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ORIGINAL PAPER

Fed batch bioconversion of 2-propanol by a solvent tolerant strain of *Alcaligenes faecalis* entrapped in Ca-alginate gel

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Abstract A gram-negative, rod-shaped, aerobe, capable of converting 2-propanol (isopropanol, IPA) to acetone was isolated from an oil/sump, and identified by 16 S rDNA analysis as *Alcaligenes faecalis*. Investigations showed this strain to be extremely solvent-tolerant and it was subsequently named ST1. In this study, *A. faecalis* ST1 cells were immobilized by entrapment in Ca-alginate beads (3 mm in diameter), and used in the bioconversion of high concentration IPA. The biodegradation rates and the corresponding microbial growth inside the beads were measured at four different IPA concentration ranges from 2 to 15 g l^{-1}. The maximum cell concentration obtained was 9.59 g dry cell weight (DCW) l^{-1} medium which equated to 66 g DCW l^{-1} gel, at an initial IPA concentration of 15 g l^{-1} after 216 h of incubation. A maximum biodegradation rate of 0.067 g IPA g $cells^{-1}$ h^{-1} was achieved for 5 g l^{-1} IPA where an increase in IPA concentration to 38 g l^{-1} caused reduction in bead integrity. A modified growth medium was developed which allowed repeated use of the beads for more than 42 days without any loss of integrity and continued bioconversion activity.

Keywords Bioconversion · *Alcaligenes faecalis* · Isopropanol (2-propanol) · Immobilized cells · Solvent tolerant

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Introduction

2-Propanol, or isopropyl alcohol (IPA), production worldwide exceeds 1.8 million metric tonnes per annum [1]. It is used particularly as a solvent in quick-drying oils, inks, cosmetics, anti-freeze compositions, and as a cheap replacement for ethanol [2]. Since it is widely used industrially, this has resulted in an increased production of organic solvent waste streams, where it can be shown that IPA represents the largest volume solvent release compound in the UK, at nearly 7% of the total emission [3].

Investigation of immobilized cell systems has rapidly expanded and many new techniques for their preparation have been developed over several decades. Immobilization of biocatalysts has received increasing interest in recent years and has been extensively investigated [4–6], the reason being that immobilized systems offer potential for the improvement of bioprocess efficiency. In comparison with free cells, immobilized cell technology has several advantages such as increased overall biodegradation rate through higher densities of specialized cells [7], increased biocatalytic and operational cell stability, tolerance to toxic compounds [8], ease of cell handling and separation from reaction mixture [9], protection against cell wash-out [10], improved efficiency increasing overall productivity and minimizing production costs [11], and capability of re-use [12]. The potential of using immobilized cells in industrial processes is considerable [13]. Cells at different stages have been successfully entrapped in various matrices [14, 15] and it has been widely applied in the treatment of numerous toxic compounds such as phenol [16], chlorobenzoates and benzene derivatives [17], pentachlorophenol [18], 3-chloroaniline [19], 4-chlorophenol [20], pyridine [21], 2,4-dichlorophenoxyacetic acid [22], naphthalene [23], 2-methylnaphthalene [24], phthalic acid ester [25], quinoline [26] and polychlorinated biphenyls [7].

Common methods of whole cell immobilization include entrapment and encapsulation. The entrapment matrix should cause as little trauma to the cells as possible, there should be reduced shock to the cells from change in temperature, osmotic pressure, chemical environment, and chemical reaction [27]. In this study and among the various matrices available for immobilization, Ca-alginate beads stand out as the most commonly used and versatile method to date, where it fulfils the previously discussed criteria, and their production method does not require extreme reaction conditions. Ca-alginate entrapment is a simple low cost option using readily available material with low toxicity [11], and it is a promising method for enhanced microbial degradation of toxic substances [28]. This immobilization method is not directly toxic to the cells, and furthermore the dissolution of gel particles, and thus the liberation of the immobilized cells may be carried out easily and rapidly if required.

