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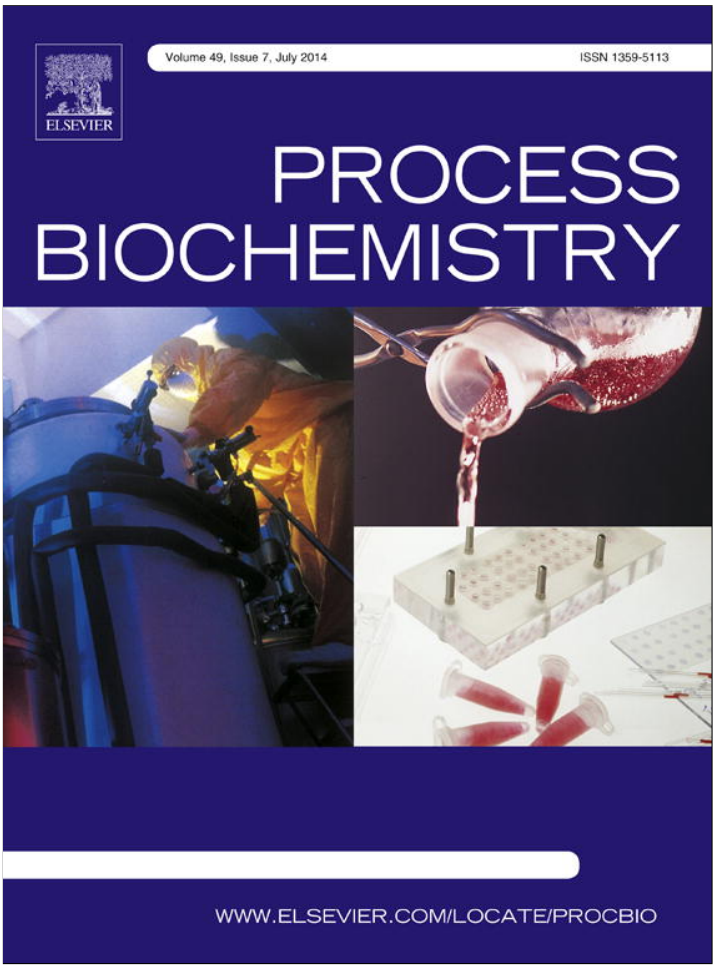

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An efficient biocatalytic system for floxuridine biosynthesis based on *Lactobacillus animalis* ATCC 35046 immobilized in Sr-alginate

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ABSTRACT

An efficient and green immobilized biocatalyst is herein reported to obtain 5-fluorouracil-2′-deoxyriboside (5FUradRib), an antimetabolite known as Floxuridine, used in gastrointestinal cancer treatment.

Alginate is a natural polysaccharide used in the pharmaceutical industry due to its physicochemical properties, biocompatibility and non-toxicity. Multivalent cations, exposure time and cross-linking solution concentration were optimized, being Sr^{2+}, 2 h and 0.2 M the best immobilization conditions. Furthermore, compression strength, swelling ratio and fracture frequency were evaluated, improving the mechanical stability of the biocatalyst favoring a future scale-up.

On the other hand, the reaction parameters for 5FUradRib biosynthesis were optimized in order to obtain an immobilized biocatalyst with enhanced activity. Thus, *Lactobacillus animalis* ATCC 35046 immobilized in Sr-alginate showed yields of 96% at short reaction times.

The obtained biocatalyst was stable for more than 25 days in storage conditions (4 °C) and could be reused at least 10 times without loss of its activity.

Additionally, Sr-alginate biocatalyst stability was evaluated in different organic solvents to obtain hydrophobic compounds such as 5-bromouracil-2′-deoxyriboside (5BrUradRib), an effective radiosensitizing agent used in anti-cancer therapy, being hexane the best co-solvent.

Finally, a smooth, cheap and environmentally friendly method to obtain anti-cancer drugs was developed in this study.

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1. Introduction

Microorganism stabilization has received considerable attention in the recent decades due to the fact that bioprocesses using immobilized microorganisms are a promising production methodology that plays a central role in the development of modern industrial biotechnology.

The main advantages of whole cell immobilization is that it allows easy separation and reuse of the biocatalyst, higher operational stability [1], high-cell density and scale-up feasibility [2]. Moreover, microorganisms can be protected from severe environmental conditions such as acid or basic pH values, high temperatures and organic solvents.

Entrapment methods are the most widely used for whole cell immobilization; these techniques are based on the inclusion of bacteria within a rigid network to prevent their release into the surrounding medium, while still allowing mass transfer of nutrients and metabolites [3]. The most commonly used supports for this methodology are agar, agarose, alginate, κ-carrageenan and polyacrylamide [4,5].

Alginate is a natural anionic biopolymer typically obtained from brown seaweed [6]. In molecular terms, it is an unbranched binary copolymer of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers [7]. The gelation behavior of alginates is based on the affinity of these polymers toward certain ions and on the ability to bind to them selectively and cooperatively [8].

