I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil

Evaluation in modified SBA-15 immobilization for ethyl ester production

Carina A. Akemi [1], Sílvio M. P. Marcucci[2], Pedro A. Arroyo[1] & Gisella M. Zanin[1*]

[1]*Engenharia Química, Centro de Tecnologia/Departamento de Engenharia Química/Mestrado em Bioenergia, Universidade Estadual de Maringá, Maringá, Brasil*
[2]*FEITEP – Educação Presencial e a Distância, Maringá, Brasil*
** Corresponding author's email address: giselladeq@gmail.com*

ABSTRACT

In this wok, pore-expanded SBA-15 was synthesized and modified with inorganic and organic compound, and used as a biocatalyst for ethyl ester production. Results of small-angle X-ray scattering (SAXS) and nitrogen physisorption showed that the supports possessed the SBA-15 structure with a hexagonal pore array and cylindrical pores. Additionally, it was demonstrated that the pore diameter of the supports was sufficient to accommodate lipase inside the carriers. The catalytic performance of non-polar organic supports was better in the ethyl ester production, indicating lower mass transfer restriction. The interaction between enzyme and supports was tested, and the results showed that there was no lipase leaching, demonstrating a strong interaction between lipase and carrier surfaces.

Keywords: *Burkholderia cepacia*. Mesoporous Silica. Textural Parameters. Stability.

1 INTRODUCTION

Lipases are enzyme that act as catalyst in hydrolysis, esterification, and transesterifications reactions. They can be used for biofuel production, such as biodiesel, which serves as a partial substitute of diesel from petroleum [1]. The use of immobilized enzymes on a solid carrier presents several advantages compared free lipase, such as a greater resistance to the reaction medium, easy enzyme recovery, and the possibility for use in continuous reactors [1].

The methods of enzyme immobilization can be classified into physical, involving immobilization via adsorption and entrapment and chemical methods, which are based on covalent bonds between the enzyme and support and crosslinking between enzymes [2]. Each method presents advantages and disadvantages [2]. For example, when comparing adsorption and covalent bonding, the latter causes more severe conformational modification of the enzyme molecule. However, the operation stability of covalent bond is higher, and enzyme leaching is lower compared to adsorption [2].

The support used in immobilization is another crucial parameter in enzyme immobilization. In this scenario, mesoporous silicas emerge as good support due to the high specific area, the possibility adjusting pore size, and surface modification with several organics or inorganics compounds for physical or chemical immobilization [3]. Therefore, the objective of this work was to synthesize pore-expanded SBA-15, a mesoporous silica, and modify its surface with 1,1,3,3,3- hexamethyldisilazane (HMDS), with stannous chloride, and with stannous chloride followed by modification with HDMS, for lipase immobilization to ethyl ester production.

2 MATERIAL & METHODS

Pore-expanded SBA-15 (S20) was synthesized according to Calin et al [4] and Marcucci et al [5]. SBA-15 was modified with stannous chloride (SnS20) and HDMS (SS20) as reported by Wang et al [6] and Zola et al [7], respectively. SnS20 was further modified with HDMS and classified as SnSS20.

The pore-expanded SBA-15 (S20) and the supports SnS20, SS20 and SnSS20 were characterized by small-angle X-ray scattering (SAXS) and nitrogen physisorption using ASAP 2020 Micromeritics for the textural parameter's determination. The BET method was employed for specific area determination, and BJH was used to evaluate pore mean size. These supports were used for the immobilization of lipase from *Burkholderia cepacia*, employing 0.5 g of carrier, 20 mL of phosphate buffer (10 mM, pH 7,0) and 0.9 g of enzyme powder. The biocatalysts were classified as BSnS20, BSS20 and BSnSS20.

The reactions for ethyl ester production were conducted in a jacked glass reactor containing 6 g of soybean oil, 1,28 g of ethanol (with an oil to ethanol ratio of 1:4), 1 wt. % of water relative to oil, and held at 25 ºC. During the reaction, aliquots were withdrawn and analyzed using gas chromatography with methyl tricosanoate as the internal standard. The analysis was performed in a GC2010-PLUS Shimadzu equipped with a GC capillary column (Agilent DB-23; 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness; USA), and a flame ionization detector (FID). The injector and detector temperatures were set to 260°C and 220°C, respectively. The oven temperature was initially held at 150°C for 5 min, then increased from 150°C to 240°C at a rate of 5°C/min and maintained at this temperature for 10 min.

