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Enzyme-Catalyzed, Gas-Phase Reactions

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ABSTRACT

Dehydrated preparations of alcohol oxidase adsorbed on DEAE-cellulose vigorously catalyze a gas-phase oxidation of ethanol vapors with molecular oxygen. The gas-phase reaction is strongly dependent on the water activity of the system. The enzymatic activity is severely inhibited by the product hydrogen peroxide. This inhibition can be alleviated, however, by an addition of catalase or peroxidase to the dry preparation. Such dehydrated, bienzymic catalysts afford a complete and selective conversion of the substrate to acetaldehyde. Dry alcohol oxidase is much more thermostable than in aqueous solution. The results of this work suggest that dehydrated enzymes have potential applications in the analysis of gaseous compounds and in the development of novel gas–solid bioreactors.

Index Entries: Enzyme catalysis of ethanol, by oxidation in the gas phase; enzymatic oxidation of ethanol, in the gas phase; oxidation of ethanol in the gas phase, by enzymes; ethanol, enzymatic oxidation in the gas phase of; gas-phase oxidation of ethanol, by enzymes; gas-phase reactions, catalyzed by enzymes.

INTRODUCTION

A remarkable specificity of enzymes has created a considerable interest in their application as analytical reagents in complex systems. In particular, enzymes have been used in clinical chemistry for routine analysis of a wide variety of compounds present in biological fluids. Some examples include creatinine, glucose, ethanol, urea, cholesterol, and amino

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acids (*1*). In all those cases, an aqueous solution containing the chemical of interest is contacted with enzymes that are in a dissolved form (*2,3*) or coated onto electrodes (*1,4,5*) or dry test strips (*6,7*).

Recent studies indicate that enzymatic catalysis can be applied to reactions taking place in organic solvents instead of water (*8*). This approach has been applied to the analysis of compounds poorly soluble in water (e.g., cholesterol) (*9*).

We have reasoned that it may also be possible to conduct enzyme catalysis involving reactants present in the *gas phase* and that in analytical applications this approach would allow the direct determination of gases and vapors. This could be accomplished by using dehydrated enzymes mixed with an appropriate chromogenic reagent, thus avoiding the need for sample pretreatments, such as absorption of the gas in liquids or in porous solids with subsequent removal for analysis and other costly and time-consuming sequential manipulations. If feasible, the use of dehydrated enzymes should find a large number of applications. Of particular interest would be the determination of toxic vapors in restricted environments. For instance, formaldehyde has been implicated as a potential carcinogen (*10,11*), and its presence in industrial and home environments, where it is released from foam insulation and wood products bonded with formaldehyde resins (*12*), has fostered an interest in its quantification. In this context, we have recently filed a patent application for an enzyme-based device that can be used for the analysis of compounds in the gaseous phase, in particular for the determination of alcohol in the breath or formaldehyde in the air (*13*). This method could be employed for the determination of any analyte in the gaseous phase if it can be selectively transformed by an appropriate enzymatic system producing a detectable change in a parameter of the system (e.g., color). For instance, it can be applied to such toxic gases as carbon monoxide, hydrogen cyanide, ethylene oxide, and the like.

One can also envision potential industrial applications of enzymes acting on gaseous substrates. Some examples include purification of gas streams and modification of compounds generated as vapors. The gas-phase reaction may have substantial advantages in reduced diffusion resistance and facilitated recovery of the reaction products.

This paper reports the results of our study on the behavior of dehydrated enzymatic systems acting on gaseous substrates. The enzyme alcohol oxidase was selected as a model system using ethanol as a substrate. These results constitute a basis for potential applications as a novel analytical technique and in new types of heterogeneous (i.e., gas–solid) bioreactors.

MATERIALS AND METHODS

Alcohol oxidase (EC 1.1.3.13) from *Pichia pastoris,* catalase (EC 1.11.1.6) from *Aspergillus niger,* peroxidase (EC 1.11.1.7) from horserad-

ish roots, and DEAE-cellulose (microgranular form) were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals used were obtained from commercial suppliers and were of analytical grade.