Nowadays, the use of alginate hydrogels in biotechnology, medicine and the pharmaceutical industry is widespread due to their unique properties such as high biocompatibility, non-toxicity and biodegradability [9,10]. Furthermore, the physical and chemical properties of alginates (e.g. porosity) can be easily modified in mild conditions [11]. Other properties of alginate gel beads, such as their mechanical strength, susceptibility to shrinkage, and stability toward antigelling cations and chelating agents depend upon both

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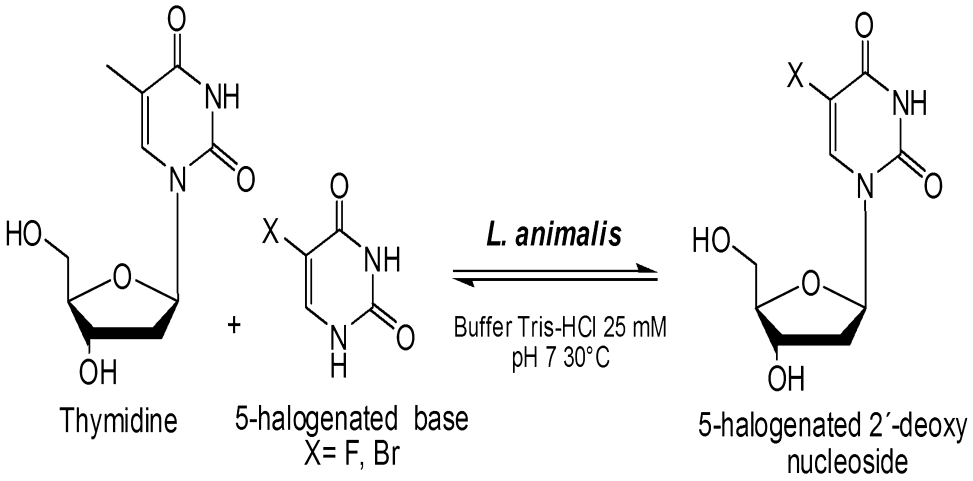

Scheme 1. Biosynthesis of halogenated 2′-deoxyribosides using *L. animalis* ATCC 35046. Model reaction using Thymidine, as sugar donor, and different 5-halogenated bases (5-fluorouracil and 5-bromouracil) to obtain 5-fluoruracil 2′-deoxyriboside (5FUradRib) and 5-bromouracil 2′-deoxyriboside (5BrUradRib).

the chemical characteristics of the alginate and the type and concentration of the cross-linking solution [12]. Consequently, altering the gel core properties is a useful strategy to increase biocatalyst stability [13].

Nucleoside analogs are widely used as antiviral and antitumoral agents and are principally synthesized by chemical methods; however, biosynthesis is a promising alternative [14,15].

Most microorganisms catalyze the biosynthesis of nucleoside analogs in two steps in the presence of inorganic phosphate [16,17]. However, lactic acid bacteria present 2′-N-deoxyribosyltransferase activity and these compounds can be synthesized in the absence of phosphate buffer [18]. In this work, a bioprocess using stabilized lactic acid bacteria (LAB) in alginate was developed. This biocatalytic system is based on immobilized *Lactobacillus animalis* ATCC 35046 in Sr-alginate to obtain halogenated pyrimidine nucleosides (Scheme 1) through a simpler and environmentally friendly methodology.

2. Materials and methods

2.1. Materials

Nucleosides and bases were purchased from Sigma Chem. Co. (Brazil). Culture media compounds were obtained from Britania S.A. (Argentina). Chemicals were purchased from Sigma Chem. Co. (Brazil). Sodium alginate was purchased from Saporiti S.A.C.I.F.I.A (Argentina). HPLC-grade solvents used were from Sintorgan S.A. (Argentina).

2.2. Lactic acid bacteria preparation

L. animalis ATCC 35046 was grown until saturation in stationary phase, harvested by centrifugation during 10 min at 10,000 × g, washed with tris(hydroxymethyl) aminomethane–HCl (Tris–HCl) buffer 25 mM pH 7 and stored at 4 °C until use. Media contained tryptone 15 g/L, soy peptone 5 g/L, NaCl 5 g/L pH 7.3.

2.3. Lactic acid bacteria immobilization by entrapment

L. animalis ATCC 35046 (1×10^{10} CFU) was immobilized by entrapment in sodium alginate as described by Trelles [19]. The entrapment method with $CaCl_2$ used as a control was described by Britos et al. [18].

2.4. Optimization of immobilization conditions

Different parameters of immobilization such as ion cross-linking (Ca^{2+}, Ba^{2+}, Sr^{2+} and Al^{3+}), gelling ion concentration and exposure time to divalent cations were analyzed. Immobilized biocatalyst activity was evaluated by biosynthesis of 5FUradRib from 6 mM thymidine (dThd) and 2 mM 5-fluoruracil (5FUra) at 2 h of reaction in Tris–HCl 25 mM at 30 °C and 200 rpm.

