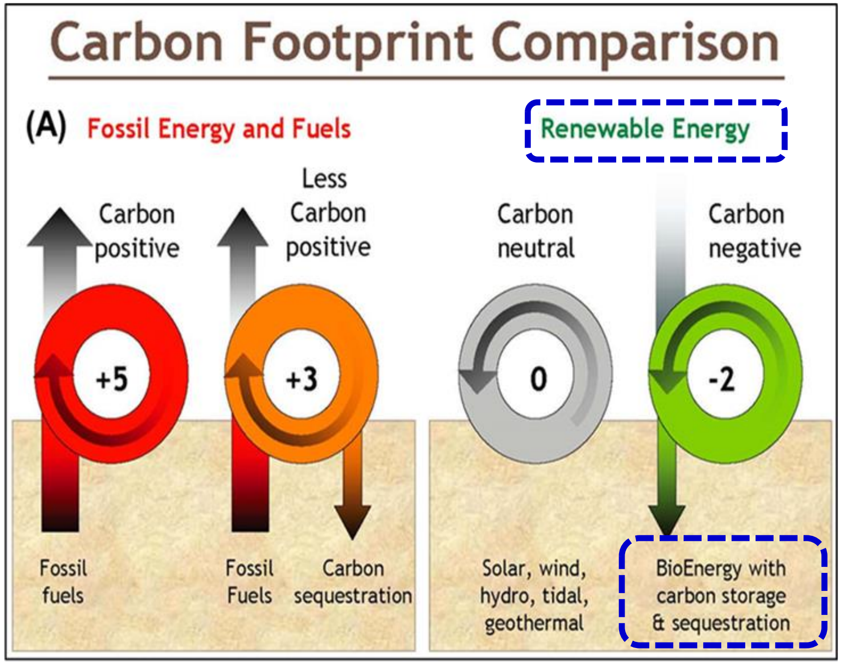
Exploiting diversity and synthetic biology for the production of algal biofuels

D. Ryan Georgianna[1] & Stephen P. Mayfield[1]

Modern life is intimately linked to the availability of fossil fuels, which continue to meet the world's growing energy needs even though their use drives climate change, exhausts finite reserves and contributes to global political strife. Biofuels made from renewable resources could be a more sustainable alternative, particularly if sourced from organisms, such as algae, that can be farmed without using valuable arable land. Strain development and process engineering are needed to make algal biofuels practical and economically viable.

Introduction:
Carbon foot-print from fossil fuel & Renewable energy

Renewable energy



Microalgae

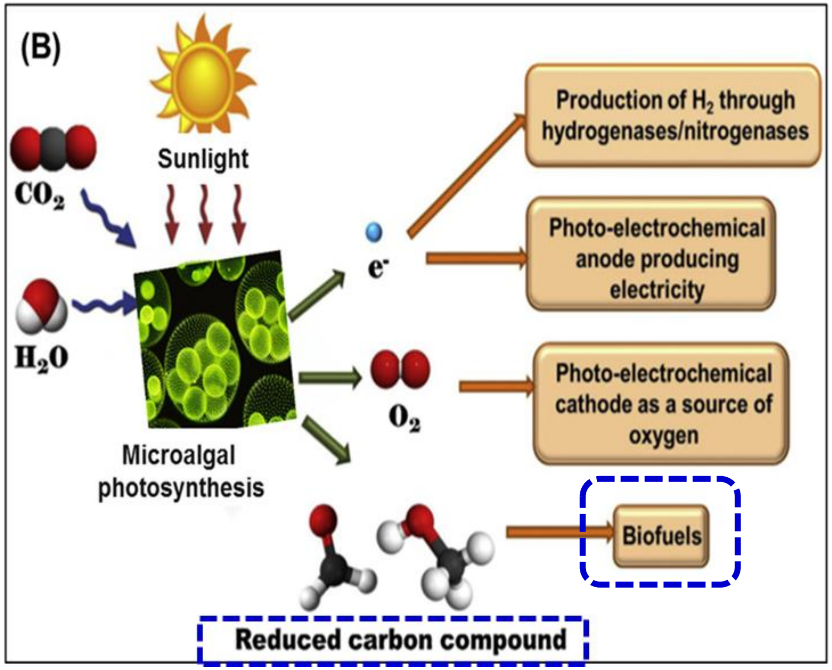


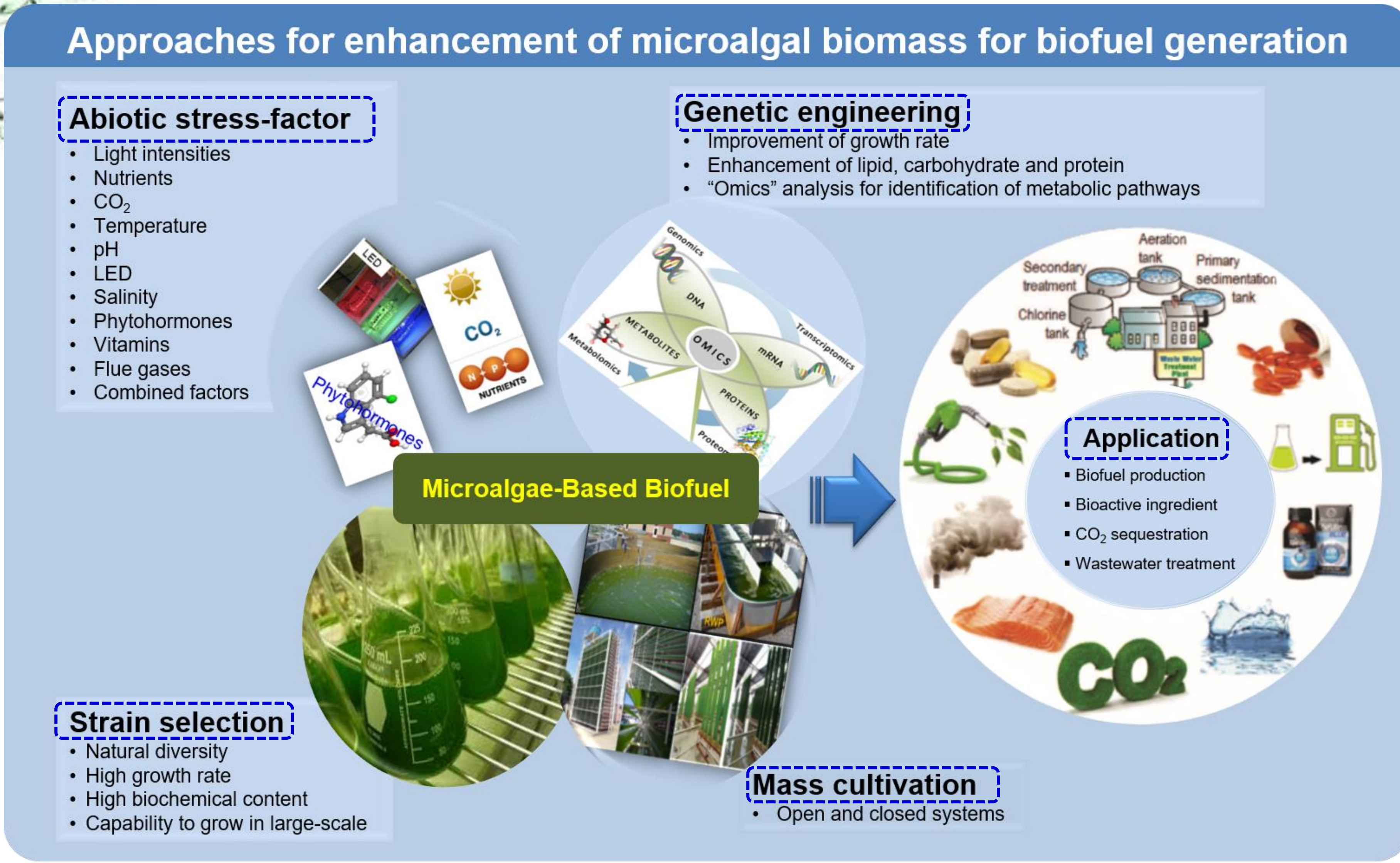
Figure. The **carbon foot-print** from **fossil** and **renewable energy** (**A**). The raw materials for the microalgal photosynthesis are **solar energy**, **CO_2** and **H_2O** and the products are **reduced carbon compounds** and **O_2** (**B**).

