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Nanotechnology Applications for Food and Bioprocessing Industries

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Abstract

Nanoscience is an emerging technology today with great application in different fields. Biocatalysts design, identification of different bacterial strains, monitoring the quality of food by different biosensors, food packaging with smart systems, active, intelligent and nano-encapsulation of bioactive food compounds are examples of some of these applications in the food industry. In this paper, some topics related to the potential of nanotechnology in the food industry are updated. In addition, some concerns about nanotechnology application in this popular industry are also discussed.

Keywords: Nanotechnology; Bioprocessing industries; Bio sensors

Introduction

Food, food-biocatalists and bioprocessing industries face great challenges in order to develop and establish systems to develop high quality, safety foods, as well as feeds and other industrial goods, environmentally acceptable and in a sustainable way [1]. Recently, an ideal immobilized biocatalyst using calcium alginate beads was described for the industrial-scale production of invert sugar for food and beverages industry [2,3]. However, despite the advantages of currently used immobilization materials like calcium alginate, nanostructure materials exhibit, a higher catalytic efficiency over other traditional materials, greater surface reaction activity, strong adsorption ability, and thermal stability [4-6]. On the other hand, the use of nanostructure carriers increase life cycles of the biocatalyst thus allowing its reuse and reducing the bioprocess cost [7]. All these features are highly desired for industrial purposes. Thermal stability increment was described in the case of *Candida rugosa* lipase immobilized on polylactic acid nanoparticles [8]. On the other hand, keratin, a non-food applied enzyme, also improved its stability by the use of a nanoscaled support [9]. According to these reports, we can assume that nanomaterials large surface area provides a better matrix for enzyme immobilization, improve the enzyme loading per nanoparticles mass unit and its catalytical properties. Not only the enzyme field with application in food has pointed to nanotechnology, the food and beverage industry has also been pointed to nanomaterial applications. According to some authors, it is a newly emerging technology, predicted to continually increase [10] since more than 400 companies worldwide develop different applications of nanotechnology in food and food packaging [11]. Some institutes dedicated to the nano-market research for food and beverages, estimated that it will grow to $6.5 billion in 2013, $1 trillion by 2015 [12], and will grow with a compound annual growth rate (CAGR) of 12.7% to reach about $15.0 billion in 2020 [13]. On the other hand, other economic studies carried by other institutions like the European Institute for Health and Consumer Protection, postulates that the use of nanomaterial's in the food packaging market will reach $20 billion by 2020. This paper contributes to a broader understanding of the main applications of nanotechnology in food fields like biocatalists and food and feed industries by openly discuss these issues using balanced information and scientific findings in a simple technical language.

Materials and Methods

We are not attempting to provide a comprehensive summary of analytical techniques used for nanotechniques applied to different research fields, thus, the goal of this report is to present a brief review of methods developed in our own and other laboratories and lessons gathered from our experiences that may be useful to other researchers. For this review, we used peer-reviewed, published papers that were identified by electronic searches in Medline and Google Scholar. The search strategy included different combinations of key words such as "nanotechniques," "enzyme immobilization", "nanotechnology in foods and feeds", "nanomaterials", "nanotechnology concerns", etc. No language limits were applied. Search period was not longer than 10 years. In addition, we performed manual searches in reference lists from specific own or other published papers to find additional pioneer and older studies that could have been overlooked by the electronic search.

Equipments, microorganism and fed-batch fermentation, cell disruption to get enzymes of interest, preparation and storage of calcium alginate beads, enzyme assays for free and immobilized enzymes, protein and carbohydrate analysis were conducted as described in our previous papers [2,3].

Nanotechnology Today

The use of new technologies based on nanomaterials created great expectation and interest since the past century [14]. Nanotechnology is considered nowadays an interdisciplinary field that works tightly with other scientific disciplines like physics, chemistry, biology as well as engineering. Working at the nanoscale level is exiting because this nano level offers different physical, chemical, and biological properties of structures and systems to those displayed by its macro-scale similar due to the interactions of individual atoms and molecules. This means that unique novel functional applications can be offered by working at the nanoscale level.

