VALORIZATION OF WASTEWATERS VIA BIOENERGY AND BIOPRODUCTS USING CARBOHYDRATES FROM MICROALGAE

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INTRODUCTION

In the last decades, several renewable sources, mainly wastes, are under research to cope with the increase of global raw material and energy demand and the crisis of fossil fuel. Therefore, the use of microalgae has been suggested as a feasible alternative for several industrial applications: wastewater treatment, nitrogen and phosphorous recovery, biogas upgrading, production of biofuels, biofertilizers, animal and fish feed, etc (Martín-Juárez et al., 2016). As with the treatment of pig manure, these are one of the major environmental problems of livestock in our countries at present. This waste can be converted into a renewable resource of great economic potential. The integrated study of the manure treatment with consortia of microalgae-bacteria and the subsequent valorization of the produced biomass through "green" process remains a challenge nowadays (Acién., 2012).

The chemical composition of this microalgae biomass depends on the several factors as: microalgae species and cultivation conditions as medium, temperature, residence time, nutrient starvation. Consequently, the biomass has a wide range of compositions: proteins (15% - 52%), lipids (5% - 10%) and carbohydrates (10% - 50%). Different pretreatments are required to release these added-valuable compounds from the cells with the aim to valorise the microalgae biomass, and hence, cell wall composition and structure is determining in this step (D'Hondt et al., 2017). Among the possible alternatives for the valorization of carbohydrates, depending on the bacteria action, can highlight the fermentation of sugars released by hydrolysis to produce bioalcohols or that of sugars and acids to produce polyesters (PHAs) for bioplastics (Azizi et al., 2017). Diverse residues or by-products are generated after the pretreatment and the extraction of its fractions, that can be used as substrate to produce biogas. The digestate after biogas production can be used as fertilizer considering its quality.

This work aims to the valorization of microalgae biomass from the pig manure wastewater treatment to produce bioenergy and bioproducts applying a biorefinery concept. Optimization of different pretreatments as a disinfectant effect is evaluated to disrupt the cell and release the sugars and, hence, the effect on the other fractions. A study about the kinetic of enzymatic hydrolysis and its models is assessed throughout the time with pretreated and raw material biomass. Subsequently, batch fermentation tests are carried out according to the compounds obtained in the previous steps, for bioethanol or PHAs. Finally, the effect of the same pretreatments to improve the biogas production, and finally, the use of the digestate as fertilizer are also studied.

MATERIAL AND METHODS

Fresh microalgae biomasses are cultivated in a thin-layer photobioreactor with a volume of 1200L fed with pig manure wastewater diluted at 10%. The biomasses are collected at different times of the year, being *Scenedesmus* the principal microalgae species. All the biomasses are kindly supplied by Cajamar Foundation (Almeria, Spain) and refrigerated at 4ºC prior to use.

The pretreatments applied are alkali (0.5 and 2 M NaOH), acid (0.5 and 2 M HCl), peroxide-alkaline (0.5 and 7.5% H_2O_2), ultrasounds (5 and 21 min), steam explosion (130 and 170ºC) and bead mill (5 and 60 min) at 5% (w/w) dry algae. After the pretreatments, the solid and liquid fractions are centrifuged at (10000 rpm, 10 min) and are stored at 4ºC for further composition analysis. The solid fractions and the whole fractions are stored for enzymatic hydrolysis and biogas production. The total solids, ash and sugars are analysed in the solid and liquid fractions. The proteins and lipids are also analysed in the solid fractions.

Enzymatic hydrolysis assays of all biomass are performed in 100 mL Erlenmeyer flasks containing 6% w/w dry solid or whole pretreated microalgae with citrate buffer 1M. The pH is adjusted at 4.9 ± 0.1. The assays are

carried out in a rotatory shaker at 50 ºC and 300 rpm for 48 h. The experiments are performed in duplicate for each taken sample at different times. The commercial enzymes used are cellulases (Celluclast 1.5L, Novozyme 188 and Viscozyme L), proteases (Alcalase 2.5 L and Flavourzyme), and lipase (Lecitase Ultra), kindly supplied by Novozymes (Denmark). In addition, controls (without enzymes and non-treated microalgae) are performed for all biomass. After the enzymatic hydrolysis, the solid and liquid fractions are separated by centrifugation (10 min, 10000 rpm). Both fractions are stored at 4ºC for further composition analysis of total solids, ash and sugars. Additionally, TNK and lipids are analysed in the solid fractions.