- The **reduced carbon compounds** serve as a source of **microalgal biofuel**

Source: Salama et al. (2016), in preparation for Energy and Environmental Science

Introduction:

Approaches for improvement of microalgal growth for biofuel production



Source: Salama et al. (2016), in preparation Energy and Environmental Science

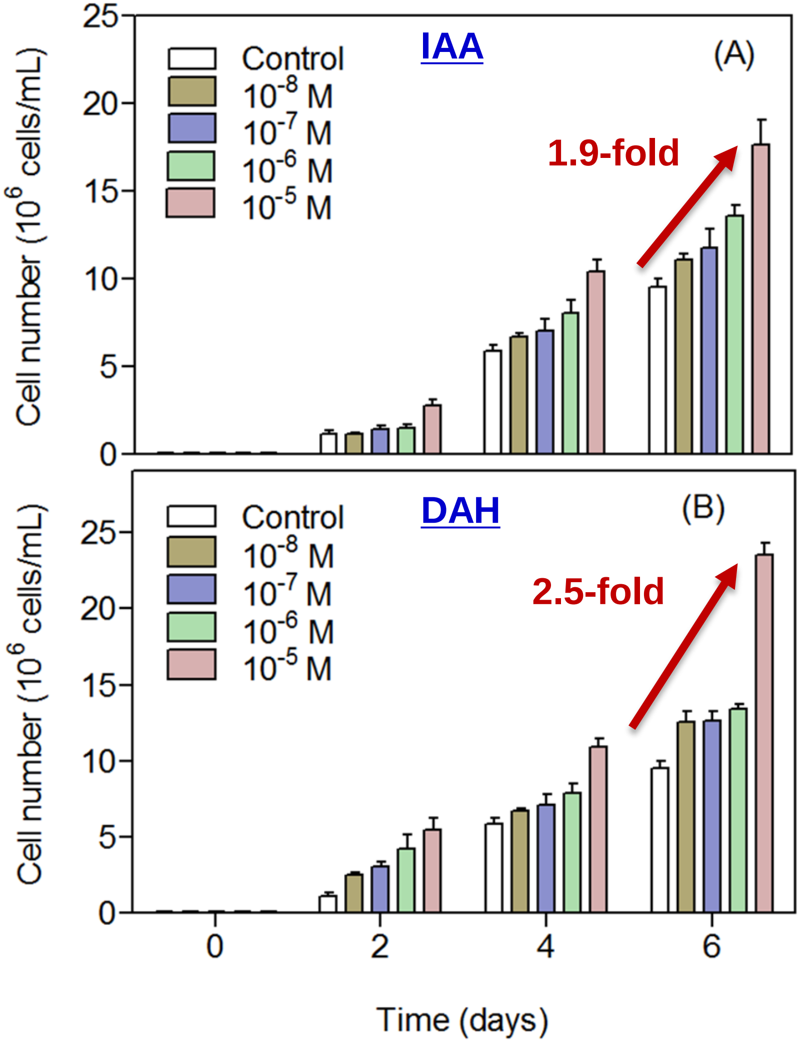


Figure. Effect of different concentrations of **phytohormones** and on the growth of *S. obliquus*.

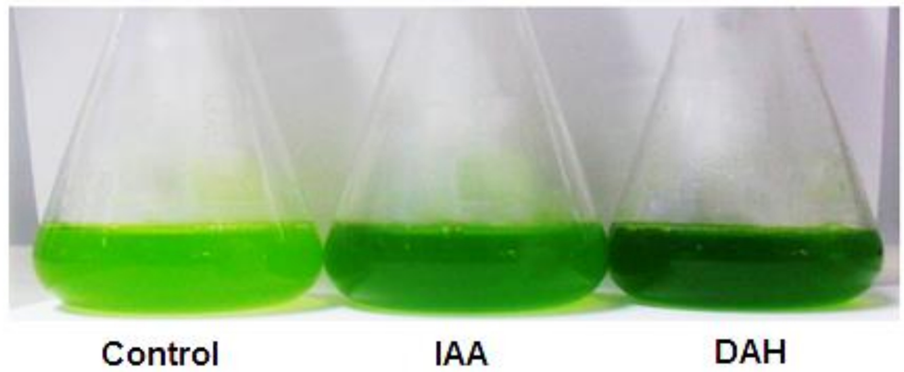
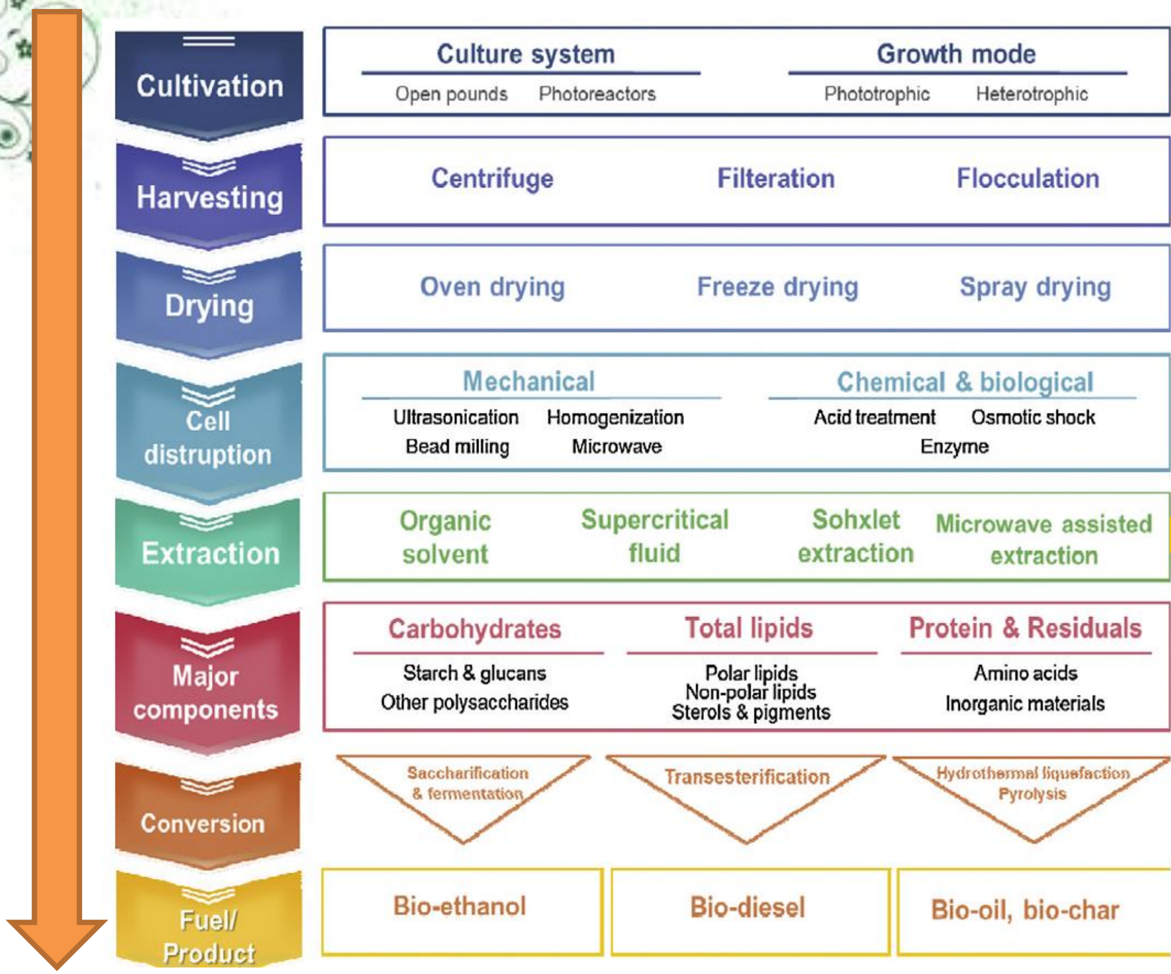


Figure. Photographic image of *S. obiliquus* cultivated in BBM, in absence (**control**) and presence of **10^{-5} M Indole-3-acetic acid** (**IAA**) and **Diethyl aminoethyl hexanoate** (**DAH**).

