Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
An efficient method to produce 1,4-pentanediol from the biomass of the algae *Chlorella ohadi* with levulinic acid as intermediate

Elena Benisvy-Aharonovich\textsuperscript{a,1}, Anat Zandany\textsuperscript{b,1}, Abed Saady\textsuperscript{a,1}, Yael Kinel-Tahan\textsuperscript{b}, Yaron Yehoshua\textsuperscript{b}, Aharon Gedanken\textsuperscript{a,⁎}

\textsuperscript{a} Department of Chemistry and Bar-Ilan Institute for Nanotechnology & Advanced Materials, Bar Ilan University, Ramat-Gan 52900, Israel
\textsuperscript{b} The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

**A R T I C L E   I N F O**

**Keywords:**
- *Chlorella ohadi*
- Levulinic acid
- 1,4-Pentanediol
- NMR
- Organic matter
- Green cytometry

**A B S T R A C T**

Today, the development of innovative methods for production of organic compounds from natural resources is an essential topic for many research groups in the worldwide. Levulinic acid is a platform for many important organic processes in the synthesis of natural products, pharmaceuticals, plasticizers, drugs and various other additives. In addition, 1,4-pentanediol which is a product of reduction of levulinic acid, is a valuable raw material in the chemical industry. Here, we report a highly efficient method for the production of levulinic acid from *Chlorella ohadi* algae using hydrothermal hydrolysis process by using HCl. Our methodology shows that the levulinic acid can be obtained in almost 90% molar yield compared to the glucose in *Chlorella ohadi*. Finally, we describe a one step reaction for the completely conversion of levulinic acid into 1,4-pentadiol in water using *S. cerevisiae* yeast as a catalyst.

1. Introduction

Our society is extremely dependent on fossil resources (petroleum, coal, natural gas) to meet the basic needs of energy, fuels, petro-chemicals etc. (Lucia et al., 2007; Pfaltzgraaf and Clark, 2014; Pinazo et al., 2015). The constant increase in demand for energy is driven mainly by the growth of global population and industrialization (Lee and Chang, 2008). These increasing energy demands in combination with the gradual depletion of fossil resources has led, before the break of the covid-19 coronavirus, to a steady rise of crude oil prices and, together with the need to diminish greenhouse gas emissions, are the main motivators for the exploration of renewable resources for the sustainable production of energy. Biomass is the only abundant and concentrated source of non-fossil carbon that is available on earth. It is a promising sustainable feedstock, that also fixes the CO\textsubscript{2} levels in the atmosphere through photosynthesis. A large amount of carbon-based chemicals is directly or indirectly produced from fossil resources (Yan et al., 2015a). The conversion of biomass to bio-based chemicals and biofuels has been the focus of intense research and development activities in the past decade (Yan et al., 2015a), with the major objectives to develop effective and environmentally benign technologies, as well as to stimulate the agricultural sector by, for instance, promoting the use of agricultural and forestry residues as input (Alonso et al., 2013; Ruiz et al., 2010; Tang et al., 2014; Yan et al., 2015b).

Among biomass, algae (macro and microalgae) usually have a higher photosynthetic efficiency and growth rate than other land plants, they do not need soil or land and many species can grow in salt water or waste water, this way its cultivation does not compete with agricultural resources such as freshwater and cropland that are usually used for food production. Recent studies show that different types of algae can produce a wide variety of organic materials such as; proteins (20–60% of the cell content), lipids (15–60%), polysaccharides (10–50%), etc. (Armenta and Valentine, 2013; Mata et al., 2010) and therefore, can be utilized as a source for the production of several types of renewable biofuels. These include methane, bioethanol, biodiesel and biohydrogen. One of the barriers to realizing the economical goal of fuel production based bio-refinery, is that the fuel, although being consumed in large volumes, is a low value product. Therefore, the return on investment in a bio-fuel only operations, does not compete with inexpensive fossil fuel production.

