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Microwave Radiation Can Increase The Rate of Enzyme-Catalysed Reactions in Organic Media

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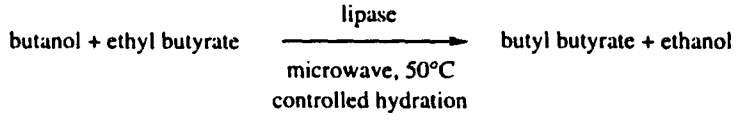
Abstract: Irradiating a hydrated lipase enzyme suspended in organic media using microwaves (2.45 GHz, 50°C) enhanced the reaction rate by 2-3 fold over classical heating and the apparent non-thermal effects observed were dependent on the hydration state of the enzyme in the organic medium.

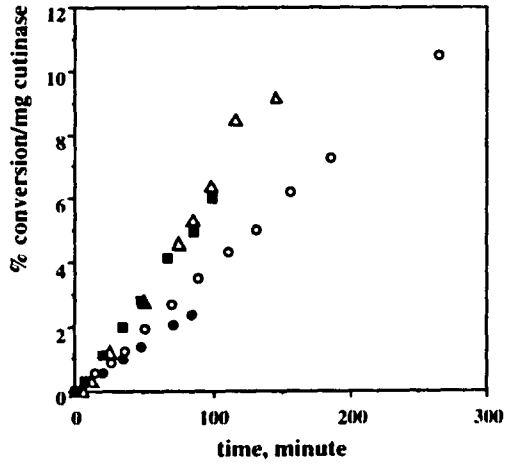
The study of microwave action on biological systems is an area of intense interest.[1-8] Studies in this area have mainly focused on safety aspects related to the microwaving of food where the reduction or enhancement of enzyme activity is an important factor in determining food processing methods. Several researchers have observed effects of microwave irradiation on enzymes in aqueous media which they have concluded to be non-thermal in origin.[2-4] In organic synthesis, microwave radiation can result in higher reaction rates and higher product yields being obtained compared to that of conventional heating.[9-12] However, in many systems the reasons for this are not well understood.



The observation that many enzymes exhibit good catalytic activity in organic media has considerably increased the range of potential applications in synthetic chemistry.[13,14] This has led to interest in understanding what factors influence the rate of catalysis, substrate specificity, synthetic yield and the enantioselectivity of enzyme action under these unusual conditions. The study of enzymes in non-aqueous media can also provide interesting insights into general mechanisms of catalysis.[15,16] We have studied the influence of microwave radiation on the reaction rate of an enzyme-catalysed transesterification reaction in organic media. In studying the effect of microwave action on enzyme catalysis, we can gain several advantages by working in organic media as opposed to aqueous solution. Most importantly, small incremental changes to the enzyme bound water content can be controlled *i.e.* as a function of thermodynamic water activity (a_w).[17,18,23] The corresponding reaction rate was measured where both the solvent water content and

the enzyme were equilibrated to the same a_w.[19, 20] This, therefore enables us to study the effect of microwave radiation on enzyme activity as a function of enzyme hydration. Protein hydration processes have been studied extensively[21,22] and the hydration process is accompanied by changes to the protein's dynamic and structural properties.[22] Therefore, we were interested to observe the effect of microwave irradiation on the hydrated enzyme activity.

The enzyme cutinase was suspended in the solvents, which were the substrates for the reaction: ethyl butyrate and butanol. Both the substrates and the enzyme powder had been equilibrated to the same a_w.[19] and the reaction mixture was either irradiated to 50°C in the microwave reactor or else in a jacketed glass vessel maintained at the same constant temperature. The progress of the transesterification was monitored by gas chromatography (GC).[20]



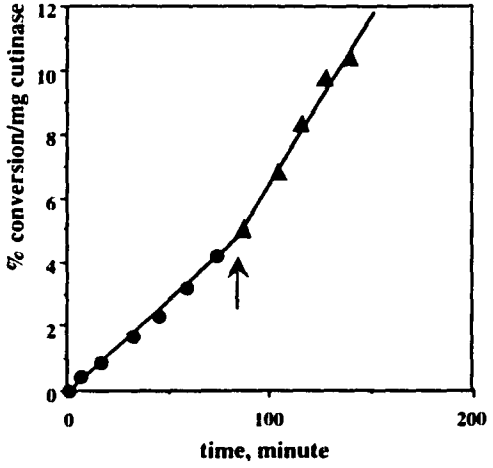


Figure 1 (left). Reaction rate for the cutinase catalysed transesterification between butanol and ethyl butyrate (50°C). Measured at a_w 0.58; classical heating (○), microwave irradiation (△) and at a_w 0.69; classical heating (●) and microwave irradiation (■).

