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Potential and Metabolic Pathway Analysis of Marine Microorganism Fermentation in Bioethanol Production

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Abstract The study found that marine yeasts, such as *Wickerhamomyces anomalus* M15, exhibit high tolerance to salt and inhibitors, making them suitable for seawater fermentation. Additionally, the use of macroalgae and microalgae, such as *Ulva fasciata* and *Chlorella vulgaris*, demonstrated significant potential for bioethanol production, with chemical hydrolysis being the most effective pretreatment method. The integration of advanced techniques like artificial neural networks with genetic algorithms (ANN-GA) further optimized the fermentation parameters, enhancing bioethanol yield. Moreover, the study highlighted the importance of specific microbial strains, such as *Saccharomyces cerevisiae*, in efficiently converting carbohydrates to ethanol. The findings suggest that marine microorganisms and biomass hold substantial promise for sustainable bioethanol production. The high tolerance of marine yeasts to saline conditions and the effective use of macroalgae and microalgae as feedstocks can lead to greener and more efficient bioethanol production processes. The optimization of fermentation parameters through advanced modeling techniques can further enhance ethanol yields, making marine-based bioethanol production a viable alternative to traditional methods.

Keywords Marine microorganisms; Bioethanol production; Fermentation; Metabolic pathways; Marine biomass; *Saccharomyces cerevisiae*; *Wickerhamomyces anomalus*; Algae hydrolysis; ANN-GA modeling

1 Introduction

Bioethanol, a form of ethanol produced from biomass, has emerged as a promising renewable energy source in response to the growing concerns over climate change and the depletion of fossil fuels. Unlike traditional fossil fuels, bioethanol is derived from biological materials such as sugar, starch, and lignocellulosic biomass, making it a more sustainable and environmentally friendly option. The production of bioethanol from renewable sources not only helps in reducing greenhouse gas emissions but also provides a viable alternative to petroleum-based fuels, thereby addressing the energy needs of the future (Robak and Balcerek, 2018; Dave et al., 2019).

Marine microorganisms, including microalgae and macroalgae, present a unique and largely untapped potential for bioethanol production. These organisms are capable of accumulating high levels of carbohydrates, which can be converted into fermentable sugars for ethanol production. Unlike terrestrial biomass, marine biomass does not compete with food crops for land and water resources, making it a more sustainable option (Harun and Danquah, 2011; John et al., 2011; Osman et al., 2023). Certain species of marine algae can produce ethanol directly through dark-anaerobic fermentation, while others can be metabolically engineered to enhance ethanol yield (Takeda et al., 2011; Dave et al., 2021). The high growth rates and abundance of marine biomass in coastal regions further underscore its potential as a renewable feedstock for bioethanol production (Borines et al., 2013; Osman et al., 2023).

The primary objectives of this study are to explore the potential of marine microorganisms in bioethanol production and to analyze their metabolic pathways. This includes identifying and evaluating various marine microorganisms that can be used for bioethanol production, investigating the metabolic pathways involved in the fermentation process of these microorganisms, assessing the efficiency and feasibility of using marine biomass as a feedstock for bioethanol production, and exploring the potential for co-fermentation of different marine biomass types to enhance bioethanol yields and reduce production costs. By achieving these objectives, this study aims to contribute to the development of more efficient and sustainable bioethanol production processes using marine microorganisms.

2 Marine Microorganisms in Bioethanol Production

2.1 Overview of marine microorganisms

Marine microorganisms, including various species of algae, bacteria, and yeasts, have shown significant potential in bioethanol production. Notable examples include marine yeasts like *Wickerhamomyces anomalus* and bacteria such as *Sphingomonas* sp. A1. Additionally, microalgae such as *Chlorella vulgaris* and *Navicula* sp. are also utilized due to their high carbohydrate content, which can be converted into fermentable sugars (Takeda et al., 2011; Kim et al., 2014; Greetham et al., 2018; Turner et al., 2022; Telussa et al., 2023).

