

Cost-Effective and Regenerating Porous Polysulfone-Based Beads Extraction for in Situ Microbial 2-Phenylethanol Recovery

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# Cost-Effective and Regenerating Porous Polysulfone-Based Beads Extraction for in Situ Microbial 2-Phenylethanol Recovery

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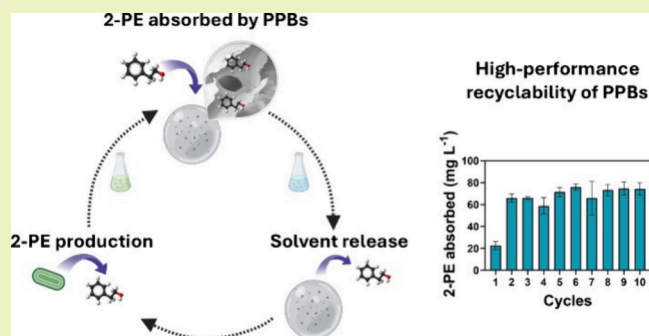
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**ABSTRACT:** 2-Phenylethanol (2-PE) is a multipurpose aromatic molecule, approved as GRAS, largely used across various industries, including cosmetics and pharmaceuticals. Growing demand and concerns about petroleum-based synthesis drive the need for sustainable alternatives. Microbial biotechnologies offer ecofriendly solutions with high specificity and mild conditions. In this work, porous polysulfone beads (PPBs) are presented as novel 2-PE recovery adsorbents. PPBs show selective absorption of 2-PE without affecting microbial growth, and they are inexpensive, reusable, and sterilizable. According to comparative testing, PPBs achieve high absorption properties reaching  $0.004 \text{ mg}_{2\text{-PE}} \text{ mg}_{\text{PPB}}^{-1}$  in 24 h when using 200 mg of PPBs with 10 mL of an aqueous  $150 \text{ mg L}^{-1}$  2-PE solution. Moreover, the majority is absorbed in the first 5 h, while after 24 h, the absorption is minimal. 2-PE affinity for PPBs in water solution is demonstrated by its partition coefficient  $K$  of 8.24, which explains its effective absorption and poor release in water. Ethanol was utilized to desorb 2-PE from PPBs more effectively, and in just 24 h, 98.8% of 2-PE was liberated. Additionally, the material retains selectivity in a complex growth medium and performs consistently throughout several usage cycles. Even at laboratory scale, the life cycle assessment (LCA) of this process shows that it overcomes the industrial extraction methods in terms of environmental impact. These results establish PPBs as viable options for scalable and sustainable 2-PE bioproduction.

**KEYWORDS:** Green extraction, Ethanol, LCA, LCI, HPLC, Downstream, Biotechnology



## INTRODUCTION

2-Phenylethanol (2-PE) is an aromatic compound characterized by a pleasant rose-like scent, and it is employed in several applications such as food and beverage, cosmetics, pharmaceuticals, and chemical industries.<sup>1,2</sup> Particularly, being that rose is one of the most popular and appreciated fragrances, 2-PE has a huge market demand, which is additionally enhanced by its approval as a GRAS molecule (Generally Recognized As Safe) by several international organizations.<sup>1,3</sup> Indeed, its market value surpassed 255 million USD in 2021 and is estimated to grow at over 370 million USD by 2028.<sup>4</sup> Today, the majority of 2-PE is chemically synthesized by using petroleum-derived feedstocks. Nevertheless, chemical synthesis involves the use of hazardous chemicals, which raises human and environmental health and safety issues.<sup>5,6</sup> Alternatively, 2-PE can be extracted from various flowers,<sup>7</sup> resulting in a low-concentrated essential oil and requiring complicated and costly downstream processes.<sup>6</sup>

A sustainable, greener alternative is biotechnological 2-PE production. Currently, microbial production of 2-PE relies on both bacteria<sup>8,9</sup> and yeast<sup>10,11</sup> offering several advantages, such as mild reaction conditions, reduced energetic requirements, higher substrate selectivity and stereoselectivity of the target

product, enhancing environmental compatibility. Besides the use of a sustainable synthesis of 2-PE, developing a low-energy technique for selective extraction of 2-PE from the growth medium is crucial for the manufacturing process.<sup>12</sup> Either the traditional method of liquid–liquid separation using organic solvents like 2-octanone, 2-methyl-3-isobuten-2-ol, and 2-methyl isobutyl ketone<sup>12,13</sup> or the use of ionic liquids<sup>14</sup> is optimal and consolidated solutions. However, while these extraction methods are cost-effective, they also have a substantial environmental impact.<sup>15,16</sup>

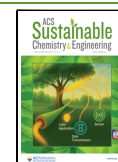
Interestingly, Amberlite interacts with organic molecules through  $\pi$ - $\pi$  contact<sup>17–21</sup> (Scheme 1) and is currently the most effective material for solid adsorbent systems for 2-PE.<sup>22</sup> Despite its great efficiency, this material is costly, with a market price of \$300 to \$500 per kilogram.<sup>17,23</sup> Additionally, this resin undergoes degradative processes in an oxidizing environment,

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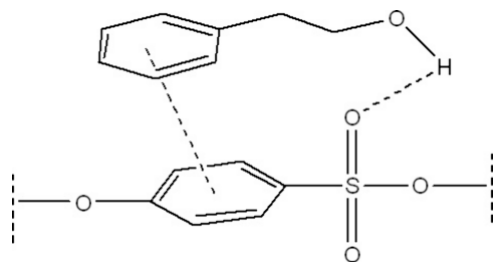
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## Scheme 1. Interaction between 2-PE and Amberlite



and while sterilization is feasible, the procedures are costly and time-consuming.<sup>24</sup>

As a feasible alternative, PSF is a commercially competitive material, having a market price ranging from \$100 to \$300 per kilogram<sup>25</sup> with several consolidated applications in many fields, such as gas separation, metal harvesting, and hemodialysis for blood purification.<sup>26</sup> PSF, a glassy polymer classified as a foam polymer, creates micrometer-sized holes inside the material;<sup>27,28</sup> the size and shape of these pores depend on the solidification technique employed.<sup>25,29</sup> This polymer displays excellent tolerance to both basic and acidic pH<sup>30</sup> and has high mechanical properties and structural robustness. Additionally, polysulfone possesses a wide range of uses in membrane development due to its chemical and thermal stability, high mechanical strength, and ability to maintain performance under harsh conditions.<sup>31</sup>

Here we report for the first time the preparation and characterization of a spherical PSF-based material that is easy to use, quick to sterilize, regenerable, and does not affect the growth of microorganisms or the ability to synthesize 2-PE. In order to assess in situ and in vivo recovery of the bioproduced 2-PE, we exploited a metabolically engineered cyanobacterial strain already developed in our laboratory.<sup>32,33</sup> Thus, we demonstrated that our material, porous PSF beads (PPBs), can overcome the limitations of traditional adsorbent materials, such as Amberlite, maintaining the extraction performance, making PPBs a sustainable alternative for in situ extraction from microbial-driven bioproduction. Furthermore, after proving the technical feasibility of the process, the environmental impact was evaluated by comparing the biological route with the conventional fossil-based route by adopting the Life Cycle Assessment (LCA) methodology (ISO 14040–44:2006).

