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Continuous bio-ethanol fermentation from food industry waste under no sterile conditions

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Bio-ethanol is probably going to be a practicable alternative to fossil fuels in the next future. However, at the moment the main costs deriving from its production seems to be the substrates, the recovery of ethanol from the fermentation broth and the maintenance of sterility during the fermentation. This is particularly true for a continuous process. This paper highlights the possibility to work with a un-expensive substrate, as can be a waste of the food industry. In this study we operated a continuous reactor under no sterile conditions reaching a good specific productivity (0.43 g Ethanol/g utilized sugars).

Introduction

Now the world economy bases itself upon the utilization of the energy produced from the fossil fuels (like oil, carbon, gas and their by-products). Unfortunately, considering the actual oil production and costs and the need to reduce the CO_2 emissions, it is necessary to find out and to reconsider the possibility to produce energy from renewable sources (Kennedy and Turner, 2004).

A possible and practicable alternative for the energy production seems to be the bio-ethanol production through bio-process (aerobic fermentations in control reactors). This involves either the energy production either the fuel production (Farrell et al., 2006).

Bio-ethanol is a renewable resource with a clean combustion, a high octane number (103), is chemically stable, no toxic and biodegradable. Moreover, bio-ethanol is an energy carrier that has the characteristics to be optimum for vehicles transport, as mixture with petrol or pure; in fact, as mixture does not require specific changes on the engine, while when used pure only small changes are needed.

Finally, the CO_2 emissions produced by the bio-ethanol combustion is "environmental friendly", because deriving by a renewable energy source and after combustion enter again in the natural biocycle of carbon.

Today the 80% of the ethanol produced in the world is from the micro-aerobic fermentation of sugary substrates (generally from cereals or sugar cane) by yeast like Saccharomyces Cerevisiae under sterile conduction. These facts represent the major costs, joined with the energy spent for breaking the water-ethanol azeotrope in ethanol recovery. A recent research (Tao et al., 2005) however has shown the possibility to operate under no-sterile condition with high ethanol production (0.488g ethanol/g glucose) working with proper hydraulic retention time and at pH 4.5.

Farrell et al. (2006) have highlighted that the ethanol production from cereal by-products is a process economically and energetically feasible when supported by the sell

of the by-products (like fodders) or by the utilization of them for the recovery of energy (via anaerobic fermentation or combustion).
Under these statements we focused on the research of better process conditions that can permit to work with a waste of the food industry which is to say with substrates at low cost, under no-sterile condition and in a continuous stirred tank reactor (CSTR).
In fact, the waste of the food industry represent a resource, an energy source and a reservoir of valuable chemicals compounds that could not be digest like simple rubbish.

Materials and Methods

We used a glass reactor 15 litres volume completely stirred. The temperature was maintained constant at 35°C with a heating-jacket. The reactor had worked during all the period in anaerobic conditions. The biogas was measured via a Milligas-counter (MGC-1) so to allow to close the mass balance of the process.
We monitored the system, after it reached steady state conditions at different dilution rates (from 0.1; 0.2; 0.3; 0.5; 0.67; 0.75; 1.33 d^{-1}).
As substrate we used an agro-waste residue (Table 1) and its composition was constant during the experimentation. We added $K_2S_2O_5$ to the substrate at the concentration of 0.1 g/L to reduce contamination of other micro-organisms present in the inlet substrate.
At the beginning of the work the reactor was inoculated with Saccharomyces Cerevisiae (strain EC1118).

Table 1- Inlet substrate composition

Parameters	Unit	Value
COD	g/L	60
Sucrose	g/L	15.75
Glucose	g/L	24.1
Fructose	g/L	14.34
Glycerol	g/L	0.17
Volatile Fatty Acids (as COD)	g/L	1.1
Lactic Acid	g/L	0.30
Total solids	g/L	2
pH	-	6.9

During the experimentation we monitored the main parameters as: biogas production and composition, ethanol, sucrose, glucose, fructose, glycerol, lactic acid and VFA (volatile fatty acids from C2 to C7) as well as the biomass concentration. We used to monitor the solids content and to count colonies formed on selective Petri plates. For growth medium we MRS Agar, Plate Count Agar (PCA) and YPD Agar.
The MRS Agar is a growth medium for Lactobacillus; PCA is a no selective medium for the growth of micro-organisms and YPD is a medium usually used for the growth of yeasts. For the determination of the total biomass present inside the reactor both counted Petri plates and dry sample in oven (at 105°C for 48 hours).

Results and Discussion

All the analysis where considered after the system reached the steady state conditions; the average of the main parameters with their relative standard deviation at stationary state for the entire set of dilution rates analyzed are reported on Table 2.