2.2 Advantages of marine microorganisms

Marine microorganisms offer several advantages for bioethanol production:

High Tolerance to Saline Environments: Marine yeasts and bacteria can thrive in high-salinity conditions, making them suitable for fermentation processes using seawater, which reduces the need for freshwater resources (Greetham et al., 2018; Turner et al., 2022).

Unique Enzymes and Metabolic Capabilities: These microorganisms possess unique enzymes that can degrade complex marine biomass components, such as alginate from brown algae, into fermentable sugars (Takeda et al., 2011; Reisky et al., 2019).

Adaptability and Stress Tolerance: Marine microorganisms like *Wickerhamomyces anomalus* exhibit high tolerance to various stress factors, including high glucose, xylose, and ethanol concentrations, enhancing their efficiency in bioethanol production (Turner et al., 2022).

2.3 Current research and findings

Recent studies have explored the potential of marine microorganisms in bioethanol production with promising results:

Marine Yeasts: *Wickerhamomyces anomalus* M15 has been characterized for its high tolerance to inhibitors and its ability to produce significant ethanol concentrations from seaweed-derived media. Adaptive strains like *W. anomalus* M15-500A have shown even higher ethanol yields, indicating their industrial potential (Turner et al., 2022).

Metabolically Engineered Bacteria: *Sphingomonas* sp. A1 has been genetically modified to efficiently convert alginate, a major component of brown algae, into ethanol. This engineered strain accumulated 13.0 g/L of ethanol in 3 days using alginate as the sole carbon source (Takeda et al., 2011).

Microalgae: Studies on microalgae such as *Chlorella vulgaris* and *Navicula* sp. have demonstrated their potential as bioethanol feedstocks. For instance, nutrient stress-induced *C. vulgaris* showed enhanced carbohydrate content, leading to high saccharification and ethanol yields (Kim et al., 2014; Telussa et al., 2023).

Enzymatic Pathways: Research on the marine bacterium *Formosa agariphila* has elucidated the enzymatic pathways involved in degrading ulvan, a polysaccharide from green algae, into fermentable sugars. This knowledge can be applied to optimize the fermentation process for bioethanol production (Reisky et al., 2019).

These findings highlight the potential of marine microorganisms in creating a sustainable and efficient bioethanol production process, leveraging their unique properties and metabolic capabilities.

3 Fermentation Processes

3.1 Overview of fermentation in bioethanol production

Fermentation is a biochemical process that converts sugars into bioethanol through the action of microorganisms. The basic principles involve the breakdown of complex carbohydrates into simpler sugars, which are then metabolized by microorganisms to produce ethanol and carbon dioxide. The key steps in the fermentation process include substrate preparation, inoculation with a suitable microorganism, fermentation under controlled conditions, and ethanol recovery. Marine microorganisms, such as certain strains of yeast and bacteria, have shown potential in bioethanol production due to their ability to tolerate high salinity and other harsh conditions (Greetham et al., 2018; Turner et al., 2022).

3.2 Fermentation pathways in marine microorganisms

Marine microorganisms utilize various fermentation pathways to convert substrates into bioethanol. For instance, the marine flavobacterium *Formosa agariphila* degrades the algal polysaccharide ulvan into fermentable monosaccharides through a series of enzymatic reactions involving polysaccharide lyases, sulfatases, and glycoside hydrolases (Reisky et al., 2019). Another example is the thermophilic bacterium *Geobacillus thermoglucosidasius*, which ferments both C5 and C6 sugars via glycolysis, the pentose phosphate pathway, and the TCA cycle, producing ethanol, lactate, acetate, and formate under different growth conditions (Tang et al., 2009). Additionally, marine yeasts like *Wickerhamomyces anomalus* M15 have been shown to effectively ferment seaweed-derived sugars into ethanol, demonstrating high tolerance to various inhibitors present in the medium (Turner et al., 2022) (Table 1).

