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Effect of medium salt concentration on the performances of an ethanol biofilter

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P. Christen[1*], F. Domenech[2], R. Auria[1,3], S. Revah[3]

1. Microbiology Lab., IRD, Université de Provence, Case 925, 163, Av. Luminy, 13009 Marseille, FRANCE.
2. Bio-engineering Dpt, ICIDCA, La Habana, CUBA
3. Dpt of Process Engineering, UAM-Iztapalapa, AP 55-534, 09340 Mexico DF, MEXICO

ABSTRACT

Ethanol biofiltration was studied with a system utilizing a *Candida utilis* strain grown on sugar cane bagasse complemented with a mineral salt solution (MS). Experiments were conducted in a tubular reactor (Vol. = 0.35 l) filled with 15.75 g of dry matter (d.m.). Initial conditions were : pH, 6; temperature, 30°C; moisture content, 65%; packing density, 0.21 g/ml; inoculum size, 6.9×10^7 cells / g d.m. The MS included NH_4NO_3, KH_2PO_4, $MgSO_4$ and Ca, Fe, B, Cu, Zn, Mn Mo, Co and I. Three total salt concentrations were used; MS1, MS2 = 5xMS1, MS3 = 10xMS1, corresponding to 0.53 gN /l , 2.65 gN /l, and 5.3 gN /l, respectively. At the beginning, ethanol was fed at L1=120 g/h.m^3 (Cin = 18 mg/l, air flow rate = 2.3 l/h) for 5 days; increased to L2=160 g/h.m^3 for six days, and increased again to L3=200 g/h.m^3 for 8 days. For MS1, ethanol removal efficiency (ERE) was 100 % but began to decrease to 95 % for L2 and to 80 % for L3. An addition of a 4-times concentrated MS1 to the medium allowed to restore an ERE of 100 %. For both MS2 and MS3, ethanol removal was complete throughout the experiment. CO_2 production was proportional to the ethanol load. When ERE was lower than 100 %, other volatil organic compounds such as acetaldehyde and ethyl acetate appeared in the outlet gas stream, due to incomplete ethanol oxidation. In the three cases, final pH was around 3.5. An experiment made with MS1 complemented with oyster shell powder (5% w/w), showed that this complement avoided the pH drop, but no gain was observed in terms of ERE. Final population varied from 2.54×10^9 cells per g/d.m. (for MS1) to 4.08 $\times 10^9$ cells per g/d.m. (for MS3).

INTRODUCTION

As a response to increasingly strict legislation for volatile organic or inorganic compound (VOC or VIC) emissions, researches have been conducted to develop efficient and cost-

effective technologies. Among them , biological processes have been increasingly studied in the past decades[1,2]. Nowadays, biofiltration is regarded as one of the most promising and cheapest technology for odour abatement and VOCs removal from contaminated air stream[3]. Full-scale biofilters have been applied successfully for controlling emissions from printing and food industry, or wastewater treatment plants[1,3,4]. Among the VOCs treated by biofiltration, ethanol was reported as a relatively toxic pollutant, subject to regulatory control, and principally emmited by bakeries, distilleries, and foundries[5].

In recent studies, high ethanol elimination rates have been reported in bench-scale experiments with mixed cultures immobilized on different solid media[6,7,8,9]. In previous experiments, we demonstrated that it was possible to implement a biofilter using a *Candida utilis* strain, previously selected for its relative tolerance to ethanol[10], grown on sugar cane bagasse as porous matrix. This system allowed both to treat efficiently ethanol loads of 93.7 g/h.m^3 during several weeks, and to obtain a bagasse enriched with up to 7.2 mg single cell protein per g of dry matter[11]. In order to improve the yeast growth, and hence the performances of the biofilter, we studied the influence of the initial nitrogen concentration of the medium, as it has been reported as a key parameter in other studies[12,13]. Particular attention was given to the ethanol removal efficiency (ERE), the final biomass obtained and the control of the pH, according to the ethanol load and the initial nitrogen concentration.