## 2. MATERIALS AND METHODS

To have a good understanding of the different chemicals used, we divided all materials between chemicals of synthesis, medium, and growth media composition. All solvents and reagents were used as received without further purification.

### 2.1. Chemicals

**2.1.1. Preparation of PPB.** *N*-Methylpyrrolidone purity 99.7%, PSF average Mw ~ 35,000 by LS, and average Mn ~ 16,000 by MO pellets (Transparent), Methanol purity ≥ 99.8%, ACS, and Ethanol purity ≥ 99.8% were purchased from Merck.

**2.1.2. Medium and Absorption Studies.** The standards 2-phenylethanol purity > 99% and *L*-phenylalanine purity 98.5–11.0% were purchased from Merck.

**2.1.3. Growth Media: BG11 and LB.** CaCl<sub>2</sub>·2H<sub>2</sub>O purity ≥ 99%, Na<sub>2</sub>EDTA·2H<sub>2</sub>O purity 99–101.0%, MnCl<sub>2</sub>·4H<sub>2</sub>O purity ≥ 98%, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O purity ≥ 99.5%, ZnSO<sub>4</sub>·7H<sub>2</sub>O purity > 99%, CuSO<sub>4</sub>·5H<sub>2</sub>O purity 99–100.5%, Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O purity ≥ 98%, FeCl<sub>3</sub>·6H<sub>2</sub>O purity ≥ 99–102%, NaCl purity ≥ 99%, and Yeast extract for

use in microbial growth medium were purchased from Merck. NaNO<sub>3</sub> purity ≥ 99%, MgSO<sub>4</sub>·7H<sub>2</sub>O purity ≥ 99%, K<sub>2</sub>HPO<sub>4</sub> purity ≥ 99%, H<sub>3</sub>BO<sub>3</sub> purity ≥ 99.8%, TES purity ≥ 99%, and Tryptone for microbiology were purchased from Carl ROTH.

**2.1.4. Sterilization.** Oxonia Active LS was purchased from Ecolab, and sodium metabisulfite purity ≥ 99% was purchased from Merck.

**2.1.5. HPLC Eluents.** Acetonitrile, for HPLC-GOLD ultragradient grade, purity 99.9% was purchased from Carlo erba, and Sulfuric acid purity 95–97% was purchased from Merck.

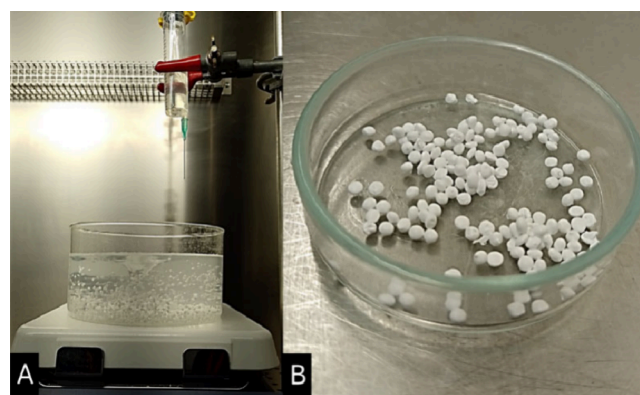
### 2.2. Instruments

2-PE concentration in the extracted samples was calculated using an ultrahigh-performance liquid chromatograph (UHPLC UltiMate 3000, Thermo Fisher, Waltham, Massachusetts, United States) equipped with a Hypersil GOLD C18 reversed phase column (250 × 4.6 mm, 5 μm; Thermo Fisher Scientific) and a Hypersil GOLD Amino column (120 × 4.6 mm, 5 μm; Thermo Fisher Scientific). A UV detector was set at 205 nm for 2-PE and 210 nm for *L*-phenylalanine.

Specific surface area of the samples was measured by means of N<sub>2</sub> sorption at −196 °C on a Tristar II micrometric instrument (Micromeritics Instrument Corporation, USA). The Brunauer–Emmett–Teller (BET) model was applied. Morphology was studied by using a field emission scanning electron microscope (FESEM, Zeiss SupraTM40, Oberkochen, Germany).

### 2.3. PPBs Preparation

The porous PSF spheres were made using the phase inversion technique according to the method of solvent casting and solvent inversion. This approach takes advantage of the polymer's insolubility in a specific solvent, in this case, the insolubility of PSF with methanol. By precipitation of the polymer dissolved in a solvent in which it is not soluble, it solidifies to create a highly porous structure. This porosity varies depending on the solvent, temperature, and concentration at which the polymer is dissolved in the solvent in which it is soluble.<sup>31,34</sup> PSF pellets were dissolved in a solution of NMP at a concentration of 15% m/v at room temperature. After total dissolution, the solution was placed in a syringe with a needle with a cavity 800 μm in diameter. This solution was dripped into a 500 mL crystallizer containing methanol under constant stirring (Figure 1a).



**Figure 1.** PPBs preparation by the Precipitation method (A) and dried PPBs (B).

As the PSF solution solidified upon contact with methanol, solid PSF spheres with an average diameter of 2.102 μm were formed (Figure 1a). Constant agitation prevents the spheres from sticking together, while the NMP residue within the pores escapes and is replaced by methanol. The spheres in methanol were stirred slowly overnight and then were filtered through a Buckner funnel and washed with methanol to remove impurities on the surface. The spheres were subsequently dried under the fume hood until they had a constant weight.

