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Optimization of immobilization conditions for vinegar production. Siran, wood chips and polyurethane foam as carriers for *Acetobacter aceti*

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Abstract

A complete experimental design has been developed to study the properties of three different solid carriers (Siran, wood chips and polyurethane foam) in the immobilization of acetic acid bacteria. Temperature-controlled reactors of 450-ml volume were employed to compare a standard immobilization procedure consisting of consecutive discontinuous acetic acid fermentations in the presence of the carrier. On reaching the final saturation condition, the different immobilized carriers were removed and introduced into identical sterilized reactors. These were then submitted to several semi-continuous fermentation cycles with the aim of characterising and comparing their acetification properties. Immobilization and acetification data obtained in this study have been evaluated in order to determine the best carrier material on the basis of several technical criteria. Polyurethane foam was the most successful because it allows a huge number of immobilized cells in the shortest time and leads to the highest acetification rate of the three assayed carriers.

Keywords: Immobilization; *Acetobacter aceti*; Acetic acid fermentation; Siran; Wood chips; Polyurethane foam

1. Introduction

In recent years, cell immobilization techniques have become increasingly important and are being successfully applied in industrial processes such as the production of alcohols (ethanol, butanol and isopropanol), organic acids (including malic, citric, lactic and gluconic acids), enzymes (cellulase, amilase, lipase and others) and the biotransformation of steroids for hormone production, wastewater treatment, and food applications (beer, wine, meat, sugars) [1]. In general terms, freedom in cellular movement can be restricted in two main ways: adsorption of cells (either between the cells themselves or to a solid carrier surface) with physical and chemical bonds, or physical entrapment of cells within the carriers [2].

Very little development work has been undertaken using these techniques in the vinegar industry and, in this respect, there are great expectations for the near future. In addition, only a few papers have been published that describe the application of these techniques to fermentation processes that involve the use of acetic acid bacteria. In this case, the microorganism is strictly aerobic and it is therefore very important to consider the mechanical resistance of supports to oxygen transfer, a property that becomes the most limiting factor [3,4]. In any case, it has been widely reported in the literature that immobilization imparts a special stability to the bacteria against the negative effects of temperature, pH, ethanol and acetic acid concentrations [5] as well as avoiding the washing-out of the population of cells upon using high dilution rates in the continuous operation mode.

Adsorption to surfaces and encapsulation within gels or porous materials (a particular type of physical entrapment) have been the most widely studied methods for the immobilization of acetic acid bacteria (Table 1). These techniques represent a particular form of cellular adhesion based on the ability of certain microorganisms to fix themselves to solid surfaces by means of the secretion of polymucosaccharides [20]. When the pore size of the matrix is small, bearing in mind the dimensions of the cell, the adsorption occurs only at the surface, as in the case of diatomaceous earth, clays and other related materials. However, when the carrier has pores that are large relative to the cell dimensions, it is possible to find adhesion within the pores. This situation occurs

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Table 1
Several of the main papers published on *Acetobacter aceti* immobilization

Author	Year	Carrier	Method	Bio-reactor
Kennedy et al. [6]	80	Hydrous titanium(IV)	Adsorption/aggregation	Tower fermenter
Ghommidh et al. [3]	81	Ceramic monolith	Adsorption	Packed column
Okuhara [7]	85	Polypropylene fibres	Adsorption	Packed column
Nanba et al. [8]	85	Polypropylene fibres	Adsorption	Packed column
Adams and Twiddy [9]	87	Wood chips	Adsorption	Packed column
Garg et al. [10]	95	Wood chips	Adsorption	Erlenmeyer flask
		Calcium alginate gel	Entrapment	Erlenmeyer flask
Lotong et al. [5]	89	Cotton towel cloth	Adsorption	Rotating disc reactor
Sueki et al. [11]	91	*Aphrocell* ceramic	Adsorption	Packed column
Horiuchi et al. [12,13]	00, 01	Charcoal pellets	Adsorption	Packed column
Krisch and Szajáni [14,15]	96, 97	Cellulose beds	Adsorption	Erlenmeyer
		Calcium alginate gel	Entrapment	Erlenmeyer
Osuga et al. [4]	84	χ-carrageenan gel	Entrapment	Fluidized bed
Mori [16]	85	χ-carrageenan gel	Entrapment	Fluidized bed
de Araujo and Santana [17]	96	Calcium alginate gel	Entrapment	Stirred tank
Levitsky et al. [18]	98	Calcium alginate gel	Entrapment	Bubble column
Ikeda et al. [19]	97	Dialysis membrane	Membrane separation	Electrocatalytic reactor

in materials such as active carbon, polyurethane foam and sintered glass.

Adsorption techniques reduce the problems associated with oxygen diffusion and do not suffer from the scale-up drawbacks experienced with encapsulation matrices.