Table 1 Ethanol productions in fermentations using natural and concentrated semi-synthetic seaweed hydrolysate media (Adopted from Turner et al., 2022)

	Glucose (g/L)	Glucose+galactose (g/L)	Total sugar (g/L)	Ethanol (g/L)	Yield based on glucose (%)	Yield based on glucose + galactose	Yield based on total sugar (%)
L. digitata							
YPD	20	20	20	9.8±2.03	95.7	95.7%	95.7
Natural	1.32	1.87	20.14	1.13±0.04	167.2	118.0%	11.0
5x	6.6	9.35	100.7	5.41±0.97	160.1	113.0%	10.5
7.5x	9.9	14.03	151.1	7.87±2.19	155.3	109.6%	10.2
10x	13.2	18.7	201.4	5.79±1.37	85.7	60.5%	5.6
U. linza							
YPD	20	20	20	10.03±0.52	97.9	97.9%	97.9
Natural	8.16	8.83	16.61	4.26±0.48	102.0	94.2%	50.1
5x	40.8	44.15	83.05	20.43±2.80	97.8	90.4%	48.0
7.5x	61.2	66.23	124.6	34.7±4.40	110.7	102.3%	54.4
10x	81.6	88.3	166.1	45.04±4.40	107.8	99.6%	53.0
P. umbilicalis							
YPD	20	20	20	10.31±1.36	100.7	100.7%	100.7
Natural	3.52	9.81	13.08	3.39±0.28	188.1	67.5%	50.6
5x	17.6	49.05	65.4	7.49±2.26	83.1	29.8%	22.4
7.5x	26.4	73.58	98.1	13.61±0.37	100.7	36.1%	27.1
10x	35.2	98.1	130.8	19.85±2.64	110.1	39.5%	29.6

3.3 Factors influencing fermentation efficiency

Several factors influence the efficiency of the fermentation process in marine microorganisms. Temperature is a critical factor, as many marine microorganisms are thermophilic and exhibit optimal ethanol production at elevated temperatures (Tang et al., 2009; Niu et al., 2015). pH levels also play a significant role, with different microorganisms requiring specific pH ranges for optimal activity. Salinity is another important factor, as marine microorganisms must be able to tolerate high salt concentrations to thrive in seawater-based fermentation processes (Greetham et al., 2018; Turner et al., 2022). Nutrient availability, including the presence of essential minerals and vitamins, can significantly impact microbial growth and ethanol yield. Additionally, the presence of inhibitors, such as salts and other by-products, can affect fermentation efficiency, necessitating the use of tolerant strains or adaptive evolution techniques to enhance performance (Turner et al., 2022).

4 Metabolic Pathway Analysis

4.1 Glycolysis in marine microorganisms

Glycolysis is a fundamental metabolic pathway that converts glucose into pyruvate, generating ATP and NADH in the process. In marine microorganisms, glycolysis operates similarly to terrestrial organisms but with adaptations to their unique environments. For instance, the cyanobacterium *Synechococcus* sp. PCC 7002 has been shown to maintain high glycolytic activity even under the stress of ethanol production, which is crucial for bioethanol synthesis (Kopka et al., 2017). Additionally, the overexpression of transcription factors such as ZNF1 in *Saccharomyces cerevisiae* has been demonstrated to enhance glycolytic flux, thereby improving bioethanol productivity under high glucose concentrations (Songdech et al., 2020). These findings suggest that marine microorganisms can be engineered to optimize glycolysis for efficient bioethanol production.

4.2 Pyruvate decarboxylation and ethanol production

The conversion of pyruvate to ethanol involves two key enzymes: pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH). In marine microorganisms, this pathway is often engineered to enhance ethanol yield. For example, the introduction of PDC and ADH from *Zymomonas mobilis* into *Synechocystis* sp. PCC 6803 has enabled the photoautotrophic conversion of CO_2 to ethanol (Dexter and Fu, 2009). Similarly, overexpression of these enzymes in *Escherichia coli* has resulted in high ethanol production, although it also led to significant metabolic rewiring (Yang et al., 2014). These modifications highlight the potential of marine microorganisms to be tailored for efficient ethanol production through targeted metabolic engineering.