The fermentations tests are carried in 100 mL Penicillium flasks containing whole slurries of hydrolysates with 10% of inoculum of *S. cerevisiae* or *Cupriavidus necator* at 30 °C under 175 rpm, for 24 h. All the experiments are conducted in duplicate. Fermentations are centrifuged at 10000rpm for 10min, supernatants are analyzed for sugars and ethanol content; and the pellet solid for PHAs production. The anaerobic digestion tests are performed with the solid and whole fractions in 300 mL Borosilicate bottles with 0.5 g VS substrate/g VS inoculum, at 37ºC, for 30-40 days. The biogas composition is analyzed by GC-MS.

RESULTS AND DISCUSSION

The biochemical composition of microalgae biomass changes over the months during the year, that the months of March and May achieve high carbohydrates content, and low proteins. The chemical pretreatments solubilize high amounts of carbohydrates up to 90% with HCl, while the physicals do not reach these yields. In terms of enzymatic hydrolysis, the pretreatment of NaOH (0.5M) achieves the highest sugar release (63%) using the whole fractions with the minimum degradation, and high yields are reached with HCl. The proteins and lipids are also affected with the same behaviour by the chemical pretreatments. However, they are less influenced by some of the enzymes used.

High volatile solids solubilization is found for all the pretreatments. Results show that the alkali pretreatment enhanced the methane production in all the assays, but with a clear lag effect. The highest increase respect to the untreated microalgae biomass is obtained using the whole fraction at NaOH 2M (130% of increase). Alkali-peroxide pretreatment rise biogas production and kinetic, but only when using the whole pretreated sample. Bead mill and steam explosion remarkably increase the anaerobic degradation kinetic but not the biogas production. Ultrasound and acid pretreatment do not improve the biogas production and, hence, they caused lag phases of around 6-10 days.

CONCLUSIONS

The use of whole fractions after the pretreatment improve the sugar yields of the next steps and, hence, low residues are generated. Chemical pretreatments applied have a disinfectant effect and at low concentrations report the best results in all the steps. The work concludes different feasible possibilities to use the whole fractions. The enzymatic hydrolysis at shorter times (<6h) achieve the maximum sugar recovery with the minimum degradation. The production of biogas and biofertilizers is the other alternative that chemical pretreatments enhance respect the untreated biomass.

ACKNOWLEDGEMENTS

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Valorization of Wastewaters via Bioenergy and Bioproducts using Carbohydrates from Microalgae

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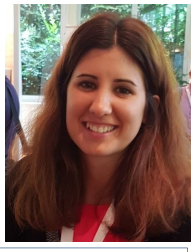
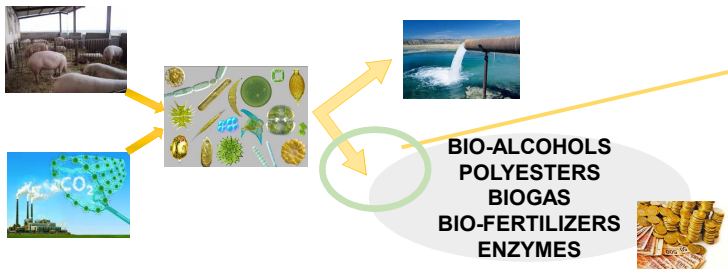
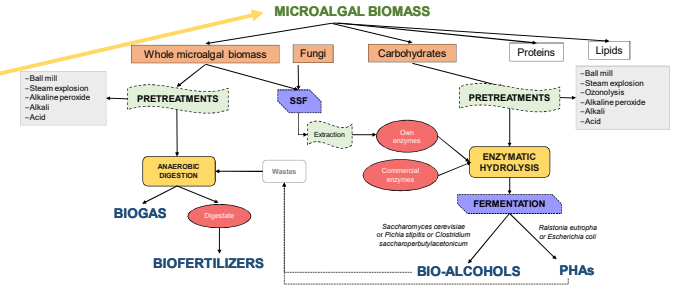
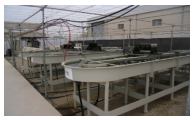