The work described here involved the immobilization of *Acetobacter aceti* bacteria by adsorption on three different solid carriers: Siran, wood chips and polyurethane foam. These carriers were chosen by taking into consideration the possibilities for scaling-up the procedure for application in industrial vinegar processes. The aim of the work was to analyse the cellular adhesion properties in each case and assess the fermentation properties of the immobilized carriers obtained.

Of the three solid supports proposed, only wood chips have been previously assayed for the immobilization of acetic acid bacteria [9,10] and good results were obtained in these studies ($\approx 10^8$ immobilized cells/g of carrier; $\approx 1^\circ$/day of acetification rate).

2. Materials and methods

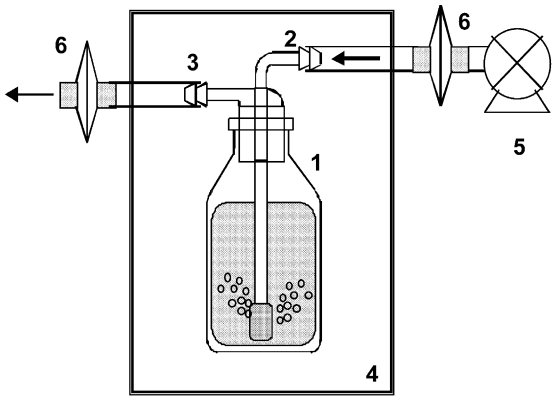
2.1. Medium, microorganism and carriers

A young wine from the Jerez-Xèrés-Sherry area was used as the initial medium. The ethanol concentration ranged between 71 and 79 g/l and the total acidity was in the range 2.5–5 g/l, expressed as acetic acid concentration. The strain responsible for the fermentations described here was a mixed culture fully adapted to industrial fermentation conditions, with the main prevailing proportion being *Acetobacter aceti* (ATCC15973).

The carriers used were units of Siran, wood chips and polyurethane foam. Their main characteristics are summarized in Table 2.

2.2. Reactor

The equipment used for immobilization and subsequent fermentation is represented in Fig. 1 and consisted of 6×500-ml reactor units (commercially available from Schott) (1) : they are cylindrical glass vessels with an upper closing cover. Each reactor was equipped with a gas inlet (2) connected, through an internal rod submerged in the liquid, to a sintered glass air diffuser (medium pore size: 100 μm). The latter device allows the dispersion of the air in the form of small bubbles and also avoids bubble coalescence. The reactor also incorporated an upper gas flow outlet (3).



1. Glass reactor with 500 mL total capacity and 450 mL working volume
2. Gas inlet
3. Gas outlet
4. Thermostatic rotary shaker
5. Aeration pump
6. Amicrobic filters for gases with 0.22 μm pore diameter

Fig. 1. Acetic acid fermentation equipment at laboratory scale (working volume 450 ml).

Table 2
Summary of main characteristics for the assayed carriers

	Siran	Wood chips	Polyurethane foam
Trade mark	SCHOTT-Sikug (012/02/300/A)		
Material	Sintered glass	Oak chips	Commercial PUF
Shape	Spheres	Slightly curved	Cubes
Size	1–2 mm	1.5 mm length; 1 mm width	1 cm length
Density	0.7–1.2 g/ml	1.092 g/ml	0.02 g/ml
Porosity	60%	96.7%	97%
Average pore size	Small pores: 10–20 μm Large pores: 60–300 μm	15 μm	400 μm
Application/reference	Nitrifying bacteria [21]	Acetic acid bacteria [9,10]	Pseudomonas sp. [22]
Scanning electron micrographs	Fig. 2	Fig. 3	Fig. 4

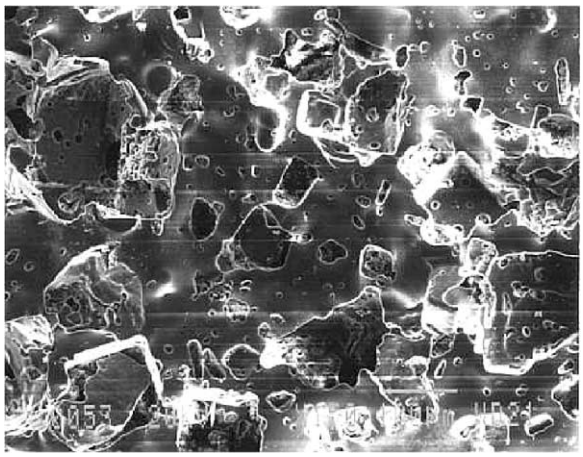


Fig. 2. Photomicrograph (×150) of pore distribution in a SIRAN particle, showing macro and micropores.

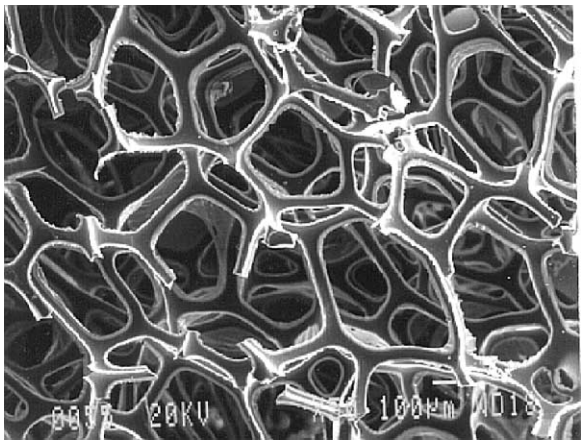


Fig. 4. POLYURETHANE FOAM internal structure (×50).