4.3 Alternative pathways

Marine microorganisms may also utilize alternative metabolic pathways for bioethanol production. For instance, the thermophilic bacterium *Geobacillus thermoglucosidasius* employs a mixed acid fermentation process under anaerobic conditions, producing ethanol along with lactate, acetate, and formate (Tang et al., 2009). This bacterium's ability to ferment both C5 and C6 sugars and tolerate high ethanol concentrations makes it a promising candidate for bioethanol production. Additionally, the rerouting of glycolytic carbon to alternative products such as lactate and glycerol has been observed in *Chlamydomonas reinhardtii* mutants lacking pyruvate formate lyase and alcohol dehydrogenase, indicating the flexibility of metabolic pathways in response to genetic modifications (Catalanotti et al., 2012). These alternative pathways provide insights into the diverse metabolic capabilities of marine microorganisms and their potential applications in bioethanol production.

By understanding and manipulating these metabolic pathways, researchers can enhance the efficiency and yield of bioethanol production in marine microorganisms, contributing to the development of sustainable biofuels.

5 Technological Integration and Optimization

5.1 Bioreactor design for marine microorganisms

Designing bioreactors that can support marine microorganisms involves addressing several unique challenges. Marine microorganisms often require specific environmental conditions, such as high salinity and particular temperature ranges, which must be maintained consistently within the bioreactor. The use of seawater as a medium, as highlighted in recent studies, can be beneficial due to the natural tolerance of marine microorganisms to salt and inhibitors, making them suitable for seawater fermentation (Greetham et al., 2018). Additionally, the integration of advanced control systems, such as optogenetic tools, can enhance the precision of metabolic control within the bioreactor. For instance, light-controlled transcription can be used to shift cells between growth and production phases, optimizing the fermentation process (Zhao et al., 2018). Furthermore, the co-culture of different engineered organisms within the same bioreactor can be employed to distribute metabolic pathways, thereby enhancing the production of complex metabolites (Zhou et al., 2015).

5.2 Genetic engineering approaches

Genetic engineering plays a crucial role in enhancing ethanol yield and efficiency in marine microorganisms. By modifying metabolic pathways, it is possible to increase the tolerance of microorganisms to high ethanol concentrations and improve their overall productivity. Techniques such as metabolic engineering and synthetic biology have been extensively used to redirect carbon fluxes towards the desired products. For example, engineering microbes to balance cellular redox metabolism can optimize microbial production by overcoming cellular redox limitations (Kracke et al., 2018). Additionally, the development of microbial cell factories through systems metabolic engineering can lead to the construction of industrial strains with optimized pathways for ethanol production, increased tolerance to inhibitors, and enhanced genetic stability (Gustavsson and Lee, 2016). The use of computational tools like Flux Balance Analysis (FBA) can further aid in designing efficient metabolic networks by predicting biomass growth and metabolic flux distribution under various environmental conditions (Sen, 2022).

5.3 Process optimization

Optimizing the fermentation process for higher yield and lower costs involves several strategies. One effective approach is the integration of artificial neural networks with genetic algorithms (ANN-GA) to model and predict optimal fermentation conditions. This method has been successfully applied to marine macroalgal biomass, resulting in significant improvements in bioethanol yield (Dave et al., 2021). Additionally, the use of high-throughput, small-scale fermentation techniques can accelerate the screening and characterization of engineered strains, thereby identifying the most promising candidates for large-scale production (Raj et al., 2021) (Figure 1). The combination of metabolic flux analysis tools with bioreactor control algorithms can also help in fine-tuning the fermentation process, ensuring optimal conditions for microbial growth and product formation (Hollinshead et al., 2014). By employing these advanced techniques, it is possible to achieve a more efficient and cost-effective bioethanol production process using marine microorganisms.

