Fermentation of sucrose solution into volatile fatty acids and alcohols by a mixed bacterial culture in up-flow packed bed reactors

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Abstract
The main objective of this study was to evaluate the volatile fatty acids and alcohol production by a fermentative mixed bacterial culture from natural environment. The experiment took place in an acclimatized chamber set to 25°C using a continuous upflow anaerobic reactor and packed bed with recycled polyethylene pellets for production of alcohols as methanol, ethanol and butanol, via anaerobic fermentation of sucrose solution. The quantity of ethanol and methanol in the effluent expressed in COD equivalent corresponded to 51.8% (w/w) and 39.1% (w/w) of the COD-sucrose consumed. The process investigated demonstrated be feasible, 91% of influent sucrose expressed in chemical oxygen demand (COD) equivalent was converted into alcohols ethanol and methanol.

Keywords
Bionergy, bioethanol, biofuel, anaerobic digestion.

INTRODUTION

In anaerobic digestion process from organic compounds, bacteria pertaining to Clostridia class accomplish the acidic fermentation. These microorganisms can utilize a broad variety of organic substrates as pentoses, hexoses, monosaccharides and polysaccharides. These compounds are present in several industrial wastewaters, including those generated by ethanol production from sugar cane, dairy, brewery, paper-mill, poultry slaughterhouses and domestic sewage.
The anaerobic fermentative process results in the production of organic acids as formic, propionic, butyric, isobutyric, valeric, caproic and isovaleric. Besides the production of organic acids, the metabolic versatility and availability of the clostridia in water and soil has been the subject of researches with the purpose of biofuel production, mainly hydrogen, ethanol and butanol. *C. acetobutylicum*, *C. beijerinckii*, *C. saccharobutylicum* and *C. saccharoperbutylacetonicum* can produce ethanol and butanol by through a two-phase anaerobic bioprocess, which involves the production and assimilation of organic acids, mainly acetic and butyric, H_2 and CO_2 (Lee et al., 2008). In this context, this study evaluated the volatile fatty acids and alcohols production by an anaerobic natural mixed bacterial culture in up-flow packed bed reactor.

MATERIAL AND METHODS

Experimental apparatus

It was used a bench scale acrylic reactor with overall volume of 3.17 L divided into three sections: bottom (0.36 L) to influent distribution, mid (2.45 L) filled with support material, and top (0.36 L) for the separation of the liquid and gaseous phases. The mid-section was filled with recycled polyethylene pellets and it was separated from top and bottom sections by stainless steel screens of

3 mm opening. The void volume in the mid-section occupied by support material has resulted in 1.81 L. The reactor was installed inside a chamber with controlled temperature at 25°C.

Substrate

The sucrose-based synthetic substrate was prepared containing; COD-sucrose (2000 $mg.L^{-1}$), COD-urea (32.1 $mg.L^{-1}$), sodium bicarbonate (500 $mg.L^{-1}$) nickel sulfate (1.00 $mg.L^{-1}$), ferrous sulfate (5.00 $mg.L^{-1}$), ferric chloride (0.50 $mg.L^{-1}$), calcium chloride (4.12 $mg.L^{-1}$), cobalt chloride (0.08 $mg.L^{-1}$), selenium oxide (0.072 $mg.L^{-1}$), monobasic potassium phosphate (10.7 $mg.L^{-1}$), dibasic phosphate potassium (2.60 $mg.L^{-1}$) and dibasic sodium phosphate (5.52 $mg.L^{-1}$). The substrate was stored in a reservoir maintained into a refrigerator with temperature set at about 4°C in order to reduce the fermentation effects from outside the reactor. The pH, COD, and sucrose concentration resulted in 7.1±0.1, 2011 ± 248 mg/L and 1395 ± 133 mg/L, respectively.

Inoculum

The inoculation was provided by means of an auto-fermentation strategy according to the technique proposed by Leite et al. (2008). The substrate was maintained during 3 day in contact with the extern environment allowing the grow of microorganisms present in the atmosphere. Afterwards, the fermented substrate was recycled to the reactor in a closed circuit for 48 hours with flow rate of 910 $mL.h^{-1}$.