Dry enzymatic preparations were obtained by the general method described by Kazandjian and Klibanov (*14*). Four grams of DEAE-cellulose prewashed with 0.01*M* phosphate buffer (pH 7.5) was suspended in 20 mL of distilled water. A certain amount of alcohol oxidase (and catalase or peroxidase in the coimmobilization experiments) was added, and the suspension was stirred gently at 5°C. After half an hour of contact, the suspension was spread on a watch glass and left to dry at room temperature under flow of air, with occasional mixing, until a visibly dry powder was obtained. Prehydration of the enzymatic powder, in preparation for the gas-phase reaction, was accomplished by exposure to the overhead space of saturated aqueous solutions of various salts (*15*) covering a wide range of water activities. The water content after prehydration was measured by weight difference after incubation at 110°C for 12 h and also by the modified Fisher method (*16*). Comparable results were obtained by both independent methods.

The retention of enzymatic activity of dry preparations of alcohol oxidase was determined by resuspending them in an aqueous solution and measuring the rate of formation of hydrogen peroxide by a modification of the method of Hildebrandt (*17*). An aqueous sample (0.6 mL) containing hydrogen peroxide was mixed sequentially with 1.2 mL of distilled water, 0.2 mL of TCA (6% w/v), 0.4 mL of 10 m*M* aqueous ferrous ammonium sulfate, and 0.2 mL of 2.5*M* aqueous KSCN. After incubation at room temperature for 10 min, the absorbance at 480 nm was detected using a Perkin-Elmer Hitachi 200 spectrophotometer and compared to the standard curve. Similar procedures were followed to establish the activity of the free (i.e., nonimmobilized) enzyme.

For gas-phase reaction experiments, a specific amount of enzymic powder was placed in a 15-mL flask. Two microliters of ethanol was injected into a small glass ampoule, which was then carefully placed inside the reaction vessel to prevent direct contact of the liquid substrate with the enzyme. The flask was then sealed with a rubber septum and placed inside a temperature-controlled oven. Agitation was provided by a wrist-action shaker. Changes in the concentration of ethanol and acetaldehyde in the headspace were determined by periodically withdrawing samples with a gas-tight syringe (Precision Sampling Co.) and injecting them into a Sigma III Perkin-Elmer gas chromatograph equipped with an 80/120 Carbopack B/3% SP-1500 column (Supelco Inc.).

RESULTS AND DISCUSSION

Alcohol oxidase (EC 1.1.3.13) oxidizes alcohols according to the following scheme:

alcohol + oxygen → aldehyde + hydrogen peroxide

The enzyme displays a rather broad specificity, but the activity decreases precipitously with the length of the alcohol chain beyond ethanol (*18*). It also oxidizes aldehydes, but at a significantly lower rate than alcohols. Catalytic activity for acetaldehyde oxidation is markedly lower than for ethanol, methanol, or formaldehyde (*19*). The use of alcohol oxidase in enzymatic electrodes for the determination of ethanol, methanol, and oxygen has been explored (*20,21*). This enzyme is readily available commercially and represents an excellent model catalyst to study gas-phase reactions because both ethanol and acetaldehyde show high vapor pressures being easily vaporized at mild temperatures (30–60°C).

The ability of purified hydrogenase from *Desulfovibrio desulfuricans* (*22,23*) or immobilized whole cells (*24,25*) to catalyze gas-phase reactions have been previously demonstrated. In a brief note, Cedeño and Waissbluth (*26*) reported an optimum temperature above 80°C for freeze-dried yeast cells containing alcohol oxidase activity packed in a gas–solid bioreactor. Piezoelectric crystals coated with antibodies are capable of selectively binding gaseous antigens (*27*); this methodology constitutes a different and interesting approach to the exploitation of biological activity for the direct measurement of gaseous compounds.

It is well established that dehydration enhances the thermostability of enzymes (*28*). This suggests that it should be possible to conduct reactions in the gas phase at higher temperatures than those attainable in aqueous solutions. Therefore, we started investigating the thermostability of dry alcohol oxidase immobilized on DEAE-cellulose. Samples of dry enzymic powder were incubated at 60°C, and the remaining activity was determined following resuspension in an aqueous solution. The free enzyme and a dry powder presuspended in an aqueous buffer prior to incubation at 60°C were used as controls. The results are shown in Fig. 1, indicating that dehydration indeed dramatically enhances the thermoresistance of alcohol oxidase: The half-life at 60°C increases from less than 2 min for the enzyme (free or immobilized) in water to about 3 h for the dry enzyme. Furthermore, 25% of the initial activity is still present after heating the dry enzyme for 20 h at 80°C (*29*).