Bio-based fine chemicals provide the incentive needed for the bioindustry by maximizing biomass valorization and consequently improving the economic viability of bio-refineries. It is possible to extract...
small organic materials from algae that will be a platform for other organic materials (Ventura et al., 2017) such as biopharmaceuticals, bionutrients, biocosmetics, biochemicals and biofertilizers. Important chemicals which can be derived from cellulosic biomass provide direct substitutes for existing petrochemicals, exhibit strong potential as a primary building block for the production of a wide range of intermediates or end-products. These include: glucose, sorbitol, mannitol, sorbitan, isosorbide, furfural, HMF, levulinic acid, 2,5-furan dicarboxylic acid, succinic acid, xylitol, erythritol, glycerol, propylene glycol, methanol, ethanol, ethylene glycol, formic acid, glycol aldehyde etc. Nevertheless, further research is still needed to improve the production and use of these chemicals from plants.

Levulinic acid (also known as 4-oxopentanoic acid or δ-ketovaleric acid) is considered one of the important platform chemicals (Fig. 1) (Bozell et al., 2000; Ghorpade and Hanna, 1997). It is also a promising organic intermediate for the synthesis of several chemicals such as: levulinate esters, γ-valerolactone (GVL), acrylic acid, 1,4-pentadiol, angelica lactone, 2-methyltetrahydrofuran (MTHF), 5-amino levulinic acid (DALA), and so on. Literature shows potential uses as a plasticizer, textile, animal feed, coating, and as an antifreeze (Cha and Hanna, 2002; Khan et al., 2018). Additionally it is widely applied in the fields of fuel additives, fragrances, inks, batteries, chemical solvents, spices and pharmaceutical synthesis.

In spite of its great potential, levulinic acid (LA) has never been produced in significant volume. Levulinic acid can be produced by high temperature acid hydrolysis of carbohydrates, such as glucose, galactose, sucrose, fructose, citrate and also from biomeric material such as wood, starch and agricultural wastes (Bozell et al., 2000; Chang et al., 2007; Fang and Hanna, 2002; Rackemann and Doherty, 2011). Isolation of levulinic acid can be accomplished either by partial neutralization, filtration of humins and vacuum steam distillation, or by solvent extraction. Levulinic acid is a highly versatile chemical with several industrial uses (Ghorpade and Hanna, 1997).

The key factor that makes levulinic acid important is the presence of two reactive functionalities, namely, the carboxylic acid (–COOH) and the ketone (CO) groups, in the same molecule. This structural advantage facilitates the transformation of levulinic acid to various other chemicals (Fig. 2), like diphenolic acid, succinic acid, 1,4-pentanediol, etc. (Bozell and Petersen, 2010).

1,4-Pentanediol (PDO) Fig. 3, is a valuable raw material in the chemical industry for the production of organic solvents and polymers, and an important intermediate in the production process of medicines, pharmaceuticals, cosmetics, or fine chemicals. Total European and USA production was 18,000 tons in 2010 (Liu et al., 2020; Schiottmann and Lendle, n.d.).

The preparation of 1,4-pentadiol from levulinic acid is usually accomplished by a multi-step synthesis of hydrogenation processes and the use of various organic reagents and catalysts resulting in several byproducts (Li et al., 2014; Mizugaki et al., 2015; Patankar and Yadav, 2015). For example, Patankar and Yadav (2015) recently reported the use of the multi-catalyst Pd – Cu/ZrO2 to convert levulinic acid into 1,4-pentanediol in a single-step synthesis (Fig. 4).

It is worth mentioning that the economic value of 1,4-pentanediol is more than 500 times higher than that of levulinic acid.

Herein, we report in the first step: The successful Chlorella ohadi biomass conversion into levulinic acid. It should be emphasized that producing levulinic acid from biomass is attractive from economic as well as environmental aspects. The second step: levulinic acid conversion into 1,4-pentanediol in a single-step synthesis by using Saccharomyces cerevisiae as a safe, environmentally friendly, metal free, and non-toxic biocatalyst. The uniqueness of our work is based on the high molar yield of levulinic acid from the algae in a short period of time. In addition, the highly efficient and environmentally benign conversion process of levulinic acid into 1,4-pentanediol.