## 2.4. Sterilization and Biocompatibility

Oxonia Active LS from Ecolab was used to provide a chemical sterilization treatment for PPBs following a modified protocol from Ecolab.<sup>35</sup> PPBs were immersed in a 2% v/v aqueous solution of Oxonia Active up to 3 h to guarantee that the solvent completely penetrated the pores of PPBs. After determining the hydrogen peroxide concentration using a peroxide test from Spelco, 0.5 mol of metabisulfite was added for every mol of hydrogen peroxide to neutralize it. Subsequently, PPBs were shaken overnight in sterile water to eliminate any traces of solvents. The water was then drained, and the PPBs were stored at room temperature in sterile bottles.

PPBs were then placed in Erlenmeyer's flasks filled with 30 mL of LB medium (prepared by mixing 10 g L<sup>-1</sup> Tryptone, 10 g L<sup>-1</sup> Sodium Chloride, and 5 g L<sup>-1</sup> Yeast Extract) and kept in a shaker incubator set at 37 °C and 100 rpm. A reference was obtained by using LB without PPBs, and three replicates were performed for each condition. The experiment lasted 7 days, and samples were observed daily under a microscope. To assess PPBs biocompatibility with cyanobacteria, the engineered strain of *Synechococcus elongatus* PCC 7942, i.e. 2PE\_aroK,<sup>33</sup> was cultivated in standard medium BG11, with the following composition, as already reported:<sup>36</sup> 1.5 g L<sup>-1</sup> NaNO<sub>3</sub>, 0.075 g L<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.004 g L<sup>-1</sup> FeCl<sub>3</sub>·6H<sub>2</sub>O, 0.04 g L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 0.036 g L<sup>-1</sup> CaCl<sub>2</sub>, 0.024 g L<sup>-1</sup> Na<sub>2</sub>EDTA·2H<sub>2</sub>O, 2.86 mg L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub>, 1.81 mg L<sup>-1</sup> MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.39 mg L<sup>-1</sup> Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.22 mg L<sup>-1</sup> ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.05 mg L<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O, and 0.03 mg L<sup>-1</sup> Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O and buffered to pH = 8 with 6.05 g L<sup>-1</sup> TES (2-([Tris(hydroxymethyl)methyl]amino)ethane-1-sulfonic acid sodium salt).<sup>32</sup> *S. elongatus* was inoculated in flasks using 30 mL of BG11 with and without PPBs. The inoculum was performed to obtain an initial biomass of around 0.05 gDW L<sup>-1</sup> (grams of dry biomass per liter). Flasks were incubated at 30 °C, 130 rpm, and 30 μmol photons m<sup>-2</sup> s<sup>-1</sup>. For each condition, three biological replicates were established. The measure of optical density at 730 nm was performed to monitor the growth during the 16 days of the experiment.

## 2.5. PPB Performances: 2-PE Uptake and Release, Selectivity, and Recyclability

The maximum quantity of PPBs for absorption was investigated by using 200, 500, and 700 mg of PPBs immersed in 10 mL of a 2-PE aqueous solution at an initial concentration of 150 mg L<sup>-1</sup>. For further tests, a fixed amount of 200 mg of PPBs has been used. 2-PE absorption kinetics was assessed by immersing PPBs in a 10 mL solution of 2-PE of different concentrations –120, 250, and 300 mg L<sup>-1</sup>– for 48 h at room temperature and monitoring the 2-PE concentration in the solution. To assess the release of 2-PE by PPBs, 200 mg of it was immersed into 10 mL of a 150 mg L<sup>-1</sup> 2-PE solution for 24 h, and then PPBs were transferred into 10 mL of deionized water or ethanol. Moreover, the competition of 2-PE with the compounds in the cultivation medium (BG11) and with L-phenylalanine was tested by immersing 200 mg of PPBs in BG11 with 150 mg L<sup>-1</sup> 2-PE and 150 mg L<sup>-1</sup> L-phenylalanine (added to BG11 to enhance the production of 2-PE). The recyclability of PPBs was checked by immersing 200 mg of PPBs into 10 mL of 150 mg L<sup>-1</sup> 2-PE for 24 h and then transferring PPBs into a new vial containing 10 mL of ethanol. This process was repeated five times. During all the tests mentioned, samples for the HPLC analysis of 2-PE and L-phenylalanine were collected. HPLC analysis 2-PE and L-Phe were measured following earlier reports.<sup>33</sup> To sum up, ultrahigh-performance liquid chromatography (UHPLC UltiMate 3000) was used to evaluate 2-PE concentration. A Hypersil GOLDTM C18 Reversed Phase Column (250 × 4.6 mm, 5 μm; Thermo Fisher Scientific, Waltham, Massachusetts, United States) was utilized, along with acetonitrile and H<sub>2</sub>O (50:50) at a flow rate of 1 mL min<sup>-1</sup> at 40 °C and UV detection at 205 nm. The Hypersil GOLDTM Amino column (120 × 4.6 mm, 5 μm; Thermo Fisher Scientific, Waltham, Massachusetts, United States) was used to measure L-phenylalanine at 210 nm. The column oven was adjusted to 50 °C, and 9 mM sulfuric acid was pumped at a rate of 0.8 mL min<sup>-1</sup>. For HPLC analysis, 20 μL of each sample was injected. Using HPLC-grade

standards, 2-PE and L-Phe were externally calibrated (Sigma-Aldrich Inc., St. Louis, MO, United States).

## 2.6. Environmental Evaluation

Life Cycle Assessment (LCA) was conducted following ISO 14040–44:2006, using the Ecoinvent 3.8 database and SimaPro 9.5.0.2 software. The LCA was performed according to its four phases (goal and scope, inventory, impact assessment, and interpretation of the results). The goal of this study was to compare the environmental impacts of biological 2-PE production and extraction in situ with PPBs with those of fossil-based current production. The scope of the LCA was to identify which process route exhibited the lowest environmental impact, thereby assessing the feasibility of scaling up biologically synthesized 2-PE from the laboratory to pilot and ultimately industrial production.