Recently, a 2-propanol utilizing bacterial strain termed *Alcaligenes faecalis* ST1 was isolated and enriched from oil-contaminated soil where this strain was subsequently capable of utilizing IPA as its sole carbon source [29]. In our study, bioconversion of 2-propanol (IPA) by *A. faecalis* ST1 entrapped in Ca-alginate gel beads was investigated, and the effect of substrate concentration on conversion rate and bacterial growth evaluated. No previous reports exist concerning biodegradation of high concentration 2-propanol (IPA) using immobilized *A. faecalis* ST1 in alginate gel to date in the literature.

Materials and methods

Chemicals

Trizma Base (minimum 99.9%), Trizma Hydrochloride (minimum 99%) and ethanol (absolute), were purchased from the Sigma Chemical Company (UK). Sodium alginate and calcium chloride were purchased from Fisher Scientific (UK). 2-propanol and acetone were purchased from the Aldrich Chemical Company (UK). Glutaraldehyde (25% EM grade) and sodium cacodylate were purchased from Agar Scientific Limited (UK). Unless otherwise stated, all other chemicals were of the highest purity available and purchased from either Sigma Chemical Company (UK), or BDH Laboratory Suppliers (UK).

Microorganism

A bacterial strain of *A. faecalis* termed ST1 was used in this work. Details concerning isolation, general characteristics, cultural conditions and its 2-propanol (IPA) degradation capacities have been discussed previously [30].

Medium

Two media types were used in this study. Medium I was a mineral salt medium (MSM), used for inocula production and preparation of both freely suspended and immobilized cells. This medium contained the following constituents per litre of deionized water, 3 g $NaHCO_3$, 1 g NH_4HCO_3, 0.2 g K_2HPO_4, 102.5 mg $MgSO_4 \cdot 7H_2O$, 36.75 mg $CaCl_2 \cdot 2H_2O$, 10 mg $FeSO_4$, and 1 ml trace elements solution at pH 6. The formulation of MSM was adapted from Angelidaki et al. [31].

In order to improve the strength of the calcium alginate beads, and the salt composition in the medium for the repeated use of the immobilized cells, a modified medium was used (Medium II). The medium composition contained per litre of Tris–HCl buffer (50 mM, pH 8.0): 0.02 g K_2HPO_4, 0.10 g KCl, 0.10 g $MgSO_4 \cdot 7H_2O$, 1.50 g $CaCl_2 \cdot 7H_2O$, 1 g NH_4Cl, and 1 ml of trace element solution. This medium composition was adapted from Ferschl et al. [19].

Immobilization

The pre-culture for immobilization protocols was prepared as follows: 10 ml of actively growing *A. faecalis* ST1 culture at late-exponential growth phase was transferred into a 500 ml Erlenmeyer flask containing 200 ml MSM (Medium I), containing 7.9 g l^{-1} IPA. Cultures were grown on a KS250 orbital shaker (IKA Werke, Germany) at 150 rpm and 18 °C and cells were harvested by centrifugation at 6,000 rpm for 10 min at 4 °C in a Mark IV refrigerated centrifuge (Baird and Tatlock, England). Supernatant was discarded, and the pellet washed twice with sterile distilled water. Approximately 5 g wet weight was obtained (equivalent to the pellet obtained by centrifugation of 200 ml culture broth with O.D. at $\lambda_{450nm} = 0.900$). Cells were immobilized in calcium alginate according to the following procedures [32]; the cell pellet obtained after centrifugation was suspended into 5 ml of sterile distilled water, then the cell suspension was added to 2% (w/v) sodium alginate solution and mixed thoroughly until a homogenous mixture was obtained. The mixture was extruded drop-wise through a nozzle into 1 l of 0.1 M $CaCl_2$ solution to form beads of average particle size 3 mm in diameter. The beads were left to harden for 1 h with gentle stirring before being washed three times with sterile distilled water to remove excess calcium ions and free cells.