Once a thermostable dry preparation was available, a gas-phase reaction was conducted at 60°C and compared to appropriate controls, as indicated in Fig. 2. It can be seen that the enzyme is capable of oxidizing ethanol vapors at 60°C, although the activity disappears after 4 h of reaction. Two possible explanations for this behavior are thermoinactivation in the presence of the substrate (which in turn may be a function of the water content) and inactivation of alcohol oxidase by the product hydrogen peroxide. We investigated those possibilities in detail.

An important variable in studying the catalytic behavior of dry enzymes is the water activity of their environment. Water affects not only

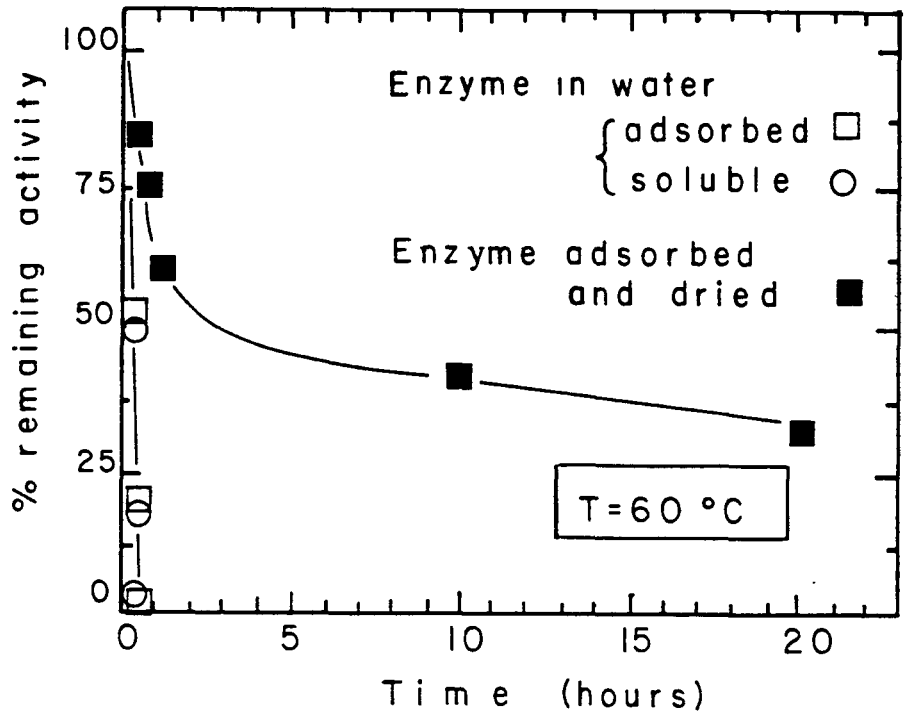

Fig. 1. Effect of dehydration on the thermostability at 60°C of alcohol oxidase adsorbed on DEAE-Cellulose. Conditions: for the dry enzyme, 100 mg of the powder (containing 1.6% of alcohol oxidase) was incubated at 60°C; for the enzyme in water, 25 mg of the dry powder or 0.3 mg of the soluble enzyme were suspended in 12 mL of aqueous phosphate buffer (0.01*M*, pH 7.5) prior to the heat treatment. Activity was measured by the H_2O_2 formation as indicated in the text.

thermostability, but also the rate of enzymatic reactions (*30,31*). Accordingly, we examined the effect of controlled levels of hydration on the rate of the gas-phase reaction and the maximal time of sustained activity. Samples were prehydrated for 12 h at 20°C to different water activities and employed as catalysts of the gas-phase reaction at 50°C. The results are shown in Fig. 3. The importance of the water content for the initial rate of reaction is evident; an increase in the level of hydration from 6 to 27% water accelerates the reaction by more than an order of magnitude. In regard to the maximal time of sustained activity, in all instances the reaction stops after about 3 h of reaction, suggesting that an increase in the water content does not promote thermal inactivation of the enzyme.

