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Detection of glucose in the growth media of *Ulva lactuca* using a Ni-Cu/TiO_2/Ti self-assembly nanostructure sensor under the influence of crude oil

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ABSTRACT

Pollution of the marine environment by crude oil is considered as a significant problem. Interestingly, the existence of algae in the marine ecosystem contributes significantly to maintaining the equilibrium of marine life and, consequently, has the ability to alert the ecosystem to the pollution by using their waterborne molecules including the photosynthetic products. The main aim of this work is to develop an electrochemical sensor (EC) for the detection of the concentration of glucose found in the growth media of *Ulva* sp. as a photosynthetic product or decomposed substance under polluted conditions. A Ni-Cu/TiO_2/Ti array electrode was fabricated, where highly-ordered self-organized nanocrystalline TiO_2 was prepared via anodization and annealing processes on a Ti substrate and Ni-Cu alloy nanoparticles were electrodeposited by linear sweep voltammetry. The chemical composition, structure and morphology characterization were carried out by high-resolution scanning electron microscopy and energy dispersive X-ray spectroscopy analyses. The ideal non-enzymatic sensor with large and constant sensitivity (402 μA mM^{-1} cm^{-2}) and low detection limit (495 μM) was successfully employed to detect glucose excreted by *Ulva lactuca* under oil pollution under alkaline condition. The present study succeeded to combine between the ecological role of algae and electrochemical sensors to be used collectively as an indicator of the oil spillage into the seawater.

1. Introduction

The marine environment is a great mystery, dramatically stimulating research work. In the last few years, the exponential use of marine areas covering 71% of the Earth's surface and growing human activities, has led to an imbalance in the ecosystem. Petroleum hydrocarbons are important contaminants of sea and marine life. The origin of hydrocarbons are either biogenic (endogenic), which are produced by marine organisms, or exogenic, due to oil pollution accumulated by marine organisms [5]. Oil pollution is one of the main causes of such disequilibrium in the marine environment that causes alterations to standard water characteristics. As a general rule, the improvement of the global marine system should be recognized, encouraged and implemented globally, in particular finding ways to speed the progress in establishing good ecological systems and a well-managed marine environment via the implementation of international law that is based on the availability of the best scientific research. Thus, controlling marine oil pollution has become an important goal for international organizations such as the International Maritime Organization (IMO), through the issuance of legislation or the use of the best scientific research by researchers in all disciplines to monitor and identify marine pollution areas.

Marine organisms, especially algae, can be used as indicators to provide information about the naturalness and the richness of the surrounding environment, and they are considered to be key links acting as time-integrators of pollutants [6–8]. Algae are organisms similar to plants, widely spread and contributing significantly to marine life equilibrium and effectively used to indicate harmful elements, such as heavy metals, in both estuarine and coastal waters [9–12]. From the viewpoint of algal nutrition, algae are autotrophic and synthesize their food, usually glucose, glycerol and amino acids, from simple inorganic materials, such as carbon dioxide, water and minerals, by means of the photosynthesis process [4,13–17]. Ecologically, as much as 90% of the organic material produced photosynthetically by algae [18]. The photosynthetic macronutrients carbohydrates, amino acids and glycerol are released into the environment [1–4]. Thus, algal community provides a carbon source for heterotrophic organisms in aquatic systems through primary production, and this carbon source is essential and important for supporting the microbial food web. Therefore, the marine environment is highly multifarious and the variation in species compositions,

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abundance and competition over space and nutrients are fundamental features of marine life. It is worth mentioning that algae are capable of withstanding the changes taking place in the environment [19–22] and contribute in the degradation of some pollutants, such as oil [23]. On the other hand, algae are well known to be sensitive to the changes in the ambient marine environment caused by pollution [24], which are significant in affecting the release of nutrients and infochemicals by algae [25]. Consequently, *Ulva* spp., as macroalgae, have become a model system for ecological study based on their short and simple life cycle and ease of induction of gametes [26]. Waterborne molecules excreted by algae can also be used as an indicator to estimate physiological processes of the symbionts, such as growth and development [27–31], in addition to estimate the alteration in environmental factors or nutrients and the presence of pollution [32]. Furthermore, oil spills can cause a broad series of impacts in the marine environment and some of the symptoms of the resulting pollution are failures in physiological functions of marine organisms, loss of habitat and changing the natural community that causes algal tide blooms.

The Red Sea is rapidly developing as one of the world's largest offshore oil production areas and also comprises a wide range of marine habitats, many of which are internationally recognized for their conservation, scientific, economic, or recreational value. Oil production, refining and transport have resulted in chronic pollution due to most oil floating on the sea surface, which can spread over wide areas by waves, winds and currents. The low viscosity of oil facilitates its rapid spread and its concentration increases with the length of time of oil spillage. Conventional analysis methods, such as ultraviolet fluorescence (UVF), gas chromatography-flame ionization detection (GC-FID) and liquid and gas chromatography–mass spectrometry (LC-MS and GC–MS), provide us with the most accurate and sensitive data about waterborne metabolites [28], but their drawback are that they have considerable limitations because of expensive sample clean-up and extraction, as well as being time consuming.

