to determine the maximum efficiency for n-hexadecane (and other n-alkanes) conversion to wax ester.

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Developing a New Industrial Enzyme Application: A Strategy

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ABSTRACT

Many companies view industrial enzymes as potential tools for making technological breakthroughs in their industries. Some have created special biotechnology groups to pursue this aim. Too often their projects are terminated before the intended goals are met. A major reason is too much time was consumed for too little perceived gain. A strategy is proposed to help R&D groups and their managers conserve valuable resources. It focuses on the essentials in evaluating new enzyme applications and in better developing discoveries.

ENZYME BENEFITS

Biotechnology—enzymes, in particular—is a new technology for the fats and oils industry. Enzymes are very familiar to those in the detergent and vegetable protein industries. There is much excitement in the business and R&D sectors over the potential for biologically modifying fats and oils for profit. At some point someone will ask, "Are such great things really possible, or is it just 'pie in the sky'?"

The answer to this question is, "Both." That is, there are objectives for which the basic technology exists today, and details must be developed. There are other objectives which will require many years of basic research to ensure success.

Enzymes are definitely "real": enzymes perform valuable tasks in many industries today. Some of these may be readily adaptable to the fats and oils industry. A few examples of how enzymes are benefiting their users can be discussed.

Many enzymes can cause viscosity reductions on materials through hydrolysis. Fishmeal processors recently have learned that a protease can save them money in drying costs (1). The protease is used to hydrolyze the protein in stickwater produced in the Menhaden process. The viscosity reduction that results leads to improved drying efficiency later in the process.

Enzymes are used to degrade the pectin and cellulose in apple juice processing. Here, too, there is a viscosity reduction; this time there is an improvement in filterpressing of the juice from the fruit. More importantly the hydrolysis improves the extraction of the juice (2). This gives the processor more saleable product and increases his return on his feedstock.

One of the most familiar uses of enzymes is in the production of corn syrups. Three enzymes are used to convert corn starch into high fructose corn syrup. In this case enzymes achieve higher conversion to end product with less by-product formation than chemical processes. Also, enzymes give producers the flexibility to make syrups to meet specific customer needs at a competitive price (3,4).

The decolorization of slaughterhouse blood is an example of where an enzyme can affect a separation. In this case a protease extensively hydrolyzes the hemoglobin in the blood. When the pH is lowered, the pigment precipitates. This can be removed using a centrifuge or a filter press. The light colored broth can be used in processed meats in Europe (5,6).

The functional properties of soy protein can be modified using enzymes. Through controlled hydrolysis with proteases soy protein whipping agents can be prepared. The protease causes the soy protein to become more soluble and to have foam expansion and stability after whipping. Thus, enzymes are able to get a value-added return on this vegetable protein (7,8).

Enzymes can be used to affect the flavor of foods. For example, the piquant flavor note in certain Italian cheeses is caused by the action of lipases or pregastric esterases. Also, a key flavor note in cheddar cheese is due to protease action. Law and others have described how enzymes can be added to hasten the cheese aging process (9,10,11). Accelerated cheese ripening saves producers money in inventory costs. Enzyme modified cheese (EMC) is becoming an item of commerce in the flavor industry.

Some hydrolytic reactions can be made to run in reverse in organic solvents. Therefore, some enzymes can be used to synthesize compounds. For example, Strobel et al demonstrated how a crude enzyme preparation could be used to make terpene esters in high yields (12). Another plus in this case is that the enzyme is specific in terms of which stereoisomer is produced, unlike chemical methods which produce racemic mixtures. This is beneficial in the flavor and fragrance industry, where one stereoisomer may be valuable and the other not.

Lastly, amylase and protease enzymes have been added to pre-soak detergents for more efficient cleaning and stain removal (13). Such preparations have been used widely for years in the detergent industry.

Larry Posorske, my colleague, discusses emerging enzyme applications for modifying fats and oils in his paper (14). The rest of this paper will outline a strategy for developing your own enzyme application. It focuses on the essentials in evaluating new enzyme concepts and in developing discoveries into beneficial applications. It will help R&D groups and their managers to conserve valuable resources and to avoid the major pitfalls of enzyme projects.

PITFALLS

The major pitfall in developing a new enzyme application is that the project can run on too long. This results in many enzyme projects being terminated prematurely. There are two situations which tend to make enzyme projects run longer than management would like.

The first is when enzymes are new to the applications scientist. There is a natural tendency to spend some research time gaining familiarity with an enzyme. Unfortunately, time spent in a familiarization phase with a model system too often does not move the project forward. This can be improved if the development scientist combines his learning with concept feasibility testing.