- **IAA** and **DAH** enhanced ***S. obliquus*** growth at all concentrations during the cultivation time.
- IAA and DAH enhanced the *S. obliquus* growth by **1.9-** and **2.5-fold**, respectively at 10^{-5} M .
- The **maximum specific growth rates** were estimated to be **2.03** and **2.35 1/day** at **2 day** with 10^{-5} M IAA and DAH, respectively.

Source: Salama et al. (2014), published in Bioresource Technology

Nanotechnology:
Application of nanomaterials in the microalgal biofuel production



- **Silica**
- Metal oxide **nanoparticles (**iron oxide and AgI/TiO_2)
- **Single-walled carbon nanotubes**

- **Nano clay**

Nanomaterials used as carrier of chemicals which perform as organic solvents:
- Nano-catalysis

- Lipase immobilized onto **carbon nanotubes (CNTs)**

➢ **Nanotechnology application** in **biodiesel production from microalgae** mainly includes **nanomaterial utilization** on **lipid accumulation**, **extraction** and on the **transesterification process** as **catalyst support** or **catalyst** (Zhang et al., 2013).

Source: Zhang et al. (2014), published in Renewable and Sustainable Energy Reviews

Table. Nanomaterial application in lipase immobilization (Zhang et al., 2013).

Lipase source	Nanomaterials	Activity remaining (%)	Times of IRTA[a] of immobilized to free lipase	Times of TCR[b] of immobilized to free lipase	Reuse ability
Candida rugosa	Carbon nanotubes	97	2.2-14	4.44	—
Candida rugosa	Nanogel	85	—	7.67	—
Candida rugosa	Fe_3O_4 nanoparticles	80	110	20.5	4
Candida rugosa	ZrO_2	214	—	3.3	8
Candida rugosa	γ-Fe_3O_4 nanoparticles	< 100	—	—	—
Candida antarctica	Fe_3O_4 nanoparticles	200	—	—	4
Candida antarctica	Polystyrene nanoparticles	204	—	—	—
Pseudomonas cepacia	ZrO_2		—	3.6	-
Thermomyces lanuginosus	Nanosized silica	93	—	—	—
Thermomyces lanuginosa	Fe_3O_4 nanoparticles	70	—	1.05	4

[a]Initial rates of transesterification activity.
[b]Transesterification conversion rate.

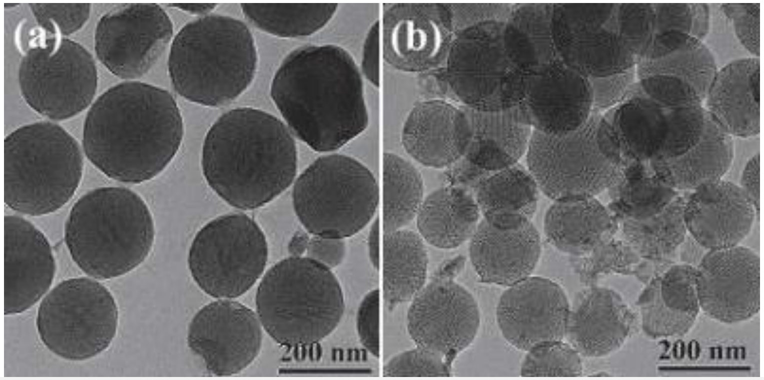
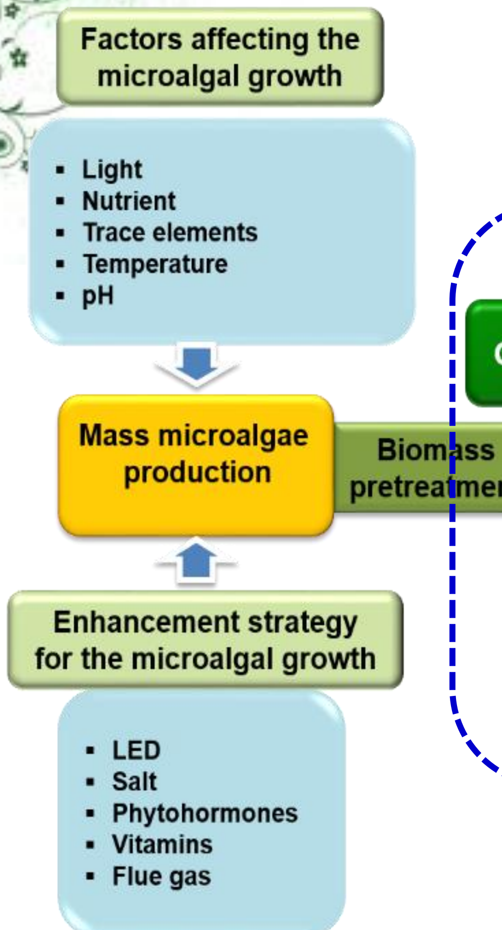


Figure. Nano-particles harvesting oil from algae without harming the organism

Source: Prabu et al. (2016), published in International Journal of Advanced Research in Biology Engineering Science and Technology (IJARBEST

- All **biochemical content of** microalgal biomass **can be transformed** to **biofuel**.