Degradation experiments

Batch conversion of 2-propanol

Two hundred and fifty-millilitres Erlenmeyer flasks were set up containing four concentrations of IPA in duplicate

flasks (concentration ranges between 2 and 15 g l^{-1}). Each flask contained 100 ml of MSM (Medium I). In addition, 10 g wet weight of calcium alginate beads were added to each flask, where the initial cell concentration was in the range 0.87–1.16 g dry cell weight (DCW) l^{-1} medium (which is equivalent to 6–8 g DCW l^{-1} gel). In order to correct for volatilization/evaporation, multiple control samples were also set up in parallel using the four corresponding concentrations IPA, in addition to 100 ml MSM (Medium I), inoculated with 10 g wet weight alginate beads on a cell-free basis. Flasks were stoppered with foam bungs and placed on a KS250 orbital shaker (IKA Werke, Germany) at 150 rpm and 18 °C for the duration of the experiment. Well mixed samples were taken from each flask at 24-h intervals for solvent concentration and bacterial growth measurement occurring in the medium. A number of beads were withdrawn periodically from the reaction to determine the immobilized cell concentration.

2-Propanol degradation and reusability of immobilized cells

To establish the long-term stability of immobilized cells in the calcium alginate matrix during IPA conversion, batch experiments were repeated with previously used immobilized cells as follows: 250-ml Erlenmeyer flasks were set up containing 100 ml of MSM (Medium II), inoculated with 10 g wet weight of calcium alginate beads, with an initial cell concentration of 0.87 DCW l^{-1} medium (equivalent to 6 g DCW l^{-1} gel), where IPA was added at a concentration of 4 g l^{-1}. The flasks were stoppered with foam bungs and placed on a KS250 orbital shaker (IKA Werke, Germany) at 150 rpm and 18 °C for the duration of the experiment. After IPA and its main metabolite (acetone) were depleted from the reaction mixture, the spent medium was decanted and beads were washed with distilled water and transferred into 250-ml Erlenmeyer flasks containing 100 ml fresh MSM (Medium II), and 4 g l^{-1} IPA. The biodegradation process was rerun under the same previous conditions. Solvent evaporation was corrected by running controls under the same conditions on a cell-free basis in parallel where samples were drawn periodically for solvent concentration analysis, and cell concentration determinations. Each experiment was repeated twice and the average recorded.

Analytical methods

Microbial growth measurements

In order to estimate the growth of immobilized cells within the matrix, the following procedures were adapted from [32]; cells were first released from the calcium alginate beads by placing a known number of beads in a beaker containing 2 ml potassium phosphate buffer (0.1 M, pH 7.4), which was shaken at 30 °C for 40 min, until the beads had dissolved fully. The optical density (OD) of the solution was measured in 1 cm pathlength cuvettes at a wavelength of 450 nm (λ_{450}) using a DR/2000 spectrophotometer (Hach, Germany), where the relationship of OD versus DCW was obtained as described previously [30].

The immobilized cell concentration was expressed as g DCW l^{-1} gel and was also linked to the medium volume and expressed as g DCW l^{-1} medium. One unit OD was found to be equivalent to 5.37 g DCW l^{-1} medium, and 37 g DCW l^{-1} gel.

Solvent concentrations determination

A 1 µl sample was extracted from well-mixed flasks by syringe for analysis with a GC-17A Gas Chromatograph (Shimadzu, Japan). This was equipped with a flame ionization detector (FID) in order to determine the concentration of IPA and its metabolite acetone. The GC was equipped with a Carbowax BP20 column (length = 15 m, 1 µm film) (Burke analytical, UK). The flow rate of the He carrier gas was 10 ml min^{-1}, the temperature of the FID system was 300 °C, whereas that of the liquid injection point was 250 °C, and the oven temperature was 70 °C. The run time was 2 min.

Each sample was injected twice and the solvent concentration calculated from the subsequent peak area using standards of known concentration.