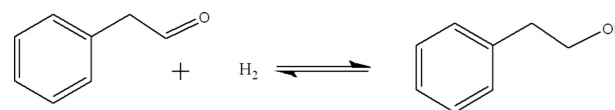
To the best of the authors' knowledge, no prior studies have assessed the environmental implications of biologically and fossil-based synthesized 2-PE in the scientific literature. Since biologically synthesized 2-PE represents an emerging technology, LCA was employed as a well-established methodology for evaluating the environmental impact of such systems. LCA studies typically focus on fully developed market systems or operational plants. However, emerging technologies pose unique challenges due to their smaller scale, lack of full-market data, uncertainties regarding system characteristics, potential efficiency losses during scale-up, and environmental impact variations.<sup>37</sup> Recognizing these challenges, the LCA research community developed the early integration of quantitative assessment tools to support technology development.<sup>38</sup>

Early stage LCA assists in understanding the environmental consequences of design decisions, minimizing unnecessary environmental burdens, reducing costs, and anticipating regulatory requirements. In this study, LCA is applied as an "ex-ante" analysis to predict the future large-scale implementation of this emerging technology. This proactive approach enables the testing of various alternatives, validation of sustainability claims, and early design improvements.<sup>39</sup>

The functional unit (FU), which serves as the reference based on which system inputs, outputs, and environmental impacts are quantified, must align with the study's objectives.<sup>40</sup> In this case, the FU was defined as 316 mg of synthesized 2-PE, as this was the target product for which the process was designed. The synthesized 2-PE was considered the main product, while coproducts were managed through mass allocation following ISO 14040–44.

A cradle-to-gate LCA approach was adopted (Figure S4) to consider the impacts of 2-PE preparation and extraction. In detail, the preparation and sterilization of PPBs, fermentation, 2-PE uptake and release, and selectivity were considered for the biological route. The styrene hydrogenation process was considered based on Scheme 2 for the fossil-based route.

### Scheme 2. Styrene Hydrogenation



The hydrogenation of styrene oxide is catalyzed by Pd/C or Rh, leading to selective reduction of the epoxide ring. The reaction proceeds in three steps. The first is catalyst activation, where hydrogen gas dissociates on the metal surface, generating active hydrogen species. The second one is the epoxide ring opening, where the activated hydrogen attacks the electrophilic oxygen of the epoxide, facilitating the cleavage of the strained three-membered ring. The last step consists of the formation of 2-PE where the protonation and subsequent rearrangement yield the final product, 2-PE (C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>CH<sub>2</sub>OH). This method provides a highly selective and efficient route to 2-PE under mild conditions, making it a preferred approach in industrial applications.<sup>41</sup>

Infrastructure impacts were excluded from the study as they were determined to have a negligible effect on the overall scenario

evaluation. Instead, the analysis focused solely on the direct environmental consequences of 2-PE synthesis.<sup>42</sup> The study included both the foreground and background systems. The foreground system encompassed the reference flow, while the background system accounted for energy and chemical supply chains associated with the foreground system.<sup>42</sup>

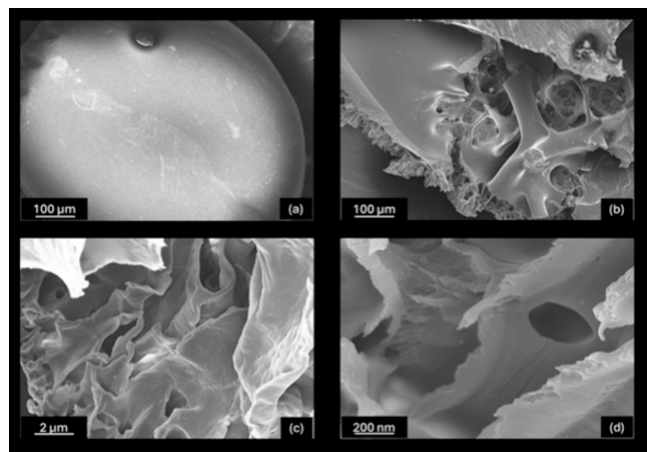
The Life Cycle Inventory (LCI) was based on primary experimental data, and an attributional LCA approach was applied. Inventory data are presented in the Supporting Information in Table S1. The Life Cycle Impact Assessment (LCIA) was conducted using the ReCiPe 2016 Midpoint (H) method to evaluate impacts across multiple categories, with a particular focus on climate change (kg CO<sub>2</sub> equiv) as an indicator of global warming potential (GWP) due to greenhouse gas emissions.

The final stage of LCA involved result interpretation and sensitivity analysis to assess potential variations in environmental impacts. Sensitivity analysis was performed by altering fermentation conditions (Conditions A and B, as described in Table S1) following previous studies on biological processes.<sup>43</sup>

### 3. RESULTS AND DISCUSSION

#### 3.1. Morphology and Surface

FE-SEM analysis was performed on the PPBs sample to obtain a full morphological characterization of the beads, as shown in Figure 2. First, the average PPBs size was calculated, obtaining an average radius of  $1.05 \pm 0.07 \mu\text{m}$  (Figure 2a) with an average sphere surface area of  $13.85 \mu\text{m}^2$ , on a sample of 100 beads.



**Figure 2.** FE-SEM images of PPBs, outer surface (a) view of transversely sectioned PPB (b), and morphological view of pores present on the surface at magnitude 5.0 KX (c) and at 50.0 KX (d).

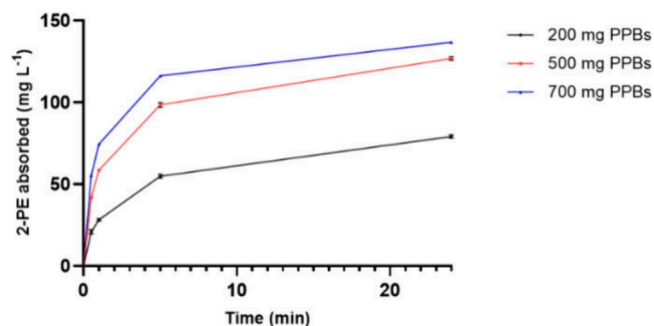
Porous networks were diffused from the exterior to the interior of the sphere, as shown in Figure 2b-c, with an average submicrometer diameter (Figure 2d). This pore size is ideal for keeping single-cell organisms like yeasts, cyanobacteria, and algae from penetrating the beads.<sup>44</sup> The cross-section shows that the dimensions of the porous cavities gradually increase from the outer to inner shell of the sphere. By combining the FE-SEM analysis with the BET analysis, we have a more accurate measurement of the total surface area, obtaining a surface area of  $4.81 \text{ m}^2 \text{ g}^{-1}$ . Additionally, the pore size is consistent with that one that was observed with the FE-SEM lower and equal to  $96.09 \text{ nm}$  (Figure 2d).

