Applications of microwaves in biological sciences

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Microwave irradiation can alter the rate of enzyme-catalysed reactions, although the role of any non-thermal factors in such processes is controversial. While the studies in aqueous buffers have been understandably limited to thermostable enzymes, higher stability of enzymes in anhydrous media allows the use of a variety of enzymes. The microwave-assisted applications described in this review include use of lipases for esterification and transesterification reactions, protease for peptide synthesis and again, use of two well-known proteases, α-chymotrypsin and subtilisin Carlsberg in esterification and transesterification reactions respectively. In the last system, it has been shown that the so-called non-thermal effects increase the reaction rates by 2-5 fold at all water-activity levels. It is also established that microwave irradiation can be used in conjunction with other strategies (like pH tuning and salt activation) for enhancing reaction rates. In addition, some miscellaneous applications in bioanalysis, food processing and extraction of biological materials have been described. Two illustrations for possible use of microwaves in medicine have been briefly discussed. Finally, the review also discusses the recent work on enhancing the rates of enzymatic reactions by pretreatment of insoluble substrates with controlled microwave irradiation.

TECHNOLOGY Vision 2020 of the US Chemical Industry believes that microwave irradiation will replace traditional heating in chemical synthesis[1,2]. Thus, it is not surprising that microwave-assisted chemical reactions are being increasingly carried out in the context of a variety of applications. These have been reviewed in a number of journals[3–5]. The present review focuses on microwave-catalysed enzymatic reactions. In addition to some miscellaneous applications in the area of bioanalytical applications, food processing and upstream processing of biological material and possible uses in medicine have also been mentioned. Finally, the review also discusses the exciting possibility of substrate pretreatment for improving the efficiency of bioconversion processes.

Thermal vs non-thermal effects

Microwaves (0.3–300 GHz) lie between infrared and radiofrequency electromagnetic radiations[3–5]. It is generally believed that the microwave irradiations accelerate chemical reactions by the heating effect. Irradiated with microwaves, a molecule of the medium continually realigns itself with the changing field. This causes electromagnetic energy to get converted into heat energy. The dielectric constant, the ability of a molecule to be polarized by an electric field, dictates the capacity of the medium to get heated with microwave irradiation. Thus, solvents such as water, methanol and dimethyl formamide get heated easily; microwaves have no effect on hexane, toluene and diethyl ether. However, some workers believe that there are non-thermal effects[3]. The two conflicting viewpoints have been nicely summarized in a recent write-up[6]. Whittaker and Mingos[7] believe that 'the rate of a reaction in many syntheses is so high that it cannot be accounted for by heating effects alone'. On the other hand, Kappe[8] asserts that 'there are no general non-thermal effects', and that the so-called non-thermal effect is due to superheating of solvents above their boiling points. The main difficulty in resolving this controversy is the fact that it has been difficult to experimentally distinguish between thermal and non-thermal effects. During the last decade or so, microwaves with temperature control have become commercially available. This has led to some studies where it is possible to distinguish between thermal and non-thermal effects. Whether such non-thermal effects originate simply in superheating, is not yet clear. However, that is not restricting the ever-increasing large number of applications. Microwave irradiation is also being used for accelerating enzymatic reactions.

Microwave-assisted enzymatic reactions in water

Porcelli *et al.*[9] tried to distinguish between thermal and non-thermal effects of microwave irradiations on enzymes in aqueous buffers. The authors chose the thermostable enzymes, *S*-adenosylhomocysteine hydrolase and 5′-methylthioadenosine phosphorylase from a thermophile *Sulfolobus solfataricus* for this study. Choosing thermostable enzymes is necessary in aqueous buffers so that one can work at higher temperatures (like 70–90°C). Normally, enzymes are not stable beyond 30–40°C (ref. 10). Thermal denaturation of enzymes/proteins is an extensively studied phenomenon[10]. The denaturation starts with reversible unfolding of polypeptide chains and if the thermal stress continues, irreversible denaturation sets in. Various mechanisms (viz. aggregation, conversion of asparagine to aspartate residue, hydrolysis of the peptide

