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Preface

We would like to present, with great pleasure, the inaugural volume-4, Issue-10, October 2018, of a scholarly journal, *International Journal of Engineering Research & Science*. This journal is part of the AD Publications series *in the field of Engineering, Mathematics, Physics, Chemistry and science Research Development*, and is devoted to the gamut of Engineering and Science issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

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Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with IJOER. We are certain that this issue will be followed by many others, reporting new developments in the Engineering and Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOER* readers and will stimulate further research into the vibrant area of Engineering and Science Research.

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Comparison of carbon nanotube and soot reinforced rubber mixtures and their mechanical-morphological properties

TAMÁSI, Kinga[1]; SÁNDOR, Zoltán Barnabás[2]

Department of Ceramic and Polymer Engineering, University of Miskolc, Hungary

Abstract— *In our study, we investigated 2 different natural rubber mixtures with CNT's and shoot. The aim was to compare the results of samples and their mechanical effects with a shore. A hardness tester, a tensile strength instrument before and after vulcanisation (t=30; 60; and 90minutes) during the UV-ageing processes. The hardness results represented the different after the degradation process that was 10 % different between the samples.*

Keywords— Rubber, CNT, UV light, degradation, soot, composites.

I. INTRODUCTION

Elastomers have become a widely used technical material of our time. This is due, among other things, to the ability to produce significant reversible deformations of up to 100% by virtue of low tensions, and their mechanical and other properties can be altered in a universal spectrum by the correct selection of base materials and other ingredients. Their physical and mechanical properties can be greatly improved with – for example – fillers or reinforcing agents, and by adding the proper plasticizer they can be more or less easily used technologically. (e.g. acting as a slider). Although different additives play a key role regarding the properties of the finished rubber, the quantity and ratio of the additives cannot be freely altered since each component has a positive (soot as a filler improves abrasion resistance) effect, or greatly reduce certain properties (e.g. over-vulcanization with increased sulfur content):

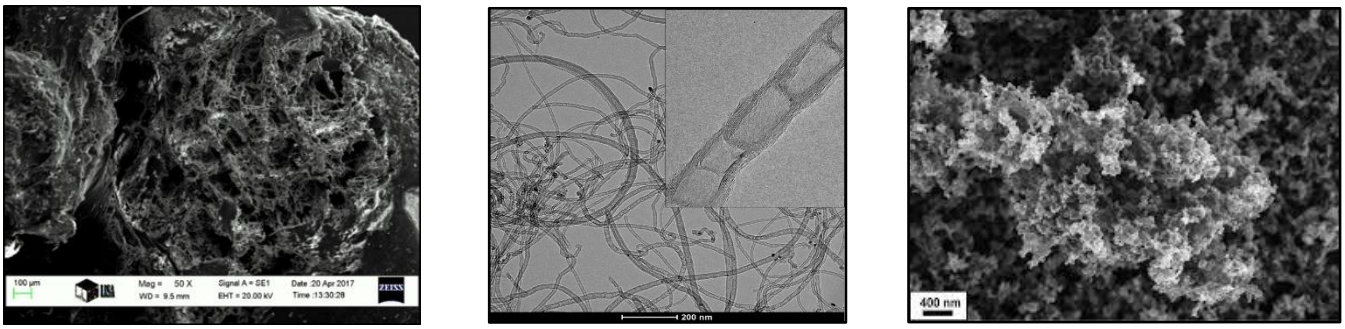

FIG. 1. SEM-Image of raw caoutchouc (50x magnification), CNT and Soot

Filler materials are isodynamic solid, matrix insoluble particle systems forming a separate solid phase. Their use has a remarkable past, and from the first quarter of the 19th century there are already references to the use of fillers in the caoutchouc. As a reinforcing filler, one of the largest quantities and the oldest used in the rubber industry are various soots. The most commonly used method for classifying them today is the 4-tag ASTM D1765 [1]. In the course of my investigations, we selected the 326 N type, in Table 1.

TABLE 1
PROPERTIES OF THE 326N TYPE SOOT ADDITIVE [1]

ASTM Classification	**OAN no., compaction** [10^{-5} m^3/kg]	**Coloring** [%]	**Density** [kg/m^3]	**Modulus** [MPa]
N326	68-69	110-113	446-470	-4,2 and -3,0

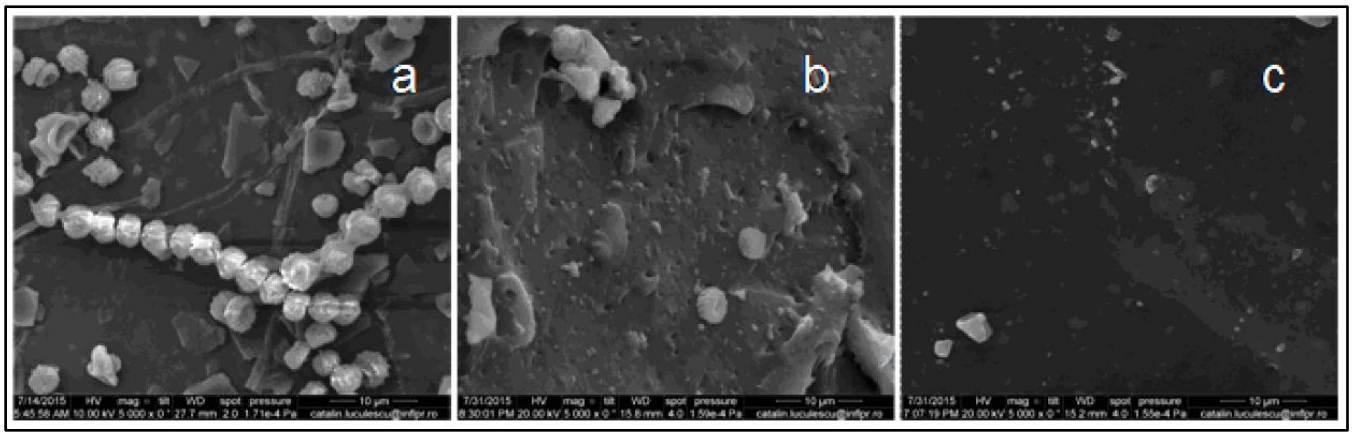

FIG. 2. SEM-image of NR-rubber mixtures cured with active fillers (10 µm) [2]

II. CARBON NANOTUBE-REINFORCE DRUBBER MIXTURES

Compared to the century-long history of carbon black, the intensive research of the possibilities of using modern nano reinforcements are limited to the last 1-2 decades. These fillers may be active or inactive, mineral, carbon based or biological, and are also distinguishable as plate, spherical and fibrous structures. Lopez-Manchado et al. [4] investigated nano composites produced by the NR matrix SWCNT reinforced laminated blending process, where it was found that SWCNT had a significant acceleration effect on vulcanization and also had a strengthening effect resulting from increased storage modulus and increased glass transition temperature of nano composites. These effects can be observed in most soot fillers, but on the basis of the results of the researchers, much more to SWCNT filling. Bokobza et al. [5] produced NR-MWCNT nano composites with toluene and then performed mechanical and conductivity tests, using unchanged mixtures as a basis.

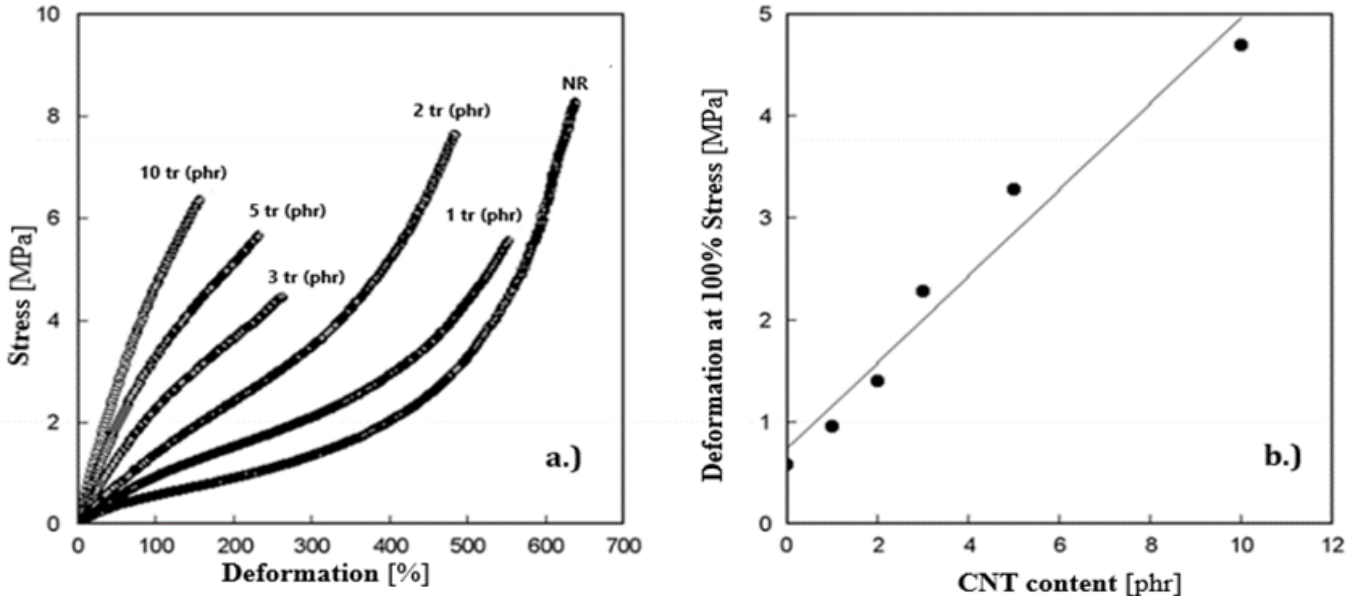

FIG. 3. Tension-deformation curves (a), and the tension-deformation curves of the NR-MWCNT composite according to carbon nanotube content (b) [2]