6 Case Studies

6.1 Successful implementation examples

Several case studies have demonstrated the successful use of marine microorganisms in bioethanol production. For instance, the marine yeast *Wickerhamomyces anomalus* M15 has shown significant potential in fermenting seaweed-derived media, producing up to 92.7 g/L ethanol from 200 g/L glucose (Turner et al., 2022). Another example is the marine yeast *Saccharomyces cerevisiae* AZ65, which achieved an ethanol concentration of 113.52 g/L using seawater-based media (Zaky et al., 2020). Additionally, the marine flavobacterium *Formosa agariphila* has been utilized to degrade ulvan from Ulva species into fermentable monosaccharides, showcasing the potential of marine bacteria in bioethanol production (Reisky et al., 2019). Furthermore, the use of fungal pretreatment of marine macroalgae has been shown to increase ethanol yields by up to 38.23% (Sulfahri et al., 2020). Lastly, the marine microalgae *Navicula* sp. strain TAD has been successfully cultivated and fermented to produce bioethanol, indicating the viability of microalgae as a feedstock (Telussa et al., 2023) (Figure 2).

6.2 Comparative analysis

Comparing these case studies reveals different approaches, microorganisms, and outcomes. *Wickerhamomyces anomalus* M15 and *Saccharomyces cerevisiae* AZ65 both demonstrated high ethanol production, but the former showed better performance in concentrated seaweed hydrolysates (Zaky et al., 2020; Turner et al., 2022). The enzymatic degradation pathway of *Formosa agariphila* highlights a bacterial approach, focusing on breaking down complex polysaccharides into fermentable sugars (Reisky et al., 2019). In contrast, the fungal pretreatment method emphasizes the use of fungi to enhance saccharification and nutrient supplementation, leading to higher ethanol yields (Sulfahri et al., 2020). The use of *Navicula* sp. strain TAD represents a microalgal approach, focusing on the cultivation and hydrolysis of microalgae for bioethanol production (Telussa et al., 2023). Each method has its unique advantages and challenges, such as the need for specific pretreatment processes or the tolerance of microorganisms to high salt concentrations.

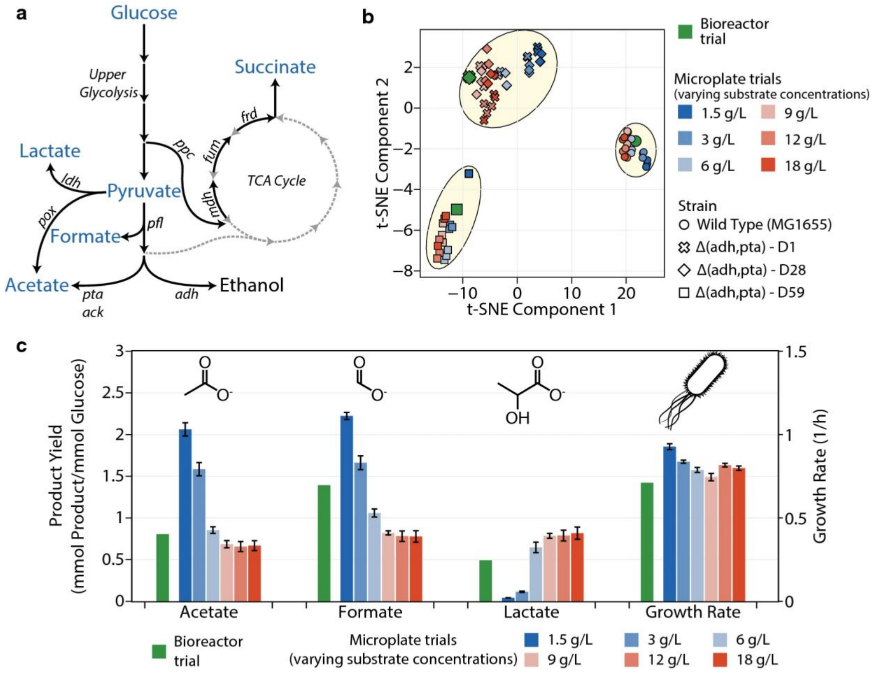

Figure 1 Comparison of *E. coli*'s anaerobic phenotype in bioreactors and microplates (Adopted from Raj et al., 2021)

