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Using Synthetic Biological Approaches to Solve Yellow Water Wastage Problems

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Abstract. This project is focused on engineering the pET-32(a) vector to insert the CDS region, enabling the expression of TAG lipase in *E. coli* BL21 (DE3). The primary objective is to produce TAG lipase in large quantities, which can then be used to catalyze the esterification of alcohols and organic acids present in yellow water, converting them into ester flavor compounds. This conversion process aims to reduce waste and enhance the flavor profile of fermented products. The project employs benching software to accurately manipulate the pET-32(a) vector in the intended insertion and expression of the target gene. In this way, through the improved generation of TAG lipase, it provides a green solution to the yellow water waste issue in Baijiu production and also has a wide application in the field of aroma improvement and waste minimization in the fermentation industry.

Keywords: Synthetic Biology, Yellow Water, E. coli BL21 (DE3), TAG Lipase, Esterification.

1. Introduction

Under yellow water wastage, potential environmental and economic impacts can be seen, especially concerning the production of the sauce-flavored Baijiu. Yellow water contains organic acids, alcohol, aldehydes, esters and other components which contribute the Baijiu aroma and taste. However, its disposal presents a very environmental challenge because of the high level of organic material content. Conventional ways of handling this waste are usually inefficient, hence resulting in pollution and wastage of resources.

2. Problem Statement

Sauce-flavored Baijiu produced by multiple cycles of fermentation requires a relatively larger amount of yellow water compared to other kinds of Baijiu.[2] This not only augments the volume of waste but also amplifies the task of tackling this nuisance in an ecological way. There is therefore a great need for coming up with the most efficient and sustainable methods for recycling or reusing yellow water. This

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report responds to the need of improving the efficiency of selected microbial strains for the bioconversion of yellow water into valuable products, towards the minimization of waste in the process.

3. Objectives and Significance

Therefore, this report seeks to understand synthetic biological solutions in addressing the yellow water wastage in the Baijiu production process. The objectives include:

1. Analyzing the composition of yellow water: Being familiar with the most important components that can be exploited for bioconversion.
2. Evaluating microbial strains: Finding and selecting more effective microbial strains to bioconvert the yellow water into valuable products.
3. Designing a bioconversion pathway: Suggesting a method for the bioconversion of yellow water in the presence of efficiently selected microbial strains.
4. Assessing the environmental impact: Analyzing the possibility to minimize waste and impact on the environment if the given approach is going to be implemented.

The importance of this report is in its ability to present a semi-ultimate fix for the issue of yellow water, which will ultimately lead to optimizing Baijiu manufacturing process along with lessening the industry's impact on the environment.

4. Results

4.1. Product

Some synthetic biological outputs that can be produced in combating yellow water wastage include the following: Significant outcome include ester formation; esters are organic compounds which are formed from the reaction of an alcohol with an acid. [3] Esters generally have the molecular formula RCOOR' and are famous for their pleasant smell and taste which are usually fruity. [4]

When considering the YWM process, the application of synthetic biology can be used to produce microbial strains that can transform waste elements, such as urea and ammonia, into esters. [5] Thus, specific enzymatic pathways necessary for ester synthesis can be introduced into these microorganisms and the inherent metabolic processes can be utilized. The resulting esters can act as flavoring agents usually in food products as well as in the production of beverages. These esters are responsible for the flavors and or aroma of wines, beer, spirits and other alcoholic drinks and hence, have the potentiality of giving added value to what would otherwise be considered waste.

Apart from using esters in their functions as flavors, they possess other uses in industries. They can be used in power generation as source of energy in cases where they are used as bio-fuels; or in the chemical industry as solvents, which avoid the use of chemicals derived from petroleum. [6] Synthetic biology makes the use of yellow water waste to produce high value products apart from solving the environmental issues of waste management, the it generates economic solutions as well. This double role of waste elimination and product creation highlights the applicability of synthetic biological processes in dealing with yellow water wastage issues.

