Hydrogen production and partial characterization of a bacterial consortium obtained from lagoon sediment

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INTRODUCTION

Energy is an essential tribute for economic development.

The increasing demand for energy and the decreasing of its conventional sources lead to the rising of oil prices.

There is a growing environmental impact caused by conventional energy sources, as greenhouse effect and global warming.

As a consequence searching for of renewable energy sources becomes imperative .

INTRODUCTION

Hydrogen is a promising energy source since:

- ✓its combustion produces only water .
- ✓is not toxic or corrosive,
- ✓its leakage during its transport would not culminated in an environmental catastrophe, and
- ✓its combustion would not generate the greenhouse gases responsible for global warming

Source: Int. J. Hydrogen Energ 2010; 74:16-26

INTRODUCTION

Biological hydrogen (BIOHYDROGEN) production is considered a promising process owing to:

✓ Sustainable and carbon neutral
✓ Added benefit of waste remediation
✓ Fermentative technology already available
✓ Valuable co-products e.g. organic acids, alcohols etc.
✓ These co-products can be used in a number of other energy generation systems or chemical feedstocks.
✓ Two stage could enhance the stability or efficiency of conventional anaerobic digestion.

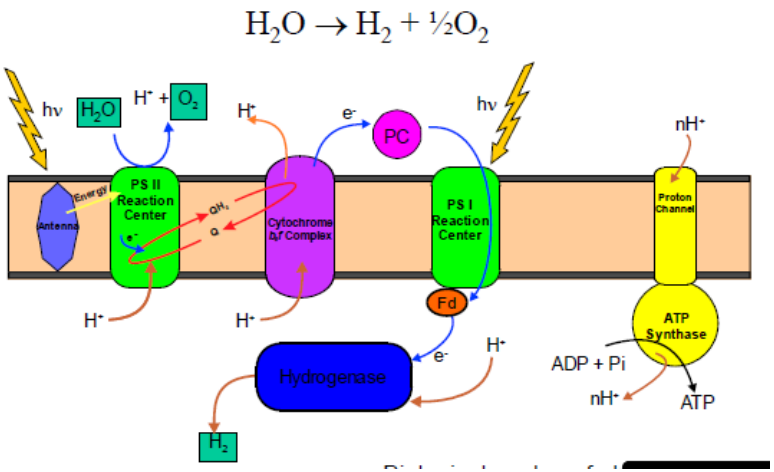
INTRODUCTION

The main biological processes employed for hydrogen production are:

- ✓ **biophotolysis** of water by algae and cyanobacteria,
- ✓ **photo-fermentation** by photoheterotrophic bacteria,
- ✓ **dark fermentation** by fermentative bacteria capable of accomplishing anaerobic digestion of organic compounds

INTRODUCTION

✓ **Biophotolysis** of water by algae and cyanobacteria, dependent on light



In this process one molecule of water is broken down to H2 and O2 using the bacterial photosynthesis apparatus.