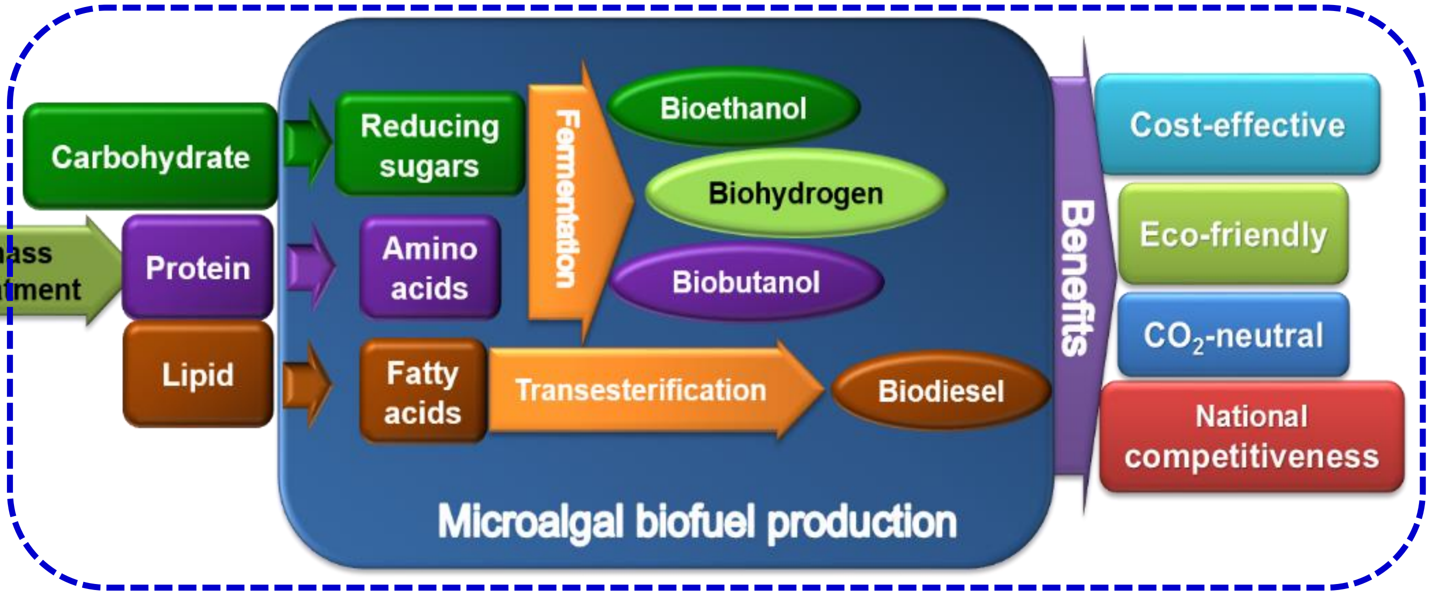


Figure. An extended schematic model for **a new biomass-energy conversion pathway** in which all **biochemical content** of microalgal biomass can be transformed to **biofuel for improving the economic feasibility of microalgae biofuel industry**.

Source: Salama et al. (2016), in preparation for Energy and Environmental Science

Fermentation:
Pretreatment of microalgal biomass (SHE)

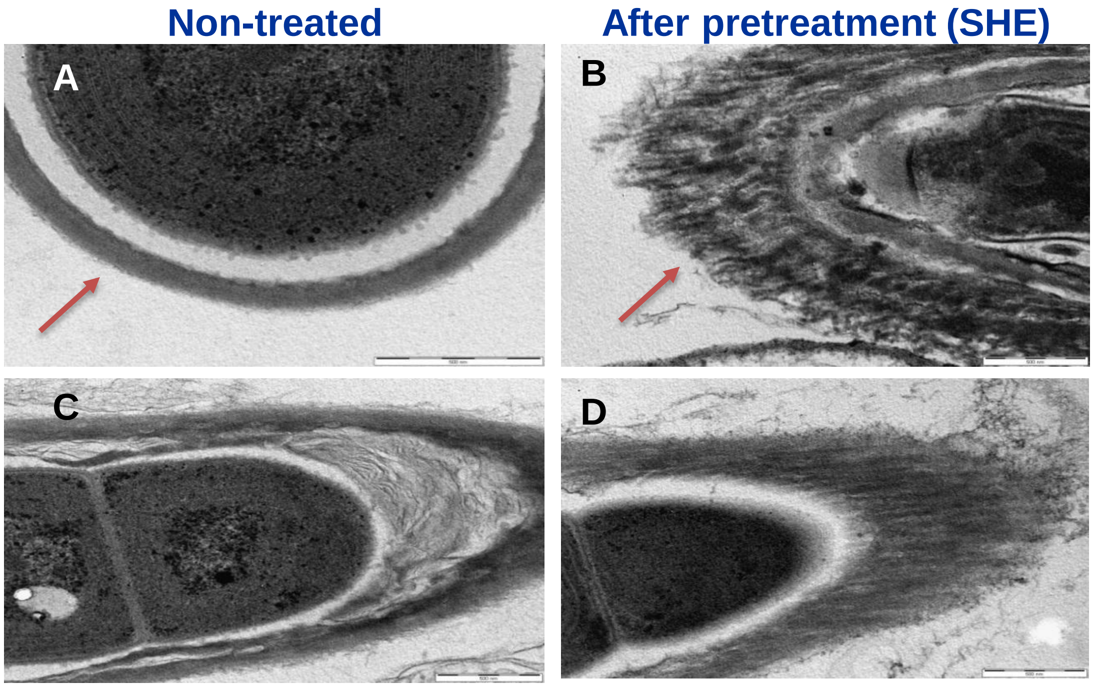


Figure. TEM images showing the destruction of *C. vulgaris* and filamentous *U. belkae*. (**A, C**) Non-treated algal cell while (**B, D**) combined pretreated (**sonication + enzyme + heat**) algal cell.

- The nucleus materials in the nucleus membrane were **clearly visible** and well defined in both non-pretreated cyclotella and filamentous algae (Fig. A and C).
- The cytoplasm and nucleus materials of both cyclotella and filamentous algae after the **SHE treatment** (Fig. B and D). presumably spread outside the cell due to **complete cell lysis**, which coincides with a significant **increase in the dissolved fraction of carbohydrates** under the same conditions.

Source: Hwang et al. (2016), published in Algal Research

Fermentation:
Ethanol production during 7-cycles (Immobilized cells)

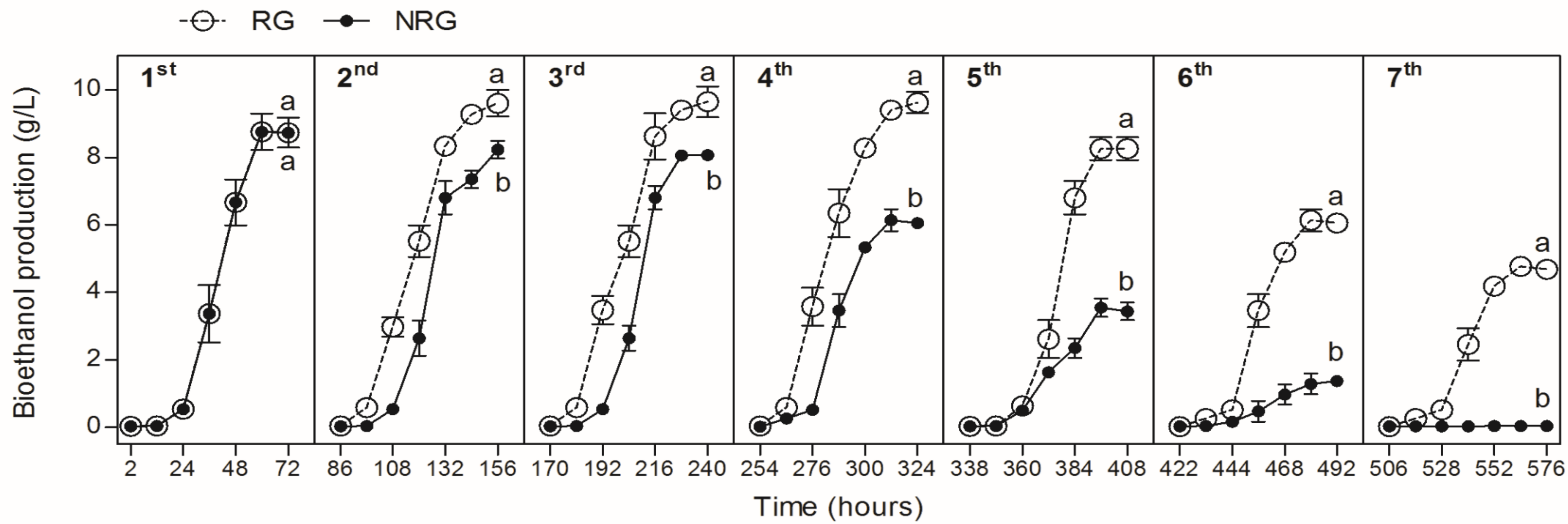


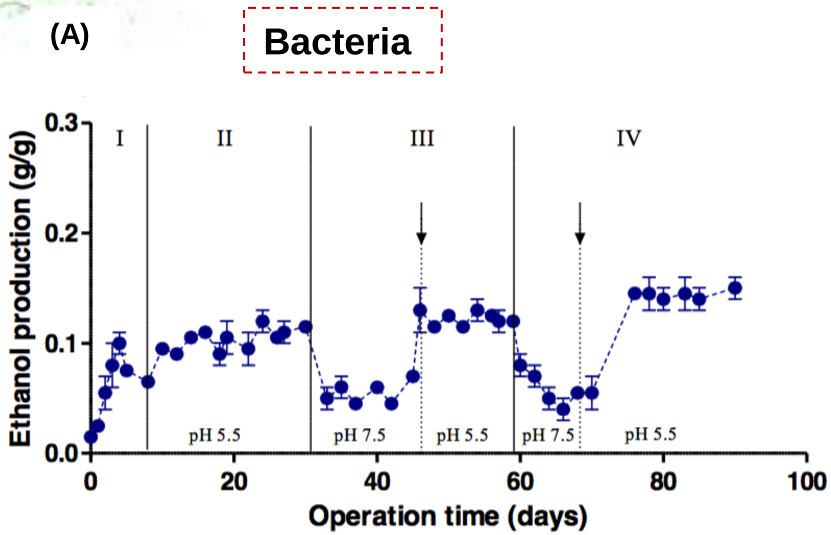
Figure. Cumulative bioethanol production from *Chlamydomonas mexicana* through **7-cycles** of repeated fermentation using **immobilized** yeast cells. **RG:** regenerated; **NRG:** non-regenerated beads