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bond next to an aspartate residue) at the molecular level have been identified. It is also now established that thermostable enzymes use a variety of strategies for achieving stable structures[11]. Porcelli *et al.*[9] found that 10.4 GHz microwave irradiation caused a non-thermal, irreversible and time-dependent inactivation of both enzymes (viz. *S*-adenosylhomocysteine hydrolase and 5′-methylthioadenosine phosphorylase). Conformational changes detected by fluorescence spectroscopy and circular dichroic spectroscopy 'suggest that microwaves induce structural rearrangements not related to temperature'[9]. This was based upon the fact that these enzymes were quite stable when subjected to thermal stress alone by conventional heating at 70–90°C. The authors also cite earlier work wherein thermal and non-thermal effects have not been distinguished as responsible for inactivation of enzymes/proteins by microwave irradiation. However, Yeargers *et al.*[12] showed that 'there is no discernible difference in the performance of the enzyme, whether heated by the microwave source or by conventional methods'. A later publication describes similar results with alcohol dehydrogenase from *S. solfataricus*[13].

Earlier, Kabza *et al.*[14] compared the microwave-irradiated and non-irradiated systems in which cellobiose hydrolysis by cellulase from *Penicillium fumiculosum* was carried out in aqueous buffers. They failed to find any difference in the rates or overall conversion, but were not sure whether this was due to lack of sensitivity of the analytical method which was used for estimating the product (glucose).

Microwaves in non-aqueous enzymology

Enzymes can be used in low water-containing organic solvents. There are a number of advantages associated with the use of enzymes in such media[15–18]. The result is a number of novel and interesting applications (Table 1). There are two reasons why microwave-irradiation is relevant to non-aqueous enzymology: (i) the enzymes in nearly anhydrous media are extremely thermostable. In many cases, keeping them at 100°C for an extended period of time does not cause inactivation[19]. This is attributed to the fact that during lyophilization, drying removes water molecules which were H-bonded to many surface residues. It results in these side chains interacting with each other and creating a rigid structure. When such lyophilized powders are used in aqueous buffers, rehydration reverses such changes[20]. On the other hand, when used in low water-containing organic solvents, rehydration is not possible and the structure remains highly thermostable. This means that one does not have to worry about thermal inactivation while using microwaves if the medium is nearly anhydrous. In practice, one may have to optimize water-activity level for best yields. Hence, one should evaluate the enzyme stability in the specific medium at a specific water-activity level. In general, thermal stability is higher than in aqueous buffers.

While enzymes are being increasingly used in organic solvents for a variety of applications, their low activity continues to be of major concern[21]. An idea about this can be gathered from the activity of subtilisin Carlsberg in aqueous buffer and organic medium (catalytic efficiency of $5.94 \times 10^3\ M^1\ s^{-1}$ for hydrolysis and $1.04 \times 10^{-1}\ M^1\ s^{-1}$ for transesterification). In fact, this low activity originates in the rigidity of protein molecules taking place during lyophilization, as described above. A number of strategies have been described to accelerate the rate of enzymatic reactions in such media. These include ligand-induced conformational changes[22,23], salt activation[24], use of cross-linked enzyme crystals[25], addition of lyoprotectants during lyophilization[21] and using propanol-rinsed enzyme precipitates[26]. The last approach consists of de-

Table 1. Applications of enzymes in non-aqueous media

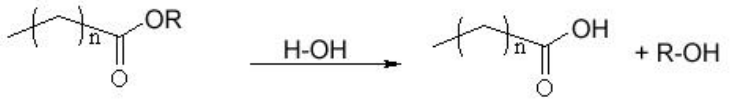
Application	Enzyme	Solvent	Reference
Production of biosurfactants	Lipase	Pyridine	63
Production of biodiesel	Lipase	1,4-Dioxane	64
Synthesis of aspartame precursor	Thermolysin	Ethyl acetate	65
Synthesis of (*S*)-naproxen ester prodrug	Lipase	Iso-octane	66
Synthesis of 'delicious' octapeptide	Papain	Acetonitrile	67
Synthesis of a flavour compound, cis-3-hexen-1-yl acetate	Lipase	Hexane	68
Production of confectionary fats	Lipase	Solvent-free system	69
Synthesis and modification of polymers	Lipase, protease and cellulase	Different solvent and cosolvent systems	70
Oxidation of benzylamine	Amine oxidase	Different organic solvents	71
Transphosphatidylation of alcohols	Phospholipase D	Chloroform	72
Synthesis of *b*-amino acids and *b*-lactams	Lipase	Diisopropyl ether	73
Synthesis of non-ionic surfactants	Lipase and protease	Different organic solvents	74
Acylation of	Lipase		
secondary amines		Hexane	75
flavonoid (naringin)		2-Methyl-2-butanol	76
Preparation of chiral amides	Lipase	Hexane	77