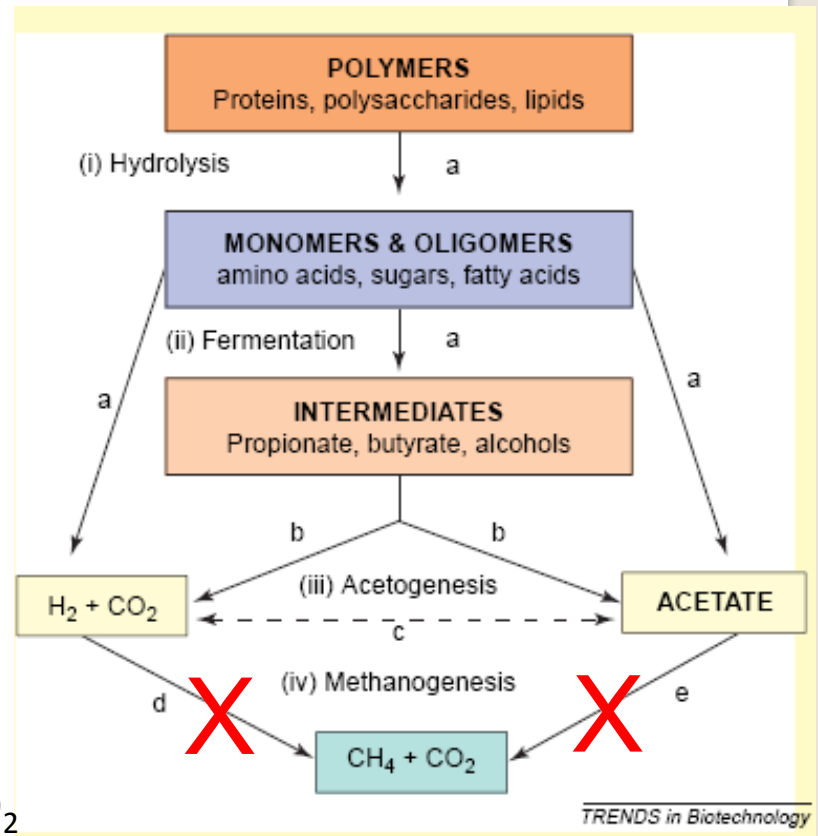
INTRODUCTION

Dark-fermentation is an anaerobic digestion process that includes hydrolysis/acidogenesis of organic compounds and methanogenesis. Hydrolysis and acidogenesis steps produce hydrogen, carbon dioxide gases and organic acids.

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 4\ H_2 + 2\ CH_3COOH + 2\ CO_2$$

or

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2H_2 + CH_3CH_2CH_2COOH + 2\ CO_2$$



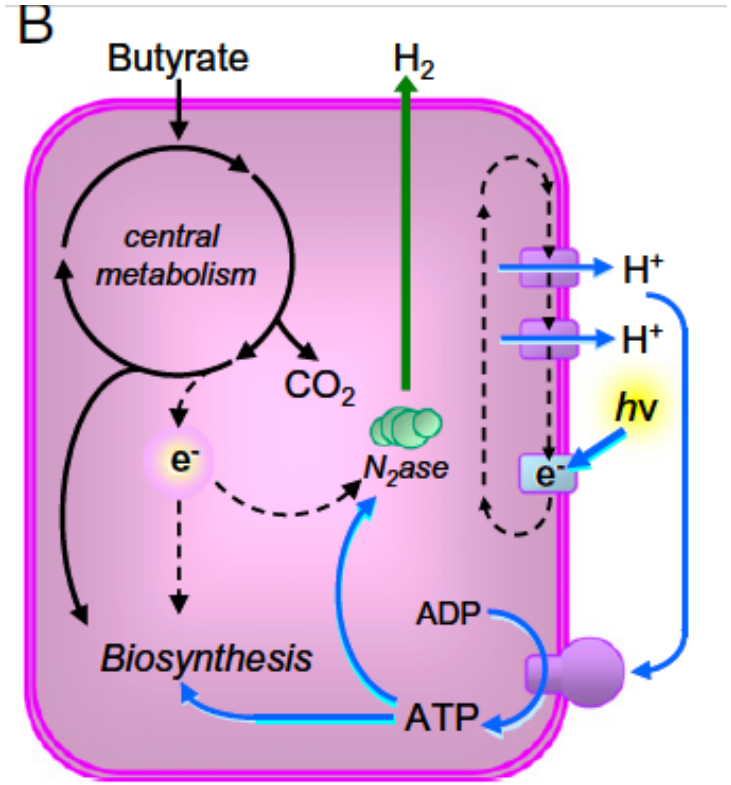
Methanogenesis - undesired steps

INTRODUCTION

Photo-fermentation can be performed by purple non-sulfur (PNS) bacteria which can utilize small chain organic acids as electron donors and light energy producing H_2 when growing under anaerobic condition .

$$CH_3COOH + 2\ H_2O + light \rightarrow 4\ H_2 + 2\ CO_2$$

In this process a nitrogenase drive the excess of reducing power generated by substrate oxidation to H2 production.



Source: McKinlay JB and Harwood CS Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria Proceedings of the National Academy of Sciences of the United States of America, 106 (29) 2010, 11669-11675.