Monitoring

After inoculation the substrate was pumped with flow rate of 910 $mL.h^{-1}$. The acidogenic condition was verified by the decrease of the pH in the effluent, since beside pH sucrose concentrations and, COD were monitored in the influent and effluent at least twice a week. Organic acids and alcohols were also analyzed in the influent of the reactors. For all analyses, except pH, samples were filtered in 1.2 µm membrane.

The values of pH and COD were determined according the methods described by APHA (1998). Sucrose, organic acids and alcohols were quantified by high performance liquid-chromatography (HPLC) in a single chromatographic run using an Aminex HPX-87H column, UV-DAD detector for acids connected in series with a RID detector for the other substances. The eluent was sulphuric acid solution 0.01N, column oven temperature of 43^{o}C and flow rate of 0.5 $mL.min^{-1}$. This method was developed and validate by Penteado et.al. (2012).

After the operation of the reactor, at each step, samples from support material were collected for the determination, measured as total volatile suspended solids, and molecular characterization. The analyses of total and volatile suspended solids were carried out according to Standard Methods for Examination of Water and Wastewater (APHA 1998). Part of the detached biomass was subjected to the molecular characterization. The genomic DNA of the microorganisms was phenol-chloroform extracted according to Griffiths et al. (2000), and used for amplification of a 16S rRNA fragment by polymerase chain reaction (PCR), employing specific primer for the Bacteria domains. The amplified DNA fragments were separated by denaturing gradient gel electrophoresis (DGGE) according to Muyzer et al., (1993). Electrophoresis gel was made with acrylamide/bis 40% and the denaturant gradient of DNA (urea/formamide) 45- 65% for the domain Bacteria. The electrophoresis was conducted at 65°C, 75 V for 16 hours. After being stained with ethidium bromide for 20 min, the gel was transferred to an Eagle Eye TM III UV chamber (Stratagene) coupled to a computer with Eagle light UV software for visualization of the bands. The dendrogram was built using the Bionumeric version 2.5 software and the Pearson's correlation coefficient was used for analysis of the phylogenetic similarity of the microorganisms.

RESULTS AND DISCUSSION

Initially the acid fermentation was poor, verified by the similarities observed for the values of pH in the influent and effluent streams. Only after 9 days of operation the values of effluent pH were significantly lower ($p<0.05$) than those observed in the influent, indicating acid production. Since then, the concentrations of sucrose, short chain volatile acids, ethanol, methanol and n-butanol, besides COD were measured. The steady state in sucrose conversion to organic acids and solvents was verified after 37th day operation, than the reactor was monitored at 56th day. The dates are show in the Table 1.

Between 37th and 56th days the average efficiency in converting sucrose was 74.9%. The average pH dropped from 7.1 in the influent to 5.0 in the effluent. The main organic acids detected in the effluent were lactic, formic, acetic and iso-butyric acids. The quantity of ethanol and methanol in the effluent corresponded to 51.8% (w/w) and 39.1% (w/w) of the COD-sucrose consumed, respectively. Therefore, 91% (w/w) of COD-sucrose was consumed for alcohols production. The presence of n-butanol was not detected in the effluent.

The volatile suspended solid concentration in the top, mid and bottom of the reactor were 245 mg. L^{-1}, 585 mg.L^{-1} and 2450 mg.L^{-1}, respectively. The acid production was more intense near the bottom of the reactor. In the mid of the reactor and near the top was observed reduction in the biomass concentrations probably associated with the transition stage between the acids and solvents production. The molecular characterization by DGGE results indicated that microorganism living on the top, and mid region of the reactor, presented 75% of similarity and these was only 38% similar to microorganism that inhabited the bottom (Figure 2). The phylogenetic differences among microorganisms that inhabited the bottom, compared with the ones from the top and middle of the reactor was caused by the ascending flow, which favored the acidogenic reactions in the lower part, and solventogenic reactions on the surface of the reactor. The biomass that developed in the bottom was more concentrates and distinct from those in the mid and top regions of the up-flow anaerobic reactor; thus, demonstrating a microbial arrangement for acids and solvent production.

