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Harnessing Enzymes from Halophiles for Sustainable Biofuel Production

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Abstract

Halophiles are a class of extremophiles that growin salty environments. They have emerged as a valuable repository of many polyextremophilic enzymes, finding diverse industrial applications. Enzymes used in industry must remain stable in challenging operating environments. In this context, the enzymes from extremophiles can be of great use because they are resistant to and capable of catalysis under extreme physical conditions. Halophilic enzymes show great potential for use in industrial applications that involve high salt or hypersaline conditions. Additionally, they have been optimized to function under polyextreme conditions, such as high pH, temperature, and the presence of hydrophobic solvents. Numerous studies have emphasized the wide range of biotechnological applications for these enzymes.Cellulase, xylanase, laccase, lipase, and amylases from halophiles have shown potential for biofuel production. Halophilic cellulase is a primary enzyme that can help in the bioconversion of lignocellulosic biomass into biofuel under harsh conditions. They have also shown tolerance to ionic liquids that can be utilized for the efficient conversion of biomass. Other than halophilic enzymes, halophilic biomasses of microbes, algae, seaweed, and halophytes hold great potential for biofuel production. Bioethanol, biodiesel, biogas, biomethane, and biohydrogen production have been reported by the utilization of biomass of halophiles. Thus, halophiles and their polyextremophilic enzymes hold good potential for biofuel production, and further systematic research and development can be useful for the biorefinery approach.

Keywords: Extremophiles; Halophiles; Enzymes; Cellulase; Laccase; Biofuels

Introduction

Extremophiles belonging to the halophile class have the unusual capacity to flourish in conditions with elevated salt concentrations.Halophiles have evolved their physiological and metabolic processes to operate in high-salinity environments. Salted foods, salt lakes, soda lakes, salty soil, saline water, and salterns are among the habitats from which halophiles have mostly been isolated and described (1-5). They are prominently featured groups in the universal phylogenetic tree of life and can be found within all three domains: Archaea, Bacteria, and Eukarya.

Halophilic organisms regulate their osmotic balance in high-salinity environments through two main strategies. In the compatible-solute approach often seen in halotolerant and moderate halophiles, osmotic balance is achieved by the synthesis or accumulation of low molecular-weight organic osmotic compounds such as ectoine, betaine, glycine, glycerol, and sucrose. The "Salt-in" strategy, primarily utilized by halophilic Archaea, entails the selective uptake of K^+ ions into the cytoplasm, where they accumulate (usually balanced with Cl^-) to levels that are isotonic with the surrounding environment (6).Their proteins exhibit distinct amino acid compositions and structures that enable them to thrive in high-salinity environments and avoid aggregation. Halophilic proteins differ from those of mesophilic organisms by having a higher proportion of acidic amino acids on their surface, lower lysine content, a relatively

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reduced hydrophobic core, and an increased number of salt bridges. The elevated levels of acidic residues significantly contribute to the binding of vital water molecules and salt ions, which helps prevent protein aggregation and enhances the flexibility of the protein structure through electrostatic repulsion (7-8).

They are promising candidates for use in bioprocesses because they can adapt to significant fluctuations in external salt concentrations. Due to their potential applications in sectors such as the food industry, enhanced oil recovery, biodegradation of harmful pollutants, and as a source of organic osmotic stabilizers, bioplastics, enzymes, exopolysaccharides, and bacteriorhodopsin, there has been substantial interest in researching halophiles (2,5,9-12).

Enzymes sourced from halophiles are a valuable resource for diverse bioprocesses. Enzymes used in industry must remain stable in challenging operating environments. In this context, the enzymes from extremophiles can be of great use because they are resistant to and capable of catalysis under extreme physical conditions.Halophilic enzymes exhibit stability even in the presence of elevated salt concentrations, unlike their non-halophilic counterparts. Despite the high salt levels, these enzymes can carry out the same functions. The extensive review conducted on halophilic enzymes has highlighted their immense potential (2,13-15). Some notable characteristics they possess are (i) their ability to maintain optimal activity and stability even in elevated salt concentrations, (ii) the shielding function of salt in preserving their structure, (iii) their improved resistance to denaturation, and (iv) their competence to catalyze reactions in low water or non-aqueous environments (5,16,17).

Several fascinating characteristics have been documented in amylases, xylanases, proteases, cellulases,nucleases, chitinases, esterases, and lipases derived from halophiles. These enzymes have primarily been investigated in the *Haloarcula*, *Halobacterium*,*Haloferax*, *Marinococcus*, *Micrococcus*, *Halobacillus*,*Bacillus*, and *Halothermothrix* genera. It is evident that these enzymes exhibit remarkable stability even in diverse extreme environments.Hence, these are the preferred catalysts for various purposes such as (i) hypersaline waste management, (ii) peptide production, (iii) detergents, (iv) textile manufacturing, (v) pharmaceuticals, and (vi) food (2,13,15,18). Several studies have been carried out related to the isolation of enzyme-producing halophiles, optimization of enzyme production, purification, characterization, and potential application of halophilic enzymes.

Halophilic enzymes show great potential for use in industrial applications that involve elevated salt or hypersaline conditions. Additionally, they have been optimized to function under polyextreme conditions, such as high pH, temperature, and the presence of hydrophobic solvents. Numerous studies have emphasized the wide range of biotechnological applications for these enzymes.Halophiles and their enzymes have been potentiated for biofuel production (19,20).

Halophilic enzymes for potential applications in Biofuel production

Cellulases

Among various halophilic hydrolases, cellulases are industrially useful enzymes with widespread applications, particularly in the valorization of lignocellulosic biomass to biofuel (Ethanol). The need for stable and effective enzymes in the synthesis of biofuels is increasing as interest in sustainable energy sources spreads around the world. Because of their innate ability to withstand harsh environments, halophilic cellulases offer a viable way to enhance enzyme-based biomass breakdown. However, a lack of thorough research on the isolation of potent cellulase producer, structural and functional characteristics, and applications has limited their commercial application.