III. PRODUCTION RECAPTURES AND THE PRODUCTION OF SPECIMEN

Fillers, depending on their activity, alter the viscosity of the mixture, active soots and silica, increase viscosity. The first and definitive step of making the raw mix was the accurate measurement of the basic and additives at room temperature (T = 25°C ± 1°C) and normal relative humidity (50% ± 1%). The raw mixtures were homogenized in a cylinder at 35-40°C and the vulcanization process was carried out at T = 145°C, p = 220 bar and t = 10 minutes.

TABLE 2
OWN RECIPE BASED ON LITERATURE AND EMPIRICAL EXPERIENCES

Appliedmaterials	**Measuredquantities** [phr]	**Mixing Phases** [min.]
NR	100	60
Stearicacid	3	5
ZnO	5	5
ALTAX (MBTS)	0,6	5
Sulfur	2,5	5
Soot/CNT	50,7	20
NO	10	20

NO: Vegetable Oil ***120 min/batch***

IV. APPLIED TESTS

4.1 Mechanical testing: "Shore A" Hardnes test and Tensile strenght

Generally it can be stated that reinforcing fillers require substantially less per unit of hardness than half-active or inactive fillers. Soots enhance hardness more effectively than white fillers. Roughly speaking, fillers with smaller particle diameters and larger specific surface are more effective than fillers of larger diameter and smaller specific surface. The fillers effect on hardness is also dependant of the type of caoutchouc used. Caoutchouc with higher density requires smaller amounts of filler for a unit of hardness increase, than smaller density caoutchouc base materials. As described in the previous chapters, theoretically the oils, softeners, internal plasticizers reduce the hardness of the rubber which can be increased by adding more sulfur.

TABLE 3
TEST RESULTS BEFORE- AND AFTER THE AGING PROCESS

	Time	**0 min.**	**30 min.**	**60 min.**	**90 min.**
Soot	Average value	42,80	37,90	39,30	42,30
	Min. value	42,00	36,70	35,70	40,90
	Max. value	43,40	39,50	41,20	44,10
Nano	Average value	33,10	35,90	38,50	40,20
	Min. value	32,30	33,30	36,10	38,60
	Max. value	33,90	37,00	39,60	41,30

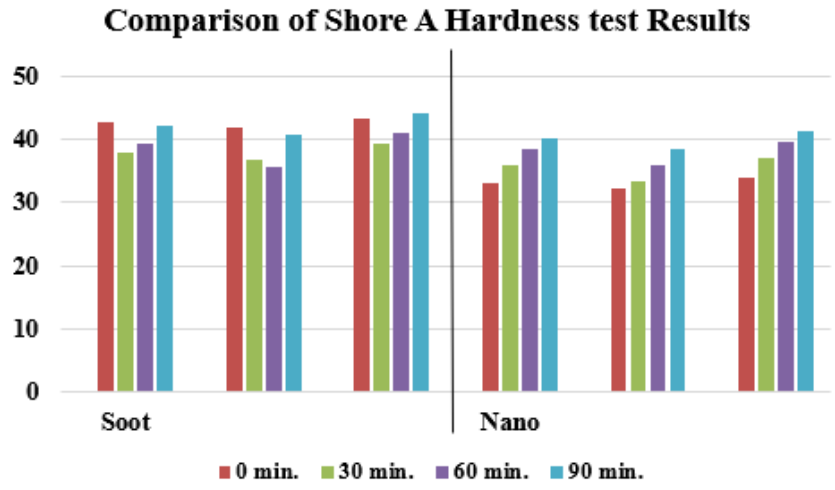

FIG. 4. COMPARISON OF INDIVIDUAL MIXTURES' SHORE A HARDNESS TESTS

4.2 Morphological and surface measurements: FT-IR spectroscopy and SEM microscopy

The next step in our investigations was surface-microscopic control of vulcanizates. Our goal was to observe the diffusion, possible migration of additives, and to follow up on aging processes. We have successfully determined that the efficiency of miscibility (mixing) has been successful; both filling systems are compatible with the raw rubber. We used a Bruker Tensor FT-IR for surface analysis and a Zeiss SEM-EDS for processes requiring higher resolution.

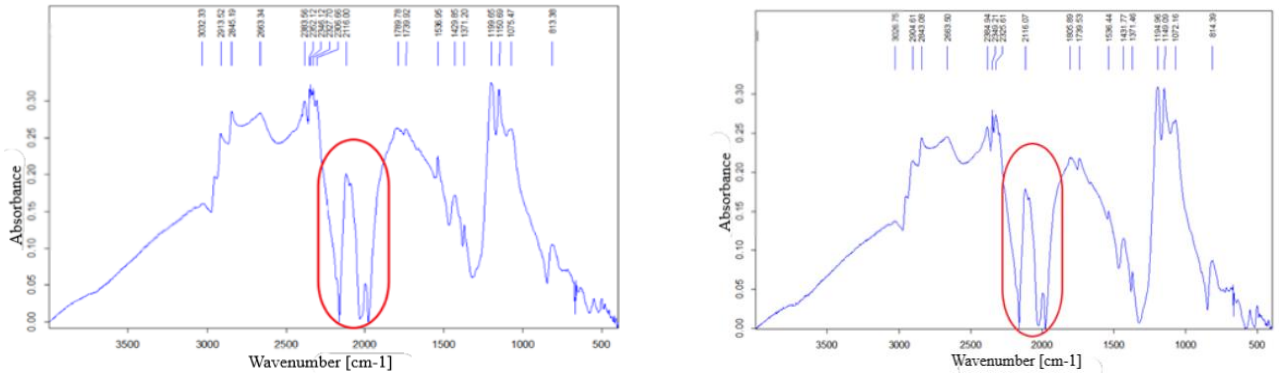

FIG. 5. Absorbance wave number of soot-cured,(a), nanotube-cured (b) vulcanised specimen before aging

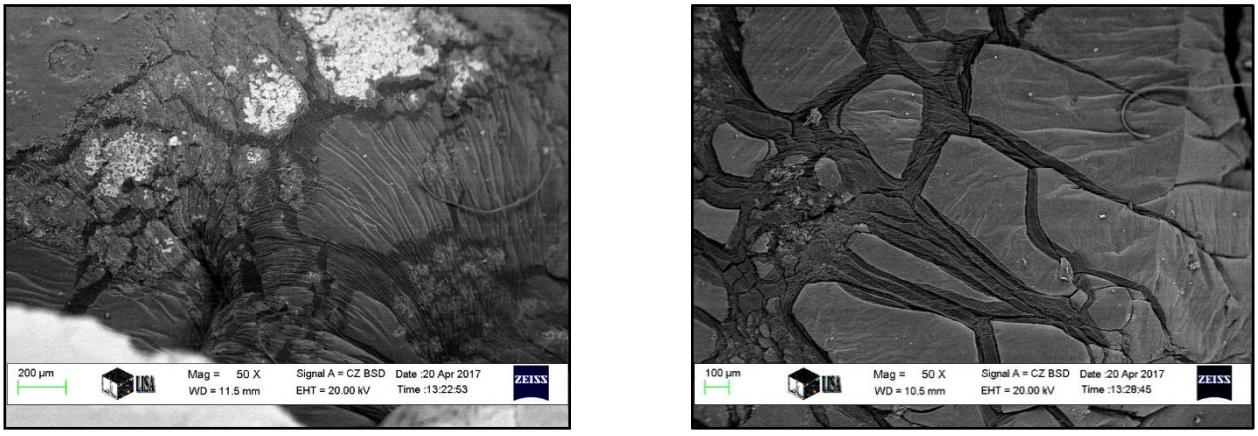

FIG. 6. SEM Images of soot-cured (a) and nanotube-cured (b) vulcanized specimen (50x magnification)

As shown on the figures above, the aging process had effect on both of the samples which appeared in the form of cracks on the surface, also gray-white areas are visible on the soot-treated specimen that may be caused by deta chingstearic acid. This is supported by SEM microscopic shots at 50x magnification, while the FT-IR device has only demonstrated the presence of fillers in the system, it cannot provide an adequate quantitative value, which is certain that a peak of similar intensity of 2000 cm^{-1} for both samples. This is the wavelength range and the vibration frequency of the carbon atoms in the carbon nanotube.