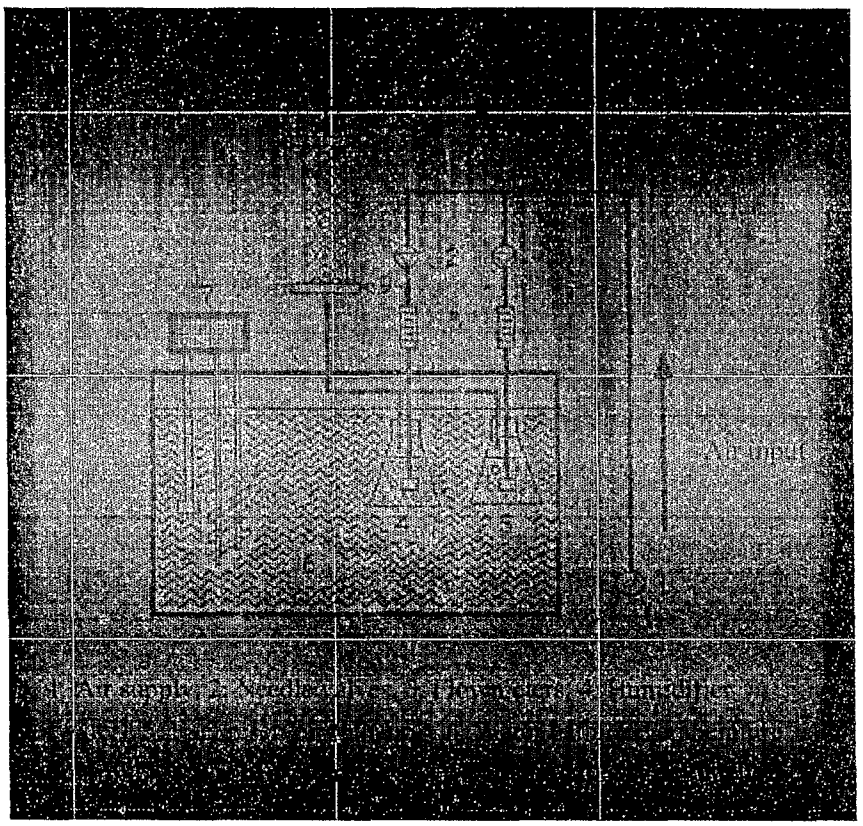
EXPERIMENTAL AND ANALYTICAL METHODS

The yeast *Candida utilis* L/375-1 from the ICIDCA (Cuba) collection was previously selected among various strains for its best elimination capacity and its relative tolerance to ethanol (up to 30 g/l in liquid culture)[10]. It was maintained on Potato Dextrose Agar (PDA) slants for 48 h at 30°C and then stored at 4°C. The inoculum was prepared in a 150 ml Erlenmeyer flask containing 50 ml of a solution of glucose (20 g/l) and malt extract (20 g/l) with shaking at 200 rpm at 30°C during 14 h.

The support used was sugar cane bagasse, an abundant by-product of the sugar industry. It was sieved through 0.4-0.8 cm screens, washed with distilled water, oven dried at 80°C for 24 h, and sterilized at 15 psi for 15 min. It was then mixed, under aseptic conditions, with the inoculum and a mineral salt solution (MS) with the following composition: NH_4NO_3, 1.5 g/l ; KH_2PO_4, 2.5 g/l ; $MgSO_4$, 0.24 g/l ; $CaCl_2.2H_2O$, 58.8 mg/l ; $FeCl_3.6H_2O$, 27.0 mg/l ; $ZnSO_4.7H_2O$, 25 mg/l ; $MnCl_2.4H_2O$, 4 mg/l ; $CuSO_4.5H_2O$, 2.5 mg/l, $NaMoO_4.2H_2O$, 0.4 mg/l ; $CoCl_2.6H_2O$, 0.4 mg/l ; H_3BO_3, 1.5 mg/l ; KI, 0.3 mg/l. This medium was used at three

concentrations: MS1, whose composition is given above, MS2, and MS3, corresponding to 5 and 10 times the concentrations used for MS1, respectively.