Image caption: A schematic showing typical fermentation pathways in *E. coli*. Typical products of mixed acid fermentation on glucose are shown in the pathway along with key fermentation reactions shown in italics. The metabolites measured in this study are shown in blue. b Microbial phenotypes reduced to two components through t-distributed stochastic neighbors embedding (t-SNE) performed on the metabolite (acetate, formate, lactate, pyruvate, and succinate) yields and growth rates of *E. coli* strains grown in rich defined media in a bioreactor and microplates with different initial concentrations of substrate (glucose). Cluster boundaries were drawn manually for illustrative purposes. c A comparison of WildType *E. coli*'s growth rate and metabolite yields on glucose obtained from a bench-top 0.5 L bioreactor and 96-well microplates with different initial concentrations of substrate (glucose) (Adopted from Raj et al., 2021)

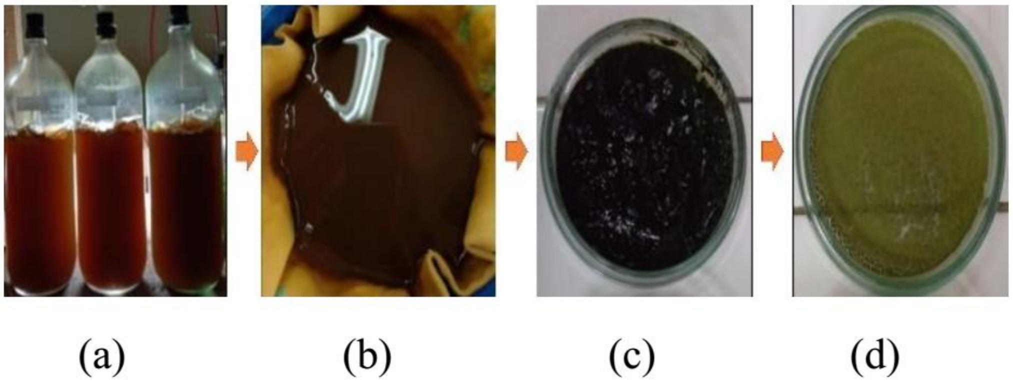

Figure 2 The process of harvesting biomass of *Navicula* sp. strain TAD (Adopted from Telussa et al., 2023)

Image caption: (a) Culture on the 7th day, (b) Filtration process, (c) Base biomass, and (d) Dry biomass (Adopted from Telussa et al., 2023)

6.3 Lessons learned

Key takeaways from these case studies include the importance of selecting microorganisms with high tolerance to inhibitors and the ability to thrive in seawater-based media. For example, *Wickerhamomyces anomalus* M15 and *Saccharomyces cerevisiae* AZ65 both demonstrated high osmotic tolerance, which is crucial for efficient bioethanol production in marine environments (Zaky et al., 2020; Turner et al., 2022). The enzymatic pathway elucidated by *Formosa agariphila* provides valuable insights into the biochemical processes required for converting complex algal polysaccharides into fermentable sugars (Reisky et al., 2019). The success of fungal pretreatment methods underscores the potential of integrating pretreatment and nutrient supplementation strategies to enhance bioethanol yields (Sulfahri et al., 2020). Additionally, the cultivation and fermentation of marine microalgae like *Navicula* sp. strain TAD highlight the feasibility of using microalgae as a sustainable feedstock for bioethanol production (Telussa et al., 2023). These lessons emphasize the need for continued research into optimizing fermentation processes, improving microorganism strains, and developing cost-effective pretreatment methods to advance the field of marine-based bioethanol production.

