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REVIEW

Seawater – a sustainable solution for the freshwater drain in bioethanol industries

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ABSTRACT

Consuming fresh water for the production of bioethanol cannot be considered as a sustainable approach due to the worldwide threats on the availability of fresh water in the future. Seawater has been studied as a reaction medium by several research groups in fermentative, enzymatic and chemo-catalytic applications. Several reports concluded that the utilization of seawater would be highly promising and hence the prospects at the interface between biology and chemistry are predictable for holistic innovations and further research. The policies made by several countries have also recommended the implementation of alternative technologies to reduce the consumption of fresh water in industries. This serious situation warrants the development of efficient, ecofriendly, sustainable practices for achieving the sustainability in the global fine and specialty chemicals' industry.

1. Introduction

Lignocellulosic biorefinery approaches are expected to reduce the problems related to depletion of fossil fuel resources occurring due to large-scale production of chemicals and biofuels in the future. Systems with improved ecological footprints are gaining attention in the production of value-added chemicals including biofuels from biomass (Indira et al., 2016). It is estimated that 1.9 to 5.9 m3 fresh water is utilized for the production of one m^3 of bioethanol during the large-scale operation. Most of the water on the planet, you will know, is saltwater 2/3 of our planet is covered by ocean. Distrubution of earth's water presented in Figure 1, and facts about water energy nexus shown in fiure 2. A very small percent of that, is actually fresh water (Indira et al., 2018). By 2030 about 8 percent of U.S. freshwater might go toward biofuels. The Water crisis is the most pervasive, most severe, and most invisible dimension of the ecological devastation of the earth. Runoff from lawns, roads and farms is the primary source of ocean pollution.

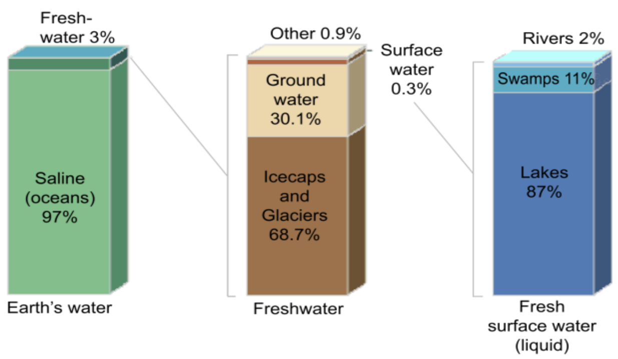

Figure 1. Distrubution of earth's water

The pollution that reaches the ocean creates a "dead zone" in the Gulf of Mexico, where farm fertilizer and industrial runoff deposited by the Mississippi River depletes the ocean of oxygen needed for marine life. One of the major findings of the Pew Oceans Report is that oceans don't start at the coastline. "There are 41 states and two Canadian provinces that cause the dead zone in the gulf, so everyone's in the ocean business." There are now some 50 'dead zones' in the world's coastal areas. The largest in the Western Hemisphere is the one in the Gulf of Mexico, caused by excess nitrogen and phosphorus flowing down the Mississippi River (WWAP, 2014). The Pew Oceans Commission-with a nonpartisan membership including fishermen, scientists and elected officials-recommended "a serious rethinking of ocean law, informed by a new ocean ethic." The report concluded that having "focused on oceans as a frontier with vast resources … we have failed to conceive of the oceans as our largest public domain, to be managed holistically for the greater public good." " What we once considered inexhaustible and resilient is, in fact, finite and fragile."

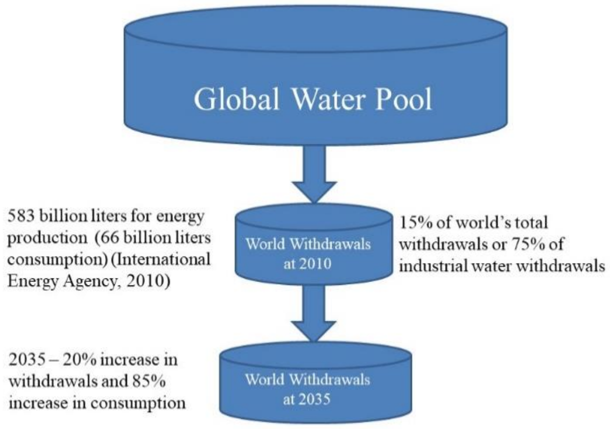

Figure 2. Facts about water energy nexus

There are solutions: better public and community control of water utilities; repairing old water systems, using less water for agriculture by using drip irrigation, stopping polluting the water we do have, increasing water conservation and focusing resources on watershed management. Lester Brown says to create the needed changes, we must eliminate subsidies that create artificially low prices of water, and raise water prices to the point where they will reduce pumping to a sustainable level. Low-income urban consumers can be protected with "lifeline rates" that provide for basic needs at an affordable price (Yang et al., 2013).