hydrating enzymes after immobilizing them on solid supports. This is followed by decanting-off the excess aqueous buffer, taking care that the enzyme remains fully wetted. Rapid dehydration is then carried out by rinsing the immobilized enzyme with a suitable water-miscible organic solvent, usually *n*-propanol, containing low levels of water. This method of dehydrating enzymes overcomes the detrimental effects of drying enzymes/proteins by lyophilization. Microwave irradiation has also been used and is yet another valuable strategy to obtain the much needed enhancement in reaction rates. There are two reasons why these studies have been rather few in number. (i) Commonly available microwave ovens with the provision of temperature control have become available only in the last few years and (ii) there are not many suppliers of such instruments. Some pioneering work was done with ovens from Prolabo, France. The company is no longer selling these microwave reactors.

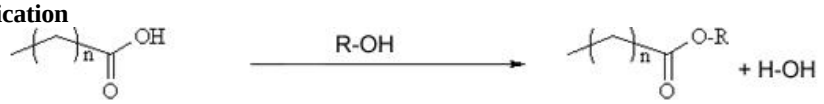
In almost all cases, the microwave-assisted reaction type studied has been lipase-catalysed transesterification. This is not surprising since lipases are the most extensively used enzymes in non-aqueous media. Both their broad specificity and easy availability (a large number of lipases are available in free as well as immobilized forms) have contributed to their extensive use. In organic media, lipases can catalyse various types of reactions (Box 1). Carrillo-Munoz *et al.*[27], in probably one of the earliest applications, used microwave-catalysed reaction for resolution of (±)-1-phenylethanol. Two commercially available lipases from *Pseudomonas cepacia* and *Candida antarctica* were used, both enzymes are known to be successful in catalysing transesterification reactions. Immobilization is generally used to improve the storage stability, operational stability and ease of handling[28]. Increase in operational stability and easy recovery of solid catalyst (from the reaction mixture) imply improvement in reusability. Even in organic solvents, immobilized enzymes are frequently used[29,30]. Carrillo-Munoz *et al.*[27] tried four matrices: Florisil™, Celite, HyfloSuper Cel™ and Accurel™. The first three matrices are minerals and the fourth one is macroporous polypropylene resin. Florisil was found to absorb microwaves and hence was rejected. HyfloSuper Cel™ and Accurel™ were found to be better choices and were used in the detailed study. Lately, 'solvent free' enzymatic reactions have been reported where either one of the substrates acts as the solvent medium or the reaction is carried out in the liquid phase associated with the reactant and enzyme particles[31]. The work of Carrillo-Munoz *et al.*[27] used the innovative approach of solubilizing the substrates in organic solvents and then 'impregnating on the enzyme-loaded supports by subsequent evaporation of these solutions'. A microwave (Synthewave 402 from Prolabo, France) fitted with a stirring system and an infrared temperature detector was used. The energy distribution was also reported to be more homogeneous and efficient than in a domestic microwave

Box 1. Types of reactions catalysed by lipases in non-aqueous media.

Hydrolysis



Esterification



Transesterification

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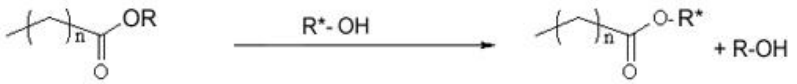
Alcoholysis



Acidolysis

oven. The immobilized enzyme could be reused three more times. The initial rates and enantiomeric ratios *E* were significantly higher with microwave assistance. Selectivity was higher with isopropenyl acetate than with the ethyl ester, presumably since higher polarity led to greater response to microwave irradiation with the former substrate. In the case of resolution of (±)-1-phenylethyl valerate by transesterification with butanol, quantitative yield of 50% was obtained compared to 47% (even with a longer reaction time) with conventional heating. The authors attribute the increased specificity of the enzyme-catalysed reactions to the shifting of reaction equilibria by microwave irradiation by evaporation of light polar molecules (viz. water or ethanol molecules) formed as by-products[27].