Results

Effect of 2-propanol on *A. faecalis* ST1 growth

In order to determine the effect of immobilization on IPA degradation activity and the tolerance of immobilized *A. faecalis* ST1 to high concentration IPA, growth was studied at initial IPA concentration ranges between 2.4 and 15 g l^{-1}, under aerobic conditions in MSM (Medium I). The initial cell concentration examined was in the range 0.87–1.16 g DCW l^{-1} medium (equivalent to 6–8 g DCW l^{-1} gel). Figure 1 shows the time course of growth of *A. faecalis* ST1 in flasks with biomass expressed as a function of medium volume. Figure 1 demonstrates that the lag phase for cell growth was prolonged with an increase in initial IPA concentration. For example, for 2.4 g l^{-1} IPA the lag time was found to be 24 h, which increased to 50 h at an initial substrate concentration of 7.34 g l^{-1}, indicating an inhibitory effect on *A. faecalis* ST1 growth at elevated IPA concentrations.

Figure 1 also demonstrates that the cell concentration inside the beads increased exponentially following lag

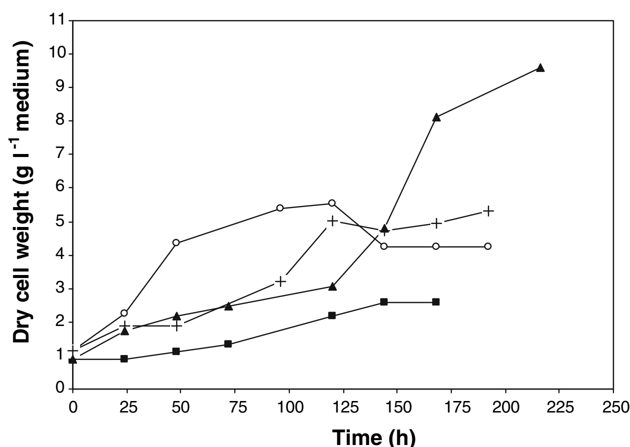

Fig. 1 Growth of *A. faecalis* ST1 entrapped in Ca-alginate beads based on medium volume at an initial IPA concentration of 2.4 (*filled square*), 4.15 (*open circle*), 7.34 (*plus*), and 15 g l^{-1} (*filled triangle*)

phase. The maximum cell concentration obtained was 9.59 g DCW l^{-1} medium (66 g DCW l^{-1} gel), for an initial IPA concentration of 15 g l^{-1} after 216 h of incubation, in comparison to a maximum cell concentration of 2.6 g DCW l^{-1} medium (17.9 g DCW l^{-1} gel), which was obtained after 144 h of cultivation of *A. faecalis* on 2.4 g l^{-1} IPA. To study the effect of initial 2-propanol concentration on the growth of the bacteria, the specific growth rate (μ) was calculated for the range of IPA concentrations studied (Table 1).

Table 1 indicates that the specific growth rate (μ) increased with increasing initial concentration of IPA until a maximum value was achieved for a concentration of 4.15 g l^{-1} with a corresponding value of 0.007 h^{-1}. Increasing the IPA concentration to 15 g l^{-1} lowered μ to 0.005 h^{-1}, and this may be as a result of growth inhibition at higher substrate concentration. Furthermore, after prolonged incubation of the beads, and owing to the rapid growth of cells near the gel surface, the gel matrix appeared to lose mechanical rigidity, and consequently, cavities near the matrix surface expanded, releasing a proportion of cells into the medium as visualized by microscopy (images not shown). At all IPA concentrations examined, the total biomass released to the medium was approximately 15% of the total cell mass entrapped inside the beads, and this was taken into account during the analysis of the specific conversion rates.