In addition, the ester production from yellow water also provides an effective way to recycle nitrogen and phosphorus, which could play an important role in sustainable agricultural resource use. [7] It will be seen that the conversion of these nutrients into esters precludes their discharge into the environment where the problems of eutrophication and others might exacerbate. However, these nutrients are rather intercepted and recycled, which complies with the concept of circular economy. [8]

4.2. Approach/Pathway

In liquor production, yellow water is a liquid by-product of the fermentation and distillation processes.[9] During fermentation, grains (such as sorghum and wheat) are converted into alcohol and flavor compounds through the action of microorganisms in the fermentation mash.[10] The liquid by-products accumulate in the fermenter, with some seeping into the fermentation matrix and settling at the bottom of the tank; this liquid is known as yellow water. After fermentation, the mixture is distilled, and the

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alcohol and volatile compounds are evaporated and condensed to form white spirit. The liquid residue that is not distilled, known as "lees water," includes yellow water.[11]

To address the issue of yellow water waste, we adopted a synthetic biology approach using engineered microorganisms to produce lipase. Lipase catalyzes the esterification of organic acids (-COOH) and alcohols (-OH) in yellow water to form ester compounds, with water produced as a by-product.[12] Lipase reduces the activation energy of the esterification reaction, enabling it to occur under mild conditions (such as neutral pH and lower temperatures). During this process, the lipase active site binds to the substrates and facilitates the formation of ester bonds between carboxyl and hydroxyl groups, producing ester compounds with fruity aromas.[13] These ester flavor compounds are highly valuable in the flavor and fragrance industry.[14]

Through lipase catalysis, we convert yellow water into four main products: esters (with fruity aromas, highly valued in the flavor and fragrance industry),[13] fatty acids (which can be further processed into biofuels or industrial products),[15] glycerol (widely used in the pharmaceutical, cosmetic, and food industries),[16] and fatty amides (with potential applications in chemical and industrial sectors).[17] By efficiently expressing lipases in engineered microorganisms, we not only enhance the utilization of yellow water but also reduce waste and increase the economic value of the white spirit production process.[18] This approach not only addresses environmental issues but also opens up new avenues for by-product utilization, promoting a circular economy in the baijiu industry.

4.3. Host

E. coli BL21 (DE3) is a widely used host strain for recombinant protein expression and is known for its unique and superior properties. The strain contains the T7 RNA polymerase gene, which is capable of activating the lac promoter,[19] an inducible promoter capable of initiating the expression of target genes upon IPTG induction.[20] This property makes it valuable for research and industrial uses, particularly where high levels of recombinant proteins must be generated. Furthermore, BL21(DE3) is a strain that possesses the endA1 mutation this minimizes the presence of endonuclease I which in turn leads to minimized digesting of the plasmid DNA hence better plasmid stability. [21]

BL21(DE3) has some advantages over other E. coli strains: it lacks Lon and OmpT proteases, which can degrade exogenous proteins in other E. coli strains. [22] For this reason, BL21(DE3) is ideal for expressing proteins that are prone to degradation at high levels, such as TAG lipase, to equally retain the structures and activities of the expressed proteins.

Data on the compatibility between the BL21(DE3) strain and the pET-32a vector reveal that this pair is easily compatible with the lac promoter and can effectively express genes of interest under IPTG-induction conditions. This would provide good stability, solubility, and high activity of the produced proteins suitable for further downstream applications like structure determination, enzyme catalytic activity assay, and the production of therapeutic proteins.

In our opinion, BL21(DE3) offers distinct advantages in terms of protein expression compare to other commonly used E. coli strains for plasmid replication like DH5α and XL1-Blue. They have high yield and are consistent for protein production in situations that need high quality and yield of proteins and where stability of the proteins and their activity is of extreme importance [24][25].

4.4. Feedstock & Process

In the case of our synthetic biology framework, the feedstock used is yellow water which is waste product produced during white wine production. This waste stream is full of a lot of organic compounds and nutrients. For this feedstock to be processed, we use LB (Lysogeny Broth) culture medium consisting of Genetically Engineered Escherichia Coli (E. Coli) strains that synthesise TAG lipase an enzyme that plays a central role in the esterification of the organic constituents present in the yellow water.

The process starts with the growth of E. coli in LB medium. In this nutrient rich medium, required amino acids, peptides, vitamins and growth factors needed for the growth and metabolic function of the bacteria can be found. [26] When the genetically modified E. coli strains have grown to the desired

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phase which is the stationary phase, TAG lipase is produced and is then collected for use in the yellow water treatment process.

TAG lipase is involved in the bioconversion process, especially esterification reactions that occur during the breakdown of TAG. This enzyme facilitates the process of reaction between fatty acids found in yellow water and alcohols which produces ester compounds. [27] This bioconversion essentially recycles the waste material yellow water by converting the organic to ester-based flavor compounds.