Around the same time, Parker *et al.*[32] reported the data on microwave-assisted transesterification (conversion of ethyl butyrate to butyl butyrate by butanol) with cutinase (a lipase with specificity towards cutin in water). The enhancement in reaction rates by 2–3 fold over classical heating was dependent on the water-activity during transesterification. A curious result was that at water-activity of 0.97, microwave-assisted reaction rates decreased drastically and was in fact lower by 2× as compared to conventional heating. It was also confirmed by the authors that microwaves do not affect the inherent enzyme activity since the enzyme during reuse, under classical heating conditions, did not show the rates of microwave-assisted reactions.

Thermostable enzyme preparations such as crude homogenate of *S. solfataricus* and recombinant *b*-glucosidase from *Pyrococcus furiosus* have been used for transglycosylation reactions[33]. Lin and Lin[34] used porcine pancreatic lipase (PPL) to acylate (*R*)-stereoisomer of 1,2,3,4-tetrahydro-1-naphthol (*R*-1) present in its racemate (rac-1) to recover (*S*)-stereoisomer with high selectivity. Vinyl acetate and vinyl butyrate were used as acyl donors and microwave irradiation led to increase in reaction rates and stereoselectivities by 4-6 and 3-9 fold respectively. Similarly, acylation of rac-1-indanol (rac-3) to recover *S*-stereoisomer showed increased stereoselectivities and reaction rates by 3-5 fold and 1-14 fold by microwave irradiation in benzene. The authors believe that 'the rate enhancements from microwave irradiation of PPL-catalysed reactions are also probably due to the locally high pressures or the increase in usable surface area for catalysis…'[34]. This explanation needs to be verified experimentally. It is however known that in some cases, microwave irradiation of enzymes suspended in nonaqueous media did not cause any irreversible changes in the catalytic performance of the enzyme[32].

More recently, Bradoo *et al.*[35] described a rapid scanning procedure for lipase specificities using microwave irradiation. A domestic microwave oven (Model No. BMC 900T, BPL Sanyo, India) was used. Commercial preparations of lipases from porcine pancreas, *Mucor miehei*, *Candida rugosa*, *P. cepacia* and two laboratory isolates were evaluated for triolein hydrolysis and esterification (of sucrose, methanol and ascorbic acid) with different fatty acids. All reactions were carried out at 1.35 kW for 30 s. The hydrolysis rates increased by 7-12 fold and esterification was obtained within 30 s with some lipases. The selectivities of lipases did not change as a result of the irradiation in either hydrolytic or esterification reactions. No comparisons *vis-à-vis* conventional heating were provided, since no temperature control was applied during microwave irradiation. In fact, the authors found that the reaction temperature rose to 80°C within 30 s.

Chen *et al.*[2] have provided some useful tips for carrying out microwave-assisted enzymatic reactions. These protocols deal with Alcalase™-catalysed peptide-bond formation. Alcalase is a subtilisin Carlsberg preparation sold by Novozymes, Denmark. The enzyme-catalysed transesterification of Cbz-Phe-OBzl with ethanol to produce Cbz-Phe-OEt in 2-methyl-2-propanol was monitored by HPLC using an RP-18 column. The time course of alcalase inactivation was followed up to 30 min. The microwave irradiation led to the temperature of the system rising to 50°C (from an initial 25°C) and only 50% of the original activity was left. A control at 25°C gave 75% remaining activity whereas conventionally heated incubation at 50°C for 30 min resulted in 44% of residual activity. Thus, the authors concluded that inactivation was largely due to thermal factors. Unfortunately, no water-activity data were provided since under low water-activity conditions, one expects the enzyme to be stable at 50°C. The protocol for alcalase-catalysed preparative-scale peptide-bond formation between Cbz-Ala-Phe-OMe and pro-NH_2HCl, has also been described[2]. The temperatures of the reaction system after 5, 10, 15 and 20 min were 35, 38, 42 and 45°C respectively. Nevertheless, all the Cbz-Ala-Phe-OMe was consumed within 20 min with several-fold increase in conversion rates compared to non-irradiated reaction where time courses at 25 and 45°C have been shown.