The stability test of the interaction between lipase and the support was performed during the reaction. After 2 hours of reaction, the biocatalysts were removed, and the reaction mixture was returned to the reactors, where the reaction continued for the same duration as the reactions with the biocatalysts.

3 RESULTS & DISCUSSION

The results of SAX and physisorption analysis for the pore-expanded SBA-15 and for the supports used in the lipase immobilization are presented in Figure 1.

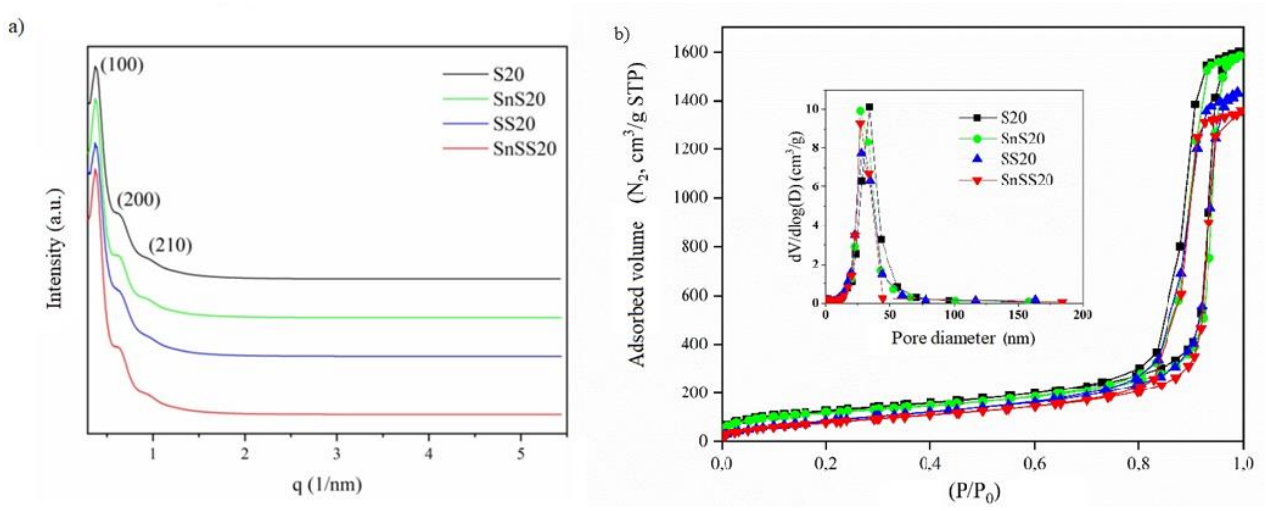

Figure 1 a) SAX analayis and b) physisorption alaysis.

In Figure 1a), its is possible to observe the peaks indexed to the planes (100), (200) and (210), which are characteristic of the hexagonal arranjemet of the pores of SBA-15 for all samples, as reported by Callin et al [4]. Regarding physisorption alaysis (Figure 1 b), type IV isotherms with H1-type hyesterese can be observed, confirming the mophology characteristic of a hexagnol pore arranjement and paralel cilindrical pores [8]. The specific areas (m^2/g) of S20, SnS20, SS20 and SnS20 were 441, 434, 322 and 318, respectively, indicating a reduction in area due to surface modification. Additionally, the mean pore sizes for the supports SnS20, SS20 and SnS20 were 23, 21, and 20 nm, repectively. The pore sizes are sufficient to accommodate the lipase inside the support, as lipase has globular dimansions of 3 nmx 4 nmx 5 nm [9].

Figure 2 ilustrates the ethyl ester yield in the reactions and the stability test regarding the interaction between lipase and the carrier.

