Scientific Research Publishing

Materials Sciences and Applications, 2022, 13, 389-400
https://www.scirp.org/journal/msa
ISSN Online: 2153-1188
ISSN Print: 2153-117X

# Electrospinning Polylactic Acid Polymer Membranes as Biological Sieve for Yeast and Bacteria

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**How to cite this paper:** Suaste-Gómez, E., Rodríguez-Roldán, G., Pérez-Solis, I., Torres-Huerta, A.L., Cruz-Cruz, C. and Tapia-Ramírez, J. (2022) Electrospinning Polylactic Acid Polymer Membranes as Biological Sieve for Yeast and Bacteria. *Materials Sciences and Applications*, **13**, 389-400.
https://doi.org/10.4236/msa.2022.136022

**Received:** May 12, 2022
**Accepted:** June 19, 2022
**Published:** June 22, 2022



## Abstract

Vaginal infections are suffered by millions of women of reproductive age. Recent research has shown that timely diagnosis of these infections avoids complications in the patient's health. Since the symptoms of infections caused by yeast and bacteria are similar, the patient is not always treated properly. Membranes with three different thicknesses (66 μm, 83 μm, 128 μm) were produced by electrospinning of polylactic polymer (PLA) and were used as a biological sieve to detect microorganisms present in vaginal infections, bacteria or yeasts. Electrospinning technique is a technique that allows the generation of membranes from nanofibers of material polymeric and therefore, obtaining porous structures. The size of the fibers was observed as well as the morphology and the size of the pores, using the scanning electron microscopy (SEM). The effectiveness of these membranes was proven by the cultivation of the bacteria that passed through the polymeric membrane and the yeasts retained by it. The membranes allowed the retention of yeasts of *Candida albicans* type with dimensions of 7 μm, which allowed the separation of *Brevibacterium ravens purgense* bacteria with dimensions of 1.4 μm, thus demonstrating their effectiveness as biological sieve.

## Keywords

Biological Sieve, Electrospinning, Polylactic Acid, Vaginal Infections

## 1. Introduction

The symptoms of vaginal problems such as discharge, itching and odor, occur frequently in millions of women of reproductive age [1] [2] [3]. This type of condition alters the vaginal microflora, favoring the growth of microorganisms in the vaginal tract. The most common vaginal infections are due to bacterial vaginosis (BV), *Vulvovaginal candidiasis* and other etiological agents [4]. It has been reported that bacterial vaginosis occurs from 40% to 50% of cases with vaginal infection, while *Vulvovaginal candidiasis* from 20% to 25% [5] [6].

Women often self-diagnose and treat themselves inappropriately. Timely diagnosis and initiation of targeted therapy are essential for successful clinical management [7].

Bacterial infection is associated with taking antibiotics, douching, the use of intrauterine devices or ETS [7]. The composition of bacteria in bacterial vaginosis can vary between women and the most frequent associated microorganisms are *Gardnerella*, *Atopobium*, *Mycoplasma*, *Prevotella*, *Bifidobacterium*, *Megasphaera*, *Leptotrichia*, *Sneathia*, *Dialister* and *Clostridium* [8]. In the case of *Candida* infections, predisposing factors such as menstruation, pregnancy and diabetes have been described, as well as the use of contraceptives and some broad-spectrum antibiotics [7] [9].

*Vulvovaginal candidiasis* is caused by *Candida* species, predominantly *C. albicans*, which represents 85% to 90% of all cases, and the rest is attributed to *C. glabrata*, *C. krusei*, *C. famata* and *C. tropicalis* [10].

*C. Albicans* is a type of dimorphic fungus belonging to the *Phylum Ascomycota* and conformed by pseudohyphae, hyphae and sub-spherical blastoconidia ((3 - 8) × (2 - 7) μm) [11]. This type of yeast has the ability to lodge and colonize mucosal surfaces [12] when there are changes in the pH or temperature of the vaginal microbiota [9] [13].

