

**Microwave-assisted synthesis of levulinic acid from low-cost, sustainable feedstocks using organic acids as green catalysts**

Short title: Green synthesis of levulinic acid from biomass without pre-treatment

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### Abstract

**BACKGROUND:** Modern day scientific endeavour strives towards global sustainability through the smart utilisation of renewable resources as base materials for chemicals. Until now, the most common commercial process to produce levulinic acid (a mass-produced platform chemical) depends on a two-stage mineral acid-catalysed reaction, which generates harmful environmental waste. In this work, an environmentally friendly levulinic acid production route using less harmful organic acids assisted by microwave heating from biomass feedstocks is reported for the first time.

**RESULTS:** Using aluminium sulfate as a green Lewis acid catalyst and seven organic acids, levulinic acid was successfully produced from barley straw under microwave heating, with maleic acid giving the highest catalytic conversion. A Response Surface Methodology (RSM) approach was used to rapidly and effectively examine the effect of five reaction variables on the productivity of the levulinic acid. A wide range of different biomass wastes (barley straw, brewery waste, olive cake, spent tea leaves and potato, tomato and mandarin peels) were subsequently screened to produce the levulinic acid. The highest yield of 86 wt% based on cellulose content from mandarin peel (a value comparable to a lengthier ‘non-green’ route) was

achieved under the following optimized reaction conditions: 180 °C, 38 min, 2 M maleic acid concentration, 0.1 g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> and 1:22 biomass:maleic acid ratio (g mL<sup>-1</sup>).

**CONCLUSIONS:** The proposed method is a promising new route towards the green, high yield production of levulinic acid from a variety of agricultural and household lignocellulosic biomass wastes, without the need for pre-treatment.

## INTRODUCTION

The world is witnessing great economic advances in both the chemical and polymer industries to meet the requirements of modern society. Historically, industrial chemical production has depended heavily on fossil fuels, but due to the associated environmental problems, limited supply of raw materials, increasing energy demand and energy security, there is a pressing need to find alternative resources. Lignocellulosic biomass is one of the most promising candidate feedstocks because it is natural, renewable, inexpensive and abundant.

Among the diverse range of chemicals that can be derived from biomass, levulinic acid (LA) and its derivatives have received significant attention because they are a precursor to various high-value products such as polymers, resins, succinic acid, antifreeze materials, plasticizers, antimicrobial agents, food additives, flavouring agents, herbicides, solvents, textile dyes, agro-chemicals, green surfactants, chemical intermediates and fuel additives.<sup>1-6</sup> It is one of the US Department of Energy's top 12 bio-derived feedstocks and is the product of the acid hydrolysis of cellulose.<sup>7</sup> Several LA production routes employing different carbohydrate substrates, from

simple sugars to whole lignocellulosic biomass, have been explored over the last few years (see Table 1). These routes use homogenous acid catalysts, such as HCl and H<sub>2</sub>SO<sub>4</sub>,<sup>8-11</sup> fluorinated solvents/ acids, sulfonated solid catalysts, heteropoly acids, metal catalysts, carbonaceous materials, biphasic media and/or ionic liquids, *etc.*<sup>2,5</sup> At commercial scale, Biofine<sup>TM</sup> technology (Biofine Technology LLC, Brookline, MA, USA) is one of the most promising LA production processes to date and it involves a two stage mineral acid-catalysed reaction.<sup>2</sup>

The conversion of lignocellulosic biomass to LA occurs through a cascade of reactions illustrated in Fig.1, starting with biomass decomposition. The cellulose fraction is hydrolysed to glucose, which in turn isomerises to fructose under acidic conditions. This isomerisation step is crucial in attaining high yields of 5-hydroxymethylfurfural (HMF) in the next step, since HMF is produced via dehydration of a monosaccharide with a five-membered ring (furanose).

Conventionally, the isomerisation step can be catalysed by Lewis acids or Brønsted bases, such as Sn-Beta, HUSY zeolites, organic amines, ion-exchange resins, hybrid solid bases of mesoporous silica molecular sieves, group 4 and 5 metal oxides, transition metal oxides and chlorides (CrCl<sub>3</sub>, FeCl<sub>3</sub> and CuCl<sub>2</sub>) or group 13 metal chlorides (AlCl<sub>3</sub>), *etc.*<sup>5,12-14</sup> A metal-based Lewis acid acts as an electron acceptor during the isomerisation step.<sup>15</sup> Fructose dehydration to 5-HMF and the subsequent rehydration to LA are both catalysed by Brønsted acids. Commonly, strong mineral acids are used because they are efficient and inexpensive, but they can corrode equipment and generate harmful environmental by-products.<sup>16</sup>

Subsequent hydration of 5-HMF produces LA and formic acid.<sup>17</sup> During this process, furfural is formed as a by-product from pentoses released from the acid hydrolysis of hemi-cellulose. Acetic acid is created from the hydrolysis of acetyl groups derived from hemi-cellulose.<sup>18,19</sup> In addition, the acid soluble fraction of lignin can be hydrolysed forming various by-products, while the insoluble component will decompose and re-polymerize as part of humin,<sup>2,20</sup> a by-product of the condensation of HMF, fructose, glucose and other intermediates (see Fig. 1). These unwanted reaction pathways hinder LA production in significant quantities because they result in increased recovery costs and low product yields.<sup>2,21</sup>

Organic acids have been proposed as promising substitutes to mineral ones, because they are less corrosive, more selective, decrease humin production and can be degraded to nontoxic molecules.<sup>22,23</sup> For example, high and selective HMF yields of up to 64 wt% were achieved from hexose using carboxylic acids, such as acetic, formic and lactic acids, at 150 °C for 2 hours.<sup>24</sup> Moreover, Zang *et al.* reported that maleic acid can selectively dehydrate hexose to HMF and then LA, decreasing the amount of humin produced by 50% compared to mineral acids.<sup>14</sup>

In parallel, microwave irradiation has become an efficient heating technique to overcome the drawbacks of conventional heating, such as extensive heating time, use of high temperatures and insufficient energy transfer to the reaction.<sup>25-27</sup> For example, microwave irradiation combined with sulfuric acid, has been shown to decrease the reaction time from 8 h to 30 min with a 31.4 wt% LA yield from wheat straw,<sup>28</sup> whereas, even higher yields (up to 67 mol%) have been reported in the presence of metal halides and phosphoric acid.<sup>29</sup>

Herein, we report the production of LA from whole lignocellulosic biomass using organic acids instead of mineral acids under microwave heating for the first time. A Response Surface Methodology (RSM) analysis has been employed to examine the effect of reaction parameters on final LA yields and efficiently identify the optimum conditions for increased productivity based on a sustainable and more environmentally friendly process.

Seven biomass feedstocks were initially screened to identify the one with the highest percentage of cellulose (details provided in the Electronic Supporting Information, ESI), as this is the key material in the LA production. The best candidate (barley straw) was subsequently treated with seven different organic acids in the presence of aluminium chloride catalyst. These acids were chosen due to their low  $pK_a$  values, as most reported studies depend on strong homogenous acid catalysts, such as HCl and  $H_2SO_4$  to produce LA.<sup>2</sup> The most effective organic acid in converting cellulose to LA (*i.e.* maleic acid) was employed in a carefully designed optimization study using Response Surface Methodology (RSM). The effect of the reaction variables (*i.e.* temperature, time, biomass to acid ratio ( $g\ mL^{-1}$ ), acid concentration (M), and  $AlCl_3$  loading (g)) on the LA yield was investigated, and the most promising conditions were applied to three other ‘greener’ catalysts, namely aluminium phosphate, zeolite Y and aluminium sulfate. Finally, the best combination of reaction conditions and type of catalyst were used with the remaining six feedstocks, in order to demonstrate the wider applicability, scope and potential of our new synthetic approach for the production of LA from different low-cost starting materials.