V. SUMMARY

The purpose of our research was to examine the mechanical and aging properties of two rubber mixtures, one of which was filled with a common industrial carbon, while the other was filled with a single-walled carbon nanotube.Mechanical testing was a Shore A hardness measurement series that was repeated before aging and after aging (0, 30, 60 and 90 minutes). The results supported our expectations that the surface degradation processes caused by UV light and ozone can be traced by changing the mechanical properties of rubber mixtures. In addition, surface morphological devices have been able to confirm the deterioration, which were present in the form of cracks on sample surfaces. At the same time, an unexpected problem occurred in the case of the soot cured mixture, in the presence of gray "blossoms" on the SEM images, which were suspected to have detected stearic acid stabilizer in the future more seriously. In the future, we would like to take a more serious look at this problem, since the compatibility of the stabilizer and the raw material, has impact on the initiation of the accelerated aging of the rubber, which can lead to high performance deterioration and reduced life expectancy.

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Simultaneous analysis of nitro compounds by Voltammetric method combined with the principal component regression (PCR)

Khuat Hoang Binh[1], Tran Van Chung[2], Nguyen Thu Huong[3], Ta Thi Thao[4]

[1,2,3]Institute of Chemistry and Material, Viet Nam
[4]VNU - University of Science, Viet Nam

Abstract— *The ability to simultaneously determine 6 nitro compounds including nitrobenzene (NB), 2-nitrophenol (2-NP), 3-nitrophenol (3-NP), 4-nitrophenol (4-NP), 2,4- dinitrophenol (DNP) and trinitrophenol (TNT) in the same samples was investigated by differential pulse Voltammetry on hanging mercury drop electrodes (HMDE), in acetate buffer of pH 4.6. It was found that peak potentials of voltammetric reduction waves of NB, 2-NP, 3-NP and 4-NP appeared at - 0.297, - 0.251, - 0.267 and - 0.337 V respectively. Under the same conditions there are three peak potentials at -0.076, - 0.161, - 0.267 V for TNT and two peaks at tại - 0.176, - 0.282V for DNT. The peaks of these compounds are very close together. Due to the serious overlapping of Voltammetric peaks of these compounds in the mixtures, so that by a conventional Voltammetry it not possible to individually determine each compound. In this study, the linear multivariate regression method such as principal component regression (PCR) was used to resolve the overlapped Voltammograms. The obtained relative standard error (RSEt) of method is less 10%. The percent of recoveries were within ± 10% of the target value. The developed method was then applied to the analysis of these nitro-substituted aromatic compounds in field samples with similar satisfactory results.*

Keywords— Nitrobenzene, 2-nitrophenol, 3-nitrophenol, 4-nitrophenol, Dinitrotoluene, Trinitrotoluene, PCR.

I. INTRODUCTION

The simultaneous determination methods of the nitro - compound mixtures in the environmental samples have received considerable attention, because they are widely used to in industries to control the pollution of individual pollutants present in waste water. The high level toxicity of nitro- compounds and their propagation through environment are capable of polluting land, water, air and affecting on human, animal health, fish, aquatic organism and other life forms [7]. There is a variety of analytical methods applied to determine these compounds including performance liquid chromatography (HPLC), UV-vis spectrophotometry and electrochemistry. These methods are often complex and time consuming and requiring sample pretreatments involving separation, extraction before analyzing [3,4,5]. For the Voltammetric methods, due to the serious overlapping of their reductive peak potentials caused by the general structural formula, the nitro-compounds cannot be quantitatively determined individually. Therefore these methods are usually limited to analyzing a single chemical composition or determining the sum of the nitro compounds in the mixtures [6-8].

In this study, linear multivariable regression method which is principal component regression (PCR), (applying mathematical, statistical, graphics methods, etc) were applied for experimental planning, optimization of obtained experimental data used to resolve the overlapped Voltammograms of nitro-compounds [3]. Based on the PCR model, each nitro compound such as NB, 2-NP, 3-NP, 4-NP, DNT and TNT was simultaneously determined from Voltammograms of their mixtures. In order to eliminate background effect, multivariable regression equation using the measured signal when carry out the experiment on the sample base instead of only using distilled water.

II. EXPERIMENTAL PART

2.1 Chemicals and apparatus

2.1.1 Chemicals

Chemicals such as TNT, DNT, 2-NP; 3-NP; 4-NP (in solid form) and NB (in liquid) with analytical purity imported from China. The stock solutions contained 100 mg /L of the nitro-compounds (TNT, DNT, 2-NP; 3-NP; 4-NP) were prepared by

accurately weighing 0.100 g of each dissolved them into 1 liter volumetric flask with twice distilled water. NB solution was prepared by adding 83.40 mL NB into 1 liter volumetric flasks with twice distilled water.

The other chemical solutions: NH_4OH, CH_3COOH, CH_3COONH_4 with the analytical purity were available in the LAB.

2.1.2 Apparatus

- Metrohm 797 Computed Electron Analyzer using three-electrode cell, including an HMDE, an Ag–AgCl reference electrode and platinum were auxiliary electrode.
- pH measurements were made with pH INOLAB (Germany).

2.2 Experimental procedures

Preparation of the sample base: Use some collected samples such as surface water, ground water and waste water, mixed together to obtain a mixed solution (A). Accurately determine the concentration of 6 study compounds including NB, 2-NP, 3-NP, -NP, DNT and TNT in the real sample by HPLC or LCMS / MS.

Add a suitable volume of solution each containing a nitro-compound, 0.5 mL of acetate buffer solution (pH 4.6) and 5 mL A into an electrochemical cell and diluted to 10 mL with twice distilled water. The solution was purged with pure nitrogent for 120 s to remove soluble oxygent before analyzing. The electrochemical behaviors as well as optimal condition for the determination by Voltammetry of some nitro- compounds were studied in the previous work [1]. The DPV was applied with the parameters such as the potential range from 0,0 to - 0.6 V; scan rate of 12.5 mV/s; pulse amplitude of 50 mV; stirring rate of 2000 rpm, 4- mercury drop size;10 s rest. The measured data were sampled by a computer at 120 potential points, in the range of 0 and −600mV with 5mV intervals. The analytical samples were prepared including 6 nitro compounds with the predetermined concentration. The concentration of each of nitro-compound denoted independent variables X, the analytical information of maximum peak current at different potential denoted Y parameters respectively. Basing on the obtained data, a relation function between Y and X was established used for analyzing each component in their mixtures [4]. The PCR is used to process with the signal matrix to find the principle component (PC) number. For each multivariate regression model, its correctness was established by the following expression.

2.2.1 Relative standard error (RSE):

$$RSE(\%) = 100\sqrt{\frac{(\sum_{j=1}^{N} C_j - \hat{C}_j)^2}{\sum_{j=1}^{N} (C_j)^2}}$$

Here N is the number of samples, C_j is the concentration of the j^{th} compound in the mixture, $\hat{C}_j$ is the concentration calculated from the regression equation

The sum of the relative standard error (RSEt) of N samples is given as following expression.

$$RSE_t = 100\sqrt{\frac{\sum_{i=1}^{M}\sum_{j=1}^{N} (C_{ij} - \hat{C}_{ij})^2}{\sum_{i=1}^{M}\sum_{j=1}^{N} (C_{ij})^2}}$$

C_{ij} is the concentration of the component i in the sample, $\hat{C}_{ij}$ is the concentration calculated from the regression equation.