INTRODUCTION

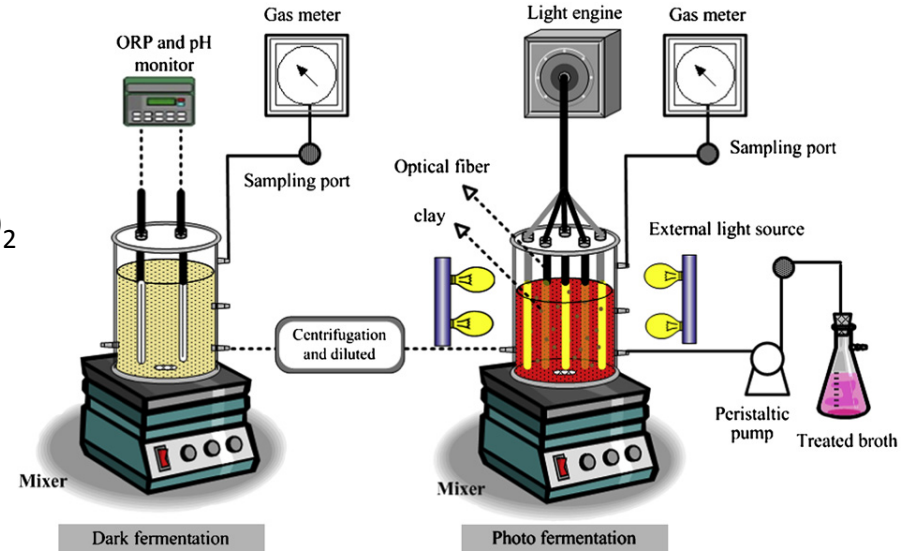
Integrated use of two fermentation processes can produces a maximum theoretical yield of 12 mol H_2/mol glucose.

STEP 1 – DARK FERMENTATION

$C_6H_{12}O_6 + 2\ H_2O \rightarrow 4\ H_2 + 2\ CH_3COOH + 2\ CO_2$

STEP 2 – PHOTO-FERMENTATION

$2CH_3COOH + 4\ H_2O + light \rightarrow 8\ H_2 + 4\ CO_2$



Source: Chen, C Y et al - I n t e r n a t i o n a l Journa l o f Hydrogen Energy 33 (2 0 0 8) 4 7 5 5 – 4 7 6 2.

INTRODUCTION

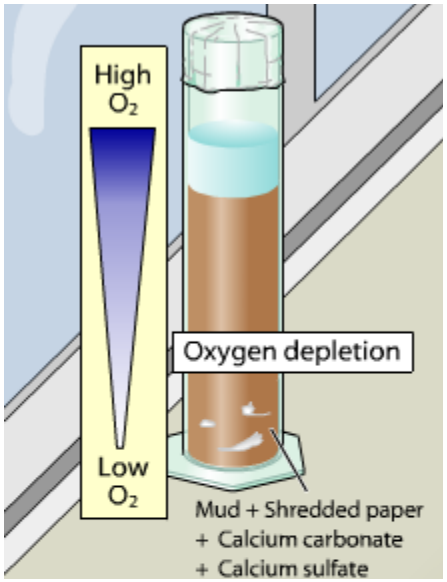
Traditional methods of isolation and enrichment with bacteria from the environment are time-consuming, especially when slow growing bacteria like PNS are concerned.

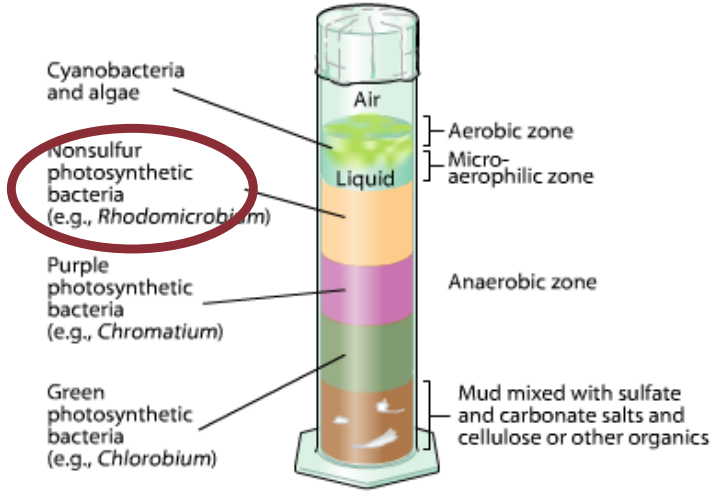
Winogradsky column is a classic demonstration of the metabolic diversity of prokaryotes and it can be used as a tool to enrich and isolate certain prokaryotic species, depending on the availability of light, carbon, and oxygen.

In these columns there is a gradient of free oxygen (O_2) ongoing from the top of the column, where oxygen is the abundant, to the bottom of the column, where oxygen is absent.

