

SUPPORTING INFORMATION

Novel nanobiocatalyst constituted by lipase from *Burkholderia cepacia* immobilized on graphene oxide derived from grape seed biochar

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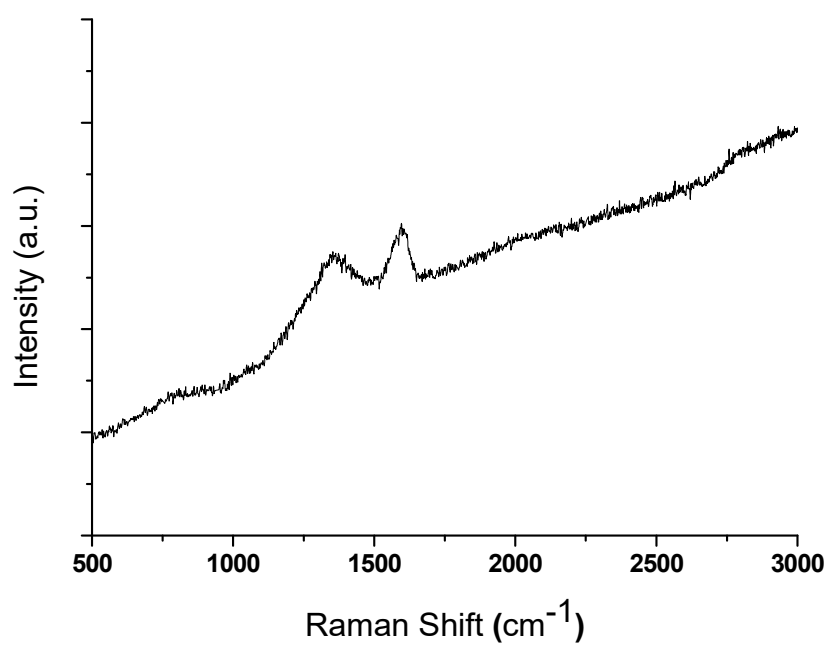


Figure S1. Raman spectrum of GO obtained from grape seed biochar. For Raman spectra were recorded from 750 to 3500 cm^{-1} on a Raman Renishaw 2000 Microprobe Confocal (Renishaw Instruments, England) using a 514.5 nm argon ion laser.

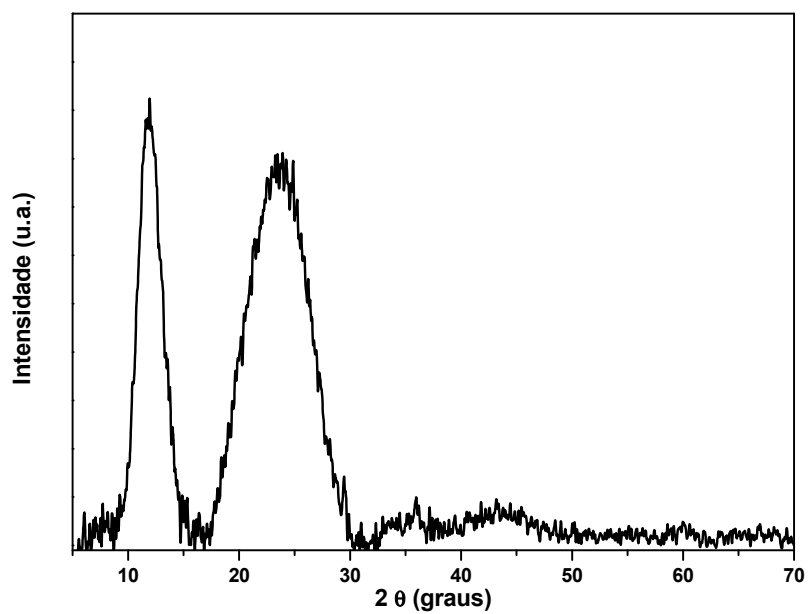


Figure S2. X-ray diffraction (XRD) pattern of graphene oxide from grape seed biochar. For recording XRD patterns, a diffractometer with Cu K α radiation operating at 40 kV with 40 mA was used while 2θ values tested for ranged between 5–80°.