The second required change, Brown says, is to stabilize population, especially in water-short countries. Most of the (at least) 3 billion people to be added worldwide by mid-century, will be born in countries already experiencing water shortages. Without these changes, he says, "there may not be a humane solution to the emerging world water shortage." Some suggest that we use desalination, but in their article "Who Owns Water," Barlow and Clark say that desalination "is prohibitively expensive, highly energy intensive using the very fossil fuels that are contributing to global warming and produces a lethal byproduct of saline brine that is a major cause of marine pollution when dumped back into the oceans at high temperatures." Water and energy challenges shown in Figure 3, and alternatives source of water Figure 4.

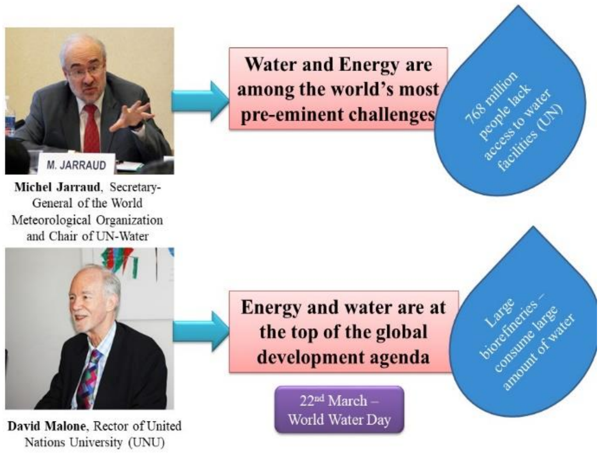

Figure 3. Water and energy challenges

2. Bioethanol's thirst for water

One of the major concerns in ethanol plants is the amount of water consumed in the process of ethanol production. Use of fresh water in ethanol plants results in depletion of a precious natural resource, which cannot be sustained in the long term. Thus, water management technologies are critical for the successful operation of an ethanol plant. One third of the water coming in the plant is used in ethanol production process and two third[s] is used in utility systems. In addition to the quantity of water used in the plants, another major concern is the quality of discharge of wastewater streams from these ethanol plants and its impact on the environment (Lingaraju et al., 2013).

- Irrigation - Growing biomass (corn, sugarcane, and other plants). Switch grass – drought tolerant – but may require water to increase yield and require water for processing
- Corn ethanol consumes 85 liters to 330 liters of water per 1 liter of ethanol (the range is due to different irrigation requirements),
- Gasoline consumes 14 to 27 liters, and switch grass consumes 8 to 34 liters (the range is due to different production technologies).
- Production cost of bioethanol will be reduced from ₹45 per liters (2012) to ₹29 / liter (2020) – Production will get increased.

Water is consumed in biofuel production mainly for growing the energy crops and processing of biofuel. Water for growing the plants yielding fermentable sugar is supplied either by precipitation or irrigation (Keeney and Muller, 2006). Replacing the sugar-rich crops with lignocellulosic biomass has reduced the water consumption in biofuel industries. Reports from U.S. Department of Energy (DOE) and U.S. Department of Agriculture (USDA) states that there is an availability of more than a billion tonnes of biomass for fuel production. Seawater-based biorefinery strategy is seen as an alternative to the freshwater-based biorefinery and is expected to create a considerable impact in these areas aiming at small carbon footprint, more efficient and cost-effective processes.

