Evaluation of obtaining biohydrogen by different fermentation methods

Evaluación de la obtención de biohidrógeno por diferentes métodos de fermentación

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Abstract

There are a lot of projects around the methods of obtaining renewable fuels that are less harmful to the environment. In that sea of investigations, one of the less explored and with greater potential to become a principal biofuel is biohydrogen production through fermentations. However, biohydrogen technologies production has limitations such as low productivity, therefore are not profitable yet. Through this project, the search for the evaluation of practice to attain biohydrogen to determine how feasible the development of said technology, as well as the efficiency as a process is the main goal. In this project, development of multiple fermentations, analysis of growth curves in different growth mediums such as nutrient agar, BBM and TAP mediums in addition with microalgae *Chlamydomonas*, were made.

Biohydrogen, Investigation, Fermentation; Microalgae *Chlamydomonas*

6 Introduction

The project called Evaluation of Obtaining Biohydrogen by Different Fermentation Methods was developed with the objective of analyzing the current methods of biohydrogen production by different fermentations and to develop a system that operates optimally and then compare with different fermentation methodologies. In addition, the project aims to give a clearer understanding of the processes of obtaining hydrogen and the different factors that affect its production.

In previous works, the analysis of the evaluation of production of biohydrogen depends mainly on different mediums of cultivation and the use of different microorganisms.

Throughout the project, analyses of bacterial samples in different cultivation mediums were performed. These analyses comprise the growth and count of each bacterial group in different cultivation medium, analysis of the type of fermentation and biohydrogen production. First, a group of bacteria was selected; i) *Escherichia coli*, ii) *Enterobacter* sp. and iii) *Citrobacter freundii*, and two commercial cultivation medium; a) nutrient broth and b) tripticasein soy broth. The analysis period comprised 96 hours in which the samples were incubated in 250ml flasks (Pyrex) with a working volume of 150ml inside an incubator (Gutstark) at 37°C. Spectrophotometer (jf721) measurements were performed at 600 nm wavelength every 2 hours for 96 hours. Afterwards, fermentation and growth of the bacteriological strains was carried out in a new medium: Microalgae. This medium adds microalgae of the Chlamydomonas type in nutrient broth, keeping them under constant agitation.

The main reason for the project is to advance biofuel technology, focused on the production of biohydrogen by fermentative means, so that it will be possible to contribute to current research with the data obtained. At present, there is a limited database on the use of bacteria with microalgae to create consortium-type systems for biohydrogen production, which is why it was decided to perform an analysis of this type of systems and to be able to complement future research focused on this topic.

It was hypothesized that there would be an exponential growth indicated in the growth curves, as well as the obtaining of biohydrogen at the end of fermentation.

6.1 Methodology

We began with the selection of bacteria as biohydrogen generation medium in our system, which were three: i) *E. coli*, ii) *Enterobacter* sp. and iii) *C. freundii*. These bacteria were selected due to their characteristic that within their metabolism they synthesize biohydrogen. Each strain was isolated using the radial quadrant streak seeding technique. The strains were then inoculated in nutrient broth and trypticasein soy broth (STC) and subjected to fermentation in an incubator at 37°C for 96 hours. Subsequently, spectrophotometer measurements were performed at a wavelength of 600 nm in absorbance mode every two hours during the 96 hours. The data obtained from the spectrophotometer were used to plot the growth kinetics of each of the bacteria.

To obtain and store biohydrogen, flasks were used for each of the cultivation mediums. For biogas storage, a balloon was used as a container for the gasses produced, as well as syringes.

When the data recording was complete, a consortium was made between each bacterium, in order to analyze their behavior and joint growth, using the same parameters.

After performing the analysis with the nutritive cultivation medium, the same steps were repeated, but the cultivation medium was changed to microalgae, which requires a different treatment during the growth stage than the one already used.

In their case, it was kept in agitation for the first 24 hours and then measurements were taken at a wavelength of 750 nm every 2 hours for 3 days. In between, the samples were shaken again.

The method for analyzing gas generation was the same as for the previous medium: by volumetric visualization in balloons. In the case of microalgae, the samples were in *Chlamydomona* algae medium with BBM medium and TAP medium along with bacteria. The ratio of microalgae to medium was 1/10, in 100 mL total cultivation medium. A constant pH of 6 was maintained in all cultivation mediums at 29°C (room temperature).