The second occurs when the project objective and scope are too broad. In this situation the applications scientist is faced with many choices. For example:

- What enzyme?
- What conditions (temperature, pH, etc.) to use?
- Where in the processing line to carry out the enzyme reaction?
- How long to run the reaction?

The scientist may be tempted to explore too many alternatives before arriving at a final process. In short it is easy to get bogged down trying to pursue too many loose ends. A few key decisions to define project limits at the start are helpful. When the scope is narrowed, it is easier and quicker for the scientist to evaluate new enzyme concepts for industry.

DEVELOPMENT TEAM

The first place to start is to assemble a well balanced project team. Some companies hire a biochemist when starting work with enzymes. The biochemist's presence alone does not guarantee a successful R&D effort. The reason is easy to see if we review the essentials to developing a new enzyme application:

- Have a clear cut goal.
- Choose the material to be modified.
- Identify the enzyme(s).
- Determine how much to modify.
- Monitor the changes in the material.

Of the five elements, the biochemist's expertise is helpful for only one—identifying the enzyme. Also, if the biochemist is not experienced in the industry, he may have difficulty keeping the project directed toward a successful commercial conclusion.

The other elements are best addressed by product development and/or operations personnel. These are the people most likely to know what needs improvement in a commercial product or process or how to follow changes in the material of commerce, the proposed substrate. What these people lack is familiarity with industrial enzymes. Therefore, it is clear that an interdisciplinary team is best. Product development and/or operations personnel are needed to keep the project directed toward a commercial goal. An enzyme expert is needed to ensure that the experimental conditions do not exceed the enzyme's capability to do the task required. By putting their talents together such a team should be able to evaluate concepts with commercial potential quickly and efficiently.

DEVELOPMENT GOAL

It's obvious that the successful enzyme development project must have a clear cut goal. Of the five elements for developing a new enzyme application this probably is the most important. So spend time on it. Most good R&D organizations identify a general business goal for all of their development projects. In the case of enzymes it is helpful to narrow the scope. This is done by putting the goal in terms of a biochemical and product development objective. For example, "identify a quality improvement" may become "use a lipase to generate free fatty acids to improve flavor". "Develop a low-cost process" may become "use an enzyme to reduce viscosity for improved drying efficiency".

Refining the definition of the goal restricts the scope of the project. This allows the applications scientist to focus on evaluating whether or not an enzyme can do a specific task or set of tasks. Therefore, management is more likely to have an answer, good or bad, within an acceptable period of time.

DEVELOPMENT CHECKLIST

The other elements can be expanded and formed into the Development Checklist presented below:

- Identify the material/component.
- Choose an enzyme class.
- Review the literature.
- Conduct feasibility check.
- Plan in-depth experiment.
- Prepare samples.
- Evaluate material in product; evaluate processing benefit.
- Repeat positive results.

Identify the Material to be Modified

This usually is easy to do. However, if the raw material is heterogeneous (e.g., soy meal) one will want to focus attention on a particular component. For example, the true target for modification in the soy meal may be the storage polysaccharide or the protein.

Choose an Enzyme Class

This can be a little more difficult, although in some cases the class of enzyme is quite obvious. For example, lipases would be the class of choice for hydrolyzing a vegetable oil to free fatty acids and glycerol. On the other hand, improving the extractability of an oilseed would entail looking at several enzymes capable of attacking the many components of plant cell wall.

In thinking about enzymes for the fats and oils industry it might be helpful to consider the types of reactions effected by enzymes of commerce. After all, these are the enzymes that are available in large enough quantities for an industry that processes massive amounts of feedstock each year.

Hydrolytic Enzymes. The most ubiquitous are hydrolytic enzymes. These hydrolyze or break up larger biopolymers into smaller units. For example, proteases break down proteins into polypeptides and amino acids. Amylases, pectinases, cellulases and lipases work similarly on their respective substrates.

Non-hydrolytic Enzymes. There are only a few commercial applications which involve non-hydrolytic enzymes. One example is glucose oxidase. It is used to scavenge oxygen in fruit juice or to oxidize glucose in egg whites before spray drying. Glucose oxidase is unique in that it can reduce molecular oxygen directly. Most other oxidases require elaborate biological electron transport systems to do this. Another non-hydrolytic enzyme is glucose isomerase. It rearranges glucose to form fructose in one enzymatic step without cofactors.