2.5. Determination of mechanical properties

2.5.1. Compression strength evaluation

The compression strength of beads was determined using a Universal Testing Machine TC-500 series II (MegaTest). The Young' modulus was calculated using the following equation:

$$\text{Young's Modulus}\,(E) = \frac{F \times D_0}{A \times \Delta D}$$

where F is the force imposed on gel beads, D_0 is the initial bead diameter, A is the gel bead area and ΔD is the difference between initial and final bead diameter.

2.5.2. Characterization of swelling behavior

Gel beads with a uniform diameter were placed in an excess of Tris–HCl buffer 25 mM pH 7 at room temperature. Bead volume change during course of swelling was monitored by measuring the weight using an analytical balance. The swelling factor (SR) was determined using the following equation:

$$\text{Swelling ratio}\,(\text{SR}) = \frac{(M_t - M_d)}{M_d} \times 100$$

where M_t is the mass of swollen hydrogel at a given time during swelling and M_d is the dried mass of the hydrogel. The mass was measured at specific times after removing excess water [20].

2.5.3. Fracture frequency

Mechanical resistance of alginate gel beads was performed according to the modified method described by Kathiravan et al. [21]. Beads were placed in a container with Tris–HCl buffer 25 mM in operating condition (30 °C and 200 rpm during 40 h). Gel beads were filtered and counted. The resistance was expressed in terms of fracture frequency, which is given below:

$$\text{Fracture frequency}\,(\%) = \frac{N_t}{N} \times 100$$

where N is the number of fractured beads and N_t is the total number of gel beads.

2.5.4. Microorganism release

The retention capacity of microorganisms of Sr-alginate gel beads was determined by optical density at 600 nm using a T60 UV–vis spectrophotometer (PG Instruments Limited) as a measure of cell release to the reaction medium. Release percentage was calculated from the difference between initial and final amount of cells in reaction medium.

2.5.5. Determination of bead shape

Bead shape was quantified using the sphericity factor (SF) resulting from the following equation:

$$\text{Sphericity factor}\,(\text{SF}) = \frac{(d_{\max} - d_{\min})}{(d_{\max} - d_{\min})}$$

where $d_{\max}$ is the largest diameter and $d_{\min}$ is the smallest diameter perpendicular to $d_{\max}$ [22].

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2.6. Biological activity test

Biosynthesis of 5FUradRib was carried out using 1×10^{10} CFU 6 mM dThd and 2 mM 5FUra in Tris–HCl buffer 25 mM pH 7 at 30 °C and shaking at 200 rpm as standard reaction condition. Volume reaction was 1 mL.

Different reaction conditions, such as pH (5, 6, 7, 8 and 9), temperature (20, 30, 45 and 60 °C) and number of cells (1×10^8, 1×10^9, 5×10^9, and 1×10^{10}), using immobilized *L. animalis* ATCC 35046 in Sr-alginate were evaluated.

2.7. Storage stability

Immobilized biocatalysts were stored at two temperatures (4 °C and 30 °C) in Tris–HCl buffer 25 mM pH 7. Then, 5FUradRib biosynthesis was evaluated at different times to ensure the biocatalyst stability. Storage stability was defined as the relative activity of 5FUradRib conversion between the first and the successive reactions.

2.8. Reusability

Reusability of immobilized whole cells in Sr-alginate and Ca-alginate was evaluated using 5FUradRib biosynthesis as standard reaction. The used beads were filtered at the end of each 4 h-cycle and washed three times using buffer.

2.9. Biocatalytic activity in non-conventional media

Biosynthesis of 5BrUradRib using immobilized *L. animalis* ATCC 35046, from dThd and 5-bromouracil (5BrUra) in Tris–HCl 25 mM at 30 °C and 200 rpm was evaluated using 20% (v/v) of different co-solvents such as dimethyl sulfoxide (DMSO), ethylene glycol, methanol and hexane. Additionally, different hexane concentrations (5%, 10%, 20%, 30% and 40% (v/v)) were assayed. 5BrUradRib conversion was calculated using the following equation:

$$5\text{BrUradRib conversion}\,(\%) = \frac{[5\text{BrUradRib}]}{[5\text{BrUradRib} + 5\text{BrUra}]} \times 100$$

where [5BrUradRib] is product concentration and [5BrUra] is 5-bromouracil concentration.

2.10. Green chemistry

Green parameters such as E-factor, C-efficiency and Atom economy were evaluated by biosynthesis of nucleoside analogs using equations described by Sheldon [23].