For the purpose of improving the stability and activity of TAG lipase, we apply covalent immobilization method. In this approach, the TAG lipase is immobilized on a support matrix by the formation of covalent linkages between the enzyme's amino acid side chains and functional groups of the carrier material. [28]. Functional groups such as hydroxyl, carboxyl, imidazole, and phenol, which are present on residues like lysine, cysteine, aspartic acid, and glutamic acid, facilitate these chemical bonds.[29] This covalent attachment ensures the enzyme's stability and structural rigidity, rendering it resistant to denaturing agents such as heat, organic solvents, and extreme pH conditions.

Further optimization is achieved using cross-linking agents, with glutaraldehyde being a common choice. The aldehyde groups of glutaraldehyde form stable amide bonds with amino groups on both the enzyme and the carrier material.[30] While this method significantly improves enzyme-carrier binding, it may alter the enzyme's active site, potentially affecting its catalytic efficiency.[31] Nonetheless, this approach substantially enhances the stability and performance of TAG lipase, facilitating effective esterification in yellow water treatment and contributing to waste reduction and resource recycling.

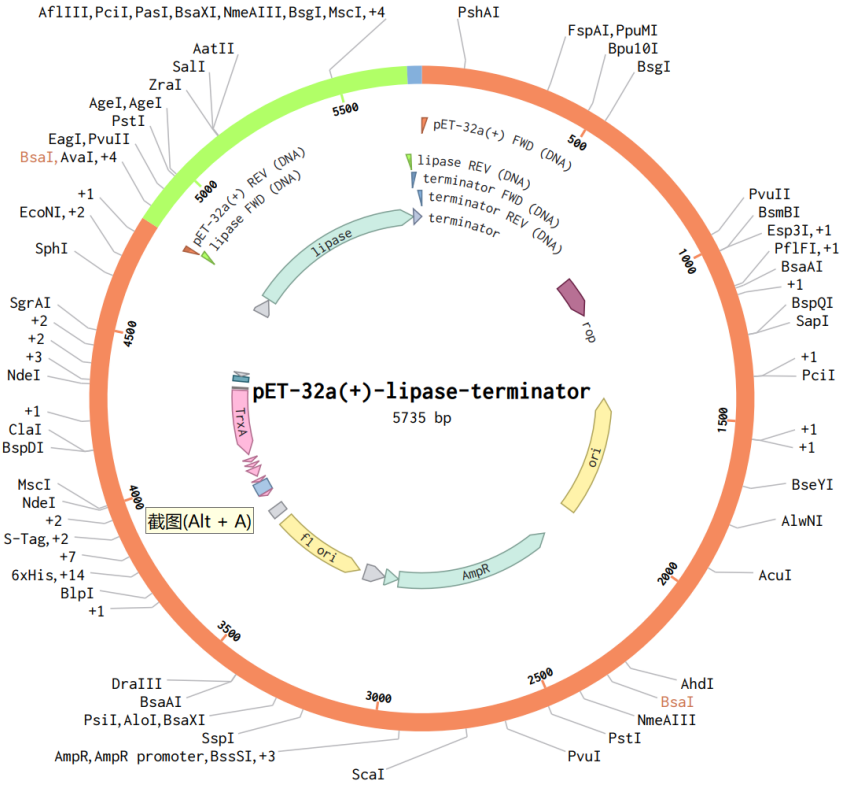


Figure 1. pET-32a(+)-lipase-terminator (5735 bp)

4.5. Design Strategy

To optimize the genetic design of the synthetic biology process, we implemented a series of strategies to ensure the efficient production of TAG lipase. The first strategic choice involved the selection of the pET-32(a) vector, a widely utilized vector due to its robust expression capability in Escherichia coli (E. coli), and the pET-32(a) vector(figure1) is equipped with His-tag and S-tag, which are integral for protein purification, a critical step in the high-yield production of enzyme proteins.[23]

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To drive the expression of TAG lipase efficiently, we employed the lacI promoter, a strong promoter regulated by the LacI repressor.[32] Induction with IPTG releases this repression, thereby activating the promoter and inducing the production of significant quantities of TAG lipase in E. coli.[20] The selection of an efficient promoter like lacI is pivotal for achieving high levels of gene expression, directly influencing protein yield. The use of the lacI promoter ensured sufficient TAG lipase production to facilitate the esterification reactions, thereby improving the efficiency of yellow water transformation.