Electrochemical sensors have been used for monitoring heavy metal pollutants [33,34]. On the other hand, marine algae could be suitably utilized for monitoring the heavy metal contamination of the coastal marine environment [35]. Although there are many studies that have taken into account the measurement of oil pollution in the marine environment, there is no research that has been exploited algae photosynthesis products to refer to the oil pollution.

Hence, electrochemical detection systems are a promising current alternative to the conventional methods, as they are simple and small-scale portable analysis techniques, providing rapid and real-time feedback information [36–39]. Indeed, electrochemical sensors are considered to be fast, cost-effective, accurate and sensitive analytical systems and give information about water quality by detecting a wide range of molecules [40]. They convert a biochemical phenomenon into a detectable and measurable electrical signal that is proportional to the analyte concentration and provide a fast detection of a sample's contents. There are many types of electrochemical sensors, i.e. amperometric, potentiometric and conductometric. The operating principle of amperometric biosensors depends on the variation of a potential and recording the corresponding current to oxidation and reduction of electro-active species on the electrode surface, which reflects the biochemical phenomena taking place [41].

Self-assembly nanostructured materials have been attracting significant attention among researchers for use in sensors applications, like algal metabolites and water quality [42]. Glucose is one of the most important photosynthetic waterborne products to be used as an indicator to the quality of a water body in the marine system, as will be discussed in the present study. Glucose biosensors have been investigated as the most popular biosensors due to their important applications in clinical diagnosis, pharmaceutical analysis and the food industry [43,44]. Owing to the instability of the active enzyme part in these biosensors and being easily influenced by temperature, humidity and the environmental factors (e.g., pH), the enzymatic sensors have limited uses [45]. To address this issue, recently, non-enzymatic glucose sensors consisting of economical transition metals, such as Ni, Cu, Mg, Mn, Co, Fe, etc., supported on TiO_2 nanostructured array electrodes as a metal [46], alloy [47] or metal oxide [48], were used for sensor applications due to their excellent stability, chemical activity and resistance to poisoning [49], and they showed high electro-catalytic activity for the oxidation of glucose [50–57].

Although trends of environmental studies are currently facing a strong push for the development of the techniques and methods for determination of oil pollution, unfortunately using algal exudates as indicators is still in the early stages. The quantity of glucose found in the growth media can be used as a pointer of the physiological state of algae. It has been demonstrated that glucose is considered as a major free monosaccharide which is present in surface water [1,58,59]. The present work is based on the fact that the decrease of glucose concentration can be used as a biomarker of the negative outcome of an oil spill, which can cause a wide range of detrimental impacts in the marine environment. A stable and active bimetallic catalyst, Ni-Cu, based on $aTiO_2$/Ti nanoporous substrate, was fabricated and used for the first time to measure glucose secreted by *U. lactuca* into the growth media to detect the degree of pollution resulting from crude oil under laboratory conditions, which might be applicable in field experiment under specific conditions. The electro-catalytic activity of the Ni-Cu/TiO_2/Ti electrode with respect to the glucose in the absence and in the presence of crude oil was studied in an alkaline medium. The morphology, chemical composition and the properties of the sensor based on this electrode, such as the sensitivity and detection limit, in terms of glucose detection, were investigated.

2. Materials and methods

2.1. Collection and cultivation of U. lactuca in bioreactors

U. lactuca was collected from the coast of the Red Sea in Jeddah, KSA, on 17 March 2016 (299° SW; 21°37′11″ N 39°6′11″ E; 10 m elevation). *Ulva* was transported immediately on ice to the laboratory at Taif University, Taif, KSA. *Ulva* was thoroughly washed with running water, and three times with distilled H_2O to remove epiphytes and small organisms. The final density of 60 g of *Ulva* (wet weight) was cultivated in a bioreactor filled with 2 L of silicate-free artificial SW (Instant Ocean salt, Aquarium System Inc., Sarrebourg, France) dissolved in bidistilled water (30 g/L). The standard culture conditions were 25 °C, illumination for 12 h from 6:00 to 18:00 (Middle East standard (summer time)) at 60–120 μmol photons$\cdot m^{-2}\cdot s^{-1}$ (50% GroLux, 50% daylight fluorescent tubes; OSRAM, T8 36 W 840, Germany). Cultures were continuously aerated to avoid algae accumulation at the bottom, which would significantly affect algal growth and to ensure the distribution of oil in the growth media in the case of treatments with crude oil. Four bioreactors were set up to determine the effect of crude oil pollution on the concentration of glucose found in the growth media of *U. lactuca*. These bioreactors were as follows: control containing only *U. lactuca*, and *U. lactuca* with 10, 20 and 30 mg of crude oil/L of artificial seawater. After 24 h and one week intervals, 3×20 mL of growth media in each bioreactor was sampled and the pH was adjusted immediately to 12.00 by 1 M NaOH.

2.2. Sensor material fabrication

Titanium foil (99.7% pure, 0.25 mm thick) was purchased from Sigma Aldrich (Steinem, Germany) and cut into 10×15 mm samples. It was then used throughout this study as the base substrate. Prior to each experiment, the samples were mechanically polished using superior grades of silicon carbide papers ever-increasing gradually from 400 to 1200 grit. After polishing, the samples were consecutively cleaned with acetone, alcohol, and dH_2O in an ultrasonic bath. Once cleaning process were performed, modification steps were taken to prepare the sensor in