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Thin bacteria/Layered Double Hydroxide films using a layer-by-layer approach

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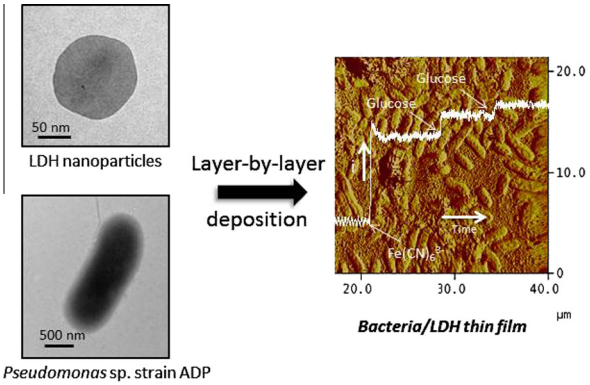
HIGHLIGHTS

- Preparation of $\{bacteria/LDH\}_n$ thin films on ITO electrode by a layer-by-layer deposition method.
- Thin films are formed by electrostatic interactions between *Pseudomonas* ADP and LDH nanosheets.
- UV–Vis spectroscopy shows a progressive assembly process.
- The metabolic activity of immobilized ADP is determined by chronoamperometry using $Fe(CN)_6^{3-}$.

GRAPHICAL ABSTRACT



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ABSTRACT

This paper reports the design of thin bacteria/Layered Double Hydroxides (LDH) films in which bacterial cells of *Pseudomonas* sp. strain ADP were assembled alternatively with $Mg_2Al\text{-}NO_3$ LDH nanosheets by a layer-by-layer deposition method. The UV–Vis spectroscopy was used to monitor the assembly process, showing a progressive increase in immobilized bacteria amount upon deposited cycles. The $\{ADP/LDH\}_n$ film was characterized by X-ray diffraction, infrared spectroscopy, scanning electron microscopy and atomic force microscopy. The metabolic activity of immobilized bacteria was determined using chronoamperometry by measuring the biochemical oxygen demand in presence of glucose using an artificial electron acceptor ($Fe(CN)_6^{3-}$) at 0.5 V/Ag-AgCl. A steady current of 0.250 µA cm^{-2} was reached in about 30 s after the addition of 5 mM glucose.

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1. Introduction

The last decade has seen an increasing interest in the design of thin biohybrid films because of their potential application in the fields of biology and medicine [1]. Different types of biological material have been incorporated into thin films such as nucleic acids, DNA, antigens, antibodies, enzymes, or bacteria developing

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effective biosensor devices [2]. To enhance the level of immobilization and promote film formation and adhesion, additional chemical compounds can be involved ranging from polymers [3] or biopolymers [4], to nanomaterials [5] or sol-gel inorganic materials [6], among which Layered Double Hydroxides (LDH) appear as promising candidates [7]. LDH are lamellar materials based on the stacking of positively charged sheets $[M^{II}_{1-x}M^{III}_{x}(OH)_2]^{x+}$ [8]. Anions and water molecules are located in the interlamellar domains to maintain the overall electroneutrality. From a general point of view, the increasing interest in LDH arises from their versatile properties in terms of chemical composition of both layer and interlayer, their high and tuneable layer density and morphology. LDH have proved to be highly effective as host structures for biomolecules, such as amino acids, DNA, proteins, enzymes and whole cells [7,9,10]. It is reported that the LDH-based biohybrid materials have functionalities for gene delivery in therapeutic applications, for bioremediation or bioconversion applications and in enzyme-based biosensors [9]. Indeed, such layered structures promote favorable electrostatic interactions with biomolecules bearing an overall negative charge at neutral or basic pH and display good permeability for the substrate which contribute to improvement of the biosensors [7].

Enzymes are mainly used as recognition elements in biosensors due to their good selectivity [7]. Nevertheless, purification of enzymes is costly and time-consuming. Algae and bacteria offer an alternative in the elaboration of biosensors in particular for water quality assessment because of large test populations and a massive production through cell-culturing [11–13]. Guidelines in the development of whole-cell biosensors or biofilm based anodes in biofuel cells are the choice of appropriate bio-organisms and the immobilization strategy maintaining the biological activity. The main methods for bacteria immobilization on electrode are adsorption, entrapment and self-assembly [12]. Association of the bacteria with nanomaterials, e.g. carbon nanotubes [14] or clays [15], can promote cell adhesion and biological activity. For instance, in our previous works [10,16,17], we have shown that *Pseudomonas* sp. strain ADP cells immobilization on LDH particles improves its biodegradative activity towards a persistent herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-s-triazine).