## **EXPERIMENTAL**

### **Materials**

Barley straw, brewery waste and olive cake were provided by the Energy and Bioproducts Research Institute (EBRI, Aston University, Birmingham, UK), whereas spent tea leaves (black tea, UK brand), potato peel, tomato peel and mandarin peel were collected from domestic use. Hydroxymethylfurfural (5-HMF, 98%, Acros Organics<sup>TM</sup>); formic acid (99%, Acros Organics<sup>TM</sup>); levulinic acid (LA, >98%, Acros Organics<sup>TM</sup>); maleic acid (extra pure, Fisher Scientific); sodium hydroxide (certified AR for analysis, Fisher Scientific); malonic acid (99%, Fisher Scientific); tartaric acid (99%, Fisher Scientific); DL-malic acid (>99%, Acros Organics<sup>TM</sup>); aluminium chloride (98.5%, anhydrous, Acros Organics<sup>TM</sup>); oxalic acid (98% anhydrous, Alfa Aesar<sup>TM</sup>); aluminium phosphate (Acros Organics<sup>TM</sup>); zeolite Y (hydrogen, Alfa Aesar<sup>TM</sup>) and aluminium sulfate hydrate (98%, Honeywell<sup>TM</sup> Fluka<sup>TM</sup>) were purchased from Fisher Scientific (Loughborough, UK). Acetone ( $\geq 99.5\%$ ) was obtained from VWR International (Lutterworth, UK). Cellulose powder and ammonium fluoride ( $\geq 98\%$ ) were acquired from Sigma-Aldrich Company Ltd (Gillingham, UK). All purchased chemicals were of analytical grade and used without further purification (unless stated otherwise).

### **Biomass pre-treatment and determination of cellulose content**

Prior to determining their cellulose content by Thermogravimetric Analysis (TGA), the feedstocks (barley straw, brewery waste, olive cake, spent tea leaves, tomato peel, potato peel and mandarin peel) underwent pre-treatment to remove compounds that may interfere with the results. This involved drying, Soxhlet extraction, and treatment with sodium hydroxide (further details are provided in the ESI). The biomass with the highest cellulose content (*i.e.* barley straw) was used for subsequent optimisation experiments without any pre-treatment.

### **Production of LA from barley straw using different organic acids**

Maleic, acetic, oxalic, malonic, tartaric, formic and citric acids were selected as homogeneous acid catalysts and AlCl<sub>3</sub> was chosen as Lewis acid co-catalyst to promote glucose to fructose isomerisation. In each experiment, 17.5 mL of organic acid (1 M in water) and 0.11 g AlCl<sub>3</sub> were added to 0.7 g of dried barley straw (Biomass to acid ratio is 1 g to 25 mL) in a 50 mL glass vessel containing a PTFE coated magnetic stirrer bar. The reaction mixture was then heated to 175 °C (250 W, 220 psi, medium stirring) for 60 min under microwave irradiation using a CEM Discover S-Class microwave (CEM Corporation, Matthews, NC, USA). At the end of the reaction, the vessel was cooled to room temperature and centrifuged (Centrifuge 5804 fitted with a F-34-36-38 rotor, at 11000 rpm for 15 min at 25 °C; Eppendorf AG, Hamburg, Germany) to separate the liquid from the solid phase. The liquid phase was analysed by <sup>1</sup>H NMR spectroscopy to elucidate the composition of the reaction mixture (*i.e.* levulinic acid, formic acid, acetic acid, furfural and HMF) and the identified products were quantified using High Performance Liquid

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Chromatography (HPLC), whereas the solid phase (humin) characterisation was conducted using TGA and FTIR.

### *<sup>1</sup>H NMR analysis*

NMR analysis was carried out on an Avance-300 MHz spectrometer (Bruker, Billerica, MA, USA) at room temperature, recorded using a 5 mm normal dual detection probe and zg30 pulse program with 16 or 128 scans and referenced to the DMSO residual solvent peak at 2.50 ppm. Samples were prepared at 5% in deuterated dimethyl sulfoxide ( $d_6$ -DMSO).

### *HPLC analysis*

HPLC analysis was performed on an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) using a Hi-Plex H analytical column (Agilent Technologies, Santa Clara, CA, USA) operating at 50 °C, constant flow rate of 0.55 mL min<sup>-1</sup>, 210 and 267 nm UV detection wavelengths, and with a 25 µL injection volume. The mobile phase was 0.01 M sulfuric acid.

### *Calculation of the LA yield*

The LA yield based on cellulose content was calculated using Equations 1-4 (the example for barley straw is given):

$$W_1 = W_2 \times 40/100 \quad (\text{Equation 1})$$

where  $W_1$  is the weight of cellulose in the specific weight of the barley straw feedstock, g;  $W_2$  is the weight of barley straw that has been used in the reaction mixture, g; 40% is the percentage of cellulose in barley straw calculated by TGA.

$$W_3 = \left(\frac{W_4}{1000}\right) \times X_5 \times W_6 \quad (\text{Equation 2})$$

where  $W_3$  is the weight of LA in the whole liquid solution resulting from the reaction mixture after centrifuge, g;  $W_4$  is the weight of LA in the HPLC sample calculated using the calibration standard, mg;  $X_5$  is number of HPLC sample dilutions;  $W_6$  is the weight of the resulting solution, calculated by:

$$W_6 = W_7 - W_8 \quad (\text{Equation 3})$$

where  $W_7$  is the weight of the reaction mixture, g;  $W_8$  is the weight of the humin, g.

$$LA \% = 100 \times W_3/W_1 \quad (\text{Equation 4})$$

### **Response Surface Methodology (RSM)**

Design-Expert software version11 (Stat Ease Inc., Minneapolis, MN, USA) was used for the RSM analysis. Central Composite Design (CCD) was chosen as a standard RSM design for this experiment to study the effect of each factor alone, quadratic and two-way interactions between them. Five variables were used: temperature, °C; time, min; biomass to acid ratio, g mL<sup>-1</sup>; amount of catalyst, g; and acid concentration, M; with three levels for each. Numeric optimization was applied to maximize the yield of LA and identify the optimum conditions. p-values <0.05 were considered significant. The lignocellulosic biomass, acid and catalyst were

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barley straw (0.7 g), maleic acid and aluminium chloride, respectively, and remained unchanged for all RSM experiments.

### **Impact of different catalysts and feedstocks on LA yield**

Three more catalysts (namely aluminium phosphate, zeolite Y, and aluminium sulfate) were tested as alternatives to aluminium chloride, using barley straw. The most promising catalyst was subsequently tested with the following substrates: glucose; cellulose; brewery waste; olive cake; spent tea leaves; tomato peel; potato peel; and, mandarin peel. The microwave reactions were carried out according to the procedure described previously, using the optimum conditions identified from the RSM analysis for barley straw. Additional control experiments were conducted when no catalyst or acid was added. All feedstocks were used without any pre-treatment.

### **Catalyst recycling**

The aluminium content of the liquid and solid (humin) phases was determined by elemental analysis using a Thermo Fisher iCAP 7000 ICP-OES (Thermo Fisher Scientific, Waltham, MA, USA). The liquid sample was injected after filtration and 10 times dilution in deionised water. The solid sample was ‘digested’ prior to analysis in a mixture of nitric acid (70% v/v) and sulfuric acid (>95% v/v), using a CEM SP-D discover microwave at 175 °C for 30 min.

Ammonium fluoride was added to generate hydrofluoric acid *in situ* before neutralisation with boric acid (4% v/v). The sample was subsequently diluted (10x) with deionised water.

In order to recycle the catalyst, ion exchange resins were used to recover the aluminium from the reaction mixture. AMBERLITE™ IRC120 H ion exchange resin (DuPont de Nemours Inc, USA) was dried in a convection oven (SciQuip Oven-80 HT) at 100 °C for 12 hours, and then placed in a desiccator (Nalgene™ Transparent Polycarbonate Classic Design Desiccator, Fisher Scientific, Loughborough, UK) to cool down. A certain amount of resin (based on the aluminium content measured in the liquid) was subsequently packed into a glass column (12 ml capacity, 205 mm height; Sigma-Aldrich Company Ltd, Gillingham, UK) fitted with a stopcock. The reaction mixture was loaded to the column, and left for one hour before draining the liquid under vacuum (200 mbar). Sulfuric acid (first 0.6 M and then 3 M) was added to the resin in order to recover any bound aluminium (from one cycle). All liquid fractions were collected and analysed with ICP-OES.