The possibility that the longevity of alcohol oxidase was limited by product (hydrogen peroxide) inactivation was then investigated. To that end, catalase, which decomposes hydrogen peroxide, was included in the dry preparation. The dry powder was hydrated over the vapor of pure water and used in the gas-phase reaction at 50°C. A sample with no

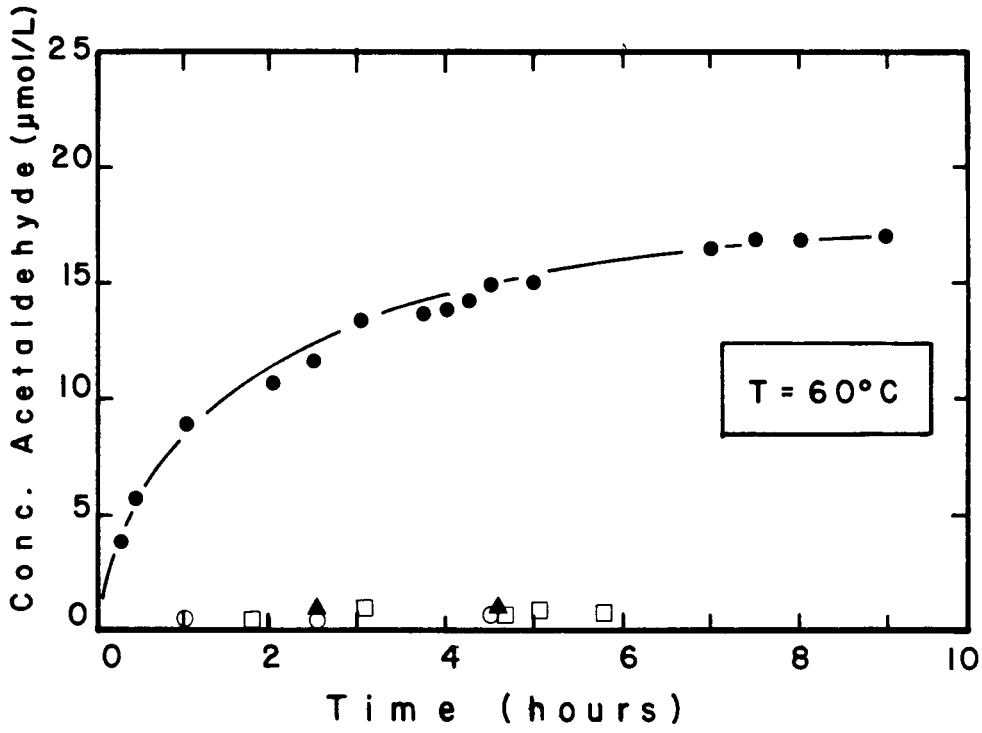

Fig. 2. Gas-phase reaction catalyzed by alcohol oxidase adsorbed on DEAE-Cellulose at 60°C. (●) Active enzyme; (○) enzyme preinactivated at pH 2 for 2 h; (▲) enzyme prethermoinactivated at 100°C for 6 h; (□) DEAE-cellulose with no enzyme. Conditions: 100 mg of dry solids (containing 1.6% enzyme) were placed in 15-mL reaction vessel; 2 m*M* ethanol in the gaseous phase.

catalase, but otherwise subjected to the same treatment, was used as a control. The results obtained are shown in Fig. 4. It can be observed that the *complete* conversion of ethanol to acetaldehyde was observed after about 1.5 h when catalase was present. This result convincingly demonstrates that dehydrated alcohol oxidase in indeed sensitive to hydrogen peroxide inactivation and that, once a mechanism to eliminate this limitation is provided, the enzyme is capable of acting efficiently on ethanol in the gas phase. No other products of oxidation (e.g., acetic acid) were observed in the reaction system during the experiment, indicating that the acetaldehyde produced is stable at the conditions used.