2.11. Analytical methods

Biosynthesis of nucleoside analogs was qualitatively evaluated by TLC Merck Silica gel 60 F_{254} in chloroform/methanol 80:20 (v/v) as mobile phase. Quantitative analysis was performed by HPLC (Gilson) at 254 nm (Detector UV/Vis 156, Gilson) with a Agilent Zorbax Eclipse XDB C-18 column (5 μm, 150 mm × 5 mm). 5FUradRib biosynthesis: Isocratic mobile phase was water/methanol (94:6, v/v), flow rate was 1.2 mL/min and retention times were 5FUra (1.49 min), Thy (2.65 min), 5FUradRib (3.90 min) and dThd (7.06 min).

5BrUradRib biosynthesis: Isocratic mobile phase was water/methanol (90:10, v/v), flow rate was 0.9 mL/min and retention times were Thy (6 min), 5BrUra (7 min), dThd (8.5 min) and 5BrUradRib (11.1 min).

Product identification was performed by MS-HPLC LCQ-DECAXP4 Thermo Spectrometer with Electron Spray Ionization methods (ESI). Phenomenex C-18 column (5 μm, 100 mm × 2 mm) and Xcalibur 1.3 software (Thermo-Finnigan, USA) were used. Mobile phase used for 5FUradRib (M^+: 246.9) and 5BrUradRib (M^+: 308.0) biosynthesis was 95:5 (v/v) water/methanol + 0.1% acetic acid and flow rate was 200 μL/min.

2.12. Statistical analysis

All experiments were performed in triplicate. One-way analysis of variance (ANOVA) was performed to determine significant differences among variables. Differences with a probability value of <0.05 were considered significant and all data were reported as mean ± SD. Statgraphics Centurion XV program (version 15.1.02) was used.

3. Results and discussion

3.1. Optimization of immobilization conditions

In order to determine the best immobilization conditions to stabilize *L. animalis* in alginate, different cross-linking solutions were evaluated through 5FUradRib biosynthesis. When a trivalent cation was used (Al^{3+}) in the solution, the matrix was rapidly disintegrated. The use of divalent cations as Ca^{2+}, Ba^{2+} and Sr^{2+} allowed to obtain stable beads [24]. It was observed that reaction conversion was influenced by the kind of cation which is used in the cross-linking solution (Fig. 1A). In all cases, the best results were obtained at 2 h exposure times. Sr^{2+} and Ba^{2+} were selected as cross-linking cations for subsequent assays. Besides, the lowest ion concentration (0.2 M) significantly enhances conversion (Fig. 1B). These results are consistent with a higher degree of cross-linking in presence of an elevated cation concentration, which causes a reduction of matrix permeability [25]. In both cases (0.2 and 2 M), the best results were obtained when Sr^{2+} was used as cross-linking cation. Additionally, analysis of results showed that the positive effect on performance biosynthesis is a unique combination between the best cross-linking solution and the best exposure time.

3.2. Characterization of Sr-alginate gel beads

Different parameters to evaluate Sr-alginate gel bead stability were determined and compared with the immobilization control conditions (Table 1).

3.2.1. Swelling ratio

Thus, a swelling ratio five times lower than Ca-alginate gel beads was obtained when using strontium as cation. It is known that osmotic swelling, a process that occurs in the core bead, is the principal cause of alginate polycation bead breakage. The reduction of the degree of swelling could be controlled by kinetics of capsule formation, for example by changing the exposure time [26].

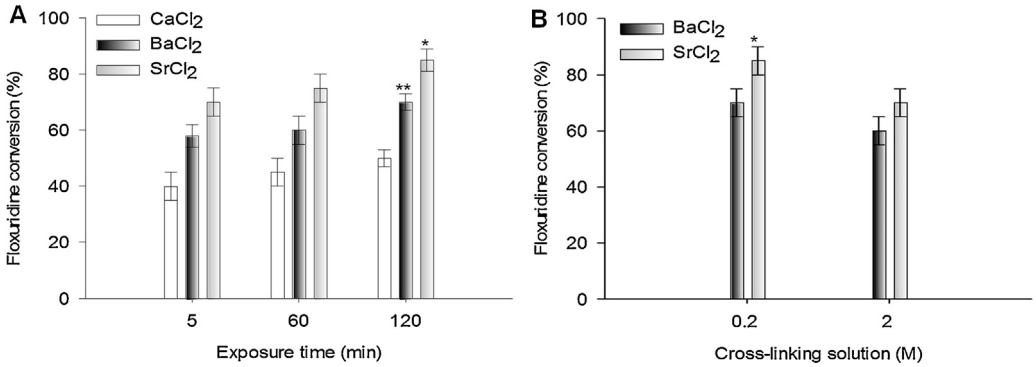

Fig. 1. Optimization of *L. animalis* immobilization conditions. Floxuridine biosynthesis was used as standard reaction (6 mM dThd and 2 mM 5FUra in 1 mL of 25 mM Tris–HCl at 30 °C and 200 rpm during 2 h). (A) Evaluation of cross-linking solutions effect at different exposure times. Significant differences when $BaCl_2$ (**$p < 0.0001$) and $SrCl_2$ (*$p < 0.0001$) were used. (B) Different concentrations of cross-linking solutions were assayed on the immobilized biocatalyst activity. Significant difference when $SrCl_2$ 0.2 M was used (*$p < 0.01$). Each assay was performed three times.