The esterification (of *N*-acetyl phenylalanine with ethanol to form *N*-acetyl phenylalanine ethyl ester) and transesterification (of *N*-acetyl phenylalanine ethyl ester with propanol to form *N*-acetyl phenylalanine propyl ester) with *a*-chymotrypsin and subtilisin Carlsberg respectively have been carried out[36] with a microwave oven equipped with a non-contact infrared continuous feedback temperature control. This allowed determining 'non-thermal' effects of microwave irradiation by running controls in which conventional heating was employed for fixing the same temperature. Six different solvents of differing polarities were used and it was found that in all cases, microwave irradiation increased the initial reaction rates by 2.1–4.7 times at all attempted water activities during the reaction. Enhancements of reaction rates in the microwave-assisted esterification were observed with un-

tuned, pH-tuned[37] and salt-activated enzymes[24]. It was found that microwave irradiation could be used in conjunction with the strategies of pH tuning and salt activation for enhancing initial reaction rates. For example, whereas untuned subtilisin showed a transesterification rate of 0.5 mmol h^{-1} at 25°C in *n*-octane [with 0.3% (v/v) added water], the salt-activated and pH-tuned subtilisin gave a microwave-assisted reaction rate of 11.76 mmol h^{-1} (with the same level of water; Figure 1). This was about 20 times increase in the initial rate. These results clearly established that increase in reaction rates observed in the case of microwave-assisted reactions was not due to thermal effects alone. It is also interesting to note that medium effects dominate over other factors. The esterification rate in toluene in case of microwave-assisted reaction with pH-tuned and salt-activated α-chymotrypsin (1.10 mmol h^{-1}) is still much lower than just pH-tuned α-chymotrypsin in *n*-octane (5.31 mmol h^{-1})[36].

Miscellaneous applications

Microwave irradiation has also been used for numerous applications which are relevant to enzymology but may or may not involve acceleration of enzyme-catalysed reactions. These are briefly reviewed here, since they may also be of interest to biochemists belonging to various sub-disciplines.

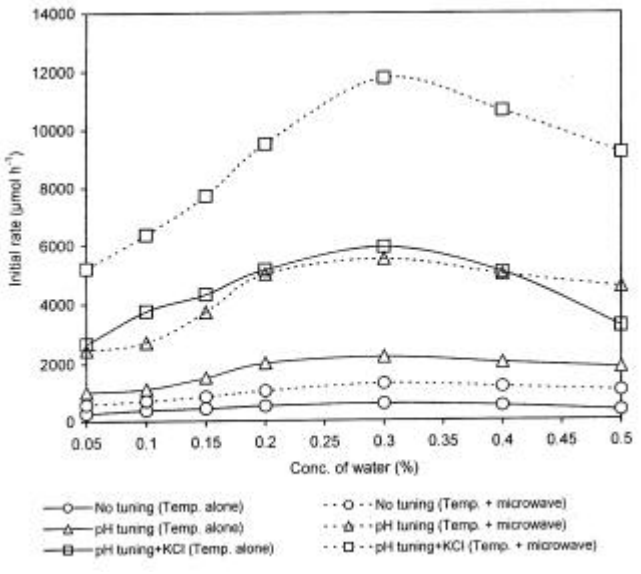


Figure 1. Effect of pH-tuning and salt activation on rate of transesterification by subtilisin Carlsberg in *n*-octane at 25°C. For pH tuning, subtilisin (100 mg) was dissolved in 10 ml of 20 mM Tris HCl, pH 7.8 and the solution was lyophilized. The lyophilized powder was then suspended in organic solvents and the reactions carried out. For salt activation, 10 mg of the pH-tuned enzyme was added to 10 mg K_2HPO_4 and 0.98 g KCl and the solid was dissolved in 40 ml distilled water and the pH adjusted to 7.8. This solution was then lyophilized and used further. Reproduced from ref. 36.

A combined microwave and heat treatment was found to inactivate polyphenol oxidase completely in a short time in whole, edible mushrooms (*Agaricus bisporus*)[38]. This prevented the enzymatic browning reactions during their storage and transport. This way of blanching produced decrease in the loss of antioxidant content, weight loss and shrinkage. The authors developed a microwave applicator in which irradiation conditions could be regulated and heating could be monitored. A similar kind of application is the prevention of oxidative rancidity in rice bran during storage[39]. It was found that raw rice bran with 21% moisture content treated in a microwave oven (850 W for 3 min), packed in closed bags and stored at 4–5°C up to 16 weeks did not develop rancidity. This could be correlated with the significant decrease in lipoxygenase activity as a result of the treatment.