These processes were repeated 3 times each to obtain more accurate results and to be able to verify in case something went wrong.

At the beginning, since the necessary equipment was not available to perform the measurements in the renewable energy laboratory, the chemistry laboratory had to be used every two hours, with the permission of the laboratory manager.

Once the spectrophotometer and incubator were obtained, these trips were avoided.

All the cultivation mediums were made with materials found in the renewable energy laboratory, except for the algae, which were already in an active cultivation medium. From this medium only 10 mL was removed per cultivation medium to be made.

The bacteria were obtained from the renewable energy laboratory, which were already being used by the renewable energy team.

The hours of measurements began at 7 AM in order to conclude at 7 PM (opening and closing time of the Tecnológico de La Laguna) in this way, it was possible to obtain a 24-hour curve by making two measurements starting at different time periods.

Consisting of a grill to sterilize the cultivation medium, the flasks used and lighters for streaking.

The measurements were performed in groups of 6 samples, 3 for each cultivation medium.

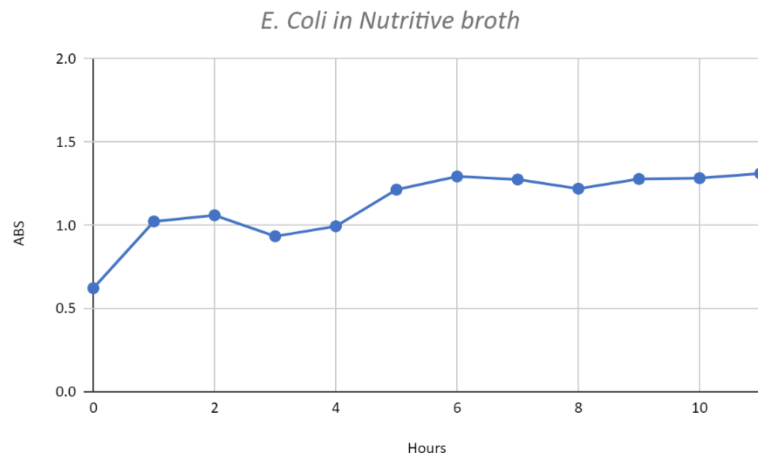
Stirring is a necessity for proper growth of microalgae.

6.2 Results obtained

For the measurement of the growth curves, the bacterial strains were reactivated in the medium indicated below. Measurements were taken with a spectrophotometer at different wavelengths (600 for nutrient broth and trypticasein soy, 750 for microalgae in BBM and TAP) in two-hour periods for three days in order to analyze their growth.

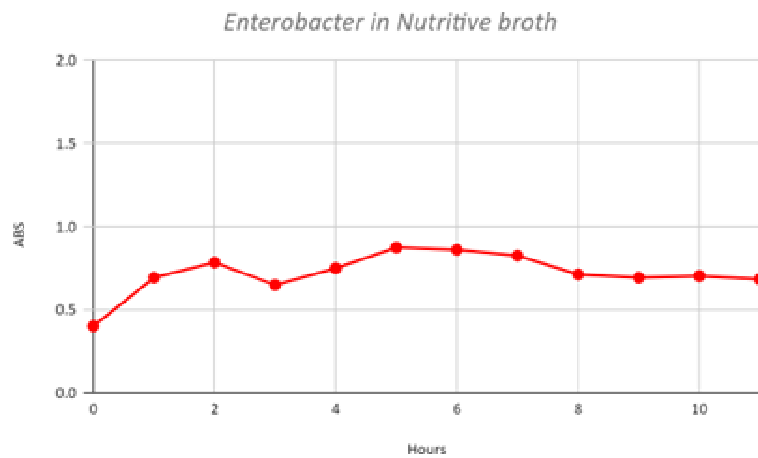
The growth graphs obtained from the nutrient and stc cultivation medium are shown below:

Graphic 6.1 *E. coli* growth curve in nutritive broth



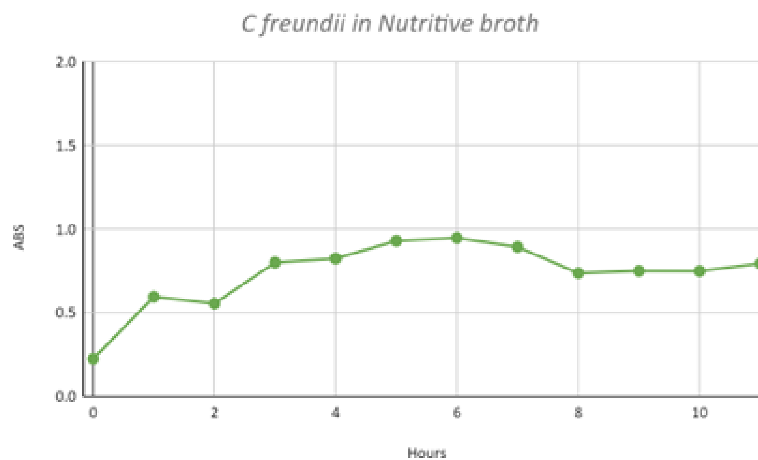
This graphic depicts the growth curve of *E. coli* bacteria in a nutrient broth medium specified for bacterial growth and other microorganisms that can be worked with. As can be seen, the bacteria are in a period of growth after 12 hours of incubation, which is beneficial for the analysis of growth in this and other mediums. In this way, we can determine whether or not the medium is suitable for the bacteria, which is our main source in this process.

Graphic 6.2 *Enterobacter* growth curve in nutrient cultivation medium



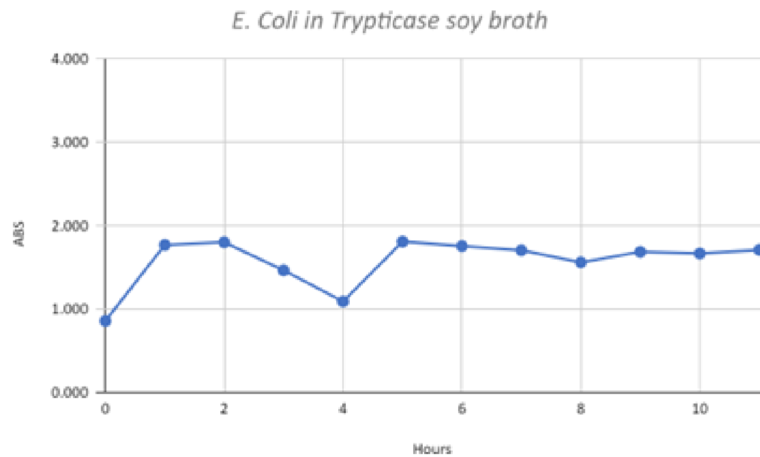
Graph depicting the growth of *Enterobacter* bacteria in the nutrient broth medium in the indicated period.

Graphic 6.3 *C. freundii* growth curve in nutrient cultivation medium



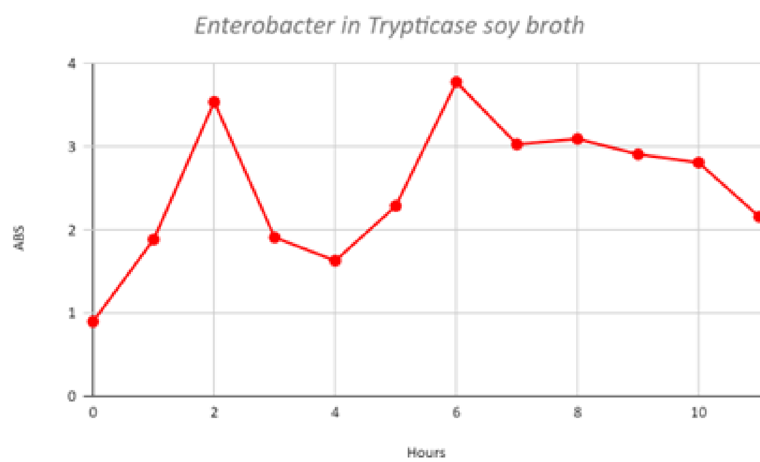
Graphic depicting the growth of *C. freundii* bacteria in the nutrient broth medium in the indicated period.