### Humin characterization

Humin was separated from the liquid component at the end of the reaction and characterized using TGA and FTIR. Results are discussed in detail in the ESI (see Figs S15 and S16).

## RESULTS AND DISCUSSION

Out of the seven different biomass feedstocks tested in this work (barley straw, brewery waste, olive cake, spent tea leaves, tomato peel, potato peel and mandarin peel). Barley straw contained the highest percentage of cellulose (~40 wt%) and was therefore carried forward for subsequent process optimisation experiments (see ESI for cellulose percentages for other feedstocks).

### Production of LA from barley straw using different organic acids

To date, most studies aimed to produce LA have depended upon strong homogenous acid catalysts, such as HCl and H<sub>2</sub>SO<sub>4</sub>.<sup>2</sup> In an attempt to replace these environmentally harmful reagents with greener alternatives, strong organic acids (based on their low pK<sub>a</sub> values) were chosen to catalyse the synthesis of LA from barley straw. Following microwave heating and solid-liquid separation, the liquid fraction was analysed using <sup>1</sup>H NMR and HPLC to identify and quantify the components of the reaction mixture. According to literature the acid hydrolysis of lignocellulosic biomass produces furfural, formic acid, LA and acetic acid.<sup>30,31</sup> Fig. 2 shows the <sup>1</sup>H NMR spectrum for the maleic acid-catalysed reaction (refer to the ESI, Figs S8-S13 for the <sup>1</sup>H NMR spectra from the other organic acids). There are three signals from LA; one singlet at 2.06 ppm (CH<sub>3</sub> group), one triplet at 2.35 ppm (CH<sub>2</sub> group) and a triplet at 2.63 ppm (CH<sub>2</sub> group), the latter partially overlapping with a signal from malic acid (identified using a malic acid standard). It has been reported that fumaric acid and malic acid by-products can be obtained from an aqueous solution of maleic acid under high pressure and temperature,<sup>32</sup> while it is also

known that maleic acid can be converted into malic acid by hydration.<sup>33</sup> The solid fraction of the reaction mixture formed a fine brown powder after drying. Literature reports have shown that insoluble residues (humins) can be created during the acid-catalysed hydrolysis of lignocellulosic biomass as a result of polymerisation of the reaction intermediates.<sup>31,34,35</sup> Fig. 3 summarises the final LA yields based on cellulose content obtained from the different acids studied. Of these, citric and maleic acids produced the highest percentage of LA, *i.e.* 35 wt% (14±0.4 wt% based on raw biomass), whereas oxalic acid gave just under 10 wt% (4±0.1 wt% based on raw biomass) and no LA was detected when malonic acid was used. These low values are attributed to the low dissipation factors ( $\tan \delta$  values) of oxalic and malonic acids, resulting in the reaction only reaching 150 °C, rather than 175 °C attained for the other organic acids. Potential by-products were analysed and are discussed in detail (with corresponding quantities of each) in the ESI (See Table S1).

### **Optimization of LA production from barley straw using maleic acid and aluminium chloride**

Response surface methodology (RSM) is a statistical approach to experimental design that is used to study the effect of several variables and their interaction on the response ‘target of the experiment’. It enables the identification of the optimal conditions, in as few experiments as possible, for the best value of the response.<sup>36</sup> According to the findings described earlier in this work, maleic acid is the best organic acid to produce LA with the aid of microwave heating. The

reactions were therefore further optimized by RSM using Design Expert 11 to study the effect of five variables (acid concentration, M; amount of catalyst ( $\text{AlCl}_3$ ), g; reaction time, min; temperature of reaction,  $^{\circ}\text{C}$ ; and biomass to acid ratio, g  $\text{mL}^{-1}$ ) on the LA yield from barley straw. The factors and levels used are listed in Table S2. The RSM design resulted in 32 runs (Table S3) and run 17 showed the highest yield of LA, reaching almost 41.5 wt% (17.2 wt% based on raw biomass) (see Table S3). The yield of LA was the response factor and fitted to a second order polynomial model, as shown in Equation 5.

$$\gamma = \beta_0 + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_3 \chi_3 + \beta_4 \chi_4 + \beta_5 \chi_5 + \beta_{11} \chi_1^2 + \beta_{22} \chi_2^2 + \beta_{33} \chi_3^2 + \beta_{44} \chi_4^2 + \beta_{55} \chi_5^2 + \beta_{12} \chi_1 \chi_2 + \beta_{13} \chi_1 \chi_3 + \beta_{14} \chi_1 \chi_4 + \beta_{15} \chi_1 \chi_5 + \beta_{23} \chi_2 \chi_3 + \beta_{24} \chi_2 \chi_4 + \beta_{25} \chi_2 \chi_5 + \beta_{34} \chi_3 \chi_4 + \beta_{35} \chi_3 \chi_5 + \beta_{45} \chi_4 \chi_5 \quad (\text{Equation 5})$$

where  $\gamma$  is the response factor (*i.e.* wt% yield of LA);  $\chi_1, \chi_2, \chi_3, \chi_4$  and  $\chi_5$  are the independent factors;  $\beta_0$  is the model coefficient;  $\beta_1, \beta_2, \beta_3, \beta_4$  and  $\beta_5$  are the linear coefficients;  $\beta_{11}, \beta_{22}, \beta_{33}, \beta_{44}$  and  $\beta_{55}$  are the quadratic coefficients; and,  $\beta_{12}, \beta_{13}, \beta_{14}, \beta_{15}, \beta_{23}, \beta_{24}, \beta_{25}, \beta_{34}, \beta_{35}$  and  $\beta_{45}$  are the interaction coefficients.

The resulting final equation (Equation 6) in terms of actual factors can be used to make predictions about the response for given levels of each factor:

$$\begin{aligned} \gamma_{\text{LA yield}} = & -164.95330 + 3.41065_{\text{Acid}} + 0.143722_{\text{Time}} + 10.05463_{\text{Catalyst}} + 1.90885_{\text{Temp}} - \\ & 0.878181_{\text{Ratio}} - 2.48557_{\text{Acid} * \text{Catalyst}} - 0.014971_{\text{Acid} * \text{Temp}} - 0.000962_{\text{Time} * \text{Temp}} + 0.001556_{\text{Time} * \text{Ratio}} - \\ & 0.086609_{\text{Temp} * \text{Catalyst}} + 0.004414_{\text{Temp} * \text{Ratio}} + 23.80991_{\text{Catalyst}^2} - 0.005370_{\text{Temp}^2} \end{aligned} \quad (\text{Equation 6})$$

The accuracy of the model was verified by analysis of variance (ANOVA) testing (see Table S4). The model F-value achieved was 23.10, implying that the model is significant and that most of the variables have significant effects relating to the response (LA yield). Any factors that were calculated to be insignificant (*i.e.* p-value >0.05) were excluded from the model to improve it. These were: (i) acid with time; (ii) ratio with acid; (iii) time with catalyst; (iv) catalyst with ratio; (v) acid<sup>2</sup>; (vi) time<sup>2</sup>; and (vii) ratio<sup>2</sup>, resulting in a new ANOVA (Table S5). These quadratic and two-way interactions have no effect on the LA yield, whereas the rest of the factors have been shown to contribute to the enhancement of LA production (as their values have increased; see Table S5). The influence of temperature was found to be the most significant factor (highest coefficient value), followed by acid concentration, as shown in Table S6. In short, these findings show that the LA yield will increase with increase in temperature and acid concentration, until optimum values are attained. Model reduction improves the R-Squared (R<sup>2</sup>) statistics to 0.77 for predicted values, which is in reasonable agreement with the adjusted ones of 0.90; *i.e.* comfortably within 0.2 of one another (whereas the difference was >0.2 before deducting the insignificant terms; see Table S7). The ‘lack of fit’ was shown to be insignificant by ANOVA as revealed in Tables S4 and S5, which means that we can successfully use the design model as a response predictor. Fig. 4 summarizes these results and confirms that the actual experimental data are very close to the predicted values of our new model.

