Enzyme Immobilization Technology in Biofuel Production: A Review

M I Shamsudin, L S Tan*, T Tsuji

SHIZEN Conversion & Separation Technology iKohza, Department of Chemical Process Engineering, Malaysia-Japan International Institute of Technology (MJIT), Universiti Teknologi Malaysia, Kuala Lumpur, Malaysia

*Corresponding author: tan.liansee@utm.my

Abstract. The application of enzymes as biocatalyst is well recognized in the field of green engineering. Due to their outstanding properties such as inconsumable in chemical reaction, highly specific in action, and speeding up the reaction rate, they are widely used in the conversion of various renewable sources into biofuel. Biofuel, in recent years, had shown such great potential in becoming the alternative for the petrol-derived fuel since it is generated from biomass origins. Researchers currently had introduced few enzyme modifications such as gene editing and supercritical fluid extraction techniques in order to maximize their catalytic performances. However, all of those methods are more tedious and still lack in maintaining the regenerative ability of the enzymes as well as their stabilities. Hence, the technique of enzyme immobilization is currently applied in the biofuel production in improving the enzymes’ performances by providing extra physical support known as backbone to the enzyme to speed up the reaction. Thus, this review aims to provide better insight on the current immobilization technology as well as the enzyme immobilization itself in biofuel generation. Different types of biofuel produced in the industry is also reviewed. The working principles, mechanisms, characteristics, and advantages of the enzyme immobilization technique is also conferred. Besides, comparisons between mobilized, extracellular immobilized and intracellular immobilization will also be reviewed along with the types of matrices used in the adsorption immobilization methods. Lastly, some issues regarding this technique are also highlighted in improving the enzyme performance itself.

1. Introduction

The era of petroleum-based fuel nowadays is getting ferociously critical with the dwindling number of their sources periodically. This fuel was proven by [1] that is predominantly consumed in the field of transportation, accounting for 57.7% of total fossil fuel consumption [3] to cause major worldwide air pollution. Classified as non-renewable energy, it would keep diminishing to meet the demand of earth’s population especially in urban areas. According to the report from [2], approximately 80% of fossil fuel share still monopolized in the energy market. However, this number should show a drastic decrease in less than a century later [3]. Thus, the introduction of renewable energy alternatives is required for man in fulfilling the populations’ criteria as well as providing a more sustainable environment for a better ecosystem. For instance, the application of photovoltaic energy in the Los Angeles port was directed in engendering 20 years of continuous electricity supply [4]. The production of syngas from biomass in the gasification process [5] also yield cleaner gas and greater efficiencies of electricity.

Besides, rendering to study in [3], the most significant prospective of energy generation originated
essentially from renewable categories in the form of wastes, contributing around 10.1% compared to other forms of energies. Wastes currently are also being developed by researchers to be converted into biofuels.

Some technologies, including the enzyme immobilization, had also been introduced in biofuel synthesis to augment as well as improve the economic viability in the process itself. Thus, dependency on fossil fuel would be decreased with this great potential of biofuel in the global energy market demand.

2. Biofuels

Biofuels are defined as the combustible substances in the state of solid, liquid, or gas made up of biologically derived resources in nature to be converted into a sufficient amount of energy [8]. The sustainability value of biomass thus enables them to transpire into treasured provenance in the field of green engineering in the envisioned future.

In first generation of biofuels, they are synthesized from edible sources including crops and oil from plants, as well as fats from animals [7]. Bioethanol, as the most renowned fuel in this category, is mainly made up of corn maize, sugar beet, and sugarcane from the fermentation process with starch as the substrate. As for the plant oils and animal fats, they would become the feedstock for biodiesel production through a transesterification reaction. Studies from [9, 10] also indicated some of those materials ranging from Yellow horn seed oil to chicken fats in recent years. In this fuel category, their resources should not become a problem due to their abundance in nature apart from human agricultural activity. However, one such common limitation is that the existence of competition between food and fuel production. The utilization of land for crop purpose also bring about water contamination and soil erosion matters.