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Cellulases from halophilic microorganisms represent a specialized group of enzymes capable of hydrolyzing cellulose, the β-1,4-glucan backbone of plant cell walls, under extreme saline conditions. There are a number of practical restrictions on the commercial usage of cellulases in the manufacture of biofuel, especially when it comes to preserving enzyme effectiveness under less-than-ideal circumstances. In high-salt industrial settings, conventional cellulases frequently lose their activity, necessitating enzyme stabilization procedures, which raises production costs. Among the few reports of halophilic cellulases, *Haloarcula* sp. LLSG7 cellulase was active and stable over a wide range of temperatures, pH, and salt concentration. It was effectively used for the enzymatic saccharification of alkali-treated rice straw, and the hydrolysate was fermented into 10.7 g/L of bioethanol by *Saccharomyces cerevisiae* (21).Purification and characterization of *Thalassobacillus* sp. LY18's salt, solvent, and alkaline-stable cellulase have been completed (22). It has also been shown that halophilic cellulase has the ability to saccharify lignocelluloses that have been treated with ionic liquids (23). Ionic-liquid pretreated bamboo was effectively saccharified using a coculture of 2 halophilic fungi, *Aspergillus flavus* and *Aspergillus penicillioides*.This co-culture exhibited FPase,CMCase, xylanase, and β-xylosidase activity. These enzymes were polyextremophilic with activity in pH 9.0-10.0, at temperatures 50-60 °C, and in 15–20% NaCl(24). In a recent study, inhibition-tolerant cellulase from *Halomonas elongata* has been reported and potentiated for biofuel production (25). Cellulase from sponge-associated *Marinobacter* sp. MSI032 has been purified and characterized. Thisalkaline-stable cellulase has been potentiated for the conversion of cellulosic waste material into biofuels (26).Polyextremophilic cellulase from the halophilic fungus *Aspergillus flavus*has been purified and characterized. This cellulase was potentiated for bioethanol production and was active at a temperature of 60 ºC, pH of 10, and in the presence of 150 g L^{-1} NaCl(27). In another study, a halo- and thermo-stable cellulase from *Virgibacillussalarius* BM-02 was found to saccharify many untreated agricultural biomasses such as wheat bran, sugarcane bagasse, and corn stover(28). The saccharifying efficiency of agricultural biomass was increased by the use of cellulase from *Hahella* sp. CR1. This cellulase was also halo- and thermo-stable, and activity was enhanced in the presence of divalent metal ions and Tween 40 (29).

Despite these few reports of cellulase from halophiles, its application has not been widely developed for industrial usage. Considering their polyextremophilic nature of stability at high temperature, and in the presence of salt and ionic liquids, they can be developed for the enzymatic saccharification of a wide range of lignocellulosic biomass into biofuels. The applied potential of halophilic cellulases lies in their compatibility with saline biomass processing, such as marine algae and seagrass, facilitating bioethanol production in coastal regions where saline feedstocks predominate.

β-Glucosidase

β-glucosidase is an essential requirement for successful cellulose hydrolysis; however, its industrial utilization is often hampered by salt, temperature, and glucose sensitivity. Mangrove ecosystems with an immense variety of microorganisms, including *Trichoderma harzianum*, serve as a high source of β-glucosidase enzymes with enhanced activity in increased salt concentration, indicating halophilic property(30). Likewise, a β-glucosidase from *Pseudoalteromonas* sp. demonstrated enhanced hydrolytic activity against isoflavonoidsdaidzin and genistein, with NaCl activating up to 6.79-fold. This is among the highest NaCl activations reported for β-glucosidases, highlighting their potential in

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food processing and industrial applications (31). From these observed properties, cellulases and β-glucosidaseproduced from halophiles under high salt concentration are an ideal choice for biomass degradation and biofuel generation, reducing fermentation costs through the utilization of cost-effective substrates.

Xylanases

Lignocellulosic biomass has a high content of xylan, second only to cellulose. Xylanase is thus crucial in the transformation of this biomass into biofuel. Xylanase converts xylan into xylooligosaccharides, xylobiose, and xylose subunits, which can be further fermented into biofuels. Stability of xylanase, particularly thermal and a wide pH range, is a challenge for its effective utilization for biofuel production (32,33). A halophilic xylanase was cloned and expressed from the camel rumen metagenome and had similarity with the glycosyl hydrolase of *Ruminococcusflavefaciens*. The activity of this xylanase was activated in the presence of artificial seawater and a low water activity environment and was potentiated for biofuel production (34). Furthermore, a thermostable and halophilic xylanase from *Thermoanaerobacteriumsaccharolyticum* NTOU1 was cloned and expressed. This was active in the pH range of 5.5–8.0 and showed enhanced activity in the presence of salt (35). Another industrially useful xylanase from *Trichoderma asperellum* ND-1 was cloned and expressed in *Pichia pastoris*. It showed remarkable activity in the presence of 4.28 M NaCl and 10% ethanol. It showed the highest activity towards beechwood xylan and effectively hydrolyzed xylan into xylotriose and xylobiose (36).

Amylases

Another significant commercial enzyme that breaks down α-1, 4-glycosidic bonds in starch and similar polysaccharides is α-amylase.Because it is essential to the metabolism of carbohydrates, it is found in all living organisms, including bacteria, plants, and animals.One of the most popular commercial enzymes, α-amylase, has potential uses in numerous industries, including the hydrolysis of starch, the synthesis of biofuel, and the paper industry.Since halophilic amylases remain active and stable in the face of changing salinity concentrations, they are superior to mesophilic amylases. Though less research has been done on them, halophile amylases have the advantage of being more effective in saline solutions.It's interesting to note that a large number of halophilic amylases have industrially significant polyextremophilic characteristics. This class of amylases is known for their stability under alkaline pH settings, high temperature tolerance, and low water activity (the presence of organic solvents)(5,37,38).