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Table 1
Mechanical properties of alginate hydrogels. Different cross linking solutions ($SrCl_2$, $CaCl_2$ and $BaCl_2$) was evaluated using 0.2 M gelling ion concentration and 2 h of exposure time.

	Swelling ratio (%)	Fracture frequency (%)	Sphericity factor (SF)	Cell release[a] (%)
Sr-alginate	2.8	3	0.06	<5
Ca-alginate	15	13	0.09	<5
Ba-alginate	−10	3.5	0.05	<5

[a] Release values lower than 5% are not significant.

In this work, the optimum relation among alginate, cation cross-linking concentration and exposure time was determined to obtain Sr-alginate gel beads with an elevated physicochemical stability and improved substrate and product permeability due to a film thickness [27]. Optimization of these variables can improve the mechanical stability of the biocatalyst favoring a future scale-up.

On the other hand, a particular behavior in Ba-alginate gel beads known as syneresis was observed, which is a macroscopic phenomenon characterized by a slow time-dependent deswelling of a gel resulting in an exudation of liquid. The shrinkage in alginate gel could be ascribed to water evaporation or intermolecular interactions leading to water exudation as a result of an elevated cation concentration [25]. Barium has an atomic radius greater than that of calcium and strontium that could affect the final structure of the alginate gel bead. This behavior is counterproductive for the interchange of substrate and product through the gel film during a reaction. Therefore, the use of barium as cross-linking solution was ruled out to form alginate gel beads.

3.2.2. Fracture frequency

The fracture frequency obtained for Sr-alginate gel beads was four times lower than control using Ca^{2+}; this result is related to matrix reinforcement using Sr^{2+} as cross-linking solution because this cation presents greater affinity for the alginate [28,29]. Moreover, swelling behavior may affect gel bead fracture due to the decreased stability of the matrix [27].

On the other hand, fracture stability could be enhanced by high alginate concentration during gel bead formation [30]. In this work, 4% (w/v) alginate concentration was used for *L. animalis* entrapment. Furthermore, an increase in exposure time leads to stronger beads and offers a more stable gel configuration [31].

3.2.3. Sphericity factor

Spherical and regular-shaped beads were obtained by using ionotropic gelation; the average diameter was 3 mm. The sphericity factor was used to represent this characteristic because it can detect shape changes more efficiently than other dimensionless indicators. The sphericity factor varies from zero for a perfect sphere to the unit for an elongated bead [22]. Comparable values in all cases were obtained, demonstrating that this entrapment technique allows to obtain regular spheres.

3.2.4. Cell release

Cell release was evaluated to determine the capacity for stabilizing of biocatalyst (1×10^{10} CFU/mL) during an extended time. After seven days in operating conditions, release was lower than 5% in all cases. A greater degree of cross-linking and aggregation of alginate dimmers can be obtained using high concentrations of gelling cations. Thus, an elevated concentration of cross-linking solution allowed to obtain gel beads with a smaller pore size and the resulting matrix presents a slower drug or cell release pattern [32,33].

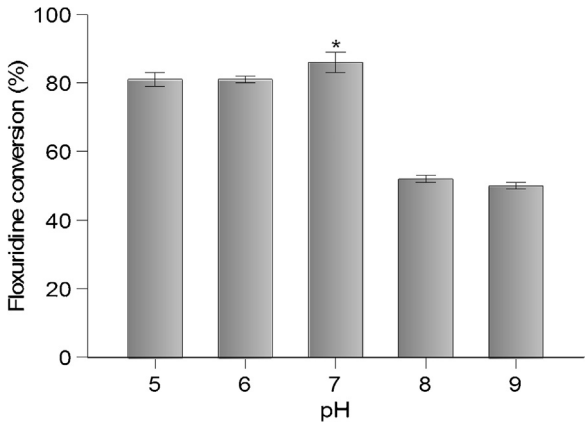

Fig. 2. Effect of pH on floxuridine conversion using Sr-alginate immobilized *L. animalis*. Standard reaction was performed during 2 h using 6 mM dThd and 2 mM 5FUra in 25 mM Tris–HCl at 30 °C, 200 rpm and different pH values (5, 6, 7, 8 and 9). Significant differences when pH was 5, 6 and 7 (*$p < 0.005$). Each assay was done three times.