Stirring and heating were facilitated by placing the reactors into an orbital thermostatic shaker (4). This apparatus has the ability to maintain a constant temperature of 30 °C, recommended in Ref. [23], and an orbital stirring regime of 200 rpm.

O_2 saturation conditions were guaranteed by an air pump that supplied a constant volumetric air flow rate of 0.2 vvm [24]. The air pump (5) was connected to the reactor inlet through a pipe containing an inserted amicrobic gas filter of 0.22 μm of pore size (6). A similar filter was also inserted in the gas outlet pipe.

Sampling, medium charge and discharge, charge of the immobilization carrier, maintenance and cleaning labours were made possible by uncoupling the upper cover.

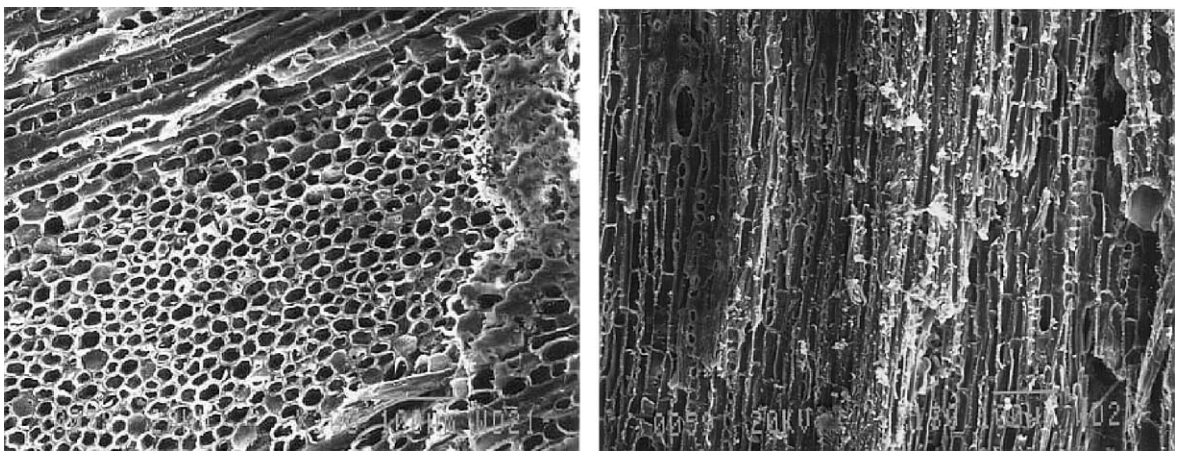


Fig. 3. Longitudinal (×130) and transversal (×200) cuts in WOOD CHIPS units assayed.

2.3. Immobilization

In a first stage, the same weight (4 g) of carrier was used in all experiments in order to obtain comparable results. Initially, the reactor containing 4 g of carrier was sterilized using wet heat at 120 °C during 20 min in a conventional sterilization system. Wine (the substrate) was filtered and, consequently, sterilized through a membrane of 0.22-μm pore size. The use of this method avoids the heating involved in the conventional sterilization process, the effect of which could lead to undesirable effects in the substrate.

In a second stage, inoculum (450 ml) was inserted into each reactor. The inoculum was a selected culture with uniform characteristics: a natural fermentation medium (vinegar) with an ethanol concentration range of 23–32 g/l, an acetic acid concentration between 40 and 50 g/l and a large cellular population of *Acetobacter aceti* in its exponential growth phase ($>500\times10^6$ cell/ml). In general terms, inoculation and start-up of the acetification reactor was based on a protocol published previously [25].

The immobilization process was finally developed by successive acetic acid fermentation cycles. One fermentation cycle ranged from 40 to 80 g/l (in acetic acid concentration) from start to finish. The whole process was developed with the carrier inside of the reactor.

Initially, the reactor contained 450 ml of medium with 40–50 g/l total acidity and the ability to produce a further 40 g/l. Under these conditions (stirring rate: 200 rpm; aeration: 0.2 vvm; temperature: 30 °C) fermentation developed quickly and the medium reached 80 g/l of total acidity in 72–96 h. At this point, the first immobilization cycle was taken to be finished.

The second cycle began with the removal from each reactor of a calculated volume of the product in such a way that, after replacement with sterile fresh wine, a decrease in the acetic acid concentration (to 50 g/l total acidity) was registered; in this case, approximately 170 ml. This process represents the start of a new semi-continuous fermentation cycle in which further biomass immobilization takes place. The whole immobilization process involved successive semi-continuous cycles, with acetic acid concentration values in the range 50–80 g/l, until the adhered biomass analysis showed that saturation of the carrier had been achieved (stabilisation of the number of immobilized cells).