### *LA experimental yields under optimized conditions*

In order to prove the accuracy of the model, an experiment was conducted using the reaction variables from the RSM analysis (numerical optimization) which predicted the maximum attainable yield of LA with this new system (*i.e.* 45 wt%). These variables were 180 °C, 0.1 g aluminium chloride, 38 min, 2 M maleic acid, and 1 g of barley straw per 22 mL of maleic acid. LA yield of 42 wt% based on cellulose content (18 wt% based on raw biomass) was achieved, which is very close to the predicted optimum value. Furthermore, this was compared to values obtained using conventional heating (oil bath) instead of MW heating. Samples were taken and analysed after 38 min (the optimum time for the MW reaction) and 2.5 hours of reaction time. Data from HPLC and <sup>1</sup>H NMR showed the absence of LA at both time points. In line with previous work, MW heating decreased the reaction time from 8 h to 30 min during the production of LA from wheat straw.<sup>28</sup> Furthermore, in addition to the high amount of maleic acid by-product (which was expected since it was used as a solvent), conventional heating resulted in the production of 19 wt% fumaric acid, 29 wt% furfural and 2 wt% HMF which is significantly higher than the MW result.

### *The influence of reaction parameters on the LA yield*

The effect of linear variables on the LA yield is shown in Fig. 5, whereas Fig. 6 shows the most significant two-way interaction terms, *i.e.* the combined variable pairings that have the best results on product yield, reaching over 40 wt%. In this work, the temperature and acid

concentration are shown to have the most considerable effect on the production of LA, followed by catalyst dosage and biomass to acid ratio, whereas no remarkable influence of reaction time was revealed (notionally ascribed to the efficient heat transfer associated with MW heating). The LA yield increased with increasing temperature and acid concentration until an optimum value was reached (see Fig. 5 and Fig. 6(II)), in line with the findings of others.<sup>1,12</sup> This was as expected, since both of these parameters can enhance the hydrolysis of the lignocellulosic biomass. However, a decrease in the yield was recognized when temperature exceeded an optimum value (180 °C, see Fig. 5 and Fig. 6(III)), due to the formation of insoluble humin that can obstruct the reaction.<sup>37</sup> Catalyst dosage also had an influence on product formation especially at both low and relatively high doses (see Fig. 5). Figs 6(I) and (V) illustrate the significant interaction of catalyst loading with acid concentration and temperature, respectively. This is due to the positive effect of both increased temperature and acid concentration on cellulose degradation, which, in turn, requires a threshold catalyst quantity to follow the reaction pathway.<sup>17</sup>

Although higher LA yields could be achieved when smaller biomass to acid ratios are used,<sup>38,39</sup> the presence of excess water can inhibit the hydrolysis process as there is less catalyst for each mole of biomass to activate the reaction,<sup>10</sup> as shown in Fig. 5. The relationship between temperature and biomass to acid ratio in Fig. 6(VI) reveals a significant impact on LA yield when the reaction temperature was between 170 °C and 180 °C. Finally, the time of reaction appeared to only have a small effect on the final product compared to the other variables, and

good yields were achieved both after 30 and 60 minutes, as shown in Fig. 6(IV). The optimum time required to reach the highest LA yield depends heavily upon the other variables that were tested, as confirmed elsewhere.<sup>8</sup>

#### *Effect of catalyst on LA yield*

A preliminary study was carried out to examine the effect of different catalysts on the LA production from barley straw, under the optimum reaction conditions revealed by the RSM analysis (*i.e.* 180 °C, 0.1 g catalyst, 38 min, 2 M maleic acid and 1 g of biomass per 22 mL of acid). Aluminium chloride was used initially as a cheap, low-toxic and effective Lewis acid catalyst to accelerate reactions containing substrates with carbonyl groups (glucose and fructose in our case).<sup>13</sup> However, towards the aim of having a greener catalyst than aluminium chloride and at the same time keeping the advantageous characteristics of the aluminium metal as Lewis acid,<sup>15</sup> three other aluminium-based catalysts were tested: aluminium phosphate; zeolite Y; and aluminium sulfate. As can be seen from Fig. 7, all three candidates were able to catalyse the conversion of barley straw to LA, with aluminium sulfate resulting in a yield as high as aluminium chloride. This result further supports the creation of a green manufacturing route for LA production, since Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> is considered safer than AlCl<sub>3</sub> and is already used in the food industry and in some USA licensed vaccines (in the form of aluminium salts) (FDA (<https://www.fda.gov>)).

### *Recycling of the catalyst*

To strive towards a truly green process, recycling of the catalyst is important. As a proof-of-concept, the potential to recycle the catalyst was verified in our new process for the run carried out using the optimum reaction conditions (*i.e.* 1 g of barley straw, with 22 mL of 2 M maleic acid and 0.1 g aluminum sulfate, under MW conditions at 180 °C for 38 min). Elemental analysis showed the presence of aluminium in the liquid phase (~672 mg L<sup>-1</sup>), but not in the solid component (humin). An ion exchange resin was shown to bind all of the aluminium present in the crude LA product, as confirmed by ICP analysis, and, even after just one elution cycle, over 50 wt% of it was recovered as aluminium sulfate (0.6 M sulfuric acid released 1 wt%, whilst a stronger 3 M solution released 56 wt%). The eluted fractions were dried in a convection oven (SciQuip Oven-HT) at 100 °C until white crystals were formed, and after two additional washing and drying cycles, the recycled catalyst was ready to be used in further experiments. This proof-of-concept study shows that it is possible to recover the catalyst in our new process and needs further optimization with greener reagents.

### **Impact of feedstock type on LA yield using optimum reaction conditions for maleic acid and aluminum sulfate**

In order to demonstrate the wider applicability of the new LA synthetic route, a series of experiments were carried out involving six other biomass feedstocks, as well as glucose and cellulose substrates, in addition to barley straw, and the results are presented in Fig. 8. The

highest percentage of LA was produced from mandarin and potato peels, reaching 87 wt% and 65 wt% (based on cellulose content), and 19 wt% and 25 wt% (based on dry raw biomass) respectively. It is important to point out that the cellulose content of the mandarin peel used in this study was almost half of that measured in the barley straw sample, and that of the potato peel was very similar to the barley straw, however the final LA yields did not follow the same trend. Although cellulose is considered to be the main precursor for LA production, feedstock composition can have an influence on LA yield. This is further supported by Tukacs and co-workers,<sup>28</sup> who reported the negative effect of ash (inorganic content) and the positive effect of low molecular weight carbohydrates on the LA process yield from various biomass wastes. Furthermore, LA was not detected when no acid or catalyst was added to the reaction, which confirms the importance of combining both of them in the final process. Table 1 summarizes LA production yields reported in the literature to date, using various starting materials and reaction conditions and compares them with the best result obtained in this work. The highest yield (based on cellulose content) achieved from a process starting from a complex substrate (*i.e.* 71 wt% from orange peel) is still ~22% lower than the 87 wt% (based on cellulose content) from mandarin peel obtained in this work, with the additional benefit of the use of environmentally friendlier reaction conditions (*i.e.* organic acids *c.f.* mineral acids).

## CONCLUSION

A novel green route using organic acids and microwave heating has been designed and developed for the production of LA from low cost industrial and household lignocellulosic biomass wastes. Maleic and citric acids proved to be effective substitutes for the more conventional mineral acids, which are currently employed for the acid hydrolysis of biomass feedstocks to LA. Reduced reaction time, through the incorporation of microwave heating, combined with the use of a food grade, recyclable catalyst, ensured that our new synthetic route is environmentally friendly, whilst at the same time delivers the high process yields required. RSM analysis was proven to be a useful and reliable tool for the quick optimization of LA production, and was key to the development of the optimum reaction conditions, that when applied to different starting materials, resulted in the successful production of LA.

### **Author contributions**

KA did the experimental work. KD helped with the TGA and HPLC experiments. AO helped with microwave and ICP experiments. JW helped with the RSM. KA, PDT and ET wrote the manuscript and all authors edited it.