3.2.5. Mechanical stability

Young' modulus or elastic modulus is the mathematical description of a substance's tendency to be deformed elastically when a force is applied on it. The elastic modulus is defined as the slope of its stress–strain curve in the elastic deformation region [34]. This parameter allows to determine the mechanical stability of alginate gel bead. The effect of Sr^{2+} as cross-linking solution before and after two days in operative conditions was evaluated. Both conditions showed similar behavior, indicating that mechanical stability under operational conditions was preserved. The determined elastic modulus of Sr-alginate beads was 24 kPa. In previous works, conventional Ca-alginate beads showed an elastic modulus equal to 17 kPa. Therefore, the Young's modulus values obtained of the Sr-alginate gel beads were 40% greater than conventional ones. Bead stiffness could be reinforced by increasing alginate concentration and using a novel gelling ion, such as strontium. Both conditions allowed to increase cross-linking density and uniform alginate distribution [22].

3.3. Biological activity test

When pH effect was assayed on 5FUradRib conversion, an almost two-fold decrease was observed at basic pH with respect to pH 5, 6 and 7 (Fig. 2). This behavior could be due to two synergic processes: an increment in alginate physical instability at basic pH that affects the microorganism viability or an unsuitable internalization of substrate because of their ionization [24,35].

When temperature was evaluated, the biocatalyst showed a conversion higher than 80% at 30 °C (Fig. 3), while 5FUradRib conversion was less at 20 °C (75%) and decreased significantly at 45 and 60 °C being 60% and 57%, respectively. This effect could be explained by biocatalyst instability due to elevated temperature which favors polymer degradation [36].

Smaller concentrations of free microorganisms (1×10^8 and 1×10^9) showed low activity. Moreover, using 1×10^{10} CFU/mL, 5FUradRib yield close to 100% was observed at 4 h (Fig. 4). Quantitative determination of 5FUradRib conversion was performed by HPLC (Fig. 5).

3.4. Storage and reusability assay

Stability of an immobilized biocatalyst is defined as the time that such biocatalyst keeps 50% of its initial activity. Different storage temperatures were analyzed to ensure biocatalyst stability. The obtained biocatalyst retained its activity for more than 25 days

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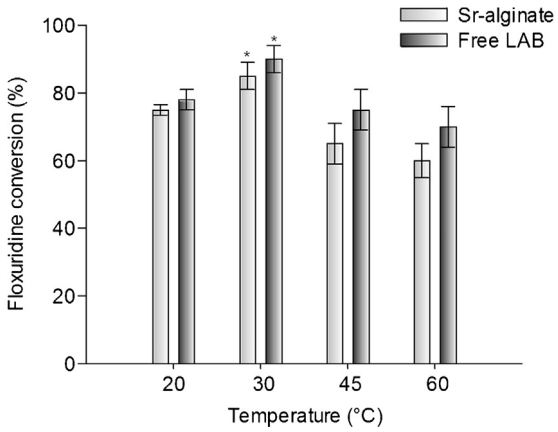

Fig. 3. Effect of temperature on floxuridine conversion using Sr-alginate immobilized *L. animalis*. Reaction conditions were 6 mM dThd and 2 mM 5FUra in 1 mL of 25 mM Tris–HCl at different temperatures (20, 30, 45 and 60 °C) and 200 rpm. Significant difference when 30 °C was used (*$p < 0.005$). Each assay was performed three times.

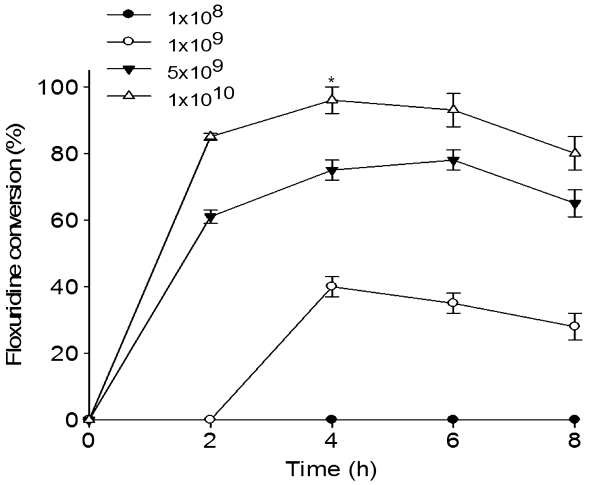

Fig. 4. Biosynthesis of floxuridine using different amounts of *L. animalis*. Reactions were carried out three times at 30 °C and 200 rpm in 1 mL of 25 mM Tris–HCl (pH 7), using 6 mM dThd and 2 mM 5FUra as subtracts. Significant difference when 1×10^{10} CFU/mL was observed (*$p < 0.05$).