The generation of second biofuel generation is predominantly initiated by the food vs fuel factor in the previous generation. As it principally exploits the non-edible materials as substrates, those drawbacks could be minimized. The discarded materials from crops, including corn stover, wheat straw, and sorghum stalk [11] are converted into biobutanol or bioethanol through enzymatic hydrolysis and chemical techniques. Chemical techniques especially are essential in breaking down the lignin wall surrounding the cellulose component in the plant biomass [11] to extract the valuable starch fuels in it. The production of syngas as depicted by [12] is also classified in this generation with the synthesis of biomethane for an instance. Enzymatic hydrolysis, on the other hand, is a much greener option due to its ability in reducing the intake of energy and less risk of corrosion exposure [14] but with slower reaction and higher cost. Besides, the application of this type of biofuel could also lead to greater production cost, more advanced production equipment in a chemical plant, and as a result, big-scale plant amenities would be built [7]. Microbial species nowadays had been recognized by researchers as the third generation of biofuel. They comprise of algae, fungi, bacteria, archaea, protozoa, and viruses. A study in [15] had enlisted algae as the most sustainable sources as they are easily grown on used land and pretreated lignocellulosic biomass. Besides, microalgae are known to possess a high amount of lipids [16] proteins, and carbohydrates [7] with high biofuel production apart from a faster growth rate. As reported in [17], Scenedesmus obliquus was able to remove around 45% of ofloxacin in wastewater in BG11 medium after increasing the lipid content by 50%. Under salinity stress also [18], Chlorella Vulgaris YH703 [19] was proven to escalate the biofuel generation by 2.5 times after being treated with NaCl in the cultivation medium. Miscellaneous techniques could even be applied including pyrolysis, fermentation, gasification, liquefaction, extraction, transesterification, and anaerobic digestion [7] to transform microalgae into biofuels. They are also able to alleviate CO2 gas in a higher amount to reduce greenhouse gases [20] emission. Nevertheless, the investment for this fuel type is still high compared to other types of fuel. Lipid accumulation could also lead to disruption in cell activities which limits the productivity of biomass apart from the impotence of many microbial species to be applied in industrial cultures [21].

3. Enzymes as Immobilized Biocatalysts in Biofuel Production

In industry, enzymes are undeniably renowned for their propensities in speeding chemical reactions with specified circumstances. These molecules are naturally fabricated from microbial organisms, plants, and
tissues [24]. Acting as natural biocatalysts, these protein structures are inclined to perform optimally under mild conditions in high selectivity to lessen the number of undesired reactions and products [22]. Thus, energy usage and production costs will be lower and more feasible. In terms of biofuel production, its generation can be classified into two; chemical and biochemical techniques. Chemical techniques usually require hazardous chemicals and tedious equipment handling while the application of biocatalysts is greener and less complicated in handling. But, they are not yet fully utilized in the industry in an account of their instability in an organic solvent and greater cost. The presence of enzymes in the reaction medium as a colloidal solution also makes it hard for them to be separated from the mixture [27]. Interestingly, these limitations could be minimized through the method of immobilization that can also enhance other characteristics of the enzymes [23]. Enzyme immobilization can be defined as the technology in which the enzymes are encapsulated in specified regions of supporting materials to maintain their stabilities as well as regenerative abilities so they can be used repeatedly [23,24]. Besides, this technology also provides a thorough separation of enzymes from the reaction mixture [25] and aiding in downstream processing [26].

In enzyme immobilizations, their main mechanisms are determined by the support (matrix) materials, attachment techniques, and the enzyme itself [25]. For instance, an excellent matrix should provide a large surface area, sufficient porosity, hydrophilic tendency, defiance towards microbial attack, and indolence towards enzymes [28]. The ideal attachment method is also essential in enabling the optimal enzyme activity to occur whether it is done through physical or chemical techniques depending on the nature of enzymes and matrix themselves. Different types of enzymes from different species would also yield different performances. In case when lipase B extracted from Candida antarctica is consumed in a transesterification reaction, a high yield of biodiesel of greater than 90% was obtained [29] whereas when the enzyme is extricated from Pseudomonas capacia [30], only 78% of biodiesel yield was produced [30]. As stated in [25, 30], enzymes should be in the immobilized from in purpose of avoiding them from being too sensitive towards pH and temperature and disturbance towards the substrates’ purities.