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## REFERENCES

- 1 Fang Q and Hanna MA, Experimental studies for levulinic acid production from whole kernel grain sorghum, *Bioresour Technol* **81**:187-192 (2002).
- 2 Rackemann DW and Doherty WO, The conversion of lignocellulosics to levulinic acid, *Biofuels Bioprod Biorefin* **5**:198-214 (2011).
- 3 Freitas FA, Licursi D, Lachter ER, Galletti AMR, Antonetti C, Brito TC, Nascimento RSV, Heterogeneous catalysis for the ketalisation of ethyl levulinate with 1,2-dodecanediol: Opening the way to a new class of bio-degradable surfactants, *Catal. Commun.* **73**:84–87 (2016).
- 4 Licursi, D, Antonetti C, Fulignati S, Giannoni M, and Galletti AMR, Cascade strategy for the tunable catalytic valorization of levulinic acid and  $\gamma$ -valerolactone to 2-methyltetrahydrofuran and alcohols, *Catal* **8**:277 (2018).
- 5 Chen, SS, Maneerung T, Tsang C D, Ok S Y, and Wang H C, Valorization of biomass to hydroxymethylfurfural, levulinic acid, and fatty acid methyl ester by heterogeneous catalysts, *Chem. Eng. J.* **328**:246-273 (2017).
- 6 Biancalana, L, Fulignati S, Antonetti C, Zacchini S, Provinciali G, Pampaloni G, Galletti AMR, and Marchetti F, Ruthenium p-cymene complexes with  $\alpha$ -diimine ligands as catalytic precursors for the transfer hydrogenation of ethyl levulinate to  $\gamma$ -valerolactone, *New J Chem* **42**:17574-17586 (2018).

- Accepted Article
- 7 Werpy T and Petersen G, Top value added chemicals from biomass: Volume I – Results of screening for potential candidates from sugars and synthesis gas, *Technical report* (2004).
  - 8 Chang C, Cen P and Ma X, Levulinic acid production from wheat straw, *Bioresour Technol* **98**:1448-1453 (2007).
  - 9 Girisuta B, Janssen L and Heeres H, Green chemicals, *Chem Eng Res Des* **84**:339-349 (2006).
  - 10 Yan L, Yang N, Pang H and Liao B, Production of levulinic acid from bagasse and paddy straw by liquefaction in the presence of hydrochloride acid, *Clean: Soil, Air, Water* **36**:158-163 (2008).
  - 11 Galletti AMR, Antonetti C, Ribechini E, Colombini MP, Di Nasso NN, and Bonari E, From giant reed to levulinic acid and gamma-valerolactone: A high yield catalytic route to valeric biofuels, *Appl. energy* **102**:157-162 (2013).
  - 12 Peng L, Lin L, Zhang J, Zhuang J, Zhang B and Gong Y, Catalytic conversion of cellulose to levulinic acid by metal chlorides, *Molecules* **15**:5258-5272 (2010).
  - 13 Tang J, Guo X, Zhu L and Hu C, Mechanistic study of glucose-to-fructose isomerization in water catalyzed by  $[Al(OH)_2 \text{ (aq)}]^+$ , *ACS Catal* **5**:5097-5103 (2015).
  - 14 Zhang X, Hewetson BB and Mosier NS, Kinetics of maleic acid and aluminum chloride catalyzed dehydration and degradation of glucose, *Energ Fuel* **29**:2387-2393 (2015).
  - 15 Carey FA and Sundberg RJ, Advanced Organic Chemistry, Part A: Structure and Mechanisms, 5<sup>th</sup> edition, Springer Science & Business Media, Berlin (2007).

- Accepted Article
- 16 Kootstra AMJ, Mosier NS, Scott EL, Beeftink HH and Sanders JP, Differential effects of mineral and organic acids on the kinetics of arabinose degradation under lignocellulose pretreatment conditions, *Biochem Eng J* **43**:92-97 (2009).
  - 17 Ahlkvist J, Formic and Levulinic Acid from Cellulose via Heterogeneous Catalysis, *PhD Thesis*, Umeå University, Umeå (2014).
  - 18 Danon B, Van der Aa L and De Jong W, Furfural degradation in a dilute acidic and saline solution in the presence of glucose, *Carbohydr Res* **375**:145-152 (2013).
  - 19 Fengel D and Wegener G, Wood: chemistry, ultrastructure, reactions, Walter de Gruyter, Berlin (2011).
  - 20 Licursi D, Antonetti C, Bernardini J, Cinelli P, Coltelli MB, Lazzeri A, Martinelli M and Galletti AMR, Characterization of the Arundo Donax L. solid residue from hydrothermal conversion: Comparison with technical lignins and application perspectives, *Ind. Crops Prod.* **76**:1008-1024 (2015).
  - 21 Hayes DJ and Hayes MH, The role that lignocellulosic feedstocks and various biorefining technologies can play in meeting Ireland's biofuel targets, *Biofuels Bioprod Biorefin* **3**:500-520 (2009).
  - 22 Kootstra AMJ, Beeftink HH, Scott EL and Sanders JP, Optimization of the dilute maleic acid pretreatment of wheat straw, *Biotechnol Biofuels* **2**:31 (2009).
  - 23 Lu Y and Mosier NS, Biomimetic catalysis for hemicellulose hydrolysis in corn stover, *Biotechnol Prog* **23**:116-123 (2007).

- Accepted Article
- 24 de Souza RL, Yu H, Rataboul F and Essayem N, 5-Hydroxymethylfurfural (5-HMF) production from hexoses: limits of heterogeneous catalysis in hydrothermal conditions and potential of concentrated aqueous organic acids as reactive solvent system, *Challenges* **3**:212-232 (2012).
  - 25 Adlington K, McSweeney R, Dimitrakis G, Kingman SW, Robinson JP and Irvine DJ, Enhanced ‘in situ’catalysis via microwave selective heating: catalytic chain transfer polymerization, *RSC Adv* **4**:16172-16180 (2014).
  - 26 Azcan N and Danisman A, Microwave assisted transesterification of rapeseed oil, *Fuel* **87**:1781-1788 (2008).
  - 27 Kappe CO, Microwave dielectric heating in synthetic organic chemistry, *Chem Soc Rev* **37**:1127-1139 (2008).
  - 28 Tukacs JM, Hollo AT, Retfalvi N, Csefalvay E, Dibó G, Havasi D and Mika LT, Microwave-Assisted Valorization of Biowastes to Levulinic Acid, *ChemistrySelect* **2**:1375-1380 (2017).
  - 29 Qin K, Yan Y, Zhang Y and Tang Y, Direct production of levulinic acid in high yield from cellulose: joint effect of high ion strength and microwave field, *RSC Adv* **6**:39131-39136 (2016).
  - 30 Jönsson LJ and Martín C, Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects, *Bioresour Technol* **199**:103-112 (2016).

- Accepted Article
- 31 Rackemann DW and Doherty WO, A review on the production of levulinic acid and furanics from sugars, *Int Sugar J* **115**:28-34 (2013).
  - 32 Gao Z, Chen W, Chen X, Wang D and Yi S, Study on the isomerization of maleic acid to fumaric acid without catalyst, *Bull Korean Chem Soc* **39**:920-924 (2018).
  - 33 Amoa K, Catalytic hydrogenation of maleic acid at moderate pressures, *J Chem Educ* **84**:1948 (2007).
  - 34 Hayes DJ, Fitzpatrick S, Hayes MH and Ross JR, The biofine process—production of levulinic acid, furfural, and formic acid from lignocellulosic feedstocks, in *Biorefineries—Industrial Processes and Products: Status Quo and Future directions*, ed by Kamm B, Gruber PR and Kamm M. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 139-164 (2006).
  - 35 Hoang TMC, Van Eck E, Bula W, Gardeniers JG, Lefferts L and Seshan K, Humin based by-products from biomass processing as a potential carbonaceous source for synthesis gas production, *Green Chem* **17**:959-972 (2015).
  - 36 Bezerra MA, Santelli RE, Oliveira EP, Villar LS and Escalera LA, Response surface methodology (RSM) as a tool for optimization in analytical chemistry, *Talanta* **76**:965-977 (2008).
  - 37 Wang P, Zhan SH and Yu HB, Production of levulinic acid from cellulose catalyzed by environmental-friendly catalyst, *Adv Mater Res (Durnten-Zurich, Switz)* **96**:183-187 (2010).