Currently, vaginal infection detection methods are based on laboratory techniques such as cultures, microscopic analysis, molecular tests and biochemical assays [6] [14]. These procedures are expensive and time-consuming, and they also require specialized equipment and trained personnel. The development of biosensors facilitates detection and diagnosis at the point of care. Several biosensors have been developed for the detection of certain vaginal infections through the study of pH variation, biochemical reactions, intravaginal temperature, among other aspects [15] [16] [17] [18] [19]. However, in many cases there is a diagnosis of nonspecific vaginitis, as the itching, pain, discharge and odor, can be a symptom of both infections caused by bacteria and yeast so they are easily confused. The most frequent method for diagnosing bacterial vaginosis is Amsel's clinical criterion, which turns out to be subjective because it is based on the presence of various symptoms [20]. The aspects to be evaluated consist of the observation of the pH of the vaginal discharge (above 4.5), the positive Whiff test (fish smell due to the mixture of vaginal discharge with 10% potassium hydroxide), the presence of key cells through examination microscopic (epithelial

cells covered by bacteria) and the appearance of vaginal discharge (amount of discharge, color, viscosity and odor) [21] [22]. Another widely used analysis is the Nugent scale, a microscopy method that quantifies 3 different types of bacterial morphotypes in a vaginal smear using a Gram stain [23]. However, the evaluation of the samples is subjective and dependent on the laboratory technician. Although both methodologies are easy and quick to perform, they do not provide an effective diagnosis of bacterial vaginosis and when combined they have a sensitivity and specificity of 81% and 70%, respectively [20].

This is the reason why in this work a new method has been developed to detect the origin of the vaginal infection (bacterial or yeast). The nanometric membranes as sieve were manufactured with polylactic acid (PLA) and the electrospinning technique in order to obtain an accurate diagnosis that will give an adequate treatment to the patient. The electrospinning technique is a cost-effective and versatile technique that is used to make nanofibers from a wide variety of materials [24]. The membranes of nanofibers are used in areas such as health, textiles, energy and electronics with applications such as filters and sensors due to their nanoporosity [25] [26] [27]. PLA is a polymer derived from 100% renewable resources such as corn and sugar beet [28] and is widely used for biomedical applications [29] [30] [31] like a versatile, biodegradable, biocompatible and non-toxic material [32].

## 2. Materials and Methods

### 2.1. Materials

Polylactic acid (PLA) filament from Makerbot (USA) was used and NN'-dimethylformamide (DMF, 99.9%) from Sigma-Aldrich was used as the solvent.

### 2.2. Preparation of PLA Solutions

The PLA filament was cut into pellets approximately 3mm long. Subsequently, DMF with a composition of 20% by weight was added to dissolve the PLA. This mixture of PLA/DMF (80/20 wt%) was left to stand until the DMF completely dissolved the PLA. This processed took approximately 24 hours. Finally, to obtain a homogeneous mixture, the solution was sonicated in an ultrasonic bath for 10 minutes.

### 2.3. Electrospinning Process

The PLA/DMF solution (80/20 wt%) was placed in a syringe with a volume of 5 ml. The manufacturing process of the PLA membranes by electrospinning was carried out at room temperature, a voltage of 20.5 kV DC and a circular copper collector plate with a diameter of 5 cm covered with aluminum foil were used. The distance between the tip of the syringe (anode) and the collector plate (cathode) was 7 cm. The PLA nanofibers were deposited on the aluminum sheet, generating the membrane. The membrane was removed from the foil and cut

into a square of 1.5 cm per side. The square was placed in the flexible acetate with a 1 cm diameter hole as shown in Figure 1. Finally, the organic sample (bacteria or yeast) was placed there.

## 2.4. Biological Test

### 2.4.1. Preparation of Membranes

A square of Whatman 3 MM paper was placed under each of the proposed active polymer membranes. Subsequently, the membranes were placed inside a Petri dish, fixing them with adhesive tape horizontally to the surface of the box as shown in Figure 2. This whole process was carried out under sterile conditions.

The strain of *Candida albicans* ATCC 60193 and the bacterium *Brevibacterium ravens purgense* were used. These microorganisms were isolated from a patient who presented vaginal infection and were characterized by biochemical and molecular tests by the National Collection of MicrobialStrains and Cell Cultures (CDBB) of the CINVESTAV. From a culture containing $1 \times 10^8$/ml yeast in YPD medium, 20 µl was taken and placed on the membranes for 30 minutes as seen in Figure 3. In the case of the bacteria, 20 µl of a culture with $1 \times 10^8$/ml bacteria were used in LB medium and they were also placed on the membranes for 30 minutes.