III. RESULTS

3.1 Electrochemical characteristics of the reduction process of nitro - compounds

3.1.1 Electrochemical characteristics

The Voltammograms of the nitro-compounds were measured resulting in (Fig,1)

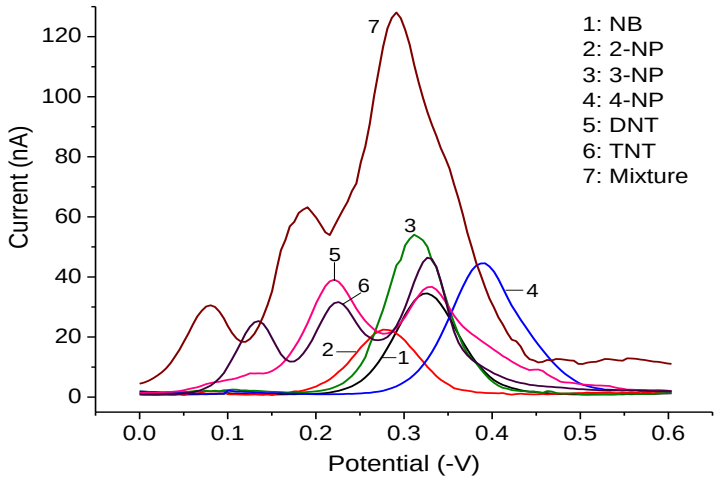

FIG 1: The Voltammograms of NB, 2-NP, 3-NP, 4-NP, DNT, TNT (1ppm) and their mixtures

The Fig.1 showed that electrochemical behaviors of the studied nitro - compounds under the concrete conditions appeared the maximum peak currents such as NB, 2-NP, 3-NP, 4-NP at - 0.297, - 0.251, - 0.267 and - 0.337 V, three TNT peaks at - 0.076 (TNT1), - 0.161 (TNT2), - 0.267 V (TNT3) and two DNT peaks at - 0.176 (DNT1), - 0.282 V (DNT2), respectively.

It can be seen that peak potentials of these components are very close together and there is serious overlapping between the Voltammogram lines so each component cannot be individually identified in the same mixture unless combined with the use of linear multivariate regression to simultaneously determine them.

3.1.2 Determination of the linear concentration range of nitro - compounds

Basing on the linear calibration of peak maximum height versus concentration of single nitro – components (not given here), and using the 6.0 Origin software the analytical characteristics of 6 nitro - compounds are presented in Table 1.

TABLE 1
THE LINEAR CONCENTRATION RANGE OF EACH NITRO – COMPOUND

Nitro-compounds	NB	2-NP	3-NP	4-NP	DNT (2peaks)		TNT (3peaks)		
					DNT1	DNT2	TNT1	TNT2	TNT3
Linear range, ppm	0.01-5.0	0.01-5.0	0.01-5.0	0.01-5.0	0.05-5.0	0.05-5.0	0.05-5.0	0.05-5.0	0.05-5.0
R^2	0.998	0.998	0.999	0.998	0.998	0.999	0.999	0.999	0.989
LOD, ppm	0.0045	0.0061	0.0033	0.0021	0.0062	0.004	0.0136	0.0053	0.0064
LOQ, ppm	0.0149	0.0202	0.0111	0.0071	0.0208	0.0132	0.0454	0.0176	0.0215

3.2 Multivariate regression equation

The multivariate regression equation was established based on PCR model. The concentration matrix (60 x 6) was established from the experimental data of 75 standard samples (including 60 standard samples and 15 standard samples for testing matrix) simultaneously containing 6 nitro- compounds such as NB, 2-NP, 3-NP, 4-NP, DNT and TNT with the concentration range of 0.5-2.5ppm respectively. Sample matrix concentration and standard sample size (60 x 6) and (15 x 6). The Voltammetric currents corresponding analyzed concentration were measured at a given potential from 0 to -0.6 V, then basing on the measured signal matrix (60 x 120) and (15 x 120) and using Matlab software the electrolyte concentration in the mixtures would be calculated.

3.3 Evaluation of the validity of the multivariate regression model

3.3.1 Selection of the principle components (PC) of multivariate regression

The selection of PC (n-Factor) was based on building a test matrix containing standard tests with nitro – compounds as following.

A test matrix was constructed to check the validity of the multivariate regression model. The standard tests of 15 samples containing all inclusive NB, 2-NP, 3-NP, 4-NP, DNT and TNT with their known concentrations corresponding the multivariate calibration, Table 2.

TABLE 2
CALIBRATION CONCENTRATION OF NITRO-COMPOUNDS

Samples	Concentrations (Co-ppm)					
	NB	2-NP	3-NP	4-NP	DNT	TNT
1	2.50	2.00	1.50	1.00	1.06	1.52
2	2.50	2.50	2.00	1.50	1.56	2.02
3	2.00	1.80	1.50	2.00	1.06	1.52
4	1.50	1.50	1.00	1.50	1.06	1.52
5	1.50	1.50	1.50	1.00	0.56	1.02
6	1.00	2.00	1.50	1.50	0.56	1.52
7	1.50	2.00	1.50	2.00	2.06	2.02
8	2.00	1.50	1.00	1.50	1.06	2.02
9	1.60	1.20	1.20	1.20	1.20	1.20
10	2.00	1.60	0.80	0.80	0.80	0.40
11	1.00	1.50	2.00	0.50	1.00	0.50
12	1.60	1.30	1.00	1.00	1.50	1.50
13	1.60	1.90	1.00	1.50	1.50	1.00
14	2.00	1.90	1.50	1.00	2.00	1.50
15	1.20	1.20	1.20	0.80	1.20	0.80

The experimental data represented in the signal matrixes PCR (15 x 120) and PLS (15 x 120) and with the MATLAB software were used to calculate the concentration of each nitro - compound in the mixtures.

The selection of the principle components was based on the dependence of RSEt (%) versus n factors (Fig.2).

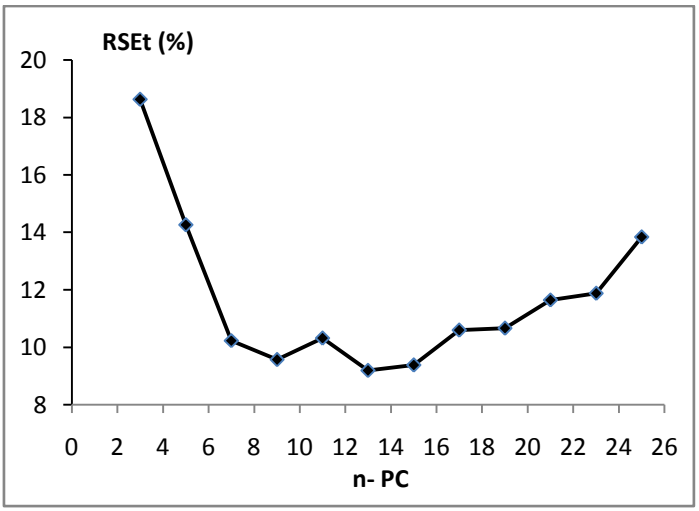

FIG. 2. Dependence of RSEt on PC wint PCR

The Fig.2 showed that RSEt (%) values of PCR model lower sharp from PC 6 to 15. The lowest RSEt can be obtained when n = 13 that was selected for further experiments.

3.3.2 Evaluation of the validity of the multivariate regression model

Using PC = 13 PCR model the relative error was calculated resulting in Table 3.

TABLE 3
THE OBTAINED RELATIVE ERRORS CORRESPONDING PC=13

Sample		Concentration (Co-ppm)					
		NB	**2-NP**	**3-NP**	**4-NP**	**DNT**	**TNT**
	RSE (%)	9.3	8.1	7.7	3.4	8.7	8.2
	Re(%)	100.6	101.2	101.6	99.6	94.9	102.4
	RSEt (%)	8.0					

$$R : \text{Recoveries } (\%) = 100 \times \sum_{i=0}^{n} (C \text{ found}/Co)/n$$

The experimental data in Table 3 exhibited that multivariate regression method using PCR model showed good results. When PC = 13 was selected, the RSE(%) relative error of PCR components ranged from 3.4 to 9.3% and RSEt is 8.0%.

3.4 Application to environmental water and industrial waste-water samples

Analysis results of some samples collected by Von-Ampe method combined with multivariate regression using the PCR model are shown in Table 4.

TABLE 4
FIELD WATER SAMPLES PREDICTION BY PCR

		Found (ppm)	Added (ppm)	Found after added (ppm)	R (%)
Sample 1	NB	0.13	1	1.29	116
	2-NP	ND	1	0.96	96
	3-NP	ND	1	0.84	84
	4-NP	0.10	1	1.08	98
	DNT	0.01	1	0.87	86
	TNT	0.04	1	0.92	88
Sample 2	NB	0.51	1	1.48	97
	2-NP	0.70	1	1.61	91
	3-NP	ND	1	0.96	96
	4-NP	0.05	1	0.99	94
	DNT	0.24	1	1.16	92
	TNT	1.09	1	2.05	96

In general, most recoveries are in the range of 90-100%, the poorer recoveries (ca. <95 or >105%) tend to coincide with the 'not detected' or very low estimates of the amount of analyte present in the actual sample or indeed in the standard added.