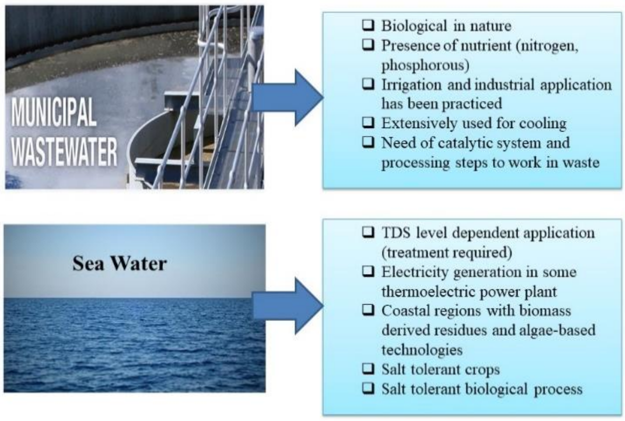

Figure 3. Alternatives source of water

3. Sea water based approach

Utilization of seawater for biofuel production reduces stress on freshwater resources while enabling the cultivation of biomass, saccharification, and processing of biofuel over a common platform (Dev et al., 2019). The current issue warrants designing of sustainable and efficient practices to curb freshwater usage for industrial purposes. Utilization of seawater for biomass-based industries like the biofuel industry can reduce the dependence on freshwater up to a larger extent, and halotolerant enzymes can play a major tool for the same. However, the employment of marine biomass and saline system for saccharification and fermentation insists on halotolerant enzymes. In contrast to normal cellulases, the halotolerant variants are tolerant to high salt concentrations and ionic solvents and utilization of salt tolerant cellulases in the pretreatment of biomass in biofuel industry shall lead to the promotion of utilization of seawater/brackish water (Lima and Porto., 2016) Seawater does not have any substantial negative effect on ionic liquid pretreated biomass or on enzymatic hydrolysis. Sea water based approach Figure 4.

Development of a seawater-based biorefinery strategy could make a strong impact in these areas with a holistic utilization of seawater, aiming at more efficient, low-cost, and small water footprint processes. The concept of water footprint emerged in the early 2000s to describe the volume of water used in the entire production process and the overall supply chain (Oren, 2008). The water footprint of bioethanol production ranges varies between 1300 and 9812 l of water per liter of ethanol, where the major portion of supply is used for cultivation of biomass (Oren, 2010). The global freshwater resources being limited may trigger a debate on food and land usage with an allocation of such large-scale supply of freshwater for the bioethanol industry (Dev et al., 2019). The use of marine biomass and replacement of freshwater with seawater are few approaches to reduce the water footprint of bioethanol production (Zaky, 2016).

There are reports for the use of seawater in enzymatic hydrolysis of lignocellulosic biomass (Nobre et al. 1999; Ren et al., 2016; Dev et al., 2019), fermentation process using halotolerant yeasts (Nobre et al. 1999) also, few marine yeasts were isolated and tested for their fermentation capacity in seawater (Gerbens-Leenes and Hoekstra, 2012). Utilization of seawater for biofuel production reduces stress on freshwater resources while enabling the cultivation of biomass, saccharification, and processing of biofuel over a common platform. Fermentation of ethanol in seawater using *S. cerevisiae* has reported the production of 0.5 g ethanol per gram of glucose Indira et al. (2016;2018).

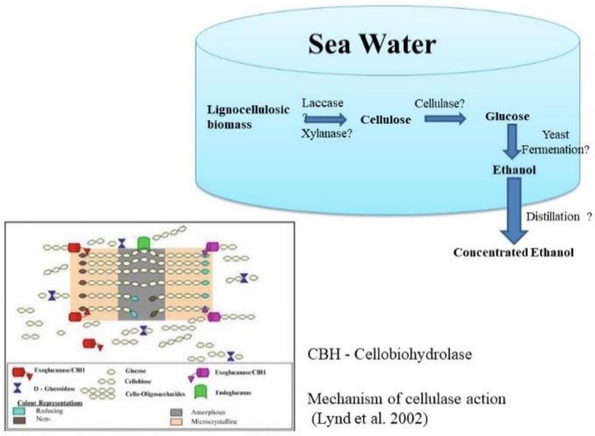

Figure 4. Sea water based approach

Composition varies based on geographical areas. Some other components (e.g., strontium) have also been reported. The pH of seawater ranges from 7.6 to 8.15 sage in saccharification of acid and alkali treated lignocellulosic Usage in saccharification using organic solvent which increases the solubility of non-polar substrates. Reduce the need for high temperature and pH neutralization for pretreated biomass before fermentation (Kucharska et al. 2018). There is expanding knowledge on halophilic enzymes and organisms capable of effectively treating marine biomass. A recent review on industrial and environmental applications of halophilic microorganism's states that demand of salt tolerant enzymes and microorganisms is still limited (Oren, 2010), nevertheless, continuous efforts in this field and favorable results may change the scenario. Estimated average composition of sea water Table 1.