2. Materials and methods

2.1. Hydrothermal synthesis of levulinic acid from algal biomass (Chlorella ohadi)

The reaction was carried out in a cylindrical stainless-steel reactor with an internal polytetrafluoroethylene lined cell to resist corrosion by the acid catalyst. Typical batch process comprised loading 2.0 g of dry algal biomass Chlorella ohadi and 50 mL HCl (1 M) to the reactor. The reaction was heated to 200 °C for 1.5 h and subsequently quenched by immersing the reactor in an ice-cold water bath. The hydrolysate was collected and separated from the unreacted residual biomass by vacuum filtration through a filter paper. The residues were washed with excess distilled water and dried overnight at 60 °C and weighed. The difference between the initial and final weight of the biomass is a measure of the amount of biomass converted to the reaction products. The conversion percentage of the overall biomass (Chlorella) to hydrolyzed products mixture is approximately 79%. The isolation of levulinic acid from the obtained mixture was carried out by phase extraction in dichloromethane, and further rotor evaporation of the solvent and unnecessary byproducts. Levulinic acid was extracted from the oily residue with the addition of distilled water.

2.2. Evaluation of the glucose content in Chlorella ohadi biomass

The calculation of glucose level in algae was performed according to the protocol (Thayermanavan and Sadasivam, 1984). This test allows the estimation of the relative amounts of glucose that could be found in the algae as a polysaccharide or mono-sugar. Chlorella ohadi culture was grown in double concentrated Bristol medium (UTEX) (Bold, 1949), cultivated in 3L tubular photobioreactors (PBRs) under outdoors conditions (throughout the months of September and October). Algal biomass was chemically tested for its glucose content on a dry weight basis using the Anthrone method, in which the polysaccharides are hydrolyzed into simple sugars by acid hydrolysis. Glucose is then dehydrated to hydroxymethyl furfural in hot acidic media, this compound forms with Anthrone a green colored product. Estimation of the glucose content is made by measuring the absorbance of the solution in a spectrophotometer using a calibration curve. This test allows the estimation of the amount of levulinic acid that could be produced in the hydrothermal process. Namely, 1 g of glucose will theoretically yield 0.64 g of levulinic acid. Glucose content in Chlorella ohadi was measured to be 42.2 wt%.

2.3. Calculation of the molar yield for levulinic acid production from algae

At the end of the hydrothermal hydrolysis, filtration was done to separate the unreacted algae from the reaction mixture. The initial amount of reactive algae was 2 g in all the experiments. 42% of the reacted mass is glucose that has hydrolyzed. After the final separation of levulinic acid, and full drying of the product, we calculated the molar yield of the reaction by the ratio of levulinic acid and the reactive amount of glucose.
2.4. The characterization of levulinic acid by HPLC

Samples of commercial levulinic acid and the reaction product were used for HPLC analysis at room temperature using an ELITE LaChrom model analyzer, equipped with a RP-HPLC on a C-18 column (Phenomenex, Kinetex 2.6u C18 100A, 100 × 4.6 mm). The mobile phase was a 95:5 mixture of 0.01 M triethylammonium acetate (pH 7) and acetonitrile, and the flow rate was 1 mL/min. The detection wavelength was 220 nm for both the commercial levulinic acid and the reaction product. The two samples were dissolved in HPLC grade water,
and a sample of this solution was injected into the HPLC analyzer.

2.5. The characterization of levulinic acid using NMR

The samples were characterized by nuclear magnetic resonance using Bruker DPX-300 spectrometers. $^1$H and $^{13}$C NMR spectra were measured at 300 MHz. Chemical shifts are expressed in ppm, downfield from Me$_4$Si (TMS), used as internal standard.

2.6. The characterization of levulinic acid using mass spectroscopy

The samples were analyzed under ESI (electron spray ionization) conditions on a Q-TOF micro-instrument (Waters, UK).