Graphic 6.4 *E. coli* growth curve in STC cultivation medium



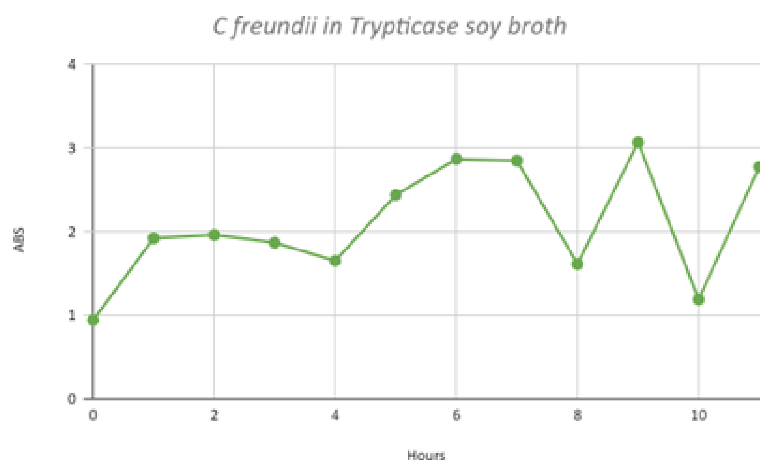
Graphic depicting the growth of *E. coli* bacteria in trypticase soy broth medium, a nutrient alternative, but similar in function to nutrient broth.

Graphic 6.5 Growth curve of *Enterobacter* in STC cultivation medium



Graphic showing the growth of *Enterobacter* bacteria in trypticase soy broth medium at the indicated time.

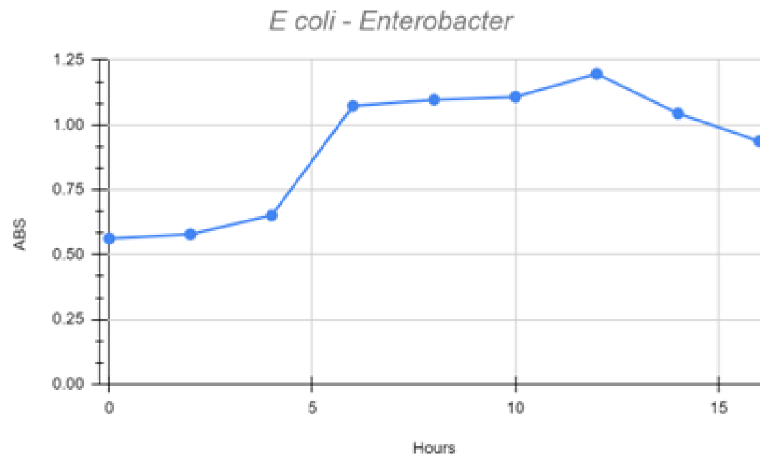
Graphic 6.6 Growth curve of *C. freundii* in STC cultivation medium



Graphic showing the growth of *C. freundii* bacteria in the trypticase soy broth medium at the indicated time.

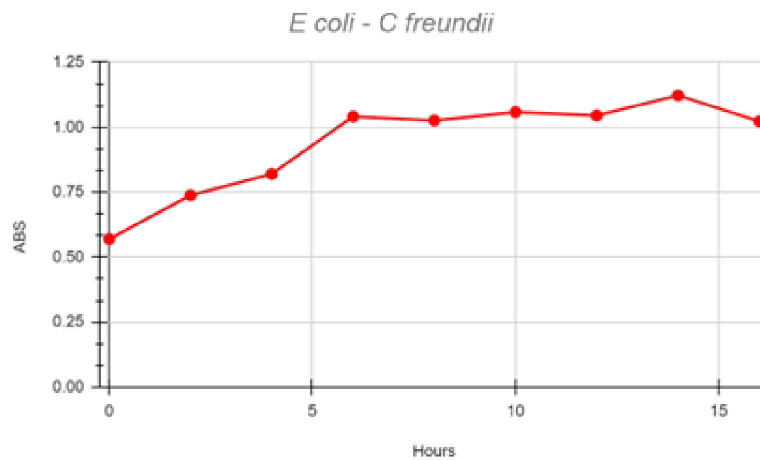
The growth curve of the bacteria in nutrient cultivation medium, but in the form of a consortium, is presented below:

Graphic 6.7 Growth curve of *E. coli* and *Enterobacter* in nutrient cultivation medium