Halophile's α-amylase is utilized in starch hydrolysis and maltooligosaccharide synthesis. Among initial studies, the amylase from *Halomonas meridiana* has been discovered to possess alkaline and salt stability (39). For the hydrolysis of starch, *Nesterenkonia* sp. strain F amylase was employed(40). Similarly, the α-amylase from *Nesterenkonia*sp. strain F, a moderately halophilic bacterium, not only hydrolyzes starch but also directly produces bioethanol and biobutanol under aerobic and anaerobic conditions, offering a unique advantage over traditional fermentation organisms like *Clostridia*. From 50 g/L glucose, it produced 105 mg/L butanol under anaerobic conditions and 66 mg/Lbutanol and291 mg/L ethanol under aerobic conditions (41). *Marinobacter* sp. EMB8 is established to be a good source of salt and solvent-stable α-amylase. The enzyme is characterized in detail, and its structural features are correlated with its novel properties and application in various industries (42).The bioremediation of starch and solvent-containing wastes has been effectively carried out using the polyextremophilic amylase derived from *Aspergillus gracilis* (43). In a recent study, anovel halo-acid-alkali-tolerant and

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surfactant-stableamylase produced from halophile *Bacillus siamensis* F2 was utilized in waste valorization for bioethanol production(44).

The other class of amylase is β-amylase,which is an exo-acting enzyme that releases maltose units by cleavingα-1,4-glycosidic bonds from the non-reducing ends through hydrolysis. It's greatly influenced by factors such as thermostability, salt tolerance, and metal ion sensitivity in industrial applications. Research indicates that halophilic microorganisms like *Halobacillus* species have extensively produced extracellular β-amylase. Studies suggest that *Halobacillus* sp. LY9 β-amylase is stable in high salt concentration, suggesting promising action in industries where harsh conditions pertain(45). A halophilic, thermotolerant, solvent-tolerant glucoamylase from *Halolactibacillus* sp. SK71was used for bioethanol production upon raw starch hydrolysis. In this study, 0.365 g of ethanol/ g of raw corn starch was produced with 71.6% theoretical yield (46).

Halophilic amylase has been studied from diverse microbial resources and has been shown to have significant stability and activity characteristics under high salt, pH, temperature, and organic solvent conditions. Their applications have also been highlighted for different industrial sectors. Despite having a good toolbox of halophilic amylases, it has not been much used for biofuel production. Halophilic α-amylases are significant enzymes representing potential applications in biofuels because of their ability to readily hydrolyze starchy biomass into fermentable sugars at moderate to high salinity. It must be tried particularly for saline starchy biomass or waste for the generation of fermentable sugars, which can then be used for biofuel production.

Laccase

Laccase, an enzyme that is classified as a multicopper oxidase, is highly essential for the breakdown of lignin, which is a key process in the effective utilization of lignocellulosic biomass for the production of biofuels. Halophilic microorganisms, especially haloarchaea like *Haloferaxvolcanii*, have emerged as valuable sources of salt-tolerant laccases that can maintain their stability and activity even in extreme environments that can be characterized by high salinity and temperatures,as well as exposure to organic solvents. For example, the laccase (LccA) derived from *H. volcanii* demonstrated significant activity at salt concentrations that reached up to 1.4 M NaCl and temperatures nearing 45°C, positioning it as an ideal candidate for industrial applications that involve hypersaline waste streams, especially in the pulp and paper sectors. This enzyme's ability to oxidize substrates like syringaldazine and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) without requiring additional cofactors enhances its potential for lignin modification, thereby facilitating the release of fermentable sugars for bioethanol production (19,47). Halophilic laccases have presented us with a sustainable solution to address the challenges posed by lignin, which is a significant obstacle in the efficient production of biofuel. These enzymes can facilitate effective biomass pretreatment in the extreme conditions that are typically found in biorefinery environments. Halophilic laccase from *Aquisalibacillus elongatus*was found to be effective in the bioconversion of lignocellulosic biomass. It was found to be effective in lignin removal from peanut shell waste. In the presence of 50% [Bmim][PF_6], 87% lignin removal was achieved with this enzyme (48). Polyextremotolerant laccase from *Aquisalibacillus elongatus* was found to be stable in the presence of salt, organic solvents, inhibitors, and surfactants. It was effective in the delignification of sugar beet in an ionic liquid (49). In another study, laccase from halophilic bacteria *Chromohalobacter salexigens* was found to be beneficial in the delignification of almond shell waste (50). Laccase of *Pseudoalteromonaspeptidolytica* was also found to be efficient in

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bioconversion of lignocellulosic biomass and yielded 91.9 µg glucose/mg biomass (51).

Lipases

Lipases are essential enzymes for the synthesis of biofuels because they catalyze the conversion of triglycerides into glycerol and biodiesel (fatty acid alkyl esters). Unlike chemical catalysts, which consume a significant amount of energy and generate hazardous byproducts, they can operate in mild conditions and handle a range of feedstocks, including waste oils. Extremophilic lipases, particularly from halophiles,have great potential for biofuel production (13,52).

Microbial lipases, especially those derived from extremophiles like *Haloarculamarismortui*and *Haloarcula* sp. G41 has more industrial applicability since it is stable in extreme conditions, including high salinity and organic solvents(53,54). For instance, even in methanol-rich settings, *Haloarcula* sp. G41 lipase efficiently solves a typical enzyme inhibition issue by converting soybean oil to biodiesel(54). Halophilic, cold-active, and solvent-tolerant lipase from *Bacillus licheniformis* KM12 was competent in biodiesel production. Non-edible Myrtus oil was converted to biodiesel in the presence of methanol with 78% yield (55). Another halophilic and solvent-tolerant lipase from *Idiomarina* sp. W33 has been used for biodiesel production from *Jatropha* oil. With free and immobilized lipase, 84% and 91%,respectively, yields were obtained from *Jatropha* oil (56). Cold-active halophilic lipase of *Halocynthiibacter arcticus* has also been used for biodiesel production (57).

Lipase-mediated biodiesel production offers reduced energy needs, feedstock flexibility, and environmental sustainability. Still, problems like costly enzymes and methanol-induced inactivation remain. Immobilisation methods, which raise lipase stability and reusability, help to solve scalability problems (58).