2.7. Conversion of levulinic acid into 1,4-pentanediol

A mixture of 100 ml of 0.1 M solution of industrial levulinic acid (98%) and 3 g Saccharomyces cerevisiae was stirred on a hot plate (−34 °C) in a Schott Duran bottles under aerobic and anaerobic conditions, for 44 days. Samples were extracted at regular time intervals and taken for $^1$H and $^{13}$C NMR analyses. Monitoring the reaction’s progress was performed for all samples by comparing the resulting peaks in $^{13}$C NMR. In addition, mass spectrometry and HPLC were tested to verify the product’s receipt in the various examples.

3. Results and discussion

As previously described, production of levulinic acid was carried out in a cylindrical stainless-steel reactor with an internal polytetrafluoroethylene lined cell to resist corrosion by the acid catalyst. All reactions performed, started with two grams of the microalgae and 50 ml HCl (1 M). The described process was repeated three times on three different days. Table 1 presents our attempts to optimize the efficient reaction conditions.

3.1. $^1$H and $^{13}$C NMR of commercial levulinic acid

In order to determine the presence and purity of the levulinic acid at each stage of our process, we examined the hydrogen and carbon NMR spectrum for commercial levulinic acid so that we can compare it with the product obtained from the described process. The commercial product was tested using CDCl$_3$ as described, (see Supplementary information). $^1$H NMR of levulinic acid shows three different peaks, two doublets with integration of 2, and one singlet with integration of 3. However, as expected, $^{13}$C NMR shows five different peaks at 213.399, 177.186, 37.824, 29.211 and 27.887 ppm.

Table 1

| Starting weight of the algae (gr) | Temperature (°C) | Time (h) | Weight of product (mg) | HPLC purity level (%) | Molar yield$^a$ |
|---------------------------------|-----------------|----------|------------------------|-----------------------|----------------|
| 2                               | 200             | 1.5      | 299                    | 90                    | 72             |
| 2                               | 180             | 1.5      | 270                    | 88                    | 65             |
| 2                               | 160             | 1.5      | 248                    | 89                    | 58             |
| 2                               | 140             | 1.5      | 195                    | 81                    | 46             |
| 2                               | 120             | 1.5      | –                      | –                     | –              |
| 2                               | 100             | 1.5      | –                      | –                     | –              |

$^a$ RP-18 reverse phase column, applying isotropic elution with 95% triethylammonium-acetate (0.01 M, pH 7.4): 5% acetonitrile.

3.2. NMR test for the product of reaction after the first step

After the reaction was completed, a small sample was taken for NMR testing to verify the presence of the product levulinic acid. $^1$H and $^{13}$C NMR spectra show very clearly that we have obtained levulinic acid. Specifically, the successful formation of levulinic acid from the reaction mixture, is presented in the supplementary information, revealed by the appearance of a singlet signal at 2.2 ppm, two triplets at 2.65 and 2.78 ppm. $^1$H NMR spectrum of commercial levulinic acid procured from Sigma Aldrich is also presented here as a reference for our product.

Additionally, $^{13}$C NMR spectra showed several peaks that match those of levulinic acid at 28.38, 29.65, 38.29, and 177.99 ppm.

Moreover, we have performed a mass spectroscopy study for the reaction mixture in order to identify the presence of levulinic acid by measuring its molecular mass. Mass spectroscopy result showed the presence of the levulinic acid as described in the supplementary information.

Next, in order to evaluate the purity of the resulting levulinic acid we performed HPLC analysis compared to the commercial levulinic acid. Table 1 presents the purity of the levulinic acid in each sample determined by HPLC analysis. As described in Table 1, the resulting levulinic acid is obtained in a high degree purity (90%) from the reaction at 200 °C, which of course can be further purify by a series of acidic extractions.

After separation in HPLC, we repeated the $^1$H and $^{13}$C NMR for the resulting collected compound, and the resulting material is 98% pure.

3.3. Evaluation of the glucose content in Chlorella ohadi

As described in the Materials and methods section, we have evaluated the glucose level in the Chlorella ohadi. This calculation was performed in order to optimize the molar yield and the efficiency of our process. Our results show that every 2 g of algae has (42%) glucose, which is 0.84 g of glucose.