Terminators play an equally crucial role in gene design. We selected the trpA terminator, derived from the tryptophan operon of E. coli. [33]This strong terminator effectively halts transcription, preventing read-through into adjacent genes and ensuring the stable expression of the target gene.[34]

Additionally, our design incorporated two specific restriction sites with the sequences‘gttg’ and ‘acac’. These sites were carefully chosen to facilitate the precise insertion of the TAG lipase gene into the vector, ensuring correct orientation and reading frame alignment. This step is essential not only for accurate gene expression but also for enabling subsequent genetic modifications.

5. Conclusions

Our project uses the approach of engineering vector pET32(a) to express TAG lipase in E. coli, to illustrates the potentials of solving the waste of yellow water in the process of brewing the baijiu by the approach of biosynthesis.

A efficient way is given by this research, which transforms organic acids and alcohol from yellow water to easters which is exactly what we are expect to gain. This process reduces not only the loss of environment but also the economy.

The use of Benchling make sure the exact gene modification, which makes this project a hopeful remedy to reduce loss in the field of brewing engineer.

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Runlin Liu, and Jun Chen contributed equally to this work and should be considered co-first authors.

References

[1] Zhang, H.Y., Liu, J.W. (2011). "Progress in research on the composition and properties of yellow water of white wine and its treatment method." Food Industry Science and Technology, 32(6), 310-314.

[2] Ministry of Ecology and Environment of the People's Republic of China. (2016). Environmental Protection Regulations. Available at: https://www.mee.gov.cn/gkml/hbb/bgth/201611/W020230203355321728743.pdf

[3] Yang, Z., Peng, M., Li, Y., Wu, X., Gui, T., Li, Y., Zhang, F., Chen, X., Kita, H. (2022). Improved Esterification of Citric Acid and n-Butanol Using a Dense and Acid-Resistant Beta Zeolite Membrane. Membranes (Basel), 12(12):1269. doi: 10.3390/membranes12121269.

[4] LibreTexts. (2023). Carboxylic Acids and Esters. Available at: https://chem.libretexts.org/Bookshelves/Introductory_Chemistry/Chemistry_for_Changing_Times_(Hill_and_McCreary)/09%3A_Organic_Chemistry/9.08%3A_Carboxylic_Acids_and_Esters

[5] McCarty, N.S., Ledesma-Amaro, R. (2019). Synthetic Biology Tools to Engineer Microbial Communities for Biotechnology. Trends in Biotechnology, 37(2), 181-197. doi: 10.1016/j.tibtech.2018.11.002.

[6] Neupane, D. (2022). Biofuels from Renewable Sources, a Potential Option for Biodiesel Production. Bioengineering (Basel), 10(1):29. doi: 10.3390/bioengineering10010029.

[7] Anjum, N., Hoque, T., Sarkar, D., Datta, R., Kibria, M., Ullah, R., Ahmed, N., Hossain, M.A., Masood, A. (2023). Sustainable Management of Phosphorus in Agriculture for Environmental Conservation. IntechOpen. doi: 10.5772/intechopen.113086.

[8] U.S. Environmental Protection Agency (EPA). (2015). Nutrient Economics Report. Available at: https://www.epa.gov