Another useful application has been in enhancing the sensitivity of protein estimation methods[40]. Recently, this has been applied to ELISA format for carrying out Lowry's estimation[41]. Thus, small sample volumes of as low as 5 µl could be handled and the assay time was reduced to 20 s from the normal 30 min. The microwave-based assay also tolerated the presence of higher concentrations of various potential interfering substances. This makes it possible to assay proteins if such substances are present within limits. Also, as the assay is carried out using an ELISA plate reader, the method lends itself to automation.

Improvement in PCR technology will increase the utility of this technique in numerous fields (e.g. basic biological research, clinical diagnosis, combinatorial chemistry, etc.). Fermer *et al.*[42] have reported the use of single-mode microwave cavity which allows the placement of a sample at a fixed position of much higher continuous electric field strength than can be obtained in a multi-mode reactor (e.g. a domestic microwave oven). Microwave amplification almost reached the same efficiency as conventional PCR. The ramping time is substantially reduced since the irradiation is directed specifically at the reaction liquid and neither the blocks nor the tubes have to be heated. Moreover, heating to the desired temperature is almost instant which allows incubation time to be shortened, since in the traditional PCR equipment, this time includes equalization of the temperature. The authors also showed that the Taq polymerase was intact after more than 25 amplification cycles using microwave heating.

Finally, it may be worth mentioning that microwave irradiation has been found useful in extraction of specific substances from biological sources. Cho *et al.*[43] have reported its usefulness in extraction of chitin from red crab *Chinoecetes japonicus*. Chitin is extracted at present using concentrated HCl or NaOH. Microwave irradiation was found to lower the necessary concentration (especially HCl) of these harsh chemicals.

There has been concern about the effect of microwaves on mammalian systems and their possible use in medi

cine. We will limit ourselves to two illustrative examples. Patients with cirrhosis and hepatocellular carcinomas were subjected to transcatheter arterial chemoembolization, followed by (within 1–2 days) ultrasonographically guided percutaneous microwave coagulation therapy[44]. Dynamic computed tomography showed complete necrosis of their tumour lesions. Ryan *et al.*[45] reported that sustained exposure of rats to 35 GHz produced hyperthermia and subsequent circulatory failure. Studies repeated with rats administered with nitric oxide synthesis inhibitor *N*-ω-nitro-L-arginine methyl ester showed that chronic NO synthesis inhibition reduced the ability of rats to withstand microwave irradiation. The authors concluded that NO does not mediate the hypotension produced by hyperthermia.

Substrate engineering

In the case of insoluble substrates, the accessibility of target bonds often limits the extent of bioconversion. For example, Esteghlalian *et al.*[46] have found that drying of Douglas fir kraft pulp fibres in the oven even at 50°C, significantly reduced the number of large pores and consequently reduced the susceptibility of dried pulps to hydrolysis by a mixture of cellulase and β-glucosidase. Thus, lignin in paper pulp, which as it is, happens to be a difficult material to hydrolyse enzymatically, became more intractable. It is not unusual in many such cases to pretreat these insoluble materials by physical and chemical methods to increase their enzymatic hydrolysis[47]. A large number of such pretreatments have been described for cellulosic and hemicellulosic materials. Recently, microwave pretreatment has been found to increase the conversion yields in hydrogenolysis of lignins[48]. A patent for an 'apparatus and method for cellulosic processing using microwave pretreatment' has been granted to NASA (USA)[49]. A feed mixture of cellulose and dilute acetic acid is irradiated with microwaves at a superatmospheric pressure in an autoclave reaction vessel and the treated cellulose was found to be hydrolysed by enzymes with enhanced reaction rates as a result of the pretreatment. As the patent abstract mentions, 'High yield, low hazard potential, low energy usage and ready preparation in space of acetic acid and the enzyme makes the present invention well suited for use on long duration space missions'[49].

Microwave pretreatment of chitin was also found to improve the efficiency of its enzymatic hydrolysis. Chitin [(1 → 4)-2-acetamido-2-deoxy-β-D-glucan] is an insoluble polysaccharide that occurs in insect exoskeletons, crustacean shells and fungal cell walls[50]. Large amount of chitin is generated as solid waste from seafood processing. The hydrolysis of chitin not only solves this waste-disposal problem, it also produces value-added soluble carbohydrates[51]. Chitinases are enzymes which can hydrolyse chitin and have been isolated from a number of sources[51]. Response surface analysis[52,53] was used to determine the optimum conditions [2% (wv^{-1}) chitin, 57.5°C, 38 min] for microwave pretreatment of chitin[54]. A microwave oven (operating at 2.5 GHz) with an inbuilt magnetic stirrer and non-contact infrared continuous feedback temperature system was used, so as to irradiate the sample at a fixed temperature. V_{max}/K_m of cabbage chitinase towards untreated and pretreated chitin was found to be 21 nmol h^{-1} mg^{-2} ml and 32 nmol h^{-1} mg^{-2} ml respectively. This is largely due to reduction in K_m value for the chitinase for the pretreated substrate. Scanning electron micrography (SEM) (Figure 2) and powder diffraction data showed changes in the morphology of chitin. While the control sample (just heated, not irradiated) shows continuous 'splattered' appearance (Figure 2 *a*),