Note: The fermentation was performed using **immobilized** yeast cells.

- Immobilized yeast cells enabled **repetitive production** of ethanol for **7 cycles** displaying a fermentation efficiency up to **~80%** for five consecutive cycles.
- The ethanol concentration was equal for both RG and NRG beads in the **1st cycle** (**8.73 g/L**), while in the **2nd and 3rd** cycles, RG beads showed higher bioethanol production (**9.6 and 9.64 g/L**, respectively) compared to NRG beads (**8.23 and 8.1 g/L**, respectively).
- Being supplied with the nutrients in this period, the yeast cells in RG beads regained their cell integrity and catalytic efficiency in terms of cell multiplication, production of enzymes, and metabolic activities.

Source: El-Dalatony et al. (2016), published in Bioresource Technology

Fermentation:
Effect of pH and pretreatment condition on ethanol production



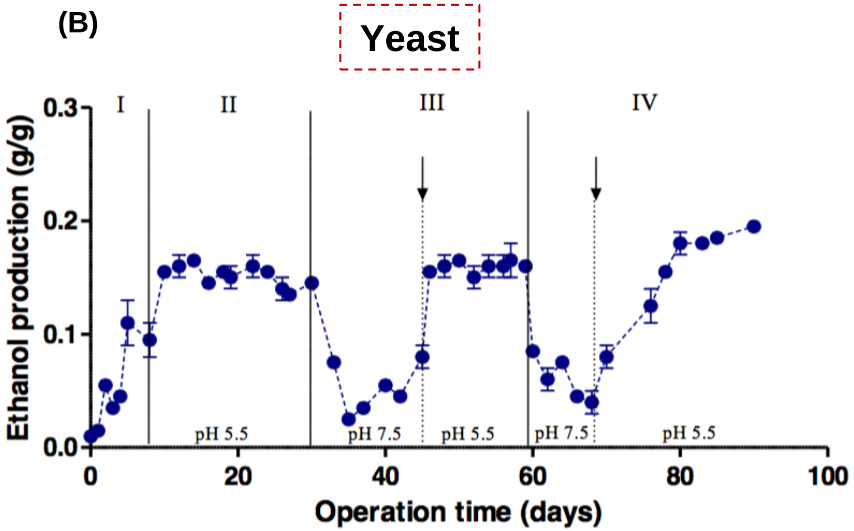


Figure. Effect of **pH** and **pretreatment condition** on continuous ethanol production**. (A) Fermenting bacteria (B) Yeast (Phase I: Stabilization stage, II: Sonication, III: SE, IV: SHE).**

- Ethanol production by fermentative **bacteria** increased from 0.05 to 0.13 g g^{-1} with a decrease in **pH from 7.5 to 5.4** for **SE** pretreated biomass.
- A decrease in ethanol production from 0.14 to 0.06 g g^{-1} was observed in both phases III and IV with increase in pH from 5.4 to 7.5.
- Higher amount of ethanol was observed in **yeast** fermentor compared to dark **bacterial** fermentor, which might be due to **higher bioactivity** of alcohol fermentation by *Dekkera bruxellensis*.

Source: Hwang et al. (2016), published in Algal Research

Fermentation:
Bioethanol production from microalgae

Table. Comparison of **bioethanol production** from microalgae and **other feedstocks**.

Substrate	Immobilization carrier	Number of cycles	Fermentation strategy	Bioethanol yield (g/g)	References
Corn meal	Ca-alginate	1	*SHF	0.430	Nikolić et al. (2009)
			*SSF	0.510	
Cassava hydrolysate	Fibrous matrix	7	SSF	0.136	Liu et al. (2015)
Sweet sorghum juice	Corncobs	8	-	0.480	Ariyajaroenwong et al. (2012)
Glucose	Ca-alginate	6	-	0.460	Sree et al. (2000)
Glucose	Ca-alginate	8	-	0.640	Duarte et al. (2013)
	Chitosan-covered calcium alginate beads	8		0.610	
Sugarcane bagasse	Sugarcane bagasse stalks	10	SHF	0.440	Singh et al. (2013)
	Ca-alginate	4	SHF	0.380	
	Agar-agar	4	SHF	0.330	
C. mexicana	**Ca-alginate**	**7**	**SSF**	**0.500**	**El-Dalatony et al. (2016)**
Corn meal	Ca-alginate	2	SHF	0.574	Rakin et al. (2009)
	Polyvinyl alcohol	5		0.396	
Sugar beet molasses	Alginate-maize stem ground tissue matrix	1	-	0. 493	Razmovski & Vucurovic, (2011)
Inverse sugar from cane molasses	alginate-loofa matrix	3	-	0.440	Phisalaphong et al. (2007)
	Ca-alginate	3		0.460	
Cashew apple juice	Cashew apple bagasse	10	-	0.490	Pacheco et al. (2010)
Blackstrap molasses	A thin-shell silk cocoon	5	-	0.470	Rattanapan et al. (2011)
Glucose	Lyophilized hydroxyl-ethyl-cellulose gels	3	-	0.400	Winkelhausen et al. (2010)
S. obliquus YSW15	-	-	-	0.164	Lee et al. (2010)
Mixed algae (*C. vulgaris* YSL001 and *U. belkae* YSL010)	-	-	-	**0.180**	**Hwang et al. (2016)**

* SHF = Separate hydrolysis and fermentation process

**SSF = Simultaneous saccharification and fermentation

Source: Salama et al. (2016), in preparation for Energy and Environmental Science

