Repeated-batch fermentation of microalgal biomass utilizing immobilized yeast cells for bioethanol production

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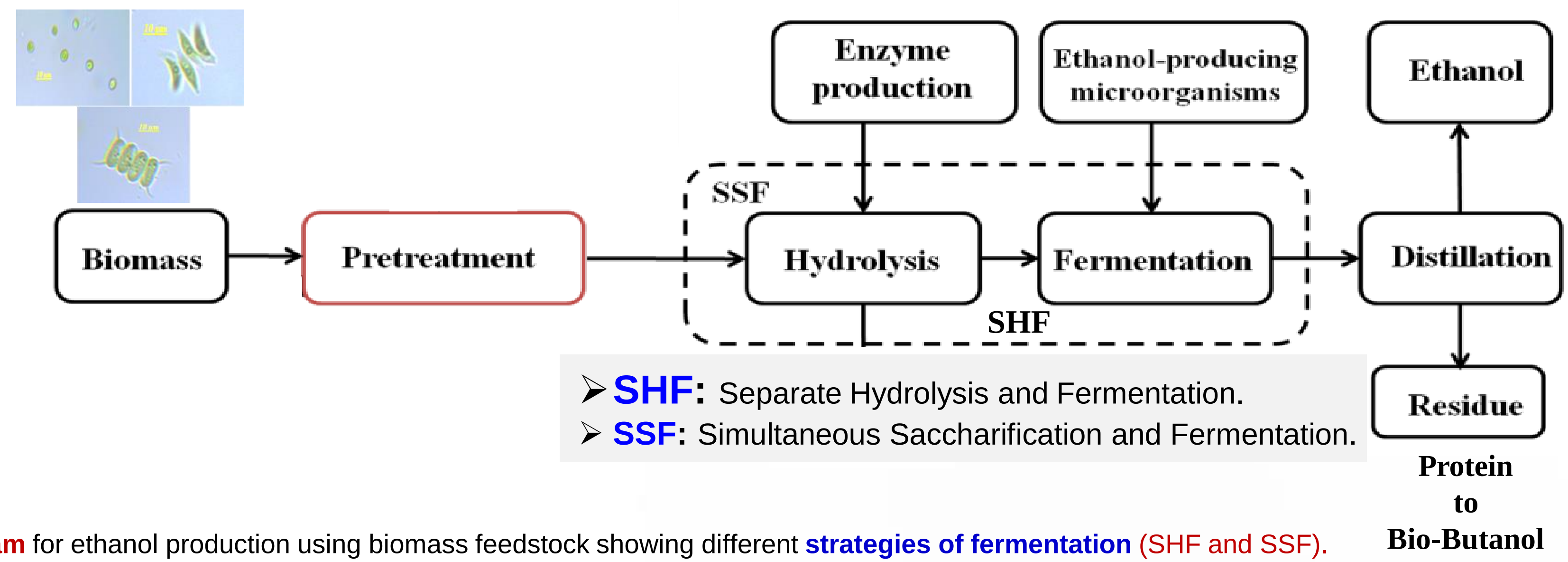
Introduction:
Serial steps to produce fuel from biomass

- **Microalgae** are sustainable biomass feedstocks that **grow faster**, **fix CO_2** and have possess **high amounts of carbohydrates** (~50%) in the form of **starch** and **cellulose**, which can be fermented to bioethanol (Gnansounou et al., 2013).

- **Pretreatments** (sonication) of biomass **enhances the rate of hydrolysis** to fermentable sugar as it **increases the surface area**, enhances the **sugar solubility**, improves the **substrate digestibility** and **weakens the cell wall for enzymes to be accessible** (Jeon et al., 2013).

- **Enzymatic hydrolysis** has **higher selectivity** and production of **low toxic hydrolysates** compared to acid hydrolysis (El-Dalatony et al., 2016).



- **SHF:** Separate Hydrolysis and Fermentation.
- **SSF:** Simultaneous Saccharification and Fermentation.

Flow diagram for ethanol production using biomass feedstock showing different **strategies of fermentation** (SHF and SSF).

Source: da Silva, A.S.A., Teixeira, R.S.S., de Oliveira Moutta, R., Ferreira-Leitão, V.S., de Barros, R.d.R.O., Ferrara, M.A., da Silva Bon, E.P. **2013**. Sugarcane and woody biomass pretreatments for ethanol production, ISBN, pp. 978-953.

Research objectives

In this study, we aimed to:

- Find an economic approach for enhanced bioethanol production from microalgae.
- Investigate two different fermentation approaches (**SHF** and **SSF**) on sonicated microalgal biomass for bioethanol production.
- Explore the efficiency of long-term production of bioethanol through repeated batch fermentation employing immobilized yeast cells.