7 Challenges and Future Perspectives

7.1 Technical challenges

The utilization of marine microorganisms for large-scale bioethanol production presents several technical challenges. One significant limitation is the need for effective pretreatment methods to break down the complex cell walls of marine biomass, which is crucial for efficient saccharification and fermentation processes (Soliman et al., 2018; Sulfahri et al., 2020). Additionally, the presence of various inhibitors in seaweed hydrolysates, such as salts, can negatively impact the fermentation efficiency of marine yeasts (Turner et al., 2022). Another challenge is the metabolic engineering required to enable microorganisms to degrade and assimilate specific polysaccharides found in marine biomass, such as alginate from brown algae (Takeda et al., 2011). The development of robust microbial strains that can tolerate high concentrations of glucose, xylose, and ethanol is also essential for improving bioethanol yields (Turner et al., 2022).

7.2 Environmental and economic considerations

Marine-based bioethanol production has the potential to be more environmentally sustainable compared to traditional bioethanol production methods. Utilizing marine biomass, such as algae, does not compete with food crops for land and freshwater resources, thereby reducing the environmental footprint (Greetham et al., 2018; Ramachandra and Hebbale, 2020). However, the economic viability of this approach is still a concern. The high costs associated with pretreatment and nutrient supplementation during fermentation need to be addressed to make marine bioethanol production commercially feasible (Sulfahri et al., 2020). Additionally, the large-scale harvesting of marine biomass must be managed sustainably to avoid negative impacts on marine ecosystems (Soliman et al., 2018; Reisky et al., 2019).

7.3 Future research directions

Future research should focus on developing more efficient and cost-effective pretreatment methods to enhance the breakdown of marine biomass. Exploring alternative nutrient supplementation strategies, such as the use of fungal biomass, could also improve the economic viability of marine bioethanol production (Sulfahri et al., 2020). Further studies are needed to understand the metabolic pathways of marine microorganisms better and to engineer strains with enhanced capabilities for bioethanol production (Takeda et al., 2011; Adegboye et al., 2021). Additionally, research should investigate the potential of using seawater as a fermentation medium to reduce freshwater consumption and improve the sustainability of the process (Greetham et al., 2018; Zaky et al., 2020). Finally, comprehensive life cycle assessments and economic analyses are essential to evaluate the overall feasibility and environmental impact of marine-based bioethanol production (Ramachandra and Hebbale, 2020).

8 Concluding Remarks

This research has explored the potential of marine microorganisms in the fermentation process for bioethanol production. Key findings include the successful use of both macroalgae and microalgae as feedstocks, with marine yeasts demonstrating high tolerance to salt and inhibitors, making them suitable for seawater fermentation. Metabolically engineered bacteria, such as *Sphingomonas* sp. A1, have shown promise in converting alginate from brown algae into ethanol, achieving significant ethanol yields. Additionally, the marine yeast *Wickerhamomyces anomalus* M15 has been characterized for its high tolerance to various inhibitors and its potential for industrial bioethanol production using seaweed-derived feedstocks. The enzymatic hydrolysis and fermentation of microalgae like *Chlorella vulgaris* have also been highlighted as effective methods for bioethanol production.

The utilization of marine microorganisms and biomass presents a sustainable and environmentally friendly alternative to traditional bioethanol production methods. Marine yeasts and bacteria can thrive in saline conditions, reducing the need for freshwater resources and making the process more sustainable. The ability to metabolically engineer bacteria to degrade complex polysaccharides from marine biomass into fermentable sugars opens new avenues for efficient bioethanol production. Furthermore, the development of robust marine yeast strains capable of high ethanol yields from seaweed hydrolysates suggests that marine biomass could meet industrial bioethanol production thresholds, potentially reducing reliance on land-based biomass and mitigating food vs. fuel conflicts.

The future of bioethanol production using marine microorganisms and biomass is promising but not without challenges. Key areas for future research include optimizing the metabolic pathways of marine microorganisms to enhance ethanol yields, improving the efficiency of saccharification processes, and developing cost-effective and scalable fermentation technologies. Addressing these challenges will be crucial for the commercial viability of marine-based bioethanol production. Continued interdisciplinary research and collaboration will be essential to unlock the full potential of marine resources in the biofuel industry, contributing to a more sustainable and carbon-neutral future.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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