Halotolerant enzymes are one of the promising candidates to be used in seawater based systems. Halotolerant enzymes can also be used for saccharifying the biomass, which are pre-treated with ionic liquids. Odisha being a coastal state with a coastline of 450 Km and with a mangrove ecosystem has potential to evidence halotolerant microorganisms, which can produce salt tolerant enzymes.

Table 1. Estimated average composition of sea water

Component	Composition in sea water (g/L)
NaCl	27.133
$MgCl_2$	2.504
$MgSO_4$	3.382
$CaCl_2$	1.167
KCl	0.742
$NaHCO_3$	0.207
NaBr	0.085
Total salts	35.220
Remnant water	964.780

4. Salt tolerant microogranisms and enzymes

Marine esterases play an important role in marine organic carbon degradation and cycling. Halotolerant esterases from the sea may have good potentials in industrial processes requiring high salts.

Table 2. Isolation and screening of halotolerant bacterial cellulase from Gopalpur, Odisha

S. No.	Samples	Isolated colonies on CMC agar plates	Negative for cellulase activity
1	Wood pieces from boats on the shore	1.1, 1.2, 1.3, 1.4, 1.5	1.1, 1.2, 1.4, 1.5
2	Seawater	2.1, 2.2, 2.3, 2.4, 2.5	2.2, 2.3, 2.4
3	Algal mass growing from boat base	3.1, 3.2, 3.3, 3.4, 3.5, 3.6	3.1, 3.2
4	Sediment from beach	4.1	4.1
5	Sediment above the beach area	5.1, 5.2, 5.3, 5.4, 5.5, 5.6	5.2

Although a large number of marine esterases have been characterized, reports on halotolerant esterases are only a few. Research reports available for marine microbial enzymes, growing microorganisms in sea (Hutcheon et al., 2005). Marine environments usually contain ~3.5% (w/v) NaCl, and in some salterns, the salinity can even reach as high as 37% (w/v). Many microbial enzymes of marine origin have evolved to be halotolerant or halophilic. Several halotolerant or halophilic lipolytic enzymes have been discovered from marine environments, including a halotolerant esterase (Est10) from *Psychrobacter pacificensis*, a halophilic esterase (LipC) from *Haloarcula marismortui*, a halotolerant esterase (ThaEst2349) from *Thalassospira* sp., and a halophilic lipase (LipBL) from *Marinobacter lipolyticus*. Isolation and screening of halotolerant bacterial cellulase from Gopalpur, Odisha shown in Table 2; sampling location indicated in Figure 5.

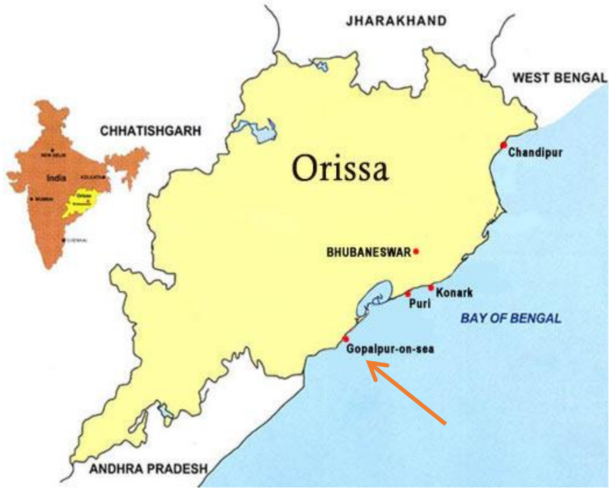

Figure 5. Sampling zone Gopalpur, Odisha (19.27°N 84.92°E)