Table 1. Values in COD equivalent (mg.L^{-1}) of Organic Compounds

				Organic Compounds in the Effluent (COD Equivalent)					
	COD-sucrose			Volatile Fatty Acids				Alcohols	
Day	Influent	Effluent	Conversion (%)	Lactic	Formic	Acetic	Iso-butyric	Ethanol	Methanol
37	1,640	556	66.1	35.8	11.5	61.4	32.4	539	506
40	1,804	563	68.8	43.5	0	53.5	30.6	456	487
43	1,712	666	61.1	44.8	22.9	55.3	20.6	562	407
47	1,586	76	95.2	0	9.3	73.1	40.6	674	470
50	1,571	181	88.5	0	8.1	65.6	49.1	637	509
54	1224	391	68.1	2.0	22.6	69.2	12.7	689	370
56	1151	121	89.5	11.6	21.8	75.8	8.9	785	524
Mean	1599	403	74.9	38.1	11.2	47.1	28.0	620	468
Standard deviation	182	214	12.6	38.3	8.0	18.8	20.3	110	58

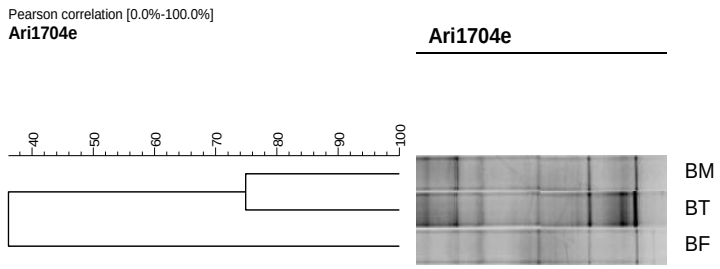

Figure 2. Dendrogram of Phylogenetic Symilaritie Betwem the Biomass that Grow in the Three Regions of the Reactor. The scale above indicate the percentage of symilaritie between the microorganisms, BM, BT and BF is the biomass developed in the mid, top and bottom, respectively.

CONCLUSION

The anaerobic fermentation process of a sucrose-based synthetic wastewater by a natural mixed bacterial culture, with purpose of volatile fatty acids and alcohols production was tested. This process demonstrated be feasible because the fermentative bacteria used 91% of the COD-sucrose for ethanol and methanol production. There was an arrangement of the microorganisms in the reactor indicating that acidogenic bacteria inhabited the bottom and solventogenic bacteria were concentrated in the mid and top.

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REFERENCES

APHA (1998). Standard method for the examination of water and wastewater. American Public Health Association, American Water Works Association, Water Environmental Federation, 20 th edition. Washington, USA.

Griffiths R.I.; Whiteley A.S.; O'donnell, A.G.; Bailey M.J. (2000). Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA and r-RNA- based microbial community composition. Applied and Environmental Microbiology vol. 66(12), pp. 5488-5491.

Lee, S.Y.; Park, J.H.; Jang, S.H.; Nielsen, L.K.; Kim, J.; Jung, K.S. (2008). Fermentative Butanol Production by Clostridia. Biotechnology and Bioengineering, Vol. 101, No. 2.

Leite, J.A.C.; Fernandes, B.S.; Pozzi, E.; Barboza, M.; Zaiat, M. Application of an anaerobic packed-bed bioreactor for the production of hydrogen and organic acids. International Journal of Hydrogen Energy, 33 (2008) 579 – 586.

Mood, A.M. (1954). On the asymptotic efficiency of certain nonparametric two-sample tests. Institute of Mathematical Statistics is collaborating with JSTOR to digitize, preserve, and extend access toThe Annals of Mathematical Statistics

Nielsen A. T.; Liu W.; Filipe, C.; Grady Jr. L..; Molin S.; Stahl D. (1999). Identification of a novelgroup of bacteria in sludge from a deteriorated biological phosphorus removal reactor. Applied and Environmental Microbiology, vol. 65 (3): 1251.

Penteado E. D.; Adorno M.A.T.; Zaiat, M."Simple and accurated method for determination of fatty acids, alchools and carbohydrates by HPLC with UV/DAD and RID detectors". Presented in the 38th International Symposium on High. PerformanceLiquid Phase Separations and Related Techniques (HPLC 2012), 16-21 June of 2012, California, USA.