2.4. Fermentation

Once the final saturation point had been reached, all carrier particles were removed from their immobilization reactors and introduced into the fermentation reactors. These reactors were identical to those used for the immobilization stage. Fermentation reactors were completely sterile and contained 450 ml of a filtered vinegar (50 g/l acetic acid concentration and 32 g/l ethanol concentration). Thus, the acetic acid fermentation (stirring rate: 200 rpm; aeration: 0.2 vvm; temperature: 30 °C) is considered to be carried out exclusively by the adsorbed bacteria, without any initial contribution from the submerged biomass.

When the acetification cycle had finished (acetic acid concentration: 80 g/l; ethanol concentration $\leq$8 g/l) the whole carrier of every reactor was taken out and introduced into new sterile reactors. Subsequently, a new acetic acid fermentation cycle was started.

2.5. Analyses

Ethanol was analysed using a HEWLETT PACKARD 5890 Series II gas chromatograph with a capillary column (Carbowax 20M on Chromosorb 0.2 μm) and a FID detector.

Acetic acid was measured taking into consideration that other organic acids are present in vinegar in negligible quantities [26], so it is reasonable to suppose that total acidity is a good indicator of the real acetic acid concentration. This concentration was determined by titration with NaOH with phenolphthalein as an indicator.

Total biomass was determined by statistical re-count with a Neubauer chamber and by optical microscopy.

Immobilized biomass concentration was measured using a technique developed specifically for the work described here. In the first stage, 100 mg of colonized carrier was removed from the reactor and submerged in an Erlenmeyer flask containing 25 ml of sodium acetate buffer solution (pH 4.6). In the second step, the flask was placed in an ultrasonic bath at room temperature for 15 min. These conditions led to the total desorption of adhered cells. In the last stage, the Neubauer chamber re-count method for the submerged cells was carried out on the liquid phase. The carrier was subsequently removed from the flask and dried in an oven at 80 °C during 24 h. It was then possible to calculate the number of immobilized cells per milligram of carrier (cell/mg). This technique has been previously validated by developing experiments concerned with cellular resistance to ultrasonic treatment and studying the desorption efficiency.

All photomicrographs presented in this paper were taken using a Jeol JSM-820 scanning electron microscope, following a previously standardized physicochemical treatment of samples: fixation with glutaraldehyde (2.5%) at 4 °C for 2 h, cacodylate salt (0.1 M, pH 7.0) for 30 min, osmium tetroxide (1%) for 1 h, dehydration with acetone and drying, and metallization with gold.

3. Results and discussion

3.1. Immobilization process

Figs. 5–7 show data for the bacterial immobilization cycles for each of the six reactors (experiments in duplicate), using identical conditions for the three carriers: Siran, wood chips and polyurethane foam. Acetic acid and submerged biomass concentrations were measured during the

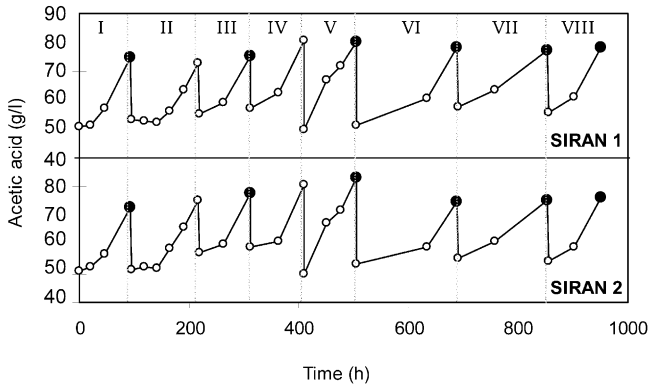


Fig. 5. Experimental data of acetic acid concentration vs. time during the immobilization cycles (I–VIII) in the laboratory reactors (SIRAN). The black dots represent the sampling points for desorption and re-count.

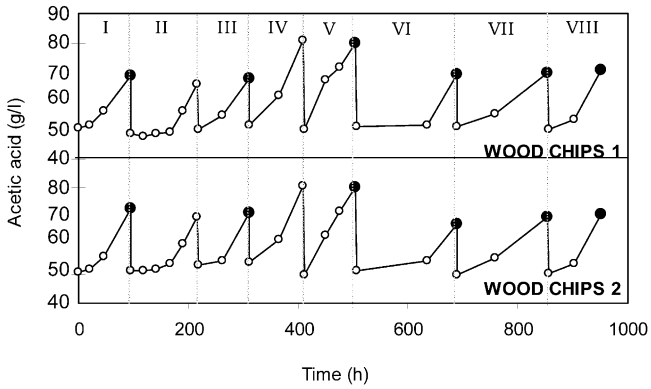


Fig. 6. Experimental data of acetic acid concentration vs. time during the immobilization cycles (I–VIII) in the laboratory reactors (WOOD CHIPS). The black dots represent the sampling points for desorption and re-count.