The species present in the water and sediment colonize different parts of the column, depending on the environment and nutrients available .

The column promotes enrichment of bacteria present at low concentration and could favor the isolation of bacterial consortia.



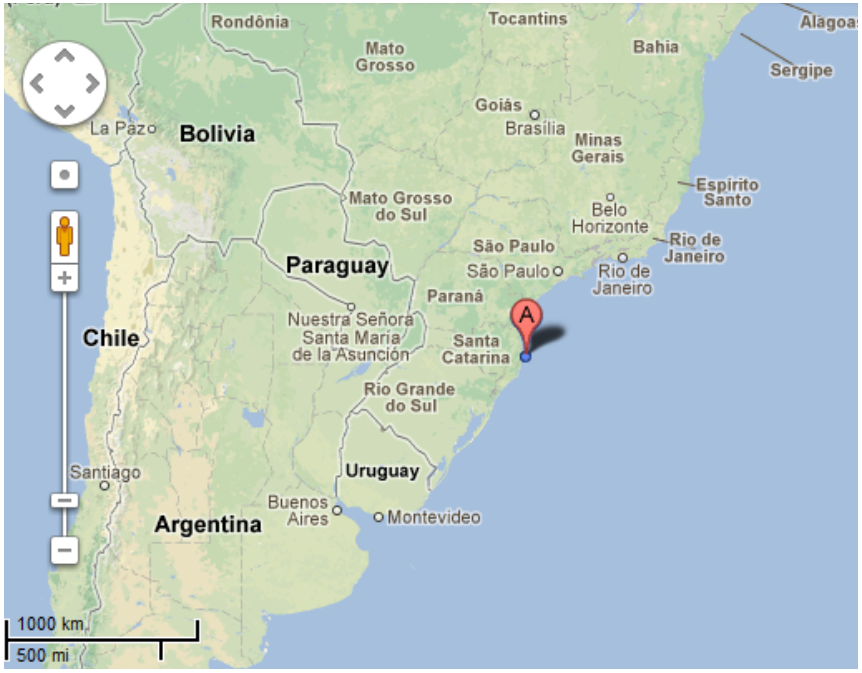


This study aimed to:

- ✓ evaluate the potential of a bacterial consortium isolated from a Winogradsky column obtained from water and sediment of Lagoa da Conceição – Florianopolis/SC - Brazil for biological hydrogen production.
- ✓ perform a partial characterization of the consortium by molecular analysis of bacterial abundance accomplished by polymerase chain reaction/denaturing gradient gel electrophoresis (PCR/ DGGE).

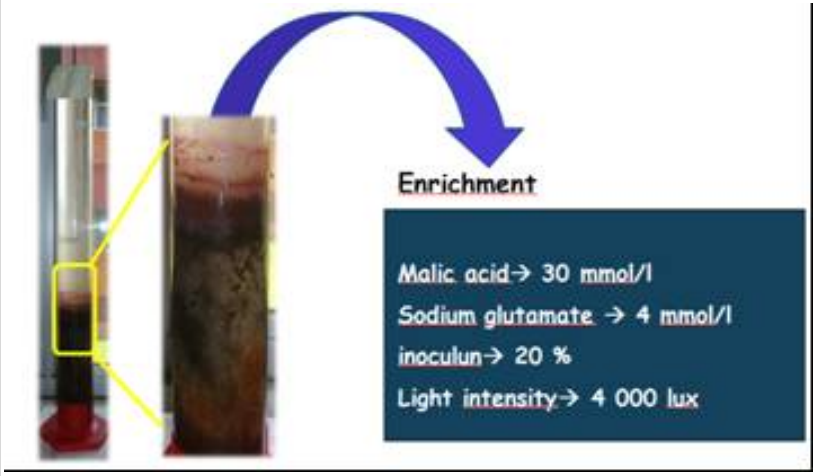
MATERIAL AND METHODS

The Winogradsky column was prepared by using water and sediment from Lagoa da Conceição, a lagoon situated in the city of Florianópolis, State of Santa Catarina, Brazil.



MATERIAL AND METHODS

The sediment was collected at a depth of 6 m, supplemented with minerals, cellulosic material and incubated for 90 days under natural light exposure in order to accomplish the stratification of microorganisms, according with their metabolism.