We can summarize that levulinic acid was synthesized from algal biomass, and successfully isolated from the product mixture by extraction in dichloromethane and rotor evaporation (evaporation under reduced pressure) techniques. We separated the LA after the first process, by extraction with dichloromethane and water. Then we used preparative HPLC in order to improve the quality of the separation as well as to evaluate the efficiency and purity of the product. The separation was performed in order to eliminate the residue (HCl). In addition, in an ideal form of work, it is generally better to separate the products of the reaction, after reactions in acidic or basic conditions in order to avoid side products in the next steps. The separation is necessary in our case for two reasons; 1) evaluate the efficiency and purity of the product. 2) avoid side product in the next step, especially, levulinic acid contains a ketone functional group, which maybe undergo Aldol condensation in the presence of acid catalyst.

Next, we have converted levulinic acid into 1,4-pentadial as described in the Materials and methods section. $^1$H and $^{13}$C NMR spectroscopy were exploited for the identification and estimation of 1,4-PDO. Initially, we recorded $^1$H and $^{13}$C NMR spectra of commercial 1,4-pentadial as a comparison. As described in the supplementary information. 1,4-Pentadial have five different peaks in $^1$H NMR, 4.03, 3.80, 1.46, 1.40, and 1.23 ppm. In addition, $^{13}$C NMR shows five different peaks at 22.469, 28.230, 34.862, 62.246, and 68.172 ppm.

The reaction was performed under different conditions (aerobic and anaerobic) for 44 days with the monitoring of the progress of the reaction. Table 2 summarizes the progress of the reaction.

As mentioned above, the reaction duration was about 44 days, and we started to detect the product appearance (1,4-PDO) on the 24th day. Fig. S10 shows a congruence between the commercial and synthesized 1,4-PDO samples, indicated by the appearance of peaks at 68.284, 62.356, 34.876, 28.251, and 22.492 ppm. The peaks of a levulinic acid
(214.744, 179.12, 38.763, 29.694, 29.312 ppm) and ethanol (58.064, 17.450 ppm) are also present. However, the weak intensity indicated very low remains of the starting materials.

As it was mentioned before, the reaction continued after the first appearance of 1,4-PDO for a subsequent 20 days. Our aim was to keep track of the reaction progress and the consumption of the reactant, levulinic acid. The metabolites mixture remained the same (for both aerobic and anaerobic conditions) for the additional 20 days.

Finally, HPLC separation was performed to verify the acceptance of the material (compared to commercial material), and certainly no levulinic acid remained. The result for the example after 24 days shows the 91% purity of 1,4-PDO and after 44 days shows the 100% purity of the 1,4-PDO.

It is worth noting, that the reduction of LA and other acids into the corresponding alcohol through green and simple chemistry has been a challenge for researchers in recent years (Liu et al., 2018). Moreover, selective conversion of LA into 1,4-pentadiol in high-efficiency in aqueous solution is an even more challenging (Li et al., 2014). The advantage of our methodology, is being able to achieve this goal, namely, complete conversion of LA into 1,4-pentadiol in aqueous solution by using *Saccharomyces cerevisiae*. Our hypothesis is that this method can be effective and applicable for other organic acids, to obtain selectively the corresponding alcohol in excellent yields.

Our results show that LA was completely converted into 1,4-pentadiol. These results were confirmed by several analytical methods. NMR and HPLC showed the absence of the starting material (LA), indicating at the end of the reaction. On the other hand, 1,4-pentadiol was the sole product. In addition, weight experiments showed that 0.9 g of 1,4-pentadiol (0.01 mol) were obtained from the reaction of 1.02 g of LA (0.01 mol).