Future advances in genetic engineering and enzyme optimisation might help to confirm the function of lipases in the manufacture of sustainable biofuel by further reducing costs and boosting efficiency. Studies indicate they might replace chemical processes, which would be consistent with the world's objectives for renewable energy. Figure 1 gives a schematic representation of the use ofhalophilic enzymes in the conversion of biomass to biofuels. Table 1 summarizes the potential use of halophilic enzymes in biofuel production.

Halophilic biomass for biofuel production

Halophilic organisms have been utilized to synthesize an array of biofuels such as bioethanol, biodiesel, biogas, biomethane, biohydrogen, etc. Diverse types of halophilic microbes, algae, seaweed, and halophytes have been involved in the synthesis of all these types of biofuels. Halophilic marine microbes, primarily comprising Archaea,have been used for methane synthesis from brown algae (68). Marine microalgae have been used to produce methane by anaerobic digestion at different salinity levels (69). Biogas has been produced by anaerobic digestion of oil-extracted halophilic algae *Scenedesmus obliquus* (70).*Clostridium bifermentans* 3AT-ma, a halophilic hydrogen-producing bacterium (HHPB), has been utilized for hydrogen production in glucose medium with 2% NaCl. Hydrogen was produced from saline substrate with a yield of 1.1 mol-H_2/mol-glucose (71). Halophile *Halanaerobium saccharolyticum* subspecies saccharolyticum and senegalensis synthesized hydrogen from glycerol. Concomitant to hydrogen production, 1,3-propanediol (1,3-PD), butyrate, and ethanolwere also produced by subspecies saccharolyticum.In this study highest hydrogen production yield of 2.5g/L glycerol was achieved (72).

Seaweed is also an important marine biomass that can be utilized for the synthesis of varied compounds as well as the production of biofuels such as ethanol (73). Conversion of seaweed biomass to simpler sugars is easier than compared of agricultural biomass as it doesn't contain lignin (74). Using halophyte

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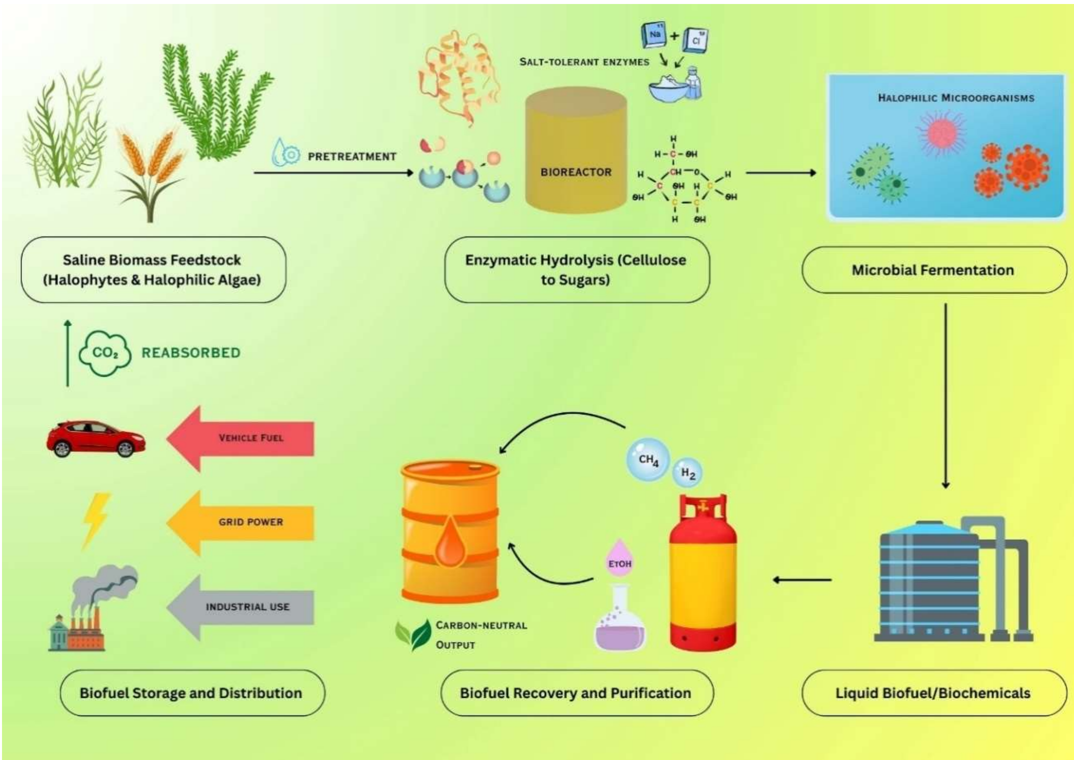

Fig. 1: Halophilic enzymes in biomass to biofuel conversion

Table 1: Halophilic enzymes for biomass degradation and biofuel synthesis

Enzymes	Sources	Uses in biofuel production	References
α-Amylase	*Haloferaxmediterranei* *Haloarcula* sp. *Haloarcula hispanica* *Marinobacter* sp. *Halobacillus* sp.	Starch is hydrolyzed to produce glucose, which could be fermented further to bioethanol	(42,59-62)
β-Amylase	*Halobacillus* sp. *Salimicrobiumhalophilum*	Hydrolyzes starch by removing maltose from the non-reducing end of the starch.	(45,63)
β-Glucosidase	*Pseudoalteromonas* sp. GXQ-1 *Trichoderma harzianum*	Hydrolyzing cellobiose into glucose	(30,31)
Cellulase	*Haloarcula* sp. strain LLSG7 *Halomonas elongata* *Natronobiformacellulositropha*	Enzymatic hydrolysis, fermentation, and pretreatment of cellulose into biofuel	(20,21,25,64)

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Xylanase	*Halorubrumsaccharovorum* *Gracilibacillus*sp.	Corncob and lignocellulosic materials can be used as a substrate for xylooligosaccharides production, thereby offering a highly promising biofuel application	(65,66)
Laccase	*H. volcanii*	Laccase is involved in the breakdown ofpolyphenolic compounds; Laccase is used in biofuel cells for biofuel electrocatalysis	(19,47)
Esterase/ Lipase	*Haloarculamarismortui* *Haloarcula* sp. G41 *Halobacterium salinarum*	Hydrolyze triglycerides into glycerol and fats; transesterification of plant fats for biodiesel production	(19,53,54,58, 67)