Table 2- Output reactor parameters and their relative standard deviation at stationary state

D	Ethanol	Sucrose	Glucose	Fructose	Biomass	Biogas
d-1	g/L	g/L	g/L	g/L	g/L	L/d
0.10	17.4 ± 0.7	0.8 ± 0.8	1.4 ± 1.2	2.8 ± 0.6	0.6 ± 0.1	6.7 ± 0.0
0.20	19.4 ± 0.6	0.9 ± 0.7	0.6 ± 0.4	0.9 ± 0.7	0.9 ± 0.4	24.6 ± 1.9
0.30	18.7 ± 2.4	3.9 ± 1.0	1.0 ± 1.0	2.3 ± 0.5	1.0 ± 0.1	37.7 ± 3.1
0.50	13.0 ± 1.4	10.0 ± 0.8	5.9 ± 0.5	6.7 ± 0.5	1.1 ± 0.2	43.4 ± 1.0
0.67	10.8 ± 0.2	11.8 ± 0.2	9.4 ± 0.2	8.3 ± 0.3	1.1 ± 0.1	45.6 ± 1.4
0.75	10.1 ± 0.3	11.9 ± 0.3	9.8 ± 0.2	8.6 ± 0.4	1.0 ± 0.1	48.3 ± 1.3
1.33	3.5 ± 0.4	14.9 ± 0.3	15.8 ± 0.1	12.8 ± 0.2	0.7 ± 0.1	38.3 ± 1.4

Using the selective Petri plate we monitored that during the entire experimentation there was no contamination of the reactor by other micro-organism; and the initial yeast strain remained always the dominant micro-organism. Figure 1 shows the profiles of the main compounds found in the reactor for different dilution rates applied to the CSTR.

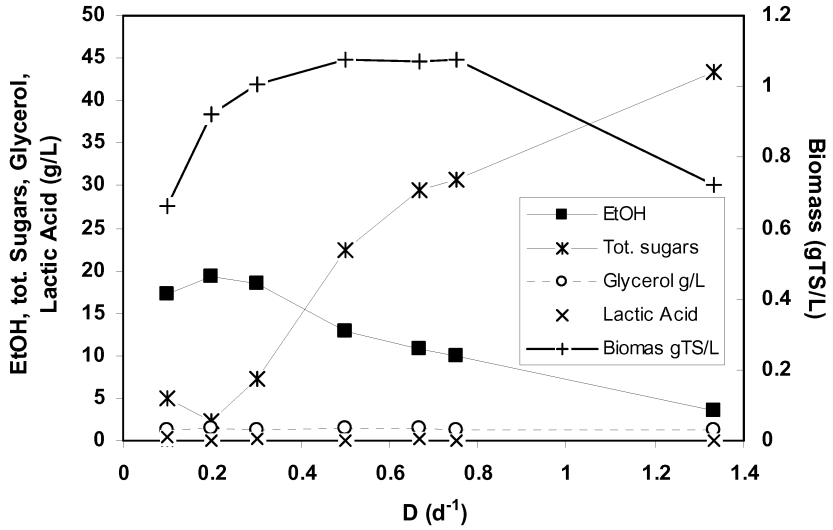

Figure 1- Output reactor parameters for the different stationary state

Biomass was relatively constant for dilution rates up to 1 d^{-1} while ethanol production reached its maximum concentration for low dilution rates (less than 0,5 d^{-1}) while sugars were effectively used in the same conditions. After a dilution rate of 0,5 d^{-1} the concentration of sugars in the effluent increased up 20-30 g/L, sucrose being the main

compound. Lactic acid and glycerol were virtually absent while VFA were not found at all.

From the mass balance calculated on carbon (figure 2) it is possible to notice how at low dilution rates the system use almost totally the inlet substrate; and about the 45% of this is converted into ethanol; in the meantime a considerable fraction of sugars is converted into undesired by-products (glycerol, lactic acid,etc...). On the other hand, when the dilution rates start to increase upon 0.3 d^{-1} the system changes its behaviour: a higher quantity of inlet sugars is not utilized, the fraction of inlet carbon convert to ethanol progressively reduces and there is the disappearance of by-products. During all the periods analysed the fraction of carbon converted into glycerol is below the 3% (w/w).

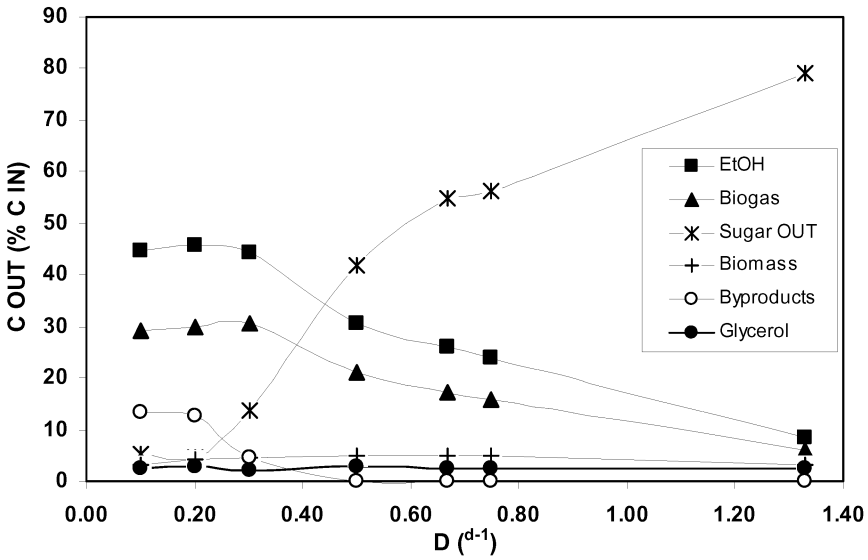

Figure 2-Mass balance of the different steady states in term of inlet carbon

If we focus our attention to the ratio ethanol produced/sugar utilized as a function of the dilution rates (figure 3) we can observe that it presents a maximum (0.43 g/g) for values of the dilution rates of some 0.7 d^{-1}.