Experiments were conducted in tubular columns (Vol = $0.35x10^{-3}$ m^3) filled with 15.75 g of dry matter (d.m.). The experimental set up is shown in Figure 1.



Initial conditions were: pH adjusted to 6 before sterilization of the liquid medium; temperature, 30°C; moisture content, 65 %; and inoculum size, $6.9x10^7$ cells /g d.m.

For all experiments, ethanol was fed to the reactor at increasing loads: L1 = 120 g/h.m^3 (Ci = 18 g/m^3, F = $2.3x10^{-3}$ m^3/h) for five days, then L2 = 160 g/h.m^3 (Ci = 18 g/m^3, F = $3.1x10^{-3}$ m^3/h) for six days and L3 = 200 g/h.m^3 (Ci = 18 g/m^3, F = $3.9x10^{-3}$ m^3/h) for eight days.

The inlet concentration (Ci) in the gas phase was maintained at 18 g/m^3, which corresponds to a concentration in the water + bagasse phase of 30 g/l, the upper ethanol threshold that the yeast can stand[11].

Cell counting was made with a Neubauer chamber, after sample dilution (1 g in 25 ml of 1 % NaCl solution). The cell number was then transformed into dry weight biomass with the following relationship: 10^7 cells = 0.158 mg, obtained from previous experiments[14].

Respirometry was followed by measuring the CO_2 produced by gas chromatography (GC). The apparatus (Chrompack CP 9000, The Netherlands) was equipped with a concentric column CTR1 (Alltech, USA) and a thermal conductivity detector. Results were expressed as g CO_2 /h.m^3 reactor.

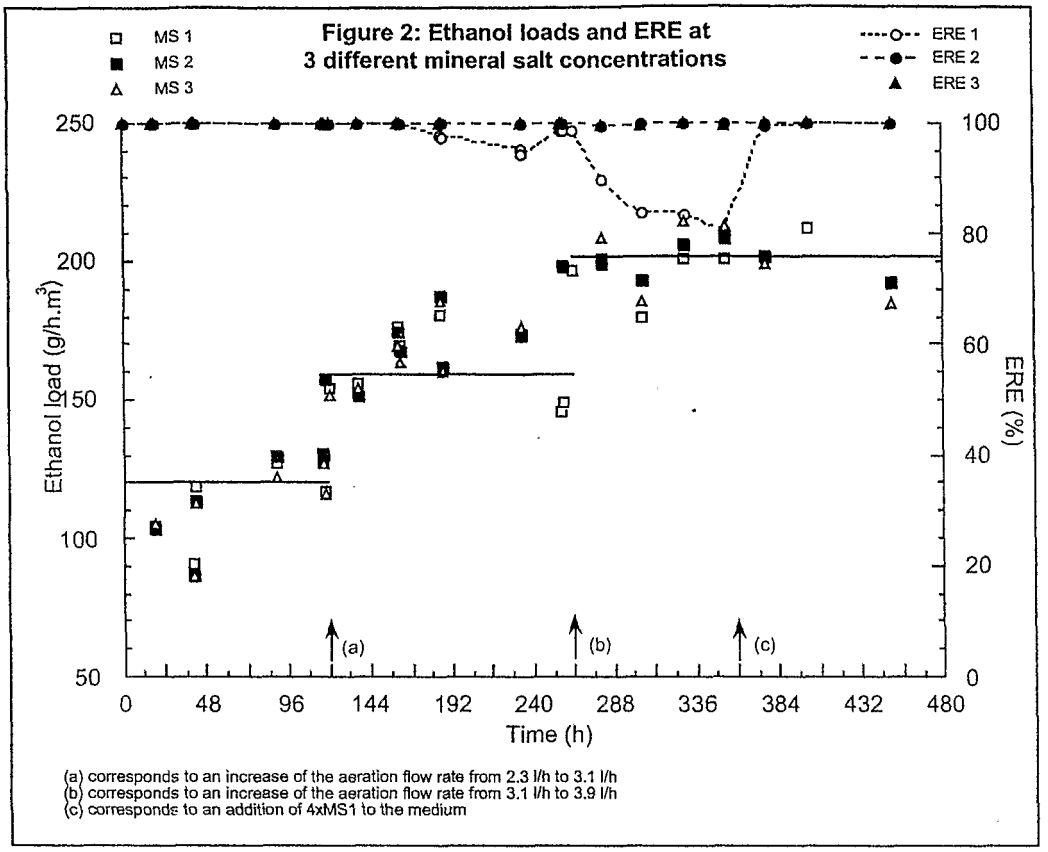
Ethanol and, eventually acetaldehyde and ethyl acetate, were analysed by GC with a Hewlett-Packard 6900 chromatograph equipped with a HP-5 column (length, 30m; inner diameter, 0.32 mm; film thickness, 0.25 µm) and a flame ionization detector. Ethanol removal efficiency (ERE) and elimination capacity (EC) were expressed as follows :

