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Microbial Lipases Production: A review

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ABSTRACT: ***This systematic review gives an outline of the major elements of the synthesis of microbial lipases. The most significant microbial lipase-producing strains for submerged and solid-state fermentations, as well as the most common substrates, such as agro industry wastes, are discussed. Current process methods are described, as well as the most common bioreactor designs. In addition, the current review article provides a broad overview of the evolution of mathematical models for lipase production. Finally, future prospects on lipase production are addressed, with a focus on lipase engineering and the use of mathematical models for process optimization and control. Lipases also have enantioselective characteristics and catalyze the hydrolysis and transesterification of other esters as well as the production of esters. Lipases have become more popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical sectors due to their capacity to conduct highly precise chemical transformations.***

KEYWORDS: ***Lipase production, Bioprocess modeling, Process conduction, Substrates***

1. INTRODUCTION

Lipases are hydrolases that catalyze the conversion of triglycerides to glycerol and long chain fatty acids at the oil-water interface. Lipases have emerged as one of the most important biocatalysts, with shown potential to contribute to the multibillion-dollar underutilized lipid technology bio-industry, and have been employed in in situ lipid metabolism and ex situ diverse commercial applications. Since the 1980s, the number of lipases accessible has grown. This is mostly due to significant progress in the cloning and expression of enzymes from microorganisms, as well as rising demand for biocatalysts with new and particular characteristics such as specificity, stability, pH, and temperature. Animals, plants, and microbes all generate lipases[1]. Because of their stability, selectivity, and wide substrate specificity, microbial lipases have gotten a lot of interest in the industry. Extracellular lipases are produced by a variety of microbes, including bacteria, yeast, and fungus. Solid-state fermentation is preferred for fungal species, whereas submerged fermentation is preferred for bacteria and yeast. The recent publication of a large number of papers demonstrates the significance of lipases. In reality, the number of papers relating to industrial applications of lipase-catalyzed reactions conducted in ordinary organic solvents, ionic liquids, or even non-conventional solvents has steadily increased in recent years[2]. The purpose of this study is to describe the major microorganisms, substrates, and process activities utilized in lipase synthesis.

1.1 Sources of Microbial Lipases:

Lipases are generated by a variety of plants, animals, and microbes in nature. In biotechnological applications and organic chemistry, microbial lipases are the most frequently utilized class of enzymes. In both submerged and solid-state fermentations, a review of the most current promising microorganisms for lipase synthesis is presented. Before 2004, the primary microorganisms utilized had previously been extensively characterized[3].

- *Filamentous Fungi are fungi*

Rhizopus sp., Aspergillus sp., Penicillium sp., Geotrichum sp., Mucor sp., and Rhizomucor sp. are some of the most significant economically important lipase-producing fungus. The strain, the content of the growth medium, culture conditions, pH, temperature, and the kind of carbon and nitrogen sources all influence lipase production in filamentous fungus. The isolation and selection of novel strains is prompted by the industrial need for new sources of lipases with various catalytic properties. Lipase-producing microorganisms have been found in a variety of environments, including industrial waste, vegetable oil processing plants, dairy plants, and soil contaminated with oil and oilseeds, among others. Using enrichment culture techniques, researchers isolated lipase-producing fungal strains from Brazilian savanna soil. In the main screening test, fungi were isolated and grown on an agar plate medium containing bile salts and olive oil emulsion. The ratio of the lipolytic halo radius to the colony radius was used to select twenty-one strains. The most prolific strain was Colletotrichum gloeosporioides, which was chosen from a total of eleven strains. In another study, a strain of Aspergillus sp. was isolated from soil samples from several parts of Turkey and shown to have expressive activity[4].

- *Yeast*

In the Arabian Sea, marine soil samples were collected near an oil production platform. After colony isolation, the colonies were transferred to plates containing 2% tributyrin and cultured for 3–4 days at 35 °C. The colonies with the most extensive hydrolysis halos zone were chosen. Rhodotorula mucilaginosa was selected as the most efficient strain for lipase production based on its phenotypic features. In Delphi, I isolated 15 yeasts from petroleum and oil sludge regions. Purification and lipolytic potential testing were performed on the isolates. Based on the greatest halo of lipolysis among these yeast strains, one was chosen for future research. This strain was identified as belonging to the Trichosporon asahii genus based on sequence homology, and it has 99 percent identity with the current database. It was discovered that newly produced olive oil is contaminated by a diverse microflora capable of altering the oil's physicochemical and organoleptic properties by producing enzymes. Several yeast strains were discovered among the microorganisms isolated from this oil, including Saccharomyces cerevisiae, Candida wickerhamii, Williopsis californica, and Candida boidinii, with S. cerevisiae and W. californica showing high ability to generate lipase. Lipase activity was shown to be intracellular in S. cerevisiae and extracellular in W. californica. Olive oil mill wastewater is a dark-colored effluent produced by the three-phase olive oil extraction process. The value of OMW was investigated by using it as a potential growth medium for the microbial synthesis of extracellular lipase.