The next logical step was to determine whether the dry enzymic preparation consisting of alcohol oxidase and catalase could be applied for analytical purposes. This requires a preparation that permits *in situ* monitoring of the gas-phase reaction when the gas is contacted with the catalyst without further treatment of the sample. For that purpose, the enzyme peroxidase represents an alternative to catalase, for it utilizes hydrogen peroxide for the oxidation of a chromogen that produces a color

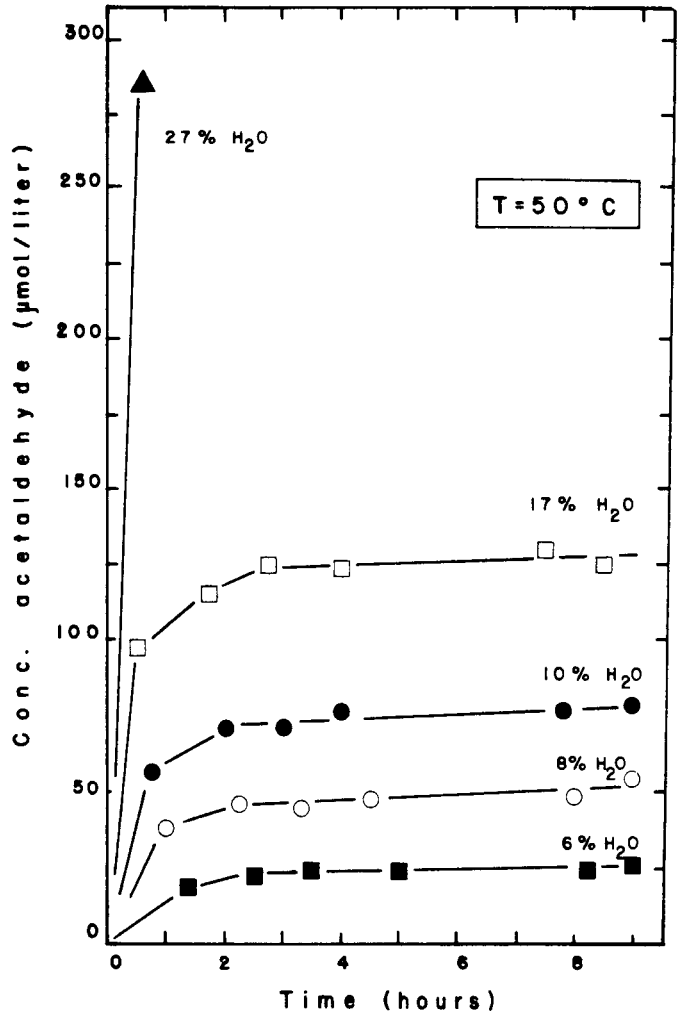

Fig. 3. Effect of the water content on the gas-phase enzymatic reaction at 50°C. Conditions: 100 mg of dry solids (containing 2.7% enzyme) were placed in 15-mL reaction vessel; 2 m*M* ethanol in the gaseous phase.

change related to the rate of the reaction. Therefore, the behavior of alcohol oxidase coimmobilized with peroxidase on DEAE-cellulose and in the presence of guaiacol, a well-known substrate of peroxidase (32), was compared to the alcohol oxidase/catalase couple. Also, since operating at ambient temperatures would simplify the system, thus making it more amenable as an analytical technique, this experiment was conducted at 25°C, and the results obtained are shown in Fig. 5. It can be seen that peroxidase indeed prevents product inactivation of alcohol oxidase with the efficiency similar to that of catalase. A clear and distinct change in color was observed for the sample containing peroxidase and guaiacol,