Nanotechnology is then generally defined, according to the explained above, as the production, design and structures application, devices, and systems with materials size and shape in the order of 10−9. The National Nanotechnology Initiative (Arlington, USA) defines that nanotechnology is 'the understanding and control of matter at dimensions between 1-100 nm, where unique phenomena enable novel applications'. Cosmetic Regulation define a nanomaterial as an insoluble, biopersistent intentionally manufactured material with one or more external dimensions, or an internal structure, on a scale from 1 to 100 nm [15].

Thanks to their unique multifunctionality, nanomaterials are built by polymeric materials, ceramics or metals, [16] and a wide spectrum of them are in use for consumer products and several new products containing nanomaterials have been launched in the market. Computer electronics, communication, energy production, medicine, biocatalysts and the food industry use widely these materials in their designs, applying them in various fields. Interestingly, the nanoscale devices are generally built trying to imitate nano biomolecules found in nature such as proteins, DNA or biological membranes [14].

Nanobiocatalyst advancements for bioprocessing for food applications

Both, Nanotechnology together with Biotechnology have produced the so called nanobiocatalyst (NBC) science that is considered an emerging and innovative branch of the nanosciences. The improvement of enzyme activity, stability, capability and engineering performances in bioprocessing applications promises exciting advantages in this field [17]. Integration of biological entities for biocatalysis (i.e. enzyme) with a nanomaterial carrier with unique electronic, optical, magnetic and external-stimuli-responsive properties constitutes the NBCs core.

Several nanomaterials that include polymers, silicas, carbons and metals are currently used in NBCs for enzymes immobilization or encapsulation, together with the development of techniques and methodologies to successfully retain these biological reagents into the nanomaterials. Generally, these techniques and methods include physical adsorption through electrostatic interactions, hydrophobic interactions, hydrogen bonding or van der Waals forces. Other methods widely used are covalent binding, cross-linking or physical enzymes entrapment or encapsulation. Another useful approach employed in the formation of NBCs is the use of functional groups on the surfaces of enzymes and nanocarriers [18].

As the first step in this technology, it is important the production of specific and processable enzymes in a cost-effective way by different techniques such as genetic engineering, directed evolution, etc. [19,20]. Once vast amounts of enzyme are available, nanocarriers for immobilization must be developed. The unique nanocarriers structures and properties have become the main motive of the great interest raised by NBCs and so, these particular features constitutes the driving force for its fabrication.

Mission et al. [17] established that the development of novel nanocarriers with unique functions and characteristics comprises (i) the introduction of functional groups on its surface that allow enzymes immobilization or respond to external stimuli, (ii) increment its surface area to facilitate substrate diffusion, nanocarriers recycling etc, by the construction of special structures able to confine the enzymes inside nanocages (iii) improvement of mechanical and thermal stability of nanocarriers. Advanced nanocarriers such as nanofibers (NFs), nanopores and nanocontainers can improve possitively the engineering performances of enzymes together with other functional nano-materials used to date like scaffolds [21], nanotubes (NTs) [22], nanocomposites (NCs) [23], and nanosheets (NSs). The use of such technologies, have proved to shelter and/or stabilize enzymes against chemical and environmental attacks. Interestingly, the immobilized enzymes could be recovered and re-used in a large-scale continuous process [24]. A 2014 review paper [25] describes and discuss in detail the mechanisms of enzyme activities enhancement, when immobilized in nanocarriers. The multiple point covalent bonding through short spacer arms on nanocarriers is also a popular and powerful strategy to improve enzyme activity [23-26]. Enzyme activity significantly increases when physical adsorption onto nanocarriers through hydrophobic interactions is carried out. Under these conditions, the open form of the enzyme used for catalysis is stabilized due to exposure of the large hydrophobic groups of the enzyme to the nanocarriers hydrophobic surfaces as evidenced by Palomo et al. [27], and Chen et al. [28]. One of the most important advantages of enzyme immobilization using nanostructure carriers is the fact that significantly increase life cycles of the biocatalyst allowing its reuse and so, reducing the overall cost of the biocatalytic process. Localized nano-environment is another important approach to the improvement of enzyme activity or its catalytical properties. Enzyme deactivation might be influenced by the nano-environment surrounding the enzyme molecules. For example, reduced concentration of hydrophobic organic solvents or some gases near to the enzyme creating a hydrophilic environment may reduce or prevent the loss of enzyme activity. On the other hand, hydrophobic environment may tremendously reduce the detrimental effect of hydrogen peroxide, a very hydrophilic deactivating molecule [29].

