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Biofiltration of volatile ethanol using sugar cane bagasse inoculated with *Candida utilis*

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Abstract

Candida utilis (*C. utilis*) growing on sugar cane bagasse complemented with a mineral salt solution was studied for gaseous ethanol removal in a biofilter. Ethanol loads from 93.7 to 511.9 g/h m^3 were used, by varying both inlet ethanol concentration (9.72 to 52.4 g/m^3) and air flow rate (1.59×10^{-3} to 2.86×10^{-3} m^3/h). At a loading rate of 93.7 g/h m^3, a steady-state was maintained for 300 h. Ethanol removal was complete, and 76.3% of the carbon consumed was found in carbon dioxide. At an higher aeration rate (ethanol load = 153.8 g/h m^3), the biofilter displayed an average removal efficiency (RE) of 70%, and an elimination capacity (EC) of 107.7 g/h m^3. Only 64.4% of the carbon consumed was used for CO_2 production. Acetaldehyde and ethyl acetate in the outlet gas attained 7.86 and 20.4% in terms of carbon balance, respectively. In both cases, the transient phase was less than one day. At a high inlet ethanol concentration (52.4 g/m^3), no steady-state was observed and the process stopped during the third day. In the three cases, final biomass was poor, ranging from 10.5 to 14.8 mg/g dm. Final pH 4.0–4.6, indicated that acidifying non-volatile metabolites, such as acetate, accumulated in the reactor.

Keywords: Biofiltration; *Candida utilis*; Ethanol; Mass balance; Sugar cane bagasse

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1. Introduction

Emissions of volatile organic compounds (VOCs) have become subject to strict regulations, and processes using microorganisms have been largely studied in the past few years to control these emissions [1–3]. Among the most common VOCs, ethanol is considered as a relatively toxic pollutant, principally originating from bakeries, distilleries, and foundries [4,5]. Most studies about ethanol biotreatment used bacteria consortia from activated sludge [6], sewage treatment plant [7], VOC treating biotrickling filter [8], or soil from petroleum refinery [9]. As an example of industrial achievement, an ethanol biofilter constituted with sewage sludge compost and pine bark was reported to eliminate 80–90% of total VOCs (mostly ethanol) emitted by a foundry plant at an inlet concentration up to 3 g/m^3 [10].

The selection of the support is an important step for high efficient biofiltration. The packing material must have the following properties: optimal microbial environment, high specific surface area, structural integrity, high moisture retention, and high porosity [11]. Packing materials used for biofiltration include polystyrene particles [5,7], peat [8,12,13], compost [9], granular activated carbon [14], or porous inorganic matrix [13], sometimes coated with activated carbon [15]. However, these supports pose the problem of their disposal after utilization. An alternative lies in the use of agro-industrial by-products such as cassava bagasse or sugar cane bagasse, whose biotechnological valorization has been demonstrated [16,17]. Among the numerous microorganisms able to grow on these natural supports [18], *Candida utilis* (*C. utilis*), a yeast with a high protein content, is also able, in liquid culture, to assimilate ethanol as sole carbon source [19]. From a previous screening, a *C. utilis* strain from the ICIDCA collection was selected in liquid medium for its best ethanol elimination rate [20,21]. Besides, the growth of *C. utilis* on sugar cane bagasse complemented with glucose, in small packed bed reactors, has been characterized previously [22].

The purpose of this work was to study ethanol biofiltration at high loads, in a packed bed bioreactor filled with sugar cane bagasse, inoculated with a pure culture of *C. utilis*. Special interest has been given to ethanol elimination capacity (EC), carbon dioxide production and volatile intermediate generation. It also considers carbon and nitrogen balances, and biomass build up.

2. Material and methods

2.1. Microorganism

C. utilis L/375-1 from the collection of ICIDCA (Cuba) 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.

2.2. Support preparation

Sugar cane bagasse, a by-product of the sugar industry, was used as support. It was sieved through 0.4–0.8 cm screens, washed with distilled water, dried at 80°C for 24 h, and sterilized at 15 psi for 15 min.