Table 1 Specific growth rate (μ), IPA bioconversion rate (q), and volumetric IPA bioconversion rate (r), for immobilized *A. faecalis* ST1

IPA concentration (g l^{-1})	μ (h^{-1})	q (g IPA g cells^{-1} h^{-1})	r (g l^{-1} h^{-1})
2.40	0.0040	0.0009	0.0145
4.15	0.0070	0.0045	0.0366
7.34	0.0060	0.0082	0.0519
15.00	0.0050	0.0046	0.0587

Batch bioconversion of 2-propanol by immobilized *A. faecalis* ST1

The effect of initial IPA concentration (2.4–15 g l^{-1}) on conversion rate by immobilized *A. faecalis* ST1 in calcium alginate beads was investigated. Figure 2a shows the IPA concentration profiles (corrected for evaporation) when experiments were carried out in MSM (Medium I). Figure 2a shows that complete conversion of IPA was achieved in the range of concentrations studied. Full conversion was achieved after 96 h for all initial IPA concentrations except 15 g l^{-1}, which was fully depleted following 168 h. Furthermore, acetone was produced as a key intermediate during IPA degradation, and its concentration profiles are plotted in Fig. 2b where the maximum yield of acetone obtained was 0.82 g acetone g IPA^{-1}, at an initial IPA concentration of 15 g l^{-1}.

To clearly show the effect of immobilization on IPA degradation, the specific bioconversion rate (q) and the volumetric bioconversion rate (r) were calculated according to equations given by Fogler [33] and the values shown in Table 1. The results demonstrate an increase in specific bioconversion rate (q) with increasing IPA concentration, until a maximum value of 0.0082 g IPA g cell^{-1} h^{-1} was achieved for 7.34 g l^{-1} IPA and a decrease was subsequently observed at higher IPA concentrations where 0.0046 g IPA g cell^{-1} h^{-1} at concentration of 15 g $l^{-1}$was demonstrated. A minimum specific bioconversion rate of 0.0009 g IPA g cell^{-1} h^{-1} was obtained for 2.40 g l^{-1} IPA. In comparison, the volumetric bioconversion rates (r) were higher than the specific IPA bioconversion rates (q), and showed an increase with increasing IPA concentration, where a rise from 0.0145 to 0.0587 g l^{-1} h^{-1} at IPA concentrations of 2.4 and 15 g l^{-1}, respectively, was evident.

Fed-batch conversion of 2-propanol by immobilized *A. faecalis* ST1

In order to test the feasibility of applying immobilized cells for long-term IPA conversion, the reusability of immobilized cells was tested as follows; batch experiments were conducted with an IPA concentration of 4 g l^{-1} and initial cell concentration of 0.87 g DCW l^{-1} medium (Medium II) where 1.5 g l^{-1} of $CaCl_2 \cdot 2H_2O$ was added to the medium to enhance the physical strength of calcium alginate beads. Following complete degradation of each IPA batch, and depletion of its main metabolite (acetone) from the reaction mixture, the spent medium was decanted and the beads washed in sterile distilled water, and re-suspended in fresh

