Enzyme and Microbial Technology 95 (2016) 13–27

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Noble metal, oxide and chalcogenide-based nanomaterials from scalable phototrophic culture systems

Si Amar Dahoumane[a], Evan K. Wujcik[b], Clayton Jeffryes[c,*]

[a] School of Life Science and Biotechnology, Yachay Tech University, San Miguel de Urcuquí, Ecuador
[b] Materials Engineering and Nanosensor (MEAN) Laboratory, Dan F. Smith Department of Chemical Engineering, Lamar University, Beaumont, TX, USA
[c] Nanobiomaterials and Bioprocessing (NAB) Laboratory, Dan F. Smith Department of Chemical Engineering, Lamar University, Beaumont, TX, USA

ARTICLE INFO

Article history:
Received 30 January 2016
Received in revised form 10 May 2016
Accepted 12 June 2016
Available online 15 June 2016

Keywords:
Algae
Bioprocess
Biosensors
Nanobiomaterials
Nanoparticles
Photobioreactors

ABSTRACT

Phototrophic cell or tissue cultures can produce nanostructured noble metals, oxides and chalcogenides at ambient temperatures and pressures in an aqueous environment and without the need for potentially toxic solvents or the generation of dangerous waste products. These "green" synthesized nanobiomaterials can be used to fabricate biosensors and bio-reporting tools, theranostic vehicles, medical imaging agents, as well as tissue engineering scaffolds and biomaterials. While successful at the lab and experimental scales, significant barriers still inhibit the development of higher capacity processes. While scalability issues in traditional algal bioprocess engineering are well known, such as the controlled delivery of photons and gas-exchange, the large-scale algal synthesis of nanomaterials introduces additional parameters to be understood, i.e., nanoparticle (NP) formation kinetics and mechanisms, biological transport of metal cations and the effect of environmental conditions on the final form of the NPs. Only after a clear understanding of the kinetics and mechanisms can the strain selection, photobioreactor type, medium pH and ionic strength, mean light intensity and other relevant parameters be specified for an optimal bioprocess. To this end, this mini-review will examine the current best practices and understanding of these phenomena to establish a path forward for this technology.

1. Introduction

Photosynthetic organisms have proven to be promising platforms for the production of nano-sized objects owing to their ability to produce shapes and materials such as spheres, triangles, plates or rods and of materials such Au^0, Ag^0, Pt^0, Au^0-Ag^0 alloy, FeO, CuO, β-HgS, or CdS among others [1–7]. Additionally, these production processes are carried out at ambient temperatures and pressures and in an aqueous environment without using potentially toxic solvents or generating harmful waste products [8–10]. In view of the total process, the cell culture acts as a bioreactor in which the organisms catalyze the reduction of metallic salts into the aforementioned materials using photons as the energetic input [11]. Furthermore, it is proposed that each cell acts as a micro-bioreactor and there may be many nano-bioreactors within each cell converting photonic energy to reducing equivalents which drive the metallic ion to nanomaterial transformation while simultaneously limiting the nanoparticle (NP) size distribution by synthesis within an intramembrane space [12]. Algal production platforms can also reduce costs owing to their ability to produce NP from impure feedstocks [13].

However, while numerous materials have been synthesized by these systems and some of the mechanisms are suspected, the translation of this existing knowledge into scalable systems has remained elusive. This is in part due to the many production parameters which remain unknown, such as: the role of charge interaction with the cell surface and the extracellular matrix (ECM) of polyanionic polysaccharides on metal ion uptake; the diffusive, active, and facilitated transport phenomena of both the metal ions and the NPs through the stagnant liquid boundary layer around each cell and across the cell membrane; metal ion and NP transport within the cell; the yields and kinetics of photon conversion to reducing equivalents and then to nanomaterials; and the importance and impact of the photobioreactor design on these processes. Indeed, except for a few cases, the fields of algal nano- and nanobiomaterials and algal bioprocess engineering for the production of nanobiomaterials within scalable algal cell culture systems have rarely overlapped [14–17]. The combination of the mechanisms and first principles could reveal the path forward to the large-scale "green" synthesis of these materials.

* Corresponding author.
E-mail address: cjeffryes@lamar.edu (C. Jeffryes).

http://dx.doi.org/10.1016/j.enzmictec.2016.06.008
0141-0229/

2. Photobioreactors (PBRs)

Conventional methods to produce NPs are often energy intensive, expensive and employ harmful chemicals. Such methods include lithography, laser ablation, gas phase aerosol generation, or high-temperature thermo-reductive processes [18]. While the trend among the community of nanomaterial scientists is towards more eco-friendly practices in nanoparticle design [19], we believe it is important to continue to investigate environmentally benign methods for nanoparticle production for mainly one reason: exploring the capabilities of natural resources to promote the biosynthesis of valuable nanomaterials and, consequently, the extent of the scalability of such process. For example, the scalable, "green" synthesis of NPs from cell cultures can be accomplished at ambient temperatures and pressures in simple cell culture media [20]. In the case of simple algal cell cultures, the cultivation requirements are even "greener," with only photons and CO_2 as the required primary substrates for the production of biomass and to drive the biocatalytic and bioreductive machinery which results in the production of NPs and other nanostructured materials once these entities are challenged with the corresponding salts [14].