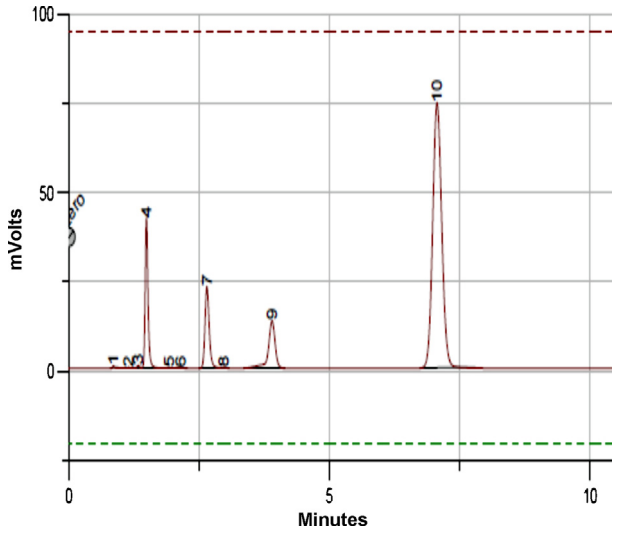

Fig. 5. Quantitative analysis of 5FUradRib conversion by HPLC. Water/methanol was used as isocratic mobile phase (94:6, v/v), flow: 1.2 mL/min. Peak number 4: 5FUra (1.49 min); 7: Thy (2.65 min); 9: 5FUradRib (3.90 min) and 10: dThd (7.06 min).

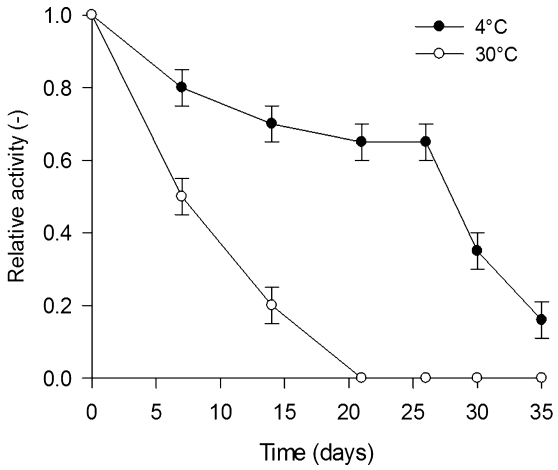

Fig. 6. Storage stability of the immobilized biocatalyst. *L. animalis* immobilized in Sr-alginate were stored at 4 °C and 30 °C. After different storage times, the biocatalyst was evaluated for 5FUradRib conversion. Relative activity was calculated with respect to the reaction carried out in the previously optimized conditions.

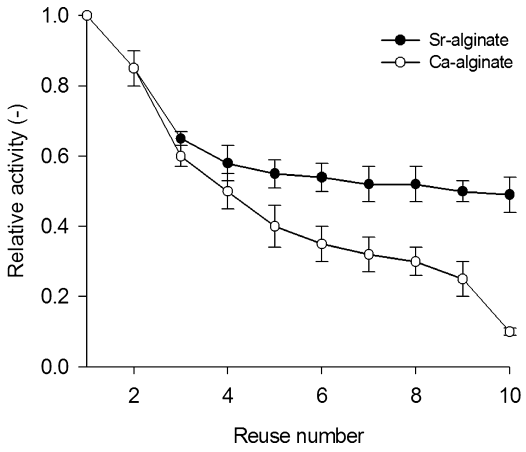

Fig. 7. Operational stability of *L. animalis* immobilized in Sr-alginate and Ca-alginate. Biocatalysts reusability was evaluated through successive 5FUradRib biosynthesis and reuse number was determined. Each reuse was performed during 4 h in previously optimized conditions. Relative activity with respect to the first reuse was determined. Every assay was carried out three times.

when was stored at 4 °C However, the biocatalyst was inactive after 5 days when was stored at 30 °C (Fig. 6).

The reusability test of *L. animalis* stabilized in Sr-alginate showed 10 successive batches without significant loss of its activity. This biocatalyst had greater operational stability than that obtained using Ca^{2+} as cross-linking solution (Fig. 7). Ca-alginate lost its activity after 4 reuses, similarly to previous reports by Tan and Ting [37].

Reusability of Sr-alginate biocatalysts was 40% greater than previously reported (Table 2). It is noteworthy that such biocatalysts have an additional stabilization step by using different polymers such as chitosan and polyethyleneimine [38–40].

3.5. Biocatalytic activity of Sr-alginate immobilized in non-conventional media

The stability of this biocatalyst was evaluated in different organic solvents. This non-conventional medium favors solubilization of bases and nucleosides such as 5BrUra. 5BrUradRib conversion was assayed using different solvents, of which hexane was the best one (Fig. 8A). In this case, 5BrUradRib yield close to 80% was observed. This behavior can be explained in terms of polarity

