



Review

A review on enzyme and ultrasound: A controversial but fruitful relationship



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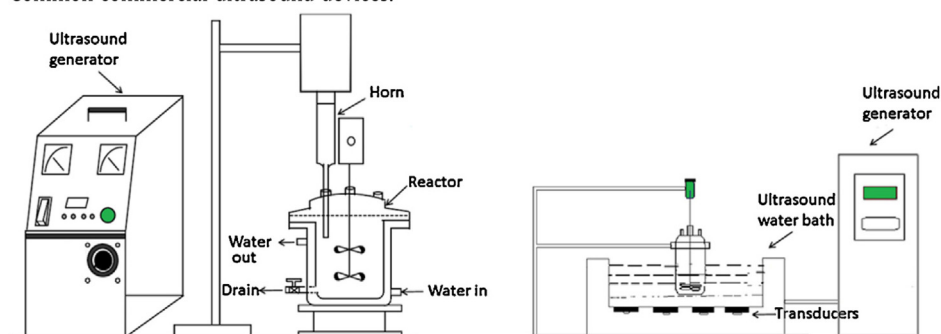
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HIGHLIGHTS

- The effect of ultrasound (US) on enzymes and their action is critically discussed.
- The scant knowledge of US users on this energy difficult its correct utilization.
- The lack of information on the US–enzyme–working conditions is in depth discussed.

GRAPHICAL ABSTRACT

Common commercial ultrasound devices.



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ABSTRACT

A critical review on the effect of ultrasound (US) on enzymes and their biocatalytic action is presented here. Discussion on the information users of US acquire before utilizing the different devices, and the importance they give to US frequency is constant along the review. The authors have gone into the different areas in which the US–enzyme binomial has been applied. The lack of enough information on the US–enzyme–working conditions under which each piece of research has been developed, and the necessity to provide complete information on the data and metadata to give enough light on each piece of research (and thus on the potential comparison of results from different studies) are critically exposed. With this aim, the study has been divided into the positive effect of US on enzymes to favor the production of metabolites, polymers or proteins; and the degradation, inhibition or activation of the

Abbreviations: ACE, angiotensin I-converting enzyme; BD, biodiesel; CAPE, caffeic acid phenethyl ester; CD, circular dichroism; DAG, diacylglycerol; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging; DWGP, defatted wheat germ protein; EAE, enzyme assisted extraction method; EDP, 2-ethylhexyl 4-(N,N-dimethylamino)benzoate; FA, fatty acid; FAME, fatty acid methyl ester; FT-IR, Fourier transform infrared analysis; GC–FID, gas chromatography–flame ionization detector; GC–MS, gas chromatography–mass spectrometry; GHz, gigahertz; GlyC, glycerol carbonate; HHP, high hydrostatic pressure; ¹H NMR, proton nuclear magnetic resonance; HPLC, high-performance liquid chromatography; ICP, inductively coupled plasma; IL, ionic liquid; k_A , apparent breakdown rate constant; kHz, kilohertz; K_M , Michaelis–Menten constant; LC-MS/MS, liquid chromatograph–triple quadrupole mass analyzer; LTQ–Orbitrap, linear trap quadrupole; MAD, microwave-assisted digestion; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MHz, megahertz; MS, mass spectrometry; MTS, nano-thermosonication; MW, microwaves; OPO, 1,3-dioleoyl-2-palmitoylglycerol; RP-HPLC, reverse phase high-performance liquid chromatography; RSM, response surface methodology; SC-CO₂, supercritical carbon dioxide; SDS PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SEM, scanning electron microscopy; SPE, solid-phase extraction; TC, tetracycline; TPP, three phase partitioning; US, ultrasound; USAEE, US-assisted enzymatic extraction; W, watt; XRD, X-ray diffraction.

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Metabolites
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 Enzymatic activation–inhibition

biocatalyst under US application. Also the effect of US on enzyme production and the main fields of application of the US–enzyme binomial are discussed.

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1. Introduction

Ultrasound (US) is a type of no radiant energy (therefore, it is incorrect to express its application as “US irradiation”) each time more used by analytical chemists despite the knowledge some of them show about it has not grown at the same rhythm as its use. Cavitation, the most named phenomenon produced by US [1], is highly dependent on the frequency of US (property scantily considered by some of its users, even sometimes forgotten when US equipment is described). The cavitation phenomenon contributes to the US effect on enzymes through three main mechanisms, the effect of which is considered to act separately or combined, by either improving enzyme catalysis or degrading the biocatalyst. One of the effects is purely thermal, due to the enormous micro-zone temperatures achieved during cavitation. Other effect is due to free radicals generated by ultrasonolysis in either water or other polar liquids. The third effect is caused by the mechanical forces, shear forces, created by microstreaming and shock.

Present literature regarding the time US is applied to enzymatic reactions can be categorized into two main groups: the first makes use of US sample preparation prior to the enzymatic step [2]. In this case, reduction in particle size and a consequent increase in the catalytic surface area are useful to reduce mass transfer limitations. The second approach involves the use of US throughout the enzymatic reaction. Here, the cavitation energy is thought to accelerate the reaction rate, yet the mechanism by which this occurs is unclear

(perhaps by increasing the movement of liquid molecules, the substrate's access to the active site is increased). The second approach has demonstrated either to accelerate enzymatic reactions or to inactivate the biocatalyst [2,3], but in the former case with a dependence of the enzymatic enhancement with the intensity of ultrasonication rather than with its frequency [4]. Apparently, cavitation promotes an increment in reaction rates rather than a change in the reaction constants.

In old literature, authors working with US have attributed its action to different effects. This is the case with acceleration of enzymatic hydrolysis, which is mainly caused, in the Tuulmetts and Raik's opinion [5], by temperature gradients in the cavitation medium; while Vulfson et al. did not attribute to cavitation the effect of power US of 25 kHz (frequency no specified in the publication) on interesterification, which is also a hydrolytic reaction [6]. In dealing with enzyme degradation, the influence of moderate pressures on the system subjected to US is considered either to exert an enormous effect [7], or to cause little effect on enzyme degradation [8]. The main causes of controversial results can be attributed to the different US frequencies, intensities and application conditions (no specified in the publication), and/or to the different types of enzymes on which US energy is applied and even to the different working conditions.

This review article is mainly aimed at showing, (i) the different areas in which the US–enzyme binomial has been applied, (ii) the lack of enough information provided on the US–enzyme-working

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