23% of biomass was converted into energy (Bioethanol).

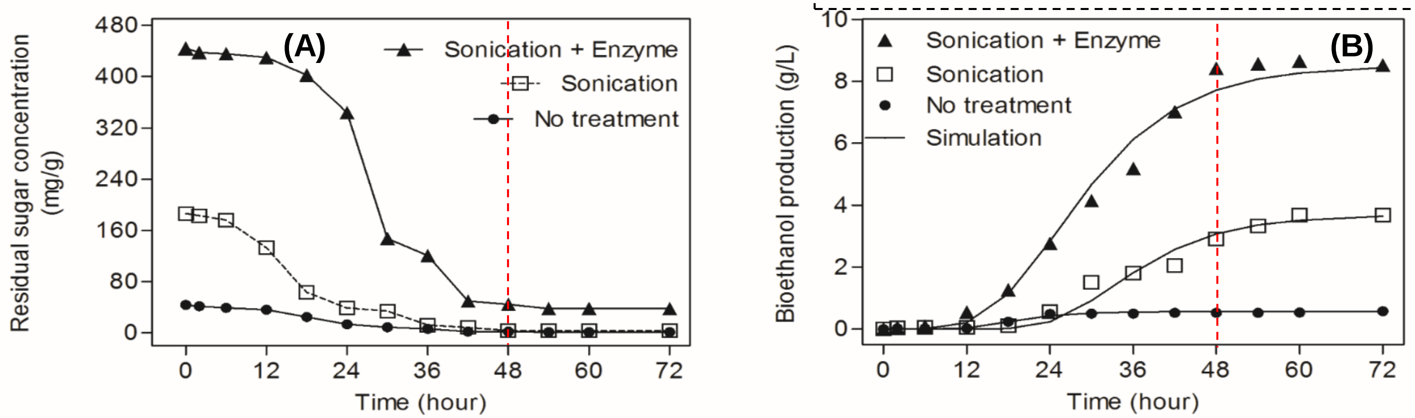


Figure. Residual sugar concentration **(A)**, Cumulative bioethanol production **(B)**, during **SHF** of microalgae *C. mexicana* for 3 days.

28% of biomass was converted into energy (Bioethanol).

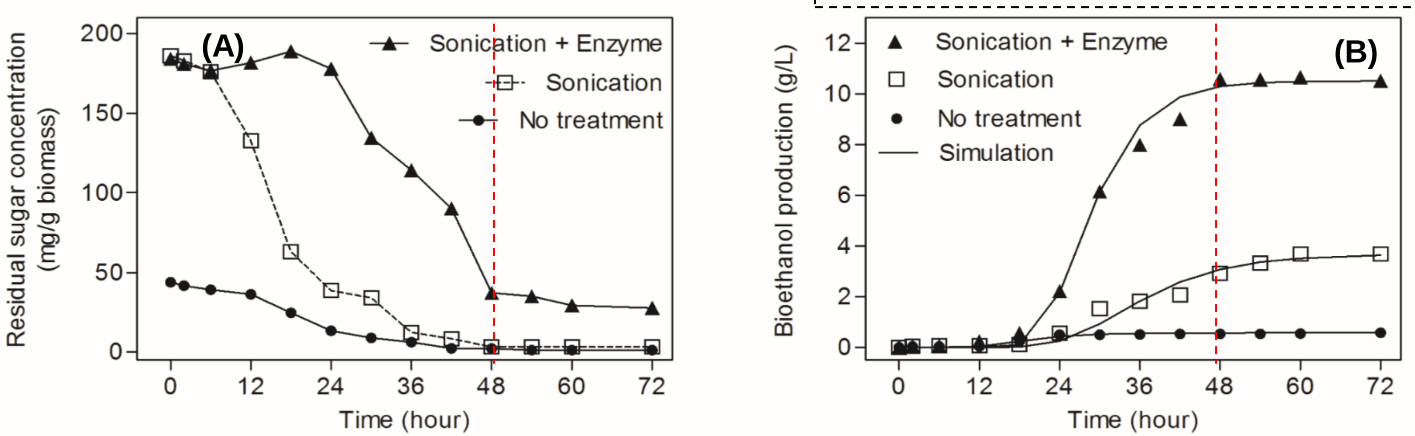


Figure. Residual sugar concentration **(A)**, Cumulative bioethanol production **(B)**, during **SSF** of microalgae *C. mexicana* for 3 days.

