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Nitrate biosensors and biological methods for nitrate determination

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ABSTRACT

The inorganic nitrate (NO_3^-) anion is present under a variety of both natural and artificial environmental conditions. Nitrate is ubiquitous within the environment, food, industrial and physiological systems and is mostly present as hydrated anion of a corresponding dissolved salt. Due to the significant environmental and toxicological effects of nitrate, its determination and monitoring in environmental and industrial waters are often necessary. A wide range of analytical techniques are available for nitrate determination in various sample matrices. This review discusses biosensors available for nitrate determination using the enzyme nitrate reductase (NaR). We conclude that nitrate determination using biosensors is an excellent non-toxic alternative to all other available analytical methods. Over the last fifteen years biosensing technology for nitrate analysis has progressed very well, however, there is a need to expedite the development of nitrate biosensors as a suitable alternative to non-enzymatic techniques through the use of different polymers, nanostructures, mediators and strategies to overcome oxygen interference.

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1. Introduction

Nitrogen and phosphorous are amongst the essential elements for all living organisms. All proteins and DNAs need both of these elements. However, living organisms cannot use nitrogen directly from the atmosphere. Nitrogen needs to be fixed or converted into useful nitrogen compounds through atmospheric phenomenon, certain microorganisms and industrial activities. Nitrate, a major source of nitrogen for plants, is usually assimilated through the roots and converted to ammonia, which is incorporated into the life cycle via the Krebs cycle for the formation of amino acids [1]. Thus, small quantities of nitrate (along with phosphate), in all aquatic systems, are necessary to maintain the growth and metabolism of aquatic organisms. However, at concentrations beyond

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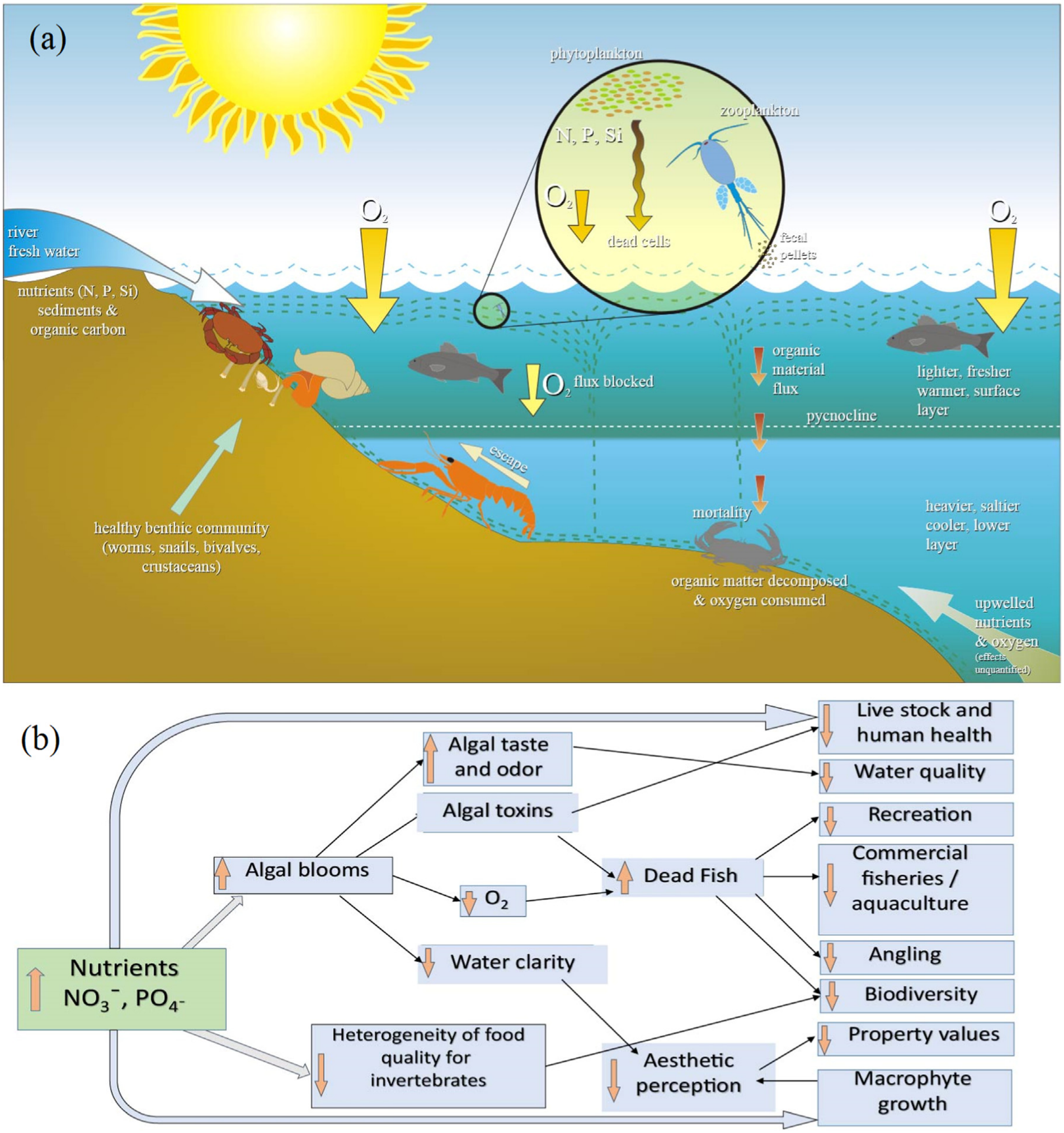