semi-continuous cycles for every reactor, along with the quantity of biomass adsorbed onto the carriers by means of re-counts at the end of every cycle. However, only the data for acetic acid concentration are shown in the figures and those data that correspond to any immobilized bacteria re-count are represented as black dots. The numerical

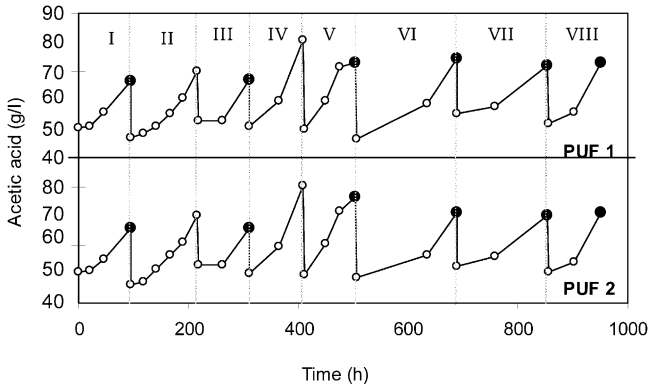


Fig. 7. Experimental data of acetic acid concentration vs. time during the immobilization cycles (I–VIII) in the laboratory reactors (POLYURETHANE FOAM). The black dots represent the sampling points for desorption and re-count.

Table 3
Average concentration of immobilized biomass in each essayed carrier (Siran, Wood chips and Polyurethane foam), in Mcel/mg (millions of cells/mg of carrier) as a function of time

Semi-continuous cycles	Time (h)	Siran Mcel/mg	Wood chips Mcel/mg	Polyurethane Mcel/mg
0	0	0	0	0
1	94	1.35	3.06	0.50
3	311	2.12	5.97	10.42
5	505	4.40	8.27	12.44
6	689	1.45	7.05	9.30
7	854	16.39	6.26	10.99
8	950	13.00	10.00	10.25

results of such re-counts, expressed in relation to the number of milligrams of carrier and averaged for the assays in duplicate, are shown in Table 3. From the data given in Table 3 it can be seen that since the process already lasted 800 h, saturation of the carriers was reached under the conditions used (stirring, aeration, carrier quantity, etc.). The development of the quantity of immobilized biomass in every carrier as a function of time is represented in Fig. 8.

It can be seen from Figs. 5–7 that eight semi-continuous acetification cycles were carried out for every reactor. Acetic acid concentrations in the medium were controlled between 50 g/l (at the start of each run) to 70–80 g/l (at the end) with an average time of nearly 110 h (4.6 days). A high degree of reproducibility in the results is evident for every reactor and, as a consequence, the developed cycles in the six different reactors show essentially the same behaviour regardless of the carrier used.

Each cycle starts with a short lag phase in which the growth and production are low as a consequence of the change in the medium conditions and the dilution of the submerged biomass. The length of this phase generally depends on the initial concentrations of ethanol and acetic acid and it is in the range 40–100 h. Once this lag phase has finished, the culture begins a phase of exponential growth

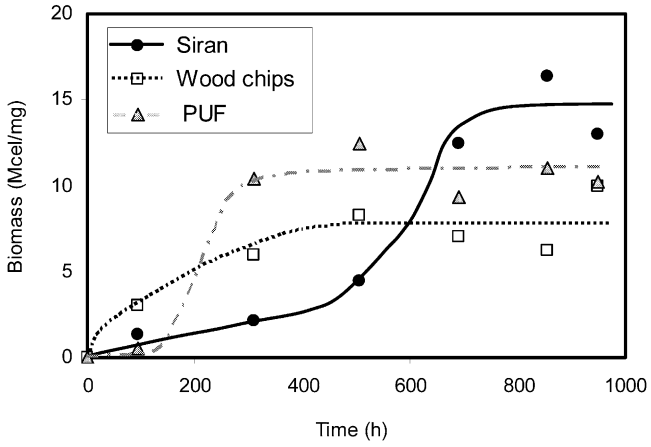


Fig. 8. Experimental data of immobilized biomass in Mcel/mg (millions of cells per milligram of carrier) vs. process time (h), for the three essayed carriers.

and maximum production occurs, reaching 70–80 g/l. This is the most interesting phase from the point of view of cellular multiplication and, of course, in terms of immobilization of the biomass on the carriers. The acetification rates found in each cycle vary and oscillate between 4.6 and 5.0 g/l day. This rate does not depend on the type of carrier submerged in the liquid but does depend on the state of activity of the submerged biomass. Thus, prior to the immobilization, acetification in this phase is mainly produced by the submerged biomass and the adsorption of the biomass onto the carrier does not markedly affect the developing cycles.