NPs helped to create an adequate environment regarding optimal pH for some enzymes in comparison to its bulk pH solution, thus reaction rates of those immobilized in NPs were 3–180 times higher than non-immobilized controls [30].

Different strategies for producing NBCs with enzymes used in food industry have been developed for a wide range of bioprocess applications. β-galactosidase used in galactoligosaccharides (GOS) production or lactose hydrolysis has been covalently immobilized with glutaraldehyde in both, polyaniline–magnetic and polysiloxane/polyvinyl alcohol–magnetic NPs [31,32]. β-galactosidase immobilization efficiency of about 93% was obtained linking the enzyme onto magnetic NPs via glutaraldehyde activation [33]. This enzyme has also been immobilized by electrostatic interaction in magnetic NPs-chitosan [24] or by physical adsorption using zinc oxide NPs [34]. Bioaffinity has been another successful β-galactosidase immobilization approach using concanavalin A-magnetic NPs described by Ansari and Husain [35]. Glucoamylase-based nanobiocatalyst used in industrial glucose production was built immobilizing the enzyme covalently with glutaraldehyde and retained by electrostatic interactions to graphite NSs [36] or to carboxyl-functioned magnetic NPs [37], respectively. NPs and α-amylase conjugates have been used for fast starch degradation [38]. This enzyme, through a gelatinization and liquefaction process, hydrolyses firstly starch at 105ºC [39].

A NBC for starch hydrolysis has been built using α-amylase linked to silver NPs through thiol linkages [40]. Interestingly, Wang et al. [41]

demonstrated that α-amylase binding through allosteric modulation to CaHPO4 nanocrystals used as nanocarriers, dramatically enhanced enzymatic performance and changed positively the catalitycal constants Km, displaying greater affinity by the substrate than that showed by its no-immobilized counterpart. Despite that immobilized enzymes have been used for large-scale industrial processes in the food industries such as invertase or glucose isomerase for production of fructose corn syrup (107 tons per annum) [2,3,42], great efforts have been done to use NBC in large scale industrial processes. Industrial bioprocesses based on industrial NBCs are not described in the literature so far. However, development and application of nanocarrier-based NBCs for bioprocesses are carried out in the laboratory-scale bioreactor or biodevice systems. Nevertheless, NBC technology offer great perspectives, exciting challenges and a bright future always with the joint of interdisciplinary collaboration of biochemists, biologists, engineers and material scientists.

Nanotechnologies in food industry

The food and beverage industry is a focus for nanomaterial applications and strategies. There were in 2006 about 400 agricultural and food companies worldwide conducting nanotechnology research and development. These companies numbers increased to date by more than those predictions [11-43]. Potential nanotechnologies application areas includes organic and inorganic nanoadditive, food with nanoparticles, nanosensors for food quality control and smart packaging, nanocoating and nanofilms for kitchenware and foodstuffs, antimicrobial, hygiene coatings, detection of pathogens in food and beverages, self-sanitizing surfaces, polymeric films for food packaging with high antibacterial properties, nanoscale freshness indicators, nanoemulsions for fat reduction, etc.

Organic and inorganic nanoadditive, food with nanoparticles, nanosensors for food quality control and smart packaging, nanocoating and nanofilms for kitchenware and foodstuffs, antimicrobial, hygiene coatings, detection of pathogens in food and beverages, self-sanitizing surfaces, polymeric films for food packaging with high antibacterial properties, nanoscale freshness indicators, nanoemulsions for fat reduction are all included as potential nanotechnologies application areas.