Consortium harvesting and enrichment

- A sample was taken from anaerobic zone of column presenting red color;
-Transferred to a selective medium (RCV medium) containing malate (30 $mmol.L^{-1}$) and sodium glutamate (4 $mmol.L^{-1}$), as carbon and nitrogen sources, respectively.
-The cultivation was performed in transparent glass bottles containing 30 mL of enrichment medium and illuminated with fluorescent lamps (4,000 lux) for 72 h.

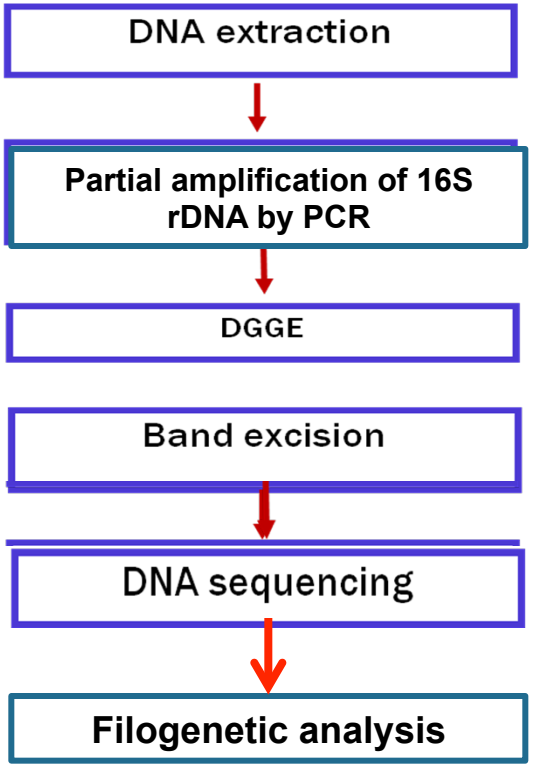
MATERIAL AND METHODS

Hydrogen production experiments

After the enrichment, the culture was centrifuged at 5000 rpm for 20 min and resuspended in RCV medium containing 2mM glutamate, 20% (v/v) of resuspended inoculum was transferred to RCV medium containing one of the carbon sources: 15mM butyric acid or 30 mM acetic acid. The experiments were conducted in 12 mL tightly closed glass tube, containing 10 ml of medium. The cultivations were carried out at 30 °C and 7000 lux, during 12 days. Each day two tubes were harvested in order to measure gas hydrogen production, final pH and dry cell weight.

MATERIAL AND METHODS

Aiming to know the abundance of species present in obtained and enriched consortium PCR/ DGGE, a finger printing culture-independent methods for the characterization and monitoring of total microbial populations was used.



DNA was extracted from enriched sample by using a commercially available extraction kit (MO BIO Ultraclean kit).

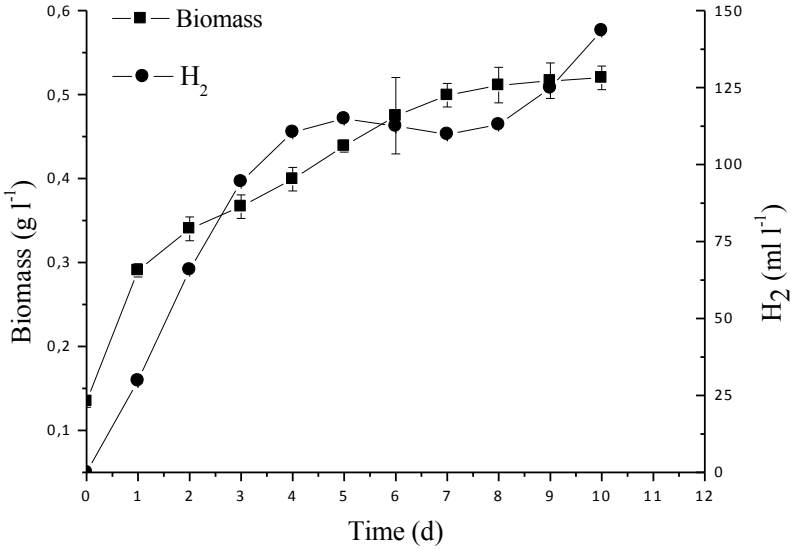
Extracted DNA was used as template to PCR partial amplification of 16S rDNA using the universal primers GC358F and 517R.