Table 1
Feeding conditions used for the three independent runs

Experimental conditions	Run 1	Run 2	Run 3
Aeration flow rate (m^3/h)	1.59×10^{-3}	1.61×10^{-3}	2.86×10^{-3}
Inlet ethanol concentration (g/m^3)	9.72	52.4	8.87
Ethanol load (g/h m^3 reactor)	93.7	511.9	153.8

2.3. Experimental conditions

Sugar cane bagasse was mixed with the inoculum and a mineral salt solution, described by Thomas and Dawson [23], with the following concentrations in the water phase: $(NH_4)_2SO_4$, 2.5 g/l; KH_2PO_4, 2.5 g/l; $MgSO_4$, 0.24 g/l; $CaCl_2{\cdot}2H_2O$, 58.8 mg/l; $FeCl_3{\cdot}6H_2O$, 27.0 mg/l; $ZnSO_4{\cdot}7H_2O$, 25 mg/l; $MnCl_2{\cdot}4H_2O$, 4 mg/l; $CuSO_4{\cdot}5H_2O$, 2.5 mg/l, $NaMoO_4{\cdot}2H_2O$, 0.4 mg/l; $CoCl_2{\cdot}6H_2O$, 0.4 mg/l; H_3BO_3, 1.5 mg/l; KI, 0.3 mg/l. The reactor was packed with 40.5 g of the mixture. Initial conditions were: pH adjusted to 6 with NaOH (0.5 M), temperature: 30°C; moisture content: 63% (w/w); inoculum size: 1×10^7 cells per gram of dm, wet packing density: 0.25 g/ml. Aeration rate and inlet ethanol concentration were as reported in Table 1.

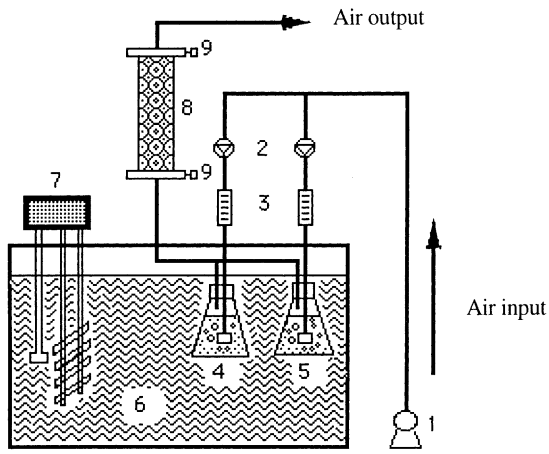
2.4. Experimental set-up

The bioreactor consisted in a glass module (diameter: 4 cm; length: 13.1 cm; total working volume: 0.165 l) equipped with a double jacket in order to control the temperature within the reactor (Fig. 1). Filtered air flow was splitted between a water column to pre-humidify it and an ethanol column to load the air with ethanol vapors (Fig. 1). Air flow in the ethanol column was adjusted to obtain the ethanol concentration required, and air flow in the water column was adjusted to obtain the total air flow desired. Both air flows were mixed before entering the biofilter.

2.5. Analytical methods

Cell number was determined by dispersing the samples (1 g in 25 ml) in an NaCl (9 g/l) solution and counting in a Neubauer chamber. The count was then transformed in biomass dry weight on the basis of 10^7 cells = 0.158 mg, obtained from a previous experiment. The pH was determined by mixing 1 g of sample with 25 ml of distilled water and homogenizing for 5 min. Water content of the medium was obtained by drying the sample in an oven at 105°C during 24 h.

CO_2 and O_2 were simultaneously evaluated in the effluent gas with a gas chromatograph (Gow Mac, USA) equipped with a thermal conductivity detector and a concentric column CTR1 (Alltech, USA). The production rate of CO_2 was then expressed as g/h m^3. The respiratory quotient (RQ) is defined as the ratio between CO_2 produced and O_2 consumed (mol/mol). The CO_2 yield coefficient is defined as the ratio between CO_2 produced and ethanol consumed (g/g).



1. Air supply, 2. Needle valves, 3. Flowmeters, 4. Humidifier (water), 5. Fuel reservoir (ethanol), 6. Water bath, 7. Temperature controller, 8. Packed bed reactor, 9. Inlet and outlet sampling ports

Fig. 1. Experimental set-up.

Acetaldehyde, ethyl acetate, and ethanol were measured by gas chromatography using a Hewlett-Packard 5890 apparatus equipped with a flame ionization detector and an HP-1 Megabore column (length, 5 m; i.d., 0.53 mm). Acetaldehyde and ethyl acetate production rates were expressed as g/h m^3 in order to compare the results for each run. RE and EC were defined as follows:

$$\mathrm{RE} = 100 \times \frac{C_\mathrm{i} - C_\mathrm{o}}{C_\mathrm{i}} \text{ and } \mathrm{EC} = \frac{F}{V} \times (C_\mathrm{i} - C_\mathrm{o})$$

where RE is the removal efficiency (%); EC the elimination capacity (g/h m^3); C_i the inlet ethanol concentration (g/m^3); C_o the outlet ethanol concentration (g/m^3); F the aeration flow rate (m^3/h); V the bioreactor volume (m^3).