Figure 2 (right).The same transesterification as in Figure 1. The sample (a_w=0.97) was initially irradiated by microwaves at T=50°C (●) for a period of 80 minutes, after which time the reaction vessel was removed (indicated by the arrow) and the same sample heated by classical means (▲) at 50°C.

The cutinase catalysed reaction rates for the transesterification between ethyl butyrate and butanol are shown in Figure 1, where the initial a_w of the system is 0.58. Results obtained for another reaction, at an initial a_w of 0.69, are also shown. At these water activities, the reaction rate measured for the microwave irradiated sample is approximately double that observed for the classically heated system. The enzyme catalysed reaction rates were also measured at temperatures of 60°C and 70°C. The alcohol substrate was also replaced by a longer chain alcohol, 1-pentanol (Table 1). Consistently, we found that at water activities 0.58 and 0.69, microwave irradiation of the enzyme-solvent mixture can increase the catalytic rate of transesterification by 2-3 fold. However, we found that when the water activity was increased to a_w 0.97, the

reverse effect was observed. Interestingly, at this high water activity, microwave irradiation resulted in significantly lower reaction rates compared to those observed by classical heating, as detailed in Table 1. For all the experiments performed, the relative differences in enzyme catalytic rate remained the same, regardless of batch to batch preparations, although there were differences in the absolute rates measured.

Table 1. Cutinase-catalysed transesterification rates.

Water activity, a_w	T(°C)	Heating method	Rate, $\mu mol.min^{-1}.mg^{-1}.ml^{-1}$
0.58†	50	classical	1.23
0.58†	50	microwave	2.94
0.58	60	classical	2.45
0.58	60	microwave	5.64
0.58	70	classical	6.37
0.58	70	microwave	14.9
0.97	60	classical	6.37
0.97	60	microwave	2.94

† 1-butanol replaced with 1-pentanol

The effects that we have observed are reversible. A sample, equilibrated to a_w 0.97, was placed in the microwave reactor and irradiated at 50°C and the reaction rate monitored over a period of 80 min. After this time the vessel was removed and heated by classical means at the same temperature. We observed a significant step-change to the enzyme catalytic rate that occurs upon removal of the sample from the electric field. This result is shown in Figure 2.

We are currently investigating the effect of microwaves on the activity of different enzymes.

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19. The cutinase (*Fusarium solani* expressed in *E. Coli*) and substrates were hydrated to the desired a_w using a series of saturated salt solutions of known water partial pressure. The enzyme and substrates were equilibrated in separate sealed containers at room temperature (20°C), the solvents for 24 h and the enzyme for 2-3 days.
20. **Typical experiment** (*e. g. aw* 0.58): *a) Classical heating*. The temperature of a jacketed glass vessel was maintained constant using water circulating from a heated water bath. Hydrated *n*-butanol (1 ml, 11 mmole) and 1 equiv. of ethyl butyrate (1.45 ml) were added to the vessel. The temperature of the solvent mixture was measured and then hydrated enzyme (1 mg, 3.7 10^{-5} mmole) was immediately added. The reaction was stirred (magnetic stirrer) for approximately 2 h. Periodically, samples were withdrawn (5µl) and added to dichloromethane (200 µl). Samples were analysed by gas chromatography (GC) injected onto a fused silica capillary column. *b) Microwave irradiation*. Identical substrate volumes as those detailed above, were placed in a quartz vessel (capacity 20 ml). To these, the enzyme was immediately added and the reaction mixture placed in the microwave oven (Synthewave S402™ Prolabo, Paris, France, operating frequency $2.45.10^9$ Hz). A glass paddle stirrer was used to stir the reaction with a stirring rate similar to that for the classically heated system. The temperature of the reaction mixture was typically set at 50°C and measured using a non-contact infra-red (IR) continuous feed-back temperature system. Parameters (*e.g.* temperature, reaction period, stirring rate and power input) were computer controlled. The sampling procedure and volumes were as for the classical reaction.
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