The PCR product was analyzed by DGGE, which was accomplished in an electrophoresis system DCode (Bio-Rad) using 8% polyacrylamide gel with a linear concentration of denaturant from 25 to 55%, where 100% denaturanting agent was composed of 42% urea (w/v) and formamide 40% (v/v).

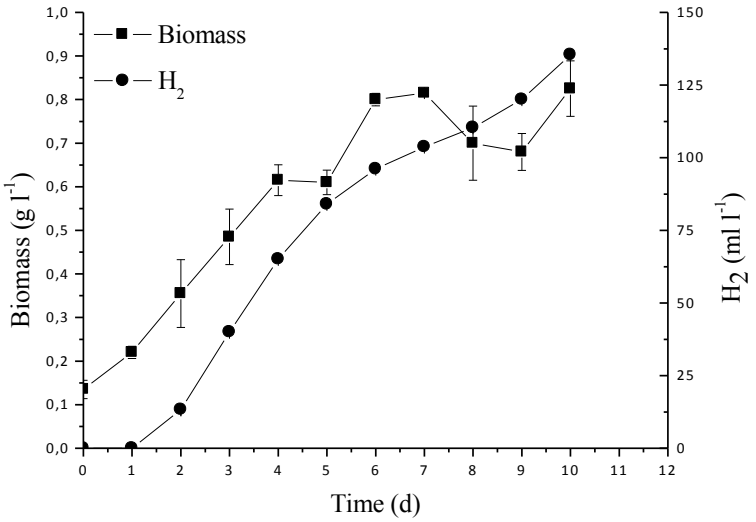
RESULTS AND DISCUSSION

There was simultaneous hydrogem production and cellular growth supported by both substrates.

(a)



(b)



Time course of photohydrogen production (-●-) and biomass production (-■-) by the mixed photoheterotrophic culture obtained from a Winogradsky column, using as carbon substrate (a) 30 mmol.l^{-1} acetate, (b) butyrate 15 mmol.l^{-1} and nitrogen source 2 mmol.l^{-1} glutamate, under anaerobic conditions, at 30 °C.

RESULTS AND DISCUSSION

Table 1 - Hydrogen production, hydrogen production rate, hydrogen yield and conversion efficiency using acetate and butyrate as substrate, in 10 days of culture.

Substrate	H_2 ml l^{-1}	Hydrogen rate ml l^{-1} h^{-1}	Yield[a] molH_2/molSubstrate	Efficiency[ab] (%)
Acetate	143.56	0.60	0.21	5.33
Butyrate	135.41	0.56	0.40	4.03

[a]After 10 day of cultivation;
[b] Considering initial concentration of substrate

Hydrogen production and the hydrogen production rate are about the same for both substrates, the molar yield obtained in the presence of butyrate is almost twice that achieved with acetate.

The efficiency of hydrogen production is about 20% higher when acetate is utilized as substrate.

RESULTS AND DISCUSSION

Molecular Analysis

Fig 2a shows a DNA 100bp ladder (as standard) and in second lane a single 200 bp band, corresponding to partial amplification of 16S rDNA.

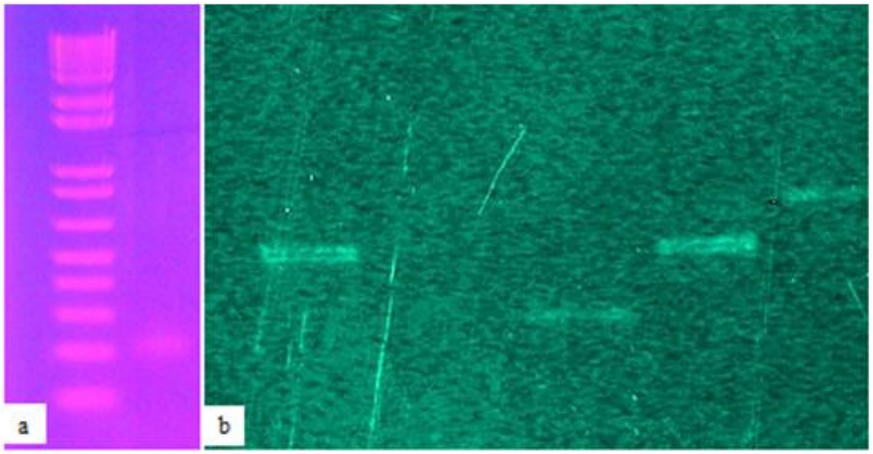


Figure 2b illustrates an image of the DGGE gel after the separation of the only 4 bands obtained from DGGE.