As can be seen from the relevant data in Table 3, the reproducibility obtained in the re-count data for each pair of reactors is satisfactory for a given carrier. A non-sensible disparity can be observed due to the unique circumstances of each fermentation and the analytical errors inherent in the use of microscopy. In graphic terms, an arithmetic mean from every pair of values has been obtained in order to describe the quantity of total immobilization vs. time. Given that the process had already been in operation for 800 h, any variation in the immobilized biomass trend would be observed, and this was seen not to change during the last 200 h of operation. It is necessary to take into account the influence that the high stirring rate has on the maximum immobilization limit. For the reactor described above, the stirring rate (200 rpm) was considered to be the most suitable to establish a satisfactory balance between two effects; on the one hand, vigorous stirring improves the general conditions for mass transfer to submerged biomass and, as a consequence, increases the total biomass population and the number of cells adsorbed onto the carrier. On the other hand, erosion effects, collision tensions and other phenomena can be produced by turbulent stirring, possibilities that can seriously hinder surface cell adsorption processes [16]. Such properties could even lead to the partial desorption of immobilized population [27] as well as an increase in total evaporation losses from the open system.

An extensive analysis of the immobilization for each carrier is necessary in order to establish their characteristic properties. The immobilization process of biomass in the Siran vs. time fits a sinusoidal curve in which two phases can be clearly distinguished (see Fig. 8). It can be seen that the biomass adheres to Siran in a progressive (but slow) way up to a process time of 500 h. This zone may well correspond to a superficial adhesion of bacteria to the external surface—i.e. macropores (60–300 μm), which are the most accessible zones of the carrier. Once the maximum immobilization capacity in the macropores has been reached, the large micropores begin to be colonized (10–20 μm diameter). The total adhesion capacity of micropores exceeds that of macropores. The appearance of an inflexion zone in the trend of the curve is essential to guarantee a maximum adsorption of the carrier. If the process would have been stopped before this time the real potential of the carrier would have been wasted. Under the experimental conditions used in this study, the maximum quantity of biomass

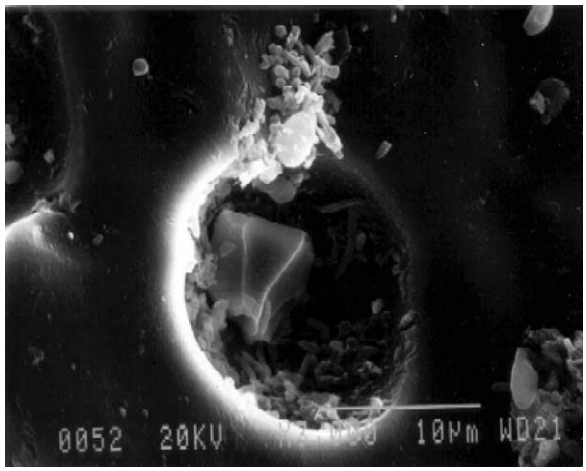


Fig. 9. Photomicrograph (SEM ×3000) of a micropore structure in a unit of SIRAN with immobilized acetic acid bacteria.

that can be immobilized by the carrier is about 13 Mcel/mg (millions of cells per milligram of carrier) and an operation time of 800 h (34 days) would be required to ensure maximum cell adsorption. Fig. 9 shows a scanning electron micrograph (SEM ×3000) of one unit of Siran and a micropore structure containing adhered acetic acid bacteria (bacillus 2–3 μm length) can be clearly observed.

The progress of immobilized biomass on wood chips vs. time (see Fig. 8) is perceptibly different from that registered for Siran. The number of cells in this carrier increases progressively with time, fitting a logarithmic trend in accordance with typical adsorption profiles. This situation is characteristic of regular surfaces in which the pore structures do not vary significantly in size, but all the pores in the carrier have a similar accessibility. In this case, carrier saturation is the key factor and points to the asymptotic limit of maximum immobilization capacity. Under the experimental conditions used in this study, the maximum quantity of biomass that wood chips can immobilize is about 8.25 Mcel/mg after 500 h of operation (21 days). Fig. 10 shows a SEM (×3500) of one of the units.

Fig. 10. Photomicrograph (SEM ×3500) of a WOOD CHIP surface with immobilized acetic acid bacteria.

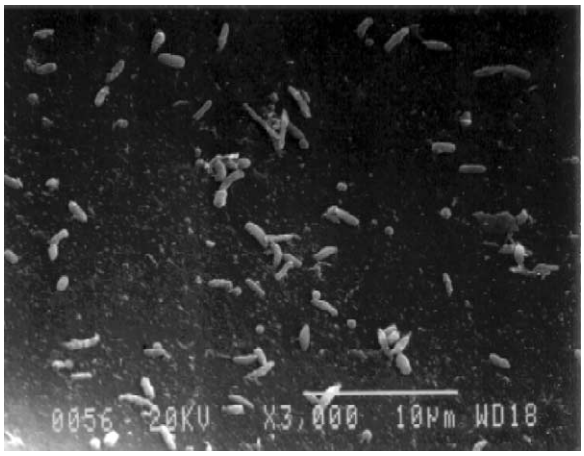


Fig. 11. Photomicrograph (SEM ×3000) of the surface of POLYURETHANE FOAM with immobilized acetic acid bacteria.