Achnatherum splendens L. biochar, bio-oil, and syngas production was achieved by pyrolysis (75). Halophytes and marine algae are good sources for biofuels. While halophytes can be converted to bioethanol, microalgae can be used for biodiesel (76). Halophytes can be sustainably used for biofuel production. Also, in the enzymatic hydrolysis, salt-tolerant enzymes would be useful (77). Halophytes have been reviewed as an energy crop for the production of oil and lignocellulosic biomass that can be converted to bioethanol or other fuels and chemicals after fermentation (78).The varied economic use of salt-tolerant plants has been analyzed as listed in the database eHALOPH, and one of the highlighted uses is in biofuel production. (79). Using seaweed for biofuel production has various benefits. The cellulose content of seaweed can be converted to simpler sugars and utilized for ethanol production. Conversion of seaweed biomass to simpler sugars is easier than compared of agricultural biomass as it doesn't contain lignin (74). Also, cultivation of seaweed doesn't require arable land and captures a great amount of carbon dioxide, thus helping in climate protection. Thus, it helps to develop a biorefinery approach for the production of biofuel and will serve to achieve many sustainable development goals.

Conclusion and future perspectives

Halophiles are an important repository of robust enzymes. The hydrolases sourced from them are not only salt-stable but have also shown polyextremophilic properties, such as stability at high pH, temperature, and activity and stability in the presence of organic solvents. Amidst halophiles, various enzymes such as amylases, laccase, lipase, xylanase, and especially cellulase, have great promise for the generation of biofuel.Besides halophilic enzymes, the highly saline biomass of microorganisms, algae, seaweeds, and halophytes are utilized for biofuels production. The halophilic biomass has been reported to produce bioethanol, biodiesel, biogas, biomethane, and biohydrogen. Further research needs to be carried out to source novelbiofuel-producing enzymecandidates by culture-dependent and independent approaches. The whole genome and metagenome of halophiles need to be explored to find newer and more robust enzymecandidates for biofuel production. Furthermore, by mutagenesis, the features of halophilic enzymes could be imparted to their mesophilic counterpart to increase the application paradigm. As more research is published, the use of halophilic enzymes in biorefinery applications will lead to increased

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sustainable biofuel production and decreased dependence on freshwater and non-renewable resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Ventosa, A., Nieto, J.J. and Oren, A., 1998. Biology of moderately halophilic aerobic bacteria. *Microbiology and molecular biology reviews*, *62*(2), pp.504-544.
2. Oren, A., 2010. Industrial and environmental applications of halophilic microorganisms. *Environmental technology*, *31*(8-9), pp.825-834.
3. Karan, R., Kumar, S., Sinha, R. and Khare, S.K., 2012. Halophilic microorganisms as sources of novel enzymes. In *Microorganisms in sustainable agriculture and biotechnology* (pp.555-579).Springer, Dordrecht.
4. Kumar, S., Karan, R., Kapoor, S., Singh, S.P. and Khare, S.K., 2012. Screening and isolation of halophilic bacteria producing industrially important enzymes. *Brazilian Journal of Microbiology*, *43*, pp.1595-1603.
5. Kumar, S., Das, B.B., Srivastava, N. and Khare, S.K., 2023. Biochemistry and Biomolecules of Halophiles: Recent Trends and Prospects. In *Some Key Topics in Chemistry and Biochemistry for Biotechnologists* (pp. 91-116). CRC Press.
6. Oren, A., 2013. Life at high salt concentrations. In *The prokaryotes*(pp. 421-440). Springer, Berlin, Heidelberg.
7. Siddiqui, K.S. and Thomas, T., 2008. *Protein adaptation in extremophiles*. Nova Publishers.
8. Kumar, S., Khan, R.H. and Khare, S.K., 2016. Structural elucidation and molecular characterization of *Marinobacter* sp. α-amylase. *Preparative Biochemistry and Biotechnology*, *46*(3), pp.238-246.
9. Ventosa, A. and Nieto, J.J., 1995. Biotechnological applications and potentialities of halophilic microorganisms. *World Journal of Microbiology and Biotechnology*, *11*, pp.85-94.
10. Margesin, R. and Schinner, F., 2001. Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles*, *5*(2), pp.73-83.
11. Litchfield, C.D., 2011. Potential for industrial products from the halophilic Archaea. *Journal of Industrial Microbiology and Biotechnology*, *38*(10), p.1635.
12. Karan, R., Capes, M.D., DasSarma, P. and DasSarma, S., 2013. Cloning, overexpression, purification, and characterization of a polyextremophilic β-galactosidase from the Antarctic haloarchaeon *Halorubrumlacusprofundi*. *BMC Biotechnology*, *13*, pp.1-11.
13. Qiu, J., Han, R. and Wang, C., 2021. Microbial halophilic lipases: A review. *Journal of Basic Microbiology*, *61*(7), pp.594-602.
14. Hmad, I.B. and Gargouri, A., 2024. Halophilic filamentous fungi and their enzymes: potential biotechnological applications. *Journal of Biotechnology*, *381*, pp.11-18.
15. Yavari-Bafghi, M. and Amoozegar, M.A., 2025. Pharmaceutical applications of halophilic enzymes. *Heliyon*, *11*(4).
16. Antranikian, G., Vorgias, C.E. and Bertoldo, C., 2005. Extreme environments as a resource for microorganisms and novel biocatalysts. *Marine biotechnology I*, pp.219-262.
17. Tokunaga, H., Arakawa, T. and Tokunaga, M., 2008. Engineering of halophilic enzymes: two acidic amino acid residues at the carboxy-terminal region confer halophilic characteristics to *Halomonas* and *Pseudomonas* nucleoside diphosphate kinases. *Protein Science*, *17*(9), pp.1603-1610.
18. Delgado-García, M., Valdivia-Urdiales, B., Aguilar-González, C.N., Contreras-Esquivel,

Vol. 19 (Supplementary Issue 3A), September 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.3s.11

J.C. and Rodríguez-Herrera, R., 2012. Halophilic hydrolases as a new tool for the biotechnological industries. *Journal of the Science of Food and Agriculture*, *92*(13), pp.2575-2580.