1 BIOREFINERY CONCEPT



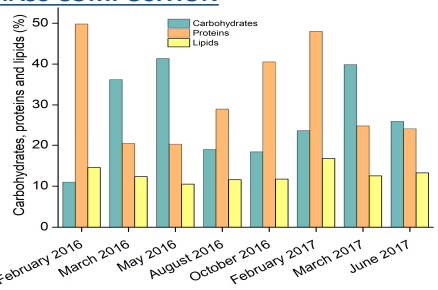


2 MICROALGAE BIOMASS COMPOSITION

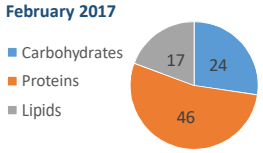


Pig manure treatment

Scenedesmus almerienses + Bacteria

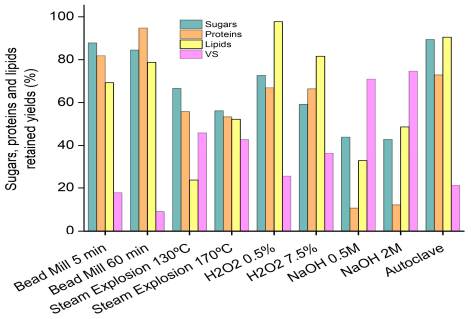


3 PRETREATMENTS



NaOH and steam explosion → release more sugars, proteins and lipids

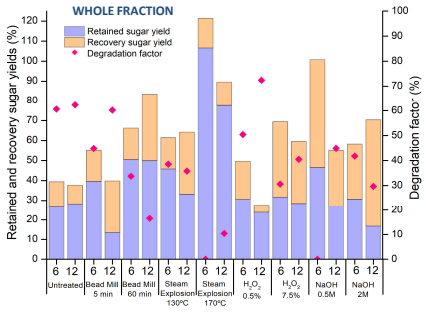
Non-exclusive pretreatments for a single fraction



4 ENZYMATIC HYDROLYSIS

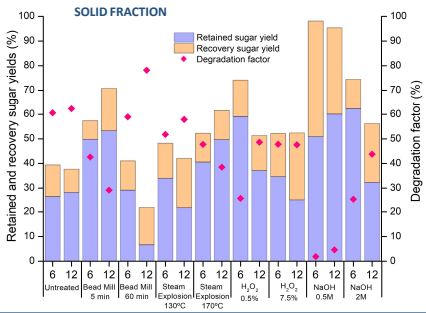
February 2017

- 6 % (w/w) dried algae
- Whole (solid and liquid) or only solid fraction
- Celluclast 1.5L (10 FPU/g)
- 50°C, 300 rpm, 6 and 12h



NaOH 0.5M
- ↑↑ sugar release
- No degradation

Other conditions and pretreatments:
- ↑ sugar release
- Higher degradation

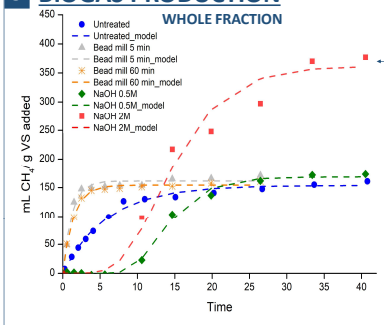


NaOH 0.5M
- ↑↑ sugar release
- No degradation

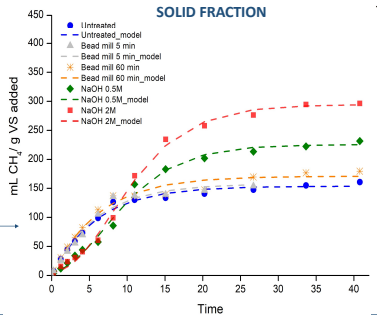
Other conditions and pretreatments:
- ↑ sugar release
- Higher degradation

Only solid fraction were used → lower yields

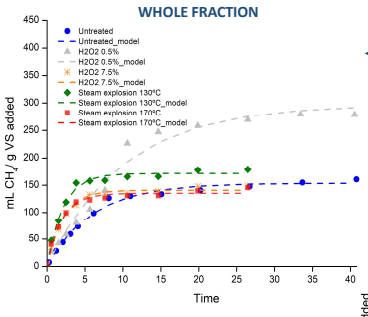
5 BIOGAS PRODUCTION



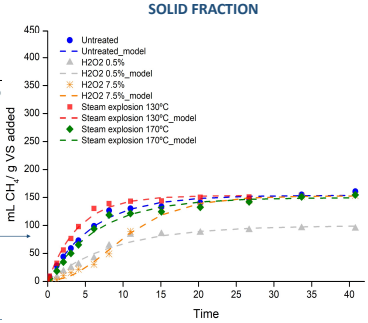
- Rapid hydrolysis → bead mill
- Lag phase ↔ inhibition → NaOH
- ↑↑ methane production → NaOH 2M



- ↑↑ methane production → NaOH
- Only with solid fraction → lower yields



- Rapid hydrolysis → steam explosion
- ↑↑ methane production → H_2O_2 0.5%



- No improvement over untreated biomass

6 CONCLUSION

- Microalgae composition: ↑carbohydrates, ↓ proteins, ~ lipids.
- Chemical pretreatments → ↑ ↑ release sugars to the liquid fraction.
- Whole fractions → feasible and useful.
- Degradation factor → key elemental
- Chemical pretreated samples → ↑ ↑ release sugars to the hydrolysate.
- ↑ ↑ methane production → chemical pretreatments with lag phase.

7 ACKNOWLEDGMENT

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