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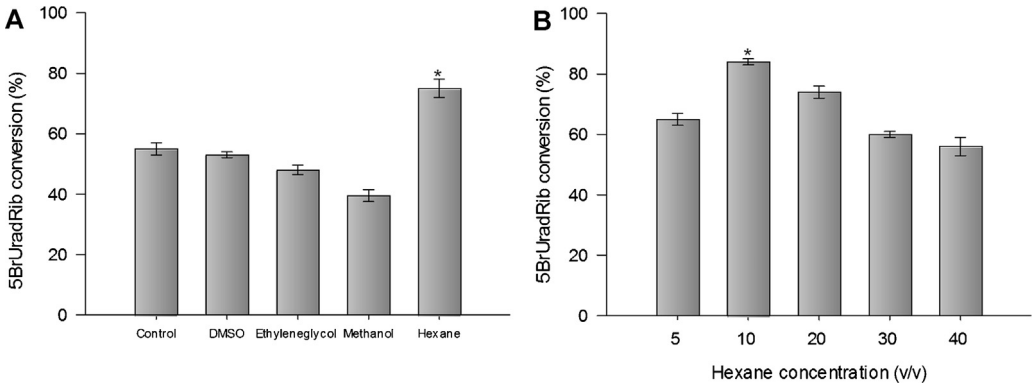

Fig. 8. Biosynthesis of 5BrUradRib using *L. animalis* immobilized in Sr-alginate. (A) Evaluation of different co-solvents (20%, v/v) in reaction medium. Significant difference when hexane was used (*$p < 0.005$); (B) Effect of different hexane percentages (5%, 10%, 20%, 30% and 40%, v/v). Biosynthesis was carried out at 30 °C and 200 rpm in 1 mL of 25 mM Tris–HCl using 1×10^{10} CFU/mL, 6 mM dThd and 2 mM 5BrUra. Significant difference when 10% (v/v) was used (*$p < 0.005$). Each assay was performed three times.

Table 2
Reusability of different alginate immobilized biocatalysts. Reusability was evaluated using 5FUradRib biosynthesis as standard reaction.

Biocatalysts	Reuses[b]	References
Ca-alginate	3	[36]
Ba-alginate-chitosan	5	[37]
Ca-alginate-gelatin	6	[38]
Ca-alginate-PEI[a]	4	[39]
Sr-alginate	10	This study[c]

[a] Polyethyleneimine.
[b] Batch reaction with total substrates renovation.
[c] Reuse: Standard reaction was carried out during 4 h with Tris–HCl 25 mM at pH 7, 30 °C and 200 rpm using 6 mM dThd and 2 mM 5Fura.

of substrates; more hydrophobic drugs, like 5BrUra (log *P* greater than zero) showed greater solubility in this non-polar solvent, being able to diffuse more easily into alginate matrix [41]. On the other hand, polar solvents such as methanol could diffuse into the beads affecting microorganism viability and reducing enzymatic activity. Additionally, the evaluation of different hexane percentages in reaction medium allowed to determine that elevated concentrations reduce significantly 5BrUradRib conversion (Fig. 8B). Similar behavior was also reported in a previous work related to a negative effect on enzimatic activity [42]. Furthermore, cell release in non-conventional media was not detected when this parameter was evaluated. These results can help to use stabilized *L. animalis* in Sr-alginate to obtain nucleoside analogs from a wide variety of water insoluble bases and nucleosides.

3.6. Green chemistry parameters

Environment-factor (E-factor) is a measure of environmental impact generated by industries. E-factor values are around 25–100 for pharmacological compounds.

In this work, E-factors lower than 6.3 for 5FUradRib and 5BrUradRib biosynthesis were obtained. These results show mass utilization efficiency and a significant decrease of waste production. Carbon efficiency (C-efficiency) and Atom economy (A-economy) were designed as parameters to evaluate the efficiency of chemical synthesis. For both biotransformations, C-efficiency was 64% in both cases and A-economy values ranged from 66% to 71% (Table 3). Therefore, a smooth and green bioprocess was designed for the obtention of anti-tumoral compounds.

Table 3
Green chemistry parameters for halogenated 2′-deoxyribosides biosynthesis by *L. animalis* ATCC 35046 immobilized in Sr-alginate. Biotransformations were carried out using 6 mM dThd, and 2 mM of different 5-halogenated bases (5-FUra and 5-BrUra) at 30 °C, buffer Tris–HCl pH 7 and 200 rpm.

Product	E-factor	C-efficiency	A-economy
5FUradRib	6.3	64	66
5BrUradRib	6.2	64	71

4. Conclusions

L. animalis ATCC 35046 immobilized in Sr-alginate is a novel biocatalytic system based on the use of natural hydrogels. This is the first time that a natural matrix was developed and improved using strontium as cross-linking solution. This immobilized biocatalyst was used to biosynthesize 5FUradRib. Additionally, stabilized *L. animalis* ATCC 35046 was able to obtain 5BrUradRib in non-conventional media at short reaction times. These results indicate that Sr-alginate is an alternative to stabilize microorganisms and could be used to produce a broad spectrum of nucleoside analogs from substrates with low solubility in water, employing an environmentally friendly methodology.

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