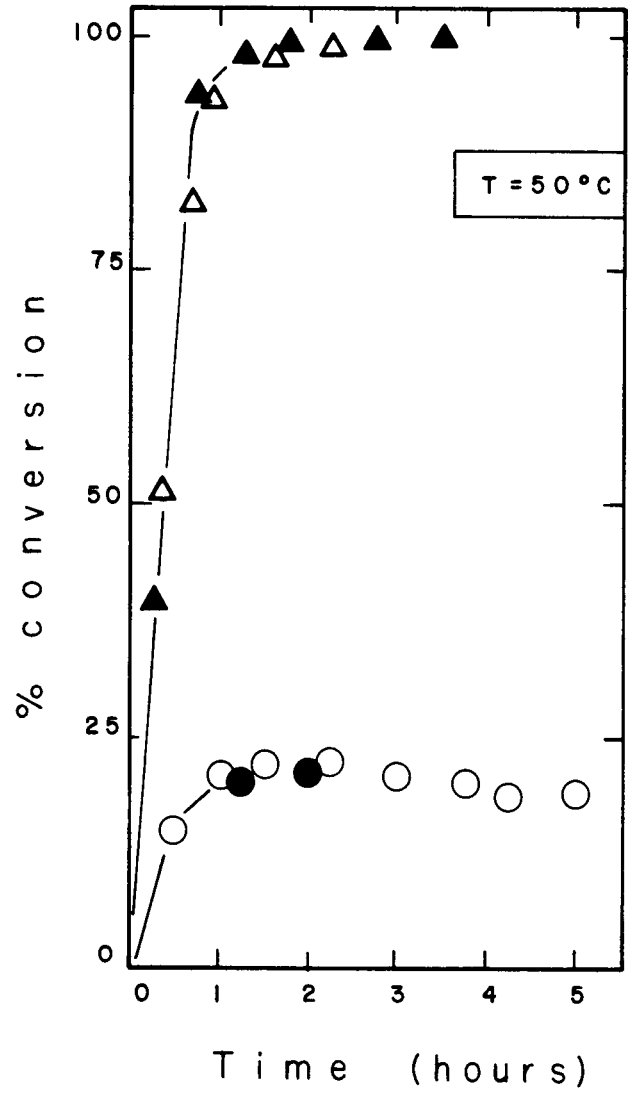

Fig. 4. Effect of catalase on the gas-phase enzymatic reaction at 50°C. (△,▲) in the presence of catalase; (○,●) in the absence of catalase. Open and closed symbols represent duplicate experiments. Conditions: 100 mg of dry solids (containing 2.7% alcohol oxidase and 0.4% catalase for the coimmobilized preparation), prehydrated to 28% water, were placed in 15-mL reaction vessel; 2 m*M* ethanol in the gaseous phase.

thereby pointing to an important area of the use of dehydrated enzymes for the direct analysis of compounds in the gas phase.

CONCLUSION

The present study demonstrated the feasibility of enzyme-catalyzed conversion of gaseous substrates and the existence of application in

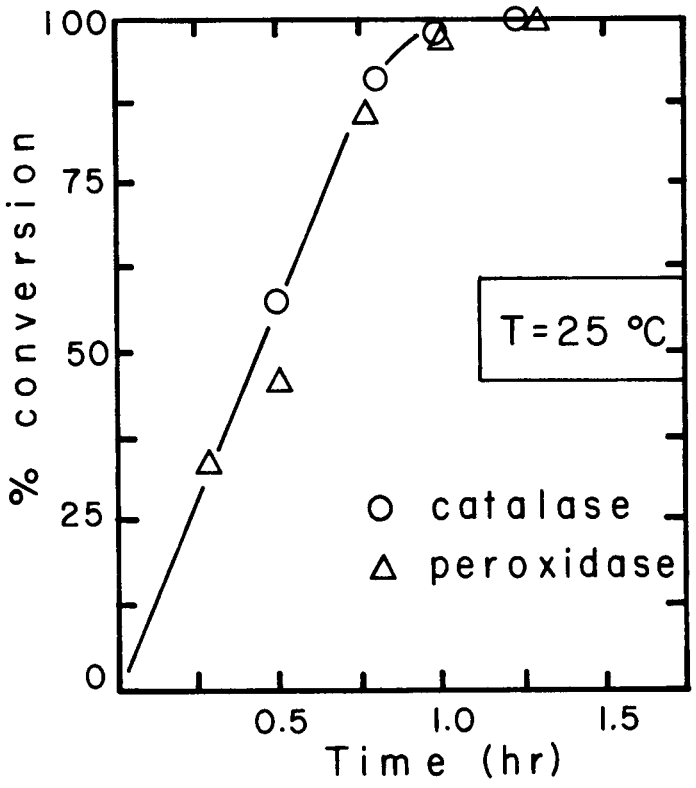

Fig. 5. Effect of catalase and peroxidase on the gas-phase enzymatic reaction at 25°C. Conditions: 100 mg of dry solids containing 2.7% alcohol oxidase and 0.4% of catalase, or 0.6% peroxidase plus 2% (v/w) guaiacol, prehydrated to 28% water; 15-mL reaction vessel; 2 m*M* ethanol in the gaseous phase.

analysis. Further research is needed to obtain a mechanistic description of the catalytic properties of enzymes acting on gaseous substrates. This may lead to other applications in the chemical industry in which the development of gas–solid bioreactors may represent an attractive alternative to conventional processes.

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