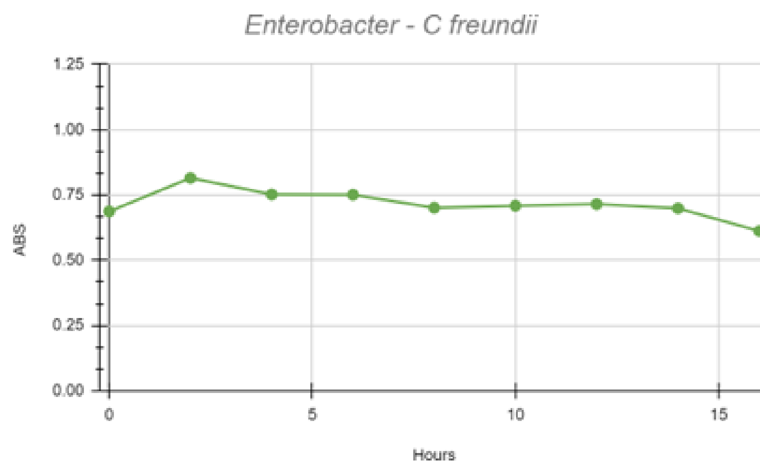
For these curves, it was decided to make consortia between the available bacteria in order to induce a state of stress that would promote their development and, thus, the production of biohydrogen.

Graphic 6.8 Growth curve of *E. coli* and *C. freundii* in nutrient cultivation medium



Graphic showing the growth of *E. coli* and *C. freundii* bacteria in consortium, in nutrient broth medium, in the indicated time.

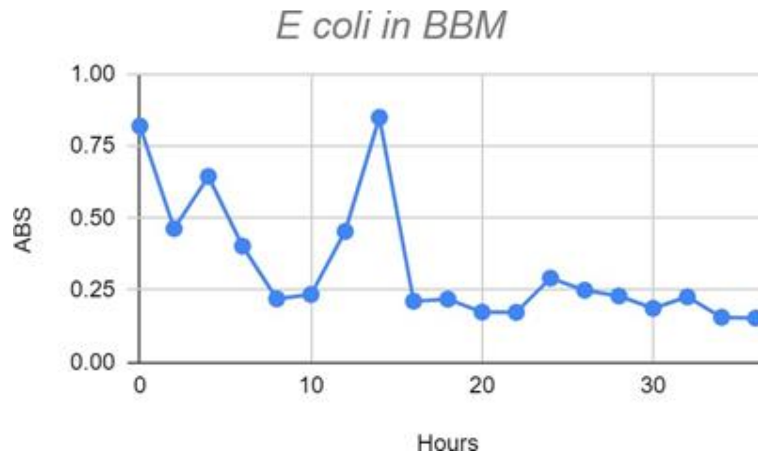
Graphic 6.9 Growth curve of Enterobacter and *C. freundii* in nutrient cultivation medium



Graphic depicting the growth of *Enterobacter* and *C. freundii* bacteria in consortium, in nutrient broth medium, in the indicated time.

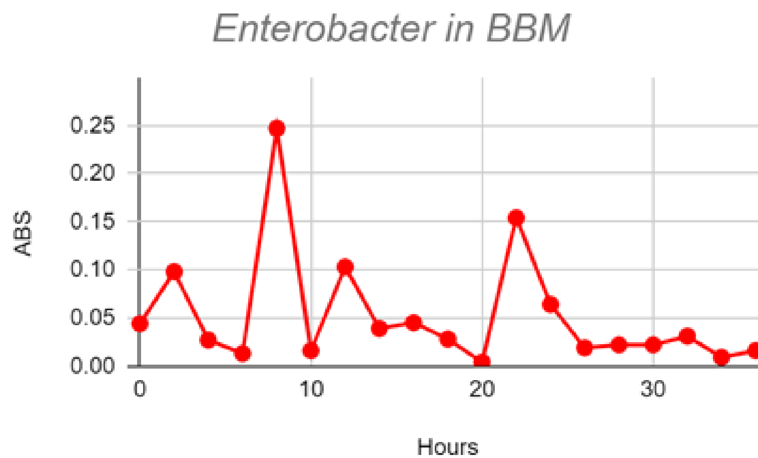
The growth curves of the bacteria in consortium with the microalgae in BBM medium are presented below:

Graphic 6.10 Growth curve of *E. coli* in *Chlamydomonas* BBM cultivation medium



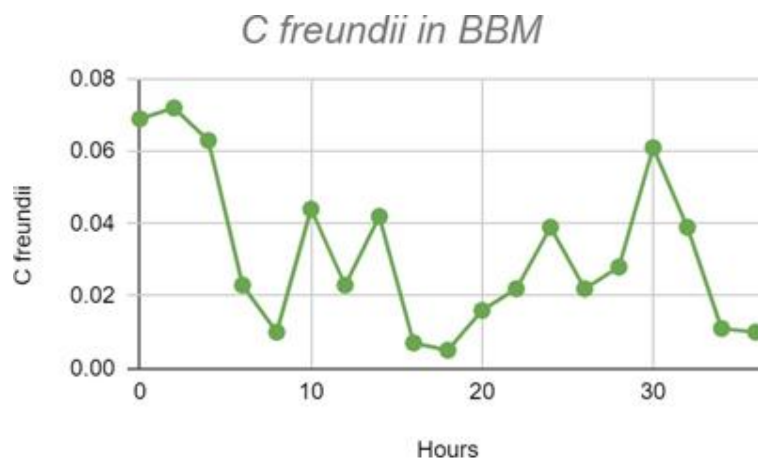
Graphic showing the consortium between *E. coli* and the microalgae *Chlamydomona* in BBM medium, an ideal medium for the development of microalgae. From these graphs, the analysis of the theory of coexistence between bacteria and microalgae for their growth and development of biohydrogen was proposed.

Graphic 6.11 Growth curve of *Enterobacter* in *Chlamydomonas* BBM cultivation medium



Graphic depicting the consortium between Enterobacter and the microalgae *Chlamydomona* in BBM medium, at the indicated time.

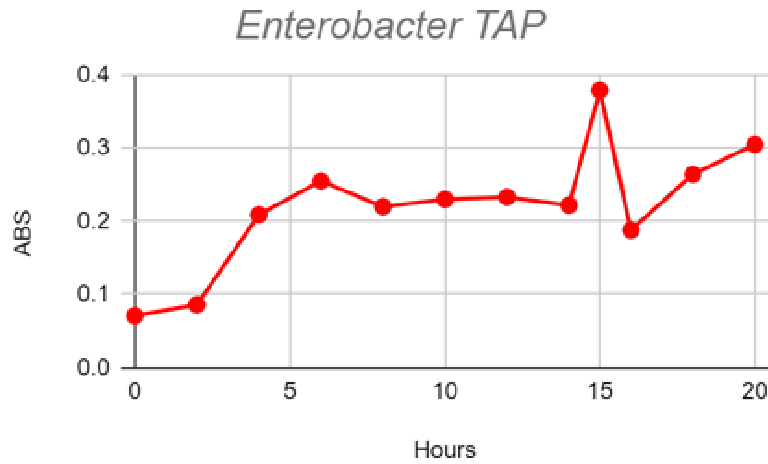
Graphic 6.12 Growth curve of *C. freundii* in *Chlamydomonas* BBM cultivation medium



Graphic showing the consortium between *C. freundii* and the microalgae *Chlamydomonas* in BBM medium, at the indicated time.

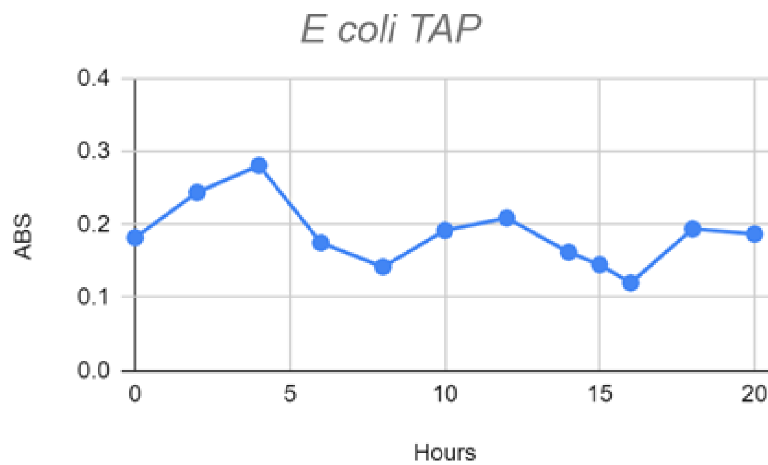
Finally, growth curve of the bacteria on *Chlamydomonas* in TAP medium:

Graphic 6.13 *E. coli* growth curve in *Chlamydomonas* TAP cultivation medium



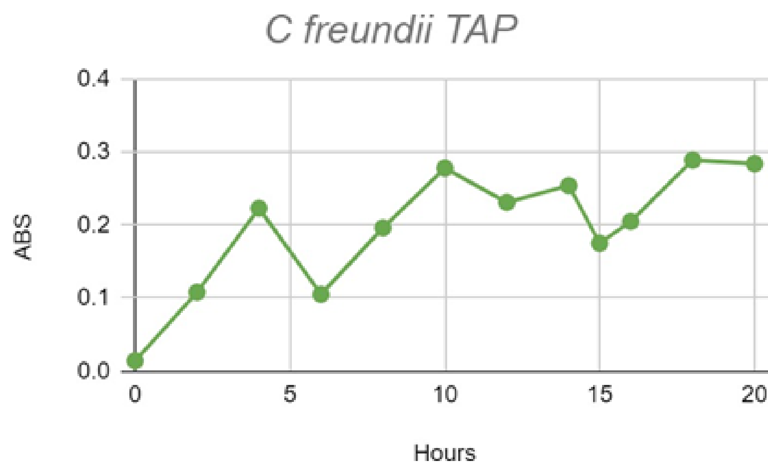
For this curve, the growth of bacteria in consortium with Chlamydomonas was analyzed, but in a different cultivation medium than BBM, TAP medium. The difference between these two mediums is the nutrients inside.

Graphic 6.14 Enterobacter growth curve in *Chlamydomonas* TAP cultivation medium



Graphic depicting the consortium between Enterobacter and the microalgae *Chlamydomona* in TAP medium, at the indicated time.

Graphic 6.15 Growth curve of *C. freundii* in *Chlamydomonas* TAP cultivation medium



Graphic showing the consortium between *C. freundii* and the microalgae *Chlamydomona* in BBM medium, at the indicated time.

As can be seen, the growth of the bacteria is highly dependent on the medium in which they are found. This is due to differences between each medium, such as nutrients.

One of the major factors that could interfere with bacterial growth would be temperature. Among the nutrient medium and consortia of microorganisms, the temperature varied from 35° to 29°C. This affects the growth of bacteria and thus, the production of biohydrogen.

As for biohydrogen production, only one sample was able to effectively inflate a balloon, that being Enterobacter in nutrient cultivation medium. This is due to multiple reasons, one of which would be fermentation time. By having more time, fermentation is carried out properly, allowing the rest of the cultivations to perform the metabolism necessary to process the nutrients and thus release the hydrogen.

Another factor that can be determinant would be the incubation conditions of both the cultivation medium, as well as of the fluted petri dishes. A couple of times it was detected that there were temperature increases inside the incubator, this could cause the bacteria to suffocate and a higher stress to the bacteria does not always translate as something positive.

On the other hand, it is suspected that the constant movement of the samples through the Tecnológico de La Laguna may have been enough to stress the bacteria and thus affect the fragile development in cultivation.

One of the major factors with which we were competing was the time between each process, and this is a variant which can definitely be altered for greater probability of success in this type of project.

Another thing to keep in mind regarding this type of consortia is that microalgae are very aggressive with bacteria, so much so that in many water purification and treatment plants, the use of microalgae is employed to deal with these types of contaminants. Clearly there is a relationship between bacteria and microalgae for the production of biohydrogen since, theoretically, these two can share a symbiotic relationship. However, there are factors such as type of microalgae or bacteria, temperature, agitation state, exposure to illumination, fermentation period, which could make a big change in these projects and should definitely be analyzed and considered in the future.

Objectively, the analysis of the fermentations can be done correctly, it can be deduced exactly what factors affected these cultivations and if the project would be replicated, more knowledge would be available regarding this area of biofuels.

6.3 Acknowledgments

The authors would like to thank the Renewable Energy Laboratory for the financial support that made possible the development of this project.

6.4 Conclusions

All the samples showed exponential growth at some point, with the exception of the BBM medium with *Chlamydomonas,* which had the lowest growth rate. The rest showed a much higher growth of at least 2% every two hours, giving the conclusion that the bacteria did grow in the prepared medium. As for the biohydrogen production, it could be observed in the Enterobacter sample on nutritive agar and in the three TAP mediums with *Chlamydomona*, with an index of 60% of biogas being biohydrogen, giving positive results.

As for growth, it is quite apparent that it was much better in a medium specifically made for bacterial growth than in one in which it must compete against microalgae because of their antagonistic relationship. Also at issue is the time between each process. As already mentioned, this can be optimized by allowing cultivations and fermentations to be carried out with greater margin.

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