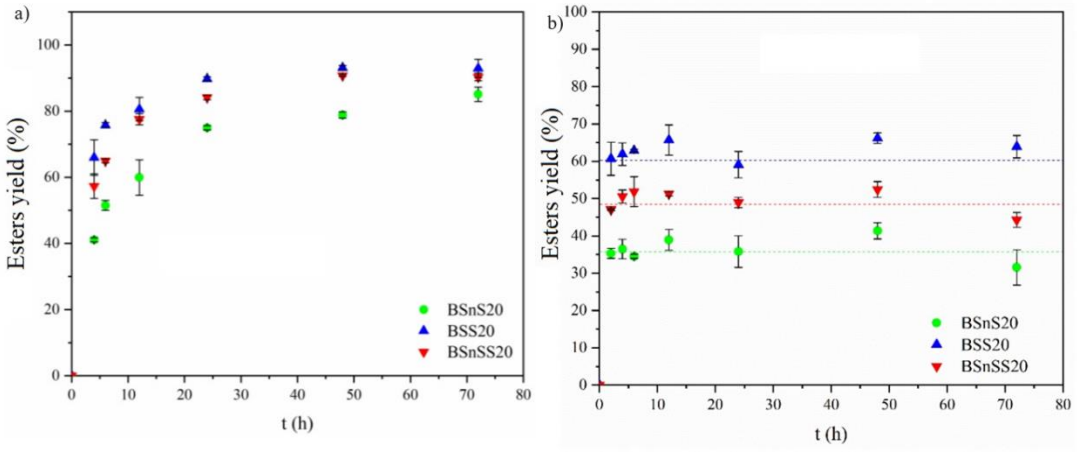

Figure 2 a) Ethyl ester yiedl and b) Stability test.

As can be seen in Figure 2 a), after 72 h, the ethyl ester yield was similar for all biocatalysts, with ester yield of 85 ± 2.2%, 93 ± 2.8% and 90 ± 1.2% for BSnS20, BSS20 and BSnSS20, respectively. However, the hydrophobic supports (SS20 and SnSS20) showed higher catalytic activity, likely due to the mass transfer and access to the lipase active site in these systems. This is because the non-polar nature of the materials minimized the accumulation of glycerol (hydrophilic) within the biocatalysts [10]. For the stability test (Figure 2 b)) it is possible to observe that the ester yield does not change after biocatalyst removal, indicating that there is no enzyme leaching and a strong interaction between the supports and the lipase.

4 CONCLUSION

Pore-expanded SBA-15 was synthesized and modified with inorganic and organic compounds. The structure of the hexagonal pore array was detected in SAX analysis and in nitrogen physisorption. Results demonstrated that the pore diameter of all supports was sufficient to accommodate the lipase inside the carrier. The biocatalyst's performance was better for organic a no-polar supports. According to the stability tests, there was a strong interaction between lipases and support.

REFERENCES

1 Y. R. Maghraby, R. M. El-Shabasy, A. H. Ibrahim, H. M. E. S. Azzazy, *ACS Omega* **2023**, *8*, 5184.

2 H. T. Imam, P. C. Marr, A. C. Marr, *Green Chem.* **2021**, *23*, 4980.

3 A. Costantini, V. Califano, *Catalysts* **2021**, *11*.

4 N. Calin, A. Galarneau, T. Cacciaguerra, R. Denoyel, F. Fajula, *Comptes Rendus Chim.* **2010**, *13*, 199.

5 S. M. Parmegiani Marcucci, G. M. Zanin, P. A. Arroyo, *Microporous Mesoporous Mater.* **2022**, *337*, 111951.

6 X. Wang, N. Li, L. D. Pfefferle, G. L. Haller, *Microporous Mesoporous Mater.* **2013**, *176*, 139.

7 A. S. Zola, L. S. Da Silva, A. L. Moretti, A. Do Couto Fraga, E. F. Sousa-Aguiar, P. A. Arroyo, *Top. Catal.* **2016**, *59*, 219.

8 M. Thommes, K. Kaneko, A. V. Neimark, J. P. Olivier, F. Rodriguez-Reinoso, J. Rouquerol, K. S. W. Sing, *Pure Appl. Chem.* **2015**, *87*, 1051.

9 S. M. P. Marcucci, C. A. Araki, L. S. da Silva, G. M. Zanin, P. A. Arroyo, *Brazilian J. Chem. Eng.* **2021**, *38*, 511.

10 D. Cazaban, A. Illanes, L. Wilson, L. Betancor, *Process Biochem.* **2018**, *74*, 86.

ACKNOWLEDGEMENTS

The authors would like to thank CAPES and Departamento de Engenharia Química da UEM (DEQ/UEM) for their support.