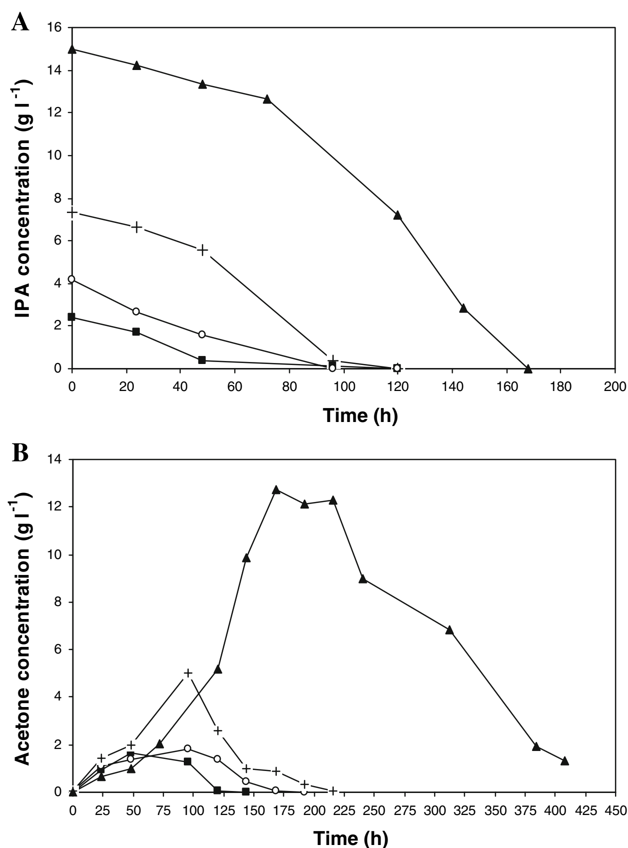

Fig. 2 **a** IPA conversion by immobilized *A. faecalis* ST1 at initial concentration of 2.4 (*filled square*), 4.15 (*open circle*), 7.34 (*plus*), and 15 g l^{-1} (*filled triangle*). **b** Acetone production by immobilized *A. faecalis* ST1 at IPA initial concentration of 2.4 (*filled square*), 4.15 (*open circle*), 7.34 (*plus*), and 15 g l^{-1} (*filled triangle*)

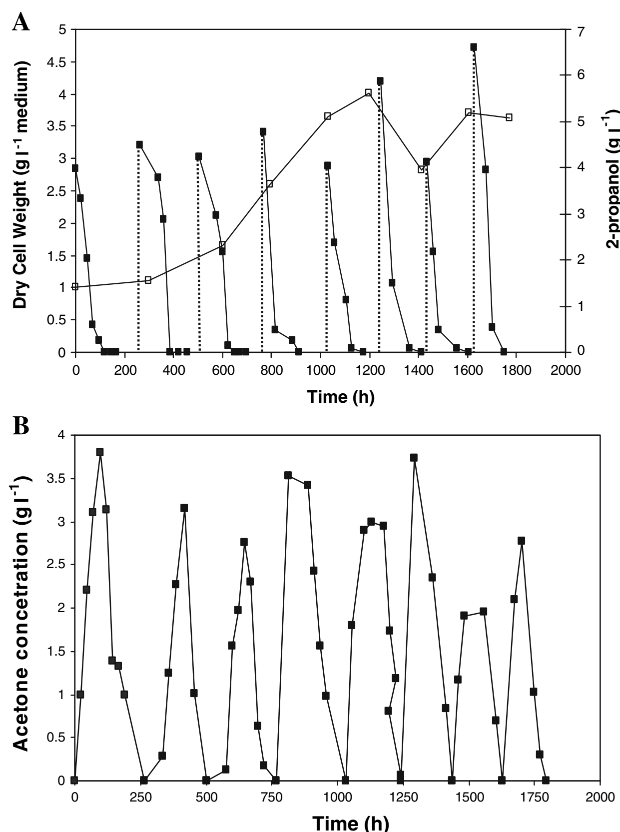

Fig. 3 **a** Bioconversion of 2-propanol by immobilized *A. faecalis* ST1 during consecutive batch cultivation: immobilized cell concentration (*open square*), and 2-propanol concentration (*filled square*). **b** Production of acetone during 2-propanol conversion by immobilized *A. faecalis* ST1 in semi-continuous cultures

medium containing IPA. The cell concentrations inside inside the gel were measured at the start and end of each run, as shown in Fig. 3a maintenance of the cells in Medium II enhanced the rigidity and stability of the alginate beads, and allowed the subsequent utilization for approximately 1800 h over eight batches. Conversely reactions carried out in Medium I only facilitated two successive cycles over a 314-h period which was then followed by rupture of the gel beads and release of biomass into the medium (images not shown). Figure 3a also shows that the cell concentration inside the beads increased from 0.87 g DCW l^{-1} medium at initiation of the first cycle up to 3.63 g DCW l^{-1} medium at completion of the eighth cycle. Continued reuse of the beads resulted in worsening integrity and increased cell leakage into the external medium. Initiation of significant leakage was demonstrated during the fourth cycle, where it reached 1.1 DCW g l^{-1} medium following completion of the fifth cycle. Figure 3a shows the IPA concentration profile during repeated use of the cells immobilized in calcium alginate beads in Medium II. Use of this modified medium allowed the repeated use of the same beads for up to eight cycles, with a stable bioactivity of immobilized cells over the 1800 h in the repeated batch cultivation and without any major loss in the volumetric bioconversion rate values (r). The average r value obtained was 0.038 g IPA l^{-1} h^{-1}. Acetone was also produced as a result of 2-propanol metabolism and its production is plotted in Fig. 3b, with an average peak concentration of 3.08 g l^{-1} and an average yield of 0.738 g acetone g IPA^{-1}.