Naturally produced as well as intentionally man-made materials are the main categories of nanomaterials used in foods. Intentionally added nanomaterials, are not generally present in a food substance and may come from engineered material or naturally occurring sources [44]. Casein micelles in the animal milk and pectin nanostructures in fruits are examples of natural nanoscale components respectively [45,46] while, on the other hand, nanocapsules that carry vitamins through the human stomach via the bloodstream is an example of engineered material source [47]. Functional additives like silver nanoparticle (AgNP), nanoclay, nano-zinc oxide (nano- ZnO), nano-titanium dioxide (nano-TiO_2), and titanium nitride nanoparticle (nano-TiN) are currently engineered nanomaterials used in food packaging [48,49], giving new distinct properties to the host and so, leading to different new functional applications [50]. Several examples of natural and engineered food encapsulators have been described [51]. Lipid based nanoencapsulation e.g. nanoliposomes, nanocochleates, and archaeosomes have been reported as nano-delivery systems for nutraceuticals, enzymes, food additives, and antimicrobials [52,53]. Nanoencapsulation of probiotics to be targeted to specific region in GI tract has been also achieved [54]. On the other hand, quality assurance in food and bioprocessing industry together with nanomaterials, is an important nanoscience field of action because consumers demand safe and wholesome food so, stringent regulations to ensure food safety are nowadays of great demand. Indeed, tailor-made nanosensors for food analysis, flavours or colours, drinking water and clinical diagnostics have been already developed [55]. Since very early in the 2000s nanoscience application through nanosensors and biochips applied to food quality assurance captured the researches attention [56]. A very sensitive nanosensor to detect gases release by foods and it spoils was developed by Ruengruglikit et al. [57]. A simple color change indicated whether the food was fresh or not. Nanosensors based on conducting polymer nanoparticles for grain quality monitoring to detect volátiles spoilage and analytes have been developed by Neethirajan et al. [58]. A smart or intelligent packaging to enhance communication aspect of the package was developed more recently by Mihindukulasuriya and Lim [59]. In this nanosensor, the response generated due to changes related with internal or external environmental factor is recorded through a specific sensor. Nanosensors to detect bacterial pathogens in food have also been developed [60-67]. Reflective interferometry and nanotechnology combination allow the Escherichia coli detection in food samples. This technique was based in measuring and detecting light scattering by cell mitochondria [68]. The principle of this sensor is simple since a protein of a known and characterized bacterium set on a silicon chip can bind with any other *E. coli* bacteria present in the food sample. Finally, a nanosized light scattering detectable by analysis of digital images is obtained. In order to identify a broad range of food related pathogens, such as *Salmonella* spp. and *E. coli*, a low cost nano-bioluminescent spray [69], has been developed that produce a visual glow very easy to detect. Conventional lab-tests based on bacterial cultures are time-consuming however, biosensor with fluorescent dye particles attached for example to anti -salmonella antibodies on a silicon/gold nano- arrays proved to be a fast and accurate for such pathogens detection [70] . Bacterial toxins have also been used in this fast response nanoparticle technology [71]. Wang et al. [72] developed microcantilevers bionanosensors to measure specific binding of *Salmonella* spp. to peptides derived from phage display libraries. These phage-derived peptides for *Salmonella* spp. and other pathogens like *L. monocytogenes* and *E. coli* are highly selective, accurate and sensitive, able to detect a single pathogen cell. Figure 1 summarizes this technique.

Another interesting application other than pathogens detection biosensors is the antimicrobial activity of different nanomaterials able to act against foodborne pathogens and food spoilage bacteria, as mentioned above. Among this antimicrobial nanoparticles mainly used in food packaging we can find MgO [73], Cu_2O [74,75], ZnO [76], and chitosan [77,78], as well as carbon nanotubes [79,80]. Natural phytoglycogen nanoparticles has also showed enhanced antimicrobial activity against L. monocytogenes when nisin was used [81] while bactericidal action against L. monocytogenes was obtained through the use of functionalized lysozyme-coated polystyrene nanoparticles with selective antibodies [82]. Nanosilver particles for example, are typically used for its antimicrobial effect. While more than 650 disease-causing pathogens can be killed by silver molecules in 6 minutes of contact, general antibiotic kills only 5 to 6 disease-causing pathogens [83,84]. Other interesting approach is related to improvement of food storage in which several worldwide food companies have been involved. Food deterioration due to oxidation of fats and oils together with microbial activities is caused mainly by internal presence of oxygen in the food packaging. Also, discoloration, changes in texture, rancidity, off-odor and flavor problems are consequences of the oxygen presence inside packages. Selective control of oxygen transmission and aroma affecting enzymes in the packaging films were built based on nanotechnologies approaches [85] (Figure 2).