19. Kasirajan, L. and Maupin-Furlow, J.A., 2021. Halophilic archaea and their potential to generate renewable fuels and chemicals. *Biotechnology and Bioengineering*, *118*(3), pp.1066-1090.

20. Amoozegar, M.A., Safarpour, A., Noghabi, K.A., Bakhtiary, T. and Ventosa, A., 2019. Halophiles and their vast potential in biofuel production. *Frontiers in Microbiology 10,* p. 1895.

21. Li, X. and Yu, H.Y., 2013. Halostable cellulase with organic solvent tolerance from *Haloarcula* sp. LLSG7 and its application in bioethanol fermentation using agricultural wastes. *Journal of Industrial Microbiology and Biotechnology*, *40*(12), pp.1357-1365.

22. Li, X., Wang, H.L., Li, T. and Yu, H.Y., 2012. Purification and characterization of an organic solvent-tolerant alkaline cellulase from a halophilic isolate of *Thalassobacillus*. *Biotechnology Letters*, *34*, pp.1531-1536.

23. Gunny, A.A.N., Arbain, D., Gumba, R.E., Jong, B.C. and Jamal, P., 2014. Potential halophilic cellulases for in situ enzymatic saccharification of ionic liquids pretreated lignocelluloses. *Bioresource Technology*, *155*, pp.177-181.

24. Zhao, B., Al Rasheed, H., Ali, I. and Hu, S., 2021. Efficient enzymatic saccharification of alkaline and ionic liquid-pretreated bamboo by highly active extremozymes produced by the co-culture of two halophilic fungi. *Bioresource Technology*, 319, p.124115.

25. Narayanan, A., Jeyaram, K., Prabhu, A.A., Krishnan, S., Kunjiappan, S., Baskaran, N. and Murugan, D., 2025. Cellulase from *Halomonas elongata* for biofuel application: enzymatic characterization and inhibition tolerance investigation. *Preparative Biochemistry & Biotechnology*, pp.1-18.

26. Shanmughapriya, S., Kiran, G.S., Selvin, J., Thomas, T.A. and Rani, C., 2010. Optimization, purification, and characterization of extracellular mesophilic alkaline cellulase from sponge-associated *Marinobacter* sp. MSI032. *Applied Biochemistry and Biotechnology*, *162*, pp.625-640.

27. Bano, A., Chen, X., Prasongsuk, S., Akbar, A., Lotrakul, P., Punnapayak, H., Anwar, M., Sajid, S. and Ali, I., 2019. Purification and characterization of cellulase from obligate halophilic *Aspergillus flavus* (TISTR 3637) and its prospects for bioethanol production. *Applied Biochemistry and Biotechnology*, *189*, pp.1327-1337.

28. Yousef, N.M. and Mawad, A.M., 2023. Characterization of thermo/halo stable cellulase produced from halophilic *Virgibacillussalarius* BM-02 using non-pretreated biomass. *World Journal of Microbiology and Biotechnology*, *39*(1), p.22.

29. Tan, M.C.Y., Zakaria, M.R., Liew, K.J. and Chong, C.S., 2023. Draft genome sequence of *Hahella* sp. CR1 and its ability in producing cellulases for saccharifying agricultural biomass. *Archives of Microbiology*, *205*(8), p.278.

30. Sun, N., Liu, X., Zhang, B., Wang, X., Na, W., Tan, Z., Li, X. and Guan, Q., 2022. Characterization of a novel recombinant halophilic β-glucosidase of *Trichoderma harzianum* derived from Hainan mangrove. *BMC Microbiology*, *22*(1), p.185.

31. Qu, X., Ding, B., Li, J., Liang, M., Du, L., Wei, Y., Huang, R. and Pang, H., 2020. Characterization of a GH3 halophilic β-glucosidase from *Pseudoalteromonas* and its NaCl-induced activity toward isoflavones. *International Journal of Biological Macromolecules*, *164*, pp.1392-1398.

32. Yang, C.C., Lin, Y.J. and Wen, F.S., 2025. Novel xylanase activity and biofuel production in *Lacrimisporaxylanolytica* isolated from termite guts symbionts. *Bioresource Technology*, *429*,p.132489.

33. Chaudhary, R., Kuthiala, T., Singh, G., Rarotra, S., Kaur, A., Arya, S.K. and Kumar, P., 2021. Current status of xylanase for biofuel production: a review on classification and characterization. *Biomass Conversion and Biorefinery*, pp.1-19.

Vol. 19 (Supplementary Issue 3A), September 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.3s.11

34. Ghadikolaei, K.K., Sangachini, E.D., Vahdatirad, V., Noghabi, K.A. and Zahiri, H.S., 2019. An extreme halophilic xylanase from camel rumen metagenome with elevated catalytic activity in high salt concentrations. *AMB Express*, *9*, pp.1-11.

35. Hung, K.S., Liu, S.M., Tzou, W.S., Lin, F.P., Pan, C.L., Fang, T.Y., Sun, K.H. and Tang, S.J., 2011. Characterization of a novel GH10 thermostable, halophilic xylanase from the marine bacterium *Thermoanaerobacterium saccharolyticum* NTOU1. *Process Biochemistry*, *46*(6), pp.1257-1263.

36. Zheng, F., Zhang, H., Wang, J., Chen, J., Zhuang, H. and Basit, A., 2024. Expression and characterization of a novel halophilic GH10 β-1, 4-xylanase from *Trichoderma asperellum* ND-1 and its synergism with a commercial α-L-arabinofuranosidase on arabinoxylan degradation. *International Journal of Biological Macromolecules*, *282*, p.136885.