The internal structure of polyurethane foam could explain the characteristic shape of the immobilized biomass experimental data (Fig. 8). After an initial stage of 100 h without any appreciable immobilization, a sudden increase in adhered biomass is observed and, within a few hours, the maximum colonization of the carrier is reached. From this point on, further adsorption is not registered. This situation could be directly related to the hydrodynamic behaviour of polyurethane foam submerged in the liquid phase. During the first cycles the particles of carrier remain dry, but as the process continues they gradually become completely wet by capillary action. After this point the cellular colonization of the carrier begins at a high rate (slope of the curve ≈0.06 Mcel/mg h). It is feasible that this colonization would have been even faster if a device for the soaking of foams had been employed from the start of the process. Nearly every immobilization is produced in an operation time of 215 h (from 94 to 311 h), a fact that is key for the success of the global process. The highly porous structure of this carrier (over 97%) facilitates the total exposure of the surface and precludes problems associated with accessibility for the cells after soaking. As a consequence, a high homogeneity for the bacterial adhesion is observed with this material. The maximum immobilization capacity is reached (about 10.5 Mcel/mg) after a process time of about 300 h (13 days) and beyond this point there is no significant increase. Fig. 11 shows a SEM (×3000) of one polyurethane foam carrier, which was removed from the reactor. The appearance of

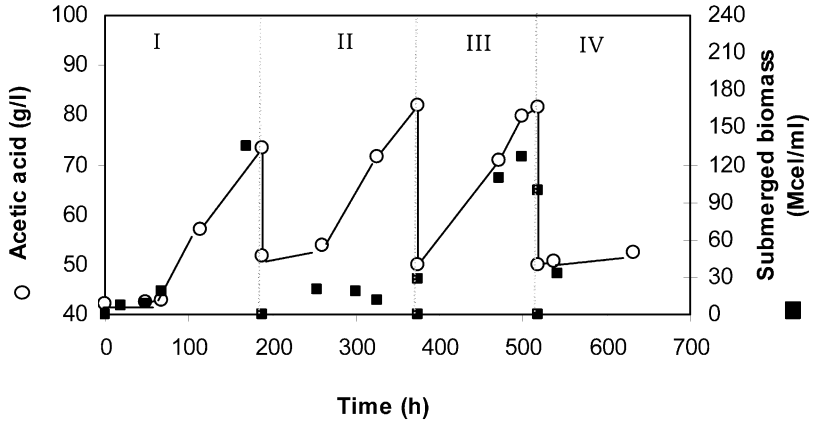


Fig. 12. Experimental data of acetic acid concentration (○) and submerged biomass (■) vs. time during the fermentation cycles with immobilized acetic acid bacteria on SIRAN.

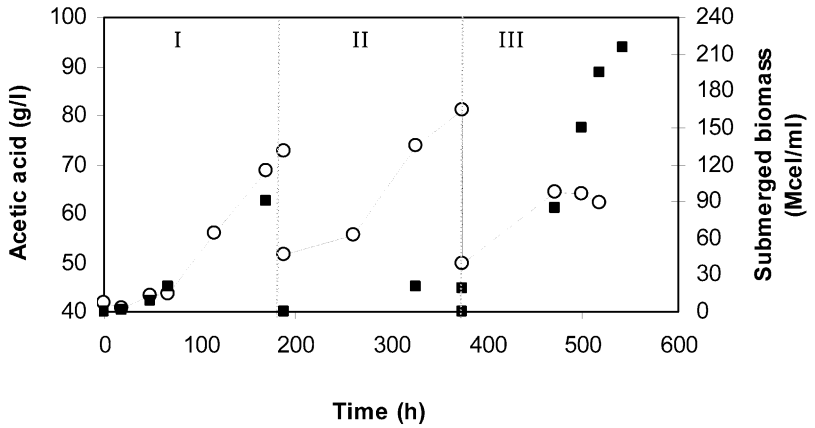


Fig. 13. Experimental data of acetic acid concentration (○) and submerged biomass (■) vs. time during the fermentation cycles with immobilized acetic acid bacteria on WOOD CHIPS.

acetic acid bacteria adhered to the surface of the particle can clearly be seen.

3.2. Fermentation with immobilized biomass

In this section the experimental data of acetic acid and submerged biomass concentrations are discussed. These data correspond to fermentation cycles developed by using immobilized biomass on the three assayed carriers (Figs. 12–14). A number of important conclusions can be drawn from the data obtained in these experiments:

Experimental data show that it is possible to obtain a complete set of reproducible fermentation cycles for each of the carriers studied. The activity of the immobilized biomass remains steady with constant acetification rates observed. Polyurethane foam stands out from the other two assayed supports in that it leads to the maximum acetification rate (over 5 g/l day). Nevertheless, it is important to note the overall decrease in the fermentation yield as these cycles progress, a decrease that is due to evaporation losses (ranging from 5 to 15% in every cycle).