Biodiesel is listed by researchers as the most produced biofuel types in the industry. Biodiesel has little toxicity in nature, greater flashpoint, prone to biodegradability, less carbon emission, sufficient lubricity, and insignificant content of sulfur compared to other types of biofuel [31, 32]. Nevertheless, other types of biofuel are also being synthesized through enzyme immobilization technology. Table 1 thus summarizes each distinct type of immobilized enzymes with their reaction conditions respectively in current years for each distinct biofuel generation.
| Types of enzymes | Attachment mode | Matrices' Types | Reaction Conditions (Temperature, Time) | Performance | Types of biofuels | Reference |
|------------------|-----------------|-----------------|----------------------------------------|-------------|-----------------|-----------|
| Lipase from *Candida rugosa* and *Rhizopus oryzae* | Cross-linking | Functionalized Activated Carbon | 60 °C, 4 h | Yield = 99% | Biodiesel | [31] |
| Lipase from *Pseudomonas cepacia* | Covalent bond | Epoxy-acrylic resin support | 37 °C, 3 h | Conversion = 47% | Biodiesel | [33] |
| Lipase from *Thermomyces lanuginosus* and *Rhizomucor miehei* | Cross-linking | Divinylbenzene | 35 °C, 8 h | Yield = 75% | Biodiesel | [34] |
| Protease from bacterial strain | Cross-linking | Sodium alginate and sodium chloride solution | 35 °C, More than 24 h | Methane yield = 0.24 g COD/COD | Biomethane | [35] |
| Accelleraseβ-glucosidase (BG) | Adsorption and covalent bond | Polyacrylic-resin and glyoxyl agarose | 50 °C, 96 h | Conversion = 40% | Bioethanol | [36] |
| De-oiled jatropha waste enzyme hydrolysate | Entrapment | Hybrid bacterial cells | 37 °C, 12 h | Gas Yield = 1.58 kJ ± 0.13 VS | Biohydrogen | [37] |
| Lipozyme RM IM | Cross-linking | Anionic resin | 40 °C, 24 h | Yield = 95% | Biolubricant | [51] |
| Lipozyme RM IM | Cross-linking | Sodium alginate | 30 °C, 48 h | Yield = 89% | Bioethanol | [38] |
| Lipase from *Pseudomonas capacia* | Covalent bond | Hybrid-bio support catalyst | 50 °C, 24 h | Yield = 75% | Biodiesel | [29] |
| Monooxygenase | Whole-cell immobilization | Modified chitosan structure | 30 °C, 24 h | Methanol yield = 6.92 m.mol L⁻¹ | Biomethanol | [46] |
| Lipase from *Pseudomonas sp.* | Covalent bond | Activated biochar | 30°C, 3 h | FAME yield = 97.41% | Biodiesel | [49] |
| Lipase from *Candida rugosa* | Ionic affinity | Magnesium modified Fe₂O₃ nanoparticles | 45 °C, 48 h | Yield = 98% | Biodiesel | [13] |
| *Porcine pancreas lipase* | Cross-linking | p-nitrobenzyl cellulose xanthate | 45°C, More than 24 h | Conversion higher than 96.5% | Biodiesel | [6] |
| *Candida antarctica lipase B* | Cross-linking | Modified magnetic cellulose beads | 50°C, 8 h | Yield = 92.3% | Biodiesel | [9] |
3.1. Mode of attachment in enzyme immobilization technique

Several techniques and mechanisms had been proposed by researchers in the immobilization technology of enzymes. Nevertheless, most of them mainly focused on the extracellular immobilized enzymes which are also defined as the type of enzymes that resided inside the cell body itself. They thus will be immobilized after being extracted with intricate procedures in specified conditions. Enzymes in their mobilized forms are usually not preferred because they lack in physical stability, more costly, less thermal stability, limited range of pH, the loss in regenerative ability as well as a strenuous separation between the enzymes and the final products [41]. Whole-cell immobilization is even not favored since it can cause the biomass substrate to insufficiently carrying out the reaction, slow reaction rate, higher cost, lower product purity, limited diffusion barrier, overgrowth of support by other microbes in the cells, and loss of stability [48]. Biofuel yield (e.g., biodiesel) also would become low if this method is applied due to its lower coherence with oleaginous substrates [45]. This section will then review some of the common immobilization techniques applied in biofuel production.

The first method, or known as adsorption is classified as the physical technique since it just involves the reversible attachment of the enzymes onto solid support known as the matrix. The mechanism is initiated through the action of hydrogen bonds, ionic salt linkages, dipole-dipole interaction, and weak van der Waals forces between the enzymes and the matrix that will lead to more ordered and active enzymes’ structures [25, 41]. The adsorption process is commenced as soon as the matrix is soaked in the enzymes solution in specified conditions. After specified retention times, the excess and unreacted enzymes will be removed from the surface of the matrix [25] using a buffer solution. Another type of adsorption method is called affinity immobilization. Its mechanism works in two ways; (1) the attachment of support (e.g., lectin) onto a specific ligand (e.g., histidine) for a specific enzyme or (2) the linkage of the enzyme to a unit having an affinity towards the support [42]. The technique was applied in the biodiesel synthesis as in [47]. In terms of biofuel production, the adsorption method is not so preferred since the methanol addition may inhibit the immobilized enzymes’ activity in biodiesel synthesis [32] and the enzyme may suffer from great loss after the washing process thus making it difficult to be regenerated. Such examples of enzymes that are usually applied lipases immobilized on matrices such as acrylic resin and macroporous ZIF-8 [43].