37. Kumar, S., Grewal, J., Sadaf, A., Hemamalini, R. and Khare, S.K., 2016. Halophiles as a source of polyextremophilic α-amylase for industrial applications. *AIMS Microbiology*, *2*(1), pp.1-26.

38. Kumar, S. and S.K. Khare. 2021. Recovery and purification of industrial enzymes. In Enzymes (pp. 59-75). Academic Press.

39. Coronado, M.J., Vargas, C., Hofemeister, J., Ventosa, A. and Nieto, J.J., 2000. Production and biochemical characterization of an α-amylase from the moderate halophile *Halomonas meridiana*. *FEMS Microbiology Letters*, *183*(1), pp.67-71.

40. Shafiei, M., Ziaee, A.A. and Amoozegar, M.A., 2010. Purification and biochemical characterization of a novel SDS and surfactant stable, raw starch digesting, and halophilic α-amylase from a moderately halophilic bacterium, *Nesterenkonia* sp. strain F. *Process Biochemistry*, *45*(5), pp.694-699.

41. Amiri, H., Azarbaijani, R., Parsa Yeganeh, L., Shahzadeh Fazeli, A., Tabatabaei, M., Hosseini Salekdeh, G. and Karimi, K., 2016. *Nesterenkonia*sp. strain F, a halophilic bacterium producing acetone, butanol, and ethanol under aerobic conditions. *Scientific Reports*, *6(1)*,p. 18408.

42. Kumar, S. and Khare, S.K., 2012. Purification and characterization of maltooligosaccharide-forming α-amylase from moderately halophilic *Marinobacter* sp. EMB8. *Bioresource Technology*, *116*, pp.247-251.

43. Ali, I., Akbar, A., Yanwisetpakdee, B., Prasongsuk, S., Lotrakul, P. and Punnapayak, H., 2014. Purification, characterization, and potential of saline waste water remediation of a polyextremophilic α-amylase from an obligate halophilic *Aspergillus gracilis*. *BioMed Research International*, *2014*(1), p.106937.

44. Rathod, B.G., Pandala, S. and Poosarla, V.G., 2023. A novel halo-acid-alkali-tolerant and surfactant stable amylase secreted from halophile *Bacillus siamensis* F2 and its application in waste valorization by bioethanol production and food industry. *Applied Biochemistry and Biotechnology*, *195*(8), pp.4775-4795.

45. Li, X. and Yu, H.Y., 2011. Extracellular production of beta-amylase by a halophilic isolate, *Halobacillus* sp. LY9. *Journal of Industrial Microbiology and Biotechnology*, *38*(11), pp.1837-1843.

46. Yu, H.Y. and Li, X., 2014. Characterization of an organic solvent-tolerant thermostable glucoamylase from a halophilic isolate, *Halolactibacillus* sp. SK 71 and its application in raw starch hydrolysis for bioethanol production. *Biotechnology Progress*, *30*(6), pp.1262-1268.

47. Uthandi, S., Saad, B., Humbard, M.A. and Maupin-Furlow, J.A., 2010. LccA, an archaeal laccase secreted as a highly stable glycoprotein into the extracellular medium by *Haloferaxvolcanii*. *Applied and Environmental Microbiology*, *76*(3), pp.733-743.

48. Rezaie, R., Rezaei, S., Jafari, N., Forootanfar, H., Khoshayand, M.R. and Faramarzi, M.A., 2017. Delignification and detoxification of peanut shell bio-waste using

Vol. 19 (Supplementary Issue 3A), September 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.3s.11

an extremely halophilic laccase from an *Aquisalibacillus elongatus* isolate. *Extremophiles*, *21*, pp.993-1004.
49. Rezaei, S., Shahverdi, A.R. and Faramarzi, M.A., 2017. Isolation, one-step affinity purification, and characterization of a polyextremotolerant laccase from the halophilic bacterium *Aquisalibacillus elongatus* and its application in the delignification of sugar beet pulp. *Bioresource Technology*, *230*, pp.67-75.
50. Jafari, N., Rezaei, S., Rezaie, R., Dilmaghani, H., Khoshayand, M.R. and Faramarzi, M.A., 2017. Improved production and characterization of a highly stable laccase from the halophilic bacterium *Chromohalobactersalexigens* for the efficient delignification of almond shell bio-waste. *International Journal of Biological Macromolecules*, *105*, pp.489-498.
51. Johnson, J. and Choi, K.Y., 2021. Enzymatic utilization of oil and lignocellulosic biomass using halophilic marine bacteria *Micrococcus luteus* and *Pseudoalteromonaspeptidolytica*. *3 Biotech*, *11*(7), p.360.
52. Vivek, K., Sandhia, G.S. and Subramaniyan, S.J.B.A., 2022. Extremophilic lipases for industrial applications: A general review. *Biotechnology Advances*, *60*, p.108002.
53. Camacho, R.M., Mateos, J.C., González-Reynoso, O., Prado, L.A. and Córdova, J., 2009. Production and characterization of esterase and lipase from *Haloarculamarismortui*. *Journal of Industrial Microbiology and Biotechnology*, *36*(7), pp.901-909.
54. Li, X. and Yu, H.Y., 2014. Characterization of an organic solvent-tolerant lipase from *Haloarcula* sp. G41 and its application for biodiesel production. *Folia Microbiologica*, *59*, pp.455-463.
55. Malekabadi, S., Badoei-Dalfard, A. and Karami, Z., 2018. Biochemical characterization of a novel cold-active, halophilic and organic solvent-tolerant lipase from *B. licheniformis* KM12 with potential application for biodiesel production. *International Journal of Biological Macromolecules*, *109*, pp.389-398.
56. Li, X., Qian, P., Wu, S.G. and Yu, H.Y., 2014. Characterization of an organic solvent-tolerant lipase from *Idiomarina* sp. W33 and its application for biodiesel production using Jatropha oil. *Extremophiles*, *18*, pp.171-178.
57. Le, L.T.H.L., Yoo, W., Jeon, S., Lee, C., Kim, K.K., Lee, J.H. and Kim, T.D., 2020. Biodiesel and flavor compound production using a novel promiscuous cold-adapted SGNH-type lipase (Ha SGNH1) from the psychrophilic bacterium *Halocynthiibacter arcticus*. *Biotechnology for Biofuels*, *13*, pp.1-13.
58. Hama, S., Noda, H. and Kondo, A., 2018. How lipase technology contributes to evolution of biodiesel production using multiple feedstocks. *Current Opinion in Biotechnology*, *50*, pp.57-64.
59. Pérez-Pomares, F., Bautista, V., Ferrer, J., Pire, C., Marhuenda-Egea, F.C. and Bonete, M.J., 2003. α-Amylase activity from the halophilic archaeon *Haloferaxmediterranei*. *Extremophiles*, *7*, pp.299-306.
60. Fukushima, T., Mizuki, T., Echigo, A., Inoue, A. and Usami, R., 2005. Organic solvent tolerance of halophilic α-amylase from a Haloarchaeon, *Haloarcula* sp. strain S-1. *Extremophiles*, *9*, pp.85-89.
61. Hutcheon, G.W., Vasisht, N. and Bolhuis, A., 2005. Characterisation of a highly stable α-amylase from the halophilic archaeon *Haloarcula hispanica*. *Extremophiles*, *9*, pp.487-495.
62. Amoozegar, M.A., Malekzadeh, F. and Malik, K.A., 2003. Production of amylase by newly isolated moderate halophile, *Halobacillus* sp. strain MA-2. *Journal of Microbiological Methods*, *52*(3), pp.353-359.
63. Li, X. and Yu, H.Y., 2012. Purification and characterization of novel organic-solvent-tolerant β-amylase and serine protease from a newly isolated *Salimicrobiumhalophilum* strain LY20. *FEMS Microbiology Letters*, *329*(2), pp.204-211.
64. Sorokin, D.Y., Khijniak, T.V., Kostrikina, N.A., Elcheninov, A.G.,