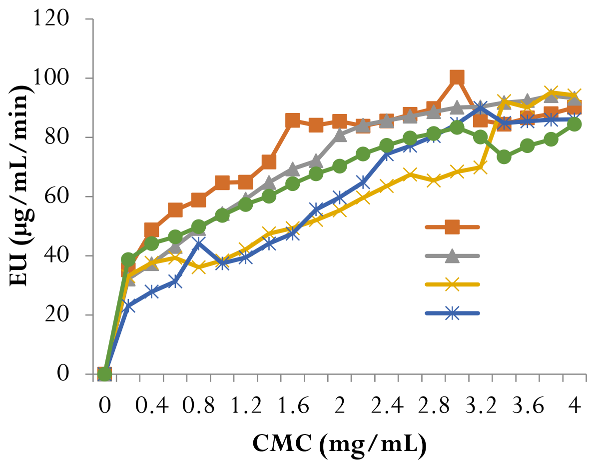

Figure 6. Effect of substrate concentration on cellulase activity

Selected cellulose digesting bacteria (106 cells inoculated in 100 mL media) were cultured on enzyme production media at 37°C, 150 rpm at pH 7.4-7.8 for 5 days. Cell free supernatant obtained after centrifugation at 5000 g for 15 min at 4°C was stored as crude enzyme extract at 4°C (Moumita et al., 2018). Determination of enzyme activity is measured using methods suggested by International Union of Pure and Applied Chemistry (IUPAC) (Ghose and Bisaria, 1987). Crude cellulase was characterized for determination of its optimal pH and temperature with CMC as substrate for hydrolysis. An enzyme with a low Km is easily saturated with substrate, and will act at a constant rate, regardless of variations in the concentration of substrate (Dev et al., 2019). Enzyme with a high Km has a low affinity for its substrate, and requires a greater concentration of substrate to achieve Vmax. Optimization of substrate concentration results in augmentation of proper enzyme activity for maximum conversion. Effect of substrate concentration on cellulase activity shown in Figure 6.

- For GS1 (*B. oceanisediminis)* the value of Vmax was recorded as 100.3 EU and Km value of 0.5 mg/mL.
- For GS2 (*B. halotolerans)* the value of Vmax was recorded as 94.114 EU, Km value of 0.7 mg/mL.
- For GS3 *(P. celer)* value Vmax of 95.165 EU and Km value of 1.4 mg/mL.
- For GS4 *(P. aeruginosa*) cellulase was recorded as 86.119 EU and Km value of 1.3 mg/mL.
- For GS5 *(B. subtilis*) value of Vmax 83.319 EU and Km value of 0.3 mg/mL.

5. Scenario of Asia and The Pacific

European Union - replacing 10% of transportation fuel with renewable energy in 2020. United States EISA (Energy Independence and Security Act) 2007 - increase the volume of biofuel alcohol use by more than 6 times, reaching an annual use volume of 36 billion gallons by 2022. International Energy Agency (IEA) – 2030 - bioethanol and biodiesel will contribute 7% and 3% to the total bioenergy demand, respectively. The IEA predicted that in 2030, the global annual WF of biofuels will be 10 times that in 2005.

- 61% of world's population
- Expected to reach 5 billion by 2050
- 380 million people do not have access to safe drinking water
- In the year 2035, the energy consumption in the Asia-Pacific region will increase from 1/3rd of global consumption to 51-56%
- Coal - most prevalent energy product
- Very good growing market for biofuels
- Indonesia and Malaysia are the two largest producers of palm oil
- China - third largest producer of biofuels
- Threatening issue – Large water consumption

6. Conclusion

All the five strains i.e. *B. oceanisediminis*, *B. halotolerans*, *P. celer*, *P. aeruginosa* and *B. subtilis* are potential cellulase producers. The optimal pH and temperature are suitable enough to be met on industrial scales without much expense of energy (Grande et al., 2012). Seawater can be used as a supplement of freshwater both in saccharification and fermentation process. Performance of *B. halotolerans* is best among the three isolates chosen for studies. Performance of *B. oceanisediminis* can be further improved by optimization of conditions for saccharification. *P. celer* is studied for the first time for cellulase activity and further optimization of process can lead to better candidate for industrial scale. Utilization of yeast cells in seawater for fermentation is possible and the ethanol production percentage is also not much affected. Immobilization of yeast cells in beads provides screening from osmotic lysis in seawater and increases the reusability. Novel enzymes of halophilic origin will reduce the dependence on fresh water for biofuels production. Halotolerant enzymes will reduce the cost of neutralization for pretreated biomass before fermentation. Optimization of fermentation conditions in sea water will be a revolutionary step in the field of fermentation technology.