- Accepted Article
- 38 Lin H, Strull J, Liu Y, Karmiol Z, Plank K, Miller G, Guo Z, Yang L, High yield production of levulinic acid by catalytic partial oxidation of cellulose in aqueous media, *Energy Environ Sci* **5**:9773-9777 (2012).
  - 39 Shen J and Wyman CE, Hydrochloric acid-catalyzed levulinic acid formation from cellulose: data and kinetic model to maximize yields, *AIChE J* **58**:236-246 (2012).
  - 40 Galletti AMR, Antonetti C, De Luise V, Licursi D and Nassi N, Levulinic acid production from waste biomass, *BioResources* **7**:1824-1835 (2012).
  - 41 Huang Y-B, Yang T, Lin Y-T, Zhu Y-Z, Li L-C and Pan H, Facile and high-yield synthesis of methyl levulinate from cellulose, *Green Chem* **20**:1323-1334 (2018).
  - 42 Ding D, Wang J, Xi J, Liu X, Lu G and Wang Y, High-yield production of levulinic acid from cellulose and its upgrading to  $\gamma$ -valerolactone, *Green Chem* **16**:3846-3853 (2014).

**Table 1.** Summary of published literature on the production of LA and comparison with this work.

Substrate	Temperature (°C) & type of heating	Time (min)	Catalyst	LA yield (wt%) <sup>a</sup>	Compared with this work	Ref.
Orange peel	170 & Microwave	30	2 M H <sub>2</sub> SO <sub>4</sub>	71	Mineral acid	28
Potato peel	170 & Microwave	30	2 M H <sub>2</sub> SO <sub>4</sub>	20.4 <sup>b</sup>	Mineral acid	28
Wheat straw	200 & Microwave	60	37% HCl	49.3	Mineral acid, higher temperature, longer time	40
Wheat straw	200 & Microwave	25	37% HCl	52.5	Mineral acid, higher temperature	40
Wheat straw	210 & Conventional	37.6	3.5% H <sub>2</sub> SO <sub>4</sub>	68.8	Mineral acid, higher temperature	8
Cellulose	180 & Microwave	40	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	70.6 <sup>c</sup>	Simple substrate, longer time	41
Cellulose	170 & Microwave	60	1.5 M H <sub>3</sub> PO <sub>4</sub> & NaCl	67	Simple substrate, not green acid	29
Cellulose	180 & Conventional	1440	Niobium-based solid acids	52.9	Simple substrate, very long time	42
Glucose	180 & Conventional	6	Maleic acid & AlCl <sub>3</sub>	41	Very simple substrate	14
Glucose	149 & Conventional	500	1 M HCl	57	Very simple substrate, mineral acid, longer time	12
Mandarin peel	180 & Microwave	38	2 M maleic acid & Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	86.7	Organic acid, moderate conditions, lignocellulosic biomass	<b>This work</b>

<sup>a</sup> Levulinic acid yield based on cellulose content in biomass; <sup>b</sup> based on raw biomass; <sup>c</sup> Methyl levulinate

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## Figure legends

Figure 1. The reaction pathway to produce levulinic acid and other platform products from lignocellulosic biomass (reproduced from Ref 14).

Figure 2.  $^1\text{H}$  NMR spectrum (in  $\text{d}_6\text{-DMSO}$ ) of the liquid phase obtained from the reaction of barley straw with maleic acid and aluminium chloride. The structures show the potential (by-) products from the reaction: (a) levulinic acid, (b) acetic acid, (c) malic acid, (d) fumaric acid, (e) furfural, (f) formic acid and (g) maleic acid.

Figure 3. LA yields from barley straw using different organic acids with aluminum chloride. Solid bars: based on cellulose content; hashed bars: based on biomass content.

Figure 4. Predicted values of LA yield (based on cellulose content) from the RSM model versus experimental values.

Figure 5. Effect of linear variables on the LA yield. The dashed beams around the predictions show the boundaries acquired from least significant difference (LSD) calculations.

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Figure 6. 3D surface plots of the effect of two-way interaction variables on the yield of LA. Key: A is acid concentration, M; B is time, min; C is catalyst loading, g; T is temperature, °C; R is biomass to acid ratio, g mL<sup>-1</sup>.

Figure 7. LA yields using different catalysts and ‘optimum’ conditions for AlCl<sub>3</sub> as identified by RSM analysis (180 °C, 0.1 g catalyst, 38 min, 2 M maleic acid and 1 g of barley straw per 22 mL of maleic acid). Solid bars: based on cellulose content; hashed bars: based on biomass content.

Figure 8. LA produced from different biomass feedstocks under the following optimized reaction conditions (using MW heating): 180 °C, 38 min, 0.1 g Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, 2 M maleic acid, 1 g of biomass per 22 mL of maleic acid. LA yields from cellulose and glucose standards were 38 wt% and 44 wt% respectively. Solid bars: based on cellulose content; hashed bars: based on biomass content.

\*STL = spent tea leaves.

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Lignocellulosic biomass

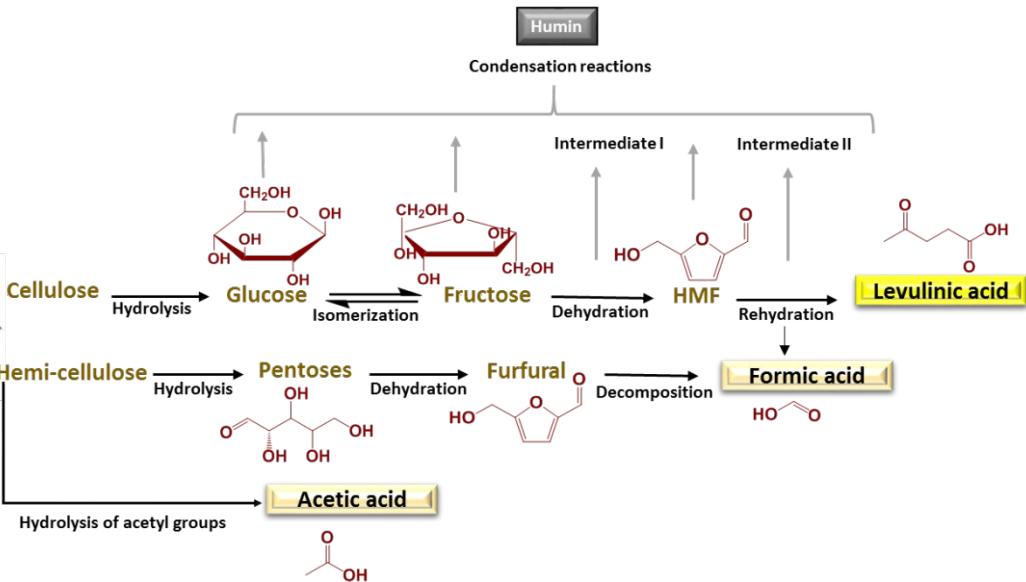
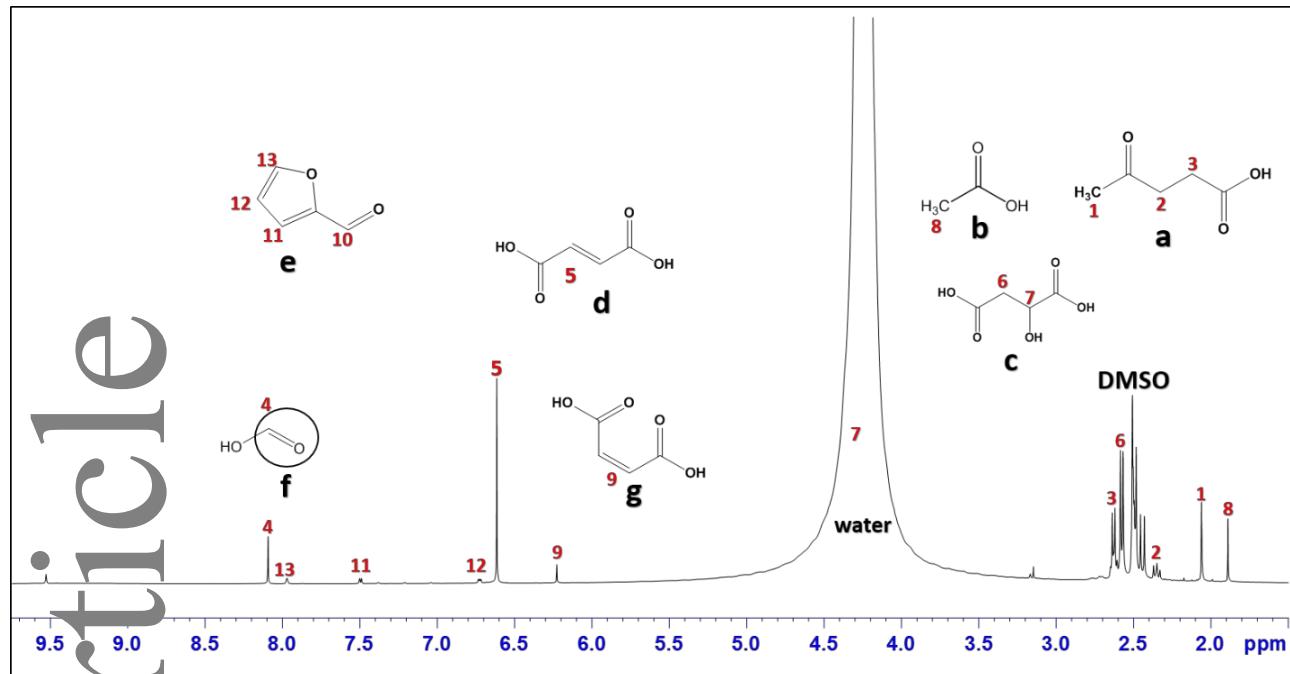


