1st Call for H.F.R.I. Research Projects for the Support of Faculty Members and Researchers and the Procurement of high-cost Research Equipment

Title:	Development of sustainable chemoenzymatic processes for optically pure amines from alcohols or alkynes
Project Acronym:	CEPOPA
Project No:	664
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Deliverable	D1.2. Catalytic characterization of GO samples

Report:

All 3 synthesized GO samples were used to prepare aqueous solutions and were tested for their catalytic oxidation activity towards **phenylethanol (PhOH)** and **phenylacetylene (PhAc)**, which according to literature give acetophenone (ACP) when oxidized.[1] Stocks of the reactants were prepared in isopropanol, which on the one hand is organic solvent and on the other hand is miscible in water (solvent of GO). The stocks were prepared in such way that there is 10% isopropanol in the reaction mixture and 100% wt reactant/catalyst content. In preliminary reactions 1 mL of each reaction mixture was placed in a thermoshaker (Cell Media TS Pro) at 80 °C and 600 rpm for 24 h and aliquots were taken (200 μL) at 1, 3, 5 and 24 h in order to check the extend of the reaction, if any. Afterwards, every aliquot was mixed with 1:1 ethyl acetate (extraction solvent) and the extracted samples were kept in the fridge until they were analyzed with gas chromatography (GC). Unfortunately, no reaction was observed for both substrates and for all 3 catalyst solutions.

Solvent-free reactions:

Due to the negative results in aqueous environment, we decided to perform the reactions in solvent-free conditions, as proposed by Dreyer et. Al.[1] In this frame, we screened the GO samples synthesized through Hummer's (**GO-HU**) and Staudenmaier's (**GO-ST**) method with both substrates. Brodie's sample was excluded for 2 basic reasons; (a) The synthetic approach is the less "green" from all 3 and (b) the majority of catalytic reactions involving GO found in the literature concern either GO-HU or GO-ST. A set of 4 reactions was screened in total, as we had two substrates (neat PhOH and PhAc) and two catalysts (GO-HU and GO-ST). The experiments were performed in 2 mL eppendorf tubes at 60ºC using 100% wt reactant/catalyst (~2 mg of each). Due to the small scale of the reaction mixture, samples were taken for GC analysis only at 24 h. The main problem was the extraction process, as every organic solvent used (dichloromethane, chloroform, ethyl acetate) seemed to dissolve GO (color change) and the samples could not be efficiently filtered, due to the nano-scaled nature of GO. In order to avoid extracting GO particles, we used 1:1 water/hexane system as GO remains in the polar phase and organic compounds are extracted in the non-polar phase. Among all 4 reactions, only the combination **GO-HU/PhAc** gave the expected acetophenone peak in the chromatogram, so we decided to focus on this material and this possible substrate. From now on we will be referred to it as **reference reaction**. However, exact conversion could not be determined, due to inefficient extraction. In an experiment (data not shown) after 7 extraction cycles, PhAc and ACP were still present in the chromatogram, giving different conversions in each extraction cycle. We attribute this fact to possible physical and/or chemical adsorption of substrate and product(s) on GO sheets. Having a positive result on the ACP oxidative production, we tried to change various conditions of the reference reaction in the frame of **optimization** and **enzyme compatibility**. The reference reaction schemes screened are presented in Table 1.1.

Table 1.1: *Reference reaction change of parameters for PhAc (or PhOH) catalytic oxidation from GO-HU.*

Substrate	%wt GO/substrate	Time (h)	Temp. (°C)	Solvent	Atmosphere	ACP	by-products
PhAc	100	24	40	-	Air	+	+
PhAc	100	24	50	-	Air	+	+
PhAc	50	24	60	-	Air	+	-
PhAc	50	5	60	-	Air	+	-
PhAc	100	24	60	EtOAc	Air	+	+
PhAc	100	24	60	H_2O	Air	-	-
PhAc	100	24	60	H_2O	O_2	-	-
PhOH	100	24	60	H_2O	O_2	-	-
PhAc	100	24	60	-	O_2	-	+
PhOH	100	24	60	-	O_2	-	+
PhAc	100	24	60	-	N_2	+	+

Despite the difficulty in accurately determining conversions, we conclude in the notion that **GO** acts rather as a **reactant** than as a **catalyst**. This becomes obvious from the fact that GO becomes more **hydrophobic** after the reaction and from the fact that in an **oxygen-free** environment ACP is produced. It is really possible that oxygen is abstracted from the abundance of oxygen functional groups on the surface of GO sheets (epoxides, hydroxyls etc).

Reusability studies:

In the frame of catalytic activity, some experiments we conducted with re-used GO. Specifically, GO dried at 100ºC after the reference reaction, was used for a second reaction cycle with fresh PhAc. Chromatograms showed both PhAc and ACP, and many by-products, after 24 h. In another experiment, already used and dried GO, was mixed with EtOAc, and the liquid extract was analysed through GC. Chromatograms showed that PhAc and ACP were still present in the flakes of GO. As a matter of fact, we still cannot be based on the above results for safe conclusions about the re-usability potential of GO. Our interest will be focused on the compatibility of enzymes and GO, as a carrier.

Literature:

1. *Dreyer D.R., Jia H.-P. & Bielawski C.W. (2010) Graphene oxide: a convenient carbocatalyst for facilitating oxidation and hydration reactions. Angew. Chem. Int. Ed. 49(38): 6813–6816.*