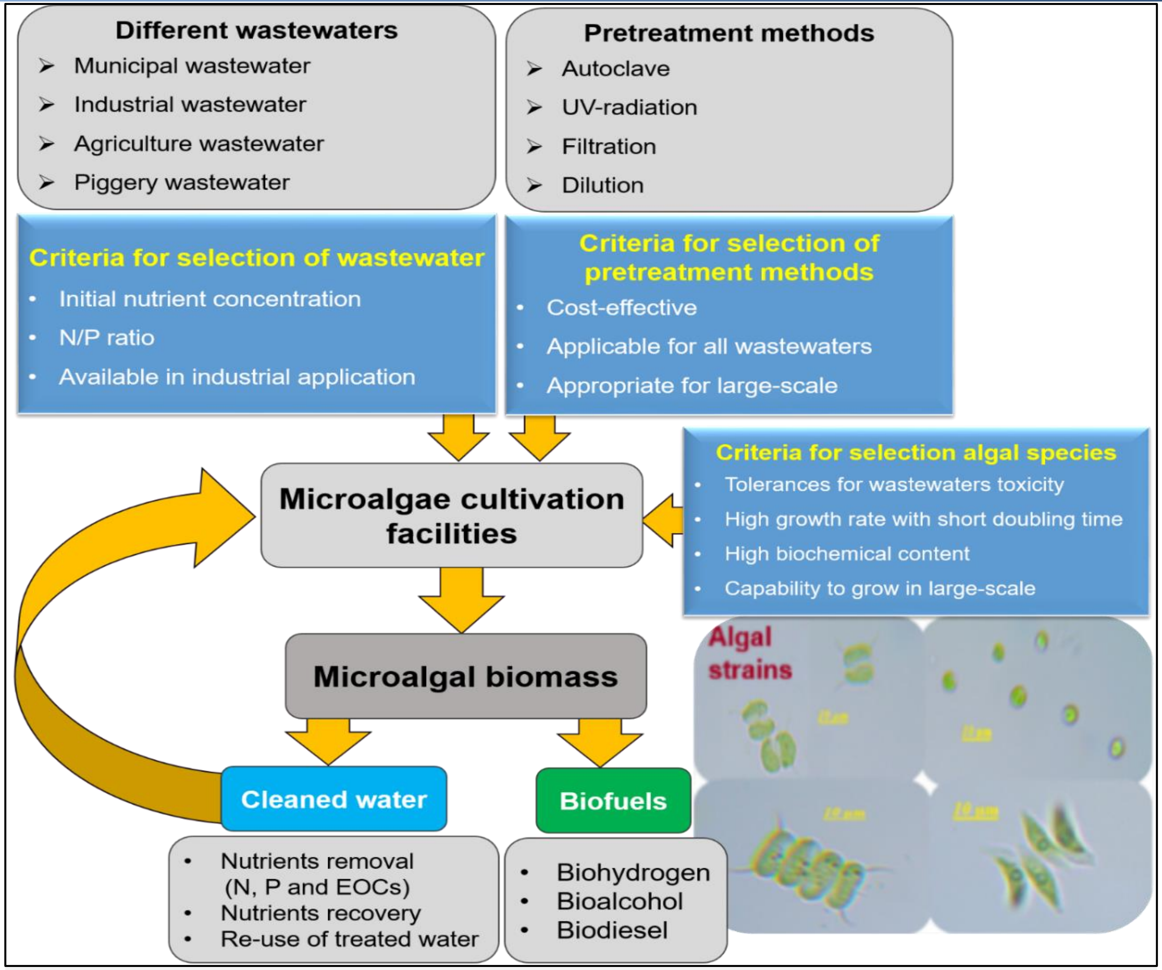


Figure. Schematic presentation of **simulations on wastewater treatment** with **microalgal biomass cultivation** for **biofuel generation**.

- **Appropriately selecting the wastewater**, **robust microalgal species**, and **pretreatment method** is critical to using **advanced wastewater treatment with microalgae cultivation** to produce biomass for **microalgae-based biofuel**.

Source: Salama et al. (2016), submitted to Renewable & Sustainable Energy Reviews

Microalgae & wastewater:
Advantages of wastewater over synthetic medium

Table. Comparison between the physical-chemical characteristics of **various wastewaters** and **a commonly used synthetic medium** (Barsanti & Gualtieri, 2014; Ji et al., 2014a; Salama et al., 2014; Tan et al., 2016).

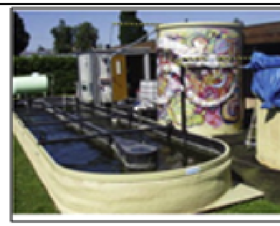
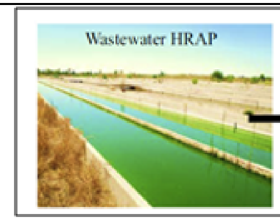
Properties	Unit	Municipal wastewater	Municipal wastewater	Concentrated municipal wastewater	Anaerobic digestion wastewater	Piggery wastewater	Bold's basal medium
pH	-	8.0	8.10	7.28	7.30-7.50	7.97	6.80
Alkalinity (total CO_3)	mg CO_3/L	-	272	-	-	-	-
Salinity	g/L	-	1.03	-	-	-	-
TSS	mg/L	-	50	-	59.35-85.26	-	-
Conductivity	mS/cm	-	2.29	-	-	-	-
COD	mg/L	63	31	-	1572.45-2265.37	37,643	-
TOC	mg/L	-	9	180.6	-	-	-
TIC	mg/L	33.4	-	80.9	-	-	-
TN	mg/L	18	27	56	537.26-702.73	2055	41.01
TP	mg/L	1.4	5.04	15.8	72.62-111.58	620	53
Microbes					-	-	-
E. coli	cfu/100 mL	-	5.4×10^6	-	-	-	-
P. aeruginosa	cfu/100 mL	-	0.2×10^6	-	-	-	-
Fecal coliforms	cfu/100 mL	-	6.2×10^6	-	-	-	-
Total coliforms	cfu/100 mL	-	75.0×10^6	-	-	-	-
Metals							-
Magnesium	mg/L	4.0	0.088	16.5	23.83-58.26	213	7
Manganese	mg/L	-	0.09	0.4	0.96-1.91	4.1	0.23
Zinc	mg/L	≤ 0.04	0.009	-	-	28.9	3.93
Copper	mg/L	≤ 0.04	-	-	0.31-0.92	10.6	0.63
Calcium	mg/L	29	29	65.6		437	7
Cobalt	mg/L	-	-	-	0.02-0.06	3.8	
Iron	mg/L	≤ 0.1	0.12	0.05	6.83-15.35	169.2	4.2
Aluminum	mg/L	≤ 0.04	0.04	0.02	-	-	-
Sulfate	mg/L	30	-	40.4	-	-	43.2
Sodium	mg/L	23	-	39.5	-	772	68
Potassium	mg/L	8.8	20	45.7	22.38-68.15	2524	34
Chloride	mg/L	68	-	-	-	-	12
Barium	mg/L	0.4	-	-	0.74-1.67	-	2.0

- The presence of **essential nutrients (including C, N, P, and trace elements)** in wastewater enables the **large-scale use of wastewater** as a culture medium for growth of microalgae.

Source: Salama et al. (2016), Renewable & Sustainable Energy Reviews

Microalgae & wastewater:
Real open ponds supplemented with wastewater

Table. Biomass production, **nutrient removal** and **CO_2 utilization** during cultivation of microalgae in **real open ponds supplemented with wastewater** as a culturing medium in **several countries during the past five years**.

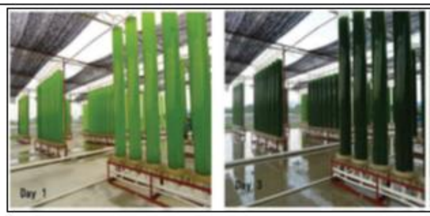
Production system	 Algal open pond reactors operated at the Lawrence WWTP, Lawrence, KS, USA (Sturm & Lamer, 2011)	 Outdoor MaB-floc SBRs treating aquaculture wastewater, Belgium (Van den Hende et al., 2014)	 A high-rate algal pond for real-scale domestic wastewater treatment operated at Monserrato, CA, Italy (Drira et al., 2016)	 Outdoor raceway pond operated at Chilgok-gun, Gyeongsangbukdo, South Korea (Hong et al., 2016)	 Raceway pond built and operated at Florence, Italy (Chiaramonti et al., 2013)
Algal strain	Natural microalgae	Microalgae and bacteria	*Chlorella* sp.	Mixed culture (*Chlorella*, *Coelastrella*, *Acutodesmus*, and *Pseudopediastrum*)	Mixed culture (*Nannochloropsis* sp. and *Tetraselmis suecica*)
Type of wastewater	Municipal wastewater	Aquaculture wastewater	Domestic wastewater	Wastewater	Cultivation media
Amount of wastewater	25,800 million gallons/day	11.959 m^3	-	> 236,000 kiloliters	200-300 L/m^2
Temperature	-	20 °C	20.2 °C	19.8 °C	20.54 °C
[a]HRT	10 days	8 days	-	-	-
Biomass yield	31,800 tons/day	25 g/m^2/day	-	2612.7 kg biomass	-
Initial nutrients	Total nitrogen = 19.5 mg/L Total phosphorus = 3.2 mg/L	Total nitrogen = 40 mg/L Total phosphorus =2.0 mg/L	Total nitrogen= 155 mg/L Total phosphor= 3.6 mg/L	[b]Total nitrogen =15-30 mg/L [b]Total phosphorus =3-6 mg/L	- -
Nutrient removal	Total nitrogen = 61.4 % Total phosphorus = 90.6 %	Total nitrogen = >50 % Total phosphorus = >50 %	- -	[b]Total nitrogen = 13.1 mg/ kg biomass [b]Total phosphorus =2.5 mg/ kg biomass	- -
CO_2 utilized	6800	89 g-CO_2/Newton meter	-	[b]4781.2/kg biomass	-