Cofactor Enzymes. Enzymes that require cofactors such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP) have not been used indus-

trially. Cofactors are involved in reactions where molecules are oxidized, reduced, rearranged or connected. Some of the more interesting reactions from an oil chemistry point of view involve cofactors. The breakdown or synthesis of fatty acids involves many enzymatic steps, all requiring cofactors. The first two reactions in fatty acid oxidation pathway are shown in Figure 1. Until someone develops an economical way to regenerate these cofactors, the cost to add them in substrate amounts will remain prohibitive. This may be where fermentations, genetic engineering or immobilized cell technology may succeed before enzymes.

Review the Literature

Don't be surprised or disappointed when you find that someone already treated your material with an enzyme years ago. The real inventions are in how the enzyme-modified material is used in a product. A good literature review may turn up information to help you in conducting a feasibility check or in selecting preliminary reaction conditions for your more in-depth experiments.

Conduct a Feasibility Check

Avoid using a model system. All the work spent learning with a model system can not satisfy management's question, "Will an enzyme make money for us in our product or process?" The point here is to work with the intended substrate and conditions most likely to be used in the plant.

It may be a little more difficult performing a "real world" feasibility check. For example, there may be an enzyme inhibitor in the raw material. Therefore, it will take more time initially to find out what the enzyme really can do. That is, one would have to look for a way to inactivate the inhibitor. However, in doing so one will have useful information about the proposed concept, not just data about a model system. In this case if a model system was explored first, the time it took would be in addition to that to solve the inhibition problem.

Planning an In-Depth Experiment

This involves several considerations discussed below.

Selecting preliminary conditions. Choosing conditions (eg., temperature, pH, moisture, at "the enzyme optimum" is not always recommended. At an enzyme optimum the enzyme is working at its fastest rate. However, it may not always be in its most stable conformation. Consider the two curves in Figure 2. The one on the left shows that the maximum activity for the alkaline protease, Alcalase, is around 60 C. One might say that the enzyme temperature optimum is at 60 C. However, in the curve on the right, the temperature stability curve for Alcalase, one sees that this enzyme is not stable at 60 C. In fact, after an hour, less than 80% of its activity remains. One would want to pick a lower temperature, e.g. 50 C, for a reaction that has to run for several hours.

Sometimes there is no leeway to adjust conditions in a plant to suit the enzyme perfectly. This doesn't mean the enzyme won't work. There are commercial processes where enzymes are working under adverse conditions. Some examples are: laundry detergents where the pH is too alkaline, fruit juice extraction and clarification where the pH is too acidic, and brewing mashes where the temperature is too high.

However, there is a trade-off when the operating conditions push the enzymes to their limits. That is, higher enzyme doses or longer processing times might have to be used. Of course, there are occasions where enzymes can exhibit a benefit with just a little hydrolysis.

Ensuring Substrate Availability. Just as important as having favorable reaction conditions is ensuring that the target substrate is available to the enzyme. Here is where one considers processing pretreatments or additional ingredients. Try to select one which will be acceptable to operations.

One example might be the hydrolysis of protein in soy meal. The grind size of the meal will affect the amount of protein that is in solution with the enzyme. Also, the protein may be in subcellular structures and surrounded by matter inert to the protease. This is another case where only part of the substrate is available to the enzyme. Grinding or a heat treatment might be desirable.

One also might consider emulsifiers to increase the effectiveness of lipases in triglyceride hydrolysis. This is because the enzyme is water soluble while the substrate is not. The emulsifier increases the surface area of the oil-water interface, thereby increasing the amount of triglyceride accessible to the enzyme.

Monitoring for Reproducibility. When the results cannot be reproduced, the enzyme project will get bogged down. Often this happens when the time/temperature profiles are used to determine how long to let the enzyme react. This methodology does not account for the variances between batches of feedstock, changes in operators, or processing history of the equipment, etc. These all have an effect on how long it takes the enzyme to modify the substrate to the desired endpoint.