4. Conclusions

We successfully obtained levulinic acid from an algae with 72% yield. Production of LA was accomplished via hydrothermal hydrolysis process. In addition, we reported our methodology of totally converting LA into 1,4-pentadiol, by using *Saccharomyces cerevisiae*. We suggest that *S. cerevisiae* participates in the reduction of the two functional groups of the levulinic acid by releasing a hydride or hydrogen into the solution (Parapouli et al., 2020; Styrger et al., 2011; Styrger et al., 2013). Applying this reaction in hot countries does not require energy investment, and since the yeast are very cheap, it can be considered a very cheap production of a costly chemical.

CRediT authorship contribution statement

Elena Benisy-Aharonovich: conducted the described experiment. Anat Zandany: conducted the described experiment. Abed Saady: investigation, methodology, recorded the NMR spectra. HPLC separation and optimization, write the article. Yael Kinel-Tahan: cultivating and providing the algae. Yaron Yehoshua: cultivating and providing the algae. Aharon Gedanken: supervised the experiments and editing of the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Research supported by The Ministry of Energy- Israel. Grant number 204512. A. Saady is supported by The Ministry of Science Technology & Space, Israel. Grant number 205272. The authors would like to thank TransAlgae Israel Ltd., a biotechnology company, for kindly giving us microalgae culture samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbitec.2020.100514.

References

Alonso, D.M., Wettstein, S.G., Dumesic, J.A., 2013. Gamma-valerolactone, a sustainable platform molecule derived from lignocellulosic biomass. Green Chem. 15 (3), 584–595.

Armanta, R.E., Valentine, M.C., 2013. Single-cell oils as a source of omega-3 fatty acids: an overview of recent advances. J. Am. Oil Chem. Soc. 90 (2), 167–182.

Bold, H.C., 1949. The Morphology of Chlamydomonas chlamydomonas, sp. nov. Bulletin of the Torrey Botanical Club. pp. 101–108.

Bozell, J.J., Petersen, G.R., 2010. Technology development for the production of biobased products from biofuels: carbohydrates—the US Department of Energy’s “Top 10” revisited. Green Chem. 12 (4), 599–554.

Bozell, J.J., Moens, L., Elliott, D., Wang, Y., Neuenschwander, G., Fitzpatrick, S., Bills, R., Jarnefeld, J., 2000. Production of levulinic acid and use as a platform chemical for derived products. Resour. Conserv. Recycl. 28 (3-4), 227–239.

Cha, J., Hanna, M., 2002. Levulinic acid production based on extrusion and pressurized batch reaction. Ind. Crop. Prod. 16 (2), 109–118.

Chang, C., Cen, P., Ma, X., 2007. Levulinic acid production from wheat straw. Bioresour. Technol. 98 (7), 1448–1453.

Fang, Q., Hanna, M.A., 2002. Experimental studies for levulinic acid production from whole kernel grain sorghum. Bioresour. Technol. 81 (3), 187–192.

Ghorpade, V., Hanna, M., 1997. Industrial applications for levulinic acid. In: Cereals. Springer, pp. 49–55.

Khan, A.S., Man, Z., Buzam, M.A., Kait, C.F., Naesrullah, A., Ullah, Z., Sarwono, A., Ahamd, P., Muhammad, N., 2018. Dicarboxylic ionics liquid as sustainable approach for direct conversion of cellulose to levulinic acid. J. Clean. Prod. 170, 591–600.

Lee, C.-C., Chang, C.-P., 2008. Energy consumption and economic growth in Asian economies: a more comprehensive analysis using panel data. Resour. Energy Econ. 30 (1), 50–65.

Li, M., Li, G., Li, N., Wang, A., Dong, W., Wang, X., Cong, Y., 2014. Aqueous phase hydrodegradation of levulinic acid to 1, 4-pentanediol. Chem. Commun. 50 (12), 1414–1416.

Liu, K.-j., Fu, Y.-l., Xie, L.-y., Wu, C., He, W.-B., Peng, S., Wang, Z., Bao, W.-H., Cao, Z., Xu, X., 2018. Green and efficient: oxidation of aldehydes to carboxylic acids and acyl hydrides with air. ACS Sustain. Chem. Eng. 6 (4), 4916–4921.