Table S1. Effect of pH variation on BCL immobilization process.

pH immobilization	Hydrolytic activity (U/g)	Relative activity (%)
4	145 ± 1	52 ± 1
6	93 ± 2	38± 2
7	52 ± 3	28± 3
8	50 ± 1	29± 1
10	5 ± 2	1± 2

Table S2. Distribution of amino acids of the BCL and their respective charges for pH 4, 7 and 10.

	ARG		ASP		GLU		HIS		LYS		TYR	
	-	+	-	+	-	+	-	+	-	+	-	+
pH 4		8	2	55	63	28	15	204		22		4
		40	21	56		35	86	286		70		9
		61	36	228		118	114		80		23	
		94	102	288		197	311		165		29	
		115	121			289			269		31	
		258	130			302			283		45	
		297	159						316		68	
		309	236								95	
		314	242								129	
			264								175	
			303								179	
											207	
											274	
										282		
pH 7	ARG		ASP		GLU		HIS		LYS		TYR	
	-	+	-	+	-	+	-	+	-	+	-	+
		8	2		28	289	15	286		22		4
		40	21		35		86		70		9	
		61	36		63		114		80		23	
		94	55		118		204		165		29	
		115	56		197		311		269		31	
		258	102		302				283		45	
		297	121						316		68	
		309	130								95	
		314	159								129	
			228								175	
			236								179	
		242								207		
		264								274		
		288								282		
		303										
pH 10	ARG		ASP		GLU		HIS		LYS		TYR	
	-	+	-	+	-	+	-	+	-	+	-	+
		8	2		28	289	15		22		4	23
		40	21		35		86		70		9	29
		61	36		63		114		80		31	
		94	55		118		204		165		45	
		115	56		197		286		269		68	
		258	102		302		311		283		95	
		297	121						316		129	
		309	130								175	
		314	159								179	
			228								207	
			236								274	
		242								282		
		264										
		288										
		303										

The amino acids are located on the surface of the enzyme molecule

Table S3. Immobilization Yield (%), Hydrolytic activity (U/g) and Relative activity (%) as a function of enzyme/support ratio during immobilization of the BCL on graphene oxide.

((g)enzyme/ (g) support)	Immobilization Yield (%)	Hydrolytic activity (U/g)	Relative activity (%)
0,15	22 ± 1	107 ± 1	23 ± 1
0,225	24 ± 2	180 ± 2	39 ± 2
0,3	32 ± 2	290 ± 2	64 ± 2
0,375	36 ± 1	457 ± 1	100 ± 1
0,45	33 ± 2	438 ± 2	96 ± 2

Table S4 pH effect on the activity the immobilized BCL by physical adsorption (PA) onto Graphene Oxide

pH	Hydrolytic activity (U/g)	Relative activity (%)
2	279 ± 3	60 ± 3
3	462 ± 2	100 ± 2
4	240 ± 2	52 ± 2
5	174 ± 3	38 ± 3
6	130 ± 2	28 ± 2
7	135 ± 2	29 ± 2
8	1 ± 2	1 ± 2
9	0	10
10	0	0

Table S5. Temperature effect on the immobilized BCL by physical adsorption (PA) onto graphene oxide.

Temperature (°C)	Hydrolytic activity (U/g)	Relative activity (%)
40	493 ± 3	74 ± 3
50	661 ± 2	100 ± 2
60	570 ± 2	86 ± 2
70	420 ± 3	63 ± 3
80	423 ± 2	64 ± 2

Table S6. Thermal stability of the immobilized biocatalyst incubated at 50° C.

Incubation time (min)	Hydrolytic activity (U/g)	Relative activity (%)
0	662 ± 1	100 ± 1
30	528 ± 2	79± 2
60	453 ± 2	68± 2
120	444 ± 3	67± 3
180	324± 1	49± 1

Table S7. Operational stability of the immobilized biocatalyst.

Cycle number	Hydrolytic activity (U/g)	Relative activity (%)
1	685 ± 3	100 ± 3
2	642 ± 2	94± 2
3	520 ± 2	76± 2
4	473 ± 3	73± 3
5	251 ± 2	69± 2