2.6. Partition coefficient study

The bioreactor was treated as a two-phase system where ethanol concentration in the gaseous phase was controlled by dispersion and transfer to the solid/water phase. It was assumed to occur instantaneously, establishing a constant concentration ratio between the solid/water phase and the air (partition coefficient). In the solid/water phase, ethanol concentration increased by transfer from the air phase and decreased by biodegradation.

The partition coefficient (K_EtOH) was determined in abiotic conditions, in 100 ml flasks with septum-covered caps containing 2 g of wet bagasse, as described previously for ethanol

[9] or toluene [24]. K_{EtOH} is defined as follows:

$$K_{EtOH} = \frac{C_{w+s}}{C_{air}}$$

where C_{w+s} is the ethanol concentration in the solid/water phase (g/m^3); C_{air} the ethanol concentration in the air phase (g/m^3). C_{air} was measured by gas chromatography and C_{w+s} was calculated from mass balance.

3. Results

3.1. Partition coefficient study

For water + bagasse:

$$K_{EtOH} = 1599 \qquad (R^2 = 0.990)$$

For water, and according to the Henry's law:

$$K_{EtOH} = 1224 \quad \text{(at } 30^{\circ}\text{C and atmospheric pressure)}$$

A good correlation was found between ethanol concentration in the air phase and in the solid/water phase (Fig. 2). The value obtained for the partition coefficient ($K_{EtOH} = 1599$) allowed to calculate ethanol concentration in the solid/water phase in further experiments. This value was lower than those obtained by Hodge and Devinny [9] for compost (4480) and activated carbon (9090) [9], probably because of a lower physical adsorption of ethanol on sugar cane bagasse than on compost or activated carbon. However, K_{EtOH} for the water/bagasse was higher than the value calculated for water.

3.2. Influence of inlet ethanol concentration

Results arise from runs 1 and 2 (Table 1), where the inlet ethanol concentration was 9.72 and 52.4 g/m^3, which corresponds to concentrations of 15.5 and 73.4 g/l in the solid/water phase respectively, according to the ethanol partitioning study.

In run 1, high initial RE was related to the absorption of ethanol. This physical elimination was coupled with a low CO_2 production, which increased after a lag phase of about 8 h. Steady conditions (constants RE and CO_2 production) were attained after one day. They were maintained for 300 h and 100% of the ethanol fed was removed. After this period, ethanol appeared in the outlet stream, indicating a loss in the biological activity of the reactor. Similarly, CO_2 production increased continuously up to 150 g/h m^3, and then decreased drastically after 250 h (Fig. 3a).

During the first 20 h, production of acetaldehyde and ethyl acetate was high, reaching 1.14 and 8.65 g/m^3 in the outlet stream, respectively (Fig. 3b). These two compounds derive from incomplete ethanol oxidation and are toxic to the yeast, particularly acetaldehyde, reported to be inhibitory at 0.3 g/l in liquid culture [25]. As reported previously [20], when ethanol is not used for biomass production, it can be oxidized into acetaldehyde, then into acetic acid,