[a]Hydraulic residence time
[b]Consumption of nutrient/kg algal biomass

Source: Salama et al. (2016), submitted to Renewable & Sustainable Energy Reviews

Microalgae & wastewater:
PBRs supplemented with wastewater

Table. Biomass production, nutrient removal and CO_2 utilization during cultivation of microalgae in **photobioreactors (BPRs)** supplemented with wastewater as a culturing medium in several countries during the past five years.

Production system	 PBRs using artificial wastewater in summer in southern China (Zhu et al., 2016)	 PBR system for wastewater-based algae cultivation in Jiangxi, China (Xin et al., 2016)	 Coupling of wastewater treatment with microalgae cultivation in PBR for nutrient removal and biomass production in Seoul, South Korea (Yang et al., 2016)	 Industrial-scale PBR of NPDEAS at UFPR in Curitiba, PR, Brazil (Silva et al., 2015)
Algal strain	*C. zofingiensis*	*Chlorella* sp.	*Chlorella vulgaris* and *Scenedesmus obliquus*	-
Type of wastewater	Artificial wastewater	Municipal wastewater	Municipal wastewater	Wastewater
Amount of wastewater	819 m^3	1200 L	160 L	9.380 m^3
Temperature	20.6-33.8 °C	-	25 °C	-
Biomass yield	1.221 g/L	1.5-2.5 g/L	0.5-0.6 g/L	1.5 kg m^{-3} day^{-1}
Initial nutrients	-	-	Total nitrogen = 12.25 mg/L Total phosphorus = 1.80 mg/L	- -
Nutrient removal	-	[b]0.058 kg TN/kg dry algae [b]0.130 kg TP/kg dry algae	Total nitrogen = 100 % Total phosphorus = 100 %	- -
CO_2 utilized	-	3.58 kg CO_2	-	-

[b]Consumption of nutrient/kg algal biomass

Source: Salama et al. (2016), submitted to Renewable & Sustainable Energy Reviews

Microalgae & wastewater:
Emerging organic contaminants (EOCs)

Biodegradation of carbamazepine (CBZ)

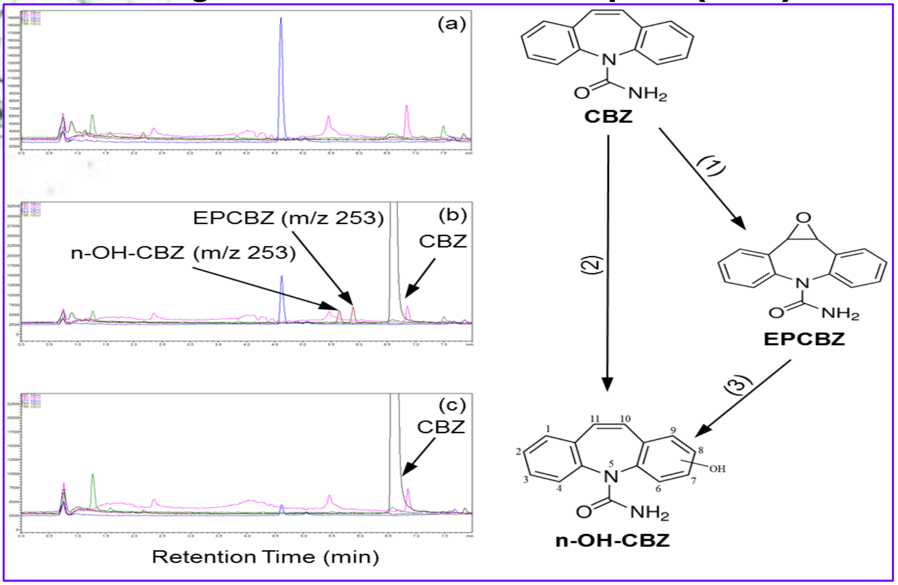


Figure. Proposed biodegradation pathway of CBZ by *C. mexicana* and **UPLC-MS chromatographs of microalgal culture without** CBZ (a), with microalgae and CBZ (b), and CBZ without microalgae (c).

Source: Xiong et al. (2016), published in Bioresource Technology

Biodegradation of Ciprofloxacin (CIP)

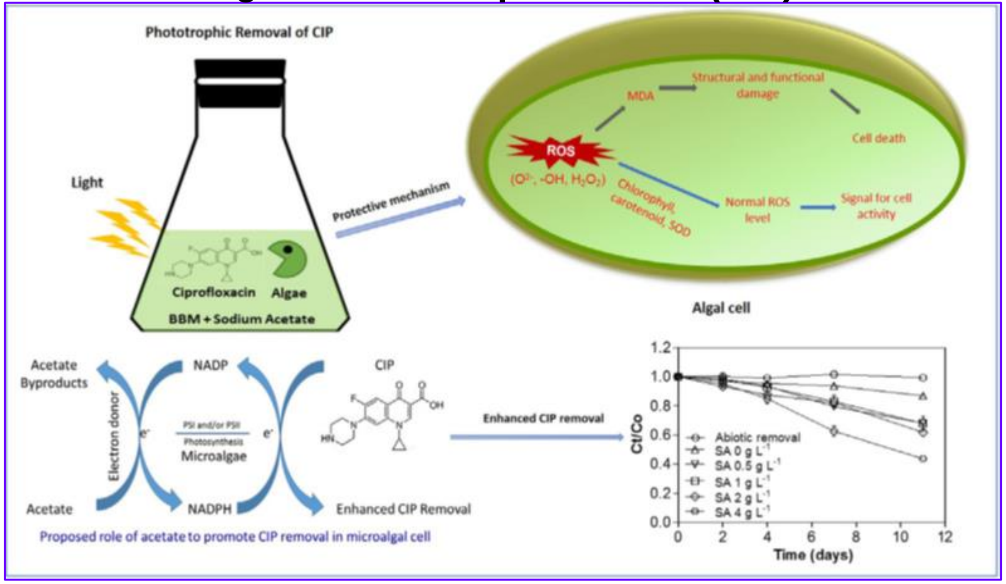


Figure. Proposed the role of **sodium acetate to promote CIP** biodegradation in *C. mexicana*

Source: Xiong et al. (2016), published in Journal of Hazardous Materials

- ***C. mexicana*** achieved a maximum of **35% biodegradation of CBZ**.
- Two metabolites (10,11-dihydro-10,11-expoxycarbamazepine and *n*-hydroxy-CBZ) were identified by **UPLC-MS**, as a result of **CBZ biodegradation** by *C. mexicana*.
- Addition of **sodium acetate** as an electron donor significantly increased the removal efficiency of CIP to 56% after 11 days of cultivation.