To avoid this pitfall it is wisest to use a monitoring technique that allows you to follow the changes of the

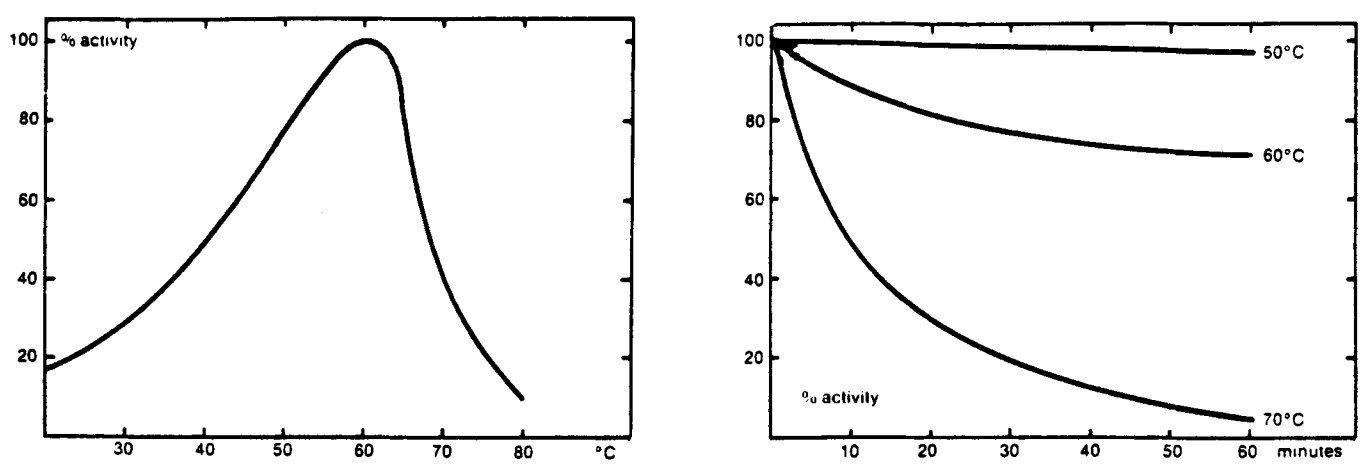

FIG. 1. Examples of Cofactor Requiring Enzyme Reactions. Such reactions have not been carried out industrially. These two reactions are the start of the fatty acid oxidation pathway.

• Where molecules are oxidized, reduced, rearranged, or connected using an enzyme and a cofactor

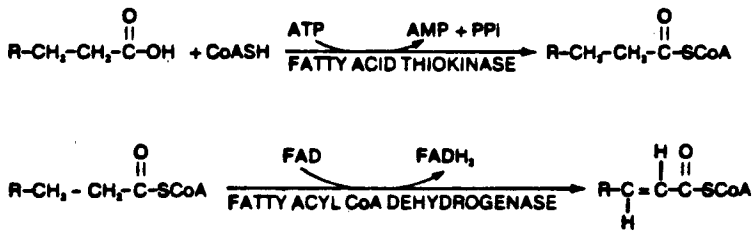

FIG. 2. Enzyme activity optimum and enzyme stability curves for Alcalase®.

material during the process. This will let the plant operator know when an enzyme reaction is ready to be terminated. Therefore, in successive experiments the material can be modified to the same extent as the material in the first.

The technique chosen should give answers in a time suitable for good control. For example, a wet chemical method to measure free fatty acids produced in a lipase reaction might take an hour to perform. Such a method would be unsuitable to control a process where the reaction will reach the desired endpoint in 3 to 5 hrs. However, it is suitable for one reaching the endpoint in 18 to 24 hrs. Examples of some techniques which give results within a few minutes are listed in Table I.

Inactivating at Endpoint. It is best to inactivate the enzyme at the end of the reaction. This will ensure that samples will not undergo additional change prior to analysis. Also, the enzyme will not act on the other components of a product after the enzyme-modified material is mixed in. In addition, if the enzyme has been inactivated during processing, often there is no labeling requirement. This is true for applications where enzymes are used as processing aids.

TABLE I

Some Methods for Monitoring Enzyme Reactions During Processing

PROTEASES
pH-Stat/pH Drop
Freezing Point Depression
Viscosity Change
HPLC
LIPASES
pH-Stat/pH Drop
GC
HPLC
Titration
CARBOHYDRASES
Freezing Point Depression
Viscosity Change
Starch-Iodine Color Change
Refractive Index
Polarimeter
HPLC
Cu-Reduction Assays

Prepare Samples and Evaluate

At this stage the objective is to determine how much to modify the substrate. Therefore, samples of the raw material modified to different extents must be prepared. These are incorporated into products and evaluated. Generally much fine tuning between trying new reaction conditions and evaluating the modified material is required before an application becomes finalized.

Repeat Positive Results

It's been said, "When it happens the first time, it's coincidence; but when it happens twice, it's science." Always make sure the positive results you observed in one experiment can be repeated. Running a duplication experiment is easier when a good method for monitoring the modification process has been chosen.

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