Figure 1

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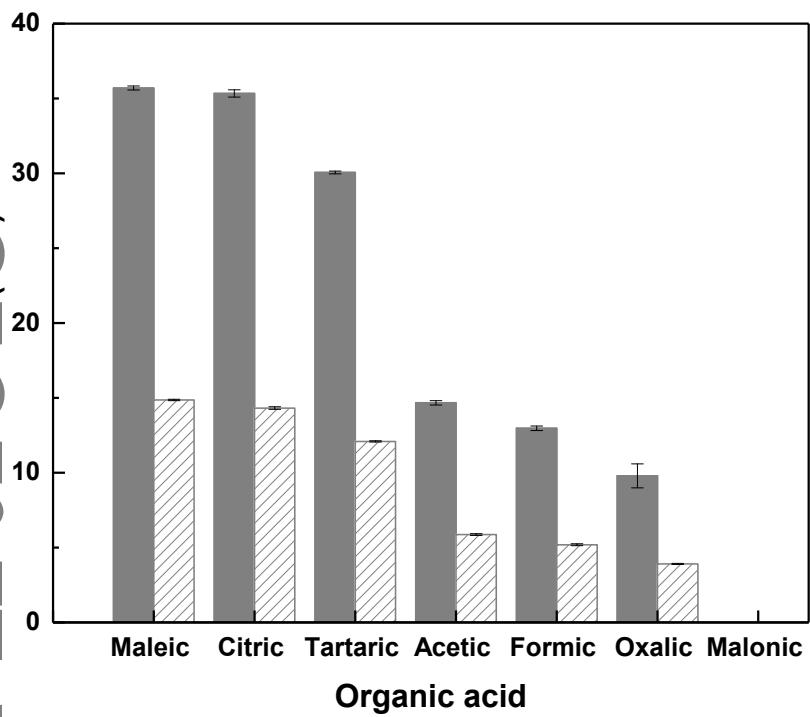
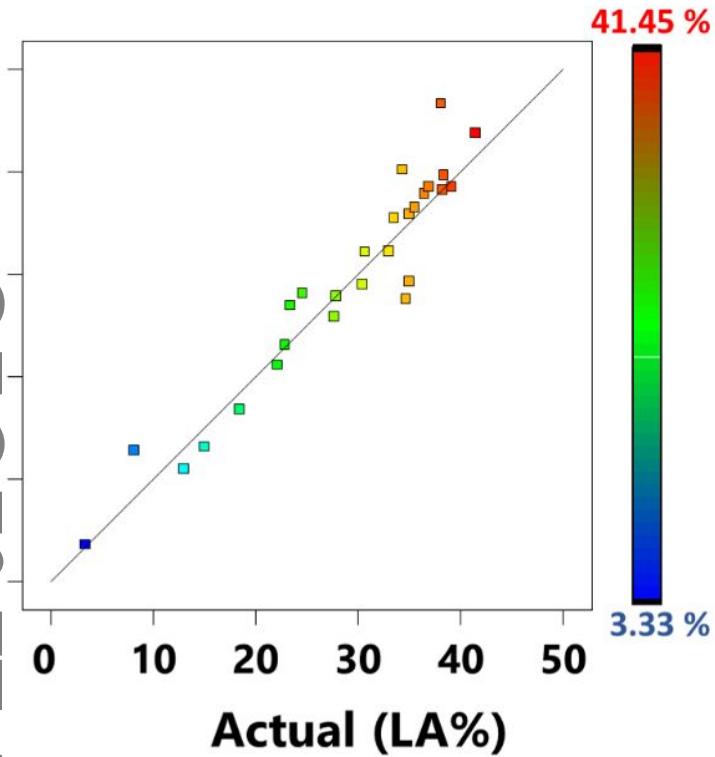


Figure 3

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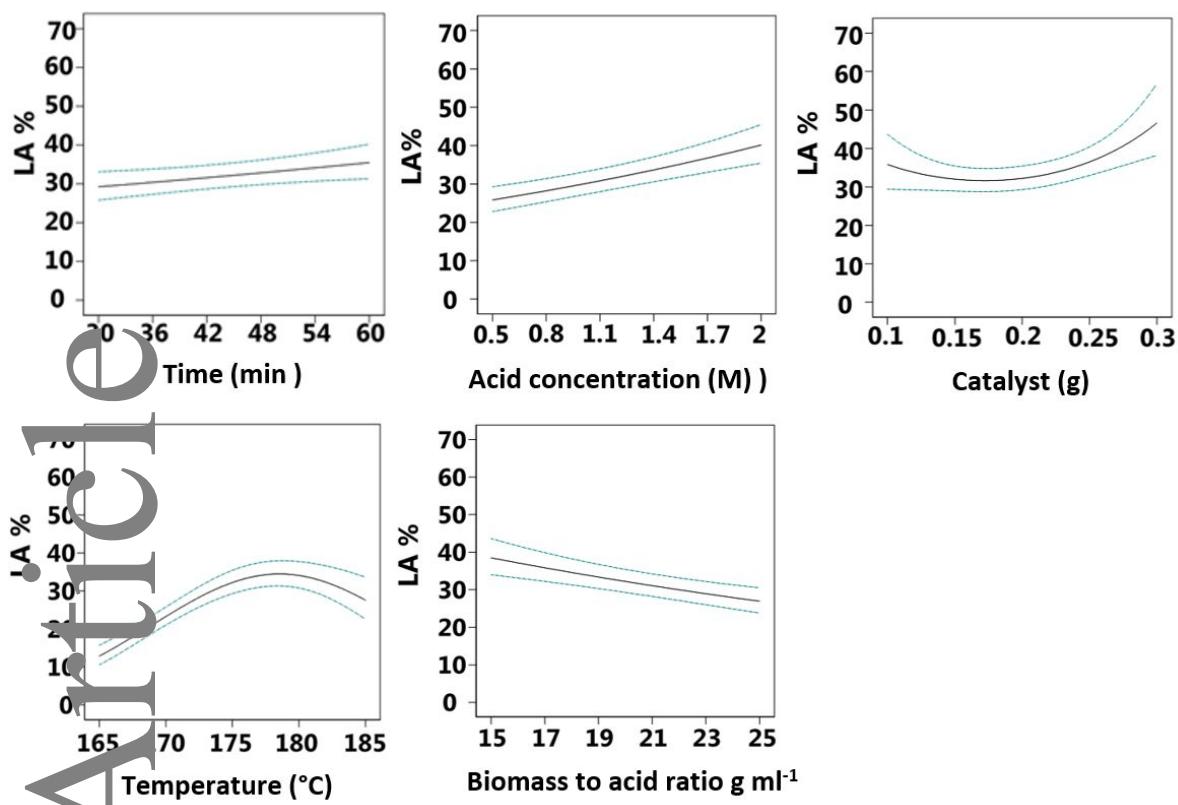