- *Bacteria*

I isolated 17 bacterial strains that could grow on OMW medium, and the most promising strain for lipase production was chosen. A Bacillus sp. strain was selected as the top lipase producer after screening on tributyrin agar medium. The intracellular activity was discovered when the medium was optimized. On tributyrin agar plates for lipase production, I isolated 57 heterotrophic bacteria from the sea sponge Dendrodoris nigra, of which 37 percent formed a distinct halo surrounding the colonies. The strain Pseudomonas MSI057, in particular, has wide clean zones surrounding the

colonies. After that, this strain was chosen for further research, and after some tweaking, a maximum lipase activity was discovered.

1.2 Substrates for Lipase Production:

Microbial lipases are mainly extracellular, and medium composition, as well as physicochemical variables like warmth, pH, and biological oxygen demand, have a big impact on their synthesis. Because lipases are inducible enzymes, the carbon supply has traditionally been described as the most important determinant in the production of lipase activity. In the presence of a lipid, such as oil, or any other inducer, such as triacylglycerol, fatty acids, hydrolysable esters, Tweens, bile salts, and glycerol, these enzymes are generated. Lipidic carbon sources seem to be necessary for high lipase yields. However, for optimal development and output, nitrogen sources and critical micronutrients should be carefully examined. Several alternative media, such as those based on defined compounds like sugars, oils, and complex components like peptone, yeast extract, malt extract media, and also agro industrial residues containing all the components necessary for microorganism development, can meet these nutritional requirements for microbial growth. Lipase may also be produced using a combination of these two types of media. The most important studies on these topics that have been published in the literature are listed below, separated by the kind of media utilized.

1.3 Synthetic Substance:

In general, culture medium improvement has resulted in excellent output. The process of determining the concentration of each component that makes up a culture medium is typically time-consuming. The traditional method of altering one variable at a time while leaving the others constant has been shown to be ineffective because it fails to account for the interaction effects between variables and their impact on the fermentation process. Plackett–Burman designs, which enable fast screening of important variables for subsequent optimization in a logical manner, are a popular and frequently used technique.

1.4 Residues from the agro-industrial sector:

Due to their potential benefits, research on the identification of appropriate substrates for fermentative processes has mostly concentrated on agro-industrial wastes in recent years. The utilization of agro-industrial wastes offers alternative substrates and may aid in the resolution of pollution issues that would otherwise arise from their disposal. The most significant element influencing fermentative processes is the substrate's nature. The substrate of choice is determined by a number of variables, the most important of which are cost and availability. As a result, process optimization may include screening a variety of agro-industrial leftovers. Many SSF studies have lately been released, with a focus on the use of agricultural by-products in the manufacture of fine chemicals and enzymes, notably lipases. P. simplicissimum lipase production utilizing soybean meal as a substrate supplemented with low-cost substrates such as soybean oil, abattoir effluent, maize steep liquor, and yeast hydrolyzed. Soybean meal without additives seems to be the most effective medium for lipase synthesis among those examined. The impact of agroindustrial residues on SSF lipase synthesis utilizing B. coagulans.

1.5 Processes of Production:

Batch, repeated-batch, fed-batch, and continuous fermentation methods have all been used. The features of the product of interest determine the method of operation to a considerable degree. In this part, we'll look at how batch, repeated-batch, fed-batch, and continuous methods have been used to make lipase in SmF and SSF.

- *Batch Processes:*

Batch mode in shaken flasks is used in the majority of publications reporting lipase production. However, a large number of research have been conducted on the utilization of bubble, airlift, and stirring bioreactors. This part will cover the main features and applications of these bioreactors, since the usage of flasks in batch mode for lipase synthesis has already been discussed in earlier sections of this study. These systems can replicate the hydrodynamic phenomena seen in large-scale equipment for the three variables of microbial growth, extracellular lipase synthesis, and activation of the gene LIP2, which codes for Y. lipolytica's primary lipase. Among the environmental variables studied, the dissolved oxygen variations produced in a controlled scale-down reactor had the most significant physiological impact, lowering the amount of LIP2 gene expression. Other environmental variables such as methyl oleate dispersion and pH variations, which were seen in a partitioned scale-down reactor, led to less severe stress, which was only interpreted as a reduction in microbial output and therefore in the extracellular lipase-specific production rate.