With a central cistern capable of holding a significant amount of 2-PE (Figure 2b), this structure acts as a sponge for the molecules. The pore surface consists of polymeric lamellae

folded on one another, giving the material a high-contact surface (Figure 2c, d).

#### 3.2. Material Performance

**3.2.1. Variation of the 2-PE Absorption Rate in Response to an Increasing Quantity of PPBs.** To determine the optimal amount of PPBs for maximizing 2-PE absorption, a 2-PE absorption test was conducted with increasing amounts of solid sorbent. 200, 500, and 700 mg of PPBs were added to 10 mL of an aqueous solution containing  $150 \text{ mg L}^{-1}$  2-PE, representing the concentration of the molecule during production by the studied cyanobacteria<sup>33</sup> and kept in constant agitation at RT. 2-PE absorption was quantified after 0.5, 1, 2, 5, and 24 h. Two replicates were performed for each condition. This experiment revealed a direct relationship between the mass of PPBs and the total amount of 2-PE absorbed from the aqueous solution. Specifically, after 24 h, the absorption rates were found to be 51.32%, 82.75%, and 89.17% for 200, 500, and 700 mg of PPBs, respectively (Figure 3), corresponding to absorption



**Figure 3.** 2-PE absorbed by different amounts of PPBs immersed in a 10 mL aqueous solution of  $150 \text{ mg L}^{-1}$  2-PE.

rates of  $0.06 \pm 0.00$ ,  $0.09 \pm 0.00$ , and  $0.10 \pm 0.00 \text{ mg L}^{-1} \text{ min}^{-1}$ , respectively, indicating that higher quantities of PPBs improve the absolute rate of 2-PE removal from the aqueous phase.

However, when normalized to the PPBs mass, the efficiency of absorption per milligram of PPBs decreased as the PPBs dosage increased. The calculated absorption efficiencies were  $3.95 \times 10^{-3} \pm 0.04 \times 10^{-3}$ ,  $2.54 \times 10^{-3} \pm 0.01 \times 10^{-3}$ , and  $1.95 \times 10^{-3} \pm 0.04 \times 10^{-4} \text{ mg}_{2\text{-PE}} \text{ mg}_{\text{PPB}}^{-1}$  for 200, 500, and 700 mg of PPBs, respectively. This trend suggests that although larger quantities of PPBs result in greater absolute absorption, the effectiveness of the absorbent material per unit weight diminishes (Table 1).

This diminishing efficiency can be attributed to saturation effects (200 mg has fewer sites, but in comparison, more 2-PE

**Table 1.** 2-PE Concentration Variation in an Aqueous Solution at Different Amounts in Milligrams of PPBs<sup>a</sup>

Time (min)	$\text{mg L}^{-1}$ 2-PE		
	200 mg PPBs	500 mg PPBs	700 mg PPBs
0	$154.23 \pm 0.00$	$153.41 \pm 0.00$	$153.41 \pm 0.00$
30	$133.25 \pm 0.87$	$111.55 \pm 0.27$	$98.08 \pm 0.12$
60	$125.97 \pm 0.57$	$94.70 \pm 0.49$	$77.40 \pm 1.40$
300	$99.34 \pm 0.83$	$54.87 \pm 1.02$	$37.07 \pm 0.24$
1440	$75.08 \pm 0.73$	$26.46 \pm 0.74$	$16.60 \pm 0.28$

<sup>a</sup>Errors are measured as standard deviation.

molecules are present per active site.) or changes in the concentration gradient driving the process as the PPBs dosage increases.

Based on these findings, 200 mg of PPBs was selected for further experiments as it offers a balance between maximizing absorption and maintaining efficiency per unit mass while minimizing material usage for practical and industrial relevance.

The partition coefficient  $K$  was calculated to quantify the affinity of 2-PE for the PPBs relative to the aqueous phase (as reported in eq 1) and yielded a value of approximately 8.24, which underscores the preferential partitioning of 2-PE into the PPBs

$$K = \frac{C_1}{C_2' - C_2''} \quad (1)$$

where  $C_1$  is the solute concentration, where 2-PE is present inside the porous system in the PPBs, and  $C_2' - C_2''$  is the variation in the solute concentration in the solution, which is aqueous in the current instance.<sup>45,46</sup> This high  $K$  value aligns with observations from release studies, which demonstrated that 2-PE exhibits significant retention within the PPBs and faces challenges in being released into water.

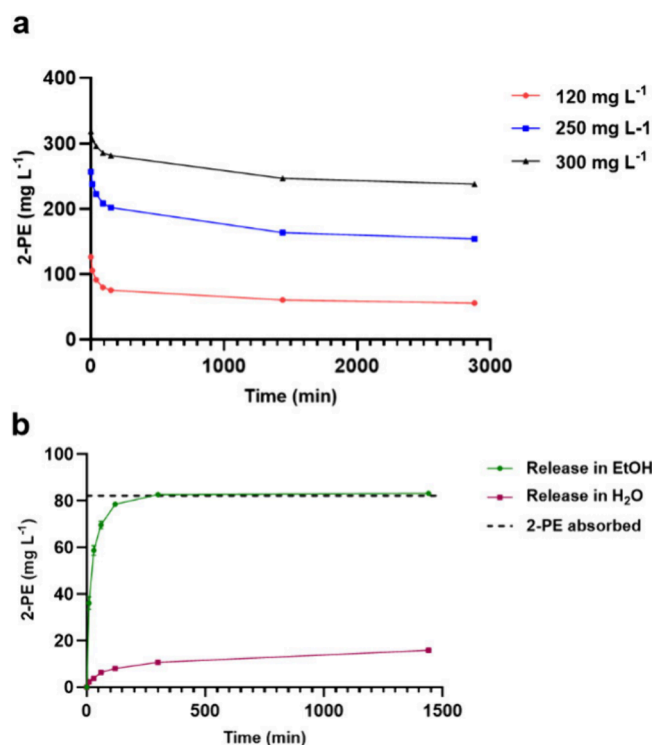
Interestingly, the release of 2-PE was significantly enhanced in polar organic solvents, e.g., ethanol, contrasting with its limited desorption into water. This suggests strong 2-PE–PPB interactions, which are disrupted in organic solvents, providing a more favorable environment for desorption.

Overall, the results highlight the need to carefully consider both absorption and release properties when designing systems for 2-PE separation or recovery, particularly in applications requiring controlled desorption for reuse or further processing.