IV. CONCLUSION

The multivariate regression using principle component regression (PCR) was successfully applied to simultaneously determine all 6 compounds: TNT, DNT, 2-NP, 3-NP, 4-NP and NB by Voltammetric using hanging mercury drop electrodes. Good validation results were obtained: RSE less 10%; recoveries (%) within 10%). As such, PCR method can be used to simultaneously analyze TNT, DNT, 2-NP, 3-NP, 4-NP and NB in the same mixture without separating them. The developed method was then applied to the analysis of the nitro-substituted aromatic compounds in field samples with similar satisfactory results.

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Isopropyl myristate continuous synthesis in a packed-bed reactor using lipase immobilized on magnetic polymer matrix

Mateus V. C. Silva[1], Caio A. P. de Souza[2], Pedro C. de Oliveira[3], Heizir F. de Castro[4], Larissa Freitas[5]

Department of Chemical Engineering, Engineering School of Lorena, University of São Paulo, Lorena, SP 12602-810, Brazil.

Abstract— *The aim of this study was to synthesize isopropyl myristate, an emollient ester, in a continuous-flow packed-bed reactor using Candida antarctica lipase immobilized on poly(styrene-co-divinylbenzene) matrix prepared by suspension polymerization and magnetized by co-precipitation of Fe^{2+} and Fe^{3+} in alkaline medium. To determine the best esterification conditions, we investigated the effects of acid/alcohol molar ratio (1:5, 1:10, and 1:15) on reaction yield in shake flasks. The three tested conditions provided similar results, esterification yields of approximately 80%. An acid/alcohol molar ratio of 1:15 was chosen for further experiments because it allowed for better operability of the bioreactor. Subsequently, we compared the reactor performance in up flow and down flow modes. This experiment showed that greater ease of operation was achieved with down flow operation. We also evaluated the influence of space time (8 and 20 h) on reaction yield and productivity. A space time of 8 h provided better results. An experimental system consisting of two bioreactors and a molecular sieve packed column was used to remove the water formed during esterification and thus increase the yield of isopropyl myristate. There was a significant improvement in performance with the use of the two-stage system, which resulted in almost complete conversion of reagents, an increase of about 150% in biocatalyst half-life, and an isopropyl myristate productivity of 25 g L^{-1} h^{-1}, confirming the beneficial effect of adding a water extraction column to the experimental system.*

Keywords— esterification, isopropyl myristate, lipase immobilized, magnetic particles, packed-bed reactor.

I. INTRODUCTION

Isopropyl myristate is an emollient ester widely used in cosmetic preparations, especially in skin care products, because of its excellent spreading properties, non-toxicity, great biocompatibility, and high skin permeation ability [1-2]. In the pharmaceutical industry, isopropyl myristate is used as a skin penetration enhancer in topical formulations for transdermal drug delivery[3].

Currently, most industrial processes for the synthesis of isopropyl myristate use conventional chemical catalysis at elevated temperatures, which affords a low-quality product with residual color and odor, demanding expensive purification steps before the product can be marketed [1]. Efforts have been intensified to replace industrial chemical processes with eco-friendly methods. A major problem in chemical industries is the use of chemical catalysts, as these compounds generate waste, have high environmental impact, and increase purification costs [4]. Bioprocesses can be a sustainable alternative to a wide variety of conventional chemical processes.

Enzymes have advantages over chemical catalysts, not only in terms of environmental impact but also in terms of productivity, specificity, toxicity, and temperature and pressure reaction conditions. Among the enzymes that are used industrially, lipases (EC 3.1.1.3) are notable for catalyzing reactions in aqueous and organic media, such as esterification and hydrolysis reactions. This class of enzymes has applications in the manufacture of pharmaceutical products, surfactants, and cosmetics [5]. Industrial biocatalysis can benefit from enzyme immobilization techniques to increase the biocatalyst's thermal stability and pH stability and allow its recovery and reuse both in batch and continuous reactors [6].

Several materials have been researched as enzyme supports. Magnetic materials are outstanding for this application because they can be easily recovered from the reaction medium, which obviates the need for centrifugation, filtration, or column separation steps [7]. When lipase is immobilized on a highly hydrophobic support, such as a poly (styrene-*co*-divinylbenzene) matrix, the hydrophobic lid, which controls access to the catalytic site, interacts with the support, exposing the enzyme's active site and increasing its affinity for the substrate, a mechanism known as interfacial activation. Another advantageous characteristic of hydrophobic supports is that they absorb less water from the reaction medium, which is desirable in esterification reactions [8].

The present work aimed to study the continuous enzymatic synthesis of isopropyl myristate in a fixed-bed reactor using lipase immobilized on a magnetic matrix as biocatalyst. The influence of the molar ratio of starting materials, feed flow direction, space time, and use of a water extraction column on reaction yield and productivity was investigated with the aim of developing a stable enzymatic process with potential industrial application.

II. MATERIALS AND METHODS

2.1 Materials

Polyvinyl alcohol (PVA) (MW 78,000, 88% hydrolyzed; Polysciences Inc.), azobisisobutyronitrile (AIBN) (MIG Química), divinylbenzene (80%; Sigma–Aldrich®), styrene (99%; Sigma–Aldrich®), heptane (95%; Cromoline), methanol (99.8%; Cromoline), and toluol (99.5%; Cromoline) were used for the synthesis of the magnetic poly(styrene-*co*-divinylbenzene) matrix. Ethyl acetate (99.5%; Cromoline), ultrapure water, sodium hydroxide (99%; Synth), iron(II) chloride tetrahydrate (99%; Sigma–Aldrich®), iron(III) chloride hexahydrate (97%; Sigma–Aldrich®), oleic acid (p.a.; Cromoline), and ethanol (96%; Cromoline) were used for the synthesis and modification of magnetite. The following materials were used for enzyme immobilization: *Candida antarctica* lipase (Sigma–Aldrich®), polyethylene glycol (PEG) (MW 1,500; Synth), and heptane (95%; Cromoline). Methyl butyrate (99%; Sigma–Aldrich®), potassium dihydrogen phosphate (99%; Cromoline), ethanol (96%; Synth), sodium hydrogen phosphate (99%; Synth), and potassium hydroxide (85%; Synth) were used for determination of enzyme activity. Pure myristic acid (Cromoline), isopropyl alcohol (99.5%; Cromoline), heptane (95%; Cromoline), and 3A molecular sieve (8–12 mesh beads; Sigma–Aldrich®) were used for the synthesis of isopropyl myristate.

2.2 Methods

2.2.1 Determination of enzyme activity

Enzyme activity was quantified by measuring the hydrolysis of methyl butyrate in 25 mmol L^{-1} phosphate buffer medium, pH 7.0, at 200 rpm and 45 °C in shake flasks, according to the modified method described by Fidalgo et al. (2016) [9]. Briefly, 300 μL of methyl butyrate and 30 mL of phosphate buffer were added to Erlenmeyer flasks. Then, 0.05 g of immobilized *C.antarctica* lipase was added to each flask. After 10 min of reaction,10 mL of ethanol was added, and samples were titrated with 0.04mol L^{-1} KOH. The mean activity of the biocatalyst was 479.12 ± 14.35 U g^{-1}.

2.2.2 Synthesis of poly(styrene-*co*-divinylbenzene) magnetized with modified magnetite

The poly (styrene-*co*-divinylbenzene) matrix (STY-DVB-M) was synthesized following the method of Bento et al. (2017) [10]. Magnetite, required for polymer synthesis, was synthesized by co-precipitation of iron ions. Iron (II) chloride (0.6 mol L^{-1}) and iron (III) chloride (1.2 mol L^{-1}) solutions were mixed and kept at 65°C under stirring. Sodium hydroxide (4 mol L^{-1}) was added slowly until pH 11. After reaching the desired pH, the ferric solution was kept at 65°C under stirring for 30 min. The flask was placed on a magnet to precipitate the magnetic particles. The resulting black material was decanted and washed successively with ultrapure water and a 1:1 ethyl acetate/water solution until the supernatant reached pH 7.0. The mixture was vacuum filtered and oven dried at 60°C for 18 h to afford magnetite particles.

Magnetite was modified with oleic acid to increase its hydrophobicity. Briefly, 3.25 g of magnetite, 40 mL of oleic acid, and 80 mL of ultrapure water were added to a beaker and kept under stirring for 15 min. The mixture was vacuum filtered, washed with ethanol to remove the residual oleic acid, and oven dried at 60 ° C for 18 h.