• *Processes with Repeated Batches:*

The benefits of fed-batch and batch processes are combined in repeated-batch operations, allowing the process to be run over longer periods of time and increasing productivity compared to batch processes. The researchers used repeated-batch fermentations to examine lipase synthesis by immobilized mycelium from R. arrhizus in submerged fermentation. The replacement time, the volume of the replaced medium, and the medium's optimum composition were all optimized. Repeated usage of immobilized cells revealed a high level of stability. In flasks, nine batches were repeated for 140 hours, and six batches were repeated in a 5-L bioreactor. A consistent cell concentration was found to be necessary for extending the number of repeated cycles, while a high cell growth rate was required for high lipase output. The authors of the previously mentioned study also confirmed that pH control had a significant impact on lipase synthesis. On the other hand, dissolved oxygen continuous feeding may be adjusted to provide for a sufficient growth rate for effective lipase synthesis.

- *Processes Using Fed-Batch:*

The fed-batch process is defined by the addition of one or more nutrients to the bioreactor throughout the fermentation process, keeping the products contained inside the bioreactor until the end of the fermentation. The fed-batch procedures are widely used to reduce the impacts of cell metabolism control and, in particular, to avoid substrate or metabolic product inhibition. Both the lab and pilot size fermentation conditions were optimized. In small-scale experiments, exponential feeding with pH control worked, while in pilot-scale fermentation, a two-stage fermentation approach that switched at 48 hours by fine-tuning the culture temperature and pH was shown to be successful.

Montesinos et al. looked at the development of extracellular and intracellular lipases in continuous cultures of C. rugosa utilizing pure or mixed carbon sources. Lipase production rose by 50% in continuous cultures compared to batch fermentation data, and was dependent on the dilution rate used. At modest dilution rates, oleic acid produced the highest yields compared to consumed substrate. Lipase activity was observed to be reduced under nitrogen restriction, according to the researchers. The results were compared to prior data from batch and fed-batch cultures in order to determine the optimum process methods for C. rugosa lipase production. The best lipase yields were achieved using oleic acid in fed-batch fermentation. Thermomyces lanuginosus produces extracellular enzymes in chemostat cultures with a dilution rate of 0.08 h1 in response to various ammonium concentrations in the feed medium. Three growth regimes were identified under steady-state circumstances, and the synthesis of numerous enzymes from T. lanuginosus was seen under various nutritional restrictions ranging from nitrogen to carbon/energy limits. From regime to regime, the spectrum and output of carbohydrate hydrolyzing enzymes and lipase increased[5].

1.6 Mathematical Modeling of Lipase Production:

Scaling up lipase manufacturing methods requires a few fundamental procedures. The first has received a lot of attention in the literature, and it involves selecting an appropriate microbe and substrates for lipase synthesis. The second stage is selecting a bioreactor configuration for process development and investigating how the altered factors influence performance at a laboratory scale. The creation and validation of mathematical models as a tool for scale-up, process control, and optimization is the third stage. Finally, the process's technical and economic feasibility must be assessed. The major features of mathematical models for lipase production published in the past 10 years are discussed in this part, as well as how these models have been utilized as a tool for process scale-up or improvement[6].

Despite the fact that screening of microorganisms that produce lipases has yielded satisfactory results to date, we believe that the use of engineering lipases will predominate in the near future, based on our experience, because the production of engineering lipases will allow the development of enzymes with new remarkable properties for a specific application. The use of agro-industrial wastes as substrates for lipase synthesis promotes, without a doubt, the decrease of substrate-related production costs. However, systematic investigations should be conducted to see whether the whole manufacturing cost, including the downstream phase, is reduced. Of However, the challenges presented by residues in the purification of the enzyme generated render its usage impractical in some situations. In terms of lipase activity and productivity, the fed-batch operating mode has produced the best results. The feeding rate was not optimized in any of the instances included in this study. The employment of an optimization technology such as dynamic optimization or optimal control may be an intriguing option[7].

This would enable the time domain to be divided into N-subintervals, each with its own feeding rate. The use of a dynamic optimization tool allows for the discovery of the optimum feeding rate profile that optimizes output or productivity. It's worth noting that using an optimization tool necessitates the development of a mathematical model that can accurately and reliably describe the process[8].

2. LITERATURE REVIEW

To get a broad knowledge of the fundamental principles and limits of the process, Becker and Markl (2000) used chemostat and batch culture to simulate olive oil degradation by the thermophilic lipolytic strain Bacillus thermoteovorans IHI-91. The Monod chemostats model was effectively expanded with parameters for maintenance needs and wall growth to explain chemostat data. A model combining growth-associated lipase synthesis and olive oil hydrolysis may predict oleic acid buildup during batch fermentation. The buildup was shown to be the cause of abrupt growth stoppage in batch fermentations with greater olive oil starting concentrations, according to simulations. The latter model also anticipated oscillatory activity, which was seen in certain chemostat studies[9].