Entrapment, on the other hand, is an irreversible immobilization method. It works in such a way that the enzymes are encapsulated within a specified region of networks that are made up of polymeric membranes and lattice material structure [25]. In this case, the movement of enzymes is restricted thus hindering the enzymes’ diffusion but still permeable to substrates and products. In [44], it is explained that the entrapment mechanism work in two steps; (1) enzyme homogenization with a monomer solution and (2) polymerization of the mixture through chemical reaction or altered conditions. Polymeric matrices such as alginate, hybrid bacterial cells, collagen, silicon rubber, polyurethane, and gelatin [25, 37] had been introduced by researchers and they were proven to reduce the leaching of the enzyme and preventing denaturation [44]. In biofuel production, for instance, de-oiled jatropha waste hydrolysate became the enzyme [37] to be entrapped within the hybrid bacterial cells in the production of biohydrogen together with lipase from Pseudomonas capacia which is encapsulated in a hybrid type of catalyst to generate biodiesel [30]. However, this method still suffers from limited mass transfer and possible leakage of the enzyme [45]. Covalent attachment is also classified as an irreversible immobilization technique. It implies the alteration of the enzyme structure itself that necessitates the binding via amino acids’ side-chain groups including lysine, cysteine, imidazole, and phenolic hydroxyl. This technique is considered better than other methods since it would create the existence of a prevailing bond between enzyme and matrices thus preventing the leakage of the enzyme to the environment [25]. It works in such a way that the strong nucleophile groups in the amino acid structure would become attracted and react to the electrophilic groups on the matrix [25]. This method should make the immobilization last longer, profound support between matrix and enzymes, and maximum stability to the enzymes [42]. The application of covalent attachment can be seen in [36] when polyacrylic resin and glyoxy1-agarose became the support for Accellerase β-glucosidase (BG) enzyme from
Trichoderma reesei in the bioethanol production.

As depicted by Table 1, it was also found that the cross-linking approach is also mostly preferred in biofuel production. This method is relatively easy and less complicated since it just entailed the reaction of cross-linking reagent and the free amino groups on the reactive site of the enzyme molecules [44].

4. Challenges and Opportunities

Despite lots of advantages offered by enzyme immobilization technology, there are also several challenges in conducting this technique. One of them is the selection in determining the most ideal immobilization technique for every substrate in biofuel production. Researchers currently still conducting trial and error experiments for different types of biofuels through an enzymatic reaction to get the most optimum condition and the highest product yield. Occasionally, the final product purity might be disrupted due to the addition of substrate onto the immobilized enzyme to become the hindrance factor for the enzymatic activity [32]. Even with their improved regenerative abilities, those enzymes are still known for their sensitivity towards pH and temperature thus make their activities easily affected by surrounding conditions by only a slight change. Besides, the procedures in synthesizing immobilized enzymes are sometimes tedious such as the extraction of protease from bacterial strain cells in biomethane generation [35] and require proper precautions in handling it. This will result in the time-consuming matter that would increase the slowness of the overall enzymatic reaction itself and result in the technique to be laborious to be implemented in large-scale production industries.

The commercialization of the enzyme immobilization technologies should be able to overcome these two main boundaries: first, the requirement in building the feasible biological reactor itself for the reaction, and second is the ability in controlling the extent of alteration in the enzymes’ structures themselves. Comparing to other conventional methods in biofuel generation, the application of enzymes is relatively expensive due to the necessity in creating a suitable environment for the enzymes as living cultured organisms in the reactor. The consumption of enzymes together with their matrices even occupies additional space in the reactor thus increasing the production cost. The mild condition of the reaction itself must be maintained because the extreme temperature would destroy the enzymes’ structure through denaturation and too low temperature would forbid high catalytic activities. The presence of matrices as impurities through the bonding process with enzyme also leads to the varying enzymes’ kinetic variations because of disruption towards the functional groups in their structures [50]. Mass transfer constraint also turns out to be the most common problem in enzymes’ immobilization owing to the existence of a barrier encapsulating the structure of the enzymes. For instance, the biobutanol and biomethane generation [39, 40] through the immobilization process is considered not economical since the retention time took hours rather than using free enzyme.