Figure 5

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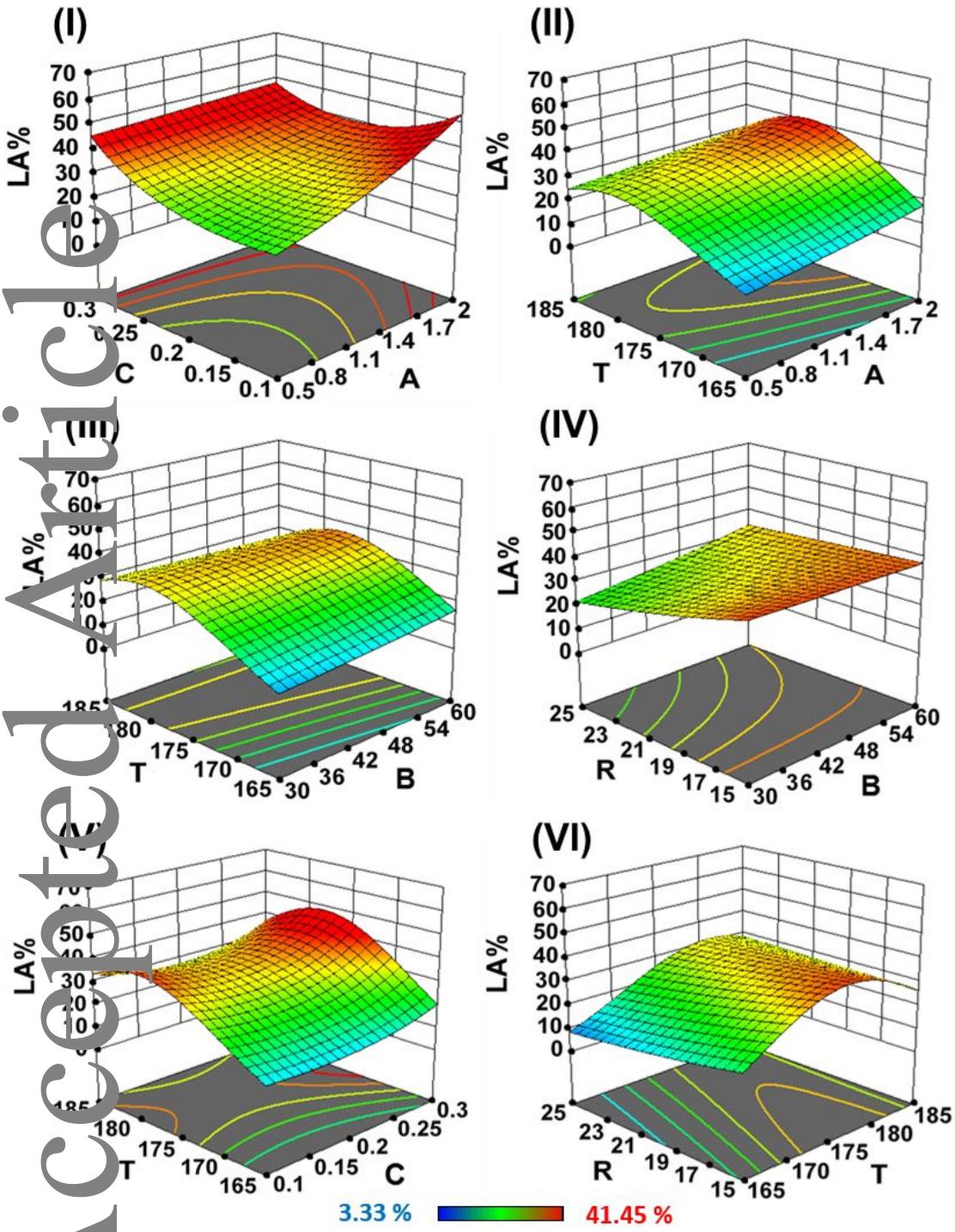


Figure 6

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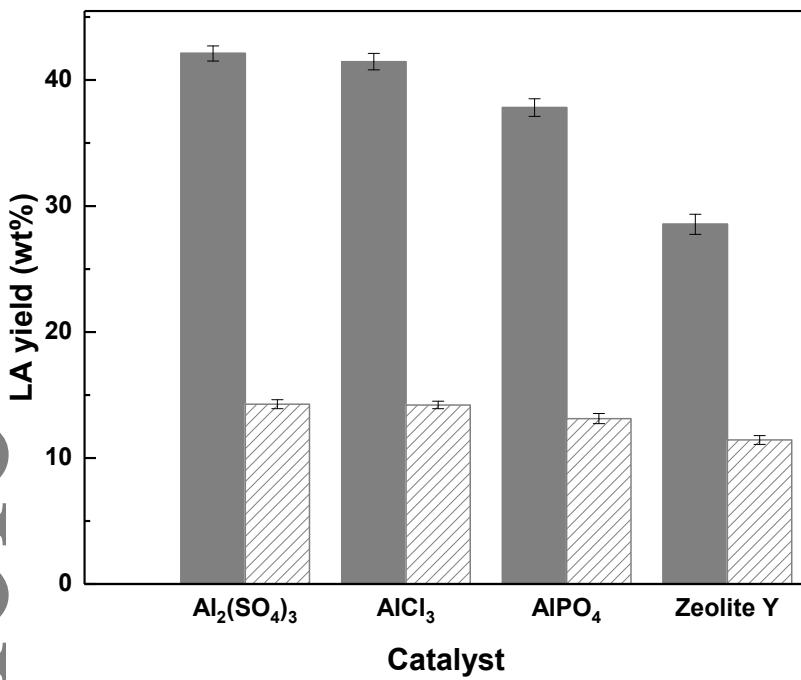


Figure 7

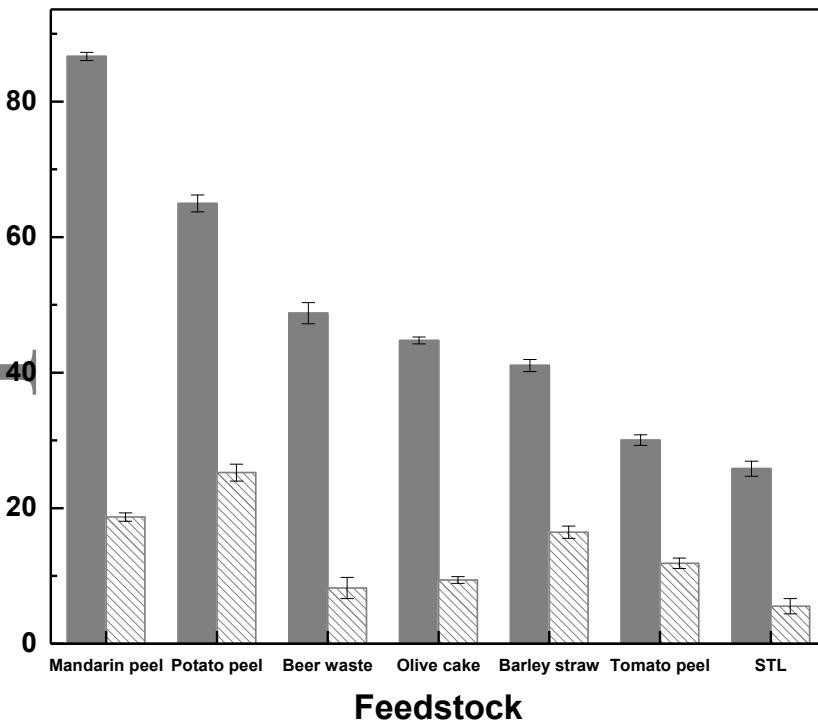


Figure 8