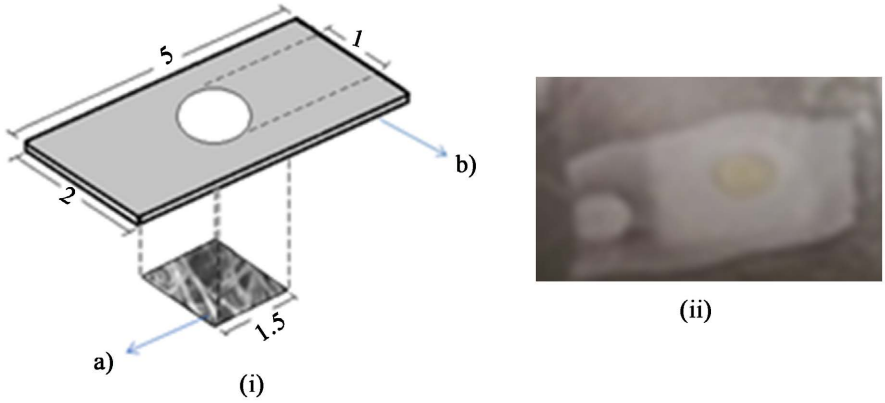



Figure 1. (i) Assembly diagram of the PLA membrane in acetate with dimensions in cm: a) Active polymer membrane, b) Flexible acetate; (ii) Photography of PLA membrane in acetate.

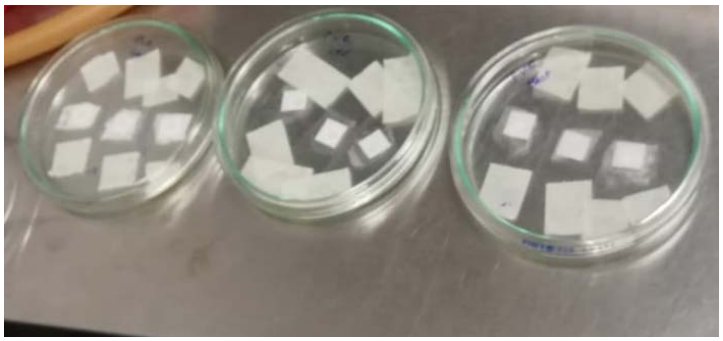


Figure 2. Membranes fixed with adhesive tape horizontally to the surface of the laboratory dish.

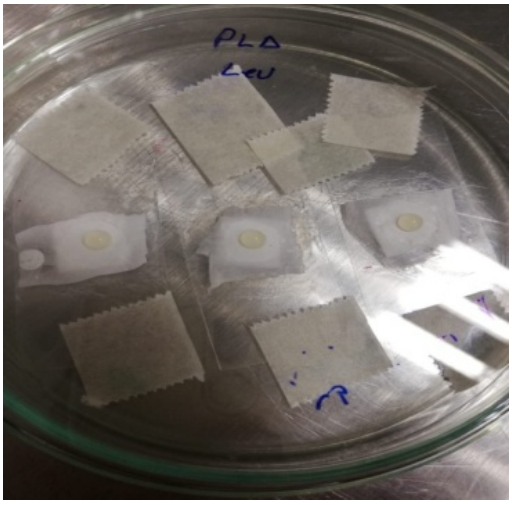



**Figure 3.** Yeast in liquid medium placed on the membranes.

#### 2.4.2. Growth of Yeast and Bacteria

To analyze the microorganisms retained by the PLA membrane, a sterile swab was used to rub the upper face of the membrane where the sample was deposited. Subsequently, each swab was deposited in a microtube with 1 ml of YPD or LB liquid medium (for yeasts or bacteria respectively). On the other hand, to analyze the filtered microorganisms, each square of Whatman paper in microtubes was placed separately with 1 ml of YPD or LB liquid medium. Finally, 100 µl of sample from each of all the microtubes was placed over the surface of solidified agar medium (YPD or LB) in a Petri dish. All plates were incubated at 37 °C for 16 hours and growth was recorded in each case.

## 3. Results

### 3.1. PLA Membranes

**Figure 4** shows the membrane cut and placed on the acetate, ready to be used. This same process was carried out with the other membranes.