[9] Xie, X., Huang, Y.J., Hong, S., et al. (2019). Study on the formation of yellow water and its effect on the flavour substances of wine spirits. Brewing, 46(4), 20-23.
[10] Li, K. (2014). Analysis of microbial community structure and diversity in yellow water of Chinese strong-flavoured liquor fermentation. Master's thesis, Xihua University.
[11] Xu, C.H., Yu, Y.G., Zhang, W.W. (2014). Progress of research on physicochemical analysis of yellow water and its application. Journal of Food Safety and Quality Testing, 5(12), 4011-4017.
[12] Yan, C., Li, Z., Zhang, J., et al. (2020). Optimisation of process conditions for lipase esterification of white wine yellow water. Modern Food Science and Technology, 36(1), 220-226.
[13] LibreTexts. (2023). Chemistry of Esters. Available at: https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Organic_Chemistry_(Morsch_et_al.)/21%3A_Carboxylic_Acid_Derivatives-_Nucleophilic_Acyl_Substitution_Reactions/21.06%3A_Chemistry_of_Esters
[14] Dong, W., et al. (2024). Characteristics and Functions of Dominant Yeasts Together with Their Applications during Strong-Flavor Baijiu Brewing. Foods (Basel), 13(15):2409. doi: 10.3390/foods13152409.
[15] Zhang, X. (2023). The Application of Synthetic Biology in the Development of New Products in the Brewing Industry. ScienceDirect. Available at: https://www.sciencedirect.com/science/article/pii/S2667041023000046
[16] Shen, Y., et al. (2023). Research progress and application of enzymatic synthesis of glycosyl compounds. Applied Microbiology and Biotechnology, 107(17), 5317-5328. doi: 10.1007/s00253-023-12652-8.
[17] Hou, Q. (2023). Applications of Synthetic Biology in the Brewing Industry. ScienceDirect. Available at: https://www.sciencedirect.com/science/article/abs/pii/B9780081002148000154#preview-section-cited-by
[18] Ziang, Z., Huang, P., Zheng, J., et al. (2023). Current status of research on yellow water, a by-product of liquor brewing, and progress of comprehensive utilisation research. China Brewing, 42(4), 20-28.
[19] Du, F., et al. (2021). Regulating the T7 RNA polymerase expression in E. coli BL21 (DE3) to provide more host options for recombinant protein production. Microbial Cell Factories, 20(1): 189. doi: 10.1186/s12934-021-01680-6.
[20] Liu, M., et al. (2024). OptoLacI: optogenetically engineered lactose operon repressor LacI responsive to light instead of IPTG. Nucleic Acids Research, 52(13), 8003-8016. doi: 10.1093/nar/gkae479.
[21] Promega Corporation. (2023). Genetic Markers of E. coli. Available at: https://www.promega.com/~/media/Files/Resources/Technical%20References/Genetic%20Markers%20of%20%20E%20Coli.pdf
[22] Ratelade, J., et al. (2009). Production of recombinant proteins in the lon-deficient BL21(DE3) strain of Escherichia coli in the absence of the DnaK chaperone. Applied and Environmental Microbiology, 75(11), 3803-3807. doi: 10.1128/AEM.00255-09.
[23] Liu, Z.Q., & Yang, P.C. (2012). Construction of pET-32 α (+) Vector for Protein Expression and Purification. North American Journal of Medical Sciences, 4(12), 651-655. doi: 10.4103/1947-2714.104318.
[24] Baneyx, F. (1999). Recombinant protein expression in Escherichia coli. Current Opinion in Biotechnology, 10(5), 411-421.
[25] Studier, F.W., & Moffatt, B.A. (1986). Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. Journal of Molecular Biology, 189(1), 113-130.
[26] Wikipedia. (2023). Lysogeny Broth. Available at: https://en.wikipedia.org/wiki/Lysogeny_broth
[27] Utama, Q.D., et al. (2019). Lipase-Catalyzed Interesterification for the Synthesis of Medium-Long-Medium (MLM) Structured Lipids - A Review. Food Technology and Biotechnology, 57(3), 305-318. doi: 10.17113/ftb.57.03.19.6025.

[28] Karimi, I., et al. (2023). Interesterification Processes in the Food Industry. ScienceDirect. Available at: https://www.sciencedirect.com/science/article/abs/pii/S1359511318306020
[29] LibreTexts. (2023). Biocatalysis. Available at: https://chem.libretexts.org/Courses/University_of_California_Davis/UCD_Chem_107B%3A_Organic_Chemistry_(Summer_2016)/Unit_VI%3A_Enzyme_and_Coenzymes/Biocatalysis
[30] López-Gallego, Fernando & Betancor, Lorena & Mateo, Cesar & Hidalgo, Aurelio & Alonso-Morales, Noelia & Dellamora-Ortiz, Gisela & Guisan, Jose & Fernández-Lafuente, Roberto. (2005). Enzyme stabilization by glutaraldehyde crosslinking of adsorbed proteins on aminated supports. Journal of biotechnology. 119. 70-5. 10.1016/j.jbiotec.2005.05.021.
[31] Homaei, Ahmad Abolpour et al. "Enzyme immobilization: an update." Journal of chemical biology vol. 6, 4 185-205. 29 Aug. 2013, doi:10.1007/s12154-013-0102-9
[32] Becker, Nicole A et al. "Mechanism of promoter repression by Lac repressor-DNA loops." Nucleic acids research vol. 41, 1 (2013): 156-66. doi:10.1093/nar/gks1011
[33] https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/regulation-of-gene-expression-and-cell-specialization/a/the-trp-operon
[34] Wu, A M, and T Platt. "Transcription termination: nucleotide sequence at 3' end of tryptophan operon in Escherichia coli." Proceedings of the National Academy of Sciences of the United States of America vol. 75, 11 (1978): 5442-6. doi:10.1073/pnas.75.11.5442