At low dilution rates the system has enough time to transform the sugars into ethanol, but at the same time the hydraulic retention time is very high and a high production of by-products is also observed; so about 15% of inlet carbon goes into by-products instead to ethanol. As the dilution rate increases the yeasts have no enough time to consume all the inlet substrate so the amount of sugars converted into ethanol starts to decrease and the by-products disappears; so the sugars are totally utilized to produce ethanol (and partially glycerol). If the dilution rate becomes higher the system achieved the condition of wash-out and this fact is well explained by the ratio Ethanol produced for Biomass produced. This is evident from figure 3 where this ratio ranges from some 25 g/g to 5g/g as the dilution rate increase.

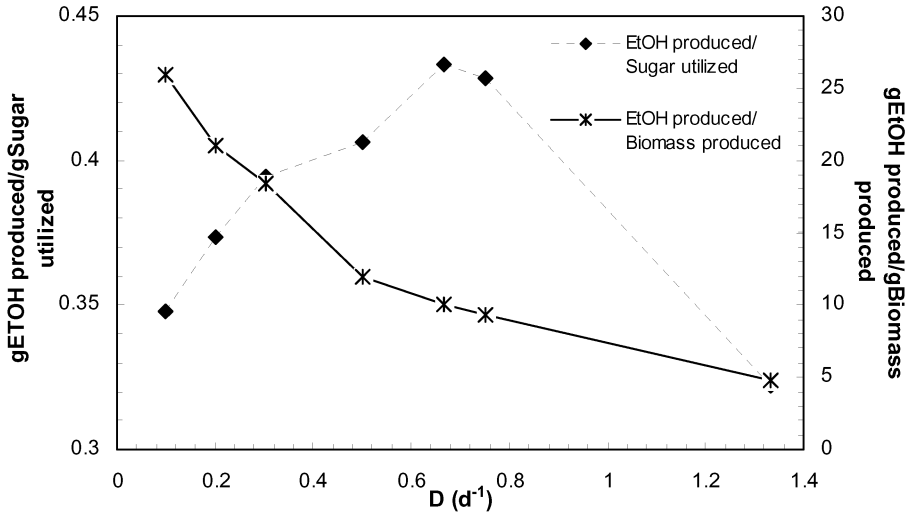

Figure 3-Mass balance of the different steady states in term of inlet carbon

Conclusion

In the experimentation carried out we investigated the fermentation of an agro-food waste under no sterile condition in a continuous stirrer reactor.

Different dilution rates (0.1; 0.2; 0.3; 0.5; 0.67; 0.75; 1.33 d^{-1}) were tested and the outlet streams of the reactor, both in terms of liquid composition and biogas were tested. Or any steady state conditions reached by the reactor.

During all the experimentation there was no contamination of the reactor by other micro-organism and a specific productivity of some 0.43 g ethanol/ g utilized sugars was reached for dilution rates of some 0,5-0,7 d^{-1}. However, in these conditions, the utilisation of sugars was relatively low: only 50% of inlet sugars was converted (in terms of carbon). Therefore, the prosecution of the study will consider the application of two CSTRs reactors in series and the application of the biomass recycle in the single reactor.

Bibliography

European Bioethanol Fuel Association (eBIO): World Biofuels 2006 - Seville, 9-11 May 2006

Farrell A. E., Plevin R. J., Turner B. T., Jones A. D., O'Hare M., Kammen D. M., 2006. *Science 311*, 506-508

Hough J. S., Gough P. E. and Davis A. D., 1962. Continuous fermentation on the pilot scale. *J. Inst. Brew.*, 68, 478

Kennedy D. and Turner J. A., 2004. Sustainable hydrogen production. *Science*, 305 (5686), 72-974

Sanchez OJ, Cardona CA Biotechnological production of fuel alcohol I: Production from different raw materials *Interciencia* **30** (11): 671+ Nov 2005.

Sanchez OJ, Cardona CA Biotechnological production of fuel alcohol II: Process integration . *Interciencia* **30** (11): 679+ Nov 2005.

Tao, F.; Miao, J.Y.; Shi, G.Y.; Zhang, K.C. Ethanol fermentation by an acid-tolerant Zymomonas mobilis under non-sterilized condition. *Process Biochemistry*, **40**(1), January, 2005, pp. 183-187.