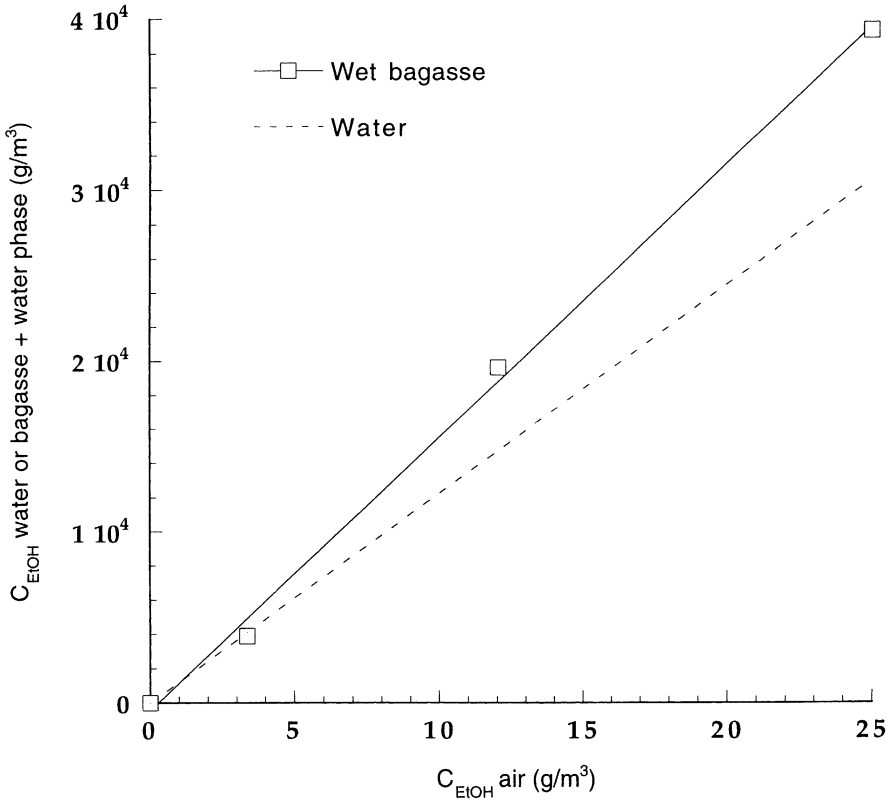


Fig. 2. Ethanol partitioning for water (theoretical value calculated from Henry's law) and wet bagasse saturated with 63% of water (experimental values).

which in turn can be esterified into ethyl acetate. This sequence and the high volatility of acetaldehyde, and at a lesser level that of ethyl acetate, explained why acetaldehyde appeared first in the outlet stream followed by ethyl acetate. These observations correspond to the transient phase during which the yeast becomes adapted to ethanol. Then, for the following 80 h, acetaldehyde and ethyl acetate were not found in the outlet stream, indicating an efficient functioning of the biofilter. The release of these two compounds at low levels corresponded to a decrease in RE and CO_2 production [15].

Results from run 1 demonstrated that a system consisting in a pure culture of *C. utilis* grown on sugar cane bagasse was adequate for ethanol biodegradation at a load of 93.7 g/h m^3.

In run 2, when the system was fed with an inlet ethanol concentration of 52.4 g/m^3, corresponding to a load of 511.9 g/h m^3, the steady-state was not attained. During the first 30 h, no ethanol was detected in the outlet gas and CO_2 production was very high (close to 300 g/h m^3). Simultaneously, acetaldehyde and ethyl acetate appeared in the outlet gas at concentrations of 5.17 and 0.6 g/m^3, respectively. Their concentration, after a short decrease, increased again after 20 h, just when CO_2 concentration began to decrease indicating that