Future direction:
"Algal omics" research

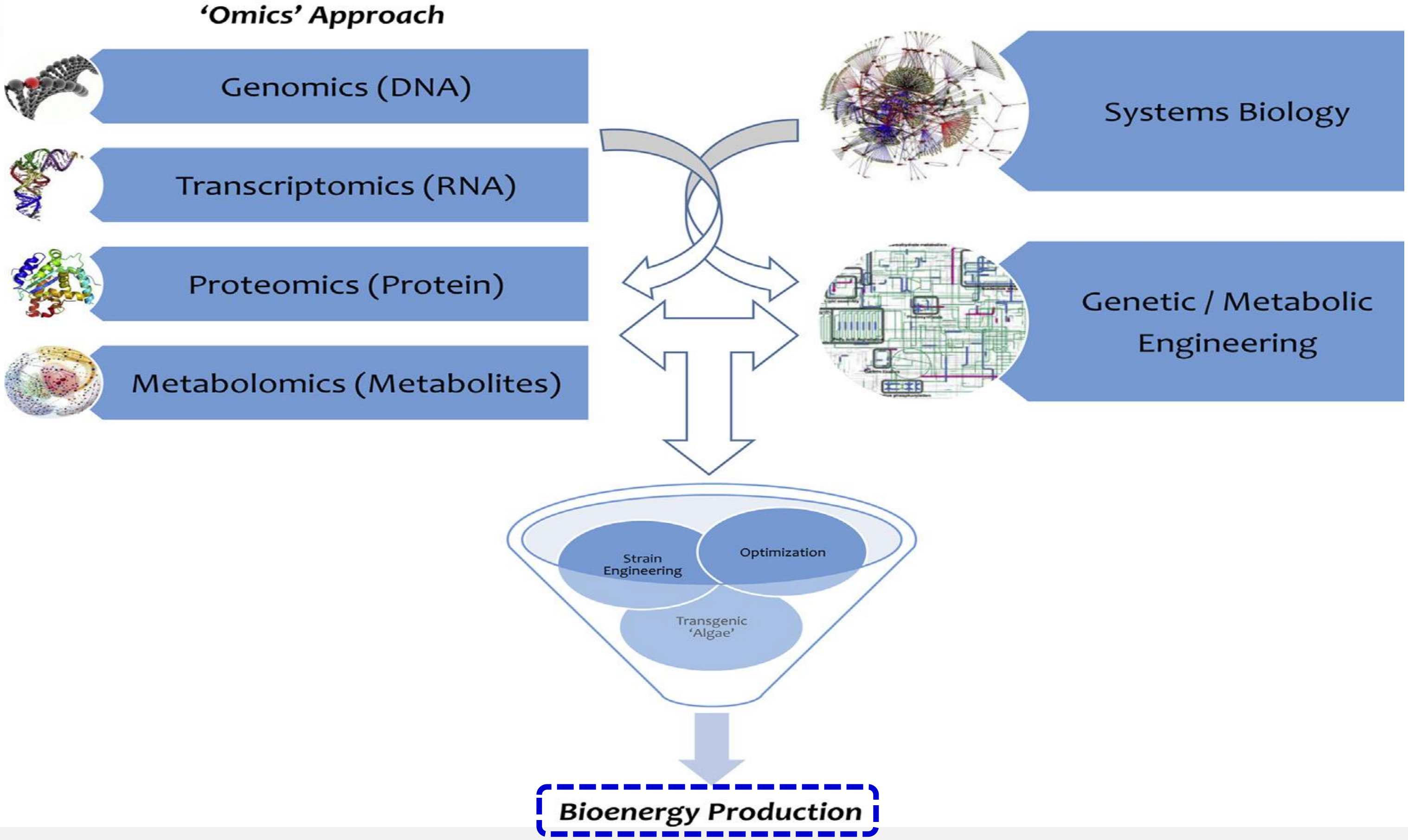


Figure. Conceptual illustration of integrative "**omics**" research with systems biology and genetic engineering approach for optimization of microalgae for bioenergy production.

- **Recent progress** in **microalgal genomics**, in conjunction with other "**omic**" strategies, has speeded the capability to **recognize genes** and **metabolic pathways** which are potential objectives in the expansion of **genetically engineered algal strains** with **optimum biochemical contents** for **biofuel production**.

Conclusions

- **Pretreatment algal** biomass reduced substrate **recalcitrance** and **enhanced accessibility** of starch to fermentative microorganisms for bioethanol production.
- **Immobilized yeast** cells were found to be superior over free yeast cells, since immobilized cells are more tolerant to ethanol and exhibit a lower degree of substrate inhibition.
- The **conversion efficiency** (**22.26-27.56%**) of *C. mexicana* biomass into biofuel revealed that approximately **one third** of the biomass has been converted into **energy** in the form of bioethanol.
- **Wastewater** supported the microalgal growth in real open ponds and photobioreactors with removal of inorganic nutrients (such as **TN**, **TP** and **TIC**).
- **Phytohormones** accelerated the microalgal growth and induced the quality and quantity of **fatty acid content** for biodiesel production.
- Microalgae were capable to biodegrade the emerging organic contaminants (EOCs) including **carbamazepine** and **ciprofloxacin**.

THANK YOU!

Acknowledgments

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References

1. Parton, W.J., Hartman, M., DeLucia, E.H. Impacts of a 32-billion-gallon bioenergy landscape on land and fossil fuel use in the US. PUBLISHED: Nature Energy. 11 JANUARY 2016 | Article number: 15005 | DOI: 10.1038/NENERGY.2015.5.
2. Georgianna, D.R., Mayfield, S.P. 2012. Exploiting diversity and synthetic biology for the production of algal biofuels. Nature, 488(7411), 329-35.
3. Salama, E.-S, Mayur B Kurade, Reda A.I. Abou-Shanab, Marwa M El-Dalatony, Il-Seung Yang, **Jeon, B.-H**, 2016. Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renewable & Sustainable Energy Reviews, submitted.
4. Salama, E.-S, Jae-Hoon Hwang, Marwa M. Eldalatony, Mayur B. Kurade, Reda A.I. Abou-Shanab, Ki-Hyun Kim, Il-Seung Yang, **Jeon, B.-H**. 2016. A review on recent progress in microalgal biofuel and omics approaches: A new biomass-energy conversion pathway for economically feasible biofuel generation. Energy and Environmental Science, in preparation.
5. Zhang, X., Yan, S., Tyagi, R., Surampalli, R. 2013. Biodiesel production from heterotrophic microalgae through transesterification and nanotechnology application in the production. Renewable and Sustainable Energy Reviews, 26, 216-223.
6. Hwang, J.-H., Kabra, A.N., Ji, M.-K., Choi, J., El-Dalatony, M.M., **Jeon, B.-H**. 2016. Enhancement of continuous fermentative bioethanol production using combined treatment of mixed microalgal biomass. Algal Research, 17, 14-20.
7. El-Dalatony, M.M., Kurade, M.B., Abou-Shanab, R.A.I., Kim, H., Salama, E.-S., **Jeon, B.-H**. 2016. Long-term production of bioethanol in repeated-batch fermentation of microalgal biomass using immobilized Saccharomyces cerevisiae. Bioresource Technology, 219, 98-105.
8. Xiong, J.-Q., Kurade, M.B., Kim, J.R., Roh, H.-S., **Jeon, B.-H**. 2016. Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga *Chlamydomonas mexicana*. Journal of hazardous materials.
9. Xiong, J.-Q., Kurade, M.B., Abou-Shanab, R.A., Ji, M.-K., Choi, J., Kim, J.O., **Jeon, B.-H**. 2016. Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana and Scenedesmus obliquus* and the determination of its metabolic fate. Bioresource Technology.
10. Salama, E.-S, Kabra, A.N., Ji, M.K., Kim, J.R., Min, B., **Jeon, B.-H**. 2014. Enhancement of microalgae growth and fatty acid content under the influence of phytohormones. Bioresour Technol, 172, 97-103.