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

- The principal benefits of performing the **enzymatic hydrolysis** together with **the fermentation,** instead of in a separate step after the **hydrolysis** are **reduced end product inhibition of the enzymatic hydrolysis**, and **reduced investment costs** (Gnansounou & Raman, 2016).

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

Results and Discussion:
Ethanol production during 7-cycles (Immobilized cells)-SSF

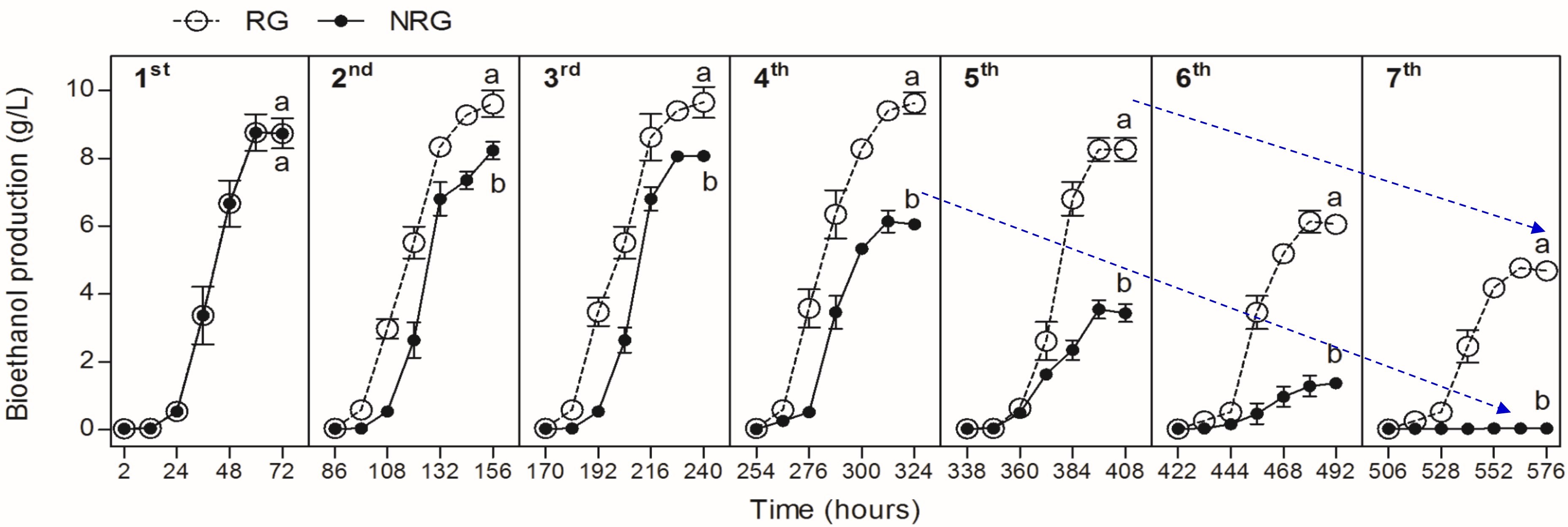


Figure. Cumulative bioethanol production from *Chlamydomonas mexicana* through **7-cycles** of repeated fermentation using **immobilized** yeast cells. **RG:** regenerated; **NRG:** non-regenerated beads