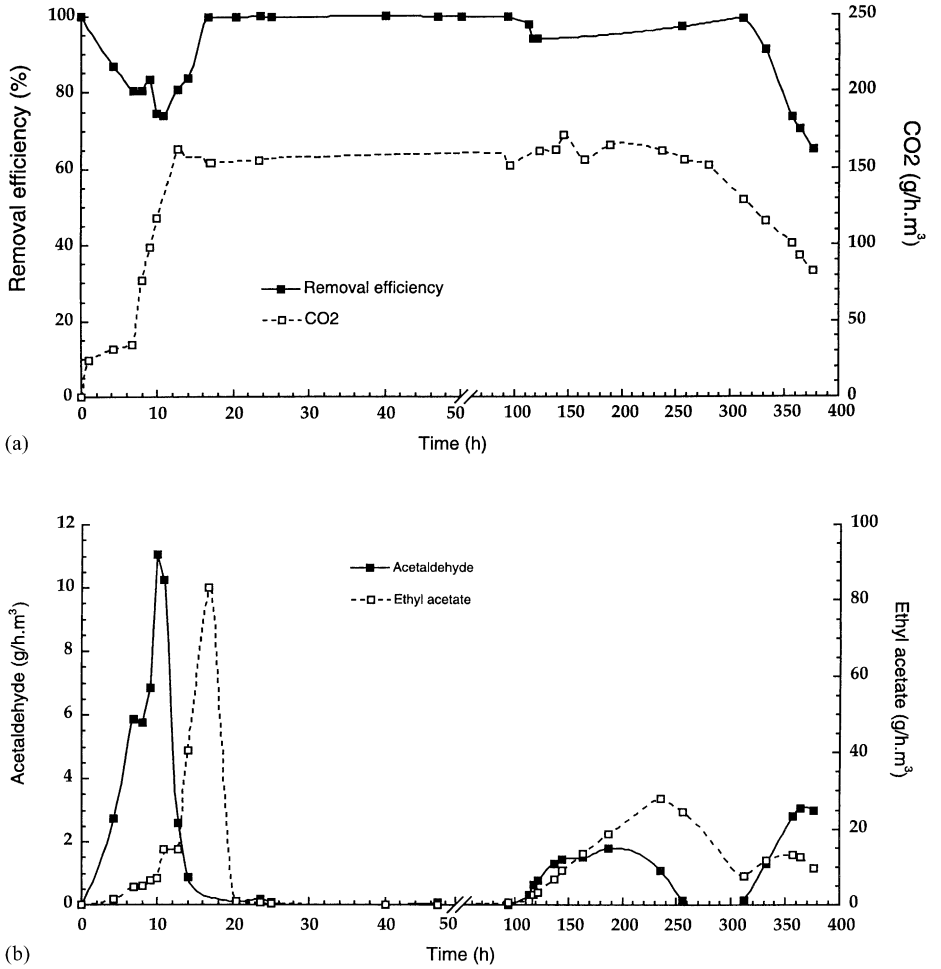


Fig. 3. (a) RE and carbon dioxide production for run 1 (ethanol load = 93.7 g/h m^3); (b) acetaldehyde and ethyl acetate production for run 1.

the biofilter could not support such a load. A few hours later, ethanol appeared in the exit gas and the process stopped (Fig. 4).

3.3. Influence of air flow rate

In run 3, a higher aeration flow rate, and the resulting shorter residence time for ethanol, affected the biofilter performance (Fig. 5a). At the beginning, RE rapidly decreased to values close to 60%. A pseudo steady-state was attained between 20 and 130 h and RE remained close to 80%, and CO_2 production kept practically constant at 200 g/h m^3. However, acetaldehyde and ethyl acetate reached values of 2.13 and 4.26 g/m^3 at the beginning of the