Currently, there are numerous studies conducted by researchers in improving those weaknesses. The most essential thing is that the propensity of the enzyme itself in maintaining its structures in catalytic activities. It is essential in determining the most suitable conditions for the enzymatic reaction to the extent of favorable of the process itself. Approaches such as the usage of blocking agents, the addition of additives to improve the enzymes’ stabilities and the introduction of cross-linking enzyme aggregate (CLEA) [50] are proven to enhance the enzymatic immobilization performance. Besides, the selection of the types of support for the enzyme even plays an important role in this matter even though further studies need to be conducted on that. In terms of biofuel production, enzyme immobilization technology would emerge as one of the most promising methods although it is yet to be studied and improved to be implemented aptly on a large industrial scale.

5. Conclusions

In biofuel production nowadays, the application of enzyme immobilization is indeed getting the attraction of industrialists in the account of its supremacy compared to other conventional methods. It undeniably possesses high efficiency, a more environmentally friendly, and greater yield of products. The risk exposure to hazardous chemicals could also be decreased. Despite greater cost in enzyme utilization, further research had been done in improving the economic factors by making use of various
microorganisms in nature as the source of enzymes and matrices apart from consuming biomass as substrates. The mild condition is also vital for the reaction to occur thus increases the sustainability value in the process aside from decreasing the risk of food vs fuel competition. Regardless of the introduction of numerous techniques in immobilization technology, lots of studies must be done to improve each of their performances since each of them possess significant disadvantages such as slow reaction and instability that could affect the economic value of immobilized enzymes. Other fields apart from chemical engineering including biology and chemistry also should work together in providing the best blueprint of immobilized enzymes especially in terms of the generation of potential renewable energies in nature for manifold applications in industries.

Acknowledgement

The authors are grateful to Universiti Teknologi Malaysia for the Collaborative Research Grant (PY/2019/03294) to finance this research work.