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

Results and Discussion:
Scanning electron micrograph SSF (Immobilized cells)-SSF

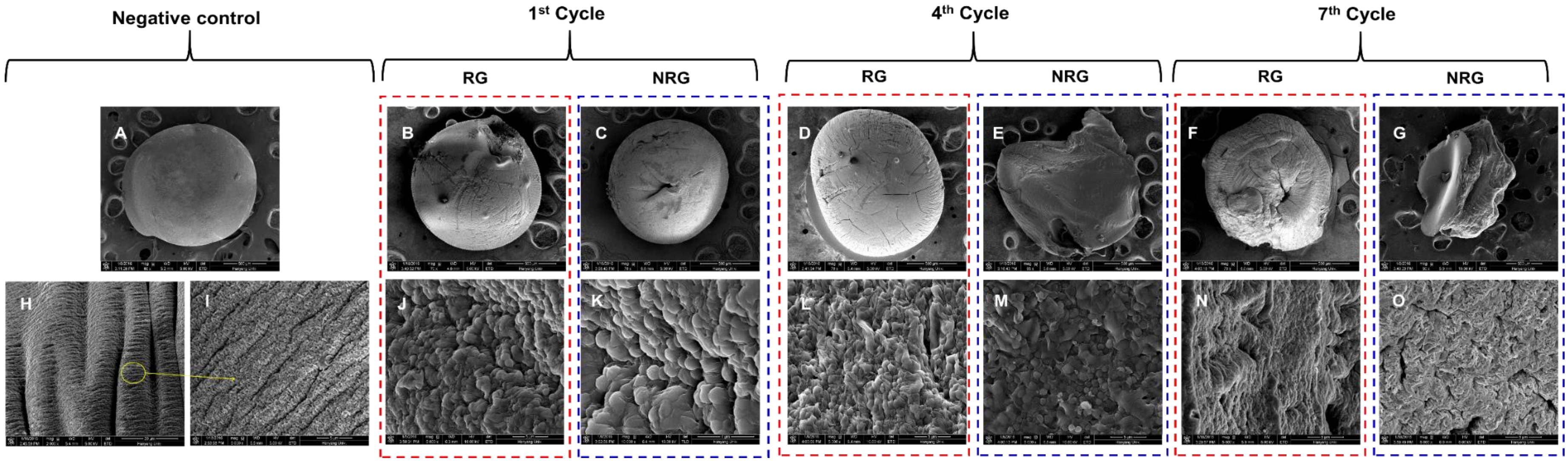


Figure. Scanning electron micrograph of Ca-alginate beads. Picture from **A** to **G** to show the whole beads and from **H** to **O** showing the cross sections of beads. **A**= Negative control bead (without yeast cells). **H** and **I** = Cross section in the negative control beads shows no yeast cells inside the beads. Whole beads and cross section for RG and NRG beads after the 1st cycle, showing yeast cells embedded inside in the bead (**B, C, J, K**), respectively. Whole beads and cross section of RG and NRG beads after the 4th cycle (**D, E, L, M**), respectively, showing that the yeast cells number are increasing in case of RG beads and decreasing for NRG beads. Soft and weak RG bead with less number of yeast cells after the 7th cycle (**F, N**). Destruction of bead with no yeast cells (**G**, **O**).

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

- SEM images of ABs without yeast cells, which exhibited better integrity and rigidity. The structure of AB carriers in the cross-section was dense and less porous.
- RG beads also showed **better** integrity and rigidity in addition to the **high** porosity and better yeast cell distribution for several cycles compared to NRG beads. While NRG beads exhibited disruption of alginate films with significant decrease in yeast cells within it after several fermentation cycles.

Conclusions

- **Sonication** combined with **enzyme hydrolysis** achieved a **445 mg/g** release of TRS.
- *Saccharomyces cerevisiae* showed TRS **consumption efficiency** of **91-98%**.
- **SSF** exhibited higher ethanol production (**10.5 g/L**) compared with SHF (**8.48 g/L**).

- **Energy recovery** improved through immobilized **repeated batch fermentations**.
- Regenerated beads (**RG**) achieved a fermentation efficiency of **79.5%** for **four cycles**.

- 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.

- These results confirmed that the repeated-batch SSF using immobilized cells was a feasible and cost-effective method for bioethanol production.

Thank You !

Acknowledgments:

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References:

1. Gnansounou, E., Raman, J.K., **2016**. Life cycle assessment of algae biodiesel and its co-products. Appl. Energy 161, 300–308.
2. Scovronick, N., França, D., Alonso, M., Almeida, C., Longo, K., Freitas, S., Rudorff, B., Wilkinson, P. **2016**. Air Quality and Health Impacts of Future Ethanol Production and Use in São Paulo State, Brazil. International Journal of Environmental Research and Public Health, 13, 695.
3. The global biotech ethanol company. **2011**, Biocarburantes Castilla y León, Abengoa Bioenergy, S. A..
4. Jeon, B.H., Choi, J.A., Kim, H.C., Hwang, J.H., Abou-Shanab, R.A., Dempsey, B.A., Regan, J.M., Kim, J.R., **2013**. Ultrasonic disintegration of microalgal biomass and consequent improvement of bioaccessibility/bioavailability in microbial fermentation. Biotechnol. Biofuels 6, 37.
5. El-Dalatony, M.M., Kabra, A.N., Hwang, J.H., Govindwar, S.P., Kim, K.H., Kim, H., Jeon, B.H., **2016**. Pretreatment of microalgal biomass for enhanced recovery/extraction of reducing sugars and proteins. Bioprocess Biosyst. Eng. 39, 95–103.
6. da Silva, A.S.A., Teixeira, R.S.S., de Oliveira Moutta, R., Ferreira-Leitão, V.S., de Barros, R.d.R.O., Ferrara, M.A., da Silva Bon, E.P. **2013**. Sugarcane and woody biomass pretreatments for ethanol production, ISBN, pp. 978-953.
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.