Vol. 19 (Supplementary Issue 3A), September 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.3s.11

Toshchakov, S.V., Bale, N.J., Damsté, J.S.S. and Kublanov, I.V., 2018. *Natronobiformacellulositropha* gen. nov., sp. nov., a novel haloalkaliphilic member of the family Natrialbaceae (class Halobacteria) from hypersaline alkaline lakes. *Systematic and Applied Microbiology*, *41*(4), pp.355-362.
65. Malik, A.D. and Furtado, I.J., 2019. Cellulase-free xylanase by *Halococcusthailandensis* GUMFAS7 and *Halorubrumsaccharovorum* GUMFAS1—bionts of a sponge Cinachyrella cavernosa. *Microbiology*, *88*, pp.212-219.
66. Giridhar, P.V. and Chandra, T.S., 2021. Xylanase production by halophilic bacterium *Gracilibacillus* sp. TSCPVG under solid state fermentation. *Research Journal of Biotechnology Vol*, *16*, p.7.
67. Fukuda, H., Kondo, A. and Noda, H., 2001. Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bioengineering*, *92*(5), pp.405-416.
68. Miura, T., Kita, A., Okamura, Y., Aki, T., Matsumura, Y., Tajima, T., Kato, J. and Nakashimada, Y., 2016. Semi-continuous methane production from undiluted brown algae using a halophilic marine microbial community. *Bioresource Technology*, *200*, pp.616-623.
69. Mottet, A., Habouzit, F. and Steyer, J.P., 2014. Anaerobic digestion of marine microalgae in different salinity levels. *Bioresource Technology*, *158*, pp.300-306.
70. Adamietz, T., Jurkowski, W., Adolph, J. and Brück, T.B., 2019. Biogas yields and composition from oil-extracted halophilic algae residues in conventional biogas plants operated at high salinities. *Bioprocess and Biosystems Engineering*, *42*, pp.1915-1922.
71. Zhang, S., Lee, Y., Kim, T.H. and Hwang, S.J., 2013. Effects of OLRs and HRTs on hydrogen production from high salinity substrate by halophilic hydrogen-producing bacterium (HHPB). *Bioresource Technology*, *141*, pp.227-232.
72. Kivistö, A., Santala, V. and Karp, M., 2010. Hydrogen production from glycerol using halophilic fermentative bacteria. *Bioresource Technology*, *101*(22), pp.8671-8677.
73. Yagi, H., Fujise, A., Itabashi, N. and Ohshiro, T., 2016. Purification and characterization of a novel alginate lyase from the marine bacterium *Cobetia* sp. NAP1 isolated from brown algae. *Bioscience, Biotechnology, and Biochemistry*, *80*(12), pp.2338-2346.
74. Tan, I.S. and Lee, K.T., 2014. Enzymatic hydrolysis and fermentation of seaweed solid wastes for bioethanol production: An optimization study. *Energy*, *78*, pp.53-62.
75. Irfan, M., Chen, Q., Yue, Y., Pang, R., Lin, Q., Zhao, X. and Chen, H., 2016. Co-production of biochar, bio-oil and syngas from halophyte grass (*Achnatherum splendens* L.) under three different pyrolysis temperatures. *Bioresource Technology*, *211*, pp.457-463.
76. Abideen, Z., Ansari, R., Hasnain, M., Flowers, T.J., Koyro, H.W., El-Keblawy, A., Abouleish, M. and Khan, M.A., 2023. Potential use of saline resources for biofuel production using halophytes and marine algae: Prospects and pitfalls. *Frontiers in Plant Science*, *14*, p.1026063.
77. Debez, A., Belghith, I., Friesen, J., Montzka, C. and Elleuche, S., 2017. Facing the challenge of sustainable bioenergy production: Could halophytes be part of the solution?. *Journal of Biological Engineering*, *11*(1), p.27.
78. Sharma, R., Wungrampha, S., Singh, V., Pareek, A. and Sharma, M.K., 2016. Halophytes as bioenergy crops. *Frontiers in Plant Science*, *7*, p.1372.
79. Garcia-Caparros, P., Al-Azzawi, M.J. and Flowers, T.J., 2023. Economic uses of salt-tolerant plants. *Plants*, *12*(14), p.2669.