This result suggests the presence of 4 different species present in the mixed culture.

The four bands were excised and sequenced in order to determine their phylogenetic affiliation.

RESULTS AND DISCUSSION *Molecular Analysis*

The closest matches (and percentages of similarity) of retrieved sequences were determined by a BLAST search (Table 1).
Similarities ranged between 91 to 96%.
The majority of retrieved sequences corresponded to uncultured microorganisms of different phyla.

Table 4. BLAST results of sequences retrieved from DGGE bands.

Band number	Most related sequence	% similarity	Source	Acession number
1	Uncultured Shewanella sp. clone QRSYY2 16S ribosomal RNA gene	96	Quinone-reducing community, environmental samples	EU919217
2	Uncultured Pseudomonas sp. clone QRSYY7 16S ribosomal RNA gene, partial sequence	93	Bacterial communities in A/O MBR with nitrogen removal, environmental samples	EU919222
3	Uncultured Wolinella sp. clone 2-1 16S ribosomal RNA gene, partial sequence	96	Reactor used to remove nitrate and sulfide simultaneously in the absence of oxygen	GQ324220
4	Uncultured alpha proteobacterium partial 16S rRNA gene, isolate DGGE band WETLE-14R	91	Microbial communities in constructed wetlands in relation to hydraulic design and plant species	FM992018

RESULTS AND DISCUSSION

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The *Shewanella* genus is Gamma Proteobacteria characterized as facultative anaerobic, Gram-negative bacteria. It is found in a variety of environments such as freshwater lakes, marine sediments, subsurface formations, and at variable depths in redox stratified aquatic systems.

Shewanella oneidensis MR-1 uses diverse compounds, such as oxygen and fumarate, as well as insoluble Fe(III) and Mn(IV) as electron acceptors.
The electron donor spectrum is more limited and includes metabolic end products of primary fermenting bacteria, such as lactate, formate, and hydrogen.

However it was report also the formation of hydrogen from pyruvate under anaerobic, stationary-phase conditions in the absence of an external electron acceptor(**Meshulam-Simon et al. 2007)**.

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Members of the genus Pseudomonas demonstrate a great deal of metabolic diversity, and consequently are able to colonise a wide range of niches.

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The genus *Wolinella* belongs to the epsilon-subclass of the proteobacteria. Together with *Helicobacter pylori* species it forms the family of the *Helicobacteraceae*. *Wollinella* is a Campylobacter-like organism and oxidase positive, this suggests that they may actually be microaerophiles (Y.-H. HAN,l R. M. SMIBERT, AND N. R. KRIEG,1991).

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Finally, one of the bands found in the samples presented similarity with a sequence of 16S rDNA gene isolated from a DGGE of environmental sample of a constructed wetland .

CONCLUSION

The Winogradisky column use was very efficient methodology for selecting microorganims with a particular feature.

According with DGGE analysis, no one of selected bacterium is known to accomplish photoheterotrophic metabolism.

However at least the genus *Shewanella* identified among the samples was found to support hydrogen production from organic acids in anerobic condictions.

The absence of PNS bacteria in consortium may explain the higher hydrogen yield when acetate was the used substrate.

REFERENCES

- Argun, H.;Kargi,F., Photo-fermentative hydrogen gas production from dark fermentation effluent of ground wheat solution: Effects of light source and light intensity, **Journal International of Hydrogen Energy** 35 (2010) 1595-1603.
- Hallenbeck, P.C., Fermentative hydrogen production: Principles, progress and prognosis, **Journal International of Hydrogen Energy** (2009) 7379-7389.
- Fang,H.H.P.;Liu,H.;Zhang,T., Phototrophic hydrogen production from acetate and butyrate in wastewater, **International of Hydrogen Energy** 30 (2005) 785-793.
- Weaver,P.;Wall,J.D.;Gest,H., Characterization *of Rhodopseudomonas capsulta*, **Archieves Microbiology** 105 (1975) 207-216.
- Lazaro, C.Z., Obtenção e caracterização filogenética de consórcio de bactérias fototróficas púrpuras não-sulfurosas consumidoras de ácidos orgânicos visando à produção de hidrogênio em reator anaeróbio em batelada, Dissertação de mestrado, Escola de Engenharia de São Carlos da Universidade de São Paulo (2009) 200.

THANKS!