Discussion

In this study, a simple and effective method of immobilization was employed to augment the potential of *A. faecalis* ST1 to convert high concentration 2-propanol (IPA). Cell immobilization is thought to be the most promising approach for application of biocatalysts in various bioprocesses. Advantages of using immobilized bacteria for bioconversion reactions have been widely reported, besides making the cells easier to handle, it allows recycling of the valuable biocatalyst, and other beneficial effects such as

increasing the resistance of entrapped cells towards toxic compounds, and protecting the biomass from being washed out of reactor systems [34].

Ca-alginate was selected to immobilize *A. faecalis* ST1, since entrapment of cells in alginate is a promising method for microbial degradation of toxic substances. The immobilization method is not toxic to the cells, and is chemically inert. These immobilized cells are also favourable for investigation of cell physiology and the dissolution of such gel particles (and thus the liberation of the immobilized cells) is easy and rapid. This technique has gained great scientific interest and has been used extensively by researchers in degradation studies of compounds such as phenols [35], pentachlorophenols [36], polychlorinated biphenyl [9], 3-chloroanilines [19], and a mixture of monochlorobenzoates and 2, 4-dichlorophenoxyacetic acid [37].

The ability of *A. faecalis* ST1 immobilized in Ca-alginate to convert IPA to acetone was investigated in our study. Batch experiments examined bioconversion of IPA initial concentration ranges from 2.4 to 15 g l^{-1}, and by focusing on the effect of immobilization on conversion activity of *A. faecalis* ST1. Ca-alginate beads are easily dissolved by treatment with calcium-chelating agents such as sodium citrate and potassium phosphate, and this allows the cell concentration inside the beads to be determined [38]. Results obtained show that cell biomass inside the beads increased with increasing initial IPA concentration, where the maximum achievable cell biomass was 9.58 g DCW l^{-1} medium at an IPA concentration of 15 g l^{-1}. On the other hand, the specific growth rate (μ) was calculated, and it was found that increasing the initial IPA to 4.15 g l^{-1} resulted in an increase in the specific growth rate to 0.0068 h^{-1}. These results may be due to substrate inhibition at high concentration and similar findings were also reported by Keweloh et al. [39] in their study of phenol degradation by immobilized *Escherichia coli* K-12 in alginate beads. These bacteria were able to tolerate phenol at concentrations up to 2 g l^{-1} although inhibition of specific growth rate at higher concentrations was demonstrated. Such an observation was also demonstrated by Shim et al. [40] during BTEX degradation by co-cultures of *Pseudomonas putida* and *P. fluorescens* immobilized in a fibrous-bed bioreactor, where the bacteria were able to tolerate benzene at concentrations up to 250 mg l^{-1} and further increase in the benzene concentration caused significant inhibition. Substrate inhibition at a concentration of 2.49 g l^{-1} was also observed by Llanes et al. [41] during conversion of sitosterol to androstenedione by *Mycobacterium* sp. NRRL B-3805 cells immobilized on celite.