The production of nanomaterials within algal cell cultures is dependent on the proper process inputs, such as the metal ion addition, temperature, pH, ionic strength of the culture medium and the light input. The latter is used as an energy source to drive metabolic reactions within the algae [21]. As part of the algal metabolism, the chloroplasts within the photosystem produce reducing equivalents and an ECM is often produced at the cell surface. Both can reduce soluble metal ions into nanomaterials. Importantly, these processes can be precisely controlled within PBRs [22].

PBRs and open ponds are the primary systems used in algal bioprocess engineering, with the latter open to the environment while the former are enclosed systems with tighter process controls. Open ponds are either unmixed or circulated by paddles in raceway ponds while the primary configurations of PBRs are airlift, bubble column, flat plate and tubular [22] or combinations of these [23]. Among these, PBRs of the tubular variety are considered the most scalable, while flat panels, because of their simple planar geometry and short light paths, are considered the most controllable with respect to photonic input [24,25]. Indeed, flat panels are also the most capable of achieving high cell densities because the delivery of photons into dense cell cultures is often the rate-limiting process in the production of algal biomass [24,25]. Additionally, the effectiveness of biofilm PBRs has been investigated for wastewater treatment and heavy metals remediation [26,27]. To date, no biofilm cultures are characterized for the production of nanomaterials, but the removal of metal cations from the aqueous phase in these cultures clearly demonstrates that the biofilm is acting as a sink for these cations; therefore, the production of metallic nanomaterials in these systems could be envisaged.

The factors affecting the photon conversion efficiency and the metabolic activity of the cell culture are photon flux incident to the cell culture, cell culture density, reactor geometry and culture specific parameters. The culture specific parameters are specific light adsorption and scattering coefficients, light saturation and inhibition constants of the photosystem and the specific maximum growth rate of the cell culture at the given temperature, pH and nutrient environment [28,29]. An adequate delivery of photons is required to meet the energetic needs of the cell culture, but when the photonic input exceeds the cellular maintenance requirements the algae can divert metabolic resources towards the production of reducing equivalents to drive the synthesis of reduced compounds such as lipids [30], or in this case, metallic nanomaterials. However, under conditions of extreme photonic excess the cells will experience photosaturation and photoinhibition, and the culture will divert metabolic resources to cellular repair [31]. This will result in a decreased ability to produce superfluous reducing equivalents which could be used for NP synthesis. This calls attention to the importance of not only photonic input, but of photonic control.

Adequate mixing and gas mass transfer are also essential to PBR operation and PBR control systems [32]. Mixing and agitation prevent sedimentation, biofilm formation and help to maintain a homogeneous culture free of cell flocs. In flocs, also known as cellular aggregates, the delivery of essential NP precursors, i.e., photons, dissolved gasses and metal ions, to interior cells is greatly reduced which will limit NP formation to only the outer cell layers.

3. Current production methods

To date, few studies have reported on the production of nanomaterials in large-scale or scalable production systems. Recent work by Satapathy et al. [33] describes a system for the extracellular production of AgNPs from *Chlorella vulgaris* in a semi-continuous, 15 L culture vessel, but the design of this system did not allow for high-density or high throughput cultures. The development of a continuous production system offers several advantages over a batch approach and, combined with a short pathlength cultivation system, higher cell densities could be achieved. This would both facilitate harvesting and reduce the chance of contamination. It would also allow tighter control on the photonic input, temperature and pH, which would enable the optimization of NP production and reduce the production costs of the biomass [34].

4. Applications of noble metal, oxide, and chalcogenide-based nanomaterials in biotechnology and biomedicine

The fields of biotechnology, biomedicine and nanotechnology have grown to encompass a wide range of technologies including implantable devices [35–37], biosensors [38–41], theranostics [42–44], medical imaging [45–47] and biomaterials [48–50]. Although yet emerging, these promising fields interconnect both fundamental–nanoscience, physics, chemistry, biology–and applied–medicine, technology, materials engineering–disciplines for state-of-the-art tools to advance science and engineering. A number of biosynthetic zero-dimensional (0D) and one-dimensional (1D) materials can result from scalable phototrophic culture systems, as discussed. These noble metal, oxide and chalcogenide-based nanomaterials have been used in numerous applications, ranging from energy storage [51–54] to biomaterials [55–57]. We now discuss breakthrough and current applications of these noble metal, oxide and chalcogenide-based nanomaterials in biotechnology, biomedicine, and nanotechnology.

4.1. Biosensors

The detection and reporting of specific analytes are applicable to a number of bio-medical related fields, including prosthetics and orthotics [58], bodily fluid tests [59] and continuous monitoring [60], among others. A number of noble metal, oxide and chalcogenide-based nanomaterials are used in biosensor applications. A protein electrochemiluminescent (ECL) immunosensor, identifying α-fetoprotein as a model analyte, has been developed by Lin et al. [61]. The layer-by-layer modification of carbon nanotubes (CNTs) using CdS quantum dots (QDs) and a capture antibody on a glassy carbon electrode (GCE) yielded the ECL sensor, which was coupled with an antibody and alkylthiol-capped bar-code G-quadruplex DNA on gold nanoparticle (AuNPs) probe. The probe amplifies the signal from the immunosenor via the interaction of hemin with the DNA to form a G-quadruplex/hemin bio-bar-code. Upon binding, the ECL is quenched, decreasing the intensity as the