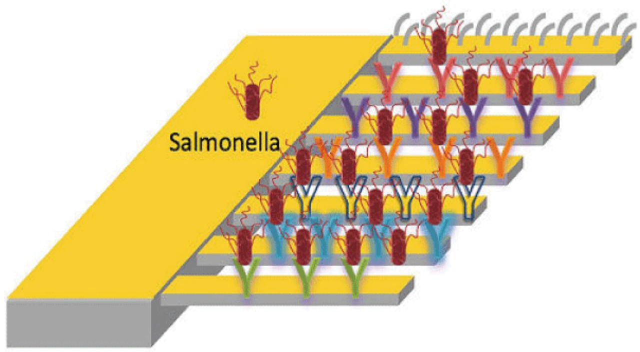

Figure 1: Example of phage display libraries peptides-based nanobiosensor for fast detection of *Salmonella* in foods according Wang et al. The yellow color designates the Gold layer deposited on a Si substrate. The "diving boards" are decorated with different unique peptides with binding affinities to different *Salmonella* strains. When a peptide catches a bacterium, the cantilever bends ever so slightly, due to a mismatch in surface stress on the top and bottom. A fine laser trained on the mechanism catches that motion and triggers the alarm. The system is sensitive enough to warn of the presence of a single pathogen.

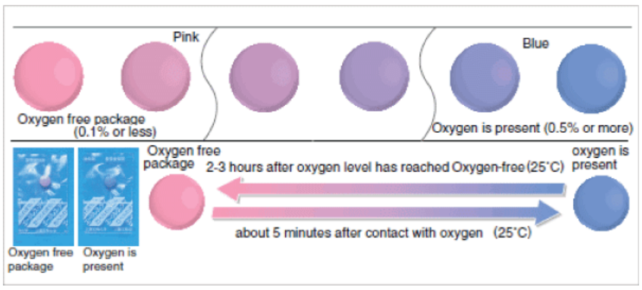

Figure 2: Nanofilm to detect Oxygen levels in food packages. Oxygen concentration ≤ 0.1 % → indicator is pink; Oxygen concentration ≥ 0.5 % → indicator is blue.

Chitin was used by Kriegel et al. [86] to developed a methodology using electro-spinning technique for making biodegradable green food packaging. The lobster shells main component is chitin which is dissolved by electro-spinning yielding a solvent useful to produce nanoslim fiber spin, with strong antimicrobial activity.

Is Nanotechnology safe for food industry: Nanotechnology success in the food industry highly depends on consumer's perception and social acceptance. Different reviews deals with possible toxicity of nanoparticles to the environment [87], to the health [88], and also those describing the different mechanism of action of nanoparticles in biological systems [89]. Due to health implications of nanoparticles entering human body, assessment of potential risks to human health is required urgently [90]. Recently, EU regulations established that any food ingredient resultant from nanotechnologies applications must undergo safety assessment before being authorized for its use [91]. Unfortunately, by now exist few human studies regarding nanoparticles potential toxicity, although preliminary studies on animals shown potential toxicity for liver, kidneys, and immune system. Therefore, risk assessment studies to show the adverse effect of nanoparticles on human health should be critically investigated.

Concluding Remarks

Nanotechnology offers great and interesting research opportunities in the food field providing new chances for innovation and bringing solutions for this and other industries. Despite that nanofoods market will increase in 2016 up to US$30 billion, it is expected that by the year 2020 more than 50% of food products will be nanotechnology based. However, it is particularly important to ensure that consumers be able to exercise choice in the use of the products of nanotechnology and that they have the information to assess the benefits and risks of such products. So that, there is urgent need for international regulation system for use of nanoparticles.

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