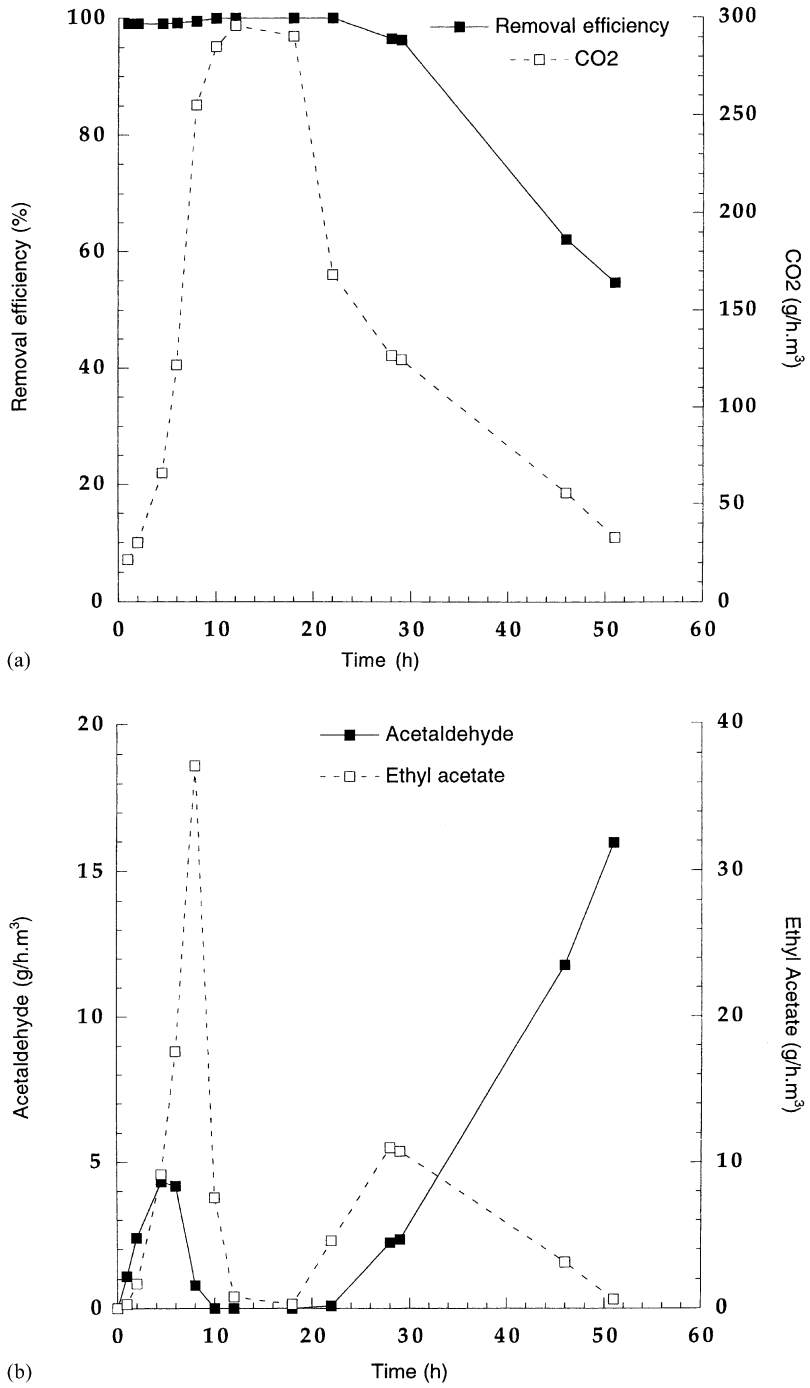


Fig. 4. (a) RE and carbon dioxide production for run 2 (ethanol load = 511.9 g/h m^3); (b) acetaldehyde and ethyl acetate production for run 2.

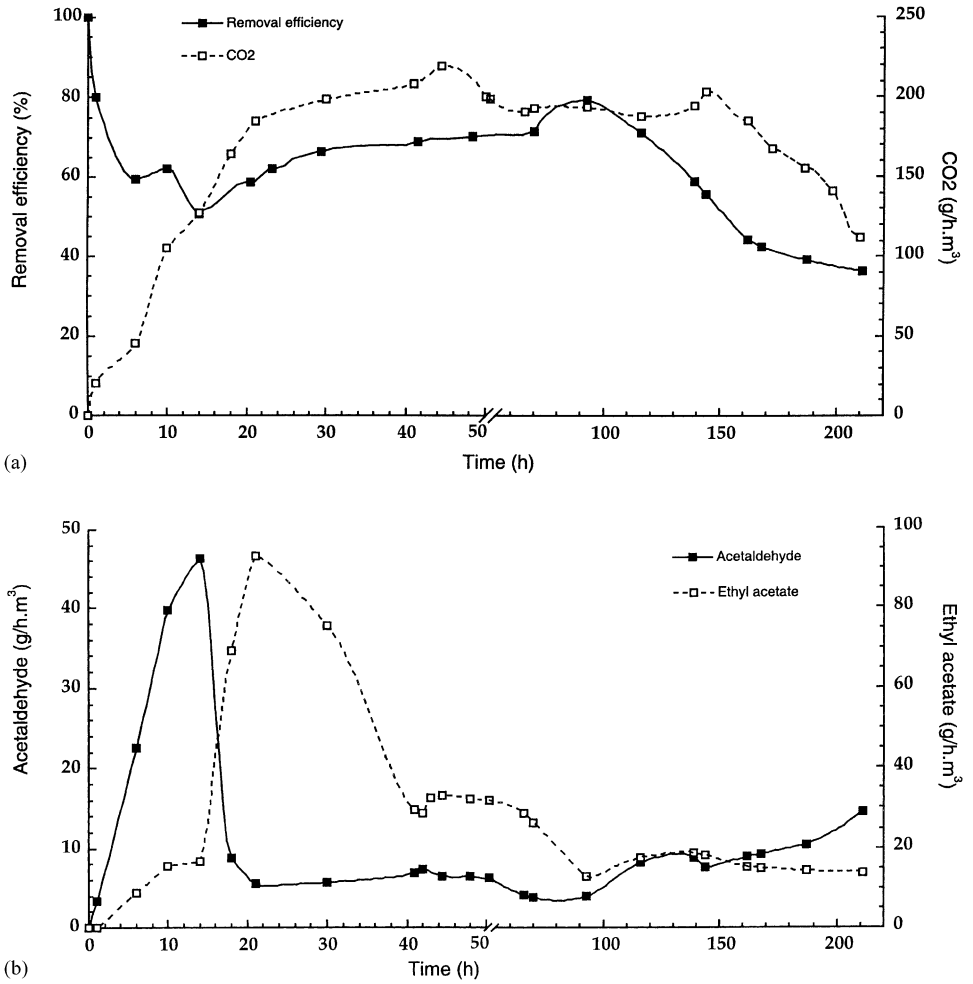


Fig. 5. (a) RE and carbon dioxide production for run 3 (ethanol load = 153.8 g/h m^3); (b) acetaldehyde and ethyl acetate production for run 3.

process and then decreased, without disappearing completely. Acetaldehyde at the exit of the reactor always remained higher than 0.2 g/m^3 and began to increase constantly at the end of the experiment. In this case, the air flow rate, and therefore the ethanol load, was too high for the system, probably because the yeast growth rate was too low or because of some nutrient limitation.