**3.2.2. Kinetics of 2-PE Absorption in 48 h.** In order to fully understand the dynamics of 2-PE absorption by PPBs, the amount of time required to reach the maximum absorption efficiency of 2-PE in an aqueous solution was investigated using a standard weight of 200 mg of PPBs, according to a previous test (Table 1). Specifically, concentrations of 120, 250, and 300 mg L<sup>-1</sup> 2-PE were tested, and samples were collected at intervals of 10, 40, 1.5, 2, and 24 h to quantify the 2-PE absorption by PPBs.

The results (Figure 4a) demonstrated that the absorption of 2-PE by PPBs increases exponentially in the first 2 h, with a consistent decrease in the concentration of 2-PE in the aqueous phase over time across all tested conditions. The absorption rates ranged from  $0.046 \pm 0.52 \times 10^{-3}$  mg L<sup>-1</sup> min<sup>-1</sup> to  $0.065 \pm 0.33 \times 10^{-3}$  mg L<sup>-1</sup> min<sup>-1</sup> for 120 and 250 mg L<sup>-1</sup> 2-PE solutions. These findings show that higher initial 2-PE concentrations stimulate faster initial absorption rates, demonstrating that the solute concentration influences the process kinetics. Indeed, the diffusion process is driven by a greater concentration gradient between the solution and the PPBs. Moreover, after 24 h, the absorption process reached a plateau for each concentration examined, indicating that the PPBs had achieved saturation threshold or that the driving force for further absorption had become negligible. This equilibrium point reflects the capacity of PPBs to absorb 2-PE under the given conditions effectively and provides insight into the kinetics and efficiency of the material as an absorbent.

**3.2.3. Release of 2-PE from PPBs into Solvents.** To investigate the release kinetics of 2-PE into a solvent, 200 mg of PPBs was immersed into 150 mg L<sup>-1</sup> 2-PE for 24 h and then



**Figure 4.** (a) Absorption kinetics at different concentrations of 2-phenylethanol: 120 (red), 250 (blue) and 300 (black) mg L<sup>-1</sup> and (b) comparison of solute release calculated with the increase of concentration of 2-PE in the solution from the material in water (green) and in ethanol (purple).

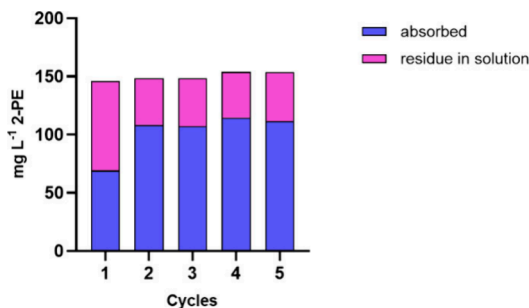
transferred into new vials containing the solvent. During the absorption-phase, PPBs were able to absorb about 80 mg L<sup>-1</sup> 2-PE. Using water as solvent, only 20% of the absorbed 2-PE was released, highlighting the inefficiency of water for this purpose (Figure 4b). This limited release can be attributed to the inability of water to adequately disrupt the interactions between 2-PE and PPBs. In contrast, ethanol demonstrated a much higher efficiency as a releasing solvent. In fact, the majority of 2-PE was desorbed within the first 2–5 h, with the process quickly reaching a plateau (Figure 4b). In an ethanol medium,  $98.85 \pm 0.21\%$  of the 2-PE initially absorbed by the PPBs was released, achieving complete desorption (Figure 4b). Compared with water, ethanol is less polar and has lower permittivity, which could easily disrupt noncovalent interactions (such as hydrogen bonding or hydrophobic interactions).<sup>47</sup> This result is also consistent with the study of Peyches-Bach et al., regarding the influence of ethanol, in contrast with water, on the extraction of aromatic alcohols such as 2-PE from wine.<sup>48</sup> From the absorption data, we calculated the partition constant  $K_{PPB/Ethanol}$  equal to 78.18 (eq 1).

The difference between ethanol and water in promoting the release of 2-PE emphasizes how important solvent characteristics are to improve the release efficiency. Thus, ethanol leads to total 2-PE desorption, allowing PPBs total regeneration and reuse, together with an efficient solute recovery and adaptability in a variety of applications.

**3.2.4. Recyclability of PPBs.** To assess the recyclability of PPBs, the absorption of 2-PE has been carried out five times using the same PPBs, according to the following procedure. 200 mg of PPBs was immersed into 10 mL of 150 mg L<sup>-1</sup> 2-PE for 24 h in continuous stirring. After that, PPBs were placed in

a new vial and left for a whole day with 10 mL of ethanol to allow complete desorption of 2-PE. Samples for the 2-PE quantification were collected in the water solution after 1, 2, 5, and 24 h.

The absorption of 2-PE was measured as 47%, 73%, 72%, 74%, and 73%, respectively, from the first to the fifth cycle (Figure 5). The low absorption the first time is not surprising,



**Figure 5.** Absorption cycles of regenerated PPBs. Consistency of performance of the reclaimed material for four Cycles.

and the causes can be related to the necessity of the material to adapt to the condition of the solution and make a “screwing” of the porous. These data highlight the possibility of using PPBs multiple times for the absorption of 2-PE without affecting efficiency.

### 3.2.5. Competition between 2-PE and Other Components of Growth Media Used for the 2-PE\_aroK Mutant.

Looking into industrial biotechnological applications, PPBs have to perform the best in exhausted growth medium, where the microorganisms, i.e. cyanobacteria, live and produce 2-PE. The growth medium is BG11, with a pH of roughly 8 and contains 150 mg L<sup>-1</sup> 2-PE, as the main bioproduct, and other aromatics as a part of the medium, such as L-phenylalanine.<sup>33</sup> To assess the selectivity of PPBs in absorbing 2-PE, 200 mg of PPBs was added to 10 mL of the growth medium, and samples were collected after 0–10–30–60–120 min.