Gordillo et al. (1998a) developed a simple structured mathematical model with a methodology to estimate biomass amounts, specific growth rate, and substrate amounts, which they applied to the production of C. rugosa lipase in batch, fed-batch, and continuous operations, resulting in a 10-fold increase in productivity over batch operations. Advanced Continuous Simulation Language was used to model the processes. The whole model was verified once the model parameters were established, and the findings were satisfactory. Different simulations of batch, fed-batch, and continuous cultures were used to determine the optimum approach for increasing lipase production. However, the greatest anticipated lipase activity was achieved in fed-batch cultures with a prefixed substrate feeding in order to keep the substrate/biomass or specific growth rate ratios constant (at their optimum values). The simulation findings matched the fed-batch process mode[10].

3. DISCUSSION

The use of agro-industrial wastes as substrates for lipase synthesis promotes, without a doubt, the decrease of substrate-related production costs. However, systematic investigations should be conducted to see whether the whole manufacturing cost, including the downstream phase, is reduced. Of However, the challenges presented by residues in the purification of the enzyme generated render its usage impractical in some situations. In terms of lipase activity and productivity, the fed-batch operating mode has produced the best results. The feeding rate was not optimized in any of the instances included in this study. The employment of an optimization technology such as dynamic optimization or optimal control may be an intriguing option. This would enable the time domain to be divided into N-subintervals, each with its own feeding rate. The use of a dynamic optimization tool allows for the discovery of the optimum feeding rate profile that optimizes output or productivity.

4. CONCLUSION

Microbial lipases are one of the most widely generated enzymes, according to a review of the literature. This study revealed that many researchers across the globe focus their efforts on finding novel lipase-producing microbes and then optimizing medium composition and operational factors. The enormous variety of lipase uses justifies all of these efforts. The screening of high lipase producers, successful substitution of synthetic medium by agro industrial residues, scale-up of process, different process operation modes, bioreactor operation strategies, and the use of mathematical models as a tool for process optimization have all seen significant progress in lipase production by bioprocesses, primarily using submerged fermentation. The findings presented in

this study clearly show that SSF produces excellent outcomes in terms of process productivity when the cultivation volume is limited to a relatively modest scale. Due of existing variations in temperature, pH, moisture, oxygen, substrate, and inoculum, SSF is difficult to scale up. Given the challenges of handling high volume bioreactors for SSF lipase production, it may be more feasible to utilize SSF for small-scale production; in the meanwhile, alternative bioreactor designs, such as spinning drums with intermittent agitation, are being explored.

REFERENCES:

[1] S. Kiran *et al.*, "Microbial Lipases : Production and Applications : A Review," vol. 1, no. 2, pp. 8–20, 2016.

[2] M. Ashfaq, "Basmati–Rice a Class Apart (A review)," *Rice Res. Open Access*, vol. 03, no. 04, pp. 88–92, 2015, doi: 10.4172/2375-4338.1000156.

[3] H. Treichel, D. de Oliveira, M. A. Mazutti, M. Di Luccio, and J. V. Oliveira, "A review on microbial lipases production," *Food Bioprocess Technol.*, vol. 3, no. 2, pp. 182–196, 2010, doi: 10.1007/s11947-009-0202-2.

[4] "Production and applications of microbial lipases: A review," *Sci. Res. Essays*, 2012, doi: 10.5897/SRE11.2023.

[5] B. Andualema and A. Gessesse, "Microbial lipases and their industrial applications: Review," *Biotechnology*, 2012, doi: 10.3923/biotech.2012.100.118.

[6] F. Hasan, A. A. Shah, S. Javed, and A. Hameed, "Enzymes used in detergents: Lipases," *African Journal of Biotechnology*. 2010, doi: 10.4314/ajb.v9i31.

[7] G. M. Borrelli and D. Trono, "Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications," *International Journal of Molecular Sciences*. 2015, doi: 10.3390/ijms160920774.

[8] J. M. Messias, B. Z. da Costa, V. M. G. de Lima, C. Giese, R. F. H. Dekker, and A. de M. Barbosa, "Lipases microbianas: Produção, propriedades e aplicações biotecnológicas," *Semin. Ciências Exatas e Tecnológicas*, 2011, doi: 10.5433/1679-0375.2011v32n2p213.

[9] A. Navvabi, M. Razzaghi, P. Fernandes, L. Karami, and A. Homaei, "Novel lipases discovery specifically from marine organisms for industrial production and practical applications," *Process Biochemistry*. 2018, doi: 10.1016/j.procbio.2018.04.018.

[10] S. Thakur, "Lipases, Its sources, Properties and Applications: A Review," *Int. J. Sci. Eng. Res.*, 2012.