$$ERE = 100 \times (Ci-Co) / Ci \qquad \text{and} \qquad EC = F/V \times (Ci-Co)$$

where ERE = ethanol removal efficiency (%)
EC = elimination capacity (g/h.m^3)
Ci = inlet ethanol concentration (g/m^3)
Co = outlet ethanol concentration (g/m^3)
F = aeration flow rate (m^3/h)
V = reactor volume (m^3)

RESULTS AND DISCUSSION

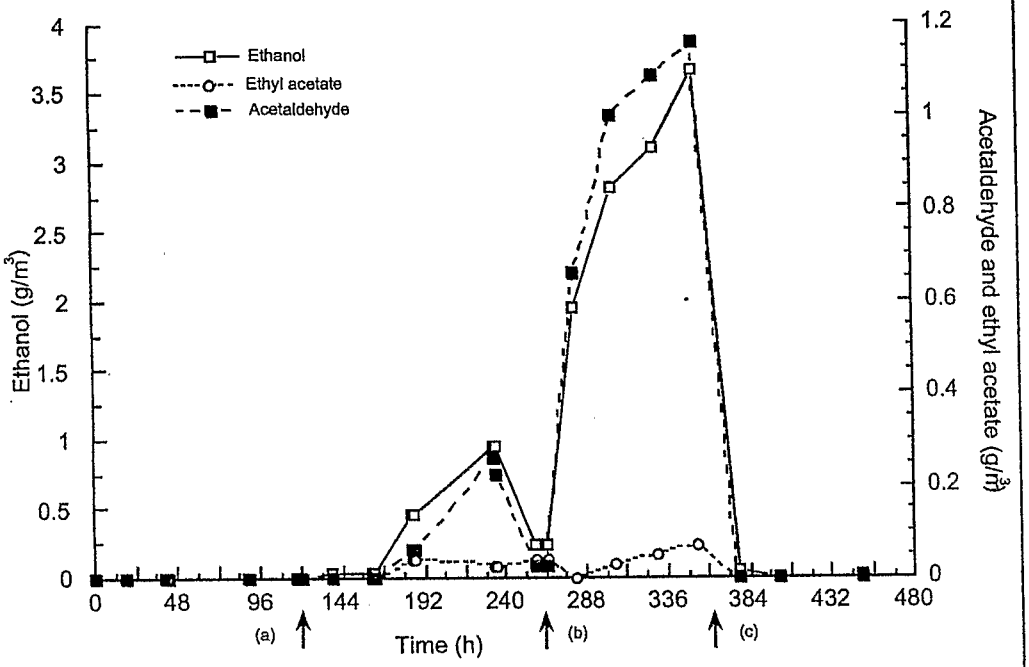
For the three media, a complete ethanol elimination was obtained for a load of 120 g/h.m^3 (Figure 2). When the load was increased to 160 g/h.m^3, ERE maintained at 100 % for MS2 and MS3, but began to decrease for MS1 around the eighth day. At a load of 200 g//h.m^3, this phenomenon became more marked and ERE felt at 80 % on the 15th day. The addition of a 4-times concentrated MS medium restored a complete elimination of ethanol. In contrast, ERE remained at 100 % throughout the experiment for MS2 and MS3. The system could treat high ethanol loads during the period studied.