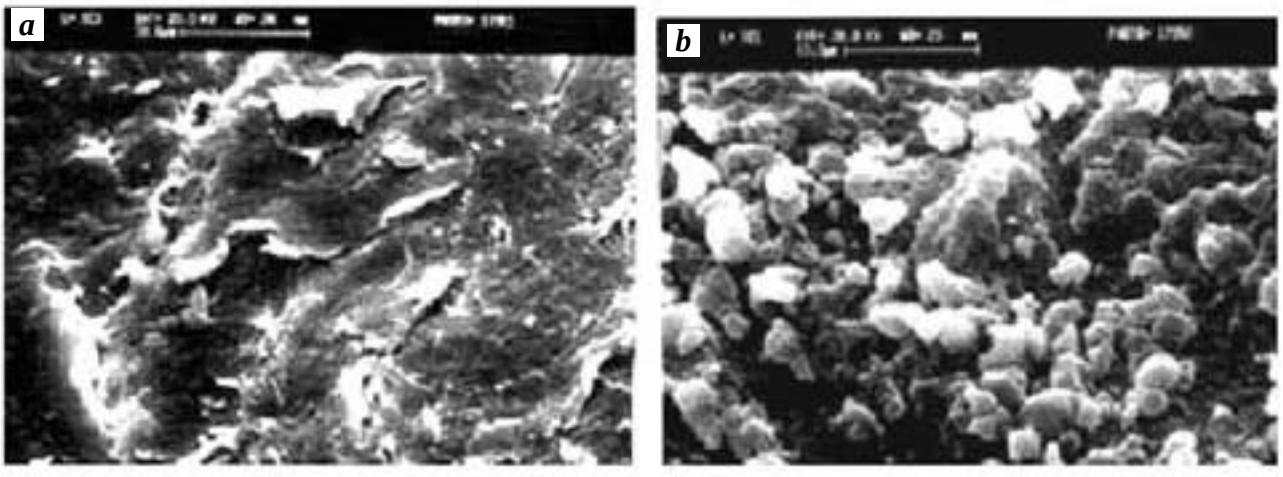


Figure 2. Scanning electron micrographs of (***a***) control and (***b***) microwave-irradiated chitin at a magnification factor of 2400. The bar represents 10 μm. Control refers to the sample heated in a water bath (under optimized conditions of chitin hydrolysis by chitinase) in an identical fashion but without microwave irradiation. Reproduced from ref. 54.

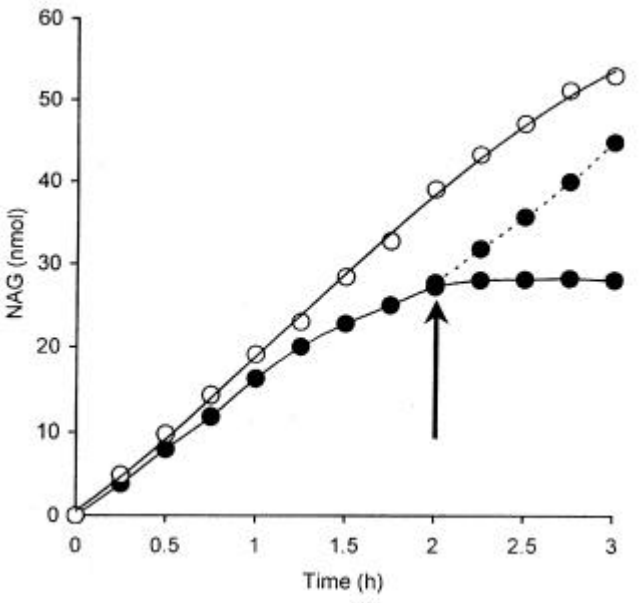


Figure 3. Hydrolysis of chitin by cabbage chitinase. The experiment was carried out by hydrolysis of microwave-treated chitin (○) and control (●) by 9.5 U of cabbage chitinase. Arrow denotes the time when fresh chitin (2%, w v^{-1}) was added to the reaction mixture in case of control. Reproduced from ref. 54.