### 3.2. PLA Membranes

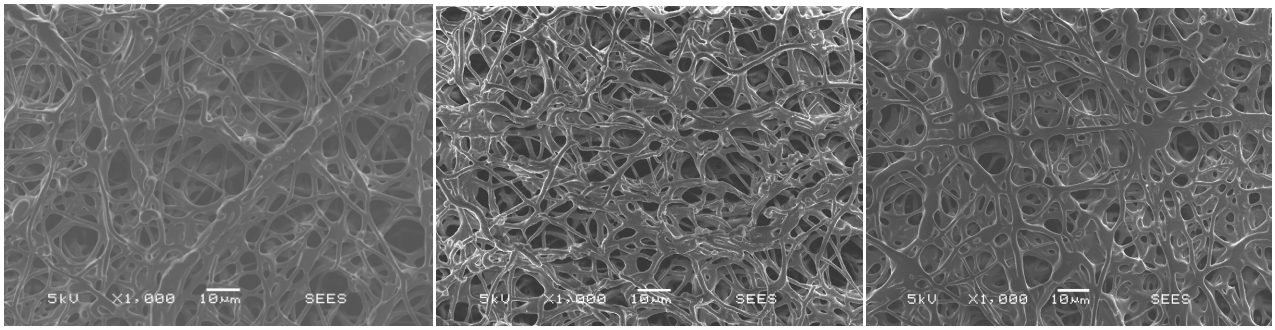
The SEM analysis of the obtained PLA polymer membranes was carried out by means of a JEOL scanning electron microscope (JMS-6360LV). **Figure 5** shows the SEM analysis of the three PLA membranes with different thicknesses (66 µm, 83 µm and 128 µm) and a magnification of 1000×. As observed, the PLA nanofibers are randomly oriented.

### 3.3. Determination of Fiber Diameter

The determination of the diameter of the fibers was carried out using the DiameterJ software [33]. **Figure 6** shows the segmentation of the image using this software.

**Figure 7** shows the diameter distribution of the fibers in the PLA membrane. It is noted that for this case, the fibers are within the range of 200 to 350 nm.

**Figure 4.** Cut-off membrane of PLA manufactured by electrospinning.




(a) (b) (c)

**Figure 5.** PLA membranes with different thicknesses at magnification of 1000×: (a) 66 µm; (b) 83 µm; (c) 128 µm.

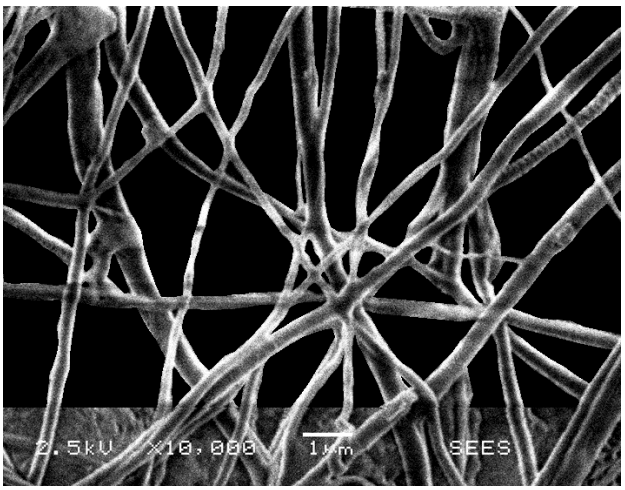



**Figure 6.** Segmented image of the membrane.

### 3.4. Biological Tests with Bacteria and Yeasts

For biological tests, yeast *C. albicans* and the bacterium *Brevibacterium ravens purgense* were used. *C. albicans* has an aspect of round or oval cells and is 7 microns in size while the bacterium has dimensions between ((0.4 - 1.4) × (0.3 - 0.50 µm) [34]. **Figure 8** shows the retention of yeasts using PLA membranes with different thicknesses: 66 µm, 83 µm and 128 µm. As observed, the membranes with thicknesses of 66 µm and 83 µm retained a large amount of yeast. On the other hand, **Figure 9** shows the yeasts that passed through the membranes. In the case of membranes with thicknesses of 66 µm and 83 µm, a minimum number of colonies (of 1 - 2) is observed, which are negligible. On the other hand,