Liu, Q., Qiao, B., Liu, F., Zhang, L., Su, Y., Wang, A., Zhang, T., 2020. Catalytic production of 1, 4-pentanediol from furfural in a fixed-bed system under mild conditions. Green Chem. 22, 3532–3538.

Lucia, L.A., Argyropoulos, D.S., Adamopoulos, L., Gaspar, A.R., 2007. Chemicals, Materials, and Energy From Biomass: A Review, ACS Publications.

Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renew. Sust. Energ. Rev. 14 (1), 217–232.

Mizugaki, T., Nagatu, Y., Togo, K., Maeno, Z., Mitsudome, T., Struzkova, K., Kanesa, K., 2015. Selective hydrogenation of levulinic acid to 1, 4-pentanediol in water using a hydroxyapatite-supported Pt-Mo bimetallic catalyst. Green Chem. 17 (12), 5136–5139.

Parapouli, M., Vasileiadis, A., Afendra, A.-S., Hatziloukas, E., 2020. Saccharomyces
cerevisiae and its industrial applications. AIMS Microbiol. 6 (1), 1.
Patankar, S.C., Yadav, G.D., 2015. Cascade engineered synthesis of γ-Valerolactone, 1, 4-Pentanediol, and 2-Methyltetrahydrofuran from levulinic acid using Pd-Cu/ZrO2 Catalyst in water as solvent. ACS Sustain. Chem. Eng. 3 (11), 2619–2630.
Pfaltzgraf, L., Clark, J., 2014. Green chemistry, biorefineries and second generation strategies for re-use of waste: an overview. In: Advances in Biorefineries. Elsevier, pp. 3–33.
Pinazo, J.M., Domine, M.E., Parvulescu, V., Petru, F., 2015. Sustainability metrics for succinic acid production: a comparison between biomass-based and petrochemical routes. Catal. Today 239, 17–24.
Rackemann, D.W., Doherty, W.O., 2011. The conversion of lignocelluloses to levulinic acid. Biofuels Bioprod. Biorefin. 5 (2), 198–214.
Ri, J., West, R., Dumesic, J., 2010. Catalytic conversion of renewable biomass resources to fuels and chemicals. Annu. Rev. Chem. Biomol. Eng. 1, 79–100.
Schlottmann, U., Lendle, H., HPVChemicals, O. SIDS Initial Assessment Report For SIAM 13.
Styger, G., Jacobson, D., Bauer, F.F., 2011. Identifying genes that impact on aroma profiles produced by Saccharomyces cerevisiae and the production of higher alcohols. Appl. Microbiol. Biotechnol. 91 (3), 713–730.
Styger, G., Jacobson, D., Prior, B.A., Bauer, F.F., 2013. Genetic analysis of the metabolic pathways responsible for aroma metabolite production by Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol. 97 (10), 4429–4442.
Tang, X., Zeng, X., Li, Z., Hu, L., Sun, Y., Lin, S., Lei, T., Lin, L., 2014. Production of γ-valerolactone from lignocellulosic biomass for sustainable fuels and chemicals supply. Renew. Sust. Energ. Rev. 40, 608–620.
Thayermannavan, V., Sadasivam, S., 1984. Determination of total carbohydrates by anthrone method. Plant Foods Hum. Nutr. 34, 253–257.
Ventura, S., Nobre, B., Ertekin, F., Hayes, M., García-Vaquero, M., Vieira, F., Koc, M., Gouveia, L., Aires-Barros, M., Palavra, A., 2017. Extraction of value-added compounds from microalgae. In: Microalgae-Based Biofuels and Bioproducts. Elsevier, pp. 461–483.
Yan, K., Jarvis, C., Gu, J., Yan, Y., 2015a. Production and catalytic transformation of levulinic acid: a platform for speciality chemicals and fuels. Renew. Sust. Energ. Rev. 51, 986–997.
Yan, K., Yang, Y., Chai, J., Lu, Y., 2015b. Catalytic reactions of gamma-valerolactone: a platform to fuels and value-added chemicals. Appl. Catal. B Environ. 179, 292–304.