the SEM of microwave-treated sample (Figure 2 *b*) shows discrete granular structure. Figure 3 shows the kinetics of hydrolysis of microwave-irradiated and non-irradiated chitin by cabbage chitinase. The higher rate obtained in the case of treated chitin presumably reflects reduction in mass transfer constraints for the enzyme accessing the substrate. The addition of fresh chitin (1%) at the end of 2 h in the case of control (non-irradiated chitin) resulted in the fresh production of *N*-acetylglucosamine, the product. Protein engineering of enzymes has been used to alter their catalytic properties[55]. In an analogous fashion, the pretreatment of substrate to improve the efficiency of enzyme-based catalytic process may perhaps be called 'substrate engineering'.

Conclusion

The recent spurt in research publications[3,4,56], books[5,57,58] and patent literature[49,59,60] on microwave-assisted reactions testifies to the growing popularity of microwave irradiation as an accepted tool in laboratories. While most of the work so far has involved chemical reactions, this review provides a glimpse of the proverbial 'tip of the ntial applications in applied enzymology and possibly in medicine. The use of microwave irradiation to enhance the rates of enzymatic reactions is bound to grow as microwave reactors with temperature control become commercially available from more vendors. Another factor which has limited such applications could be the sense of unease over the uncertainty regarding non-thermal effects. Biochemists still tend to view enzymes as fragile catalysts and are perhaps diffident about subjecting them to relatively less-understood reaction conditions. It is curious, how perceptions in science affect the choice of our tools. Biochemists have been using lyophilization for concentrating and drying proteins for decades, as the process was perceived to be an innocuous one. There were sufficient data since quite sometime ago that lyophilization causes aggregation[61]. Recent studies show that lyophilization is a harsh process which causes considerable structural changes in proteins[21,62]. Microwave irradiation could turn out to be actually much less harmful to enzymes. We need more data which will be available once people use this simple strategy that results in shorter process time in many cases.

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Antifungals from fluorescent pseudomonads: Biosynthesis and regulation

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A group of root-associated bacteria, plant growth-promoting rhizobacteria (PGPR), intimately interact with the plant roots and consequently influence plant health and soil fertility. Among these PGPR, fluorescent psuedomonads occur commonly in the rhizosphere of plants and help suppress disease establishment and spread. Psuedomonads suppress soil-borne fungal pathogens by producing antifungal metabolites such as pyoluteorin, pyrrolnitrin, phenazines, and 2,4-diacetyl phloroglucinol. In addition, psuedomonads can indirectly suppress fungal pathogens by scavenging iron in the rhizosphere environment through the release of siderophores. Considering the global significance of antifungal metabolites in disease suppression and consequent applicability of psuedomonads in biological control strategies, biosynthesis and regulation of these molecules is discussed in this review to highlight new developments in the subject.

FLUORESCENT pseudomonads are ubiquitous bacteria that are common inhabitants of the rhizosphere, and are the most studied group within the genus *Pseudomonas*. They comprise of *P. aeruginosa*, the type species of the genus; *P. aureofaciens*, *P. chlororaphis*, *P. fluorescens* (four biotypes), *P. putida* (two biotypes), and the plant pathogenic species *P. cichorii* and *P. syringae*; the latter includes a large number of nomenspecies[1].

All fluorescent pseudomonads fall into one of the five 'ribonucleic acid homology groups' as defined by rRNA–DNA competition experiments. The G + C content ranges from 58 to 68%. Most of the plant-beneficial pseudomonads are quite heterogeneous in that they comprise a collection of non-enteric Gram-negative strains, generally aerobes, which are non-fermenting and motile. These fit into one of the three categories: pathogens, biodegraders and root-colonizers/biological control agents. The last category exerts a protective effect on the roots through antagonism towards phytopathogenic fungi and bacteria. Two major mechanisms have been proposed to explain the suppressive and antagonistic effects of fluorescent pseudomonads. According to one, the pathogen is inhibited by competition for iron, since availability of Fe [III] in this soil is low (10^{-17} M). In general, most microorgan-

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