The polymer support was synthesized by suspension polymerization, in which styrene and divinylbenzene monomers comprised the organic phase, toluol and heptane were respectively the high-affinity and low-affinity solvents, AIBN was the initiator, and the aqueous phase was an aqueous PVA solution. The reaction was conducted in a 1 L glass reactor at 400 rpm and 70°C under an inert atmosphere (nitrogen gas). After polymerization, particles were vacuum filtered and washed sequentially with ultrapure water at room temperature, ultra pure water at 50°C, acetone, and ethanol to remove the aqueous phase and residual reagents. The material was then oven dried at 60°C for 18 h and sieved to 24–80 mesh size using an electromagnetic sieve shaker (Sppencer).

2.2.3 Immobilization of *C.antarctica* lipase on STY-DVB-M

STY-DVB-M was immersed in heptane at a ratio of 1:10 (w/v) and kept under stirring for 2 h on a shaker. Excess heptane was removed, and flasks received the addition of 100 μL of 5 g L^{-1}PEG 1500 and 250 μL of lipase per gram of STY-DVB-M. The mixture was homogenized and then kept still for 18 h at 4 °C. Finally, immobilized lipase was vacuum filtered and washed with heptane until the moisture content was reduced to less than 10%.

2.2.4 Substrate preparation

The substrate for isopropyl palmitate synthesis was prepared in 500 mL glass flasks using appropriate amounts of myristic acid, isopropanol, and 10% (w/v) molecular sieves previously activated.

2.2.4.1 Esterificationin shake flasks

Isopropyl myristate synthesis was carried out at 50 °C and 200 rpm in shake flasks containing 20 mL of substrate and 10% (w/v) biocatalyst. At predetermined intervals (0.15, 30, 45, 60, 90, 120, 180, and 240 min), aliquots of approximately 0.1 g were withdrawn to monitor product formation.

2.2.4.2 Continuous esterification in a fixed-bed bioreactor

Fixed-bed bioreactor experiments were carried out in a jacketed glass column (Diogolab®) with a height of 166 mm, an internal diameter of 11 mm, and an internal volume of 15.8 mL. The height/diameter relationship was defined on the basis of previous work [11]. The reactor was packed with 4.3 g of biocatalyst, and substrates were fed using a peristaltic pump (Sci-Q 400, Watson-Marlow). The reaction was conducted at 50 °C. The reaction scheme is shown in Figure 1.

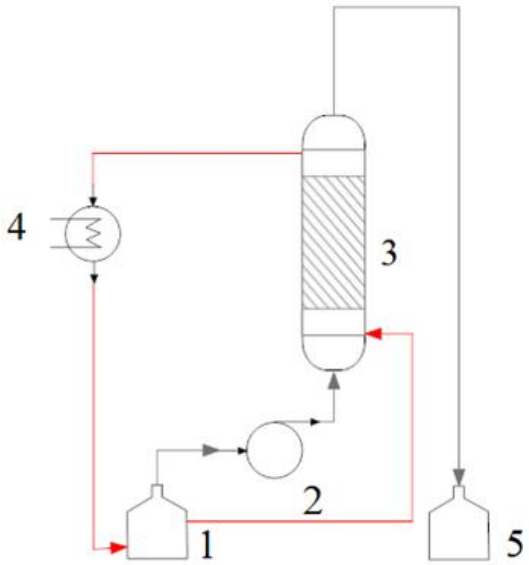

FIGURE 1 – Experimental lay out of the packed bed reactor used in the synthesis of isopropyl myristate under continuous flow, where: 1-feeding reservoir; 2-peristaltic pump; 3-glass column; 4-thermostated bath; 5 – product reservoir.

Space-time was calculated according to Equation 1:

$$\tau = \frac{V_{working}}{Q} \quad (1)$$

In which: V= Reactor working volume reactor (mL) and Q= flow rate (mL /min).

The reactor working volume was calculated as the difference between the total reactor volume and the volume occupied by the biocatalyst. The density of the biocatalyst was 1.11 g L^{-1}, calculated according to Simões et al. (2015)with modifications[12].

2.2.4.3 Strategy for water removal from the reaction medium

To remove the water formed during the esterification reaction and thus shift the equilibrium to the side of ester formation, we operated the reactor in a two-stage configuration with a water extraction column (packed with molecular sieves) between the two bioreactors.

The experimental system comprised two jacketed glass columns (166 mm in height, 11 mm in internal diameter, and 15.8 mL in internal volume), each packed with 4.3 g of biocatalyst, and a water extraction column containing 10.8 g of molecular sieves. The immobilized enzyme/molecular sieve relationship was determined by Freitas et al. (2011)[13] and corresponds to a 1:1.25 (w/w) ratio. Molecular sieves were replaced in the water extraction column every 3 days of continuous operation. Figure 2 illustrates the two-stage reactor system.

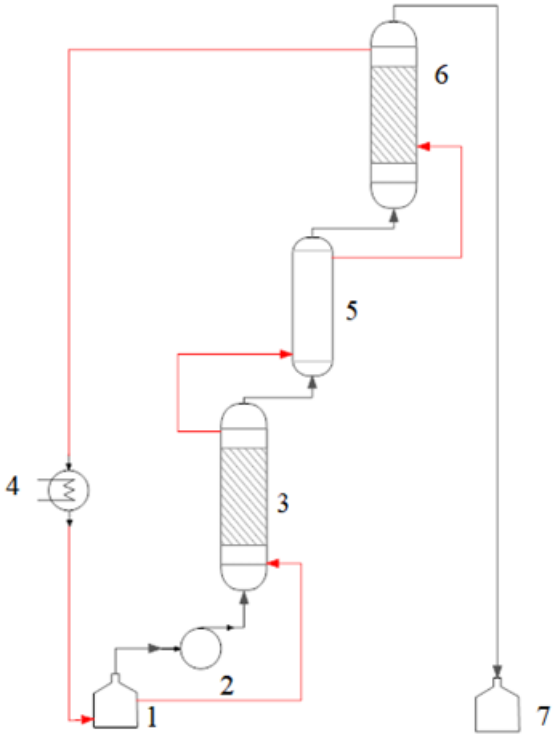

FIGURE 2- Experimental set up used for esterification of isopropanol with myristic acid in two-stage packed bed reactor connecting with water column extractor: 1-feeding reservoir; 2-peristaltic pump; 3 and 6-enzyme packed columns; 4-bath for temperature control; 5-column with molecular sieves and 7-product reservoir.

2.2.5 Determination of isopropyl myristate content

Quantification of isopropyl myristate was performed using a PerkinElmer® Clarus 580 gas chromatograph equipped with a flame ionization detector (FID) and a 5% diphenyl, 95% dimethyl polysiloxane capillary column coated with glass fiber. Isopropyl myristate standard was synthesized following the methods proposed by Vilas Bôas et al. (2017) [14]. FID was supplied with synthetic air at 400 mL min^{-1} and H_2 at 40 mL min^{-1}. N_2 was used as carrier gas at a flow rate of 0.2–1.0 mL min^{-1}. Detector and injector were maintained at 250 °C.

Temperature program was started at 105 °C for 7 min with an N_2 flow rate of 0.2 mL min^{-1}, followed by ramp to 200 °C at 20 °C min^{-1} and hold for 1 min and ramp to 280 °C at 25 °C min^{-1} and hold for 2 min, totaling 17.95 min of analysis. The injection volume was 1 µL of a 1:1 mixture of sample and internal standard (8.0 g L^{-1}hexanol in heptane medium).

2.2.6 Monitoring of esterification by quantification of the limiting reagent (myristic acid)

Concentration of myristic acid was monitored by titration with aqueous KOH solution (0.04 mol L^{-1}) using phenolphthalein as indicator. Aliquots (0.1 g) of the reaction medium were added to 10 mL of ethanol. Esterification yield was calculated on the basis of the stoichiometric ratio of synthesized ester and considering that myristic acid was completely converted to isopropyl myristate, according to Equation 2:

$$Esterification\ yield\ (\%) = \left(\frac{C_{ester}}{C_i}\right) * 100 \quad (2)$$

In which: C_i = Initial concentration of the limiting reactant (myristic acid, mmol·L^{-1}) and C_{ester} = Ester concentration at a given time (mmol·L^{-1}).

2.2.7 Operational stability of the biocatalyst

The operational stability of the biocatalyst was evaluated according to the method proposed by Pires-Cabral et al. (2010) [15]. The highest esterification yield was used as the reference activity (100% catalytic activity), and the residual activity was determined by the ratio of esterification yield at a given time to the highest esterification yield. The best linear fit to the data was calculated using Origin 9.0.

III. RESULTS

3.1 Influence of acid/alcohol molar ratio on isopropyl myristate synthesis in shake flasks

Aiming to obtain the optimal experimental condition for the synthesis of isopropyl myristate in solvent-free medium, we tested the following acid/alcohol molar ratios: 1:5, 1:10, and 1:15. The 1:5 acid/alcohol molar ratios is the minimum required for solubilization the fatty acid at 50 °C.