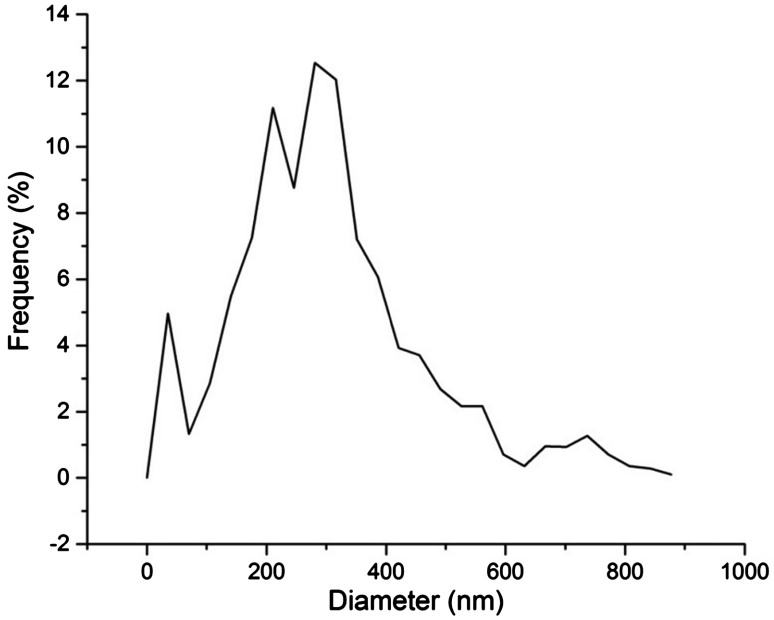



**Figure 7.** Distribution of fiber diameter in nanometers.

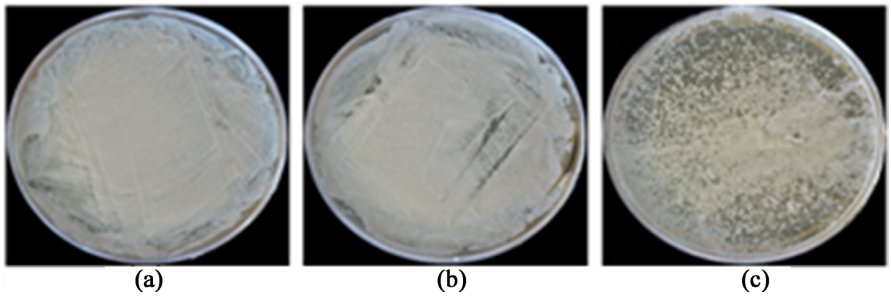



**Figure 8.** Test of retention of yeasts to PLA membranes with different thicknesses: (a) 66 μm; (b) 83 μm; (c) 128 μm.

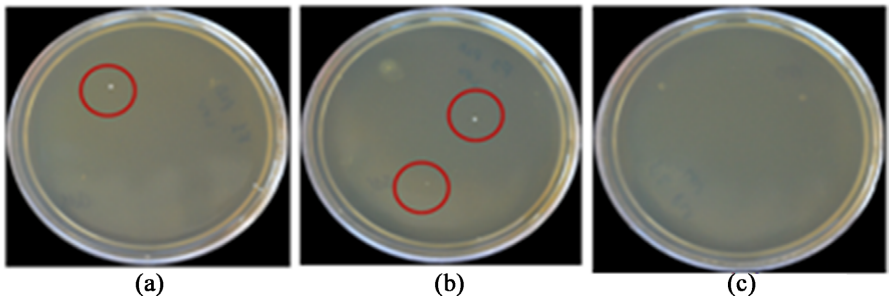



**Figure 9.** Yeasts that crossed the PLA membranes with different thicknesses: (a) 66 μm; (b) 83 μm; (c) 128 μm.

the membrane with a thickness of 128 μm completely retained the yeasts.

Figure 10 shows the retention of bacteria using PLA membranes with different thicknesses: 66 μm, 83 μm and 128 μm.

In Figure 11, it can be seen that the membrane with a thickness of 66 μm did not allow the bacteria to pass, while the 83 μm thick membrane allowed more colonies to pass, although the passage of these is not as homogeneous. On the

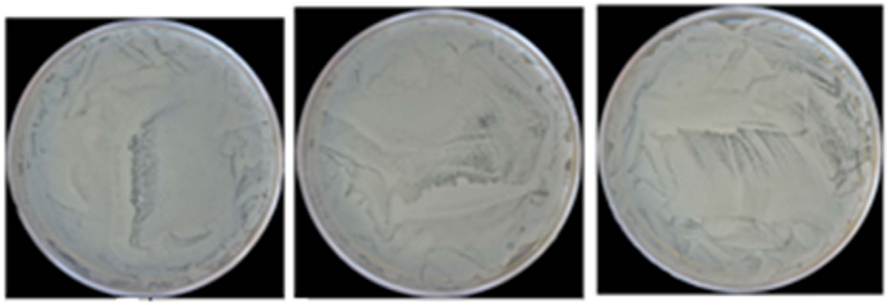



**Figure 10.** Test of retention of bacteria to PLA membranes with different thicknesses: (a) 66 μm; (b) 83 μm; (c) 128 μm.