We found that while the 2-PE concentration decreased in the medium by adding PPBs, the L-phenylalanine value remained unchanged (Table 2). Since polysulfone is a neutral

**Table 2.** Variation in the 2-PE Concentration in an Aqueous Solution Compared with the Variation in a Solute Concentration in the Presence of the Medium Components, Including L-Phenylalanine<sup>a</sup>

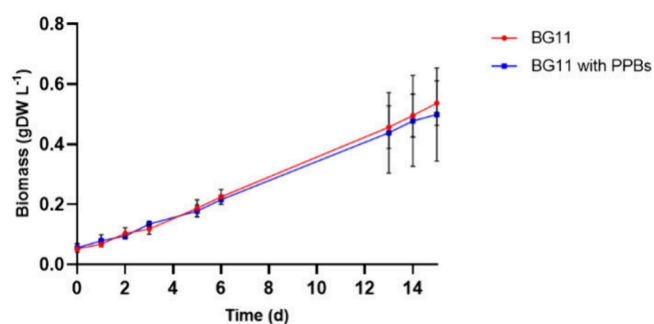
Time (min)	[2-PE] in wt (mg L <sup>-1</sup> )	[2-PE] in BG11 and L-Phe (mg L <sup>-1</sup> )
0	152.21 ± 0.60	150.02 ± 0.79
10	128.29 ± 0.51	125.77 ± 1.02
30	119.39 ± 1.28	115.69 ± 2.98
60	109.91 ± 0.41	105.91 ± 2.41
120	98.148 ± 1.87	93.59 ± 0.30

<sup>a</sup>Errors are measured as standard deviation.

aromatic polymer, its affinity for charged molecules is very low, favoring neutral aromatic molecules, i.e., 2-PE. Additionally, the presence of salts and pH 8 make the porous system selective to 2-PE. In fact, the amino acid phenylalanine is deprotonated at that pH value. Moreover, we can conclude that the concentration of L-phenylalanine is unaffected by the introduction of porous beads into the culture. Based on these data, we investigated whether the polymer system could be

introduced prior to bacterial growth and assessed its potential impact on the growth process.

**3.2.6. PPBs Biocompatibility.** To avoid competition between 2-PE-producing cyanobacteria and other biological contaminants, such as bacteria, the PPBs were sterilized as reported in subsection 2.4. The chemically sterilized PPBs were evaluated by immersing them in LB medium, a rich growth broth that allows several microorganisms to grow. No microorganisms were detected after immersion of PPBs, confirming the efficiency of the sterilization procedure. Consequently, 2-PE producing cyanobacteria were cultivated in BG11 medium with the addition of PPBs. PPBs did not affect the growth rate or biomass productivity of cyanobacteria during the 16-day experiment. In the reference condition and the PPB-containing condition, biomass yields were 0.56 ± 0.07 gDW L<sup>-1</sup> and 0.52 ± 0.14 gDW L<sup>-1</sup>, respectively, with growth rates of 0.24 ± 0.03 d<sup>-1</sup> and 0.22 ± 0.03 d<sup>-1</sup> (Figure 6).



**Figure 6.** Comparison of cyanobacteria grown in standard BG11 medium without (red line) and with the presence of PPBs (blue line). The growth curve is expressed as biomass (dried weight) of *S. elongatus* measured in 15 days of cultivation. The red line refers to the control condition, while the blue line represents the cultivation with the addition of PPBs.

Furthermore, considering these results and the pore's dimension, the cyanobacteria cannot be absorbed by the PPBs. Regarding adsorption, microscopic examination of the PPBs after cultivation revealed that no cyanobacteria attached to their surfaces, indicating that neither absorption nor adsorption occurred. These findings supported the biocompatibility of PPBs with cyanobacteria.

### 3.3. Environmental Evaluation

The goal of this study was to quantify and compare the environmental impacts of biologically synthesized 2-Phenylethanol (2-PE) production with in situ extraction using porous polysulfone-based beads (PPBs) and conventional fossil-based production consisting of the hydrogenation of styrene oxide. The scope of the LCA was to identify which process route exhibited the lowest environmental impact, thereby assessing the feasibility of scaling up biologically synthesized 2-PE from laboratory to pilot and ultimately industrial production. The LCA followed the ISO 14040–44:2006 guidelines and was performed using SimaPro 9.5.02 software and the Ecoinvent 3.8 database. Since biological 2-PE production is still in its early stages and has only been tested at the laboratory scale, the LCA was conducted using laboratory data (primary data).<sup>37</sup> The selected functional unit (FU) was 396 mg of 2-PE, based on the scale of the study performed.

To the best of the authors' knowledge, no previous studies have assessed the environmental implications of biologically

**Table 3. Environmental Impacts Calculated with the ReCiPe MidPoint 2016 (H) Method Referring to the FU = 396 mg of 2-PE<sup>a</sup>**

Impact category	Unit	4A_Step: Extraction_condition_A	4B_Step: Extraction_condition_B	Chemical route
Global warming	g CO <sub>2</sub> eq	3.245	3.260	3.94
Stratospheric ozone depletion	g CFC11 eq	$1.78 \times 10^{-06}$	$1.82 \times 10^{-06}$	$2.57 \times 10^{-06}$
Ionizing radiation	Bq Co-60 eq	0.335	0.350	0.036
Ozone formation. Human health	g NO <sub>x</sub> eq	0.006	0.006	0.008
Fine particulate matter formation	g PM2.5 eq	0.004	0.004	0.004
Ozone formation. Terrestrial ecosystems	g NO <sub>x</sub> eq	0.006	0.006	0.008
Terrestrial acidification	g SO <sub>2</sub> eq	0.010	0.010	0.009
Freshwater eutrophication	g P eq	0.001	0.001	0.001
Marine eutrophication	g N eq	0.002	0.002	0.000
Terrestrial ecotoxicity	g 1.4-DCB	5.826	5.472	19.743
Freshwater ecotoxicity	g 1.4-DCB	0.081	0.079	0.444
Marine ecotoxicity	g 1.4-DCB	0.106	0.104	0.548
Human carcinogenic toxicity	g 1.4-DCB	0.101	0.099	0.127
Human noncarcinogenic toxicity	g 1.4-DCB	1.768	1.719	2.965
Land use	cm <sup>2</sup> a crop eq	0.685	0.720	0.740
Mineral resource scarcity	g Cu eq	0.005	0.004	0.006
Fossil resource scarcity	g oil eq	1.068	1.060	1.623
Water consumption	dm <sup>3</sup>	0.058	0.056	0.037

<sup>a</sup>The analyzed configurations are biologically synthesized 2-Phenylethanol (2-PE) production with in-situ extraction using porous polysulfone-based beads (PPBs) with fermentative conditions A and B and the conventional fossil-based production consisting of the hydrogenation of styrene oxide.