Vadgma, Odaneth, and Lali (2015) [1] reported that the optimal temperature for the activity of *C. antarctica* lipase, the same lipase used in the present work, immobilized on acrylic resin (Novozym 435) was 50–60 °C. Thus, 50 °C was the reaction temperature used in the present study. Results are shown in Figure 3.

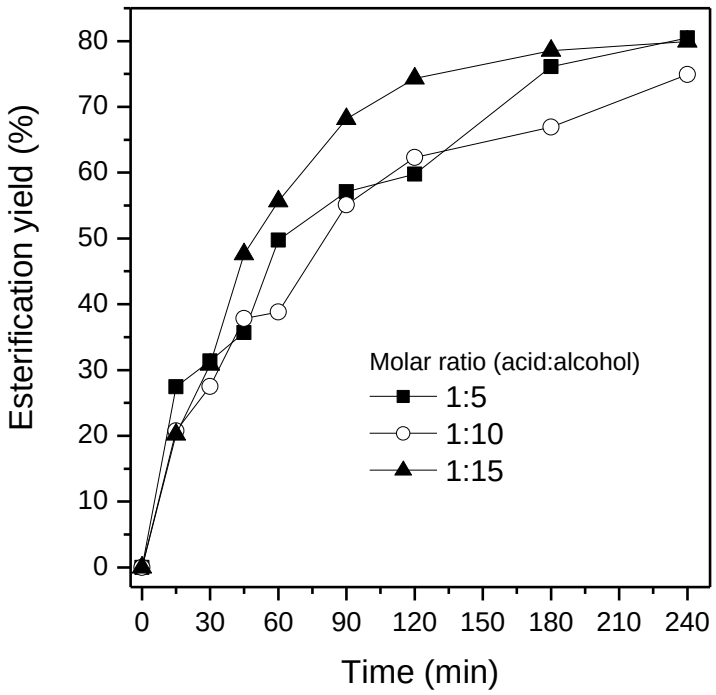

FIGURE 3–Esterification progress in the synthesis of isopropyl myristate catalyzed by CALB-STY-DVB-M at 50ºC, in shake flasks, using different molar ratio acid to alcohol and 10% (m/v) of biocatalyst in solvent free medium

No significant differences were observed among the esterification yields obtained under the three conditions. Yields of approximately 80% were obtained in 240 min of reaction. The reaction yield was not influenced by the increase in the proportion of isopropyl alcohol (reagent).

3.2 Study of flow direction in the single-stage continuous fixed-bed bioreactor

The acid/alcohol molar ratio of 1:15 was chosen for this experiment, as the viscosity of the reaction medium is proportional to the concentration of fatty acids; that is, the lower the concentration of fatty acids, the lower viscosity of the reaction medium. It is known that the use of a high-viscosity reaction medium in a fixed-bed bioreactor can cause pressure drop, affecting the substrate flow rate and thereby reducing the reaction yield [16].

Continuous esterification was carried out in a fixed-bed reactor operating in upflow and down flow modes to investigate the influence of flow direction on the synthesis of isopropyl myristate. Feed can be pumped down flow, which leads to a lower bed pressure drop as a result of the action of gravitational force, or upflow, which minimizes flow channeling. In upflow mode, however, the maximum flow rate is limited, as it cannot exceed the minimum fluidization velocity; otherwise, the bed will fluidize [16-18].A feed rate of 0.0405 mL min^{-1} was used, totaling a space time of 5 h. Results are exhibited in Figure 4.

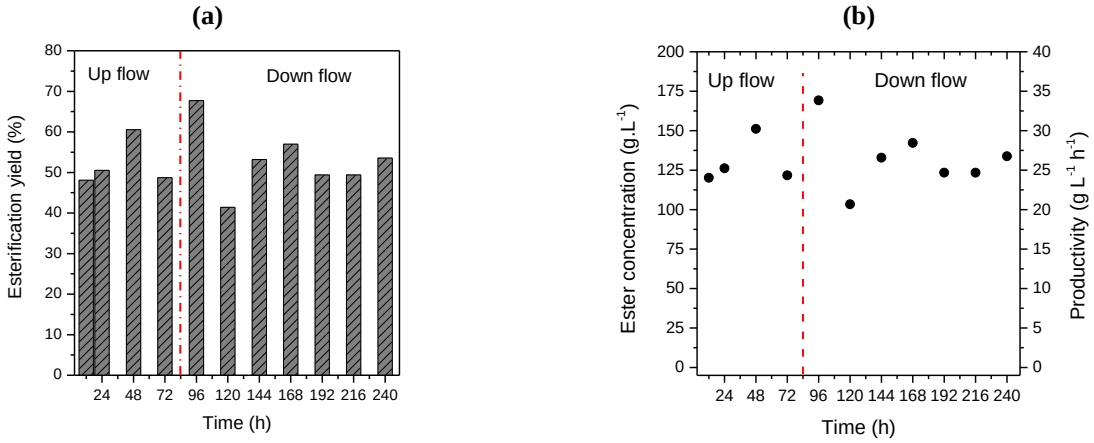

FIGURE 4 – a) Esterification yield and b) Ester concentration and volumetric productivity in the isopropyl myristate synthesis carried out under continuous flow in packed bed bioreactor catalyzed by CALB-STY-DVB-M at 50°C, using up flow and down flow rates (space-time 5h).

As shown in Fig. 4a, mean esterification yields of 55% were obtained regardless of the flow direction. However, down flow resulted in higher stability and less bubble formation inside the bed, probably because of the lower pressure drop.

Freitas et al. (2011) [13] synthesized monoglycerides from babassu oil using lipase from *Burkholderia* cepacia immobilized on SiO_2-PVA and observed significant differences in monoglyceride yieldsbetween the two reactor configurations: a 5% yield was obtained with upflow, whereas a 22% yield was obtained with downflow. According to the authors, this result was due to insufficient homogenization of the substrate when pumped upflow, which hampered catalysis. Fidalgo et al.(2016)studied the influence of flow direction on biodiesel production using Novozym 435 and obtained similar yields (approximately 90%) in both flow directions[9]. These results might be associated with the low feed rate used in each study, as was the case of the present work. The down flow mode was used in subsequent experiments.

3.3 Study of feed rate in the single-stage continuous fixed-bed bioreactor

To increase the performance of the evaluated system, we investigated the influence of feed rate on isopropyl myristate synthesis. Feed rates of 0.025 mL min^{-1} and 0.01 mL min^{-1} were tested, resulting in space- times of 8 and 20 h, respectively. Figure 5 shows the results obtained with an 8 h space-time, and Figure 6 presents the results obtained with a 20 h space-time

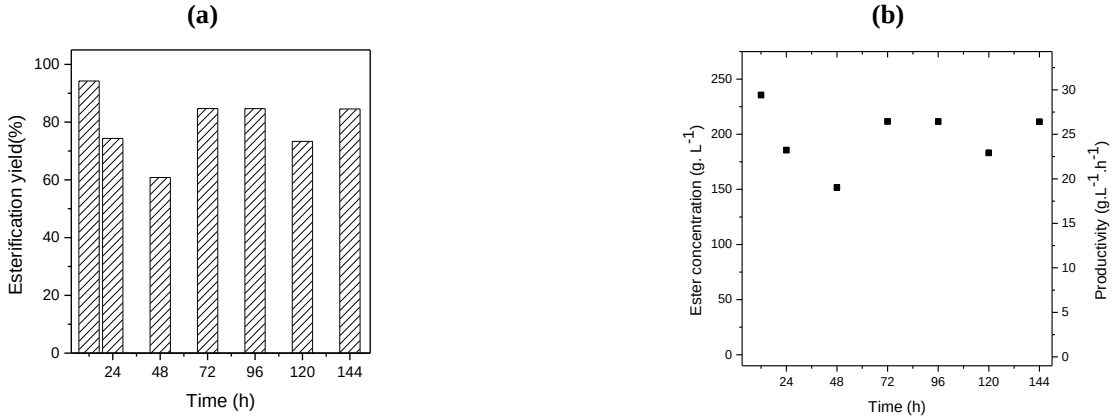

FIGURE 5 – a) Esterification yield and b) Ester concentration and volumetric productivity in the isopropyl myristate synthesis carried out under continuous flow in packed bed bioreactor catalyzed by CALB-STY-DVB-M at 50°C, using down flow rates (space-time 8h).

A space time of 8 h resulted in an 80% yield and 25 g L^{-1} h^{-1} productivity. Similar results were found by Vadgama et al. (2015) [1], who studied the continuous synthesis of isopropyl myristate by esterification of myristic acid with isopropanol in a fixed-bed bioreactor using Novozym 435 as biocatalyst. The operating conditions were acid/alcohol molar ratio of 1:15, 60°C, and space time of 5 h. Yields of approximately 80% were obtained.