It can be seen from Figs. 12–14 that, at the beginning of every cycle, no appearance of submerged biomass is observed. This phenomenon is due to the prior sterilization of the reactor, a fact that means any bacteria in the reactor should be present as immobilized biomass. After start-up of the reactor, the contact between carrier and liquid and the intense stirring lead to the progressive desorption of the bio-film. As a consequence, the presence of suspended cells is observed in the medium. This new population has a full reproductive and fermentative ability. The proportion of submerged cells to total cells (i.e. submerged+adhered cells) increases from zero to 20% in 75 h, and to 100% (the same quantity of submerged and adhered cells) at the end of every cycle. Indeed, it is possible to find a higher number submerged cells than immobilized biomass. This is the case for polyurethane foam, which contributes an additional biomass population arising from the liquid remaining within its interstitial structure. It is therefore of great interest to assess the contribution to the acetification process of each fraction of biomass (immobilized and submerged). It can be concluded that the submerged biomass is responsible for the main contribution because it does not suffer from the diffusional problems typically associated with the solid carriers and, in addition, it has a better availability of the nutrients required for its cellular metabolism. As a consequence, this double contribution can be seen as a positive point in that the main role of the bio-film would be to provide a constant source of biomass for the medium through the continuous adsorption–desorption dynamic equilibrium in the interface.

3.3. Selection of the best carrier for immobilization and fermentation

Four criteria are the most relevant to establish the appropriate comparisons between the different carriers assayed: immobilization capacity, time for immobilization process, acetification rate with immobilized biomass and, finally, mechanical stability of the carrier. Moreover, a fifth important characteristic will be considered: the economic cost of each carrier. The analysis of the aforementioned criteria applied to the assayed solid supports results:

If only the immobilization capacity is considered, it is clear that Siran would be the most suitable carrier because it allows the immobilization of a higher number of cells per milligram of solid. However, the other two materials are also more than acceptable for the immobilization as their values can also be considered high. In addition to the values discussed above it is very important to take into account the time required to reach the maximum immobilization capacity. In this sense, polyurethane foam has the ability to reach an acceptable quantity of adhered biomass in a substantially lower time than the other two solids. The porous structure is highly uniform in the foam, a fact that facilitates rapid cellular adhesion. Thus, although the quantity of immobilized bacteria in the foam is 20% less than that immobilized in Siran, the foam gives a time 70% faster than Siran, making the operation more profitable. This balance between the

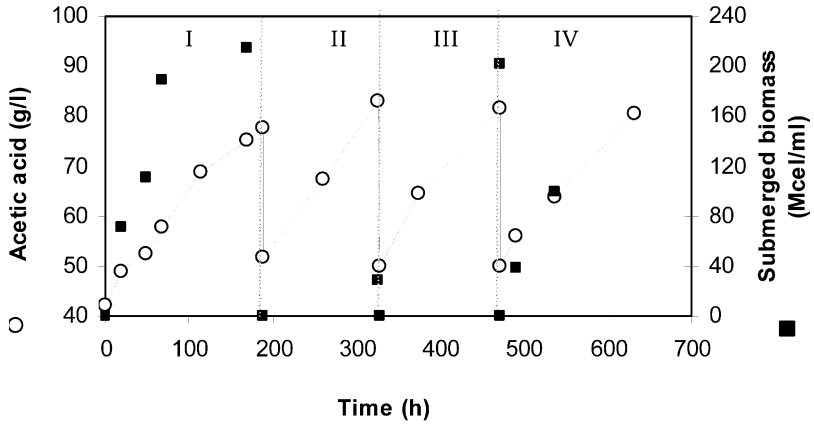


Fig. 14. Experimental data of acetic acid concentration (○) and submerged biomass (■) vs. time during the fermentation cycles with immobilized acetic acid bacteria on POLYURETHANE FOAM.

properties is even more favourable if the appropriate data for the wood chips are compared. The data obtained for the acetification rates in the process with immobilized biomass are clearly best for the polyurethane foam. Finally, other economic aspects must also be considered. Polyurethane foam is, without a doubt, the cheapest material of the three assayed and it is also readily and widely available. Wood chips can be obtained either from old ageing casks or by direct acquisition, but in both cases the chips require chemical treatment prior to immobilization, a fact that increases the cost of this option. Finally, Siran is an expensive synthetic material and is perhaps best used on the laboratory scale. Siran, however, does not seem to be a viable alternative for industrial acetic acid fermentations at the moment.

4. Conclusions

Of the three carriers assayed, the best for the immobilization of acetic acid bacteria and the subsequent acetification stage with adhered biomass on a laboratory scale is polyurethane foam. Polyurethane foam allows the immobilization of a large number of cells (about 10.5 millions/mg) in a short time (300 h). Moreover, it leads to the highest acetification rate of the three assayed carriers (4.74 g/l day), which makes the overall yields higher. Furthermore, it is an inert material and is very cheap, thus making the process potentially suitable for industrial scale-up.

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