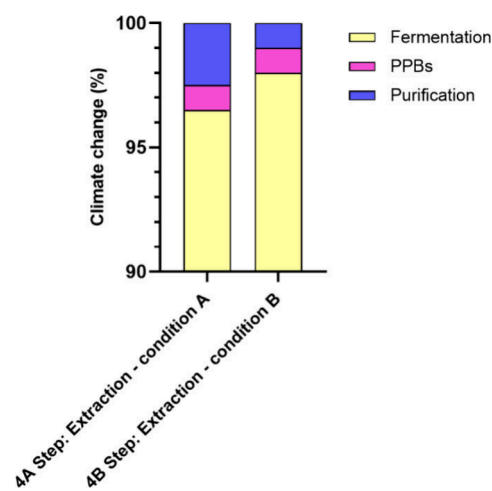
and fossil-derived 2-PE production. This study aims to fill that gap by providing insights into the investigated processes, highlighting their advantages and limitations.

Table 3 presents the environmental impacts calculated using ReCiPe MidPoint (H) 2016. Additionally, Table 3 includes a sensitivity analysis considering potential variations in the fermentative production process (Steps 3A and 3B), which differ in processing time (10 vs 16 days) and working volume (1.96 vs 2.2 L).

The results in Table 3 indicate that biological 2-PE production with in situ extraction using PPBs outperformed fossil-based production in all impact categories, except for terrestrial acidification and water consumption. The higher terrestrial acidification impact was attributed to the composition of the fermentation medium, particularly the presence of NH<sub>4</sub>. Meanwhile, the increased water consumption was due to the necessity of adding water to the medium broth to optimize the mass, nutrients, and energy transfer during fermentation.

Focusing on climate change impact, biological 2-PE production exhibited lower emissions than the fossil-based process. This is primarily due to the integration of two zero-burden inputs (sludge and washing water) into the fermentation process as carbon sources, demonstrating the benefits of waste reutilization in reducing the fossil resource dependency. Additionally, the fermentation process was carried out under mild conditions (30 °C), further minimizing its environmental footprint. These findings align with the European Green Deal's objective of achieving carbon neutrality by 2050, highlighting the potential contribution of biologically synthesized 2-PE to the decarbonization of industrial processes.

Figure 7 further delves into the climate change impact, highlighting that within biologically synthesized 2-PE production, the fermentation step accounted for 96.5–98% of total emissions, while PPBs production contributed 1%, and extraction and recovery contributed 2–2.5%.



**Figure 7.** Percentage contribution of the process steps in the climate change impact category calculated with the ReCiPe MidPoint 2016 (H) method.

By integration of the results from Table 3 and Figure 7, it is evident that biologically synthesized 2-PE represents an environmentally viable alternative to conventional fossil-based production. Even with variations in fermentation conditions (conditions A and B), the biological process consistently resulted in lower environmental impacts. The fermentation step remains the primary environmental hotspot, mainly due to energy consumption associated with prolonged processing times (10 and 16 days for conditions A and B, respectively) and the composition of the fermentation medium. These concepts related to fermentation limitations were already highlighted in the study of Hua and colleagues.<sup>7</sup>

Notably, the in situ extraction of 2-PE using PPBs exhibited minimal environmental impact, reinforcing its potential as a scalable and sustainable extraction technique. Furthermore, the

impacts of 2-PE using PPBs could be reduced by optimizing the chemical recycling (i.e, methanol and ethanol).

#### 4. CONCLUSION

The use of PPBs has proven to be an advantageous solution for the absorption and capture of hydrophobic compounds such as 2-PE from various perspectives. For industrial applications, evaluating the cost of this polymer is essential. Its price ranges from 100 up to 300 \$/kg based on the purity, which is significantly lower than other materials on the market, such as Amberlite XAD. This cost-effectiveness gives PPBs a competitive edge and underscores the importance of expanding research on this polymeric system's technical properties and potential applications.

In this study, we focused on using PSF to create spheres with an average diameter of up to approximately 2.1 mm. The ability of these spheres to absorb 2-PE, produced by a genetically modified cyanobacterium, was thoroughly investigated. The selected polymer's resistance to a wide pH range allowed for effective chemical sterilization of the PPB without altering the spheres' physical characteristics. The 2-PE absorption and release of 2-PE by the PPBs were determined. We found that 200 mg of PPBs achieved the maximum absorption efficiency, equal to 0.396 mg of 2-PE per milligram of PPBs. Complete release of 2-PE after absorption was achieved by using ethanol as a solvent, enabling the regeneration of PPBs. This regenerated material retained its effectiveness among multiple cycles, highlighting not only economic advantages but also the environmental impact. Additionally, the biocompatibility of PPBs was demonstrated through the successful cultivation of cyanobacteria with PPBs. Finally, PPBs absorb 2-PE efficiently, even in the presence of cultivation medium components, including L-phenylalanine, demonstrating their reliability for selective absorption applications.

In conclusion, PPBs act as a cost-effective and durable material for the selective absorption and release of hydrophobic compounds like 2-PE, with promising industrial applications due to its reusability, chemical resistance, and compatibility with biological systems, as demonstrated by its support for cyanobacterial cultivation and sustained efficiency over multiple cycles.

Furthermore, the environmental analysis proved that biologically synthesized 2-PE production with in situ extraction using porous polysulfone-based beads (PPBs) outperformed conventional fossil-based production consisting of the hydrogenation of styrene oxide. In detail, the innovation of this study relies on the extraction of aromatic solvents using recyclable beads, which contributes less than 4% of climate change, according to LCA. Based on these findings, extracting aromatic compounds using PPBs is a sustainable method that can be scaled-up for industries and applied to any production method.

Considering these results in the future, we will develop a process for the continuous extraction of 2-PE from *S. elongatus* culture.

#### ■ ASSOCIATED CONTENT

##### Data Availability Statement

The authors confirm that the data supporting the conclusions of this investigation are available in the paper and in the [Supporting Information](#).

#### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.5c06523>.

Additional information about the study's environmental modeling and experimental accuracy, modest error variation seen in both aqueous and ethanol solutions is highlighted by the detailed magnifications of the absorption and release curves, a comparative HPLC measurement of 2-PE and phenylalanine absorption, precise cradle-to-gate boundary conditions for the PPB-mediated fermentation process and a thorough Life Cycle Inventory (LCI) that compares our biological configurations to the conventional fossil-based hydrogenation of styrene oxide in order to support the sustainability assessment ([PDF](#))

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## Notes

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