3.4. Respiratory quotient study

In run 1, RQ was close to the theoretical value of 0.67, corresponding to a complete ethanol oxidation (Fig. 6). This indicated that the reactor mainly worked as a biofil-

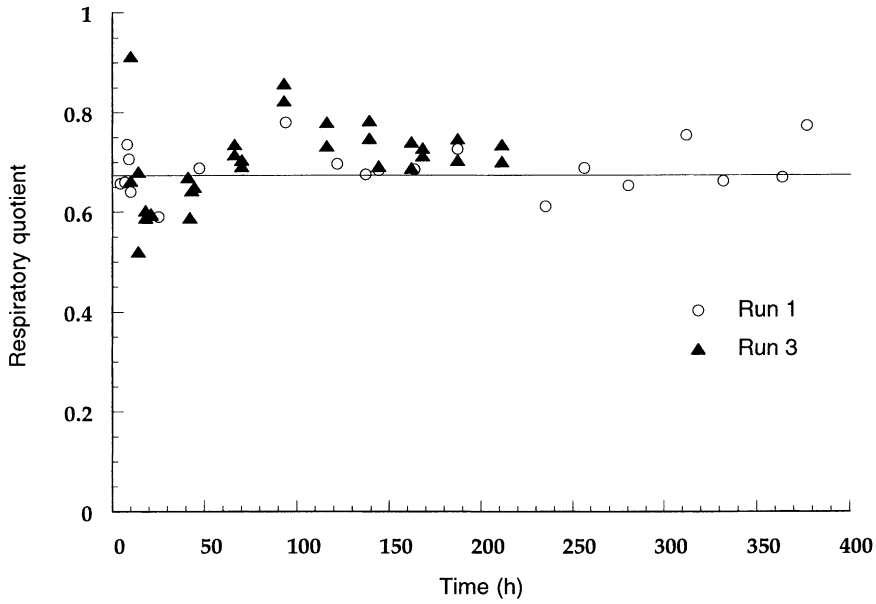


Fig. 6. RQ for runs 1 and 3. The solid line corresponds to the theoretical value.

ter sensu stricto and little of the ethanol consumed was used for biomass or metabolite production. However, in run 3, some variations of the RQ were observed. During the transient phase, RQ was below the theoretical value, meaning that part of the oxygen consumed was used for other purposes, such as biomass build up or metabolite production (acetaldehyde, ethyl acetate), resulting from an incomplete ethanol oxidation. Then, a RQ value slightly above the theoretical value indicated the possible oxidation of metabolites such as acetic acid (for which RQ = 1) accumulated during the first part of the process.

4. Discussion

4.1. Performance of the system and mass balance

As reported by other authors [5,9,14], RE is greatly influenced by the ethanol load. Different critical loading rates (CLR), i.e. the load at which 99% of the contaminant is eliminated, have been reported according to the systems (media and microorganisms) studied. For example, Leson et al. [5], reported a decrease of the RE from 100% at 70 g/h m^3 to 90% at 105 g/h m^3 [5]. Other authors reported CLR values close to 80 g/h m^3 [14,26]. Higher values have been reported with well-adapted bacteria consortia: 185 [6] and 195 g/h m^3 [7]. Our results, obtained at a bench scale, with a pure culture, are similar to those previously reported in the literature (Table 2).

Table 2
Final results and mass balances

	Run 1	Run 2	Run 3
Ethanol load (g/h m^3)	93.7	511.9	153.8
Residence time (min)	6.2	6.5	3.5
Final results			
Average RE (%)	100	–	70
EC (g/h m^3)	93.7	–	107.7
Biomass (mg/g dm)	10.5	14.8	14.0
Final moisture (%)	72.9	66.8	67.7
Final pH	4.0	4.6	4.2
CO_2 yield coefficient (g/g)	1.46	0.32	1.23
Carbon balance			
Ethanol consumed (g C)	2.772	1.610	1.743
CO_2 produced (g C) (% of ethanol consumed)	2.116 (76.3)	0.266 (16.5)	1.122 (64.4)
Acetaldehyde produced (g C) (% of ethanol consumed)	0.035 (1.26)	0.016 (0.99)	0.137 (7.86)
Ethyl acetate produced (g C) (% of ethanol consumed)	0.406 (14.6)	0.025 (1.55)	0.356 (20.4)
Biomass produced (g C)[a] (% of ethanol consumed)	0.079 (2.85)	0.092 (5.71)	0.083 (4.76)
Other (g C) (% of ethanol consumed)	0.136 (4.91)	1.211 (75.2)	0.045 (2.58)
Nitrogen balance			
Nitrogen from the medium (mg N/g dm)	1.5	1.5	1.5
Nitrogen from biomass (mg N/g dm)[a]	1.15	1.63	1.54

[a] According to [27], *C. utilis* grown on ethanol contains 50.3% of carbon and 11% of nitrogen.