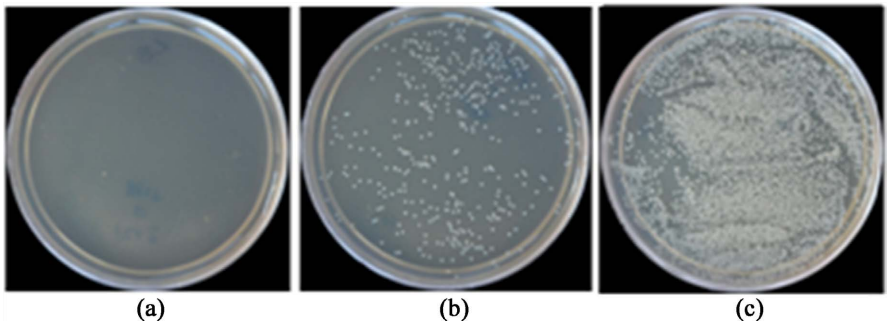



**Figure 11.** Bacteria that crossed the PLA membranes with different thicknesses: (a) 66 μm; (b) 83 μm; (c) 128 μm.

other hand, in the membrane with a thickness of 128 μm, the passage of bacteria is more homogeneous. These results confirm this last membrane is favorable for the development of ultrafilters as biosensors because they retain the yeasts and allow the passage of bacteria.

## 4. Discussion

There are commercial membranes such as microfiltration and water disinfection membranes [35] [36] with pores between 0.1 μm and 10 μm in diameter and also polyamide membranes with a pore size of 0.4 μm, a fiber diameter between 50 - 100 μm and a thickness of 120 μm. These characteristics allow the retention of microorganisms such as bacteria and yeasts [37] [38]. It was observed that the membrane with a thickness of 128 μm generated in this work behaved in a similar way, allowing the passage of bacteria and retaining a part of them. In this work several membranes with different thicknesses were generated, for this, both the manufacturing times and the distance between the syringe and the collector plate were modified. Analyzing the retention and filtration ability of the membranes in this study, a different behavior was observed between each type of microorganism. In the case of the yeasts, all the membranes allowed their retention. It was observed that although the thickness of the membrane does not seem to affect the apparent pore size, when the membrane tends to be thinner it could have holes or defects that allow the passage of some yeasts which was minimal (1 - 2 Colony Forming Units (UFC). On the other hand, in the case of bacteria, a

correlation was observed between the thickness and the passage of bacteria. Although all the membranes had the same pore diameter range (200 - 550 nm), the thicker membrane (128 μm) allowed the highest passage of bacteria compared with the membranes of 66 μm and 83 μm. Taking into account that the bacteria used (*Brevibacterium ravens purgense*) has dimensions of ((0.4 - 1.4) × (0.3 - 0.5) μm), it was expected that all membranes would allow the passage of bacteria. In addition, it is known that the transport and adhesion of microorganisms are influenced by their hydrophobic characteristics and that the topography of the surface of a membrane dictates (inhibits or promotes) its roughness and wettability [39] [40]. Additionally, cells tend to respond to the micro characteristics of a material, such as geometry, material properties, and functionalization of the surface [41].

## 5. Conclusion

In this work, membranes with different thicknesses were successfully performed to detect bacteria and yeasts as a biological sieve for diagnose vaginal infections, caused by these types of microorganisms. The polylactic acid nanofibers obtained by the electrospinning process provided porous surfaces, which allow a wide variety of applications in the biomedical sector, such as the implementation of new biosensors that have not yet been considered [42]. With the results obtained in this study, it is concluded that the membrane with a thickness of 128 μm has high potential for its use as a biological sieve, because it has the advantage of separating and concentrating the microorganisms to be analyzed. This is why it is a useful tool in the diagnosis of infections, identifying bacteria or yeast present in a vaginal discharge sample and favoring the allocation of a more effective treatment. Additionally, these membranes can be used in other types of laboratory tests, such as PCR, microscopy, cell culture, biochemical tests and more. On the other hand, these membranes achieved the isolation of bacteria or yeasts without expensive or sophisticated equipment. Additionally, the manufacture of membranes by the electrospinning method is versatile due to the ease of varying either the polymeric material or the thickness of the membrane generated and, in this way, achieving the selectivity of organic material.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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