An esterification yield of 70% and a productivity of 8 g L^{-1} h^{-1} (Fig. 6b) were obtained with a space time of 20 h. The lower yield, compared with that obtained with a space time of 5 h, can be explained by the increase in water formed during the esterification reaction, contributing to the reversion of esterification as a result of the longtime of contact between substrate and biocatalyst. An option to minimize this effect is to remove the water formed in the reaction medium.

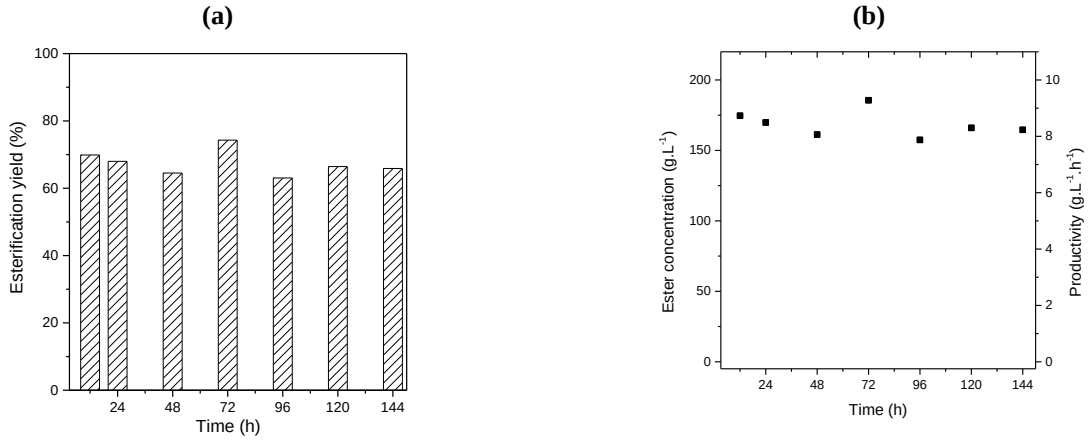

FIGURE 6– a) Esterification yield and b) Ester concentration and volumetric productivity in the isopropyl myristate synthesis carried out under continuous flow in packed bed bioreactor catalyzed by CALB-STY-DVB-M at 50°C, using down flow rates (space-time 20h).

3.4 Synthesis of isopropyl myristate in a two-stage fixed-bed bioreactor with a water extraction column

As stated earlier, the water formed during esterification affects substrate conversion; lower conversions are obtained with increased water contents [19]. Several strategies for water removal are described in the literature, such as pressure reduction; use of drying agents (molecular sieve or silica gel), hydrophilic solvents, or salt pairs; and dry air bubbling [20]. Furthermore, reduction of residual fatty acid in the reaction medium facilitates the separation and purification of the product.

We evaluated the performance of a water extraction column containing molecular sieves in the continuous system studied. A feed rate of 0.04 mL min^{-1} was used, which corresponds to a space time of 10 h. Results are shown in Figure 7.

Under these operating conditions, conversion to isopropyl myristate was almost complete. Esterification yield and productivity were 99% and 25 g L^{-1} h^{-1}, respectively. These results indicate that molecular sieves were effective in removing water from the reaction medium, which shifted the equilibrium toward ester formation and consequently increased the yield. In addition, the extraction column provided greater stability to the system. No variations were observed during 6 days of operation.

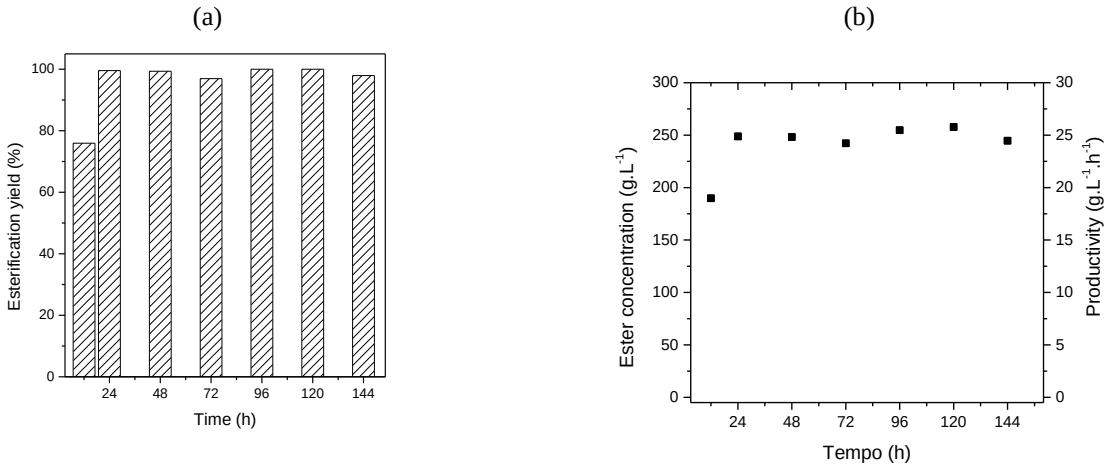

FIGURE 7 -a) Esterification yield and b) Ester concentration and volumetric productivity in the isopropyl myristate synthesis carried out under continuous flow in two stage packed bed bioreactor coupling with water extraction column catalyzed by CALB-STY-DVB-M at 50°C, using down flow rates (space-time 10h).

3.5 Operational stability of the biocatalyst

The operational stability of immobilized enzymes is a parameter of fundamental importance in reactions carried out for long periods. The operational stability of *C. antarctica* immobilized on STY-DVB-M was evaluated in the single-stage (τ = 8 h) and the two-stage (τ = 10 h) packed-bed reactor systems. Figure 8 shows the residual activity of the biocatalyst in both processes.

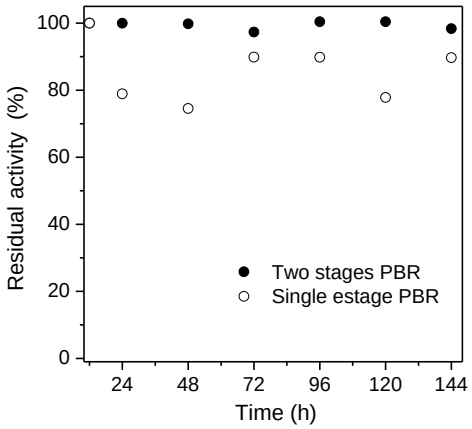

FIGURE 8 – Residual esterification activity of the lipase from *Candida antarctica* immobilized on STY-DVB-M quantified on continuous runs carried on packed bed reactors (single stage and two-stages)

The biocatalyst had a half-life of approximately 35 days (Kd = 0.00137 h^{-1}) in the single-stage reactor, whereas, in the two-stage reactor, the half-life was 51.7 days (Kd = 0.000559 h^{-1}). The use of the water extraction column significantly increased the half-life of the biocatalyst (by approximately 150%), which made the process more stable.

Similar results were observed by Freitas et al. (2011) [13]. The authors studied the synthesis of monoglycerides by esterification of oleic acid and glycerol with *Penicillium camemberti* lipase immobilized on SiO_2–PVA as biocatalyst in a continuous fixed-bed reactor. An increase in the half-life of the enzyme (total of 19 days) was achieved with the use of a water extraction column. In a study by Lee et al. (2013) [21], who used lipase Novozym 435 to synthesize erythorbyl laurate, a two-fold increase in the half-life of the biocatalyst was observed with the use of a potassium resin to remove water from the reaction medium.

The results suggest that the biocatalyst can be used for several cycles of operation without significant loss of productivity, an economic and environmental advantage. This bioprocess is a competitive alternative to conventional chemical processes.

IV. CONCLUSION

No significant differences in esterification yields were observed among the three tested acid/alcohol molar ratios (1:5; 1:10, and 1:15). Therefore, an acid/alcohol ratio of 1:15 was used for the synthesis of isopropyl myristate in a continuous packed - bed reactor, as it resulted in lower substrate viscosity and, consequently, greater operability. The down flow mode was selected because it caused a low bed pressure drop as compared with the upflow mode.

In the single-stage reactor, the highest yield and productivity were obtained with a space-time of 8 h. The extraction column used in the two-stage reactor was effective in removing the water formed during esterification and in increasing the stability of the bioprocess. Almost complete conversion of myristic acid to isopropyl myristate was obtained, and the half-life of the biocatalyst increased by approximately 150%.

The results were satisfactory, and reaction and operational conditions were established for the continuous enzyme-catalyzed synthesis of isopropyl myristate in solvent-free medium conducted in a fixed-bed bioreactor. The bioprocess is an interesting option for large-scale industrial application

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