Final pH values were low, indicating the probable accumulation of organic acids, such as acetic, in the medium. Because of this accumulation, and a subsequent drop in the pH medium, the metabolism was probably slowed down, which in turn allowed the accumulation of ethanol and organic acids in the medium. This phenomenon could also be due to the use of ammonium as nitrogen source. These conditions may be responsible for the decrease of RE observed in runs 1 and 3. Sugar cane bagasse has a relatively poor buffering capacity, and even though *C. utilis* is known to be relatively acid tolerant, an important gain in the lifetime of the biofilter can be expected if the pH is maintained at higher values by using appropriate nitrogen sources alone, or in a mixture of various sources. This was already reported for toluene biofiltration [28,29].

For a given system, there are two ways to increase the loading rate: by increasing the inlet concentration or the aeration rate. In a previous paper, we showed that *C. utilis* could not stand more than 30 g/l of ethanol in a liquid culture [20]. According to the above partition coefficient study, this corresponds to a concentration of 19 g/m^3 in the inlet stream. This agrees with our experimental results: at concentrations under 19 g/m^3 (runs 1 and 3), the system was working with a RE of 100 and 70%, while for run 2, the RE observed was mainly due to the ethanol adsorption into the packed bed, but the biological activity stopped very rapidly.

Aeration rates used in this study were low, leading to residence times higher than those commonly reported —– typically in a 15–60 s range — which means that the biofilter had a slow ethanol degrading rate. When the residence time decreased from 6.2 to 3.5 min,

RE also decreased and, consequently, metabolites resulting from the incomplete ethanol oxidation appeared in the outlet stream. Devinny and Hodge [15] previously described this phenomenon at an ethanol load of 156 g/h m^3 [15]. These authors associated it to rapid ethanol consumption. A preliminary adaptation of the yeast could probably improve the EC of the system. Another possibility is to increase the biomass within the reactor. As shown in Table 2, biomass produced was low, ranging from 10.5 to 14.8 mg/g dm. This was due to nitrogen limitation, as nitrogen balance showed that all nitrogen from the mineral medium was recovered in the biomass. An increase in nitrogen content would improve the biomass produced, and hence increase the EC of the system. Moreover, this would also improve the protein content of the support and could make the system attractive for using it, after a drying step, as cattle feed.

Final moisture remained higher than the initial one, indicating that the continuous humidification of the reactor was apparently satisfactory.

For runs 1 and 3, the CO_2 yield coefficients were 1.46 and 1.23, respectively. The deviation from the theoretical value for the complete ethanol oxidation (1.91 g/g) corresponds to the carbon used for biomass and other metabolite production. On the contrary, this coefficient was much lower for run 2, resulting in ethanol accumulation in the reactor and diversion in other metabolites [9].

In runs 1 and 3, the difference between the amount of carbon entering the biofilter and the carbon in the measured fractions (biomass, CO_2, acetaldehyde and ethyl acetate) was very low (Table 2). In run 1, we found that 76.3% of the carbon from ethanol was evolved as CO_2, which indicated an efficient functioning of the biofilter. The proportion of the evolved acetaldehyde was low and part of the acetate produced was eliminated by esterification, leading to a relatively high proportion of ethyl acetate (14.6%). In run 2, most of the carbon entering the biofilter was not found in the measured products. Part of it was probably dissolved or adsorbed in the water + solid phase of the reactor. Another part was probably transformed into an acid non-volatile form. In run 3, the proportion of carbon transformed into CO_2 was lower than in run 1, while the proportions of acetaldehyde and ethyl acetate increased, indicating that the biofilter had some difficulty to completely oxidize ethanol. This was due to the low biomass content in the reactor and a too low residence time. In all cases, the proportion of carbon used for biomass build up was low, therefore, we can expect a better biomass production and hence a higher EC by increasing mineral medium concentration.

5. Conclusion

Our results demonstrate that gaseous ethanol can be eliminated with a bagasse biofilter inoculated with a pure culture of *C. utilis*. A RE of 100% was observed with a load of 93.7 g/h m^3. The elimination of higher loads was not sustained. It is expected that increased biomass, through mineral salt addition, and a better buffering capacity of the solid bed through the addition of $CaCO_3$, would improve the time-span of the process.

Furthermore, the possibility of using the yeast-enriched support as a single cell protein source is being explored. To reach adequate levels in protein (i.e. 8% in the dry bagasse), levels of nitrogen and other salts would have to be increased.

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