This prouves that the process was sensitive to a lack in some mineral nutriment, as already reported[12,13], in particular for nitrogen. The adequate salt concentration to be used was comprised between MS1 and MS2. The system is not affected by the variations occuring in ethanol feeding.

As a consequence of the incomplete ethanol elimination, volatile intermediates of ethanol oxidation (acetaldehyde and ethyl acetate) appeared in the outlet stream (Figure 3). Acetaldehyde, a very volatile and toxic compound, results from direct ethanol oxidation, and can be oxidized into acetic acid, which in turn, can react with ethanol to form ethyl actate. This explains why these three compounds present a similar dynamics. This is consistent with the observations by Devinny and Hodge for an overloaded ethanol biofilter[15].

Figure 3: VOCs in the outlet stream



(a), (b), (c): as for Figure 2

For the three media, the CO_2 production was directly proportional to the amount of ethanol fed to the reactor (Figure 4), with the exception of a peak observed at the beginning of the experiment, probably due to the consumption of some residual sugars of the bagasse. For the first two loads, no difference was observed among the three media used, but at 200 g/h.m^3, CO_2 production increased drastically for MS2 and MS3, while it remained constant for MS1, indicating that the maximum EC was reached. However, the addition of the mineral medium at the 15th day allowed to increase CO_2 production. This was consistent with the decrease in ERE and the apparition of acetaldehyde and etyl acetate in the outlet stream.

An experiment with MS1 complemented with oyster shell powder (5 % d.m.) avoided the pH drop occuring without this complement, but no improvement in terms of ERE was observed (Table 1). Final biomass was positively correlated with the initial amount of salts added, but the relationship was not proportional (Table 1).