To investigate the effect of variation of initial IPA concentration on the conversion capability of immobilized *A. faecalis* ST1, both the specific bioconversion rate (q) and volumetric bioconversion rate (r) were evaluated during cultivation of the bacteria. The maximum specific bioconversion rate obtained was 0.0082 g IPA g $cells^{-1}$ h^{-1} at an IPA concentration of 7.34 g l^{-1}, and then a subsequent decrease to 0.0046 g IPA g $cells^{-1}$ h^{-1} at an initial IPA concentration of 15 g l^{-1} was demonstrated. In comparison with free cells, this low specific bioconversion rate [30] may be due to diffusion limitations inside the beads. Increasing the IPA concentration to 15 g l^{-1} may cause an inhibitory effect on the bacteria degradation ability due to its toxic effect at this high level. It was previously reported by Dursun and Aksu [42] that immobilized *P. fluorescens* in Ca-alginate exhibited a lower degradation rate towards ferrous (II) cyanide in comparison with free cells due to diffusional limitation within the gel matrix. Such an observation was also made Chung et al. [43], during a study of phenol degradation by *P. putida* CCRC 14365, where the immobilized cells showed an ability to degrade phenol up to 1,000 mg l^{-1}, in comparison to 600 mg l^{-1} by free cells, although the bioconversion rate was reduced following cell immobilization.

In our study, a higher volumetric bioconversion rate was still achievable, where 0.0587 g l^{-1} h^{-1} was demonstrated at15 g l^{-1} IPA. This was also observed in a study for pyridine degradation at up to 2 g l^{-1} by Ca-alginate immobilized *Pimelobacter* sp., where the volumetric bioconversion rate (r) and specific bioconversion rate (q) values obtained were 0.082 g l^{-1} h^{-1} and 0.018 g pyridine g $cells^{-1}$ h^{-1}, respectively [21]. A similar observation was made by Cho et al. [44], when they studied the effect of presence of phenol as co-substrate in the biodegradation of *p*-nitrophenol within concentration ranges from 0.1 to 0.7 g l^{-1} using calcium alginate immobilized *Nocardioides* sp. NSP41. The volumetric degradation rate values obtained ranged from 0.5 to 35 mg l^{-1} h^{-1} in comparison to specific conversion values of 0.001–0.014 *p*-nitrophenol g $cell^{-1}$ h^{-1}.

One well-documented advantage of using immobilized cells is their reuse in consecutive batch cultures. *A. faecalis* ST1 has been cultivated in MSM (Medium I) to test its stability during multiple cycles, 2.4 g l^{-1} IPA was added, but bead breakage occurred during the second biodegradation cycle. The medium composition greatly affects the stability of the beads and the presence of substances such as phosphate or citrate which have a high affinity for Ca^{2+} will sequester the cross-linking calcium ions, and therefore destabilize the gel. Consequently, a further medium (Medium II) was introduced in order to sustain the physical strength of Ca-alginate beads by increasing the $CaCl_2 \cdot 2H_2O$ 40-fold and reducing the phosphate concentration tenfold. Effect of changing the concentration of such components on cell growth has been studied previously by Lee et al. [21], where a similar medium was used during the degradation of pyridine, without detrimental effect where this medium enabled the re-use of the same beads for 18

cycles with an average volumetric degradation rate of 0.129 g l^{-1} h^{-1} at a pyridine concentration of 2 g l^{-1}.

Our results show that beads can be used repeatedly for eight consecutive batches of conversion of 4 g l^{-1} IPA over a 1800-h period without any significant loss of their performance and with an average volumetric bioconversion rate of 0.038 g l^{-1} h^{-1}. Fed-batch conversion studies revealed that Ca-alginate immobilized cells retained their effectiveness for extended periods of use. The increased mechanical stability and lower cell leakage make the beads suitable for long-term use, and from the viewpoint of the stability of the cells in repeated batches, it would appear that this method of immobilization is effective. Other studies have also described the successful application of this medium in biodegradative systems. For example, Rhee et al. [45] utilized this medium in their degradation study of pyridine, which allowed the use of the same beads in up to 16 cycles without any loss in their biodegradation performance. In conclusion, our studies demonstrate that it was possible to convert IPA at concentrations up to 15 g l^{-1} using *A. faecalis* ST1 entrapped in calcium alginate gel beads, where no previous reports have treated such high concentration of IPA with immobilized bacteria. This indicates that this system may have potential for exploitation in high solvent concentration, low water activity biocatalytic systems in the future.

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