Fig. 1. (a) Graphical representation of the process of Eutrophication and its effects on aquatic life (taken from https://commons.wikimedia.org/wiki/File:Scheme_eutrophication-en.svg), and (b) Schematic presentation of some effects of increased nutrients that could influence freshwater ecosystem (Reproduced from [5]).

a safe limit both of these nutrients have serious consequences on the environmental health. Nitrate is also a well-known contaminant of ground and surface waters. Along with phosphate, nitrate is frequently implicated in the eutrophication of lakes and coastal waterways [2]. As shown in Fig. 1, the accumulation of nitrates and phosphates promotes eutrophication of water bodies, causing a burst of excessive growth of plants, algal blooms and microorganisms that create slime layers across the whole top surfaces of the water bodies. This excessive growth of plants and phytoplankton stops the sunlight and atmospheric oxygen from entering into a water body. In the absence of light plants below the top surface of water bodies die and their subsequent decomposition by micro-organisms consumes all the dissolved oxygen. This phenomenon is called as Anoxia and it diminishes the amount of dissolved oxygen which in turn favors the growth of anaerobic organisms while aerobic organisms including fish populations and invertebrates die creating an imbalance across the whole food chain. Furthermore, water with excessive anaerobic life becomes fouled smell while algal blooms make water scummy, cloudy and soupy green in color. Decay of the dead algae and plants also favors the growth of the disease causing bacteria such as *E. coli*. All these factors make water unsuitable for the daily use. Nitrate is usually transported from the terrestrial to the aquatic ecosystems by processes such as wind erosion, surface runoff and leaching from the soil. The major nitrogen sources in the environment include the agricultural use of fertilizers and animal manure as well as atmospheric deposition [3], rock weathering and point source discharges [4,5].

Consequently, the major sources of nitrate for ingestion by humans include drinking water, fruits and vegetables. The excessive ingestion of nitrate ($>$ 44 ppm) from these sources may result in levels of human intake that are markedly greater than necessary, which may cause serious health effects [6–9]. High concentrations of nitrate ($>$44 ppm) in the body can lead to the production of other substances, such as nitrite, nitric oxide and N-nitroso compounds [10,11], which have been linked to gastric