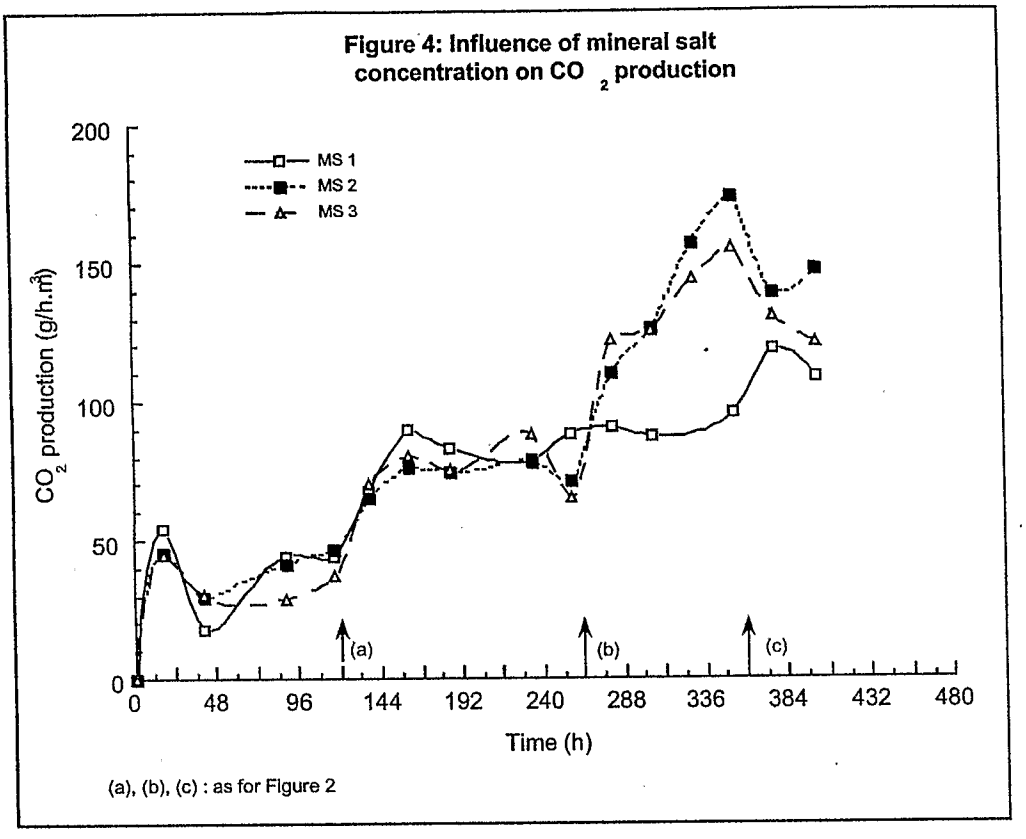


Table 1. Final values for pH and biomass. *osp : oyster shell powder

	MS1	MS2	MS3	MS1+ osp*
Final pH	4.35	3.86	3.67	6.37
Cell count (10^9 cells/g d.m.)	2.54	3.31	4.08	2.65
Biomass (mg/g d.m.)	40.1	52.3	64.5	41.9

CONCLUSIONS

The initial amount of salts in the medium is a key parameter which can affect the biomass in the biofilter and thus, the performances of the process. The elimination capacity of the system

was at least 200 g/h.m^3, a value close to the upper limits found in the literature for ethanol biofiltration[2,3,8,9]. To maintain a stable and complete ethanol degradation at a load of 200 g/h.m^3, a nutrient supply with concentrations comprised between those reported for MS1 and MS2 should be adequate. When the biofilter was overloaded, volatile intermediates of the ethanol oxidation were detected in the outlet stream and the ERE decreased. CO_2 production is a good indicator of the behaviour of the biofilter. On a long term, a decrease in pH can be expected through CO_2 production and organic acid accumulation. However, this decrease can be controlled by adding a $CaCO_3$ source (e.g. oyster shell powder) and maintaining the ethanol load at an adequate level. Besides, this yeast can stand relatively low pH values (around 4). The final yeast population obtained can also make this system attractive for direct single cell protein production on bagasse. No significant bacterial or fungal contamination was observed by microscopy.

REFERENCES

1. van Groenestijn, J.W., Hesselink, P.G.M. *Biodegradation.* **1993**, 4, 283-301.
2. Kennes, C., Thalasso, F. *J. Chem. Technol. Biotechnol.* **1998**, 72, 303-319.
3. Devinny, J.S., Deshusses, M.A., Webster, T.S., **1999**, Biofiltration for Air Pollution Control. Lewis Pub.
4. Swanson, W.J., Loehr, R.C. *J. Environ. Eng.* **1997**, 123, 538-546.
5. Leson, G., Hodge, D.S., Tabatabai, F., Winer, A.M. Proc. 86th Ann. Meet. Exhib. Air & Waste Manag. Assoc., Denver, Colorado, **1993**, 93-WP-52C.04.
6. Hodge, D.S., Devinny, J.S. *Environ. Prog.* **1994**, 13,167-173.
7. Kiared, K., Wu, G., Beerli, M., Rothenbuhler, M., Heitz, M. *Environ. Technol.* **1997**, 18, 55-63.
8. Cioci, F., Lavecchia, R., Ferranti, M.M. *Biotechnol. Lett.* **1998**, 11, 893-898.
9. Arulneyam, D., Swaminathan, T. *Bioproc. Eng.* **2000**, 22, 63-67.
10. Domenech, F., Christen, P., Paca, J., Revah, S. *Acta Biotechnol.* **1999**, 19, 27-36.
11. Christen, P., Domenech, F., Michelena, G., Auria, R., Revah, S. *J. Hazard. Mat.* **2001**, submitted.
12. Morales, M., Revah, S., Auria, R. *Biotech. Bioeng.* **1998**, 60, 483-491.
13. Gribbins, M.J., Loehr, R.C. *J. Air & Waste Management Assoc.* **1998**, 48, 216-226.
14. Christen, P., Auria, R., Vega, C., Villegas, E., Revah, S. *Biotech. Adv.* **1993**, 11, 549-557.
15. Devinny, J.S., Hodge, S. *J. Air & Waste Management Assoc.* **1995**, 45, 125-131.

KEY WORDS : biofiltration, *Candida utilis*, ethanol, mineral salts, removal efficiency.