Nomenclature and Abbreviation

EU	European Union
IEA	International Energy Agency
IUPAC	International Union of Pure and Applied Chemistry
USDA	U.S. Department of Agriculture
CMC	Carboxymethylcellulose
EISA	Energy Independence and Security Act

References

Dev B., Zaky A.S., Jayabalan, R., 2019. Bioethanol Fermentation: The Path Forward for Eco-Friendly and Sustainable Development. In Technologies for Value Addition in Food Products and Processes (pp. 233-270). Apple Academic Press.

Gerbens-Leenes W., Hoekstra A.Y., 2012. The water footprint of sweeteners and bio-ethanol. Environment International 40: 202-211.

Ghose T.K., Bisaria V.S., 1987. Measurement of hemicellulase activities: Part I Xylanases. Pure and Applied Chemistry 59: 1739-1751.

Grande P.M., De Maria P.D., 2012. Enzymatic hydrolysis of microcrystalline cellulose in concentrated seawater. Bioresource Technology 104: 799-802.

Hutcheon G.W., Vasisht N., Bolhuis A., 2005. Characterisation of a highly stable a-amylase from the halophilic archaeon Haloarcula hispanica. Extremophiles 9:487-495.

Indira D., Jijnasa B., Arati N., Moumita S., Ajay D., Eldin J., Jayabalan R., 2015. Comparative studies of ethanol production and cell viability: free cells versus immobilized cells. Research Journal of Pharmaceutical. Biological and Chemical Sciences 6(2): 1708-1714.

Indira D., Sharmila D., Balasubramanian P., Thirugnanam A., Jayabalan R., 2016. Utilization of sea water based media for the production and characterization of cellulase by *Fusarium subglutinans* MTCC 11891. Biocatalysis and Agricultural Biotechnology 7: 187-192.

Indira D., Das B., Balasubramanian P., Jayabalan R., 2018. Sea water as a reaction medium for bioethanol production. In Patra J.K., Das G., Shin H.S., (Eds.), Microbial Biotechnology: Application in Food and Pharmacology. (pp. 171-192). Singapore: Springer

Keeney D., Muller M., 2006. Water use by ethanol plants: potential challenges. Institute for Agriculture and Trade Policy, Minneapolis, MN.

Kucharska K, Rybarczyk P, Hołowacz I, Łukajtis R, Glinka M, Kamiński M. 2018. Pretreatment of lignocellulosic materials as substrates for fermentation processes. Molecules 23(11): 2937.

Lima R.N., Porto A.L.M., 2016. Recent advances in marine enzymes for biotechnological processes. In Advances in food and nutrition research (Vol. 78, pp. 153-192). Academic Press.

Lingaraju B.P., Lee J.Y., Yang Y.J., 2013. Process and utility water requirements for cellulosic ethanol production processes via fermentation pathway. Environmental progress and sustainable energy, 32(2): 396-405.

Lynd L.R., Weimer P.J., Van Zyl W.H., Pretorius I.S., 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiology and Molecular Biology Reviews 66(3): 506-57.

Moumita S., Das B., Sundaray A., Satpathi S., Thangaraj P., Marimuthu S., Jayabalan, R., 2018. Study of soy-fortified green tea curd formulated using potential hypocholesterolemic and hypotensive probiotics isolated from locally made curd. Food chemistry 268: 558-566.

Nobre A., Lucas C., Leão C., 1999. Transport and utilization of hexoses and pentoses in the halotolerant yeast *Debaryomyces hansenii*. Applied and Environmental Microbiology 65(8): 3594-3598.

Oren A., 2008. Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Systems 4(1):2.

Oren A., 2010. Industrial and environmental applications of halophilic microorganisms. Environmental technology 31(8-9): 825-834.

Ren H., Zong M.H., Wu H., Li, N., 2016. Utilization of seawater for the biorefinery of lignocellulosic biomass: ionic liquid pretreatment, enzymatic hydrolysis, and microbial lipid production. ACS Sustainable Chemistry & Engineering 4(10): 5659-5666.

WWAP (United Nations World Water Assessment Programme) (2014) The United Nations world water development report 2014: water and energy. UNESCO, Paris.

Yang H., Pfister S., Bhaduri A., 2013. Accounting for a scarce resource: virtual water and water footprint in the global water system. Current Opinion in Environmental Sustainability 5(6):599-606.

Zaky A.S., Greetham D., Louis E.J., Tucker G., Du C., 2016. A new isolation and evaluation method for marine-derived yeast spp. with potential applications in industrial biotechnology. Journal of Microbiology and Biotechnology 26(11):1891-1907.