There are two main ways to prepare LDH films on electrodes: deposition on a support or *in situ* growth onto a support. The latter is a "one-step method" where the support may be a source of metal cations which are incorporated in the LDH structure during a slow *in situ* growth process [18,19]. Electrochemically-induced precipitation is an alternative to this one-step method using the electrode as the support where electrogenerated hydroxide anions are used as precipitant agent [20]. The former is a "two-step method" generally used to form nanostructured films of hybrid or biohybrid LDH or LDH-based composites, in which a LDH suspension is first prepared and then coated on a support by means of solvent evaporation [7,18], electrophoretic deposition [21–23] or electrostatic layer-by-layer assembly (LbL) [18].

LbL self-assembly approach is mainly developed *via* electrostatic interaction and the successive inversion of a net charge by cyclic deposition on a substrate surface [24]. The LbL method can be used to optimize the inner structure and thickness of the biofilm, promoting interactions between bacteria and electrode. For instance, the LbL technique was recently used to assemble bacteria, mediators and carbon nanotubes on carbon paper electrode into a hierarchical artificial biofilm based anode [14]. In practice, for LDH 2D layers, it is conducted thanks to an alternate immersion of a support (glass or quartz slides, metallic or indium tin oxide-coated glass (ITO) electrodes) in different LDH and polyanions solutions [18,25–27] taking advantages of the LDH positively charged layer surface.

Such strategy was already applied to prepare various heterogeneous LDH thin films involving functional anionic molecules with photochemical or electrochemical properties. Hence, modified electrodes were prepared by using alternate assembly of exfoliated LDH and various electroactive metal complexes, for instance ferricyanide [28], metal *meso*-tetrakis (*p*-sulfonatophenyl) porphyrin (FeTSPP [29,30] and MnTSPP [31]), cobalt phthalocyanine tetrasulfonate (CoTSPc) [32], ruthenium(II) tris(bipyridine) ($Ru(bpy)_3$ [33] and naphthol green B (NGB) [34,35]. These as-prepared LDH-modified electrodes were mainly used in electrochemical sensors [7,36]. More recently, composite multilayer films of CoAl-LDH nanosheets and graphene oxide (GO) were also elaborated through LbL assembly and the electrochemical performances of these films were evaluated as electrode materials in supercapacitor devices [37]. In the same vein, the elaboration of LDH nanosheets/Au nanoparticles composite electrode by self-assembly was described by Zhao et al. [38]. Concerning the biomolecules, nanostructured $\{proteins/LDH\}_n$ [39] and $\{DNA/LDH\}_n$ [40] thin films were also elaborated using the LbL procedure. Nevertheless, to the best of our knowledge, LbL assembly has never been reported for the immobilization of whole cells within LDH. With the care of developing a bacterial sensor, we report here for the first time the preparation of a $\{bacteria/LDH\}_n$ thin film through the electrostatic LbL assembly. In comparison to the previous procedures used for the immobilization of enzymes or ADP cells within LDH, namely adsorption or coprecipitation [7,10,16,17], the LbL method is preferred, in the present work, since it should enhance the adhesion of ADP/LDH biohybrid on the electrode surface. Moreover this method can be adapted to a large electrode area to form a homogeneous artificial biofilm (2 cm^2). The positively charged MgAl-LDH nanosheets as building blocks were assembled alternately with negatively charged ADP cells (Fig. 1). The UV–Vis spectroscopy was used to monitor the assembly process. The $\{ADP/LDH\}_n$ thin film was characterized by powder X-ray diffraction (XRD), Infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and atomic force microscopy (AFM). The biological activity of whole cells was then analyzed by electrochemistry with glucose as substrate.

2. Experimental section

2.1. Materials and reagents

The magnesium and aluminium nitrate $Mg(NO_3)_2{\cdot}6H_2O$, $Al(NO_3)_3{\cdot}9H_2O$ and $K_3Fe(CN)_6$ salts were of analytical grade (Aldrich or Acros). Mineral water (Volvic®, France) and carbonate-free distilled water were used for all aqueous solutions and rinsing.

2.2. Cell cultures

Pseudomonas sp. strain ADP (ADP) (gift from F. Martin-Laurent from INRA Dijon, France) was grown in 100 mL portions of Trypcase Soy broth (BioMerieux) in 500 mL Erlenmeyer flasks incubated at 27 °C and 200 rpm. Cells were harvested after 24 h of culture. Samples of 20 mL of culture (45 ± 5 mg of dried cells) were centrifuged at 12,000*g* for 15 min at 4 °C. The resulting pellets were washed first with NaCl solution (8.0 g L^{-1}) and then, with Volvic® water. The cell concentration used was around 2×10^{10} colony-forming units (cfu)/mL (OD = 10 at λ = 575 nm).

2.3. Synthesis of Mg_2Al-NO_3 LDH nanoparticles

Mg_2Al-NO_3 LDH was prepared by a fast coprecipitation method followed by a controlled solvothermal treatment. A mixed salt solution (100 mL) of $Mg(NO_3)_2{\cdot}6H_2O$ (0.3 M) and $Al(NO_3)_3{\cdot}9H_2O$ (0.15 M) was prepared in methanol and rapidly added (3.57 mL/min) to a reactor containing 320 mL of NaOH methanolic solution

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