References

[1] Hosseinzadeh-Bandbafha H, Tabatabaei M, Aghbashlo M, Khanali M and Demirbas A 2018 Energy. Conver. Manage. 174 579
[2] Johnsson F, Kjärstad J and Rootzén J M 2018 Clima. Poli. 19 259
[3] Lam K M, Lee K T and Mohamed A R 2010 Biotech. Advan. 28 501
[4] Fossile D K, Frej E A, da Costa S E G, de Lima E P and de Almeida A T 2020 J. Cleaner Produc. 260 3
[5] AlNouss A, Mckay G and Al-Ansari T 2020 J. Cleaner Produc. 242 2
[6] Rial R C, Freitas O N, Nazário C E D and Viana L H 2019 Renewable Energy 149 20
[7] Singh A, Olsen S I and Nigam P S 2011 J Chem Techno. Biotecnho 86 1349
[8] Godin B, Lamaudière S, Agneessens R, Schmit T, Goffart J, Stilmant D, Gerin P A and Delcarte J 2013 Energy. Fuels 27 2589
[9] Zhang H, Liu T, Zhu Y, Hong L, Li T, Wang X and Fu Y 2020 Renewable Energy 145 1246-1248
[10] Dehghan L, Golmakani, M T and Hosseini S M H 2019 Renewable Energy 138 915
[11] Kesharwani R, Sun Z, Dagli C and Xiong H 2019 Applied Energy 242 1468
[12] Al-asadi M, Miskolczi N and Eller Z 2020 J. Cleaner Produc. 271
[13] Juliano M, Sarno M, Pasquale S D and Ponticorvo E 2020 Renewable Energy 162 124
[14] Malakar B, Das D and Mohanty K 2020 Renewable Energy 145 2724
[15] Leong W H, Lim J W, Lam M K, Uemura Y and Ho Y C 2018 Ener. Rev. 91 951
[16] Gifford M, Liu J, Ritmann B E, Vannela R and Westerhoff P 2014 Water Research 4
[17] Yang L, Ren L, Tan X, Chu H, Chen J, Zhang Y and Zhou X 2020 Bioresour. Tech. 315
[18] Yun C J, Hwang K O, Han S S and Ri H G 2019 Biomass Bioener. 127 1
[19] Thirunananthamathan R, Elango T and and Elangovan K 2020 Mater.Today.:Proceed.1
[20] Anto S, Mukherjee S S, Muthappa R, Mathimani T, Deviram G, Kumar S S, Verma T N and Pugazhendi A 2020 Chemosphere 242 10
[21] Beisson Y L and Peltier G 2013 Oilseeds & fats Crops and Lipids 20 1
[22] Wilk M, Brodzka A, Koszlewski D, Madej A, Paprocki D, Dobrowolska A Z and Ostaszewski R 2019 Biororg. Chem. 1
[23] Rodrigues R C, Virgen-Ortiz J J, Santos J C d, Murcia Â B, Alcantara A R, Barbosa O, Ortiz C and Lafuente R F 2019 Biotech. Adv. 37 746-750
[24] Vijayalakshmi S, Anand M, and Ranjitha J 2020 Microalgae Cultiv. Biofu. Prod. 251-254
[25] Mohamad N R, Marzuki N H C, Buang N A, Huynop F and Wahab R A 2015 Biotech. & Biotech. Equipment 1-6
[26] Pena S A, Carballares D, Sterlling R M, Murcia Â B, Alcãntara A R, Rodrigues R C and
Lafuente R F 2020 Biotech. Advan. 3
[27] Sarangapani P, Hudson S D, Jones R L and Douglas J F 2015 Biophys. J. 108 724
[28] Li S, Hu J and Liu B 2004 Biosyst. 77 25-26
[29] Angulo E, López C, Izquierdo Á C and Munarriz M 2018 J. of Bioosci. Bioeng. 126 1
[30] Kumar D, Das T, Girir B S and Verma B 2020 Renewable Energy 147 11
[31] Lee J H, Lee J H, Kim D S, Yoo H Y, Park C and Kim S W 2019 Bioresou. Tech. Rep 7.1
[32] Jayaraman J, Alagu K, Appavu P, Joy N, Jayaram P and Mariadoss A 2019 Renewable Energy 145 399
[33] Lopresto C G, Naccarato S, Albo L, Paola M G D, Chakraborty S, Curcio S and Calabro V 2015 Ecotox. Env. Safety 121 229-235
[34] Marín-Suárez M, Méndez-Mateos D, Guadix A, Guadix E M 2019 Renewable Energy 1
[35] Ushani U, Kavitha S, Johnson M, Yeom I T and Banu J R 2016 Env. Sci. Pollut Res. 24 813
[36] Borges D G, Junior A B, Farinas C S, Giordano R D L C and Tardioli P W 2014 Bioresour.. Tech. 167 206
[37] Kumar G, Sen B, Sivagurunathan P and Lin C Y 2016 Fuel 182 131
[38] Kim K H, Choi I S, Kim H M, Wi S G and Bae H J 2014 Bioresour. Tech. 153 47-54
[39] Schlager S, Neugabauer H, Haberbauer M, Hinterberger G and Sariciftci N S 2015 ChemCatChem 7 967-971
[40] Senko O, Gladchenko M, Maslova O and Efremenko E 2019 catalysts 9 1-13
[41] Jesionowski T, Zdarta J and Krajewska B 2014 Adsorption 1
[42] Badea M, Hayat A and Marty J L 2020 Methods in Molecular Biology (New York: Humana Press) p 189-190
[43] Hu Y, Dai L, Liu D, Dai W 2020 Biotech. Biofuels 13 1
[44] Vijalakshmi S, Anand M and Ranjitha J 2020 Microalgae Cultivation for Biofuels Production (London: Acaademic Press-Elsevier) p 256-257
[45] Thangaraj B, Solomon P R, Muniyandi B, Ranganthan S and Lin L 2019 Clean Energy 12
[46] Patel S K S, Gupta R K, Kondaveeti S, Otari S V, Kumar A, Kalia V C and Lee J 2020 Bioresour. Tech. 315 2-6
[47] Bandikari R, Qian J, Baskaran R, Liu Z and Wu G 2018 Bioresour.Tech. 249 354
[48] Es I, Vieira J D G and Amaral A 2015 Appl. Microb. Biotech. 99 2067-2068
[49] Kumar V and Thakur I S 2020 Bioresour. Tech. 307 1
[50] Fopase R, Paramasivam S, Kale P and Paramasivan B 2020 J. Env.Chem.Eng.8
[51] Aguieiras Ė C G, Cavalcanti E D C, Silva P R, Soares V F, Fernando-Lafuente R, Assunção C L B, Silva J A C and Freire 2020 Renewable Energy 148 689